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Hydration Study of Oligo-saccharides and Agarose Gel in Water by Microwave Dielectric Analysis

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We studied oligo-saccharide solutions and agarose gel using microwave dielectric spectroscopy in the range from 0.2 to 20 GHz. The measured samples were aqueous solutions of glucose, maltose, maltotriose, maltopentaose, maltoheptaose and an agarose gel. The dielectric spectra of oligo-saccharides showed two relaxation schemes at 7~8 GHz and 3~5.5 GHz. Above 5 GHz, $\Delta\varepsilon'$ was not sensitive to the molecular length of the solute. By using a single-Debye approximation on the complex dielectric constant of hydrated solutes above 5 GHz, we obtained hydration water numbers for these solutes in water. We have come to the conclusion that 7~9 water molecules are bound to a glucose unit. For an agarose-gel of 2%w/v the estimated water number per repeating unit was 9~11, which is about half of that for oligo-saccharides. This suggests bundle formation.

Key words: hydration, polysaccharide, agarose gel, microwave, dielectric spectroscopy

1. INTRODUCTION

Dynamic movements and hydration of polymer chains in solutions or gels are the fundamental interest in polymer science. Dielectric spectroscopy can provide direct information about it[1-4]. One of the authors has developed a dielectric method to measure the hydration level of proteins [5-6] and amino acids [7]. This method of analysis is based on the emulsion mixture theories of Wagner[8] and Hanai[9].

Our first aim is to obtain the microwave dielectric spectra of dilute aqueous solutions of linear sugars of different lengths, then to estimate the hydration number N_h of an oligo-saccharide molecule and the length dependence of N_h. Mashimo, et al.[4] showed two clear relaxation peaks in the dielectric absorption spectra of oligo-saccharide solutions of high concentration (10 to 75 wt%) from 1 MHz to 20 GHz. They attributed the low frequency peak around 0.03~0.2 GHz to the orientational relaxation motion of oligo-saccharide molecules, and the high frequency peak to that of bulk water at 20 GHz.

Our second aim is to obtain the dielectric spectrum of dilute polymer gel in water. By approximating the segments between neighboring crosslinking points with linear solutes, we can estimate the hydration number per repeating unit based on the result derived from oligo-saccharides. By this procedure we may obtain information about the hydration property of segments or the crosslinks.

In this study we have applied the method of hydration analysis[5] to diluted oligo-saccharide aqueous solutions and agarose gel. Our method is based on the two-component model which consists of pure solvent and hydrated solutes, and can provide a quantitative estimation of hydration level. Therefore, the concentration of the solution must be low enough to assure that the solvent can be regarded as a pure solvent. This requires a precise dielectric spectra with the resolution of 0.05 in dielectric constant in the frequency range from 2 GHz to 20 GHz. The dielectric properties in this frequency range were not as deeply examined in the previous work[4] as in the hydration analysis.

2. MATERIALS

We used D-glucose (JIS S, Wako), maltose (Wako), maltotriose (Wako), maltopentaose (Wako) and maltoheptaose (Wako) without any further purification. The concentration of these aqueous solutions was determined by a dry weight method using a vacuum chamber from 3 to 8×10^{-5} Torr at room temperature for several days until the weights became stable within 1%. Agarose I was purchased from Dojin and used without any further purification.

3. METHOD

3.1 Complex dielectric constant of mixture

The apparent complex dielectric constant ε_{ap}^* of the mixture is expressed using the dielectric constant of solvent ε_a^*, and that of dispersed spheres ε_q^*, by

$$\varepsilon_{ap}^* = \varepsilon_a^*(2(1-\phi)\,\varepsilon_a^*+(1+2\phi)\,\varepsilon_q^*)/((2+\phi)\,\varepsilon_a^*+(1-\phi)\,\varepsilon_q^*)$$
(Wagner equation for $\phi<<1$), (1)

where ϕ is the volume fraction of the hydrated solute. When the solute is a shelled sphere, ε_q^* is again expressed with the dielectric constant of hydration shell ε_h^* and that of core molecule ε_p^*.

