International Journal of Cosmetic Science, 2003, **25**, 103–112

pH-induced alterations in stratum corneum properties

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Received 10 April 2003, Accepted 23 May 2003

Keywords: surfactant irritation, mildness, pH-induced irritation, soaps, cleanser mildness

Synopsis

Skin-cleansing compositions based on alkyl carboxylates (soaps) have a higher irritation potential than those based on syndet surfactants such as alkyl isethionates or alkyl ether sulphates. Contributing factors include inherent differences in the irritation potential of soaps and syndet surfactants, pH-induced changes in surfactant solution chemistry, and the direct effects of pH on the physical properties of the stratum corneum (SC). Past work has not directly addressed the effect of solution pH on the SC itself and its potential role in cleanser-induced skin irritation. In the current work, alterations to SC properties induced by buffered pH solutions and two strongly ionizable surfactants, sodium dodecyl sulphate and sodium lauryl ether sulphate, at different pH values are measured. By utilizing optical coherence tomography (OCT) and infrared (IR) spectroscopy we have directly measured physical changes in SC proteins and lipids. Our results indicate that SC swelling, which reflects alterations to SC structural proteins, is increased significantly at pH 10, compared to pH 4 and 6.5. The transition temperature (T_m) of SC lipids is found to increase at pH 10, compared to pH 4 and 6.5, suggesting a more rigid SC lipid matrix. Surfactants cause a further increase in swelling and lipid rigidity. Some aspects of what these results mean for SC physical properties as well as their implications to potential mechanisms of surfactant-induced skin irritation are discussed.

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Résumé

Les compositions nettoyantes pour la peau à base d'alkyl carboxylates (savons) ont un potentiel irritant supérieur à celles à base de syndet tensioactifs tels que les alkyl isothionates ou les alkyl ether sulfates. Les facteurs en cause comprennent les différences de potentiel irritant inhérentes aux savons et aux syndet tensioactifs, les modifications de la chimie de la solution de tensioactif dues aux pH, et les effets directs du pH sur les propriétés physiques de la couche cornée (CC). Les travaux antérieurs n'ont pas traité directement l'effet du pH de la solution sur la couche cornée elle-même et son rôle potentiel dans l'irritation de la peau due à la solution nettoyante. Dans la présente étude on a mesuré les altérations des propriétés de la CC causées par des solutions à pH tamponné et deux tensioactifs fortement ionisables, le dodecyl sulfate de sodium et le lauryl ether sulfate de sodium, à différentes valeurs de pH. En utilisant la tomographie optique (OCT) et la spectroscopie à infrarouge (IR) on a mesuré directement les modifications physiques des protéines et des lipides de la CC. Nos résultats montrent que le gonflement de la CC, qui traduit des altérations des protéines structurales de la CC, augmente significativement à pH 10, par comparaison au pH 4 et 6.5. On observe que la température de transition (T_m) des lipides de la CC augmente à pH 10, par comparaison au pH 4 et 6.5, suggérant une matrice lipidique de la CC plus rigide. Les tensioactifs provoquent une augmentation plus importante du gonflement et de la rigidité lipidique. On aborde certains aspects de la signification de ces résultats vis-à-vis des propriétés physiques de la couche cornée ainsi que leurs conséquences sur les

mécanismes potentiels de l'irritation de la peau causée par les tensioactifs.

Introduction

It is well known that cleansers based on soaps (alkyl carboxylates) have a higher potential to irritate skin than those based on synthetic surfactants (syndets) such as sodium alkyl isethionate or alkyl ether sulphates [1–6]. Soaps are alkaline in nature while syndets (synthetic surfactant-based cleansers) are mostly close to neutral pH or slightly acidic. In principle, differences in irritation potential between alkaline soaps and neutral pH syndets can arise from: (i) inherent structural and charge density differences, (ii) direct effects of pH on the stratum corneum (SC) and/or (iii) indirect effects of pH mediating the solution chemistry of charged headgroups at alkaline and neutral pH. The relative importance of the above effects will change with physical and environmental conditions, as will any combined effects. Past work has failed to differentiate or clarify the direct versus indirect roles of pH on surfactant-induced skin irritation. Because of their complex pH-dependent phase behaviour [7], soaps cannot be used to address the direct versus indirect role of pH on surfactant-induced skin irritation. However, surfactants with strongly ionizable functional groups such as sulphates and ether sulphates are not pH sensitive, and therefore, can be used to study the direct versus indirect contributions of pH/alkalinity to differences in surfactant-induced irritation. In this paper we present new findings showing that pH has a direct role in changing SC properties and discuss how this might contribute to surfactant-induced irritation. Relevant past work on the role of pH in surfactant-induced skin irritation is discussed in the context of our new findings.

