The role of sodium dodecyl sulfate (SDS) micelles in inducing skin barrier perturbation in the presence of glycerol

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Synopsis

The stratum corneum (SC) serves as the skin barrier between the body and the environment. When the skin is contacted with an aqueous solution of the surfactant sodium dodecyl sulfate (SDS), a well-known model skin irritant, SDS penetrates into the skin and disrupts this barrier. It is well established, both in vitro and in vivo, that the SDS skin penetration is dose-dependent, and that it increases with an increase in the total SDS concentration above the critical micelle concentration (CMC) of SDS. However, when we added the humectant glycerol at a concentration of 10 wt% to the aqueous SDS contacting solution, we observed, through *in vitro* quantitative skin radioactivity assays using ¹⁴C-radiolabeled SDS, that the dose dependence in SDS skin penetration is almost completely eliminated. To rationalize this important observation, which may also be related to the well-known beneficial effects of glycerol on skin barrier perturbation in vivo, we hypothesize that the addition of 10 wt% glycerol may hinder the ability of the SDS micelles to penetrate into the skin barrier through aqueous pores that exist in the SC. To test this hypothesis, we conducted mannitol skin permeability as well as average skin electrical resistivity measurements in vitro upon exposure of the skin to an aqueous SDS contacting solution and to an aqueous SDS + 10 wt% glycerol contacting solution in the context of a hindered-transport aqueous porous pathway model of the SC. Our *in vitro* studies demonstrated that the addition of 10 wt% glycerol: (i) reduces the average aqueous pore radius resulting from exposure of the skin to the aqueous SDS contacting solution from 33 ± 5 Å to 20 ± 5 Å, such that a SDS micelle of radius 18.5 ± 1 Å (as determined using dynamic light-scattering measurements) experiences significant steric hindrance and cannot penetrate into the SC, and (ii) reduces the number density of aqueous pores in the SC by more than 50%, thereby further reducing the ability of the SDS micelles to penetrate into the SC and perturb the skin barrier.

INTRODUCTION AND SIGNIFICANCE

Human skin consists of three stratified layers, the stratum corneum, the viable epidermis, and the dermis (1). The stratum corneum (SC), which is the topmost layer of the skin, possesses an ordered brick-and-mortar structure, which consists of the flat corneo-

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cytes (the cellular bricks), interlocked with the lipid lamellae (the intercellular mortar) (2–5). Compared to the porous structure of the viable epidermis and the porous-andhydrated structure of the dermis, the rigid and ordered structure of the stratum corneum makes it a very effective permeability barrier that is primarily responsible for the skin barrier function (2–4). The lipid lamellae of the SC consist of lipid bilayers alternating with aqueous, hydrophilic layers (1–4). Under passive skin permeation conditions, permeants traverse the SC through diffusion across the lipid lamellae. Although diffusion through the "oily" lipid lamellae can explain the permeation of hydrophobic molecules across the SC, it cannot explain the permeation of hydrophilic molecules across the SC, as observed in many earlier studies (6–9).

Indeed, if no aqueous/hydrophilic transport pathways existed within the SC oily lipid domain, then aqueous/hydrophilic permeants, for example mannitol (6-9), could not traverse the SC solely through the lipoidal/hydrophobic pathways that exist in the lipid bilayer domains in the SC. The observation that hydrophilic solutes are able to permeate across the SC, even under passive skin permeation conditions, has led researchers to propose the existence of tortuous, aqueous porous pathways through the intercellular lipid lamellae in the SC. In fact, Menon and Elias (10) have established a morphological basis for the existence of a pore pathway in the mammalian SC. They applied hydrophilic and hydrophobic tracers in vivo to murine skin under passive skin permeation conditions, and also under enhanced skin permeation conditions, using chemical enhancers, a lipid synthesis inhibitor, sonophoresis, and iontophoresis, and following that, they utilized ruthenium tetroxide staining and microwave post fixation methods to visualize the resulting penetration pathways (10). Their results revealed that both the hydrophobic and the hydrophilic tracers localized to discrete lacunar domains embedded within the extracellular lipid lamellar domains (10). Menon and Elias also observed that under skin permeation enhancement conditions, the lacunar domains exhibited an increasing extent of structural continuity when compared to passive skin permeation conditions (10). Hence, structurally continuous lacunar domains have been considered by Menon and Elias as providing a physical basis for the existence of aqueous pores and polar pathways through the intercellular lipoidal mortar in the SC (10). These aqueous pores in the SC provide the primary skin barrier penetration and transport pathways for hydrophilic chemicals, which would otherwise not be able to penetrate into the skin barrier through the lipoidal, hydrophobic pathways that exist in the SC (6-11).

In general, surfactants commonly encountered in skin care formulations are known to reduce the barrier properties of the skin (11-15). It is well-accepted that surfactants have to first penetrate into the skin barrier before they can reduce the skin barrier properties. Therefore, if a formulator can minimize surfactant skin penetration, this should also minimize the ability of the surfactant to reduce the skin barrier properties. Sodium dodecyl sulfate (SDS), an anionic surfactant and a model skin irritant, penetrates into and disrupts the skin barrier upon contacting it from an aqueous solution. The SDS monomers self-assemble to form micelles at concentrations above the critical micelle concentration (CMC). Moore *et al.* (11) and others (12,13) have observed, both *in vitro* and *in vivo*, that the SDS-induced skin barrier disruption is dose-dependent, and that it increases with an increase in the total SDS concentration above the CMC of SDS. This important observation contradicts the well-accepted monomer penetration model (MPM), which attempts to explain surfactant skin penetration by considering solely the role of the surfactant monomers that can penetrate the skin barrier through the aqueous

pores in the SC (11–23). The MPM does not consider the possibility that surfactant micelles may also contribute to surfactant skin penetration, and consequently, to surfactant skin barrier disruption, since it considers the micelles to be too large to penetrate through the aqueous pores that exist in the SC. In her comprehensive review of surfactant-skin interactions, Rhein (14) stated that the observed dose dependence of surfactant-induced skin irritation beyond the CMC cannot be explained solely by the contribution of the monomeric surfactant. Indeed, Agner and Serup (13) had earlier observed that the severity of the transepidermal water loss (TEWL) induced by SDS increased as the SDS concentration increased beyond the CMC of SDS (8.7 mM) (13). In separate studies, Ananthapadmanabhan *et al.* (15) and Faucher and Goddard (16) observed that as the SDS concentration increased beyond the CMC, the extent of SDS skin penetration also increased.

Through *in vitro* SDS skin penetration studies, Moore *et al.* (11) provided substantial evidence that indicates that the amount of SDS that can penetrate into the skin barrier is dose-dependent, and furthermore, that the SDS surfactant in micellar form also contributes to SDS skin penetration. In addition, Moore *et al.* demonstrated conclusively that the contribution of the SDS micelles to SDS skin penetration dominates that of the SDS monomers at concentrations above the CMC, which are typically encountered in skin care formulations (11).

In this paper, we have further investigated, from a mechanistic perspective, how SDS micelles may contribute to SDS skin penetration, thereby leading to the previously observed dose dependence of SDS-induced skin barrier perturbation (11-23). Specifically, we will provide new evidence, through *in vitro* transdermal permeability and skin electrical current measurements, in the context of a hindered-transport porous pathway model of the SC (6–9,42), that the aqueous pores in the SC increase both in size and in number density when skin is exposed to an aqueous SDS contacting solution, such that the average pore radius is larger than the SDS micelle radius. As a result, SDS micelles, contrary to the view put forward by the MPM, are not sterically hindered from penetrating into the skin barrier through these pores.

Inspired by our proposed mechanistic understanding of how SDS micelles may contribute to SDS-induced skin barrier perturbation, we have also investigated *in vitro* whether the addition of glycerol, a well-known humectant and skin beneficial agent, to the aqueous SDS contacting solution can minimize the observed contribution of the SDS micelles to SDS skin penetration. Although not within the scope of this paper, if shown to be valid *in vivo*, such a strategy can also significantly reduce the amount of SDS that can penetrate into the skin barrier and induce skin barrier perturbation *in vivo*.

