

H. Kunieda
K. Shigeta
K. Nakamura
T. Imae

Formation and structure of reverse vesicles

Dr. H. Kunieda (✉) · K. Shigeta
Department of Physical Chemistry
Division of Materials Science
and Chemical Engineering
Faculty of Engineering
Yokohama National University
Tokiwadai 156, Hodogaya-ku
Yokohama 240, Japan

K. Nakamura
Department of Living Science
Faculty of Education
Niigata University
Igarashi Nino-cho 8050
Niigata 950-21, Japan

T. Imae
Department of Chemistry
Faculty of Science
Nagoya University Chikusa-ku
Nagoya 464, Japan

Abstract Both normal and reverse vesicles can be formed in the mixture of sucrose dodecanoates with different hydrocarbon chain number. The conditions to produce both types of vesicles are discussed using the geometrical packing model. The conditions are roughly the same for both types of vesicles. The formation of reverse vesicles is confirmed by means of video-enhanced microscopy (VEM) and cryo-transmission electron microscopy (Cryo-TEM).

Key words Reverse vesicles – sucrose alkanoates – cryo-electron microscopy – video enhanced microscopy

Introduction

Contrary to normal vesicles formed in water, recently it was found that closed bimolecular layers can be also formed in nonpolar media [1–11]. The self-organizing structure was named reverse vesicles. The orientation of amphiphilic molecules in bilayers of reverse vesicles is opposite to that in normal vesicles. Reverse vesicles were found in various mixed surfactant systems in which hydrophilic and lipophilic surfactants were combined. Correlation between the surfactant molecular shape and resulting self-organizing structure can be explained by geometrical packing model [12]. Since reverse vesicles are new self-organizing structures the packing condition of surfactant has not been reported.

Sucrose alkanoates are unique and biocompatible surfactants which possess a strong hydrophilic sucrose ring. Ordinary polyoxyethylene-type nonionic surfactants are in general completely miscible with hydrocarbons, but do not self-associate in the absence of water [13]. On the other hand, the monomeric solubilities of sucrose alkanoates in nonpolar solvents are rather low and they tend to self-associate from self-organizing structure in nonpolar media.

In this paper, the condition to produce reverse vesicles is discussed and the formation of reverse vesicles in sucrose alkanoate systems is reported. The morphology of reverse vesicles is shown by means of video enhanced microscopy (VEM) and cryo-transmission electron microscopy (Cryo-TEM).

Experimental

Materials

Sucrose dodecanoates, L-1695 and L-595 were kindly supplied by Mitsubishi Chemical Corp. L-1695 consists of 83.6 wt% sucrose monododecanoate, 15.2 wt% sucrose didodecanoate, and 1.2 wt% sucrose tridodecanoate. L-595 consists of 30.3 wt% sucrose monododecanoate, 39.3 wt% sucrose didodecanoate, and 30.4 wt% sucrose tridodecanoate. L-1695 is water-soluble whereas L-595 forms milky dispersion in water. By mixing L-1695 and L-595, we can prepare sucrose dodecanoates with different number of hydrophobic chains from 1.13 to 1.84.

Methods

Sample preparation

L-1695 and L-595 were dissolved in methanol in order to obtain a homogenized mixture. After evaporation of methanol in vacuum, isooctane was added to the dried mixture. The sample was sonicated by means of an ultrasonicator (Shimadzu, USP-50) at 9 W for 5 min. The vesicular dispersion was twice extruded using a 0.6 μm Millipore filter. Branched hydrocarbon, isooctane is used in order to avoid the crystallization of solvent when vitrifying.

Optical, and electron microscopy

VEM: A differential-interference-phase-contrast (Nomarski-type) microscope (Nikon, X2F-NTF-21) equipped with an image processor (Hamamatsu Photonics Co., Argus 10) was used for VEM observation.

Cryo-transmission electron microscopy: A droplet of vesicular dispersion was placed on a TEM grid and quickly vitrified in liquid nitrogen at its freezing point. The frozen sample was observed at X20 000 magnification on a transmission electron microscope, Hitachi H-800, operating at 100 kV.

Results and discussion

Condition to produce reverse vesicles

For normal vesicles in water, when the surfactant bilayer forms a closed structure, the interfacial part of the outer layer is the most compressed part as is shown in Fig. 1(a).

