

## Reversible Vesicle Formation by Changing pH

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Protonation of oleyldimethylamine oxide (OleylDAO) decreased the average curvature and induced a change from threadlike micelles in the nonionic state to particles (vesicles) at the half-protonated state (degree of ionization  $\alpha = 0.5$ ), concluded from viscoelastic measurements and cryoTEM observations in water at 25 °C. The viscoelastic changes were confirmed to be reversible with respect to a pH change. The times required for the solutions to recover the behavior at  $\alpha = 0.5$  after the addition of HCl to the solutions of  $\alpha = 0$  were about 25 h at 0.05 mol (kg water)<sup>-1</sup> and 14 days at 0.15 mol (kg water)<sup>-1</sup>. The correspondence between the viscoelastic properties and the cryoTEM observations was good. It was suggested from the viscoelastic properties that the threadlike micelles grew with  $\alpha$  at  $\alpha = 0.2$ , but the change from threadlike micelles to vesicles was suggested as  $\alpha$  increased further. On increasing the concentration at  $\alpha = 0.5$ , the vesicles became perforated, probably due to the increased counterion concentration. The present results indicate unambiguously the important and effective action of protonation in controlling the packing parameter, or the mean curvature, of amine oxide amphiphiles.

Vesicle formation by single-chain amphiphiles has been studied extensively in recent years.<sup>1,2</sup> Mixing of two or more single-chain amphiphiles or cosurfactants with widely different packing parameters is a popular approach.<sup>3</sup> Catanionic surfactants may be classified into this group.<sup>4–6</sup> For a spontaneous and reversible vesicle formation, tuning of the packing parameter by some physical factor is preferable, such as temperature, pressure or pH. Fatty acids have been known to form acid soaps on partial protonation and some of them form vesicles.<sup>7–9</sup> Introduction of electric charges generally increases the average curvature and facilitates the formation of vesicles from the lamellar phase.<sup>10,11</sup> In the present study, however, the introduction of electric charge decreases the average curvature and hence induces a change from threadlike micelles to vesicles. Alkylamine oxides can be protonated to various degrees. The change of the packing parameter or the average curvature by protonation has been shown to differ from the expectation based on purely electrostatic interaction: it goes through a maximum at about the half-protonated state contrary to the expectation of monotonic decrease with ionization (protonation in the case of amine oxides).<sup>12–14</sup> This characteristic behavior has been attributed to additional short-range attractive interaction between the protonated and the nonprotonated headgroups of amine oxides.<sup>12</sup> As a probable candidate, a hydrogen bond is proposed.<sup>12</sup> Recently, spectroscopic information was obtained in favor of the proposed hydrogen bond.<sup>13</sup>

Oleyldimethylamine oxide was obtained from oleyldimethylamine (Lion Akzo) through the oxidation in ethanol by hydrogen peroxide following, in most part, the procedure similar to that for other alkyldimethylamine oxides and purified by the

extraction of the unreacted amine by hexane.<sup>14</sup> The surface tension showed no minimum around the critical micelle concentration  $((8 \pm 1) \times 10^{-5} \text{ mol (kg water)}^{-1})$ . The surfactant concentration  $C$  is expressed in mol (kg water)<sup>-1</sup>, molality, and denoted with  $m$ . The viscoelastic properties were measured at  $25 \pm 0.1$  °C with a stress-controlled rheometer (Carri-MED CSL-100) with a cone plate (plate diameter 6 cm, 2° angle) and a parallel plate type geometry (plate diameter 4 cm) on OleylDAO solutions.

