RESEARCH ARTICLES

Liquid Crystals in Emulsions, Creams, and Gels Containing Ethoxylated Sterols as Surfactant

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Abstract: Depending on the concentration and the hydrophilicity of the surfactant, different mesophases have been demonstrated by X-ray analysis, polarizing microscopy and transmission electron microscopy of freeze-fractured samples. These mesophases participate in the microstructure of ternary mixtures. Mixtures of sterol-PEG5- and stereol-PEG10-ether form lamellar liquid crystals which are organized into multilamellar vesicles with a size of up to several microns. At low concentrations of the surfactant the ternary systems consist of fluid emulsions of liquid crystalline vesicles and droplets of liquid paraffine dispersed in the outer hydrous phase. With increasing concentrations of the surfactant the mixtures become creamy and semisolid but remain emulsions with an increased volume ratio of the inner phase. The phase diagram of mixtures with sterol-PEG16-ether shows three different regions of liquid crystals: lamellar liquid crystals of planar arrangement at high concentration of the surfactant, a hexagonal mesophase with dispersed liquid paraffine and a ringing gel of close-packed mixed micelles. The higher the volume fraction of the liquid paraffine the larger are the oily droplets which are dispersed in the outer liquid crystalline phase of the close-packed micelles. The phase diagram of the most hydrophilic sterol-PEG25-ether is similar to that of sterol-PEG16-ether except for the absence of lamellar liquid crystals.

Introduction

Structural studies of topical preparations containing cholesterol and fatty alcohol as rather lipophilic surfactants have demonstrated the presence of liquid crystals of the lamellar type only (1, 2). These liquid crystals arrange in multilamellar vesicles (3). In order to study the influence of more hydrophilic derivatives of cholesterol on the liquid crystal formation in ternary mixtures different sterol-PEG-ethers (HLB-value 5, 12, 15 and 17) have been investigated. The sterol-PEG-ethers of varying hydrophilicity form different liquid crystals in mixtures with water and liquid paraffine. These mesophases take part in the fine structure of topical preparations like creams, emulsions, and gels.

Materials

The following chemicals and materials were used: Soya sterol-PEG5-ether, soya sterol-PEG10-ether, soya sterol-PEG16-ether, soya sterol-PEG25-ether (Generol® 122 E5, 10, 16 and 25 of Cosmedia, Henkel), liquid paraffine DAB 8, aqua purificata Ph. Eur. I. The phytosterols consisting primarily of sitosterol, campesterol and stigmasterol were obtained from soybean oil. The main portion of the ethoxylated sterols is sitosterol (55 %).

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Methods

For binary mixtures the melted surfactant and the second component with the same temperature as the fused sterol-PEG-ether were mixed together. Then the mixture was cooled to room temperature while stirring. For ternary mixtures the ethoxylated sterol and the liquid paraffine were melted together. Water of the same temperature was added and the mixture was cooled while stirring.

X-ray analysis was performed with wide angle, Debye-Scherrer, and with small angle, according to Kiessig (4) and/or Kratky (5). A polarizing microscope, Leitz Laborlux was used with a heating stage, Kofler. Freeze-fracture technique followed the method of Moor et al. (6), and Balzers BAF 400 D was used for the preparation of replicated tissues for transmission electron microscopy, Philips EM 300.

Results and Discussion

Surfactants

Ethoxylated sterols assume semisolid or solid configurations at room temperature. Non-ethoxylated soya sterol melts at 400 K. With increasing degree of ethoxylation the melting point decreases. Sterol-PEG5-ether has its final melting point at 380 K. Sterol-PEG25-ether already melts at 320 K. The derivatives, except for sterol-PEG25-ether, form thermotropic liquid crystals of the lamellar type, that can be detected upon heating and cooling in a polarizing microscope. The shorter the PEG-chain the larger is the temperature range of the lamellar liquid crystals. This range is approximately 20 K with sterol-PEG16-ether, approximately 40 K with sterol-PEG10-ether and 70 K with sterol-PEG5-ether. Upon cooling these derivatives remain liquid crystalline down to room temperature. A further decrease of the temperature causes the derivatives with 5, 10 and 16 oxyethylene groups to crystallize, while sterol-PEG25-ether already recrystallizes at 300 K. Crystals and liquid crystals were differentiated by wide angle X-ray studies. The crystals of sterol-PEG25-ether show the typical sharp interference pattern of real crystals at the Debye-Scherrer film, while the other derivatives have only a few diffuse interferences of which the highest intensity is approximately 5.6 Å.

