JOURNAL OF COLLOID AND INTERFACE SCIENCE 170, 367–373 (1995)

Stability and Size Control of Reverse Vesicles

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Received March 14, 1994; accepted September 29, 1994

The stability and size control of reverse vesicles have been investigated for a sucrose monoalkanoate/hexaethylene glycol hexadecyl ether/decane/water system. The stability is highly dependent on the surfactant mixing ratio, amount of added water, and vesicle size. The size distribution of reverse vesicles produced by simple mixing is very large, but larger vesicles can be removed by means of the extrusion method and reasonably homogeneously size-distributed reverse vesicles can be obtained. If a probe-type ultrasonicator is used, the reverse vesicles obtained are homogeneous and of very small size (50–70 nm in diameter) and they are considered to be of the unilamellar type. © 1995 Academic Press, Inc.

INTRODUCTION

Amphiphilic bimolecular layers form closed structures in water, which are called vesicles or liposomes (1–4). Vesicles have been widely investigated, because, their structure is essentially the same as that of biological cell membranes. Recently, we found that amphiphilic bimolecular layers also form closed structures in nonpolar media and these are called reverse vesicles (5–13). In contrast to normal vesicles, polar groups of amphiphilic molecules orient inward in the bimolecular layers, and the inside and outside of reverse vesicles are nonpolar phases. Therefore, unlike the hydrophobic interactions of amphiphilic molecules in normal vesicles, hydrophilic interactions are of importance for the formation of reverse vesicles (7).

Among various systems in which reverse vesicles are formed, it was found that very stable reverse vesicles are formed in a sucrose monoalkanoate–polyethylene glycol–alkyl ether system. The formation and stability of reverse vesicles are highly dependent on the surfactant mixing ratio and amount of added water, though this point has not been fully investigated as yet.

For applications and basic characterization, it is very important to control reverse vesicle sizes and their distribution. However, in the previous study (13), we found only multilamellar-type reverse vesicles of wide size distribution. Their structures were confirmed by video-enhanced microscopy (VEM), electron microscopy (11), and small-angle X-ray scattering (SAXS) (6, 12). Formation of unilamellar reverse vesicles has not been reported to date, because it seems that our former method of preparation (5, 6, 12) is not sufficient to break the multilamellar structure into unilamellar structures.

In this context, we have investigated the effect of added water and the mixing ratio of surfactants on the formation and stability of reverse vesicles of nonionic amphiphiles

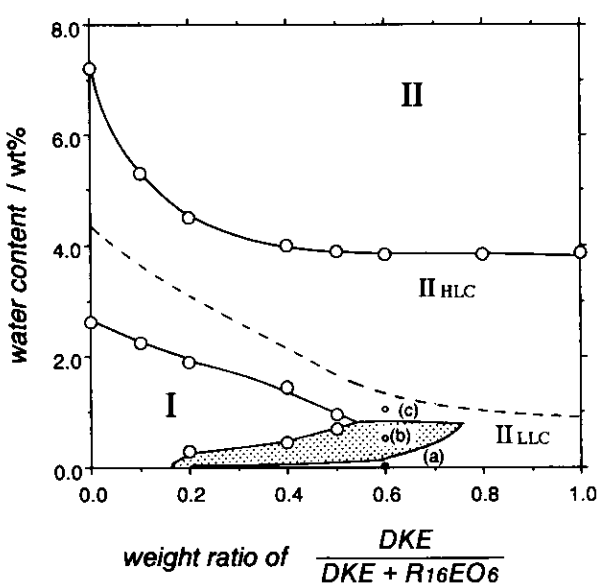

FIG. 1. Effect of added water on the formation and stability of reverse vesicles as a function of the DKE/$R_{16}EO_6$ ratio in a water/DKE/$R_{16}EO_6$/decane system at 30°C. The total amphiphile concentration is fixed at 3.0 wt%. I and II are single- and two-isotropic-phase regions. II_{HLC} denotes a two-phase system containing a normal hexagonal liquid crystalline phase. II_{LLC} denotes a two-phase system containing a lamellar liquid crystalline phase. In the shaded area of the II_{LC} region, the liquid crystal is stably dispersed and no macroscopic precipitation is observed within 3 days of preparation.

