A Fundamental Investigation of Surfactant-Induced Skin Irritation

by

Peter Nathaniel Moore

Submitted to the Department of Chemical Engineering in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemical Engineering at the MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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Abstract

Surfactants frequently come in contact with the skin in the form of personal care
products, where they are used to improve the wetting and oil solubilizing qualities of the
products. Surfactants are also known to induce skin irritation by damaging the barrier
properties of the stratum corneum, the outer layer of the skin, and denaturing proteins in
the epidermis and the dermis. The goal of this thesis has been to understand the
relationship between the physicochemical properties of surfactant solutions and their skin
irritation potential.

In vitro tests were developed to measure: (1) the effect of surfactants on the
barrier properties of the skin, (2) the concentration of surfactant in the skin, and (3)
surfactant-induced protein denaturation, all of which can be related to skin irritation. The
physicochemical properties of the surfactant solution, specifically, the concentration of
the surfactant monomers (unmicellized surfactant), the composition of the surfactant
monomers, and the size and shape of the surfactant micelles, were related to the results of
these tests.

An in vitro skin irritation test was developed that measures the electrical
conductivity of pig skin to quantify the reduction in the barrier properties of the skin, or
the skin damage, induced by surfactant solutions. Skin conductivity was found to be
directly related to the transdermal water permeability, directly relating the skin
conductivity to in vitro skin irritation. Skin conductivity was used to measure the in vitro
skin irritation potential of mixtures of the anionic surfactant sodium dodecyl sulfate
(SDS) and the nonionic surfactant dodecyl hexa(ethylene oxide) (C_{12}E_6), and a
relationship was observed between the surfactant monomer concentration and the skin
conductivity. The in vitro skin irritation test correctly ranked the in vivo irritation
potential of three mild commercial soap bars-Dove, Lever 200, and Ivory.

In order to understand the relationship between the micelle concentration and the
surfactant-induced damage to the skin, a method was developed to measure the
penetration of ^{14}C-radiolabeled SDS surfactant into pig skin. It was found that both
monomeric and micellar SDS are able to penetrate into the skin, and that the contribution
of the micellar SDS to the concentration of SDS in the skin is comparable to the
contribution of the monomeric SDS. SDS penetration into the skin was also measured in
the presence of poly(ethylene oxide) (PEO), which forms PEO-bound SDS micelles, and
C\textsubscript{12}E\textsubscript{6}, which forms SDS/C\textsubscript{12}E\textsubscript{6} mixed micelles. In mixtures of PEO-bound and free SDS micelles, the PEO-bound SDS micelles were found not to penetrate into the skin while the free SDS micelles were found to penetrate. Mixing SDS with C\textsubscript{12}E\textsubscript{6} led to a reduction in the penetration of SDS into the skin by reducing the SDS monomer concentration, as well as by reducing, or preventing altogether, the penetration of micellar SDS. The hydrodynamic radii of the free SDS micelles (21 Å), the PEO-bound SDS micelles (25 Å), and the SDS-C\textsubscript{12}E\textsubscript{6} mixed micelles (24-30 Å) were measured using dynamic light scattering. Based on these results, a new model of surfactant penetration into the skin was proposed, in which the penetration of micellar surfactant into the skin is limited by the size of the micelles, which must be small enough to penetrate into the aqueous pores in the skin. These aqueous pores have radii of 15-28 Å.

A direct correlation was observed between the \textit{in vitro} skin irritation (measured by skin conductivity) and the concentration of SDS in the skin. A Forearm Controlled Application Technique (FCAT) was used to evaluate the effect of adding PEO to a fixed concentration of SDS on skin irritation \textit{in vivo}. The addition of PEO led to a decrease in the \textit{in vivo} skin irritation, corresponding to the observed reduced penetration of SDS into the skin in the presence of PEO. Therefore, both the \textit{in vitro} and the \textit{in vivo} skin irritation results indicate that reducing the penetration of micellar SDS into the skin can lead to significant reductions in the skin irritation potential of SDS.

The zein solubilization test has been used to quantify the protein-denaturation potential of individual surfactants by measuring how much zein protein can be solubilized by a surfactant solution. In this thesis, the zein solubilization ability of SDS and mixtures of SDS with nonionic C\textsubscript{12}E\textsubscript{n} (n=4, 6, and 8) surfactants was measured. The addition of nonionic surfactants decreased the amount of zein solubilized by SDS. Specifically, less zein was solubilized when more C\textsubscript{12}E\textsubscript{n} was added and when the hydrophilic head size of C\textsubscript{12}E\textsubscript{n}, reflected in the value of n, increased from n=4 to n=8. A theory was developed to analyze the zein solubilization results, which demonstrated that as the hydrophilic head size (n) of the nonionic surfactant increased, it was less favorable for the zein to bind to the mixed micelle. The less favorable binding corresponded to a reduction in the zein solubilization potential of the surfactant mixture, and to a reduced ability to denature proteins in the skin.

Previously, it was believed that the skin irritation potential of a surfactant solution could be minimized by reducing the surfactant monomer concentration. In this thesis, we have found that skin irritation is also determined by micelle size and micelle concentration. The concept that the surfactant micelles can contribute significantly to surfactant penetration into the skin and associated skin irritation, and that this penetration can be prevented by the judicious addition of polymers or other surfactants, represents a novel approach in the design of milder surfactant formulations.

Thesis Advisor: Daniel Blankschtein
Title: Professor of Chemical Engineering
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Chapter 1

Introduction

1.1 RESEARCH MOTIVATION AND GOALS

Surfactant-induced skin irritation has several manifestations. Exposure of the skin to surfactants can cause redness, referred to as erythema, as well as inflammation, scaling, peeling, the formation of bullae, itching, or simply the feeling of tightness in the skin (1-3). The current goal of the cosmetics and toiletries industry is to formulate surfactant-containing products that are as mild to the skin as water (4). In order to create new surfactant formulations that are milder, or less irritating, to the skin, a fundamental understanding of surfactant-induced skin irritation must be developed. Such an understanding will allow the rational design of new surfactant formulations, or the modification of existing ones, based on fundamental knowledge of what will irritate the skin, and how a surfactant system can be tuned to increase the mildness of the formulation.

With this in mind, research has been conducted in the cosmetics and soap industries to compare the relative skin irritation induced by different aqueous solutions of single surfactants at fixed concentrations that are typically above the critical micelle concentration (CMC), the surfactant concentration beyond which micelles form (2, 3, 5-8). There has also been some work that has
examined skin irritation from the perspective of the physical chemistry of surfactant solutions, including understanding the roles of micellization and surfactant monomer concentration, that has yielded results indicating that the micellization of surfactants plays an important role in determining the skin irritation potential of the surfactant solution (9-11). An experimental study in which a harsh surfactant was mixed with a mild surfactant showed a strong correlation between the solution monomer concentration of the harsh surfactant and the measured skin irritation (12). Other experiments have also demonstrated that a reduction in skin irritation can be achieved by mixing surfactants, and these will be discussed in more detail in Section 1.4.3 and Chapter 5 (13). Therefore, knowledge of how to control the surfactant monomer concentration and composition appears to be useful in designing surfactant-containing products having lower skin irritation potentials.

Through previous work in our group, we have the capability of modeling the solution behavior of single and mixed surfactant systems (14, 15), as well as of single surfactants mixed with nonionic polymers (16), to predict important surfactant solution characteristics, such as the surfactant monomer concentration and composition. By combining these unique predictive capabilities with in vitro and in vivo skin irritation tests aimed at quantifying the skin irritation response to different surfactant solutions, we can then compare the results of these tests to the predicted surfactant solution properties and establish possible relations between the two. The results of this research should be applicable to the rational design and optimization of new surfactant formulations. With this background in mind, the central goal of this thesis is to develop a fundamental understanding of the connection between the solution properties of a surfactant solution and the skin irritation potential of that surfactant solution. This knowledge may then be used to rationally formulate novel surfactant-containing products in order to
minimize their skin irritation potential. This thesis goal will be achieved by pursuing the following sub-goals:

i) Determining how surfactants affect the skin permeability, and how changes in the skin permeability to ions (skin conductivity) can be used to quantify \textit{in vitro} skin irritation (discussed in Chapter 2).

ii) Determining how surfactant solution properties affect the penetration of surfactant into the skin (discussed in Chapters 3 and 5).

iii) Correlating the amount of surfactant penetrating into the skin with \textit{in vitro} and \textit{in vivo} skin irritation to relate how factors that control the penetration of surfactant into the skin can affect skin irritation (discussed in Chapter 4).

iv) Examining how different surfactant types and their solution properties determine their protein-denaturation potential, which has been shown to be related to their skin irritation potential (discussed in Chapter 6).

In order to understand the following arguments concerning how to tailor surfactant solutions to reduce surfactant-induced skin irritation, a brief review of surfactant solution phenomena is presented in Section 1.2. Section 1.3 provides a description of the structure and function of the skin. Subsequently, in Section 1.4, a summary of what is currently known about surfactant-induced skin irritation is provided. Section 1.5 presents an outline of the remaining chapters of the thesis.
1.2 SURFACTANT SOLUTION BEHAVIOR

1.2.1 Chemical Structure of Surfactants

Surfactants belong to a class of molecules called amphiphiles (17). These molecules consist of a hydrophilic, or ‘water-loving’, head, and a hydrophobic, or ‘water-fearing’, tail (see Figure 1.1). The head and the tail of the surfactant are attached by a covalent bond, resulting in a molecule with a regiospecific character. The hydrophobic tail of surfactants is typically a hydrocarbon, such as an alkyl chain or an alkyl-phenyl chain. Common tails include straight chain alkanes, benzyl-alkanes, and methyl-branched alkanes. The tails typically consist of at least 8 carbon atoms, resulting in a distinct domain that is poorly solubilized by water.

Figure 1.1 shows three broad types of surfactant hydrophilic heads: (i) ionic, (ii) nonionic, and (iii) amphoteric or zwitterionic. Ionic surfactants have a charged group, such as a sulfate ion, bonded to the tail. Nonionic surfactants have a polar group that is soluble in water, such as a poly(ethylene oxide) group, as their head. Amphoteric, or zwitterionic, surfactants have no net charge, but have an internal charge separation. For example, in a betaine head, there is a negatively-charged carboxylate group separated from a positively-charged quaternary ammonium group that is covalently bonded to the surfactant tail. In all these groups, the head has either an ionic or a polar character that renders it soluble in water. Accordingly, there are competing solubility tendencies for surfactant molecules, since the head is well solubilized by water while the tail is very poorly solubilized. These competing solubility tendencies cause the amphiphilic molecules to display interesting solution behavior in water (18).
Figure 1.1: Schematic representation of a surfactant, including the chemical formulae of the polar heads of an ionic, nonionic, and amphoteric surfactant.
1.2.2 The Critical Micelle Concentration

One of the most important characteristics of surfactants is the formation of aggregates of surfactant molecules, called micelles, above a threshold surfactant concentration, known as the critical micelle concentration or CMC (see Figure 1.2). At concentrations below the CMC, the surfactant molecules do not aggregate with one-another in appreciable quantities, and are instead free in solution. These free surfactant molecules are referred to as surfactant monomers. When the concentration of surfactant reaches the CMC, it becomes more free-energetically favorable for the surfactant molecules to aggregate into micelles than to remain as surfactant monomers (17). An interesting and important aspect associated with the CMC, shown in Figure 1.2, is that the onset of micellization is fairly sharp, such that below the CMC there are essentially no micellar species, while above the CMC micelles coexist with surfactant monomers, with the surfactant monomer concentration remaining essentially constant. Accordingly, as the total surfactant concentration is increased above the CMC, the concentration of surfactant monomers remains essentially flat, while the concentration of micellar surfactant increases steadily. However, it should be noted that this statement is not strictly correct in the case of surfactant mixtures, in which the distribution of the different surfactant types between the micellar and the monomeric fractions can lead to a gradual change in the surfactant monomer concentration and composition above the CMC (14, 15). Nevertheless, under the conditions examined in this thesis, the statement that the surfactant monomer concentration remains constant as the total surfactant concentration is increased above the CMC is adequate, and will play an important role in the discussions presented in Chapters 3, 4, and 5.

The main driving force responsible for micellization in water is a phenomenon known as the hydrophobic effect (18). The hydrophobic effect is the result of the hydrophobic tails of the
Figure 1.2: The effect of the onset of micellization at the critical micelle concentration (CMC) on the concentrations of surfactant monomers and of micellar surfactant.
surfactants disrupting the structure of liquid water, forcing the water molecules to adopt a free-
ergetically unfavorable structured shell around the tails. Micelles are formed with the
hydrophobic tails residing in the interior of the aggregates and with the hydrophilic heads
residing on the aggregate surfaces (see Figure 1.2). This minimizes the contact between the
hydrophobic tails and water, releasing the water molecules that were in the structured shell
around the hydrophobic tails, thereby increasing the entropy (or alternatively, decreasing the free
energy) of the surfactant solution (18). The free-energetic considerations that inhibit
micellization include the entropic loss of locating several surfactant molecules within the
micelle, the interfacial free energy between the hydrophobic micelle core and the water, and
repulsions of electrostatic and steric origin between the heads of the surfactants residing at the
micelle core-water interface (14, 15).

1.2.3 Surfactant Mixtures and Surfactants Mixed with Polymers

Mixed surfactant systems differ from single surfactant systems because the micellized
surfactants can interact within the mixed micelle, altering the micellar properties from those of a
single surfactant system (14, 15). These interactions are often synergistic, indicating that the
mixing of the surfactants in a micelle can further reduce the free energy of the system. An
important manifestation of this synergism is that the CMC of mixed micellar systems can be
lowered below what would be predicted by treating the surfactants as mixing ideally. For
example, mixing a nonionic surfactant, such as nonyl-phenyl 10-ethylene-oxide, with an anionic
surfactant, such as sodium dodecyl sulfate (SDS), results in favorable interactions because the
smaller heads of the anionic surfactants reduce the steric repulsions between the bulkier
ethylene-oxide heads of the nonionic surfactants, and also because the uncharged nonionic
surfactants reduce the micelle surface charge density. Both of these effects lead to more
favorable micellization in the surfactant mixture, relative to those of each individual surfactant component in the mixture, and hence bring about a reduction in the CMC of the surfactant mixtures.

Many surfactants are known to form micelle-like complexes with water-soluble polymers, in which the polymer forms a corona around the micelle (19-21). The critical aggregation concentration (CAC) is the surfactant concentration at which polymer-bound micelles first form, and is lower than the CMC of the surfactant in the absence of polymer (16). These polymer-bound micelles will only form if they are free-energetically more favorable than forming a free, or unbound, micelle. Otherwise, the CMC would be lower than the CAC, and the surfactant monomer concentration would never be high enough to make the formation of polymer-bound micelles favorable. When a water-soluble polymer is added to an aqueous surfactant solution, the surfactant monomer concentration is approximately equal to the CAC if there is an excess of polymer. However, when the polymers are saturated with micelles (an excess of surfactant), the additional surfactant will increase the surfactant monomer concentration until free micelles begin to form at the CMC. Consequently, it is possible to have mixtures of polymer-bound micelles and free micelles.

Our group has developed molecular-thermodynamic theories to describe and quantitatively predict the effects of the surfactant chemical structure and the solution conditions on the surfactant solution physical chemistry for both single and mixed surfactant systems (14, 15), as well as for surfactants mixed with nonionic polymers (16). These theories can predict the behavior of aqueous solutions containing ionic, nonionic, and amphoteric linear-chain surfactants. Of particular relevance to this thesis is the ability to predict the concentrations and compositions of the surfactant monomers and of the micelles, requiring only the molecular
structures of the surfactants involved and the solution conditions as inputs. Throughout the research presented in this thesis, our surfactant modeling capabilities were used as a tool to predict the surfactant solution conditions, such as monomer concentrations and compositions, and these conditions were related to the results of in vitro and in vivo skin irritation measurements.

1.3 STRUCTURAL AND CHEMICAL DESCRIPTION OF THE SKIN

The skin is the largest organ in the human body, and functions as a protective barrier between the internal organs and the outer environment by controlling the passage of chemicals into and out of the body (22-24). One of the main functions of the skin is to control the water content of the body, preventing it from drying out in the air, and from taking up too much water when immersed in water. Human skin consists of three layers: the stratum corneum, the epidermis, and the dermis (22, 23), as shown schematically in Figure 1.3.

1.3.1 The Three Main Skin Layers

The stratum corneum (SC) is the outer layer of the skin, and is primarily responsible for the outstanding barrier properties of the skin (2, 9, 22). It is a 20 μm thick layer composed of permanently differentiated cells without nuclei, called corneocytes, that have been completely converted into protein (22, 23). These flattened cells are disc shaped with an average thickness of 0.5-1 μm, and a radius of 15-20 μm, and are arranged in a ‘brick-wall’ structure, as shown schematically in Figure 1.3, and more realistically in the electron micrograph in Figure 1.4. These cells are held together by lipid bilayers, which form the ‘mortar’ in the brick-wall structure, and are believed to impart the outstanding barrier properties to the stratum corneum (25). The lipid bilayers differ from those of typical cell membranes in that they contain almost
Figure 1.3: Schematic diagram of the skin.
Figure 1.4: Electron micrograph of the stratum corneum, showing the ‘brick-wall’ structure of the corneocytes, and part of the viable epidermis (30). The lipid bilayers have been stained black, and the corneocytes appear white.
no phospholipids, found in most cell walls, and a far larger proportion of ceramides, two-tailed lipids with amide and alcohol groups in the head (5, 22, 26, 27). Other lipids in the stratum corneum include free sterols and fatty acids. Within the lipid bilayers are occasional discontinuities in the bilayers structure called lacunae (22, 23, 28). These lacunae are small regions that are filled with a polar material that is thought to be water (25, 28, 29), and are believed to be involved in the transport of polar materials through the stratum corneum (see Section 1.3.2).

The epidermis lies below the stratum corneum, and is responsible for making the corneocytes through a process known as keratinization (22). In this process, the various organelles and nucleic acids of the epidermal cells are broken down until the cell is only composed of keratin filaments. Likewise, the cell walls change from polar phospholipids to less polar lipids, such as ceramides. Beneath the epidermis is the dermis, which is the thickest layer of the skin. The dermis is well networked by capillaries, providing a transport path from the skin to the bloodstream, and is largely made of a collagenous-membrane. For this reason, it is generally accepted that a chemical will enter the bloodstream if it permeates through the stratum corneum and the epidermis (31, 32). As a result, when transepidermal skin diffusion is measured experimentally in vitro, often only the stratum corneum and the viable epidermis are used as the model skin membrane.

1.3.2 Transport of Permeants across the Skin

Despite being the thinnest of the skin layers (~15 μm, see Figure 1.3), the stratum corneum acts as the primary resistive barrier to chemical permeation through the skin, protecting against chemical attack and excessive water loss from the viable skin beneath it (22, 23, 33). It is
generally accepted that the lipid bilayers play the primary role in controlling diffusion through the skin (6, 9, 33). Two other possible transdermal pathways are through either the corneocytes, or through the naturally existing channels in the stratum corneum caused by the sweat ducts and hair follicles (34). However, passage through the corneocytes would require passage through solid proteins, which is not very likely for chemical permeants of significant size. The area of hair follicles and sweat ducts open to transport is extremely small compared to the area of the exposed skin, and is therefore insignificant in passive transdermal penetration. Studies involving the diffusion of drugs into the skin have found that the hair follicles only play a role in penetration when an electric current is applied to drive the molecules into the skin (34).

It has been hypothesized that there is an 'aqueous pore pathway' through the stratum corneum, a path through which polar permeants can traverse the hydrophobic environment of the stratum corneum (28, 29, 35). Aqueous pathways in the SC are believed to be located in the intercellular region, in particular, in the lacunae and other aqueous regions surrounded by polar lipids (25, 28, 29, 35). After treatment with permeability enhancers or ultrasound, these aqueous regions have been observed to form a more continuous structure as the stratum corneum is swollen by the absorption of water. Using permeability and conductivity measurements in the context of hindered-transport theories (36), several authors have attempted to determine the average radius of the 'aqueous pore pathway' in the SC, and have reported radii between 10 and 28 Å (31, 37-40).
1.4 BACKGROUND ON SURFACTANT-INDUCED SKIN IRRITATION

1.4.1 Manifestations of Surfactant-Induced Skin Irritation

A large body of literature exists that examines surfactant-induced skin irritation. These studies have examined the medical and dermatological effects of surfactants (5, 6, 8, 41-43), how the skin reacts to different surfactants and solution conditions (11, 12, 33, 44-47), as well as surfactant interactions with proteins (4, 7, 48-51) and lipid bilayers (3, 9, 10, 26, 27, 52, 53). When the skin is exposed to surfactants, various degrees of irritation can result. The severity of this irritation ranges from a feeling of tightness in the skin to severe inflammation (1, 2). This skin irritation depends on many factors, such as the particular sensitivity of an individual to surfactants, the types of surfactants used (13, 43), and the concentration of the surfactant solution applied to the skin (11, 12). The medical aspects of surfactant-induced skin irritation have been studied at both the macroscopic and the microscopic levels. At the macroscopic level, it has been found that skin exhibits irritation by localized redness (erythema) (11), decreased barrier functions (2, 44-47), scaling and eventually formation of callus and scaly, thick skin (22, 54). The reduced skin barrier properties increases the rate of transepidermal water loss (TEWL), drying the skin and causing it to crack and scale (2). Chemical irritants, including surfactants, can pass through the skin more easily and damage the viable tissue inside the body, the epidermis and dermis, causing dermatitis. Histologically, parakeratosis, the retention of the nucleus by the corneocytes, may appear (6, 8, 41-43).

At the microscopic level, irritation by sodium dodecyl sulfate (SDS) and nonanoic acid, two representative anionic surfactants, increased the proliferation of keratinocytes, the cells in the epidermis that mature into corneocytes (41-43). Parakeratosis, the formation of nuclei-retaining
corneocytes, was also found to increase with exposure to these surfactants. Both of these results may indicate that the increase in skin roughness with surfactant-induced skin irritation is a result of the localized increase in the rate of stratum corneum production, which is often the cause of parakeratosis, as well as of the production of abnormal stratum corneum.

1.4.2 Protein-Surfactant Interactions in Relation to Surfactant-Induced Skin Irritation

Protein-surfactant interactions are significant in the study of surfactant-induced skin irritation because when surfactants penetrate the stratum corneum, they interact with the keratin proteins of the corneocytes, possibly denaturing these proteins and increasing the permeability of the skin (2, 12, 48, 49, 55). In addition, when surfactants penetrate deeper into the skin and into the viable epidermis and the dermis, the denaturation of important signaling proteins may lead to improper functioning of the cells in the viable epidermis and the dermis, as described in Section 1.4.1 (6, 8, 41-43). Therefore, understanding how surfactants interact and denature proteins appears important to understanding how to tailor surfactant solutions to reduce their skin irritation potential. Denaturation of proteins by surfactants has been correlated with the skin irritation potential of single surfactants (54, 56). The quantitative measurement of protein denaturation can therefore be used to evaluate the skin irritation potential of surfactant solutions. In Chapter 6, Section 6.1, we provide extensive background on how protein-surfactant interactions have been related to the irritation potential of surfactants.

1.4.3 Interactions between Surfactants and Stratum Corneum Lipids

Conjectures that surfactants solubilize vital lipids in the stratum corneum, thereby reducing the barrier properties of the lipid bilayers, have not been confirmed by experiments (3, 5, 10).
Although experiments have shown that surfactants do not extract significant amounts of lipids from the stratum corneum, it has been found that the composition of the lipid bilayers can be affected by exposure to surfactants. Surfactants have been shown to reduce the barrier properties of the skin, and since it is believed that diffusion across the skin occurs through the lipid bilayers, changes in the transepidermal diffusive flux may indicate a strong surfactant effect on the integrity of the lipid bilayers (2, 44-47). Therefore, although the lipids of the stratum corneum are not removed by the exposure of the skin to surfactant solutions, surfactants do affect the barrier properties of the stratum corneum. In order to study the effects of surfactants on the lipid bilayers without the additional complicating effect of the skin proteins, the stratum corneum lipid bilayers have been modeled by making both lipid bilayers and vesicles, or liposomes, using lipids that were extracted from the stratum corneum (57). In addition, other bilayers have been made that approximate the composition of the lipid bilayers, containing 40% ceramides, 25% cholesterol, 25% palmitic acid, and 10% cholesterol sulfate (26, 53, 58).

Differential scanning calorimetry experiments with extracted lipids have shown that the thermal transitions of the isolated lipids differ from those in the intact stratum corneum, indicating the existence of protein-lipid interactions (59). However, the other thermal transitions are also observed in the extracted lipids, indicating that these model lipid bilayers provide a reasonable system to represent the lipid bilayers found in the intact stratum corneum. Another interesting experiment demonstrated that when permeability-enhancing chemicals were added to extracted bilayers, they had the same effect on the thermal transitions of the lipid bilayers as the addition of SDS, which increases the skin permeability as well as causes irritation (60).

Experiments were performed using liposomes made from model lipids to determine the effect of poly(ethylene oxide), sulfate, and betaine surfactants on the permeability of the bilayers (26, 53,
58). In these experiments, it was found that all the surfactants increased the permeability of the bilayers, as measured by the release of a fluorescent dye from the vesicle interior due to the presence of different surfactant concentrations. In all these experiments, the monomer concentration remained below the CMC, since the liposomes broke apart before micelles formed. Increasing the permeability of the lipid bilayers of the skin does increase transepidermal water loss, which can dry the skin, as well as allow other irritants to pass through the skin more easily (2).

1.4.4 Effect of Surfactant Solution Properties on Skin Irritation

Examining the effects of changing the surfactant solution concentration or composition on skin irritation has been another thrust of previous studies (11, 12). In this respect, the effect of micelle formation on skin irritation with increasing surfactant concentration is not well understood, and this thesis has focused on illuminating the details of this problem. The majority of the evidence demonstrates that the skin irritation induced by the surfactant solution continues to increase with increasing surfactant concentration above the CMC, albeit at a slower rate than below the CMC (2, 11, 61-64). However, it has often been proposed that only the surfactant monomers are able to penetrate into the skin, in a description that we refer to as the monomer penetration model (2, 12, 48, 61, 64-66).

The monomer penetration model is partially based on the demonstration that the diffusion of surfactants through a collagen membrane, used as a skin model, is controlled by the monomer concentration and composition (67, 68). This is relevant to the skin irritation problem, because in order to irritate the skin, the surfactant must first penetrate into the skin. If this penetration can be reduced, then the irritation potential of a surfactant solution could be reduced. According
to the monomer penetration model, reducing the surfactant penetration into the skin is simply a matter of reducing the surfactant monomer concentration, for example, through the use of surfactant mixtures.

A dramatic demonstration of this principle was provided by Rhein et al. in Ref.(12), when they mixed the mild anionic surfactant alkyl 7-ethoxy sulfate with a fixed concentration of the harsh anionic surfactant sodium dodecyl sulfate. The mildness of the ethoxy-sulfate surfactant is believed to be due to the large head size that would sterically hinder it from interacting with proteins and lipid bilayers. In spite of increasing the total concentration of surfactant, the skin irritation induced by the mixed surfactant solution was observed to decrease. Analysis of the monomer concentration and composition showed that the skin irritation was strongly correlated to the monomer concentration of SDS, the harsher surfactant. Likewise, measurements of collagen membrane swelling when exposed to a mixture of SDS and alkyl 6-ethoxy sulfate, in which the swelling indicates denaturation of the protein, have shown a good correlation with in vivo skin irritation measurements (49). The swelling due to surfactant interactions can be reduced by mixing a harsh surfactant like SDS with a mild one like alkyl 6-ethoxy sulfate.

However, if the monomer penetration model is strictly valid, then the irritation potential of a surfactant solution should not be dose-dependent above the CMC, since the surfactant monomer concentration is not affected by increasing the total surfactant concentration above the CMC (see Figure 1.2). Nevertheless, a surfactant dose-dependent skin irritation response appears to be the norm (2, 11, 61-64) rather than the exception (66), raising the validity of the monomer penetration model into question. Just as there is a dose-dependence in the skin irritation potential of surfactant solutions, it has also been observed that the penetration of SDS into the stratum corneum can behave in a dose-dependent fashion (48, 55), thus potentially connecting the skin
irritation potential of a surfactant solution with the amount of surfactant penetrating into the stratum corneum from that solution. A central result of this thesis will be the unambiguous demonstration that the micellar surfactant can affect the surfactant penetration into the skin (see Chapters 3 and 5), and that this penetration is related to the resulting skin irritation (see Chapter 4).

1.5 OUTLINE OF THESIS

The remainder of this thesis is divided into the following chapters. Chapter 2 describes the development of an in vitro skin irritation test that measures how surfactants affect the electrical conductivity of the skin, and relates this skin conductivity to the permeability of the skin to ions and other polar molecules. Chapter 3 presents measurements of the penetration of SDS into the skin, and examines how this penetration is affected by the addition of poly(ethylene oxide), a water-soluble nonionic polymer known to form complexes with SDS in aqueous solution. In Chapter 4, the concentration of SDS in the skin is related to both in vitro and in vivo skin irritation measurements. In Chapter 5, the role of surfactant mixtures in controlling the penetration of micellar SDS into the skin is examined. Chapter 6 examines the effect of mixing surfactants on the ability of SDS to denature zein proteins, in the context of surfactant-induced skin irritation. Finally, Chapter 7 presents the conclusions of this thesis, and discusses possible future research in the area of surfactant-induced skin irritation.