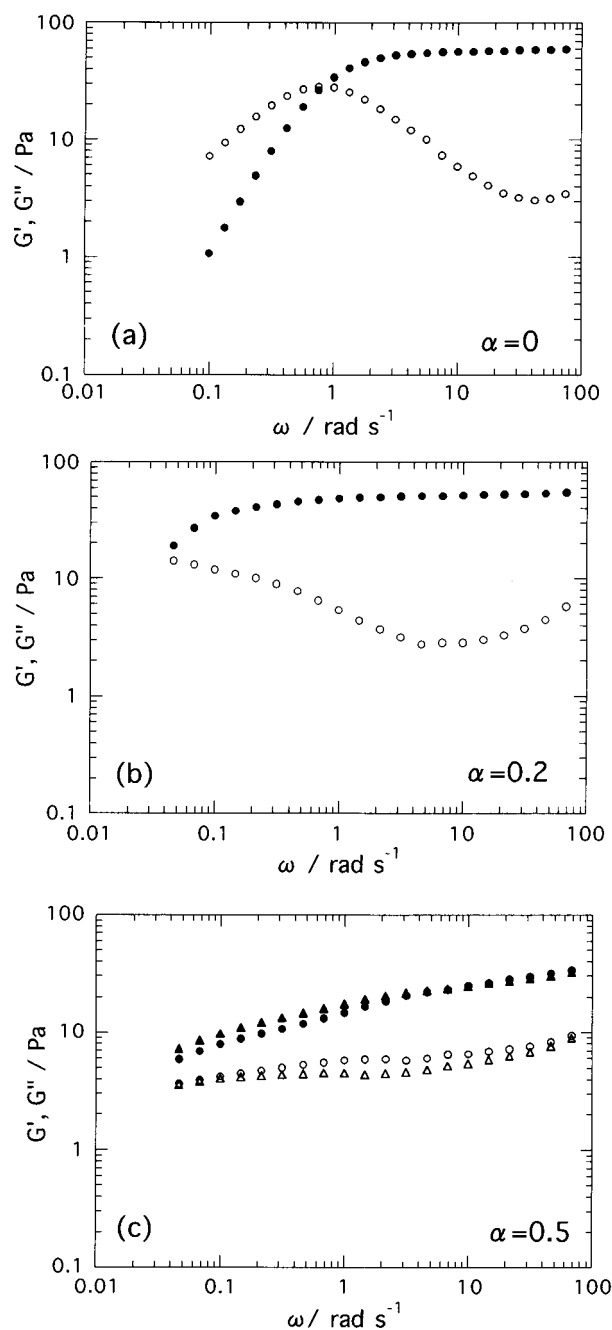
Figure 1 shows angular frequency  $\omega$  dependence of the storage shear modulus  $G'$  and the loss shear modulus  $G''$  of OleylDAO solutions at 25 °C at  $C = 0.15 m$  with different degrees of ionization  $\alpha$ . At the degree of ionization  $\alpha = 0$  (nonionic state), Figure 1a indicates the behavior characteristic of a transient network consisting of long flexible chains.<sup>14,15</sup> The slowest relaxation process is well separated from other fast relaxation processes, and it can be well described with a Maxwell model. The similar behavior was observed for tetradecyldimethylamine oxide (C14DAO) only at near the half-protonated state ( $\alpha = 0.5$ ) in 0.1 M NaCl solutions but not in the case of the nonionic state.<sup>14</sup> The effect of decreasing the average curvature of the aggregates with increasing hydrocarbon chain length is evident. The viscoelastic behavior of OleylDAO dramatically changed on protonation, as shown in Figure 1a–c. At  $\alpha = 0.5$ , Figure 1c, both the storage modulus  $G'$  and the loss modulus  $G''$  show weak dependence on the frequency with a relation  $G' > G''$ , a kind of behavior typical to gels or concentrated suspensions. The gel structure at  $\alpha = 0.5$  is expected to be of densely packed particles. When compared at an angular frequency of 32 rad s<sup>-1</sup>, values of  $G'$  were about 60 and 30 Pa for  $\alpha = 0$  and 0.5, respectively. Both solutions showed shear thinning behaviors, as shown in Figure 2. The zero-shear viscosities were approximately the same both at  $\alpha = 0.5$  and  $\alpha = 0$ , but the shear-thinning at  $\alpha = 0.5$  started already in the shear rate region of 0.01 s<sup>-1</sup> or even smaller,

