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In Vitro Penetration of Petrolatum in Stratum Corneum from Bodywash Formulation

APIPA WANASATHOP, ZHANQUAN SHI, Q. CHING STELLA, KARL S. WEI, PETER B. STYCZYNSKI, CHUIYING LI, and S. KEVIN LI, *Division of Pharmaceutical Sciences, James L. Winkle College of Pharmacy, University of Cincinnati, Cincinnati, Ohio (A.W., Z.S., S.K.L.), Procter & Gamble Co., Mason, Ohio (Q.C.S., K.S.W., P.B.S., C.L.)*

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Synopsis

Petrolatum is a mixture of hydrocarbons that is widely used as a moisturizer. It is incorporated in bodywash formulations to help hydrate and maintain healthy skin appearance. The aim of this study was to investigate skin deposition and penetration of petrolatum from an experimental bodywash system consisting of petrolatum *in vitro*. Experiments were performed using cadaver split-thickness skin and Franz diffusion cells. Radiolabeled ^{14}C-dotriacontane (C_{32}-alkane) was used as a model permeant for petrolatum. The bodywash was applied on the skin and subsequently rinsed. At predetermined time points, the skin was wiped to remove the residual material on the surface, and tape-stripping was performed. Petrolatum was observed to deposit from the bodywash when applied on split-thickness skin with simulated rinsing. Petrolatum then penetrated into the stratum corneum and was detected at the depth of 12 tape-stripping and in the epidermis. The bodywash formulation could provide significant deposition and penetration of petrolatum into the stratum corneum at 1–72 hours postapplication.

INTRODUCTION

Bodywash is a widely used cosmetic product and is directly applied to the human skin. Its purpose is to cleanse and condition the skin. The active ingredients in bodywash are surfactants that remove dirt and soil on the skin. Surfactants also remove protective oils that are naturally present. This can lead to dry and uncomfortable skin after washing (1). Therefore, moisturizing bodywashes are formulated to improve skin hydration and maintain a healthy skin surface condition.

Petrolatum is a semisolid widely used in cosmetics as a moisturizer. It is a complex mixture of hydrocarbons obtained from dewaxing paraffinic residual oil (2). It was suggested that petrolatum can accelerate the skin surface barrier recovery and replace intercellular lipids by filling in the stratum corneum interstices (3). Petrolatum was also found to induce

Address all correspondence to Apipa Wanasathop at wanasaaa@mail.uc.edu.

expression of key barrier differential markers such as filaggrin and loricrin and upregulate antimicrobial peptides and innate immune genes, modulating skin antimicrobial activity (4). Petrolatum in bodywash formulation could therefore benefit the function of the skin surface barrier.

The aim of this *in vitro* study was to investigate skin deposition and penetration of petrolatum into the stratum corneum from a bodywash system consisting of petrolatum. The experiments were performed with human cadaver skin in Franz diffusion cell *in vitro*. Radiolabeled ^{14}C-dotriacontane (C32-alkane) was the model permeant for petrolatum that was mixed with the bodywash before the study. After bodywash application and rinse-off from the skin surface, the amount of petrolatum deposited on the skin was determined by the amount in the rinse-off solution and mass balance. Tape-stripping was then performed to determine the amount and depth of petrolatum penetration into the stratum corneum. The epidermis was separated from the dermis by dissection to determine the amounts of petrolatum in these skin layers.

