J. Cosmet. Sci., **68,** 219–231 (May/June 2017)

Development of a preclinical surfactant skin penetration assay to reflect exposure times typical of consumer use

NICOLE MCCARDY, RYAN THOMPSON, MATTHEW MILLER, PETER STYCZYNSKI, STEPHANIE VENTURA, ROBERT GLENN, and GERALD B. KASTING
James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, OH (N.M., M.M., S.V., G.B.K.), and Research and Development Department, The Procter & Gamble Company, Cincinnati, OH (R.T., P.S., R.G.).

Accepted for publication May 30, 2016.

Synopsis

Mixed surfactant and surfactant–polymer compositions have been reported to decrease surfactant deposition onto and penetration into the skin relative to single surfactant compositions, potentially improving the mildness of the product. Previous workers in this area [see Moore *et al.*, *J. Cosmet. Sci.* **5**4:29–46 (2003), and subsequent publications] employed a procedure in which excised porcine skin was exposed to a surfactant solution containing radiolabeled sodium dodecyl sulfate (^{14}C-SDS) for 5 h. We have developed an improved SDS penetration assay using excised human skin that reflects typical consumer exposure times for rinse-off products. Using the new protocol, we were able to see a significant decrease in ^{14}C-SDS penetration from a sodium lauryl sulfate (SLS)/polyethylene oxide composition applied to excised skin for either 2 or 10 min, as compared to SLS only. Furthermore, differences between the SDS penetration patterns on porcine skin and human skin were seen with a second SLS/polymer system; consequently, we do not recommend porcine skin for routine mildness screening by ^{14}C-SDS penetration.

INTRODUCTION

Surfactants are amphiphilic molecules that adsorb at air–liquid, liquid–liquid, and liquid–solid interfaces, reducing surface and interfacial tensions, respectively. Surfactants have multiple industrial uses as emulsifying and cleansing agents, especially in the cosmetic and personal care industry. Human skin can be exposed to surfactants from short periods of time (for rinse-off cleansers) to long periods of time (for leave-on emulsions). Depending on how long the skin is exposed, a surfactant can penetrate to a greater or lesser extent into the layers of the skin. This penetration has been linked to skin irritation (1–3).

Sodium dodecyl sulfate (SDS) is a common anionic surfactant often found in cleansing products. It has been shown that with increasing concentrations of SDS above the critical

Address all correspondence to Gerald B. Kasting at Gerald.Kasting@uc.edu.
Present address for Nicole McCardy, Dermazone Solutions, St. Petersburg, FL 33713.

micelle concentration (CMC), there is a dose-dependent increase in the amount of SDS measured in the skin (1). When certain polymers are added to SDS and other surfactant systems, SDS penetration is reduced (1,3,4). Polymers can interact with surfactant micelles and modify the adsorption behavior of the surfactant (1,3,5,6). For such interaction, a "pearls on a string model" has been proposed, where surfactants self-assemble around the hydrophobic portions of the polymers to form hemimicelles (5,6). In general, for water-soluble polymers, the more hydrophobic the polymer, the stronger the interaction (6). In the case of SDS, the ionic repulsion between the "micellar pearls" leads to an expansion of the polymer chain, which causes an increase in blob size (i.e., the size/length of the polymer molecule). This model has been validated through viscosity measurements and neutron scattering.

When the surfactant concentration is above the CMC, micelles can begin to form. A widely accepted view of surfactant penetration through the skin, as reviewed by Moore *et al.* (1), is that "at surfactant concentrations that exceed the CMC, where surfactant micelles first form, only surfactant monomers can penetrate into the skin, because the surfactant micelles are not surface active, or they are too large to penetrate into the stratum corneum (SC)." This theory is known as the monomer penetration model (7); it is largely based on clinical observations using surfactant mixtures.

This view was challenged in 2003 by the Blankschtein group at the Massachusetts Institute of Technology (1), who showed that addition of polyethylene oxide (PEO, MW ~8000) to SDS solutions reduced the penetration of ^{14}C-radiolabeled SDS into porcine SC at levels well above the CMC (1). The Moore *et al.* study (1) and subsequent publications from this group (3) employed a 5-h exposure time of the skin to the surfactant solution.

The objectives of the present study were to confirm the effects of polymer addition on SDS penetration into human skin and to determine whether the exposure time could be further reduced to reflect conditions closer to consumer usage of rinse-off products. We furthermore sought to simplify the assay and maximize its sensitivity. Because the envisioned use of the assay was to screen prototype rinse-off product formulations, we used commercial-grade surfactants and polymers rather than highly purified materials. A limited study of the solution properties of these materials was conducted to provide partial characterization. Notably, the bulk surfactant was sodium lauryl sulfate (SLS), which contains a natural mixture of alkyl chain lengths as well as residual impurities, whereas the radiolabeled marker was the purified C_{12} homolog, SDS. We will maintain this distinction throughout the article. Experimental SDS penetration trials on human skin were then conducted using exposure times of 10 and 2 min. A simplified protocol, in which the tape-stripping step was eliminated, was employed for the 2-min exposure protocol; furthermore, a random controlled block design, followed by a two-way analysis of variance of $\log_{10}$-transformed data, was employed to increase sensitivity (8). The report presents the details of these studies and provides a recommendation for further use of this assay.

