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In Vivo Skin Permeation of Sodium Naproxen Formulated in Pluronic F-127 Gels: Effect of Azone® and Transcutol®

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ABSTRACT The objective of this study was to determine the penetration of sodium naproxen, formulated in Pluronic F-127 (PF-127) gels containing Azone® and Transcutol® as penetration enhancers, through human skin in vivo. It was found that the combination of Azone® and Transcutol® in PF-127 gels enhanced sodium naproxen penetration, with enhancement ratios of up to two fold compared with the formulation containing only Transcutol®. These results were confirmed by TEWL and ATR-FTIR spectroscopy, suggesting a synergic action for Azone® and Transcutol®. Because of the thermo-reversible behavior of Pluronic gels, the influence of the components added to the gel formulations on viscosity, as a function of temperature, was also studied.

KEYWORDS Sodium naproxen, Transcutol®, Azone®, PF-127 gel, Tape stripping, Skin permeation

INTRODUCTION

The main obstacle to topical and transdermal administration is the barrier formed by the intercellular lipid matrix of the stratum corneum (SC). Therefore, much effort has been devoted to the development of transdermal drug delivery systems able to overcome the SC barrier in a local and temporal manner.

Sodium naproxen is the salt of a potent nonsteroidal anti-inflammatory drug (NSAID) used for a variety of inflammatory conditions (Shing & Roberts, 1994). Like other NSAIDs, the most common side effect of oral naproxen is gastrointestinal irritation. Thus, the possibility of delivering naproxen through the skin for local inflammation is appealing. Penetration enhancers and appropriate vehicles/cosolvents are needed, because otherwise, only small amounts of naproxen pass through the skin (Valjakka-Koskela et al., 1998; Van den Ouweland et al., 1989).

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Combination of a traditional NSAID, such as sodium naproxen, with Azone® and Transcutol® offers a means to produce a more effective topical pharmaceutical product. The chemical structures of Azone®, Transcutol® and sodium naproxen are shown in Fig. 1. Azone® is a well-documented penetration enhancer (Afouna et al., 2003; Hadgraft et al., 1996; López et al., 2000; Williams & Barry, 2004). It appears to act by reducing the SC diffusional barrier, because it inserts itself into the lipid domain located in the intercellular channels. Consequently, the fluidity of the lipid microenvironment is increased and diffusion improves. Furthermore, Azone® has also been shown to be capable of forming ion pairs with anionic drugs (Hadgraft et al., 1985). Transcutol® is a powerful solubilizing agent used in several dosage forms, and it is attractive as a penetration enhancer due to its lack of toxicity, biocompatibility with the skin, miscibility with polar and nonpolar solvents, and optimal solubilizing properties for a number of drugs (Barthélémy et al., 1995). Recent studies have shown that Transcutol® significantly increases the percutaneous penetration of various active substances, particularly when used in combination with suitable cosolvents (Ganem-Quintanar et al., 1997; Lee et al., 2003; Mura et al., 2000; Touitou et al., 1994; Watkinson et al., 1991).

Surface-active polyoxyethylene-polyoxypropylene-polyoxyethylene (POE-POP-POE, Pluronics®) block copolymers are widely used in pharmaceutical formulations as solubilizing and wetting agents. Some Pluronics® possess special properties that make them particularly suitable for use in the formulation of topical dosage forms; these include their relatively low toxicity and their ability to form clear gels in aqueous media (Gilbert et al., 1986). Pluronic F-127 is of particular interest, because concentrated solutions (>20% w/w) of the copolymer are transformed from low viscosity transparent solutions to solid gels on heating to body temperature. This suggests that when poured onto the skin or injected into a body cavity, the gel preparation will form a solid artificial barrier and a sustained release depot (Koller & Buri, 1987; Suh et al., 1997).

This work focuses on the effects of Azone® and Transcutol® formulated in PF-127 gels, on the skin penetration of sodium naproxen in vivo. Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR/ATR) and transepidermal water loss (TEWL) were used to evaluate the effect of some of the components of the gel formulations on SC permeability properties. The viscosity of gel formulations over the 10–40°C range was also determined.

