

STRUCTURE AND FORMATION PROCESS OF CROSSLINKING REGION OF CARRAGEENAN GELS

Makoto Takemasa, Akio Chiba, and *Munehiro Date

Department of Applied Physics, School of Science and Engineering, Waseda University

3-4-1 Okubo, Shinjuku-ku, Tokyo, 169-8555, Japan

FAX: 81-3-5286-3194, e-mail: takemasa@polymer.phys.waseda.ac.jp

*Kobayasi Institute of Physical Research

Dielectric properties and DC conductivity were measured in order to clarify the formation process of the cross-link and the network structure of carrageenan gels. DC conductivity sharply decreases at the coil to helix transition temperature, and the dielectric relaxation with the relaxation time $\sim 100\mu\text{s}$ with huge dielectric strength ~ 1000 arises. This is interpreted as the counterion condensation due to an increase of the charge density during coil-helix transition. This relaxation process can be ascribed to the fluctuation of counterions along high charge density region, in other words, aggregated region of helices. The fluctuation distance d estimated from the relaxation time is correlated to the length of aggregated helical molecules. It is found that the d increases sharply at the initial stage of gelation.

Key words: Gelation, Carrageenan, dielectric properties, viscoelasticity, strain optical coefficient

1. INTRODUCTION

Carrageenans are polysaccharides extracted from red seaweed. The primary structure is based on an alternating disaccharide-repeating unit of 1,3-linked β -D-galactose and 1,4-linked 3,6-anhydro- α -D-galactose. Its solution with specific counter ions forms physically cross-linked polyelectrolyte gel. Macroscopic properties of the gel depend on the amount of added salt and the counterion species.¹⁻⁴ This indicates that the crosslinking structure and the gel network structure depend on these conditions.

We are particularly interested in the formation process of the gel network structure and the cross-linking structure. In this study, the temperature dependence of the DC conductivity and the complex permittivity of Ca- and Na-form κ -carrageenan aqueous solutions were investigated. We can obtain information about the mobility of cations passing through gel network structure and the binding state of counterions to the helical molecules.

The charge density along the carrageenan chain increases with decreasing temperature due to the temperature induced coil to helix transition and the aggregation of helices. Then, the amount of the bound counterions increases, and the binding state of counterions should change in the sol-gel transition.

It is known that in the semi-dilute region two dielectric relaxation processes, the low frequency ($\sim\text{kHz}$) and the high frequency ($\sim\text{MHz}$) processes, are observed for linear polyelectrolyte solutions, such as DNA, polyacrylic acid, and polyglutamate.⁵⁻⁷ According to Ito *et al.*, the low and high frequency relaxations arise from the counterion fluctuations in the directions parallel and perpendicular to the polymer chain axis, respectively.⁸ The low frequency relaxation is ascribed to the counterion fluctuation tightly bound to polyion and the high frequency relaxation to the fluctuation of loosely bound counter-ions spreading over the average

distance between poly-ions. When the dielectric relaxation arises from the counter-ion fluctuation, the relaxation frequency f is characterized by $f \sim d^2/\mu$, where μ is the mobility of the counterion, which is proportional to the diffusion constant, and d is the fluctuation distance of the counter-ion.⁹

In carrageenan gels, an aggregation of helical molecules acts as a cross-link. The fluctuation distance must reflect the longitudinal length of the high charge density region, in other words, an aggregated region of helical molecules.

2. EXPERIMENTALS

2.1 SAMPLES

κ -carrageenan extracted from Irish Moss was purchased from Sigma Chemical Co. Ltd. We refined the calcium and sodium-form κ -carrageenan by following method: 1. dialysis with tube, 2. de-ionize with Amberlite IR-120 (H^+) cation exchange resin, 3. neutralized with NaOH and Ca(OH)₂, respectively.

Each cation content in the purified sample was determined by inductively coupled plasma spectrometry, and the sulfur content was determined by ion chromatography. The ratio of each cation to sulfur was listed in Table I.

	Sodium	Potassium	Calcium	Cesium
Na-form	99.4%	<0.01%	0.43%	0.15%
Ca-form	2.23%	0.77%	86.7%	10.3%

Table. I The molar ratio of cation content in the purified samples to the sulfur content. The ratio for Cs is calculated by subtraction of the other ratios for Na, K, and Ca estimated by ion chromatography from total.

