

Rheological properties of concentrated solutions of fucoidan
in water and in an ionic liquid

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ABSTRACT:

Dynamic viscoelasticity has been examined for concentrated solutions of fucoidan from *Cladosiphon okamuranus* in water and in an ionic liquid 1-butyl-3-methylimidazolium acetate (BmimAc). The existence of the electrostatic interaction between fucoidan chains in the aqueous solutions and its disappearance in the BmimAc solutions have been clearly shown by the difference in the terminal flow behavior between the aqueous and BmimAc solutions. On the other hand, the rubbery plateau indicating the entanglement coupling between fucoidan chains has been observed for both solvents at rather high concentrations. The molecular weight between entanglements (M_e) for fucoidan in the BmimAc solutions has been determined from the plateau modulus at several concentrations and then M_e for fucoidan in the molten state ($M_{e,\text{melt}}$) has been estimated to be 1.0×10^4 as a measure of the specific chain characteristics of fucoidan.

1. Introduction

Fucoidan is a polysaccharide that contains repeating L-fucose units in the main chain with sulfates and other monosaccharides as the side groups. Fucoidan exists in a wide variety of brown algae, most of which have been known as conventional healthful foods, and there are a great number of structural variations in terms of the sulfate content as well as the monosaccharide species depending on the origin it is extracted from. Since brown algae range all over the world, the chemical structures of fucoidan obtained from different algae have been reported by many research groups together with the extraction techniques [1-5]. Furthermore, fucoidan has been recently paid attention due to the bioactivities such as the anticoagulation, antitumor properties, and gastric protection [3,6-8]. Relevant medical utility is still under investigation to expand the applications of fucoidan.

In spite of the long popularity as foods and the potential importance as medicines, fundamental physical properties of fucoidan have not been examined sufficiently [1-5,9]. Generally speaking, characterization of a polymer needs highly-purified samples, but it is not easy to obtain purified fucoidan samples suitable for such measurements. The above-mentioned diversity of chemical structures of fucoidan might be also responsible; the diversity makes it difficult to determine common characteristics of fucoidan. Concerning the rheological properties of fucoidan, investigations have been restricted to aqueous solutions. This is primarily because the functions

of fucoidan as medicines or foods usually emerge while in water. It has been reported that dilute aqueous solutions of fucoidan show pseudoplastic or shear-thinning behavior even at low shear rates. It has been also demonstrated that the critical concentration from the dilute to semidilute regime is apparently determined to be a very low concentration [2-5,9]. These results suggest that there is substantial electrostatic force between fucoidan chains dissolved in water, as expected of polyelectrolytes.

Although previous studies on rheology of fucoidan solutions have been confined to the dilute or semidilute regime, it is likely that fucoidan dissolves in water at much higher concentrations. It is well known that polymer chain motion in concentrated solutions is significantly affected by constraints of nearby chains and the so-called entanglement coupling dominates the rheological properties at long times [10-12]. At the present time, however, there is no information about rheological properties of concentrated aqueous solutions of fucoidan where the entanglement coupling has to be taken into consideration in addition to the electrostatic interaction mentioned above. Concerning the lack of rheological studies on fucoidan, it should be also noted that there seem no solvents other than water used for preparation of fucoidan solutions. In fact conventional solvents are not good solvents for fucoidan, but we know that some ionic liquids are possibly better solvents than water for fucoidan [13,14]. Because ionic liquids intrinsically have great ionic strength, the electrostatic interaction between fucoidan chains would be shielded once dissolved

in an ionic liquid. Therefore studies on the rheological properties of concentrated solutions of fucoidan in an ionic liquid can provide information about the effect of the entanglement coupling free of the electrostatic interaction. In addition, using an ionic liquid make it possible to perform rheological measurements in a wide range of temperature due to the very low vapor pressure, so that rheological data over a wide range of frequency or time are expected to be obtained based on the time-temperature superposition principle.

In this study, we have examined the rheological behavior of fucoidan solutions in water and in an ionic liquid. Concentrated solutions exhibiting the entanglement coupling can be prepared by using these solvents. Therefore, the difference in the flow behavior between the aqueous and ionic liquid solutions has been elucidated in the concentrated regime. Then the molecular weight between entanglements (M_e), which is a gauge of the entanglement coupling, for fucoidan has been determined from the dynamic viscoelasticity data for the ionic liquid solutions at different concentrations. The value of M_e for fucoidan in the molten state ($M_{e,\text{melt}}$) has been also estimated from the concentration dependence of M_e .

