

Rheological Behavior of Worm-Like Micelles in a Mixed Nonionic Surfactant System of a Polyoxyethylene Phytosterol and a Glycerin Fatty Acid Monoester

Kaname HASHIZAKI,*^a Nao TAMAKI,^a Hiroyuki TAGUCHI,^a Yoshihiro SAITO,^a Koji TSUCHIYA,^b Hideki SAKAI,^b and Masahiko ABE^b

^a College of Pharmacy, Nihon University; 7-7-1 Narashinodai, Funabashi, Chiba 274-8555, Japan; and ^b Faculty of Science and Technology, Tokyo University of Science; 2641 Yamazaki, Noda, Chiba 278-8510, Japan.

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In the present study, we examine a worm-like micelle consisting of a nonionic surfactant system of polyoxyethylene phytosterol (PhyEO_m)/glycerin fatty acid monoester (GFA-C_n)/Water (m=10, 20, 30; n=8, 10, 12) using rheological measurements looking towards potential application of the system as a gel base. Phase diagrams in the dilute region of the PhyEO_m/GFA-C_n/Water systems show the formation of worm-like micelles in some of the surfactant combinations. It is thought that the worm-like micelles form with the GFA-C_n solubilized in the palisade layer of a spherical or rod-like micelle consisting of PhyEO_m, resulting in a decrease in the interfacial curvature of the molecular assembly. The rheological properties of micellar solutions were examined while changing the ratio of GFA-C_n (R) with the total concentration of the surfactants (wt%) fixed. Steady-flow viscosity measurements in the region of worm-like micelle formation showed Newtonian flow in the low shear rate region and non-Newtonian flow at higher shear rates. This result shows that the network structure of worm-like micelles does not break in the low shear rate region, but does break in the high shear rate region. The zero-shear viscosity (η_0) was calculated from the steady-flow viscosity curve and was found to change dramatically with changing R value, increasing to a value 10000 times that of other values at the maximum. Thus, there is an optimal composition of surfactants which leads to the greatest entanglement of the worm-like micelles. To consider the change in η_0 in detail, dynamic viscoelasticity measurements were carried out. Consequently, the viscoelastic behavior of the worm-like micelles was found to be similar to the Maxwell model, which represents the most basic model for a viscoelastic body, and it was shown that this worm-like micelle had a single relaxation time. Moreover, it was found that the change in η_0 of a worm-like micelle was influenced by its mechanical strength.

Key words worm-like micelle; polyoxyethylene phytosterol; glycerin fatty acid monoester; rheology; phase diagram

Surfactants are amphipathic molecules which have hydrophilic and hydrophobic groups and in combination with water and oil phases they allow the formation of a great variety of molecular assemblies such as spherical micelles, rod-like micelles (worm-like micelles), vesicles, cubic liquid crystals, lamellar liquid crystals, and hexagonal liquid crystals. Among these, worm-like micelles, cubic liquid crystals, and hexagonal liquid crystals are found to form gels with high viscosity. In particular, worm-like micelles are well known to form gels even when the surfactant concentration is low. Worm-like micelles form a transient network structure in which the micelles entangle with one another. Worm-like micelles can be formed with the addition of aromatic salts such as sodium salicylate to cationic surfactants such as cetyltrimethylammonium bromide or cetylpyridinium chloride.^{1–3)} However, the preparation of worm-like micelles of high viscoelasticity using nonionic surfactants remains a challenge.

Recently, Kunieda *et al.*^{4–7)} reported that worm-like micelles of high viscoelasticity could be formed by mixing two types of nonionic surfactants with very different HLB (hydrophilic–lipophilic balance) values. For example, a worm-like micelle was prepared by the addition of a hydrophobic surfactant, polyoxyethylene dodecyl ether, to a spherical micellar solution of a hydrophilic surfactant, such as polyoxyethylene cholesterol or polyoxyethylene phytosterol. Because the hydrophobic cholesterol and phytosterol groups are of natural origin, these surfactants are not expected to irritate the skin, and as such could be useful as the base for an exter-

nally applied medicine. However, as yet there have been no investigations of such systems for this application.