The past work related to effects of pH on skin irritation can be broadly classified into *in vivo* clinical studies and *in vitro* studies. In 1994, Murahata and Aronson [8] reviewed the prior clinical work and concluded that the role of pH in cleanser irritation was inconclusive. They demonstrated that pH has an important role in determining differences in irritation potential of complex skin-cleansing formulations and attributed this to pH effects on ionizable constituents, i.e. an indirect effect. However, their study did not address potential direct effects of pH on the SC and the possible contribution of such changes to the increased irritation observed at pH 10. More recently, a clinical study of people with sensitive skin demonstrated that pH neutral bars (typically of syndet compositions) are less irritating than pH 10 bars (typical soap compositions). These authors concluded that cleansing bars could have a considerable irritation effect that is related to the pH of the product [9]. Again, however, this study was not designed to discriminate between a direct or indirect effect of pH. Further support for the important role of pH comes from a recent study [10], indicating that sustained increase in mice SC pH, for example caused by daily use of soap-based cleansers, may have a long-term effect on skin condition. This work shows that an increase in SC pH by as little as half a pH unit can affect the barrier repair mechanism [10].

In vitro studies using the tendency of surfactants to swell or denature a protein are a useful indicator of their harshness towards human skin *in vivo* [11–15]. Collagen/corneum swelling by surfactants have been shown to correlate with their tendency to irritate human skin in a soap chamber test [16] and a patch test [17]. Furthermore, surfactant-induced excess water uptake *in vivo*, often referred to as 'hyper-hydration', also correlates with the irritation potential of surfactants measured using *in vivo* patch tests [18, 19]. These and other *in vitro* studies [12, 20] could not clarify the role of pH because ionic strength and pH were not sufficiently controlled to permit unambiguous discrimination of direct versus indirect effects of pH.

While the interaction of corneum proteins with cleanser surfactants is well recognized as a potential problem that can lead to skin irritation and barrier damage, the role of surfactant interactions with lipids and their effects on skin condition has been a controversial one [15, 21–25]. It has been suggested that surfactants may intercalate into skin lipids leading to local structural changes or may even cause selective removal of certain lipid components leading to instability in the bilayer lipids. However, the effect of pH on surfactant interaction with SC lipids or the direct effect of pH on SC lipids, has not been investigated.

The SC is a complex material consisting of a connected protein network (composed of intracellular keratin, a cross-linked cellular protein envelope, and intercellular protein connections) embedded within a lipid matrix. It is expected that these SC components will respond differently to changes in pH value because the isoelectric point (IEP) of SC keratin (the major protein) is ~pH 5 while the effective pK_a of SC fatty acids in the lipid bilayer is ~pH 7. Thus, it is expected that the magnitude and specificity of pH effects upon SC physical properties will depend on the varying response of these components to pH, as will any synergistic interactions involving proteins and lipids.

In this paper, we present the results of some recent work designed to determine and separate the direct and indirect contributions of pH to altering SC properties. In particular, our experiments are designed to separately examine pH effects upon the protein and lipid components of the SC across a range of pH values. Two strongly ionizable surfactants, sodium dodecyl sulphate (SDS) and sodium laureth (lauryl ether) sulphate (SLES), which do not exhibit pH-dependent chemistry in the pH range investigated, were used in the current study. In addition, all experimental systems were buffered to ensure constant pH and ionic strength. The pH-dependence of SC swelling, in the presence and absence of surfactants was investigated using a relatively new and sensitive imaging technique, optical coherence tomography (OCT). This technique has the potential to be used *in vivo* to measure SC swelling [26–28]. Standard Fourier transform–infrared (FT-IR) spectroscopy techniques were employed in the current work to measure changes in SC lipid fluidity after treatment at different pH values, with and without surfactants.

Materials

Laboratory grade sodium dodecyl sulphate (SDS) was purchased from Sigma Chemicals Co. 'Steol CS 330'™, a sodium lauryl ether (3EO) sulphate (SLES), was obtained from Stephan Chemicals Co. pH of the solutions was adjusted using pH buffers. Specifically, potassium hydrogen phthalate–HCl buffer was used to maintain pH 4.0. NaOH–potassium dihydrogen Phosphate buffer was used to obtain pH 6.5. Borax + NaOH was used to buffer pH 10 solutions. Buffer concentration was adjusted to a constant value of 0.006 M representing ionic strength for those experiments conducted at or below the critical micelle concentration (cmc). The cmc of SDS and SLES in 0.006 M buffer solutions were 0.15% (5.2 mM) and 0.05% (~1.2 mM), respectively. The stratum corneum was from a full thickness female Yucatan by-product piglet skin purchased from Sinclair Research Centre Inc., MO. The stratum corneum was separated using a modified trypsin separation procedure [29].