1.6 REFERENCES


Chapter 2

Development and Verification of an *in vitro* Skin Irritation Test using Skin Conductivity Measurements

2.1 INTRODUCTION

The role of the stratum corneum (SC), the outermost layer of the skin, is to prevent water loss from the body, as well as to prevent the transdermal penetration of irritating and hazardous substances into the body (1, 2). Any reduction in the barrier properties of the SC will increase water loss through the skin, as well as allow more irritants to penetrate into the skin, leading to other forms of skin irritation (1-5). Although the SC acts as a barrier to all molecules, it is particularly effective in preventing hydrophilic molecules from traversing it (1-3, 6, 7). In this respect, changes in the barrier properties of the SC typically involve large changes in the ability of hydrophilic molecules to traverse the SC, while the passage of hydrophobic molecules is less affected by such changes (8).

Surfactants are known to reduce the barrier properties of the skin, and are even used as penetration enhancers in transdermal drug delivery applications (2, 3, 6, 7, 9-12). From the skin irritation point of view, the increased Transepidermal Water Loss (TEWL) due to
surfactant exposure can lead to drier skin, especially in cold and dry environments, and may cause other skin irritation symptoms, such as scaly skin or even erythema (redness of the skin), to occur (1, 13-19). The increased penetration of irritants into the skin, including irritating surfactants, resulting from a compromised skin barrier, enables the irritants to attack the viable epidermis and dermis after passing through the SC. Once in these viable skin layers, the irritants can induce several irritation symptoms, including erythema and parakeratosis (the formation of corneocytes containing nuclei) (20-23). Accordingly, whether the goal is to increase the transdermal flux of a drug, or to minimize the water loss from the skin, understanding the extent to which a surfactant solution reduces the barrier properties of the skin is a key aspect of understanding how surfactants affect the skin.

There are several testing methods that quantify the reduction in the barrier properties of the skin, as well as other symptoms of skin irritation. Wilhelm et al. compared the in vivo skin irritation response to different concentrations of the anionic surfactant sodium dodecyl sulfate (SDS) using TEWL, laser Doppler flow measurements, skin color reflectance, and visual erythema scores (19). They found that all these methods correlated well with each other, with the strongest correlation occurring between TEWL and visual erythema. TEWL is a measure of the ability of the skin to prevent water loss, with the TEWL increasing as the skin barrier is compromised. Thus, these results suggest that erythema is related to the increased permeability of the skin. There are several problematic aspects concerning in vivo skin irritation measurements, including the high cost per datum point, the inter-subject variability, and the ethical issues involved in using live animals for testing (which is balanced by the high cost of human in vivo
testing). These particular problems may be overcome using an *in vitro* skin irritation measurement, where the conditions affecting the skin can be well controlled, and the cost per sample can be lowered significantly. Although *in vitro* skin irritation testing is not a perfect replacement for *in vivo* skin irritation testing, accurate *in vitro* methods are useful in the early stages of developing commercial products as a screening approach, and provide a particularly useful tool to narrow down the range of possible formulations that needs to be subjected to *in vivo* testing.

There are several *in vitro* procedures that measure the barrier properties of the skin, including measuring the transdermal permeability of probe molecules (2, 3, 5, 6, 12, 24), and measuring the electrical properties of the skin (9, 25-29). It is well-accepted that the electrical conductivity of the skin is a quantitative measure of the permeability of the skin to ions, and it has been observed that an increase in the skin conductivity can be correlated with an increase in the permeability of the skin to hydrophilic molecules such as urea and mannitol (9, 28, 29). Surfactants have been shown to increase the permeability of the skin to hydrophilic molecules, as well as to affect the electrical properties of the skin. However, to be able to compare the irritation potential of different surfactant solutions with respect to the barrier properties of the skin, we needed to develop our own *in vitro* skin irritation test. Most of the available tests that study changes in the barrier properties of the skin are undertaken with the goal of *increasing* the skin permeability for applications in transdermal drug delivery. On the other hand, the main goal of minimizing skin irritation is to *minimize* the decrease in the barrier properties of the skin, and therefore, our *in vitro* test had to be sensitive to smaller changes in the skin barrier properties.
In this chapter, we discuss the development of such an \textit{in vitro} skin irritation test based on the measurement of the electrical conductivity of pig skin. In Section 2.3.1, we demonstrate that the skin conductivity is directly related to the transdermal water permeability (TDWP), measured using \textsuperscript{3}H-radiolabeled water, and that skin conductivity can be used in lieu of TDWP to probe changes in the skin barrier properties. In Section 2.3.2, a more sensitive impedance spectroscopy apparatus was employed to observe how surfactants affect the electrical properties of the skin in greater detail, and to confirm that our original apparatus did indeed measure the electrical resistance (conductivity) of the skin. In Section 2.3.3, the ionic strength of the surfactant solution contacting the skin, which can vary greatly between commercial products, was observed to have an effect on the measured skin conductivity, and as such, could interfere with the use of skin conductivity as a quantitative indicator of the skin barrier properties. To eliminate the ionic strength effect, in Section 2.3.4, the skin was contacted with a buffer solution following exposure to the surfactant solution. Once a reliable \textit{in vitro} skin irritation test was developed, it was used to determine the irritation potential of three commercial products (see Section 2.3.5), and was found to agree well with the available \textit{in vivo} skin irritation results.

2.2 EXPERIMENTAL

2.2.1 Materials

Sodium dodecyl sulfate (SDS), sodium chloride (NaCl), disodium phosphate (Na\textsubscript{2}HPO\textsubscript{4}), and citric acid were purchased from Sigma Chemicals (St. Louis, MO), and were used as received. Dodecyl hexa(ethylene oxide) (C\textsubscript{12}E\textsubscript{6}) was purchased from Nikko Chemicals
(Tokyo, Japan), and was used as received. Water was produced using a Millipore Academic water filter. $^3$H-radiolabeled H$_2$O was purchased from American Radiolabeled Chemicals (St. Louis, MO), and was used as received. Except where noted, a version of McIlvaine’s buffer system was used as a background electrolyte. This buffer contained 16.4 mM Na$_2$HPO$_4$ and 1.8 mM citric acid, yielding a pH of 7.1 and an ionic strength of 45 mM. Phosphate buffered saline (PBS) was used as a background electrolyte in the experiments described in Section 2.3.5, and was prepared using PBS tablets from Sigma Chemicals and Millipore filtered water (137 mM NaCl and 10 mM Na$_2$HPO$_4$, resulting in a pH of 7.2 and an ionic strength of 160 mM). In this chapter, the term ‘buffer solution’ refers to any generic buffers, ‘McIlvaine’s buffer’ refers to the citric acid-Na$_2$HPO$_4$ solution, and ‘PBS buffer’ refers to the buffer made from PBS tablets.

2.2.2 Preparation of Skin Samples and Mounting of Skin Samples in the Diffusion Cell

Female Yorkshire pigs (40-45kg) were purchased from local farms. Skin from the back of the pig was harvested within one hour of sacrificing the animal. The subcutaneous fat was trimmed off using a razor blade, and the full-thickness pig skin was cut into 2cm x 2cm pieces and stored in a –80 °C freezer until used.

After allowing the skin to thaw for a half-hour at room temperature, the pig skin was mounted in a vertical Franz diffusion cell (Permegear Inc., Riegelsville, PA), with the SC facing the donor compartment (see Figure 2.1 for a schematic representation of the diffusion cell set-up used for the TDWP and the skin conductivity measurements). The
Figure 2.1: A schematic representation of the Franz diffusion cell used in the TDWP and the skin conductivity measurements.
diffusion cells expose 1.76 cm² of the SC to the solution in the donor compartment, referred to as the contacting solution. The donor and the receiver compartments of the diffusion cell were filled with a buffer solution, and the skin was left to hydrate for 1 hour. After hydration, the skin conductivity was measured (see Section 2.2.3), and the skin was discarded if the skin conductivity was greater than 0.05 (kΩ cm²)⁻¹ (30). The buffer solution in the donor compartment was then removed, and 1.5 ml of surfactant solution was added to the donor compartment.

2.2.3 Measurement of Skin Conductivity

Prior to filling the diffusion cell with buffer solution and mounting the skin, a Ag/AgCl electrode (E242, In Vivo Metrics, Healdsburg, CA) was placed in both the donor and the receiver compartments, as shown in Figure 2.1. Once the diffusion cell was assembled, the skin conductivity was measured by applying a 100 mV AC voltage (RMS) at 10 Hz across the skin for about 10 seconds, and measuring the current with an ammeter. An AC voltage was applied because a DC voltage could induce damage to the barrier properties of the skin, and could also result in iontophoresis of surfactant ions into, or out of, the skin, while a 12.5 Hz AC voltage under similar conditions was shown not to affect the barrier properties of the skin (31). The voltage was produced with a model HP 33120A signal generator (Hewlett-Packard, CA), and the ammeter consisted of a 971A Digital Multimeter (Hewlett-Packard, CA). The skin conductivity, σ_{skin}, was determined using the following relation:

\[
\sigma_{skin} = \frac{1}{V \cdot A} \left( i_{total}^{-1} - i_{back}^{-1} \right)^{-1} = \frac{1}{R_{skin} \cdot A}
\]  (2.1)
where $\sigma_{\text{skin}}$ has units of $(k\Omega \ \text{cm}^2)^{-1}$, $V$ is the applied voltage, $A$ is the exposed area of the skin, $i_{\text{total}}$ is the current measured across the skin and the background electrolyte, $i_{\text{back}}$ is the current measured under the applied voltage $V$ in the absence of the skin, and $R_{\text{skin}}$ is the electrical resistance of the skin.\(^1\) The use of Eq. (2.1) is not entirely accurate, since Eq. (2.1) is strictly applicable in the case of a DC voltage, while $\sigma_{\text{skin}}$ is actually measured using an AC voltage. However, as will be shown in Section 2.3.2, at 10 Hz, the use of Eq. (2.1) is appropriate to characterize $\sigma_{\text{skin}}$.

### 2.2.4 Measurement of Transdermal Water Permeability

The Transdermal Water Permeability (TDWP) was determined by measuring the flux of $^3$H-radiolabeled water ($^3$H-H$_2$O) from the donor compartment into the receiver compartment. The contacting solution initially contained 1 $\mu$Ci/ml of $^3$H-H$_2$O. Starting at 20 hours after adding the contacting solution to the donor compartment, when the flux of $^3$H-H$_2$O attained a steady state, 100 $\mu$l samples were withdrawn from the receiver compartment through the sample port every 2 hours to determine the concentration of $^3$H-H$_2$O in the receiver compartment, $C_r$ (see Figure 2.1). The concentration of $^3$H-H$_2$O in the donor compartment, $C_d$, was measured at 20 hours, and was found not to change over time thereafter. Both $C_r$ and $C_d$ were determined using Hionic Fluor (Packard) as the scintillation cocktail and a Packard Tri-Carb 4350 scintillation counter (Packard). Under steady-state conditions with a fixed $C_d$ (an infinite-source condition), the TDWP was calculated as follows:

---

\(^1\) The units of conductivity are typically $(\Omega \ \text{m})^{-1}$ because they depend on the length of the conductor as well as on its cross-sectional area, $A$. However, in the case of skin, the relevant thickness is not easily accessible, since the main barrier to conductivity is the SC, which has a thickness between 10 and 20 $\mu$m. By eliminating the length dependence, the conductivity has units of $(\Omega \ \text{m}^2)^{-1}$ (32).
\[ TDWP = \frac{1}{A \Delta C} \frac{dQ}{dt} \]  

(2.2)

where \( \Delta C = C_d - C_r \), \( A \) is the skin permeation area (1.76 cm\(^2\)), and \( Q \) is the cumulative amount of \(^3\)H-H\(_2\)O transported into the receiver compartment at time \( t \).

### 2.2.5 Impedance Spectroscopy

Impedance spectroscopy of the skin was carried out using the same set-up used for the skin conductivity measurements (see Figure 2.1), but using an EG&G 1025 frequency response detector connected to an EG&G 263A potentiostat (EG&G). The measurements were made using a 20 mV AC perturbation between 1 Hz and 10 kHz. The resistance and capacitance values of the skin were determined by fitting the impedance spectrum to an equivalent circuit using the software Equivcrtpas, written by Bernard Boukamp and provided by EG&G.

### 2.3 RESULTS AND DISCUSSION

#### 2.3.1 Comparison of Transdermal Water Permeability and Skin Conductivity

We simultaneously measured the effect of adding surfactants on the TDWP and the skin conductivity, \( \sigma_{\text{skin}} \). Measuring \( \sigma_{\text{skin}} \) is essentially equivalent to determining the permeability of the skin to ions, with a higher \( \sigma_{\text{skin}} \) value indicating that ions can more easily traverse the skin. Both water and ions are polar molecules, while the SC, the main barrier to passage across the skin, is primarily a hydrophobic environment (see Section 1.3.2). As a result, we expect the water molecules and the ions to traverse the skin
through the same polar pathway (29, 33-36). To test this expectation, we exposed the skin to 50 mM aqueous mixtures of SDS and C₁₂E₆ in a McIlvaine buffer for 30 hours, the time necessary to make the TDWP measurements (see Section 2.2.4). The contacting solution also contained 1 µCi/ml of $^3$H-H₂O to permit the simultaneous determination of the transdermal water permeability (TDWP).

Figure 2.2 compares the resulting $\sigma_{\text{skin}}$ after 24 hours versus the TDWP for individual skin samples exposed to aqueous SDS/C₁₂E₆ surfactant mixtures of various surfactant solution compositions, $\alpha_\phi$ (1, 0.75, 0.5, 0.25, and 0), where $\alpha=0$ is 100% C₁₂E₆ and $\alpha=1$ is 100% SDS. For a control system of just McIlvaine buffer as the contacting solution, $\sigma_{\text{skin}}$ was found to be 0.006±0.002 (kΩcm²)$^{-1}$, and the corresponding TDWP was found to be 0.0005±0.0002 (cm/hr). Figure 2.2 clearly shows a direct correlation between $\sigma_{\text{skin}}$ and the TDWP, thereby confirming our expectation that increasing the TDWP is also reflected in an increase in $\sigma_{\text{skin}}$. This is useful, because $\sigma_{\text{skin}}$, whose measurement requires that an AC current be applied to the skin for about 10 seconds, is much easier to determine than the TDWP, which requires measuring the amount of $^3$H-H₂O traversing the skin over several hours, as well as the necessary facilities to handle radiolabeled materials. Moreover, we also found that the distribution of $\sigma_{\text{skin}}$ values for a given condition was smaller than the distribution of the TDWP values, allowing for a better discrimination between surfactant conditions when using $\sigma_{\text{skin}}$. For these reasons, we chose $\sigma_{\text{skin}}$ to quantitatively probe changes in the barrier properties of the skin induced by exposure to a surfactant solution, with an increase in $\sigma_{\text{skin}}$ reflecting a decrease in the barrier properties of the skin.
Figure 2.2: Correlation between $\sigma_{\text{skin}}$ and the TDWP for solutions containing 50 mM SDS/C$_{12}$E$_6$ surfactant mixtures in McIlvaine buffer, as well as for a control solution containing only McIlvaine buffer. The various symbols in the legend correspond to the surfactant composition, $\alpha_s$ (0=pure C$_{12}$E$_6$, 1=pure SDS), and each data point corresponds to a single skin sample.
Figure 2.3 presents the average $\sigma_{\text{skin}}$ data from Figure 2.2 as a function of $\alpha_s$, as well as the $\sigma_{\text{skin}}$ value for an $\alpha_s=0.9$ solution, and compares it to the critical micelle concentration (CMC) predicted using a molecular-thermodynamic theory of mixed surfactant micellization (37). The relationship between surfactant-induced skin irritation and the CMC of surfactant mixtures has been examined for several systems, and it was found that a decrease in the CMC upon mixing the surfactants could be correlated with a decrease in the skin irritation (38-40). As discussed in Chapter 1 (see Section 1.2.2), the CMC can be considered to be equivalent to the surfactant monomer concentration.² According to the monomer penetration model, which states that only surfactant monomers can penetrate into the SC, lowering the surfactant monomer concentration will decrease the driving force for the monomers to penetrate into the skin, and thereby reduce the skin irritation induced by the surfactant (see Chapters 3 and 5 for a more complete description of the monomer penetration model, including our new findings that challenge the validity of the monomer penetration model). Accordingly, since it is well-known that mixing SDS with $C_{12}E_6$ would lower the CMC due to the synergistic interactions between the anionic and the nonionic surfactants in the mixed micelles (37), we expected that this would lead to a decrease in the amount of surfactant penetrating into the skin. This, in turn, would reduce the dose of surfactant in the skin, thereby reducing the surfactant-induced damage to the barrier properties of the skin.

Figure 2.3 clearly corroborates our expectation, as it shows that increasing the solution composition of SDS, $\alpha_s$, leads to an increased $\sigma_{\text{skin}}$, corresponding to an increased CMC,

² However, this statement is not entirely accurate for mixtures of surfactants, in which the surfactant monomer concentration can be higher than the CMC due to complexities in the mass balances of the surfactant molecules as they distribute between monomers and micelles (37).
Figure 2.3: Average measured values of $\sigma_{\text{skin}}$ after 24 hours of exposure to a contacting solution of 50 mM SDS/C$_{12}$E$_6$ surfactant mixtures in McIlvaine buffer with increasing $\alpha_s$, and the corresponding predicted CMC of the SDS/C$_{12}$E$_6$ surfactant mixture (37). The solid circles (●) represent the average measured $\sigma_{\text{skin}}$ values of four to six skin samples, and the error bars reflect a 95% confidence interval. The line represents the predicted CMC.
reflecting an increased driving force for surfactant penetration into the skin. The fact that this effect could be observed using the $\sigma_{\text{skin}}$ measurement provided additional credibility to the ability of the new in vitro skin conductivity test to probe the surfactant-induced skin damage. However, a direct correlation between the skin irritation, reflected by $\sigma_{\text{skin}}$, and the CMC of the SDS/C$_{12}$E$_6$ surfactant mixture ignores the fact that SDS and C$_{12}$E$_6$ will have different interactions with the skin. Based on other skin irritation measurements, it is expected that SDS will induce more skin irritation, and hence, lead to a larger increase in $\sigma_{\text{skin}}$, than C$_{12}$E$_6$ (41, 42). Thus, the increase in $\sigma_{\text{skin}}$ with increasing $\alpha_s$ is expected primarily due to the increase in the concentration of the more irritating surfactant SDS. However, the fact that the increase in $\sigma_{\text{skin}}$ is not linear with $\alpha_s$, but instead follows a pattern similar to the CMC (see Figure 2.3), suggests that the micellization behavior of the SDS/C$_{12}$E$_6$ surfactant mixture also plays a role in determining the skin irritation potential of the surfactant solution. In Chapter 5, we will investigate how mixing SDS with C$_{12}$E$_6$ affects the penetration of SDS into the skin, and our findings suggest that the increase in $\sigma_{\text{skin}}$ with increasing $\alpha_s$ observed in Figure 2.3 is not solely the result of an increased SDS monomer concentration, reflected by the increased CMC, but also results from the penetration of micellar SDS into the skin. However, in this Chapter, we are more concerned with the development of the in vitro skin conductivity test, and will therefore forgo further discussion of these details until Chapter 5.
2.3.2 Impedance Spectroscopy of the Skin Exposed to Solutions of SDS and of C_{12}E_{6}

As discussed in Section 2.2.3, the skin conductivity is related to the measured currents, \( i_{\text{total}} \) and \( i_{\text{back}} \), using Eq. (2.1), which we repeat below for clarity:

\[
\sigma_{\text{skin}} = \frac{1}{V \cdot A} \left( i^{i-1}_{\text{total}} - i^{i-1}_{\text{back}} \right)
= \frac{1}{R_{\text{skin}} \cdot A}
\]  

(2.1)

Because \( \sigma_{\text{skin}} \) is measured using an AC voltage, the use of Eq. (2.1) is not entirely accurate, since Eq. (2.1) is strictly applicable to the case of a DC voltage. The skin conductivity, as determined by an AC voltage, is better described by

\[
\sigma_{\text{skin}} = \frac{1}{Z_{\text{skin}}(\omega) \cdot A}
\]  

(2.3)

where \( Z_{\text{skin}}(\omega) \) is the impedance (the AC equivalent of the resistance) of the skin for a particular applied frequency, \( \omega \). A model circuit of the skin has been proposed by several authors (25-27), and we have used the model circuit of Kalia et al. (27), because it was found most able to fit our impedance results without resorting to unexplainable circuit elements. This circuit is shown in Figure 2.4, and consists of a resistor, \( R_{\text{skin}} \), and a constant phase element (CPE) connected in parallel. Because the current must pass through a solution in the donor and the receiver compartments, a resistor representing the resistance of the solutions in the donor and the receiver compartments, \( R_{\text{soln}} \), is also connected in series in the overall circuit. For the skin, the CPE acts mostly as a capacitor with some resistive characteristics, and has been described as a ‘leaking’ capacitor, that
Figure 2.4: Model circuit used to describe the electrical properties of the skin in a diffusion cell (27).
is, as a capacitor that allows some current to pass across it (25-27). The impedance of this circuit, $Z_{cir}(\omega)$, is described by

$$Z_{cir}(\omega) = \frac{1}{\omega^\alpha Y + R_{\text{skin}}^{-1}} + R_{\text{soln}} = Z_{\text{skin}}(\omega) + R_{\text{soln}}$$

(2.4)

where $\alpha$ is an exponent between 0 and 1 that describes the degree to which the CPE acts like a capacitor (with $\alpha=1$ corresponding to a capacitor, and $\alpha=0$ to a resistor), $Y$ is the capacitance of the CPE (units of $\Omega^{-1}$), $R_{\text{skin}}$ is the electrical resistance of the skin, and $R_{\text{soln}}$ is the electrical resistance of the solutions in the donor and the receiver compartments of the diffusion cell. Based on Eq. (2.4), the effect of $\omega$ on $Z_{cir}(\omega)$ is that, at low $\omega$ values, $Z_{cir}=R_{\text{skin}}+R_{\text{soln}}$, while at very high $\omega$ values, $Z_{cir}=R_{\text{soln}}$. To determine the values of $R_{\text{skin}}$, $R_{\text{soln}}$, $Y$, and $\alpha$, $Z_{cir}$ is measured as $\omega$ changes, resulting in what is known as a Bode plot (see Figure 2.5).

Figure 2.5 is a Bode plot showing how $Z_{cir}$ of a piece of skin exposed to a contacting solution of 100 mM SDS varies with $\omega$ as the duration of the skin exposure to the surfactant solution, $t_s$, increases from 5 minutes to 90 minutes. The observed decrease in $Z_{cir}$ with increasing $t_s$ at low $\omega$ values, where $Z_{cir}=R_{\text{skin}}+R_{\text{soln}}$, reflects a decrease in $R_{\text{skin}}$ with increasing $t_s$. An interesting aspect of Figure 2.5 is observed for the $t_s=5$ minutes curve, where $Z_{cir}$ increases between $\omega=1$ Hz and 20 Hz, followed by a decrease in the impedance beyond 20 Hz. This apparent increase in $Z_{cir}$ is an artifact of the method used to measure the impedance, which starts at a high $\omega$ value and decreases to a low $\omega$ value. Measuring the impedance for the range of $\omega$ values examined requires about 5 minutes. During this 5-minute period, SDS is affecting the barrier properties of the skin as it
Figure 2.5: Bode plot showing how the impedance of the skin circuit, $Z_{cir}$, changes with increasing AC frequency, $\omega$, and increasing exposure time, $t_s$, when the skin is exposed to a contacting solution of 100 mM SDS.
penetrates into the skin. At the short $t_s$ values, the rate of change of the impedance is faster than the characteristic time associated with the impedance measurement. As a result, the value of $R_{\text{skin}}$ decreases during the measurement as the impedance is measured for decreasing $\omega$ values, leading to a noticeable change in $Z_{\text{cir}}$ as the low $\omega$ range is probed for short $t_s$ values. By fitting Eq. (2.4) to the $Z_{\text{cir}}(\omega)$ versus $\omega$ results in Figure 2.5, the changes in the electrical nature of the skin with time were quantified, and are reported in Table 2.1, which includes the results shown in Figure 2.5, as well as the $Z_{\text{cir}}(\omega)$ values measured at $t_s=3$, 5, and 10 hr (not shown in Figure 2.5). As was inferred from Figure 2.5, $R_{\text{skin}}$ is found to decrease with increasing $t_s$. Interestingly, $Y$ is found to increase with $t_s$, while $\alpha$ essentially remains constant for $t_s<3$ hours, and starts to decrease for $t_s>3$ hours.

Table 2.1: The effect of increasing the skin exposure time, $t_s$, to a 100 mM SDS contacting solution on the values of $R_{\text{skin}}$, $Y$, and $\alpha$.

<table>
<thead>
<tr>
<th>Exposure Time, $t_s$</th>
<th>$R_{\text{skin}}$ (k$\Omega$)</th>
<th>$Y$ (s$^{-1}$s 10$^6$)</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background</td>
<td>85.69</td>
<td>0.24</td>
<td>0.81</td>
</tr>
<tr>
<td>5 min</td>
<td>52.01</td>
<td>0.22</td>
<td>0.85</td>
</tr>
<tr>
<td>25 min</td>
<td>22.57</td>
<td>0.27</td>
<td>0.84</td>
</tr>
<tr>
<td>45 min</td>
<td>17.78</td>
<td>0.32</td>
<td>0.83</td>
</tr>
<tr>
<td>60 min</td>
<td>15.63</td>
<td>0.38</td>
<td>0.82</td>
</tr>
<tr>
<td>90 min</td>
<td>13.74</td>
<td>0.43</td>
<td>0.81</td>
</tr>
<tr>
<td>3 hr</td>
<td>12.15</td>
<td>0.53</td>
<td>0.79</td>
</tr>
<tr>
<td>5 hr</td>
<td>7.56</td>
<td>1.02</td>
<td>0.74</td>
</tr>
<tr>
<td>10 hr</td>
<td>3.05</td>
<td>2.89</td>
<td>0.66</td>
</tr>
</tbody>
</table>

In order to evaluate the relationship between the impedance measured by the multimeter and the impedance measured using impedance spectroscopy, the two measurements were performed on the same pieces of skin exposed to contacting solutions of either 50 mM
SDS or C_{12}E_{6}, each in 100 mM NaCl. The 100 mM NaCl was added because without it, $R_{soln}$ for the 50 mM C_{12}E_{6} solution would be very large (no ionic species would be present), and would therefore complicate the determination of $R_{skin}$, since it would be comparable to $R_{soln}$. Tables 2.2 and 2.3 show how the electrical characteristics of the skin under the different surfactant conditions (50 mM SDS in Table 2.2 and 50 mM C_{12}E_{6} in Table 2.3) vary with $t_s$. Notably, $R_{skin}$ decreases and $Y$ increases with $t_s$ in all cases, while $\alpha$ remains essentially unchanged in the presence of SDS, while it decreases slightly in the presence of C_{12}E_{6}. The observed decreases in $R_{skin}$ correspond to increases in $\sigma_{skin}$ (increased skin permeabilities), and are expected, because in Figures 2.2 and 2.3, both SDS and C_{12}E_{6} were found to increase $\sigma_{skin}$. The observed increases in $Y$ are not yet completely understood, although some authors suggest that the capacitance of the skin is related to the electrical potential of the electrical double layer associated with the aqueous pores present in the stratum corneum (25-27). The effective capacitance of an electrical double layer is directly related to $\kappa$, the Debye-Hückel parameter (43). As the ionic strength inside the aqueous pores increases due to the penetration of NaCl or SDS into the skin, $\kappa$ will increase and the capacitance of the electrical double layer will increase, potentially explaining the observed increase in the $Y$ values with $t_s$ reported in Tables 2.1, 2.2, and 2.3. In addition, surfactants are known to increase the permeability of the SC lipid bilayers (44-46). As a result, if one equates ‘leakiness’ with permeability, the penetration of the surfactant into the skin could make the effective CPE of the skin more leaky, thus explaining the observed decrease in $\alpha$. 
Table 2.2: Changes in the average values of \( R_{\text{skin}} \), \( Y \), and \( \alpha \) induced by exposure to a 50 mM SDS-100 mM NaCl solution. The ± values correspond to a 95% confidence interval based on 4 measurements.

<table>
<thead>
<tr>
<th>( t_s )</th>
<th>( R_{\text{skin}} ) (kΩ)</th>
<th>( Y (\Omega^{-1} s \times 10^6) )</th>
<th>( \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>86.2±8.5</td>
<td>0.30±0.06</td>
<td>0.80±0.02</td>
</tr>
<tr>
<td>2.5 hr</td>
<td>69.9±6.0</td>
<td>0.40±0.05</td>
<td>0.80±0.01</td>
</tr>
<tr>
<td>5 hr</td>
<td>32.6±11.6</td>
<td>0.51±0.08</td>
<td>0.78±0.02</td>
</tr>
</tbody>
</table>

Table 2.3: Changes in the average values of \( R_{\text{skin}} \), \( Y \), and \( \alpha \) induced by exposure to a 50 mM C\(_{12}\)E\(_6\)-100 mM NaCl solution. The ± values correspond to a 95% confidence interval based on 4 measurements.