In general, a water-soluble polymer such as macrogol *etc.* is used as the base in external applications. However, in their natural state these bases cannot dissolve many of the target drugs. As such, organic solvents are usually added to the base, or the drug is dispersed into the base. However, organic solvents such as ethanol may induce skin irritation and suspended drugs have low permeability from the base to the skin. In another approach, a base applied with a worm-like micelle increases the solubility of insoluble drugs. In such systems, adhesion of the base to the skin can also improve due to a reduction in surface tension. In addition, the use of a worm-like micelle is expected to become a transdermal drug delivery system (TDDS) because certain surfactants such as glycerin fatty acid monoesters have the effect of enhancing the transdermal absorption of drugs.^{8,9)}

In this study, we examined a worm-like micelle consisting of polyoxyethylene phytosterols and glycerin fatty acid monoesters using rheological measurements with a view towards potential application in a gel base.

Experimental

Materials Polyoxyethylene phytosterol (PhyEO_m, m: addition molar number of EO units, m=10, 20, 30) was purchased from Nikko Chemicals Co., Ltd. (Tokyo, Japan) and was used as the hydrophilic nonionic surfactant. The glycerin fatty acid monoester (GFA-C_n, n: carbon number of fatty acid, n=8, 10, 12) was a gift from Taiyo Kagaku Co., Ltd. (Mie, Japan) and was used as the hydrophobic nonionic surfactant. Distilled water for injection as defined by the JP (Japanese Pharmacopoeia) was purchased from Otsuka Pharmaceuticals Co., Ltd. (Tokyo, Japan).

* To whom correspondence should be addressed. e-mail: hashizaki.kaname@nihon-u.ac.jp

Sample Preparation and Phase Diagrams The required amount of PhyEO_m, GFA-C_n and water was placed in a screw-cap bottle and mixed using a magnetic stirrer. The bottles were then thermostated at 25 °C from a few days (for micellar phase) to several weeks (for the liquid crystalline phase) for equilibration. Phase diagrams of PhyEO_m/GFA-C_n/Water systems were obtained using visual observation through crossed polarizers and small angle X-ray scattering (SAXS) (Nano-STAR SSS, Bruker AXS Inc., WI, U.S.A.) analysis. Worm-like micellar solutions are optically isotropic because they do not form a crystal structure; therefore, a characteristic pattern does not appear in the polarized light image. For the same reason, a scattering peak is not present in the SAXS pattern for worm-like micelles in SAXS analysis.

Rheological Measurements Steady and dynamic rheological measurements were performed using a rotational rheometer (HAAKE RS600, Thermo Fisher Scientific Inc., MA, U.S.A.) equipped with a cone-plate geometry (three sizes: 60-, 35- and 20-mm diameters, each having a cone angle of 1°) and a Peltier-based temperature control set at 25 °C. A solvent trap was used in order to prevent evaporation.

Results and Discussion

Phase Diagrams of PhyEO_m/GFA-C_n/Water Systems

The phase states of the nonionic surfactant solutions were identified using visual observation through crossed polarizers, or using SAXS analysis. Figure 1 shows the phase diagrams in the dilute region of the PhyEO_m/GFA-C_n/Water systems. In the PhyEO₁₀/GFA-C₈/Water system, the PhyEO₂₀/GFA-C₁₀/Water system, and the PhyEO₃₀/GFA-C₁₀/Water system, the formation of worm-like micelles was confirmed based on the appearance of a highly viscous region (shaded area) in the micellar phase (Wm). All the liquids were transparent and optically isotropic when stationary, but upon movement showed a weak birefringence. Israelachvili^{10,11} defined the critical packing parameter (*CPP*) relating the shape and volume ratio of the molecules, which allows prediction of the structure of the aggregates being formed. The *CPP* value is obtained by the following equation:

