

# Stearic acid delivery to corneum from a mild and moisturizing cleanser

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## Summary

**Objective** A mild moisturizing body wash with stearic acid, a key component of corneum lipids, and emollient soybean oil has been introduced in the market place. The objectives of this study are to determine the amount and the location of the stearic acid in the corneum after *in vivo* cleansing by the formulation.

**Method** Clinical cleansing studies for one and five consecutive days were carried out with the formulation containing soybean oil or petroleum jelly (PJ). The free stearic acid in it was replaced by the fully deuterated variant. The amounts of stearic acid in 10 consecutive corneum tape strips were measured by liquid chromatograph–mass spectroscopy. Separately, electron paramagnetic resonance (EPR) measurements were taken with a porcine skin after a wash by the soybean oil formulation with its free fatty acid replaced by its spin probe analogue, 5-doxyl stearic acid.

**Results** Deuterated stearic acid was detected in all 10 consecutive layers of stratum corneum and the total amount after five washes with the soybean oil formulation was  $0.33 \mu\text{g}/\text{cm}^2$ . The spin probe in cleanser-treated skin was incorporated in a partially ordered hydrophobic region similar to corneum lipids. The probe mobility increased in the temperature region where lipid disorder was expected.

**Conclusions** The estimated total fatty acid delivered to skin from cleansing is comparable to the amount of fatty acid in a corneum layer. The delivered fatty acid is most likely incorporated in the corneum lipid phase.

**Keywords:** body wash, corneum lipid, cosmetic formulation, cutaneous bioavailability, electron spin resonance (ESR), percutaneous penetration

## Introduction

Stratum corneum, the outermost layer of the human skin, is the protective barrier to penetration of foreign substances into the body. It is 10–20 microns in thickness and consists of fully keratinized protein corneocyte

cells embedded in a lipid matrix in a “bricks and mortar” arrangement. The corneocytes are separated by highly ordered lipid lamellae consisting primarily of ceramides, cholesterol, and saturated fatty (e.g., stearic) acids.<sup>1</sup> The lipids are about 15% of the corneum by weight and are organized in predominantly rigid multilayer structures. It has been proposed that the protein-bonded ceramides interdigitate with intercellular lipids forming a scaffold for the organization and stability of the intercellular lipid lamellae.<sup>2</sup> The fatty acids play a key role in maintaining the flexibility of the lipid barrier in corneum.

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Cleansing with harsh surfactants such as soap can cause skin irritation, drying, and tightness. Surfactants interact with skin in a number of ways.<sup>3,4</sup> Strong binding of ionic surfactants to skin proteins leads to barrier damage and irritation. Nonionic surfactants can cause skin dryness and tightness by removing fatty acids from the corneum. A major breakthrough in skin cleansing was the introduction of a clinically proven<sup>5</sup> synthetic detergent (syndet) mild bar containing the surfactant sodium cocoyl isethionate and high level (around 30%) long-chain fatty acids. Recently, a liquid body wash that is not only mild but also moisturizes the skin has been introduced in the marketplace. This product has sodium cocoyl isethionate as the primary surfactant with fatty acids and soybean oil as the benefit agent emollients.<sup>6</sup>

The objectives of this study are to determine the amount and the location of the stearic acid in the corneum after *in vivo* cleansing by body wash. The amounts of stearic acid delivered to 10 consecutive layers of corneum after both one and five consecutive days of *in vivo* washes by two body washes are measured. In one clinical study, the mild moisturizing body wash with soybean oil was applied to 20 subjects. The other study was carried out with 10 subjects with a similar formulation except that soybean oil was replaced by petroleum jelly (PJ). To differentiate the exogenous fatty acid delivered from the cleanser from the endogenous one of the corneum, the free stearic acid in the formulations was replaced by fully deuterated variant. The low levels of deuterated stearic acid in the corneum layers were measured by the liquid chromatograph–mass spectroscopy (LC–MS) technique. Deposition of fatty acids to deeper layers of corneum from washing with syndet bars has been previously reported.<sup>7</sup> However, the fatty acid content of the bar was around 30% whereas the level in the body wash used in this study is only around 5%.

