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BINDING STATE OF IONS IN CARRAGEENAN GELS INVESTIGATED BY DIELECTRIC AND CONDUCTMETRIC MEASUREMENTS

Makoto Takemasa, Takaaki Sato, 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
TEL/FAX: 81-3-5286-3194, e-mail: takemasa@polymer.phys.waseda.ac.jp
*Kobayasi Institute of Physical Research
Kobayasi Institute of Physical Research, 3-20-31 Kokubunji, Tokyo, 185-8533, Japan

In order to investigate the interaction between polymer and counterions and the binding state of ions bound to the polymers, the complex permittivity spectra $\varepsilon^*(\omega)$ of Ca-form κ-carrageenan aqueous solutions were measured at 25°C (coil state) in the cooling process, in the wide frequency range 10^{-2}–$10^{10.4}$Hz. The dielectric relaxation processes with the relaxation time τ_k and the strength $\Delta\varepsilon_k$ (k=1..4) were observed at the frequencies, ~10 kHz, 1 MHz, 100 MHz, and 10GHz. We especially focus on the two low-frequency processes (k=1,2), comparing the spectrum at 25°C (coil state) with that at 5°C (helix state). The presence of polymer and ion seems not to affect the state of water evidenced by the fact that τ_4~8.32ps and $\Delta\varepsilon_4$ ~72.0 characterizing a bulk water process (k=4) are almost the same as those of pure water. The k=2 MHz relaxation can be ascribed to the fluctuation of loosely bound counterions, whose relaxation parameters obey $\tau_2 \sim C^{-1}$ and $\Delta\varepsilon_2 \sim C^0$, where C is polymer concentration, in both the coil and helix states. We concluded that the calcium is bound more tightly to polymers than potassium and sodium.

Key words: Gelation, Carrageenan, Dielectric relaxation spectroscopy, Binding state of ion

1. INTRODUCTION

Carrageenan is natural polysaccharides extracted from red seaweed, whose 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. The carrageenan aqueous solution forms physically cross-linked gel under the suitable condition, at low temperature and in the presence of specific counter ions, such as potassium, cesium, and calcium. Although the gelation mechanism of carrageenan gels is still in dispute, the commonly accepted model exists, and is as follows: 1) The coil to helix conformational transition occurs at low temperature in the cooling process. 2) An association of helices is formed. 3) This association acts as a cross-link and gel is formed.[1] The sol to gel transition temperature and the macroscopic properties, such as mechanical modulus, of the gel strongly depend on the amount of added salt and the counterion species.[2,3,4,5] These indicates that the crosslinking structure and the gel network structure are dominated by the interaction between ion and polymer, especially the high charge density region (helix) compared with the coil region.

In this paper, we focus on the different kinds of bound counterions to the carrageenan molecules by using the electrical methods. Dielectric relaxation spectroscopy (DRS) is a powerful tool for detecting the motion of ions[6] and monitoring cooperative solvent processes.[7] We can obtain information about the amount and mobility of cations passing through charged gel network by using dc conductivity, and a various kind of binding state of ions to the charged macromolecules. We investigated in the previous study the binding states of ions, focusing on tightly bound counterions to the polymer in the gel state. The tightly bound counterions exists only near the high charge density region, and fluctuate along the polymer axis, which can be detected as the dielectric relaxation in the low frequency region (~kHz). The fluctuation distance reflecting the length of the high charge density region, an aggregated region of helices, can be estimated from the relaxation time of this kHz process.

