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Influence of lamellar liquid crystal structure on percutaneous diffusion of a hydrophilic tracer from emulsions

LAURE BRINON, SANDRINE GEIGER, VALERIE ALARD, JEAN-FRANÇOIS TRANCHANT, THIERRY POUGET, and GUY COUARRAZE, *Laboratoire de Physique Pharmaceutique, URA CNRS 1218, Université Paris-Sud, 92290 Châtenay-Mabahry, France (L.B., S.G., G.C.), and Laboratoire de Recherche Christian-Dior, 45804 St. Jean de Braye, France (L.B., V.A., J.-F.T., T.P.).*

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Synopsis

The purpose of this study was to investigate the effect of a lamellar liquid crystalline phase in emulsions on percutaneous diffusion of a hydrophilic sunscreen (benzophenone-4). Six emulsions were formulated, using all the same components except the surfactants. The emulsion structures were visualized by freeze-fracture electron microscopy. *In vitro* penetration measurements were performed with static diffusion cells.

Benzophenone-4 fluxes were smallest for emulsions without liquid crystals and highest for those with liquid crystals. The permeant fluxes seemed to be modified by the surfactant organization within the emulsions.

INTRODUCTION

Surfactants are major components of cosmetic formulations. They are commonly used to stabilize the emulsions. Indeed, these molecules are able to form a monolayer at the oil–water interface, thus reducing the interfacial forces. Within the concentration range of the emulsifying agents used in topical emulsions, some surfactants may form particular colloidal aggregates such as liquid crystal structures (of lamellar, hexagonal, or cubic type). These lamellar structures, when they surround droplets in an emulsion, can enhance the emulsion stability by preventing droplet coalescence (1).

These lamellar structures are also encountered in liposomes. Indeed, a liposome is an aqueous compartment surrounded by one or more lipid bilayers. It has been demonstrated that these particular structures of lipids in the bilayer configuration may enhance the concentration of certain molecules within the skin while reducing their systemic absorption, although the mechanism of action has not yet been fully elucidated (2–5).

Many studies have evaluated the influence of different amphipathic compounds on percutaneous absorption (6–11), but generally the authors do not pay attention to the

vehicle structure. Other studies have investigated the *in vitro* release of active components (12–15) and the cutaneous permeability (16–19) as a function of the various colloidal organizations for a given composition of the vehicle. However, in general, effects are demonstrated for one surfactant system. The aim of our study was to evaluate the impact of the presence of lamellar liquid crystals within emulsions on percutaneous permeability for a large variety of surfactants, by comparing six emulsions that only differed by the surfactant used and, as a result, by their structure.

CHEMICALS AND FORMULATIONS

CHEMICALS

The permeant was benzophenone-4 (the sunscreen Uvinul®MS40). A series of six emulsions containing different surfactants but with the same aqueous and oily phases was prepared.

The oily phase was a caprilic/capric triglycerides (Miglyol®812)/octyl methoxycinnamate (Parsol®MCX) mixture. The surfactants polysorbate 60 (Tween®60); steareth-2 (Brij®72); steareth-21 (Brij®721); poloxamer 407 (Synperonic®PE F/127); sorbitan stearate and sucrose cocoate (Arlatone®2121); and triethanolamine stearate were obtained from ICI Americas. The surfactant acrylates/C_{10-30} alkyl acrylate crosspolymer (Pemulen®TR1) was obtained from Goodrich. The preservative, diazolidilyl urea (Germall®II), was obtained from Sutton. The sodium hydroxide required for aqueous phase and acrylates/C_{10-30} alkyl acrylate crosspolymer neutralization was obtained from Fluka.

FORMULATIONS

Two hundred grams of each emulsion were prepared using a standardized procedure: surfactant, aqueous phase, and oily phase were weighed into a glass container, sealed, heated to 80°C, and stirred using Istral-type mixing equipment (1050 rpm). Agitation was maintained until the emulsion cooled to room temperature. Three passes through a microfluidizer (Rannie®), set at a pressure of 600 PSI, were necessary to produce stable emulsions with polysorbate 60 and poloxamer 407. The compositions of the formulations are listed in Table I.

EMULSION STRUCTURE

Freeze-fracture electron microscopy was used to study aggregate structures in emulsions with the six different surfactants.

