

Dielectric Study on Various Aqueous Gels of Polysaccharide

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We investigated dielectric property of aqueous polysaccharide gels of carboxymethylcellulose, hyaluronic acid and agarose by using a time domain reflectometry (TDR) and an impedance material analyzer over a frequency range of 1MHz-20GHz. We assigned a relaxation process due to free water molecules around 10GHz and other relaxation processes at the lower frequency range. Unfreezable water was observed below a temperature freezing the free water T_f . Amount of the water per a saccharide was calculated and compared with that per an amide acid residue of globular protein and that per monomer unit of synthetic polymer.

Key words: dielectric relaxation, TDR, aqueous gel, polysaccharide

1. INTRODUCTION

Dielectric relaxation spectroscopy is a useful technique to investigate the molecular dynamics in a polysaccharide aqueous solution [1-3]. Debye relaxation due to the orientation of water molecules is observed in the range around 18GHz at 25°C in pure water, but its shape and position are changed by a presence of biopolymers in the solution. This effect has been discussed in view of the interaction with the polymers [3]. Dielectric studies by a time domain reflectometry (TDR) have been reported for schizophyllan under the order-disorder transition [1] and for gellan gum under the sol-gel transition [2].

In polymer solutions, water molecules could be classified into three categories in view of its dynamical property, according to our recent dielectric study [1-8]. The first is the free water, whose local structure is almost the same as that of pure water. The second is the bound water and the structured water, which is attached to biopolymers directly through hydrogen bonding. This water is unique to biopolymers, which form an ordered structure like double helix of DNA and the tertiary structure of globular proteins [5-7]. The third is the unfreezable water, which exists around polymers below the temperature freezing free water, T_f .

We already reported dielectric studies concerning the water structure in an aqueous gel of gellan gum in the

temperature range from 2.5°C to 52.5°C [2]. However, an investigation of a temperature lower than T_f has not been examined yet. We performed dielectric measurements in order to discuss the relationship between the polysaccharide structure and the dynamical properties of water in gel. This paper describes the results of hyaluronic acid, carboxymethylcellulose (CMC), and agarose. Hyaluronic acid is a major constituent of the extracellular matrices as an essential structure element in animal tissues. CMC is degradable by bacteria in a soil. Agarose gel is commonly utilized as a medium of electrophoresis.

2. EXPERIMENTAL

Hyaluronic acid of human umbilical cord was purchased from Sigma. Its repeat unit is disaccharide of D-glucuronic acid and D-N-acetylglucosamine linked through alternating beta-1,4 and beta-1,3 glycosidic bonds. Average molecular weight is about 10^6 in a state of nature. Deionized water was added to hyaluronic acid to adjust polymer concentration of 0.1wt%, 0.5wt%, 1.0wt%, 5.0wt%, 10.0wt% and 20.0wt%. These samples were kept for a few days in order to complete forming gel. Agarose was purchased from Wako, and the aqueous gel of 10wt% was prepared by the same procedure as the case of hyaluronic acid gel.

Carboxymethylcellulose (CMC) was obtained from Daicel Co. Ltd., Japan. Average molecular weight estimated from viscosity measurements is 5.2×10^5 [9]. Degree of substitution of carboxymethyl for hydroxyl is 2.2. CMC was dissolved in deionized water. The samples of 1.0wt%, 3.0wt%, 5.0wt% and 7.0wt% CMC were irradiated with gamma rays of 10kGy, 20kGy, and 30kGy [10]. According to a viscoelastic study, the sample of 7.0wt% irradiated with gamma ray at 30kGy is a gel and others are solutions.

We used TDR and Impedance Material Analyzer to measure complex permittivity. TDR system, which equipped a sampling head (Hewlett-Packard 54121A) and a digitizing oscilloscope (Hewlett-Packard 54121B), covered the frequency range from 10MHz to 20GHz. Details of TDR were reported before [11-14]. Impedance Material Analyzer (Hewlett-Packard 4291A) was utilized to cover the range between 1MHz and 1GHz. Semirigid cable, whose diameter was about 2mm, was used as the electrode. The electric length was 0.15mm, and the geometric capacitance (C_0) was 0.0101 ± 0.0004 pF.

