Vesicles From Sucrose Fatty Acid Esters

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It has been confirmed, from observations with an electron microscope after staining negatively with aqueous uranyl acetate solutions and using a flourescent microscope, that sucrose fatty acid esters form closed vesicles. The range of particle size of the vesicle, consisting of chromatographically fractionated sucrose dilaurate, was apparently 70-700 nm in the longer diameter of individual vesicles based on the transmission electron microscopic (TEM) observation. The weight-average particle size was 424 nm as shown by means of the photon-correlation method. The amounts of 6-carboxyfluorescein trapped in the vesicles of sucrose fatty acid esters were determined, and it was ascertained that the volumes of the central water phase depended upon the acyl chain lengths of fatty acid residues. Further, the effect of the additives [cholesterol and dicetyl phosphate (DCP) was examined. As an example, the vesicle of sucrose stearate had a central water phase of 1.7 one water/mol ester, and showed a slow release of 6-carboxyfluorescein from the central water phase after preparation of the vesicle.

It is noteworthy that some kinds of surfactants (1-3) and some other chemicals (4-7) as well as phospholipids form vesicles in water. We are especially interested in the vesicle-forming materials of biological origin (8-9) as replacments for phospholipids because these vesicles may be useful for drug-carrying microcapsules, biometric biological membranes and artificial cells. We first looked for the vesicle-forming properties of sucrose fatty acid esters. Then, we tried to investigate in detail their characteristics in comparison with know vesicles or liposomes. There are many kinds of application of sucrose fatty acid esters, in food emulsifiers, solubilizers, antifungicides, detergents, and so on (10-12). Therefore, we are also interested in unique applications of these functional materials in super-microcapsules and peculiar kinds of micro-emulsions.

TABLE 1

Sucrose Fatty Acid Esters

	Fatty acid composition	Ester composition mono (%)	di (%)	≧tri (%)
Octadecanoate	C_{18}70%, C_{16} 30%	28.6	37.0	≧34.4
Hexadecanoate	C_{18}30%, C_{16} 70%	28.8	39.6	≧31.6
Tetradecanoate	C_{14}≧99.5%	28.0	38.0	≧34.0
Dodecanoate	C_{12}≧99.4%	30.2	39.3	≧30.5
Decanoate	C_{10}≧99.5%	27.1	39.7	≧33.2
Octanoate	C_8≧99.5%	30.6	43.4	≧26.0

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EXPERIMENTAL

Materials. Sucrose fatty acid esters were prepared by the transesterification method of Osipow et al. (13), and their compositions are shown in Table 1. The alkyl chain lengths of the acyl groups of sucrose fatty acid esters are from stearate to octanoate. These esters are composed of monoester, diester and triester fractions. Sucrose has three primary hydroxyl groups and five secondary hydroxyl ones. The main components of all the sucrose esters are diesters. The diester content is about 30%, triester or more content is more than 30%, and monoester content is less than 30%.

The diester-enriched samples were prepared from sucrose laurate by repeating chromatographic fractionation with $CHCl_3$-CH_3OH solvent system according to the method of Otake (14). Figure 1 shows the analytical result using a TLC plate. The leftmost lane is the developing chromatogram of the mixed sucrose lautrate, and No 7 is sucrose monolaurate; the rightmost lane is sucrose trilaurate. We collected the fraction of diester just higher than monoester, namely No 32, 36, 37 to avoid contamination of the monoester and triester.

Egg yolk lecithin was purchased from Asahi Chemical Industries Co. Tokyo, Japan; dipalmitoyl phosphatidyl choline (DPPC) from Sigma Chemical Co., St. Louis, Missouri; dicetyl phosphate (DCP) from Nakarai Chemical Co. Kyoto, Japan; 6-carboxylfluorescein (CF) from Eastman Kodak Co. Rochester, New York, and other reagents from Wako Pure Chemical Industries Co. Osaka, Japan.