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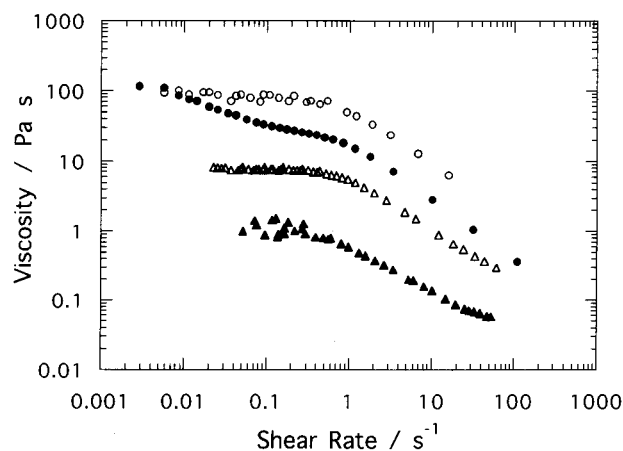
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**Figure 1.** Angular frequency  $\omega$  dependence of the storage shear modulus  $G'$  (●, ▲) and the loss shear modulus  $G''$  (○, △) of oleyldimethylamine oxide solutions at 25 °C and  $C = 0.15$  m with different degrees of ionization  $\alpha$ . (a)  $\alpha = 0$ , (b)  $\alpha = 0.20$ , and (c)  $\alpha = 0.50$ . Circles refer to the solutions prepared by dissolving the solid samples of the prescribed compositions  $\alpha$ . Triangles in Figure 1c refer to the solution prepared by the addition of HCl to the solution of  $\alpha = 0$  and incubated for 14 days.

which was about 2 orders smaller than the corresponding value at  $\alpha = 0$ . The yield stress was estimated to be smaller than 1 Pa for the solution of the half-protonated species. However, this small yield stress was found to be sufficient to prevent air bubbles from escaping the solution during the preparation. These results are consistent with the picture of densely packed particles. The viscoelastic properties at  $\alpha = 0.5$  are similar qualitatively to those observed by Hoffmann et al. on the nonionic C14DAO/cationic surfactant mixture plus a cosurfactant.<sup>16</sup>

The above results were obtained with the solutions prepared by dissolving solid samples of the prescribed  $\alpha$  values. When



**Figure 2.** Shear rate dependence of the viscosity of oleyldimethylamine oxide solutions at 25 °C. Degrees of ionization  $\alpha$ : Filled and open symbols refer to  $\alpha = 0.5$  and  $\alpha = 0$ , respectively. Concentration: circles and triangles refer to  $C = 0.15$  m and  $0.05$  m, respectively.