Binary mixtures

Varying the concentration of the binary mixtures in steps of 10 % we investigated mixtures of surfactant and water mixtures of surfactant and liquid paraffine. In the presence of water the ethoxylated sterols form different liquid crystals at room temperature. The microstructure of the different mesophases depends on the degree of ethoxylation. The non-ethoxylated sterols do not form liquid crystals with water, neither at room temperature nor at elevated temperatures as high as 370 K.

Sterol-PEG5-Ether or Sterol-PEG10-Ether with Water

Ethoxylated sterols of only 5 or 10 EO-groups are significantly more hydrophilic than non-ethoxylated sterols and form liquid crystals of the lamellar type in the presence of water. The surfactant molecules arrange in layers. With increasing content of water increasing interlayer spacings are measured by small angle X-ray technique. Water penetrates the polar layers of the surfactants and causes a definite swelling. But there is no swelling in the lateral direction from one ethoxylated molecule to the other. Only hydration of the ether groups may be possible. The short distances recognized by the wide angle X-ray technique (Debye-Scherrer) do not change with increasing water content. The main interference of the short distances is the same as in the case of the water-free ethoxylated sterols (5.6 Å). Only the large spacings change with increasing concentration of water. Fig. 1 demonstrates swelling of the lamellar layers with water by approximately 30 Å. At room temperature, more than 80 % water in a mixture with sterol-PEG5-ether and more than 70 % water in a mixture with sterol-PEG10-ether produce systems that no longer show X-ray diffraction patterns. However, small droplets with an anisotropic border that have been detected at a lower water content in the polarizing microscope are still recognizable at high water content. With increasing concentrations of water the anisotropic droplets gradually disappear, while very small isotropic droplets remain. Even below 1 % of surfactant in water the isotropic droplets can be found. These dispersions may be considered as opalescent or milky emulsions of the o/w type.

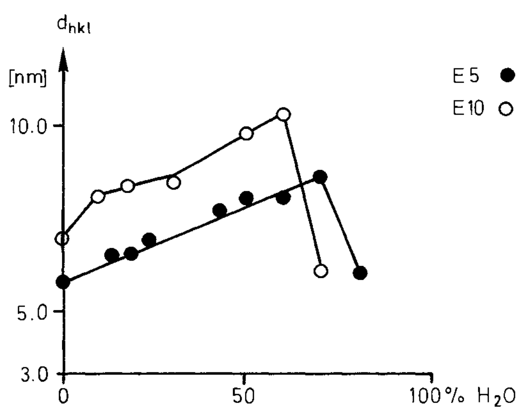

Fig. 1 Dependence of the interlayer spacings on the concentration of water in binary mixtures with sterol-PEG5- (E5) and sterol-PEG10-ether (E10).

Fromherz (7) proposes that micellar solutions represent fragments of anisotropic liquid crystalline structures. These fragments are highly dispersed and form colloidal solutions. In his view swelling of anisotropic liquid crystals should be constant after reaching a definite maximum. However, the results of our investigations show that after the swollen liquid crystals reach maximum water content the swelling decreases. The resulting interlayer spacings correspond to the spacings of the water free sterol-PEG5-ether. The connection between the strongly hydrated layers is lost, and the layers are "breaking up". The surfactant molecules have to arrange in a new manner: the opalescent dispersion arises. By increasing the temperature to approximately 30 K above room temperature, the dispersion of isotropic droplets in water is again transformed into liquid crystals because of the decrease of the hydration of the surfactant molecules with increasing temperature. The actual hydratation results in interlayer spacings that permit the formation even of liquid crystals. The layers are still connected by interlayer forces, for example by hydrogen bonds.