Information about environment of the delivered stearic acid in the corneum is inferred from the electron paramagnetic resonance (EPR) spectra of a fatty acid analogue spin probe, 5-doxyl stearic acid (DSA), where the doxyl probe group is attached at the 5th carbon. The spin probe was incorporated in the body wash formulation by replacing the free fatty acid. The EPR measurements were taken on dermatomed porcine skin surface after washing and rinsing. From a computer-aided analysis of the spectra, the mobility, environmental polarity, and local order of the probe environment are calculated. Similar or complementary information could be obtained from the direct NMR measurements of the exogenous fatty acid in skin. Unfortunately, the signal from the small amount of stearic acid deposited after

cleansing appears to be well below the sensitivity of existing NMR instruments. EPR technique is about three orders of magnitude more sensitive than NMR and has been utilized<sup>8</sup> to study lipid bilayer structure. Literature studies<sup>9</sup> have shown that at least for magnetically aligned phospholipid bilayers, the results from solid state NMR with deuterated fatty acid position agree very well with the corresponding results for EPR fatty acid probe.

## Materials and methods

### *In vivo* clinical study

All clinical studies were reviewed and approved by an institutional review board.

### Test materials

The nominal compositions of the two formulations studied are given in Table 1. In both the formulations, a part of the stearic acid is replaced by deuterated stearic acid (Aldrich Chemicals, Milwaukee, WI, USA). The only difference between the two formulations is the replacement of the emollient soybean oil (formulation A) by PJ (formulation B). Soybean oil is a natural polar oil whereas PJ is a nonpolar semi-solid mixture of hydrocarbons. These two emollients were chosen as they are often used in marketed body wash products.

### Design/study plan

The clinical studies were randomized, single-blind and controlled cleansing application in which 20 or 10 subjects completed the study. Enrollment was conducted on Thursday, and application and tape stripping were performed Monday through Friday of the following week. The product application phase lasted 5 days (Monday–Friday). Subjects arrived at the testing center for one controlled application wash per day. The following wash procedure was used: The subject's lower forearm was wetted with water, and 0.5 mL of product was then dispensed onto it by the study personnel. The

**Table 1** Primary ingredients in test products

Formulation A	Sodium cocoyl isethionate, sodium laureth sulfate with an average of 1 ethylene oxide group, cocoamidopropyl betaine, soybean oil, glycerin, lauric acid, palmitic acid, deuterated stearic acid (1.35%) and water
Formulation B	Sodium cocoyl isethionate, sodium laureth sulfate with 1 ethylene oxide, cocoamidopropyl betaine, petroleum jelly, glycerin, lauric acid, palmitic acid, deuterated stearic acid (1.25%) and water

subject washed the entire inner forearm with the product for 30 s; the lather remained for 15 s on the arm, and then was rinsed for 15 s. The site was then patted dry. The same arm was washed once each day for 5 days. On the first and final day of the study (Monday and Friday), subjects arrived at designated times for a wash. Twenty minutes after washing, the arm was tape stripped. A designated area for tape stripping was marked using a template and a skin marker. Ten 1 in  $\times$  1.5 in tape strips (Sellotape Company; <http://www.selotape.com>, sample code 680293) were taken sequentially from the same site in the stratum corneum. Study personnel wore rubber gloves and used forceps to avoid contamination of the tapes. A piece of the tape was applied to the designated area, pressed firmly onto the skin with thumbs to ensure adhesion to skin, then removed/pulled from the opposite direction from which it was applied. Visual assessments (safety checks only) were made prior to each wash, as well as before and after tape stripping. The tape strips were stored in glass scintillation vials and stored at 20 °C prior to extraction and analyses of stearic acid and triglycerides. On Friday, the tape-stripping procedure was repeated on a new site, lateral to the previous site. The first-day and 5-day sampling were randomized between inner and outer sites of the arm.

#### Extraction of stearic acid from the tapes

Ten milliliters of hexane (Aldrich Chemicals) was added to each tape strip placed in a 13-mL glass culture tube with sticky side exposed. It was then sonicated for 15 min, vortexed, and then heated for another 15 min. After cooling to room temperature, it was again vortexed four times in a time period of 2 h, the supernatant liquid was then filtered through a 0.45-micron Teflon PTFE filter.