MATERIALS AND METHODS

MATERIALS

^{14}C-dotriacontane (specific activity 10 mCi/mmol) was purchased from American Radiolabeled Chemicals (Saint Louis, MO). ^{3}H-water (specific activity 1 mCi/g) was purchased from Moravek Biochemicals (Brea, CA). Ultima Gold scintillation cocktail was purchased from PerkinElmer (Waltham, MA). Glyceryl monooleate was purchased from BASF Corporation (Florham Park, NJ). Petrolatum was purchased from Sonneborn (Petrolia, PA). An experimental bodywash base without petrolatum and glyceryl monooleate was obtained from the Procter & Gamble Co. (P&G). Hexane and bovine serum albumin, fraction V (BSA) were purchased from Fisher Scientific (Fair Lawn, NJ). Ethanol was purchased from Pharmco-Aaper (Shelbyville, KY). Polysorbate 20 (Tween 20) was purchased from Uniqema (Wilmington, DE). Sodium azide (NaN_3) was purchased from Acros Organics (Morris Plains, NJ). Phosphate-buffered saline (PBS: 0.01 M phosphate buffer, 0.0027 M potassium chloride, and 0.137 M sodium chloride), pH 7.4, was prepared using PBS tablets and deionized water as described by the manufacturer (MP Biomedicals, Solon, OH) and preserved using 0.02% NaN_3.

FRANZ DIFFUSION CELL SETUP

Posterior torso split-thickness cadaver skin (thickness ~0.01–0.04 cm) from eight skin donors was obtained from the New York Firefighters Skin Bank (New York, NY) in packages at −80°C. The ages of the skin donors were between 45 and 70 years. The split-thickness skin was thawed in PBS according to the instructions on the skin packages and stored at −20°C until use. Before use, the skin sample was cut into 1.5 × 1.5 cm pieces and equilibrated in PBS at room temperature for 2 h. The vertical Franz diffusion cell used in this study had a diffusional area of 0.71 cm^2. The fully hydrated skin sample was mounted on the cell between the donor and receptor chambers, with the stratum corneum side of the skin facing upward to the environment. The dermis side of the skin sample

was in contact with 5 mL receptor medium, which consisted of PBS containing 2% w/v BSA and 0.02% NaN_3. The use of BSA in the receptor chamber increased the solubility of lipophilic compounds in the chamber and resembled the condition of dermis *in vivo* for the penetration study (5). Each diffusion cell was placed on a thermostated heating and stirring module and maintained at 37°C at the receptor, resulting in a stratum corneum surface temperature of 34 ± 1°C. A micro magnetic stir bar was placed in the receptor chamber to ensure stirring throughout the experiments. The relative humidity level in the room during the penetration experiments was approximately 20–30%. The skin sample was equilibrated for 2 h, followed by a prescreening water permeability assay to confirm the skin integrity before the penetration experiments. Briefly, 0.15 mL of ^{3}H-water was added onto each skin in the diffusion cells. Five minutes postdosing, cotton swabs were used to remove excess ^{3}H-water from the donor chambers. After 1 h, 2-mL samples from the receptor chamber were collected in scintillation vials and the solutions were mixed with 10 mL scintillation cocktail and analyzed using a liquid scintillation counter (Beckman Coulter LS 6500 multipurpose scintillation counter, Fullerton, CA). Skin samples with water permeation values greater than 1.6 μL/cm^2 were discarded. Following the prescreening procedure, the receptor solution was replaced with fresh receptor medium twice to remove the residual radioactivity. The skin sample was then allowed to equilibrate in the heating and stirring module overnight.

FINITE DOSE SKIN PENETRATION STUDY WITH SPLIT-THICKNESS SKIN SAMPLES

Bodywash preparation. The experimental bodywash was prepared by mixing the bodywash base (ingredients include water, sodium trideceth sulfate, sodium chloride, cocamidopropyl betaine, trideceth-3, fragrance, guar hydroxypropyltrimonium chloride, sodium benzoate, xanthan gum, disodium ethylenediaminetetraacetic acid, citric acid, sodium hydroxide, acrylates/C10-30 alkyl acrylate crosspolymer, methylchloroisothiazolinone, and methylisothiazolinone) with 9.8% petrolatum, 0.2% glyceryl monooleate, and ^{14}C-dotriacontane (C_{32} alkane, as the model permeant for petrolatum). Specifically, 29.4 mg of petrolatum and 0.6 mg glyceryl monooleate were mixed with 30 μL hexane containing the radiolabeled dotriacontane in a small glass vial by hand using a stainless steel spatula for 3 min. The mixture was then left in a fume hood to allow hexane evaporation for approximately 50 min. After that, 270 mg bodywash base was added to the mixture and mixed by the spatula for 3 min. The size of petrolatum particles in the mixture was examined by light microscopy to ensure uniform sizes in the range of 0.10 ± 0.05 mm. The final concentration of petrolatum was 9.8% as particles dispersed in the bodywash base with 10 μCi ^{14}C-dotriacontane as the tracer in 300 mg bodywash.