The samples were mixed with glycerol (30% of the water content) to prevent ice crystal formation and were rapidly frozen in liquid propane (−196°C). The frozen samples were fractured in a Balzers BAF400 freeze-etching device. Platinium and carbon replicas were made. These solids were evaporated by electron beaguns. Contrast was achieved by depositing the particles of platinium at an angle of 30°. The carbon layer was deposited vertically onto the replica. The replica was transferred from one cleaning solution to another. After being washed and dried on an electron microscope grid, the replica was

Table I
Compositions of the Formulations (%)

	Emulsion						
	1	2	3	4	5	6	Reference
Oily phase							
Octyl methoxycinnamate	5	5	5	5	5	5	0
Caprilic/capric triglycerides	15	15	15	15	15	15	0
Aqueous phase							
Benzophenone-4	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sodium hydroxide	0.3	0.3	0.3	1.3	0.3	0.3	0
Diazolidilyl urea	0.2	0.2	0.2	0.2	0.2	0.2	0
Water	q.s.p. 100	q.s.p. 100	q.s.p. 100	q.s.p. 100	q.s.p. 100	q.s.p. 100	q.s.p. 100
Surfactants							
Polysorbate 60	3	0	0	0	0	0	0
Steareth-2	0	1.5	0	0	0	0	0
Steareth-21	0	1.5	0	0	0	0	0
Poloxamer 407	0	0	3	0	0	0	0
Sorbitan stearate and sucrose cocoate	0	0	0	3	0	0	0
Acrylates/C_{10-30} alkyl acryalte crosspolymer	0	0	0	0	0.5	0	0
Triethanolamine stearate	0	0	0	0	0	3	0

observed with Jeol 100SX operated at 80kV. The main steps of the freeze fracture electron microscopy technique are summarized in Figure 1.

The median sizes of the oily droplets were determined from microscopic negatives. To localize benzophenone-4 in the emulsions, benzophenone-4 was assayed in the whole emulsions and in the aqueous phases obtained after ultracentrifugation. Benzophenone-4 was quantified using reversed-phase HPLC with a column of Novopack C18 at 285 nm. The mobile phase consisted of water with 1% perchloric acid and methanol (6:4).

THE CUTANEOUS MEMBRANE

Benzophenone-4 flux was determinated across ventral skin of male 6–7-week-old hairless rats (Iffa Credo). The skin was always freshly obtained and used full-thickness, with the subcutaneous fat carefully removed.

IN VITRO PERMEATION STUDIES

The *in vitro* drug release experiments were performed with static vertical diffusion cells with an exposure area of 2.54 cm^2. Five tenths of a gram of each emulsion was applied to the rat skin surface. The receiving fluid (9 ml) was phosphate buffer, pH = 7.4, with 5% (w/w) polysorbate 80 to ensure sink conditions and with 0.5% (w/w) of antibiotics (streptomycin 10000 UI/ml and penicillin 10000 µg/ml) to prevent microbiological proliferation. During permeation experiments, the receiver phase was constantly stirred by means of a magnetic bar. The receiver medium was collected over a period of 48 hours. For each system a mean of six cells was calculated.

RESULTS AND DISCUSSION

DETERMINATION OF THE EMULSION STRUCTURE

The freeze-fracture electron microscopy consisted of observing and photographing the metallic replica of the fractured surface of the cryofixed sample. Shadows were cast by any topographical features of the specimen surface, and a three-dimensional impression of the surface was thus created.

For emulsions with polysorbate 60, poloxamer 407, and acrylates/C_{10-30} alkyl acrylate crosspolymer, all the fractured oily droplets and continuous aqueous phases were homogeneous without relief. These three emulsions did not contain any liquid crystal structure (Figure 2).

Micrographs of emulsions with triethanolamine stearate, sorbitan stearate and sucrose cocoate, and steareth-2/-21 clearly showed lamellar liquid crystals in the aqueous phase and vesicles with relief, which were oily droplets surrounded by one or more lamellar bilayers (Figure 3). Table II summarizes the emulsion structure, the medium size of oily droplets, and the distribution of benzophenone-4. The relatively amphiphilic character of benzophenone-4 may predispose it to associate with surfactant structures.