Our approach considers exposing skin *in vitro* to aqueous mixtures of glycerol and SDS. The importance of glycerol (or glycerin) in skin care products is well established, and glycerol is widely used in cosmetic and pharmaceutical formulations (24–31). To explain its *in vivo* benefits, studies have focused on its humectant and smoothing effects (25) and on its protective functions in emulsion systems against skin irritation (26). Researchers have shown that glycerol diffuses into the SC, increases skin hydration, and relieves clinical signs of dryness (27–29). One of the views regarding the effect of glycerol on the skin held by researchers today is that it may influence the crystalline arrangement of the

intercellular lipid bilayers. The bulk of the bilamellar lipid sheets are proposed to be in crystalline/gel domains bordered by lipids in a fluid crystalline state. In skin exhibiting SC barrier damage, the proportion of lipids in the solid state may be elevated, and subsequent skin exposure to glycerol may help maintain these lipids in a liquid crystalline state at low relative humidity, thereby enhancing SC barrier function and decreasing SC water permeability (30). A second prevalent view is that glycerol may increase the rate of corneocyte loss from the upper layers of the SC, through a keratolytical effect due to enhanced desmosome degradation, thereby reducing the scaliness of dry skin and maintaining the SC barrier (31). A third, more recent view advanced by Fluhr *et al.* (24) is based on the hygroscopic property of glycerol. Glycerol, by virtue of its high transdermal diffusivity, can penetrate into the SC, and, by virtue of its hygroscopic property, is able to bind water and thus reduce water evaporation. Therefore, glycerol, by absorbing water, may modulate water fluxes in the SC, which, in turn, may lead to a stimulus for SC barrier repair.

However, it is still not well understood how glycerol may mitigate surfactant-induced SC barrier perturbation induced by a formulation containing aqueous mixtures of glycerol and a surfactant, such as SDS. Most of the studies discussed above (24-31) considered the application of glycerol to forearm skin in vivo, either: (i) as dilute aqueous solutions containing 5-15 wt% glycerol or (ii) as cosmetic formulations, such as barrier creams, containing a similar range of glycerol concentrations. With this in mind, using such an aqueous mixture of SDS and 10 wt% glycerol, we will demonstrate in vitro that the addition of glycerol eliminates almost completely the contribution of the SDS micelles to SDS skin penetration. Using dynamic light-scattering (DLS) measurements, we will show that the addition of 10 wt% glycerol to an aqueous SDS contacting solution does not increase the size of the SDS micelles, which if increased, could explain the observed reduced ability of SDS (present in the larger SDS micelles) to penetrate into the skin and induce less skin barrier perturbation in the presence of glycerol. Furthermore, using surface tension measurements, we will show that the addition of 10 wt% glycerol to an aqueous SDS contacting solution does not decrease the CMC, and hence, does not reduce the concentration of the SDS monomers contacting the skin, which if reduced, could explain the observed reduced ability of SDS (present in monomeric form) to penetrate into the skin and induce less skin barrier perturbation in the presence of glycerol. Finally, using in vitro mannitol as well as skin permeability and skin electrical current measurements, in the context of a hindered-transport porous pathway model of the SC (6-9,42), we will show that a plausible explanation of our findings is that the addition of 10 wt% glycerol to an aqueous SDS contacting solution reduces the size and the number density of the aqueous pores in the SC relative to the SDS micelle size, such that the SDS micelles present in the contacting solution are sterically hindered from penetrating into the SC. This, in turn, leads to significantly less SDS-induced skin barrier perturbation upon the addition of 10 wt% glycerol.

EXPERIMENTAL

MATERIALS

Sodium dodecyl sulfate (SDS) was purchased from Sigma Chemicals (St. Louis, MO). Analytical-grade glycerol was purchased from VWR Chemicals (Cambridge, MA).¹⁴C-

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radiolabeled SDS and ³H-radiolabeled mannitol were purchased from American Radiolabeled Chemicals (St. Louis, MO). All these chemicals were used as received. Water was filtered using a Millipore Academic water filter (Bedford, MA). Phosphate-buffered saline (PBS) was prepared using PBS tablets from Sigma Chemicals (St. Louis, MO) and Millipore filtered water, such that a phosphate concentration of 0.01 M and a NaCl concentration of 0.137 M were obtained at a pH of 7.2.

PREPARATION OF SKIN SAMPLES

Female Yorkshire pigs (40–45 kg) were purchased from local farms, and the skin (back) was harvested within one hour after sacrificing the animal. The subcutaneous fat was trimmed off using a razor blade, and the full-thickness pig skin was cut into small pieces (2 cm \times 2 cm) and stored in a -80°C freezer for up to two months. The surfactant penetration experiments were performed using pig full-thickness skin, referred to hereafter as p-FTS.

IN VITRO TRANSDERMAL PERMEABILITY MEASUREMENTS

Vertical Franz diffusion cells (Permegear Inc., Riegelsville, PA) were used in the *in vitro* transdermal permeability measurements (see Figure 1). All the experiments were performed at room temperature (25°C). Prior to each experiment, a p-FTS sample was mounted in the diffusion cell with the SC facing the donor compartment. Both the donor



Figure 1. Vertical Franz diffusion cell experimental setup to measure transdermal permeability, skin electrical current, and/or skin radioactivity *in vitro*.

and the receiver compartments were filled with PBS, and the p-FTS sample was left to hydrate for one hour before the beginning of the experiment to allow the skin's initial barrier property to reach steady state. At this point, the skin electrical current across the p-FTS sample was measured (see below), and only p-FTS samples with an initial skin current <3 µA were used in the permeation studies (a well-accepted criterion for selecting suitable in vitro skin samples (7,13)). The PBS in the donor compartment was then replaced with either 1.5 ml of an SDS aqueous solution or 1.5 ml of an SDS + 10wt% glycerol aqueous solution. The solution in the donor compartment, referred to hereafter as the contacting solution, contacted the p-FTS sample for five hours. Note that a five-hour exposure of the skin was chosen because this is a sufficiently long time to allow significant SDS skin penetration, yet a short enough time to prevent the saturation of the skin with SDS. Subsequently, the contacting solution was removed and the donor compartment and the p-FTS sample were rinsed four times with 2 ml of PBS to remove any trace chemical left on the skin surface and in the donor compartment. The receiver compartment was stirred with a magnetic stirrer at a speed of 400 rpm throughout the experiment to eliminate permeant bulk concentration gradients.

Following the SDS aqueous solution and the SDS + 10 wt% glycerol aqueous contacting solution treatments of the skin, the p-FTS samples in the diffusion cells were exposed to a contacting solution of ³H-radiolabeled mannitol in PBS (1-10 µCi/ml) for 24 hours. Mannitol is: (i) a low-molecular-weight monosaccharide (MW = 182 Da) (6,7) and (ii) a highly hydrophilic (log $K_{OVV} = -3.1$) chemical (7), which is not metabolized by the body, and hence, if desired, can also be used for in vivo skin permeation studies (6,7). Being small in size and highly hydrophilic, mannitol can access similar aqueous pores as do ions in order to transport across the skin barrier. This, in turn, makes mannitol a suitable permeant to study in the context of the hindered-transport porous pathway model of the SC (6-9). Pretreatment of p-FTS with (a) SDS or (b) SDS + 10 wt% glycerol aqueous contacting solutions in this manner, followed by passive mannitol-skin permeation, allowed for a controlled comparison of the skin barrier perturbation potential of solutions (a) and (b) at fixed exposure times of five hours. Throughout these experiments, solution samples were withdrawn from both the receiver (r) and the donor (d) compartments every two hours, and the concentrations of the radiolabeled permeant (mannitol) in the two compartments (C_r and C_d , respectively) were measured using a liquid scintillation counter (Packard, Sheldon, CT). When the transport of mannitol attained steady state, the mannitol skin permeability, P_{i} was calculated as follows (6,7):

$$P = \frac{1}{AC_d} \left(\frac{d(C_r V_r)}{dt} \right) \tag{1}$$

where V_r is the volume of the receiver compartment, $A = (1.77 \text{ cm}^2)$ is the area of the SC exposed to the mannitol solution in the donor compartment, and t is the exposure time.