$$CPP = \frac{V}{a_0 l_c} \quad (1)$$

where *V* is the volume of the hydrophobic portion, *a*₀ is the effective area per hydrophilic group and *l*_c is the length of the hydrophobic group. It has been found that the preferred molecular assembly depends on the *CPP* value. For instance, *CPP* < 1/3 corresponds to a spherical micelle, 1/3 < *CPP* < 1/2 corresponds to a rod-like micelle (worm-like micelle), 1/2 < *CPP* < 1 corresponds to a vesicle, *CPP* = 1 corresponds to a planar bilayer and *CPP* > 1 corresponds to reverse aggregates. In general, in a mixed surfactant system, it is difficult to guess the form of the molecular assembly based simply on the molecular parameters of each surfactant. However, PhyEO_m is considered to give *CPP* < 1/3 because it forms a spherical micelle or rod-like micelles without entanglement,¹² while GFA-C_n gives *CPP* > 1 because it forms a reverse rod-like micelle.¹³ Therefore, when these surfactants are mixed at an appropriate ratio and the *CPP* value enters the range of 1/3 < *CPP* < 1/2, it is thought that a worm-like micelle is formed and high viscoelasticity is generated.

Accordingly, it is thought that the worm-like micelle forms such that the GFA-C_n is solubilized in the palisade layer of a spherical or rod-like micelle formed of PhyEO_m, resulting in a decrease in the interfacial curvature of the molecular assembly. Moreover, the micellar region could be observed in the systems containing GFA-C₁₂. These results showed that there was an optimal surfactant combination for formation of

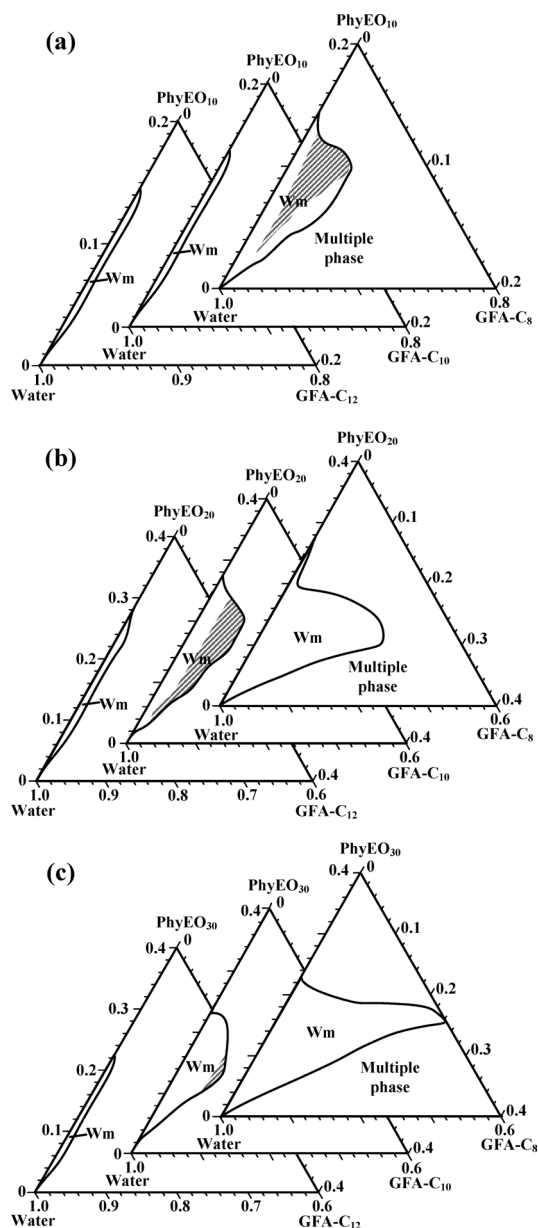


Fig. 1. Partial Phase Diagrams of (a) PhyEO₁₀/GFA-C_n/Water, (b) PhyEO₂₀/GFA-C_n/Water and (c) PhyEO₃₀/GFA-C_n/Water Systems in a Dilute Region at 25 °C

The notation Wm represents a micellar phase. The region of high viscosity within the Wm phase is shown by the shaded area.

worm-like micelles.

