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PAS-FTIR and FT-Raman qualitative characterization of sodium dodecyl sulfate interaction with an alternative stratum corneum model membrane

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The interaction of the surfactant sodium dodecyl sulfate with the stratum corneum (SC) of shed snake skin from *Bothrops jararaca*, used as a model membrane, was characterized qualitatively by FT-Raman and infrared photoacoustic (PAS-FTIR) spectroscopy, used as analytical tools. Surfactant solutions were 50.0 $g \cdot l^{-1}$ and 2.34 $g \cdot l^{-1}$ with treatment intervals of 4, 8 and 12 h. The employment of FT-Raman and PAS-FTIR indicated increased hydration of the SC with alteration of the tissue topography. The interaction of the SC with surfactant was increased by the tape-stripping process. The consequent exposure of the internal layers of the tissue intensified the effect of the anionic surfactant, indicating that this layer acted as an additional barrier.

1. Introduction

Surfactants are often used to modify the physical stability of a number of topical pharmaceutical dosage forms and such substances have an effect on the permeability of various biological membranes, including the skin. For this reason they can increase the penetration of some other components of pharmaceutical preparations (López et al. 2000).

As a fundamental stage of research into the penetration of active substances through the skin, the selection of *in vitro* models presents an important question. The ideal situation is to employ human skin for *in vitro* assessment of the permeation characteristics of drugs, although this model membrane presents limitations such as insufficient availability and problems concerning storage. Experimental animals, synthetic membranes and three-dimensional cultures, as skin equivalents, have been utilized as alternative membranes. There has been recent interest in the use of shed snake skins as an alternative model membrane (Rigg and Barry 1990; Turunen et al. 1993; Schmook et al. 2001; Widler et al. 2002; Ngawhirunpat et al. 2006).

Shed snake skin suggests itself as pure stratum corneum possessing barrier properties similar to human skin. The samples are obtained without sacrifice of the animals, are easy to store and do not suffer microbiological deterioration, and also this model membrane addresses the question of experimental ethics with animals and humans and is ecologically sound (Itoh et al. 1990).

Raman (FT-Raman) and infrared photoacoustic (PAS-FTIR) spectroscopy are useful analytical tools for the study of the alterations provoked in the structure and organization of the stratum corneum by enhancers. Knowledge of the characteristic spectrum of biomolecules that compose the stratum corneum tissue, such as proteins and lipids, allows the evaluation of the interactions of those sites with enhancers, identified by modifications to typical bands, involving intensity changes, band shifting and broadening.

FT-Raman identified characteristic spectra for the stratum corneum, as following: symmetric and asymmetric CH_2 stretching at 2883 and 2852 cm^{-1}, respectively, from hydrocarbons of lipid chains, as well C–C stretching at 1126, 1082 and 1062 cm^{-1}; another C–C stretching typically occurs at 1031 cm^{-1}. The C–H stretching at 3060 cm^{-1}, at least most of it, is from α-keratin (Williams et al. 1993; Anigbogu et al. 1995).

The FT-Raman spectra from human and shed snake skin stratum corneum are similar. These tissues show comparable constituents, but in different relative amounts. Some differences were observed, such as an absence of C–S stretching at 644 and 620 cm^{-1} and a C–H stretching at 2975 cm^{-1}, in the spectral region of 2931–2883 cm^{-1}, from the shed snake skin (Williams and Barry 1994).

This research work focused on the evaluation and qualitative characterization of the interaction of the surfactant sodium dodecyl sulfate with the stratum corneum of shed snake skin from *Bothrops jararaca*, used as a model membrane, employing FT-Raman and PAS-FTIR techniques.

