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International Journal of Pharmaceutics 317 (2006) 7–9
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Rapid Communication

Evaluation of the interaction of surfactants with stratum corneum model membrane from *Bothrops jararaca* by DSC

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Received 8 March 2006; received in revised form 12 April 2006; accepted 12 April 2006
Available online 19 May 2006

Abstract

The interaction of surfactants sodium dodecyl sulfate (SDS), cetyl trimethyl ammonium chloride (CTAC) and lauryl alcohol ethoxylated (12 mol ethylene oxide) (LAE-12OE) was evaluated on the stratum corneum (SC) of shed snake skins from *Bothrops jararaca*, used as model membrane, and thermal characterized by differential scanning calorimetry (DSC). Surfactant solutions were employed above of the critical micellar concentration (CMC) with treatment time of 8 h. The SDS interaction with the SC model membrane has increased the characteristic transition temperature of 130 °C in ≈10 °C for the water loss and keratin denaturation, indicating an augmentation of the water content. Samples treated with CTAC have a decrease of the water loss temperature, while, for the LAE-12OE treated samples, changes on the transition temperature have not been observed.

Keywords: Differential scanning calorimetry; Sodium dodecyl sulfate; Cetyl trimethyl ammonium chloride; Lauryl alcohol ethoxylated; *Bothrops jararaca*

1. Introduction

Human skin acts as an excellent barrier controlling the water loss and other constituents from the internal medium of the organism and, simultaneously, preventing the absorption of substances from the environment. The upper layer of the skin, the epidermis, specially the stratum corneum, represents the major barrier to the penetration of those chemicals (Anigbogu et al., 1995; Barry, 2001).

Surfactants are often used to enhance the physical stability of many topical pharmaceutical dosage forms and cosmetic products. These categories of substances also act over the permeability of diverse biological membranes, including the skin. They may elevate the penetration rate of some other components present on pharmaceutical preparations, acting as enhancers (Park et al., 2000; López et al., 2000).

The selection of in vitro model assay represents a fundamental step to the research of the penetration of active substances through the skin (Schmook et al., 2001). The ideal situation is the employment of the human skin. Although, this model membrane presents limitations such as insufficient availability and problems concerning the storage (Rigg and Barry, 1990).

Recently interest is being given on the use of shed snake skins, as model membrane (Turunen et al., 1993; Widler et al., 2002). The shed snake skin presents itself as a pure stratum corneum possessing a barrier property similar to the human skin. The samples are obtained without the sacrifice from the animals, are easy to store and do not suffer microbiological deterioration, besides, this model membrane contemplates the aspect of experimental ethic with animals and humans and is ecologically correct (Rigg and Barry, 1990; Itoh et al., 1990).

This research work focused on the interaction evaluation of the surfactants sodium dodecyl sulfate, cetyl trimethyl ammonium chloride and lauryl alcohol ethoxylated (12 mol ethylene oxide) with the stratum corneum of shed snake skin from *Bothrops jararaca*, used as model membrane, employing the differential scanning calorimetry.

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doi:10.1016/j.ijpharm.2006.04.006

2. Experimental section

2.1. Chemicals

All chemicals were of pharmaceutical grade obtained from commercial sources and used as received, without any further purification. The surfactants sodium dodecyl sulfate (95.80%: Stephanol ME-Dry), cetyl trimethyl ammonium chloride (50.52%: Ammonyx CETAC 50) and lauryl alcohol ethoxylated (12 mol ethylene oxide) (Polystep AE-128) were purchased from Stepan Química Ltd. (São Paulo, SP, Brazil). The surfactant samples have anionic, cationic and non-ionic properties, respectively. Mono-hydrated citric acid was obtained from Anidrol Produtos Químicas Ltd. (São Paulo, SP. Brazil). Purity of materials was as stated by the suppliers.

