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Notes

Microscopic Observation of Vesicles Formed by Alkenylsuccinic Acids

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Vesicle formation by amphiphilic molecules can be confirmed by the facts that molecular assemblies have membrane structure and that the inner aqueous phase exists in the assemblies. One useful technique for such confirmation is microscopic observation; electron micrographs have provided images of membrane layers in vesicles and inner compartments divided by membranes.[1-3]

Vesicles are generally formed in aqueous solutions of native phospholipids and artificial amphiphiles with double chains. On the other hand, it has been reported that the saturated and unsaturated fatty acids with single chains can be associated into vesicles in solutions, when those are moderately ionized.[1,3]

Alkenylsuccinic acids ($CH_3(CH_2)_{n-4}CH{=}CHCH_2CH(COOH)CH_2COOH$, C_nSA) which have carboxyl polar groups may behave like fatty acids in aqueous solutions. Dilute C_nSA solutions were opalescent (or turbid) at pH below 7, if solids were dissolved at high temperature.[4] On the other hand, aqueous $C_{16}SA$ solutions at surfactant concentrations of 1–2 wt % exhibited iridescence at high temperature.[5] It was observed by video-enhanced differential interference contrast microscopy (VEM) that vesicle-like assemblies with membrane layers and small particles existed in an iridescent solution at high temperature and in the opalescent solutions at medium pH.[6,7]

While the existence of the inner aqueous phase in C_nSA vesicles was confirmed from their significant trapping efficiency,[8] the fine structure of vesicular membrane layers and the identity of small particles were not established from VEM. For this purpose, we examine the turbid solutions of C_nSA by electron microscopy.

Experimental Section

Materials and Solutions. Dipotassium dodecenylsuccinate ($C_{12}SAK$) and alkenylsuccinic acids (C_nSA, $n = 12, 14, 16$) are the same samples as previously prepared and used.[4-8]

Aqueous $C_{12}SAK$ solutions of various surfactant concentrations in water were prepared by dissolving $C_{12}SAK$ in water and titrating with a small amount of standardized HCl solution. Aqueous C_nSA solutions of 0.2×10^{-2} g cm^{-3} in $^1/_{15}$ M phosphate buffer were prepared according to the vesicle preparation method from thin films.[8] Sonication of the resulting turbid solutions was performed twice for 1 min at 45-W output on a probe-type sonicator.

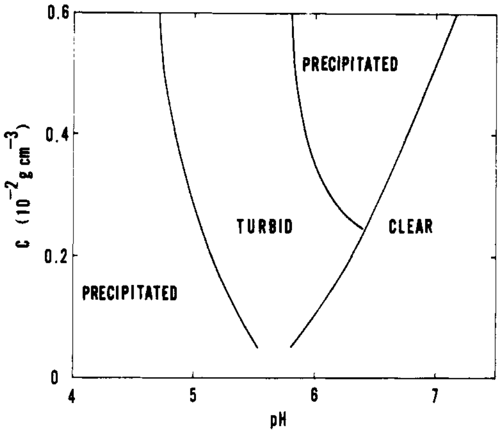

Figure 1. A phase diagram for aqueous $C_{12}SAK$ solutions in water at 25 °C.

Phase Diagrams. Phase diagrams were examined for solutions of $C_{12}SAK$ and C_nSA which had been allowed to stand for 1 day at 25 °C. They were surveyed by visual inspection and, if needed, by an optical microscope or laser light scattering. The observations were carried on for a few months at room temperature.

Optical and Electron Microscopic Observation. Optical microscopic observation of turbid solutions of $C_{12}SAK$ and C_nSA was carried out at 25 °C on an Olympus BH optical microscope.

The replica films for the transmission electron microscopic (TEM) observation were prepared on a freeze-fracture apparatus, JEOL JFD-7000 or Balzers BAF 400, by the freeze-fracture method[9] for a turbid solution of $C_{12}SA$ which was mixed with glycerol (30% v/v). The addition of glycerol was useful to obstruct the formation of ice crystals without any damage for vesicles. The replica films were observed with ×7000–×50000 magnification on a Hitachi electron microscope H-800 or H-500 operating at 200 kV.

Results and Discussion

Figure 1 shows the phase diagram for aqueous $C_{12}SAK$ solutions in water at 25 °C, which were prepared at different surfactant concentrations (*c*) and pH. Clear, turbid, and precipitated regions are observed. The turbid region at pH 5–6, where solutions are translucent or opalescent, shifts slightly to lower pH with increasing surfactant concentration. At the surfactant concentrations above 0.3×10^{-2} g cm^{-3}, a second precipitated region is observed between the turbid and clear regions. Solutions below pH 5, which were turbid just after the preparation, precipitate after 1 day. Precipitates occur in the turbid region in a period of more than 1 day, whereas the solutions at medium pH in the turbid region do not precipitate even after 1 month.