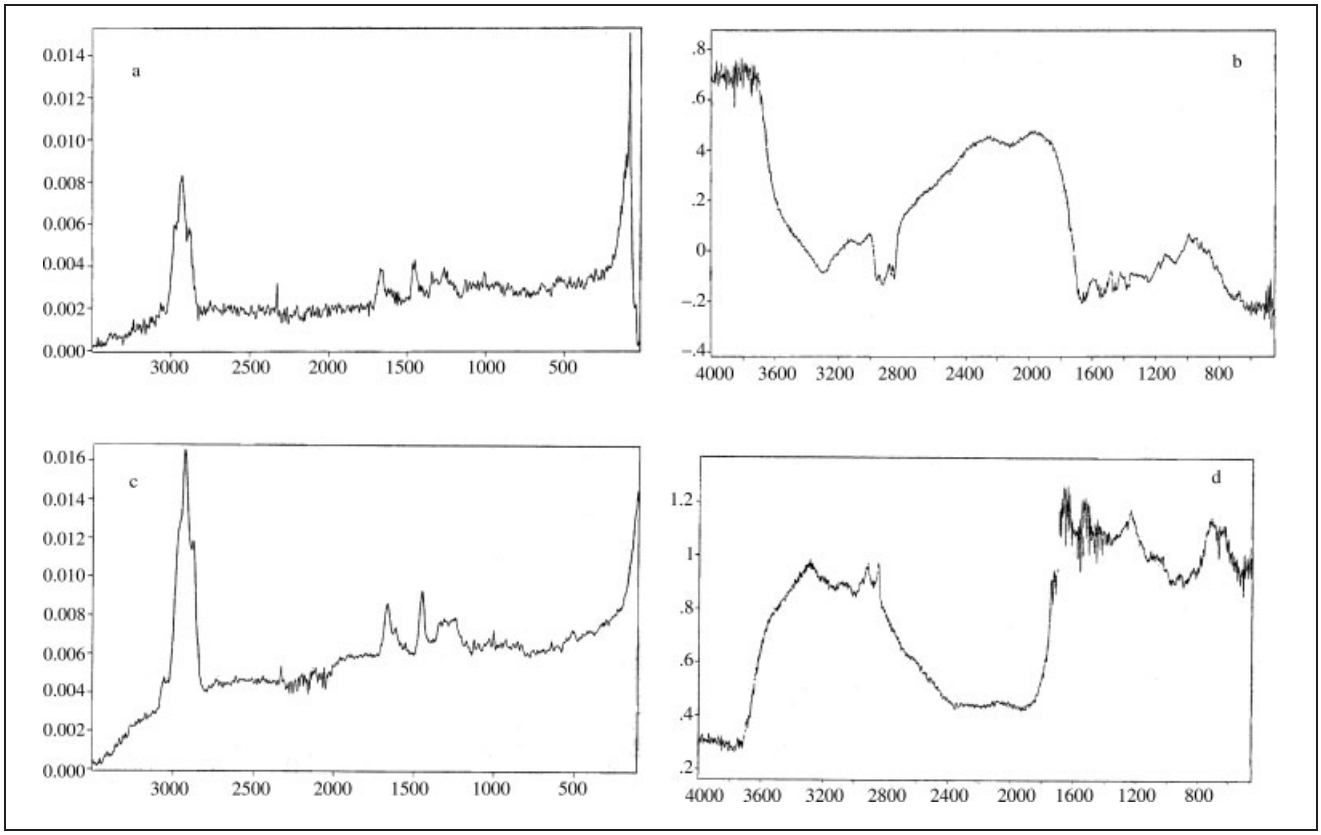

Fig. 1: FT-Raman (a, c) and PAS-FTIR (b, d) spectra of SC model membrane from *Bothrops jararaca* in water for 8 h. (a) and (b) intact SC; (c) and (d) tape-stripped samples

2. Investigations, results and discussion

Anionic surfactants interact with the SC principally through linkage with the protein fraction (keratin) by electrostatic and hydrophobic bonding (Scheuplein and Ross 1970; Rhein et al. 1986). At neutral pH, a condition maintained in this study, keratin possesses negative residual charge; interactions with anionic surfactants occur with the hydrophobic portions of both the keratin and the surfactant, exposing the negatively charged hydrophilic fraction of the molecule, which acts as a water bonding site (Breuer 1979).

As controls, SC samples in the intact and tape-stripped states were immersed in distilled water for 8 h. Spectra are shown in Fig 1.