3. RESULTS and DISCUSSION

Dielectric dispersion and absorption curves for aqueous CMC gel of 7.0wt% at 25°C were shown in Fig. 1. The curves obtained by the TDR agreed closely with those obtained by the Impedance Material Analyzer in the range from 100MHz to 1GHz. The curves were well explained by summation of three relaxation processes, which were described by KWW function and Cole-Cole equation, as follows.

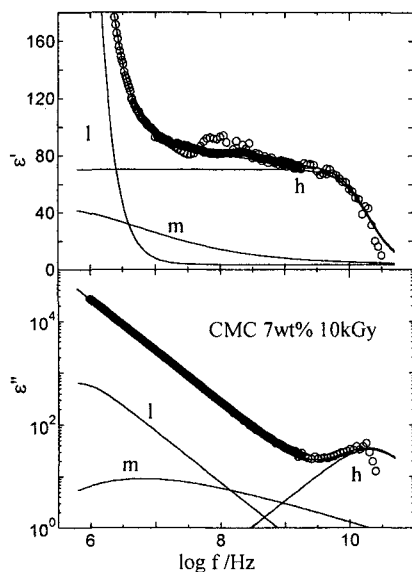


Fig. 1 Dielectric dispersion and absorption curves measured at 25°C for 7wt% CMC solution irradiated at 10kGy. Solids lines were calculated from Eq. (1).

$$\varepsilon^*(\omega) = \frac{\Delta\varepsilon_l}{1 + (j\omega\tau_l)^{\beta_l}} + \Delta\varepsilon_m \int_0^\infty \left[-\frac{d\Phi(t)}{dt} \right] \exp(-j\omega t_m) dt + \frac{\Delta\varepsilon_h}{1 + (j\omega\tau_h)^{\beta_h}} + \varepsilon_\infty + \frac{\sigma}{j\omega\varepsilon_0} \quad (1)$$

and

$$\Phi(t) = \exp \left[-\left(\frac{t}{\tau_m} \right)^{\beta_k} \right]$$

where ε_∞ is the limiting high-frequency permittivity, τ is the relaxation time, ω is the angular frequency, j is the imaginary unit, ε_0 is the permittivity of vacuum, σ is the dc conductivity, $\Delta\varepsilon$ is the relaxation strength, β_k is the parameter to describe asymmetrical broadness, β_h and β_l are the parameters to describe symmetrical broadness, and Φ is the response function.

The high frequency wing of "l" process was seen in the lower frequency in Fig. 1. This should be due to an electrode polarization caused by ions near an electrode surface, since the process was observed below the frequency of kHz region in various solutions in recent TDR study [15].

The dielectric parameters of $\Delta\varepsilon_h$, τ_h , and β_h obtained as a function of polymer concentration were plotted in Fig. 2. The concentration dependence is continuous in the whole range including pure water as 0%. The relaxation process denoted "h" is obviously due to orientation of free water.

The relaxation peak of "m" was found in a frequency

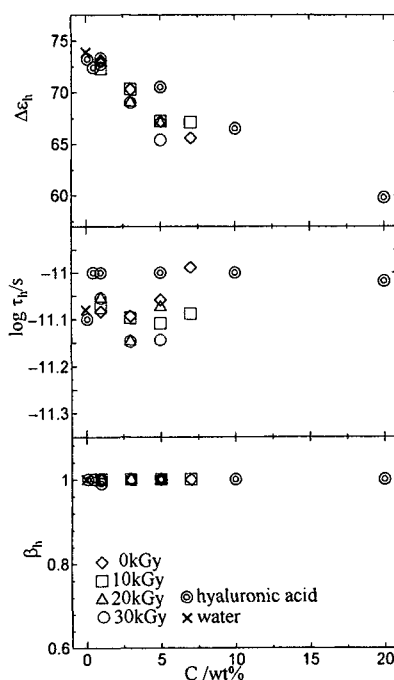


Fig. 2 $\Delta\varepsilon_h$, τ_h , and β_h as a function of polymer concentration C (wt%) of CMC solution and gel, and hyaluronic acid gel.