Methods

Optical coherence tomography: SC swelling

Swelling measurements were carried out both in the presence and absence of surfactants. All solutions were prepared as wt.% solutions. Solutions were buffered at pH values 4, 6.5 and 10. Sections of SC were cut into ~4 × 4 mm sections and placed into the bottom of the wells of a 96-well, non-culture-treated microtiter plate. They were held in place with rhinestone settings. 270 μL of each surfactant solution was added to the wells (four replicates/surfactant concentration/pH). The microtiter plates were incubated for 5 or 21 h at 37 °C. The increase in swelling was determined by measuring the increase in thickness of the section by using Optical Coherence Tomography (OCT, Case Western Reserve University). The thickness of swollen SC samples was determined from the OCT images. Resolution of the OCT technique is about 10 μm. Thickness of dry corneum could not be determined using OCT because of excessive reflection. Therefore, OCT was used only to determine the thickness of corneum treated with buffer or surfactant solutions. Thickness of dry porcine corneum as determined by interferometry is in the range of 20–30 μm.

FT-IR spectroscopy: lipid fluidity

Stratum corneum (SC) samples were soaked in buffer or buffered surfactant solution for a period of ~16 h at room temperature. The SC samples were then rinsed and dried to remove all excess water. Dried SC samples were sandwiched between CaF_2 infrared windows and placed in a Harrick temperature controlled transmission cell for FT-IR spectroscopy studies. Spectra were collected using a Nicolet Magna 750 FT-IR spectrometer equipped with a broad-band N_2 cooled MCT detector and kept under continuous dry air purge. Spectra were collected every 3 °C from 30 to 99 °C and analyzed off-line using GRAMS software. SC lipid conformational order (i.e. bilayer fluidity) was determined from the peak position of the symmetric CH_2 stretching mode of the lipid chains. The precise position of this band was determined by generating second derivative spectra. Changes in CH_2 frequency are directly correlated with the number of trans/gauche rotamers in hydrocarbon chains and therefore with the fluidity of biological membranes [30, 31].

Some preliminary studies were carried out on the feasibility of measurement of zeta potential of homogenized SC samples. These samples were cut into small pieces and treated with appropriate buffer/surfactant solutions for desired length of time. Samples were then homogenized using a tissue homogenizer to reduce their size further. Homogenized samples were filtered using a 5 μm cut off Millipore filter. The

zeta potential was measured using a commercial instrument, Zeta Plus (manufactured by Brookehaven Instruments Corp., Holtsville, NY), based on laser Doppler light scattering technique. It measures both the charge and size of the particulate materials.

Results

Stratum corneum swelling – OCT measurements

Figure 1 displays representative OCT images of SC after 21 h soaking at pH 4, 6.5 and 10. The thickness of SC samples soaked in different pH buffered solutions is plotted in Fig. 2; it clearly shows that SC swelling increases with increasing pH. These results under controlled ionic strength conditions demonstrate that there is a direct pH effect on SC swelling.

Figures 3–6 show the relative swelling of SC (scaled to that in buffer solution at pH 6.5) at two time points of swelling, 5 and 21 h, and for three concentrations of the surfactants SDS and SLES. These figures further demonstrate that SC swelling is highest at pH 10 in all cases, and also suggest an additional surfactant effect. Figure 7, which includes surfactant concentrations above the cmc, shows a clear trend for an increased swelling in the presence of SDS at all pH levels investigated. A similar trend is suggested for the milder surfactant, SLES, in Fig. 8.

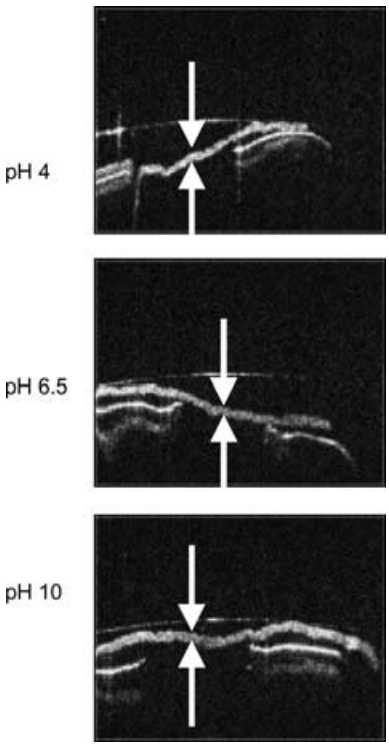

Figure 1 Optical coherence tomography (OCT) images of stratum corneum (SC). Arrows show the position and thickness of the corneum.