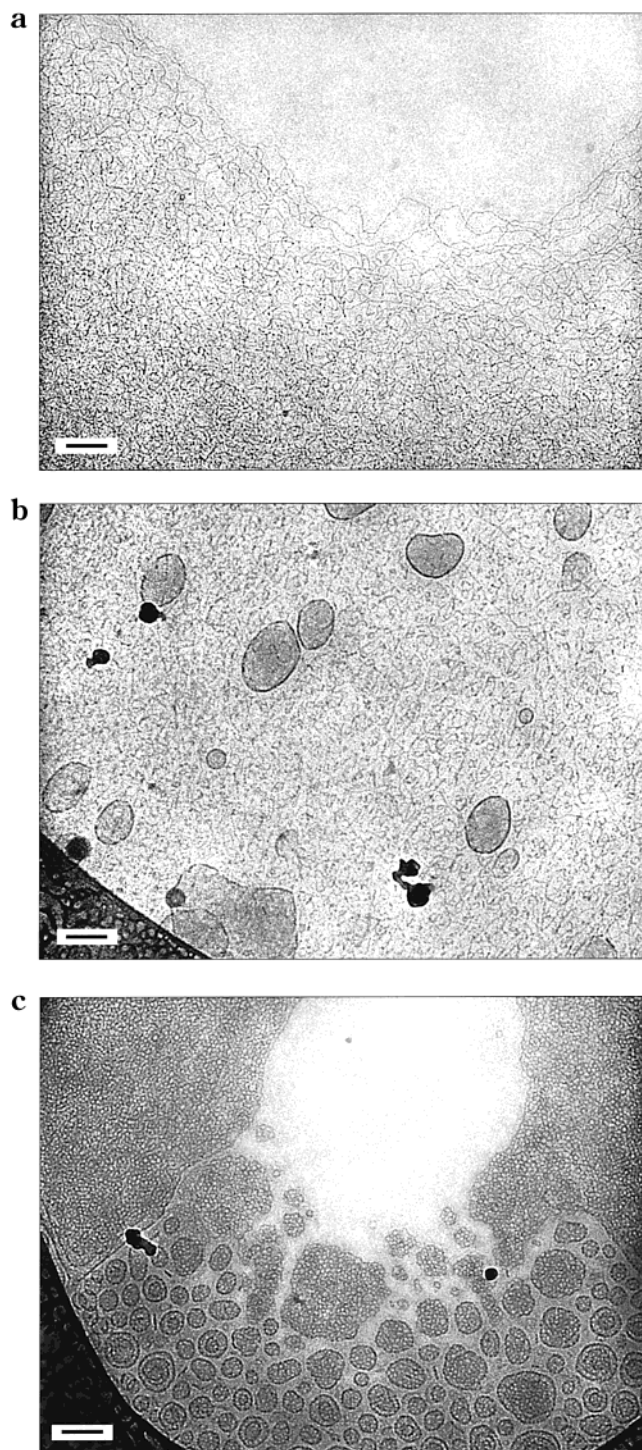
the solution of  $\alpha = 0$  was brought to  $\alpha = 0.5$  by adding HCl, the Maxwell type behavior disappeared and the solidlike behavior was observed, as shown in Figure 1c after about 14 days at  $C = 0.15$  m. At  $C = 0.05$  m, the change completed after 25 h, as shown in Figure 4. When the solution of  $\alpha = 0.5$  was brought to  $\alpha = 0$  by adding NaOH, on the other hand, the Maxwell type behavior was recovered but the complete agreement was not attained even after 3 weeks, probably because NaCl was produced in the neutralization process.

Cryotransmission electron micrographs (cryoTEM) of the two solutions ( $\alpha = 0$  and  $0.5$ ) were taken. In the solutions of the nonionic state ( $\alpha = 0$ ) at  $C = 0.05$  m, highly entangled and curled threadlike micelles were found, as shown in Figure 3a. Some of them extend over about 1000 nm. Contrary to the case of C14DAO at  $\alpha = 0.5$  ( $C = 0.1$  m in 0.1 M NaCl),<sup>14</sup> the threads do not appear to branch frequently. A TEM picture at  $\alpha = 0.5$  and  $C = 0.15$  m, Figure 3b, shows mainly perforated bilayer structures in the form of vesicles and lamellae.<sup>17</sup> The size of vesicles is in the range 50–100 nm. For the half-protonated species ( $\alpha = 0.5$ ) at  $C = 0.05$  m, the number of vesicles decreased but they are still seen in Figure 3c. These vesicles are irregular in shape and often have defects in the membrane. Their size was about 50–100 nm. Hence, the vesicle size did not change much on dilution.

Perforated vesicles were found to appear with increasing concentration  $C$  in the present study. This might appear to be inconsistent with the expectation that formation of perforated bilayers is suppressed by increasing concentration.<sup>18</sup> In the absence of supporting electrolyte, as encountered in the present study, however, the ionic strength increases significantly with  $C$  and the electrostatic repulsion is decreased by increased screening. The net repulsive interlamellar interaction is expected to decrease, if this effect is more than canceling the opposing steric repulsion that increases with  $C$ . Threadlike micelles coexisted with the vesicles at both concentrations.