$$\varepsilon_q^*=\varepsilon_h^*(2(1-\phi_p)\,\varepsilon_h^*+(1+2\phi_p)\,\varepsilon_p^*)/((2+\phi_p)\,\varepsilon_h^*+(1-\phi_p)\,\varepsilon_p^*)$$
(for $0<\phi_p<1$), (2)

where $\phi_p = v/\phi$. v is the volume fraction of the solute in solution calculated by $cM_w s_v/1000$. c, M_w and s_v are the concentration of solutes in moles/liter, the molecular weight in g/mol and the partial specific volume of solute in ml/g, respectively. This equation is used to obtain $\varepsilon_{q\infty}$, the high frequency limit ($f\to\infty$) of ε_q^*, where $\varepsilon_h^* = \varepsilon_{h\infty}$ and $\varepsilon_p^* = \varepsilon_{p\infty}=2.5$ were used at infinite frequency. $\varepsilon_{h\infty}=5.6$ was assumed to be equal to the high frequency value of bulk water[10]. The procedure to obtain ε_q^* is as

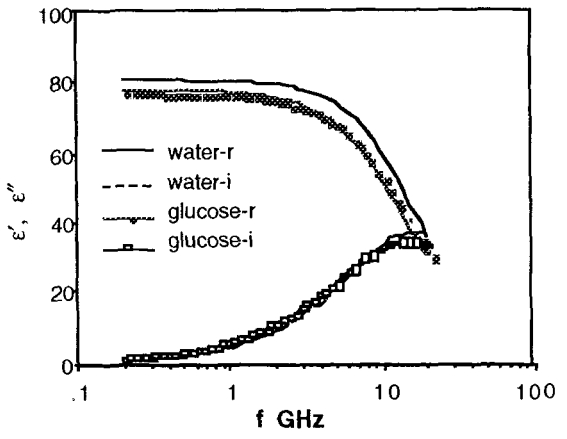

Fig.1 Dielectric spectra of water and glucose solution.

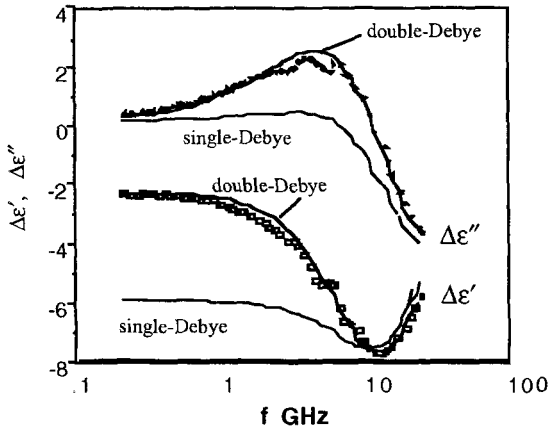

Fig.2 Difference spectrum of glucose in water. Lines were calculated with eq(1) and ε_q^* using single-Debye function eq.(3), or double-Debye function eq.(4).