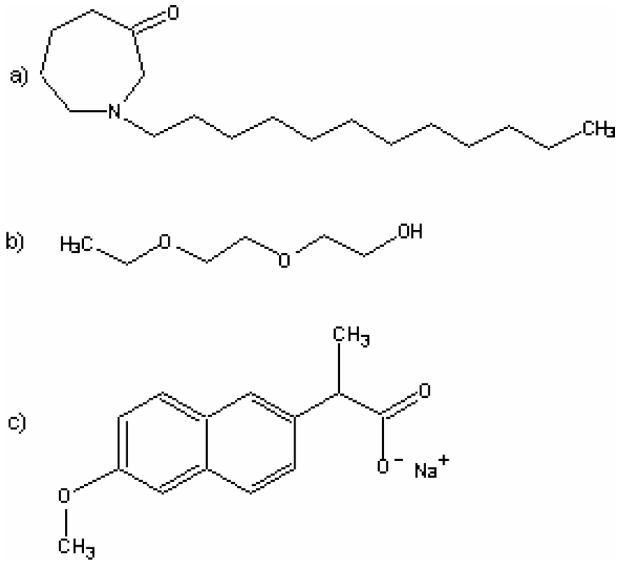

FIGURE 1 Chemical Structures of (a) Azone®, (b)Transcutol®, and (c) Sodium Naproxen.

MATERIALS AND METHODS

Materials

Sodium naproxen was kindly provided by Syntex (Mexico City). Azone® was a contribution from Whitby Research, Inc. (Richmond, USA) and Transcutol® (diethyleneglycol monoethyl ether) was a donation from Gattefossé (Noveon, Mexico). PF-127 was obtained from Sigma-Aldrich (Steinheim, Germany). Ethyl acetate (Fermont, Mexico) was used as a high-performance thin layer chromatography (HPTLC) developing solvent. Gel formulations were prepared with distilled water of Milli-Q quality (Millipore Corp., Bedford, USA).

Gel Formulations

Gels were prepared by using the "cold" method described by Schmolka (1972). Briefly, a weighted amount of PF-127 was slowly added to cold water over a period of 2–3 min with gentle mechanical stirring to make a 27% w/w mixture. PF-127 was then allowed to hydrate and disperse overnight at 4°C. On

complete dissolution of PF-127, the appropriate amounts of PF-127 gel, Transcutol®, or Azone®/Transcutol®, and sodium naproxen (3% w/v) were added to the cold solution under gentle stirring. The mixture was then incubated overnight at 30°C to facilitate the complete dissolution of the solutes. Table 1 shows the gel formulations prepared (all of them containing 3% w/v of sodium naproxen). The PF-127 percentage was adjusted to have similar viscosities among formulations within the temperature range 25–37°C. The formulation pH ranged from 6.1 to 7.5, ensuring that naproxen was fully ionized (pKa = 4.2). It was not possible to have a formulation containing only Azone® because of its very lipophilic nature; however, once Azone® was completely dissolved in Transcutol®, this mixture was easily incorporated into the PF-127 solution. The gels obtained were fully transparent homogeneous systems.

Viscosity Studies

Viscosity measurements of Pluronic gels were performed by placing 50 μL on a CAP 2000 Digital Viscometer plate (Brookfield, USA) at a temperature range from 10 to 40°C, at 50 rpm using no. 1 circular spindle. The following systems were evaluated: PF-127 gel (27% w/w), PF-127 gel containing Transcutol®, PF-127 gel containing Azone®/Transcutol®, and formulations I, II, and III.

Human Subjects

Six healthy volunteers (4 females and 2 males, aged 23–30 years, type 4 skin) with no history of dermatological disease participated in this study, after giving their written consent. The subjects were required to maintain the sites under investigation (the left and right ventral forearm) free from any application of cosmetic or pharmaceutical topical formulations for at least a week before and during the study.

In Vivo Skin Permeation

The subjects were dosed topically on the ventral forearm with either 5 mL (infinite dose) or 0.9 mL (enough to form a uniform film on the skin) of the formulations shown in Table 1, by means of a glass perfusion cell (12.56 cm^2). Each subject was treated with the complete array of formulations. At the end of the application period (90 min), the excess formulation was removed with a sterile gauze.

The sodium naproxen distribution across the SC was determined by tape stripping. Twenty sequential tape-strips were carried out by using Scotch® Book Tape No. 845 (3M, USA). The amount of sodium naproxen from each tape-strip was extracted in acetone:water (40:60) and quantified by HPTLC.

Analytical Method

Samples were analyzed for sodium naproxen content by HPTLC. The fluid extracted from the tapes was spotted on TLC plates (Alugram® Sil G/UV_{254}, Macherey-Nagel, Germany) by using the Automatic CAMAG TLC Sampler III (version 2.12, Switzerland). Standard solutions of increasing concentrations were applied for mass calibration. Separation was carried out in developing chambers (CAMAG, Switzerland) saturated at room temperature with ethyl acetate. The solvent front was allowed to migrate to 8 cm (the R_f obtained for sodium naproxen was 0.62).