Equation 1 is based on the following two assumptions: (i) the concentration of the permeant in the donor compartment is high, and does not deplete with time, and (ii) the concentration of the permeant in the donor compartment is always much higher than that in the receiver compartment. In the experiments reported here, assumptions (i) and (ii) were both satisfied because less than 2% of mannitol in the contacting solution permeated across the p-FTS samples.

IN VITRO SKIN ELECTRICAL CURRENT AND SKIN ELECTRICAL RESISTIVITY MEASUREMENTS

During each skin permeation experiment, two Ag/AgCl electrodes (E242, In Vivo Metrics, Healdsburg, CA) were placed in the donor and in the receiver compartments to measure the electrical current and the electrical resistivity across the p-FTS sample (see Figure 1). A 100 mV AC voltage (RMS) at 10 Hz was generated by a signal generator (Hewlett-Packard, Atlanta, GA) and was applied across the two electrodes for 5 s. The electrical current across the skin was measured using an ammeter (Hewlett-Packard, Atlanta, GA). This ammeter was used to measure low AC currents and was accurate in the 0.1 µA range. The electrical resistance of the p-FTS sample was then calculated from Ohm's law (7). Because the measured skin electrical resistance is the sum of the actual skin electrical resistance and the background PBS electrical resistance, the latter was subtracted from the measured skin electrical resistance to obtain the actual skin electrical resistance. The skin electrical resistivity was then obtained by multiplying the actual skin electrical resistance by the skin area (A = 1.77 cm^2). The skin electrical resistivity, being an intrinsic electrical property of the skin membrane, is a preferred measure in this analysis over the skin electrical resistance, which is an extensive electrical property of the skin membrane (33). Therefore, by using the skin electrical resistivity, it will be easier to compare differences in the electrical properties of the skin barrier upon exposure of the skin to the SDS and to the SDS + 10 wt% glycerol aqueous contacting solutions. Skin electrical current and resistivity measurements were carried out before and during the permeation experiments at each predetermined sampling point. For each p-FTS sample, an average skin electrical resistivity was determined over the same time period for which the steady-state skin permeability, P, was calculated using equation 1. This average skin electrical resistivity, R, was then analyzed along with the corresponding skin permeability, P, in the context of the theoretical framework presented below in the Theoretical section.

IN VITRO SKIN RADIOACTIVITY MEASUREMENTS

The p-FTS samples were mounted in vertical Franz diffusion cells, as was done in the case of the skin transdermal permeability measurements described above. Following a similar protocol, p-FTS samples were now exposed to aqueous contacting solutions containing 1.5 ml of SDS or 1.5 ml of SDS + 10 wt% glycerol. Each of these contacting solutions also contained about 1 μ Ci/ml of ¹⁴C-SDS.

Diffusion of SDS into the skin took place for five hours, as before, and subsequently, the aqueous contacting solutions were removed and the donor compartment and the p-FTS sample were rinsed four times with 2 ml of PBS to remove any trace chemical left on the skin surface and in the donor compartment. The p-FTS samples were then heat-stripped following a well-known procedure (11). Briefly, a p-FTS sample was placed in a water bath at 60°C for two minutes, and subsequently, the epidermis (the SC and the viable epidermis) that was exposed to the contacting solution was peeled off from the dermis. The exposed epidermis was then dried for two days in a fume hood and weighed. The dried epidermis was dissolved overnight in 1.5 ml of Soluene-350 (Packard, Meriden, CT). After the epidermis dissolved, 10 ml of Hionic Fluor scintillation cocktail (Packard) was added to the Soluene-350, and the concentration of radiolabeled SDS was determined using the Packard scintillation counter. Note that we did verify that the

concentration of radiolabeled SDS in the contacting solution did not change appreciably during the five-hour exposure to the skin. The concentration of radiolabeled SDS in the contacting solution was determined by using approximately 100 μ l of the contacting solution and assaying for the radioactivity of ¹⁴C-SDS using the scintillation cocktail assay described above.

Knowing the concentration of SDS in the contacting solution, $C_{S \triangleright S}$, the radioactivity of the contacting solution, $C_{rad,donor}$, the dry weight of the epidermis, m, and the radioactivity of the epidermis, $C_{rad,skin}$, we were able to determine the concentration of SDS in the dried epidermis, $C_{SDS,skin}$, using the following equation (11):

$$C_{SDS,skin} = \frac{C_{rad,skin} \cdot C_{SDS}}{C_{rad,donor} \cdot m}$$
(2)

DYNAMIC LIGHT-SCATTERING MEASUREMENTS

The aqueous SDS and SDS + 10 wt% glycerol solutions were prepared in Milliporefiltered water with 100 mM of added NaCl. Note that 100 mM NaCl was added to screen potential electrostatic repulsions between the negatively charged SDS micelles while performing the dynamic light-scattering (DLS) measurements (11,34,36–39). After mixing, the solutions were filtered through a 0.02-µm Anotop 10 syringe filter (Whatman International, Maidstone, England) directly into a cylindrical scattering cell to remove any dust from the solution, and then sealed until use. Dynamic light scattering (34) was performed at 25°C and a 90° scattering angle on a Brookhaven BI-200SM system (Brookhaven, Holtsville, NY) using a 2017 Stabilite argon-ion laser (Spectra Physics) at 488 nm. The autocorrelation function was analyzed using the CONTIN program provided by the BIC dynamic light-scattering software (Brookhaven, Holtsville, NY), which determines the effective hydrodynamic radius, \overline{R}_b , of the scattering entities using the Stokes-Einstein relation (35):

$$\overline{R}_{b} = \frac{k_{B}T}{6\pi\eta\overline{D}}$$
(3)

where k_B is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the aqueous salt solution, and \overline{D} is the mean diffusion coefficient of the scattering entities.

In order to measure the size of the SDS micelles in the aqueous SDS and in the SDS + 10 wt% glycerol solutions, while eliminating the effects of interparticle interactions, the effective hydrodynamic radii were determined at several different SDS concentrations, and then extrapolated to a zero micelle concentration, which corresponds to the CMC of SDS, 8.7 mM (11,34,36–39). Note that the viscosity of a 10 wt% glycerol aqueous solution is similar to that of water, and hence, viscosity effects did not play a significant role in these measurements.

SURFACE TENSION MEASUREMENTS

We used surface tension measurements to determine the critical micelle concentration, CMC, of the SDS and of the SDS + 10 wt% glycerol aqueous micellar solutions. It is well known that as the surfactant concentration, X, is increased, both the hydrophobicity

of the surfactant tails and the high water-air surface free energy promote the adsorption of the surfactant molecules onto the surface (40). The increase in the surface pressure due to surfactant surface adsorption leads to a lowering of the surface tension, σ . Beyond a certain threshold surfactant concentration, the CMC, it becomes more favorable, from a free energy point of view, for the surfactant molecules added to the solution to form micelles, rather than to continue to adsorb at the surface. This is reflected in a negligible change in surface tension, σ , with increasing surfactant concentration, X, beyond the CMC. The "break" in the σ versus X curve, therefore, approximates the concentration at which micellization first takes place (40). In order to determine this "break," the equilibrium surface tensions of SDS in water and of SDS in water + 10 wt% glycerol were measured as a function of the logarithm of the SDS solution concentration using a Kruss K-11 tensiometer (Kruss, Charlotte, NC) with a platinum plate. Additional experimental details can be found in reference 41. The experimental uncertainty in the surface tension measurements was approximately 0.05 dyn/cm. The temperature was held constant at 25.0 \pm 0.1°C by a thermostatically controlled jacket around the sample.