Rheological Behavior of PhyEO_m/GFA-C_n/Water Systems After the formation of worm-like micelles was confirmed in the micellar phases (Wm) of the phase diagrams (Fig. 1), the rheological properties were examined. In these investigations, the micellar solution was prepared such that the total concentration of the surfactants (wt%) was fixed while changing the ratio of GFA-C_n (*R* = GFA-C_n conc. (wt%)/surfactant total conc. (wt%)).

First, the steady-flow viscosity of the PhyEO_m/GFA-C_n/Water systems was measured. Figure 2 shows the results of steady-flow viscosity measurements of PhyEO₁₀/GFA-C₈/Water (5 wt%) systems as an example. In all systems, Newtonian flow was exhibited over the low shear rate range as demonstrated by a near constant viscosity (*η*) over shear

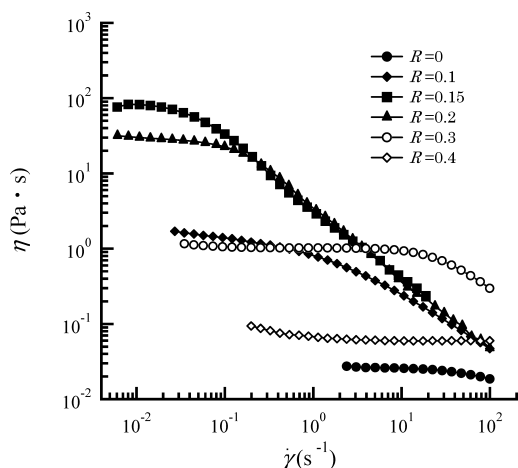


Fig. 2. Viscosity (η) vs. Steady Shear Rate ($\dot{\gamma}$) Plots of the PhyEO₁₀/GFA-C₈/Water System at 25 °C

Total surfactant concentration is 5 wt%.

rate. This indicates that the network structure^{14,15} formed by the worm-like micelles does not break in this range. At higher shear rates, the solutions displayed non-Newtonian flow, giving a decrease in η of the solution with increasing shear rate. This phenomenon may be a result of the collapse of the network structure due to disentanglement and/or breakage of the worm-like micelles as the shear rate increases. Figure 3 shows the relation between the zero-shear viscosity (η_0), given by extrapolation of the shear rate curve to zero, and the R value. In the systems in which worm-like micelles formed, there is considerable variation of η_0 with R value, with the maximum value about 10000 times the lowest. This result indicates there is an optimal composition of surfactants which leads to maximum entanglement of the worm-like micelles. In the PhyEO₁₀/GFA-C₈/Water system, the R value of maximum η_0 represents a micellar phase (Fig. 1a). On the other hand, in the PhyEO₂₀/GFA-C₁₀/Water system, the R value of maximum η_0 almost corresponds to the boundary between the micellar phase (Wm) and a multiple phase (Wm+lammellar liquid crystalline phase (L α)) (Fig. 1b). Therefore, to examine the change in η_0 in detail, dynamic viscoelasticity measurements were performed.

First, to decide the linear viscoelastic region of the solution, oscillation stress sweep measurements were performed. Figure 4 shows the shear stress dependence of the storage modulus (G'), loss modulus (G''), and strain (γ) for the PhyEO₁₀/GFA-C₈/Water (10 wt%, $R=0.2$) system as an example. Here, G' and G'' reflect the elasticity and viscosity, respectively. The worm-like micelle had a linear viscoelastic region in which G' and G'' did not change with changes in shear stress; that is, in this region the network structure of the worm-like micelle did not break with increasing shear stress. Next, an oscillation frequency sweep measurement was performed using a γ value from the linear viscoelastic region.