Applying and rinsing method. To simulate a skin washing condition, 5 μL dose of bodywash was applied to the stratum corneum side of the skin sample using a positive displacement pipette. The bodywash was spread evenly on the skin with a glass rod (5 mm diameter, with a smooth ground glass flat tip). The flat surface of the glass rod tip was moved over the skin in circular motion for 30 s. After the bodywash was applied and left on the skin for another 30 s, i.e., a total of 1-min contact, 0.5 mL water was added to the donor chamber of the Franz diffusion cell. A pipette was used to create water motion in the donor chamber by pipetting the water "in and out" of the chamber three times over 10 s (approximately one "in and out" action every 3 s). Immediately after rinsing, the solution in the donor chamber was removed and placed into a collection

vial. This rinsing step was repeated three times to simulate a washing scenario in the rinse-off protocol. After the rinsing step, the pipette tip used in this step was rinsed twice with 0.5 mL hexane and twice with 0.5 mL water. The used glass rod was also rinsed twice with 1 mL hexane and twice with 2 mL water. The rinse-off solution, the pipette tip rinsing solution, and glass rod rinsing solution were then combined and mixed with scintillation cocktail and analyzed using the liquid scintillation counter. To check the weight and amount of ^{14}C-dotriacontane in the applied dose, 5 μL of the bodywash was placed in a container and weighed with an analytical balance, and the content in the container was mixed with scintillation cocktail and analyzed using the liquid scintillation counter.

Sample collection and tape-stripping. In the penetration study, 2-mL samples of receptor medium were collected at 1, 24, or 72 h postapplication of the bodywash. After sampling, the diffusion cells were disassembled and each skin sample was wiped twice with Whatman filter paper soaked with 0.5% Tween 20 in PBS and once with filter paper soaked with 70%/30% ethanol/deionized water (v/v) to remove the remaining bodywash on the skin surface. After the filter wipes, tape-stripping was performed to remove a portion of the stratum corneum. To determine the penetration profile of petrolatum in the stratum corneum, tape-stripping was performed up to 12 times at 1, 24, or 72 h postapplication of the bodywash: in the 1-h experiments, five times tape-stripping were applied, and in selected skin samples, 12 times tape-stripping were applied; in the 24 and 72 h experiments, 5 times tape-stripping were applied. For the tape-stripping, the edges of the skin were covered with adhesive tapes (fixing tapes), leaving a central square hole of 1×1 cm^2 (the available area for tape-stripping). Standard D-Squame® disc (Cuderm Corporation, Dallas, TX), a diameter of 2.2 cm and an area of 3.8 cm^2, was pressed onto the skin for 5 s using the D-Squame® pressure instrument, and the D-Squame® disc was quickly removed. After tape-stripping, the skin was cut to isolate the diffusion region of the epidermis using a cork borer, and the epidermis was separated from the remaining skin using a pair of forceps. Each skin section was dissolved separately in 1 mL Solvable (PerkinElmer Life and Analytical Sciences, Boston, MA): each tissue was mixed with Solvable in a scintillation vial and kept in an oven at 50°C overnight for the solubilization of the sample. The donor chamber was washed with hexane to remove the residual cosmetic ingredients on its surface. The receptor collections, filter paper wipes, tapes from tape-stripping, solubilized skin sections, and donor chamber wash solutions were mixed with scintillation cocktail and analyzed separately using the scintillation counter. The samples were discarded when one of the following occurred: tearing of the skin before Tape 3 in the tape-stripping procedure or an outlier from the statistical analysis in an experiment.