A plot of σ as a function of the logarithm of the surfactant concentration, X, was generated using the procedure outlined above for the SDS and for the SDS + 10 wt% glycerol aqueous micellar solutions. Linear regression was used to determine the best fit line on either side of the break in the curve, and the value of the SDS concentration at the intersection of these two best-fit lines was taken as the experimental CMC value.

THEORETICAL

DETERMINATION OF THE RADIUS AND NUMBER DENSITY OF THE SKIN AQUEOUS PORES

Tang *et al.* (7) have recently demonstrated the existence of a linear-log relationship between the mannitol skin permeability, P, and the average skin electrical resistivity, R. Specifically, within statistical error, the following relation holds (7):

$$\log P = \log C - \log R \tag{4}$$

where C = $(k_B T/2z^2 Fc_{ion} e_0)^* (D_p^{\infty} H(\lambda_p)/D_{ion}^{\infty} H(\lambda_{ion}))$ is a constant that depends on the average skin aqueous pore radius, r_{pore} , through $H(\lambda_p)$ and $H(\lambda_{ion})$, as follows (7,8,42):¹

$$H(\lambda_i) = \phi_i (1 - 2.1044\lambda_i + 2.089\lambda_i^3 - 0.948\lambda_i^5), \text{ for } \lambda_i \le 0.4$$
(5)

where i = p (permeant, in our case, mannitol) or ion, r_{pore} = pore radius, $\lambda_i = r_i/r_{pore}$, and ϕ_i (the partition coefficient of *i*) = $(1 - \lambda_i)^2$. Note that equation 5 considers only steric, hard-sphere particle (p or ion)-pore wall interactions, and does not account for longer-range interactions, such as electrostatic and van der Waals interactions (7). Although the ions (and the permeant molecules) in the contacting solutions may be charged, Tang *et al.* have shown that equation 5 is valid provided that the Debye-Hückel screening length—the length scale associated with the screening of electrostatic interactions between the ions (or between the charged permeants) and the negatively charged skin aqueous pore walls—is much smaller than the average skin aqueous pore radius, r_{pore} (7).

¹ It is noteworthy that the skin aqueous pores have a distribution of pore radii (9). In this paper, we imply the average pore radius to be the mean of this distribution of pore radii, and denote this as the radius of the aqueous pores.

Tang *et al.* also showed that for the PBS control contacting solution containing Na⁺ and Cl⁻ ions, and also for the mannitol aqueous solution, the Debye-Hückel screening length ≤ 7 Å, which is much smaller than the typical average skin aqueous pore radii, that is, than the sizes of the aqueous pores, of approximately 15–25 Å (7). The quantities, D_p^{∞} and D_{ion}^{∞} , appearing in C refer to the permeant and to the ion infinite-dilution diffusion coefficients, respectively (note that these quantities correspond typically to the bulk diffusion coefficients of the permeant and of the ion in the dilute donor contacting solutions used in the *in vitro* transdermal permeability and electrical resistivity measurements).

According to the hindered-transport theory (42), the hindrance factor for permeant or ion transport, $H(\lambda_i)$, is a function of both the permeant/ion type and of the skin membrane characteristics. The four intrinsic membrane characteristics of the skin barrier are: (i) the porosity, ε , which is the fraction of the skin area occupied by the aqueous pores, (ii) the tortuosity, τ , which is the ratio of the permeant diffusion path length within the skin barrier to the skin barrier thickness, (iii) the average pore radius, r_{pore} , and (iv) the skin barrier thickness, ΔX . Based on these four membrane characteristics, one can express the permeability, P, of a hydrophilic permeant, such as mannitol, through the skin aqueous pores as follows (6,7,42):

$$P = \frac{\left(\frac{\varepsilon}{\tau}\right) D_{\rho}^{\infty} H(\lambda_{\rho})}{\Delta X} \tag{6}$$

Therefore, from equations 4–6, once one can determine *P* and *R* upon exposure of p-FTS to contacting aqueous solutions of SDS and SDS + 10 wt% glycerol, one can also determine the radius of the aqueous pores as the average skin pore radius, r_{pore} , and the ratio of porosity-to-tortuosity, defined as ε/τ , if all the other parameters, such as ΔX , are known (see Appendix, where we illustrate how to deduce r_{pore} and ε/τ when p-FTS is contacted with SDS aqueous solutions). The porosity-to-tortuosity ratio, ε/τ , corresponds to the number of tortuous aqueous pores per unit volume of the SC, that is, to the pore number density (6,7,42). In the context of the hindered-transport aqueous porous pathway model of the SC, an increase in the porosity, ε , and/or a decrease in the tortuosity, τ , which increases the porosity-to-tortuosity ratio, ε/τ , of the aqueous pores, can be interpreted as an increase in the number of aqueous pores per unit volume of the SC (7–9,42).

A harsh surfactant like SDS can induce skin barrier perturbation by modifying the SC aqueous porous pathways as follows: (i) increasing the size of the existing aqueous pores in the SC, and/or (ii) increasing the number density of the existing aqueous pores in the SC, or both. It then follows, in the context of the hindered-transport aqueous porous pathway model, that mechanism (i) involves increasing r_{pore} , while mechanism (ii) involves increasing ϵ/τ [6–9,42]. In Table I, we report r_{pore} values resulting from the exposure of p-FTS to contacting solutions of: (a) SDS in water, (b) SDS + 10 wt% glycerol in water, (c) PBS control, and (d) 10 wt% glycerol in water. Note that in Table I, we have reported the ϵ/τ values resulting from the exposure of p-FTS to the contacting solutions (a–d) normalized by the ϵ/τ value resulting from the exposure of p-FTS to contacting solutions (ϵ/τ)_{normal}. It then follows that when (ϵ/τ)_{normal} > 1, it indicates that the contacting solution creates more aqueous pores in the

Skin Aqueous Pore Characteristics Induced by Various Skin Contacting Solutions		
Type of aqueous contacting solution	Average pore radius, r _{pore} (Å)	Normalized pore number density, (ε/τ) _{normal}
(a) SDS	33 ± 5	7 ± 1
(b) SDS + 10 wt% glycerol	20 ± 5	3 ± 1
(c) PBS control	20 ± 3	1
(d) 10 wt% glycerol	11 ± 4	0.5 ± 0.1

 Table I

 Skin Aqueous Pore Characteristics Induced by Various Skin Contacting Solutions

The hindered-transport aqueous porous pathway model was used, along with the *in vitro* mannitol transdermal permeability and average skin electrical resistivity measurements, to determine the average pore radius, r_{pore} , and the pore number density, ε/τ , resulting from skin exposure to the four aqueous contacting solutions considered: (a) SDS, (b) SDS + 10 wt% glycerol, (c) PBS control, and (d) 10 wt% glycerol. Note that we have reported ε/τ values resulting from the exposure of p-FTS to the contacting solutions a–d normalized by the ε/τ value resulting from the exposure of p-FTS to contacting solution (c), which we have denoted as $(\varepsilon/\tau)_{normal}$.

SC relative to those created by the PBS control, while when $(\epsilon/\tau)_{normal} < 1$, it indicates that the contacting solution creates fewer aqueous pores relative to those created by the PBS control.

RESULTS AND DISCUSSION

EFFECT OF GLYCEROL ON SDS-INDUCED SKIN BARRIER PERTURBATION

In order to quantify the effect of the SDS concentration in the skin aqueous contacting solution on the skin barrier in the absence and in the presence of glycerol, we utilized the *in vitro* transdermal permeability and the skin electrical current measurements discussed above. The physical basis for these measurements is as follows: a large skin electrical current or transdermal permeability, which results from a high transfer rate of permeant molecules (mannitol in our case) or of ions, respectively, across the skin, is indicative of a large extent of skin barrier perturbation *in vitro* (1–7). Therefore, if upon exposure of the skin to an aqueous contacting solution of SDS or of SDS + 10 wt% glycerol, one observes a high skin electrical current (corresponding to a low average skin electrical resistivity) or permeability, one may conclude that the contacting solution has induced skin barrier perturbation, thereby compromising the skin barrier.