Figure 5 shows the variation in G' and G'' as a function of the frequency (ω) using the PhyEO₁₀/GFA-C₈/Water (10 wt%) system as an example. G' and G'' intersect under a certain ω ; at high frequencies the elasticity is predominant ($G' > G''$) whereas at low frequencies the viscosity is predominant ($G'' > G'$). Moreover, the G' and G'' of the PhyEO₁₀/GFA-C₈/Water system increased overall with increasing R

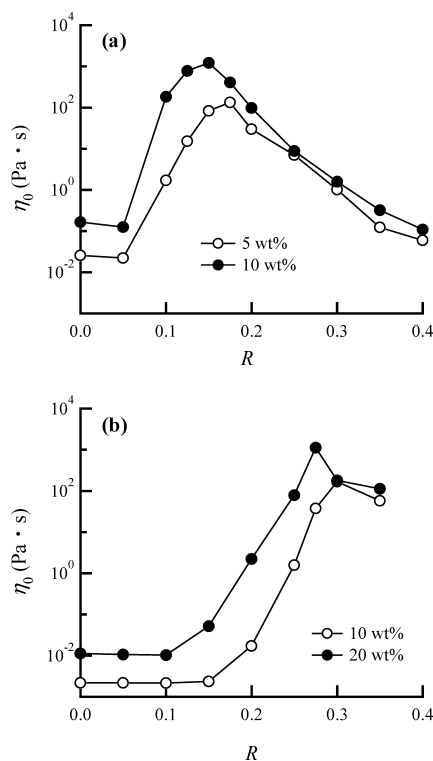


Fig. 3. Relation between Zero-Shear Viscosity (η_0) and Ratio of GFA-C_n (R) in (a) PhyEO₁₀/GFA-C₈/Water and (b) PhyEO₂₀/GFA-C₁₀/Water Systems at 25 °C

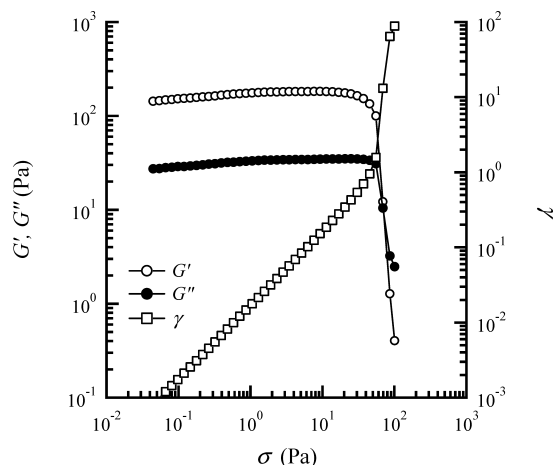


Fig. 4. Shear Stress (σ) Dependence of G' , G'' and γ for the PhyEO₁₀/GFA-C₈/Water System ($R=0.2$) at 25 °C

Total surfactant concentration is 10 wt%.

value. This suggests that the network structure in the solution strengthens with growth of the worm-like micelles as the GFA-C₈ concentration increases. Figure 6 shows the Cole-Cole plots (relation between G' and G'') based on the results of the oscillation frequency sweep measurements. The Cole-Cole plot of the experimental results is a complete semicircle, following the Maxwell model with a single relaxation time,²⁾ and is a simple way to check whether a result is consistent with the single Maxwell model. Incidentally, this relaxation time indicates the time that the stress of the entanglement point of worm-like micelles relaxes and reflects the disentanglement time of worm-like micelles. Since the

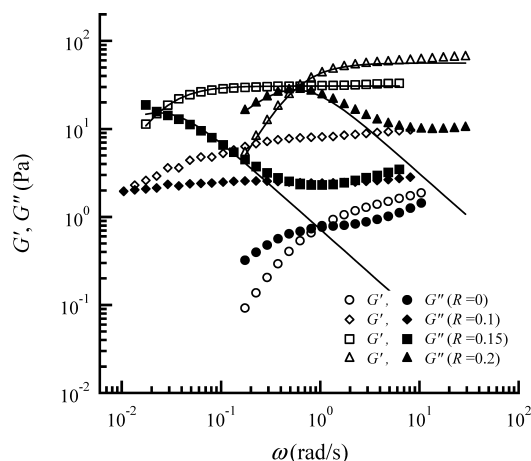


Fig. 5. Variation of G' and G'' as a Function of ω Obtained by Oscillation Frequency Sweep Measurements at Different R Values in the PhyEO₁₀/GFA-C₈/Water System at 25 °C

Total surfactant concentration is 10 wt%. The Maxwellian fittings to the experimental data are shown by a solid line.