DATA ANALYSIS

Figure 1A summarizes the experimental procedure in the present study. The net applied dose of dotriacontane was the remaining amount of dotriacontane on the skin before the rinse-off and was calculated by the difference between the total applied dose and the amount remaining on the glass rod (i.e., the net applied dose of dotriacontane = the total applied dose of dotriacontane minus the amount in the glass rod rinse). The total applied dose of dotriacontane was determined by mixing 5-μL dose of bodywash directly with the scintillation cocktail. The % applied dose in each compartment was calculated by the amount

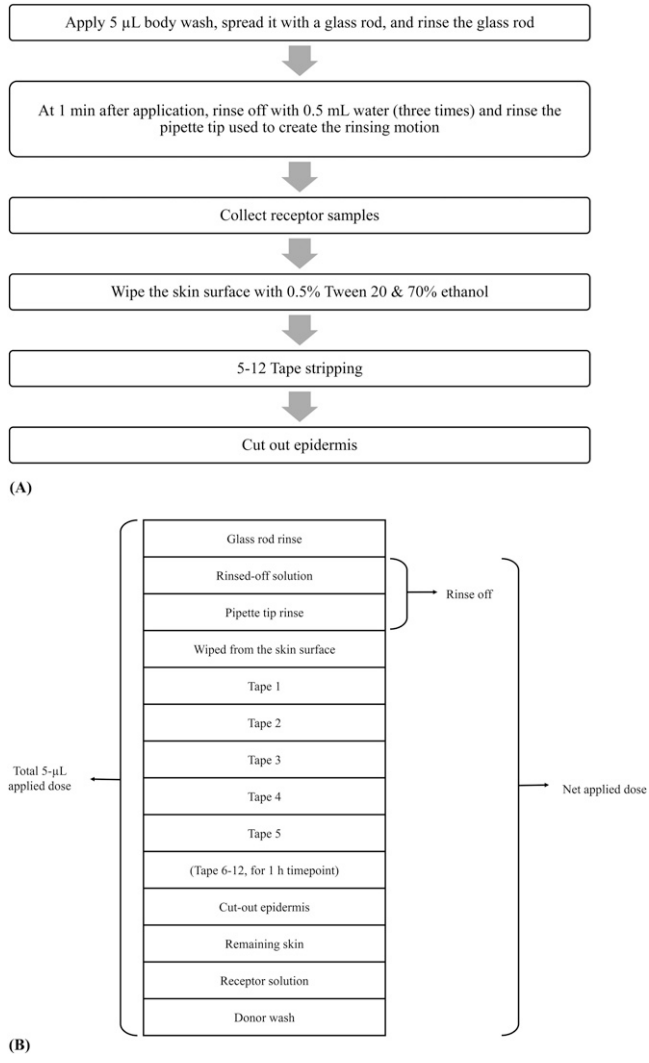

Figure 1. (A) Schematic diagram of the experimental procedure to evaluate petrolatum deposition on the skin surface and its penetration into the stratum corneum from the bodywash formulation, and (B) the amount of petrolatum measured in different compartment of the rinsed-off solution, the net applied dose, and the total 5-µL applied dose in the experiments.

of dotriacontane in the compartment normalized by the total applied dose of dotriacontane (total applied radioactivity in 5 µL from the pipette). The amount of petrolatum normalized by the skin area was calculated by the amount of dotriacontane in each compartment, specific activity of dotriacontane for petrolatum, weight of petrolatum in the 5-µL dose bodywash formulation, and the diffusion area of the Franz diffusion cell (i.e., the amount of petrolatum in µg = the amount of dotriacontane divided by the specific activity of dotriacontane and 0.71 cm^2). Total recovery was calculated using mass balance. Figure 1B summarizes the compartments to be investigated in the present petrolatum penetration study. All experiments were performed with at least four skin donors for each condition.