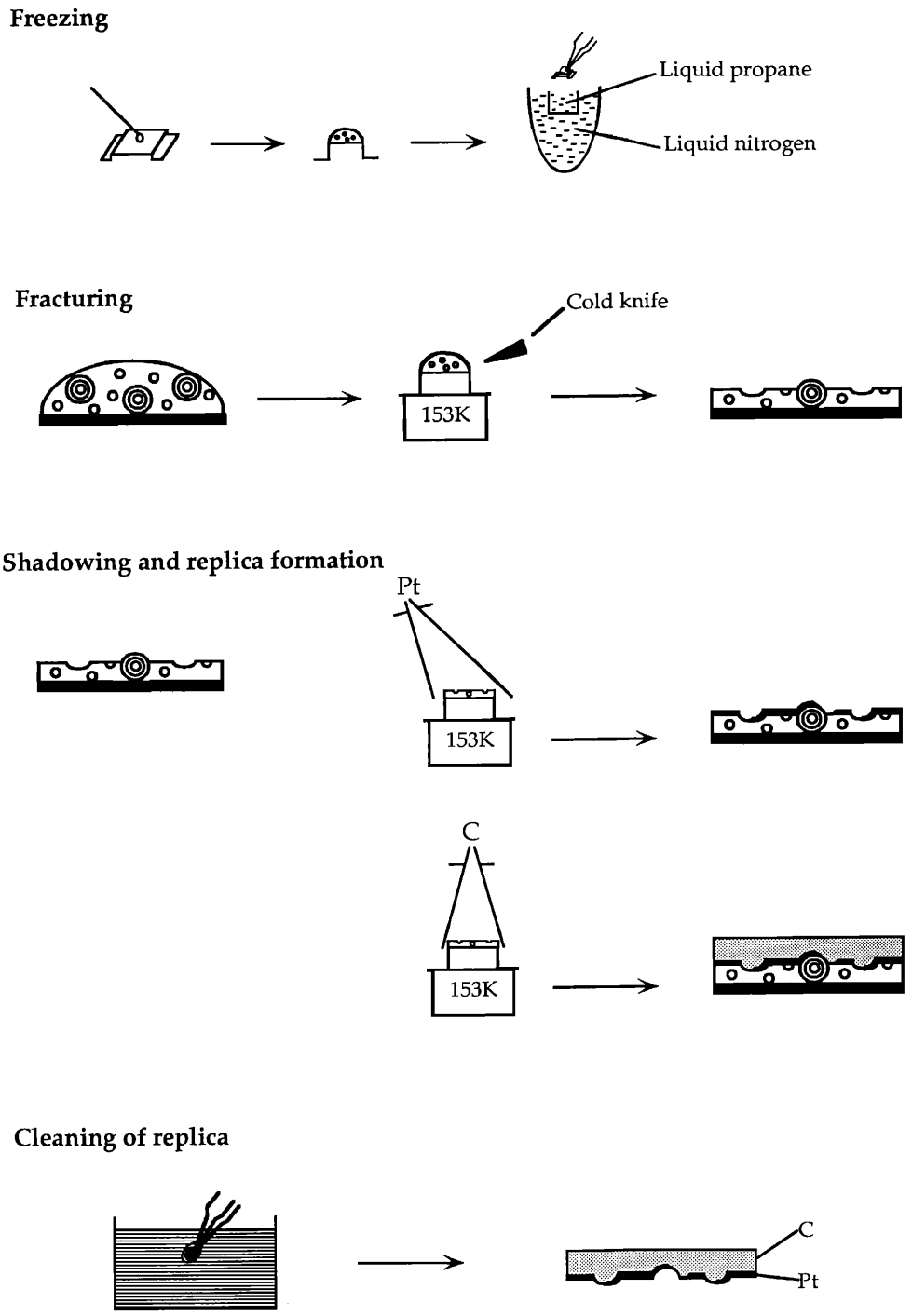

Figure 1. The main steps of the freeze-fracture electron microscopy technique.