MATERIALS AND METHODS

Aqueous solutions of SLS (50 mM), SLS with 2% polyethylene glycol (PEG 8000, hereafter referred to as PEO) and SLS with 2% polyvinyl alcohol (PVA) were provided by the Procter & Gamble (P&G) Company (Cincinnati, OH). The SLS sample was a commercial-grade material showing evidence of surface-active impurities. The PVA raw material had

an average molecular weight of 30,000 Da and 17% of unhydrolyzed acetate groups. Common commercial PVAs have an acetate content of 4–12% (9). Radiolabeled SDS (^{14}C-SDS, 55 mCi/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO). Tritiated water ($^{3}H_2O$, 1.0 mCi/ml) and the tissue-dissolution reagents Soluene®-350 and Solvable™ were obtained from Perkin Elmer (Waltham, MA). Dulbecco's phosphate-buffered saline (PBS) and sodium azide were obtained from Fisher Scientific (Pittsburgh, PA). Deionized (DI) water was prepared by ultrafiltration. D-Squame™ tapes were obtained from CuDerm (Dallas, TX). Pig skin was obtained from a local slaughter house and dermatomed to a thickness of ~800 µm. Human cadaver skin, dermatomed to a thickness of 300–400 µm, was obtained from the New York Firefighters Skin Bank (New York, NY). A different donor was used in each experimental trial. The source and identity of each human donor skin sample (age, ethnicity, gender, date of death, and cause of death) was documented. The pig skin studies were approved by the P&G Institutional Animal Care and Use Committee, and work on de-identified human tissues was exempted from human subjects' categorization by the University of Cincinnati Academic Health Center Institutional Review Board.

PREPARATION OF SKIN MEMBRANES

Human skin was stored at −80°C until use. On the morning before the study, the skin was thawed rapidly by immersing the sealed packet in warm water. It was then rinsed with distilled water and cut into 2 × 2 cm pieces using a scalpel. Porcine skin taken from the belly area was obtained from a slaughterhouse, stored in chilled saline, and used within 24 h of collection.

IN VITRO STATIC DIFFUSION CELLS

The skin membranes were mounted in Franz diffusion cells (0.79 cm^2) (10) with the SC facing the donor chamber. The receptor solution (~5 ml) was Dulbecco's PBS (pH 7.4) to which 0.02% w:v sodium azide had been added to retard microbial growth. The receptor fluid was continuously stirred using a magnetic stir bar. The cells were maintained at 37°C in a thermostatted aluminum block, yielding a skin-surface temperature of 32°C. Low glass tops with no occlusion were used for this study.

HUMAN SKIN MEMBRANE INTEGRITY ASSESSMENT

The integrity of each of the human skin membranes was assessed by $^{3}H_2O$ penetration (8). The skin was mounted and allowed to equilibrate for about 1 h. A 150 µl aliquot of $^{3}H_2O$ (0.4 µCi/ml) was applied using a pipette and allowed to remain on the skin surface for 5 min. It was then removed with a cotton-tipped swab, which was placed on the skin surface for 30 s. The receptor solution was collected 60 min after dose and replaced with PBS. The collected samples were analyzed for ^{3}H in Ultima Gold XR cocktail (Perkin Elmer, Waltham, MA) by liquid scintillation counting (LSC) using a Beckman LS 6500 counter (Beckman Coulter, Inc., Indianapolis, IN). They were counted for 1 min, and the results

were reported as µl $^3H_2O/cm^2$. Samples with water permeation greater than 2.0 µl $^3H_2O/cm^2$ were discarded. The remaining cells were ranked in order of increasing water permeability to facilitate the random controlled block experimental design (8). The receptor exchange procedure was repeated, and the cells were allowed to wash out overnight. A final exchange was performed in the morning before dosing.

Porcine skin integrity was assessed visually to ensure the absence of large hair follicles.

SURFACTANT PENETRATION PROTOCOL—PORCINE SKIN

^{14}C-SDS solutions (50 mM SLS + 6.7 µCi/ml ^{14}C-SDS) in DI water, with and without 2% w/w of added polymer, were prepared and shaken to ensure homogeneity. The SLS concentration corresponds to 1.44% w/v, about 10-fold lower than typical anionic surfactant concentrations in a shampoo or shower gel. This is a commonly accepted dilution factor for consumer exposures. The test concentration was furthermore about 16-fold higher than the apparent CMC for the SLS sample, so most of the SLS in these formulations existed in either micellar or polymer-bound micellar form. A 150 µl aliquot (10 µCi) of the surfactant solution was pipetted onto each skin membrane. Skin from one donor was exposed to the surfactant solution for 10 min (n = 6/treatment).