2. Experimental section

2.1 Materials

A purified fucoidan from *Cladosiphon okamuranus* was supplied by Marine Products

Kimuraya (Japan). The appearance of the sample was amber powder. It was reported that fucoidan from *Cladosiphon okamuranus* contains a glucuronic acid unit as the monosaccharide side group every six fucose units (available at <http://www.jhnfa.org/kikaku9.pdf>, p 24) and no branch architecture. According to the manufacturer's data, the molecular weight (in g/mol) was 3×10^5 , and the sulfate and water contents were 14.2% and 16.5% by weight respectively. Aqueous solutions of fucoidan were prepared by adding the fucoidan sample as it is into distilled water taking the water content into account. Several hours of stir with a magnetic stirrer at room temperature gave yellowish solutions. On the other hand, concentrated solutions of fucoidan in an ionic liquid were prepared in the following way. The received sample was dried under vacuum at 80 °C for 3 days, so that the weight became constant after reducing by about 16wt%. Preliminary experiments to obtain concentrated solutions of fucoidan showed that among several kinds of ionic liquids 1-butyl-3-methylimidazolium acetate (BmimAc) gave yellowish solutions up to the fucoidan concentration of $3.9 \times 10^2 \text{ kgm}^{-3}$. BmimAc (Sigma-Aldrich, USA) was used without further purification. The dried fucoidan was added to BmimAc in a dry glass vessel, and the mixture was quickly stirred with a stainless spatula on a hot plate around 80 °C. Then, the mixture was stirred with a magnetic stirrer at 80 °C for about 1day. The concentration of fucoidan (c) ranged from 2.0×10^2 to $4.9 \times 10^2 \text{ kgm}^{-3}$ for the aqueous solutions, and from 3.1×10^2 to $3.9 \times 10^2 \text{ kgm}^{-3}$ for the BmimAc solutions. In the calculation of c, $1.0 \times 10^3 \text{ kgm}^{-3}$ was assumed for

the density of fucoidan, which was actually unknown, and the reported value of $1.055 \times 10^3 \text{ kgm}^{-3}$ was used for BmimAc.

2.2 Rheological measurement

Dynamic viscoelasticity was examined for the fucoidan solutions. An ARES rheometer (now TA Instruments, USA) was used with a cone-plate geometry having a diameter of 25 mm and a cone angle of 0.1 rad. The frequency (ω) dependence of the storage modulus (G') and the loss modulus (G'') for the solutions was measured in the ω range of 0.1 to 100 s^{-1} with a strain amplitude (γ) of 0.1. The measurements for the aqueous solutions were carried out at 17 °C without temperature control of the apparatus. Regarding the BmimAc solutions, temperature (T) ranged from 20 to 80 °C; actually, frequency sweep tests were conducted at 20, 40, 60, and 80 °C.

3. Results and discussion

Fig. 1 shows the ω -dependence curves of G' and G'' for the aqueous solutions of fucoidan. It seems that each pair of the G' and G'' curves almost corresponds to the flow region, and the typical terminal behavior $G'' \propto \omega$ is observed at low ω for $c = 2.0 \times 10^2$ and $2.9 \times 10^2 \text{ kgm}^{-3}$. However, the terminal behavior for $c = 3.9 \times 10^2$ and $4.9 \times 10^2 \text{ kgm}^{-3}$ is not observed within the experimental condition: the G'' curves exhibit slopes of less than unity even at low ω , namely $G'' \propto \omega^n$ ($n < 1$).

Here, to illustrate the flow behavior of the aqueous solutions clearly, the dynamic viscosity (η' ; $\eta' = G''/\omega$) has been estimated from the dynamic viscoelasticity data and is double-logarithmically plotted against ω in Fig. 2. The values of η' for $c = 2.0 \times 10^2$ and $2.9 \times 10^2 \text{ kgm}^{-3}$ are constant at low ω and then decrease slightly as ω increases, which is similar to the common flow behavior of polymer solutions. On the other hand, η' for $c = 3.9 \times 10^2$ and $4.9 \times 10^2 \text{ kgm}^{-3}$ decrease with increasing ω over the entire range of measurement. This probably corresponds to the pseudoplastic or shear-thinning behavior in the steady shear flow measurements reported in the earlier rheological studies cited above [2,4,5,9,15]. If the dynamic viscoelasticity data are obtained down to lower ω and/or at higher temperature, η' for $c = 3.9 \times 10^2$ and $4.9 \times 10^2 \text{ kgm}^{-3}$ will also become constant at low ω , though neither is possible using our apparatus due to evaporation of solvent water. Apart from the terminal flow behavior, it is also noteworthy that the G' and G'' curves for $c = 4.9 \times 10^2 \text{ kgm}^{-3}$ apparently show part of the rubbery plateau region at high ω , where $G' > G''$. This can be interpreted as a sign of the entanglement coupling between fucoidan chains dissolved in water at the high concentration.

