Effects of Sucrose Oleate and Sucrose Laureate on *in Vivo* Human Stratum Corneum Permeability

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Purpose. The purpose of this work was to 1) investigate the effect of sucrose esters (sucrose oleate and sucrose laureate in water or in Transcutol®, TC) on the stratum corneum (SC) barrier properties *in vivo* and 2) examine the impact of these surfactant-like molecules on the *in vivo* percutaneous penetration of a model penetrant 4-hydroxybenzonitrile (4-HB).

Methods. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy and transepidermal water loss measurements were used to evaluate the sucrose oleate- and sucrose laureate-induced biophysical changes in SC barrier function *in vivo*. In addition, the effect of the enhancers on 4-HB penetration was monitored *in vivo* using ATR-FTIR spectroscopy in conjunction with tape-stripping of the treated site.

Results. Treatment of the skin with 2% sucrose laureate or sucrose oleate in TC significantly increased the extent of 4-HB penetration relative to the control. Furthermore, when skin treated with these formulations was examined spectroscopically, the C-H asymmetric and symmetric stretching bands of the lipid methylene groups were characterized by 1) decreased absorbances and 2) frequency shifts to higher wavenumbers. These effects on the SC lipids and 4-HB penetration were more pronounced for sucrose laureate when combined with TC.

Conclusions. A combination of sucrose esters (oleate or laureate) and TC is able to temporally alter the stratum corneum barrier properties, thereby promoting 4-HB penetration. These molecules are worthy of further investigation as potential candidates for inclusion in transdermal formulations as penetration enhancers.

KEY WORDS: skin penetration; penetration enhancement; sucrose esters; ATR-FTIR; transepidermal water loss.

INTRODUCTION

Among the most commonly studied enhancers for skin application are surfactants and bile salts. Typically, both cationic and anionic surfactants are more potent enhancers than their nonionic counterparts, but they are also more toxic. Nonionic surfactants, which are less irritating, have been shown to increase the absorption of numerous drugs (1–6) and are widely used in dermatological products and cosmetics.

Surfactants have been shown to influence skin permeability in a number of ways. Many surfactants penetrate into the skin and act directly on skin components, sometimes inducing a loss of membrane integrity, e.g., by lipid or protein extraction or by protein denaturation. The penetration-enhancing ability of a surfactant is dependent not only on its interaction with membrane components but also on its concentration (7,8). At concentrations below the critical micelle concentration (CMC), higher levels of surfactant monomers are available to penetrate the skin and increase permeability (2,7). In addition, if the drug is solubilized by the surfactant micelle, transport of the active species may be reduced as a result of an unfavorable micelle/vehicle partition coefficient and lower thermodynamic activity. The nature of the vehicle also plays an important role in the interaction between the surfactant, the drug and the skin. Not only may the presence of a cosolvent change the solubility of a solute, altering its activity and consequently the skin/vehicle partition coefficient (1), but it may also promote the absorption of a surfactant into the skin (2), favoring the interaction of the surfactant with the stratum corneum lipids. Sarpotdar and Zatz (2,3) demonstrated that the effect of polysorbates on lidocaine and hydrocortisone penetration through the skin, was significantly enhanced when the content of propylene glycol was increased.

Structural parameters, such as chain length, degree, and position of unsaturation, and the nature of substituents can influence the ability of surfactants to act as skin penetration enhancers. It is well accepted that a linear alkyl chain of 12 carbon atoms maximizes the effect of a surfactant on membrane permeability. The C_{12} chain has an intermediate oil/water solubility and is able to penetrate the lipid bilayer (1,6,9). Among a series of polyoxyethylene ethers, the lauryl (C_{12}) ether was reported to be the most effective enhancer for ibuprofen, followed by oleyl (C_{18}) ether (6). Although saturated C_{18} fatty acid analogues (e.g., stearic acid) have been shown to be ineffective, numerous reports agree on the disrupting effect of oleic acid, a monounsaturated C_{18} fatty acid, particularly on the stratum corneum lipid domains (7,10–13). It has been proposed that oleic acid induces phase separation, thus reducing membrane resistance (14,15).

