Studies on the micro-phase separation in mixed carrageenan gels using particle tracking

Dissertation Summary

STUDIES ON THE MICRO-PHASE SEPARATION IN MIXED CARRAGEENAN GELS USING PARTICLE TRACKING

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I. Purpose

Biopolymers as food hydrocolloids are generally utilized in food industries to control the texture of food products to meet the sensory preference of targeted costumers. Food hydrocolloids such as carrageenans are commonly as rheology modifier i.e. thickener, stabilizer and gelling agent. In addition to pure carrageenans, carrageenan mixtures are also utilized which provides greater controllability of the physical properties and texture. The three widely used carrageenans are the κ- (KC), ι- (IC) and λ- (LC) carrageenan. These carrageenans differ in the number of sulfate groups: one (G4S-DA) for KC, two (G4S-DA2S) for IC, and three (G2S-D2S,6S) for LC [1]. In aqueous environment, KC and IC exhibit gelling properties influenced by temperature and the presence of cations (especially K^+ and Ca^{2+}), while LC yields viscous solutions [2].

Due to its importance in industries, several studies ranging from macroscopic to molecular level has been performed to understand the gelation mechanism of these carrageenan gels as well as its mixtures. However, the gelation mechanism and network structure at microscopic point of view is still not well understood. In the present study, the gelation mechanism and network structure of pure carrageenan gels (KC, IC, and LC) as well as its mixtures were studied at the microscopic observation using particle tracking. In addition, the phase separated structure of mixture solutions of KC/IC and KC/LC were investigated.

II. Methodology

Sodium type KC and IC powder (Tokyo Chemical Industry Co., Ltd. Tokyo, Japan) was dialyzed against aqueous NaCl solution and subsequently against deionized water to obtain Na^+ type carrageenan. The concentrations of cations in these sample, analyzed by inductively coupled plasma atomic emission. For KC, Na^+ and K^+, were 0.4% and 0.11% while 0.63% and 0.105% for IC. Meanwhile, no Mg^{2+} or Ca^{2+} were detected in both dialyzed samples. LC was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used without further purification, except for preparation of pure LC solutions, as described below. The concentrations of cations were 4.71, 4.72 and 0.65% for Na^+, K^+ and Ca^{2+}. Samples for the particle tracking experiments and rheological measurements were prepared using the same procedure described

elsewhere [3,4]. Thereafter, fluorescent labeled probe particles were added to the solution at ~ 0.01% w/w (0.1 μm, Green, ThermoScientific). For preparation of pure LC solutions at 15 mM K^+ concentration, a small amount of the LC powder had to be dialyzed against pure water and mixed with untreated LC.

Mixture solutions of KC/IC and KC/LC were prepared by mixing pure KC and IC or LC solutions at different mixing ratios. Samples were designated using the code KC*x*(IC or LC)*y* (*e.g.*, KC50(IC or LC)50), where *x* and *y* indicate the percentage of KC and IC or LC out of the total polysaccharide content ($x + y = 100$), which was fixed to 1.5%. Samples for the rheological measurements were prepared using the same procedure except for the addition of probe particles.

Rheological properties were measured using HAAKE MARS II rheometer (Thermo Scientific, Waltham, MA, USA) equipped with serrated parallel plates (diameter 35 mm) with a one-mm gap. Hot sample solutions were loaded on preheated plates at 80 °C and covered with oil to avoid water evaporation. The storage modulus (G'), loss modulus (G'') and complex modulus (G^*) were monitored on cooling from 80 °C to 5 °C at a rate of 1 °C/min with a frequency of 1 rad/s and at strain of 1%.

Particle tracking measurements of fluorescent particles added to the carrageenan solutions were carried out using a BZ-9000 inverted microscope (Keyence Corp., Osaka, Japan) equipped with a PlanFluor 100 × NA 1.30 oil-immersion objective (Nikon Corp. Inc., Japan) and a temperature-controlled microscope stage (ALA Scientific Instruments Inc., New York). Hot sample solutions were placed in a custom-made sample chamber equipped with a temperature sensor (CENTER 309, Center Technology Corp., Tokyo) as described elsewhere [3,4]. Movies of the diffusing fluorescent-labeled particles were recorded using a built-in 2/3-inch, 1.5 megapixels, 12-bit, monochrome cooled CCD camera (Keyence Corp., Osaka, Japan) at a rate of 7.5 frames per second for 110 seconds. In cooling experiments, the particle tracking was performed at different temperatures from 50 °C to 10 °C. For measurement at low temperatures (gel state), a total of 60–80 particles were tracked simultaneously. For measurement at high temperatures, particles were observed to have high mobility and occasionally moved in-and-out of focus during the tracking and fewer particles (≈20–40 particles) were successfully tracked throughout the measurement. In storage experiments, hot solutions of carrageenan were cooled down from 50 °C to 5 °C at a rate of 1 °C/min in a temperature-controlled incubator to form a gel and stored at 5 °C prior to the particle tracking experiments, also done at 5 °C.

