Participation of Macrogolstearate 400 Lamellar Phases in Hydrophilic Creams and Vesicles

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In binary Macrogolstearate 400 (MS 400)/water systems, lamellar surfactant arrangements can be detected by polarized light and transmission electron microscopy. As demonstrated by X-ray diffraction and differential scanning calorimetry, the alkyl chains of the emulsifier are in the crystalline state. Ternary systems with liquid paraffin represent optically isotropic, homogeneous o/w creams for a wide composition range. Incorporation of up to 50 mol% cholesterol into the MS 400 lamellar structures leads to a gel–liquid crystalline phase separation within the bilayer, thus enabling the formation of spherical nonionic vesicles. The transition enthalpy of the samples decreases linearly with increasing cholesterol concentrations. The Macrogolstearate 400/cholesterol vesicles proved to be stable in hydrophilic cream systems. Cationic vesicles can be prepared using cetyltrimethylammonium bromide (CTAB) as a charge inducer. Low-CTAB portions are inhomogeneously distributed within the bilayer, as detected by DSC. The results also indicate a perturbation of the alkyl chains packing for the positively charged vesicles.

KEY WORDS: nonionic surfactants; Macrogolstearate 400; lamellar phase; hydrophilic creams; gel–liquid crystalline phase separation; nonionic surfactant vesicles; cationic surfactant vesicles.

INTRODUCTION

Structural investigations of hydrophilic creams and vesicular systems such as liposomes reveal lamellar surfactant arrangements (1–3). As proposed by Barry (4,5), o/w creams contain networks built of mixed emulsifiers, associated in a "frozen" lamellar liquid crystal. Generally, the addition of water to the surfactant mixture at an increased temperature leads to the formation of a swollen liquid crystalline lamellar mesophase (6). Upon cooling, the alkyl chains crystallize in a regular lattice while water is still present between the polar groups, and the lamellar gel phase is obtained (6). For some special cases a specific liquid crystalline microstructure has been proposed (2).

In lipid bilayer spheroids, termed vesicles, both the gel and the liquid crystalline state may be observed at ambient temperature, depending on factors such as the length and degree of saturation of the hydrocarbon chains, nature of the polar head group, and presence of cholesterol (7).

At a given surfactant concentration, more lipophilic emulsifiers tend to organize in vesicles, while more hydrophilic ones form planar lamellar phases (8). Due to the hydrophilic gel network, the latter systems show a higher consistency and a creamy appearance.

In creams containing liposomes, stability problems have been reported (9), due to vesicle solubilization by the emulsifiers. Incorporation of detergents in bilayers leads to a transition of the lamellar phase into a solution of mixed micelles (10). Therefore, formulation of creams and vesicles using the same surfactant is expected to overcome these problems. In this paper, the application possibilities for a nonionic surfactant, Macrogolstearate 400, are investigated. In addition, non-ionic surfactant vesicles are regarded as a novel drug delivery system, possessing some advantages over liposomes (11).

We also address the question whether charged vesicles can be obtained by combining a nonionic and a cationic surfactant. Positively charged vesicles are expected to interact readily with negatively charged biological membranes.

MATERIALS AND METHODS

Materials

The commercial quality o/w surfactant polyoxyethylene-9-stearate (Cremophor S9, BASF, Ludwigshafen, Germany) corresponds to Macrogolstearate 400 DAB 9. For the preparation of creams, purified water (DAB 9) and Paraffinum subliquidum (DAB 9, Mainland, Frankfurt/Main, Germany) were used. Cholesterol (USP XXII, Merck, Darmstadt, Germany) and cetyltrimethylammonium bromide (Fluka Chemie, Buchs, Switzerland) were used for preparing vesicles.

Methods

The cream systems were prepared by melting together MS 400 and paraffin oil at 70°C and slowly adding water of the same temperature while stirring at 300 rpm. Stirring was continued until room temperature was reached.

Vesicle dispersions were prepared according to the film method described by Bangham *et al.* (12).