It is known that the other diffusive modes causing the dielectric relaxation appear observed at the frequencies ~ 1MHz for linear polyelectrolyte solutions, such as DNA, polyacrylic acid, and polyglutamate,[8,9,10] and surfactants, such as hexadecyltrimethylammonium bromide, aqueous solutions.[11] According to Ito *et al.*, as for these polyelectrolyte solutions, the high frequency (~MHz) processes is ascribed to the induced polarization perpendicular to the polyion axis due to the fluctuations of loosely bound counterion vertical to the polyion axis.[12]

We performed broadband dielectric relaxation measurements. A concentration dependence of the $\varepsilon^*(\omega)$ spectra of Ca-form aqueous κ-carrageenans was examined focusing on the MHz relaxation process in order to clarify a peculiar binding nature of calcium ion, which had been expected from the most conspicuous behavior of the low frequency kHz relaxation.

2. EXPERIMENTALS

2.1 SAMPLES

We purchased κ-carrageenan extracted from Irish

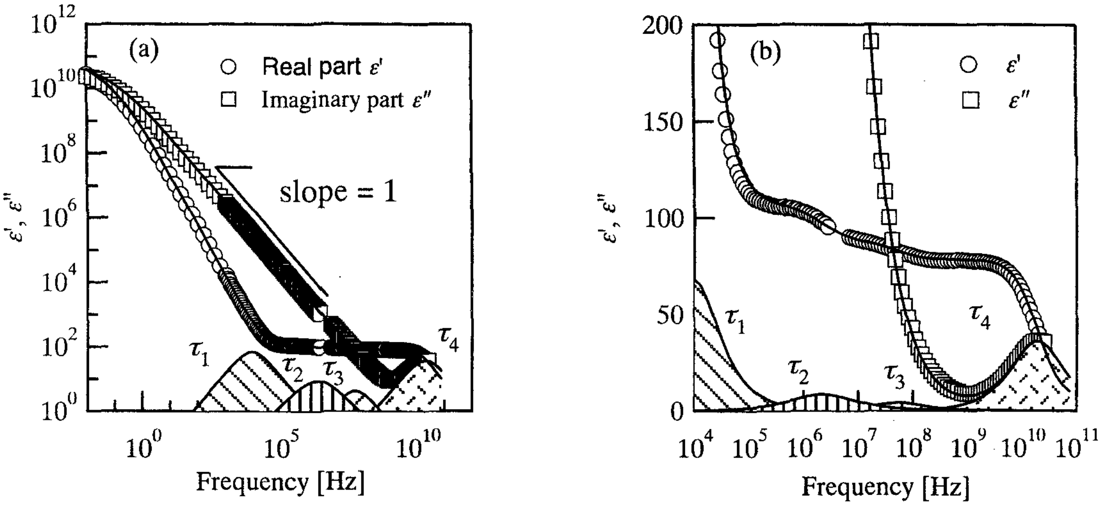

Figure 1 The dielectric dispersion and the loss spectrum of 50mM Ca-form κ-carrageenan at 25°C (the concentration is the monomer concentration). (a) A double logarithmic plot for overall view. (b) A magnified view in the linear scale of the vertical axis. The solid line was calculated from eq 3 by the least square fitting procedure.

Moss from Sigma Chemical Co. Ltd., and refined the calcium-form κ-carrageenan by following method: 1; dialysis with a cellulose tube, 2; de-ionize with Amberlite IR-120 (H^+) cation exchange resin, 3; neutralized with $Ca(OH)_2$. The content of various cations in the purified sample was checked by inductively coupled plasma spectrometry (ICP), and the sulfur content was checked by ion chromatography. The ratio of each cation to sulfur is listed in Table I.

2.2 APPARATUS

We performed the broadband dielectric measurements in the frequency range 10mHz-25GHz by the combinational use of the custom-built dielectric spectrometer in 10mHz-100kHz,[6] in 1kHz-1MHz Hewlett Packard HP4284A precision LCR meter, in 1kHz-10MHz HP4192A LF impedance analyzer, in 1MHz-1GHz HP4191A RF impedance analyzer, and in 100MHz-25GHz HP54121A/HP54120B time domain reflectometer (TDR). We used the parallel plate electrodes for the impedance analyzer, and the coaxial and flat-end capacitor cells for TDR. A temperature control was made by LAUDA RCS with an accuracy of ±0.1°C. The sample was poured into the cell in the sol state.