Methods. The vortexed suspensions were prepared as usual (15). We observed the vesicle structure using an

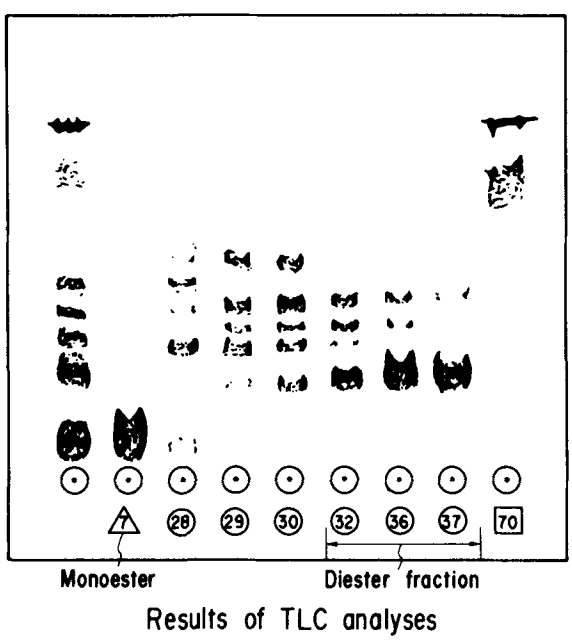


FIG. 1. Chromatographic preparation of the diester-enriched sample of sucrose laurate.

electron microscope, Hitachi HU-12A, after staining negatively with uranyl acetate, etc., and then using a fluorescent microscope after staining with 2,8-bis (dimethylamino)-10-dodecylacridinium bromide as a fluorescent probe into the lipid bilayer. Entrapment of CF in the vesicles was determined using a fluorescence spectrophotometer, Hitachi MPF-4. Phase transition temperatures of vesicles and the corresponding anhydrous esters were measured using a differential scanning calori-meter, Mettler TA-3000, by elevating the crucible temperature at 1.0°C/min. Measurements on anhydrous samples and aqueous suspensions were run using a standard pan and an air-tight, pressure-endurable crucible, respectively. Particle sizes and size distributions of the vesicles were determined at a room temperature of 20°C by means of the photon-correlation method using a Coulter N-4 submicron particle analyzer. REV (Reverse-phase evaporation) vesicles were also prepared as usual.

Determination of the volumes of trapped central water phase. Lipid films of sucrose fatty acid esters were spread on the bottom of the 100-ml, round-bottomed flask from the mixed $CHCl_3$-CH_3OH solvent, and were dried. Then, aliquots of aqueous CF solution and phosphate buffered saline at pH 7.2 were added to the flask, and the flask was vortexed for more than 15 min above the vesicles' phase transition temperatures. CF of the bulk phase was eliminated by centrifuging. CF concentrated in the central water phase didn't show fluorescence because of self-quenching, but fluorescence appeared again by destroying the vesicle suspension with Triton X-100. The difference in fluorescent intensity between the values with and without Triton X-100 was measured by exciting at 490 nm and monitoring the intensity at 520 nm. Phosphate buffered saline was prepared by mixing 8.0 g of NaCl, 0.20 g of KH_2PO_4 and 1.15 g of Na_2HPO_4 in water, and by adjusting pH 7.2 with the additon of hydrochroic acid and finally by filling up to 1 liter in a volumetric flask.

RESULTS AND DISCUSSION

In order to confirm the vesicle formation of the vortexed suspension, we first tried to observe their vesicle struc-