The systems were studied by polarized light microscopy (PM) (IMT-2, Olympus Optical Co., Ltd., Tokyo), hot-stage polarized microscopy (Superspan, Leitz, Wetzlar, Germany), differential scanning calorimetry (DSC) (TA 2000, Mettler, Gießen, Germany) at a scanning rate of 5°/min, and wide-angle X-ray diffraction (WAXD) (X-ray generator PW 1730, Cu-K α radiation of 1.5418-Å wavelength, vertical goniometer PW 1050, proportional counter PW 1965/60, step motor PW 1394; Phillips, Kassel, Germany). Transmission electron microscopy (TEM) of freeze-fractured samples (BAF 400 D, Balzers GmbH, Liechtenstein) was performed on an Elmiskop 102 (Siemens, Erlangen, Germany) according to a technique described elsewhere (13,14).

RESULTS AND DISCUSSION

Cream Systems

First, binary mixtures of MS 400 with water, which appear as white semisolid systems over a wide concentration

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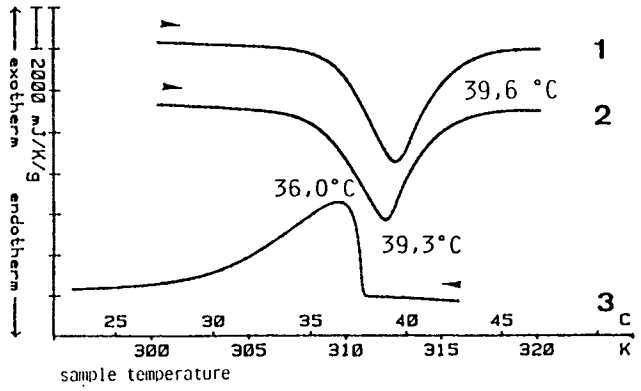

Fig. 1. DSC profiles for a binary MS 400/water (40/60) mixture: (1) first heating curve; (2) second heating curve; (3) cooling curve.

range (3–60%, w/w, MS 400), were studied. PM shows the formulations to be nearly isotropic up to 15% MS 400 and an increasing anisotropy as well as planar lamellar arrangements at higher MS 400 contents. TEM of a freeze-fractured replica of a binary MS 400/water (10/90) system also reveals planar lamellar structures (Fig. 2).

WAXD patterns of the binary systems show one sharp 4.2-Å (21.2° 2θ) reflection, which is characteristic for ordered crystalline alkyl chains (15,18). Based on these results the optically detected lamellar arrangements may be identified as a lamellar gel phase.

DSC heating experiments indicate a gel–liquid crystal phase transition with a maximum at 39.6°C (Fig. 1). Upon cooling, recrystallization of the fatty acid chains is detected at 36.0°C. Microscopically, growth of myelin structures similar to those described for lecithin (16) as a result of surfactant swelling is observed above this temperature. As the second heating curve appears unchanged, no polymorphic transitions take place.

In the ternary phase diagram of MS 400, water, and paraffin oil, a region of optically isotropic hydrophilic creams is found (Fig. 3). Figure 4 represents the microstructure of a formulation containing 25% liquid paraffin. Dispersed oil droplets are immobilized in a lamellar network, similar to that observed for binary MS 400/water systems.

Fig. 2. TEM micrograph of a binary MS 400/water (10/90) system. 1 cm = 250 nm; reduced to 55% for reproduction.

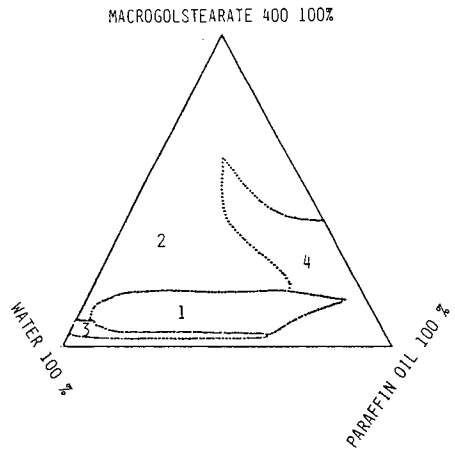

Fig. 3. Ternary phase diagram of MS 400, liquid paraffin, and water: (1) optically isotropic hydrophilic creams; (2) anisotropic turbid gels; (3) o/w emulsions; (4) separation of an emulsion and a clear oil phase.

