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Comparing Surfactant Penetration into Human Skin and Resulting Skin Dryness Using *In Vivo* and *Ex Vivo* Methods

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Synopsis

Numerous tests have been developed to estimate a surfactant's mildness in rinse-off formulations. In this study, mixed surfactant systems were examined for their impact on surfactant penetration into the skin and skin hydration using *in vivo* and *ex vivo* methods. A forearm controlled application test (FCAT) was conducted, and skin hydration was evaluated using corneometry and visual dryness grading. Tape strip and cup scrub extractions were completed within the FCAT to examine the penetration of five individual surfactants into the skin *in vivo*. The ratio of surfactant mass extracted by five pooled tape strips to surfactant mass extracted by cup scrubs was found to be in the range of 40–59%. Furthermore, cup scrub collection and analysis was less time-consuming and less expensive to conduct than tape stripping. Thus, we recommend cup scrub extraction as a suitable substitute for tape stripping in future surfactant skin penetration analyses. *In vivo* results were compared with *ex vivo* ^{14}C-sodium dodecyl sulfate (^{14}C-SDS) penetration into human cadaver skin from the same surfactant systems. *In vivo* measurements conducted in the FCAT, including corneometer reading, visual dryness score, and individual surfactant (sodium laureth (1) ether sulfate and cocamidopropyl betaine) extracted from the skin, were found to correlate well with ^{14}C-SDS penetration into the skin *ex vivo* for anion-based surfactant systems. Thus, ^{14}C-SDS skin penetration may be a useful preclinical test for skin dryness induced by rinse-off products containing anionic surfactants.

INTRODUCTION

Surfactants are commonly used to provide the cleansing action in rinse-off personal care products such as body washes and shampoos. These products are designed to cleanse the

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skin and hair of dirt, sweat, and oils, and then be rinsed away with water. They contact the skin for a short period of time, typically from tens of seconds up to 2 min per application; nevertheless, the surfactants penetrate the skin and can denature proteins (1–5) and remove and/or disrupt skin lipids (1,3,6,7). Numerous tests have been developed to estimate a surfactant's irritation potential or "mildness" (8,9). Surfactant mildness in cosmetics and personal care products is best evaluated under conditions typical of consumer use. However, consumer use conditions do not usually elicit an acute irritation response which can be differentiated between varying products. Rather, more subtle changes in skin condition are observed following prolonged use (e.g., changes in moisture levels, visual dryness, or tightness). Therefore, these products tend to be tested using exaggerated protocols over a short period of time to bring out mildness differences. In this study, a forearm controlled application test (FCAT) was conducted to demonstrate the relative mildness of a variety of mixed surfactant and surfactant–polymer systems. This method has been shown to produce results that correlate with actual consumer response (10). In addition, both tape stripping and cup scrub methods were used to examine the penetration of five individual surfactants into the skin from mixed surfactant systems and to determine whether cup scrub measurements could replace tape stripping in future surfactant-skin penetration analysis. Tape stripping is a common method that can be used to examine depth profiles of stratum corneum contents. However, when a depth profile is not required, cup scrub collection is less time-consuming and less expensive to conduct than tape stripping.

Surfactant penetration into excised skin has previously been examined. Blankschtein et al. (1,11–13) have implemented an *ex vivo* method in which pig skin was exposed to surfactant solutions containing ^{14}C-sodium dodecyl sulfate (^{14}C-SDS) for 5 h. James-Smith et al. (14) used a similar method using pig skin and a 2.5-h surfactant exposure. In this study, these methods have been replaced with a 2-min surfactant exposure on human skin, which is more consistent with consumer use of personal care rinse-off products (15). Results from *in vivo* and *ex vivo* methods are compared and reported here.

MATERIALS AND METHODS

TEST FORMULATIONS

The seven test formulations examined in this study were obtained from the Procter & Gamble Company (Cincinnati, OH) (Table I). The surfactants and polymers used in these formulations include the following: sodium laureth (1) ether sulfate (SLE_1S), cocamidopropyl betaine (CAPB), cocamide monoethanolamine (CMEA), sodium cocoyl glycinate (SCG), sodium laureth (3) ether sulfate (SLE_3S), sodium lauroamphoacetate (NaLAA), polyvinyl alcohol (PVA), cationic polyvinyl alcohol (CPVA), and EcoSense (Dow Chemical Company, Midland, MI). The PVA raw material had 12% unhydrolyzed acetate groups. Formulations A–F are anion-based surfactant systems, whereas formulation G is nonionic based. Individual surfactants quantified in the tape strip/cup scrub extraction procedures are also listed in Table I. Two surfactants per formulation were quantified.