2.2 APPARATUS

We used the parallel plate electrodes made of platinum black. In the case of the solutions with high DC conductivity, the loss tangent $\tan\delta$ increases exponentially with decreasing frequency, since the imaginary part of the complex permittivity ε'' obeys σ/ω . Thus, in the lower frequency, accurate measurements become more difficult. In this study, $\tan\delta$ reaches over 10^3 near the frequency 10^4 Hz. We tried to cancel the DC conductivity component before the amplification and digitizing the signal in order to measure the real part of complex permittivity more accurately, and then amplified the signal. The dielectric measurement was performed in the frequency range 10mHz-100kHz with this apparatus, and for 10^3 Hz- 10^6 Hz with Hewlett-Packard HP4284A impedance analyzer.

Temperature control was made by LAUDA RCS with an accuracy of $\pm 0.1^\circ\text{C}$. The sample was poured into the cell in the sol state, and keep at 90°C in 30min to dissolve completely.

3. RESULT AND DISCUSSIONS

Figure 1 shows the temperature dependence of dielectric dispersion ε' and absorption ε'' spectrum of 1.0wt% Ca-form κ -carrageenan solution in the frequency range $10^2 - 10^6$ Hz in the temperature range $2^\circ\text{C} - 60^\circ\text{C}$. The frequency dependence of ε' shows the characteristic behavior in the temperature range lower than 9.6°C , corresponding to coil-helix transition temperature. In contrast, in the case of the samples with non-gelling promoting cations of Na, the coil-helix transition does not occur in this ionic concentration, and the characteristic behavior was not observed.¹⁰

Figure 2 shows the spectrum of ε' and ε'' of 1.0wt% Ca-form κ -carrageenan solution at 60°C (a) and 5°C (b), corresponding to the coil (sol) and helix state (gel). The ε'' obeys $\varepsilon'' \sim 1/\omega$ in the wide frequency range 10^0 Hz- 10^6 Hz, which indicates that DC conductivity is a dominant effect in this frequency region.

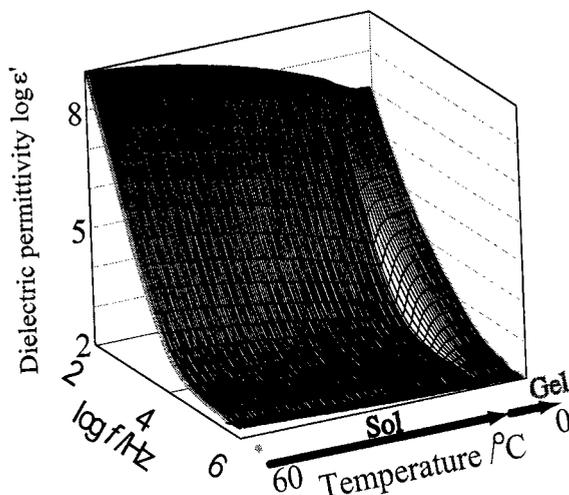


Figure 1 Temperature dependence of the dielectric properties of Ca-form κ -carrageenan in the cooling process.

The electrode polarization effect ε_{el}^* is represented by the empirical function,¹¹

$$\varepsilon_{el}^* = \frac{\varepsilon_{ep}}{(i\omega)^\gamma}, \quad (1)$$

where ω is the angular frequency, ε_{ep} and γ are the electrode polarization parameter.

The ε' exponentially increases with decreasing frequency below $\sim 10^3$ Hz due to an electrode polarization, as shown in Figure 2. We used the equation including the effects of DC conductivity and electrode polarization to reproduce the experimental data,

$$\varepsilon^* = \varepsilon_\infty + \frac{1}{\frac{i\omega}{\sigma} + (i\omega)^\gamma}, \quad (2)$$

where ε_∞ is the instantaneous permittivity and σ the DC conductivity.

We can well reproduce the data at 60°C (coil state) with eq 2 as a result of the non-linear least square fitting procedure, as shown in Figure 2, which indicates that DC conductivity and electrode polarization are dominant in this temperature. However, at 5°C we cannot reproduce the data with eq 2. We found that the systematic deviation between ε' and the fitting curve calculated from eq 2 in the frequency range $10^{2.5}$ - $10^{4.5}$ Hz though, due to the large $\tan\delta$, clear deviation was not observed. Only an addition of the dielectric relaxation term gave an excellent fit,

$$\varepsilon^* = \varepsilon_\infty + \frac{\Delta\varepsilon}{1+(i\omega\tau)^\beta} + \frac{1}{\frac{i\omega}{\sigma} + (i\omega)^{1-\gamma}}, \quad (3)$$

where $\Delta\varepsilon$ is the relaxation strength defined as $\varepsilon_0 - \varepsilon_\infty$, τ the relaxation time, and β the distribution parameter of relaxation time in Cole-Cole relaxation function. The residue resulting from the fitting procedure with eq 3 is much smaller than that with eq 2 at low temperatures below T_{CH} , as shown in Figure 3.