The plates were dried at room temperature for 10 min and scanned with a CAMAG TLC Scanner 3 at 237 nm. The heights and areas of the peaks were obtained by using the CATS software (version 4.06). The assay was linear ($r^2 > 0.9973$) in the amount range 40–200 ng. The detection limit was of 20 ng.

Transepidermal Water Loss (TEWL) Measurements

To assess the effect of the formulations on skin barrier function, TEWL was measured with a

TABLE 1 Composition of PF-127 Gel Formulations

	Amount (% v/v)		
Constituents	Formulation I	Formulation II	Formulation III
PF-127 (27%)	100	75.2	73.5
Transcutol®	—	24.8	24.8
Azone®	—	—	1.7

Courage+Khazaka Tewameter TM 210® (Cologne, Germany). TEWL measurements were carried out by placing the probe gently on the skin application sites, and leaving it there until a constant value was established (≈3 min).

All measurements were performed in a single ventilated room with an ambient temperature of 23±2°C and relative humidity of 50–70%.

Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) Spectroscopy

Infrared spectra were recorded with a Shimadzu 8300 FTIR spectrophotometer equipped with a standard detector. An ATR-trapezoidal crystal (ZnSe, dimensions: $80 \times 10 \times 4$ mm with and incident angle of 45°) enabled horizontal positioning of the subject's forearm. The spectra obtained represent an average of 45 scans, collected at a resolution of 1 cm^{-1} and a sensitivity of 0.1 cm^{-1}.

The frequencies and intensities of the peaks assigned to the C-H_2 symmetric and asymmetric stretching vibrations of the SC lipid alkyl chains (2850 cm^{-1} and 2920 cm^{-1}, respectively) were examined.

RESULTS AND DISCUSSION

Viscosity Measurements

PF-127 gels exhibit an interesting reverse thermal gelation behavior. This characteristic has allowed PF-127 to be used as a carrier for several administration routes, such as oral, intranasal, vaginal, rectal, ocular, parenteral, and topical [e.g., DiBiase & Rhodes (1996), Jain et al. (1991), Li & Sung (2000), Miyazaki et al. (1984), Ryu et al. (1999)]. These gels can be applied as a solution at room temperature, gelling at body temperature and forming a depot from which the drug can be released. Gelation can be explained as a desolvation and swelling process of the copolymer to form cross-linked aggregates. In aqueous solution at low temperature, a hydration layer surrounds copolymer. However, as the temperature rises, the hydrophilic chains of the copolymer are desolvated as a result of breakage of the hydrogen bonds that have been established between the solvent and these chains. This phenomenon favors hydrophobic interactions among the polyoxypropylene domains and leads to gel formation (DiBiase & Rhodes, 1996; Gilbert et al., 1986). This behavior is clearly seen in Fig. 2 for a PF-127 solution (27% w/w), where an increase in the temperature produces an increase in the viscosity of the gel.

It is well documented (Koller & Buri, 1987) that the solutes added to the PF-127 solution will affect its rheological behavior. With this in mind, the viscosity of formulations I, II, and III (all of them containing sodium naproxen) was recorded and compared with a PF-127 solution and PF-127 solutions added with Transcutol® or Azone®/Transcutol®. As shown in Fig. 2, between 25 and 40°C, all the solutes decreased PF-127 gel viscosity in the following order: Azone®/Transcutol® > Transcutol® > formulation II ≥ formulation III > formulation I. It is known that the addition of acids or alcohols decreases the rigidity of Pluronic gels and increases the transition temperature, causing a reduction in viscosity values due to the formation of oxonium ions (Schmolka, 1974) and a reduction of the hydrophobic interactions, or by the formation of mixed micelles (BASF Wyandotte Corp; Industrial Chemical Group, 1973a,b; Schmolka, 1974). In this sense, Transcutol®, and in particular the Azone®/Transcutol® combination, result in a dramatic loss of viscosity. With the exception of formulation III, all the other systems presented the same profile. Formulation III showed an unexpected behavior, with high viscosity values at low temperatures and a rapid decrease in viscosity as the temperature was raised. This formulation is the only system that combines sodium