#### Measurement technique

The low levels of deposited deuterated stearic acid (molecular weight 318) in the extracted liquid were measured by the liquid chromatograph–mass spectroscopy (LC/MS) technique. Samples were analyzed using a Micro mass Quattro Ultima equipped with 4.1 Mass

Lynx software. A concentration level of 0.01 ppm can be measured by the LC–MS technique. The calibration curve of d35-stearic acid in methanol was linear at these low concentrations. To verify that the MS peak at mol.wt. 318 was indeed that of d-stearic acid, an identical experiment was carried out with skin treated with formulation with regular stearic acid (not deuterated). The absence of a MS peak of mol.wt. 318 in this experiment (data not shown) indicated that d-stearic was found in corneum only after the application of the formulation containing d-stearic acid.

#### Determination of % recovery

Twenty-five microliters of d-stearic acid in methanol at 211 ppm or 505 ppm concentration was applied *in vivo* to a 9.67-cm<sup>2</sup> area. After the solvent was evaporated, nine consecutive tape strips were taken. From the tapes, d35-stearic acid was extracted using the hexane solvent and quantified by the LC–MS method. The recovery experiments were carried out four times and the average recovery was 82%.

#### Electron Paramagnetic Resonance (EPR)

##### Probe

The EPR probe 5-doxyl stearic acid was purchased from Aldrich chemicals. The ionizable 5-doxyl stearic acid (5-DSA) (Fig. 1a) is an analogue of stearic acid (Fig. 1b) and is commonly used to probe the membrane lipid–water interface. The nitroxide doxyl group (stable radical) is attached in a rigid, stereo-specific manner to the 5th carbon atom of the stearic acid so that the motion of the nitroxide group directly reflects the motion of the labeled part of stearic acid.

##### Skin

Skin from approximately 10-week-old female pigs was obtained from Thomas D Morris (4001 Millender Mill Rd; Reisterstown, MD, USA). The pigs were sacrificed for research purposes unrelated to Unilever or to the present study. The 2.5  $\times$  8-inch skin strips were lightly clipped, dermatomed to 500-micron thickness, and shipped frozen on saline-soaked gauze.



**Figure 1** The chemical structures of (a) 5-doxyl stearic acid (5-DSA) and (b) stearic acid.

### Sample preparation

For the EPR study, the free stearic acid in the formulation was replaced by 5-DSA probe. The procedure to incorporate the probe into the formulation is as follows: 25 mg of 5-DSA probe in a vial is dissolved in 2 mL of methanol. The solution is poured in a wide bottom beaker and kept overnight for the solvent to evaporate. Following that, 1.85 g of the cleansing formulation without the stearic acid was added to the film. The formulation with the probe was then placed in a water bath at 60 °C and stirred for 15 min.

### Washing protocol

After the skin was thawed, the remaining hair on the surface was removed very carefully by a pair of scissors making sure not to cause any incision on the skin. It was then washed under running water for 30 s, patted dry and cut into a number of pieces of dimension of 1.5 × 1 inch. Selected skin pieces were then placed on different glass slides in different Petri dishes. 0.5 mL of formulation was then rubbed with gloved fingers on a piece of skin for 30 s. The resulting lather was allowed to remain on the skin for 15 s. It was then rinsed off the skin by a 15 s wash under running tap water. The washed skin was allowed to dry for 20 min. A single tape strip was then taken to remove the top layer of the skin. This was carried out to remove any surface deposition. The samples were stored in a refrigerator overnight before the EPR measurements were taken.

### Instrumentation

The continuous wave EPR spectra were recorded by means of an EMX-Bruker spectrometer, operating at X band (9.5 GHz) interfaced to a PC-IBM computer (Bruker software) for data acquisition and handling. The temperature was controlled with a Bruker N2 Temperature controller cooled with liquid nitrogen vapor.

### Simulation of EPR spectra

The observed EPR spectra are matched to simulated spectra generated by computer-assisted analysis of the line shape employing the well-established procedure of Budil and Freed.<sup>10</sup> The g tensor components for the coupling between the electron spin and the magnetic field are assumed to be constant with  $g_{ii} = 2.0085, 2.006, 2.002$ . From the input to the simulated spectra the mobility of the probe, the environmental polarity, and local order of the probe environment are obtained. The main parameters from the simulation are:

A<sub>ii</sub>: diagonal components of the hyperfine tensor for the coupling between the electron spin and the nitrogen nuclear spin: The trace  $\langle A \rangle = 1/3 (A_{xx} + A_{yy} + A_{zz})$

is an indicator of the polarity of the probe environment. In nonpolar mineral  $\langle A \rangle$  is 14 gauss, in microsomal lipid it is around 15 gauss, and in ethanol:water (1:1) it is 15.3 gauss.<sup>11</sup>

$\tau_{\text{perp}}$ : perpendicular component of the correlation time for the rotational diffusion motion of the probe (corresponding to rotation around the long fatty chain axis): an increase corresponds to the decrease in rotational mobility. The EPR time scale varies from  $10^{-12}$  to  $10^{-7}$  s.