Fig. 1 Schematic representation of normal and reverse vesicles. A_s is an interfacial area per one hydrophilic group (sucrose ring) and l is hydrocarbon chain length

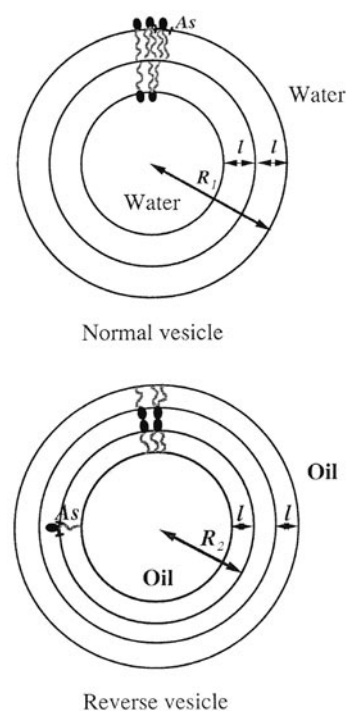
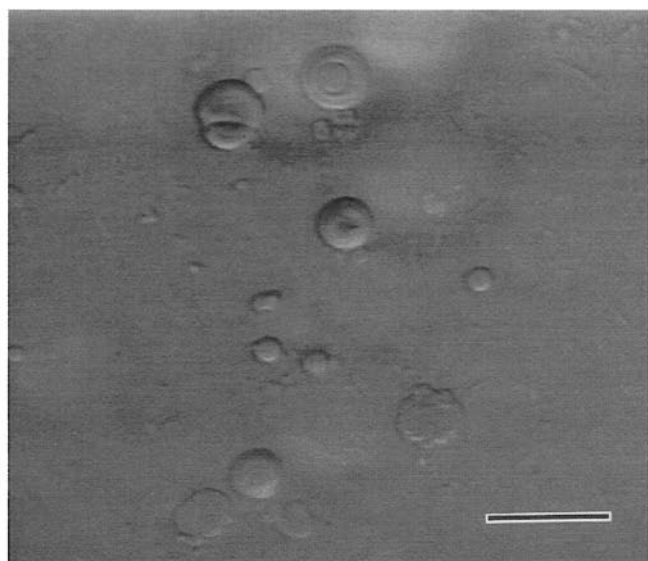
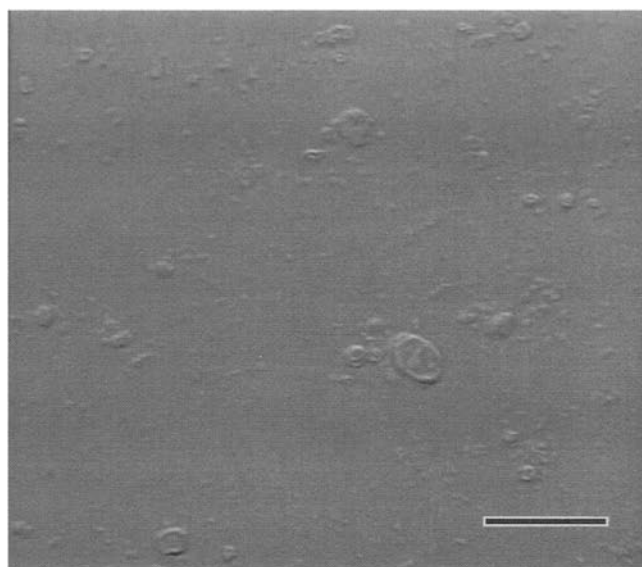


Table 1 Packing parameter and formation of vesicles

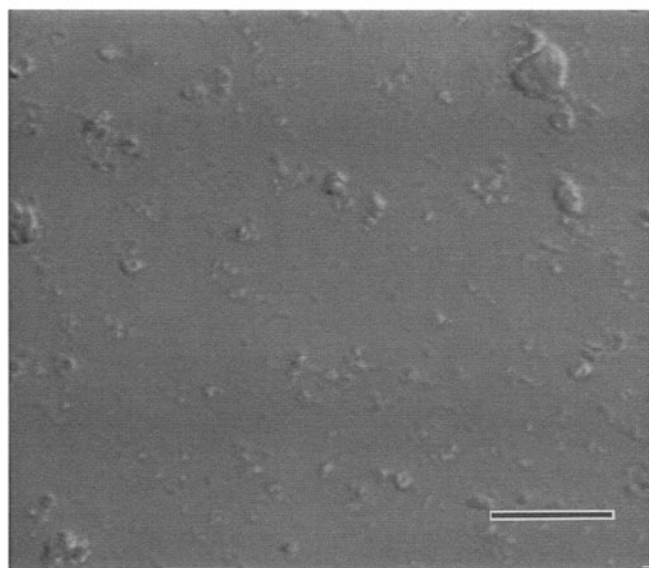
SE1	SE2	Average number of hydrocarbon chains	Normal vesicles in water			Reverse vesicles in oil		
			$v/A_s \cdot l$	R (nm)	Formation	$v/A_s \cdot l$	R (nm)	Formation
100 wt%	0 wt%	1.13	0.49	—	M	0.55	3.4	RV
90	10	1.19	0.52	—	M	0.58	3.7	RV
80	20	1.25	0.55	—	M	0.61	4.0	RV
70	30	1.31	0.57	—	M	0.64	4.3	RV
60	40	1.38	0.60	3.9	NV	0.67	4.7	RV
50	50	1.45	0.63	4.2	NV	0.71	5.2	RV
40	60	1.52	0.66	4.6	NV	0.74	5.9	RV
30	70	1.59	0.70	5.2	NV	0.78	7.0	RV
20	80	1.67	0.73	5.7	NV	0.81	8.1	RV
10	90	1.75	0.77	6.6	NV	0.86	11	RV
0	100	1.84	0.80	7.8	NV	0.90	15	RV