Sucrose fatty acid esters (SE), which are nonionic surfactants with a sucrose substituent as the polar head group (Fig. 1), have also recently been investigated as enhancers. For a series of SE tested, only the sucrose laureate was found to increase the passage of lidocaine hydrochloride through buccal and palatal mucosae (16). SE are nontoxic and biodegradable surfactants approved by FAO/WHO as food additives (5). Because they are nonirritants to skin, they are suitable not only for foods but also for therapeutic and cosmetic applications (5,17). Their application in dermatology has been further accentuated by the ability of some SE, such as laurate and ricinoleate, to form liquid crystals and microemulsions (5,18,19).

Biophysical evidence on the extent and duration of the effect of an enhancer can be provided through measurements of transepidermal water loss (TEWL) and attenuated total

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reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. TEWL has been shown to be useful in assessing macroscopic changes in the functional state of the skin barrier and contributes to the prediction of percutaneous penetration (20,21). However, ATR-FTIR allows conformational changes in stratum corneum lipid and protein domains to be detected, and has proved to be a useful tool for monitoring the percutaneous penetration of drugs or other constituents such as cosolvents and enhancers (11,12,21–24). TEWL and ATR-FTIR share the advantage of being noninvasive and enabling experiments to be conducted *in vivo*, in human subjects.

The aim of this study was to evaluate in human skin *in vivo* 1) the effect of two SEs, sucrose oleate and sucrose laureate, on skin barrier function and 2) to examine the impact of these nonionic surfactants on the percutaneous penetration of 4-hydroxybenzonitrile (a model penetrant), which was selected on the basis of its intense C≡N stretching absorbance at 2230 cm^{-1}, permitting it to be monitored by ATR-FTIR spectroscopy.

MATERIALS AND METHODS

4-hydroxybenzonitrile (4-HB) was purchased from Aldrich (St. Louis, MO, USA). Transcutol® (TC) was a gift from Gattefossé (Saint-Priest, France). Sucrose oleate (O, Ryoto Sugar Ester® O-1570) and sucrose laureate (L, Ryoto Sugar Ester® L-1695) were generously donated by Selectchemie AG (Zürich, Switzerland). Scotch® Book Tape 845 was purchased from 3M (St. Paul, MN, USA). Aqueous solutions were prepared using deionized water supplied by a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA).

Treatment of the Skin

Six healthy adults (female, aged 20–30 years) who were receiving no medication and had no history of skin disease participated in the study (approved by the local ethics committee) after giving their written consent. During the study, volunteers were required to maintain the experimental skin sites on the midventral forearm free from application of any skin care products. The skin sites were gently cleansed with water and dried using a gauze pad. Immediately thereafter, the subjects were dosed topically with the following solutions: 2% w/v or 10% w/v of O or L in water or in TC. Transcutol, a monoethyl ether of diethylene glycol (Fig. 1), was chosen because of its well-known ability to increase drug solubility in the skin (25). A volume of 1.5 mL of the formulations was applied via filter paper (11.5 × 4.5 cm), which was affixed to the skin with an occlusive film. At the end of the application period (1 h), the filter paper was removed and the skin surface was wiped clean with a water-soaked cotton ball and dried gently with a gauze pad. The application site was delineated with a felt-tip marker to allow repositioning of the area for each measurement. Each subject was treated with the complete array of formulations. ATR-FTIR spectra and TEWL measures were recorded before treatment, post-treatment (approx. 5 min after the cleansing) and then periodically (each hour) over the next 4 h.