After the surfactant exposure, the dose solution was removed using a transfer pipet. The surface of the skin was rinsed three times with 0.5 ml of tap water for 10 s, and the rinses were collected and pooled. The receptor solution was collected, and each skin sample was wiped two times with Whatman filter paper (GE Healthcare Life Sciences, Pittsburgh, PA) soaked with PBS/Tween 20 and once with 70%/30% ethanol/water to remove unabsorbed (residual) product. Wipes were collected and pooled for mass balance determination. After surface rinsing, the surfaces of the skins were dried, and 10 tape strips (D-Squame™) were collected. The tapes were placed directly into Ultima Gold XR cocktail to be analyzed individually. After tape stripping, the remaining epidermis was dissected from the dermis, and the skin sections were dissolved in 0.50–1.25 ml Soluene-350™ at 50°C overnight. Radioactivity in receptor collections, surface rinses, filter paper wipes, tape strips, and solubilized tissue sections was determined using LSC. Results were expressed as µg/cm^2 ^{14}C-SDS equivalents or % of applied radioactive dose. The arithmetic mean and standard error mean (SEM) were reported for each treatment.

SURFACTANT PENETRATION PROTOCOL—HUMAN SKIN

^{14}C-SDS solutions (50 mM SLS + 6.7 µCi/ml ^{14}C-SDS) in DI water, with and without 2% w/w of an added polymer, were prepared and shaken to ensure homogeneity. A 150 µl aliquot (10 µCi) of the surfactant solution was pipetted onto each skin membrane, which were rank ordered in terms of permeability based on the ^3H_2O prescreening results. The rankordering and subsequent randomization by treatment were key elements in maximizing the sensitivity of the assay (8).

Two sets of experiments were conducted. In Experiment 1, skin from three donors was exposed to the surfactant solution for 10 min (n = 4–6/donor). The total sample size was n = 14–15/treatment. In Experiment 2, skin from four donors was exposed to the surfac-

Table I
Dose Solutions and Sample Sizes for Human Skin ^{14}C-SDS Penetration Studies

	Sample size (n)[a]	
Treatments	Expt. 1 10-min	Expt. 2 2-min
50 mM SLS (control)	14	20
50 mM SLS + 2% PEO	15	21
50 mM SLS + 2% PVA	14	21

[a]Sum of replicate samples from 3 to 4 donors.

tant solution for 2 min (n = 2–7/donor). The total sample size was n = 20–21/treatment. The treatment groups are summarized in Table I.

The same method of collection was used as with porcine skin after the surfactant exposure. For Experiment 1, 4–10 tape strips were performed on each sample. The tapes were put directly into Ultima Gold XR cocktail and analyzed individually. The residual epidermis and the dermis were also physically separated and dissolved overnight in Solvable™. For Experiment 2, neither tape stripping nor physical separation of the skin layers was conducted; instead, the rinsed skin samples were removed from the diffusion cells and directly dissolved in 2 ml of Solvable™. This choice followed from the fact that Experiment 1 showed most of the residual radioactivity in the skin samples to be recovered in the first three tape strips and only very low radioactivity levels in the lower skin layers. All samples were analyzed by LSC for 5 min or until 2% accuracy [2 relative standard deviations (SDs)] was reached. Results were expressed as microgram per square centimeter ^{14}C-SDS equivalents in the various samples, after background subtraction. The deposited dose was calculated as the total amount of ^{14}C-SDS in skin plus the receptor solution.

HUMAN SKIN STATISTICAL ANALYSIS

Outliers were detected using Dixon's test on the full dataset following a logarithmic transformation of the individual sample values (8). Outlying results were rejected if they exceeded the 95% confidence limit. The least squares mean and standard error for each skin donor were calculated; these values were then averaged arithmetically over donors to obtain the final mean and standard error.

Results were expressed as microgram per square centimeter ^{14}C-SDS equivalents in the various samples, after background subtraction. Statistical comparisons between treatments were made via two-way ANOVA on the ($\log_{10}$)-transformed values, using skin donor and treatment as the blocking variables. There was a significant statistical difference between skin donors for both Experiments 1 and 2, yielding $p < 0.001$ and $p = 0.020$, respectively. The SEM between donors ranged from 0.052 to 0.086 μg/cm^2 for Experiment 1 and from 0.061 to 0.110 μg/cm^2 for Experiment 2. Differences between treatments were highly significant, with $p < 0.001$ for Experiment 1 and $p = 0.009$ for Experiment 2. There were no significant interactions between skin donor and treatment for either study. Therefore, differences attributed to skin donor did not depend on treatment and vice versa.