It is an interesting finding that model creams with 10% MS 400 possess much lower melting enthalpies (3 J/g) compared to "classical" creams based on mixed emulsifiers, such as the nonionic hydrophilic cream DAB 9 (11 J/g). These results indicate a low content of crystalline material for the MS 400 creams, allowing prediction of good stability and optimum properties (2).

Nonionic Surfactant Vesicles

In diluted MS 400/water systems (≤2%, w/w), polyhedrally shaped anisotropic particles are observed microscopically. The samples represent milky dispersions of vesicles with a polygonal form resulting from lipid bilayer crystallization (17). At dilutions beyond 0.01% MS 400 a transition into a clear isotropic micellar solution takes place.

To modify the physical state of MS 400 bilayers we tried incorporation of cholesterol (Table I) using the film method. For formulations with 3% (w/w) total lipid, increasing cho-

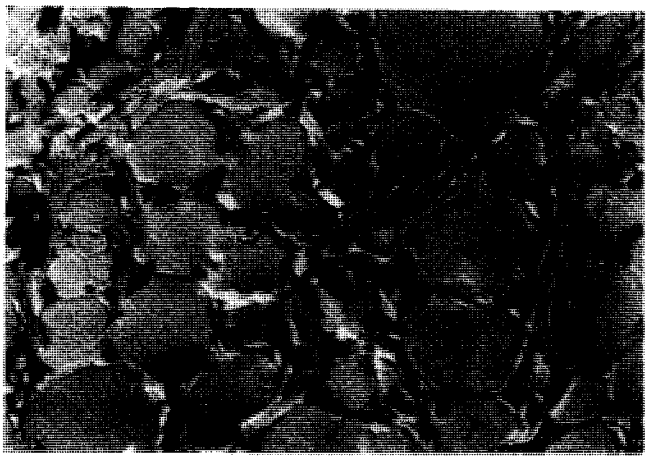

Fig. 4. TEM micrograph of a hydrophilic cream containing 10% MS 400, 25% liquid paraffin, and 65% water. 1 cm = 250 nm; reduced to 55% for reproduction.

Table I. Composition of Cholesterol-Containing MS 400 Dispersions

Relation (w/w) MS 400:cholesterol	Relation (mol:mol) MS 400:cholesterol	Cholesterol concentration (mol%)
1:0	—	—
1:0.14	4:1	20
1:0.21	2.7:1	27
1:0.29	2:1	34.0
1:0.35	1.6:1	38.0
1:0.435	1.3:1	43.3
1:0.57	1:1	50.1
1:0.86	1:1.5	60.2

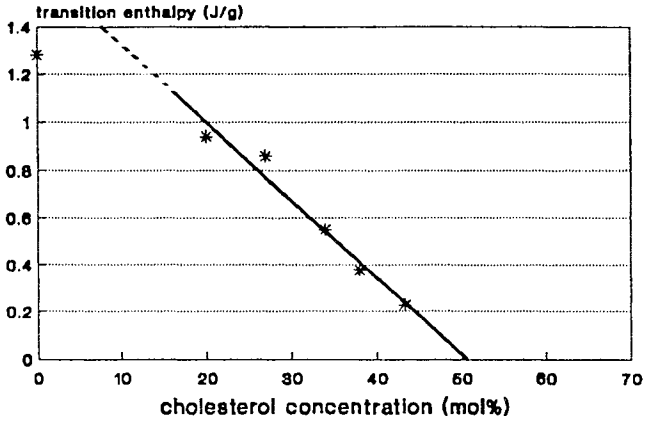

Fig. 6. Transition enthalpy of MS 400/cholesterol dispersions with 3% total lipid as a function of cholesterol concentration.