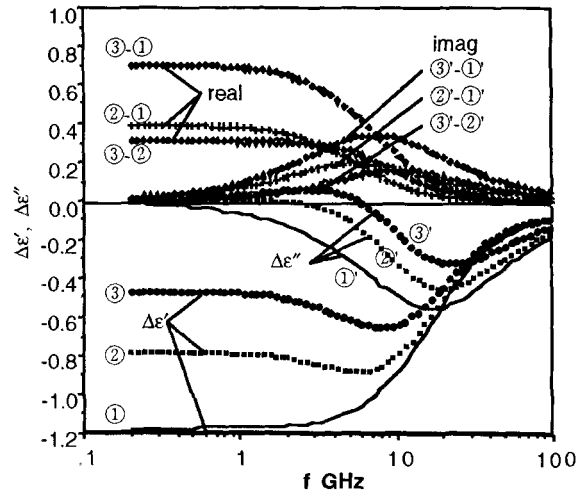

Fig..3 Simulation of difference dielectric spectra.
Zero level: pure water (ε_w', ε_w''). $\Delta\varepsilon'=\varepsilon'-\varepsilon_w'$, $\Delta\varepsilon''=\varepsilon''-\varepsilon_w''$. $\varepsilon^*=\varepsilon'-j\varepsilon''$ was calculated by eq(1) and (4) with ϕ=0.01, $\varepsilon_{q\infty}$= 1. ①,①': air, $\delta_1=\delta_2=0$; ②,②':δ_1=20, f_{c1}=5GHz, δ_2=0; ③, ③': δ_1=20, f_{c1}=5GHz, δ_2=20, f_{c2}=8GHz.

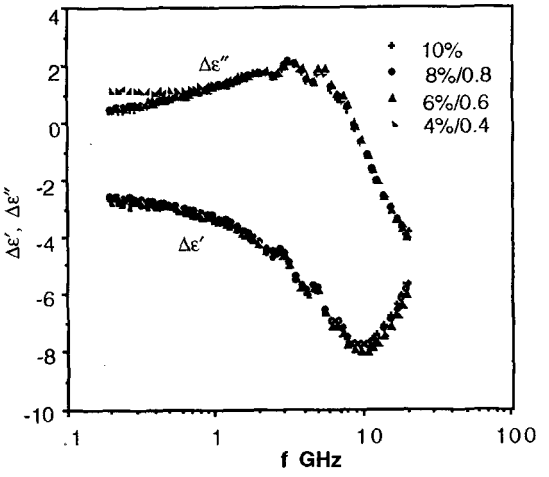

Fig. 4 Difference dielectric spectra of maltose solution showing the proportional relationship between (Δε′,Δε″) and the concentration.
Sample concentrations were: 10.0, 8.0, 6.0, 4.0 %w/v, and the indicated values of Δε′and Δε″ at each concentration for all frequency points were magnified by: 1.0, 1.25, 1.67, 2.5, respectively.

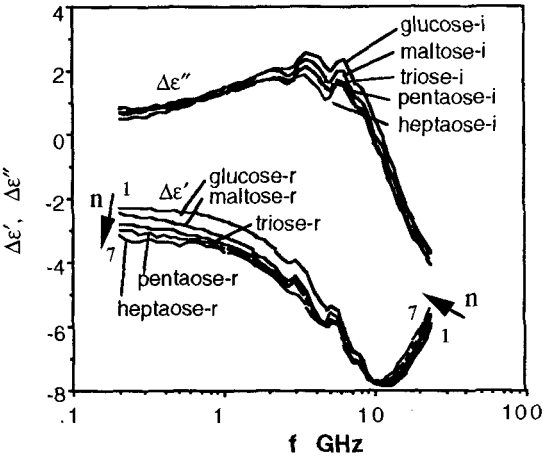

Fig.5 Difference dielectric spectra of sugars in water
Zero level: pure water. For comparison each spectrum was numerically magnified to 10 %w/v equivalently. Actual concentrations: glucose (10.0%), maltose (10.0%), maltotriose (9.65%), maltopentaose (5.02%), maltoheptaose (5.0%). "n" indicates the degree of polymerization.

Table 1 Hydration properties of oligo-saccharides

sugar	N_h	f_{c1} (GHz)	δ_1	$\varepsilon_{q\infty}$
glucose	14.4	8.1	59	4.6
maltose	23.4	7.2	57	4.4
maltotriose	31.4	7.1	56	4.4
maltopentaose	51.1	7.2	55	4.4
maltoheptaose	66.9	7.2	54	4.3

(error of N_h: 7%)

follows: First, starting with the initial value of ϕ, the dielectric spectrum of hydrated solute ε_q^* was calculated by using equation (1). Second, the obtained ε_q^* was fitted by a single Debye relaxation function in the high frequency range from 5 to 20GHz,

$$\varepsilon_q^* = \varepsilon_{q\infty} + (\varepsilon_{qs}-\varepsilon_{q\infty})/(1+ j\,(f/f_c)) \quad (3)$$

This equation approximates the dielectric relaxation of hydrated solutes in solution. If the dielectric constants of organic solute molecules are constant (around 2~3) in this frequency range, f_c represents the orientational relaxation frequency of the hydrating water molecules on a solute molecule.