FOREARM CONTROLLED APPLICATION TEST (FCAT)

The FCAT study was conducted over a 5-d period in 2015 in the Winnipeg, Manitoba, area. A total of 70 healthy female subjects between the ages of 18 and 55 years (inclusive)

Table I
Compositions and Codes of the Test Formulations Examined in this Study

Code	Total composition (% w/v)	Surfactants quantified using tape strips and cup scrubs
A	SLE_1S (12), CAPB (2), CMEA (1)	SLE_1S, CAPB
B	SLE_1S (9), CAPB (5), CMEA (1)	SLE_1S, CAPB
C	SLE_1S (9), SCG (5), CMEA (1)	SLE_1S, SCG
D	SLE_1S (11), SLE_3S (1.5), NaLAA (8.15)	SLE_1S, NaLAA
E	SLE_1S (11), SLE_3S (1.5), NaLAA (8.15), PVA (8)	SLE_1S, NaLAA
F	SLE_1S (12), CAPB (2), CMEA (1), CPVA (2)	SLE_1S, CAPB
G	EcoSense (12), CAPB (2), CMEA (1)	EcoSense, CAPB

with Fitzpatrick skin types I–IV were recruited. Subjects who had recently (within 3 weeks of enrollment) participated in another forearm study at this or any other facility were excluded from participation. In addition, subjects who were allergic to the ingredients of personal care products or to tapes/adhesives, who had active eczema or psoriasis on any portion of the body, who had a history of cancer, with recent use of topical medications on the forearms, with chronic use of any medication that could inhibit the appearance of irritation, who had a history of diabetes, with use of moisturizers on the forearms 24 h before baseline measurements, with demonstration of abrasions or scarring of the forearms, with any immunologic or infectious disease, who were pregnant or lactating, who had insufficient forearm length to accommodate the number and size of test sites, and/or who were an employee of the sponsor or testing site were excluded from participating in this study. Because of the cosmetic nature of this study, the use of non-regulated test articles and/or monograph ingredients, and the low risk to study subjects (normal expectation), an institutional review board (IRB) was not used for review/approval of this clinical study. This decision conformed to the sponsor's standard operating procedure on IRB review.

The FCAT was a randomized, double-blinded (subject and grader/instrument operator), round-robin design. Fifteen test formulations were randomized to six sites per subject (three on each forearm), yielding $N = 28$/formulation. After reviewing study deviations, subject medications, adverse events, and subject/test site drops, 22–24 measurements were completed for each formulation. Tape strip extractions were only completed on a subset of seven of the total 15 formulations as it was not possible to analyze tape strip samples from all 15 formulations in regard to time and cost. The subset of formulations chosen (listed in Table I) comprises mild and harsher treatments, including both internal controls and a sampling of the competitive market at the time of the study. Cup scrub extractions were completed on all study participants, but the results shown here are of a subset matching the tape stripped population. Thus, because of the incomplete block design and our interest in certain formulations over others, sample sizes vary across measurements completed in the FCAT.

The method was adapted from Ertel et al. (9) with deviations highlighted below. Each study participant had three application areas (5 cm × 5 cm) marked off on the volar surface of each forearm with a laboratory marking pen, totaling six test sites. A clinical assistant wetted the participant's volar forearm with warm tap water (35°C) and then applied the test formulation, beginning with the site nearest the elbow, by dispensing the appropriate amount of test formulation into the center of the marked area. Test formulations varied

in concentration, but each formulation was applied such that 37.5 mg of total surfactant was delivered to each area. The assistant then used a gloved hand to lather by a circular motion within the test site for 10 s. The lather remained on each site for 90 s, after which the site was rinsed with warm water for 15 s. The formulation application and rinse procedure was repeated on the remaining five test sites of one subject, with each test site receiving a different test formulation according to the study randomization. After all test sites were rinsed, the clinical assistant patted the subject's arm dry. This procedure was immediately repeated on the same subject. Thus, each wash visit comprised two washes per test site. Wash visits occurred twice a day for the first 4 d and once on the final day for a total of 18 applications. Wash visits were spaced by a minimum of 3 h.