When the surfactant concentration  $C$  was decreased to 0.05 m at  $\alpha = 0.5$ , the solidlike viscoelastic properties disappeared, as shown in Figure 4, while the solutions of the nonionic species maintained its Maxwell behavior. Viscosities also showed a marked decrease on dilution at  $\alpha = 0.5$  (Figure 2). The concentration effect at  $\alpha = 0$  is consistent with the characteristics of the phantom network structure. The relaxation times scarcely changed (0.8–1 s), consistent with the concentration-independent nature of the chain-crossing relaxation mechanism proposed previously.<sup>14</sup> On the other hand, the plateau modulus

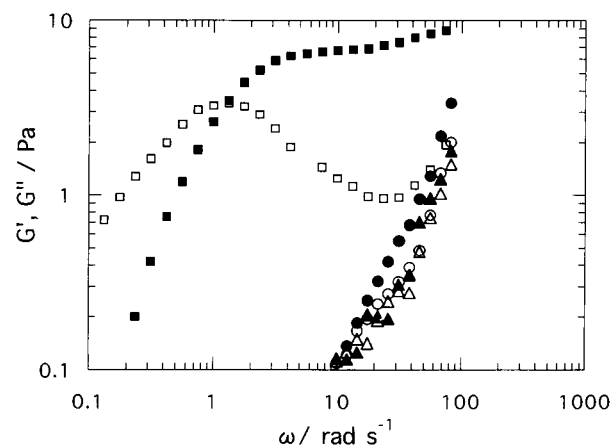




**Figure 3.** Cryoscopic transmission electron micrographs of oleyldimethylamine oxide solutions in water. A bar in each figure corresponds to 100 nm. Key: (a)  $\alpha = 0$ ,  $C = 0.05$  m, (b)  $\alpha = 0.5$ ,  $C = 0.15$  m, and (c)  $\alpha = 0.5$ ,  $C = 0.05$  m.

increased with  $C$  by about 9 times (7 and 60 Pa), as expected from the relation  $G' \sim C^{2-2.2}$  for the network of flexible chains.

The marked decrease of the viscoelasticity at  $\alpha = 0.5$  by the 3-fold dilution is reminiscent of the breakdown of a colloidal crystal when the ionic strength parameter  $\kappa$  of the media exceeds a critical value.<sup>19</sup> In this example, the breakdown was considered to take place when the effective particle radius  $R_{\text{eff}}$  (=particle radius  $R + \kappa^{-1}$ ) becomes smaller than the half of the average interparticle distance  $d$ . In the present study, however, randomly and closely packed colloid particles instead of a crystalline structure are expected to give a solidlike nature. On dilution,  $d$



**Figure 4.** Angular frequency  $\omega$  dependence of the storage shear modulus  $G'$  and the loss shear modulus  $G''$  of oleyldimethylamine oxide solutions at 25 °C and  $C = 0.05$  m. The solutions of  $\alpha = 0$  prepared by dissolving the solid samples in water:  $G'$  (■) and  $G''$  (□). The solutions of  $\alpha = 0.5$  prepared by dissolving the solid samples in water:  $G'$  (○) and  $G''$  (△). The solutions of  $\alpha = 0.5$  prepared by the addition of HCl to the solutions of  $\alpha = 0$  and incubated for 25 h:  $G'$  (●) and  $G''$  (▲).

is expected to pass through the critical value  $2R_{\text{eff}}$  and the critical concentration was estimated to be in the range 0.1–0.15 m, on the basis of the yield values and the shear moduli.

The viscoelastic behavior did not change monotonically with the degree of protonation  $\alpha$ , as shown in Figure 1. For example, zero-shear viscosities were about 100, 640, and 100 Pa s for  $\alpha = 0, 0.2$ , and 0.5, respectively. We regard the behavior at  $\alpha = 0.2$  (Figure 1b) to be not gellike, but the Maxwell behavior with a longer relaxation time  $\tau$  (longer than 10 s) than that of the nonionic state ( $\sim 1$  s). As observed in the case of C14DAO,<sup>14</sup> protonation of amine oxides makes the rodlike micelles tougher and longer. The rodlike micelles cannot accommodate themselves to a further increase of  $\alpha$  (decrease of average curvature), however, and vesicles characterized by smaller mean curvatures begin to form on further increase of  $\alpha$ . Coexistence of threadlike micelles and vesicles has been observed earlier.<sup>6,20,21</sup>