The anisotropic droplets in mixtures of water and sterol-PEG-ethers with 5 or 10 EO-groups show a special organization of the lamellar liquid crystals. With polarizing microscopy we have already shown that the lamellar liquid crystals are not in a planar arrangement but organize themselves into multilamellar vesicles. These were detected by transmission electron microscopy of replicated tissues. Since the mixtures contain water, it is not possible to watch the samples directly in the TEM. Therefore, replicated tissues of these mixtures were produced by the freeze-fracture technique of Moor et al. (6). Because of the very short time of freezing in slash N_2 at 63 K the original structures of the samples are preserved. In particular, there is no risk of ice recrystallization that could destroy the original structures. Electron micrographs of the replicated tissues show the hydrated surfactant layers arranged in multilamellar vesicles (Fig. 2). The size of these vesicles ranges to several microns.

Fig. 2 Multilamellar vesicle of 75 % sterol-PEG5-ether and 25 % water, TEM, magnification 125000 x, bar 500 nm.

Sterol-PEG16-Ether with Water

The lipophilicity of the sterol-PEG16-ether surfactant decreases in comparison with that of the sterol-PEG5- and sterol-PEG10-ether. A hexagonal arrangement of the liquid crystals occurs at water concentrations of 20 to 50 %. Below 20 % water there is a lamellar liquid crystalline phase of mainly planar arrangement. Vesicles are not detected. Above 50 % water a micellar solution exists at room temperature which transforms into a mesophase with increasing temperature. When comparing sterol-PEG16-ether and the more lipophilic surfactants, one recognizes that the range of water concentrations decreases over which the mesophase exists (Figs. 1 and 3). After reaching maximum swelling the connection between the hydrated molecules breaks up.

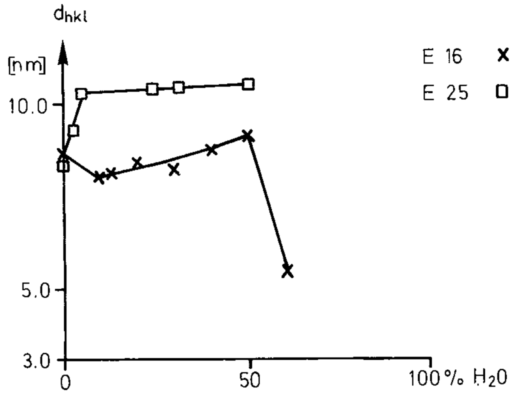

Fig. 3 Dependence of the interlayer spacings on the concentration of water in binary mixtures with sterol-PEG16- (E16) and sterol-PEG25-ether (E25).

Sterol-PEG25-Ether with Water

Because of its strong hydrophilicity sterol-PEG25-ether exclusively forms hexagonal liquid crystals. The limiting concentration of water is 50 %. The hexagonal arrangement can be directly visualized by freeze-fracture and electron micrographs of the replicated tissues. The distance between the axis of the cylinders was estimated by small angle X-ray technique. The ratio of the measured interlayer spacings is $1 : 1/\sqrt{3} : 1/\sqrt{4} : 1/\sqrt{7}$. According to Luzatti et al. (8) this ratio is typical for hexagonal liquid crystals.

Fig. 3 demonstrates the largest spacings measured by small angle X-ray technique. To calculate the exact cylinder distance one has to multiply the largest spacing with the factor of $2\sqrt{3}$. The result of 120 +/− 6 Å is in good correlation with the electron micrographs (Figs. 4 and 5). Mixtures of more than 50 % water are micellar solutions at room temperature.