<table>
<thead>
<tr>
<th>( t_s )</th>
<th>( R_{\text{skin}} ) (kΩ)</th>
<th>( Y (\Omega^{-1} s \times 10^6) )</th>
<th>( \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr</td>
<td>87±12</td>
<td>0.20±0.05</td>
<td>0.82±0.03</td>
</tr>
<tr>
<td>2.5 hr</td>
<td>41±9</td>
<td>0.49±0.09</td>
<td>0.75±0.03</td>
</tr>
<tr>
<td>5 hr</td>
<td>16±5</td>
<td>0.64±0.11</td>
<td>0.73±0.02</td>
</tr>
</tbody>
</table>

Besides studying the electrical properties of the skin in more detail, as stated in Section 2.1 and at the beginning of this section, an important goal of the impedance measurements was to determine whether Eq. (2.1) can be used in the case of the 10 Hz AC voltage. Equation (2.1) essentially assumes that the impedance of the skin, \( Z_{\text{skin}} \), is determined solely by \( R_{\text{skin}} \), and that the CPE has little or no effect at 10 Hz. To compare \( R_{\text{skin}} \) determined using the multimeter and \( R_{\text{skin}} \) determined using impedance spectroscopy, \( \sigma_{\text{skin}} \) was measured with the multimeter for the systems shown in Tables 2.2 and 2.3. The multimeter and the impedance spectroscopy measurements were conducted simultaneously on the same piece of skin. Figure 2.6 compares the \( R_{\text{skin}} \) values
Figure 2.6:  Comparison of $R_{\text{skin},m}$, determined using a multimeter and Eq. (2.5), with $R_{\text{skin}}$, determined by impedance spectroscopy and Eq. (2.4), for contacting solutions of either 50 mM SDS+100 mM NaCl, or of 50 mM C$_{12}$E$_6$+100 mM NaCl. The dotted line represents the best regression fit with an intercept of zero. The slope of this line is $0.96\pm0.04$, with an $r^2$ of 0.96
of the individual skin samples used to arrive at the average values reported in Tables 2.2 and 2.3, using the impedance spectroscopy measurements, with the $R_{\text{skin}}$ values determined using the multimeter ($R_{\text{skin,m}}$) utilizing the following relation (see Eq. (2.1)):

$$R_{\text{skin,m}} = \frac{1}{A \cdot \sigma_{\text{skin}}} = V \cdot \left( \frac{1}{i_{\text{total}}^{-1}} - \frac{1}{i_{\text{back}}^{-1}} \right)$$  \hspace{1cm} (2.5)

A linear regression analysis of the results presented in Figure 2.6 yields a slope of 0.96±0.04, with an $r^2$ of 0.96. In other words, $R_{\text{skin}}$ determined using impedance spectroscopy can be equated to $R_{\text{skin,m}}$. For convenience, the multimeter was used to measure $R_{\text{skin,m}}$ in all subsequent experiments.

### 2.3.3 Effect of Ionic Strength on the Skin Conductivity Test

When measuring the effect of surfactants on $\sigma_{\text{skin}}$, in general, we observed that the ionic strength of the surfactant solution contacting the skin could affect the measured value of the skin conductivity. Specifically, increasing the ionic strength of the surfactant solution, at a fixed surfactant concentration, led to an increase in $\sigma_{\text{skin}}$. An illustration of this ionic strength effect is shown in Figure 2.7 and explained below. To understand this apparent increase in the damage to the skin barrier with increasing ionic strength, it is necessary to realize that $\sigma_{\text{skin}}$ is a function of both the ability of the ions to traverse the skin, which is a direct measurement of changes in the barrier properties of the skin, as well as of the number of charge carriers present in the skin (27, 32). The higher skin conductivities observed in Figure 2.7 at the higher ionic strengths could result from an increase in the permeability of the skin to the ions, from an increase in the ionic content of the skin, or from both, thus making it unclear whether the observed increase in $\sigma_{\text{skin}}$
Figure 2.7: The effect of the background electrolyte on the measured value of $\sigma_{\text{skin}}$ after 5 hours of exposure to solutions containing 50 mM $\text{C}_{12}\text{E}_6$. The electrolyte solutions are McIlvaine buffer (ionic strength of 45 mM), 100 mM NaCl, and 200 mM NaCl. The error bars reflect a 95% confidence interval based on 6 samples at each condition.
with ionic strength is due to an increased actual damage to the skin barrier, or it is an artifact of the higher ionic content in the skin. We are only interested in measuring the permeability of the skin, and it was therefore necessary to determine how large of an effect the ionic strength of the donor solution has on $\sigma_{\text{skin}}$, as well as whether the observed increases in $\sigma_{\text{skin}}$ with increasing ionic strength reflect an actual change in the permeability of the skin resulting from the surfactant-induced reduction in its barrier properties.

With this in mind, $\sigma_{\text{skin}}$ was measured for a contacting solution containing 50 mM of the nonionic surfactant C$_{12}$E$_6$, in a background electrolyte of McIlvaine buffer (ionic strength of 45 mM), or 100 mM NaCl, or 200 mM NaCl. The solution behavior of C$_{12}$E$_6$ is not strongly affected by the ionic strength of the solution (47), and therefore, any observed increase in $\sigma_{\text{skin}}$ with increased ionic strength should result from an increase in the number of charge carriers in the skin. The resulting skin conductivities for a contacting time $t_c=5$ hours are shown in Figure 2.7, clearly demonstrating that increasing the ionic strength of the contacting solution increases $\sigma_{\text{skin}}$, despite the fact that C$_{12}$E$_6$ should not be affected by the ionic strength. The surfactant exposure time, $t_s$, was set to 5 hours because this contacting time was found to be long enough to induce measurable damage to the skin, while still being short enough to approach the relatively short surfactant exposure times that occur in typical surfactant uses, which are on the order of minutes. (Note that we found that for surfactant systems that were not strongly damaging to the skin, it was necessary to increase $t_s$ to 24 hours, see Section 2.3.5.)
To determine whether the increase in $\sigma_{\text{skin}}$ observed in Figure 2.7 was the result of an increase in the number of charge carriers in the skin, or due to an actual increase in the skin permeability, the TDWP was measured for the C$_{12}$E$_6$+100 mM NaCl and for the C$_{12}$E$_6$+200 mM NaCl cases. Note that the TDWP measurements were different from those used to collect the data shown in Figure 2.2. In the present case, the contacting surfactant solutions were removed after 5 hours of exposure and replaced with McIlvaine buffer containing $^3$H-H$_2$O, while in the experiments presented in Figure 2.2, the surfactant solution remained in contact with the skin for 30 hours. The reason behind this change in protocol is explained in Section 2.3.4, where the purpose of exposing the skin to the buffer without surfactant is to moderate the ionic strength of the skin. The TDWP after 24 hours of skin exposure to the buffer solution was found to be\(^3\)

$$\text{TDWP (50 mM C}_{12}\text{E}_6 + 100 \text{ mM NaCl)} = 0.0032 \pm 0.0023 \text{ cm/hr}$$

$$\text{TDWP (50 mM C}_{12}\text{E}_6 + 200 \text{ mM NaCl)} = 0.0036 \pm 0.0013 \text{ cm/hr}$$

The observed equality of the TDWP values for the C$_{12}$E$_6$+100 mM and for the C$_{12}$E$_6$+200 mM NaCl contacting solutions clearly demonstrates that the observed increase in $\sigma_{\text{skin}}$ after 5 hours (see Figure 2.7) is probably due to an increase in the concentration of charge carriers in the skin as the NaCl concentration in the contacting solution increases from 100 to 200 mM. This result emphasizes the importance of controlling the ionic strength of the contacting solution when comparing different surfactant systems, and highlights a potential difficulty in using the skin conductivity method to compare the skin damage

---

\(^3\) In Chapter 4, we will show that, for skin exposed to a contacting solution of SDS, even after contacting the skin with a buffer solution for 24 hours, the skin retains a large amount of SDS in the epidermis. Therefore, we expect that the additional exposure of the skin to the buffer solution does not remove enough C$_{12}$E$_6$ to invalidate the \textit{in vitro} skin irritation test.
induced by different surfactant solutions. This difficulty is particularly important for solutions of commercial surfactants, which are typically impure, and where the added salt concentrations may vary widely from commercial surfactant to commercial surfactant.

2.3.4 Compensating for the Effects of Salt in the Contacting Solution

In view of our finding about potential ionic strength effects in Section 2.3.3, a modification was implemented in the skin conductivity protocol in order to reduce, or eliminate altogether, the observed effect of the ionic strength on $\sigma_{\text{skin}}$ (see Figure 2.7). Specifically, after exposing the skin to the surfactant solution for 5 hours, the surfactant solution was removed, and it was subsequently replaced with a contacting buffer solution for 24 hours. The purpose of contacting the skin with the buffer solution for 24 hours was to generate a similar ionic strength environment for the skin, regardless of the ionic strength of the surfactant solution that comes in contact with the skin, thus allowing for a more controlled comparison of the surfactant-induced damage to the barrier properties of the skin for surfactant solutions having different ionic strengths by reducing, or eliminating altogether, any effect associated with the ionic strength of the contacting solution.

The choice of the skin contact time with the buffer solution, $t_b$, was made based on our prior knowledge that 20 hours was required to achieve a steady-state diffusion of water across the skin (see Section 2.3.1). The purpose of contacting the skin with the buffer solution was to allow the ionic concentration to equilibrate between the skin and the buffer solution in both the donor and the receiver compartments of the diffusion cell. Skin conductivity measurements for $t_z=5$ hours and different $t_b$ values, shown in Figure

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2.8 for two individual skin samples, indicate that $\sigma_{\text{skin}}$ continues to increase even for $t > t_b = 24$ hr, but that the rate of this increase is smaller than at the shorter $t_b$ values (compare the local values of the slopes at the shorter and the longer $t_b$ values in Figure 2.8). It was felt that waiting more than 24 hours would prolong the duration of the experiment too much, which could, in turn, result in a significant fraction of the surfactant leaving the skin. Consequently, a value of $t_b = 24$ hr was adopted for the \textit{in vitro} skin irritation (conductivity) test.

Figure 2.9 shows the measured value of $\sigma_{\text{skin}}$ for the 50 mM C$_{12}$E$_6$ systems of different ionic strengths shown previously in Figure 2.7, both before (open bars) and after (solid bars) contacting the skin with the buffer solution for 24 hours. Clearly, contacting the skin with the buffer solution for 24 hours dramatically reduces the observed differences in $\sigma_{\text{skin}}$ induced by the ionic strength of the contacting surfactant solution, even to the point of reducing $\sigma_{\text{skin}}$ for the 200 mM NaCl case. One might expect that contacting the skin with the buffer solution would lower $\sigma_{\text{skin}}$ because the concentration of surfactant in the skin would be decreased. However, once the skin has been permeabilized by the penetration of surfactants, water and ions may more easily penetrate into the skin, causing it to swell and increasing the permeability of the skin to ions (48). Although there was an increase in $\sigma_{\text{skin}}$ when the skin was exposed to a solution of 50 mM C$_{12}$E$_6$ in McIlvaine buffer for 5 hours, followed by exposure to the buffer solution alone for 24 hours, skin exposure solely to the McIlvaine buffer did not induce large changes in $\sigma_{\text{skin}}$, presumably because the skin was never permeabilized in the absence of the surfactant (C$_{12}$E$_6$ in this case).
Figure 2.8: Variation of the measured $\sigma_{\text{skin}}$ values with the skin contact time to the buffer solution, $t_b$, for two skin samples after 5 hours of skin exposure to a solution of 50 mM C$_{12}$E$_6$ and McIlvaine buffer as the background electrolyte. The solid circles (●) and the open circles (○) each represent a single skin sample. These two skin samples were chosen as being representative of the change in $\sigma_{\text{skin}}$ with $t_b$. The numbers adjacent to the various lines through the data points (drawn to guide the eye) correspond to the local rate of change in $\sigma_{\text{skin}}$ with $t_b$. 
Figure 2.9: The effect of the background electrolyte on the measured $\sigma_{\text{skin}}$ value after 5 hours of skin exposure to solutions containing 50 mM C$_{12}$E$_6$, with $t_p=0$ hours (open bars) and $t_p=24$ hours (solid bars). The error bars correspond to a 95% confidence interval based on 6 samples at each condition.
2.3.5 Application of the *in vitro* Skin Irritation (Conductivity) Test to Commercial Products

The *in vitro* skin irritation (conductivity) test described in Section 2.3.4 was applied to three commercial soap bars: Dove, Lever 2000, and Ivory. The *in vivo* skin irritation impact of these three soap bars was measured independently by several methods, and revealed that Dove is the least irritating to the skin while Ivory is the most irritating (49). To evaluate our *in vitro* skin irritation test, it was necessary to compare the results of our *in vitro* skin irritation measurements with the known *in vivo* skin irritation results (49).

A 5 wt% solution of each product was prepared by homogenizing grated Dove, Lever 2000, or Ivory soap bars in distilled water using a Silverson L4RT-A Lab Mixer at 3000 rpm for 2 minutes, resulting in an opaque and viscous suspension. Each soap bar solution was added to the donor compartment of the diffusion cell, removed after 5 hours of exposure to the skin, and replaced with a phosphate buffered solution (PBS).⁴ Applying our new *in vitro* skin irritation test to the three commercial soap bars illustrates another benefit of measuring the skin conductivity with reference to a standard solution (the buffer), because the three soap bar suspensions displayed varying solution properties, including viscosity and emulsion stability, thus complicating the measurement of the ionic strength of each suspension. Indeed, by replacing each soap bar solution with a standard PBS solution, we effectively created a known and consistent electrolyte on both sides of the skin for the skin conductivity measurements, as discussed in Section 2.3.4.

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⁴ Note that, in these experiments, we used a PBS buffer instead of the McIlvaine buffer used previously. This replacement buffer is easier to prepare and has an ionic strength more similar to that of the body than the McIlvaine buffer.
Figure 2.10: Measured values of $\sigma_{\text{skin}}$ after $t_r=24$ hours for 5 wt% solutions of three soap bars: Dove, Lever 2000, and Ivory. The open bars represent cases for which $t_r=5$ hours, and the solid bars represent cases for which $t_r=24$ hours. The error bars correspond to a 95% confidence interval based on 6 samples at each condition.
As discussed above, $\sigma_{\text{skin}}$ was measured after $t_s=24$ hours, and the results are shown in Figure 2.10 for the three soap bars studied. Unfortunately, the results for $t_s=5$ hours (see open bars in Figure 2.10) did not yield a statistically significant difference between the Lever 2000 and the Ivory soap bars. To ameliorate this, we increased $t_s$ to 24 hours, also shown in Figure 2.10 (see solid bars), hoping that the more exaggerated exposure time would increase any differences between the soap bar solutions. Indeed, increasing $t_s$ had the desired effect, and the skin conductivity results rank in the expected skin irritation order based on the in vivo skin irritation results (49), with Ivory increasing $\sigma_{\text{skin}}$ the most, and Dove increasing $\sigma_{\text{skin}}$ the least. These results raise the question of what value of $t_s$ should be used for the in vitro skin irritation test. Low $t_s$ values are closer to actual surfactant exposure times that are typically encountered in daily washing situations, while high $t_s$ values can exaggerate the damage to the skin, potentially clarifying differences between surfactant systems. Given our findings in Figure 2.10, use of a high $t_s$ value is recommended for surfactant systems that are expected to exhibit similar skin irritation potentials (like Lever 2000 and Ivory).

2.4 CONCLUSIONS

An important manifestation of skin irritation is the reduction in the barrier properties of the skin, which increases the water loss through the skin, and increases the amount of irritants penetrating into the skin. Surfactants are known to increase the skin permeability—an inverse quantitative measure of the skin barrier strength. Accordingly, quantifying the skin permeability induced by contact of the skin with various surfactant solutions can allow a comparison between the skin irritation potential induced by the
various surfactant solutions. Based on the assumption that water and ions will traverse the skin through the same polar pathways, we compared how the TDWP and $\sigma_{\text{skin}}$ are affected by contacting the skin with solutions of SDS/C$_{12}$E$_6$ surfactant mixtures. We found that $\sigma_{\text{skin}}$, a much easier quantity to measure, was well correlated with the TDWP. Therefore, skin conductivity was chosen as the experimental method for quantifying the surfactant-induced damage to the barrier properties of the skin. The direct correlation found between the TDWP and $\sigma_{\text{skin}}$ supports the expectation that the transdermal movement of the water molecules, reflected in the TDWP measurement, and the transdermal movement of the ions, reflected in the skin conductivity measurement, are controlled by similar mechanisms.

Impedance spectroscopy was used to more closely probe the changes induced by surfactants to the electrical characteristics of the skin. We found that both SDS and C$_{12}$E$_6$ had similar effects on the electrical resistance of the skin, $R_{\text{skin}}$ (the inverse of $\sigma_{\text{skin}}$), as well as on the behavior of the CPE in the model circuit of the skin, including an increase in the effective capacitance of the skin. A comparison between $R_{\text{skin}}$, determined using impedance spectroscopy, with $R_{\text{skin,m}}$, determined using a signal generator and multimeter, revealed that the two determined values are essentially the same.

A complication encountered using an electrical measurement to quantify the barrier properties of the skin became apparent when we discovered that the effect of the ionic strength of the contacting solution can affect the measured value of $\sigma_{\text{skin}}$. Specifically, $\sigma_{\text{skin}}$ was found to increase with both the ionic permeability of the skin and with the ionic strength of the contacting solution, which could both increase the number of charge
carriers in the skin. The purpose of the *in vitro* skin irritation (conductivity) test that we aimed to develop was to determine the surfactant-induced damage to the barrier properties of the skin, and consequently, it became necessary to minimize, or eliminate altogether, the ionic strength effect on $\sigma_{\text{skin}}$. This was achieved by contacting the skin with a buffer solution for 24 hours, following the exposure of the skin to the surfactant solution of interest, in order to normalize the ionic strength in the skin for different surfactant samples.

The final *in vitro* skin irritation (conductivity) protocol involved a 5-hour or 24-hour exposure of the skin to the surfactant solution, with the 24-hour exposure recommended for the milder surfactant solutions, followed by contacting the skin to a buffer solution for 24 hours to remove any effect of the ionic strength of the surfactant solution on $\sigma_{\text{skin}}$. Following the 24-hour contact of the skin with the buffer solution, $\sigma_{\text{skin}}$ was measured using a 10 Hz signal. The purpose of any *in vitro* skin irritation test is to model the *in vivo* skin irritation results. Using the skin conductivity as a metric to quantify the irritation potential of the soap bars Dove, Lever 2000, and Ivory, the order of increasing skin conductivity, determined *in vitro*, matched the known order of increasing skin irritation potential, as determined *in vivo*. The success of our newly-developed *in vitro* skin irritation (conductivity) test in the case of the three commercial soap bars examined is very encouraging, and indicates the potential utility of this relatively simple *in vitro* method in enabling a comparison of the skin irritation of surfactant-containing formulations that can affect the barrier properties of the skin.
2.5 REFERENCES


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49. S. Puvvada, Unilever Home and Personal Care, NA. *Personal Communication.*
Chapter 3

Challenging the Surfactant Monomer Skin Penetration Model: Penetration of Sodium Dodecyl Sulfate Micelles into the Epidermis

3.1 INTRODUCTION

The investigation of surfactant-induced skin irritation has been extensive, including: (i) which surfactants are most irritating (1-8), (ii) how mixing surfactants can reduce skin irritation (9-11), and (iii) how surfactants can lead to changes in the permeability of the skin (12-18). All the proposed mechanisms for surfactant-induced skin irritation involve the penetration of surfactants into the skin, where they can: (i) denature proteins (2, 4, 19-21), (ii) remove lipids from the stratum corneum (SC) (22-25), or (iii) disrupt the integrity of the lipid bilayers in the SC and the viable epidermis (12, 16-18, 26, 27). The widely-accepted view regarding surfactant-induced skin irritation is that, at surfactant concentrations that exceed the critical micelle concentration (CMC), where surfactant micelles first form, only surfactant monomers can penetrate into the skin, either because surfactant micelles are not surface active, or because they are too large to penetrate into the SC (2, 5, 7, 9, 10, 15, 28, 29). This view is based primarily on experimental observations using mixtures of surfactants, where surfactant-induced skin irritation was
correlated with the CMC of the surfactant mixtures examined (5, 9-11). This correlation is viewed as evidence that only the surfactant monomers are responsible for skin irritation, because the CMC is approximately equal to the surfactant monomer concentration. We call this widely-accepted view the *surfactant monomer skin penetration model*, and will refer to it hereafter as the *monomer penetration model*.

If surfactant-induced skin irritation is controlled solely by the monomeric surfactant, then it would naturally follow that there should be *no effect* of increasing the total surfactant concentration beyond the CMC, since the surfactant monomer concentration should remain approximately constant in that case. Instead, it has been observed experimentally that as the total surfactant concentration is increased beyond the CMC, the surfactant-induced damage to the skin increases as well (1-3, 7, 10, 13, 15). For example, Agner and Serup found that the severity of the transepidermal water loss (TEWL) induced by the anionic surfactant SDS increased as the SDS concentration increased beyond the CMC of SDS (8.7 mM) (1). In other studies, it was found that as the SDS concentration increased beyond the CMC, the amount of SDS that penetrates into the stratum corneum also increased (2, 28, 29).

Yet another illustration of the effect of increasing the total surfactant concentration above the CMC is provided by Rhein et al.(10) Specifically, these authors demonstrated that mixing SDS with a second, milder anionic surfactant, alkyl 7-ethoxy sulfate (AEOS-7), led to a reduction in the irritation potential of SDS that correlated with a reduction in the CMC of the surfactant mixture, thus providing evidence for the monomer penetration model. However, the authors also noted that when the total surfactant concentration was increased, the irritation also increased, a finding that is not consistent with the monomer penetration model, and suggests a more complex mechanism of surfactant-induced skin irritation.
Various explanations have been put forward to rationalize the observed dose-dependent effect of surfactants on the skin at surfactant concentrations above the CMC, including: (i) that the monomer activity increases above the CMC (15), and (ii) that the micelles in the contacting solution solubilize lipids present in the skin (22, 28). However, regarding explanation (i), various measurements, including surface tension and solution conductivity, clearly indicate that the surfactant monomer activity remains fairly constant, or even decreases, as the surfactant concentration increases beyond the CMC (30, 31). With regards to explanation (ii), there is evidence that micelles are able to remove some lipids from the skin, but the observed changes in the skin lipid concentration and composition are rather small and difficult to interpret (22-25). According to explanation (ii), an increase in the number of micelles above the CMC should lead to an increase in the lipid solubilization capacity of the contacting solution, thus explaining the increased damage to the skin, but not explaining the observed increased penetration of SDS into the stratum corneum observed by Faucher and Goddard (29), and by Ananthapadmanabhan et al. (28). In this chapter, we will therefore focus on investigating the factors controlling the penetration of surfactant into the epidermis.

With this in mind, we have measured the amount of SDS that penetrates into the epidermis, consisting of the SC and the viable epidermis, after 5 hours of exposure to aqueous SDS solutions of increasing SDS concentration (see Section 3.3.1). We have found that the concentration of SDS in the epidermis is directly related to the concentration of free SDS micelles in the contacting solution. We have also found that, in the presence of poly(ethylene oxide), the SDS that complexes with PEO in the form of PEO-bound SDS micelles cannot penetrate into the epidermis, while the free, or unbound, SDS micelles can (see Section 3.3.2). Using Dynamic Light Scattering (DLS), we will show that a plausible explanation of our
findings is that the free SDS micelles can penetrate into aqueous pores present in the SC, while the PEO-bound SDS micelles are sterically hindered from penetrating into the SC (see Sections 3.3.4 and 3.3.5).

### 3.2 EXPERIMENTAL

#### 3.2.1 Materials

Sodium dodecyl sulfate (SDS) and poly(ethylene oxide) (PEO) (molecular weight of 8000 g/mol) were purchased from Sigma Chemicals (St. Louis, MO) and used as received. Water was produced using a Millipore Academic water filter. $^{14}$C-radiolabeled SDS was purchased from American Radiolabeled Chemicals (St. Louis, MO) and used as received. Phosphate buffered saline (PBS) was prepared using PBS tablets from Sigma chemicals and Millipore filtered water.

#### 3.2.2 Preparation of Skin Samples

Female Yorkshire pigs (40-45kg) were purchased from local farms, and the skin (back) was harvested within one hour after sacrificing the animal. The subcutaneous fat was trimmed off using a razor blade, and the full-thickness pig skin was cut into small pieces and stored in a −80°C freezer for up to 2 months. The surfactant penetration experiments were performed using full-thickness pig skin.

#### 3.2.3 Experimental Protocol

The pig skin was mounted in a vertical Franz diffusion cell (Permegear Inc., Riegelsville, PA), with the SC side facing the donor compartment. Phosphate buffered saline (PBS; phosphate concentration, of 0.01 M; NaCl concentration of 0.137 M; Sigma Chemical Company, St. Louis, MO) was added to the donor and the receiver compartments of the diffusion cell, and the skin
was allowed to hydrate for 1 hour. The PBS in the donor compartment was removed, and 1.5 ml of surfactant solution was added to the donor compartment. The solution in the donor compartment, referred to hereafter as the contacting solution, contained mixtures of SDS and PEO, each with about 0.5 µCi/ml of $^{14}$C-SDS. We verified that the concentration of radiolabeled SDS in the contacting solution did not change appreciably during the 5 hour exposure to the skin.

Diffusion into the skin took place for 5 hours, and subsequently, the contacting solution was removed and the donor compartment was rinsed 4 times with 2 ml of PBS. A 5 hour exposure was chosen because this was a sufficiently long time to allow significant SDS penetration into the skin, but a short enough time to prevent the saturation of the skin with SDS. The skin was then heat-stripped by placing it in a bath of water at 60°C for two minutes, and then peeling off the epidermis (SC and viable epidermis) that had been exposed to the contacting solution from the dermis. The exposed epidermis was then dried for two days in a fume hood and weighed. The dried epidermis was dissolved overnight in 1.5 ml of Soluene-350 (Packard, Meriden, CT). After the epidermis dissolved, 10 ml of Hionic Fluor scintillation cocktail (Packard) was added to the Soluene-350, and the concentration of radiolabeled SDS was determined using a Packard Tri-Carb 4350 scintillation counter (Packard). Knowing the concentration of SDS in the contacting solution, $C_{SDS}$, the radioactivity of the contacting solution, $C_{rad,donor}$, the dry weight of the epidermis, $m$, and the radioactivity of the epidermis, $C_{rad,skin}$, it was possible to determine the concentration of SDS in the dried epidermis, $C_{skin}$, using the following equation:

$$C_{skin} = \frac{C_{rad,skin} \cdot C_{SDS}}{C_{rad,donor} \cdot m}$$

(3.1)
3.2.4 Dynamic Light Scattering

The SDS and SDS+PEO solutions were prepared in Millipore filtered water with 0.1 M NaCl. After mixing, the solutions were filtered through a 0.02 μm Anotop 10 syringe filter (Whatman International, Maidstone, England) directly into a cylindrical-scattering cell to remove any dust from the solution, and then sealed until use. Dynamic Light Scattering (DLS) (32) was performed at 25°C and a 90° scattering angle on a Brookhaven BI-200SM system (Brookhaven, Holtsville, NY) using a 2017 Stabilite argon-ion laser (Spectra Physics) at 488 nm. The autocorrelation function was analyzed using the CONTIN program provided by the BIC Dynamic Light Scattering software (Brookhaven, Holtsville, NY), which determines the effective hydrodynamic radius, $\overline{R}_h$, using the Stokes-Einstein relation (33):

$$\overline{R}_h = \frac{k_B T}{6\pi \eta \overline{D}}$$

(3.2)

where $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, $\eta$ is the viscosity of the salt solution, and $\overline{D}$ is the mean diffusion coefficient of the scattering species.

In order to measure the size of the micelles, including free SDS micelles and PEO-bound SDS micelles, and eliminate the effects of interparticle interactions, the effective hydrodynamic radii were determined at several different SDS concentrations, and the average effective hydrodynamic radii were extrapolated to a zero micelle concentration (32, 34-37). 100 mM NaCl was added to screen the intermicellar electrostatic interactions in the DLS measurements (32, 35-37).
In the presence of PEO, the concentration of PEO was adjusted such that only PEO-bound SDS micelles were predicted to exist (38). This was done to minimize the scattering from either the free SDS micelles or the free PEO molecules, both of which have $\bar{R}_h$ values that are similar to that of the PEO-bound SDS micelles. The concentrations of SDS and PEO utilized in the DLS studies are listed in Table 3.1.

Table 3.1: Concentrations of SDS and PEO used in the Dynamic Light Scattering experiments to minimize the concentrations of free SDS micelles and free PEO molecules in the scattering solution.