The Raman spectra obtained with the SC model membrane treated with SDS 50.0 $g \cdot l^{-1}$ for 4 and 8 h (Fig. 2) showed an elevated signal intensity at 3100–2700 cm^{-1} when compared with the control sample treated with water, while the 8 h treatment resulted in a more intense signal, demonstrating that modifications of the topography of the SC occurred. The interaction level of the surfactant with the SC model membrane depended, besides other factors, on the contact period (Ananthapadmanabhan et al. 1996).

The 3100–2700 cm^{-1} signal is characteristic of the stretching vibration of CH from CH_3 and CH_3 symmetric and asymmetric lipids. The elevated Raman signal after SDS treatment possibly reflected the interaction of the anionic surfactant with keratin, since the hydrophobic chain of the SDS molecule (C12) exhibits absorption bands of symmetric and asymmetric CH_2 stretching at the same wavelengths as the lipids.

The interaction of the anionic surfactant with the SC samples, without penetrating the internal layers, could explain

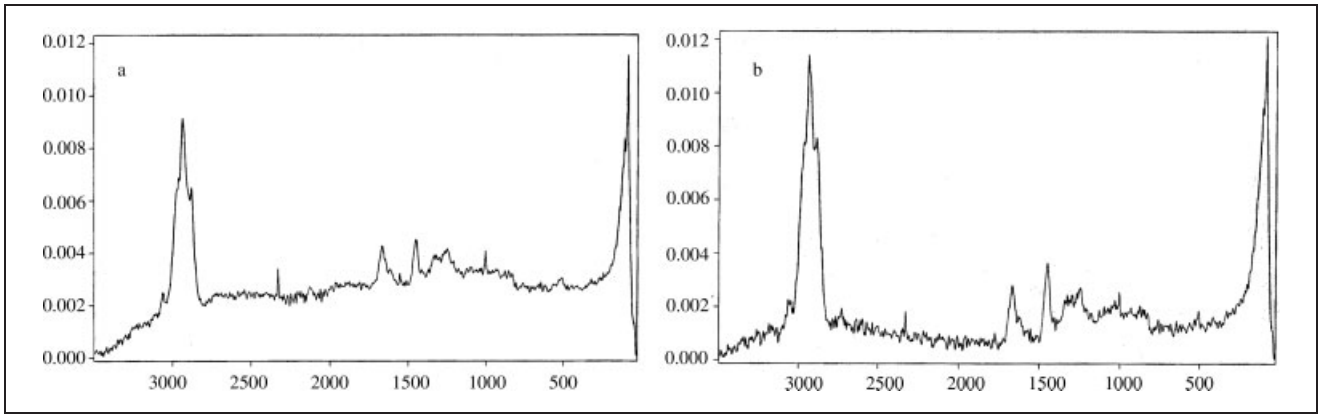

Fig. 2: FT-Raman spectrum of SC model membrane from *Bothrops jararaca* treated with SDS 50.0 $g \cdot l^{-1}$ for (a) 4 h and (b) 8 h