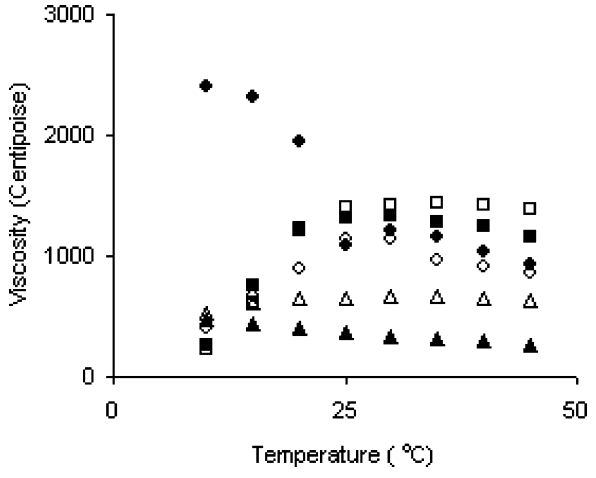

FIGURE 2 Viscosity Measurements (n=3) of a PF-127 Gel (27% w/v, □), Formulation I (■), Formulation II (◇), and III (◆) and Effect of Components (Transcutol 24.8% △, and Azone 1.7%/ Transcutol 24.8% ▲) on the Viscosity of the PF-127 Gel.

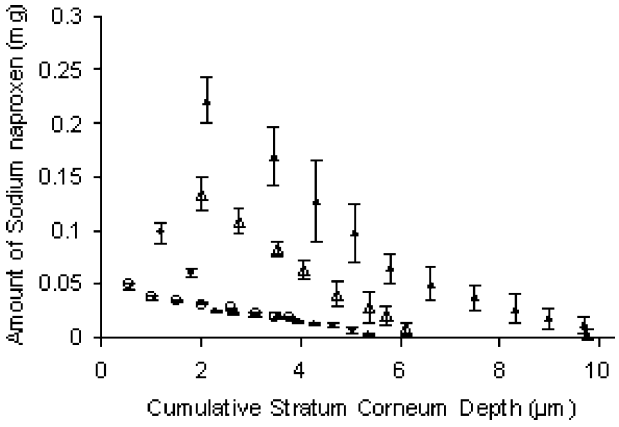

FIGURE 3 **Penetration Profiles Across Human Stratum Corneum of Sodium Naproxen Formulated in PF-127 Gels Applying an Infinite Dose (Formulation II ●, Formulation III ▲), and a Film (Formulation II ○, Formulation III △) (Mean ± SD; n = 6).**

naproxen and Azone®. Considering that formulation III has a pH of 6.1, it is very likely that both Azone® and sodium naproxen are ionized (protonated Azone® and sodium naproxen as a carboxylate). Therefore, an ionic interaction between Azone® and sodium naproxen could contribute to the increased viscosity at low temperatures. At temperatures above 25°C, the viscosity of formulation III decreased but was higher than that for the system combining Azone®/Transcutol without sodium naproxen. Moreover, it has been reported that the incorporation of mineral salts results in an increase in the viscosity of Pluronic gels (Koller & Buri, 1987); in this sense, sodium naproxen could act by counteracting the effect of Transcutol® or Azone®/Transcutol on the viscosity.

DiBiase & Rhodes (1991) previously emphasized the importance of viscosity on the release rate of drugs from PF-127 gels. Therefore, in the present work, the percentages of PF-127 gels and Transcutol® were adjusted to have similar viscosities among formulations I–III at physiological temperature (~37°C).

In Vivo Skin Permeation

The penetration profiles of sodium naproxen across the SC following passive diffusion from PF-127 gels are shown in Fig. 3. In formulation I (without Transcutol® and Azone®), sodium naproxen was not detected in the strips. Because it is ionized in the gel, this may contribute to its inability to permeate the skin. On the contrary, it was clearly confirmed that the presence of enhancers promoted sodium naproxen permeation. The inclusion of about 24.8% Transcutol® (formulation II) allowed sodium naproxen to penetrate into the skin. The enhancing ability of Transcutol® has been attributed to its ability to pass through the skin (Ganem-Quintanar et al., 1997) being incorporated into the multiple-lipid bilayers, thereby swelling the intercellular lipids (Godwin et al., 2002). This enhancer has also shown skin accumulation of topically applied compounds without a concomitant increase in transdermal permeation (Ritschel et al., 1991). The combination of Azone® and Transcutol® (formulation III) produced an approximately two- to three-fold increase in drug penetration with respect to formulation II, suggesting a synergistic effect between Azone® and Transcutol®. Synergy between these two enhancers has been shown for different substances such as prostaglandins (Watkinson et al., 1991) and cyanophenol (Harrison et al., 1996). On the other hand, it is well known that Azone® is capable of enhancing the permeation of naproxen by inserting itself into the lipid bilayers and, thus, creating a disruption in their stacking (Degim et al., 1999).