<table>
<thead>
<tr>
<th>Concentration of SDS (mM)</th>
<th>Concentration of PEO (wt%)</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>0.14</td>
</tr>
<tr>
<td>20</td>
<td>0.29</td>
</tr>
<tr>
<td>30</td>
<td>0.45</td>
</tr>
<tr>
<td>40</td>
<td>0.60</td>
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</tbody>
</table>

3.2.5 Determination of the Aqueous Pore Radius in the Stratum Corneum

Although we did not measure the aqueous pore radii ourselves, we believe that a brief discussion of the measurement method is in order. Using hindered-transport theories (39), several authors have measured the average aqueous pore radius in the SC (12, 40-43). In this method, the transdermal permeabilities of two types of polar molecules, or the transdermal permeability of a polar molecule and the transdermal conductivity of an ion, are measured. The permeants are either both radiolabeled molecules (12, 42) or a radiolabeled molecule and an ion (41, 43), which have different hydrodynamic radii. The effect of the skin pore radius on the diffusion of the permeant depends on the ratio of the permeant hydrodynamic radius to the pore radius, $\lambda$, with an increase in the hindrance to the diffusion of the permeant across the skin as $\lambda$ approaches 1.
Accordingly, by comparing the hindrance to diffusion across the skin of the two molecules, one can determine the effective aqueous pore radius of the skin.

3.3 RESULTS AND DISCUSSION

3.3.1 Dose-Dependent Penetration of SDS into the Epidermis

We first investigated whether increasing the total SDS concentration in the contacting solution above the CMC led to an increase in the concentration of SDS measured in the epidermis. An increase in the SDS concentration in the epidermis could explain much of the skin irritation data reported in the literature, since a larger 'dose' of the SDS irritant in the skin would lead to greater skin irritation. Figure 3.1 shows that as the concentration of SDS in the contacting solution increased from 8.7 mM (the CMC of SDS) to 200 mM (about 20 times the CMC of SDS), the concentration of SDS measured in the epidermis increased in a linear manner, similar to the increased penetration reported by others (28, 29). This finding clearly contradicts the monomer penetration model, according to which the amount of SDS penetrating into the epidermis should remain constant as the SDS concentration increases beyond the CMC. Instead, Figure 3.1 vividly demonstrates that SDS present in micellar form must be contributing to SDS penetration into the epidermis, since the SDS monomer concentration is constant over the experimental range examined, while the concentration of SDS micelles is increasing.

The concentration of SDS in the epidermis measured here (2-9 wt%) compares well with the concentrations reported by others (4.3 wt%) (44). This large partitioning of SDS into the skin has been viewed as reflecting the binding of SDS molecules to the keratin protein in the corneocytes found in the SC (28, 29, 44). A mass balance of the amount of SDS found in the epidermis
Figure 3.1: Effect of increasing the SDS concentration in the contacting solution on the amount of SDS that penetrates into the epidermis. The vertical dashed line indicates the CMC of SDS (8.7 mM), and the solid line through the data points is shown to guide the eye. The error bars reflect a 95% confidence interval based on 6 samples at each SDS concentration.
relative to the initial amount of SDS indicates that less than 1% of the SDS in the initial contacting solution penetrates into the skin for all the contacting solutions used in Figure 3.1. Accordingly, one can assume that the contacting solution provides an infinite reservoir of SDS for penetration into the skin.

Although the results in Figure 3.1 contradict the monomer penetration model, they do not contradict many observations reported in the literature where a surfactant dose-dependent skin irritation response was observed (1-3, 7, 10, 13, 15). Moreover, if the surfactant-induced damage to the skin is related to the actual amount of SDS in the skin, then the increased concentration of SDS in the epidermis shown in Figure 3.1 can be related to the increased skin irritation induced by SDS, observed by many researchers, as the SDS concentration in the contacting solution is increased (1-3, 7, 10, 13, 15).

3.3.2 Effect of PEO on the Penetration of Micellar SDS into the Epidermis

Having demonstrated that the amount of SDS penetrating into the epidermis above the CMC is related to the total SDS concentration (micellar and monomeric) in the contacting solution, instead of only to the SDS monomer concentration, we examined the effect of adding PEO to the contacting solution. PEO is known to form micelle-like complexes with SDS, with the PEO forming a corona around the SDS micelles (34, 37, 45). The critical aggregation concentration (CAC) is the surfactant concentration at which polymer-bound micelles first form, in our case PEO-bound SDS micelles, and is lower than the CMC of the surfactant in the absence of PEO (38). When PEO is mixed with a solution of SDS, if there is an excess of PEO, the SDS monomer concentration is approximately equal to the CAC. Consequently, we anticipated that the contribution of the SDS monomers to skin penetration would be diminished in the SDS-PEO...
solutions because the SDS monomer concentration would be lowered. However, it was unclear how the SDS in the PEO-bound SDS micelles would behave when exposed to the skin, namely, whether this SDS could contribute to SDS penetration into the epidermis.

Initially, we prepared solutions containing either 50 mM or 100 mM SDS as well as 2.0 wt% PEO. According to a molecular-thermodynamic theory developed recently (38), there is an excess of PEO at the two SDS concentrations examined. As a result, any micelles that form are bound to the PEO, and the SDS monomer concentration should be approximately equal to the CAC. Moreover, for PEO having a molecular weight of 8000 g/mol, we would expect, according to the theory (38), that there would be a maximum of 1 SDS micelle per PEO molecule. Figure 3.2 shows how the concentration of SDS measured in the epidermis was affected by the presence of PEO. Two aspects of Figure 3.2 should be noted:

i. The addition of PEO at a given SDS concentration (50 mM or 100 mM) in the contacting solution leads to a significant reduction in the concentration of SDS measured in the epidermis.

ii. The difference between the concentrations of SDS in the epidermis at 50 mM and 100 mM SDS concentrations in the contacting solution is not statistically significant in the presence of PEO, while it is statistically significant in the absence of PEO.

According to the monomer penetration model, the reduction in the SDS monomer concentration upon the addition of PEO should lead to a reduction in the amount of SDS penetrating into the epidermis, thus potentially explaining (i). However, the more interesting aspect of Figure 3.2, (ii), is that SDS bound to PEO contributes very little, if at all, to the amount of SDS measured in the epidermis as the SDS concentration increases from 50 mM to 100 mM, while the SDS in free
Figure 3.2: Effect of adding 2 wt% PEO to a solution of SDS (50 mM and 100 mM) on the amount of SDS that penetrates into the epidermis. The empty bars indicate SDS penetration in the absence of PEO, and the solid bars indicate SDS penetration in the presence of 2 wt% PEO. The error bars reflect a 95% confidence interval based on 6 samples at each condition.
micelles does. Instead, it is apparent that increasing the SDS concentration in the presence of PEO has no effect on the amount of SDS that penetrates into the epidermis. Therefore, the addition of PEO modifies the penetration behavior of the micellar SDS.

To further study the relationship between the various forms of SDS present in the contacting solution (SDS monomers, free SDS micelles, and PEO-bound SDS micelles) and the SDS concentration measured in the epidermis, the skin was exposed to solutions of 100 mM SDS and different PEO concentrations. The PEO concentration in the contacting solution was varied such that there would be different proportions of free SDS micelles and SDS micelles bound to PEO, thereby controlling the concentration of SDS in free micelles while maintaining a constant total SDS concentration, including SDS monomers, free SDS micelles, and PEO-bound SDS micelles. The effect of increasing the total PEO concentration on the distribution of SDS between free SDS micelles and PEO-bound SDS micelles is shown schematically in Figure 3.3, with Figure 3.4 showing how the predicted concentrations of free SDS micelles and PEO-bound SDS micelles vary with increasing PEO concentration at 100 mM SDS (38). Figures 3.3 and 3.4 reveal that as the concentration of PEO increases, the concentration of free SDS micelles decreases as more SDS forms PEO-bound SDS micelles. However, as long as free SDS micelles are able to form, the SDS monomer concentration is predicted to remain constant with increasing PEO concentration. This is because once PEO is saturated with SDS micelles, the SDS monomer concentration should increase until the CMC is reached, beyond which free SDS micelles will form (38). However, above a concentration of 1.75 wt% PEO, no free SDS micelles are present, and the SDS monomer concentration is equal to the CAC of SDS in the presence of PEO, ~5mM (38). This was, in fact, the condition under which the measurements reported in Figure 2 were actually carried out.
Figure 3.3: Schematic representation of the effect of increasing the PEO concentration in a SDS-PEO solution on the distribution of SDS in monomeric form, in free SDS micelles, and in PEO-bound SDS micelles at a fixed SDS concentration. With no added PEO, only free SDS micelles and SDS monomers are present. With intermediate concentrations of added PEO, there are both free SDS micelles and PEO-bound SDS micelles, as well as SDS monomers. With excess added PEO, only PEO-bound SDS micelles and SDS monomers are present, as well as free PEO molecules.
Figure 3.4: Predicted distribution of SDS in free SDS micelles (empty bars) and PEO-bound SDS micelles (solid bars) as a function of PEO concentration at 100 mM SDS (38).
Figure 3.5 clearly shows that the concentration of SDS in the epidermis decreases as the concentration of PEO in the contacting solution increases, even though the total concentration of SDS is fixed at 100 mM. Figure 3.5 also compares this experimentally observed decrease with the *predicted* decrease in the concentration of SDS in free micelles with increasing PEO concentration, also shown in Figure 3.4 (38). The observed correlation between the concentration of free SDS micelles and the concentration of SDS measured in the epidermis is apparent, clearly demonstrating that SDS in the form of free micelles contributes to the penetration of SDS into the epidermis, while SDS in the form of PEO-bound SDS micelles does not. Although PEO prevents the SDS bound to it from penetrating into the epidermis, the SDS monomer can still penetrate into the epidermis, leading to the plateau observed at PEO concentrations above 1.75 wt%.

### 3.3.3 Regression Analysis of the Contributions of SDS Monomers, Free SDS Micelles, and PEO-Bound SDS Micelles to SDS Penetration into the Epidermis

Figures 3.1, 3.2, and 3.5 indicate that there is a relationship between the concentration of SDS that is not bound to PEO and the amount of SDS that penetrates into the skin after 5 hours of skin exposure to an SDS+PEO solution. With this in mind, we wanted to quantify the relative contributions of SDS monomers, free SDS micelles, and PEO-bound SDS micelles in the contacting solution to the SDS concentration measured in the epidermis after 5 hours of exposure. A multiple linear regression was used for this quantification, using all the experimental data, prior to averaging, that was presented above relating the SDS concentration in the epidermis to the SDS concentration in the contacting solution. The simplest relationship
Figure 3.5: The measured decrease in the concentration of SDS in the epidermis with increasing PEO concentration is compared with the predicted decrease in the concentration of SDS in free micelles. The closed circles (●) represent the concentration of SDS in the epidermis, and the solid line (—) represents the predicted concentration of SDS in free micelles (38). Above 1.75 wt% PEO (indicated by the vertical dashed line), no free SDS micelles are present, and therefore, only the SDS monomers contribute to the penetration of SDS into the epidermis. The error bars reflect a 95% confidence interval based on 6 samples at each PEO concentration. The arrows direct the reader to the relevant axis.
between the three contributions to SDS penetration into the skin is a linear one, since for Fickian diffusion from an infinite reservoir with a large concentration difference, the net permeant flux at a given time is directly proportional to the initial permeant concentration (46). This leads to the following relation that formed the basis of our regression analysis:

\[
C_{\text{skin}} = a \cdot C_{\text{monomers}} + b \cdot C_{\text{free micelles}} + c \cdot C_{\text{PEO-bound micelles}}
\]  

(3.3)

where a, b, and c are weighting factors that were determined by the regression analysis, \(C_i\) is the concentration of SDS in monomeric, free micellar, or PEO-bound micellar form, and \(C_{\text{skin}}\) is the concentration of SDS in the dry epidermis, having units of mmols of SDS per gram of dry epidermis. For the regression analysis, \(C_{\text{monomers}}\) was set to 8.7 mM, the CMC of SDS, when there were free SDS micelles, and to 5 mM, the CAC of SDS+PEO, when only PEO-bound SDS micelles were present, based on data for the CMC and the CAC of SDS in the presence of PEO (38). \(C_{\text{PEO-bound micelles}}\) was determined using the predictive theory (38), and \(C_{\text{free micelles}}\) was determined from the mass balance on SDS (\(C_{\text{SDS}}-C_{\text{PEO-bound micelles}}-C_{\text{monomers}}\)), where \(C_{\text{SDS}}\) is the total SDS concentration in the contacting solution.

The regression analysis yielded a value of \(c\) that is two orders of magnitude smaller than that of \(a\) or \(b\), and was not significantly different from zero. Accordingly, a second regression analysis was performed where \(c\) was set equal to zero. Removing the \(c\) parameter from the regression analysis led to a smaller confidence interval for the value of \(a\), while the regressed value of \(b\) remained the same. The second regression analysis yielded:

\[
a = 0.14 \pm 0.04 \frac{C_{\text{skin}}}{C_{\text{monomers}}}
\]

\[
b = 0.043 \pm 0.006 \frac{C_{\text{skin}}}{C_{\text{free micelles}}}
\]
The important conclusion that can be drawn from the regression analysis is that SDS in free micelles contributes to the amount of SDS measured in the epidermis, while SDS in PEO-bound micelles does not. The regression analysis also shows that the contribution of free SDS micelles to $C_{\text{skin}}$ is independent of the presence of PEO, because the value of the parameter $b$ is the same whether or not the data with PEO is included. When one considers that in surfactant-containing washing formulations the concentration of micelles is typically much higher than that of the monomers, it is evident that, with regards to the penetration of surfactant into the skin, it is important to consider the contribution of the micelles in addition to that of the monomers.

3.3.4 Dynamic Light Scattering Results

Figure 3.6 shows the results of the DLS measurements, with the micelle size being determined by extrapolation to a zero micelle concentration, which occurs at the CMC (about 0.9 mM SDS for free SDS micelles) or at the CAC (about 0.6 mM SDS for PEO-bound SDS micelles) of the surfactant solution. Using a linear regression analysis, we have determined that the average radius of the free SDS micelles is 20 Å and that the PEO-bound SDS micelles have an average radius of 25 Å.

3.3.5 New Proposed Models of Surfactant Penetration into the Epidermis

We have demonstrated that the amount of SDS that penetrates into the epidermis is determined by both the concentrations of SDS monomers and of free SDS micelles in the contacting solution, and that the concentration of SDS bound to PEO has no effect on SDS penetration into the skin. Because we have shown that the monomer penetration model cannot explain the experimental observations in Figures 3.1, 3.2, and 3.5, we next attempted to identify a surfactant
Figure 3.6: Measured effective radii of SDS micelles in the absence and in the presence of PEO plotted versus the SDS concentration minus the CMC, or the SDS concentration minus the CAC respectively, using DLS measurements at 25°C. The closed circles (●) represent the effective radii in the absence of PEO, and the open squares (□) represent the effective radii in the presence of PEO. The micellar radii were determined using a CONTIN analysis. The error bars reflect a 95% confidence interval based on 8 samples at each SDS concentration.
skin penetration model that would be consistent with our experimental observations. We believe that the key to the new surfactant skin penetration model lies in the different behaviors observed for SDS in free micelles and in SDS micelles bound to PEO, since the former contribute to surfactant penetration into the epidermis while the latter do not contribute, as shown by the regression analysis. Below, we discuss two possible surfactant skin penetration models to explain our experimental observations: (i) micelle kinetics affect the rate of surfactant monomer replenishment, and (iii) micelles can penetrate into the skin, but there is a size limitation.

Penetration model (i) utilizes the kinetics of micelle dissolution (47, 48) to explain the observed increase in SDS penetration into the epidermis with increasing SDS concentration in the contacting solution, and is an extension of the original monomer penetration model. In this model, as the SDS monomers in the contacting solution penetrate into the SC, they must be replenished, either by the break-up of SDS micelles into monomers, or by the diffusion of other SDS monomers present in the contacting solution farther away from the SC. As the SDS micelle concentration in the contacting solution increases, the argument goes, the rate of monomer formation through micelle dissolution should increase, and hence, the concentration of SDS monomers adjacent to the SC should be higher in the presence of more SDS micelles. As appealing as this model appears initially, it fails in several respects. First, it is well-known that as the SDS concentration in pure water increases from 50 to 200 mM, the rate of monomer formation through micelle dissolution actually decreases, with the SDS micelles at 200 mM being the most stable (49). Therefore, as the concentration of SDS in the contacting solution is increased, the rate of SDS monomer formation should decrease. According to this kinetic model, the SDS penetration into the epidermis should actually decrease, because the concentration of
SDS monomers adjacent to the SC will be lower as the concentration of SDS in the contacting solution increases.

Another problem with this micelle-kinetic model is that the addition of PEO to a SDS solution has been shown to increase the rate of micelle dissolution dramatically (50, 51). Therefore, if micelle kinetics controlled the penetration of SDS into the epidermis in the presence of PEO, the penetration of SDS into the epidermis should increase as more SDS is added. Accordingly, one should observe an increase in the SDS concentration in the epidermis in Figure 3.2 as one goes from 50 mM SDS to 100 mM SDS in the presence of PEO. Instead, PEO prevents the PEO-bound SDS from penetrating into the skin, as determined by the regression analysis presented above, thus making penetration model (i) inconsistent with the experimental observations.

Penetration model (ii) assumes that, contrary to the monomer penetration model, SDS micelles are actually able to penetrate into the SC. Although it is not expected that a SDS micelle would pass through the SC without breaking-up due to its self-assembling nature, if the SDS micelles could penetrate even partially into the SC, then the concentration of SDS in the epidermis could be increased dramatically. If the free SDS micelles could penetrate into the SC while the PEO-bound SDS micelles could not, then the concentration of SDS in the epidermis would be related to the concentration of SDS in the free micelles and not to the concentration of SDS bound to PEO, as we observed experimentally in Figures 3.1, 3.2, and 3.5. Therefore, our hypothesis that some micelles can penetrate into the SC, while others cannot, appears to be consistent with our experimental observations.

Due to the hydrophilic nature of micelles, micelles would be expected to enter the SC through aqueous pathways (pores) rather than through non-polar pathways (52). Aqueous pathways in
the SC are believed to be located in the intercellular region, in particular, in the lacunae and other aqueous regions surrounded by polar lipids (52-55). The characteristic radius of the aqueous pores in the skin has been determined using hindered-transport theories (12, 41-43). Typical values for the aqueous pore radii of human skin range from 10 to 25 Å (12, 42, 43). For the Yorkshire pig skin used in this chapter, the characteristic aqueous pore radius was determined to be 28 Å (41).

In Section 3.4.4, we determined that the average radius of the free SDS micelles is 20 Å. These micelles are small enough that they could conceivably penetrate into the aqueous pores of the skin. However, according to our light scattering studies, the PEO-bound SDS micelles have an average radius of 25 Å, and would be sterically hindered from passing into most aqueous pores in the skin since they are of about the same size as these pores. Consequently, according to our hypothesis, the ability of the micelles to affect the amount of surfactant penetrating into the skin is determined by the size of the micelle relative to the aqueous pore size. In this respect, it should also be recognized that both the micelles and the aqueous pores exhibit a range of sizes (31, 56). It is expected that the micelles will only be able to penetrate into the skin when there is an overlap in these size ranges. Accordingly, although some aqueous pores may be large enough to allow the penetration of PEO-bound SDS micelles, such events would be relatively rare.

In general, one should keep in mind that the aqueous pores in the skin are negatively charged (12, 40). As a result, in addition to the micelle steric hindrance considerations put forward in this chapter, there may be cases where electrostatic interactions between charged micelles and the charged aqueous pores need to be considered to determine the ability of charged micelles to penetrate into the aqueous pores. However, such electrostatic effects are not expected to play a role in the studies reported in this chapter because the free SDS micelles and the PEO-bound
SDS micelles should have the same net negative charge, since PEO is a neutral molecule and its complexation with SDS does not screen the negatively-charged sulfate groups of SDS. In addition, PEO is not expected to modify the negative charge of the aqueous pores. Consequently, the observed exclusion of the PEO-bound SDS micelles from the aqueous pores, coupled with the observed inclusion of the free SDS micelles into the aqueous pores, should result entirely from the proposed micelle steric hindrance mechanism embodied in penetration model (ii).

3.4 CONCLUSIONS

According to a widely-accepted view, surfactant micelles cannot penetrate into the skin, due to size limitations or other factors, and as a result, surfactant-induced skin irritation should be determined solely by the concentration of surfactant monomers (2, 5, 7, 9, 10, 15). We have shown that this is not the case for SDS. Instead, for a fixed amount of time (5 hours), we found that the amount of SDS that penetrates into the skin is directly related to the concentration of SDS micelles present in the solution contacting the skin. When PEO is added to the SDS solution, SDS in micelles that are bound to PEO does not penetrate into the skin, while monomeric SDS and SDS in free micelles do penetrate into the skin. A regression analysis of our experimental data indicated that the monomeric SDS penetrates into the skin between two and three times faster than the SDS in free micelles, while SDS bound to PEO does not penetrate into the skin significantly. However, at the relatively high surfactant concentrations typically encountered in practical applications, 5-10 wt% (where 1 wt% SDS corresponds to 35 mM), the micellar contribution will overwhelm that of the monomers.
A new model of surfactant penetration into the skin was required to explain these results, since the current monomer penetration model was unable to do so. The model had to be able to explain both why SDS from free micelles is able to penetrate into the skin, and why SDS from PEO-bound SDS micelles is unable to penetrate into the skin. We proposed a new model to explain these results in which the free SDS micelles are able to penetrate into the skin, while the PEO-bound SDS micelles are not. SDS micelles are very hydrophilic, such that any penetration into the stratum corneum will require a hydrophilic pathway. Such a pathway exists in the aqueous pores that are present in the stratum corneum (52-55). In our new surfactant skin penetration model, the free SDS micelles can access the aqueous pores while the PEO-bound SDS micelles cannot. We propose that the ability to access the aqueous pores is determined by the size of the SDS micelle, or of the PEO-bound SDS micelle, relative to the skin aqueous pore size.

Several researchers have measured the average aqueous pore radius of the skin, and found it to be between 10 and 28 Å (12, 41-43). We have measured the average radius of the SDS micelles, both in the presence and in the absence of PEO, and found that the free SDS micelles have a radius of about 20 Å, while the PEO-bound SDS micelles have a radius of about 25 Å. Therefore, the free SDS micelles are small enough to access the aqueous pores, while the PEO-bound SDS micelles are sterically hindered from penetrating into the aqueous pores of the skin.

Although our results contradict the widely-accepted view that surfactant in micellar form does not contribute to surfactant penetration into the skin, there is ample evidence in the literature that the damage to the skin is related to the concentration of micellar SDS in the contacting solution (1-3, 7, 10, 13, 15). We have shown that this damage can be explained by the increased
penetration of SDS into the skin, and we have also shown that this penetration can be reduced by changing the solution characteristics of the SDS micelles through the addition of PEO.

Our findings and proposed new model of surfactant penetration into the skin should also be useful from a practical viewpoint, as they provide a new operational strategy for reducing the penetration of a known irritant (SDS) into the skin, which will hopefully reduce the damage caused by SDS through a reduction in its dose in the skin. In this new strategy, one must consider the possible contribution of the surfactant micelles, in addition to the contribution of surfactant monomers, to the penetration of the surfactant into the skin. As stressed earlier, depending on the type of surfactant system contacting the skin, electrostatic effects between charged micelles and the charged aqueous pores may need to be considered, in addition to the micelle steric hindrance considerations put forward in this chapter, to elucidate the ability of charged micelles to penetrate into the epidermis.

In Chapter 4, we will investigate the relationship between the penetration of surfactant into the epidermis and the actual damage induced to the barrier properties of the skin. In Chapter 5, we will examine how mixing surfactants affects surfactant penetration into the skin, since results reported in the past using mixed surfactant systems have been utilized as evidence to support the monomer penetration model (5, 9-11), which we have shown not to be applicable in the case of SDS.

3.5 REFERENCES


Chapter 4

Relationship between the Penetration of SDS into the Epidermis and \textit{in vivo} and \textit{in vitro} Skin Irritation

4.1 INTRODUCTION

Throughout this thesis, we have proposed that surfactant-induced skin irritation is related to the concentration of surfactant that penetrates into the skin. In Chapter 3, we reported on the measurement of the concentration of sodium dodecyl sulfate (SDS) in the epidermis ($C_{\text{skin}}$), and showed how it is affected by the total SDS concentration, as well as by the concentrations of free SDS micelles and SDS monomers, in the solution contacting the skin (referred to hereafter as the \textit{contacting solution}). A review of the \textit{in vivo} skin irritation literature reveals that the SDS dose-dependent skin irritation above the critical micelle concentration (CMC) (1-4) may be related to the increase in $C_{\text{skin}}$ with increasing SDS concentration above the CMC reported by us and by other researchers (5-7). However, a direct measurement relating the skin irritation to $C_{\text{skin}}$, using the same surfactant solution, would eliminate the concern expressed by some researchers that minor differences in the purity of the surfactant solutions used are responsible for the
dose-dependent results observed (8, 9). With this in mind, in this chapter, we establish an experimental correlation between $C_{skin}$ and the \textit{in vitro} skin irritation measurements (see Section 4.3.1), as well as between $C_{skin}$ and \textit{in vivo} skin irritation measurements (see Section 4.3.2).

In addition to comparing $C_{skin}$ with both the \textit{in vitro} and the \textit{in vivo} skin irritation measurements, we wanted to test whether the addition of poly(ethylene oxide) (PEO) to the SDS solutions contacting the skin would lead to a reduction in skin irritation, as expected based on the results of Chapter 3. To the best of our knowledge, PEO has not been used in the past for the specific purpose of reducing the skin irritation potential of SDS. Using \textit{in vivo} skin irritation methods, we will show that a reduction in skin irritation does indeed occur (see Sections 4.3.2 and 4.3.3). In Chapter 3, we demonstrated that adding PEO to an SDS contacting solution prevented the micellar SDS from penetrating into the epidermis, thereby eliminating the SDS dose-dependence of SDS penetration into the skin that was observed in the absence of PEO. In this chapter, we report on the measurement of the effect of the addition of PEO on the SDS dose-dependence of \textit{in vivo} skin irritation in order to determine whether there is a correlation with the SDS dose-independence of SDS penetration into the skin in the presence of PEO reported in Chapter 3 (see Section 4.3.3).

4.2 EXPERIMENTAL

4.2.1 Materials

For a description of the materials used, see Chapter 3, Section 3.2.1.
4.2.2 Preparation of Pig Skin

For a description of the preparation of the pig skin used in the skin conductivity measurements, see Chapter 2, Sections 2.2.2 and 2.2.3.

4.2.3 Measurement of in vitro Skin Conductivity

The following protocol was carried out for the skin conductivity measurements. After mounting the skin on the diffusion cell and allowing it to hydrate in a PBS solution contacting the skin in both the donor and the receiver compartments of the diffusion cell for 1 hour, the PBS solution in the donor compartment was replaced with 1.5 ml of a SDS solution. After 5 hours of exposure, the SDS contacting solution was removed, the donor compartment was rinsed four times with 1.5 ml of PBS solution to remove any unabsorbed traces of SDS from the skin surface, and then 1.5 ml of a PBS solution was added to the donor compartment. After 24 hours of exposure to this PBS contacting solution, the skin conductivity, $\sigma_{\text{skin}}$, was measured at 10 Hz and 0.1 mV (see Chapter 2, Sections 2.2.3 and 2.3.4).

4.2.4 Measurement of SDS Concentration in the Epidermis

The concentration of SDS in the epidermis of the pig skin, $C_{\text{skin}}$, was measured after the skin was exposed to a SDS solution for 5 hours, followed by a 24 hours exposure to a PBS solution to allow the simultaneous measurement of the skin conductivity. $C_{\text{skin}}$ was determined using $^{14}$C-radiolabeled SDS and implementing the method described in Chapter 3, Section 3.2.3.
4.2.5 Measurement of *in vivo* Skin Irritation using a Forearm Controlled Application Technique

The *in vivo* skin irritation was determined using a Forearm Controlled Application Technique (FCAT). A detailed description of the FCAT test is presented in Ref. (10). The *in vivo* skin irritation was evaluated using Transepidermal Water Loss (TEWL), skin conductance (a measurement of skin hydration), corneometry (a measurement of the skin capacitance), visual skin dryness, and visual skin erythema (redness of the skin). The measurement of skin conductance *in vivo*, it should be noted, is different from the measurement of the skin conductivity *in vitro*. The *in vivo* skin conductance measurement is carried out on dry skin, with a low conductance value indicating less hydrated skin, and hence, more irritated skin (11), while the *in vitro* skin conductivity measurement is performed on skin in contact with a PBS solution, as detailed in Section 4.2.3. In all cases reported, the test values correspond to the deviation of the test score from the baseline measurement (a measurement taken prior to the skin treatment) after 5 days of performing the FCAT protocol. The test was carried out by Unilever Home and Personal Care, North America on 45 volunteers.