STATISTICAL ANALYSIS

The means ± standard errors of the means (SEM) of the data are presented. Data analyses and statistical tests (one-way Analysis of variance, ANOVA) were performed using Microsoft Excel (Redmond, WA) and GraphPad InStat (San Diego, CA), respectively, and a difference of $p < 0.05$ was considered statistically significant. Outlier data points (~9% of the data) of the amounts of dotriacontane (as % applied dose) in the tapes and epidermis in each set of experiments were identified using an outlier test (Outlier calculator, GraphPad Software, La Jolla, CA).

RESULTS

DEPOSITION AND PENETRATION OF PETROLATUM INTO THE STRATUM CORNEUM

The average weight of bodywash formulation (5 μL) applied *in vitro* on the skin (a diffusion area of 0.71 cm^2) was 2.38 ± 0.05 mg (mean ± SEM, $n = 54$ total samples, from $n = 3$ in each set of experiments). Dotriacontane was used as the model compound for the penetration of petrolatum into the stratum corneum. Table I shows that the majority, 61–66% (mean values), of the dotriacontane in the bodywash formulation was removed by the rinse-off protocol. Approximately 8–14% (mean values) of the dotriacontane in the bodywash remained on the skin surface that was removed by the filter wipes before the tape-stripping evaluation. Figure 2 shows the amounts of dotriacontane as % applied dose and amounts of petrolatum (μg/cm^2) in the first five tapes after the application of the bodywash system and rinsing. The amounts of dotriacontane in the stratum corneum, collected by tape-stripping, decreased from 2.9% ± 0.3% in the first tape to 0.33% ± 0.05% (mean ± SEM) in the fifth tape at 1 h postapplication. At 24 and 72 h after bodywash

TABLE I
The Amount of Dotriacontane (Petrolatum Probe) as Percent of Bodywash Net Applied Dose Recovered from Different Compartments at 1, 24, and 72 h after Application and Rinsing in the Skin Penetration Study. Mean ± SEM, $n = 7–11$

Compartment	1 h	24 h	72 h
Rinse-off[a]	61 ± 5	61 ± 4	66 ± 5
Skin surface wipe	14 ± 1	8 ± 1	10 ± 1
Tape-stripping[b]	8 ± 1	9 ± 1	10 ± 1
Epidermis below Tape 5 (cut out epidermis)	0.7 ± 0.1	3.0 ± 0.4	5 ± 1
Remaining skin (after tape-stripping and cut out epidermis)	0.8 ± 0.2	1.6 ± 0.3	2.0 ± 0.3
Buffer in receptor	0 ± 0[c]	0 ± 0[c]	0 ± 0[c]
Donor wash[d]	10 ± 2	10 ± 2	9 ± 1
Total	95 ± 5	92 ± 3	103 ± 4
Range	(82–129)	(84–104)	(87–112)

[a]Rinse-off solution was collected immediately after rinsing, which was performed at 1 min after dosing.
[b]Amounts of dotriacontane on the fixing tapes used in the tape-stripping procedure are less than 1% applied dose of dotriacontane.
[c]Mean values of dotriacontane in the receptor chamber <0.1% applied dose.
[d]Residual dotriacontane on the glass surface of the donor chamber. The residual amount was determined by washing the donor chamber cap with hexane and collecting the wash solution. This amount was only used for recovery calculation.