Each test site was divided into four quadrants to accommodate multiple evaluations. Although the entire test site received product application, a designated quadrant within the test site underwent specific evaluations. Evaluations in the FCAT were conducted twice, once at baseline before formulation application and again 3 h after the final wash. The order of evaluations was as follows: subject acclimation in a temperature and humidity controlled environment for 30 min, visual dryness assessed by an expert grader, instrumental measurement of skin hydration conducted using a Corneometer® CM 825 (Courage + Khazaka electronic GmbH, Cologne, Germany) operated with a multipronged probe (model MT-8C; Measurement Technologies, Inc., Cincinnati, OH), cup scrub sample collection, and tape strip sample collection. At the baseline visit, the full test site was evaluated for visual dryness, the upper left quadrant underwent tape strip sample collection, the upper right quadrant underwent cup scrub sample collection, and the lower left quadrant underwent instrumental evaluation. Three hours after the final wash, the entire test site was evaluated for visual dryness, the lower left quadrant underwent instrumental evaluation followed by tape strip sample collection, and the lower right quadrant underwent cup scrub sample collection. Each quadrant was only sampled with tape strips/cup scrubs one time throughout the study so that the test site was not compromised before each sample collection.

TAPE STRIP AND CUP SCRUB EXTRACTIONS

Preliminary work showed that 85–90% (results not shown) of the applied surfactant could be accounted for using five sequential tape strips. Thus, we chose to collect five tape strips in this study. Each D-squame tape (22 mm in diameter; Cuderm Corp., Dallas, TX) was pressed on the skin with a constant pressure for 5 s using a D-500 D-squame pressure instrument (CuDerm Corp.). The tape was gently peeled away from the skin with blunt-tipped forceps and another tape was placed in the same location. This process was repeated with approximately 1 min between tape placements until five tapes were collected. Individual tapes were placed in vials and extracted using 2 mL of a 50/50 v/v mixture of water and methanol.

Cup scrub samples were collected by placing a sterile glass cylinder (2 cm diameter) on the participant's forearm, pipetting 1 mL of a 50/50 mixture of 100 proof ethanol and high performance liquid chromatography (HPLC) grade water into the cylinder, and scrubbing with moderate pressure for 30 s using a sterile glass rod. The ethanol/water mixture was then collected, and this procedure was repeated with another 1 mL of ethanol/water on the same sample area. The ethanol/water samples were pooled.

Tape strip and cup scrub samples were analyzed for two individual surfactant components for each test formulation by stable isotope-based reversed-phase HPLC with tandem mass spectrometry using multiple reaction monitoring. Standard curves were constructed based on the peak area ratio of each analyte to the stable isotope internal standard versus the concentration of the standard. The concentration of the analyte in the cup scrub solution was then determined by the peak area ratio of the sample by interpolation from the regression curve. Tape strip results were then pooled.

^{14}C-SODIUM DODECYL SULFATE (^{14}C-SDS) SKIN PENETRATION *EX VIVO*

This method closely follows that described by McCardy et al. (16). Split-thickness human cadaver skin was obtained from the New York Firefighters Skin Bank (New York, NY) and stored at −80°C until use. Excised skin was cut into small pieces approximately 1–1.5 cm^2 in size and mounted in Franz diffusion cells (area = 0.79 cm^2) with the stratum corneum facing up. Skin samples were allowed to equilibrate in phosphate-buffered saline (PBS; Sigma Aldrich, St. Louis, MO) with 0.02% w/v sodium azide (NaN_3; Fisher Scientific, Pittsburgh, PA) for 1–2 h and then integrity of the skin membranes was assessed by tritiated water (^3H_2O; Perkin-Elmer, Waltham, MA) permeation using the Kasting et al. method (16). Skin samples with water permeation greater than 2 μL/cm^2 were discarded. Test formulations were assigned to skin samples using a complete randomized block design with ^3H_2O permeation as the blocking factor.