2.2. Surfactant solutions

Aqueous solutions of surfactants were $50.0\,g\,l^{-1}$ of active material for concentration above the CMC. The CMC values for SDS, CTAC and LAE-12OE were: 8.2×10^{-3}, 1.3×10^{-3} and 14.0×10^{-5} mol l^{-1}, respectively (Lodén, 1990). The pH of the solutions was adjusted at 6.5–7.0 with citric acid 10.0% (w/v) in deionized water.

The optimal pH value of the surfactant solutions was set at the range described above to avoid modifications on the profile adsorption of the surfactants with the SC samples. Variations of the pH values may affect the surfactant molecule, modifying the ionic sites, and the adsorption mechanism, which might occur through hydrogen bonds (Rosen, 1989; Martin, 1993).

2.3. Preparation of SC model membranes from B. jararaca

Ventral portions of shed snake skins were obtained from *B. jararaca* (Viperidae family), kindly given by Instituto Butantã (São Paulo, SP, Brazil). Ventral portions were chosen due to the adequate piece dimension, besides the uniformity and homogeneity of the segments that possess potential to be employed as alternative membrane to skin permeation studies. SC model membranes were cut and washed in current distilled water at room temperature ($25.0 \pm 1\,^{\circ}C$), followed by immersing the samples at the surfactant solutions (concentration above the CMC: $50.0\,g\,l^{-1}$) separately. The contact time of the samples with the solutions was 8 h, and at the end of the period, the SC model membranes were dried employing quantitative filter paper (CAAL n. 1541) under gentle compression.

2.4. Differential scanning calorimetry (DSC)

A DSC 10-TA instruments-differential scanning calorimeter was used to evaluate the interaction of the surfactants with pieces of SC model membrane of *B. jararaca*. The analysis was conducted in nitrogen atmosphere ($100\,ml\,min^{-1}$) between 10 and 160 °C with a constant rate of heating of $10\,^{\circ}C\,min^{-1}$. For all cases, pans of aluminium partially closed were used and the weight of the SC model membranes, previously prepared, were ≈2.0 mg. Transition temperatures were determined considering the minimum values of the endothermic peaks observed at the DSC curves.

3. Results and discussion

Alterations of the characteristic temperatures of the endothermic events were observed, such as modifications of the phase transition related to the lipid section and the dehydration and denaturation of the protein fraction (Lin et al., 1996).

The surfactants interact with the SC, providing alterations on the phase transition temperatures of the endothermic peaks. For the lipids, the temperature values reduce or suffer an attenuation of the peaks caused by the disorganization of the lamellar bilayers (Ashton et al., 1992; Shin et al., 2001).

Surfactant solutions with concentration above the CMC value present colloid structures denominated as micelles. This attribute is a fundamental property of the surfactants. Interfacial phenomena, such as detergency and solubilization, depend on the micelle formation (Rosen, 1989). Solubilization may be defined as the spontaneous dissolution of a substance in a solvent, obtaining a thermodynamically stable solution with reduced thermodynamic activity of the material solubilized. The solubilization occurs through micellar interactions of the surfactants in solution (Attwood and Florence, 1985; Miller and Neogi, 1985).

Isolated human SC presents four characteristic endothermic temperature transitions: at 40 °C—lipid bilayers transition to crystalline state for a gel-like structure; at 75 and 85 °C – transition of gel-like lipids of the membrane to a liquid state; and at 105 °C – representing the dehydration and keratin denaturation of the protein fraction of the SC. During the last transition temperature, some water must be present at the sample to show this occurrence (Ashton et al., 1992; Golden et al., 1986; Golden et al., 1987; Leopold and Lippold, 1994).