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0021-9797/95 $6.00
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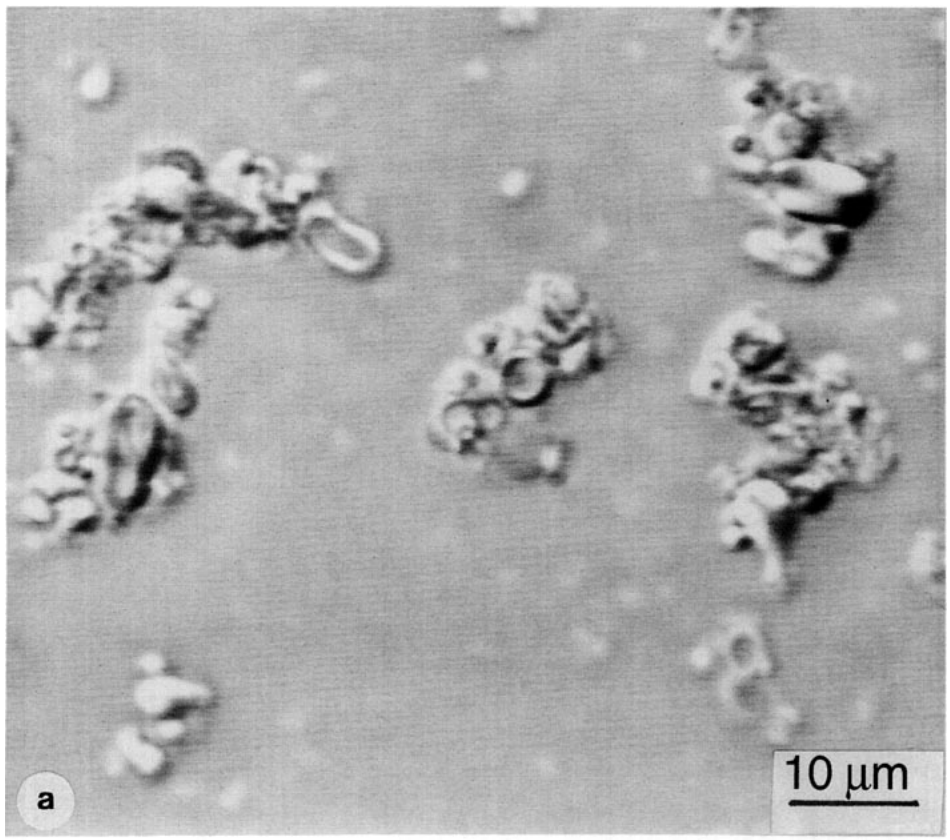


FIG. 2. VEM images of reversed vesicles in a water/DKE/$R_{16}EO_6$/decane system at 30°C without extrusion. The composition is total amphiphile concentration 3.0 wt%, DKE/$R_{16}EO_6$ mixing ratio 60/40, and water content (a) 0 wt%, (b) 0.6 wt%, and (c) 1.0 wt%. Each composition is indicated in Fig. 1.

(hexaethylene glycol hexadecyl ether and sucrose monoalkanoate) in decane. Both the extrusion method (13, 14) and a probe-type ultrasonicator (15, 16) were used to control the size of reverse vesicles and to form unilamellar reverse vesicles.