Intrinsic line width: line broadening arises from spin-spin interactions which may be because of (a) paramagnetic species, like oxygen, close to the nitroxide moiety; (b) probes close to each other.

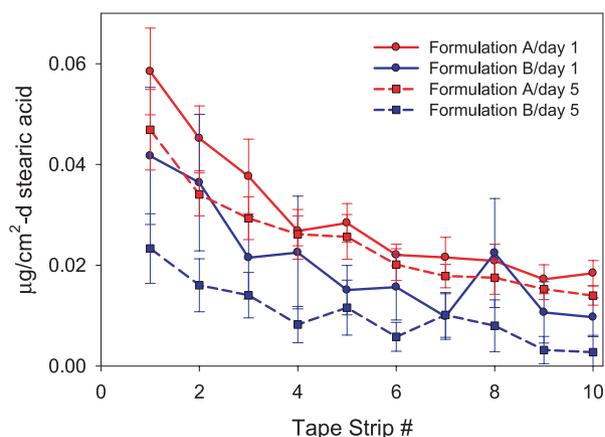
S: microscopic order parameter which measures the wobbling motion of the probe inserted into a lipid layer; for a macroscopically random sample, S can vary between 0 and 1 which corresponds to a completely disordered to a fully ordered probe environment.

## Results and discussions

### In vivo stearic acid deposition

The amount of d35-stearic acid in ten consecutive tape strips after one and five washes of formulation A and B were measured. To compare the results from the two studies, the data are divided by the percent of d-stearic acid in each formulation (1.35 or 1.25) and shown in Figure 2.

The depth profile of the deposited stearic acid in corneum is approximately exponential. It is interesting to note that the amount of stearic acid deposition does not increase but decreases with the number of washes.

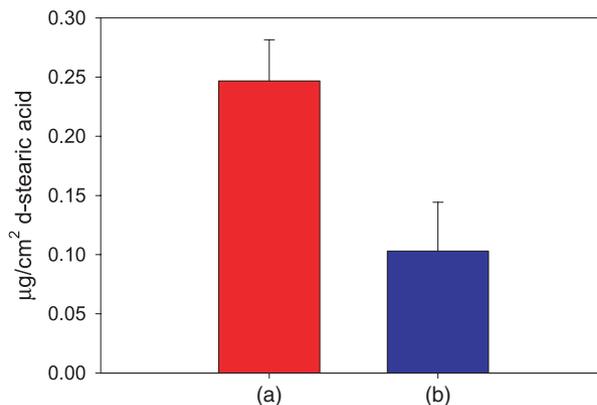


**Figure 2** Normalized d35-stearic acid deposition at each tape strip after 1- and 5-day wash by the two formulations. The bars represent standard error of mean.

The reduced penetration of stearic acid with number of washes is possibly because of enhanced skin moisturization following regular repeated washing with the moisturizing cleanser. In a separate study, consumer self-perception questionnaire scores on the effects of the moisturizing cleanser on leg feel indicated enhanced moisturization after 1 week of application<sup>6</sup> (Fig. 7b). It is known that tape strips extract more materials from a dry corneum than from a moisturized one. The data (Fig. 2) indicate the greatest differences between the washes are at or near the top layer where the effect of dryness would be most pronounced. The results suggest that, in the future, penetration data using tape strips should be normalized by the mass of the material (e.g. the amount of protein) on the tapes.

Replacement of soybean oil by the viscous emollient PJ in formulation B reduces the level of d-stearic acid, especially after five washes. The penetration of stearic acid into skin takes place within a minute. The penetration depth is much more than what could be achieved by simple diffusion through lipid bilayers. Assuming a diffusivity  $D$  of  $10^{-11}$  cm<sup>2</sup>/s, in one minute ( $T$ ) stearic acid would penetrate to a distance  $L$  of 0.3 micron ( $L^2 = 2DT$ ) which is less than one tape strip depth. It is likely that stearic acid is transported into the deeper layers by convective diffusion of the liquid through hydrophilic defects in the corneum.