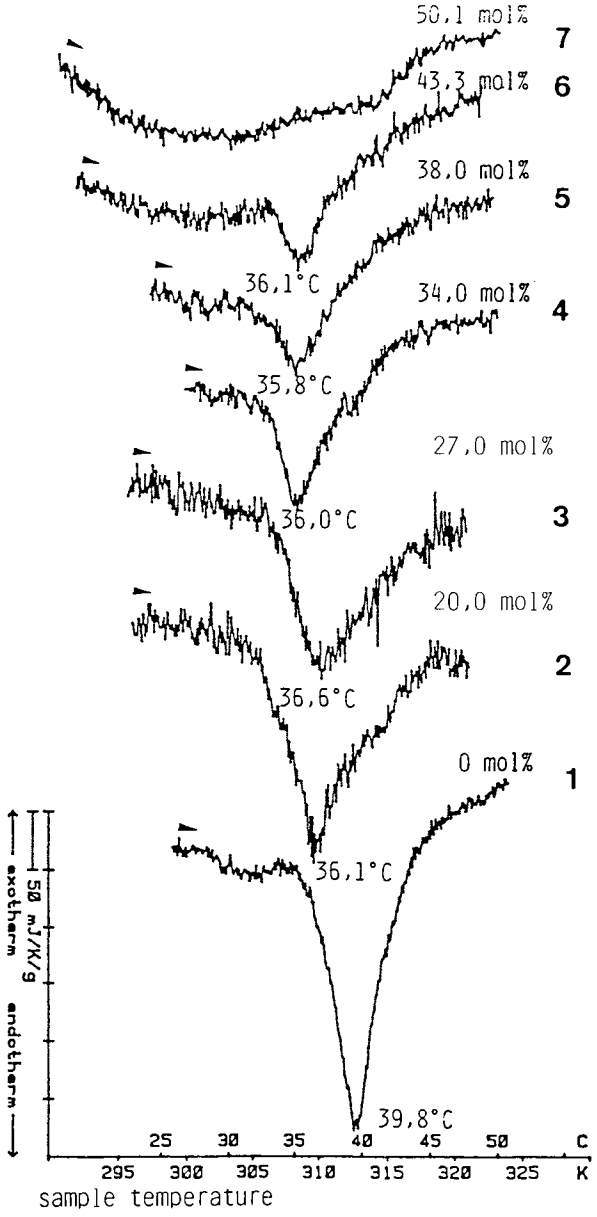

Fig. 5. DSC heating curves of MS 400/cholesterol dispersions with 3% total lipid and increasing cholesterol amounts (mol%): (1) 0; (2) 20.0; (3) 27.0; (4) 34.0; (5) 38.0; (6) 43.3; (7) 50.1.

lesterol portions (20–50 mol%) lead to disruption of the gel network and gradual formation of multilamellar vesicles, which can be easily identified by the "Maltese cross" (14). Excess cholesterol can be detected microscopically as anisotropic plate-like crystals at higher cholesterol concentrations (60 mol%).

DSC measurements (Fig. 5) show decreased phase transition temperatures for the cholesterol-containing samples

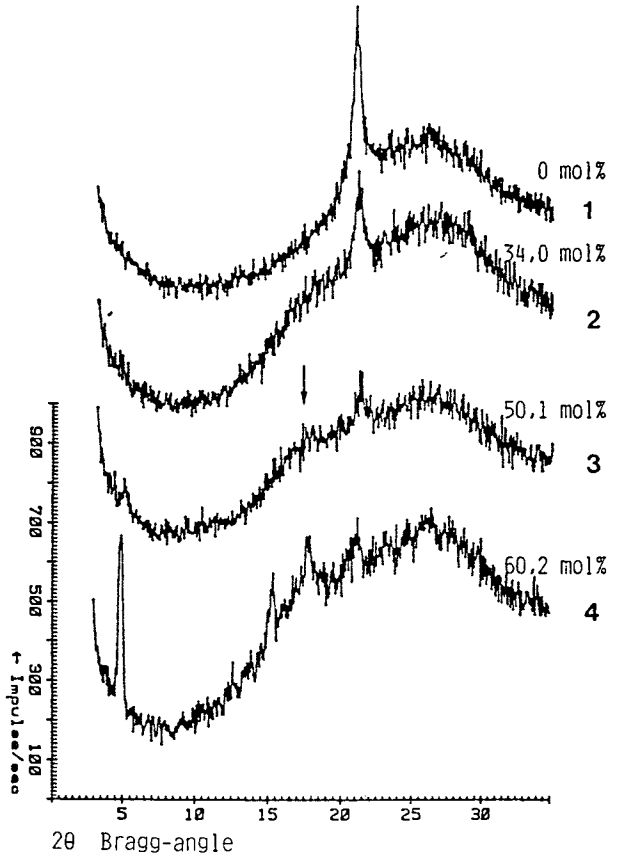

Fig. 7. WAXD patterns of MS 400/cholesterol dispersions with 40% total lipid and increasing cholesterol amounts (mol%): (1) 0; (2) 34.0; (3) 50.1; (4) 60.2.