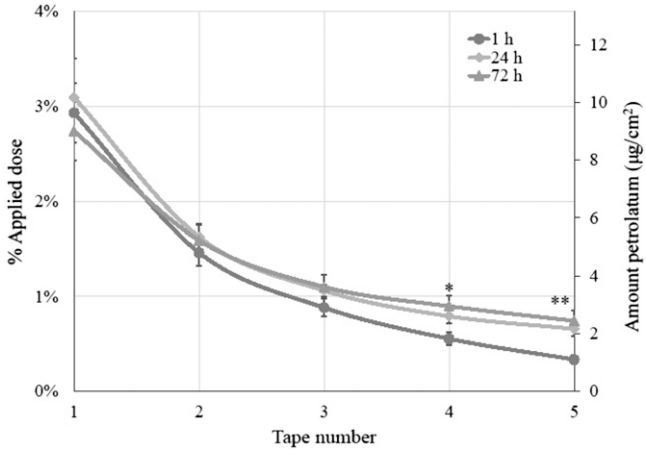

Figure 2. Amount of dotriacontane (% applied dose) and calculated petrolatum amount (µg/cm^2) in each tape, from Tape 1 to 5, at 1 h (circles), 24 h (diamonds), and 72 h (triangles) after bodywash application and rinsing. Mean ± SEM (n = 8 for 1 h, n = 7 for 24 h, and n = 11 for 72 h). * denotes significant difference between the data at 1 and 72 h ($p < 0.05$, ANOVA). ** denotes significant difference between the data at 1 and 24 h and between the data at 1 and 72 h ($p < 0.05$, ANOVA).

application and rinsing, the amount versus tape number profiles of dotriacontane (from Tape 1 to Tape 5: 3.1% ± 0.4% to 0.66% ± 0.07% and 2.7% ± 0.3% to 0.74% ± 0.11% at 24 and 72 h, respectively, mean ± SEM) were similar to that at 1 h with slightly larger amounts of dotriacontane in the later tapes (Tapes 4 and 5) compared with the 1 h data.

PENETRATION DEPTH OF PETROLATUM INTO THE STRATUM CORNEUM

A more complete stratum corneum penetration profile of dotriacontane was obtained by using up to 12 tapes in the tape-stripping at 1 h after bodywash application and rinsing. The amounts of dotriacontane in this profile are shown in Figure 3 as % applied dose. Because of the small amounts of dotriacontane in the deeper layers of the stratum corneum, the later tapes (Tapes 7–12) were combined in pairs: Tapes 7 and 8, Tapes 9 and 10, and Tapes 11 and 12. Although only a small % of dotriacontane was found in Tapes 7–12, the data support the hypothesis that petrolatum can penetrate into the deeper layers of the stratum corneum after bodywash formulation application and rinsing.

PENETRATION OF PETROLATUM INTO THE DEEPER SKIN LAYERS

Figure 4 shows the amount of dotriacontane beyond the first five tapes in the epidermis (stratum corneum and viable epidermis excluding the first five tape-stripping) as % applied dose. Considerable amount of dotriacontane was found in the deeper layers in the stratum corneum and epidermis. The % applied dose of dotriacontane in the deeper layers of epidermis at 1 h after application and rinsing was 0.78% ± 0.17% (mean ± SEM), corresponding to 2.6 ± 0.6 µg/cm^2 petrolatum. This amount tripled at 24 h to 2.3% ± 0.3% and then increased to 3.9% ± 0.4% applied dose at 72 h. The amounts of dotriacontane recovered in the dermis (remaining skin in Table I) were 0.8% ± 0.2% at 1 h, 1.6% ± 0.3% at 24 h, and 2.0% ± 0.3% at 72 h (mean ± SEM). Together, these results suggest

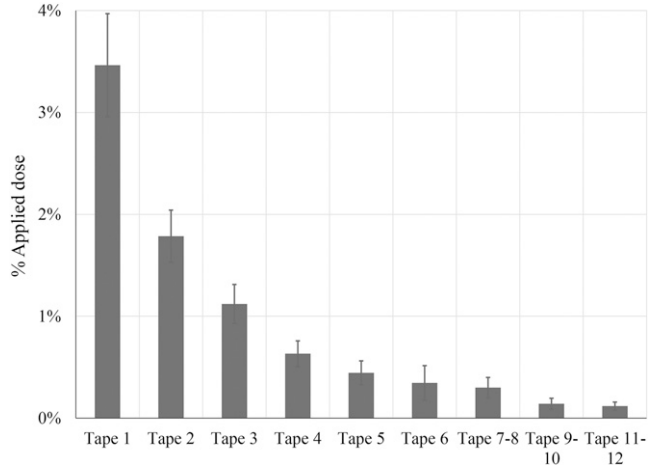

Figure 3. Amount of dotriacontane (% applied dose) in each tape, from Tape 1 to 12, at 1 h after bodywash application and rinsing. Mean ± SEM ($n = 7$ for Tapes 1–8, $n = 5$ for Tapes 9–10, and $n = 4$ for Tapes 11–12). The amounts between Tape 1, Tape 2, and other tapes are considered significantly different ($p < 0.05$, ANOVA).