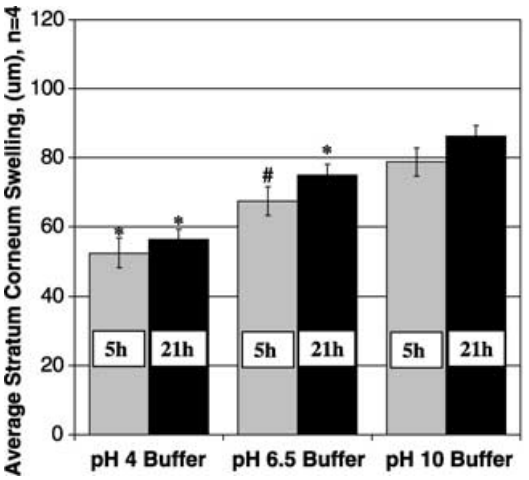

Figure 2 pH-dependence of swelling of stratum corneum (SC) in buffered solutions at 5 and 21 h. Buffer strength 0.006 M. (*) Shows significance at the $P < 0.05$ level compared to pH 10. (#) Shows significance at the $P < 0.1$ level compared to pH 10.

Preliminary studies of SC zeta potential measurements (Fig. 9) indicate that in the presence of SLES a significant increase in negative charge is observed at pH 4 and 6.5 and even at 10 at high surfactant levels. This would lend support to the idea that both surfactant adsorption and increased pH raise the charge of SC proteins, consistent with the observed increased swelling.

It is interesting that at high pH and in the presence of higher concentrations of surfactant, swelling can also induce significant mechanical stresses, manifested in curling of SC samples (see Fig. 10).

Lipid behaviour – FT-IR spectroscopy

Typical FT-IR spectrum of SC at different temperatures is shown in Fig. 11. The methylene (CH_2) stretching frequency, an indicator of lipid conformation/fluidity [30, 31], is also plotted in this figure. An increase in (CH_2) stretching frequency with increase in temperature shows the increasing fluidity of the lipid region at high temperatures.

Figure 12 displays the thermotropic behaviour of the (CH_2) stretching frequency, arising from SC lipids in samples soaked at pH 4, 6.5, and 10. The transition temperature (T_m), defined as the mid-point of the

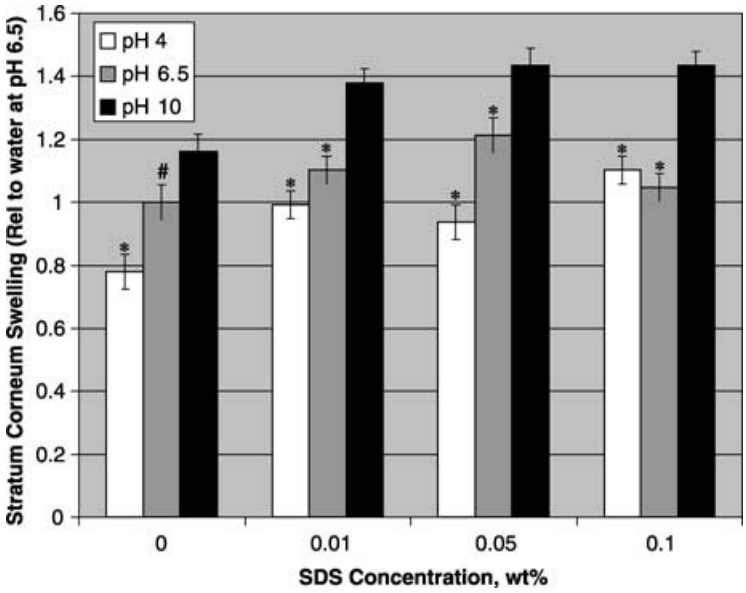

Figure 3 Stratum corneum (SC) swelling after 5 h of exposure to sodium dodecyl sulphate (SDS) solutions of various concentrations at three different pH values. Buffered systems (0.006 M buffer), $n = 4$. (*) Shows significance at the $P < 0.05$ level compared to pH 10. (#) Shows significance at the $P < 0.1$ level compared to pH 10.

thermotropic curve, undergoes a shift from ordered rigid bilayers to fluid (liquid crystalline) bilayers and increases ~3 °C (70–73 °C) between SC soaked at pH 4 versus 6.5. A much greater increase in T_m to ~87 °C is observed for SC treated at pH 10. This figure demonstrates clearly that the fluidity of SC lipids can be impaired at high pH. Therefore, high pH can be detrimental to the organization of SC lipids as well as SC protein structure (increased swelling, see Fig. 2).