EXPERIMENTAL

Materials

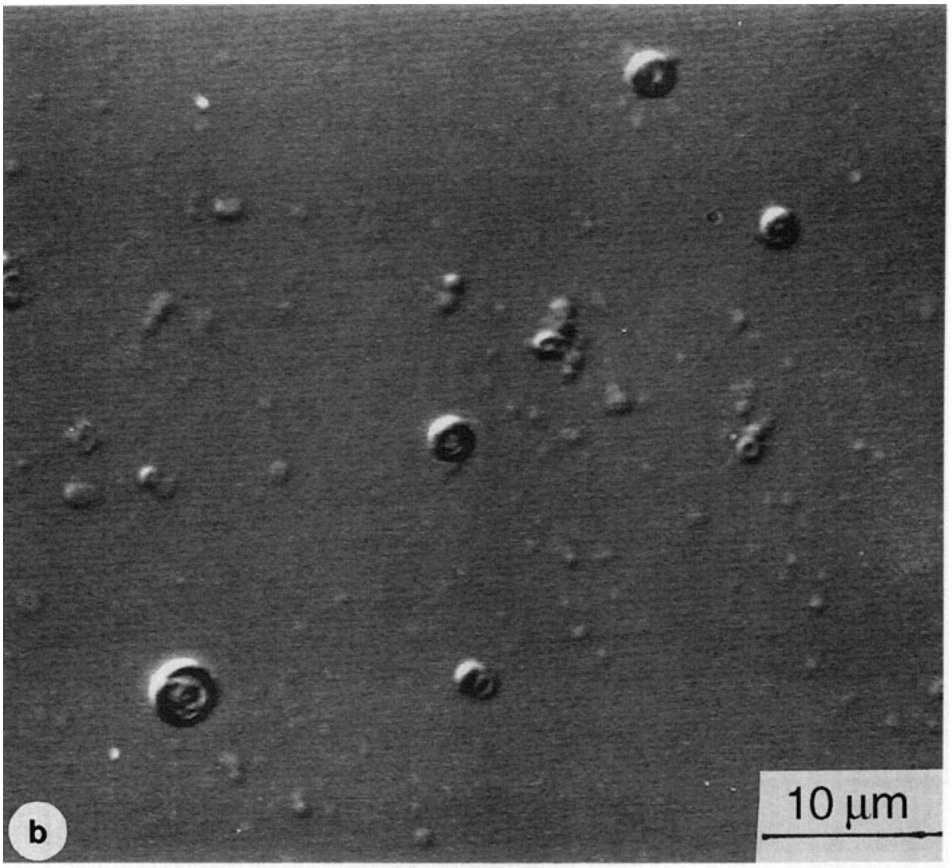
Sucrose monoalkanoate (fatty acid content: C_{14}, 10%; C_{16}, 40%; C_{18}, 50%, abbreviated DKE) was supplied by Dai-ichi Kogyo Seiyaku Company. Homogeneous hexaethylene glycol hexadecyl ether ($R_{16}EO_6$) was kindly supplied by Nikko Chemicals Company. Extra-pure-grade decane was obtained from Tokyo Kasei Kogyo Company. All chemicals were used without further purifications, and the water used was always doubly distilled.

Phase Diagram

One part of DKE was weighed and dissolved in 10–15 parts of pure methanol, and various amounts of $R_{16}EO_6$ were added and mixed thoroughly. After slow vacuum evaporation of the solvent, the amphiphile mixture was dried and again kept under vacuum with P_2O_5. Various amounts of the mixed amphiphile, water, and decane were weighed and capped in test tubes. The samples were hand-stirred to a temperature above 50°C, and kept at 30°C in a thermostat for several hours to days to equilibrate. Phase boundaries were determined by visual observation. Lamellar liquid crystals were detected with the help of crossed polarizers and a polarizing microscope (Olympus BH-2); the determinations were made according to a method described earlier (12, 17, 18).

Preparation of Reverse Vesicles

Extrusion method. Various amounts of mixed amphiphile, water, and oil were weighed and capped in test tubes. Vortex mixing at around 50°C was used to obtain complete dispersion of the mixed amphiphile, water, and decane. The turbid dispersions were then sonicated by a water bath-type sonicator (Kaijo Denki Co., 38 kHz, 250 W). The sonicated dispersions were extruded through polycarbonate mem-