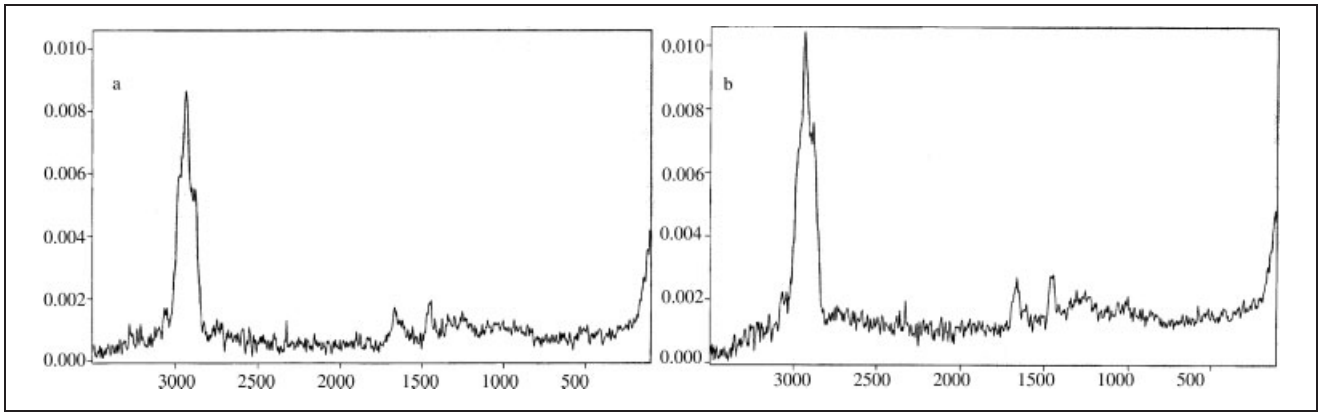

Fig. 3: FT-Raman spectrum of tape-stripped SC model membrane from *Bothrops jararaca* treated with SDS (a) 50.0 g · l^{-1} and (b) 2.34 g · l^{-1} for 12 h

the absence of water afflux to the tissue, as was expected on treatment with SDS. The additional layer of β-keratin of the SC of shed snake skins from *Bothrops jararaca* would be the potential reason for the superficial interaction with SDS (Williams and Barry 1994).

Although the FT-Raman technique showed a low sensitivity to the presence of water, the increase in water content was observed indirectly through the alterations in the superficial topography of the SC, once dilated of the higher water content. This behavior was also observed by Baby et al. (2006a), who employed differential scanning calorimetry (DSC) to characterize the interaction of anionic surfactant with a stratum corneum model membrane from shed snake skin, which identified an elevation of the temperature that caused water loss and keratin denaturation from 130 to 140 °C. This occurrence indicated an increase of water content in the tissue.

After the tape-stripping process, the SC of shed snake skins from *B. jararaca* treated with solutions of SDS at 50.0 g · l^{-1} and 2.34 g · l^{-1} for 12 h showed a reduction in the Raman signal in the range from 2800–3000 cm^{-1}, which was more intense for the samples treated with the solution above the CMC, as shown in Fig. 3.

The reduction of Raman signal intensity is related to a lower density of chemical groups that disperse light, present per unit area of surface, indicating possible expansion of the SC decurrently to the water content of the tissue, which caused the increase in volume of the samples. This effect was observed markedly in the SC treated with the higher concentration of SDS (50.0 g · l^{-1}), suggesting that not only the monomers of the surfactant were interacting with the SC, but also the micellar species. The tape-stripping process removed the superficial layers of the tissue and facilitated the interaction of the surfactant with the deeper α-keratin layer and the lipid matrix of the SC samples (Scheuplein and Ross 1970; Rhein et al. 1986).

The FT-Raman results permitted the observation that the supplementary layer of β-keratin in the SC of shed snake skin acted as an additional barrier to the penetration of external substances (Itoh et al. 1990; Rigg and Barry 1990; Williams and Barry 1994).

The PAS-FTIR spectra obtained for the SC of shed snake skins from *B. jararaca* treated with 50.0 g · l^{-1} SDS solution for 4 and 8 h (Fig. 4) did not suffer significant alterations in the region of 3600–3300 cm^{-1}.

The 8 h treatment caused an intensification of the signal at 2920–2850 cm^{-1}, the stretching region of lipid CH, that identified the presence of water in the superficial layer of the SC samples, detected through the C12 chain of the SDS and confirming the results obtained with the FT-Raman technique. The contact time has been shown to be a meaningful parameter in the process of the interaction of SDS with keratin (Ananthapadmanabhan et al. 1996).

PAS-FTIR of tape-stripped SC samples treated with 50.0 g · l^{-1} and 2.34 g · l^{-1} (Fig. 5) SDS solutions showed enhanced hydration identified by the broad bands in the region 3600–3300 cm^{-1}. Both concentrations gave similar results, indicating that, even though the interaction with the surfactant involved the monomer species that is smaller and penetrates through the SC, micelles may have acted as reservoir of monomers, keeping their concentration in the solution constant (Vaddi et al. 2001). After removing the superficial layers of the SC samples by the tape-stripping process, the interaction of SDS with the