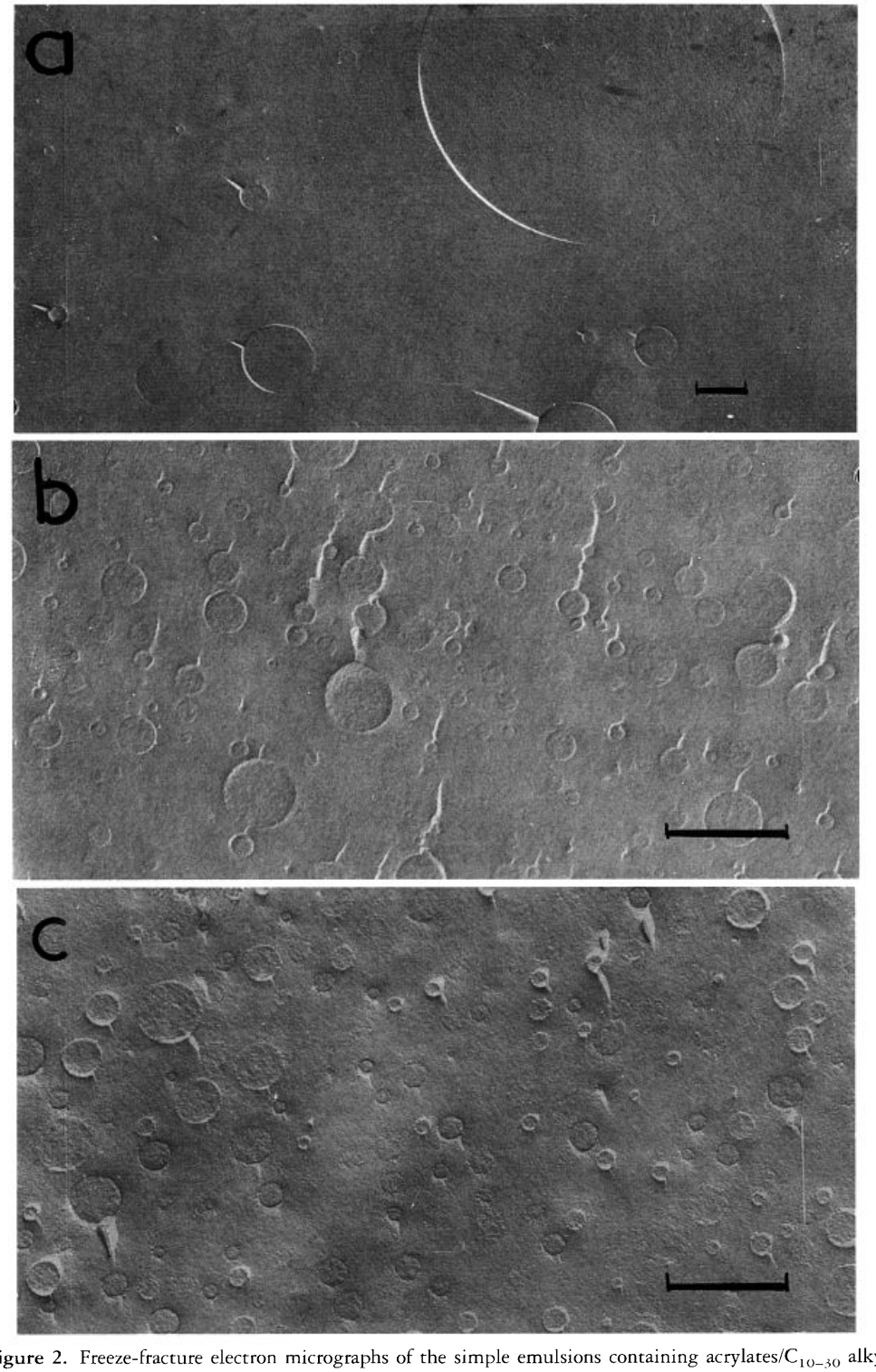

Figure 2. Freeze-fracture electron micrographs of the simple emulsions containing acrylates/C_{10-30} alkyl acrylate crosspolymer (a), polysorbate 60 (b), and poloxamer 407 (c). The scale bar indicates 0.5 μm.