Effect of SE on the *in Vivo* Penetration of 4-HB

In a separate series of experiments, the application site was pretreated topically with either the L or O solutions, in water or in TC (2 and 10% w/v), for 1 h (following the procedure described above). After this pretreatment, the skin surface was wiped clean with a water-soaked gauze pad and 1.5 mL of a saturated aqueous solution of 4-HB was applied to the skin via a segment of filter paper (11.5 × 4.5 cm) attached with an occlusive film. After 1 h, the skin surface was cleansed with water and dried. To determine the distribution of 4-HB across the stratum corneum, IR spectra of the dosed site were recorded and 16 sequential tape-strippings were obtained using Scotch® Book Tape No. 845 (3M). An infrared spectrum was obtained for each tape. The amount of 4-HB in each tape-strip was quantified spectroscopically by virtue of the intense C≡N stretching absorbance of 4-HB at 2230 cm^{-1}. A calibration curve was constructed by adding known amounts of 4-HB to tapes (with stratum corneum) before recording a spectrum. Each tape was analyzed 10 times. The penetration depth of 4-HB was calculated assuming a constant tape-stripped area and a density of approximately 1 $g \cdot cm^{-3}$ for the stripped stratum corneum. In this way, the mass of stratum corneum removed (determined by weighing the tapes before and after stripping), was converted to the depth of SC sampled (mass of SC = area × depth × density).

ATR-FTIR Spectroscopy

FTIR spectra were recorded using a Shimadzu FTIR-8300 spectrophotometer (Kyoto, Japan) equipped with a trapezoidal ATR crystal (80 × 10 × 4 mm, 45° bevel). Each measurement represented an average of 45 scans with a resolution of 1 cm^{-1}. To determine the effect of the SE, the frequencies and intensities of the peaks assigned to the $C\text{-}H_2$ symmetric and asymmetric stretching vibrations of the stratum corneum lipid alkyl chains (approx. 2850 cm^{-1} and 2920 cm^{-1}, respectively) were examined in the acquired spectra.

TEWL Measurements

TEWL was measured with a Tewameter TM 210 (Courage+Khazaka electronic GmbH, Cologne, Germany), with a resolution of 0.1 $g/h \cdot m^2$, in accordance with recommended guidelines (26). Measurements were carried out in a ventilated Plexiglas chamber, and volunteers remained undisturbed and relaxed during the experiment to avoid erratic fluctuations in TEWL.

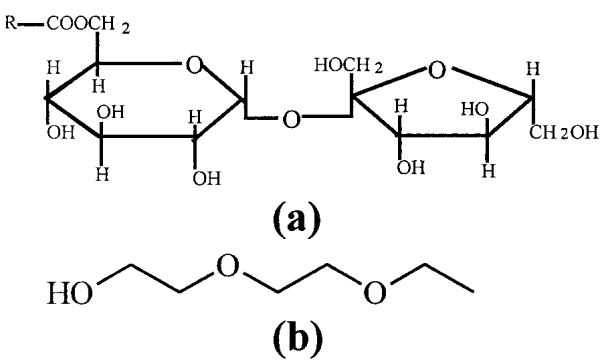

Fig. 1. Chemical structures of (a) sucrose monoester [R = fatty acid] and (b) Transcutol (diethylene glycol monoethyl ether).