3. RESULT AND DISCUSSIONS

In Figure 1, we present the $\varepsilon^*(\omega)$ spectrum, the dielectric dispersion ε' and absorption ε'', of 50mM Ca-form κ-carrageenan aqueous solution at 25°C in the frequency range 10^{-2} – $10^{10.4}$Hz. The concentration is the monomer concentration.

	Na	K	Ca	Cs and others
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.

The "apparent" dielectric relaxations observed at the low frequencies with a huge dispersion amplitude result from the electrode polarization and dc conductivity. The behavior of ε'' obeying $\varepsilon'' \sim \omega^{-1}$ observed in the wide frequency range 10^0Hz-10^7Hz indicates dc conductivity, which is represented by,

$$\varepsilon^* = \frac{\sigma}{i\omega}, \tag{1}$$

where the σ is the dc conductivity. The increase of ε' with decreasing frequency at the low frequencies below $\sim 10^3$Hz is apparently due to an electrode polarization, whose the contribution ε_{el}^* is empirically described by,[13]

$$\varepsilon_{el}^* = \frac{\varepsilon_{ep}}{(i\omega)^{\gamma}}, \tag{2}$$

where ω is the angular frequency, ε_{ep} and γ are the electrode polarization parameters. The apparent dielectric relaxation observed at lowest frequencies with

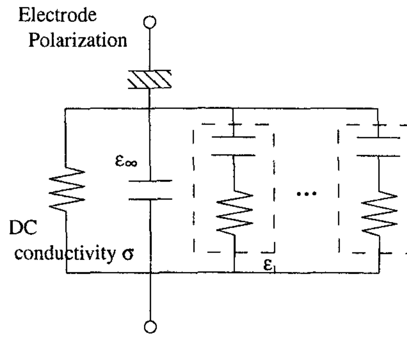

Figure 2 An equivalent circuit of equation 2.

relxation process	1	2	3	4
Δε	137	21.5	8.17	72.0
τ	17.0μs	71.4ns	2.91ns	8.32ps

Table II The dielectric relaxation strength Δε and the relaxation time τ of the 25mM Ca-form κ-carrageenan at 25°C.

the huge dispersion amplitude results from the electrode polarization and dc conductivity.

The other "true" relaxations, which we will show originate in motions of counterions and water, were observed at ~ 1MHz, 100MHz, and ~10GHz as shown in the magnified view, Figure 1(b). We used the model of considering the effects of dc conductivity and electrode polarization as illustrated in Figure 2, which is represented by the equation,

$$\varepsilon^* = \frac{1}{\dfrac{1}{\varepsilon_\infty + \sum_{k=1}^{4} \frac{\Delta\varepsilon_k}{1+(i\omega\tau_k)^{\beta_k}} + \dfrac{\sigma}{i\omega}} + \dfrac{1}{\dfrac{\varepsilon_{ep}}{(i\omega)^\gamma}}}, \tag{3}$$

$$\Delta\varepsilon_k \equiv \varepsilon_k - \varepsilon_{k-1},$$

where $\Delta\varepsilon_k$ is the relaxation strength, τ_k the relaxation time ($\tau_k > \tau_{k+1}$), and β_k the distribution parameter of relaxation time in a Cole-Cole relaxation function for the kth relaxation process, respectively, and ε_∞ is the instantaneous permittivity. The estimated dielectric relaxation parameters τ_k, β_k, $\Delta\varepsilon_k$, and $\varepsilon_\infty$of 50mM Ca-form κ-carrageenan aqueous solution using a least-squares fitting procedure are presented in Table II. Figure 3 shows the temperature dependence of the dc conductivity σ estimated by using eq 3. Based on the temperature dependence of σ, which can be used for detecting the coil-helix transition temperature and the helical fraction,[14] it is confirmed that the sample is in the coil state at 25°C for all the concentration in this study.