If two independent relaxation processes are involved in the hydrated solute, ε_q^* can then be expressed with a double-Debye function by eq.(4),

$$\varepsilon_q^* = \varepsilon_{q\infty} + \delta_1 /(1+ j\,(f/ f_{c1})) + \delta_2 /(1+ j\,(f/ f_{c2})). \quad (4)$$

The first term is the high frequency value relating to the electronic polarization of the solute molecule. The second term is for the hydrated solute, and the third term for the orientation of solute molecules or other origins such as conformational change of linear solute molecules. For example, in the case of glycine in water[6] $\varepsilon_{q\infty}$ =4.4, δ_1=20, f_{c1}=8 GHz, δ_2=980, f_{c2}=0.7 GHz. In general, ε_q^* may be written as a sum of several Debye functions with the relaxation frequency f_{cn} ($\leq f_c$) and an additional ionic conduction term which is in the form of $j\sigma_{qc}/(2\pi f)$ relating to the solute-solvent interface. However, if f is high enough, the terms other than f_c can be ignored.

This method enabled us to measure the number of hydrating water molecules which have a lower mobility than that of bulk water with a relaxation frequency of 17 GHz at 20°C. When we analyze the spectra below 5 GHz, we should detect a lower level of hydration along with lower relaxation frequencies f_{cn}.

Third, ϕ was iteratively adjusted in equations (1) - (2) until $\varepsilon_{q\infty}$ in equation (3) agreed with the value obtained. Thus, we obtained f, f_c and ε_{qs}. The number of bound water molecules per monomer molecule N_h was then calculated by using eq.(5).

$$N_h = 1000(\phi - v)\rho_0/(18c) \quad (5)$$

The density of hydration shells ρ_0 is 1000 kg/m^3 whenever we use s_v's to calculate v by its definition.

3.2 Experimental Procedure

The dielectric spectra were obtained using a microwave network analyzer, Hewlett Packard 8720C, and an open-end flat-surface coaxial probe. To avoid accumulation of microbubbles, the probe was fixed in an upward position in a glass cell controlled at 20.0 ± 0.01°C in a Neslab thermobath. The cell was a cylinder with a conical top for stirring space, with dimensions of 17mm inner diameter; 20mm in height; and 3ml in volume. The solution temperature was monitored using a platinum-resistor thermosensor by using the four-terminals method. The cell was filled with a sample solution that was degassed under reduced pressure. Microwaves in the frequency range from 0.2 to 20 GHz were introduced into the cell through the probe. The calibration was done by a procedure consisting of open-circuit in air, short-circuit with mercury and pure water at 20.0±0.01°C. The reflected waves were taken in a network analyzer and then converted into a complex dielectric spectra with HP85070A software using the Nicolson-Ross method. Since the oscillator of the network analyzer is sensitive to temperature changes, especially above 50°C, we kept the inside of the network analyzer below 35°C by using a forced air flow, and suppressed the total drift of the dielectric constant within 0.025 for 1 - 10 GHz and 0.015 for 2.5 - 8 GHz which is one order of magnitude smaller than the commercial grade. For each sample, ten dielectric spectra of fifty-one frequency points were sequentially obtained every 10 seconds at 20.0±0.01°C and then averaged.

All the measurements except for the gel were made by repeating one-set measurements of water and sample solution several times. This enabled us to distinguish between signals and sudden drifts which should be omitted.