The mean deposition of d35 stearic acid from the formulation A (with soybean oil) was  $0.4 \pm 0.04$   $\mu\text{g}/\text{cm}^2$  after one wash and  $0.33 \pm 0.05$   $\mu\text{g}/\text{cm}^2$  after five washes. The error bounds were standard error of the mean. The deposition was first calculated for each subject by summing up the amounts in the ten tape strips and from that data the mean and the standard error were calculated. The total deposition of d35 stearic acid from the formulation B (with PJ) was  $0.26 \pm 0.1$   $\mu\text{g}/\text{cm}^2$  after one wash and  $0.13 \pm 0.05$   $\mu\text{g}/\text{cm}^2$  after five washes. Using the measured extraction efficacy of d-stearic acid from tape strip of 82%, the actual depositions of d-stearic acid from formulation A are 0.49 and 0.40  $\mu\text{g}/\text{cm}^2$  after one and five washes, respectively. The observation that a measurable amount of stearic acid was found even in the 10th tape suggests an even deeper penetration of stearic acid into skin and that the estimate of total deposition is a conservative one. If we assume that all the stearic/palmitic (C18/C16) acid in the formulation penetrates the corneum with the same efficiency as the deuterated stearic acid, the estimated total deposition of C18/C16 fatty acid after 1 and 5 days would be 1.6  $\mu\text{g}/\text{cm}^2$  and 1.3  $\mu\text{g}/\text{cm}^2$ , respectively. This is comparable to the amount of fatty acid present per layer of corneum and to the amount of lipid removed by a mild cleanser.<sup>7,12</sup>



**Figure 3** Total amount of d-stearic acid in the corneum after five washes by the cleanser formulation with soybean oil (a) or petroleum jelly (b). The data are normalized by the percent of d-stearic acid in each formulation. The error bars represent the standard error of mean.

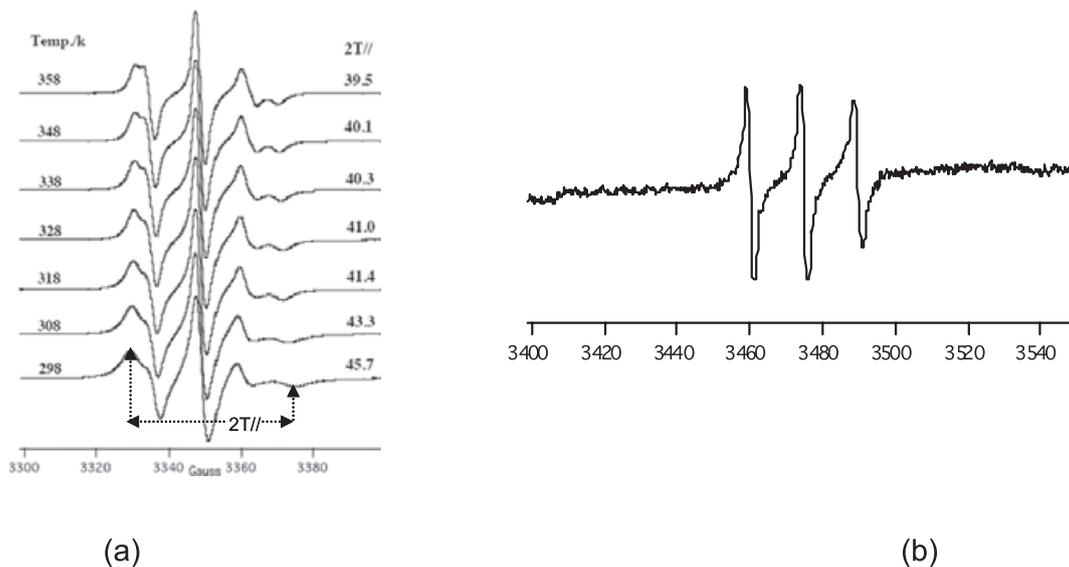
The comparison between the estimated total fatty acid delivered to skin from cleansing and the amount of fatty acid in a corneum layer is based on the following simple calculation. We assume a corneum of 10-micron thickness and 15 layers containing 15% lipids of which about 9% is fatty acid.<sup>13</sup> If we assume a density of 0.9 gms/mL for lipids, each corneum layer would then contain around 0.81  $\mu\text{g}/\text{cm}^2$  of fatty acid which is comparable to calculated amount of total fatty acid of 1.6  $\mu\text{g}/\text{cm}^2$  that could be delivered from the body wash with soybean oil formulation.