With the above expectation in mind, we conducted skin electrical current measurements for aqueous contacting solutions of SDS ranging in SDS concentrations from 1 mM to 200 mM.² The results of these measurements are shown as striped bars in Figure 2. As can be seen, the extent of skin barrier perturbation, quantified in terms of the skin electrical current, continues to increase, with an increase in the SDS concentration in the contacting solution above the CMC of SDS (8.7 mM).³ According to the monomer

 $^{^2}$ Note that 1 wt% SDS = 35 mM, and that the CMC of SDS = 8.7 mM = 0.25 wt%.

³ Recall that the CMC is the threshold total surfactant concentration above which the concentration of the surfactant monomers remains approximately constant, while that of the surfactant micelles increases upon increasing the total surfactant concentration. This is because, above the CMC, any new surfactant molecules added to the solution self-assemble to form micelles, a process that is thermodynamically more favorable than to remain as free monomers in the surfactant solution.



Figure 2. Comparison of the *in vitro* skin electrical currents induced by SDS aqueous contacting solutions (striped bars) and by SDS + 10 wt% glycerol aqueous contacting solutions (filled bars). The error bars represent standard errors based on 6-10 p-FTS samples.

penetration model (MPM) adopted by many researchers in the past, only the surfactant monomers are able to penetrate into the skin barrier and induce skin barrier perturbation, while the micelles, due to their larger size relative to that of the monomers, are not able to do so. Hence, according to the MPM, the skin barrier perturbation induced by a surfactant contacting solution should not increase significantly upon increasing the total surfactant concentration above the CMC.⁴ However, our skin electrical current results clearly show that an increase in the SDS concentration in the contacting solution above the CMC induces a significant increase in the skin electrical current (see Figure 2). This observation is consistent with the results reported by other researchers in previous studies (11-19). For example, Moore et al. (11) found that SDS micelles contribute to SDS skin penetration. Therefore, it is natural that SDS micelles should also contribute to skin barrier perturbation, as we have demonstrated experimentally through these skin electrical current measurements. Indeed, these measurements indicate unequivocally that SDS micelles contribute to skin barrier perturbation, as reflected in the observed increase in the skin electrical current above the CMC.⁵ Next, we measured skin electrical currents upon exposing p-FTS to aqueous contacting solutions of SDS (1-200 mM) + 10 wt% glycerol. The results of these measurements are shown as filled bars in Figure 2, which clearly shows that the filled bars (corresponding to the skin electrical currents induced by the SDS + 10 wt% glycerol aqueous contacting solutions) are much shorter than the striped bars (corresponding to the skin electrical currents induced by the SDS aqueous contacting solutions). This important finding clearly shows that the addition of 10 wt% glycerol to an SDS aqueous contacting solution significantly reduces SDSinduced skin barrier perturbation, as quantified by the skin electrical currents.

⁴ This statement implies that a surfactant monomer, or a micelle, has to first penetrate into the skin barrier in order to induce skin barrier perturbation. Consequently, if one can minimize, or prevent altogether, penetration of surfactant into the skin, one should be able to minimize skin barrier perturbation induced by the surfactant monomers or by the micelles.

 $^{^5}$ It is noteworthy that the skin electrical current induced by PBS (phosphate-buffered saline), which served as the control for these experiments, was 11 ± 4 μA , which is comparable to that induced by a 1 mM SDS solution (see Figure 2).

Finally, we measured *in vitre* mannitol skin permeabilities upon exposing p-FTS samples to aqueous contacting solutions of SDS (1-200 mM) and of SDS (1-200 mM) + 10 wt% glycerol. The results of these measurements are shown in Figure 3, in which the diamonds correspond to the permeability values resulting from exposure to the SDS aqueous contacting solutions, and the triangles correspond to the permeability values resulting from exposure to the SDS + 10 wt% glycerol aqueous contacting solutions. These measurements seem to indicate that: (i) the SDS micelles, in general, do contribute to skin barrier perturbation, as reflected in the increasing *P* values with increasing SDS concentration above the CMC of SDS (8.7 mM), and (ii) the addition of glycerol minimizes SDS micelle-induced skin barrier perturbation, as reflected in the triangles lying below the diamonds in Figure 3.

EFFECT OF GLYCEROL ON SDS SKIN PENETRATION

We developed the skin radioactivity assay discussed above to directly quantify the amount of SDS that can penetrate into the skin barrier from an SDS aqueous contacting solution in the absence and in the presence of 10 wt% glycerol. Use of this assay allowed us to directly measure the contribution of the SDS micelles, in the absence and in the presence of 10 wt% glycerol, to SDS skin penetration. The results of our measurements are shown in Figure 4.

The concentrations of SDS in the skin barrier (in wt%) resulting from the exposure of p-FTS to aqueous contacting solutions of SDS (1–200 mM) correspond to the diamonds in Figure 4. One can clearly see that upon increasing the total SDS concentration in the contacting solution above the CMC (8.7 mM), the concentration of SDS in the skin



Total SDS Concentration in the Aqueous Contacting Solution (mM)

Figure 3. Comparison of the *in vitro* mannitol skin permeability induced by SDS aqueous contacting solutions (diamonds) and by SDS + 10 wt% glycerol aqueous contacting solutions (triangles). The dotted vertical line at an SDS concentration of 8.7 mM denotes the CMC of SDS. The error bars represent standard errors based on 6–10 p-FTS samples.



Figure 4. Comparison of SDS skin penetration *in vitro* induced by aqueous contacting solutions of SDS (diamonds) and of SDS + 10 wt% glycerol (triangles). The dotted vertical line at an SDS concentration of 8.7 mM denotes the CMC of SDS. The dashed line passing through the diamonds is drawn as a guide to the eye. The error bars represent a standard error based on 6–10 p-FTS samples.

barrier increases significantly. In Figure 4, the contribution of the SDS monomers to SDS skin penetration above the CMC remains approximately constant above 8.7 mM (the CMC value), and corresponds to the horizontal solid line. On the other hand, the total SDS contribution to SDS skin penetration increases above the CMC, and corresponds to the dashed line, drawn as a guide to the eve. Clearly, the difference between the dashed and the solid lines at any given total SDS concentration corresponds to the contribution of the SDS micelles to SDS skin penetration. Note that below the CMC, only the SDS monomers are available for penetration into the skin. Consequently, the diamonds and the triangles overlap below the CMC (see Figure 4). These results are in excellent agreement with the SDS skin penetration results reported by Moore et al. (11). Indeed, these authors showed earlier that: (i) there is a significant SDS micellar contribution to SDS skin penetration, and (ii) the SDS micellar contribution increases with an increase in the total SDS concentration above the CMC. However, in this paper, we have demonstrated in vitro, for the first time, that the significant SDS micellar contribution to SDS skin penetration also leads to a large extent of SDS skin barrier perturbation, as quantified by the observed increases in the skin electrical currents and in the mannitol transdermal permeabilities (see Figures 2 and 3, respectively). These in vitre results suggest, from a practical, formulation design point of view, that any strategy designed to minimize skin barrier perturbation induced by surfactants like SDS, in addition to minimizing the penetration of the surfactant monomers into the skin, as was done in the past, may also benefit from minimizing the penetration of the surfactant micelles into the skin. In this paper, we have investigated in vitro such a simple and useful practical strategy by using mixtures of SDS and glycerol, which we discuss next.