4. RESULTS

4.1 Oligo-saccharides

As a main component of polysaccharides, a glucose aqueous solution of 10.0 %w/v was examined first. The dielectric spectrum of glucose solution compared with the water spectrum is shown in Fig. 1.

The difference spectrum of glucose solution $\Delta\varepsilon'=\varepsilon'-\varepsilon_w'$ and $\Delta\varepsilon''=\varepsilon''-\varepsilon_w''$, where w denotes water, are presented in Fig. 2, where solid lines noting single-Debye are based on eq.(3) with $\varepsilon_{q\infty}$ =4.6, $\varepsilon_{qs}-\varepsilon_{q\infty}$=59, f_c=8.1 GHz . Then we obtained N_h=14.4. Here, the small humps and dips between 2 and 6 GHz are due to machine noise.

As a reference, the double-Debye function gives wide-range fitting with

$$\varepsilon_q^* = 4.5 + 25/(1+ j\,(f/7.8)) + 38/(1+ j\,(f/5.5)) \quad (6).$$

It gave N_h=13.5. Single-Debye and double-Debye methods resulted in similar estimates on N_h. For the sake of understanding, simulated $\Delta\varepsilon'$ and $\Delta\varepsilon''$ made with eq.(1) and eq.(4) are shown in Fig. 3 for three typical examples: 1)air bubbles in water; 2)solute with a single relaxation process with δ_1=20, f_{c1}=5 GHz; and 3) solute with two relaxation processes with δ_1=20, f_{c1}=5 GHz, δ_2=20, f_{c2}=8 GHz. ϕ -value was 0.01 for the three cases. The difference curves (2-1) and (3-2) for the real part and curves (2'-1') and (3'-2') for the imaginary part became single-Debye-like. The difference curve (3-1) is not made by a simple addition of curve (2-1) and curve (3-2) because of the nonlinear formulation of eq.(1). The situation is the same in curve (3'-1'). From eq.(5) the hydration number N_h is determined by ϕ. And ϕ is determined by the high frequency value of $\Delta\varepsilon'$ where curve-(2) and curve-(3) converge with curve-(1). It shows the importance of precise measurement at high frequencies.

From these arguments, the single-Debye method may produce a fitting-error in hydration number N_h of about 7% (simply by 14.4/13.5). This error is equivalent to the error by the baseline drift of the instrument at a high frequency. Therefore, the double-Debye method does not improve the result at this stage.

The concentration dependence of the absorption peak frequency is another important factor. For example, Fig. 4 shows the case of maltose, the values of $\Delta\varepsilon'$ and $\Delta\varepsilon''$ at different concentrations were compared after dividing the values of $\Delta\varepsilon'$ and $\Delta\varepsilon''$ for 10.0, 8.0, 6.0, 4.0%w/v

solutions with 1.0, 0.8, 0.6, 0.4, respectively. For all sugars examined in this experiment the values of $\Delta\varepsilon'$ and $\Delta\varepsilon''$ were proportional to the concentration. There was no concentration dependence in the absorption peak frequency. We found the absorption peak frequency at 5.5GHz for glucose, 4GHz for maltotriose and 3GHz for both maltopentaose and maltoheptaose. The molecular length dependence of this frequency was not strong compared to the orientational relaxation frequency of oligo-saccharides below 200MHz, as shown by Mashimo et al.[4]

In Fig.5 $\Delta\varepsilon'$ and $\Delta\varepsilon''$ plots are shown summarizing the results for different sugars at equivalent concentrations after their conversion to 10%w/v. Actual concentrations of glucose, maltose, maltotriose, maltopentaose and maltoheptaose were 10.0, 10.0, 9.65, 5.02, 5.0%w/v, respectively. Below 5 GHz in $\Delta\varepsilon'$ one notices a clear dependence of excess polarization on the number n of glucose rings which are linearly bound. $\Delta\varepsilon'$ decreased with n, and became insensitive to n for n>3. On the other hand, above 5 GHz the values of $\Delta\varepsilon'$ of each sugar were very close. This showed that the dielectric excluded volume is insensitive to the polymerization degree.