Test formulations were prepared at 1.5% w/v total surfactant to simulate realistic cleansing exposures. Shampoos are typically formulated with approximately 15% w/v total surfactant, and we have estimated that consumers remove 90% of that material upon initial rinsing. Test formulations were spiked with 10 μCi/mL of radiolabeled ^{14}C-SDS (American Radiolabeled Chemicals, St. Louis, MO). On the morning of the study, receptor solutions were replaced with 4.25 mL of fresh PBS + 0.02% NaN_3 and 150 μL of radiolabel-spiked test formulation was dosed into each donor chamber. After 2 min, excess formulation was removed and collected. The surface of the skin was rinsed with three aliquots of 0.5 mL Millipore water by sequential up/down pipetting three times each, and these rinses were collected into one vial. The receptor solution was collected and then the skin sample was wiped once with Whatman filter paper (GE Healthcare Life Sciences, Issaquah, WA) soaked with PBS and then three times with Whatman filter paper soaked with 70% (w/w) ethanol to remove any residual test formulation. The first three wipes were collected together and the last wipe was collected into a separate vial to ensure the radioactive test formulation had been sufficiently removed from the skin surface. Skin samples were dissolved in 2 mL Solvable (Perkin-Elmer) at 50°–60°C overnight. Ultima Gold XR scintillation cocktail (Fisher Scientific) was added to each component, and these solutions were analyzed for radioactivity in disintegrations per minute using liquid scintillation counting via an LS 6500 Beckman counter (Beckman Instruments, Hebron, KY). Results were reported as percent of applied radioactive dose penetrated into the skin, including material that permeated through the skin into the receptor solution. A total of six skin donors were used. Each product was tested on two to three samples of each of five skin donors, and four of the seven products (C, D, E, and G) were additionally tested on one sample of a sixth skin donor.

DATA AND STATISTICAL ANALYSIS

SAS (SAS, Cary, NC), JMP™ (SAS), and SigmaPlot (Systat Software, San Jose, CA) software were used for statistical analyses. A p-value ≤ 0.05 was used to determine statistical significance. The two clinical skin hydration measures (corneometer reading and visual dryness by expert grader) conducted in the FCAT were analyzed using a mixed model analysis of covariance. Analyses modeled subject as a random factor, and side, site, and treatment as fixed factors. Baseline measurement and age were tested for significant effects as covariates for both clinical measures. Based on these results, baseline measurement was used as a covariate for corneometer readings, whereas baseline measurement and age were used as covariates for visual dryness. Studentized residuals beyond four standard deviations were assessed as possible outliers; however, no outliers were removed in the final analyses. Results are reported as adjusted mean change from baseline (CFB). Individual surfactant components extracted from either tape strips or cup scrubs in the FCAT study were calculated as masses extracted, and arithmetic means were reported. Ratios of masses extracted by tape strips to masses extracted by cup scrubs were calculated for each subject, and medians were reported. Results from the *ex vivo* surfactant-skin penetration study were log10-transformed to achieve normality, and a two-way analysis of variance (ANOVA) with treatment and skin donor as the factors was used to obtain the least squares mean and standard error of the mean (SEM) for each test formulation. The geometric mean and standard error were calculated from these values and reported. Comparisons across the *in vivo* and *ex vivo* methods were compared using linear regression. Pairwise correlations using means were determined using Pearson's Chi-squared test.

RESULTS

Results from the FCAT and ^{14}C-SDS skin penetration studies are provided in Table II. Clinical mildness is associated with higher corneometer readings (skin hydration) and lower visual dryness scores. The use of all test formulations led to significant reductions in skin hydration and significant increases in visual skin dryness versus baseline values. The mildest formulation (G) demonstrated a 5.2 decrease in corneometer reading with a 0.7 increase in dryness score, whereas the least mild formulation (A) demonstrated a 13.1 decrease in corneometer reading and a 1.5 increase in skin dryness score. Mean corneometer and visual dryness scores yielded similar rankings with respect to formulation-induced skin dryness. The clinical measures were found to correlate well with ^{14}C-SDS skin penetration *ex vivo* (corneometer reading, $R^2 = 0.75$, $p < 0.05$; visual dryness scores, $R^2 = 0.78$, $p < 0.01$).