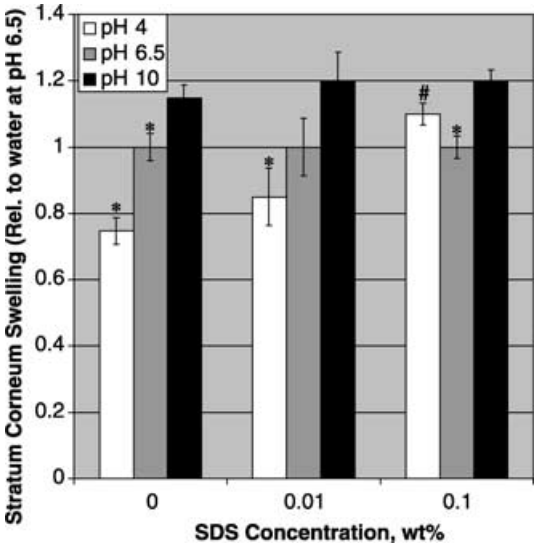

Figure 4 Stratum corneum (SC) swelling after 21 h of exposure to sodium dodecyl sulphate (SDS) solutions of various concentrations at three different pH values. Buffered systems (0.006 M buffer), $n = 4$. (*) Shows significance at the $P < 0.05$ level compared to pH 10. (#) Shows significance at the $P < 0.1$ level compared to pH 10.

Figure 13 shows that surfactants can increase SC lipid stiffness. It is interesting to note that exposure to SDS at pH 6.5 causes an increase in stiffness of similar magnitude to that obtained for surfactant-free pH 10 buffer solution. In Fig. 14, we show the results of a systematic study of the effects of pH and surfactant concentration on the transition temperature, T_m. As can be clearly seen, the lipid transition shifts to higher temperatures with increasing pH. Moreover, the effect is further increased by surfactants.

Discussion

The current experiments were specifically designed to address the question of whether pH has a direct role in altering SC physical properties. The results provide novel and unambiguous evidence that pH has a direct role in changing SC properties, and in particular, reveals that both SC proteins and lipids are affected. In addition, our results clearly indicate that surfactants can add to the effect of pH.

Swelling of the SC is known to be influenced by local swelling of keratin structures and subject to electrostatic factors [12, 32]. It is expected that SC swelling will increase with pH given that keratin has an IEP of pH 4.5–5. As the pH increases above keratin's IEP, the protein will acquire more net charge resulting in an expansion of its structure. The extent of swelling will be determined by the pH-dependent charge of the protein and the concentration of

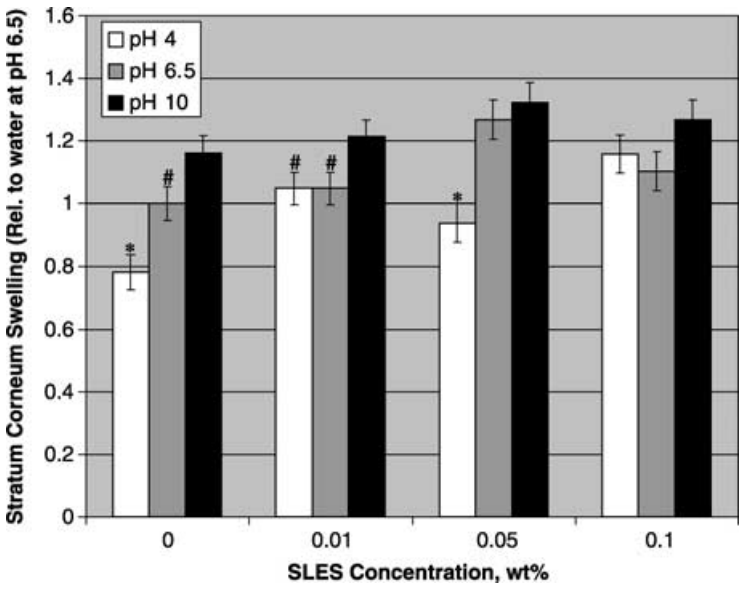

Figure 5 Stratum corneum (SC) swelling after 5 h of exposure to sodium laureth (lauryl ether) sulphate (SLES) solutions of various concentrations at three different pH values. Buffered systems (0.006 M buffer), $n = 4$. (*) Shows significance at the $P < 0.05$ level compared to pH 10. (#) Shows significance at the $P < 0.1$ level compared to pH 10.

electrolyte. Maximum swelling will be limited by the nature of the permanent cross-linking within the SC structure.

In addition to pH, the net charge of keratin can be affected by adsorption of surfactants and it is well known that anionic surfactants bind significantly to keratin. In general, it is well known that anionic surfactants can also bind significantly to proteins well above their IEP [33]. This process can be expected to lead to an overall increased charge on the protein and cause further swelling.