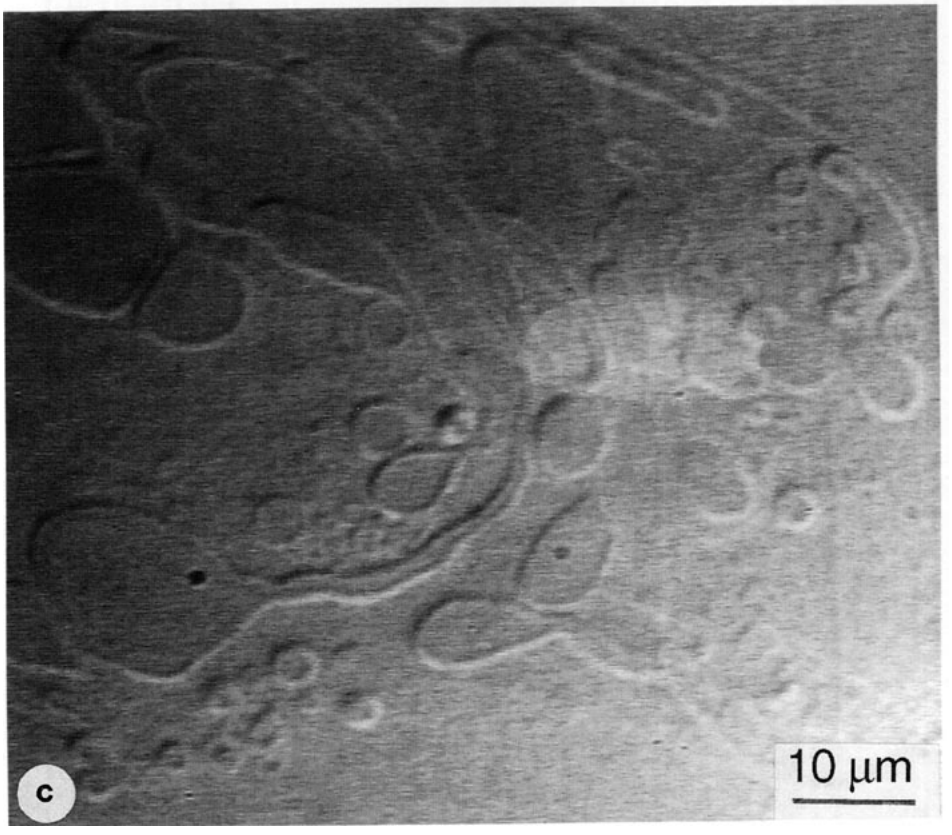


FIG. 2—*Continued*

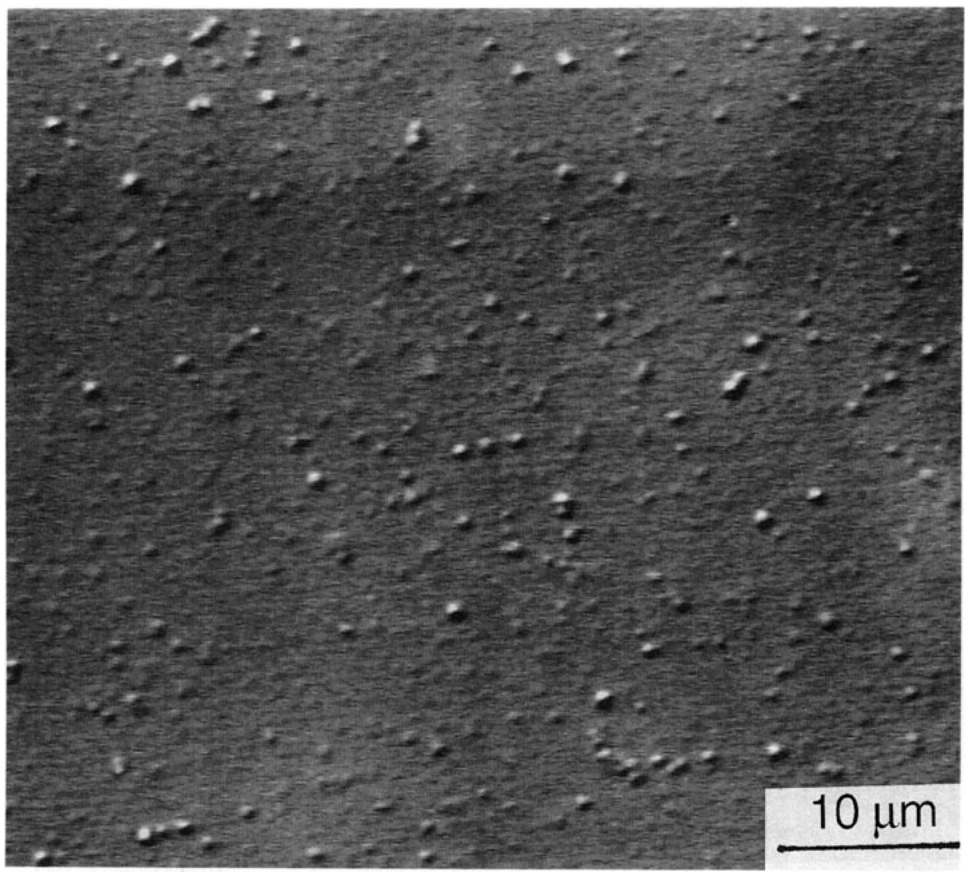


FIG. 3. VEM image of reverse vesicles extruded (five times) through a 0.6-μm polycarbonate membrane. The composition is the same as that in Fig. 2b.