Transepidermal water loss (TEWL) was measured using a Servomed Evaporimeter EP1 or EP2. The Servomed probe was held over the skin test site for approximately 60 seconds (a 30 second stabilization time followed by a 15-30 second reading time). Stratum corneum hydration was determined as a measure of both skin conductance, using a SKICON-200 instrument with a MT-8C probe, and skin capacitance, using a Corneometer CM820. Both the visual skin dryness and the visual skin erythema scores were determined by a trained evaluator, and were rated on a scale of 0.0 to 6.0, with the
lowest response corresponding to 0.0. The treatment of a skin site was discontinued if the visual skin dryness score exceeded 4.0 and/or if the visual skin erythema score exceeded 3.0. Table 4.1 presents the criteria used to evaluate the visual skin dryness and the visual skin erythema scores.

Table 4.1: Score system used to determine the visual skin dryness and the visual skin erythema as part of the FCAT test (12).

<table>
<thead>
<tr>
<th>Score</th>
<th>Visual Skin Erythema Scale</th>
<th>Visual Skin Dryness Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>1.0</td>
<td>Barely detectable redness</td>
<td>Patches of slight powderiness, and occasional patches of small scales may be seen. Distribution generalized.</td>
</tr>
<tr>
<td>2.0</td>
<td>Slight redness</td>
<td>Generalized slight powderiness. Early cracking or occasional small lifting scales may be present.</td>
</tr>
<tr>
<td>3.0</td>
<td>Moderate redness</td>
<td>Generalized moderate powderiness, and/or heavy cracking and lifting scales.</td>
</tr>
<tr>
<td>4.0</td>
<td>Heavy or substantial redness</td>
<td>Generalized heavy powderiness, and/or heavy cracking and lifting scales.</td>
</tr>
<tr>
<td>5.0</td>
<td>Extreme redness</td>
<td>Generalized high cracking and lifting scales. Powderiness may be present, but not prominent. May see bleeding cracks.</td>
</tr>
<tr>
<td>6.0</td>
<td>Severe redness</td>
<td>Generalized severe cracking. Bleeding cracks may be present. Scales large, may be beginning to disappear.</td>
</tr>
</tbody>
</table>
The FCAT wash procedure is described in Ref. (10), and is repeated below for completeness:

“Subjects meeting the enrollment criteria had four application areas (1.25” diameter) marked off on the volar surface of each forearm with a template and a laboratory marking pen. This clearly identified each application area throughout the study. A technician washed each marked area with the appropriate test product following the procedure outlined below:

1. The subject wet his/her left forearm with warm (90-100°F) tap water.

2. A technician wet a piece of Masslinn ® Towel with warm water, then squeezed the towel gently to remove excess water.

3. The moistened towel was rubbed in a circular motion on a wetted test bar for six seconds to generate lather.\(^5\)

4. The technician rubbed the lathered towel in a circular motion on the application area nearest the elbow for ten seconds.

5. The lather remained on the application area for ninety seconds.

6. The application area was rinsed with warm tap water for fifteen seconds.

This procedure was repeated for each application area on the left forearm and the arm was patted dry. The procedure was then repeated on the right forearm. After both arms had been washed once, each treatment was applied a second time, i.e., application areas

\(^5\) In our case, because a test bar was not used, the surfactant solution was applied directly to the skin test site on the arm in lieu of step 3.
were washed twice at each visit. Wash visits occurred twice daily on the first four days of the study, once on the final day. Visits on any given day were spaced by a minimum of three hours.”

For a comparison of the test conditions, an analysis of variance (ANOVA) technique was used to compare the extent of change from the baseline among the test conditions (10).

4.3 RESULTS AND DISCUSSION

4.3.1 Relationship between the Measured Concentration of SDS in the Epidermis and the in vitro Skin Conductivity

Individual pieces of pig skin were exposed to contacting solutions of SDS and of SDS mixed with PEO in the diffusion cells (see Section 4.2.3). The concentrations of SDS used were 50, 100, and 200 mM, and the PEO concentrations used were 0 wt% and 2 wt%. The skin was exposed to the surfactant solution for 5 hours, after which the surfactant solution was removed from the donor compartment and replaced with a PBS solution, which remained in contact with the skin for 24 hours. After 24 hours, the skin conductivity was measured, the skin was removed from the diffusion cell and heat-stripped, and finally, $C_{skin}$ was measured for each piece of skin (see Section 4.2.4).

Figure 4.1 presents a comparison of the values of $C_{skin}$ measured with (solid bars) and without (empty bars) contacting the skin with a PBS solution for 24 hours. As can be seen, contacting the skin with the PBS solution for 24 hours does decrease $C_{skin}$, reducing the $C_{skin}$ values by approximately 50% for all the cases examined, except for the 50 mM
Figure 4.1: Decrease in $C_{\text{skin}}$ after contacting the skin with a PBS solution for 24 hours, following exposure of the skin to different contacting solutions of SDS, and to a solution of SDS + 2wt% PEO. The empty bars correspond to $C_{\text{skin}}$ values after 5 hours of exposure to the SDS contacting solutions, and the solid bars correspond to $C_{\text{skin}}$ after 5 hours of exposure to the SDS contacting solutions followed by 24 hours of exposure to the PBS solution. The error bars reflect a 95% confidence interval based on 5 or 6 skin samples.
SDS solution. Note, however, that even after a 24-hour exposure to the PBS solution, the same trend of increasing $C_{\text{skin}}$ with increasing SDS concentration is observed (compare the empty bars and the solid bars in Figure 4.1).

The implication of the findings shown in Figure 4.1 is quite important, clearly showing that SDS found in the epidermis is not easily removed from the skin, even after 24 hours of exposure to the PBS solution, suggesting that SDS is tightly bound to the epidermis. If surfactant (SDS in this case) is not easily removed from the epidermis, then it will continue to compromise the functions of the skin. Reported values of the amount of SDS that penetrates into the stratum corneum (SC) indicate that the partitioning into the SC from a contacting solution is quite large (6, 7), with up to 50 wt% SDS found in the SC. Reported values of the amount of SDS that penetrates into the epidermis, that is, into the SC and the viable epidermis, include 4.3 wt% SDS found in human epidermis (13), which is consistent with the values shown in Figure 4.1. This large partitioning of SDS into the skin has been viewed as reflecting the binding of SDS molecules to the keratin proteins found in the corneocytes of the SC (6, 7, 13). Regarding our results, the "tenacity" displayed by SDS to remain in the epidermis, even after exposing the skin to the PBS solution for 24 hours, is consistent with the view that SDS binds strongly to the corneocytes. As a result of this strong binding, the only way for SDS to be removed from the SC is by desquamation, where the SC with the SDS bound to it is removed by the natural growth and removal of the SC, a process which takes about 10-15 days (14).

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6 We believe that the lack of a decrease in $C_{\text{skin}}$ for the 50 mM SDS solution in Figure 4.1 after 24 hours of contact with the buffer is simply the result of a statistical variation in the measurements rather than the result of a statistically significant change in the pattern of the trend.
Figure 4.2 shows how the measured skin conductivity increases with an increase in the measured value of $C_{skin}$ for the various SDS contacting solutions examined. Each data point shown in Figure 4.2 corresponds to a single skin sample, while the data presented in Figure 4.1 corresponds to an average of the data for each SDS contacting solution examined. In Chapter 2, Section 2.3.5, we related the skin conductivity to skin irritation. Clearly, increasing $C_{skin}$ reduces the barrier properties of the skin, as reflected by the observed increase in the skin conductivity, confirming our hypothesis presented in Section 4.1 that SDS must penetrate into the skin in order to induce skin irritation (reflected by the skin conductivity measurement). Generalizing the findings in Figure 4.2, in order to minimize surfactant-induced skin irritation, one must reduce the amount of surfactant that penetrates into the skin. As will be shown here and in Chapter 5, and has already been shown in Chapter 3, reducing the amount of surfactant that penetrates into the skin can be achieved by enlarging the micelles present in the contacting solution (through mixing with polymers (see Chapter 3) or with other surfactants (see Chapter 5)), as well as by reducing the surfactant monomer concentration.

Using the raw skin conductivity data in Figure 4.2, the average skin conductivities after 24 hours of contacting the skin to the PBS solution, $\sigma_{skin}$, for the various SDS solutions examined are shown in Figure 4.3. Figure 4.3 demonstrates the ability of the *in vitro* skin irritation test to capture the dose-dependent effect of SDS on skin irritation. This dose-dependent effect is a well-known characteristic of the skin irritation induced by several surfactants (1, 4, 15-19). Figure 4.3 also shows the average skin conductivity for a contacting solution of 50 mM SDS and 2 wt% PEO, clearly demonstrating that the addition of PEO leads to a large reduction in the *in vitro* skin irritation (which was related
Figure 4.2: A comparison of the measured skin conductivity with the corresponding concentration of SDS measured in the epidermis, $C_{\text{skin}}$. Each data point represents a single skin sample exposed to a solution of SDS (50, 100, or 200 mM SDS), or to a solution of 50 mM SDS + 2.0 wt% PEO, for 5 hours, followed by a 24-hour exposure to a PBS solution. In each case examined, the skin conductivity was measured after the 24 hours exposure to the PBS solution. The dashed line is drawn to guide the eye.
Figure 4.3: The SDS dose-dependence of the average skin conductivity, $\sigma_{\text{skin}}$. The effect of increasing the SDS concentration in the contacting solution on the in vitro skin irritation, as measured using the skin conductivity method. The closed circles (○) correspond to contacting solutions having SDS concentrations of 50, 100, and 200 mM, and the open circle (○) corresponds to a contacting solution of 50 mM SDS and 2wt% PEO. The error bars reflect a 95% confidence interval based on 5-6 samples.
to the skin conductivity in Chapter 2, Section 2.3.5). Based on the results of Figures 4.1, 4.2, and 4.3, it is clear that the observed SDS dose-dependent increase in the permeability of the skin, reflected in the observed increase in the average skin conductivity, is directly correlated to the amount of SDS present in the skin, $C_{\text{skin}}$. Moreover, reducing the value of $C_{\text{skin}}$ through the addition of PEO to the contacting surfactant solution, as shown in Figure 4.1 and in Chapter 3, can lead to a significant reduction in the damage to the barrier properties of the skin, shown in Figure 4.3. However, although the in vitro skin irritation test agrees with the expectations of the $C_{\text{skin}}$ results, both in terms of the SDS dose-dependent skin irritation and in terms of the ability of PEO to reduce this irritation, there is always a concern that an in vitro test does not measure what actually occurs under in vivo conditions. Accordingly, an in vivo skin irritation test is required to convincingly confirm our findings obtained using the in vitro skin conductivity test.

### 4.3.2 Relationship between the Penetration of SDS into the Epidermis and in vivo Skin Irritation: Effect of Adding PEO to a Contacting Solution of Fixed SDS Concentration

In Chapter 3, Section 3.3.2, we demonstrated that increasing the concentration of PEO in a contacting solution having a fixed SDS concentration of 100 mM led to a decrease in $C_{\text{skin}}$, as shown in Figure 4.4. According to Figures 4.2 and 4.3, the reduction in $C_{\text{skin}}$ with increasing PEO concentration observed in Figure 4.4 should also manifest in a reduction in the SDS-induced skin irritation. To test this expectation, in vivo skin irritation experiments were carried out for contacting solutions of 100 mM SDS and increasing PEO concentrations: 0, 0.5, 1, 2, and 2.5 wt%.
Figure 4.4: The measured decrease in the concentration of SDS in the epidermis, $C_{\text{skin}}$ (●) with increasing PEO concentration in a contacting solution having a fixed concentration of 100 mM SDS. Above 1.75 wt% PEO (indicated by the vertical dashed line), no free SDS micelles are present, and therefore, only the SDS monomers contribute to the penetration of SDS into the epidermis. The error bars reflect a 95% confidence interval based on 6 samples at each PEO concentration.
The *in vivo* skin irritation tests measured TEWL, skin conductance, corneometry (skin capacitance), visual skin dryness, and visual skin erythema (see Section 4.2.5). The results of the visual skin dryness test did not reveal any discernable trends, except that the skin exposed to the various SDS contacting solutions was significantly drier than the skin that was only exposed to water. As a result, we will not discuss the visual skin dryness results any further.

Figure 4.5 shows how the average TEWL score decreases with increasing PEO concentration in the contacting solution. TEWL is a measure of how easily water passes through the skin, and irritated skin is typically characterized by a higher TEWL score (1, 2). Figure 4.6 shows how the average skin conductance score increases with increasing PEO concentration in the contacting solution. The measurement of skin conductance *in vivo*, it should be noted, is different from the measurement of skin conductivity *in vitro* (see Section 4.2.3). The *in vivo* skin conductance measurement is carried out on dry skin, *with a higher skin conductance value indicating more hydrated skin, and hence, less irritated skin* (11). The *in vitro* skin conductivity measurement is carried out on skin that has been exposed to buffered water, making the skin super-hydrated, and measures the ability of ions to pass through the hydrated skin, *with a high skin conductivity value reflecting a low skin barrier property, and hence, more irritated skin* (see Chapter 2). Figure 4.7 shows how the corneometer reading increases with increasing PEO concentration in the contacting solution. The corneometer measures the capacitance of the skin, which is an alternative measure of skin hydration. Therefore, a higher corneometer reading indicates that the skin is better hydrated, and hence, less irritated (20). Finally, Figure 4.8 shows how the average value of the visual skin erythema score
Figure 4.5: Average TEWL score for contacting solutions of 100 mM SDS having increasing PEO concentrations, as well as for a tap water control.
Figure 4.6: Average skin conductance score for contacting solutions of 100 mM SDS having increasing PEO concentrations, as well as for a tap water control.
Figure 4.7: Average corneometer reading for contacting solutions of 100 mM SDS having increasing PEO concentrations, as well as for a tap water control.
Figure 4.8: Average visual skin erythema score for contacting solutions of 100 mM SDS having increasing PEO concentration, as well as for a tap water control.
decreases with increasing PEO concentration in the contacting solution. A lower visual skin erythema score reflects a decreased skin irritation.

According to Figures 4.5-4.8, it is clear that increasing the PEO concentration, while maintaining a fixed 100 mM SDS concentration in the contacting solution, reduces the measured in vivo skin irritation (as reflected in its various manifestations). In Figures 4.9 and 4.10, the in vivo skin irritation results (see Figures 4.5-4.8) are compared with the corresponding $C_{skin}$ values shown in Figure 4.4. Figures 4.9 and 4.10 strongly suggest that the reduction in skin irritation results from a decrease in the amount of SDS that penetrates into the skin (as quantified by $C_{skin}$). In fact, not only is there a general decrease in the in vivo skin irritation responses with increasing PEO concentration, but also the TEWL scores (see Figure 4.5) and the visual skin erythema scores (see Figure 4.8) appear to plateau for the 2 and 2.5 wt% PEO solutions examined, mirroring a similar plateau in the values of $C_{skin}$ at these two PEO concentrations (see Figure 4.4).

4.3.3 Relationship between the Penetration of SDS into the Epidermis and in vivo Skin Irritation: The SDS Dose-Dependent Effect in the Presence and in the Absence of PEO in the Contacting Solution

In addition to the effect on $C_{skin}$ of adding PEO to a contacting solution having a fixed concentration of SDS examined in Section 4.3.2, another interesting aspect that was studied in Chapter 3 was the examination of how increasing the total SDS concentration in the contacting solution affected the value of $C_{skin}$, referred to as the SDS dose-dependent effect. In this respect, Figure 3.2 in Chapter 3 shows that increasing the SDS concentration in the contacting solution from 50 mM to 100 mM leads to an increase in
Figure 4.9: A comparison of the concentration of SDS in the epidermis, $C_{skin}$, with the \textit{in vivo} TEWL (●) and the visual skin erythema (□) scores. The $C_{skin}$ values correspond to the average $C_{skin}$ values reported in Figure 4.4, and the TEWL and visual skin erythema scores correspond to the values reported in Figures 4.5 and 4.8, respectively.
Figure 4.10: A comparison of the concentration of SDS in the epidermis, $C_{\text{skin}}$, with the *in vivo* skin conductance (●) and the corneometer reading (□) scores. The $C_{\text{skin}}$ values correspond to the average $C_{\text{skin}}$ values reported in Figure 4.4, and the skin conductance and the corneometer reading scores correspond to the values reported in Figures 4.6 and 4.7, respectively.
$C_{\text{skin}}$ in the absence of PEO. However, in the presence of 2 wt% PEO in the contacting solution, no SDS dose-dependent increase in $C_{\text{skin}}$ is observed. The *in vivo* skin irritation induced by these solutions was also measured, using the same techniques described in Section 4.2.2. Once again, the trends for visual skin dryness did not show any significant deviations between the various contacting solutions examined (50 mM SDS, 100 mM SDS, 50 mM SDS+2wt% PEO, and 100 mM SDS+2wt% PEO), apart from a consistent increase in the visual skin dryness score for the contacting solutions containing SDS relative to the tap water control. Table 4.2 summarizes the average values of the *in vivo* skin irritation measurements for the four contacting solutions examined, as well as the average $C_{\text{skin}}$ values corresponding to each case.

Table 4.2: The average values of $C_{\text{skin}},$ as well as of the *in vivo* skin irritation scores, for contacting solutions of 50 or 100 mM SDS in the absence and in the presence of 2 wt% PEO.

<table>
<thead>
<tr>
<th>Contacting Solution</th>
<th>$C_{\text{skin}}$ (wt% SDS)</th>
<th>TEWL Score</th>
<th>Conductance Score</th>
<th>Corneometer Reading</th>
<th>Visual Erythema Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM SDS</td>
<td>2.6</td>
<td>5.64</td>
<td>16.5</td>
<td>-5.82</td>
<td>0.89</td>
</tr>
<tr>
<td>100 mM SDS</td>
<td>4.5</td>
<td>8.21</td>
<td>-18.92</td>
<td>-9.38</td>
<td>1.29</td>
</tr>
<tr>
<td>50 mM SDS+2 wt% PEO</td>
<td>0.8</td>
<td>3.89</td>
<td>10.83</td>
<td>-5.23</td>
<td>0.78</td>
</tr>
<tr>
<td>100 mM SDS+2 wt% PEO</td>
<td>1.0</td>
<td>4.88</td>
<td>4.33</td>
<td>-7.39</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Table 4.2 vividly shows that both in the absence and in the presence of 2wt% PEO, increasing the concentration of SDS in the contacting solution leads to an increase in the *in vivo* skin irritation (as reflected in the increases in the TEWL scores, the decreases in the conductance scores, the decreases in the corneometer readings, and the increases in the visual skin erythema scores). The observed increase in the *in vivo* skin irritation was
expected in the absence of PEO, since there is a large increase in the measured $C_{\text{skin}}$ values (from 2.6 wt% to 4.5 wt%), as well as because the SDS dose-dependent skin irritation is a well-documented phenomenon (1-4). The observed increase in the \textit{in vivo} skin irritation in the presence of PEO was unexpected, since in Chapter 3 and Table 4.2, we found that under these conditions, $C_{\text{skin}}$ is statistically dose-independent when SDS is mixed with PEO. However, using an ANOVA analysis, only the increase in the TEWL scores in the presence of PEO is significant, while the observed increases in the \textit{in vivo} skin irritation scores from 50 mM to 100 mM SDS without PEO are statistically significant in all cases. Moreover, the magnitudes of the increases in the \textit{in vivo} skin irritation responses are lower in the presence of PEO than in the absence of PEO, suggesting that, even if the addition of PEO does not completely eliminate the SDS dose dependence, which we believe is related to the ability of the micellar SDS to penetrate into the skin (see Chapter 3), it does reduce the ability of the PEO-bound SDS micelles to penetrate into the skin \textit{in vivo}.

Another aspect of the effect of adding PEO to the SDS contacting solution is also shown in Table 4.2. Specifically, Table 4.2 shows that for every case examined, except for the conductance of the 50 mM SDS solution, there is a decrease in the \textit{in vivo} skin irritation response when PEO is added to a fixed SDS concentration. Overall, the results in Table 4.2 show that the addition of PEO to the SDS contacting solution does reduce the skin irritation potential of SDS, and that it also reduces the magnitude of the increase in skin irritation with an increase in the SDS concentration in the contacting solution.
4.4 CONCLUSIONS

In Chapter 2, we developed an *in vitro* skin irritation test based on the measurement of skin conductivity that was used in this chapter to demonstrate a direct correlation between the concentration of SDS in the epidermis ($C_{skin}$) and the skin conductivity $\sigma_{skin}$, which has, in turn, been correlated to skin irritation *in vivo*. This is an important result, because it confirms our hypothesis that reducing the penetration of SDS into the skin will lead to a reduction in the skin irritation potential of SDS. The *in vitro* skin conductivity test demonstrated the dose-dependence of the skin irritation induced by SDS, and it also demonstrated that the addition of PEO to the SDS contacting solution led to a dramatic reduction in the *in vitro* skin irritation potential of SDS. In conducting the *in vitro* skin conductivity test, it was noted that exposing the skin to a PBS contacting solution for 24 hours, following the exposure of the skin to a SDS contacting solution for 5 hours, reduced $C_{skin}$ approximately by 50%, demonstrating that a significant fraction of SDS is not easily removed from the epidermis. This finding suggests that preventing the penetration of SDS and other surfactants into the epidermis is very important, since once these surfactants have penetrated into the epidermis they will only be removed through desquamation.

The $C_{skin}$ values reported in Chapter 3 for contacting solutions of SDS mixed with PEO were shown to be closely correlated with various manifestations of the *in vivo* skin irritation induced by these SDS/PEO contacting solutions. This unambiguously demonstrated that not only is $C_{skin}$ a good quantitative predictor of the *in vivo* skin irritation, but also that the addition of PEO to a contacting solution of SDS can lead to a
significant reduction in the in vivo skin irritation. The in vivo skin irritation tests were found to be SDS dose-dependent both in the absence and, to a lesser extent, in the presence of PEO. The magnitude of the dose-dependence was lower in the presence of PEO than in the absence of PEO, demonstrating that even if the addition of PEO did not completely eliminate the contribution of micellar SDS to skin irritation, it did significantly reduce the skin irritation potential of the micellar SDS. Consequently, in general, we have shown that the addition of PEO to a solution of SDS will lead to a reduction in the skin irritation potential of the surfactant solution.

In the context of the steric model of micelle penetration into the skin, advanced by us in Chapter 3, increasing the micelle size should reduce the ability of the micelles to access the skin pores. Therefore, the in vivo skin irritation results presented in this chapter are consistent with the steric model of micelle penetration. The overall implication of the results presented in this chapter is that SDS-induced skin irritation is directly related to the penetration of SDS into the skin. If these results are generalized to all surfactants, they indicate that preventing the penetration of surfactants into the skin, both in micellar and in monomeric form, should represent a central goal in formulating surfactant systems exhibiting a reduced skin irritation potential.

### 4.5 REFERENCES


12. A. Shiloach, Unilever Home and Personal Care, NA. *Personal Communication*.


Chapter 5

Penetration of Mixed Micelles into the Epidermis: Effect of Mixing Sodium Dodecyl Sulfate with Dodecyl Hexa(Ethylene Oxide)

5.1 INTRODUCTION

The study of why and how surfactants induce skin irritation and skin damage has broad implications, from the design of mild personal care products to assisting the transport of therapeutic drugs across the stratum corneum (SC) (1-12). Previous studies have compared the irritation potential of different surfactants (3, 8, 10, 11, 13-16), and have also determined how different surfactants can lead to changes in the permeability of the skin (1, 2, 9, 17-20). Although various mechanisms have been invoked to explain surfactant-induced skin irritation, in the majority of these mechanisms the surfactant must penetrate into the skin in order to induce irritation (1, 3, 7, 9, 10, 19-23). Accordingly, a simple way to reduce the skin irritation potential of a surfactant solution involves reducing the amount of surfactant that penetrates into the skin.

A widely-accepted view regarding surfactant-induced skin irritation is that, at surfactant concentrations exceeding the critical micelle concentration (CMC), where surfactant
micelles first form, only surfactant monomers can penetrate into the skin, either because surfactant micelles are not surface active, or because they are too large to penetrate into the SC (3, 6, 14, 16, 18, 24, 25). This description of surfactant monomer penetration into the skin will be referred to hereafter as the monomer penetration model. The monomer penetration model is based primarily on experimental observations using mixtures of surfactants, where surfactant-induced skin irritation was correlated with the CMC of the surfactant mixtures examined (6, 24, 26). The surfactant monomer concentration is approximately equal to the CMC (27), and therefore, according to the premise of the monomer penetration model, only the surfactant monomers should contribute to the observed surfactant-induced skin irritation.

In Chapter 3, we challenged the monomer penetration model by unambiguously demonstrating that micelles of the anionic surfactant sodium dodecyl sulfate (SDS) contribute significantly to SDS penetration into the epidermis at SDS concentrations exceeding the CMC. The fact that SDS micelles were found to contribute to SDS penetration into the epidermis clearly contradicts the monomer penetration model, which predicts that the micellar surfactant should have no effect on surfactant penetration into the epidermis. In addition, we demonstrated that the SDS micelle contribution to skin penetration can be eliminated by mixing SDS with poly(ethylene oxide) (PEO), a nonionic polymer known to bind to SDS micelles, to form PEO-bound SDS micelles. To explain both findings, we proposed a new model of surfactant penetration into the skin, in which the free SDS micelles are sufficiently small to access the aqueous pores of the SC, while the PEO-bound SDS micelles are sterically hindered from doing so due to their larger size. In contrast to the monomer penetration model, the new surfactant skin
penetration model highlights the potential importance of the micelles in determining surfactant penetration into the skin. If the micellar surfactant is able to penetrate into the skin, then one predicts the commonly reported dose-dependent skin irritation response to surfactants (2, 3, 8, 13, 16, 18), as well as provide an explanation for the increased penetration of surfactants into the skin beyond the CMC (25, 28, 29). The monomer penetration model fails to predict this observed dose-dependence because at surfactant concentrations exceeding the CMC, where the concentration of surfactant monomers is constant, there should be no effect of increasing the total surfactant concentration on the surfactant-induced skin irritation.

An important question that arose from the results of Chapter 3 is whether mixing surfactants will have an effect on the ability of the micellar surfactant to penetrate into the skin. It is well-known that mixing surfactants can lower the surfactant monomer concentration (24, 30, 31), and in Chapter 4, we demonstrated that there is indeed a correlation between the amount of SDS that penetrates into the skin and the skin irritation induced by SDS. The relationship observed between the reduction in the surfactant monomer concentration due to mixing surfactants and the resulting skin irritation reduction was used as the basis for the monomer penetration model (6, 24, 26). However, having demonstrated that the micellar surfactant can contribute to surfactant penetration into the skin (see Chapter 3), it became important to determine whether mixing surfactants could also reduce the penetration of micellar surfactant into the skin in addition to reducing the surfactant monomer penetration. In this respect, it also became important to determine the relative contributions of the monomeric and the micellar
surfactant fractions to surfactant penetration into the skin, including quantifying which one is reduced the most by mixing surfactants.

With this in mind, we measured the amount of SDS that penetrates into the epidermis from aqueous mixtures of SDS and the nonionic surfactant dodecyl hexa(ethylene oxide) \((C_{12}E_6)\) after 5 hours of exposure (see Sections 5.3.1 and 5.3.2). We found that SDS in SDS/C_{12}E_6 mixed micelles is less able to penetrate into the epidermis than SDS in pure SDS micelles. We also found that the majority of SDS penetrating into the skin from SDS/C_{12}E_6 mixtures results from the monomeric fraction (see Section 5.3.3). Dynamic Light Scattering (DLS) measurements indicated that mixing SDS with C_{12}E_6 leads to an increase in the micelle size (see Section 5.3.4). We therefore propose that it is the increased micelle size that reduces, or prevents altogether, the penetration of the SDS/C_{12}E_6 mixed micelles into the epidermis. Furthermore, we propose that, in general, surfactant mixtures that obey the monomer penetration model contain mixed micelles that are too large to be able to penetrate into the epidermis.

5.2 EXPERIMENTAL

5.2.1 Materials

Sodium dodecyl sulfate (SDS) and sodium chloride (NaCl) were purchased from Sigma Chemicals (St. Louis, MO) and used as received. Dodecyl hexa(ethylene oxide) \((C_{12}E_6)\) was purchased from Nikko Chemicals (Tokyo, Japan) and was used as received. Water was produced using a Millipore Academic water filter. \(^{14}\)C-radiolabeled SDS was purchased from American Radiolabeled Chemicals (St. Louis, MO) and was used as
received. Phosphate buffered saline (PBS) was prepared using PBS tablets from Sigma Chemicals and Millipore filtered water.

5.2.2 Preparation of Skin Samples

Female Yorkshire pigs (40-45kg) were purchased from local farms. Skin from the back of the pig was harvested within one hour of sacrificing the animal. The subcutaneous fat was trimmed off using a razor blade, and the full-thickness pig skin was cut into 2cm x 2cm pieces and stored in a –80 °C freezer until used.