(a)



(b)



(c)

Fig. 2 VEM pictures of normal (a) and reverse (b and c) vesicles. (a) 3 wt% L-595, 97 wt% water. (b) 1 wt% L-1695, 99 wt% isooctane. (c) 1 wt% of equal weight mixture of L-1695 and L-595, 99 wt% isooctane. The bar indicates 25 μm

By using this consideration, Israelachvili proposed the following equation to determine the minimum possible radius of normal vesicles.

$$R_1 = \frac{l}{1 - v/A_s l}, \quad (1)$$

where l is the hydrophobic chain length of surfactant, v is the volume of hydrophobic chain and A_s is the interfacial area per surfactant molecule. Thus, in order to form normal

vesicles in water, $v/A_s l < 1$ is the condition for surfactant. If this value is less than 1/2, it is suggested that normal micelles form [12].

In the case of reverse vesicles, the orientation of amphiphilic molecules in the bilayer is opposite and the hydrophilic group is oriented toward the inside of the bilayer as is shown in Fig. 1(b). When the bilayer forms the closed structure, the interfacial part of the inner layer must be compressed. Therefore, as well as normal vesicles, the condition for the minimum size of reverse vesicles can be represented by

$$R_2 = \frac{l}{1 - v/A_s l}. \quad (2)$$

Namely, the conditions to produce normal and reverse vesicles are similar. If the $v/A_s l$ exceeds unity, reverse micelles tend to form [16]. However, the lower limiting value for reverse vesicles has not been known yet.

Sucrose dodecanoate system

Monomeric solubilities of sucrose alkanates in both water and oil are very small. Hence, it is considered that most of the surfactant molecules form self-organizing structure if the surfactant concentration is well above the CMC. Sucrose alkanates have the same hydrophilic group and sucrose rings, and only the number of fatty acids attached to it is changed. By using Eqs. (1) and (2), we calculated the possible minimum radius of normal (R_1) and reverse (R_2) vesicles in the sucrose dodecanoate systems and the result are shown in Table 1. The calculated packing parameters are also shown.