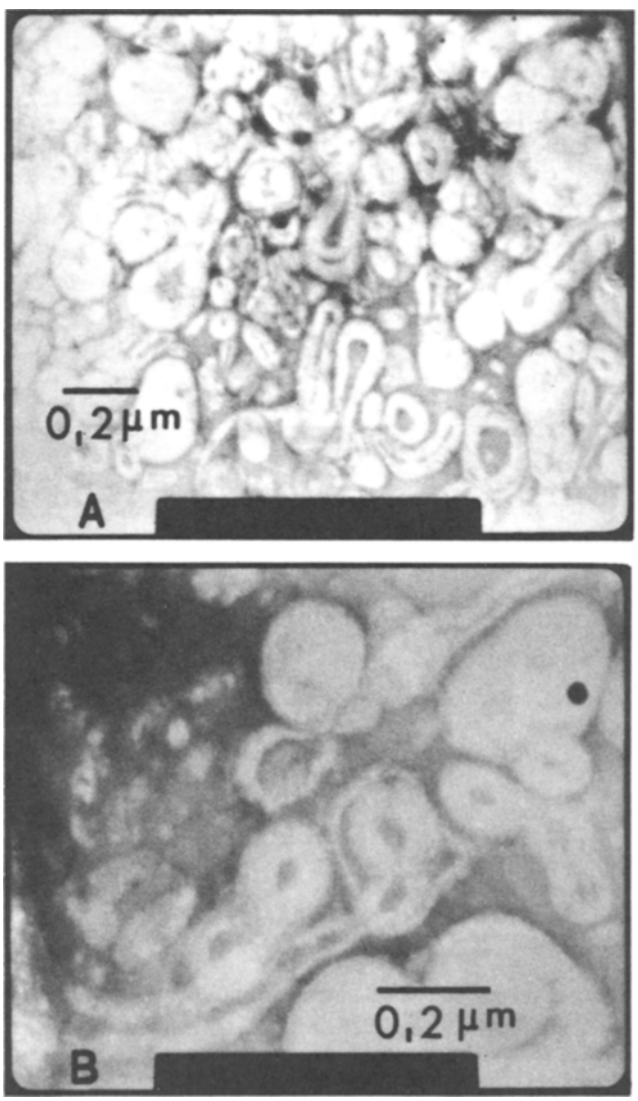


FIG. 2. Negative stain electron microscopy of vortexed sucrose dilaurate vesicles (A, top, and B).

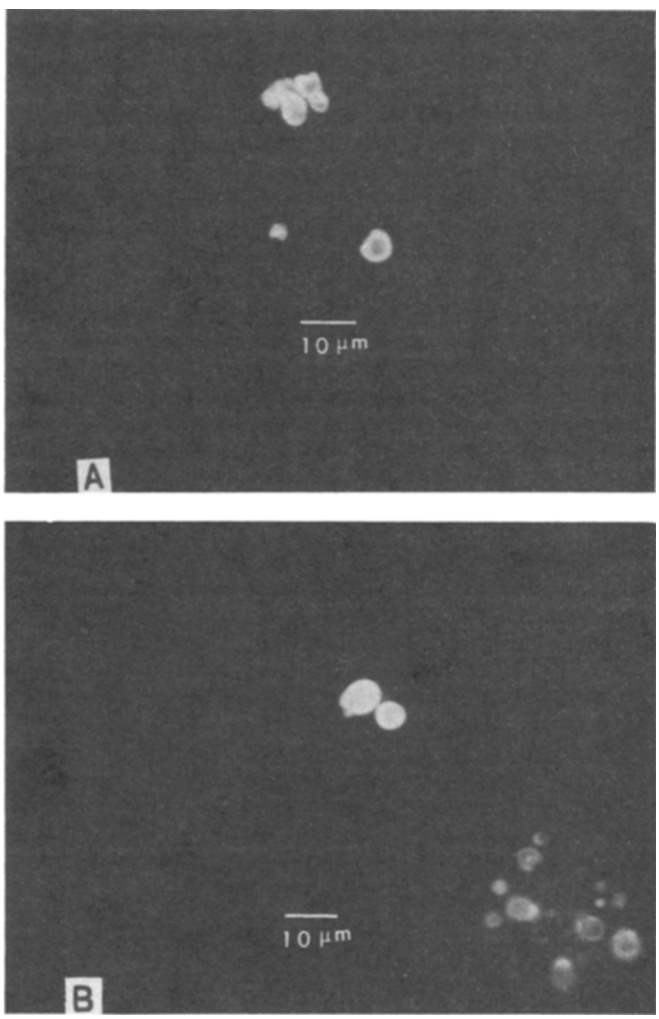
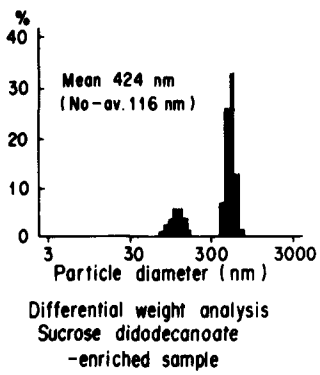


FIG. 3. Fluorescnet micrographs of sucrose palmitate vesicles stained with 2,8-bis(dimethylamino)-10-dodecylacridinium bromide (A, top, and B).



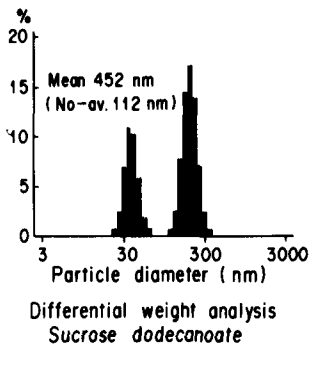
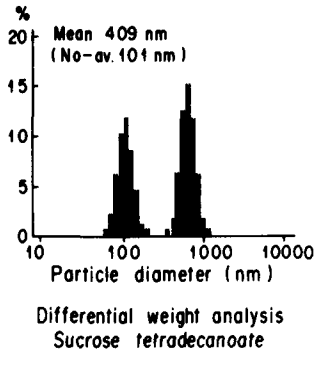


FIG. 4. Particle sizes and their distributions of the vesicles composed of sucrose dilaurate-enriched sample and sucrose laurate, respectively, by means of the photon-correlation method at 25°C.