As indicated in Materials and Methods, all of the formulations were applied in two ways: as an infinite

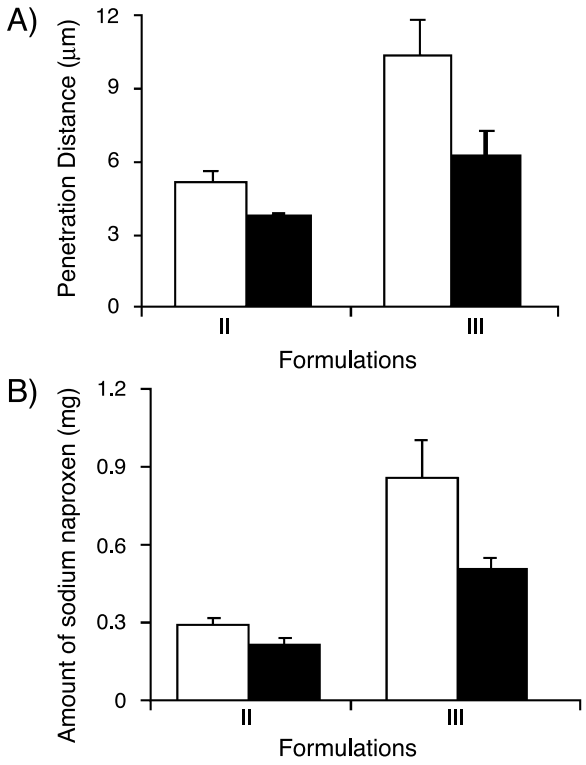

FIGURE 4 **Penetration Distance (A) and Total Amount of Sodium Naproxen Recovered in the Stratum Corneum (B) for Formulations II and III with an Infinite Dose (□) and by Applying a FIlm (■) (Mean ± SD; n = 6).**

dose and as a film. The calculation of the penetration distances and the total amount of permeated drug (Fig. 4) indicated that, irrespective of the application mode, formulation III always showed a greater penetration (in both depth and the total amount permeated) than formulation II. On the other hand, although the infinite dose favored a greater penetration than the film application, as shown in Fig. 4, the difference between the infinite dose and the film application is greater for formulation III. Therefore, to compare the total amount permeated and the penetration depth for these four systems (formulations II and III, infinite dose, and film), an analysis of variance was performed, followed by Tukey's test. Significant differences between formulation III-infinite dose and the other systems were found, regarding the total amount permeated ($F = 78.9$; $F_{0.05/2;3,20} = 3.1$) and the penetration depth ($F = 57.9$; $F_{0.05/2;3,20} = 3.1$). Summarizing, the deepest penetration (Fig. 4A) and the greatest amount permeated (Fig. 4B) were achieved with formulation III by applying an infinite dose.

TEWL Measurements

Under controlled environmental conditions, an elevated TEWL is predictive of altered barrier properties and has been used to verify skin integrity in vivo when a formulation is applied (Pu & Lyn, 1998; Tanojo et al., 1997). The TEWL mean value for the control site (without treatment) was 10.4 ± 1.1 g/h·m^2, which is well in agreement with previous studies of control readings in human skin (Fang et al., 2003; Takeuchi et al., 1995). Treatment of the skin with formulation II increased the value of TEWL to 20.6 ± 1.7 g/h·m^2, and with formulation III, TEWL reached 33.5 ± 2.6 g/h·m^2. These results correlate well with those found in penetration studies, where Transcutol® enhanced penetration of sodium naproxen, and a synergistic effect was proposed for the Azone®/Transcutol® mixture (formulation III). Furthermore, in addition to its ability to disrupt the structured lipids of the SC, Azone® has been reported to increase water retention in the SC lipid matrix (Takeuchi et al., 1995). These effects may have considerably contributed to the highest TEWL with formulation III compared with the other formulations and the control (Fig. 5).

The statistical analysis (one-way ANOVA, followed by Tukey's test) revealed a significant difference in water loss between formulations II and III and between these formulations and the control ($F = 215.5$, $F_{0.05/2;2,15} = 3.7$). No difference was found between formulation I and the control.