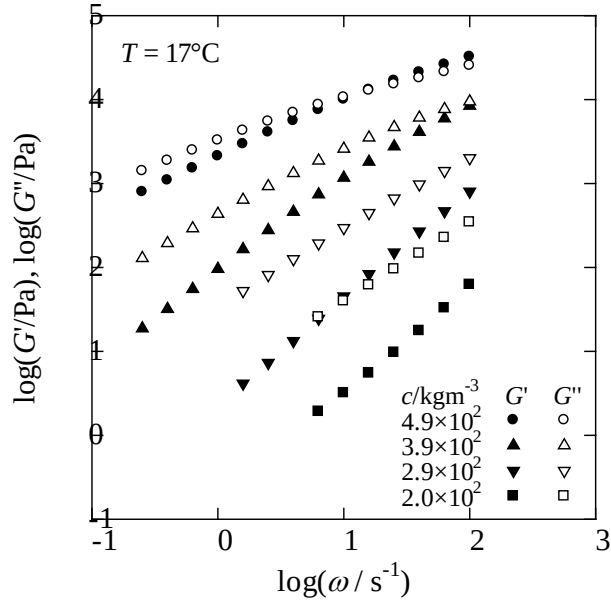


Fig. 1. Plots of ω dependence of G' and G'' for aqueous solutions of fucoidan of $c = 2.0 \times 10^2 - 4.9 \times 10^2 \text{ kgm}^{-3}$ at 17°C .

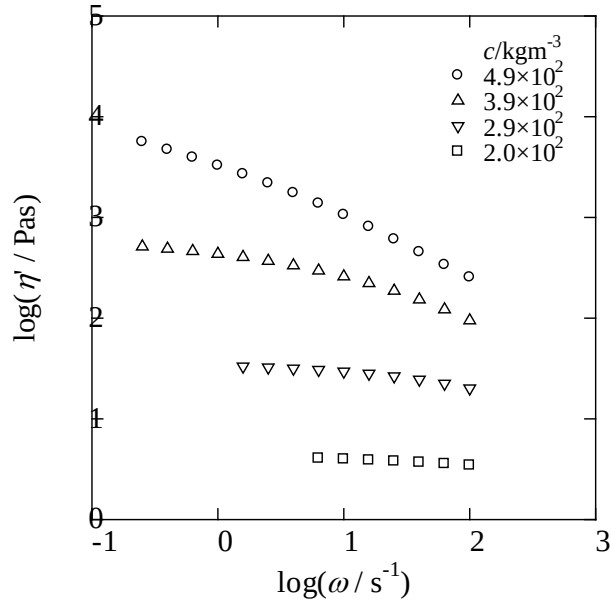


Fig. 2. Double-logarithmic plot of η' against ω . η' has been estimated from the dynamic viscoelasticity data in Fig. 1.

Fig. 3 shows the master curves of G' and G'' for the BmimAc solutions of fucoidan. These curves were made by applying the time (angular frequency)-temperature superposition principle to the G' and G'' curves at each T with the reference temperature (T_r) of 60 °C. The horizontal shift factor is designated by a_T . As demonstrated in the figure, the superposition principle holds well. Here c has been chosen to be 3.1×10^2 and $3.9 \times 10^2 \text{ kgm}^{-3}$, which are practically the lowest c exhibiting the rubbery plateau and the solubility limit respectively. Comparison has been made between $\gamma = 0.1$ and 0.01 for the $3.9 \times 10^2 \text{ kgm}^{-3}$ solution at 60 °C, as shown in the inset to Fig.3. The coincidence of the data points clearly demonstrates that the measurements have been conducted within the linear viscoelastic regime.