branes of varying pore size (13). Samples were extruded five times using a 0.6-μm Millipore and then five times each using 0.2- and 0.1-μm.

Probe-type ultrasonication method. After mixed amphiphile, water, and oil were mixed with a Vortex mixer, the resulting turbid dispersion was ultrasonicated twice for 1 min at 45-W output, on a probe-type sonicator equipped with titanium tip (ϕ = 5 mm, Ohtake, Japan).

Optical and Electron Microscopy

Video-enhanced microscopy. Reverse vesicles were observed by means of VEM. A differential-interference phase-contrast (Nomarski-type) microscope (Nikon, X2F-NTF-21) (11, 12, 19, 20) equipped with an image processor (Hamamatsu Photonics Co., Argus 10) was used for this purpose.

Freeze-fracture electron microscopy. Replica films for freeze-fracture transmission electron microscopy (FFTEM) were prepared by means of a freeze-fracture apparatus (Balzer BAF 400) (21, 22). A droplet of vesicular dispersion was placed on the specimen holder and quickly vitrified in liquid nitrogen at its freezing point which was prepared under reduced pressure. The replica films were made of platinum (thickness 2–2.5 nm) and graphite (20–25 nm) and were observed at ×50,000 to ×120,000 magnification on a transmission electron microscope (JEOL-2000 FX-2) operating at 100 kV.

Light Scattering

Dynamic light scattering (DLS) measurements were performed with a Malvern 4700c PS/MW spectrometer equipped with a computer-controlled stepping motor-driven goniometer with a temperature-controlled cell holder, a multibit correlator (Malvern, Model 7032-ES/136c), and a He–Ne laser (NEC Gas Laser, λ = 632.8 nm). All measurements were made at a scattering angle of 90° and at a constant temperature of 30 ± 0.1°C. High-precision 10-mm Burchard cells were used. The measured intensity autocorrelation functions were analyzed by the method of cumulants. From this analysis, z-averaged diffusion coefficients were derived and used to calculate the z-average mean diameter according to the Stokes–Einstein equation (23).

RESULTS AND DISCUSSION

Effect of Added Water and Surfactant Mixing Ratio on Phase Behavior

The temperature at which the solid-to-isotropic liquid phase transition occurs for the nonionic surfactant, $R_{16}EO_6$, in decane is about 25°C (12). Addition of DKE to this system slightly decreased the phase transition temperature. Hence, the partial phase diagram of a water/DKE/$R_{16}EO_6$/decane system as a function of the water content of the system and the weight fraction of DKE in the DKE/$R_{16}EO_6$ mixture was determined at 30°C for a concentration of total amphiphile in decane of 3 wt% (Fig. 1). In the binary decane–$R_{16}EO_6$ system, there is no self-organizing structure (24, 25). By adding water or DKE, reverse micelles are formed in the single isotropic region (region I) in Fig. 1 (12).

In the absence of water, the single isotropic phase is present up to a DKE/$R_{16}EO_6$ ratio of 20/80. On addition of a small amount of water, the isotropic phase region extends toward higher DKE/$R_{16}EO_6$ weight ratios and reaches a maximum (50/50) at 0.8 wt% water. With an increase in water content and DKE/$R_{16}EO_6$ ratio, the lamellar liquid crystalline phase appears beyond the boundary of the single isotropic phase, and a two-phase region ($\mathbf{II}_{LLC}$) is formed, as shown in Fig. 1. At a high DKE/$R_{16}EO_6$ weight ratio, a lamellar liquid crystal is formed in the absence of water. With further increase in water content, the lamellar liquid crystal changes to an isotropic surfactant-rich phase via a normal hexagonal liquid crystal, and eventually, the isotropic surfactant-rich phase coexists with an excess oil phase. As a result, two isotropic phases are observed in a water-rich region (the **II** region in Fig. 1). Since both surfactants are hydrophilic at this temperature, the surfactant-rich phase in the **II** region is an aqueous micellar solution phase (16). A small amount of water can be incorporated into reverse micelles or liquid crystals. However, hydrophilic smaller aggregates, micelles, are formed at higher water contents.

