Abstract
Surfactant-based cleansing products can cause skin damage and/or irritation due to surfactant-skin interactions, which can compromise skin barrier function. In this work, skin impedance measurements were conducted in vitro on porcine skin using vertical Franz diffusion cells to investigate the impact of surfactants, as well as skin cleansing formulations, on skin barrier integrity and function. This method can guide the development of milder cleansing formulations resulting in less or no skin damage/irritation. Examples of some beneficial formulation additives are illustrated and discussed. The study demonstrates that skin impedance is a useful proxy for skin barrier function and can be utilized as a routine approach to screen surfactant containing formulations for their propensity to compromise the skin barrier.

INTRODUCTION
Stratum corneum (SC), the outermost layer of the skin, plays a critical physiological role in protecting the body from stresses in the external environment [1]. However, cleansing the skin with soap bars and other surfactant-based cleansing products can reduce skin barrier function, as frequent exposure to surfactants leads to various degrees of skin damage and irritation, including dry and itchy skin. It is widely established that the cleansing process also causes damage to the skin by denaturing proteins and/or solubilizing or disrupting the organization of stratum corneum (SC) lipids [2-3]. As the result, skin is often left dry and flaky after frequent cleansing as surfactants interact with the skin and weaken its natural defensive SC barrier function. The undesirable effects of surfactants on skin also include poor skin appearance and increased skin tightness. These effects are greater when the ambient temperature and humidity levels are relatively low. An additional negative effect of cleansing is that surfactant molecules may penetrate into the SC and induce further skin irritation; such effects are greater for individuals with sensitive skin. All these undesirable side effects are interrelated. Many methods, both in vivo and in vitro, have been reported for assessing skin barrier properties. [4-5]. Skin’s electrical impedance is a direct measure of its permeability and can be used to evaluate skin integrity and skin barrier damage and recovery [6]. Skin barrier perturbation due to exposure to different chemicals can be quantitatively expressed by measuring changes in skin impedance. When the skin barrier is perturbed, whether by a physical or chemical stress, its electrical impedance decreases since the transport rate of ions flowing through the skin is higher. It has been shown by several research groups, and for different applications, that skin impedance correlates well with skin permeability [6-8].

To mitigate the negative effects, and to develop improved and milder cleansing technologies, it is clearly desirable to modify the cleansing formulations with materials that will reduce or alleviate surfactant related problems resulting from the cleansing process. Emollients and other formulation components such as glycerin are often added to cleansing formulations to enhance their mildness via a variety of direct and indirect mechanisms. Often the biggest challenge with this approach is preventing components intended for skin deposition from being washed away when the cleanser is rinsed off [9].

In this work, in vitro skin electrical current (impedance) measurements were conducted to quantitatively monitor changes in skin barrier integrity as a function of surfactant chemistry and concentration. The beneficial effect of introducing some ingredients to surfactant solutions and skin cleansing formulation were also investigated and demonstrated using skin impedance measurements.

MATERIALS AND METHODS
Materials
Sodium dodecyl sulfate (SDS), phosphate-buffered saline (PBS) tablets, and glycerin were purchased from Sigma-Aldrich (St. Louis, MO). PBS solution was prepared using PBS
tablet and DI water following the instruction. Sodium lauryl ether sulfate (SLES, Standopal ES-3, ~28.5%) solution were purchased from Cognis (now BASF) and diluted with DI water. Diluted NaOH and H₂SO₄ (both from Sigma-Aldrich) were used to adjust the pH of solutions, if necessary. Maleated castor oil (MCO) and lightly cross-linked PVP (LC-PVP) were obtained from Ashland Specialty Ingredients (formerly International Specialty products). A clear shower gel formulation (Table I) was prepared in the laboratory of Ashland Inc. Commercial cleansing products (body wash and soap bars) were purchased.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition, wt. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocamide dimethyl taurate</td>
<td>20.0</td>
</tr>
<tr>
<td>Maleated Castor Oil</td>
<td>0.55</td>
</tr>
<tr>
<td>Deethanol EDIHA</td>
<td>0.1</td>
</tr>
<tr>
<td>Glycerin</td>
<td>6.0</td>
</tr>
<tr>
<td>Sodium Laureth Sulfate</td>
<td>20.0</td>
</tr>
<tr>
<td>Propylene Glycol, Disodium/</td>
<td>1.0</td>
</tr>
<tr>
<td>Methy/laurate, and Propy/laurate</td>
<td></td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>3.0</td>
</tr>
<tr>
<td>Water</td>
<td>49.35</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