Regarding the connection between pH and the lipid organization, several factors probably play a role. The increase in SC lipid T_m at pH 10 may be a result of a rearrangement of lipids in the lamellar bilayers producing a more cohesive and rigid structure. As the effective pK_a of fatty acids within the SC bilayers is ~pH 7, there should be increased electrostatic (and altered hydrogen bonding) interactions above that pH as the fatty acids become ionized. A rearrangement of the complex hydrogen bonding network involving both SC fatty acids and ceramides

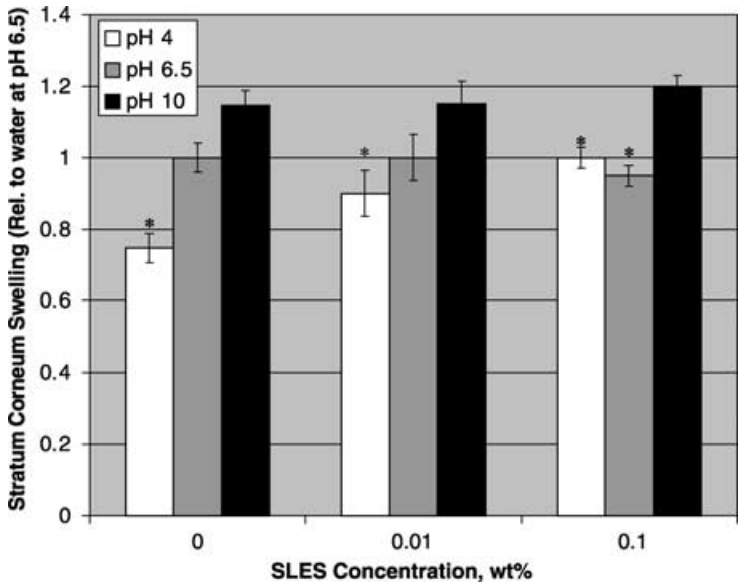

Figure 6 Stratum corneum (SC) swelling after 21 h of exposure to sodium laureth (lauryl ether) sulphate (SLES) solutions of various concentrations at three different pH values. Buffered systems (0.006 M buffer), $n = 4$. (*) Shows significance at the $P < 0.05$ level compared to pH 10. (#) Shows significance at the $P < 0.1$ level compared to pH 10.

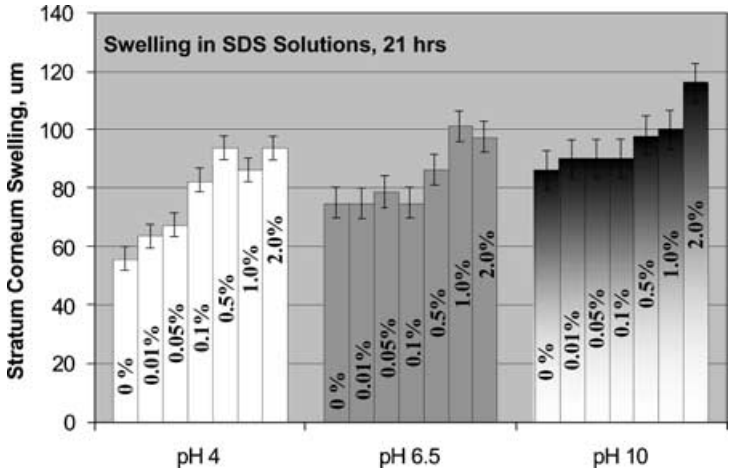

Figure 7 Stratum corneum (SC) swelling after 21 h of exposure to sodium dodecyl sulphate (SDS) solutions of various concentrations at three different pH values (percentages of SDS are shown in the figure). Swelling increases with increase in SDS concentration at all three pH values.

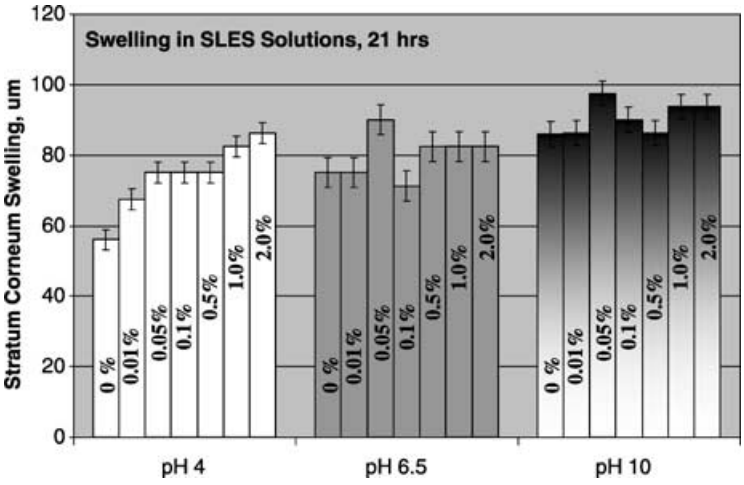

Figure 8 Stratum corneum (SC) swelling after 21 h of exposure to sodium laureth (lauryl ether) sulphate (SLES) solutions of various concentrations at three different pH values (percentages of SLES are shown in the figure).

could certainly increase T_m and fundamentally alter the physical properties of the SC lipid matrix. At pH values of 4 and 6.5 such effects would be much less as the majority of SC fatty acids are protonated. A second, and potentially compounding, factor in altering SC physical properties could be the selective removal of more fluid lipids from the SC lipid matrix, especially at surfactant levels above their cmc. This would result in an increase in T_m of the remaining lipids within the SC lipid matrix.