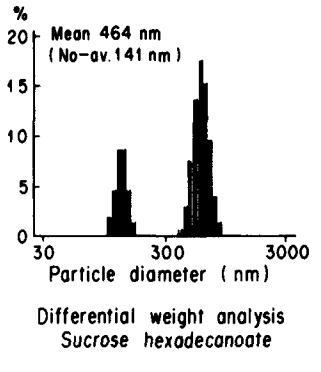


FIG. 5. Particle sizes and their distributions of the vesicles composed of sucrose myristate and palmitate, respectively, by means of the photon-correlation method at 20°C.

tures using a transmission electron microscope (TEM) after staining 1% sucrose fatty acid ester suspensions negatively with aqueous 1% uranyl acetate or sodium phosphotungstate (16). We succeeded in observing the presence of vesicles as circular and tubular rings for the first time. These micrographs are shown in Figure 2. The micrographs were taken at magnifications of 50-(A) and 80-(B) thousand, and they were then enlarged about twofold. These are very organized steructures with different sizes. The sizes range from less than 100 nm to more than 500 nm in the long diameter.

Next, we observed the vesicles of these sucrose fatty acid esters at the 200-fold magnification using a light microscope with the 410 nm filter on the light source and staining with 2, 8-bis (dimethylamino)-10-dodecylacridinium bromide as a fluorescent dye in the lipid bilayer. These micrographs of sucrose palmitate are enlarged and are shown in Figure 3. In this case, individual vesicles are alive in water because there was no drying process; this is different from the electron microscopic observation of anhydrous shadows of inorganic metals. Particle size and size distribution of vesicles composed of sucrose laurate were determined by the photo-correction method. There are two peaks in particle size frequencies in both cases, sucrose dilaurate and mixed laurate, as shown in Figure 4. The size distribution is remarkably wide and is independent of the preparation techniques. The weight-average mean diameter of dilaurate is 424 nm in the REV vesicle, while that of sucrose laurate is 452 nm in the vortexing vesicle. Sucrose myristate and palmitate give results similar to Figure 4 and are shown in Figure 5. Table 2 shows the relation between the alkyl chain length of sucrose fatty acid ester and the weight-average particle size of vesicles. Sucrose palmitate, myristate and laurate are about 450 nm. On the other hand, vesicles from stearate have a value of 1080 nm, and those from decanoate have a value of 290 nm. Vesicle sizes of egg yolk lecithin as prepared by the conventional vortex method were 100-1000 nm from TEM, while av.340 nm from the photon-correlation method.

The volume of the central water phase within closed vesicles depends upon the acyl chain length of sucrose fatty acid esters. This may be observed from Figure 6. It is also apparent that the bilayer membrane of sucrose fatty acid esters havung acyl chain lengths longer than lauroyl groups exert a barrier function, separating strongly

TABLE 2

Average Particle Sizes of Vortexed Sucrose Ester Vesicles[a]

Sucrose ester	Particle size (nm)
Stearate	1080
Palmitate	464
Myristate	506
Laurate	452
Fractionated dilaurate	453
Decanoate	292

[a]By means of the photon-correlation method at 25°C.