The phase regions after 1 day were examined at 25 °C for aqueous C_nSA solutions of 0.2×10^{-2} g cm^{-3} in $^1/_{15}$ M phosphate buffer. The regions are not significantly different for sonicated and unsonicated solutions, although the sonicated solutions are less turbid. While the turbid solutions are obtained below pH 6.3, the solutions below pH 5 precipitate after 1 day. The turbid regions after 1

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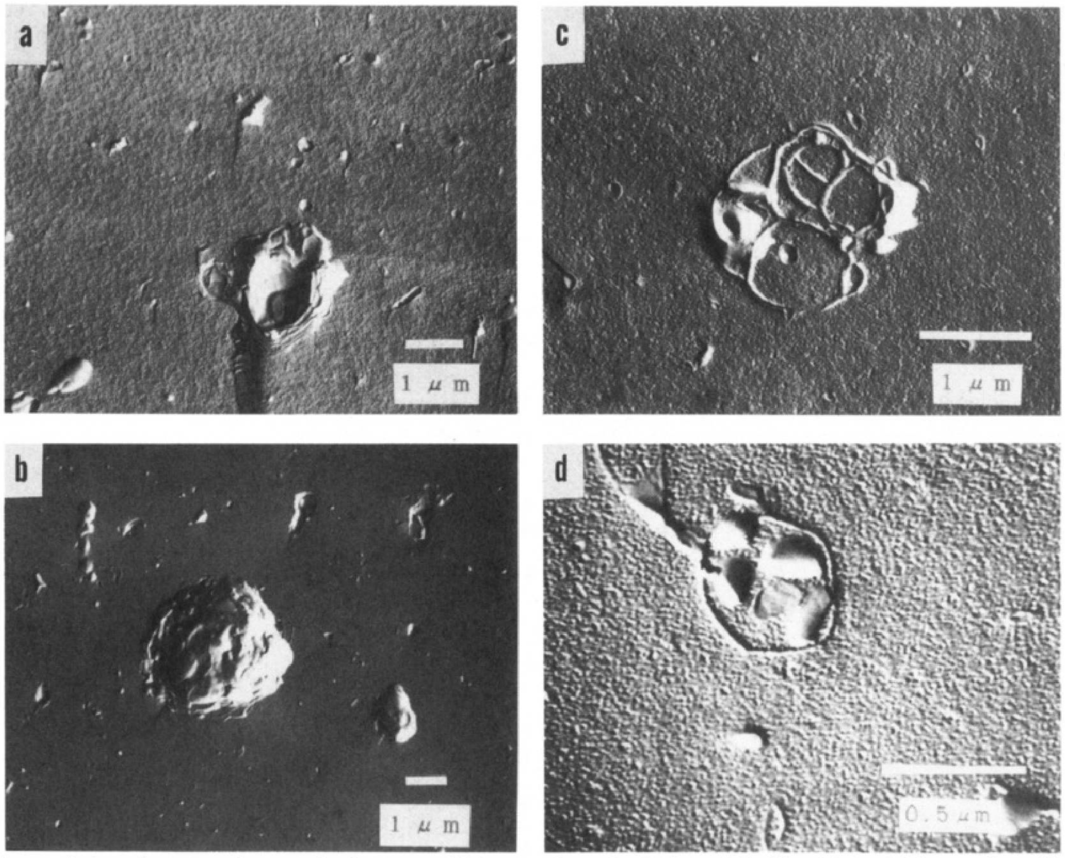

Figure 2. Transmission electron micrographs from a turbid, unsonicated solution of $C_{12}SA$ of 0.2×10^{-2} g cm^{-3} at pH 5.85 in $^{1}/_{15}$ M phosphate buffer.

day become slightly narrower with increasing hydrocarbon chain length of surfactant. The solutions at medium pH in the turbid regions are still turbid even after 2 months, but even such solutions precipitate after several months.

The turbid, unsonicated solutions of C_nSA at pH 5.28–5.85 were observed at 25 °C on an optical microscope. There are a number of spherical particles with diameters below 10 μm in all solutions. This is consistent with the VEM observation.[6] However, the significant images on an optical microscope are not observable for the sonicated solutions. Spherical particles are also observed in the turbid solutions of $C_{12}SAK$ without phosphate buffer.