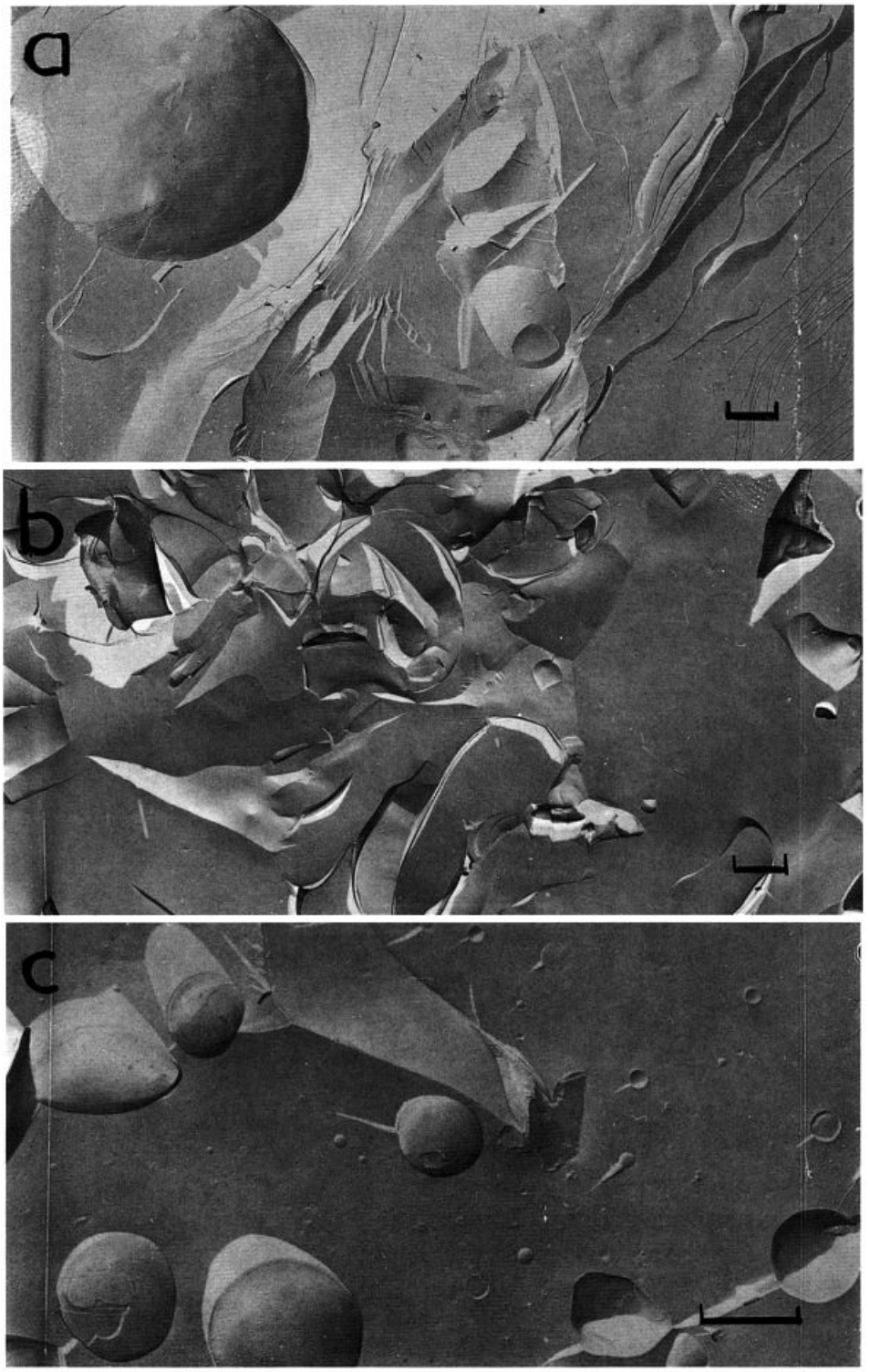

Figure 3. Freeze-fracture electron micrographs of the structured emulsions containing steareth-2/-21 (a), sorbitan stearate and sucrose cocoate (b), and triethanolamine stearate (c). The scale bar indicates 1 μm.

Table II
The Structural Properties of the Different Emulsions

Surfactants	Presence of lamellar liquid crystals	Medium-size oily droplets (μm)	Benzophenone-4 partition between oil/water (%) (w/w)
Polysorbate 60	No	0.1 ± 0.1	21/79
Poloxamer 407	No	0.1 ± 0.2	8/92
Acrylates/C_{10-30} alkyl acryalte crosspolymer	No	3.1 ± 4.6	28/72
Sorbitan stearate and sucrose cocoate	Yes	10.1 ± 11.3	21/79
Steareth-2/-21	Yes	6.4 ± 9.4	25/75
Triethanolamine stearate	Yes	0.4 ± 0.3	11/89
Aqueous reference	No		—/100

IN VITRO PERMEATION

As benzophenone-4 is a predominantly hydrophilic drug, its permeation from the continuous aqueous phase of emulsion was compared with permeation from an aqueous solution.

Figure 4 represents the *in vitro* permeation profiles of benzophenone-4 from the six emulsions studied and from an aqueous solution as reference (water with 2.5% benzophenone-4). The corresponding steady-state fluxes and the amounts of benzophenone-4 that diffused after 48 h are summarized in Table III.

Permeation from the emulsion with anionic surfactant (triethanolamine stearate) was much higher than that from those with nonionic surfactants (the five other surfactants used). This result is in agreement with results concerning anionic and nonionic enhancer permeation effects (20–21).

According to the differences obtained between the emulsions and the aqueous solution, the surfactants seemed to have influenced benzophenone-4 transport from the vehicle to the receiver medium. Moreover, the benzophenone-4 fluxes were the smallest for emulsions without liquid crystals (termed simple emulsions) and the highest for those with liquid crystals (called structured emulsions), and thus the permeant fluxes also seemed to be modified by surfactant organization in emulsions.

The overall effect of surfactant on membrane permeability is the result of two opposing events (22–23): on one hand, interaction with cutaneous membrane enhances the permeation, whereas, on the other hand, association of the permeant molecules with the surfactant into micelle-like structures increases permeant solubility in the vehicle, and so its partition coefficient towards the skin is reduced and permeability decreases (21,24–26).

The flux from the simple emulsion with poloxamer 407 was significantly lower than that of the aqueous reference (Student's t-test, $p = 0.05\%$). For emulsions containing poloxamer 407, an interaction of benzophenone-4 with surfactant micelles could be preponderant over a possible interaction of the surfactant with the skin, and this could lead to a decrease in benzophenone-4 permeation.