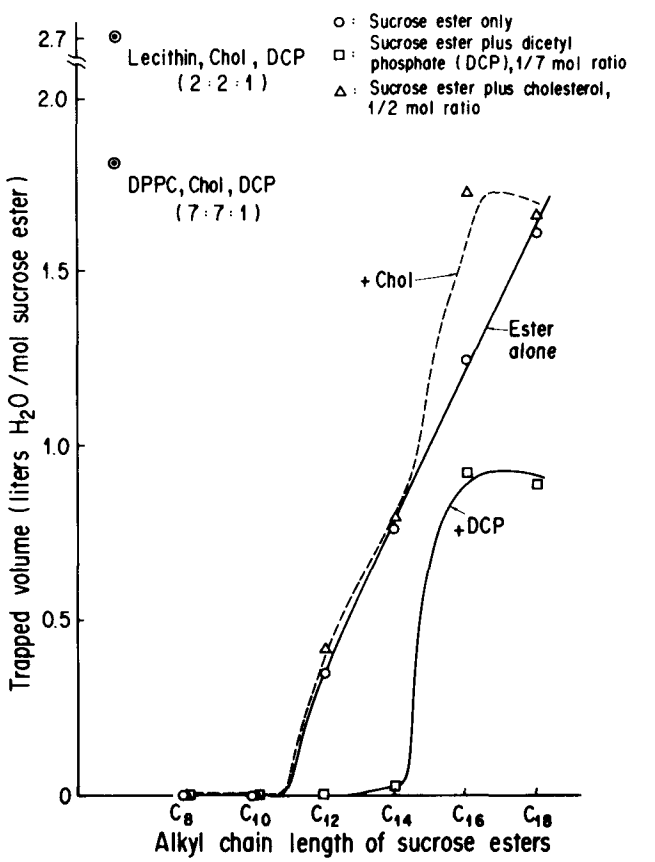


FIG. 6. The relation between trapped volumes (central water phase) and the acyl chain lengths of sucrose esters at 25°C.

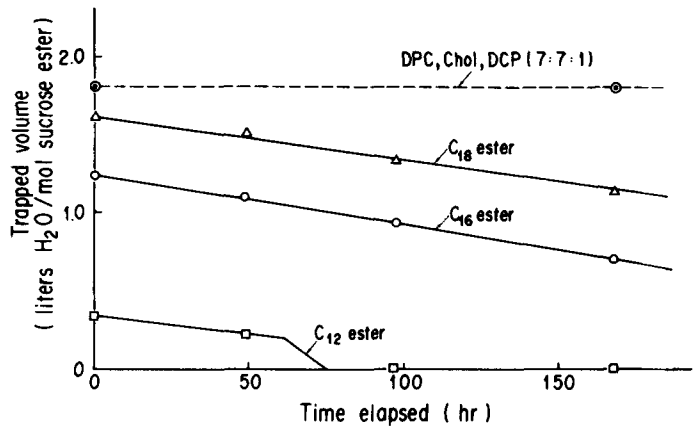


FIG. 7. The release of 6-carboxyfluorescein (CF) from sucrose ester vesicles with time at 25°C.

between their central water and bulk phases. However, sucrose decanoate formed closed vesicles in water, based on electron microscopic observation. So, the vesicle of sucrose decanoate cannot have a bilayer as the separating wall to prevent the leakage of trapped CF. The vesicle composed of sucrose stearate has the largest volume of 1.7 liter water/mol ester comparable to the vesicle of DPPC, Chol, DCP (7:7:1), based on the experiments of CF entrapment. On the other hand, the effects of the additives on the volume of the vesicles consisting of sucrose fatty acid esters also were examined. The addition of cholesterol gives a rather large central water phase, while the addition of DCP reduces the central water phase considerably, as is shown in Figure 6. The effect of cholesterol could be attributed to the mutual penetration of the molecules of sucrose fatty acid ester and cholesterol. The effect of DCP may be anticipated as giving anionic charges to nonionic sucrose fatty acid ester vesicles. Then the hydrophilic and lipophilic balance (HLB) of the mixed vesicle system of sucrose fatty acid ester and DCP may shift to larger water affinity.

Subsequently, the CF release from the vesicles of sucrose fatty acid esters with time is illustrated in Figure 7. The rate of CF leakage depends upon the acyl chain length. The stearate ester vesicle releases 40% of its CF after a week, while the laurate ester vesicle loses all of its CF after three days. The vesicle of DPPC, cholesterol, DCP (7:7:1) is unchanged even after a week.

Phase transition behavior of sucrose fatty acid ester was determined by a differential scanning calorimeter (DSC). The endothermal heat vs temperature plot of sucrose palmitate is illustrated in Figure 8.

The endothermal peak of anhydrous sucrose palmitate is 54.3°C, while in the vortexed suspension it is 22.5°C. So, it was also confirmed calorimetrically that the aqueous suspension of sucrose pamitate formed liposomes after vortexing its solution.

These DSC results are summarized in Table 3. From the stearate to decanoate, all the sucrose ester suspensions have similar phase transition temperatures in agreement with the similarity of phase transition temperatures among the anhydrous material. This similarity in phase transition temperatures of sucrose esters is different from that of synthetic L-α-lecithin homologues (17). Phase transition temperatures of these sucrose vesicles are 23.0°C for stearate ester, 19.1°C for decanoate ester and so on, and are similar to each other.