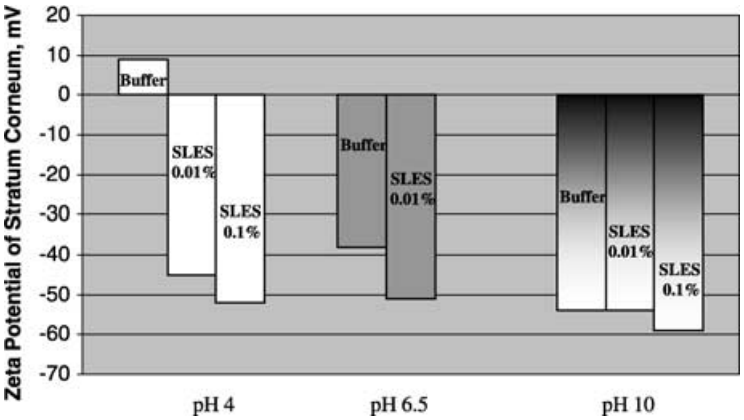

Figure 9 Zeta Potential of stratum corneum (SC) at three different pH values in the presence and absence of 0.01 and 0.1% sodium laureth (lauryl ether) sulphate (SLES). In the presence of SLES a significant increase in negative charge is found at pH 4 and 6.5. The observed increase in negative charge even at pH 10 in the presence of SLES suggests interaction of anionic surfactant even when the corneum is highly negatively charged.

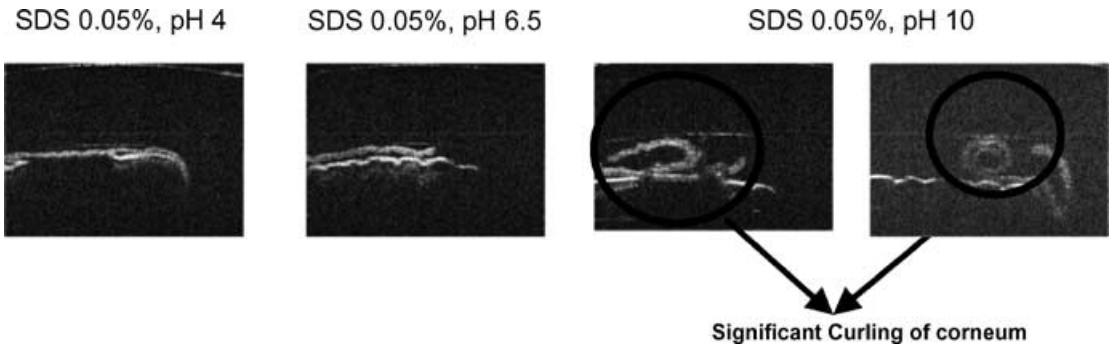

Figure 10 Optical coherence tomography (OCT) Images of stratum corneum (SC) after treatment with sodium dodecyl sulphate (SDS) at 0.05% level (21 h, 37 °C). SDS at pH 10 shows significant curling.

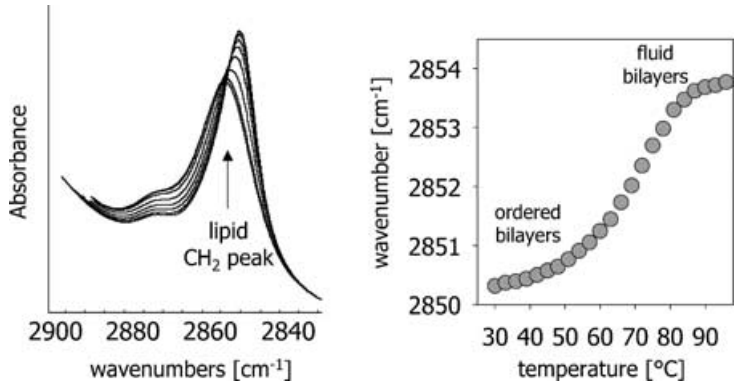

Figure 11 Typical FT-IR spectra of stratum corneum (SC) in the $-CH_2-$ stretching region. Changes in lipid conformation, i.e. fluidity, can be directly measured from frequency shifts in lipid CH_2 stretching peak. Spectra shown are at 30–93 °C (every 9 °C)

It is worth noting that several proposed models of the SC lipid matrix stress both the non-equilibrium nature of SC lipid organization and the importance of the heterogeneous composition and organization in providing the required SC physical properties, including sufficient pliability [34–36]. It is, therefore, quite clear that our observed, significant alteration in SC lipid conformational order (T_m) at pH 10 would significantly alter the SC lipid barrier function.