Temperature dependence of the estimated dielectric relaxation parameters τ , β , $\Delta\varepsilon$, and ε_∞ of Ca-form κ -carrageenan estimated by the least square fitting procedure is shown in Figure 3. The electrode polarization parameters ε_{ep} and γ become nearly constant in the entire temperature.¹⁰ Since the $\tan\delta$ becomes huge value $\sim 10^3$ near the 10kHz as already mentioned, the change of ε'' near the frequency $\sim 1/2\pi\tau$ cannot be clearly observed. The accurate estimation of the relaxation time τ becomes more difficult in lower frequency range, since an increased electrode polarization effect at low frequencies hid the dielectric relaxation process.

DC conductivity measurement has been used for detecting the conformational transition. According to Rochas and Landry, DC conductivity can be used to estimate the helical fraction, just corresponding to the optical rotation results.¹²

According to the Manning's counterion condensation theory, the bound counterion to the polyion is classified by the dimensionless charge density parameter ξ defined as,

$$\xi = \frac{e^2}{\epsilon b k T}, \quad (4)$$

where b is the distance between polyion, e the elementary of electric charge, ϵ the dielectric constant of solvent, k the Boltzmann constant, and T the absolute temperature.¹³ In the case of monovalent cation, if $\xi < 1$, bound counterion is classified to the loosely bound counterion. In the case that $\xi > 1$, the counterion is classified to the tightly bound counterion to polyion. The critical value is $1/z$, z is the counterion valence.

During coil-helix conformational transition, the charge separation distance b becomes shorter, which induces the increase of ξ . The b of κ -carrageenan at coil and helical state are estimated by, the chemical structure of the monomers and the helical structure using X-ray crystallographic data, respectively. In this way, we obtained $\xi_{coil} = 0.68$ and $\xi_{helix} = 1.65$.¹⁴⁻¹⁶

Resulting from the increase of the charge density during the coil-helix transition, bound counterions arise and cause the dielectric relaxation instead of decreasing the contribution to conductivity. Dielectric studies of bound counterions offer information of high charge density region, in other words, aggregated helical molecules. As mentioned in the introduction, tightly bound counterions can fluctuate along polymer axis, and can cause the dielectric relaxation.

The DC conductivity σ was estimated from the fitting procedure with eq 3. Figure 4 shows the temperature dependence of the σ of Ca and Na-form κ -carrageenan. The DC conductivity decreases with decreasing temperature for both samples. A sharp decrease was observed in a few degree of temperature just below the coil-helix transition temperature T_{CH} for the 1.0wt% Ca-form κ -carrageenan. The 1.0wt% Na-form κ -carrageenan aqueous solution is in coil state

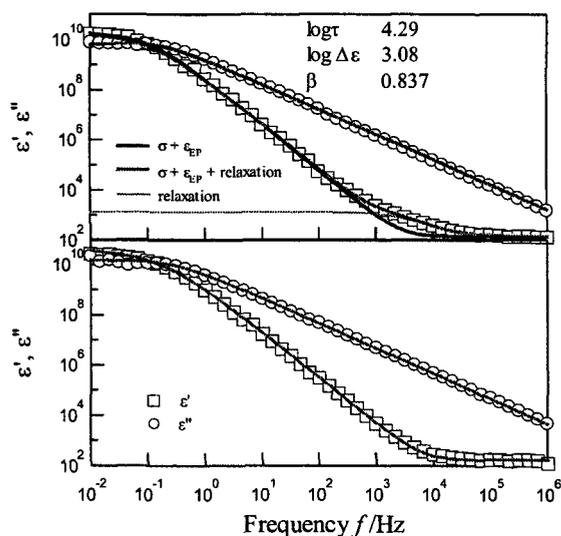


Figure 2 Frequency dependence of the dielectric properties of 1.0wt% Ca-form κ -carrageenan at 5°C and 60°C, corresponding to the sol and gel state. The solid curve in the sol state represents the superposition of two terms, DC conductivity and electrode polarization (see eq 2).

under this experimental condition in the entire temperature range. The σ decreases with decreasing temperature, and no significant change of ϵ' was observed in the entire temperature region, as shown in Figure 4. This means that only the diffusion coefficient decreases with decreasing temperature, and binding nature of counterions does not change.¹⁷ On the other hand, the solution exhibiting coil-helix transition, Ca-form κ -carrageenan, the sharp decrease of σ at the T_{CH} , which indicates that the change of the binding state of counterions. In fact, the dielectric relaxation with relaxation frequency ~ 10 kHz were observed. The dielectric relaxation function is needed to reproduce the data only at the temperatures lower than T_{CH} .