In the DSC curve of control, SC treated with distilled water immersion for 8 h, represented in Fig. 1, not all characteristic endothermic transition peaks for the lipids were observed,

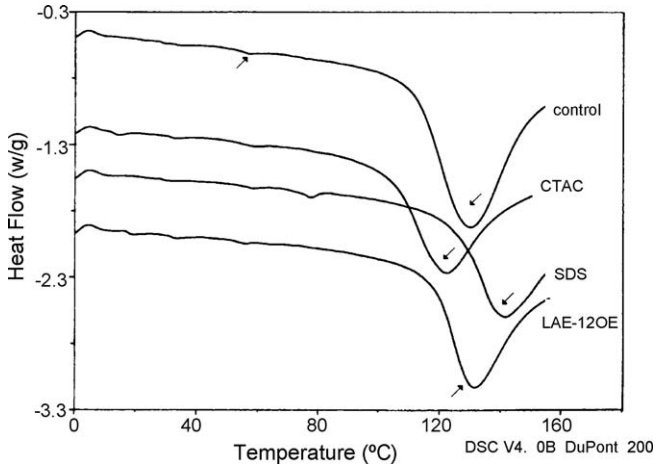

Fig. 1. DSC curves of the SC model membranes from *B. jararaca* treated with sodium dodecyl sulfate (SDS), cetyl trimethyl ammonium chloride (CTAC) and lauryl alcohol ethoxylated (12 mol ethylene oxide) (LAE-12OE) solutions of $50.0\,g\,l^{-1}$ for 8 h.

although, a weak endothermic transition could be identified at ≈58 °C. The peak related to the endothermic transition involving the dehydration and keratin denaturation occurred clearly at 130 °C.

On the SC of shed snake skins from *B. jararaca*, treated with SDS solution, an endothermic transition at 58 °C could be observed similar to the control sample. The temperature, which provoked water loss and keratin denaturation presented an elevation from 130 to 140 °C. This behavior indicated an increase of the water content at the sample decurrently from the interaction of the anionic surfactant with the protein portion of the SC model membrane.

Anionic surfactants interact with the SC providing an increase on the local water concentration with consequent swelling and expansion of the thickness of the tissue (Breuer, 1979; Scheuplein and Ross, 1970). The elevation of the aqueous content at the SC reduces the transition temperature as a result of the disorder caused on the lipid chains. A possible interpretation for this effect is the presence of water on the polar region of the lipid bilayers, reducing the cohesion among them (Golden et al., 1986).

Cationic surfactants, like wise the anionic, may interact with the SC through electrostatic and hydrophobic links. Although the interaction of non-ionic surfactants with the protein fraction of the SC is also possible through hydrophobic interactions with keratin, due to the absence of a charge of the molecule, the interaction occurs probably with the lipid fraction of the tissue (Waters et al., 1988; Ananthapadmanabhan et al., 1996).

Samples treated with CTAC showed a decrease of the water loss temperature, indicating a lower aqueous content on the SC membranes, while, for the LAE-12OE treated samples, changes on the transition temperature have not been observed. The absence of alterations compared with the control probably signified that the non-ionic surfactant presented a low level of interaction with the lipid bilayers and the keratin which did not affect the water content of the SC model membrane.

4. Conclusions

The SDS promoted an elevation of the water content of the tissue, while the CTAC has dehydrated the SC model membrane. The LAE-12OE presented a not significant interaction with the samples.

Acknowledgement

This work was supported by National Council for Scientific and Technological Development (CNPq), foundation linked to the Ministry of Science and Technology (MCT), to support Brazilian research and CAPES.

References

Ananthapadmanabhan, K.P., Yu, K.K., Meyers, C.L., Aronson, M.P., 1996. Binding of surfactants to stratum corneum. J. Soc. Cosmet. Chem. 47, 185–200.

Anigbogu, A.N.C., Williams, A.C., Barry, B.W., Edwards, H.G.M., 1995. Fourier transform Raman spectroscopy of interactions between the penetration enhancer dimethyl sulfoxide and human stratum corneum. Int. J. Pharm. 125, 265–282.

Ashton, P., Walters, K.A., Brian, K.R., Hadgraft, J., 1992. Surfactant effects in percutaneous absorption I. Effects on protein and lipid structure of the stratum corneum. Int. J. Pharm. 87, 261–264.

Attwood, D., Florence, A.T., 1985. Surfactant Systems: their Chemistry, Pharmacy and Biology. Chapman and Hall, New York, pp. 1–29.