Table I. Original formulation of clear shower gel with MCO.

Skin impedance measurements
Detailed skin impedance measurement procedures are available elsewhere (10). In short, full-thickness skin samples from Yucatan pigs were cut into small pieces (3 cm × 3 cm), flash frozen, and stored in a -16°C freezer for up to two months. The skin was allowed to thaw at room temperature for one hour before use. The skin samples were secured in vertical Franz diffusion cells serving as a diffusion barrier between the two compartments, with the SC side facing the donor compartment. Both chambers were initially filled with PBS solution (pH 7.25) avoiding any air gaps or trapped air bubbles. The skin sample was soaked in the PBS solution for one hour before measuring the initial skin impedance. An AC sinusoidal signal, 100 mV RMS (root mean square) at a frequency of 10 Hz was then applied across the skin with a wave generator. Sintered Ag/AgCl reference electrodes were placed in the donor and the receptor compartments (through the sampling port) to measure the electrical potential and current across the skin samples. The electric current was measured using a multimeter. After measuring the initial skin impedance (calculated using Ohm’s law), the solution in the donor compartment was replaced with an experimental solution (e.g., surfactant or formulation solutions) and the skin was soaked for 5 hours. After 5 hours the solution in the donor cell was removed and the donor cell and the SC were rinsed thoroughly with the PBS solution three times. Skin impedance was then measured with PBS solution in the donor cell after one hour equilibration. Each experiment was conducted on 4-12 skin samples. Relative normalized impedance is defined and calculated as the ratio of the skin impedance after and before solution treatment and is a direct measure of skin permeability and hence, barrier function. In these studies the higher the relative normalized impedance (RNI), the less the barrier damage is.

RESULTS AND DISCUSSION
Surfactant systems
Surfactant-skin interactions with different surfactants (SDS and SLES) was first studied using the skin impedance measurement method. SLES is a frequently used surfactant in personal cleansing products and is generally considered to be a milder and less irritating surfactant than SDS.

Figure 1 compares the relative normalized skin impedance of skin exposed to SDS and SLES at similar concentrations and different pH values. The data show that SLES perturbs barrier function to a much lesser extent than SDS. This correlates well with the milder characteristics of SLES reported in clinical studies and agrees with other in vitro results (3,11). This positive correlation between the relative harshness of these surfactants supports the use of skin impedance measurements to study barrier function and its disruption by surfactants and cleansing formulations. In addition, Figure 1 shows the impact of the pH of the surfactant solutions on the skin barrier. To investigate the role that pH plays in surfactant penetration into SC, the pH of an SDS solution was adjusted to pH 4 and the pH of an SLES solution was also adjusted from its normal pH 4 to pH 7. It is clear from Fig. 1 that pH plays an important role in regulating surfactant penetration onto the skin. Increasing the pH of a surfactant solution from pH 4 to 7 increases the barrier damage induced by the surfactant and vice versa. The observation that surfactants with higher pH (more alkaline) are less mild is not surprising and is consistent with previous reports (12). It has been proposed that since the SC pH is acidic (generally around pH 5), which helps protect the skin from microorganisms, the attraction and penetration of basic surfactant solutions is more favorable. However, under basic pH conditions anionic surfactants bind primarily by hydrophobic functional groups to hydrophobic sites on the skin to minimize the repulsion of negative charges (13). Another factor to consider is SC swelling. Higher pH values in cleansing products are often associated with significantly higher swelling and skin irritancy (3,14).