Since the increase of the dielectric relaxation strength and the decrease of the DC conductivity were observed at the temperatures lower than T_{CH} , as shown in Figure. 4, it is reasonable to consider that the dielectric relaxation process arises resulting from the increase of ξ . According to Ito *et al.*, the dielectric relaxation process at low frequency (\sim kHz) of linearly charged polyelectrolyte aqueous solution is ascribed to the fluctuation of counterions tightly bound to polyions

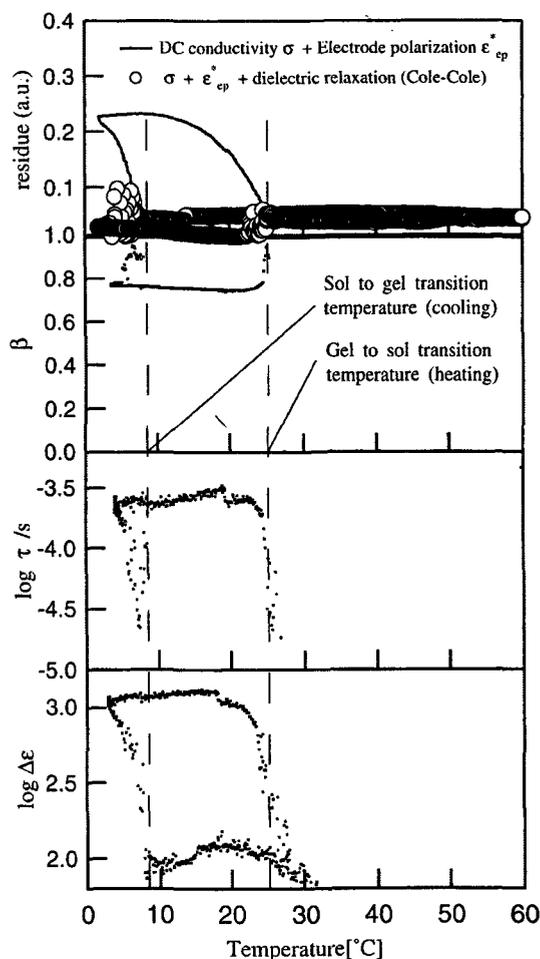


Figure 3 Temperature dependence of the residue resulting from the least square fitting procedure and the estimated dielectric relaxation parameters, τ , β , $\Delta\epsilon$, and ϵ_∞ for 1.0wt% Ca-form κ -carrageenan

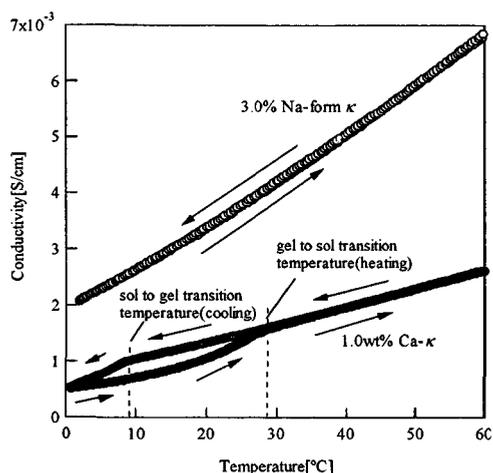


Figure 4 Temperature dependence of DC conductivity of 1.0wt% Ca- and Na-form κ -carrageenan. The cooling rate is $0.2^{\circ}\text{C}/\text{min}$.

along the polyion axis.⁸ When the dielectric relaxation arises from the counter-ion fluctuation, the relaxation frequency f is given by,

$$f \sim \frac{d^2}{\mu}, \quad (5)$$

where μ is the mobility of the counter-ion and d is the fluctuation distance of the counter-ion.⁹

Since the crosslinking mechanism of carrageenan gels has been considered as an association of helical molecules¹⁸, it is reasonable to consider that the fluctuation distance of counterions corresponds to the longitudinal length of the high charge density region, reflecting the associated region of helical molecules.