We believe that our new results could have important implications for understanding the differences in the irritation potential of surfactant types and the role of alkaline pH. It is generally accepted that skin

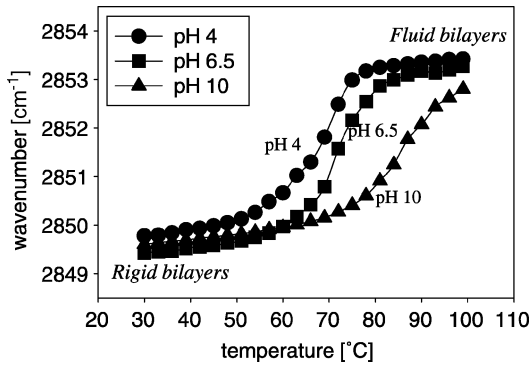

Figure 12 Change in methylene stretching frequency as a function of temperature in stratum corneum (SC) treated with pH 4, 6.5 and 10 buffers. Lipid T_m increases with increase in pH.

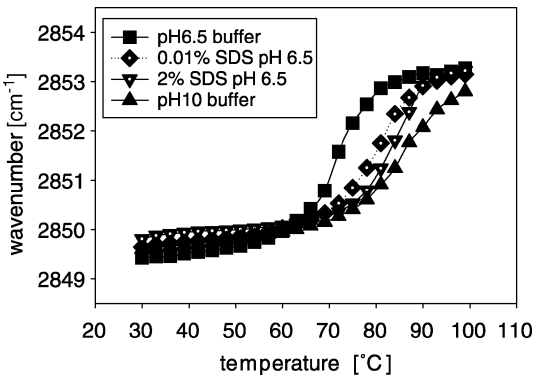

Figure 13 $-CH_2-$ stretching frequency as a function of temperature at pH 6.5, and 10 along with the data for 0.01 and 2% sodium dodecyl sulphate (SDS) at pH 6.5. Results show an increase in lipid stiffness after exposure to pH 10 buffer similar to effect of SDS at pH 6.5.

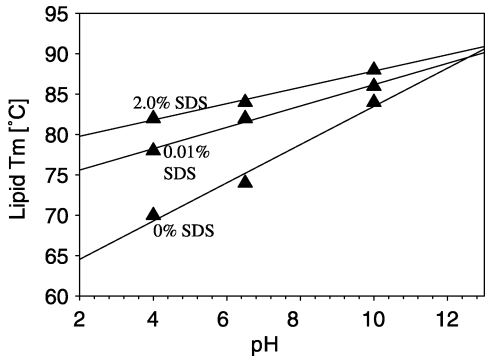

Figure 14 Change in T_m of stratum corneum (SC) samples obtained from FT-IR spectra of SC treated with sodium dodecyl sulphate (SDS) solutions at two concentrations. Also included is the data for the three pH buffers at pH 4, 6.5 and 10. Increase in T_m shows that lipid rigidity increases with pH. At a given pH, SDS increases lipid rigidity further.

irritation by surfactants is caused by penetration of surfactants into living epidermis and the subsequent disruption of epidermal cells [15, 21]. Factors that enhance the penetration of surfactants can be expected to increase surfactant-induced irritation. Thus, a swollen corneum will allow increased penetration of the surfactant into deeper layers. The ability of a surfactant to swell the corneum is an indication of its ability to enhance its own penetration into deeper layers and disrupt the cells in the living layer. This is consistent with the established correlation between the ability of surfactants to swell the corneum and its irritation potential. If the swelling occurs by other mechanisms such as increase in the protein negative charge because of high-solution pH, penetration of surfactants can also be expected to be enhanced under these conditions. Thus, direct effect of pH 10 by itself on the corneum could contribute to increased surfactant irritation. Changes in lipid layers at pH 10 may also have an impact on irritation in that their increased rigidity may make them more vulnerable to cracking and debonding from the corneocytes and thereby permitting penetration of irritants.

Conclusion

This study demonstrates that there is a direct effect of pH on SC protein swelling and lipid rigidity. Both are observed to be greater at pH 10 than at pH 6.5. The study also shows an additional, concentration-dependent effect of surfactants on both protein swelling and lipid organization.

Acknowledgements

The authors wish to acknowledge the contributions of T. Patel for help with experiments, M. Matzke for help with the statistical analysis of the data and Dr I. R. Scott for helpful discussions.

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