MICELLE/MACROMOLECULE SIZE ANALYSIS

A pilot study of micelle, polymer, and/or surfactant/polymer complex size in the test formulations was conducted by dynamic light scattering. Approximately 1.5 ml of test formulation (50 mM SLS with or without added polymer) was filtered through a 0.1-μm syringe filter. The first 0.25 ml was discarded to avoid contamination. The filtered solution was poured into a disposable polystyrene cuvette and analyzed via a 173°-backscatter measurement using a Zetasizer (Malvern Instruments Ltd., Malvern, UK). Results were reported as scattering intensity versus hydrodynamic radius, r_h. Because the SLS–polymer formulations contained an excess of each polymer relative to its ability to bind SLS [*cf.* Cabane (11) for the SDS/PEO system; see also Goddard (6)], and the SLS concentrations were high relative to its tendencies to self-aggregate and bind to polymers (see next section), it is probable that the r_H values obtained for the SLS–polymer compositions represent a weighted average of polymer and SLS/polymer aggregate radii. In the absence of polymers, the measurements represent the characteristic size of SLS micelles for a representative commercial SLS sample.

CMC AND RELATED SURFACE TENSION MEASUREMENTS

The surface tension, γ, of each test formulation as a function of concentration was determined using a Krüss K100 tensiometer (Krüss USA, Matthews, NC) fitted with a Wilhelmy plate. The measurements were made by sequentially diluting the test compositions with DI water; thus, the ratio of SLS and polymer was maintained constant. Apparent CMC for SLS and critical aggregation concentration (CAC) for the SLS–polymer mixtures were determined from inflection points on a plot of γ versus log concentration as described later. These methods are approximate and should not be construed to replace more precise measurements made using pure SDS and constant polymer concentrations (11,12). Test formulation solutions were prepared with Millipore water (17.8 MΩ) and studied 1–3 times.

RESULTS

^{14}C-SDS PENETRATION INTO PORCINE SKIN

Figure 1 shows the ^{14}C-SDS penetration results on porcine skin. Total recovery of radiolabel in the SC after a 10-min exposure to the 50 mM SLS control was ~100 μg/cm^2 ^{14}C-SDS equivalents, corresponding to 3.7% of the applied radioactive dose. Addition of 2% PEO to this formulation reduced penetration by ~80%. This result is consistent with values previously reported by Moore *et al.* (1). Addition of 2% PVA yielded a similar result.

^{14}C-SDS PENETRATION INTO HUMAN SKIN

Figure 2 shows ^{14}C-SDS penetration into and through human skin after a 10-min exposure. Nearly all of the recovered radioactivity was found in the tissue, with ≤1% penetrating into

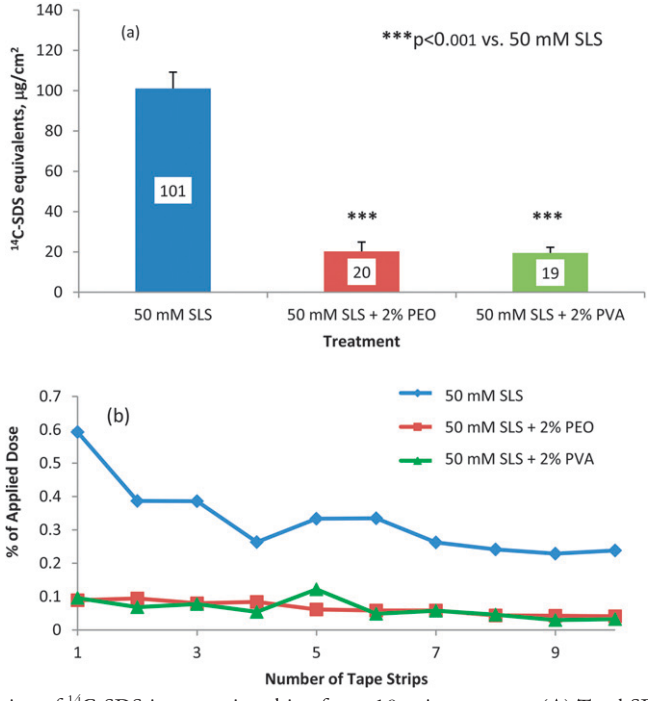

Figure 1. Penetration of ^{14}C-SDS into porcine skin after a 10-min exposure. (A) Total SDS recovered in the porcine SC; (B) Penetration profile into SC obtained from D-Squame tape strips. The applied dose corresponded to 2740 µg/cm^2 of ^{14}C-SDS equivalents.

the receptor solutions. Tabular results showing the distribution are given in the Appendix. The 50 mM SDS control showed ~16 µg/cm^2 ^{14}C-SDS equivalents was recovered from the human skin membrane–receptor solution, an approximately sixfold reduction from the value measured in porcine skin. The treatment with 2% PEO was observed to significantly reduce skin penetration. Unlike the porcine skin result, reduction of ^{14}C-SDS skin penetration by 2% PVA was not statistically significant.