The fluxes from the simple emulsions with polysorbate 60 and with acrylates/C_{10-30} alkyl acrylate crosspolymer were smaller than that of the aqueous reference, whereas the flux from structured emulsions with sorbitan stearate and sucrose cocoate was higher,

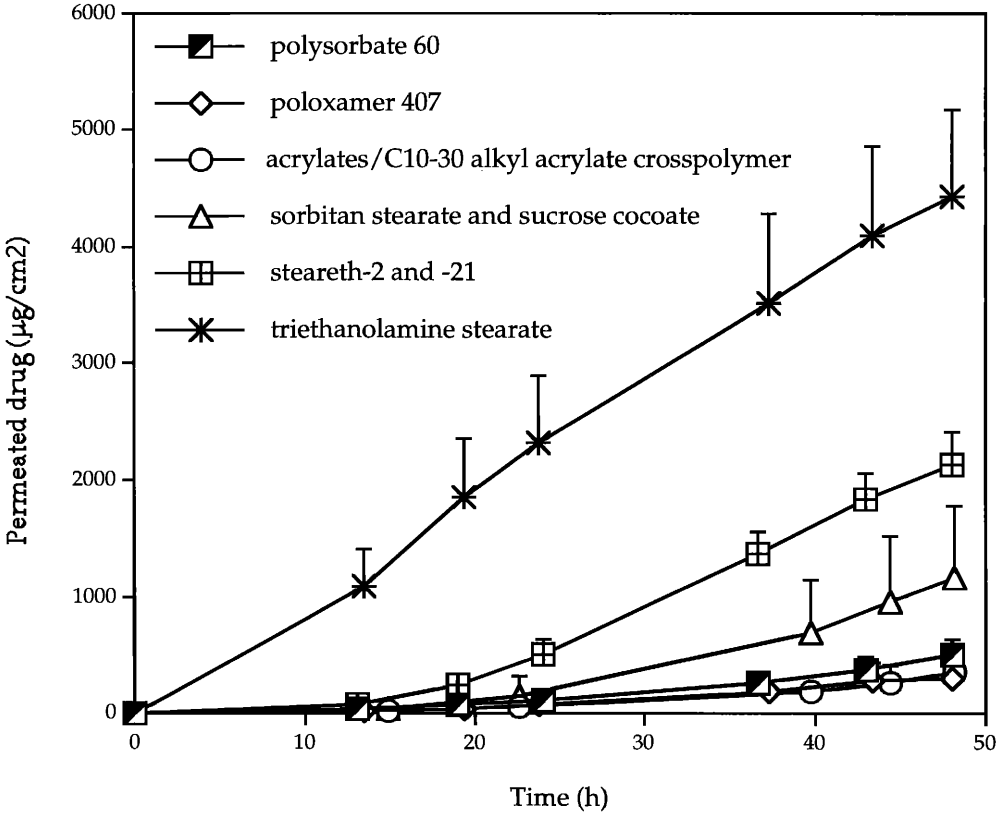

Figure 4. Transcutaneous permeation profiles of benzophenone-4 from the six emulsions and from the reference.

Table III
Transcutaneous Permeation Parameters for Benzophenone-4

	Benzophenone-4 flux ($\mu g/cm^2/h$)	Benzophenone-4 diffused amount ($\mu g/cm^2$)
Emulsions		
Polysorbate 60	21 ± 7	504 ± 138
Poloxamer 407	10 ± 6	317 ± 156
Acrylates/C_{10-30} alkyl acrylate crosspolymer	19 ± 13	367 ± 200
Sorbitan stearate and sucrose cocoate	54 ± 24	1155 ± 621
Steareth-2/-21	67 ± 12	2140 ± 268
Triethanolamine sterate	86 ± 7	4433 ± 744
Reference		
Aqueous solution	32 ± 24	602 ± 357

but these differences were not significant (t-test, p = 0.05%). For emulsions containing polysorbate 60 or acrylates/C_{10-30} alkyl acrylate crosspolymer or sorbitan stearate and sucrose cocoate, a possible interaction of benzophenone-4 with surfactant organization could partially counterbalance a possible interaction of the surfactant with the membrane, resulting in slight modifications of benzophenone-4 permeation.

The fluxes from the structured emulsions with steareth-2/-21 or triethanolamine stearate were significantly higher than that of the aqueous reference (t-test, p = 0.05%).

The main barrier for the percutaneous absorption of almost all compounds is the stratum corneum, which is composed of keratinized cells embedded in lamellar lipid layers (27). Most intercellular lipids are in a gelified crystalline state, but some of them may be in a liquid crystalline state (28–30). It is generally accepted that these intercellular domains constitute the primary route for the passive permeation of many molecules through the skin. The effective barrier property of the stratum corneum intercellular lamellae has been attributed to the highly ordered bilayer structures in the intercellular spaces.