region between 1MHz and 100MHz in CMC. The parameters of $\Delta\epsilon_m$, τ_m , and β_m as a function of polymer concentration were plotted in Fig. 3. The origin of the process can be presumed either migration of counter ion, orientation of bound water, or rotation of the local chain. τ_m was decreased with decreasing concentration. This dependence is consistent with the effect of dilution on the chain motion related to free volume. The process was explained by KWW function, which was commonly employed to express the relaxation caused by the local chain motion [16]. The "m" process can be assigned to the molecular motion of polysaccharides. The dependence of irradiation was not clear in Fig. 3. It may be related to scission of polymer chains by gamma ray. The details will be discussed in another paper concerning rheological and hydrodynamic properties of CMC [9].

The dielectric measurements of hyaluronic acid gel and agarose gel were also performed at 25°C, 5°C and -7°C. Dielectric dispersion and absorption curves for aqueous gel of hyaluronic acid 20% at 25°C and agarose 10% at 5°C and -7°C were shown in Figs. 4 and 5, respectively. The obtained spectra at 25°C were explained by Eq. (1). $\Delta\epsilon_h$, τ_h , and β_h as a function of polymer concentration were also plotted in Fig. 2. Abrupt change occurred in dispersion and absorption at cooling process from 5°C to -7°C in hyaluronic acid 20% gel and agarose 10% gel (not shown), so that the free water froze around -5°C. The relaxation in ice is observed in the range around kHz region. The relaxation peak observed below -5°C in Fig. 5 is due to the unfreezable water that

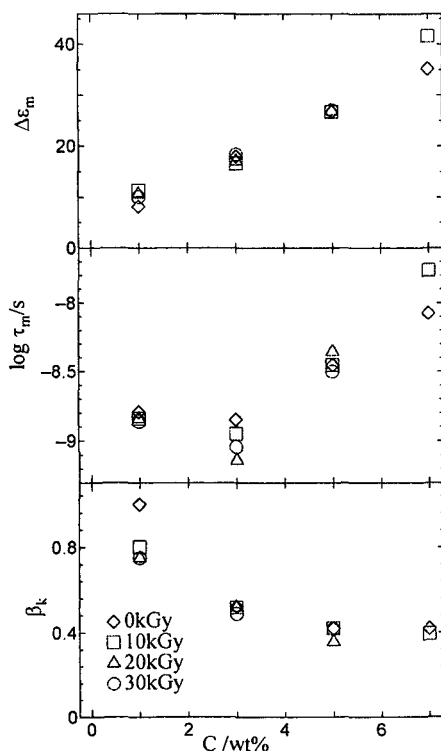


Fig. 3 $\Delta\epsilon_m$, τ_m , and β_m as a function of polymer concentration C (wt%) of CMC solution and gel.

located near polysaccharide molecules. This water has been found already in synthetic polymers, globular proteins and other polysaccharides by dielectric studies.

Content of the unfreezable water, c_{ufw} , just below T_f can be obtained as,

$$c_{ufw} = \left(\frac{\Delta\epsilon_h^i}{\Delta\epsilon_h^s} \right) \left(\frac{100 - C}{C} \right) \quad (2)$$

where C is the polymer concentration (wt%), $\Delta\epsilon_h^i$ is the relaxation strength at a temperature slightly below T_f and $\Delta\epsilon_h^s$ is that slightly above T_f . The estimated content, c_{ufw} , is 0.76 [g water / g polymer] for agarose, and 0.85 [g water / g polymer] for hyaluronic acid.

By TDR studies before, c_{ufw} was estimated in synthetic polymer solutions of poly (vinyl pyrrolidone) (PVP) [4], poly (ethylene glycol) (PEG) [17], poly (vinyl methyl ether) (PVME) [17], and poly (vinyl alcohol) (PVA) [17], in globular protein solutions of albumin [5] and trypsin [6], in heat denatured gel of ovalbumin [18], and in polysaccharide solution of schizophyllan [1].