that the dotriacontane and petrolatum remaining on the skin after bodywash application and rinsing were able to penetrate the stratum corneum into the deeper skin layers over time.

PENETRATION OF PETROLATUM THROUGH THE SPLIT-THICKNESS SKIN AND TOTAL PERCENT RECOVERY

No dotriacontane was detected in PBS in the receptor samples, suggesting that the petrolatum cannot penetrate through the dermis to reach the systemic circulation at a significant quantity under the condition studied. The total % recovery of dotriacontane at

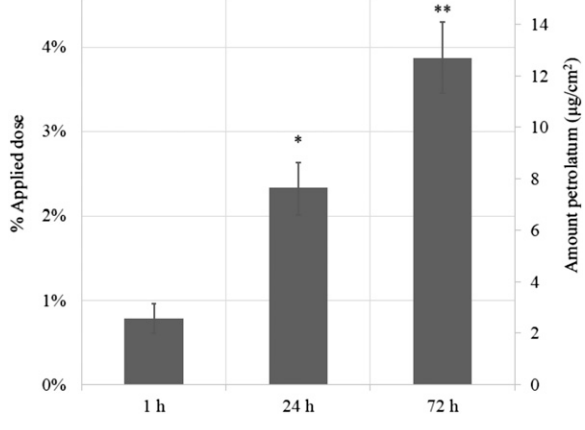

Figure 4. Amount of dotriacontane (% applied dose) and calculated petrolatum amount (µg/cm^2) in the stratum corneum and epidermis beyond the 5 tape-stripping (i.e., epidermis minus the first 5 tapes) at 1, 24, and 72 h after bodywash application and rinsing. Mean ± SEM ($n = 8$ for 1 h, $n = 7$ for 24 h, and $n = 11$ for 72 h). * denotes significant difference compared with the data at 1 h ($p < 0.05$, ANOVA). ** denotes significant difference compared with the data at 1 h ($p < 0.01$, ANOVA).

all time points is summarized in Table I. The average total recovery was between 92% and 103% (range: 82–129%).

DISCUSSION

APPLYING AND RINSE-OFF METHOD

A previous study investigated the penetration of petrolatum into the stratum corneum after skin application (6). The differences between the previous Franz diffusion cell and tape-stripping study and the present study include the methodology (with and without simulated rinsing) and the use of the bodywash formulation. In the present study, the bodywash formulation was applied to the *in vitro* skin and spread in circular motions using a smooth ground flat surface glass rod. A rinse-off protocol was then applied to remove the bodywash from the *in vitro* skin surface. Nguyen et al. (7) studied the methods to simulate rubbing of a gel topical formulation on skin and found that using the vertical glass rod method resulted in spreading of the gel to the entire area and facilitated a rapid onset of product delivery at 4 h. The glass rod method was the method of choice for applying topical formulations in skin penetration studies *in vitro*. Using the present method of bodywash application and rinsing, a significant portion of petrolatum was deposited on the skin. Particularly, the rinse-off data in Table I show that approximately 1/3 of the petrolatum in the bodywash formulation was deposited on the skin after the rinse-off protocol. Approximately 1/3 of the petrolatum in the bodywash remaining on the skin (~1/9 of the total petrolatum in the bodywash) was removed by the filter wipes at the end of the penetration study. The relatively large amount of petrolatum deposited on the skin surface after rinsing (i.e., ~1/3 of the dose of petrolatum) could lead to better petrolatum penetration into the stratum corneum with the bodywash formulation.