To test an exposure time closer to consumer use, a 2-min study was conducted. Figure 3 shows the penetrated ^{14}C-SDS dose; the distribution is given in the Appendix. The SLS control formulation yielded an average of 7.3 µg/cm^2 ^{14}C-SDS equivalents in the human skin membrane–receptor solution, with only 0.1% of this total found in the receptor solution. In the presence of 2% PEO, the amount of SDS recovered in the skin was significantly reduced. The presence of 2% PVA did not yield a statistically significant reduction in penetration.

MICELLE/MACROMOLECULE SIZE ANALYSIS

The 50 mM SLS composition yielded a scattering intensity distribution peaked at a hydrodynamic radius (r_h) of 1.14 ± 0.27 nm (mean ± SD, n = 6). SLS + 2% PEO yielded a bimodal intensity distribution with the smaller component centered at r_h = 1.52 ± 0.10 nm (n = 3). SLS + 2% PVA yielded a bimodal size distribution with the

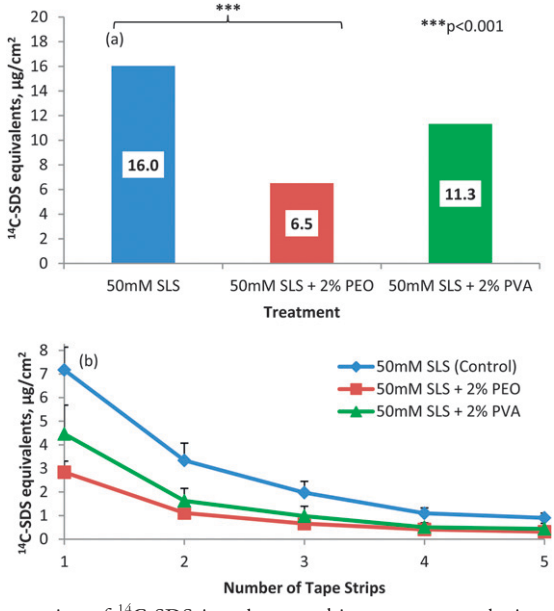

Figure 2. (A) Total penetration of ^{14}C-SDS into human skin + receptor solution after a 10-min exposure (Experiment 1); (B) Penetration profile into human SC obtained from D-Squame tape strips.

smaller component centered at $r_h = 1.28 \pm 0.03$ nm ($n = 2$). Additional details as well as small angle neutron scattering results are available from the authors (13). The SLS micelle radius was approximately one half that of the SDS micelle radius of 2.1 nm reported by Moore *et al.* (1), which was measured in 100 mM NaCl. The difference may be largely attributed to the effect of added salt, which is well known to swell SDS micelles (14).

CMC AND RELATED SURFACE TENSION MEASUREMENTS

The SLS sample yielded a surface tension profile characteristic of an anionic surfactant containing one or more highly surface-active impurities (Figure 4). A minimum value of $\gamma = 19.9$ mN/m was obtained at an SLS concentration (C) of 3.1 mM. For comparison, pure SDS yields $\gamma \approx 38$ mN/m at its CMC of 8.2 mM (15). There was an inflection point

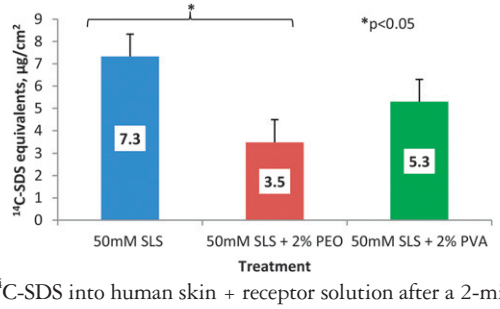

Figure 3. Penetration of ^{14}C-SDS into human skin + receptor solution after a 2-min exposure (Experiment 2).

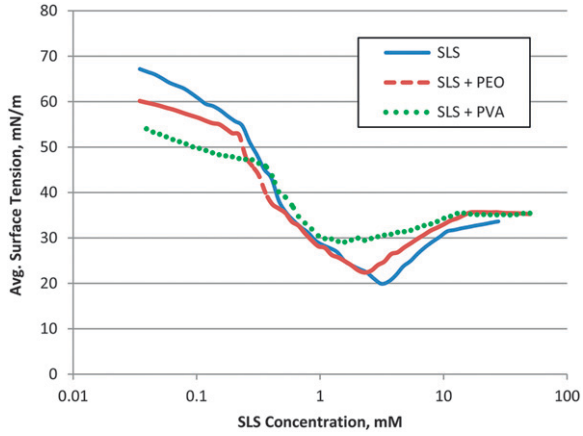

Figure 4. Surface tension of SDS and SDS/polymer solutions obtained by sequentially diluting the compositions described in Table I with Millipore™ water.

in the plot of γ versus log C at a concentration of 0.23 mM that can be interpreted on the basis of the Gibbs adsorption isotherm as tighter packing at the air–liquid interface for concentrations above this value (15).