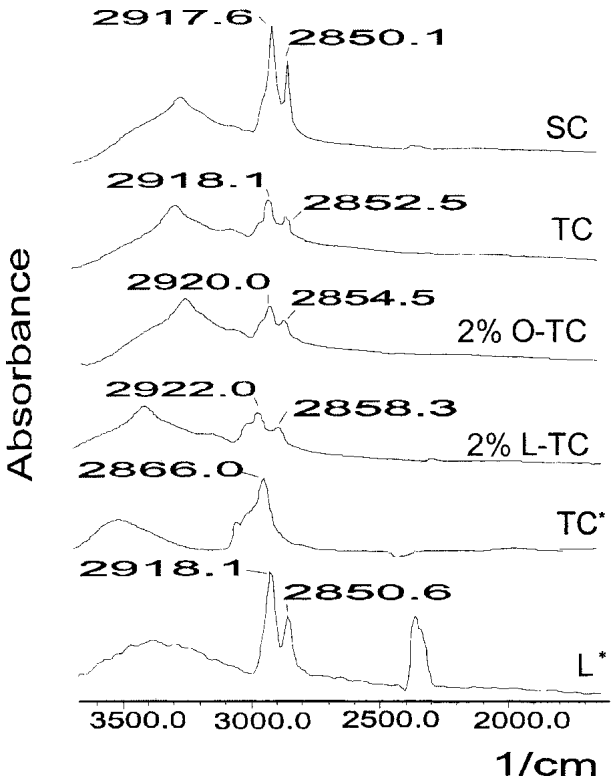

Fig. 2. Representative ATR-FTIR spectra in the spectral range 3500–2000 cm^{-1} for skin treated with Transcutol (TC), 2% O-TC, and 2% L-TC. For comparison, the spectra of untreated stratum corneum (SC), neat TC (TC*) and neat sucrose laureate (L*) are also shown. The IR spectrum of sucrose oleate (not shown) is very similar to that of sucrose laureate.

Surface Tension Measurements

The surface tension of the solutions were determined using the Wihelmy-Noüy method. Distilled water was used as a reference standard.

Statistics

The results are expressed as the mean ± standard deviation (SD). Student's *t* test or analysis of variance was performed to test the level of significance. Duncan's procedure for the separation of means was used to locate the source of the difference when a significant *F* value was found.

RESULTS AND DISCUSSION

As discussed earlier, surfactants may enhance drug transport by a number of mechanisms. First, they may partition into the intercellular domains and possibly increase fluidity and/or extract barrier lipids. Second, they may penetrate into the corneocytes and disrupt the keratin filament network, thereby rendering the corneocytes more permeable (6). In the case of SE, the potential exists for their long hydrocarbon chain (Fig. 1) to be inserted between the lipophilic tails of the bilayer, allowing the sucrose ring to interact with the polar head groups of the lipids. To probe the effect of SE on the barrier properties of the stratum corneum, treated SC was examined *in vivo* by ATR-FTIR. Of particular interest in lipid studies are the IR absorbances originating from the $C\text{-}H_2$ symmetric and asymmetric stretching vibrations of the stratum corneum lipid alkyl chains (approx. 2850 cm^{-1} and 2920 cm^{-1}, respectively). The ATR-FTIR spectra of the stratum corneum treated for 1 h with 2% SE in Transcutol are illustrated in Fig. 2. As seen in the spectra, treatment with 2% L-TC and 2% O-TC resulted in a significant decrease in the area and height of these peaks. Indeed, subsequent to treatment with 2% L-TC, the lipid absorbances almost entirely disappear. This observation suggests that the 2% SE-TC formulations are able to interact with the lipid structures, resulting in lipid dissolution and extraction from the SC (21). In contrast, when the skin was treated with aqueous or TC formulations containing 10% SE, this phenomenon was not observed (data not shown). Although treatment with TC alone showed a decrease in the height of these peaks (Fig. 2), the effect was not as marked as with the 2% SE-TC solutions.