PENETRATION OF PETROLATUM INTO THE STRATUM CORNEUM

In a previous study with a heat-separated human epidermal membrane, when petrolatum was directly deposited on the skin surface using a volatile solvent, hexane, a trace amount of petrolatum (<2% applied dose) was found to penetrate the membrane into the receptor chamber of the diffusion cell at 72 h postapplication and no detectable penetration of petrolatum was found in the receptor chamber when split-thickness skin was used (<0.05% applied dose, estimated from the detection limit) (6). This result indicates that petrolatum could penetrate across the epidermis but not the split-thickness dermis, possibly because of the high lipophilicity of petrolatum. This also suggests that petrolatum likely would not penetrate across the dermis and enter the systemic circulation from each application under the *in vivo* condition. Petrolatum is a mixture of C12–C85 long-chain aliphatic hydrocarbons (8). It was suggested that highly lipophilic compounds such as petrolatum can accumulate in the stratum corneum (9). It was also suggested that the shorter chain hydrocarbons in petrolatum were able to dissolve and penetrate into the deeper layers of the epidermis, whereas the longer chain hydrocarbons were "trapped" in the upper layers. ^{14}C-dotriacontane is a C_{32} alkane and is one of the main components of petrolatum (6). It was used as the radiolabeled probe

(i.e., a model permeant) of the petrolatum in the present study. The study results show that dotriacontane was mainly found in the upper layers of the stratum corneum (e.g., Tapes 1 and 2) and was traceable in the stratum corneum deeper layers (e.g., in 12 tape-stripping study, Figure 3). The petrolatum detected in the stratum corneum from tape-stripping could be related to the ridges and furrows (10) and/or desquamated layers of the skin. A comparison of these tape-stripping results with those from petrolatum deposition using a volatile solvent in a previous study (6) shows that the amounts of petrolatum (as % applied dose) penetrated into the stratum corneum from the solvent deposition method were ~5× larger than those from the bodywash. The difference is likely attributed to the (a) different dosing methods, (b) different amounts of petrolatum deposited on the skin surface possibly relating to the dosing methods, (c) different data analyzes including the number of compartments used in the calculations, and/or (d) skin-to-skin variability. Despite these differences, when the data were compared based on the total amounts of petrolatum penetrating the stratum corneum, taking into account the differences due to water rinsing, skin wiping, and donor chamber recovery wash, the two skin deposition methods provided similar % amount penetration profiles in the stratum corneum.

In addition to the present *in vitro* study, previous clinical data have suggested that the penetration of petrolatum into the stratum corneum is related to the effect of glycerol monooleate (11). Glycerol monooleate is a glyceryl fatty acid ester that has a *cis* C=C double bond at the C_9 position. It is widely used in cosmetic products as an emulsifier and absorption enhancer. The Food and Drug Administration (FDA) has classified this ingredient as generally recognized as safe in the FDA Inactive Ingredient Guide. It is considered nontoxic, biodegradable, and biocompatible (12). The study concluded that petrolatum bodywash with glyceryl monooleate clinically improved skin appearance, skin surface barrier function, and hydration in humans compared with bodywashes without glyceryl monooleate (11). The results in the present study support a possible relationship between petrolatum skin penetration and the observed clinical benefits, and the increase in petrolatum penetration depth by glyceryl monooleate in the stratum corneum (6) could be one of the factors leading to these benefits. Further studies will be needed to explore the relationship and mechanism of petrolatum and glyceryl monooleate.

CONCLUSION

The bodywash formulation with petrolatum could provide significant deposition and penetration of petrolatum into the stratum corneum (~0.03–0.05 mg/cm^2 from 5 µL bodywash formulation) at 1–72 h postapplication. Specifically, petrolatum was observed to deposit from the bodywash when applied on the split-thickness skin with simulated rinsing. The petrolatum then penetrated into the stratum corneum and was detected at the depth of 12 tape-stripping and further in the epidermis.

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