The electron micrographs from a turbid, unsonicated solution of $C_{12}SA$ are given in Figure 2. The images of spherical particles with various diameters from a few micrometers to 100 nm are observed. Some of the large particles have the multilamellar membrane structure (Figure 2a), and the fractured surface of the particle is smooth (Figure 2b). The latter implies that the bilayer was fractured at the weakly interacted hydrophobic boundary of two monolayers.[2] The other large particles have some compartments filled with aqueous medium (Figure 2c) or encapsulate some small particles (Figure 2d). It is confirmed that the large particles are vesicles with the bilayer membrane structure and the inner aqueous phase. The images on micrographs were very similar to those of vesicles of oleic and linoleic acids.[1,2]

Multilamellar vesicles are generally formed by swelling thin films. They are converted into the unilamellar vesicles after sonication.[10] The electron micrograph from a turbid, sonicated solution of $C_{12}SA$, which is shown in Figure 3, displays particles with relatively uniform diameter, about 100 nm. This means that the sonication diminishes and homogenizes the particles, similar to that for common vesicles.[10] Such small particles cannot be observed by an optical microscope.

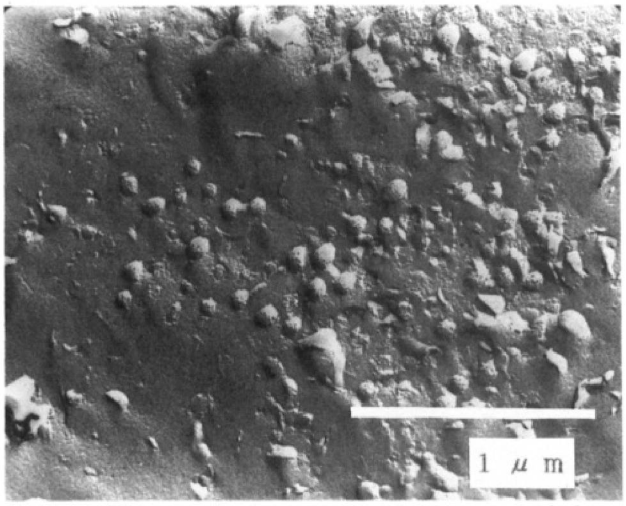

Figure 3. Transmission electron micrograph from a turbid, sonicated solution of $C_{12}SA$ of 0.2×10^{-2} g cm^{-3} at pH 5.85 in $^{1}/_{15}$ M phosphate buffer.

The aqueous solutions of $C_{12}SAK$ and $C_{16}SA$ have been examined by VEM.[6,7] Vesicles and small particles with diameters less than 7 μm were observed in their opalescent solutions at medium pH.[6] The electron micrographs in the present work confirm that small particles are small vesicles with about 100-nm diameter, although it cannot be decided from the electron microscopic observation whether those are unilamellar or not.

Gebicki and Hicks[11] have reported that, although the vesicle suspension of oleic acid changed to a transparent

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(clear) solution by the sonication, the solution became turbid after a few minutes and uniform vesicles of small size were not obtained. It was presumed that vesicle fusion quickly occurred.[12] It should be noticed that small C_nSA vesicles do not fuse easily after the sonication, different from oleic acid vesicles.

A turbid solution of $C_{16}SA$ of 0.5×10^{-2} g cm^{-3} displayed the viscoelastic character. The quantitative examination of viscoelasticity was reported by Tsujii et al., although they estimated an entanglement coupling or a flexible network structure formation of long cylindrical micelles.[13] On the other hand, the trapping efficiency was about 30% at 25 °C for a turbid solution of $C_{16}SA$ of 0.5×10^{-2} g cm^{-3} after sonication.[8] This value can be regarded as the volume percentage of vesicles in whole volume. Since the vesicle diameter is about 100 nm, the approximate calculation provides the average distance between vesicle centers of ~150 nm. Then the distance between two vesicle surfaces is only about 50 nm. Such short distances between vesicles may participate in the viscoelastic behavior.

The van der Waals attraction between vesicles and the electrostatic repulsion by vesicle surface charges can act significantly in the case described above, although two interactions are neglected between vesicles at a long distance from each other. The vesicle surface charges are low at pH 5–6, since C_nSA vesicles are moderately ionized. Then the attractive interaction is dominant, and the flocs with the loose pseudonetwork structure are formed. Such flocculation structure of vesicles may induce the rheological behavior. The behavior is similar to that of latex dispersions;[14] the latex particles with low surface charge in polyacrylamide solutions formed aggregates, which induce the viscoelasticity.

Registry No. $C_{12}SAK$, 57170-07-7; $C_{12}SA$, 13877-83-3; C_{14}-SA, 455-95-8; $C_{16}SA$, 61412-52-0.

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