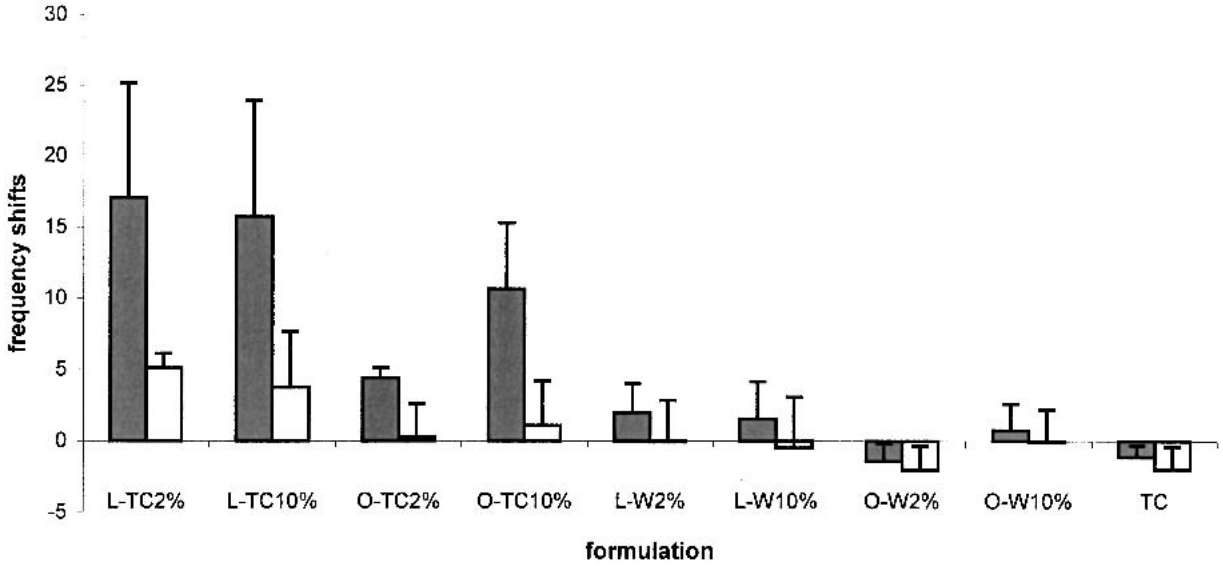

Fig. 3. Frequency shifts of the $C\text{-}H_2$ symmetric (■) and asymmetric (□) stretching vibrations of the stratum corneum lipid acyl chains (n = 6 ± SD). L, laureate; O, oleate; TC, Transcutol; W, water.