Judging from the phase rule, a three-phase region is considered to exist between each two-phase region; however, the phase boundary is too narrow to determine. Consequently, lamellar liquid crystal is present over a wide range of surfactant mixing ratios in a decane-rich region if the water content is not very large.

Stability of Reverse Vesicles

In the $\mathbf{II}_{HLC}$ region, the dispersion of hexagonal liquid crystal is extremely unstable and precipitates within 1 h. The rather stable dispersion of lamellar liquid crystal in decane (reverse vesicles) is obtained in the $\mathbf{II}_{LLC}$ region (6–13). However, its stability is highly dependent on the amount of added water and the surfactant mixing ratio. The effect of both these parameters on the stability of the dispersion of liquid crystals was determined by mixing compositions of the two-phase system of Fig. 1 with a Vortex mixer and a water bath-type ultrasonicator. No extrusion method was used.

In most of the $\mathbf{II}_{LLC}$ region, the dispersion of liquid crystal is unstable, and separates into bulk lamellar liquid crystalline and excess oil phases within 3 days. In the shaded area in Fig. 1, however, the dispersion is very stable and no precipitation was observed within 3 days of preparation.

VEM images of reverse vesicles at different water contents are shown in Fig. 2. The DKE/$R_{16}EO_6$ weight ratio is 60/40. At 1 wt% water, slightly out of the shaded area in Fig. 1, the vesicles become too soft to maintain the vesicular structure, as is shown in Fig. 2c. The dispersion of liquid crystal easily coalesces and reverts back to the bulk phase. As mentioned earlier, the mixed surfactant forms an aqueous micellar solution phase at higher water contents. Therefore, it is considered that on addition of relatively large amounts of water, the bilayer in the liquid crystal swells with water and becomes too flexible.

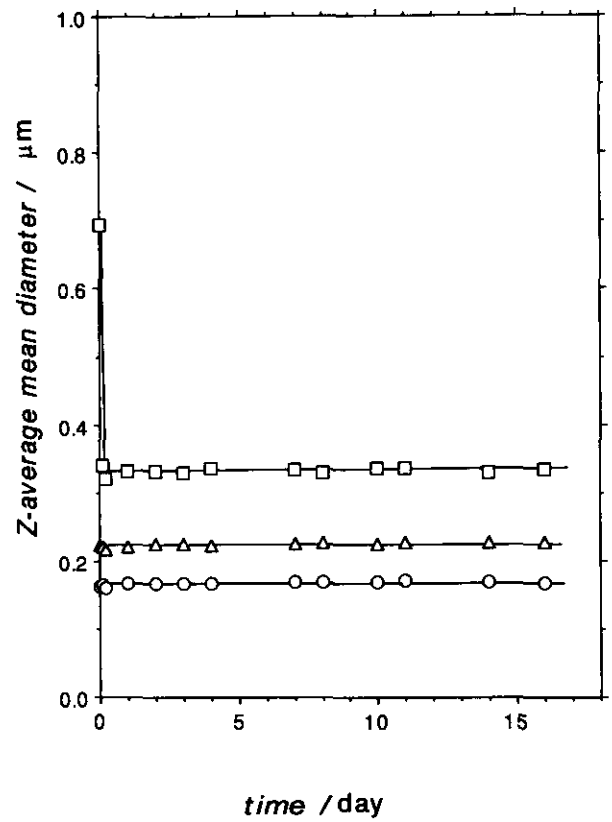


FIG. 4. Change in reverse vesicle size (z-average mean) as a function of time measured using DLS. The composition is total amphiphile concentration 3.0 wt%, DKE/$R_{16}EO_6$ mixing ratio 60/40, and water content 0.2 wt%. The samples were extruded using 0.1-μm (○), 0.2-μm (△), and 0.6-μm (□) polycarbonate membranes.