The SLS + polymer systems both yielded lower surface tension at low concentrations than did SLS alone; thus, the polymers were surface active. The PVA employed in this study was more surface active than PEO, consistent with its high level of unhydrolyzed acetate groups. Inflection points at 0.22 mM (SLS + PEO) and 0.36 mM (SLS + PVA) may partly reflect the influence of the SLS impurities, but also the advent of surfactant/polymer binding. It is tempting to describe these values as "apparent CACs." However, working at a constant PEO concentration of 2 g/l or 0.2% w/v, Cabane (11) identified the CAC for the SDS/PEO system as about 5.5 mM (see Figure 1 in Ref. 11), some 25-fold higher than the inflection point in Figure 4. Consequently, Figure 4 should be interpreted cautiously. But, it does provide some evidence that, for the materials employed in this study, PEO interacted more strongly with SLS than did PVA.

DISCUSSION

SDS evidently penetrates the upper layers of the SC quite rapidly, as shown by the tape-strip results in Figures 1 and 2. It binds primarily to keratin and gradually swells and disrupts the tissue (16). In the short exposures employed in this study, very little (<1%) of the deposited ^{14}C-SDS permeated through the skin into the receptor solutions. In human skin, it was predominately found on the first three tape strips (Figure 2B). A comparison of Figures 1 and 2 suggests that free SLS penetrated into porcine skin much more rapidly than it did into human skin. The ^{14}C-SDS penetration reported in Figure 1A for a 10-min exposure of porcine skin to 50 mM SLS approaches that observed by Moore *et al.* (1) in a 5-h exposure. If one estimates the dry weight of the epidermis in the Moore *et al.* study to be 6.5 mg/cm^2, then the SDS concentration of 2.6 wt% reported by these workers corresponds to ~170 µg/cm^2, about 70% higher than that shown in Figure 1A. The comparison suggests that the rapid ^{14}C-SDS penetration observed in the present studies slows considerably during longer exposures.

Human skin treated for either 2 or 10 min also yielded a statistically significant difference between SDS and SDS + PEO, although the magnitude of inhibition by the polymer was smaller than in porcine skin. The third treatment, 50 mM SLS + 2% PVA, yielded a statistically significant reduction in penetration versus SLS for porcine skin, but not for human skin. The reason for this difference is not known. However, the interaction of PVA with anionic surfactants is generally considered to be weaker than that of PEO; this belief is encoded in an affinity sequence PVA < PEO < MeC < PVAc ≤ PPO ~ PVP originally attributed to Breuer and Robb (17) and republished frequently since that time, e.g., (6,18). One might anticipate from this sequence that PVA would have less impact on surfactant penetration into skin than PEO at comparable concentrations, as it binds the surfactant less tightly. The tensiometry data in Figure 4 support this hypothesis. In the present study, the PVA test material was not fully hydrolyzed, potentially pushing it closer to polyvinyl acetate (PVAc) in the surfactant affinity sequence. Furthermore, the surface activity of the PVA material was higher than that of PEO. Positioning the effectiveness of this material to inhibit surfactant penetration was an objective of the test.

Statistical differences between PEO and the control were stronger for the 10-min exposure time than for the 2-min exposure. This could be due to the fact that the skin used for the 10-min study had more consistent $^{3}H_2O$ permeation results than that used for the 2-min study. This difference in consistency is highlighted in Table II; the SD was lower for the 10-min exposure than for the 2-min exposure. Despite this difference, both experiments revealed a similar pattern of surfactant skin penetration. Figure 5 displays the distribution of the $^{3}H_2O$ permeation values for the membranes used in both experiments, which further emphasizes the difference in $^{3}H_2O$ permeation.

Unlike porcine skin, the treatment with 2% PVA did not statistically reduce ^{14}C-SDS skin penetration in either human skin experiment. This could be due to the fact that porcine skin does not contain eccrine sweat glands, which is an additional route of entry for excipients, or to differences in pore structure that excluded SLS/PVA complexes from pig skin but not from human skin. However, it could simply result from chance. We are not convinced that the pore structure of the substrate is the major determinate of surfactant penetration and offer the following thoughts on this subject, without claiming to know the answer.