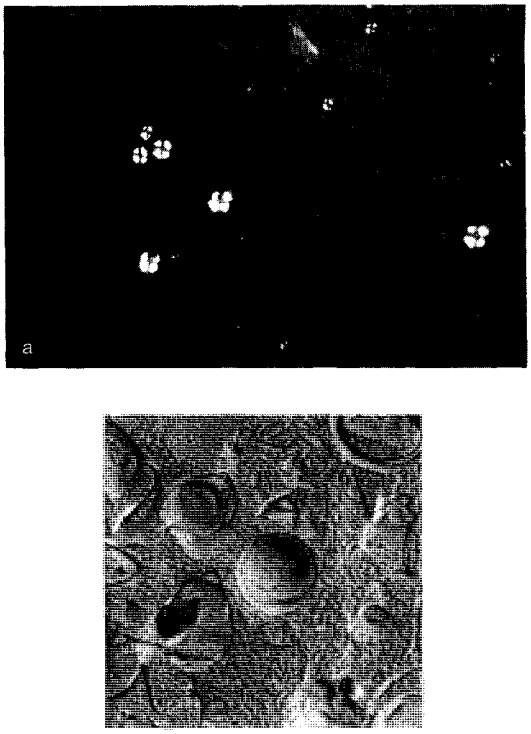

Fig. 8. PM (a) and TEM (b) micrograph of a vesicle containing cream [DAC Basiscreme (23)]. (a) 1 cm = 20 μm, (b) 1 cm = 350 nm; reduced to 55% for reproduction.

(around 36.0°C) compared to the cholesterol-free MS 400 bilayers (39.8°C).

There is also a dramatic decrease in the transition enthalpy and a linear correlation is observed (Fig. 6). The *X*-axis intercept indicates a maximum incorporation capacity of about 50 mol% cholesterol.

WAXD measurements were performed on samples with 40% total lipid, which represented concentrated vesicular dispersions. The diffraction patterns are shown in Fig. 7. As described above, the sharp 4.2-Å (21° 2θ) interference is characteristic for the lamellar gel phase with crystalline hydrocarbon chains, while the diffuse 4.6-Å (18° 2θ) reflection is caused by lipids in the liquid crystalline state (18). Upon the addition of cholesterol the intensity of the 4.2-Å reflection decreases. Concominantly we observe an increase in the diffuse 4.6-Å signal. Therefore, a lamellar gel–liquid crystalline phase separation may have formed, the presence of the liquid crystalline phase with fluid alkyl chains enabling curvature of the bilayer sheets and thus the formation of spherical vesicles. In this way domains of gel and liquid crystalline phases coexist in the MS 400/cholesterol bilayer. These results are in good agreement with the findings of Funk *et al.* (26) for thermotropic phase separation phenomena in microsomal membrane vesicles. In samples with a high cholesterol content (60 mol%), excess crystalline cholesterol can be identified by the typical reflections at 17.1 Å (5.2° 2θ), 5.7 Å (15.6° 2θ), and 5.2 Å (17.1° 2θ) (19). These observations are in good agreement with the microscopic and calorimetric results.

MS 400 vesicles containing 38 mol% cholesterol proved to be stable after incorporation in MS 400 creams, as well as in a POE-20-glycerol monostearate-containing cream (DAC Basiscreme) (20) over a period of 12 months as detected by PM and TEM (Fig. 8). There is an interesting parallel to bacterial membranes, where the coexistence of gel and liquid crystalline domains even at optimal growth temperature is supposed to be responsible for the required membrane stability (21).

Cationic Surfactant Vesicles

For the preparation of cationic MS 400 vesicles, cetyltrimethylammonium bromide (CTAB) was used as a charge inducer. Homogeneous MLV dispersions containing 3% (w/w) total lipid were obtained using the film method. The bilayer composition is shown in Table II. A maximum incorporation capacity of 50 mol% CTAB was estimated by gel electrophoresis (19).