One could hypothesize that surfactants that have formed liquid crystals in the emulsions, diffusing as single molecules through the intercellular lipid lamellae, are able to preferentially transform the gelified crystalline lipid packing into the liquid crystalline lipid packing and create a more fluid, permeable, membrane.

French *et al.* (31) have shown that incorporation of surfactants (dodecyl ether ethoxylate type) into multilamellar vesicles of distearylphosphatidylcholine (model of stratum corneum lipids) disrupted the highly ordered packing of the lipid chains, causing increased fluidity within lipid bilayers.

For the aqueous solution and for simple emulsions, benzophenone-4 partitioned between the stratum corneum and water, in which the diffusible permeant concentration can be modified by micellar solubilization. For structured emulsions, the case could be more complex. Indeed, it has been demonstrated that when some suspensions of nonionic vesicles (32) or liposomes (33) (e.g., an aqueous compartment surrounded by lamellar layers) were put on the skin, vesicles aggregated and fused, thus depositing lamellar structures on the surface of the stratum corneum. If a similar phenomenon occurred with an oily droplet surrounded by lamellar layers or with lamellar layers in the aqueous phase, benzophenone-4 partitioning between water and stratum corneum could be replaced by partitioning between lamellar liquid crystals and stratum corneum. If the partition between stratum corneum and lamellar liquid crystals were more favorable to the stratum corneum than water, permeation could be enhanced.

REFERENCES

(1) S. Friberg, Three-phase emulsions, *J. Soc. Cosmet. Chem.*, **30**, 309–319 (1979).
(2) G. Couarraze and J. Wepierre, "Topical Applications of Liposomes," in *Liposomes, New Systems and New Trends in their Applications,* F. Puisieux, P. Couvreur, J. Delattre, and J. P. Devissaguet, Eds. (Editions de santé, Paris, 1995), pp. 615–644.
(3) H. Komatsu, H. Okamoto, K. Miyagawa, M. Hashida, and H. Sezaki, Percutaneous absorption of butylparaben from liposomes *in vitro, Chem. Pharm. Bull.*, **34**, 3423–3430 (1986).
(4) A. Vermoken, M. Hukkelhoven, A. Vermeesch-Markslag, C. Goos, P. Wirtz, and J. Ziegenmeyers, The use of liposomes in the topical application of steroids, *J. Pharm. Pharmacol.*, **36**, 334–336 (1984).
(5) M. Mezei and V. Gulasekharam, Liposomes, a selective drug delivery system for the topical route of administration, (2) Gel dosage form, *J. Pharm. Pharmacol.*, **34**, 473–747 (1982).
(6) C. B. Lalor, G. L. Flynn, and N. Weiner, Formulation factors affecting release of drugs from topical vehicles. II. Effect of solubilities on *in vitro* delivery of a series of n-alkyl p-aminobenzoates, *J. Pharm. Sci.*, **84**, 673–676 (1995).
(7) P. Ashton, K. A. Walters, K. R. Brain, and J. Hadgraft, Surfactant effects in percutaneous absorption. I. Effects on transdermal flux of methyl nicotinate, *Int. J. Pharm.*, **87**, 261–264 (1992).