In conclusion, protonation of OleylDAO induced a change from threadlike micelles in the nonionic state to particles (vesicles) in the half-protonated state. The viscoelastic nature of the solutions changed with the protonation in a manner consistent with the transformation of long fibrous micelles into vesicular particles, as confirmed by cryoTEM observations. The change of the viscoelastic properties was reversible with respect to a pH change. The present results indicate unambiguously the important and effective action of protonation in controlling the packing parameter, or the average curvature, of amine oxide amphiphiles. The action is a result of the interplay between the short-range attractive interaction and the electrostatic repulsion, both are introduced on protonation. The hydrogen bond between the nonionic and the cationic headgroups has been proposed as a probable candidate of the short-range attraction.<sup>12,13</sup>

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## References and Notes

- (1) Engberts, J.; B. F. N.; Kevelam, J. *Curr. Opin. Colloid Interface Sci.* **1996**, *1*, 779.
- (2) Khan, A.; Marques, E. F. *Curr. Opin. Colloid Interface Sci.* **2000**, *4*, 402.

- (3) Hoffmann, H.; Munkert, U.; Thunig, C.; Valiente, M. *J. Colloid Interface Sci.* **1994**, *163*, 217.
- (4) Kaler, E. W.; Murthy, A. K.; Rodriguez, B. E.; Zasadzinski, J. A. *N. Science* **1989**, *245*, 1371.
- (5) Kaler, E. W.; Herrington, K. L.; Murthy, A. K.; Zasadzinski, J. A. *N. J. Phys. Chem.* **1992**, *96*, 6698.
- (6) Yacilla, M. T.; Herrington, K. L.; Brasher, L. L.; Kaler, E. W.; Chiruvolu, S. *J. Phys. Chem.* **1996**, *100*, 5874.
- (7) Gebicki, J. M.; Hicks, M. *Nature* **1973**, *243*, 232.
- (8) Edwards, K.; Silvander, M.; Karlsson, G. *Langmuir* **1995**, *11*, 2429.
- (9) Blochiner, F.; Blocher, M.; Walde, P.; Luisi, P. L. *J. Phys. Chem. B* **1998**, *102*, 10283.
- (10) Edwards, K.; Gustafsson, J.; Almgren, M.; Karlsson, G. *J. Colloid Interface Sci.* **1993**, *161*, 299.
- (11) Oberdisse, J.; Couve, C.; Appell, J.; Berret, J. F.; Ligoure, C.; Porte, G. *Langmuir* **1996**, *12*, 1212.
- (12) Maeda, H.; Kakehashi, R. *Adv. Colloid Interface Sci.* **2000**, *88*, 275.
- (13) Kawasaki, H.; Maeda, H. *Langmuir* **2001**, *17*, 2278.
- (14) Maeda, H.; Yamamoto, A.; Kawasaki, H.; Souda, M.; Hossain, K. S.; Nemoto, N.; Almgren, M. *J. Phys. Chem. B* **2001**, *105*, 5411.
- (15) Shikata, T.; Kotaka, T. *Langmuir* **1987**, *3*, 1081.
- (16) Hoffmann, H.; Thunig, C.; Schmiedel, P.; Munkert, U. *Langmuir* **1994**, *10*, 3972.
- (17) Gustafsson, J.; Orädd, G.; Nyden, M.; Hansson, P.; Almgren, M. *Langmuir* **1998**, *14*, 4987.
- (18) Bagdassarian, C. K.; Roux, D.; Ben-Shaul, A.; Gelbart, W. M. *J. Chem. Phys.* **1991**, *94*, 3030.
- (19) Matsumoto, T.; Okubo, T. *J. Rheology* **1991**, *35*, 135.
- (20) Edwards, K.; Gustafsson, J.; Almgren, M.; Karlsson, G. *J. Colloid Interface Sci.* **1993**, *161*, 299.
- (21) Gustafsson, J.; Orädd, G.; Almgren, M. *Langmuir* **1997**, *13*, 6956.