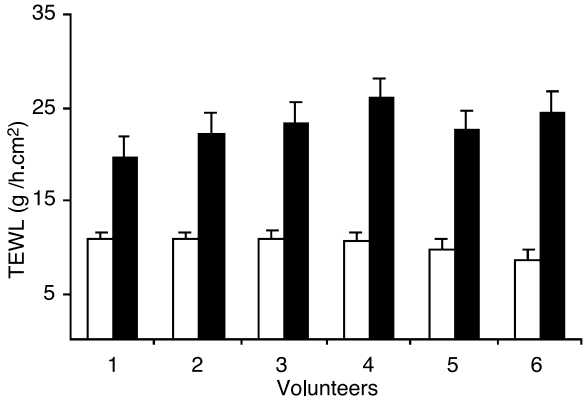

FIGURE 5 **Absolute TEWL (Each Formulation Value Minus Control Value) Values After Applying Sodium Naproxen Formulated in PF-127gels. Formulation II (□) and Formulation III (■) (Mean ± SD; n = 6).**

ATR-FTIR

With the exception of a few homonuclear molecules, all molecular species absorb infrared radiation. ATR-FTIR spectroscopy records the molecular vibrations of absorbing species. Although the number of possible vibrations is large with respect to the stratum corneum, the CH_2-stretching vibrations originating from the alkyl chain of the intercellular lipids have been the most widely studied. Two peaks near 2850 cm^{-1} (symmetric stretching vibration) and 2920 cm^{-1} (asymmetric stretching vibration) are good indicators of the conformational nature of the alkyl chains. Generally, a shift of these stretching vibrations toward higher wavenumbers is indicative of an increase in the gauche rotational isomers along the alkyl chains, and thus, of a greater motional freedom, commonly called "fluidization" (Naik & Guy, 1997). In this work, the effect of Azone® and Transcutol® included in formulations II and III was examined in vivo by ATR-FTIR.

The SC uptake of the hydrocarbon (H/C) base components in both formulations was monitored by ATR/FTIR, because the effect of the formulations' constituents produces a significant increase in the methylene (CH_2) group vibrations located at the 2850–2920 cm^{-1} region of the IR spectrum (Casal & Mantsch, 1984).

As shown in Fig. 6, a shift toward a higher wavenumber was observed for the asymmetric stretching vibration at 2850 cm^{-1} only in the case of the

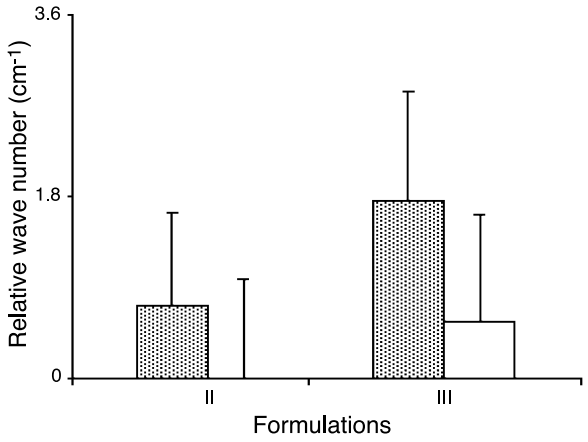

FIGURE 6 **Frequency Shifts of the C-H_2 Asymmetric (■) and Symmetric (□) Stretching Vibration of the Stratum Corneum Lipid Acyl Chains After Application of the Sodium Naproxen Gel Formulations (II and III) (Mean ± SD; n = 6).**

Azone®/Transcutol® mixture (formulation III). The statistical comparison (one-way ANOVA) of the data supported this finding. A significant difference was found for the symmetric band located at 2850 cm^{-1} ($F = 10.5$, $F_{0.05/2;2,15} = 3.7$) with respect to the control, when the skin was treated with formulation III. However, no difference was observed for the asymmetric band at 2920 cm^{-1} ($F = 0.9$, $F_{0.05/2;2,15} = 3.7$). As explained earlier, it is possible to associate these results with the fluidization of lipids and, consequently, with an increased permeability. These observations are in agreement with the results of penetration and TEWL presented above and confirm the enhancing ability of the Azone®/Transcutol® mixture in formulation III, suggesting a synergic relationship between the two enhancers.

CONCLUSIONS

The experiments demonstrated that the inclusion of an enhancer (Transcutol® or Azone®/Transcutol®) promotes sodium naproxen permeation across the skin. However, the Azone®/Transcutol combination appears to act synergistically. This fact was confirmed by TEWL and ATR-FTIR. A marked increase in TEWL and a shift toward a higher wavenumber for the symmetric stretching vibrations of the lipid alkyl chains were found when the two enhancers acted together.