5.2.3 Experimental Protocol

After allowing the skin to thaw for a half-hour at room temperature, the pig skin was mounted in a vertical Franz diffusion cell (Permegear Inc., Riegelsville, PA), with the SC facing the donor compartment. The donor and the receiver compartments of the diffusion cell were filled with phosphate buffered saline (PBS; phosphate concentration of 10 mM M; NaCl concentration of 137 mM; Sigma Chemical Company, St. Louis, MO), and the skin was left to hydrate for 1 hour. The PBS in the donor compartment was removed, and 1.5 ml of surfactant solution was added to the donor compartment. The solution in the donor compartment, referred to hereafter as the contacting solution, contained mixtures of SDS and C_{12}E_{6}, each with about 0.5 µCi/ml of ^{14}C-SDS and 100 mM NaCl. We verified that the concentration of radiolabeled SDS in the contacting solution did not change appreciably during the 5 hour exposure to the skin. A 5 hour exposure was chosen because this time was sufficient to enable significant penetration of SDS into the skin, but short enough to prevent the saturation of the skin with SDS.
After 5 hours of exposure, the contacting solution was removed and the donor compartment was rinsed 4 times with 2 ml of PBS to remove any SDS that was not bound to the skin. The skin was subsequently heat-stripped by soaking it in a bath of water at 60°C for two minutes, and the epidermis (SC and viable epidermis) was separated from the dermis. The exposed epidermis was then dried in a fume hood for two days and weighed. The dried epidermis was dissolved in 1.5 ml of Soluene-350 (Packard, Meriden, CT). 10 ml of Hionic Fluor scintillation cocktail (Packard) was added to the Soluene-350 after the epidermis was dissolved, and the concentration of radiolabeled SDS was determined using a Packard Tri-Carb 4350 scintillation counter (Packard). Knowing the concentration of SDS in the contacting solution, $C_{SDS}$, the radioactivity of the contacting solution, $C_{rad,donor}$, the dry weight of the epidermis, $m$, and the radioactivity of the epidermis, $C_{rad,skin}$, it was possible to determine the concentration of SDS in the dried epidermis, $C_{SDS,skin}$, using the following equation:

$$C_{SDS,skin} = \frac{C_{rad,skin} \cdot C_{SDS}}{C_{rad,donor} \cdot m}$$ (5.1)

### 5.2.4 Dynamic Light Scattering

The SDS and SDS/C$_{12}$E$_6$ solutions were prepared in Millipore filtered water with 100 mM NaCl. The 100 mM NaCl was added to the surfactant solution to screen electrostatic intermicellar interactions in the DLS measurements (32-35). To prevent dust from interfering with the light scattering measurements, the surfactant solutions were filtered through a 0.02 μm Anotop 10 syringe filter (Whatman International, Maidstone, England) directly into a cylindrical-scattering cell, and sealed until use. DLS was
performed at 25°C and a 90° scattering angle on a Brookhaven BI-200SM system (Brookhaven, Holtsville, NY) using a 2017 Stabilite argon-ion laser (Spectra Physics) at 488 nm. The autocorrelation function was analyzed using the CONTIN program provided by the BIC Dynamic Light Scattering software (Brookhaven, Holtsville, NY), which determined the effective hydrodynamic radius, \( \overline{R}_h \), using the Stokes-Einstein relation (36):

\[
\overline{R}_h = \frac{k_B T}{6 \pi \eta D}
\]  \hspace{1cm} (5.2)

where \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature, \( \eta \) is the viscosity of the salt solution, and \( \overline{D} \) is the mean diffusion coefficient of the scattering species. In order to eliminate the effects of intermicellar interactions when measuring the hydrodynamic radii of the micelles, the effective hydrodynamic radii were determined at several different total surfactant concentrations having a fixed solution composition, and the average effective hydrodynamic radii were extrapolated to a zero micelle concentration (32-35, 37).

5.2.5 Micellization Behavior of the SDS/C\(_{12}\)E\(_6\) Surfactant Mixtures

In this chapter, \( \alpha_x \) denotes the fraction of the total surfactant that is SDS, referred to as the SDS composition, and is defined as follows:

\[
\alpha_x = \frac{[SDS]_x}{[SDS]_x + [C_{12}E_6]_x}
\]  \hspace{1cm} (5.3)
where \([\text{SDS}]\) denotes the concentration of SDS, \([C_{12}E_6]\) denotes the concentration of \(C_{12}E_6\), and the subscript \(x\) refers to the monomeric fraction \((x=1)\), to the micellar fraction \((x=m)\), or to the overall solution \((x=s)\). Accordingly, \(\alpha_s=0.83\) implies that 83\% of the surfactant in the contacting solution is SDS, and the remaining 17\% \((1-\alpha_s=0.17)\) is \(C_{12}E_6\).

Recently developed molecular-thermodynamic theories of micellization (30, 31) were used to predict the micellization behavior of the SDS/\(C_{12}E_6\) surfactant mixtures. Specifically, the concentration and the composition of the surfactant monomers and of the mixed micelles were predicted as a function of the total surfactant concentration and solution composition. The resulting predicted values of \(\alpha_1\), \(\alpha_m\), and the total surfactant monomer concentration, \(C_1\), for the contacting solutions examined are reported in Tables 5.1 and 5.2.

Table 5.1: Predicted values of \(\alpha_1\) and \(\alpha_m\) for mixtures of SDS and \(C_{12}E_6\) in 0.1 M NaCl at the various SDS concentrations and solution compositions \((\alpha_s)\) used for the SDS skin penetration experiments (30, 31).

<table>
<thead>
<tr>
<th>(\alpha_s)</th>
<th>25 mM SDS (\alpha_1), (\alpha_m)</th>
<th>50 mM SDS (\alpha_1), (\alpha_m)</th>
<th>100 mM SDS (\alpha_1), (\alpha_m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1, 1</td>
<td>1, 1</td>
<td>1, 1</td>
</tr>
<tr>
<td>0.83</td>
<td>0.96, 0.83</td>
<td>0.96, 0.83</td>
<td>0.96, 0.83</td>
</tr>
<tr>
<td>0.50</td>
<td>0.925, 0.50</td>
<td>0.925, 0.50</td>
<td>0.925, 0.50</td>
</tr>
</tbody>
</table>
Table 5.2: Predicted total surfactant monomer concentration, \( C_I \) (mM), for the mixtures of SDS and \( C_{12}E_6 \) in 0.1 M NaCl at the various SDS concentrations and solution compositions (\( \alpha_s \)) used for the SDS skin penetration experiments (30, 31).

<table>
<thead>
<tr>
<th>( \alpha_s )</th>
<th>25 mM SDS</th>
<th>50 mM SDS</th>
<th>100 mM SDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.850</td>
<td>0.864</td>
<td>0.877</td>
</tr>
<tr>
<td>0.83</td>
<td>0.683</td>
<td>0.695</td>
<td>0.707</td>
</tr>
<tr>
<td>0.50</td>
<td>0.266</td>
<td>0.270</td>
<td>0.276</td>
</tr>
</tbody>
</table>

5.3 RESULTS AND DISCUSSION

5.3.1 Effect of Adding \( C_{12}E_6 \) at a Fixed SDS Concentration on the Penetration of SDS into the Epidermis

It is well known that when two surfactants which interact synergistically are mixed, the surfactant mixture often exhibits lower skin irritation than either of the individual surfactants (6, 24, 26). It is also known that SDS and \( C_{12}E_6 \) interact synergistically to reduce the CMC of the surfactant mixture (30, 31). SDS is a model skin irritant (10, 13, 15, 16, 26, 38), while \( C_{12}E_6 \) is thought to be a mild surfactant, although it may lead to skin dryness (3, 39). The system of SDS and \( C_{12}E_6 \) was chosen as a model surfactant mixture because of the synergy that it exhibits, as well as because the skin irritation potential of this surfactant mixture is expected to result primarily from the action of the irritating surfactant SDS. This, in turn, allows us to relate the penetration of SDS into the epidermis to skin irritation, while neglecting the irritation potential of \( C_{12}E_6 \).
Evidence for the relationship between the concentration of SDS in the epidermis and the skin irritation induced by SDS was presented in Chapter 3, in which the concentration of SDS in the epidermis was observed to be dose-dependent for $\alpha = 1$, corresponding to the dose-dependent SDS skin irritation potential observed by other researchers (2, 3, 8, 13, 16, 18). However, it should be noted that, in this chapter, we have not measured the amount of $C_{12}E_6$ that penetrates into the epidermis. Therefore, we did not ascertain whether the interaction between $C_{12}E_6$ and the SC is indeed mild. In this respect, experiments by de la Maza et al. have shown that nonionic surfactants can have a strong effect on reducing the barrier properties of SC lipid bilayers (19, 40). However, other researchers have observed that nonionic surfactants tend to have a smaller effect on the skin than SDS (3, 39, 41). Therefore, although the assumption that $C_{12}E_6$ is benign irritation-wise may not be entirely accurate, it is expected that the skin irritation potential of SDS should overwhelm that of $C_{12}E_6$. An investigation of the skin irritation potential of $C_{12}E_6$ is underway, and the results of this investigation will be reported elsewhere.

Based on the premise that the skin irritation induced by SDS is related to the concentration of SDS in the epidermis, we measured whether adding $C_{12}E_6$ to a fixed SDS concentration (50 mM) in the contacting solution would reduce the concentration of SDS in the epidermis after 5 hours of exposure, $C_{skin}$, and consequently, reduce the skin irritation potential of the surfactant solution. The purpose of conducting the experiments at a fixed SDS concentration is to ensure that any observed decrease in $C_{skin}$ upon the addition of $C_{12}E_6$ would not result from the decrease in the total SDS concentration in the contacting solution, but instead would be related to changes in the solution behavior of SDS. Figure 5.1 shows that as $\alpha$ is decreased by adding more $C_{12}E_6$ to the contacting
Figure 5.1: The effect of decreasing the composition of SDS, $\alpha_s$, in the contacting solution on the concentration of SDS in the epidermis after a 5-hour exposure ($C_{skin}$) for solutions containing 50 mM SDS and increasing concentrations of $C_{12E_6}$. The error bars reflect a 95% confidence interval based on 6 samples at each composition.
solution, $C_{skin}$ decreases. The observed decrease in $C_{skin}$ as $\alpha_s$ decreases is consistent with reported observations of the reduced skin irritation potential of surfactant mixtures, provided that $C_{skin}$ is related to the observed skin irritation (6, 24, 26).

\subsection*{5.3.2 Effect of Increasing $\alpha_s$ on the Ability of Micellar SDS to Penetrate into the Epidermis}

There are two plausible mechanisms responsible for the decrease in $C_{skin}$ observed in Figure 5.1: (i) the addition of C$_{12}$E$_6$ reduces the SDS monomer concentration, as predicted by the monomer penetration model, and (ii) the addition of C$_{12}$E$_6$ reduces the ability of the micellar SDS to penetrate into the epidermis, as predicted by our proposed micelle penetration model from Chapter 3. It is entirely possible for both mechanisms to act simultaneously. In view of that, we conducted the following experiments to clarify whether mechanism (ii) was involved in the reduction of $C_{skin}$ observed in Figure 5.1.

We tested whether mixed micelles present in the SDS/C$_{12}$E$_6$ surfactant mixtures could penetrate into the epidermis by maintaining a constant $\alpha_s$ value and increasing the total surfactant concentration in the contacting solution. In general, the ability of micelles to penetrate into the skin can be determined by measuring how increasing the total surfactant concentration beyond the CMC, at a fixed $\alpha_s$ value, affects the amount of surfactant penetrating into the epidermis (see Chapter 3). If the surfactant concentration in the epidermis is found to increase, then surfactant in micellar form contributes to surfactant penetration into the epidermis. Conversely, if the surfactant concentration in the epidermis is found to remain constant, then surfactant in micellar form does not
contribute to surfactant penetration into the epidermis, in which case the surfactant penetration should obey the monomer penetration model.

The SDS/C₁₂E₆ surfactant mixtures that were investigated had solution compositions of \( \alpha_s = 1, 0.83, \) and 0.50. Figure 5.2 shows the effect of increasing the total SDS concentration in the contacting solution (from 25 mM to 100 mM) on \( C_{skin} \) at these three fixed \( \alpha_s \) values. As shown in Tables 5.1 and 5.2 (30, 31), for each value of \( \alpha_s \) over the range of surfactant concentrations examined, \( \alpha_m = \alpha_s, \) \( \alpha_1 \) is constant, and \( C_I \) is approximately constant. Therefore, any observed increase in \( C_{skin} \) as the total SDS concentration increases for each \( \alpha_s \) value examined can only be attributed to the penetration of micellar SDS into the epidermis, because only the micelle concentration is increasing. (Recall that the SDS monomer concentration is equal to \( \alpha_1 C_I \), which remains constant, while the concentration of SDS in micellar form is equal to \( \alpha_m (C_I - C_I) \), where \( C_I \) is the total surfactant concentration, which increases in this case.)

The increase in \( C_{skin} \) with increasing total SDS concentration observed in Figure 5.2 for \( \alpha_s = 1, 0.83, \) and 0.50 clearly indicates that the micelles present in these solutions do contribute to SDS penetration into the epidermis, with their contribution decreasing as \( \alpha_s \) decreases. Specifically, by comparing the observed increase in \( C_{skin} \) as the SDS concentration in the contacting solution is increased from 25 mM to 100 mM (\( \Delta C_{skin} \)) for each \( \alpha_s \) value examined, it is clear that the pure SDS micelles (\( \alpha_s = 1 \)) contribute more to \( C_{skin} \) (\( \Delta C_{skin} \approx 2.3 \)) than the mixed micelles corresponding to \( \alpha_s = 0.83 \) (\( \Delta C_{skin} \approx 0.9 \)) and to \( \alpha_s = 0.50 \) (\( \Delta C_{skin} \approx 0.6 \)). This is clear evidence that changing \( \alpha_s \), and hence \( \alpha_m \), can affect the ability of the micellar SDS to penetrate into the epidermis, because for each \( \alpha_s \) value
Figure 5.2: The effect of lowering $\alpha_s$ (increasing the $C_{12E6}$ concentration) on the concentration of SDS in the epidermis after a 5-hour exposure ($C_{skin}$) for increasing SDS concentrations in the contacting solution. For each composition ($\alpha_s=1, 0.83, \text{and } 0.50$), the concentrations of SDS in the contacting solution are 25 mM (empty bars), 50 mM (solid bars), and 100 mM (striped bars). The error bars reflect a 95% confidence interval based on 6 samples at each SDS concentration.
examined, the SDS concentration in the contacting solution increases by the same amount (from 25 to 100 mM), but the effect on $\Delta C_{\text{skin}}$ is found to decrease as $\alpha_s$ is decreased. Although this simple analysis, based on the experimental results presented in Figure 5.2, clearly demonstrates that adding $C_{12}E_6$ to the SDS solution reduces the ability of the micellar SDS to penetrate into the epidermis, as proposed in mechanism (ii), a more quantitative analysis, presented in Section 3.3, is required to determine the contribution of mechanisms (i) and (ii) to SDS skin penetration. It should be kept in mind that the ability to reduce the penetration of the micellar SDS into the skin by mechanisms (i), (ii), or both should have a pronounced effect on reducing the skin irritation induced by SDS.

In Chapter 3, we demonstrated that the contribution of the micellar SDS to $C_{\text{skin}}$ is comparable to the contribution of the monomeric SDS at low SDS concentrations. However, because the concentration of SDS micelles increases as the total SDS concentration increases beyond the CMC, while the concentration of SDS monomers remains constant, we concluded that it is the penetration of the micellar SDS that leads to the dose-dependent skin irritation response observed by many researchers (2, 3, 8, 13, 16, 18). Indeed, we found that the SDS micellar contribution overwhelms the SDS monomeric contribution at the higher SDS concentrations. Accordingly, reducing, or preventing, the contribution of micellar surfactant to $C_{\text{skin}}$ by mixing surfactants should lead to a reduction in the skin irritation potential of the surfactant mixture, in addition to any beneficial effect due to a reduction in the surfactant monomer concentration.
5.3.3 Regression Analysis of the Contributions of Micellar and Monomeric SDS to $C_{skin}$ from Solutions of SDS/C$_{12}$E$_6$

Figure 5.2 shows that as $\alpha_s$ decreases, the contribution of the SDS/C$_{12}$E$_6$ mixed micelles to $C_{skin}$ decreases. To quantify the relative contributions of SDS in mixed micelle form ($\alpha_m=1$, 0.83, and 0.50) and in monomeric form to $C_{skin}$, we carried out a multiple linear regression analysis using all the experimental data, prior to averaging, that was utilized to generate Figure 5.2. The simplest relationship between the SDS concentrations in micellar and monomeric form to $C_{skin}$ is a linear one. The basis for this linear relationship is that in Fickian diffusion from an infinite reservoir with a large concentration difference, the net permeant flux at a given time is directly proportional to the initial permeant concentration (42). With this assumption in mind, we fitted $C_{skin}$ to the following expression:

$$C_{skin} = a \cdot C_{1,SDS} + b \cdot C(\alpha_m = 1) + c \cdot C(\alpha_m = 0.83) + d \cdot C(\alpha_m = 0.50)$$

(5.4)

where $a$, $b$, $c$, and $d$ are the regression coefficients that were determined from the regression analysis, $C_{1,SDS}$ is the SDS monomer concentration, $C(\alpha_m)$ is the SDS concentration in micelles of composition $\alpha_m$, and $C_{skin}$ is the SDS concentration in the epidermis (in units of mmols of SDS per gram of dry epidermis). For the regression analysis, $C_{1,SDS}=\alpha IC_I$ and $C(\alpha_m)=\alpha_m(C_I - C_I)$ using the appropriate values of $\alpha_1$, $\alpha_m$, and $C_I$ reported in Tables 5.1 and 5.2 (30, 31). In this manner, we were able to isolate the contributions to $C_{skin}$ due to the micellar SDS for the three micelle compositions examined (reflected in $b$, $c$, and $d$), as well as due to the monomeric SDS (reflected in $a$). The following values of $a$, $b$, $c$, and $d$ were obtained from the regression analysis:
\[ a = 4.1 \pm 1.0 \ C_{\text{skin}}/C_{1, SDS} \]

\[ b = 0.032 \pm 0.014 \ C_{\text{skin}}/C(\alpha_m=1) \]

\[ c = 0.003 \pm 0.012 \ C_{\text{skin}}/C(\alpha_m=0.83) \]

\[ d = 0.0009 \pm 0.0092 \ C_{\text{skin}}/C(\alpha_m=0.50) \]

According to these regression results, the \( \alpha_m=0.50 \) micelles do not contribute to \( C_{\text{skin}} \) at all, because \( d \) is essentially equal to zero. The \( \alpha_m=0.83 \) micelles contribute very little or not at all to \( C_{\text{skin}} \), because although the average value of \( c \) is not zero, the 95\% confidence interval includes zero. On a per SDS molecule basis, the contribution of the SDS monomers is quite large, with one SDS molecule in monomeric form being 130 times more skin penetrating than one SDS molecule in a pure SDS micelle (\( \alpha_m=1 \)). However, at the higher SDS concentrations, there is significantly more micellar SDS than monomeric SDS, and as a result, the net contribution to \( C_{\text{skin}} \) due to the micellar SDS may overwhelm that due to the SDS monomers.

To more clearly describe the relative contributions of the monomeric SDS and the micellar SDS to skin penetration, Figures 5.3, 5.4, and 5.5 show the total contributions of the monomeric and the micellar fractions of SDS to \( C_{\text{skin}} \) for \( \alpha_s=1 \), 0.83, and 0.50 respectively, based on the regression data given above. Specifically, the SDS monomeric contribution to \( C_{\text{skin}} \) is \( a \cdot C_{1, SDS} \), and the three micellar contributions to \( C_{\text{skin}} \) are \( b \cdot C(\alpha_m=1) \), \( c \cdot C(\alpha_m=0.83) \), and \( d \cdot C(\alpha_m=0.50) \). Figures 5.3-5.5 clearly show that the SDS monomers make a contribution to \( C_{\text{skin}} \) that is larger than that of the micellar SDS for the three \( \alpha_s \) values examined, as seen by the empty bars (representing the monomeric...
Figure 5.3: The contribution of monomeric SDS (open bars) and micellar SDS (solid bars) to $C_{skin}$ based on the multiple linear regression analysis for $\alpha_s=1$. Adding up the contributions from the two bars yields the combined contribution of the SDS monomers and the micellar SDS to $C_{skin}$. 
Figure 5.4: The contribution of monomeric SDS (open bars) and micellar SDS (solid bars) to $C_{skin}$ based on the multiple linear regression analysis for $\alpha_c=0.83$. Adding up the contributions from the two bars yields the combined contribution of the SDS monomers and the micellar SDS to $C_{skin}$. 
Figure 5.5: The contribution of monomeric SDS (open bars) and micellar SDS (solid bars) to $C_{\text{skin}}$ based on the multiple linear regression analysis for $\alpha_s=0.50$. Adding up the contributions from the two bars yields the combined contribution of the SDS monomers and the micellar SDS to $C_{\text{skin}}$. 
contribution) always being larger than the solid bars (representing the micellar contribution). It is only for \( \alpha_s = 1 \), the pure SDS case, that the micelles make a large contribution to \( C_{\text{skin}} \), particularly at the highest surfactant concentrations examined (see Figure 5.3). In Figures 5.4 and 5.5, which correspond to the \( \alpha_s = 0.83 \) and 0.50 surfactant mixtures, the micellar contribution is almost non-existent. Indeed, considering the confidence interval for the coefficients \( c \) and \( d \), it is apparent that the micellar contributions include the possibility of a zero contribution to \( C_{\text{skin}} \). Therefore, the monomer penetration model represents a reasonable approximation for the two SDS/C_{12}E_6 surfactant mixtures examined, where the micellar SDS does not penetrate appreciably into the epidermis, for the SDS concentrations examined (25, 50, and 100 mM). However, the reduction in \( C_{\text{skin}} \) observed with decreasing \( \alpha_s \), shown in Figures 5.1 and 5.2, results from both the reduction in the SDS monomer concentration, as well as from the reduction in the ability of the micellar SDS to penetrate into the epidermis.

Generalizing the observations made in the case of the SDS/C_{12}E_6 surfactant mixtures to other surfactant mixtures, it is plausible that the observed reduction in skin irritation upon mixing surfactants reported by several researchers occurs because both the monomeric and the micellar surfactant penetrations into the skin are diminished (6, 24, 26). At the high total surfactant concentrations commonly utilized in commercial surfactant products, the micellar contribution can be quite large, as demonstrated by the dose-dependent surfactant-induced skin irritation results reported in the literature (2, 3, 8, 13, 16, 18). Consequently, any reduction in the ability of the micellar surfactant to penetrate into the skin, as reflected by lower values of the regression coefficients (such as \( b \), \( c \), and \( d \)), should have a significant impact on \( C_{\text{skin}} \) at high total surfactant concentrations. In other
words, reducing the micellar contribution to $C_{skin}$ should lead to a reduction in the skin irritation potential of the surfactant system contacting the skin.

### 5.3.4 Dynamic Light Scattering Determination of SDS/C$_{12}$E$_6$ Mixed Micelle Sizes

In Figure 5.6, the hydrodynamic radii of the SDS/C$_{12}$E$_6$ mixed micelles are determined using DLS by extrapolating the effective hydrodynamic radii of these micelles to a zero micelle concentration. At the surfactant concentrations corresponding to Figure 5.6, $\alpha_m$ is predicted to be approximately equal to $\alpha_s$, and therefore, one can treat the micelles as having a constant $\alpha_m$ value over the entire surfactant concentration range examined (see Table 5.1). This is important, because a change in $\alpha_m$ could lead to a change in the hydrodynamic radius of the micelles. The hydrodynamic radii of the micelles determined using this method are reported in Table 5.3. According to the surfactant penetration model advanced in Chapter 3, the size of the micelle determines its ability to penetrate into the SC. (Note that the discussion in Section 5.3.5 below introduces the caveat that electrostatic interactions may also play a role.) The micelle penetration model is based on the premise that only micelles that are small enough to access the aqueous pores in the SC can contribute to surfactant penetration into the epidermis. Other researchers have determined the average aqueous pore radius in the skin using permeability and/or conductivity measurements in the context of hindered-transport theories, and have reported radii values between 10 Å and 28 Å (9, 12, 43-45).
Figure 5.6: Measured effective hydrodynamic radii of SDS/C_{12}E_{6} mixed micelles. The symbols refer to SDS/C_{12}E_{6} compositions of $\alpha_m=1$ (●), $\alpha_m=0.83$ (□), and $\alpha_m=0.50$ (△) as a function of the concentration of micellar SDS (that is, the SDS concentration minus the predicted SDS monomer concentration $\alpha_1 C_1$, see Tables 5.1 and 5.2) (30, 31) using DLS at 25°C. The micellar radii were determined using a CONTIN analysis. The error bars reflect a 95% confidence interval based on 8 samples at each SDS concentration. The actual hydrodynamic radius is equal to the intercept.
Table 5.3: The micelle hydrodynamic radius determined using a CONTIN analysis of the correlation function. The actual hydrodynamic radius of the micelle is determined by extrapolating the effective hydrodynamic radii in Figures 5.3-5.5 to a zero micelle concentration. The error values reflect a 95% confidence interval.

<table>
<thead>
<tr>
<th>$\alpha_\text{m}$</th>
<th>$R_H$ (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20±1</td>
</tr>
<tr>
<td>0.83</td>
<td>24±1</td>
</tr>
<tr>
<td>0.50</td>
<td>27±3</td>
</tr>
</tbody>
</table>

Based on the hydrodynamic radii reported in Table 5.3 and a purely steric model of micelle penetration into the skin that ignores electrostatic interactions (discussed in Section 3.5), and considering a skin aqueous pore radius of at most 28Å, the $\alpha_\text{m}=1$ micelles should be able to penetrate into the SC more easily than the $\alpha_\text{m}=0.83$ and the 0.50 micelles. This conclusion is consistent with the results of the multiple linear regression analysis presented above, and lends greater validity to the idea put forward in Chapter 3 that steric factors can play a key role in determining whether micellar surfactant can penetrate into the skin.

5.3.5 Possible Electrostatic Effects on SDS Skin Penetration

Interestingly, the $\alpha_\text{m}=1$ micelles have an equal, or slightly lower value, of the regression coefficient, $b$ (0.032±0.014), than the one reported in Chapter 3 (0.043±0.006), while the SDS monomers penetrate into the epidermis much more readily according to the results reported in this chapter ($a = 4.1±1.0$ here versus $a = 0.14±0.04$ in Chapter 3). The main
difference in the conditions corresponding to the two sets of experiments is the presence of 0.1 M NaCl in the systems examined in this chapter, compared to no added salt in Chapter 3. It is known that the skin carries a net negative charge (9), and that the addition of salt screens this negative charge. Screening the negative charge would make it easier for negatively-charged SDS monomers to approach the skin surface, which could explain the observed increase in the value of $a$. However, the same argument should apply to the $\alpha_m=1$ micelles, which are also negatively charged. Nevertheless, the SDS micelles do not show a significant change in their contribution to SDS penetration upon the addition of salt. In fact, the pure SDS micelles appear to be somewhat less able to contribute to $C_{skin}$ in the presence of salt ($b=0.032$) than in the absence of salt ($b=0.043$ in Chapter 3). It is important to keep in mind, however, that the addition of salt may lead to some micelle growth (32, 46). As a result, applying our model of micelle penetration, the larger micelles in the presence of salt may be less able to penetrate into the skin, thus counteracting the effect of any decrease in the electrostatic repulsions between the skin and the SDS micelles.

The discussion above about potential electrostatic effects affecting surfactant penetration into the skin indicates that steric hindrance may not be the only factor determining whether a micelle can penetrate into the aqueous pores of the skin. Iontophoresis experiments with charged permeants have shown that the aqueous pores in the SC are charged, and that positively-charged permeants traverse the skin more easily than negatively-charged permeants (9, 44). However, it is also known that the size of the permeant relative to that of the aqueous pore affects the penetration of the permeant into the skin (9, 43). If the permeant is larger than the aqueous pore size, then electrostatic
effects should be irrelevant, since the steric hindrance would prevent any access into the pore. However, when the permeant is physically small enough to access the aqueous pores, then the electrostatic interactions between the permeant and the pores, as well as the steric interactions between the permeant and the pore wall, will both play a role in the transport of the permeant across the skin (9, 12, 43, 45, 47).

In our experiments, all the micelles are negatively-charged due to the presence of SDS, but the surface charge density of the SDS/C_{12}E_{6} mixed micelles decreases as α_m decreases. This reduction in surface charge density should make it easier for the less negatively-charged mixed micelles to access the negatively-charged skin pores. However, the addition of C_{12}E_{6} also causes the micelles to grow and sterically hinders their access to the skin pores, thereby counteracting this surface charge reduction effect.

5.4 CONCLUSIONS

It is well known that mixing surfactants can lead to a reduction in the skin irritation potential of a surfactant system (6, 24, 26). Based on the premise that the irritating surfactant must penetrate into the skin to induce skin irritation, we tested whether mixing the irritating surfactant SDS with C_{12}E_{6} affected the amount of SDS penetrating into the epidermis (C_{skin}). We found that increasing the concentration of C_{12}E_{6} in the contacting solution, while maintaining a constant concentration of SDS, led to a decrease in C_{skin}. Provided that the skin irritation induced by SDS is related to C_{skin}, these findings are consistent with the expectation of reducing skin irritation by mixing surfactants.