A comparison of the normalized deposition results from the two clinical studies (Fig. 3) suggests that replacement of soybean oil by PJ reduces the amount of d-stearic acid delivered to corneum after five washes by about 50%. The higher penetration with formulation A may be because of solubilization of fatty acid in the soybean oil. However, this hypothesis needs to be validated by comparing the effects of other polar and nonpolar oils on fatty acid penetration into corneum. In addition, the semi-solid hydrophobic PJ may create a better barrier against penetration into corneum. We should note, however, that for a more definitive conclusion, the two formulations need to be compared in the same study.

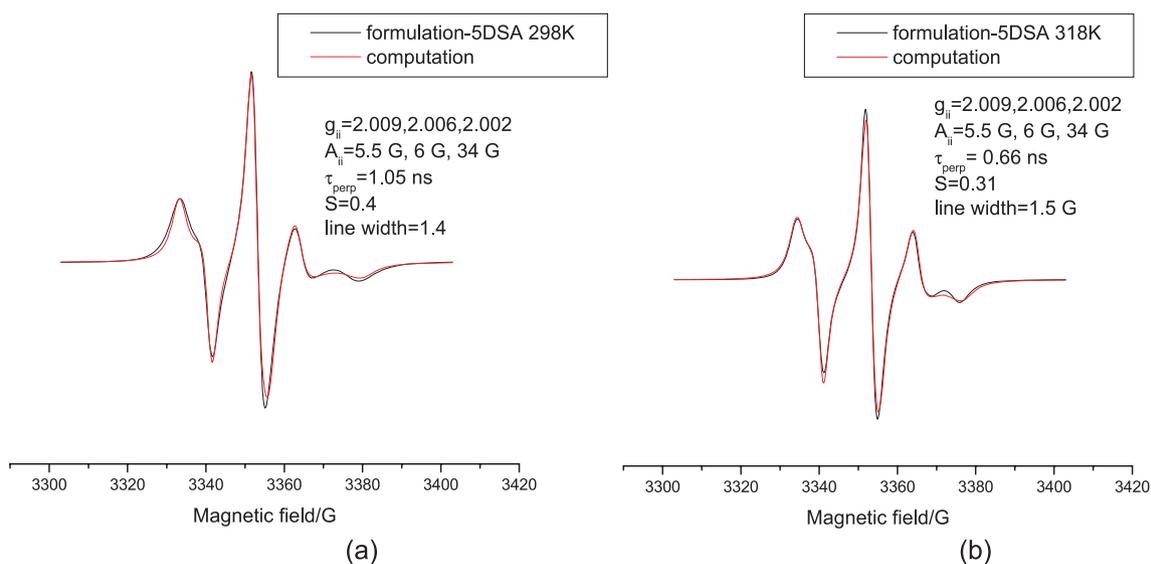
### Electron paramagnetic resonance studies

#### Probe in formulation

The EPR spectra of 5-DSA in formulation A at a series of temperatures are shown in Figure 4a, and the spectrum in ethanol at 298°K is shown in Figure 4b. The broad structure of the EPR spectra of 5-DSA probe in the formulation indicates that probe is moving relatively



**Figure 4** Electron paramagnetic resonance spectra of 5-doxyl stearic acid (DSA) in (a) cleansing formulation A and in (b) ethanol.



**Figure 5** Experimental and simulated electron paramagnetic resonance spectra of 5-doxyl stearic acid (DSA) in cleansing formulation with soybean oil at (a) 298°K and (b) 318°K.

slowly in an anisotropic environment. On the other hand, the probe molecule undergoes a rapid rotation in the low viscosity unstructured ethanol solvent. Consequently, the anisotropic interactions of the unpaired electron in the doxyl group with the external magnetic field is averaged out resulting, as expected, in three sharp peaks.

The experimental spectra at 298 and 318°K and the corresponding spectral simulations are shown in Figure 5. The main parameters used for computation are

shown in the figure as well. These parameters indicate that the probe in the formulation is in a partially ordered (possibly chain-melted liquid crystalline) structure tumbling with a mobility at the limit between fast and slow motion. With increasing temperature, the probe rotates faster (decreasing  $\tau$ ) in a more disordered environment (decreasing order parameter,  $S$ ). The polarity of the probe environment remains invariant with temperature with an  $\langle A \rangle$  value of 15.17 gauss suggesting a moderately polar environment.