Specifically, we conducted skin radioactivity assays using 14 C-SDS in the presence of 10 wt% added glycerol in aqueous solution to measure the amount of SDS that may

penetrate into the skin barrier in the presence of glycerol (corresponding to the triangles in Figure 4). It is interesting to observe that the triangles and the diamonds overlap below the CMC in Figure 4. At an SDS concentration below the CMC of SDS (8.7 mM), the SDS aqueous contacting solution essentially consists of SDS monomers contacting the skin. Therefore, upon adding 10 wt% glycerol to the SDS aqueous contacting solution, one can observe that the SDS monomers are not hindered from penetrating into the skin. However, the addition of 10 wt% glycerol to the SDS aqueous contacting solution at concentrations above the CMC significantly impacts SDS skin penetration. Indeed, as can be seen, the presence of 10 wt% glycerol in the SDS contacting solution eliminates almost completely the amount of SDS that can penetrate into the skin barrier from the high SDS concentration contacting solutions. The significant difference between the diamonds (or the dashed line) and the triangles (which lie very close to the SDS monomer contribution corresponding to the solid line) clearly shows that SDS micelles, which would have contributed to skin penetration in the absence of 10 w% glycerol, cannot do so in the presence of 10 wt% glycerol in the contacting solution. These in vitro results suggest that the addition of 10 wt% glycerol to the SDS contacting solutions may also represent a simple, yet very useful, practical strategy to mitigate SDS-induced skin barrier perturbation in vivo by preventing the SDS micelles from penetrating into the skin barrier.

In the following section, we put forward several hypotheses to explain, from a mechanistic viewpoint, why glycerol, without affecting the skin penetration ability of the SDS monomers, is able to significantly reduce the ability of the SDS micelles to contribute to SDS skin penetration *in vitro*.

POSSIBLE HYPOTHESES TO EXPLAIN THE EFFECT OF GLYCEROL ON THE OBSERVED *IN VITRO* DOSE INDEPENDENCE OF SDS SKIN PENETRATION

Using micelle stability arguments put forward by Patist *et al.* (43), Moore *et al.* (11) have shown that the kinetics of micelle dissolution cannot be invoked to explain the observed dose dependence of SDS skin penetration. Moore *et al.* have also compared the time constant for the breakup of SDS micelles to replenish the decreased SDS monomer supply to the SC as the SDS molecules penetrate into the skin with the time constant for SDS diffusion across the skin. This comparison has unambiguously shown that the rate-determining step for SDS skin penetration is governed by the diffusion, or the penetration, through the SC and not by the micelle kinetics (11). Furthermore, Moore *et al.* have shown that micelle disintegration upon impinging on the SC and subsequent absorption by the skin barrier also does not seem to be a plausible mechanism to explain the observed dose dependence of SDS skin penetration (11,44,45). With all of the above in mind, according to Moore *et al.*, a consistent hypothesis to explain the observed dose dependence of SDS skin penetration (11). Without directly measuring the skin aqueous pore radius, r_{pore} , and the pore number density, ϵ/τ , Moore *et al.* hypothesized⁶

⁶Note that Moore *et al.* (11), to their credit, compared micelle sizes for free SDS micelles and PEO-bound SDS micelles, using DLS measurements similar to those reported here, and found that the PEO-bound SDS micelle had a larger hydrodynamic radius than the free SDS micelle. This observation, along with the observation that the PEO-bound SDS micelle, unlike the free SDS micelle, did not contribute to SDS skin (continued on p. 124)

that a free SDS micelle, being smaller than the aqueous pore, is able to penetrate into the SC, while a PEO-bound SDS micelle, being larger then the aqueous pore, is not able to do so. Our hypothesis to explain the observed dose-dependence of SDS skin penetration in the absence of glycerol is similar to that of Moore *et al.* (11), the difference being that we have further substantiated this hypothesis by directly determining the average skin aqueous pore radius, r_{pore} , and the pore number density, ε/τ , of the skin aqueous pores induced by SDS.

Considering the skin penetration of both the SDS monomers and the SDS micelles, we have investigated *in vitro* the following three hypotheses to explain the ability of glycerol to minimize the contribution of SDS micelles to SDS skin penetration: (i) the addition of 10 wt% glycerol to the SDS aqueous contacting solution reduces the concentration of the SDS aqueous contacting solution increases the SDS micelle size relative to that of the skin aqueous pores, such that the larger SDS micelles can no longer penetrate through these aqueous pores into the SDS micelles, which are on average larger than the skin aqueous pores, such that the SDS micelles, which are on average larger than the skin aqueous pores, the decrease in the number density of the aqueous pores should further limit the ability of the SDS micelles to penetrate into the SC through these aqueous pores.

We have investigated hypothesis (i) by conducting surface tension measurements to deduce the CMC of SDS in aqueous solution in the absence and in the presence of 10 wt% glycerol. Hypothesis (ii) was investigated through DLS measurements to determine the SDS micelle hydrodynamic radius in aqueous solution in the absence and in the presence of 10 wt% glycerol. Finally, we investigated hypothesis (iii), by determining the radius and the number density of the skin aqueous pores induced by aqueous SDS contacting solutions in the absence and in the presence of 10 wt% glycerol through our average skin electrical resistivity and mannitol transdermal permeability measurements, in the context of the hindered-transport porous aqueous pathway model. We discuss the results of studies (i–iii) above in the following three sections.

(i) Results from the surface tension measurements to determine the CMC. Recall that the CMC of a SDS aqueous contacting solution is the threshold total SDS concentration above which the concentration of the SDS monomers remains approximately constant, while that of the SDS micelles continues to increase upon increasing the total SDS concentration. Therefore, if the addition of 10 wt% glycerol to the SDS aqueous contacting solution results in a lowering of the CMC, one may conclude that the number of SDS monomers contacting the skin decreases in the presence of glycerol, which may explain why glycerol reduces SDS skin penetration. However, our surface tension results indicate that the CMC of SDS in the presence of 10 wt% glycerol is 9.2 mM, which is slightly larger than the CMC of SDS in the absence of glycerol (8.7 mM). Our CMC value in

(continued)

penetration, formed the basis for their hypothesis that SDS micelles can penetrate into the SC, based on a size limitation. However, Moore *et al.* did not measure the effect of SDS on the radius and on the number density of the skin aqueous pores directly, as is done here.

the presence of glycerol is in excellent agreement with previously reported CMC values of SDS in water/glycerol binary mixtures (46). Therefore, based on the CMC values of SDS in water and in a 10 wt% glycerol aqueous solution, one may conclude that hypothesis (i) is not valid, and therefore, cannot explain the observed ability of glycerol to reduce SDS skin penetration.

(ii) Results from the dynamic light-scattering (DLS) measurements to determine the size of the SDS micelles. Using DLS, we determined the sizes of the SDS micelles in aqueous solutions, in the absence and in the presence of 10 wt% glycerol. Figure 5 shows the results of the DLS measurements in terms of the SDS micelle hydrodynamic radii in: (a) water and (b) 10 wt% glycerol aqueous solutions. The SDS micelle hydrodynamic radii were determined by extrapolation to a zero micelle concentration, which corresponds to the CMCs of SDS solutions corresponding to (a), 8.7 mM (see the diamonds in Figure 5), and to (b), 9.2 mM (see the triangles in Figure 5). Using a linear regression analysis, we determined that the hydrodynamic radius of the free SDS micelles corresponding to (a) is 19.5 ± 1 Å, while that corresponding to (b) is 18.5 ± 1 Å. The SDS micelle hydrodynamic radii corresponding to (a) reported here are in excellent agreement with the values reported previously by Moore et al. (11) and by Almgren and Swarup (47). Therefore, these results indicate that the SDS micelle size is slightly smaller, not larger, in the SDS aqueous solution with 10 wt% added glycerol, and hence, cannot explain how glycerol minimizes the SDS micellar contribution to SDS skin penetration. In other words, hypothesis (ii) is not valid either.