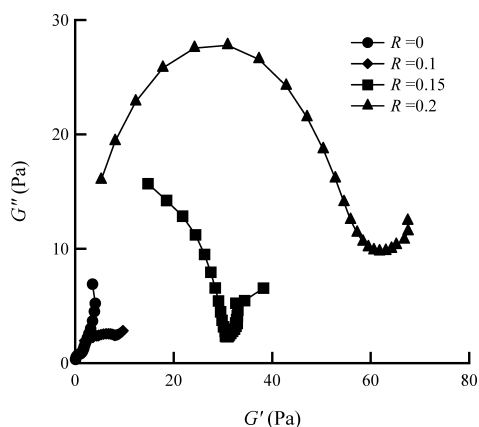


Fig. 6. Cole-Cole Plots of G' and G'' at Different R Values in the PhyEO₁₀/GFA-C₈/Water System at 25 °C

Total surfactant concentration is 10 wt%.

Cole-Cole plot approaches a semicircular curve with increasing R values, it was confirmed that the relaxation time takes a single value with the formation of worm-like micelles. In general, the relaxation time of linear polymers has a wide distribution because they disentangle *via* reptation (reptile-like motion) and the relaxation time is proportional to the third power of the molecular weight.^{16,17} On the other hand, the rheological behavior of worm-like micelles is decided by the balance between reversible breaking and reptation of the micelles, and single Maxwellian behavior is generally observed when the reversible breaking time is sufficiently shorter than the reptation time.¹⁾ In this situation, the relaxation time is given by a geometric mean of the breaking time and reptation time. Assuming that a worm-like micelle formed in this system follows a single Maxwell model, curve fitting can be performed based on Eqs. 2 and 3.

$$G' = \frac{\omega^2 \tau^2}{1 + \omega^2 \tau^2} G_0 \quad (2)$$

$$G'' = \frac{\omega \tau}{1 + \omega^2 \tau^2} G_0 \quad (3)$$

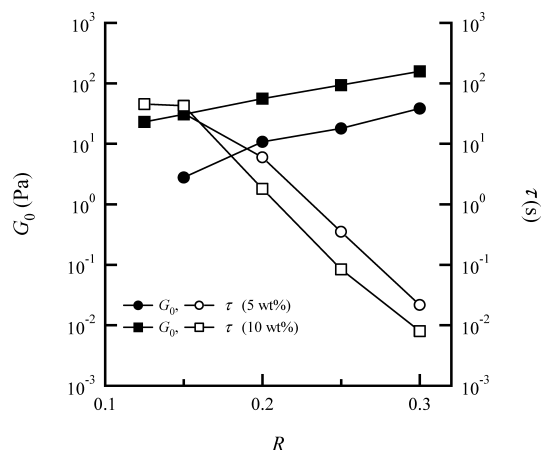


Fig. 7. Variation of G_0 and τ at Different R Values in the PhyEO₁₀/GFA-C₈/Water System at 25 °C

where G_0 is the plateau modulus reflecting the volume fraction of the entangled worm-like micelles¹⁾ and τ is the relaxation time of the worm-like micelles. The curve fitting results are shown in Fig. 5 by the solid line. The rheological behavior of the worm-like micelles deviates from the Maxwellian fittings at high frequencies. This shows that the relaxation mode of worm-like micelles formed in this system is not strictly limited to a single Maxwell model and implies that there is a faster relaxation mode in addition to relaxation *via* the reversible breaking of the worm-like micelles. It is thought that this faster relaxation mode is related to micro-Brownian motion (*i.e.*, the Rouse mode¹⁸⁾) of the worm-like micelle itself. For lower R values (*e.g.*, $R=0.10$), for which there is disagreement with the single Maxwell model, it is thought that disentanglement of worm-like micelles takes place by reptation, as occurs in linear polymer systems, because the mechanical strength of the worm-like micelles is high. Further experiments are needed to investigate the relaxation behavior of the worm-like micelles in more detail.