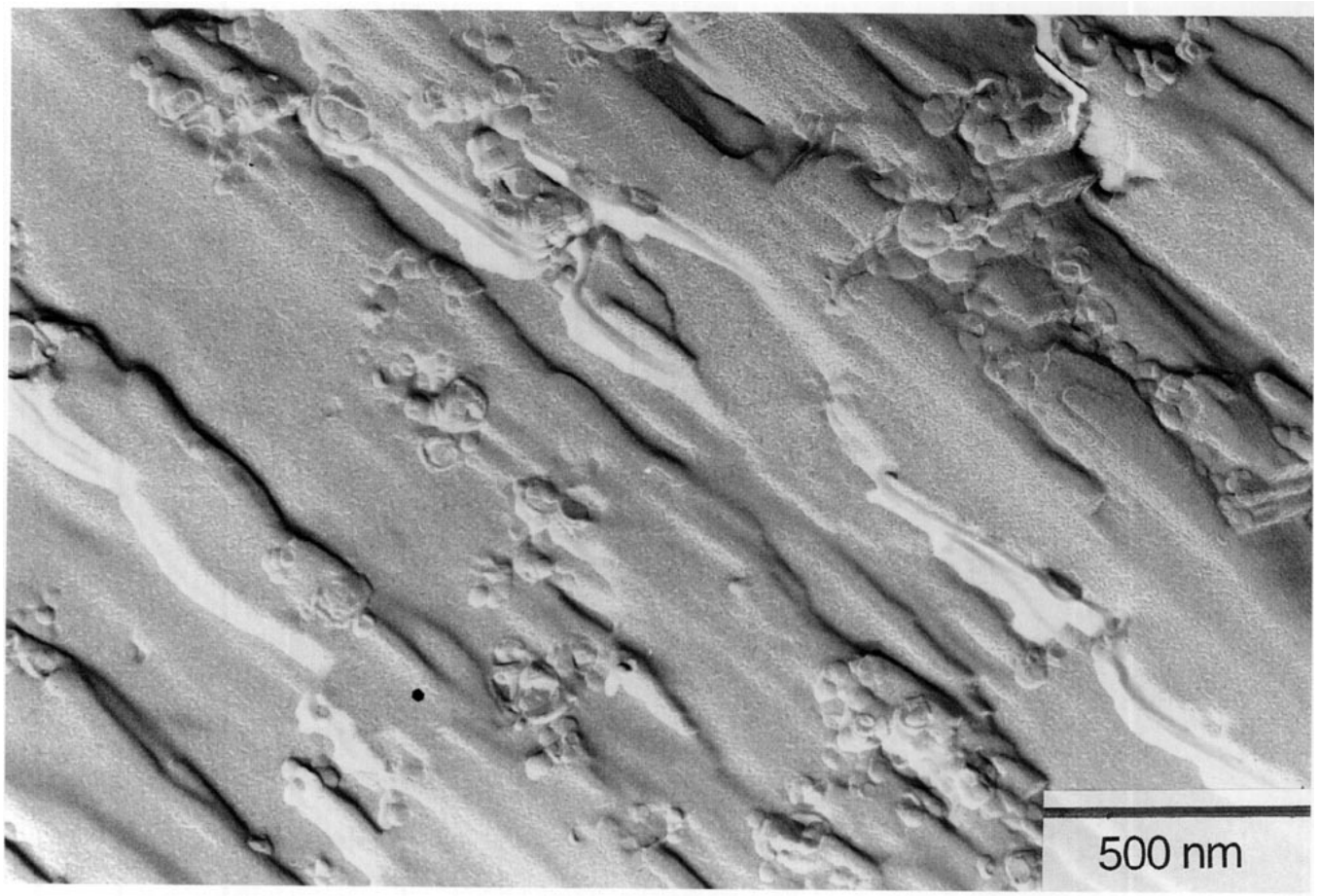


FIG. 5. Freeze-fracture transmission electron micrograph of reverse vesicles in a water/DKE/$R_{16}EO_6$/decane system. The composition is, water 1.2 wt%, total amphiphile concentration 6.0 wt%, DKE/$R_{16}EO_6$ mixing ratio 60/40.