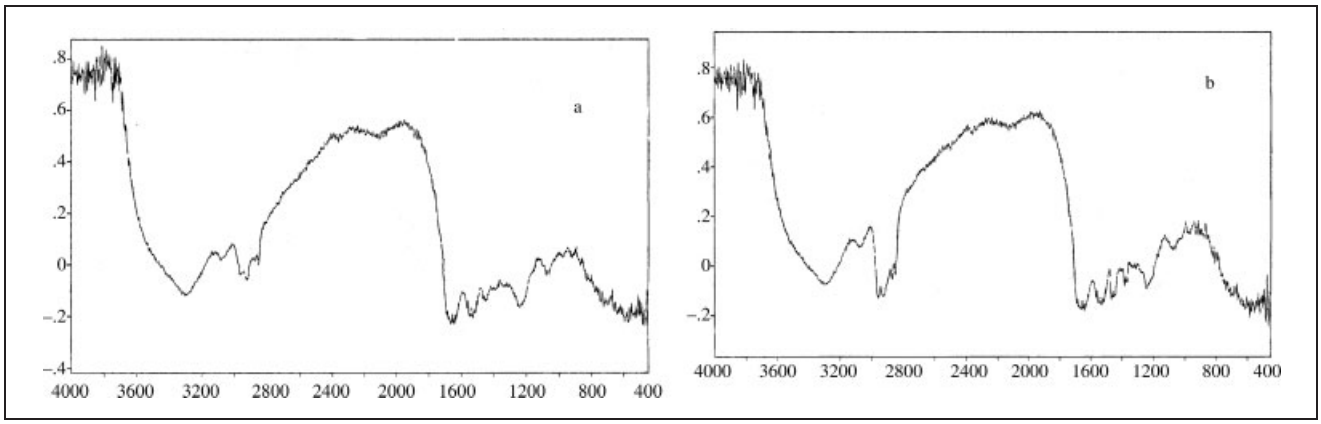

Fig. 4: PAS-FTIR spectrum of SC model membrane from *Bothrops jararaca* treated with SDS 50.0 g · l^{-1} for (a) 4 h and (b) 8 h

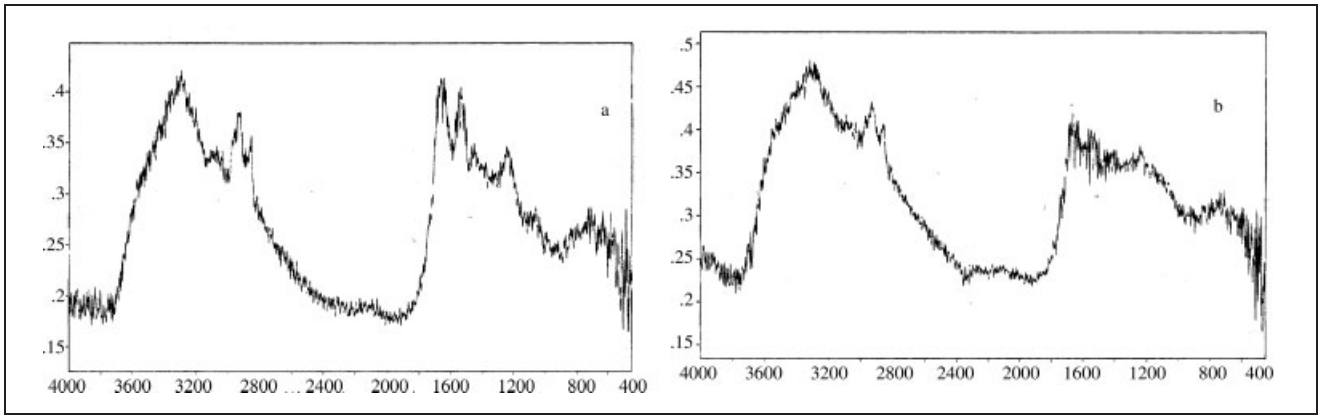

Fig. 5: PAS-FTIR spectrum of tape-stripped SC model membrane from *Bothrops jararaca* treated with SDS (a) 50.0 $g \cdot l^{-1}$ and (b) 2.34 $g \cdot l^{-1}$ for 12 h

internal protein region (α-keratin enclosed by the lipid matrix) was intensified when compared with intact SC samples.