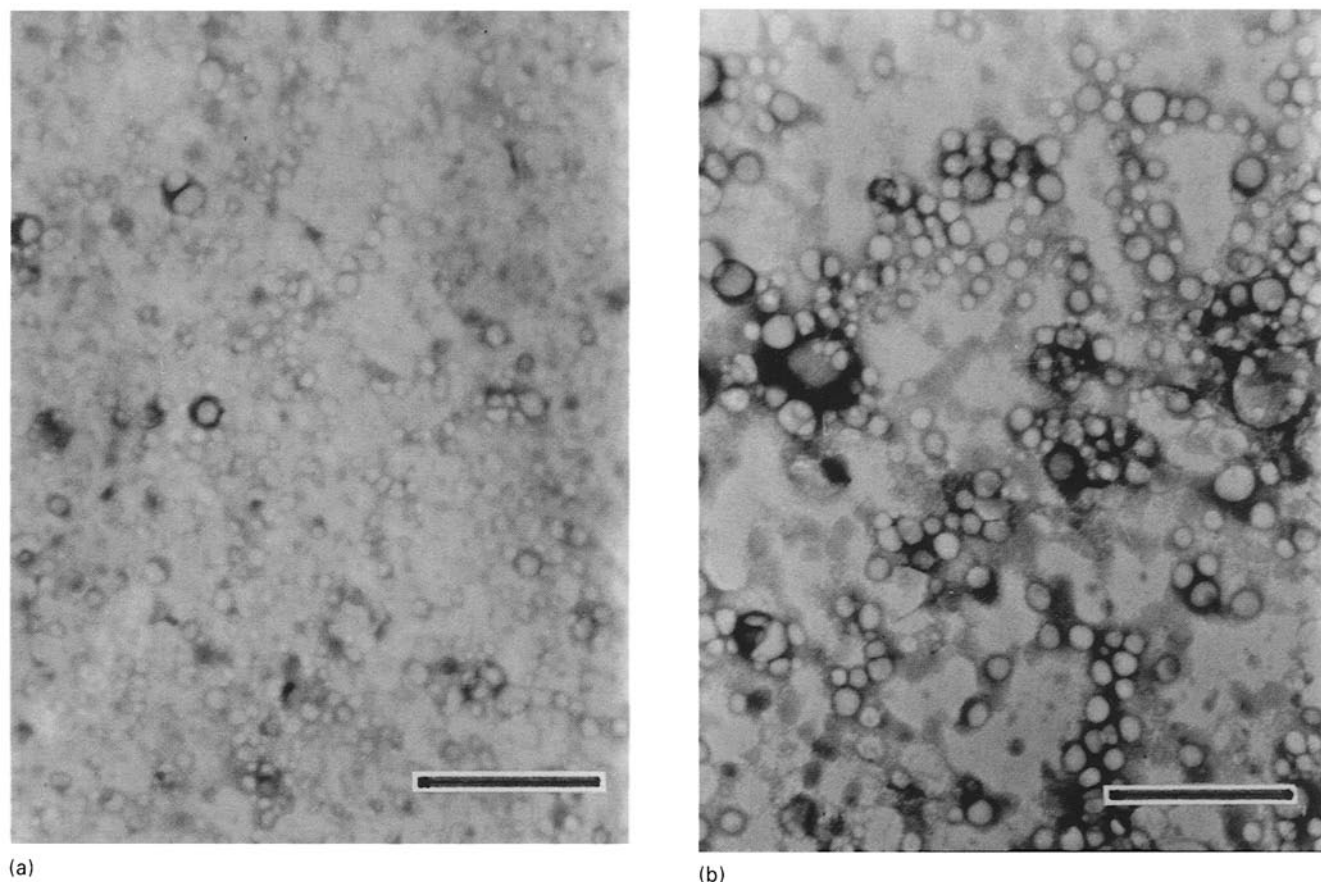


Fig. 3 Cryo-TEM picture of reverse vesicles. (a) 0.5 wt% L-1695, 99.5 wt% isooctane. (b) 0.5 wt% of equal weight mixture of L-1695 and L-595, 99.5 wt% isooctane. The bar indicates 1 μm

The volume (0.323 nm^3 per one hydrocarbon chain of dodecanoate) and length (1.54 nm) of hydrophobic chain were calculated by Tanford equation [17] and the interfacial area ($A_s = 0.48 \text{ nm}^2$ for normal vesicles $A_s = 0.43 \text{ nm}^2$ for reverse vesicles) was obtained from the previously measured SAXS data for lamellar liquid crystal [10]. It is clear from Table 1 that the minimum radius of vesicles decreases with decreasing the average hydrocarbon chain number. It is suggested from the present rough calculation that both normal and reverse vesicles can be obtained in the present system.

In the above discussion, we assume that the mixing ratio of surfactants is always unchanged in vesicles. It is possible that distribution of single- or double-chain surfactants in the inner surfactant layer is different from that in the outer layer.

VEM and Cryo-TEM pictures of vesicles

Phase behavior of sucrose dodecanoate mixtures in water or isooctane was observed and the result is also indicated

in Table 1. In the case of water system, normal vesicles are changed to micelles as the hydrocarbon chain number decreases. On the other hand, formation of reverse vesicles was observed at any sucrose dodecanoate mixing ratio as is shown in Table 1. Figure 2 shows the VEM pictures for normal and reverse vesicles.

In Figs. 2(a)–(c), we can observe reverse vesicles whose size distribution is quite large. In order to verify the close structure of small particles, we observed the solutions by means of Cryo-TEM and the result is shown in Fig. 3.

Although the preparation method is exactly the same, the diameter of most of the reverse vesicles is approximately $0.1 \mu\text{m}$ or less in both L-1695 and L-1695 + L-595 mixture systems. As predicted from Table 1, the possible minimum sizes are similar in both systems though the surfactant mixing ratios are largely different.

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