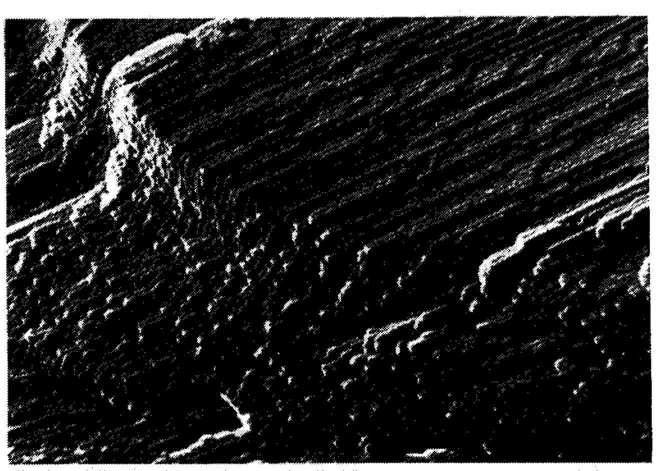

Fig. 4 Hexagonal liquid crystals of 70 % sterol-PEG-25-ether and 30 % water, TEM, magnification 108000x, bar 500 nm.

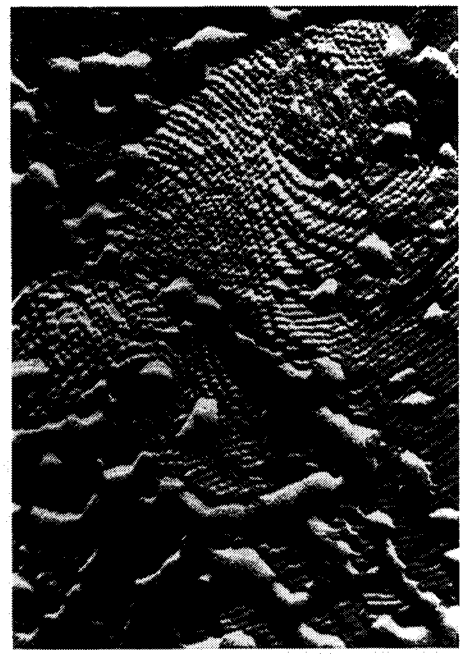

Fig. 5 Hexagonal liquid crystals of 50 % sterol-PEG25-ether and 50 % water, TEM, magnification 165000x, bar 500 nm.

Mixtures with Liquid Paraffine

Investigations of binary mixtures of sterol-PEG-ethers and liquid paraffine show that mesophases occur as well. Upon cooling the melted mixture of sterol-PEG-ether and liquid paraffine to room temperature, liquid crystals of the lamellar type appear except for the binary mixtures of sterol-PEG25-ether and liquid paraffine. In this case the melted sterol-PEG25-ether is highly dispersed in the liquid paraffine. The surfactant recrystallizes without formation of liquid crystals while cooling.

Further organization of the lamellar liquid crystals in case of sterol-PEG5- or 10-ether to multilamellar vesicles occurs in both liquid paraffine and water. The multilamellar vesicles are dispersed in the outer phase which is rich in liquid paraffine. The liquid paraffine does not participate in the structure of the vesicles. Small angle X-ray analysis demonstrated that the interlayer spacings are independent of the concentration of liquid paraffine. The spacings are identical compared with those of the pure surfactants.

Our recent investigations, however, have shown that in binary mixtures of sterol-PEG16-ether and liquid paraffine a limited swelling with paraffine occurs (data not shown). 30 % liquid paraffine in the sterol-PEG16-ether causes a swelling of the interlayer spacing of approximately 25 Å. Increasing the temperature to 310 K causes the interlayer spacing to decrease, and a homogeneous isotropic system appears. Higher contents of liquid paraffine form emulsions of two saturated phases at elevated temperature.

Ternary Mixtures

The following investigations of ternary mixtures primarily concern creamy mixtures throughout the range of a triangle diagram. If sterol-PEG5-ether or sterol-PEG10-ether take part in the mixture, only lamellar liquid crystals are found. The measured spacings do not correspond to special regions in the triangle diagram. They only depend on the ratio of water to surfactant, which is shown in Fig. 6, while the actual content of liquid paraffine has no influence. The measured spacings at varying water to surfactant ratios in the ternary mixtures agree with those of the binary mixtures of water and surfactant. However, at high water contents some deviations are observed. In ternary mixtures lamellar liquid crystals occur even at high water to surfactant ratios, while in binary mixtures of the same ratio there is a sudden decrease of the swelling with the concomitant isotropic dispersion of surfactant in water.