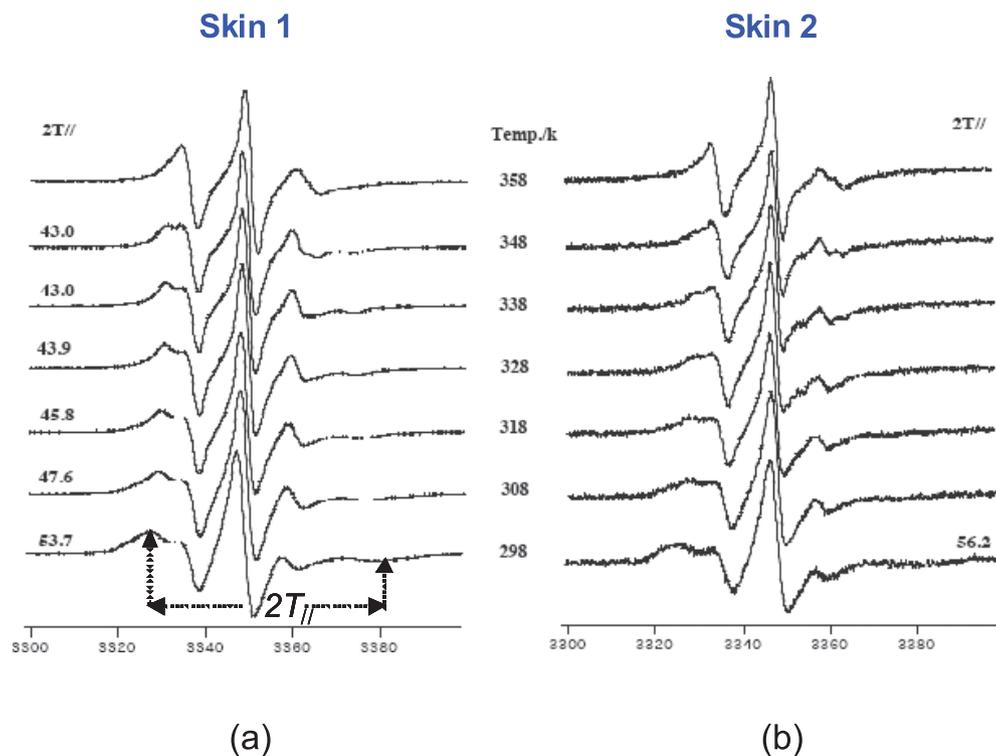
*Probe in skin*

The EPR spectra of the probe 5-DSA as a function of temperature in two skin samples (without the top layers) treated by the cleansing formulation A (with soybean oil) are shown in Figure 6. The spectral simulations were carried out for 5-DSA spectra in skin at 298 to 318°K. The experimental and computed spectra are shown in Figure 7, together with the main parameters used for computation. The matching between the experimental and the simulated spectra is good but not perfect. We believe this is a consequence of the need to match simultaneously the slow motion anisotropic probe spectra at two different temperatures. The fatty acid probe is localized in partially ordered lipid layers of a complex system. To get the information about the variation of the rotational mobility of the fatty acid probe as a function of temperature, the simulation has to include the lipid order parameter (to account for the structural factor), retain the same magnetic parameters ( $g_{ii}$  and  $A_{ii}$ ), and change only the probe mobility from 298 to 318°K.

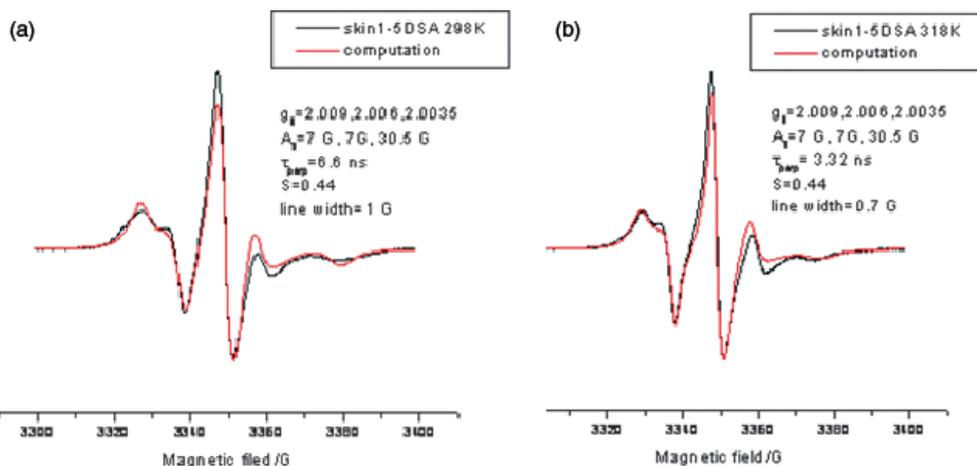
The simulation parameters indicate that the 5-DSA probe is in a partially ordered environment with  $S = 0.44$ . The order does not change when the temperature was raised from 298 to 318°K. However, the mobility of the probe increases with temperature with