On the other hand, reverse vesicles are also unstable at very low water contents. Large aggregates of vesicles are observed, as shown in Fig. 2a. It is thought that the mass of vesicular aggregates does not easily break into fine vesicles. The optimum concentration of water to produce stable vesicles was found to be between 0.4 to 0.6%. Although the size distribution is very large, shown in Fig. 2b, the dispersion is very stable. The system looks bluish white and no macroscopic phase separation was observed within 3 days.

Controlling Multilamellar Vesicular Sizes and Their Stability

When one forms reverse vesicles with a Vortex mixer, multilamellar vesicles with a large size distribution are obtained as mentioned earlier. We have applied the extrusion method normally used for preparing homogeneous normal vesicles (14) to the present reverse vesicle system. The results are shown in Fig. 3. Compared with the reverse vesicles in Fig. 2b, it is clear that reverse vesicle size does become homogeneous; however, it is thought that reverse vesicles much smaller than the pore size used cannot be eliminated by this extrusion method.

The stability of reverse vesicles is also dependent on their size. Reverse vesicles attain maximum stability at a DKE/$R_{16}EO_6$ ratio of 60/40 and water content of 0.6% (Fig. 1). All the reverse vesicles extruded successively using 0.6-, 0.2-, and 0.1-μm polycarbonate membranes were stable and precipitation was not observed for 2 weeks. Therefore, slightly unstable reverse vesicles at 0.2% water were extruded, and the change in vesicle size as a function of time was measured by DLS. The result is shown in Fig. 4.

The size of reverse vesicles extruded through the 0.6-μm membrane changes from 0.6 to 0.3 μm within 2 h; vesicle size then remains unchanged for more than 2 weeks. In fact, macroscopic precipitation was observed immediately after preparation. Even after precipitation, however, the reverse vesicles maintained their vesicular shapes, as confirmed by VEM (7, 13). Therefore, larger vesicles immediately precipitate due to the difference in densities between reverse vesicles and the continuous phase. On the other hand, smaller vesicles

are very stable for more than 14 days. Judging from these results, reverse vesicles smaller than 0.3 μm are found to be very stable. Thus the stability of reverse vesicles is highly dependent on their size.

Unilamellar Reverse Vesicles

By the extrusion method, we can control reverse vesicle size of the multilamellar type. At least, large reverse vesicles can be eliminated. This method, however, is not suitable for forming unilamellar reverse vesicles. To break the multibimolecular layers into a single bimolecular layer, we used a probe-type ultrasonicator (15, 16). After ultrasonication, the system apparently becomes a clear bluish solution. This system was observed by freeze-fracture transmission electron microscopy, and the photograph is shown in Fig. 5. The surfactant concentration in the sample is 6 wt% and the surfactant/water ratio is the same as that in Fig. 2b. Since the vitrified decane was soft whereas the reverse vesicles were stiff, only a small number of reverse vesicles were cut and the surface of decane was not completely flat. Small reverse vesicles of narrow size distribution are observed. A mass of reverse vesicles are observed in the upper right-hand area of Fig. 5. Therefore, it is possible that some reverse vesicles flocculate when the decane is vitrified. However, the continuous medium, decane, is in a glassy state because its appearance is different from that of the decane crystal. The average diameter of the reverse vesicles is about 50–70 nm although a few larger vesicles are also present. This size is in good agreement with the value calculated from the self-diffusion constant of decane in the reverse vesicle system measured by means of NMR technique (16), using a unilamellar vesicular model.

The size of unilamellar reverse vesicles is similar to that of normal vesicles formed in water (26); however, the orientation of amphiphilic molecules in the bilayers is completely opposite to normal vesicular structure. Consequently, most of the methods used to produce normal vesicles and to control their size are directly applicable to reverse vesicle systems also. Thus, we see that unilamellar reverse vesicles can be formed.

ACKNOWLEDGMENTS

The authors thank Mr. T. Iwamoto, Nagoya University, and Mr. Y. Shimada, Yokohama National University, for their technical assistance with electron microscopic observation. We also thank Mr. M. Akimaru (Nihon Surfactant Co.) for supplying homogeneous nonionic surfactant. Support by CIRIT (Grant EE92/1-38) and DGICYT (Grant PB92-0102) is gratefully acknowledged.

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