Formulation III (with Azone®/Transcutol®) forms a transparent gel at skin temperature, with an adequate viscosity, allowing sodium naproxen to be delivered into the SC. These gels are interesting systems for the delivery of anti-inflammatory drugs, improving their action.

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REFERENCES

Afouna, M. I., Fincher, T. K., Zaghloul, A. A. A., & Reddy, I. K. (2003). Effect of Azone upon the in vivo antitumoral efficacy of cidofovir or acyclovir topical formulations in treatmeny/preventation of cutaneous HSV-1 infections and its correlation with skin target site free drug concentration in hairless mice. *International Journal of Pharmaceutics*, *253*, 159–168.

Barthélémy, P., Farah, N., & Laforet, J. P. (1995). Transcutol-product profile. *Gattefossé*, Technical bulletin, 1–10.

BASF Wyandotte Corp; Industrial Chemical Group (1973a). *The Wonderful World of Pluronic Polyols*. Catalog Card No. 70-150738, BASF Corp.

BASF Wyandotte Corp; Industrial Chemical Group (1973b). *Pluronic Polyols, Toxicity and Irritation Data*. Publication No. OS-3012, BASF Corp.

Casal, H. L., & Mantsch, H. H. (1984). Polymorphic phase behaviour of phospholipids membranes studied by infrared spectroscopy. *Biochimica et Biophysica Acta*, *779*, 381–401.

Degim, L. T., Uslu, A., Hadgraft, J., Atay, T., Akay, C., & Cevheroglu, S. (1999). The effects of Azone and capsaicin on the permeation of naproxen through human skin. *International Journal of Pharmaceutics*, *179*, 21–25.

DiBiase, M. D., & Rhodes, C. T. (1991). Investigations of epidermal growth factor in semisolid formulations. *Pharmaceutica Acta Helvetiae*, *66*, 165–169.

DiBiase, M., & Rhodes, C. (1996). Formulation and evaluation of epidermal growth factor in Pluronic-127 gel. *Drug Development and Industrial Pharmacy*, *22*(8), 823–831.

Fang, J.-Y., Leu, Y.-L., Hwang, T.-L., Cheng, H.-Ch., & Hung, Ch.-F. (2003). Development of sesquiterpenes from Alpnia oxyphylla as novel skin permeation enhancers. *European Journal of Pharmaceutical Sciences*, *19*, 253–262.

Ganem-Quintanar, A., Lafforgue, A. C., Falson-Rieg, F., & Buri, P. (1997). Evaluation of the transepidermal permeation of diethyleneglycol monoethylether and skin water loss. *International Journal of Pharmaceutics*, *147*, 165–172.

Gilbert, J. C., Hadgraft, J., Bye, A., & Brookes, L. (1986). Drug release from Pluronic F-127 gels. *International Journal of Pharmaceutics*, *32*, 223–228.

Godwin, D. A., Kim, N. H., & Felton, L. A. (2002). Influence of Transcutol CG on the skin accumulation and transepidermal permeation of ultraviolet absorbers. *European Journal of Pharmaceutics and Biopharmaceutics*, *53*, 23–27.

Hadgraft, J., Walters, K. A., & Wotton, P. K. (1985). Facilitated transport of sodium salicylate across an artificial lipid membrane by azone. *Journal of Pharmacy and Pharmacology*, *37*, 725–727.

Hadgraft, J., Peck, J., Williams, G. D., Pugh, J. W., & Allan, G. (1996). Mechanisms of action of skin penetration enhancers/retarders: Azone and analogues. *International Journal of Pharmaceutics*, *141*, 17–25.

Harrison, J. E., Watkinson, A. C., Green, D. M., Hadgraft, J., & Brain, K. (1996). The relative effect of Azone and Transcutol on permeant diffusivity and solubility in human stratum corneum. *Pharmaceutical Research*, *13*, 542–546.

Jain, N. K., Shah, B. L., & Taneja, L. N. (1991). Nasal absorption of metoprolol tartrate. *Indian Journal of Pharmaceutical Sciences*, *53*, 16–19.

Koller, C., & Buri, P. (1987). Propriétés e intérêt pharmaceutique des gels thermoréversibles à base de poloxamers et poloxamines. *STP Pharmaceutics*, *3*(2), 115–124.

Lee, T. W., Kim, J. Ch., & Hwangl, S. J. (2003). Hydrogel patches containing Triclosan for acne treatment. *European Journal of Pharmaceutics and Biopharmaceutics*, *56*, 407–412.