First, the relaxation process (k=4) is assigned to the cooperative rearrangement of hydrogen-bond network of bulk water, in view of the fact that τ_4 is completely the same as that of pure water, 8.38ps.[15] We think that the k=3 relaxation process can be ascribed not to water but to a local motion of bound counterions to polymer,[16] since the $\Delta\varepsilon_4$, which can monitor the amount of bulk water, very close (~99%) to that of pure water, 72.15,[15] indicates that all water molecules in the solution contribute to the k=4 bulk water process.

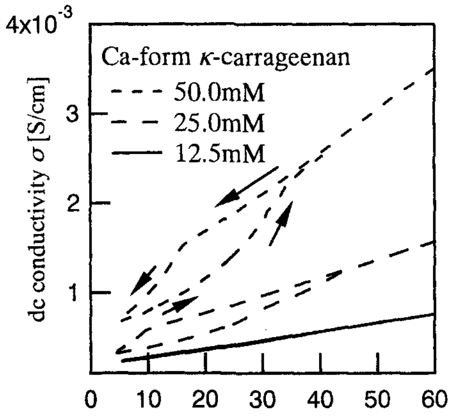

Figure 3 Temperature dependence of the dc conductivity σ of 50.0, 25.0, and 12.5mM Ca- κ-carrageenan aqueous solutions.

We showed the k=1 process in this system is ascribed to the fluctuation of the tightly bound counterions along to the polymer axis.[6] According to Ito et al., there are two kinds of dielectric relaxation observed at ~kHz and ~MHz due to the tightly and loosely bound counterions.

Generally dielectric relaxation originating in the fluctuation of the loosely bound counterions in semi-dilute region is characterized by typical power low concentration dependence of the relaxation strength and the relaxation time,

$$\Delta\varepsilon \propto C^0 N^0, \qquad \tau \propto C^{-1} N^0, \tag{4}$$

where C is the concentration and N the degree of polymerization.[17] Ikeda *et al.* showed that eq. 4 is valid

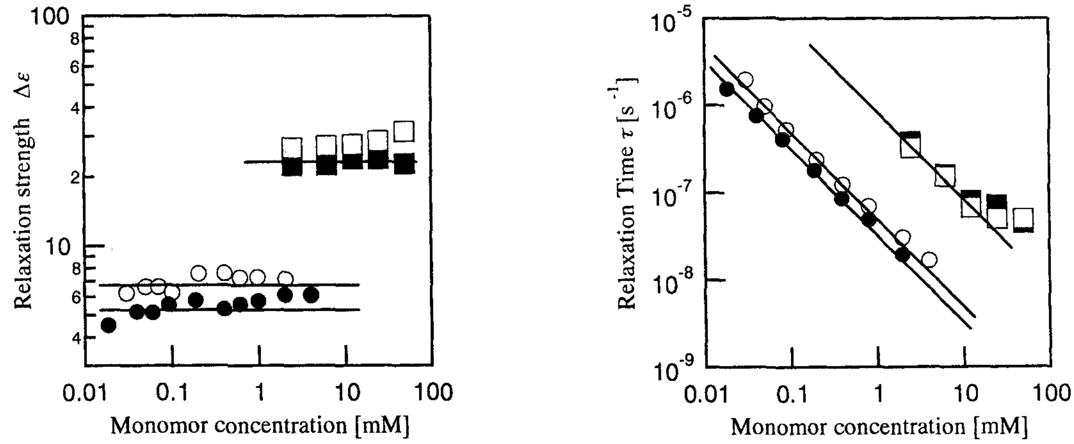

Figure 4 The concentration dependence of the estimated relaxation time τ_2 (a) and $\Delta\varepsilon_2$ (b) of Ca-form κ-carrageenan at 25°C (■) and 5°C(□). The slope of the lines, which is drawn for comparison of data with eq. 3, is 0 (a) and –1 (b), respectively. The data from the literature[18] of Na- (○) and K-form(●) κ-carrageenan measured at 25°C is also plotted for comparison.