Comparisons of tape strip and cup scrub extractions are listed in Table III. We found the ratio of surfactant mass extracted by five pooled tape strips to surfactant mass extracted by cup scrubs to be 40–59%. Quantities of individual surfactants extracted from five pooled tape strips and cup scrubs are listed in Table II and illustrated in Figure 1. SLE_1S penetration into the skin as measured by cup scrubs *in vivo* was found to correlate well with ^{14}C-SDS skin penetration *ex vivo* ($R^2 = 0.76$, $p < 0.05$) (Figure 2).

Significant and nearly significant relationships emerged between CAPB penetration into the skin from cup scrub and tape strip *in vivo* and *ex vivo* skin penetration results when examining the anion-based test formulations only. CAPB penetration into the skin as measured by cup scrubs *in vivo* approached a significant correlation with ^{14}C-SDS skin penetration *ex vivo* ($R^2 = 0.95$, $p = 0.15$) for the anion-based systems (Figure 3). CAPB

Table II
Results from the FCAT and ^{14}C-SDS Skin Penetration Studies

Measurement		Code A	B	C	D	E	F	G
Corneometer reading,[a] CFB		–13.09	–8.48	–10.92	–8.89	–8.10	–11.58	–5.16
Visual dryness score,[b] CFB		1.489	1.082	1.164	1.064	0.911	1.365	0.736
Tape strip extract (five pooled extracts),[c] µg	SLE_1S	48.4 ± 6.6	29.9 ± 3.6	30.5 ± 4.5	15.0 ± 2.0	17.8 ± 3.8	49.9 ± 7.1	–
	CAPB	23.8 ± 3.3	40.3 ± 5.5	–	–	–	22.9 ± 3.2	7.8 ± 1.4
	SCG	–	–	55.5 ± 8.8	–	–	–	–
	NaLAA	–	–	–	11.1 ± 1.7	11.8 ± 3.2	–	–
	EcoSense	–	–	–	–	–	–	25.5 ± 3.2
Cup scrub extract,[c] µg	SLE_1S	79.5 ± 7.3	45.5 ± 4.2	71.6 ± 14.1	37.1 ± 4.1	30.8 ± 3.5	87.4 ± 9.5	–
	CAPB	51.7 ± 5.3	83.9 ± 9.5	–	–	–	55.8 ± 7.2	15.9 ± 2.1
	SCG	–	–	65.7 ± 12.9	–	–	–	–
	NaLAA	–	–	–	28.9 ± 3.9	28.6 ± 3.9	–	–
	EcoSense	–	–	–	–	–	–	71.3 ± 9.8
^{14}C-SDS penetration into and through the skin,[d] % applied radioactive dose		0.247 ± 0.044	0.106 ± 0.018	0.198 ± 0.035	0.175 ± 0.030	0.087 ± 0.015	0.277 ± 0.048	0.095 ± 0.016

[a]Adjusted mean CFB; pooled SEM = 0.35, n = 22–24.
[b]Adjusted mean CFB; pooled SEM = 0.055, n = 22–24.
[c]Arithmetic mean ± SEM, n = 11–26.
[d]Geometric mean ± SEM, n = 12–14.

penetration into the skin as measured by tape stripping *in vivo* was found to correlate well with ^{14}C-SDS skin penetration *ex vivo* (R^2 = 0.996, $p < 0.05$). Interestingly, comparison of Figures 2 and 3 reveals that SLE_1S and CAPB penetration into the skin from anion-based formulations has opposite relationships with the *in vivo* skin hydration measures and with the *ex vivo* skin penetration measure. For example, higher corneometer readings are associated with decreased SLE_1S skin penetration and increased CAPB skin penetration.

DISCUSSION

The test cleansers examined in this study were formulated using strategies to maintain optimum skin hydration while still providing lather and adequate cleansing efficacy for rinse-off applications. Although none of these strategies completely offset the skin dryness induced by multiple daily exposures to the test formulations over a period of 5 d in the FCAT study, some formulations were able to mitigate adverse effects in a reproducible manner better than others. ^{14}C-SDS skin penetration *ex vivo* was found to be a good predictor of clinical skin hydration as quantitated with corneometer and visual dryness scores in the FCAT study. A 5-h exposure of pig skin to surfactant solutions containing ^{14}C-SDS used in Blankschtein et al. (11) and subsequent publications has been replaced by a 2-min exposure on human skin in this study (15). The changes not only involve exposures more consistent with consumer use of rinse-off products, but they also avoid making inferences about human exposure from pig skin, which has a different array of skin appendages than human skin (17–21). Penetration pathways for polar or amphiphilic compounds are not completely understood and may be different in porcine and human skin. The results of this study show that ^{14}C-SDS skin penetration may be a useful preclinical test for mildness of rinse-off products containing anionic surfactants.