Barry, B.W., 2001. Novel mechanism and devices to enable successful transdermal drug delivery. Eur. J. Pharm. Sci. 14, 102–114.

Breuer, M.M., 1979. The interaction between surfactants and keratinous tissues. J. Soc. Cosmet. Chem. 30, 41–64.

Golden, G.M., Guzek, D.B., Harris, R.R., McKie, J.E., Potts, R.O., 1986. Lipid thermotropic transitions in human stratum corneum. J. Soc. Invest. Dermatol. 86, 255–259.

Golden, G.M., McKie, J.E., Potts, R.O., 1987. Role of stratum corneum lipid fluidity in transdermal drug flux. J. Pharm. Sci. 76, 25–28.

Itoh, T., Xia, J., Magavi, R., Nishihata, T., Rytting, J.H., 1990. Use of shed snake skin as model membrane for in vitro percutaneous penetration studies: comparison with human skin. Pharm. Res. 7, 1042–1047.

Leopold, C.S., Lippold, B.C., 1994. An attempt to clarify the mechanism of the penetration enhancing effects of lipophilic vehicles with differential scanning calorimetry (DSC). J. Pharm. Pharmacol. 47, 276–281.

Lin, S.Y., Duan, K.J., Lin, T.C., 1996. Microscopic FT-IR/DSC system used to simultaneously investigate the conversion process of protein structure in porcine stratum corneum after pretreatment with skin penetration studies. Methods Find. Exp. Clin. Pharmacol. 18, 175–181.

Lodén, M., 1990. The simultaneous penetration of water and sodium lauryl sulfate trhough isolated human skin. J. Soc. Cosmet. Chem. 41, 227–233.

López, A., Llinares, F., Cortell, C., Herráez, M., 2000. Comparative enhancer effects of Span 20 with Tween 20 and Azone on the in vitro percutaneous penetration of compounds with different lipophilicities. Int. J. Pharm. 202, 133–140.

Martin, A., 1993. Physical Pharmacy: Physical Chemical Principals in the Pharmaceutical Sciences, 4th ed. Lea & Febiger, Philadelphia, pp. 512–542.

Miller, C., Neogi, P., 1985. Interfacial Phenomena-Equilibrium and Dynamic Effects. Marcel Dekker, New York, pp. 140–180.

Park, E.S., Chang, S.Y., Hahn, M., Chi, S.C., 2000. Enchancing effect of polyoxyethylene alkyl ethers on the skin permeation of ibuprofen. Int. J. Pharm. 209, 109–119.

Rigg, P.C., Barry, B.W., 1990. Shed snake skin and hairless mouse skin as a model membrane for human skin during permeation studies. J. Invest. Dermatol. 94, 235–240.

Rosen, M.J., 1989. Surfactant and Interfacial Phenomena. John Wiley & Sons, New York, pp. 33–205.

Scheuplein, R., Ross, L., 1970. Effects of surfactants and solvents on the permeability of epidermis. J. Soc. Cosmet. Chem. 21, 853–873.

Schmook, F.P., Meingassner, J.G., Billich, A., 2001. Comparison of human skin or epidermis models with human and animal skin in in vitro percutaneous absorption. Int. J. Pharm. 215, 51–56.

Shin, S.C., Cho, C.W., Oh, I.J., 2001. Effects of non-ionic surfactants as permeation enhancers towards piroxican from the polaxamer gel through rat skins. Int. J. Pharm. 222, 199–203.

Turunen, T.M., Buyuktimkin, S., Buyuktimkin, N., Urtti, A., Paronen, P., Rytting, J.H., 1993. Enhanced delivery of 5-fluorouracil through skin by two new transdermal penetration enhancers. Int. J. Pharm. 92, 89–95.

Waters, K.A., Walker, M., Olejnik, O., 1988. Non-ionic surfactant effects on hairless mouse skin permeability characteristic. J. Pharm. Pharmacol. 40, 525–529.

Widler, N., Sigrist, A., Gafner, F.M., 2002. Lipid analysis and transepidermal water loss in snakes. IFSCC Mag. 5, 24–29.