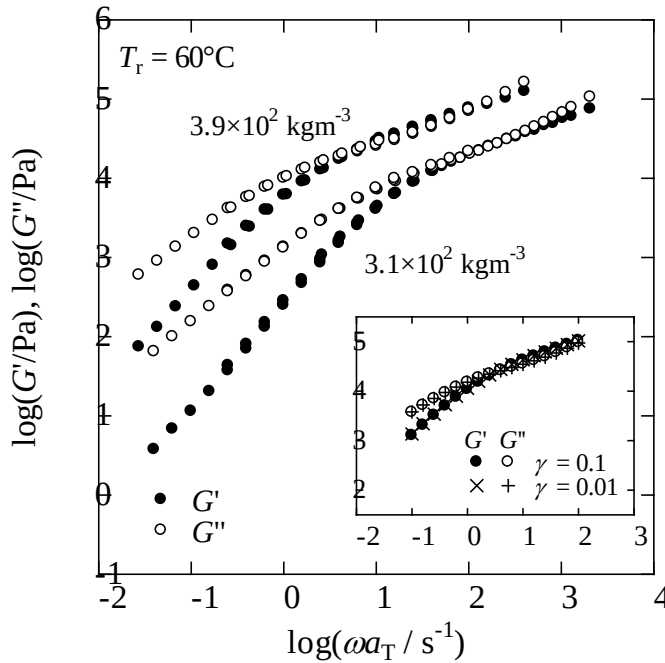


Fig. 3. Master curves of ω dependence of G' and G'' for BmimAc solutions of fucoidan of $c = 3.1$

$\times 10^2$ and $3.9 \times 10^2 \text{ kgm}^{-3}$ at $T_r = 60 \text{ }^\circ\text{C}$. Inset shows a comparison of the dynamic viscoelasticity for the $3.9 \times 10^2 \text{ kgm}^{-3}$ solution at $60 \text{ }^\circ\text{C}$ between $\gamma = 0.1$ and 0.01 .

The G' and G'' curves in the figure clearly show the terminal relation $G'' \propto \omega$ in the low ωa_T region. This is a case of polymer solutions without any intermolecular aggregation, implying that the electrostatic force is shielded due to the great ionic strength of the medium as expected. It is seen in the higher ωa_T region that there appears the so-called rubbery plateau, although the actual G' curves slope up with ωa_T probably due to the polydispersity of the fucoidan sample. The rubbery plateau arises from the entanglement coupling between fucoidan chains in BmimAc; the plateau region for the solution of $c = 3.9 \times 10^2 \text{ kgm}^{-3}$ is actually obvious compared to that for $c = 3.1 \times 10^2 \text{ kgm}^{-3}$ [12,16]. Master curves of G' and G'' for $c = 3.5 \times 10^2 \text{ kgm}^{-3}$ also show similar dynamic viscoelasticity, which is posted below.

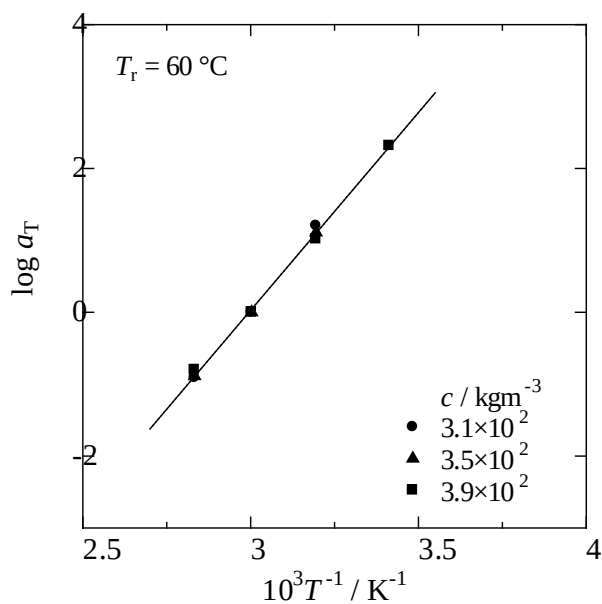


Fig. 4. Temperature dependence of a_T determined for obtaining the master curves. All data points fall on a single line.

Fig. 4 shows the temperature dependence of a_T determined for obtaining the master curves; $\log a_T$ is plotted against $1/T$ for the three BmimAc solutions with $T_r = 60\text{ }^\circ\text{C}$. It is seen that $\log a_T$ at a given T is almost the same regardless of c and that all data points can be fitted by a straight line very well. Hence the temperature dependence of a_T can be represented by an Arrhenius-type equation within the conditions employed in this study and the activation energy of viscous flow is estimated to be $1.1 \times 10^2\text{ kJ/mol}$.