Li, H., & Sung, K. C. (2000). Carbopol/pluronic phase change solutions for ophthalmic drug delivery. *Journal of Controlled Release*, *69*, 379–388.

López, A., Llinares, I., Cortell, C., & Herráez, M. (2000). Comparative enhancer effects of Span®20 with Teewn®20 and Azone on the in vitro percutaneous penetration of compounds with different lipophilicities. *International Journal of Pharmaceutics*, *202*, 133–140.

Miyazaki, S., Takeuchi, S., Yokouchi, C., & Takada, M. (1984). Pluronic F-127 gels as vehicles for topical administration of anticancer agents. *Chemical and Pharmaceutical Bulletin*, *32*(10), 4205–4208.

Mura, P., Faucci, M. T., Bramanti, G., & Corti, P. (2000). Evaluation of transcutol as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations. *European Journal of Pharmaceutical Sciences*, *9*, 365–372.

Naik, A., & Guy, R. H. (1997). *Mechanisms of Transdermal Drug Delivery: Infrared Spectroscopy and Differential Scanning Calorimetric Investigations of the Stratum Corneum Barrier Function* (pp. 87–162). New York: Marcel Dekker, Inc.

Pu, Y., & Lyn, P. (1998). Water permeation barrier in isolated cutaneous newborn rat epidermis. *Journal of Pharmacological and Toxicological Methods*, *40*, 145–149.

Ritschel, W. A., Panchagnula, R., Stemmer, K., & Ashraf, M. (1991). Development of an intracutaneous depot for drugs. Binding, drug accumulation and retention studies, and mechanisms of depot. *Skin Pharmacology*, *4*, 235–245.

Ryu, J. M., Chung, S.-J., Lee, M.-H., Chang-Kook, K., & Chang-Koo, S. (1999). Increased bioavailability of propanolol in rats by retaining thermally gelling liquid suppositories in the rectum. *Journal of Controlled Release*, *59*, 163–172.

Schmolka, I. R. (1972). Artificial skin I. Preparation and properties of Pluronic F-127 gels for treatment of burns. *Journal of Biomedical Materials Research*, *6*, 571–582.

Schmolka, I. R. (1974). Pluronic® polyols in skin lotions. *Cosmética & Perfumaria*, *89*, 63–66.

Shing, P., & Roberts, M. S. (1994). Skin permeability and local tissue concentration of nonsteroidal anti-inflammatory drugs after topical application. *Journal of Pharmacology and Experimental Therapeutics*, *268*, 144–151.

Suh, H., Jun, H. W., Dzimianski, M. T., & Lu, G. W. (1997). Pharmacokinetic and local tissue disposition studies of naproxen following topical and systemic administration in dogs and rats. *Biopharmaceutics & Drug Disposition*, *18*(7), 623–633.

Takeuchi, Y., Yasukawa, H., Yamaoka, Y., Taguchi, K., Fukushima, S., Shimouaka, Y., Nishinaga, H., & Morimoto, Y. (1995). Behaviour of propylene glycol in dermis after treatment of rat intact skin surface with fatty acids, fatty amines or Azone dissolved in propylene glycol. *Biological & Pharmaceutical Bulletin*, *18*, 304–309.

Tanojo, H., Junginger, H. E., & Boddé, H. E. (1997). In vivo human skin permeability enhancement by oleic acid: transepidermal water loss and Fourier-transform infrared spectroscopy studies. *Journal of Controlled Release*, *47*, 31–39.

Touitou, E., Levy-Schaffer, F., Dayan, N., Alhaique, F., & Riccieri, F. (1994). Modulation of caffeine skin delivery by carrier design: liposomes versus permeation enhancers. *International Journal of Pharmaceutics*, *103*, 31–136.

Valjakka-Koskela, R., Kirjavainen, M., Mönkkönen, J., Urtti, A., & Kiesvaara, J. (1998). Enhancement of percutaneous absorption of naproxen by phospholipids. *International Journal of Pharmaceutics*, *175*, 225–230.

Van den Ouweland, F. A., Eenhoorn, P. C., Tan, Y., & Gribnau, F. W. J. (1989). Transcutaneous absorption of naproxen gel. *European Journal of Clinical Pharmacology*, *36*, 209–211.

Watkinson, A. C., Hadgraft, J., & Bye, A. (1991). Aspects of the transdermal delivery of prostaglandins. *International Journal of Pharmaceutics*, *74*, 229–236.

Williams, A. C., & Barry, B. W. (2004). Penetration enhancers. *Advanced Drug Delivery Reviews*, *56*, 603–618.