The "penetration" process can be thought of as deposition and binding of surfactant onto surface keratins, leading to swelling and opening of the keratin structure, followed by more facile diffusion of unbound surfactant into underlying lipid and protein layers. In this scenario, penetration of both monomeric and micellar SLS into the outer SC is rapid because of the loss of barrier lipids in the desquamating layers. Bulky structures such as surfactant/polymer complexes diffuse from applied formulations to the skin surface more

Table II
Median, Mean, and SD Values for $^{3}H_2O$ Permeation

	10-min Expt. 1	2-min Expt. 2
Median	1.23	1.22
Mean	1.22	1.22
SD	0.34	0.68

Values reflect pooled data from 3 or 4 donors.

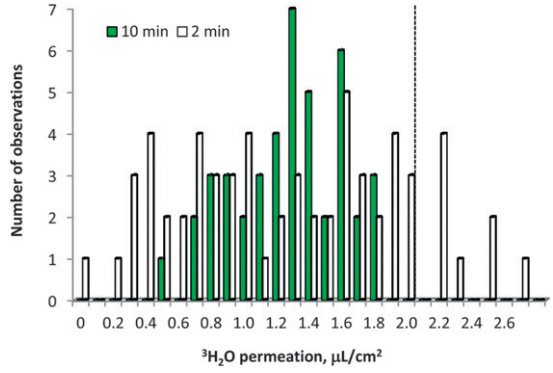

Figure 5. Distribution of ^3H_2O permeation values obtained for membranes used in the 10- and 2-min studies. Dashed line indicates cutoff of 2.0 µl/cm^2. Membranes with permeation greater than this value were discarded.

slowly than surfactant alone; their deposition onto the SC surface in a consumer-relevant 2-min exposure would thus be reduced relative to surfactant alone.

The alternative scenario of micellar penetration through pores in the SC presented by Moore *et al.* (1) and supported by other articles from this group (3,7) is not ruled out by the previous argument. But, it seems to us that it is not necessary to invoke the presence of microscopic pores of a specific size to explain the polymer impact on penetration observed in this study. There are several lines of evidence showing that PVA binds anionic surfactants less tightly than does PEO. The desquamating layers of the SC are more porous than lower SC layers, especially when swollen by SLS or other anionic surfactants. We propose that both monomeric and micellar surfactant could diffuse directly into these layers without requiring a separate pore structure. The fact that penetration appears to slow substantially after the initial deposition and swelling process (*cf.* Figure 2 (10 min) vs. Moore *et al.* (5 h)) suggests that loss of barrier lipids in the outer SC leads to rapid penetration of exogenous substances regardless of their size.

CONCLUSION

Human skin admitted substantially less radiolabeled surfactant than did porcine skin in identical exposure scenarios. The addition of 2% PEO to 50 mM SLS solution significantly lowered ^{14}C-SDS penetration for both 10- and 2-min exposures on human skin. This result mirrored that from 5-h porcine skin studies of Moore *et al.* (1) and a 10-min study in porcine skin (Figure 1). Unlike the porcine skin, addition of 2% PVA did not lower penetration significantly in either of the human skin experiments; however, the 10- and 2-min exposures revealed a similar pattern of penetration. Statistical differences between SLS + PEO and SLS were stronger for the 10-min exposure time versus 2-min; this could be due to the fact that the skin used for the 10-min study had more consistent ^3H_2O permeation results than the 2-min study. Based on these results, we recommend the 2-min human skin protocol for further studies. It provides differentiation between treatments comparable with the 10-min human skin protocol and corresponds more closely to typical consumer use time for rinse-off products. The 10-min porcine skin protocol gave a result for SLS + 2% PVA that was not confirmed in the human skin studies; furthermore, porcine skin admitted substantially more SDS than did human skin in iden-

tical exposures. Consequently, we cannot recommend porcine skin for routine mildness screening by ^{14}C-SDS penetration.

ACKNOWLEDGMENTS

Financial support for this research was provided by the Procter and Gamble Company. We thank Tiffany Brooks and Keun Il Kim for help in conducting the human skin penetration assays.

APPENDIX

Tabular results for 10- and 2-min ^{14}C-SDS penetration into human skin (Tables III and IV).

Table III
Treatments, Sample Sizes, and ^{14}C-SDS Disposition for 10-min Exposure on Human Skin (Expt. 1)

	μg/cm^2 ^{14}C-SDS equivalents				
Treatment	Wipe[a]	Receptor[b]	Penetration[c]	(+) SE	(–) SE
50 mM SLS (control) (n = 14)	8.0 ± 1.9	0.03 ± 0.01	16.0	2.3	2.0
50 mM SLS + 2% PEO (n = 15)	2.8 ± 0.6	0.05 ± 0.03	6.5	0.8	0.7
50 mM SLS + 2% PVA (n = 14)	12.9 ± 7.0	0.09 ± 0.05	11.3	1.5	1.3

[a]Radioactivity removed with wetted filter papers after the wash step.
[b]Radioactivity measured in receptor solution.
[c]Geometric mean of the total radioactivity found in all layers of skin + receptor solution.