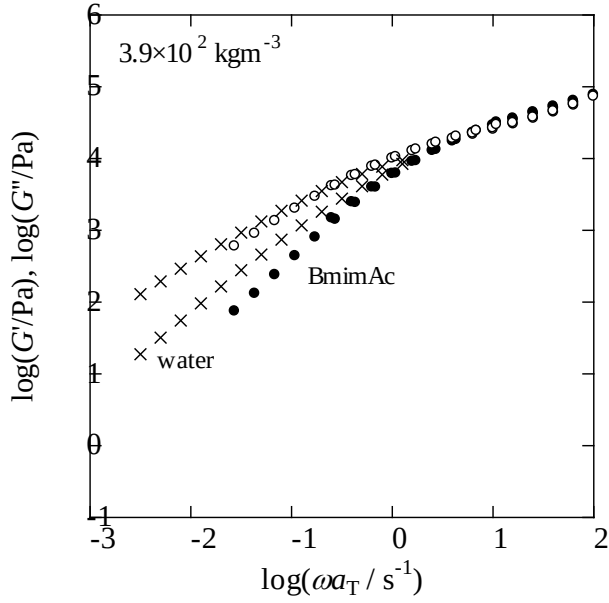


Fig. 5. Comparison of flow behavior between the aqueous and BmimAc solutions at the same concentration of $3.9 \times 10^2 \text{ kgm}^{-3}$.

The flow behavior of the aqueous and BmimAc solutions has been compared at a given concentration of $3.9 \times 10^2 \text{ kgm}^{-3}$ in Fig. 5. Here the plot for the BmimAc solution is a part of that in Fig. 3 and the plot for the aqueous solution has been shifted only horizontally so that the high ω ends of their flow regions meet. As pointed out earlier, the terminal slope of G'' for the aqueous solution is more gradual than that for the BmimAc solution. It also becomes obvious that the same goes for G' . These differences can be attributed to the electrostatic interaction between fucoidan chains in the aqueous solution, which delays the mechanical relaxation.

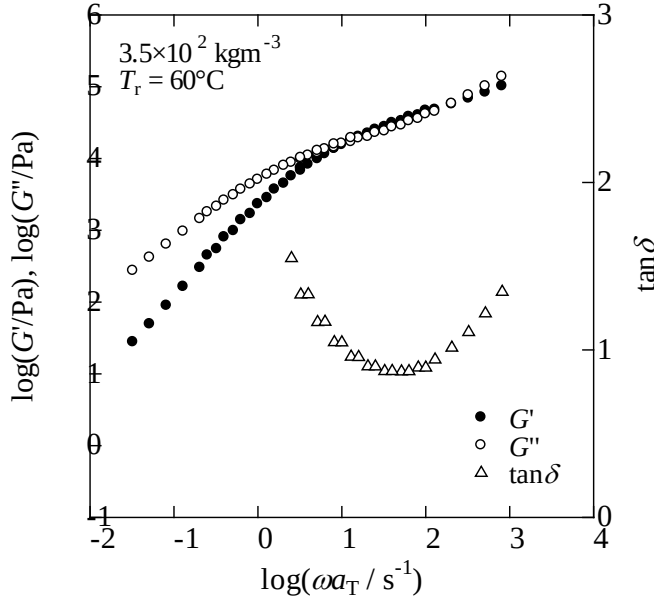


Fig. 6. Master curves of G' and G'' for $c = 3.5 \times 10^2 \text{ kgm}^{-3}$ together with $\tan\delta$ in the vicinity of the minimum.

Now, let us turn to the rubbery plateau observed for the BmimAc solutions. The plateau modulus (G_N^0) can be determined from a tilted rubbery plateau as the value of G' at ω where the loss tangent ($\tan\delta$; $\tan\delta = G''/G'$) attains the minimum. Then M_e for a polymer in a concentrated solution of c can be calculated from G_N^0 based on the analogy with rubber elasticity as

$$M_e = \frac{10^3 c R T}{G_N^0} \quad (1)$$

where R is the gas constant [10-12]. As an example, Fig. 6 shows the master curves of G' and G'' for $c = 3.5 \times 10^2 \text{ kgm}^{-3}$ together with the loss tangent ($\tan\delta$; $\tan\delta = G''/G'$) in the vicinity of the minimum. In the figure, G_N^0 can be determined to be $3.0 \times 10^4 \text{ Pa}$, which gives $M_e = 3.0 \times 10^4$ (in gram/mole). The values of M_e at other concentrations were determined in a similar manner.