Cup scrubs consistently extracted more material than did five pooled tape strips. This may simply be because surfactants are more readily extractable using liquid than tapes. The extraction liquid likely solubilized surfactant molecules that were loosely bound to proteins and those within stratum corneum (SC) lipid layers deep in the skin compared with tape strips, which only remove one SC layer at a time. In execution, the tape strip sampling procedure is easier to perform than the cup scrub collection. The cup scrub collection requires the technician to hold the cup in place with enough pressure to ensure a good seal to prevent the solution from leaking out of the cup. The potential for leakage can compromise the sample and provide opportunity for experimental error. However,

Table III
Ratio of Surfactant Mass Extracted by Five Pooled Tape Strips to Surfactant Mass Extracted by Cup Scrubs

Surfactant extracted	Tape strips/cup scrubs (%)
SLE_1S	58.8
CAPB	41.4
SCG	40.0
NaLAA	40.0
EcoSense	40.0
Total	48.9

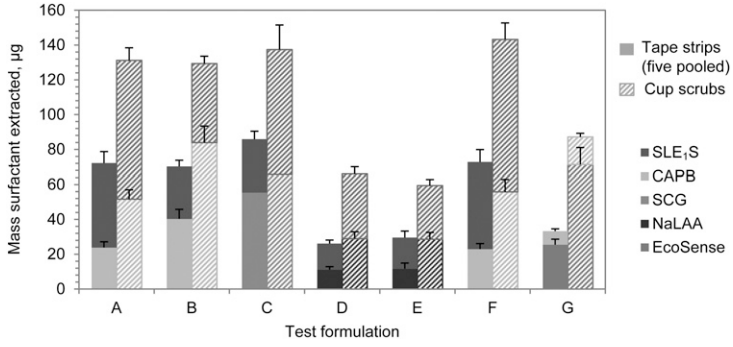

Figure 1. Masses of individual surfactants extracted from either five pooled tape strips or cup scrubs. Bars are stacked, and each bar represents the mean of 11–26 samples + SEM.

cup scrub collection takes less time in execution and analytical analysis. In the cup scrub collection procedure, there is only one sample to analyze, store, and document versus five (or more) in tape stripping. In addition, tape strips require an extraction step, whereas the cup scrub sample can be processed as is, without any additional steps. Furthermore, tape strips must be extracted individually, as they stick together if placed in the same vial. In this study, it would have taken approximately 8 weeks to analyze 2,100 tape strip samples (70 subjects, six test sites/subject, and five tape strips/site) versus 2 weeks to analyze 420 cup scrub samples (70 subjects, six test sites/subject, and one cup scrub sample/site) if all 15 formulations were tested. Cup scrub collection is also a less expensive procedure to perform, as the glass cups and stir rods can be cleaned and reused many times, whereas tape strips are consumable, expensive, and must be purchased for each use. In this study, the cost to complete the tape stripping procedure for 15 formulations would be $809 versus $187 for cup scrub collection. We, therefore, conclude that cup scrubs can be used for future surfactant skin penetration analysis, and may be the preferred method as they are less expensive and require less time for collection and analytical analysis. If a depth profile of surfactant penetration into the skin is desired, we recommend the use of tape strips.

Tape strip and cup scrub analyses of varying surfactants revealed that total surfactant penetration into the skin as measured by either extraction method did not correlate well with ^{14}C-SDS skin penetration. However, when the surfactants were examined individually, significant trends emerged. We found some of the distributions of individual surfactants extracted from tape strips and cup scrubs to be positively skewed; thus, medians are more representative for these data sets compared with means. On examination of correlations between tape strip and cup scrub extraction medians versus corneometer reading, visual dryness scores, and ^{14}C-SDS penetration means, we found similar or better R^2 values. However, because similar conclusions are found using the means, we chose to present the means here for consistency in understanding the error associated when comparing various measures.