(iii) Results from an analysis of the hindered-transport aqueous porous pathway model to determine the radius and the number density of the skin aqueous pores. We quantified the extent of skin barrier perturbation using the average aqueous pore radius and the pore number density as quantitative descriptors of the SC morphological changes upon exposure to: (a) an



Figure 5. Measured effective radii of SDS micelles in aqueous solutions in the absence (diamonds) and in the presence (triangles) of 10 wt% glycerol plotted versus the SDS concentration minus the CMC, which corresponds to the concentration of the SDS micelles, using DLS measurements at 25°C. The SDS micelle radii were determined using a CONTIN analysis. The error bars reflect standard errors based on six samples at each SDS concentration.

aqueous solution of SDS (1–200 mM) and (b) an aqueous solution of SDS (1–200 mM) + 10wt% glycerol. Specifically, an increase in the radius and/or in the number density of the aqueous pores corresponds to an increased perturbation in the skin barrier (1,6,7,10,42). The radius and the number density of the skin aqueous pores resulting from the exposure to contacting solutions (a) and (b) above were determined using the hindered-transport model of the skin aqueous porous pathways, along with the *in vitro* mannitol transdermal permeability and the average skin electrical resistivity measurements. For completeness, we also conducted similar measurements on p-FTS, which was exposed to: (c) the PBS control, and (d) 10 wt% glycerol aqueous contacting solutions.

In Figure 6, we have plotted the log of the mannitol transdermal permeability, P (cm/h), against the log of the average skin electrical resistivity, R (kohm-cm²), over the same exposure time, exhibited by p-FTS samples exposed to solutions (a), the diamonds, and (b), the triangles, above. Each diamond/triangle represents a log P value of one p-FTS sample at steady state and the corresponding log R (the log of the average skin electrical resistivity value). The slopes of the best-fit curves resulting from linear regressions, the dashed line for (a) and the solid line for (b), are not statistically different from the theoretically predicted slope value of -1, thereby indicating consistency with the hindered-transport aqueous porous pathway model analysis for p-FTS samples exposed to contacting solutions (a) and (b) above (6,7). Also, note that the dashed line has a larger intercept value than that corresponding to the solid line, which reflects a larger average pore radius, r_{pore} , for p-FTS samples exposed to (a) than to (b). Having determined r_{pore} , the pore number density was determined using equation 6, in which all the parameters, except ε/τ , the pore number density, are known in advance (recall that $\Delta X = 15 \ \mu m$)



Figure 6. Experimental correlation between the *in vitro* mannitol transdermal permeability, P (cm/h), and the *in vitro* skin electrical resistivity, R (kohm-cm²), exhibited by p-FTS samples exposed to an aqueous solution of SDS (1–200 mM), the diamonds, and to an aqueous solution of SDS (1–200 mM), the diamonds, and to an aqueous solution of P-FTS sample at steady state and the associated log R, the log of the average skin electrical resistivity value over the same time period. The slopes of the best-fit curves resulting from a linear regression are: (i) –0.9768 ± 0.06 for SDS (1–200 mM), with $R^2 = 0.9636$, shown as the dashed line, and (ii) –1.0453 ± 0.06 for SDS (1–200 mM) + 10 wt% glycerol, with $R^2 = 0.9653$, shown as the solid line. Note that these slope values are not statistically different from the theoretically predicted value of –1.

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(6,7). Using the model described above, we found that the average pore radius does not depend on the SC thickness, ΔX , while the pore number density is directly proportional to ΔX . The aqueous pore number density, ε/τ , values resulting from exposure of the p-FTS samples to contacting solutions a-d above were normalized by the ε/τ value resulting from exposure of the p-FTS samples to the PBS control solution, solution (c), which served as the baseline, and have been denoted as $(\varepsilon/\tau)_{normal}$ (see Appendix, where we illustrate how to obtain r_{pore} and $(\varepsilon/\tau)_{normal}$ for p-FTS samples exposed to (a)).

Our deduced values of r_{pore} and $(\epsilon/\tau)_{normal}$ corresponding to solutions a–d above are reported in Table I. As can be seen, the average pore radius, r_{pore} , corresponding to (a) is 33 ± 5 Å, while that corresponding to (b) is 20 ± 5 Å, which is similar to the average pore radius corresponding to (c), 20 ± 3 Å. In addition, the normalized pore number density, $(\epsilon/\tau)_{normal}$, corresponding to (a), 7 ± 1 , is about twice that corresponding to (b), 3 ± 1 . Interestingly, we also see that a 10 wt% glycerol aqueous solution (contacting solution d) reduces r_{pore} and $(\epsilon/\tau)_{normal}$ by about 50% relative to the PBS control.

The results in Table I indicate that an SDS aqueous contacting solution containing micelles, in the presence of 10 wt% glycerol, induces a lower extent of skin barrier perturbation, as reflected in the lower average pore radius and normalized pore number density, when compared to an SDS aqueous contacting solution, in the absence of glycerol. In fact, in the absence of glycerol, an SDS micelle of 19.5 ± 1 Å hydrodynamic radius experiences no steric hindrance in penetrating through aqueous pores in the SC that have an average pore radius of 33 ± 5 Å (see Table I). However, in the presence of 10 wt% glycerol, an SDS micelle of 18.5 ± 1 Å hydrodynamic radius experiences significant steric hindrance in penetrating through smaller aqueous pores in the SC that have an average pore radius of 20 ± 5 Å (see Table I). Moreover, the presence of 10 wt% added glycerol in the SDS aqueous contacting solution reduces the (ϵ/τ)_{normal} value from 7 ± 1 to 3 ± 1 , which is more than a 50% reduction in the normalized pore number density. Hence, adding 10 wt% glycerol to an aqueous SDS micellar contacting solution minimizes the micellar contribution to SDS skin penetration *in vitro* by minimizing both the average pore radius and the pore number density of the skin aqueous pores.

The results of this study indicate that the data is consistent with hypothesis (iii): Glycerol reduces both the radius of the aqueous pores in the SC relative to that of the SDS micelles, as well as the aqueous pore number density, which if not reduced, would allow SDS micelles to contribute to SDS skin penetration *in vitro*.

POSSIBLE STRUCTURAL MODES OF INTERACTION OF GLYCEROL AND SDS WITH THE SKIN BARRIER

Our results indicate that the addition of 10 wt% glycerol to an aqueous contacting solution of SDS mitigates skin barrier perturbation *in vitro* by reducing the skin aqueous pore radius and the aqueous pore number density. We propose two scenarios to rationalize these results. According to the first scenario, it is well-accepted that because of its strong hygroscopic property and ability to modulate water fluxes in the SC, glycerol can diffuse into the SC and bind water within the SC (24,28,29). In fact, researchers have observed a significant positive correlation *in vivo* between the skin-moisturizing ability of glycerol, as determined through skin conductance measurements, and the corresponding amount of glycerol found in the skin barrier (52). As a result, water binding by glycerol in the SC reduces the mobility of water within the SC. The limited mobility of

water within the SC may result in lacunar domains, as observed by Menon and Elias (10). losing structural continuity, partially or completely, within the extracellular lipid bilayers of the SC. We suggest that a partial loss in the structural continuity of lacunar domains is responsible for a reduction in the radius of the corresponding aqueous pores. while a complete loss in continuity of lacunar domains is responsible for the elimination or closing of the corresponding aqueous pores, that is, for a reduction in the overall number density of the aqueous pores in the SC. Figure 7 illustrates schematically a combination of lacunae that are continuous under normal skin hydration conditions. resulting in an aqueous pore, but may become discontinuous upon exposure of the skin to glycerol, thereby resulting in a size reduction, or a closing, of the aqueous pore. A second scenario describing how glycerol may result in partial, or complete, loss of the structural continuity of lacunar domains considers the ability of glycerol to maintain the intercellular lipid mortar in a liquid crystalline state, as opposed to a solid crystalline state (30). Froebe et al. have shown that addition of 10 wt% glycerol to a mixture of SC lipids *in vitro* inhibited the transition from liquid to solid crystals, which could maintain the intercellular lipid mortar in the SC and potentially minimize the size, as well as the continuity, of the lacunar domains within the SC (30). Most likely, we suggest that both scenarios may play a role in inducing partial, and/or complete, loss of structural conti-



Partial Elimination of Lacunar Structural Continuity – Smaller Aqueous Pore

Complete Elimination of Lacunar Structural Continuity – Closed Aqueous Pore

Figure 7. Schematic illustration of possible structural modes of interaction of aqueous lacunar domains in the hydrated skin barrier with glycerol. Aqueous lacunar domains, shown in grey, gain structural continuity in hydrated skin to form an aqueous pore. However, when glycerol is added to the hydrated skin barrier, lacunar domains, shown in black, lose structural continuity due to glycerol binding water and minimizing water mobility, either partially, resulting in a smaller aqueous pore, or completely, resulting in a closed aqueous pore.

nuity of the lacunar domains, thereby resulting in a reduction in the radius, and/or in the number density of the aqueous pores in the SC.