We have already reported that the stress inducing the polymer chain orientation, especially in helical molecules, increases with decreasing temperature during the formation process of gel network¹⁹. Although there are some possible reasons for enlarging the stress for orientation, the length and its distribution of the rigid segment, helical molecules, are important. Based on this study, the longitudinal length of the helices and their aggregated region increases in the initial stage of gelation and gradually reaches a constant value at low temperatures. This results support the suggestion that the enlargement the stress inducing polymer orientation is caused by the increase of the number of helical molecules bundled in a cross-linking region. Once the gel network is formed, it may be hard that the aggregated region grows due to the constraint by the other chains in the gel network.

4. CONCLUSIONS

The temperature dependence of the dielectric properties of κ -carrageenan aqueous solutions was investigated over the frequency range 10^{-2} - 10^6 Hz and the temperature range 2.0 - 80.0°C . In the case of solutions exhibiting the coil-helix conformational transition, just below the coil to helix transition temperature, T_{CH} , the DC conductivity sharply decreases within a few degrees of temperature. The dielectric relaxation process with the relaxation time $\sim 100\mu\text{s}$,

which can be assigned to the counterion fluctuation in the parallel direction to the helical axis, arises below T_{CH} . The relaxation strength increases sharply, reaching ~ 1000 at the temperatures far below T_{CH} . This indicates that the counterions are bound to helical molecules due to the increase of charge density along the helical axis during the coil-helix transition. The increase of the charge density due to the coil-helix transition induces the tightly bound counterions to helical molecules. The fluctuation distance estimated from the relaxation time increases sharply in the initial stage of gelation.

5. ACKNOWLEDGEMENT

The authors are grateful to Dr. Takaaki Sato for helpful discussion.

This work is partly supported by the grant-in-aid for scientific research from Japan Society for the Promotion of Science.

6. REFERENCES

- [1] L. Piculell, "Gelling Carrageenans", Food Sci. Technol. N. Y. (1995) pp. 205-244.
- [2] C. Rochas, M. Rinaudo, *Biopolymers*, **19**, 1675-1687 (1980).
- [3] M. Watase and K. Nishinari, *Rheol. Acta*, **21**, 318-324 (1982).
- [4] A. McKinnon, D. A. Rees, and F. B. Williamson, *Chem. Soc. Chem. Commun.*, 701-702 (1969).
- [5] S. Uemura, R. Hayakawa, and Y. Wada, *Biophys. Chem.*, **11**, 317-320 (1980).
- [6] F. van der Touw and M. Mandel, *Biophys. Chem.*, **2**, 231-241 (1974).
- [7] G. Muller, F. van der Touw, S. Zwolle, and M. Mandel, *Biophysical Chemistry*, **11**, 317-320 (1980).
- [8] N. Okubo, Y. Hirai, K. Ito, and R. Hayakawa *Macromolecules*, **22**, 1359-1366 (1989).
- [9] F. Oosawa, "Polyelectrolytes", Marcel Dekker, New York, (1971).
- [10] The counterion dynamics of κ - and ι -carrageenan aqueous solutions investigated by the dielectric properties, To be published.
- [11] Johnson, J. F.; Cole, R. H. *J. Am. Chem. Soc.*, **73**, 4536-4540 (1951).
- [12] C. Rochas and S. Landry, "Gums and Stabilizers for the Food Industry", IRL Press, Oxford, (1988) p 445.
- [13] G.S. Manning, *J. Chem. Phys.*, **51**, 924-933 (1969).
- [14] S. Nilsson and L. Piculell, *Macromolecules*, **23**, 2776-2780 (1990).
- [15] N. S. Anderson, J.W. Cambell, M. M. Harding, D. A. Rees, and J. W. Samuel, *J. Mol. Biol.*, **45**, 85- (1969)
- [16] S. Nilsson, L. Piculell, and B. Jonsson, *Macromolecules*, **22**, 2367-2375 (1989).
- [17] M. Ciszowska and Kotlyar, *Anal. Chem.*, **71**, 5013-5017 (1999).
- [18] E.R. Morris, D.A. Rees, and G. Robinson, *J. Mol. Biol.*, **138**, 349-362 (1980).
- [19] M. Takemasa, A. Chiba, and M. Date, *Macromolecules*, **34**, 7424-7434 (2001).

(Received January 30, 2002; Accepted April 2, 2002)