SDS adsorption to the protein portion of the SC samples intensified the PAS-FTIR bands at 2920–2850 cm^{-1}, which is related to the stretching vibrations of SC lipid CH coinciding with stretching vibration of the anionic surfactant dodecyl chain. No alterations were detected in the 1700–1600 cm^{-1} amide I band with respect to the keratin conformation, indicating that the SDS did not cause modifications to the protein molecule, which would be one of the mechanisms of action proposed for the interaction of surfactants as enhancers (Rieger 1985; Walters et al. 1988; Castellano et al. 2000).

3. Experimental

3.1. Chemicals

Sodium dodecyl sulfate (95.80% – Stephanol ME-Dry) was purchased from Stepan Química Ltd. (São Paulo, SP, Brazil). The surfactant sample has anionic properties. Mono-hydrated citric acid was obtained from Anidrol Produtos Químicas Ltd. (São Paulo, SP. Brazil). Purity of materials was as stated by the suppliers. All chemicals were of pharmaceutical grade obtained from commercial sources and were used as received, without any further purification.

3.2. Surfactant solutions

Aqueous solutions of surfactant were 50.0 and 2.34 $g \cdot l^{-1}$ of active material giving concentrations above and below the critical micellar concentration (CMC). The pH of the solutions was adjusted to 6.5 to 7.0 with 10.0% (w/v) citric acid solution in deionized water, at room temperature (25.0 ± 1 °C), when required. The CMC value for SDS is 8.2×10^{-3} $mol \cdot l^{-1}$ (Lodén, 1990; Baby et al., 2006a).

3.3. Preparation of SC model membranes from Bothrops jararaca

3.3.1. Whole SC model membrane

Ventral portions of shed snake skins were obtained from *Bothrops jararaca* (Viperidae family), kindly given by Instituto Butantã (São Paulo, SP, Brazil). SC model membranes were cut and washed in distilled water at room temperature (25.0 ± 1 °C), followed by immersing the samples in surfactant solution (concentration above the CMC – 50.0 $g \cdot l^{-1}$). The times of contact of the samples with the solution were 4 and 8 h, and at the end of those periods, the SC model membranes were dried using quantitative filter paper (CAAL n. 1541) with soft compressure.

3.3.2. Tape-stripped SC model membrane

Ventral portions of shed snake skins from *B. jararaca* were cut and washed in current distilled water at room temperature (25.0 ± 1 °C). Samples were re-hydrated in distilled water by immersion in a Petri dish for 1 h, followed by drying with quantitative filter paper, as described elsewhere (Baby et al. 2006b). The dried samples were left at room temperature (25.0 ± 1 °C) for 30 min. The tape-stripping procedure was performed once with Transpore™ adhesive tape (100 × 4.5 m – 3M, São Paulo, SP, Brazil) on the upper layer of the samples under gentle pressure to mechanically remove the superficial cells of the SC model membrane from *B. jararaca* (Baby et al. 2006b). The tape-stripped samples were placed in contact with the surfactant solutions of 2.34 (below the CMC) and 50.0 $g \cdot l^{-1}$ for 12 h and after this time, the SC model membranes were dried with quantitative filter paper.

3.4. FT-RAMAN

A Bruker RFS 100/S Raman spectrometer with OPUS software was used. Samples were analyzed under the following conditions: laser power – 250 mW; number of co-additions – 256; resolution – 4 cm^{-1}; and spectral range – 3500 to 200 cm^{-1}. Measurements were performed with three replicates.

3.5. PAS-FTIR

Samples were cut large enough to fill the whole area of the photoacoustic cell (MTEC 200) and covered with a metal device to avoid movement of the samples. A flow of helium was used for 2 min to remove water and CO_2 molecules. A rubber composite was used as standard. Conditions were: resolution – 4 cm^{-1}; number of co-additions – 64; spectral range – 400 to 4000 cm^{-1}; and velocity of mobile mirror – 0.05 cm s^{-1}. Prior to analysis, the spectrometer was evacuated and the measurements were in three replicates. Results were analysed with the BOMEM PCDA program.

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