In Chapter 3, we found that both monomeric and micellar SDS are able to penetrate into the epidermis. An important consideration in the case of SDS/C_{12}E_{6} surfactant mixtures
was whether the reduction in the amount of SDS penetrating into the epidermis was due to the reduced SDS monomer concentration and/or due to a reduction in the skin penetration ability of the micellar SDS. A regression analysis, based on our experimental results, demonstrated that only pure SDS micelles ($\alpha_m=1$) contributed to $C_{\text{skin}}$ at a level comparable to the contribution of the SDS monomers, particularly at the highest surfactant concentrations examined (see Figure 5.3). For the SDS/C$_{12}E_6$ surfactant mixtures, corresponding to mixed micelles having compositions of $\alpha_m=0.83$ and 0.50, the monomeric SDS contributed significantly more to skin penetration than the micellar SDS, which essentially did not contribute to $C_{\text{skin}}$ (see Figures 5.4 and 5.5). Consequently, mixing SDS with C$_{12}E_6$ reduced $C_{\text{skin}}$ both by reducing the concentration of monomeric SDS and by almost entirely preventing micellar SDS from penetrating into the epidermis.

Using DLS measurements, we demonstrated that the average hydrodynamic radius of the SDS/C$_{12}E_6$ mixed micelles increased as the solution composition of SDS decreased. This corresponded to the observed decreased ability of the SDS/C$_{12}E_6$ mixed micelles to penetrate into the SC. Comparing the hydrodynamic radii of the SDS/C$_{12}E_6$ mixed micelles examined (24Å for $\alpha_m=0.83$ and 27Å for $\alpha_m=0.50$) with the radii of the PEO-bound SDS micelles in Chapter 3 (25Å), the steric hindrance model for the prevention of micelle penetration into the skin remains consistent with our experimental findings in this chapter, with SDS in the larger mixed micelles not contributing to $C_{\text{skin}}$.

From our results, one can understand how the monomer penetration model was derived from mixed-surfactant skin irritation data. Mixing surfactants often leads to growth in the micelle size (30, 31). When the mixed micelles cannot penetrate into the skin, then
the surfactant penetration mechanism reduces to the monomer penetration model. In that case, since the CMC is comparable to the surfactant monomer concentration, there is a direct correlation between the CMC and the observed skin irritation. However, preventing the micellar SDS, or for that matter any micellar surfactant, from penetrating into the skin has a pronounced effect on skin irritation, because it should eliminate the dose-dependent behavior commonly observed for pure surfactant systems (2, 3, 8, 13, 16, 18). Once the micelles are prevented from penetrating into the skin, then the only other mechanism to reduce $C_{skin}$ involves a reduction in the surfactant monomer concentration.

5.5 REFERENCES


Chapter 6

The Role of the Surfactant Polar Head Structure in Protein-Surfactant Complexation: Zein Protein Solubilization by SDS and SDS/C_{12}E_{n} Surfactant Solutions

6.1 INTRODUCTION

Several mechanisms have been proposed to explain surfactant-induced skin irritation, including skin lipid solubilization (1-4), disruption of the stratum corneum (SC) lipid bilayers (5-10), and protein denaturation by surfactants (11-14). In this chapter, we examine the protein-surfactant mechanisms associated with surfactant-induced skin irritation. In this respect, it has been observed that there is often a direct correlation between the ability of a surfactant to denature a protein and the skin irritation potential of that surfactant (11, 12, 14-17). The relationship between the skin irritation potential of a surfactant system and its protein denaturation potential is believed to result from surfactant binding to proteins present in the skin, such as keratin in the corneocytes of the SC (13, 18, 19), followed by the denaturation of these proteins, leading to skin irritation and other abnormal behavior of the skin, such as parakeratosis (the retention of nuclei in
the formation of corneocytes) (20, 21). According to this view, surfactants that do not denature proteins are expected to be non-irritating to the skin.

In practice, the protein denaturation potential of surfactant solutions has been related to different manifestations of skin irritation in vivo, including erythema (redness of the skin), changes in the barrier properties of the skin, and ocular irritation (often studied in conjunction with skin irritation) (14, 15, 17). Based on this relationship, in vitro skin irritation tests have been developed that measure the protein denaturation potential of a surfactant system. In particular, the protein denaturation potential of surfactants has been determined using circular dichroism (12, 14), the extent of binding to solid proteins (11, 17, 19), and the swelling of the SC or collagen (13, 15, 16). In addition, a very popular test involves measuring the amount of zein protein solubilized by a surfactant solution, referred to as the zein solubilization test (15, 16, 19, 22-24).

Zein, or zea mais, is a corn protein which is insoluble in water (24). However, zein can be dissolved in water by adding different types of surfactants, and the ability of these surfactants to dissolve zein has been correlated to their skin irritation potential (15, 16, 22-24), as well as to the amount of surfactant penetrating into the stratum corneum (19). The zein solubilization method is particularly well-suited to ranking the skin irritation potential of anionic surfactants, since these surfactants are the strongest zein solubilizers, while amphoteric (zwitterionic) and nonionic surfactants dissolve little or no zein (15, 23, 24). This is also consistent with the low protein denaturation capacity of amphoteric and nonionic surfactants reported in the literature for various protein systems, such as Bovine Serum Albumin (BSA), Ovalbumin, and Lysolecithin phospholipidase enzyme (11, 12, 17, 25, 26). In fact, amphoteric surfactants have been mixed with the anionic surfactant
sodium dodecyl sulfate (SDS) to prevent SDS from denaturing proteins (17, 25, 26). Although the focus of this thesis is on surfactant-induced skin irritation, understanding how surfactants interact with proteins and induce protein denaturation has a broader scope, including applications in the preparation and formulation of therapeutic protein solutions using surfactants (26, 27), as well as in the use of surfactants to enhance transdermal drug delivery (9, 27-32).

In this chapter, we investigate the ability of SDS to solubilize zein. We suggest some improvements to the conventional zein solubilization protocol that allows for a better comparison between different surfactant systems. These improvements are based on a theoretical framework of zein solubilization that quantifies how SDS is distributed between solubilized zein and unsolubilized zein (see Section 6.3.2). We also demonstrate that mixing SDS with dodecyl poly(ethylene oxide), \( C_{12}E_n \) (\( n=4,6, \) and 8), nonionic surfactants can reduce the zein solubilization capacity of SDS (see Section 6.4). This reduction is then analyzed in the context of a previously developed molecular-thermodynamic theory of polymer-surfactant complexation (33) to demonstrate that the polar head size of the nonionic surfactant, determined by the degree of ethoxylation, \( n \), mixed with SDS plays an important role in reducing the zein solubilization potential of SDS (see Section 6.5).

## 6.2 MATERIALS AND METHODS

### 6.2.1 Materials

Zein protein and sodium dodecyl sulfate (SDS) were purchased from Aldrich Chemicals (St. Louis, MO), and were used as received. Dodecyl tetra(ethylene oxide) \( (C_{12}E_4) \),
dodecyl hexa(ethylene oxide) (C₁₂E₆), and dodecyl octa(ethylene oxide) (C₁₂E₈) were purchased from Nikko Chemicals (Tokyo, Japan), and were used as received. Water was produced using a Millipore Academic water filter. A BCA Protein Assay Kit (Bicinchoninic Colorimetric Assay) was purchased from Pierce (Rockford, IL).

6.2.2 Methods

A weighed amount of zein protein was mixed with 7 mL of surfactant solution. The solution was mixed for 1 hour, which was found to be sufficient to reach the equilibrium solubilization of zein (subsequent mixing for up to 12 hours led to no further zein solubilization). 1.5 ml of the solution was centrifuged for 20 minutes in a Fisher Scientific Model 59A Micro-Centrifuge (Pittsburg, PA) to sediment any solid zein particles dispersed in the solution. 1 ml of the supernatant was removed after centrifugation to determine the concentration of solubilized zein protein. The concentration of zein in the surfactant solution was measured using the BCA protein assay of Pierce (34) on a Shimadzu UV160U Spectrophotometer (Kyoto, Japan). Three repeats were made for each zein mass and surfactant concentration examined.

6.3 THEORETICAL FRAMEWORK OF ZEIN SOLUBILIZATION

6.3.1 Interactions between Water-Soluble Proteins and SDS

Much of the available literature investigating surfactant binding to proteins examines the interactions between water-soluble proteins and surfactants. The fundamental principles underlying these interactions should also apply to the interactions between zein and surfactants, particularly once zein is solubilized in water. Binding between surfactants
and water-soluble proteins occurs in two distinct stages, related to Sections (a) and (b) in Figure 6.1 (35, 36). In the first stage, (a), the surfactant binds to sites on the protein for which there is a favorable interaction between the surfactant and the site, referred to as 'site-specific binding'. An example of such a favorable interaction is the binding of an anionic surfactant to a positively-charged amino acid group on the protein. Due to the one-to-one side-chain to surfactant specificity of these interactions, and the low fraction of these sites on the protein relative to the total number of side-chains, the bound surfactant molecules are unlikely to interact with other bound surfactant molecules (35). For some surfactants, such as those of the nonionic variety, there are no site-specific interactions with the protein, since interactions between the protein and the surfactant are not free-energetically favorable in this case (11, 12, 14).

In the second stage, (b), of water-soluble protein-surfactant interactions, cooperative binding of surfactant to the protein takes place, and corresponds to the protein unfolding and losing its secondary structure (35). In this second stage, the surfactant molecules aggregate to form micellar structures that interact with the protein. The formation of micellar structures is a cooperative process, and therefore, this form of protein-surfactant interaction is referred to as 'cooperative binding'. Complexes between SDS and water-soluble proteins have been well-characterized using neutron scattering, and the resulting complexes have been shown to behave as necklace-and-bead structures, in which the micelles are the beads on a protein-backbone necklace (37-39). This structure is similar to those observed to form between surfactants and water-soluble polymers (for example, between SDS and poly (ethylene oxide) (40, 41)). A classic example of this interaction occurs in the SDS-PAGE technique, in which the binding of the protein to the SDS
Figure 6.1: Schematic adsorption isotherm for anionic surfactants binding to a water-soluble protein.
micelles leads to the loss of the secondary structure of the protein, and the electrophoretic movement of the resulting protein-SDS complex can be related to the molecular weight of the protein (42, 43). Investigations using fluorescence spectroscopy and NMR indicate that the protein surrounds the outer surface of the micelle, and that there is little penetration of the protein into the hydrophobic core of the micelle (44-46).

6.3.2 Theoretical Analysis of Zein Solubilization by SDS

Zein solubilization by SDS is believed to occur when the solid zein protein unfolds, or denatures, from the solid structure and forms a micelle-containing complex with SDS (22-24, 35, 45), corresponding to the second stage, (b), of surfactant binding described in Section 6.3.1 (see Figure 6.1). Prior to dissolving zein, it is expected that SDS will bind to the zein protein in a site-specific manner, in which individual SDS molecules bind to specific sites on the protein, such as to positively-charged side-chains, but in which there are no cooperative interactions between the SDS molecules. In our proposed model, the solubilization of zein by SDS reflects the onset of cooperative binding of the SDS molecules to the zein protein. Based on this model, a concentration balance on the total concentration of SDS ([SDS], in mM) as it is divided between the concentration of solid (undissolved) zein (s_z, in g/l) and the concentration of dissolved zein (d_z, in g/l) can be expressed as follows

\[ [SDS] = C_d d_z + C_s s_z \]  \hspace{1cm} (6.1)

where \( C_d \) denotes the milimoles of SDS required to dissolve 1 g of zein, and \( C_s \) denotes the milimoles of SDS bound to 1 g of solid zein. The initial total concentration of zein (\( t_z \) in g/l) is equal to
\[ t_z = d_z + s_z \]  \hfill (6.2)

Note that Eq. (6.1) neglects the contributions of free SDS monomers and SDS micelles. However, we have observed that, for the relatively small \( t_z \) values considered here, zein solubilization occurs at SDS concentrations as low as 1 mM (47). Based on the values of \( d_z \) and \( s_z \) reported in Section 6.4.1, under these low [SDS] conditions, most of the SDS would be bound to the solid zein, suggesting that the SDS monomer concentration is quite low relative to the distribution of SDS between \( d_z \) and \( s_z \). The formation of free SDS micelles may be neglected, because the formation of zein-SDS complexes must be more favorable than the formation of free SDS micelles; otherwise, there would be no zein solubilization and free SDS micelles would form instead. Indeed, in the case of surfactants that do not solubilize zein, it is free-energetically more favorable to form free micelles than it is to solubilize zein (36).

In our analysis of SDS interacting with zein, we assume that \( C_s \) and \( C_d \) are independent of \( d_z \), \( s_z \), and [SDS], because, for a given surfactant type (SDS in this case), \( C_s \) and \( C_d \) are determined by the molecular-level structure of the zein molecules, such as their amino acid sequence, and should therefore be unaffected by the macro-level characteristics of the system. We also assume that the SDS binding to solid zein will not be affected by the presence of other surfactant types in the system, provided that the other surfactants do not bind to zein in a site-specific manner, thus making \( C_s \) constant for all surfactant systems containing SDS. This assumption is made because the binding of SDS to solid zein is expected to be in the site-specific stage, (b), of Figure 6.1. In our experiments with surfactant mixtures, the cosurfactants examined are \( C_{12}E_n \) nonionic surfactants, which are
known to bind to proteins only in a non-specific manner (35). Accordingly, the $C_{12}E_n$ nonionic surfactants examined are not expected to interfere with the site-specific binding ability of SDS.

However, in the cooperative-binding stage, the resulting cooperatively-bound protein-surfactant complexes will include both types of surfactants when SDS is mixed with the $C_{12}E_n$ nonionic surfactants. In this cooperative-binding stage, which is necessary to dissolve the zein protein, zein and SDS may interact with the $C_{12}E_n$ nonionic surfactants as well, possibly leading to changes in $C_d$. Therefore, $C_d$ may depend on the $C_{12}E_n$ nonionic surfactant structure and on the mixture composition in the SDS/$C_{12}E_n$ surfactant mixtures examined.

The zein solubilization measurement determines $d_z$. By combining Eqs. (6.1) and (6.2) to solve for $d_z$, one obtains

$$d_z = \frac{[SDS] - C_z t_z}{C_d - C_z} = \left(\frac{-C_z}{C_d - C_z}\right) t_z + \frac{[SDS]}{C_d - C_z}$$

(6.3)

According to Eq. (6.3), $d_z$ will increase linearly as $[SDS]$ increases, since a larger concentration of SDS will lead to a greater capacity to dissolve zein. Likewise, $d_z$ will decrease linearly as $t_z$ increases, because a larger amount of SDS bound to the solid zein will leave less SDS available to dissolve zein. By measuring how $d_z$ decreases as $t_z$ increases, at a constant value of $[SDS]$, it is possible to determine the values of $C_z$ and $C_d$ using a linear regression analysis of the experimental data, where (see Eq. (6.3))
\[ \text{Slope} = \frac{-C_s}{C_d - C_s} \]  \hfill (6.4a)

and

\[ \text{Intercept} = \frac{[SDS]}{C_d - C_s} \]  \hfill (6.4b)

Rearranging Eqs. (6.4a) and (6.4b), \( C_s \) and \( C_d \) are found to be

\[ C_s = \frac{-[SDS] \cdot \text{Slope}}{\text{Intercept}} \]  \hfill (6.5a)

and

\[ C_d = \frac{[SDS]}{\text{Intercept}} (1 - \text{Slope}) \]  \hfill (6.5b)

### 6.4 RESULTS

#### 6.4.1 Zein Solubilization by SDS

As discussed in Section 6.3.2, a complication in the study of how zein interacts with the anionic surfactant SDS is that SDS binds to both solid zein and dissolved zein. To determine the distribution of SDS between the solid and the dissolved forms of zein, Figure 6.2 shows how much zein is dissolved, \( d_z \), by a 10 mM solution of SDS as \( t_z \) increases. Clearly, increasing \( t_z \) reduces the amount of zein that is dissolved by the fixed concentration of SDS, demonstrating that, as expected, the amount of SDS bound to solid
Figure 6.2: The dependence of the concentration of zein dissolved, $d_z$, by a 10 mM SDS solution on the initial concentration of zein, $t_z$. The error bars reflect a 95% confidence interval.
zein ($C_s$) is not insignificant. Equations (6.3), (6.5a), and (6.5b) were applied to the data shown in Figure 6.2 to determine the amount of SDS bound to both solid zein ($C_s$) and dissolved zein ($C_d$). The linear regression analysis determined that:

$$C_s = 0.06 \pm 0.02 \text{ mmol of SDS/g of solid zein, or } 0.017 \text{ g of SDS/g of solid zein,}$$

and

$$C_d = 0.9 \pm 0.3 \text{ mmol of SDS/g of dissolved zein, or } 0.26 \text{ g of SDS/g of dissolved zein,}$$

with $r^2 = 0.8$, clearly indicating that much more SDS is required to dissolve zein ($C_d = 0.9$) than to bind to the solid (undissolved) zein ($C_s = 0.06$).

### 6.4.2 Zein Solubilization by Mixtures of SDS and $C_{12}E_n$ ($n=4, 6, \text{ and } 8$)

$C_{12}E_n$ nonionic surfactants alone were found not to dissolve appreciable quantities of zein, while mixtures of SDS and $C_{12}E_n$ were found to dissolve zein. The inability of the $C_{12}E_n$ surfactants to dissolve zein is consistent with the known poor ability of nonionic surfactants to denature proteins (11, 12, 14). Figure 6.3 demonstrates that the addition of $C_{12}E_6$, at fixed [SDS] and $t_z$ values, leads to a decrease in $d_z$. According to Eq. (6.3), the only explanation for the observed decrease in $d_z$ is that $C_d$ and/or $C_s$ changed upon addition of $C_{12}E_6$ to the SDS solution, since [SDS] and $t_z$ were kept fixed. In Section 6.3.2, we proposed that $C_s$ should be independent of the presence of the $C_{12}E_n$ nonionic surfactant mixed with SDS, due to the lack of site-specific interactions observed between proteins and $C_{12}E_n$ nonionic surfactants, as well as due to the lack of cooperative binding involved in determining $C_s$, implying that only SDS-zein site-specific interactions are
Figure 6.3: The effect of increasing the concentration of $C_{12}E_{6}$ on the amount of zein dissolved, $d_z$, by a 10 mM SDS solution. For all the conditions examined, $t_c = 14$ g/l. The error bars reflect a 95% confidence interval.
expected. Provided that this expectation is satisfied, then the decrease in $d_c$ observed in
Figure 6.3 as C_{12}E_6 is added can only result from an increase in $C_d$ (see Eq. (6.3) with $C_s$
fixed), indicating that more SDS is required to dissolve each gram of zein, that is, that
SDS is less effective at solubilizing zein in the presence of C_{12}E_6.

To determine the effects of the C_{12}E_n nonionic surfactant polar head size, C_{12}E_4 and
C_{12}E_8 were also added to the SDS solution in lieu of C_{12}E_6, with [SDS] and $t_z$ fixed, and
the resulting measured $d_c$ values are shown in Figure 6.4. Once again, if $C_s$ is assumed to
remain constant, then only an increase in the value of $C_d$ upon addition of C_{12}E_n to the
SDS solution can explain the observed decrease in $d_c$ at each concentration of added
C_{12}E_n (5, 10, and 15 mM). By rearranging Eq. (6.3), the following expression is obtained
for $C_d$

$$C_d = C_s + \frac{[SDS] - C_s t_z}{d_c}$$  \hspace{1cm} (6.6)

allowing $C_d$ to be determined for each SDS/C_{12}E_n mixture examined. Table 6.1 reports
the values of $C_d$ determined using Eq. (6.6), with the average $d_c$ values presented in
Figure 6.4, and $C_s$=0.06 mmol SDS/g zein obtained from the regression analysis of
Figure 6.2 (see Section 6.4.1). The observed increase in $C_d$ with increasing C_{12}E_n
concentration indicates that more SDS is required to dissolve a given amount of zein in
the SDS/C_{12}E_n mixtures examined.
Figure 6.4: The effect of increasing the concentration of C\textsubscript{12}E\textsubscript{n} on the concentration of zein dissolved, $d_c$, by a 10 mM SDS solution. The empty bars correspond to n=4; the diagonally-slashed bars correspond to n=6; and the solid bars correspond to n=8. For reference, the value of $d_c$ for a pure SDS solution corresponds to the horizontally-stripped bar. For all the conditions examined, $t_c = 0.014$ g/ml. The error bars reflect a 95% confidence interval.
Table 6.1: Effect of adding C_{12}E_{n} (n=4, 6, and 8) nonionic surfactants to a 10 mM SDS solution, having \( t_{z}=0.014 \text{ g/ml} \), on \( C_{d} \). The units of \( C_{d} \) are mmol SDS/g dissolved zein, with \( C_{d} \) determined using Eq. (6.6).

<table>
<thead>
<tr>
<th>Added C_{12}E_{n} Surfactant</th>
<th>( C_{d} ) for 0 mM C_{12}E_{n}</th>
<th>( C_{d} ) for 5 mM C_{12}E_{n}</th>
<th>( C_{d} ) for 10 mM C_{12}E_{n}</th>
<th>( C_{d} ) for 15 mM C_{12}E_{n}</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{12}E_{4}</td>
<td>0.89</td>
<td>0.89</td>
<td>0.94</td>
<td>0.99</td>
</tr>
<tr>
<td>C_{12}E_{6}</td>
<td>0.89</td>
<td>1.09</td>
<td>1.31</td>
<td>3.42</td>
</tr>
<tr>
<td>C_{12}E_{8}</td>
<td>0.89</td>
<td>1.01</td>
<td>1.61</td>
<td>4.90</td>
</tr>
</tbody>
</table>

### 6.5 DISCUSSION

The interaction between zein and SDS, leading to the solubilization of zein, is controlled by several factors, including \([SDS]\), \( t_{z} \), \( C_{s} \), and \( C_{d} \). Figure 6.2 demonstrates that the linear correlation between \( d_{z} \) and \( t_{z} \) described by Eq. (6.1) adequately captures this interaction. This analysis is useful, because it allows a quantitative study of the factors controlling zein solubilization by surfactants. For example, it is known that other anionic surfactants can solubilize zein (15, 16, 19, 22-24), but, due to a lack of consistency between the different testing conditions, a comparison of their zein solubilization potentials requires performing a zein solubilization test with each individual surfactant at specific testing conditions. However, using the analysis methods embodied in Eqs. (6.1) through (6.5), one can compare the values of \( C_{d} \) and \( C_{s} \) for different anionic surfactants. A low value of
$C_d$ indicates a strong zein solubilization potential of the surfactant tested, and therefore, a strong protein denaturation potential, since little surfactant is needed to denature the protein.

For SDS, we have demonstrated that $C_d$ is approximately 10 times greater than $C_s$ (see Section 6.4.1). The solubilization of zein protein is believed to require unfolding of the protein, exposing the hydrophobic sites of the protein so that a SDS-zein micellar complex can form (15, 16, 19, 22-24). The mechanism of binding SDS to these aqueous SDS-zein complexes differs significantly from the mechanism involved in site-specific SDS binding to zein. Site-specific binding merely matches a single surfactant molecule, in this case SDS, to a site on the protein, such as a positively-charged lysine or arginine group, and requires far less surfactant to saturate the available sites than in the case of cooperative binding (35). Based on this description, it is reasonable to consider the observed 10-fold increase in $C_d$ over $C_s$ as evidence indicating a transition from site-specific binding to the solid zein to cooperative binding to the dissolved zein.

Figures 6.3 and 6.4 graphically demonstrate that adding a second surfactant, in this case a C$_{12}$E$_n$ nonionic surfactant, affects the capacity of SDS to solubilize zein. In both Figure 6.3 and Figure 6.4, [SDS] and $t_z$ are fixed, and therefore, according to Eq. (6.1), the only explanation for the observed decrease in $d_z$ involves a change in $C_s$ and/or $C_d$. As discussed in Section 6.3.2, for the site-specific binding of SDS to zein, we assume that the addition of C$_{12}$E$_n$ to the SDS solution should have little effect on the extent of SDS binding to zein. In the absence of zein, SDS and C$_{12}$E$_n$ (n=4,6, and 8) are known to interact synergistically to form mixed micelles at a lower critical micelle concentration (CMC) than that of SDS by itself (48). Accordingly, we would expect that the C$_{12}$E$_n$
nonionic surfactants would be incorporated into any available SDS-zein micellar complexes. The presence of C_{12}E_n surfactants in the cooperatively-bound surfactant-zein complexes will change the micellar environment that the zein molecules interact with, and could potentially affect the value of C_d. Based on this analysis, Table 6.1 demonstrates that both increasing the amount of C_{12}E_n, as well as increasing the head size of the nonionic surfactant, determined by the value of n, leads to an increase in the amount of SDS required to solubilize zein (C_d), vividly demonstrating that SDS is a less effective zein solubilizer in the presence of C_{12}E_n.

One possible explanation for the observed increase in C_d with increasing C_{12}E_n concentration is that the surface charge density of the micelles decreases upon incorporating the non-charged C_{12}E_n surfactant molecules into the negatively-charged SDS micelles. This occurs because as the concentration of C_{12}E_n increases at a fixed SDS concentration, the mixed micelles that are formed will have a higher composition of C_{12}E_n and a lower SDS composition (48). A lower micelle surface charge density, in turn, would reduce the magnitude of the electrostatic interactions between a negatively-charged SDS/C_{12}E_n micelle and the positively-charged sites on zein. However, the small effect on C_d upon adding C_{12}E_4, relative to the large effect upon adding C_{12}E_8, clearly demonstrates that the decreased micelle surface charge density cannot be the only important factor involved. If it was, then one would expect that the addition of any nonionic surfactant would induce a similar increase in C_d. Instead, Table 6.1 reveals that adding as much as 60% C_{12}E_4 (10 mM SDS and 15 mM C_{12}E_4) has only a small effect on
\( C_d \), from 0.89 for pure SDS to 0.99 for 60\% C\textsubscript{12}E\textsubscript{4}, which is within the 95\% confidence interval for \( C_d \) for pure SDS.\textsuperscript{7}

Table 6.1 and Figure 6.4 both demonstrate that as the size of the nonionic surfactant head increases (as \( n \) increases), \( C_d \) increases, and \( d_c \) decreases. C\textsubscript{12}E\textsubscript{n} nonionic surfactants are known to interact synergistically with SDS to lower the critical micelle concentration (CMC) of the resulting surfactant mixtures (48). Could this synergism explain the trends that we observe experimentally, as has been suggested by other researchers (17, 25, 26)? Unfortunately, a simple relationship between the CMC of the surfactant mixtures and \( d_c \) does not explain the results for \( C_d \) reported in Table 6.1. Indeed, at a given solution composition, the SDS/C\textsubscript{12}E\textsubscript{4} mixture has a lower CMC than the SDS/C\textsubscript{12}E\textsubscript{8} mixture (48). We could therefore expect that C\textsubscript{12}E\textsubscript{4} would be better able to hinder zein solubilization than C\textsubscript{12}E\textsubscript{8}, because the SDS would form free SDS/C\textsubscript{12}E\textsubscript{4} micelles at a lower SDS monomer concentration than required to form SDS/C\textsubscript{12}E\textsubscript{8} micelles, thus reducing the amount of SDS available to solubilize the zein protein. Instead, the opposite trend is observed, in that the SDS/C\textsubscript{12}E\textsubscript{4} mixture is able to solubilize more zein than the SDS/C\textsubscript{12}E\textsubscript{8} mixture at the same solution composition and total surfactant concentration (see Figure 6.4). The existence of a direct correlation between the ability to form free micelles (reflected in the CMC) and the ability to solubilize zein is not guaranteed, given that zein solubilization involves the interaction between zein and the surfactant micelles, which is not necessarily reflected in the CMC. Therefore, a closer examination of the interactions between the micelle and the protein is required to explain the effect of the

\textsuperscript{7} We have not included the 95\% confidence interval in Table 6.1 for clarity. Based on the regression data, we expect the interval to be \( \pm 30\% \) of the average.
increased C₁₂Eₙ head size on Cₐ, as well as the effect of increasing the concentration of C₁₂Eₙ on Cₐ, shown in Table 6.1.

Using NMR, fluorescence spectroscopy, and other spectroscopic techniques, it has been shown that the interaction between various water-soluble proteins and SDS micelles involves the binding of the protein backbone to the surface of the SDS micelle (44-46). This interaction is favorable because: (i) the protein backbone shields exposed hydrophobic patches at the hydrophobic core-water micellar interface from the aqueous environment surrounding the micelle, (ii) the hydrophobic protein side chains are able to penetrate into the hydrophobic core of the micelle, and (iii) any positively-charged protein side chains may interact favorably with the negatively-charged sulfate heads of SDS. The incorporation of a non-charged surfactant (C₁₂Eₙ in the present case) into the SDS micelle should affect interaction mechanisms (i)-(iii). As discussed above, a reduction in the micelle surface charge density should make the electrostatic interactions described in (iii) less favorable. A larger head size of the nonionic surfactants should shield, and therefore reduce, the hydrophobic surface area of the micelle core exposed to water, thus reducing the driving force for the protein backbone to bind to this surface, as described in (i), by increasing the steric interactions between the protein and the surfactant heads. Finally, a larger head size of the nonionic surfactants should also sterically hinder the access of the hydrophobic protein side-chains into the micellar interior, thus reducing the strength of the interactions described in (ii).