The position of each fluorescent labeled particle was determined using an algorithm that improves the accuracy of particle position using the image-intensity weighted centroid for each particle [5], from which

particle trajectories were calculated and analyzed. The time-averaged MSD for each particle (*msd*) obtained from *N* images representing a total diffusion time τ was calculated with the following equation [6]:

$$msd(\tau) = \frac{1}{N - \tau/\Delta t} \sum_{i=1}^{N-\tau/\Delta t} [r(i\Delta t + \tau) - r(i\Delta t)]^2 \qquad (1)$$

where Δt is the interval time for each frame, that is the inverse of the frame rate, and $r(\Delta t)$ is the position of the particle centroid. Particle tracking and MSD calculations were performed using a custom-made program written in Mathematica 10 (Wolfram Research, Inc., Champaign, IL) [5].

III. Experiments/Analysis

Particle tracking algorithm with improved accuracy of centroid coordinates and particle identification was developed for the microrheological experiments. In the algorithm, a new technique was introduced for the particle tracking by deciding a cut-off threshold for each particle based on the pixel intensity distribution for each image of local area around the particles. The developed algorithm was successfully utilized to track the 100 nm particle for up to 2000 s embedded in the carrageenan gels for the microrheological studies.

Using the particle tracking algorithm, the Brownian motion of 100 nm particles embedded in KC and IC gels were investigated. Based on bulk rheology measurements, KC gels exhibit characteristics of a hard and brittle gel, whereas IC gels form a soft and so-called “weak-gel”. Particle tracking revealed the differences in the local physical properties of the two gel systems, which are not accessible with the macroscopic rheological measurements. The mean square displacement (MSD) of the probe particles were investigated to characterize changes in the gel network structure on cooling and storage. The MSD of probe particles in KC demonstrated a trapped behavior within the network structures of KC gels while those in IC exhibited diffusive behavior even far below the gelling temperature, although the MSD decreased on storage. These results suggest that KC solutions formed a permanent gel network structure of KC chain aggregates that restricted the motion of particles on cooling. For IC at low temperatures, in contrast, the results suggested two possible structures: 1. clusters of IC chain aggregates, or, 2. a loose network with large pores, which allow the diffusion of particles and lead to weak-gel behavior. The aggregates further aggregate to form a more permanent gel network structure during storage.

The gelation mechanism and phase separated network structures of mixed KC and IC gels were also investigated using the particle tracking. The particle tracking of 100 nm particles revealed a high degree of heterogeneity in the microscopic rheology for the mixed gels as compared to the pure carrageenan gels. We consider that this heterogeneity came from the frozen structure on the way to phase-

separated network structure. Plotting the MSD of individual particles vs α with the MSD scaled as t^{α} and t the lag time suggested a bimodal distribution made up of fast and slow particles. The presence of two groups of particles with different mobilities suggested that mixtures of KC and IC was frozen on the way to a phase-separated network structures made of KC-rich and IC-rich domains with a size of >100 nm due to the network formation of KC and IC chains. These findings provide clarification on the phase separated structure of mixed KC and IC gels.

As a next step, the mixture of KC and LC (a non-gelling carrageenan) was investigated by macroscopic and microscopic observation using rheological measurements and particle tracking measurements. Based on the theoretical model on the rheological measurements the phase separated structure and phase transition from sea-island to bi-continuous and bi-continuous to island-sea structure were suggested. Particle tracking of individual particles following 1-day storage showed a large distribution especially for gels with low proportion of KC suggesting a microstructural heterogeneity. This heterogeneity was correlated with the phase-separated network structure made of KC-rich and LC-rich domains. These results serve as a concrete evidence on the phase separated structure of mixed carrageenan gels.

IV. Conclusion

The gelation mechanism and network structure of pure carrageenan gels as well as its mixtures were studied in the macro- and microscopic points of view using rheological measurements and particle tracking measurements. The local physical property obtained in the particle tracking were related to the overall mechanical properties of the gels.

Overall, this research focuses on the micro-rheological investigation and gelation mechanism of carrageenan gels and with an aim of understanding the interaction between different mixtures of carrageenan and ingredients, in general. In food industry, the interaction of ingredients greatly influenced the quality of foods. Thus, particle tracking provides significant information on the microscopic physical property of gels which is useful in improving and developing the food product with desired texture and physical properties to meet the consumers preference.

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