(8) M. J. Cappel and J. Kreuter, Effect of nonionic surfactants on transdermal drug delivery: I. Polysorbates, *Int. J. Pharm.,* **69,** 143–153 (1991).
(9) K. A. Walters, M. Walker, and O. Olejnik, Non-ionic surfactant effects in hairless mouse skin permeability characteristics, *J. Pharm. Pharmacol.,* **40,** 525–529 (1988).
(10) Z. T. Chowhan and R. Pritchard, Effect of surfactants on percutaneous absorption of naproxen. I. Comparisons of rabbit, rat, and human excised skin, *J. Pharm. Sci.,* **67,** 1272–1274 (1978).
(11) W. W. Shen, A. G. Danti, and F. N. Bruscato, Effect of nonionic surfactants on percutaneous absorption of salicylic acid and sodium salicylate in the presence of dimethyl sulfoxide, *J. Pharm. Sci.,* **65,** 1780–1783 (1976).
(12) H. G. Ibrahim, Release study from lyotropic liquid crystal systems, *J. Pharm. Sci.,* **78,** 683–687 (1989).
(13) H. L. G. M. Tiemessen, H. E. Bodde, C. VanMourik, and H. E. Junginger, *In vitro* drug release from liquid crystalline creams: Cream structure dependence, *Progr. Colloid. Polymer Sci.,* **77,** 131–135 (1988).
(14) C. C. Muller-Goymann and S. G. Frank, Interaction of lidocaine-HCl with the liquid crystal structure of topical preparations, *Int. J. Pharm.,* **29,** 147–159 (1986).
(15) S. Wahlgren, A. L. Linsdtrom, and S. E. Friberg, Liquid crystals as a potential ointment vehicle, *J. Pharm. Sci.,* **73,** 1484–1486 (1984).
(16) K. Kriwet and C. C. Muller-Goymann, Diclofenac release from phospholipid drug systems and permeation through excised human stratum corneum, *Int. J. Pharm.,* **125,** 231–242 (1995).
(17) I. L. Wilisch and C. C. Muller-Goymann, Correlation of colloidal microstructure, drug release and permeation through excised human skin, *Int. J. Pharm.,* **96,** 79–84 (1993).
(18) J. Swarbrick and J. Siverly, The influence of liquid crystalline phases on percutaneous drugs. II. Percutaneous studies through excised human skin, *Pharm. Res.,* **9,** 1550–1555 (1992).
(19) D. W. Osborne, A. J. I. Ward, and K. J. O'Neill, "Surfactant Association Colloids as Topical Drug Delivery Vehicles," in *Topical Drug Delivery Formulations,* D. W. Osborne and A. H. Amann, Eds. (Marcel Dekker, New York, 1990), pp. 349–379.
(20) S. B. Ruddy, "Surfactants," in *Percutaneous Penetration Enhancers,* E. W. Smith and H. I. Maibach, Eds. (CRC Press, Boca Raton, FL, 1995), pp. 245–257.
(21) P. Ashton, J. Hadgraft, and K. A. Walters, Effects of surfactants in percutaneous absorption, *Pharm. Acta Helv.,* **61,** 228–235 (1986).
(22) K. A. Walters, "Surfactants and Percutaneous Absorption," in *Prediction of Percutaneous Penetration,* R. C. Scott, R. H. Guy and J. Hadgraft, Eds. (IBC Technical Services, London, 1989) pp. 148–162.
(23) D. Atwood, A. T. Florence, *Surfactants Systems* (Chapman and Hall, London, 1983) pp. 124–228.
(24) P. P. Sarpotdar and J. L. Zatz, Evaluation of penetration enhancement of lidocaine by nonionic surfactants through hairless mouse skin *in vitro, J. Pharm. Sci.,* **75,** 176–181 (1986).
(25) M. Loden, The simultaneous penetration of water and sodium lauryl sulfate through isolated human skin, *J. Soc. Cosmet. Chem.,* **41,** 227–233 (1990).
(26) J. L. Zatz, "Modification of Skin Permeation by Surface-Active Agents," in *Skin Permeation: Fundamentals and Application,* J. L. Zatz, Ed. (Allured Publishing Corp., Wheaton, IL, 1993), pp. 149–162.
(27) P. M. Elias, Epidermal lipids, membranes, and keratinization, *Int. J. Dermatol.,* **20,** 1–19 (1981).
(28) B. Ongpipattanakul, M. L. Francoeur, and R. O. Potts, Polymorphism in stratum corneum, *Biochim. Biophys. Acta,* **1190,** 122–155 (1994).
(29) D. T. Parrott and J. E. Turner, Mesophase formation by ceramides and cholesterol: A model for stratum corneum lipid packing?, *Biochim. Biophys. Acta,* **1147,** 273–276 (1993).
(30) S. J. Rehfeld, W. Z. Plachy, S. Y. E. Hou, and P. M. Elias, Localization of lipid microdomains and thermal phenomena in murine stratum corneum and isolated membrane complexes: An electron spin resonance study, *J. Invest. Dermatol.,* **95,** 217–223 (1990).
(31) E. J. French, C. W. Pouton, and G. Steele, "Fluidization of Lipid Bilayers by Non-Ionic Surfactants: Structure-Activity Studies Using a Fluorescent Probe," in *Prediction of Percutaneous Penetration,* R. C. Scott, R. H. Guy, J. Hadgraft, Eds. (IBC Technical Services, London, 1989), pp. 308–315.
(32) H. E. J. Hofland, J. A. Bouwstra, F. Spies, H. E. Bodde, J. F. Nagelkerke, C. Cullander, and H. E. Junginger, Interactions between non-ionic surfactant vesicles and human stratum corneum *in vitro, J. Liposome Res.,* **5,** 241–263 (1995).
(33) J. Lasch and J. Bouwstra, Interactions of external lipids (lipid vesicles) with the skin, *J. Liposome Res.,* **5,** 543–569 (1995).