for carrageenan aqueous solutions with potassium and sodium ions. [18] Figure 4 shows the concentration dependence of the relaxation strength $\Delta\varepsilon_2$ and the relaxation time τ_2 of Ca form κ-carrageenan aqueous solutions. We confirmed that eq 4 is also valid as for calcium ion, and found that the relaxation time of Ca in κ-carrageenan aqueous solutions is much slower than that of Na and K reported in the literature.

When the dielectric relaxation arises from the counter-ion fluctuation, the relaxation time τ is given by,

$$\tau = \frac{d^2}{2D}, \qquad (5)$$

where D is the diffusion coefficient of bound ion and d is the fluctuation distance of the ion.[19] The fact that τ_2 of calcium ion is about ten times larger than that of K indicates that this kind of motion of Ca is much slower than that of K, suggesting that the Ca ion is bound in more restricted way or tightly bound to the polymer compared with the K ion.

In the case of the dielectric relaxation due to the loosely bound counterions, the relaxation amplitude $\Delta\varepsilon$ is expressed by,

$$\Delta\varepsilon = \frac{n\alpha}{\varepsilon_0}, \quad \alpha = \frac{\langle \mu^2 \rangle}{3kT}, \qquad (6)$$

where the n is the concentration of the bound ion, α the electric polarizability, μ the dipole moment, $<\mu^2>=q^2d^2$ the mean square fluctuation of μ, q the charge of the counterion, and d the fluctuation length, ε_0 the vacuum permittivity.

Taking the ratio between eq 5 and 6, the relation,

$$\frac{\Delta\varepsilon}{\tau} = \frac{2nq^2D}{3\varepsilon_0 kT}, \qquad (7)$$

is obtained, and we can estimate the concentration of loosely bound counterions n by substituting $\Delta\varepsilon$, τ, and D into this equation. Based on the eq 4 and 7, n is proportional to the total concentration. Under the assumption that D in eq.4 is equal to that in free medium, $D_{\mathrm{Ca}} = 0.791\mathrm{x}10^{-9}\mathrm{m}^2\mathrm{s}^{-1}$,[20] the calculated ratio between n and C, is about 0.104, which means that 10.4% of calcium ion in the system contributes this dielectric relaxation process (k=2).

At 5°C, basically τ and $\Delta\varepsilon$ satisfy eq 4 as shown in Figure 4. Although the $\Delta\varepsilon_1$ largely increases just below the coil to helix transition temperature in the cooling process as shown in the previous paper, the $\Delta\varepsilon_2$ does not show such a large difference between the coil and the helix state.

4. CONCLUSIONS

In light of the results of the broadband dielectric relaxation spectroscopy for Ca-form κ-carrageenan aqueous solutions over the range 10^{-2}-$10^{10.4}$Hz, Ca ion appears to possess a characteristic binding nature to the polymer in terms of τ_2, being consistent with the previous study. The four relaxation processes can be detected. The quite compatible values of τ_4 and $\Delta\varepsilon_4$ with those for pure water demonstrate that the presence of ions and polymer shows no significant perturbation of the state of water. In view of the relation $\tau_2 \sim C^{-1}$ and $\Delta\varepsilon_2 \sim C^0$, the MHz process ($k$=2) has been assigned to a motion of loosely bound Ca ion, likely to Na and K. The much longer τ_2 for Ca revealed in this study compared with those for Na and K, underlines a stronger and tighter binding of Ca ion to carrageenan polymer.

Thus the results are of potentially major significance for our better understanding of dynamical aspects in aqueous polyelectrolyte and in particular the various binding states of ions fluctuating at different time scales.

5. ACKNOLEDGEMENT

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