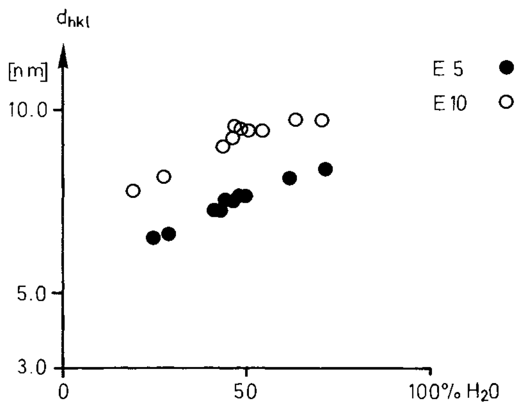

Fig. 6 Dependence of the interlayer spacings on the ratio of water to surfactant in ternary mixtures of water, liquid paraffine, and sterol-PEG5- (E5) or sterol-PEG10-ether (E10).

The swollen lamellar liquid crystals arrange in multilamellar vesicles. The hydrophilic part of the molecules of the outer layer is in contact with the surrounding water. The liquid paraffine may be in the inner core of the vesicle. The creamy mixtures with sterol-PEG5- or sterol-PEG10-ether have o/w character, although the HLB-value of sterol-PEG5-ether is 5. Creams with sterol-PEG5-ether will only be stable if the concentration of the liquid paraffine is not too high. Otherwise drops of paraffine show coalescence and creaming.

Ternary mixtures containing the more hydrophilic sterol-PEG16-ether or sterol-PEG25-ether differ from those with the other sterols. Since multilamellar vesicles do not exist, the interlayer spacings, measured by X-ray analysis, do not simply depend on the ratio of water to surfactant. The liquid paraffine influences the microstructure. At low concentration of the liquid paraffine hexagonal mesophases occur. Oily droplets are dispersed in the outer hexagonal mesophase (Fig. 7). With increasing concentration of the liquid paraffine the hexagonal liquid crystals disappear. Only a single interlayer spacing can be demonstrated by X-ray analysis. This spacing belongs to close-packed micelles. Hydrogen bonds are thought to cause the strong interaction between the micelles. These mixtures are found to yield ringing gels of high elasticity. Oily droplets of different size are dispersed in the outer gel of close-packed micelles (Figs. 8 and 9). The size of the droplets increases with increasing concentration of paraffine. In the polarizing microscope the ringing gels appear isotropic.

Fig. 7 Dispersed oily droplet in a hexagonal mesophase, ternary system of 49 % sterol-PEG16-ether, 5 % liquid paraffine, 46 % water; TEM, magnification 168000x; bar 500 nm.

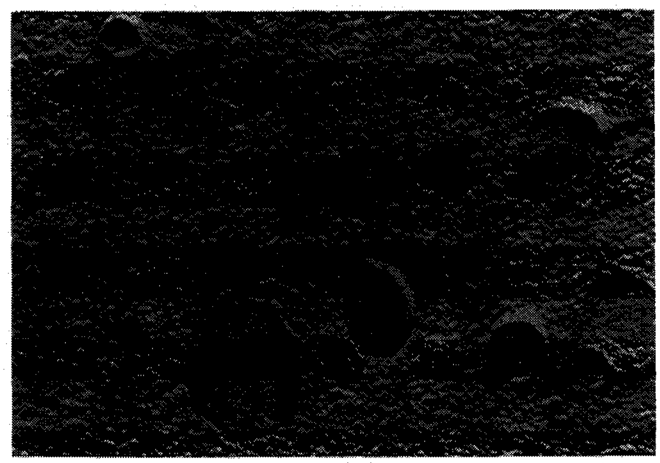

Fig. 8 Ringing gel of close-packed micelles with dispersed oily droplets, ternary mixture of 45 % sterol-PEG25-ether, 25 % liquid paraffine, 30 % water; TEM, magnification 132000x; bar 500 nm.

Fig. 9 Ringing gel of close-packed micelles with dispersed oily droplets, ternary mixture of 26 % sterol-PEG25-ether, 51 % liquid paraffine, 23 % water; TEM, magnification 81000x; bar 500 nm.