Based on the molecular structure of sucrose diester (Fig. 9A), a schematic model of sucrose dilaurate and other diester vesicles is illustrated in Figure 9B. The primary hydroxyl group of the glucose moiety of sucrose will be most reactive, and may react first with a long chain fatty acid; the primary hydroxyl group of the end of fructose moiety will react with fatty acid second. When triesters are mixed with monoesters and diesters, alkyl chains

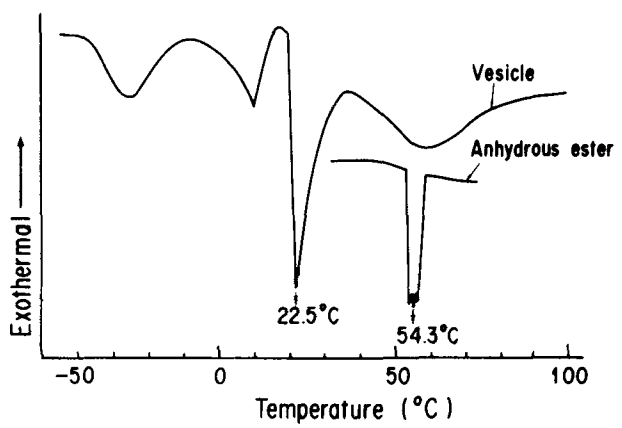


FIG. 8. Phase transition behavior of sucrose palmitate in a vesicle form (1% aqueous suspension) and the anhydrous state.

TABLE 3

Differential Scanning Calorimetry

Sucrose ester	Phase transition temperature (°C)	
	Vesicle	Anhydrous
Stearate	23.0	53.7
Palmitate	22.5	54.3
Myristate	22.0	53.1
Laurate	22.8	44.1
Fractioned dilaurate	18.9	ca 50
Decanoate	19.1	44.6

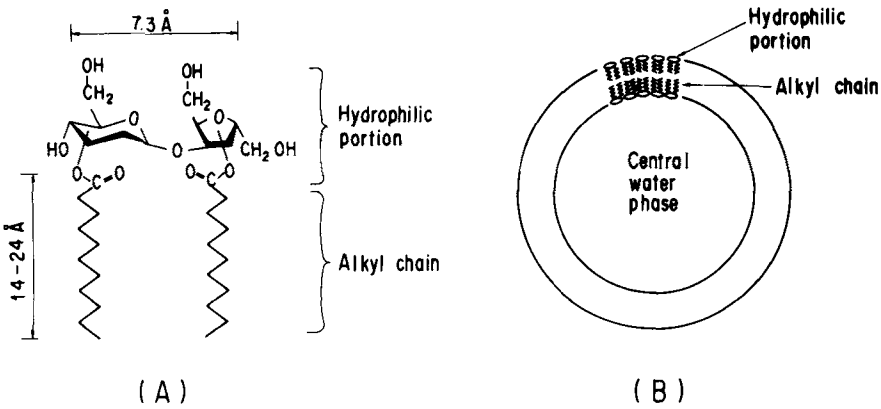


FIG. 9. Molecular structure of sucrose fatty acid diester (A) and a schematic diagram of the vesicle (B).

could coagulate together more tightly, owing to their hydrophobic interaction, and form a cork-like structure. For comparison, dipalmitoyl phosphatidycholine (DPPC) as a phospholipid homologue has a corresponding molecular structure and a molecular volume (18), and thus may be expected to form similar sizes of vesicles having av. 340 nm like Figure 9A and B. The addition of cholesterol increases the barrier function, and the addition of DCP causes a structural defect which leaks CF.

Because these mixtures of monoalkyl and polyalkyl chain compounds of the sucrose moiety (sucrose fatty acid esters) carry both bulky hydrophobic and hydrophilic parts, these monoesters form a cork-like molecular structure in water. Then, these molecules of sucrose esters would be able to form closed vesicles. Sucrose ester vesicles may be unique for having nonionic saccharide moieties as hydrophilic groups in comparison with those of phospholipid homologues. It was concluded that sucrose fatty acid (C_{10}-C_{18}) esters, especially diesters, formed vesicles in water similarly to phospholipid homologues. This finding of a new function of sucrose esters may lead to the development of new materials for superfine microcapsules, microemulsions.

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[Received August 4, 1986; accepted August 18, 1988]