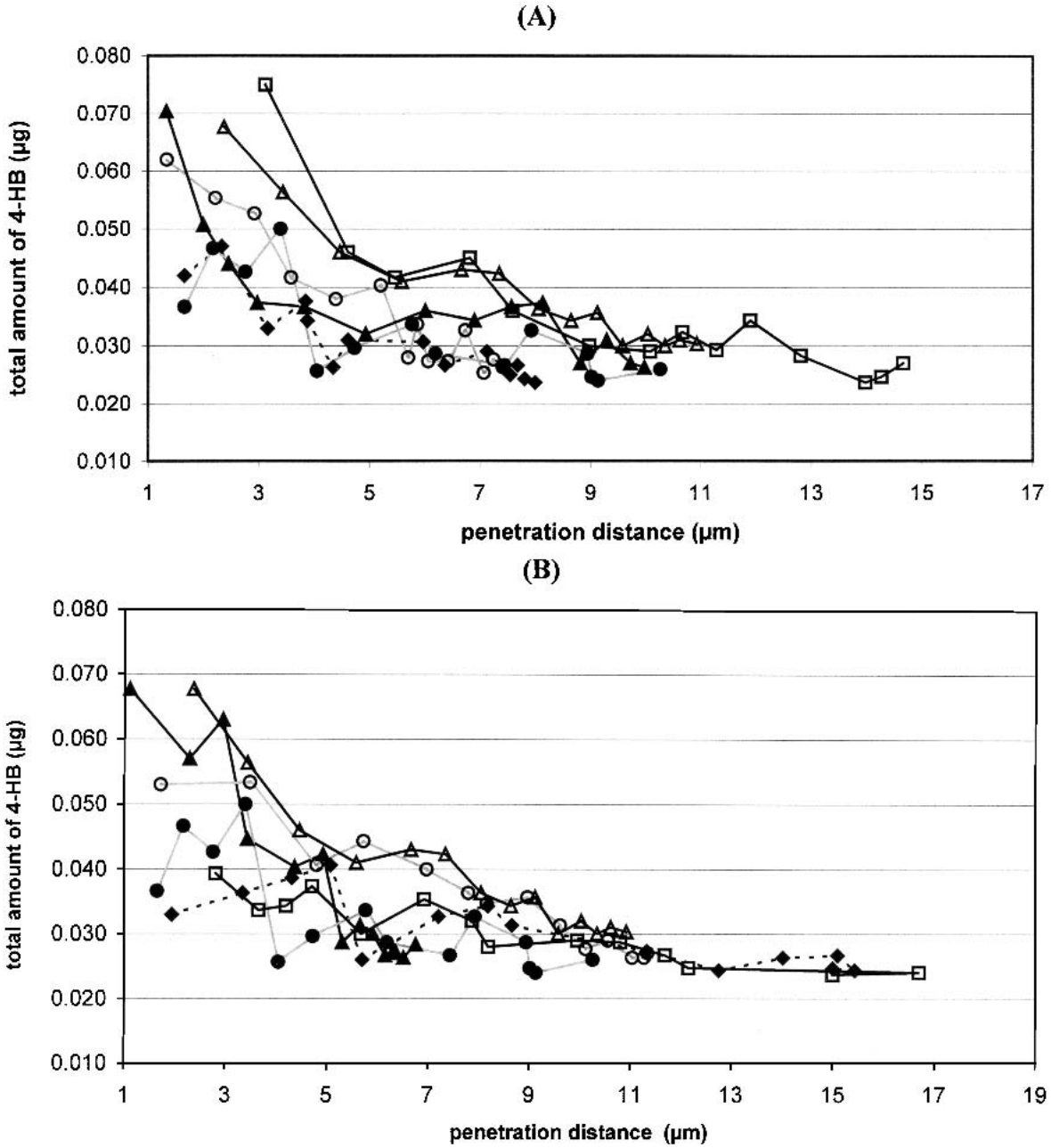

Fig. 4. Distribution profiles of 4-HB as a function of SC depth after treatment with either (A) oleate or (B) laureate: 2% in water (○) or in Transcutol (□); 10% in water (▲) or in Transcutol (♦). Transcutol (△); control (•).

Furthermore, treatment with SE-TC formulations caused the C-H_2 symmetric and asymmetric stretching vibrations to shift toward higher wavenumbers, relative to the untreated stratum corneum (Fig. 3). Treatment with 2% L-TC produced a marked shift of the asymmetric stretching frequency compared to the control ($t = 3.876$; $t_{0.05/2,5} = 2.57$), which was not mirrored by the aqueous solutions or TC alone. However, the C-H_2 symmetric stretching frequency demonstrated a blue shift with all the SE-TC solutions, but as shown in Fig. 3, the shift was greater with 2 and 10% L-TC solutions. Indeed, analysis of variance and Duncan's procedure revealed significant differences ($p < 0.05$) between 2 and 10% L-TC formulations when compared with the control, the aqueous solutions, TC alone, and the O-TC formulations. No significant differences were found when comparisons were made between the control, aqueous solutions, TC, and 2 and 10% O-TC formulations. In some instances, treatment with SE-TC provoked the appearance of a shoulder on the symmetric stretching band at 2850 cm^{-1}, which was probably associated with the incorporation of the SE into the stratum corneum. Although the methylene stretching absorbances of the formulation components themselves are expected to contribute to the total absorbance, and consequently to the measured frequency shifts (particularly to higher wavenumbers), these contributions may be assumed to be negligible based on two observations. First, the C-H_2 stretching frequencies of the excipients are not greater than those of the SC lipids, and second, given that SE solutions of higher concentrations