Skin penetration of SLE_1S or ^{14}C-SDS, two anionic surfactants, seems to be a good predictor of clinical skin hydration state for anion-based systems. Penetration of CAPB, a zwitterionic surfactant, can also be a good predictor of clinical hydration state for anion-based systems. The correlations between CAPB extractions and skin hydration measures lost significance when the nonionic formulation was included. This may indicate that CAPB is not a good predictor of nonionic surfactant penetration, but CAPB penetration from

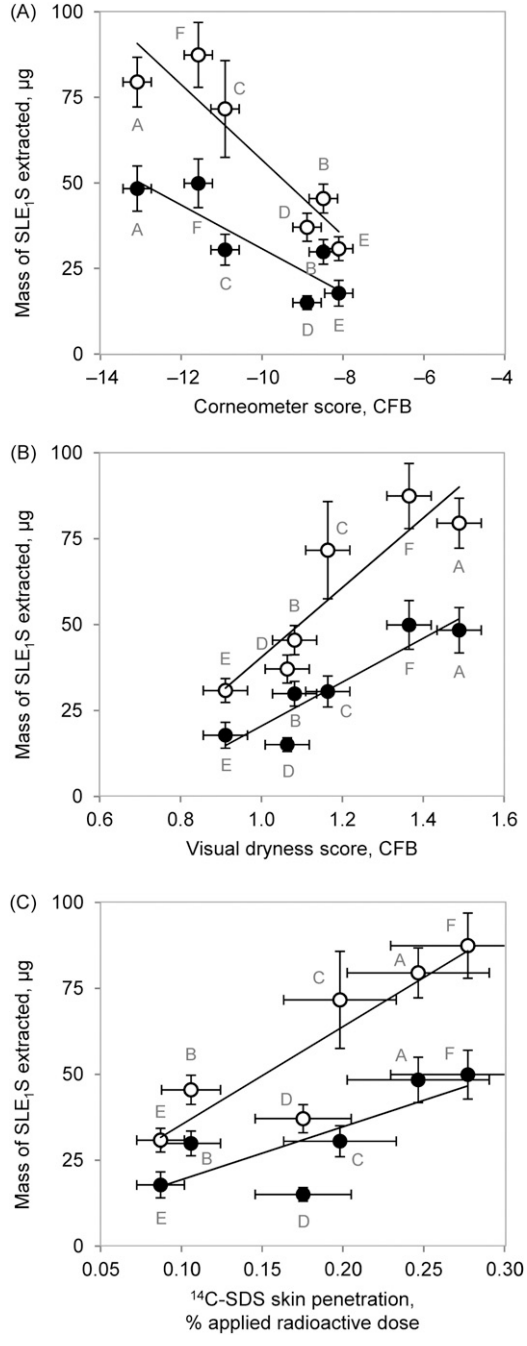

Figure 2. SLE_1S extracted from the skin using five pooled tape strips or cup scrubs *in vivo* versus (A) corneometer CFB reading determined *in vivo*; (B) visual dryness CFB scores determined *in vivo*; and (C) ^{14}C-SDS skin penetration determined *ex vivo*. Data points represent mean ± SEM. (●) Tape strip extraction (five pooled extracts); (○) cup scrub extraction.