The effect of the head size of the C₁₂Eₙ nonionic surfactants examined is clearly observed in Figure 6.4 and Table 6.1. Calculations have shown that the effective area of the
micellar core obstructed by C_{12}E_{4} (27 \, \text{Å}^2) is similar to that of SDS (25 \, \text{Å}^2) (49).\textsuperscript{8} Accordingly, the addition of C_{12}E_{4} should have little effect on \textit{C}_d because interactions (i) and (ii) should not be affected significantly. However, for both C_{12}E_{6} and C_{12}E_{8}, the head sizes (cross-sectional areas of 38 \, \text{Å}^2 and 48 \, \text{Å}^2, respectively (49)) are much larger than that of SDS (25 \, \text{Å}^2), and the favorability of interactions (i) and (ii) should decrease because it will be more difficult for the protein to shield and access the hydrophobic core of the micelle. Interaction (iii) will only depend on the composition of the micelle, that is, on the relative amount of charged to uncharged surfactant, and the head size of the nonionic surfactant is not expected to have a strong effect on this interaction. The net result is that the driving force to unfold and dissolve the zein protein so that it can interact with the SDS/C_{12}E_{n} micelles is reduced. Accordingly, more SDS is required per zein molecule to drive the zein solubilization process, which results in the increases in \textit{C}_d shown in Table 6.1.

An equation that relates \textit{C}_d to the protein-surfactant interactions involved at the molecular level is required to demonstrate how these interactions behave in a semi-quantitative manner. As a first approximation, one may model the zein-surfactant interactions as being similar to those of a polymer interacting with the micelle (35). In particular, we would like to determine primarily the relative ability of the surfactant to solubilize a zein molecule, as reflected in \textit{C}_d. To this end, we will make use of a previously-developed molecular-thermodynamic theory of polymer-surfactant complexation (33). In modeling the interactions between a polymer and a micelle, the parameter \textit{n}_b is used to denote the

\textsuperscript{8} The steric surfactant head area, \textit{a}_h, is used here because in order for protein segments to reach the hydrophobic core of the micelle, they need to get around this obstruction. In the case of water, a different area is used, since the water molecules are small relative to the surfactant head size (48, 49).
number of polymer segments bound to the micelle per surfactant molecule in the micelle. This is relevant to our analysis of zein solubilization, because $C_d$, the amount of SDS required to dissolve 1 g of zein (SDS/amount of zein), is related to $1/n_b$ (SDS/amount of polymer). Accordingly, the increases in $C_d$ observed in Table 6.1 should reflect decreases in $n_b$. In Ref. (33), the following expression was derived for $n_b$:

$$n_b = \frac{-\chi_p a - \epsilon_{ps} a_h + a \ln(1 - a_h / a)}{b^2 (1 - 2\chi)}$$

(6.7)

where $\chi_p$ represents the free-energy change associated with the adsorption of a single polymer segment at the hydrocarbon core-water micellar interface, $a$ is the area per surfactant molecule at the hydrocarbon core-water micellar interface, $\epsilon_{ps}$ is the free-energy change due to the specific interaction between a polymer segment and a surfactant head, $a_h$ is the cross-sectional area of the surfactant head, $b$ is the length of a polymer segment, and $\chi$ is the Flory-Huggins parameter describing the polymer segment-water interactions (33). Both $b$ and $\chi$ depend only on the polymer (the unfolded zein protein in the present case), and therefore, are not affected by the type of surfactant present, thus making it unnecessary to discuss the effect of changing their values on $n_b$.

The three factors that should change as the composition of the surfactant changes from pure SDS to a SDS/C$_{12}$E$_n$ surfactant mixture are $a_h$, $\chi_p$, and $\epsilon_{ps}$, which can be related to interactions (i), (ii), and (iii) respectively, discussed above. The $\chi_p$ and $\epsilon_{ps}$ values are negative (attractive), and as they become more negative (and hence more favorable), there will be an increase in the value of $n_b$. At the hydrocarbon core-water micellar interface, the protein backbone must compete for space with the surfactant heads,
represented by $a_h$. The third term in Eq. (6.7) indicates that increasing the average value of $a_h$ will decrease $n_b$, since the logarithmic term is negative, because $(1-a_h/a)$ is between 0 and 1, and will become more negative as $a_h$ increases, thus reducing $n_b$. An increase in the average $a_h$ value will occur as $C_{12}E_n$ surfactants having larger heads, such as $C_{12}E_8$, are added to the micelle, and as the head size of the nonionic surfactants increases for a given micellar composition.

Zein is a hydrophobic protein, which is why it is not soluble in water. Therefore, $\chi_p$ is expected to be negative, because there should be a favorable attractive interaction between zein and the hydrocarbon core-water micellar interface. As it becomes more difficult for the protein to approach the micelle surface, due to the presence of the larger surfactant heads, it will be less favorable for the protein to adsorb onto the micellar surface, which should lead to a decrease in the magnitude of $\chi_p$, and hence, to a decrease in $n_b$. Surfactants having larger head sizes, such as $C_{12}E_8$, will occupy a larger volume around the micellar core, and will therefore hinder the ability of the zein protein to interact with the micellar core more than surfactants having smaller head sizes. Likewise, increasing the micellar composition of the larger head size surfactants at the expense of the smaller head size surfactants will decrease the magnitude of $\chi_p$.

A favorable attractive interaction between the surfactant heads and the protein (for example, of electrostatic origin) can be represented by a negative value of $\varepsilon_{ps}$. For a mixture of SDS and $C_{12}E_n$ surfactants, with a SDS fraction $\alpha$ and a $C_{12}E_n$ fraction $(1-\alpha)$, the average value of $\varepsilon_{ps}$ can be approximated by $(\alpha\varepsilon_{ps,SDS}+(1-\alpha)\varepsilon_{ps,C_{12}E_n})$. Accordingly, a mixture of $C_{12}E_n$ nonionic surfactant heads, which do not interact with the protein
(\epsilon_{ps,C_{12}E_n}=0), and SDS will decrease the magnitude of the average \epsilon_{ps} value as \alpha decreases, that is, as more \text{C}_{12}\text{E}_n is incorporated into the micelle. This decreasing magnitude (\epsilon_{ps} approaches zero as \alpha approaches zero), should also lead to a decrease in \(n_b\) according to Eq. (6.7). However, the small change in \text{C}_d upon adding \text{C}_{12}\text{E}_4 to SDS suggests that the interactions reflected in \epsilon_{ps} may not have a strong effect on \(n_b\).

The discussion above, based on Eq. (6.7), indicates that the addition of \text{C}_{12}\text{E}_n nonionic surfactants to a solution of SDS is expected to reduce \(n_b\) in all cases, and as the size of the \text{C}_{12}\text{E}_n head increases, it will have a dramatic effect on \(a_h\) and \(\chi_p\). A reduction in \(n_b\) indicates that each surfactant molecule is able to bind less zein, leading to an increase in \text{C}_d. Therefore, the semi-quantitative predictions based on Eq. (6.7) are consistent with the experimental findings on \text{C}_d reported in Table 6.1, and provide a useful molecular-level interpretation of our results.

6.6 CONCLUSIONS

In this chapter, we investigated the ability of SDS/\text{C}_{12}\text{E}_n (n=4,6, and 8) surfactant mixtures to solubilize the protein zein, which is related to the protein denaturation ability of the surfactant mixture. We developed a simple model to analyze our results, in which the partition of SDS between the solid (undissolved) and the dissolved forms of zein is used to determine the ability of SDS to solubilize zein. This is captured by the factor \text{C}_d, which is the amount of SDS required to dissolve 1 gram of zein protein, and \text{C}_s, which is the amount of SDS that binds to 1 gram of solid zein. For solutions that contain only SDS, we found that \text{C}_d is roughly 10 times greater than \text{C}_s, justifying the concept that binding to dissolved zein is a cooperative process, while binding to solid zein is not.
When \( C_{12}E_n \) nonionic surfactants were mixed with SDS, \( C_d \) was found to increase, indicating that SDS is a less efficient zein solubilizer in the presence of \( C_{12}E_n \). This increase in \( C_d \) resulted when the nonionic surfactant concentration increased, as well as when the head size (n) of the nonionic surfactant increased. The effect of \( C_{12}E_n \) on the \( C_d \) of SDS was analyzed in the context of a previously-developed molecular-thermodynamic theory of polymer-surfactant complexation, with the unfolded zein molecule playing the role of the polymer. The observed increase in \( C_d \) with both increasing nonionic head size and nonionic surfactant concentration was explained by the increased steric repulsions between the zein backbone and the SDS/\( C_{12}E_n \) micelle surface, which reduces the driving force for complexation of the zein protein and the surfactants. The inability of \( C_{12}E_4 \) mixed with SDS to increase \( C_d \) dramatically suggests that the dilution of the micelle charge does not play a strong role in reducing the zein solubilization potential of SDS.

Our findings can be utilized to reduce the skin irritation of surfactant solutions, as well as to understand how surfactants denature proteins and how this denaturation can be minimized. Provided that there is a correlation between the zein solubilization capacity of a surfactant mixture and the skin irritation induced by that surfactant mixture, the zein solubilization test can provide a simple and useful \textit{in vitro} test that can be used to screen surfactant mixtures prior to \textit{in vivo} skin irritation testing. The theoretical framework of zein solubilization, presented in Section 6.3.2, also allows for the easy comparison of the zein solubilization potential of different surfactants. Surfactants that exhibit a high value of \( C_d \) are less denaturing than those exhibiting low \( C_d \) values. This is useful, because at the present time, each study of zein solubilization has used different surfactant concentrations and total amounts of zein, making it impossible to compare the results of
different zein solubilization tests. On the other hand, $C_d$ and $C_s$ depend solely on the surfactant system examined, and therefore, the specific conditions used for the zein solubilization test should not change their values significantly for a given surfactant system, thus significantly enhancing the value of the zein solubilization test.

6.7 REFERENCES


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Chapter 7

Conclusions and Future Work

The central goal of this thesis was to develop a fundamental understanding of how the solution properties of surfactant solutions that come in contact with the skin affect and control the skin irritation potential of the surfactants involved. This fundamental understanding, in turn, can be utilized to design surfactant-containing products that are less irritating to the skin, or to facilitate the transport of permeants across the skin in transdermal drug delivery applications. In this Chapter, Section 7.1 summarizes the main results of the thesis. Section 7.2 presents potential avenues for future research in the field of surfactant-induced skin irritation based on the results of this thesis. Finally, Section 7.3 presents general conclusions on how to minimize the skin irritation potential of surfactant solutions.

7.1 Thesis Summary

In Chapter 2, we described the development of an in vitro skin irritation test that utilized the electrical conductivity of the skin (skin conductivity) as a quantitative indicator of the permeability of the skin to ions. The permeability of the skin, in turn, is related to skin irritation, because any increase in the skin permeability will allow more water to escape

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the skin, as well as will increase the penetration of irritants into the skin. Mixtures of the anionic surfactant sodium dodecyl sulfate (SDS) and the nonionic surfactant dodecyl hexa(ethylene oxide) (C\(_{12}\)E\(_{6}\)) were used as model systems to induce skin irritation. The \textit{in vitro} skin irritation test was able to reproduce the expected correlation between the skin irritation potential of the SDS/C\(_{12}\)E\(_{6}\) surfactant mixtures and the critical micelle concentration (CMC) of the surfactant mixtures. However, it was observed that the ionic strength of the surfactant solution could lead to changes in the skin conductivity that was not necessarily related to the skin irritation potential of the surfactant solution. To reduce, or eliminate altogether, the effect of the ionic strength of the surfactant solution, the skin was exposed to a buffer solution after being exposed to the surfactant solution in an attempt to normalize the ionic strength inside the skin for different surfactant solutions. When this approach was implemented, the \textit{in vitro} skin irritation test was able to rank the skin irritation potential of three commercial soap bars (Dove, Lever 2000, and Ivory) in the correct known order of increasing \textit{in vivo} skin irritation.

In Chapter 3, the penetration of SDS into the epidermis was measured using \(^{14}\)C-radiolabeled SDS. It was found that, at surfactant concentrations that exceed the CMC of SDS, the concentration of SDS measured in the epidermis increased as the total SDS concentration in the solution contacting the skin increased, thus demonstrating that micellar SDS contributes to the penetration of SDS into the epidermis. The observed SDS dose-dependent response contradicts the widely-accepted view that only surfactant monomers penetrate into the skin, while surfactant in micellar form does not contribute to surfactant penetration into the skin. Nevertheless, this finding is consistent with previously unexplained observations of a dose-dependent damage to the skin induced by
SDS at concentrations above the CMC. When poly(ethylene oxide) (PEO) was mixed with SDS, SDS micelles bound to PEO did not contribute to the concentration of SDS in the epidermis, while SDS in free SDS micelles did. Dynamic light scattering measurements revealed an average hydrodynamic radius of 20Å for the SDS micelles, and a larger radius of 25Å for the PEO-bound SDS micelles. A comparison with typical aqueous pore radii in the stratum corneum reported in the literature (10-28Å) suggested that the SDS micelles may be able to penetrate into the skin, while the PEO-bound SDS micelles may be sterically hindered from penetrating into the skin.

In Chapter 4, our goal was to show that the skin irritation potential of SDS is related to the amount of SDS that penetrates into the skin. To achieve this goal, both in vitro and in vivo skin irritation tests were carried out with the SDS and the SDS+PEO solutions examined in Chapter 3. Using the in vitro skin irritation test developed in Chapter 2, we found a direct correlation between the amount of SDS in the epidermis and the resulting in vitro skin irritation. This result confirmed the expectation that reducing the penetration of SDS into the skin should assist in reducing the skin irritation induced by SDS. A Forearm Controlled Application Technique (FCAT) was then used to evaluate the effect of adding PEO to a fixed concentration of SDS on in vivo skin irritation. The addition of PEO led to a decrease in the in vivo skin irritation, which is consistent with the observed reduced penetration of SDS into the skin when PEO was added reported in Chapter 3. Therefore, the in vivo skin irritation results also indicate that a reduction in the penetration of micellar SDS into the skin can play an important role in reducing the skin irritation potential of SDS.
In Chapter 5, we investigated an alternative method to reduce surfactant-induced skin irritation by mixing surfactants, including an examination of the underlying mechanisms associated with this alternative method. The penetration of SDS into the epidermis from contacting solutions of SDS and C_{12}E_{6} was measured for three SDS concentrations and three SDS solution compositions. The addition of C_{12}E_{6} to the SDS solutions was found to decrease the amount of SDS penetrating into the epidermis, and therefore, according to the results of Chapter 4, should result in a reduction in the skin irritation potential of the surfactant solution. The observed decrease in the SDS concentration in the skin occurred via two mechanisms: (i) the addition of C_{12}E_{6} decreased the SDS monomer concentration, thus reducing the driving force for the penetration of monomeric SDS into the epidermis, and (ii) the addition of C_{12}E_{6} reduced, or prevented altogether, the penetration of micellar SDS into the epidermis. It is noteworthy that, at surfactant concentrations well above the CMC, mechanism (ii) will play a larger role in reducing surfactant penetration into the epidermis than mechanism (i). The hydrodynamic radii of the SDS/C_{12}E_{6} micelles were determined using dynamic light scattering. A comparison with typical stratum corneum aqueous pore radii reported in the literature suggests that the pure SDS micelles are able to penetrate into the epidermis, while the SDS/C_{12}E_{6} mixed micelles are sterically hindered from doing so, due to their larger sizes. Based on the results of Chapter 4, the observed reduced penetration of SDS into the epidermis upon the addition of C_{12}E_{6} should lead to a reduction in the skin irritation potential of SDS. Accordingly, using our new model of micelle penetration into the skin, we can explain previous results concerning the mildness of surfactant mixtures in the context of our
model, as well as make useful suggestions regarding the design of new surfactant formulations which are less irritating to the skin.

In Chapter 6, we investigated how the interactions between SDS and the protein zein are affected by the relative amounts of SDS and zein, as well as by the addition of the nonionic surfactants C$_{12}$E$_n$ (n=4, 6, and 8). These studies were driven by the possible connection between protein-surfactant interactions and skin irritation. The behavior of the SDS-zein solutions was analyzed using a mass balance approach that supported the claim that the solubilization of the zein protein occurs at the onset of cooperative binding of SDS to the zein protein. The addition of C$_{12}$E$_n$ surfactants led to a decrease in the ability of SDS to solubilize zein, with the greatest decrease occurring for C$_{12}$E$_8$, while C$_{12}$E$_4$ had the smallest effect. The reduced ability of SDS to solubilize zein in the presence of C$_{12}$E$_n$ surfactants was analyzed using a molecular-thermodynamic theory of polymer-surfactant complexation. This analysis suggested that as the head size (determined by n) and/or the concentration of the C$_{12}$E$_n$ nonionic surfactant increased, there would be an increase in the steric repulsions between the zein protein and the SDS/C$_{12}$E$_n$ mixed micelles, thus reducing the driving force to form a cooperatively-bound surfactant-protein complex, thereby reducing the driving force to solubilize the zein protein. Applying these results to protein-surfactant interactions in general, they indicate that the ability of a surfactant to denature a protein can be tailored through the appropriate mixing with other surfactants, leading to a useful vehicle to design surfactant systems that are less denaturing to proteins. With respect to skin irritation testing, the analysis of zein solubilization by surfactants presented in Chapter 6 should allow for a
more meaningful comparison of the results from different versions of the zein solubilization test.

7.2 **FUTURE WORK**

7.2.1 **Penetration of Other Surfactants into the Epidermis**

We have demonstrated that one can control the penetration of SDS into the epidermis by controlling the penetration of SDS micelles through a manipulation of the size of these micelles. However, it would be useful to test the range of applicability of this important finding further using other types of surfactants, including cationic (such as, dodecyl trimethyl ammonium bromide), nonionic (such as, C_{10}E_{4}), and zwitterionic (such as, dodecyl amidopropyl betaine) surfactants.

Using a chemical analytical method, such as a hyamine dye analysis, the concentration of surfactant that traverses the skin from the donor to the receiver compartments of the diffusion cell can be measured quantitatively. Potentially, if micellar surfactant can (cannot) traverse the skin, then there should (should not) be a dose-dependent relationship between the amount of surfactant found in the receiver compartment and the concentration of surfactant in the donor compartment. If this method could replace the use of radiolabeled surfactants to measure their penetration into and through the skin, then the penetration of a broader class of surfactants through the skin could be studied at a much lower cost. Initially, the test could use SDS, with and without PEO, to test whether the amount of SDS traversing the skin follows the same relationship with micelle size as it does in the case of SDS penetration into the epidermis.
According to our current conjecture, surfactant micelles that are smaller than SDS micelles should be able to penetrate into the skin with relative ease. To test this conjecture, one could use sodium decyl sulfate or sodium octyl sulfate for that purpose. For these two surfactants, which are expected to form micelles having a size that is smaller than that of SDS micelles, the penetration into and through the epidermis should occur in a surfactant dose-dependent manner. On the other hand, it is expected that in the case of sodium tetradecyl sulfate and sodium hexadecyl sulfate, the micelles would be too large to penetrate into the epidermis.

7.2.2 Using Other Additives to Reduce Surfactant Penetration into the Skin

Addition of Polymers or Proteins

In addition to the PEO nonionic polymer examined in Chapter 3, the addition of a cationic polymer would be expected to dramatically reduce the monomer concentration of an anionic surfactant, as well as to form large polymer-surfactant complexes that would not penetrate into the epidermis. Similarly, proteins, including ovalbumin and zein, that are known to bind cooperatively to anionic surfactants could be used. Both a reduction in the surfactant monomer concentration and/or an increase in the size of the micellar complexes should lead to a reduction in the amount of anionic surfactant that penetrates into the skin, leading to a reduction in the surfactant-induced skin irritation, which could be tested using our in vitro skin irritation test. There are several cationic polymers that could be tested, keeping in mind that it would be preferable to utilize those that possess a low linear charge density, such as Polymer JR, to prevent precipitation of
the polymer-surfactant complexes. Initial tests using zein protein to minimize the SDS monomer concentration indicate that there is a dramatic reduction in the penetration of SDS into the skin.

**Addition of Oils**

It is known that the addition of oil to a surfactant solution can improve its mildness (1). Possible mechanisms for this mildness improvement include:

(a) The oil forms a protective coating on the stratum corneum, thus preventing the surfactant from interacting with the stratum corneum.

(b) The oil replenishes lipids in the stratum corneum that are removed by the surfactant.

(c) The presence of oil in the contacting solution reduces the partitioning of surfactant into the skin, thereby decreasing the penetration of surfactant into the skin.

(d) The oil causes the surfactant micelles to swell, thus reducing, or preventing altogether, the penetration of the micellar surfactant into the skin.

It would be interesting to test mechanisms (a)-(d) experimentally. For example, if only mechanism (c) was operating, then the amount of surfactant penetrating into the skin should increase in a dose-dependent manner above the CMC, since micelles should contribute to surfactant penetration into the skin. Different oils and oil concentrations could be used to determine how the size of the surfactant-oil swollen micelles affects the amount of surfactant penetrating into the skin and the related damage to the skin. Current theoretical work in our laboratory is aimed at predicting the effect of oil solubilization on
micelle size, and should be of use in selecting the appropriate surfactant/oil systems, as well as in analyzing the experimental findings.

**Use of Complex Surfactant Systems**

As more components are added to the contacting solution, the system behavior becomes more complex. An interesting question is whether our hypothesis of micelle penetration is applicable for these more complex systems as well. The use of oils and other polymers has already been discussed in this section, but it may also be interesting to study the effects of other additives/surfactants. For example, a system where one mixes two surfactants with some oil and a polymer could be studied, after an understanding is gained of how the individual components contribute to the penetration of surfactants into the epidermis and the associated skin irritation. These more complex systems will mimic a real system, and will therefore represent a precursor to subsequent studies in a real system of practical relevance. If the "mimic system" and the real system are found to behave differently, then this would indicate that other components present in the real system, but absent in the "mimic system", have a significant effect on surfactant penetration into the skin and associated surfactant-induced skin irritation.

**7.2.3 Electrostatics and Skin Electrical Characteristics**

**Electrostatics**

In Chapter 5, it was observed that the addition of salt led to an increase in the ability of SDS monomers to penetrate into the epidermis. Other unpublished work by us, involving adjusting the ionic strength of the surfactant solution and the charge density of the
micelles, suggests that the electrostatic interactions of the surfactant monomers and the surfactant micelles with the skin can play a role in controlling the penetration of charged surfactants into the skin. A thorough examination of how the electrical characteristics of the surfactants affect their ability to penetrate into the skin would illuminate how factors other than the steric size of the penetrant control the ability of molecules to access and traverse the skin. Some experiments that could be carried out include measuring the penetration of an ionic surfactant under a systematic variation of the ionic strength of the contacting solution, and measuring the effect of the ionic strength on the separate penetration of surfactants having opposite charges. If the size of the micelles is known, then the penetration results could potentially be analyzed using hindered-transport theories (2).

**pH Effects on Surfactant-Induced Damage to the Skin**

The ionic charge of many surfactants, such as carboxylate and zwitterionic surfactants, is affected by the solution pH. Changing the solution pH can lead to changes in the aggregation number of the surfactant micelles present in the contacting solution, as well as to changes in the surfactant monomer concentration (3). It would be interesting to study the effects of pH on the barrier properties of the skin in the presence of both pH-sensitive and pH-insensitive surfactants. It appears necessary to use pH-insensitive surfactants to provide a control case to study the effects of the pH on the skin under well-defined surfactant exposure conditions. With that information, the effects of pH on the pH-sensitive surfactants could be decoupled from the direct pH effects on the skin. Current theoretical work in our laboratory is aimed at predicting pH effects on the micellization behavior of pH-sensitive surfactants, and should be useful in selecting
appropriate pH-sensitive surfactants, as well as mixtures of pH-sensitive and pH-insensitive surfactants, including an analysis of the experimental findings.

**Electrical Impedance of the Skin**

In Chapter 2, Section 2.3.2, we reported results of measurements of the effects of SDS and of C₁₂E₆ on the electrical impedance of the skin. The electrical resistance and capacitance of the skin were observed to change with time. If these changes are related to the time variation of the surfactant concentration in the epidermis, which can be measured using the technique described in Chapters 3 and 5, one could potentially utilize the variation in the skin impedance with time to observe the penetration of surfactant into the skin in real time. Measuring an impedance spectrum requires about 5 minutes, but the impedance was observed to continue to change even after several hours. Accordingly, monitoring how the impedance spectrum was influenced by both the surfactant exposure time and the concentration of surfactant in the skin could provide insight into the transport of surfactants into and through the skin.

**7.2.4 Location of Surfactants in the Stratum Corneum**

Although we can currently measure the amount of surfactant that penetrates into the epidermis, we do not know the specific location of the surfactant within the epidermis. Using two-photon fluorescence microscopy and fluorescent surfactants, one could study the location of the fluorescent surfactants in the stratum corneum, including the corneocytes, the corneocyte envelopes, and the lipid bilayers (4). In addition, using a fluorescent probe (hydrophobic and hydrophilic) on skin exposed to a surfactant solution containing that probe, one could potentially determine how surfactants affect the structure
of the SC, including the corneocytes, the lipid bilayers, and the corneocyte envelopes by visualizing the locations of the fluorescent probe within the stratum corneum. Such studies could also shed light on how surfactants affect the transport pathways (lipoidal versus aqueous pore) of the probe in the skin.

7.2.5 Skin Penetration of Fixed-Size Particles

Uncharged polymeric dendrimers can be used as probes of a fixed size (between 1 and 4 nm). If these dendrimers could be labeled with a fluorescent tag, then one could test the steric penetration model by using different dendrimer sizes and probing their location in the stratum corneum using two-photon fluorescence microscopy (4). In addition, charged polymeric dendrimers of various surface charge densities could be utilized to study the role of electrostatics on the penetration of charged solutes into the skin.

7.2.6 Theoretical Issues Associated with Surfactant Penetration into the Skin

A theoretical analysis of surfactant penetration into the skin would be useful to examine the effects of changing the surfactant solution physical chemistry, including micelle size and composition, on the surfactant penetration into the skin. The theoretical treatment would involve: (1) a molecular-thermodynamic description of how the chemical structure of the surfactant determines the partition coefficient of that surfactant from the donor solution into the stratum corneum, (2) the subsequent transport of that surfactant across the stratum corneum and viable epidermis, and (3) the possible binding of the surfactant to proteins and/or lipid bilayers in the stratum corneum and/or the viable epidermis.
7.3 SUMMARY OF CONCLUSIONS BASED ON THE IN VITRO SKIN IRRITATION TEST AND THE MEASUREMENT OF SDS PENETRATION INTO THE SKIN

i) An \textit{in vitro} skin irritation test was developed that made use of skin conductivity to quantify the skin irritation potential of surfactant solutions that contact the skin. This \textit{in vitro} skin irritation test was then successfully used to rank the known increasing \textit{in vivo} skin irritation potential of the commercial soap bars Dove, Lever 2000, and Ivory.

ii) The penetration of SDS, an irritating surfactant, into the epidermis was found to depend on the total SDS concentration, even above the CMC. This important finding contradicted the commonly-accepted surfactant monomer penetration model, and clearly demonstrated that the micellar SDS played an important role in controlling the penetration of SDS into the epidermis.

iii) The penetration of SDS into the epidermis was reduced by adding either PEO or C\textsubscript{12}E\textsubscript{6} to the SDS contacting solution. The addition of these components reduced the SDS penetration into the epidermis via two mechanisms: 1) a reduction in the concentration of monomeric SDS, thereby reducing the driving force for SDS monomeric diffusion into the epidermis, and 2) prevention of SDS in the SDS-PEO micelles or the SDS-C\textsubscript{12}E\textsubscript{6} mixed micelles from penetrating into the epidermis.

iv) A direct correlation was observed between the amount of SDS in the epidermis and the \textit{in vitro} and the \textit{in vivo} skin irritation responses. In order to minimize the skin irritation potential of a SDS containing solution, the amount of SDS penetrating into the
epidermis must be minimized. As described in (iii), this can be accomplished both by reducing the SDS monomer concentration, as well as by preventing the micellar SDS from penetrating into the epidermis through an increase in the size of the micelles.

v) To generalize the conclusions of this thesis, in the design of milder surfactant formulations, it is important to minimize the amount of surfactant penetrating into the skin. In order to achieve this goal, one must not only reduce the surfactant monomer concentration, but must also manipulate and control the solution properties of the surfactant system that comes in contact with the skin to prevent the penetration of surfactant in micellar form. For surfactant formulations having total surfactant concentrations that are well above the CMC, the contribution of the micellar surfactant to skin penetration can overwhelm the contribution of the monomeric surfactant, thus emphasizing the importance of preventing the skin penetration of the micellar surfactant.

7.4 REFERENCES