On the other hand, *in vitro* as well as *in vivo* studies document that surfactants like SDS have an opposite effect on the SC lipids and on the corneocyte keratins. SDS has been shown to induce direct alteration to the structure of the intercellular lipid mortar (48,49), as well as to disrupt the keratin structure of the corneocytes in the SC (16,50,51). Both of these effects can induce the formation of additional lacunar domains, as well as enhance the structural continuity of existing lacunar domains. This is how SDS may induce an increase in the radius, and/or in the number density, of the aqueous pores in the SC. A mixture of SDS and glycerol in an aqueous contacting solution will result in: (a) glycerol reducing and (b) SDS increasing the radius and the number density of the aqueous pores in the SC. These considerations may help rationalize how adding 10 wt% glycerol to an SDS aqueous contacting solution can reduce the radius and the number density of the aqueous pores induced by SDS in the SC.

CONCLUSIONS

According to a well-accepted view in the cosmetics literature, surfactant micelles cannot penetrate into the skin due to size limitations, and as a result, surfactant-induced skin barrier perturbation should be determined solely by the concentration of the surfactant monomers (11–23). Moore *et al.* (11) have recently shown that this is not the case for a model skin irritant, the surfactant SDS. Instead, they hypothesized that SDS micelles can penetrate into the skin barrier and induce skin barrier perturbation. In this paper, for the first time, using mannitol transdermal permeability and average skin electrical resistivity measurements in the context of a hindered-transport aqueous porous pathway model, we have demonstrated *in vitro* that SDS induces an increase in the average radius of the skin aqueous pores, from $20 \pm 3\text{\AA}$ to $33 \pm 5\text{\AA}$, such that the SDS micelles of size $19.5 \pm 1\text{\AA}$ can penetrate into the SC through these aqueous pores. In addition, SDS induces a sevenfold increase in the number density of these aqueous pores, thereby significantly enhancing the SDS micellar contribution to SDS skin penetration and to skin barrier perturbation *in vitro*.

Using *in vitro* skin radioactivity measurements, we demonstrated that adding 10 wt% glycerol to an aqueous SDS micellar contacting solution significantly reduces: (i) the total extent of SDS skin penetration and (ii) the SDS micelle contribution to SDS skin penetration. This is due to the fact that glycerol eliminates almost completely the contribution of the SDS micelles to SDS skin penetration. Through dynamic light-scattering measurements, we have verified that glycerol does not increase the size of the SDS micelles, which if increased, could have minimized the SDS micellar contribution to SDS skin penetration. In addition, through surface tension measurements that were used to determine the CMC values of SDS in water and in a 10 wt% glycerol aqueous solution, we have shown that glycerol does not reduce the concentration of the SDS monomers contacting the skin, which if reduced, could have minimized the SDS monomeric contribution to SDS skin penetration. Using *in vitro* transdermal permeability and average skin electrical resistivity measurements upon exposure of the skin to aqueous contacting solutions of SDS and of SDS + 10 wt% added glycerol, in the context of a hindered-transport aqueous porous pathway model, we have conclusively demonstrated

that the addition of 10 wt% glycerol prevents SDS micelles from penetrating into the skin barrier by: (a) reducing the radius of the skin aqueous pores induced by the SDS aqueous contacting solution, from $33 \pm 5\text{\AA}$ to $20 \pm 5\text{\AA}$, such that an SDS micelle of radius $18.5 \pm 1\text{\AA}$ in an aqueous SDS micellar solution with 10 wt% added glycerol experiences steric hindrance and cannot penetrate into the SC, and (b) reducing the number density of the skin aqueous pores by more than 50%, thereby further reducing the ability of the SDS micelles to penetrate into the SC and induce skin barrier perturbation.

APPENDIX

DETERMINATION OF THE RADIUS AND THE NUMBER DENSITY OF THE SKIN AQUEOUS PORES RESULTING FROM EXPOSURE OF $_{\rm P}\text{-}{\rm FTS}$ TO SDS AQUEOUS CONTACTING SOLUTIONS

Average skin electrical resistivities, R, and mannitol-skin permeabilities, P, were measured upon exposure of p-FTS to SDS aqueous contacting solutions, as discussed in the text, and the resulting log P vs log R plot is shown in Figure 6 (see diamonds and the dashed line).

It is noteworthy that the slope of the best-fit straight line (the dashed line) through the diamonds in Figure 6 is 0.98 ± 0.06 , which is statistically similar to the theoretical value of -1 (see equation 4). The R^2 value is 0.96, which is close to 1. Hence, these results lend further support to the validity of the hindered-transport skin aqueous porous pathway model developed by Tang *et al.* (7). The intercept value in Figure 6 is -2.90 ± 0.03 .

The infinite-dilution diffusion coefficient of mannitol, D_p^{∞} , is 0.672×10^{-5} cm²/s at 25°C (6,7). The hydrodynamic radius of mannitol, r_p, is 4.44 Å (6,7). Because skin electrical currents were measured in PBS that contained Na⁺ and Cl⁻ as the dominant ions, the Na⁺ ions were used to model the current-carrying ions present in the solution. The infinite-dilution diffusion coefficient of the Na⁺ ions, D_{ion}^{∞} , is 1.33×10^{-5} cm²/s at 25°C (7). The hydrodynamic radius of the Na⁺ ion, r_{ion} , is 2.2 Å (7). In addition, we have used the following parameter values in C (see equation 4) in the Theoretical section): $k_B = 1.38 \times 10^{-23}$ J/K (Boltzmann constant), T = 298 K, F = 9.6485 × 10⁴ C/mol (Faraday constant), z = 1 (in the PBS solution, since NaCl is the dominant electrolyte), $c_{ion} = 0.137$ M, and $e_0 = 1.6 \times 10^{-19}$ C. Using these parameter values, along with the experimentally determined value of C, we were able to determine the value of the ratio: $H(\lambda_p)/H(\lambda_{ion})$ (see the expression for C in the Theoretical section). Next, using: (i) equation 5, (ii) the hydrodynamic radii values of mannitol and Na⁺, that is, 4.44 and 2.2 Å, and (iii) the value of the ratio $H(\lambda_p)/H(\lambda_{ion})$, we were able to numerically solve for the average pore radius, r_{pore}. The average pore radius, r_{pore}, was found to be 33 ± 5 Å, which we have taken as the radius of the skin aqueous pores. Note that $H(\lambda_p)$ and $H(\lambda_{ion})$ are each less than 0.4, which is necessary for equation 5 to be valid (6,7,42). Having determined the aqueous pore radius, r_{pore} , the pore number density was determined using equation 6, in which all the parameters, except for ε/τ , the pore number density, are known in advance (since $\Delta X = 15 \mu m$) (6,7).

The aqueous pore number density, ε/τ , for p-FTS exposed to the PBS control aqueous solution was determined using a calculation similar to the one for p-FTS exposed to the

SDS aqueous contacting solutions presented in this appendix. Finally, the aqueous pore number density (ε/τ) value resulting from the exposure of p-FTS to the SDS aqueous contacting solutions was normalized by the ε/τ value resulting from the exposure of p-FTS to the PBS control aqueous solution. We calculated this normalized value, $(\varepsilon/\tau)_{normal}$, to be 7 ± 1 (see Table I).

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