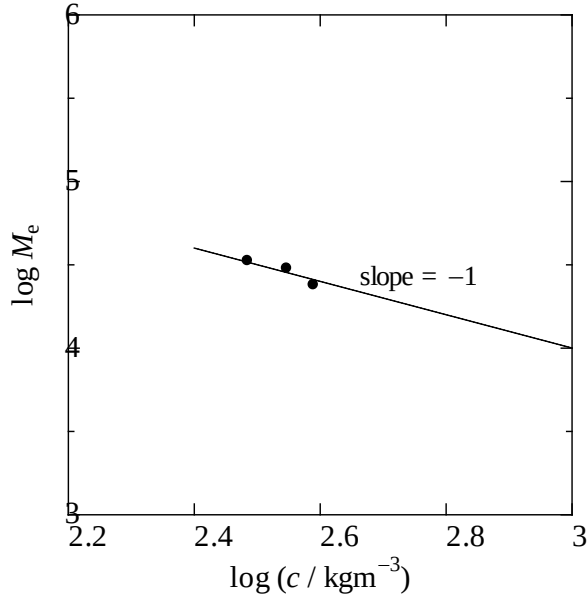


Fig. 7. Double-logarithmic plot of M_e against c for fucoidan in the BmimAc solution. The solid line is the best fit one with a slope of -1 .

Fig. 7 shows the double-logarithmic plot of the obtained M_e against c . It is well known that a relation $M_e \propto c^{-1}$ holds for a great number of concentrated polymer solutions [17-19] and therefore a straight line with a slope of -1 is also drawn in the figure to fit the plot best. It can be confirmed that the c dependence of M_e holds well for the fucoidan solutions. Based on the $M_e \propto c^{-1}$ relation and the assumption that the density of fucoidan is $1.0 \times 10^3 \text{ kgm}^{-3}$, $M_{e,\text{melt}}$ for fucoidan can be estimated to be 1.0×10^4 . Some remarks should be made about the density of fucoidan. Of course, fucoidan in the molten state is not available actually, but it is possible to assume an imaginary “molten state” of this polysaccharide. According to the fact that $M_{e,\text{melt}}$ is defined for amorphous polymers in the molten state, the imaginary “molten state” can be explained as a random assembly

of fucoidan chains without solvent molecules. The density of fucoidan is just chosen as a classic value of density of amorphous polymers in the absence of experimental data. We would claim that $M_{e,melt}$ obtained in this study is comparable with those of amorphous polymers.

Our earlier rheological studies have reported a large number of $M_{e,melt}$ for polysaccharides. Regarding the relationship between $M_{e,melt}$ and the molecular structure of polysaccharides, it has been found that the linkage between the monosaccharide repeating units of the main chain is quite influential on $M_{e,melt}$ for D-glucans [20]. It has been also shown that $M_{e,melt}$ depends much on the species of the repeating unit [18]. On the other hand, the content of monosaccharides as the side groups as well as of sulfate groups in the main chain units seems to have weaker effect on $M_{e,melt}$ according to the results for galactomannans and carrageenans [21,22]. These results suggest that $M_{e,melt}$ for fucoidan obtained in this study does not change significantly as far as the main chain mostly consists of $\alpha(1\rightarrow3)$ -linked L-fucose like fucoidan from *Cladosiphon okamuranus*. Actually, fucoidan from other origins contain $\alpha(1\rightarrow4)$ -linkage to some extent [3], but how the hetero-link affects $M_{e,melt}$ cannot be evaluated at present due to lack of suitable samples.

4. Conclusion

Dynamic viscoelasticity was examined for concentrated solutions of fucoidan from *Cladosiphon okamuranus* in water and in BmimAc. The rheological data indicates the existence

of the electrostatic interaction between fucoidan chains in the aqueous solutions, while it disappears in the BmimAc solutions. The entanglement coupling between fucoidan chains was observed as the rubbery plateau in both solvents at rather high concentrations. From the concentration dependence of M_e for the BmimAc solutions, $M_{e,melt}$ for fucoidan was estimated to be 1.0×10^4 as one of the chain characteristics. It can be concluded that this value corresponds to a material constant of fucoidan, despite the fact that fucoidan has a number of minor variations of chemical structures depending on the origin.

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