Table IV
Treatments, Sample Sizes, and ^{14}C-SDS Disposition for 2-min Exposure on Human Skin (Expt. 2)

	μg/cm^2 ^{14}C-SDS equivalents				
Treatment	Wipe	Receptor	Penetration	(+) SE	(–) SE
50 mM SLS (control) (n = 20)	3.2 ± 0.6	0.06 ± 0.04	7.3	1.2	1.0
50 mM SLS + 2% PEO (n = 21)	3.1 ± 1.5	0.07 ± 0.05	3.5	0.5	0.5
50 mM SLS + 2% PVA (n = 21)	3.2 ± 0.4	0.11 ± 0.07	5.3	0.8	0.7

Columns have the same meaning as in Table III.

REFERENCES

(1) P. N. Moore, S. Puvvada, and D. Blankschtein, Challenging the surfactant monomer skin penetration model: Penetration of sodium dodecyl sulfate micelles into the epidermis, *J. Cosmet. Sci.,* **54(1),** 29–46 (2003).
(2) C. Froebe, F. Simion, L. Rhein, R. Cagan, and A. Kligman, Stratum corneum lipid removal by surfactants: Relation to *in vivo* irritation, *Dermatology,* **181(4),** 277–283 (1990).
(3) S. Ghosh and D. Blankschtein, The role of sodium dodecyl sulfate (SDS) micelles in inducing skin barrier perturbation in the presence of glycerol, *J. Cosmet. Sci.,* **58,** 109–133 (2007).
(4) M. J. Fevola, R. M. Walters, and J. J. LBrizzi, A new approach to formulating mild cleansers: Hydrophobically-modified polymers for irritation mitigation, in Polymeric Delivery of Therapeutics, S. E. Morgan and R. Y. Lochhead, eds. (American Chemical Society, New York, 2010), pp. 221–242.

(5) R. Y. Lochhead and L. R. Huisinga, A brief review of polymer/surfactant interaction, *Cosmet. Toilet.,* **119,** 37–45 (2004).
(6) E. D. Goddard, Polymer/surfactant interaction: Manifestations, methods, and mechanisms, in Principles of Polymer Science and Technology in Cosmetics and Personal Care, E. D. Goddard and J. V. Gruber. Eds. (Marcel Dekker, New York, 1999), pp. 128–195 (online version).
(7) P. N. Moore, S. Puvvada, and D. Blankschtein, Role of the surfactantpolar head structure in protein-surfactant complexation: Zein protein solubilization by SDS and by SDS/C12E surfactant solutions, *Langmuir,* **19(4),** 1009–1016 (2003).
(8) G. B. Kasting, T. G. Filloon, W. R. Francis, and M. P. Meredith, Improving the sensitivity of in vitro skin penetration experiments, *Pharmaceut. Res.,* **11(12),** 1747–1754 (1994).
(9) T. Tadros, Polymeric surfactants: Stabilization of emulsions and dispersions, in Principles of Polymer Science and Technology in Cosmetics and Personal Care, E. D. Goddard and J. V. Gruber, Eds. (Marcel Dekker, New York, 1999), pp. 88–127 (online).
(10) E. W. Merritt and E. R. Cooper, Diffusion apparatus for skin penetration, *J. Contr. Release,* **1,** 161–162 (1984).
(11) B. Cabane, Structure of some polymer-detergent aggregates in water, *J. Phys. Chem.,* **81(17),** 1639–1645 (1977).
(12) M. J. Fevola, R. M. Walters, and J. J. LBrizzi, A new approach to formulating mild cleansers: Hydrophobically-modified polymers for irritation mitigation, in Poymeric Delivery of Therapeutics, ACS Symposium Series, S. Morgan, Ed. (American Chemical Society, Washington, DC, 2010), pp. 221–242.
(13) C. Ade-Browne, S. Qian, M. Weaver, and H. Kumari, Investigating the effect of surfactant and surfactant-polymer micellar nanoassemblies on skin penetration, unpublished data.
(14) K. Holmberg, B. Jonsson, B. Kronberg, and B. Lindman, Surfactants and Polymers in Aqueous Solution, 2nd Ed. (Wiley, West Sussex, England, 2002).
(15) P. H. Elworthy and K. J. Mysels, The surface tension of SDS solutions and the phase separation model of micelles, *J. Coll. Interface Sci.,* **21,** 331–347 (1966).
(16) L. Rhein, C. Robbins, and K. Fernee, Surfactant structure effects on swelling of isolated human, *J. Soc. Cosmet. Chem.,* **37,** 125–139 (1986).
(17) M. M. Breuer and I. D. Robb, Interactions between macromolecules and detergents, Chem. Ind. (London), 13, 530 (1972).
(18) E. D. Goddard, Polymer-surfactant interaction Part I. Uncharged water-soluble polymers and charged surfactants, *Colloid Surf.,* **19(2–3),** 255–300 (1986).