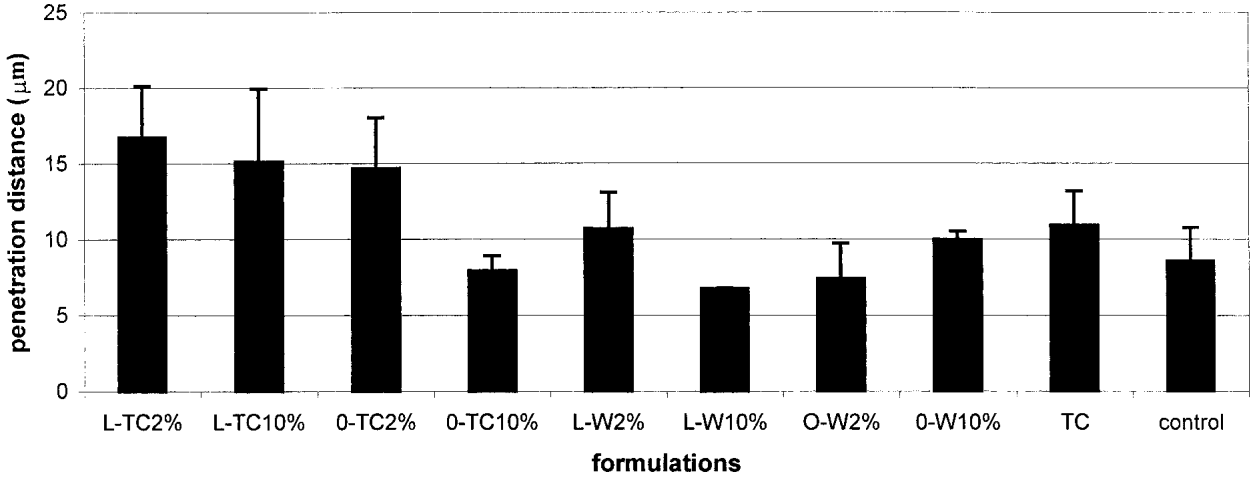

Fig. 5. Effect of SE on the penetration distance of 4-HB (n = 3 ± SD). L, laureate; O, oleate; TC, Transcutol; W, water.

(10% w/v) did not induce a hypsochromic shift, it is extremely unlikely that the marked response at lower concentrations (2% w/v) originates from the intrinsic absorbances of the excipients.

The temporal nature of these frequency shifts was monitored by recording spectra hourly over the subsequent four hours. The results demonstrated that the frequency shifts were reversible, with peaks returning to their original values after 3 h. It has been reported that shifts in these frequencies reflect increased lipid fluidity and an increase in the proportion of *gauche* conformers along the lipid hydrocarbon chains (27).

TEWL increased transiently immediately after removal of the protective film at the end of the application period, an observation which can be attributed to the evaporation of water after occlusion. However, none of the formulations caused a sustained increase in water loss, and no significant difference was found between the control value and that subsequent to treatment with any of the formulations (approx. 9–13 $1/h \cdot m^2$). Despite the alterations observed in the FTIR spectra, TEWL remained unaltered for all the tested formulations.

One objective of our study was to investigate the relationship between the degree of stratum corneum disruption induced by the SE and permeant (4-HB) penetration. 4-HB distribution profiles as a function of stratum corneum depth are depicted in Fig. 4. As shown, the depth of 4-HB penetration was modest when delivered from aqueous media. However, combination of TC and SE resulted in an increased extent of penetration, particularly at a concentration of 2%. Figure 5 presents the total penetration distance of 4-HB for the skin treated with SE in aqueous solutions and in TC. Analysis of variance of these results followed by Duncan's test revealed significant differences between 2% L-TC and the control ($F = 5.33$; $F_{0.05/2;9,20} = 2.39$). These findings are consistent with the IR spectroscopic data presented earlier, which did not indicate SC lipid modification when the skin was treated with aqueous SE formulations, but a distinct lipid fluidization in the presence of SE-TC. These results are also consistent with previous reports demonstrating that skin penetration was significantly enhanced from solvent-water mixtures (e.g., mixtures of propylene glycol (2,3), isopropyl alcohol (28)) containing a high proportion of solvent. Ganem-Quintanar *et al.* (25) have previously demonstrated that TC diffuses through the stratum corneum, establishing a constant flux within two hours. The synergistic enhancement observed between SE and TC can therefore probably be attributed to the facilitated surfactant absorption by the stratum corneum