Skin impedance measurements also demonstrate the beneficial effects that glycerin (GLY) and other additives provide in reducing skin barrier damage when added to surfactant formulations. The results shown in Fig. 2 demonstrate that addition of 10% glycerin to SDS surfactant solution reduces surfactant-induced skin barrier perturbation, as quantified by the larger relative normalized impedance. This is in agreement with previous reports (15-16).

Figure 2 also shows that addition of 1% maleated castor oil (MCO, INCI name: castoryl maleate oil) separately or combined with glycerin can further reduce the harshness of surfactant systems. MCO is a synthetically modified triglyceride molecule and made by the reaction of castor oil with maleic anhydride (17).
The effect of adding lightly cross-linked polyvinylpyrrolidone (LC-PVP) on surfactant-induced skin damage was also studied using the skin impedance method. Figure 4 shows that a significant reduction (Student t-test p < 0.05) of skin barrier damage was achieved by the addition of LC-PVP.

**Skin cleansing formulation systems**

Having established that the skin impedance approach was useful in evaluating simple surfactant formulations it was further used to evaluate some commercial skin cleansing formulations.

First it was used to compare the harshness of several soap bar products. Basically there are two types of soap bar products: those with fatty-acid soap-based surfactants, which are anionic type and referred to as regular soap “bars”, and those with non-soap-based surfactants and are referred to “syndet” (synthetic detergent-based bars) (3). Soap-based cleansers are alkaline with a pH around 10, while syndets are mostly neutral or slightly acidic (pH 7 or below). Besides the intrinsic harness of the surfactants, the pH difference between these two types of bars makes a significant difference in the extent of cleanser-induced skin damage. Figure 5 confirms that compared to regular soap bars, syndet soap bars compromise skin barrier integrity significantly less (Student’s t-test p < 0.05) even at higher concentration.

Skin impedance measurements were also conducted on a fully formulated clear shower gel formulation (Table I) with and without 0.55% MCO. The cleansing formulation with MCO was specifically designed to effectively deliver MCO from rinse-off cleansing formulations. As shown in Fig. 6, and similar to the SLES system in Fig. 3, the inclusion of MCO in the shower gel cleansing formulation dramatically reduces the surfactant-induced skin damage indicating that the clear shower gel formulation with the addition of MCO is significantly milder than the regular formulation (Student’s t-test p < 0.05). This is consistent with other in vitro biophysical

The mechanism of MCO in reducing surfactant-induced skin damage is not fully understood. From critical micelle concentration (CMC) and micelle size measurements (data not shown), as well as previous biophysical and clinical results, adding MCO to surfactant solutions does not appear to change the CMC. However, it may retard surfactant molecular penetration by increasing micelle size and provide a significant and substantial occlusive lipid/oil barrier by depositing from cleansing formulations.
and in vivo clinical and studies reported previously (18-19). Besides the aforementioned formulations, the impedance method has also been used in our laboratories to study other practical formulations and commercial products such as baby wash products, hand dish washing products and shave preparation formulations (data not shown here). The results correlate well with clinical results and consumer perception.

CONCLUDING REMARKS

The examples in the current work on surfactant solutions and skin cleansing formulations demonstrate that skin impedance measurement can be used to characterize skin barrier integrity as a function of a wide range of physicochemical variables. With relatively straightforward in vitro skin impedance studies, it is possible to quantitatively examine in situ skin barrier perturbation induced by surfactants, and other physico-chemical stresses. The data in the current study are consistent with results obtained from a range of other in vitro and in vivo studies and, as such, validate the use of skin electrical impedance to predict the impact a surfactant-based formulation will have on the skin barrier, including damage and irritation.

Using this approach it is possible to screen many formulations and dramatically reduce the time and effort needed for skin-related formulation improvement, including product development and marketing.
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