This supports our estimation of the hydration number of linear solutes in solution based on the Wagner mixture theory in the frequency range from 5 to 20 GHz, without much error. In this frequency range, one may notice that there is a slight increase of $\Delta\varepsilon'$ with n. We used s_v=0.61 ml/g for the tested oligo-saccharides (glucose to maltoheptaose) by density measurement with Anton Paar DMA58. The results including the hydration number N_h, are shown in Table 1. We obtained the following empirical equation:

$$N_h = 5.6 + 8.85\ n. \qquad (7)$$

We found that approximately 9 water molecules are bound to a glucose ring in oligo-saccharides. This number includes hydrating water molecules bound strongly and weakly on a solute molecule.

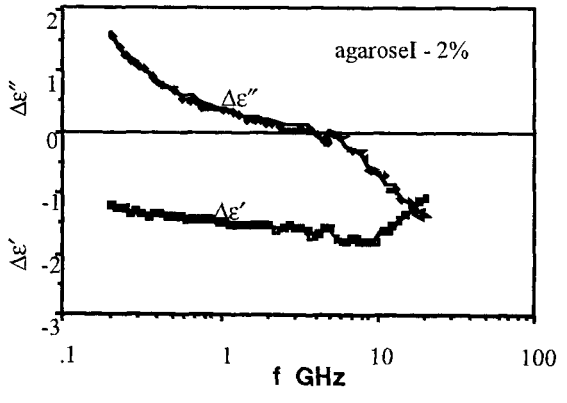

Fig.6 $\Delta\varepsilon'$ $\Delta\varepsilon''$ of agarose I gel at 20 °C.

4.2 Agarose gel

Fig. 6 shows the difference spectra of agarose gel of 2.0 %w/v. Agarose-I was dissolved in hot water and then injected into the measurement cell. The temperature became 20.0°C within 15 min. at which time gelation proceeded. Below 5 GHz there was very little increase in $\Delta\varepsilon'$ observed. By applying Wagner mixture theory to the network structure, we obtained N_h =11 per repeating unit (two rings) of agarose I (gel state) assuming s_v=0.61ml/g. This number N_h =11 is about half of that of a glucose ring in oligo-saccharides. In this study s_v of agarose could not be measured because the solution became gel during the injection into the cell of DMA58. However, as s_v of an agarose chain should not be so different from that of glucose, small values of $\Delta\varepsilon'$ strongly suggest bundle formation of agarose chains.

5. DISCUSSION

In this study the Wagner mixture theory with a spherical-solute model was applied to a hydration analysis of linear solutes. It is interesting to note the case of a prolate-spheroid model shown by Asami, et al.[11] We have examined the prolate-spheroid solute(air) in water and found that when the axial ratio q (=long axis/ short axis) is equivalent to 3 $\Delta\varepsilon'$ was 5% larger than that of a spherical air model; and that when q=100, $\Delta\varepsilon'$ was 11% larger than that of the spherical air model, with a constant ϕ=0.01. Therefore, the values of N_h for long solutes in Table 1 might be overestimated by as much as 17% by eq.(5). If we accept this argument, eq.(7) should be replaced by eq.(8).

$$N_h = 7 + 7.3\ n. \qquad (8)$$

By a similar consideration on agarose gel, N_h (=11) reduces to 9 per repeating unit.

The relaxation frequency f_{c1} for oligosaccharides was found as 7~8 GHz which is possibly due to loosely bound water molecules on a solute molecule. As shown by Mashimo, et al[4], maltotriose has an orientational relaxation time around 1ns (which is equivalent to an absorption peak at 0.16GHz). By extrapolation from their result, glucose may have a peak at 0.4 GHz. Then the 5.5 GHz relaxation peak must be due to some other reason such as structural fluctuation of glucose rings.

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