The phase diagrams of the ternary mixtures of sterol-PEG16- and sterol-PEG25-ether are plotted in Figs. 10 and 11. There are only small deviations between these two diagrams

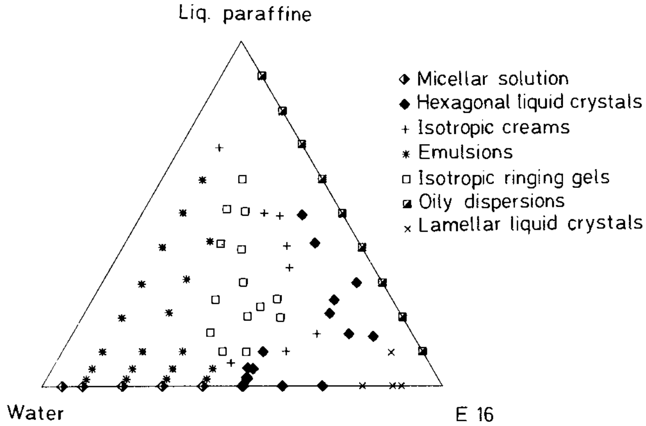

Fig. 10 Phase diagram of sterol-PEG16-ether (E16), liquid paraffine, and water.

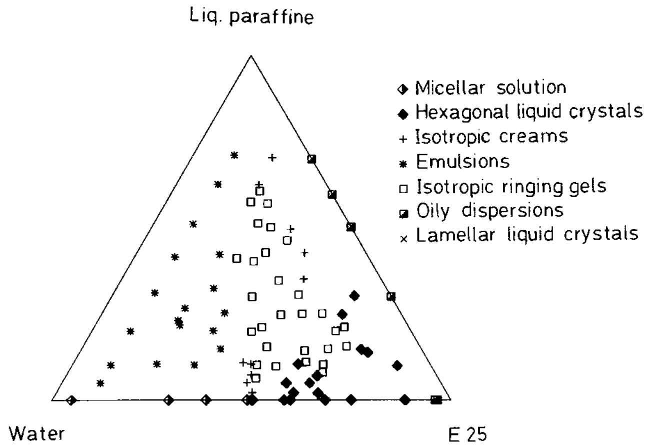

Fig. 11 Phase diagram of sterol-PEG25-ether (E25), liquid paraffine, and water.

concerning the region of the different phases. With sterol-PEG16-ether there is a small additional region of a lamellar mesophase, while sterol-PEG25-ether only forms hexagonal mesophases and isotropic ringing gels as liquid crystals together with water and liquid paraffine. The region of the ringing gel is the largest liquid crystalline region.

While the region of the different liquid crystals is rather large in both phase diagrams, the area of the emulsions exists only at or below 20 % of the surfactant, except for mixtures of very low concentration of liquid paraffine. The region of the emulsions adjoins the micellar solution on the one side and the pure liquid paraffine on the other side. The isotropic creams in the phase diagrams are in the transition region of the different liquid crystals. With the exception of the dispersed droplets of liquid paraffine both hexagonal liquid crystals and close-packed micelles of the ringing gel can thus be demonstrated by electron microscopy. If sterol-PEG16-ether is part of the mixtures, lamellar liquid crystals are also found.

Conclusion

Changes in the hydrophilicity of surfactant molecules by varying the degree of ethoxylation result in different structures of topical preparations. It is possible to produce creamy mixtures containing different concentrations of the components bound in very different structures. We found classical emulsions that consist of two phases: highly dispersed liquid paraffine in a micellar solution. The surfactants are more or less dissolved in the two phases and enriched in the interface area. According to the IUPAC definition we further showed emulsions of the o/w type with liquid crystals. In this case, the inner phase consists of multilamellar vesicles.

We further showed that droplets of paraffine were dispersed by an outer mesophase of hexagonal arrangement. Finally we found liquid crystalline ringing gels with rather hydrophilic derivatives. The microstructure of the ringing gels was found to be close-packed micelles which are in contact with each other. The different structures were characterized by electron micrographs.

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