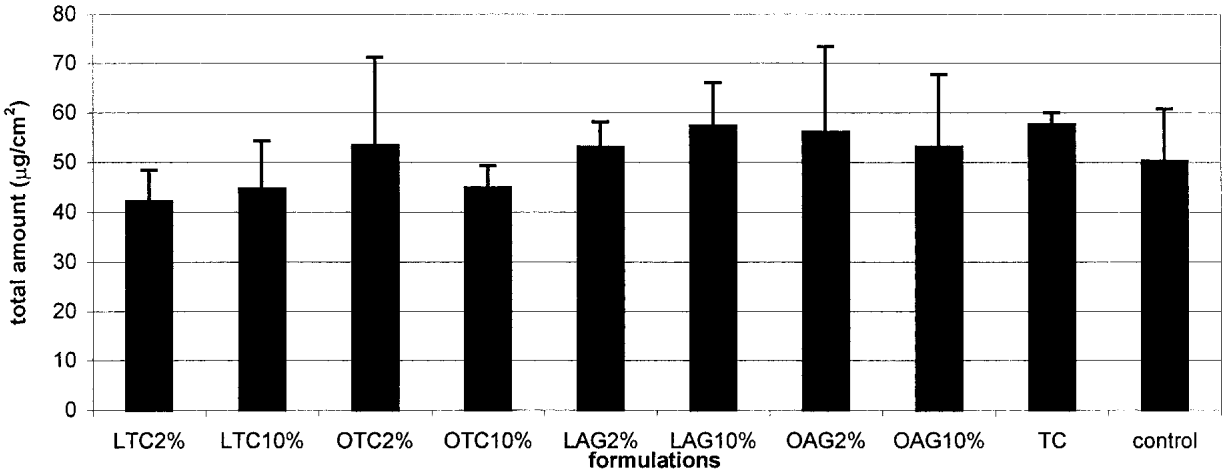

Fig. 6. Total penetrated amount of 4-HB per unit area of stratum corneum (n = 3 ± SD). L, laureate; O, oleate; TC, Transcutol; W, water.

Table I. Critical Micelle Concentration for Sucrose Laureate and Sucrose Oleate in Water and in Transcutol

	Solvent	
Sugar ester	Water	Transcutol
Sucrose laureate	0.05%	3%
Sucrose oleate	0.01%	3%

in the presence of TC. Figure 6 illustrates the total amount of 4-HB determined in the tapes, representing the quantity that penetrated through the stratum corneum. The results suggest that after an application of 1 h, the total amount of 4-HB detected in the SC is independent of the formulation. However, as summarized in Fig. 5, the extent to which the molecule penetrates is clearly influenced by the formulation, being enhanced by the incorporation of SE-TC.

CMC, as determined by surface tension measurements, rose dramatically when TC was used as the solvent. As presented in Table I, whereas in aqueous solution the CMC values were 0.05% and 0.01% for L and O, respectively, in TC the CMC increased to 3% for the two sugar esters. These results are in agreement with previous studies where propylene glycol was shown to increase the CMC of non-ionic surfactants (2,3,29). The ATR-FTIR data presented here are consistent with the concentration-dependent permeation behavior of surfactants and support the assumption that the monomer is preferentially transported across the SC (2,3,30) given that the maximal lipid-disruption was achieved by 2% SE-TC formulations at concentrations below the CMC. Moreover, despite 5-fold higher SE levels, the 10% SE formulations (concentrations above the CMC) did not produce a corresponding increase in 4-HB permeation.

CONCLUSIONS

We have demonstrated that penetration of 4-HB, a model permeant ($M_w = 119.12$; $\text{Log}K_{o/w} = 1.56$, water solubility = 10.25 mg/mL) is enhanced in the presence of SE and TC. It is apparent that TC and SE interact synergistically to modify the skin barrier and to promote the penetration of 4-HB. Of the two SEs evaluated, the laureate ester, in combination with TC, was significantly more effective than its oleate analogue. These relatively innocuous nonionic surfactant molecules justify further investigation as potential candidates for inclusion in transdermal formulations as penetration enhancers.

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