To discuss a local water structure, we defined n_{ufw} as an amount of unfreezable water per unit component of polymers,

$$n_{ufw} = \frac{M_{wr}}{M_{ww}} c_{ufw} \quad (3)$$

where M_{ww} is the molecular weight of water, and M_{wr} is the molecular weight of the repeat unit in synthetic polymer, the average molecular weight of an amino acid residue in globular proteins, and molecular weight of a saccharide in polysaccharides. Concerning the various polymers, n_{ufw} as a function of M_{wr} was shown in Figure 6.

n_{ufw} increases in proportion to an increase of M_{wr} in

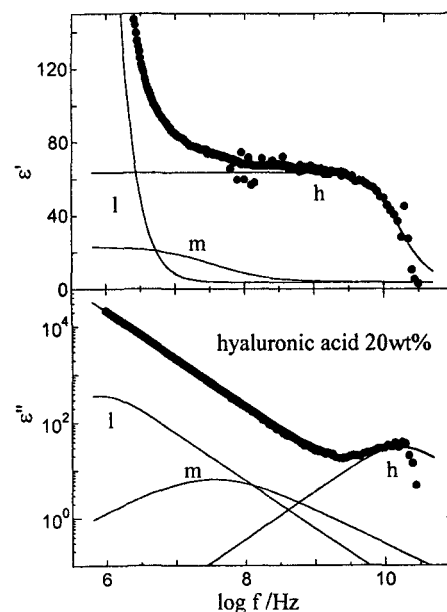


Fig. 4 Dielectric dispersion and absorption curves for aqueous gel of hyaluronan 20wt% at 25°C. Solid lines were calculated from Eq. (1).

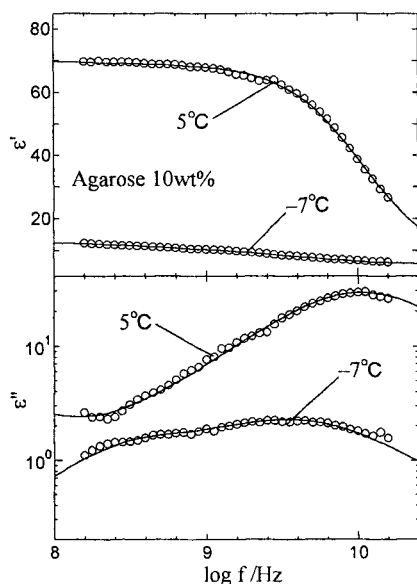


Fig. 5 Dielectric dispersion and absorption curves for aqueous gel of agarose 10wt% at 5°C and -7°C. Solid lines were calculated from Eq. (1).

the case of coiled polymer solutions of PVP, PEG, PVME and PVA, and amorphous gels of agarose and hyaluronic acid. The straight line through the origin in Fig. 6 showed this relationship. Number of unfreezable water per M_{wr} depends on the size of the component's volume. However, n_{ufw} for globular proteins was apparently smaller than the line. It can be attributed to the protein's tertiary structure of the polypeptides forming α -helix and β -sheet. Water molecules do not go into the inside of globular structure. Schizophyllan of a triple-helical polysaccharide also has smaller n_{ufw} . It is indicated that the volume of water molecules near the polysaccharide bound into the helical structure is smaller than that of the randomly coiled polymers. These results suggest that Fig. 6 does not reflect only the intermolecular interaction between water and polymer but also the macroscopic structure of polymers. It is expected that more systematic measurements and suitable estimations of volume for polymer chain will bring detailed discussion on the mesoscopic structure of water, i.e. bound water, structured water, etc., around the polymer chain.

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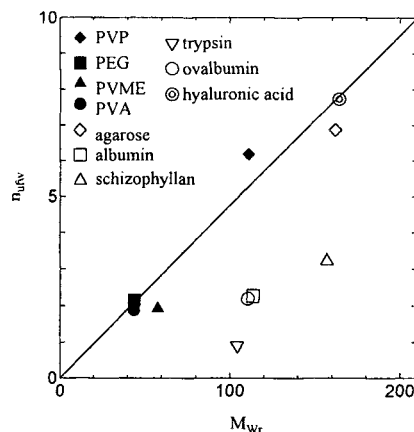


Fig. 6 Amount of unfreezable water, n_{ufw} , as a function of M_{wr} , which is the molecular weight of the repeat unit in synthetic polymers, the average molecular weight of an amino acid residue in globular proteins, and the molecular weight of a saccharide in polysaccharides.

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