the correlation time  $\tau_{\text{perp}}$  decreasing from 6.6 ns to 3.3 ns. The probe polarity also did not change at this temperature range and remained constant at  $\langle A \rangle = 14.87$  gauss. It is interesting to note that, on the basis of the EPR computation parameters, both mobility and polarity are much lower in the skin samples (Fig. 7) if compared to cleansing formulation samples (Fig. 5).

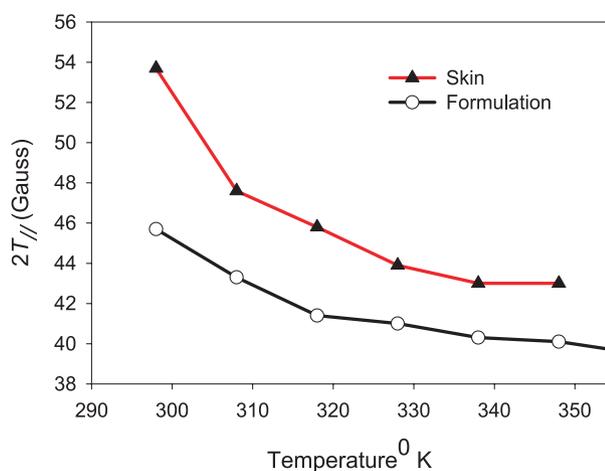
The hyperfine splitting of the outer peak,  $2T_{II}$ , of the EPR spectrum is a qualitative measure of the probe mobility. A decrease in  $2T_{II}$  indicates faster probe motion. In Figure 8, the  $2T_{II}$  values of the 5-DSA in the cleanser formulation (A) with soybean oil and in skin after a wash by the formulation are plotted as a function of temperature. The results indicate that the probe mobility in skin increases rapidly between 298 and 328°K which is the temperature region where the corneum lipids are expected to become more chain-melted and fluid. The probe spectra in skin are different from the spectra in the formulation thus ruling out the possibility that the probe in the skin is in a residual lipid bilayer phase from the formulation. Thus, the results suggest that the fatty acid analogue 5-DSA delivered from the body wash with soybean oil is incorporated in the corneum lipid phase.



**Figure 6** (a) and (b) Electron paramagnetic resonance spectra of 5-doxyl stearic acid (DSA) as a function of temperature with two skin samples after cleanser wash and the top surface removed by a single tape stripping.



**Figure 7** Experimental and simulated electron paramagnetic resonance spectra of 5-doxyl stearic acid (DSA) in skin delivered from cleansing formulation A at (a) 298 K and (b) 318 K.



**Figure 8** Hyperfine splitting of the outer peak,  $2T_{II}$ , of 5-doxyl stearic acid (DSA) spectra as a function of temperature in the cleanser with soybean oil and in skin after a wash by the cleanser.

## Conclusions

*In vivo* cleansing clinical studies with two different liquid cleansers with stearic acid and soybean oil or PJ as emollients showed that measurable amounts of stearic acid are delivered to at least 10 layers of stratum corneum after 1 and 5 days of washing. Replacement of soybean oil by PJ reduced the delivery of stearic acid to skin by about 50%. The amount of fatty acids delivered to corneum after a wash is comparable to the amount of fatty acid present per layer of corneum and to the amount of total lipids removed by a mild cleanser. Electron paramagnetic resonance measurements with *ex vivo*

porcine skin after a single wash by the cleanser formulation with soybean oil and containing the fatty acid analogue spin probe 5-DSEA suggests that the deposited fatty acid after a cleanser wash probe is incorporated in the corneum lipids in a partially ordered bilayer structure.

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