The thermal behavior of positively charged vesicle dispersions is illustrated in Fig. 9. For small CTAB amounts (14.2–25 mol%) a shoulder at 37–40°C is observed along with the main transition peak, indicating a lateral phase separation within the lamellar phase. The transition temperature of the main peak is about 3° lower than that of the CTAB free dispersion with the same cholesterol content. As the shoulder temperature corresponds roughly to the transition temperature of the binary MS 400/water mixture (39.6°C), an inhomogeneous distribution of CTAB resulting in exclusion of MS 400 molecules from the MS 400/cholesterol phase can be postulated. Phase separation phenomena and domain formation have already been described for charged bilayers (22).

Higher CTAB portions seem to be homogeneously distributed within the bilayer because only the main transition peak is detected. The shift to lower temperatures compared to the CTAB-free dispersions implies a perturbation in the alkyl chain packing resulting from electrostatic repulsion between the charged molecules. Similar findings have been reported for negatively charged phosphatidic acid bilayers (23).

Table II. Composition of Cationic MS 400 Dispersions

Relation (w/w) MS 400:cholesterol:CTAB	Relation (mol:mol) MS 400:cholesterol:CTAB	CTAB (mol%)
1:0.29:0.135	1:0.5:0.25	14.2
1:0.29:0.27	1:0.5:0.5	25.0
1:0.29:0.54	1:0.5:1	40.0
1:0.29:0.80	1:0.5:1.5	49.7
1:0.29:1.06	1:0.5:2	56.7

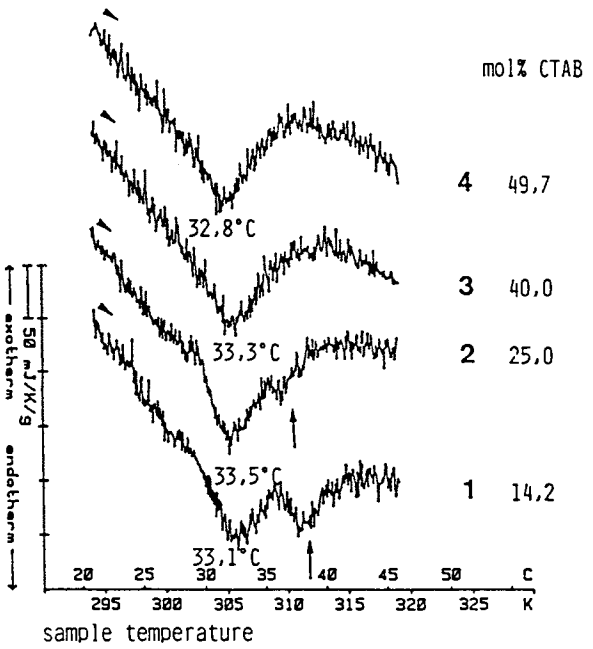

Fig. 9. DSC heating curves of cationic vesicle dispersions with 3% total lipid and increasing CTAB amounts (mol%): (1) 14.2; (2) 25.0; (3) 40.0; (4) 49.7.

CONCLUSIONS

The formation of a lamellar gel phase in binary MS 400/water mixtures allows the formulation of o/w creams under substitution of mixed emulsifiers for a single surfactant. With MS 400, very homogeneous and optically isotropic creams are obtained.

Incorporation of cholesterol in the MS 400 lamellar phase leads to modification of the bilayer fluidity and formation of spherical vesicles. With increasing cholesterol amounts the liquid crystalline bilayer fraction increases, but no complete phase transition takes place. Up to about 50 mol% cholesterol can be incorporated in the MS 400 bilayer, a value similar to that determined for phospholipid membranes (24). Unlike liposomes composed of phospholipids, nonionic MS 400 vesicles proved to be stable in hydrophilic creams over the investigation period of 12 months.

Using CTAB as a positive charged inducer cationic vesicles can be obtained. At low CTAB concentrations, phase separation due to inhomogeneous distribution of the charged molecules within the bilayer is detected by DSC.

Higher CTAB portions exert an additional fluidizing effect on the lipid bilayer. The application possibilities of the cationic MS 400 vesicles for introducing DNA into cell nuclei are the subject of another study (25).

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