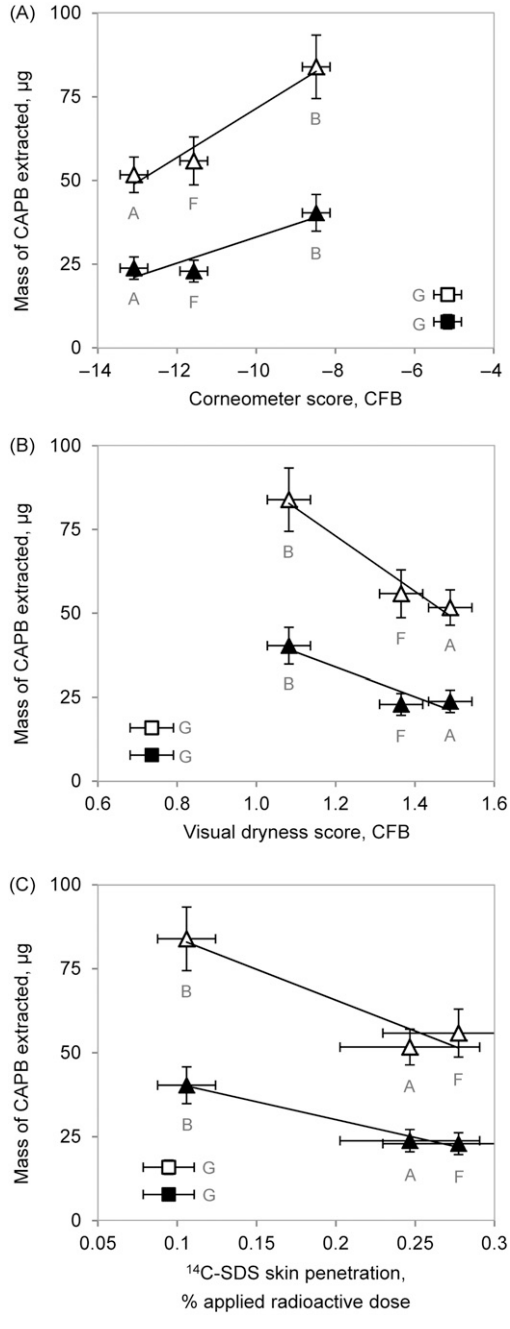

Figure 3. CAPB extracted from skin using five pooled tape strips or cup scrubs *in vivo* versus (A) corneometer CFB reading determined *in vivo*; (B) visual dryness CFB scores determined *in vivo*; and (C) ^{14}C-SDS skin penetration determined *ex vivo*. Data points represent mean ± SEM (some error bars are too small to see). (▲) Tape strip extraction (five pooled extracts) after multiple applications of an anion-based formulation; (■) tape strip extraction (five pooled extracts) after multiple applications of a nonionic-based formulation; (△) cup scrub extraction after multiple applications of an anion-based formulation; (□) cup scrub extraction after multiple applications of a nonionic-based formulation.

more nonionic-based formulations would need to be examined to investigate this theory. The fact that the correlations between CAPB extractions and the skin hydration measures only approached statistical significance is likely because of the small number of formulations tested here. A follow-up study to further examine the relationship between CAPB penetration into the skin, SLE_1S penetration into the skin, and skin hydration has been completed and will be reported separately.

The observation that SLE_1S and CAPB have opposite relationships with the clinical measures may be because of differences in these surfactants' physical properties, and in particular in their structures and/or charges. The structure–function relationships of these surfactants and how they impact the mechanisms of surfactant penetration into human skin are currently unknown. We also noted that the individual surfactants did not penetrate the skin in quantities that are consistent with their bulk solution compositions. For example, the ratio of SLE_1S to CAPB in formulation A is 6:1, but CAPB made up 33–39% of the total surfactant mass found in the skin after treatment with formulation A. It is well understood that mixed micelle composition often differs from bulk solution composition because of interactions between the surfactants (22). Our results indicate that mixed micelle composition may play a major role in the mechanism of surfactant-induced skin dryness. More work will need to be carried out in this area to fully understand this phenomenon.

CONCLUSIONS

Surfactant-induced skin dryness and individual surfactant penetration into human skin were examined using a clinical FCAT study and an *ex vivo* study using a 2-min exposure protocol. Our results indicate that cup scrub extraction is a suitable substitute for tape stripping in surfactant skin penetration analysis, and may be the preferred method as cup scrubs extract more material and the method is less time-consuming and less expensive to perform. SLE_1S and SDS were found to be good predictors of clinical hydration for the anion-based surfactant systems examined here. Furthermore, we found that ^{14}C-SDS skin penetration from surfactant systems trends with clinical skin hydration induced by the surfactant systems as assessed by corneometry and visual dryness grading, indicating that this *ex vivo* method may be a useful preclinical test for sulfate-based rinse-off products. More work will need to be completed to understand the structure–function relationships between SLE_1S and CAPB and skin penetration, which resulted in oppositely trending relationships for the two surfactants with skin hydration measures.

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