Figure 7 shows the variation in G_0 and τ as a function of the R value of the PhyEO₁₀/GFA-C₈/Water system. In this system, G_0 gradually increased with increasing R values, while τ rapidly decreased at R values 0.15 or greater. This indicates that the disentanglement time of the worm-like micelles rapidly decreases despite the increase in volume fraction of the entangled worm-like micelles with increasing R values. This result suggests that the worm-like micelles become breakable because the mechanical strength of the worm-like micelles decreases as the R values increase. Assuming the single Maxwell model, the zero-shear viscosity (η_0) is defined by the following equation:

$$\eta_0 = G_0 \tau \quad (4)$$

In this equation, η_0 is decided by the balance between the volume fraction and the disentanglement time of the entangled worm-like micelles. The η_0 values calculated from this equation corresponded to those obtained by the steady-flow viscosity measurements as shown in Fig. 3a. As noted above, in the PhyEO₁₀/GFA-C₈/Water system, the R value of the maximum η_0 existed in the micellar phase, and the change in η_0 can be explained from the relation between G_0 and τ as follows. That is, η_0 is thought to increase with increasing R

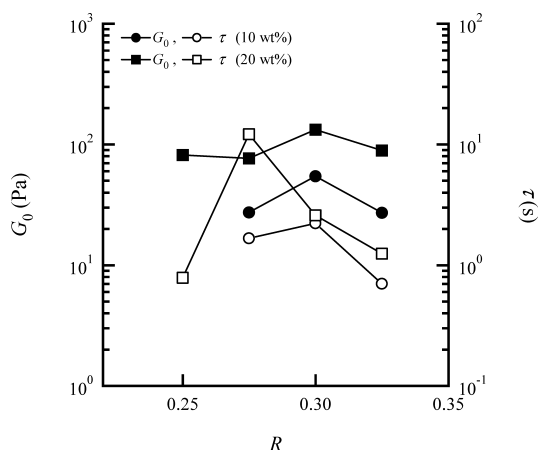


Fig. 8. Variation of G_0 and τ at Different R Values in the PhyEO₂₀/GFA-C₁₀/Water System at 25 °C

value, as shown in Fig. 3a, not only because of the increase in the volume fraction of the entangled worm-like micelles but also because the worm-like micelles are not easily broken. Conversely, it is thought that η_0 decreases with increasing R values because the worm-like micelles rapidly become more breakable as the volume fraction of the entangled worm-like micelles increases.

Figure 8 shows the variation in G_0 and τ as a function of R value of the PhyEO₂₀/GFA-C₁₀/Water systems. For each surfactant concentration, G_0 showed a maximum in the vicinity of $R=0.30$, which almost corresponds to the boundary between the micellar phase and a multiple phase (Wm+L α) (Fig. 1b). This result indicates that the increase in G_0 originates in the growth of the worm-like micelles, and the decrease in G_0 arises from a change in morphology from a worm-like micelle to a lamellar liquid crystal. Moreover, the maximum value of τ is given for $R=0.30$ in the 10 wt% system and $R=0.275$ in the 20 wt% system. In addition, the η_0 obtained by Eq. 4 was consistent with that observed by the steady-flow viscosity measurement. From these results, it is thought that the increase in η_0 with R value, as shown in Fig. 3b, is due to an increase in the volume fraction and mechanical strength of the worm-like micelles. Conversely, the decrease in η_0 with R values is considered to be due to a decrease in the volume fraction of worm-like micelles upon

formation of lamellar liquid crystals in addition to a decrease in the mechanical strength of the worm-like micelles.

Overall, the viscosity of the worm-like micelle could be controlled by varying the composition and concentration of the surfactants, suggesting its utility as a new gel base for pharmaceuticals. Future research will examine the properties of gel bases formed by worm-like micelles with respect to their solubilizing ability, adhesion and transdermal absorption.

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