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Comparative enhancer effects of Span®20 with Tween®20 and Azone® on the in vitro percutaneous penetration of compounds with different lipophilicities

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Abstract

Sorbitan monolaurate (Span®20) was used in this study to analyze the influence of the polar functional group on the effects that non-ionic surfactants have on skin permeability. Its ethoxylate derivative polysorbate 20 (Tween®20) and Azone®, both with the same C12 alkyl chain as Span®20, were used for comparative purposes. We evaluated the relative potency of the three molecules as enhancers in the permeability of a series of compounds with lipophilicities ranging from log Poct = −0.95 to log Poct = 2.33. The influence of the enhancer concentration was also studied. For this purpose the epidermis of Wistar rat was pretreated with ethanolic solutions (1 and 5%, w/v) of each enhancer. Our results indicate that the nature of the enhancer head group greatly influences cutaneous barrier impairment. The enhancer concentration must also be taken into account, even though the influence of the concentration seems to depend on the lipophilicity of the penetrant assayed.

Keywords: Azone®; Span®20; Tween®20; Non-ionic surfactants; Enhancer concentration; Skin permeability

1. Introduction

Transdermal administration of many drugs is generally a problem because of the stratum corneum barrier. To reduce this diffusional barrier penetration enhancers which usually disrupt the highly ordered membrane structure can be added to formulations (Walters and Hadgraft, 1993). Ideally, these enhancers are pharmacologically inert and have an immediate but reversible effect on the stratum corneum (Barry, 1983).

As surfactants are used as physical stabilizing agents in many topical pharmaceutical formulations, the extent to which human skin is exposed to these chemicals becomes a relevant question. Moreover, it is well known that surfactants have effects on the permeability characteristics of several biological membranes, including skin (Florence et al., 1984), and for this reason they can enhance the skin penetration of other compounds

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present in the formulation. Non-ionic surfactants have long been recognized as those with the least toxicity and irritant potential. These properties make these compounds good candidates as potential penetration enhancers for use in transdermal delivery systems (Walters, 1990).

The hydrophobic portion of non-ionic surfactants usually consists of alkyl or acyl chains that are attached to a polar head group, which in many non-ionic surfactants is a polyoxyethylene chain. Many reports have dealt with the importance of the alkyl chain length in the potency of surfactants as penetration enhancers (Zaslavsky et al., 1978; Walters et al., 1981, 1983). From these reports, one can deduce that the strongest effects were observed with molecules that had a C12 alkyl chain. However, it is not yet clear how the polar head group influences the activity of surfactants as enhancers. For instance, reports on surfactant-induced alterations in the permeability of biological membranes for alkyl ether ethoxylates (Brij®) and nonyl phenol ether ethoxylates surfactant series indicate that the ethoxy chain length is important. In fact, the relation observed was parabolic relative to the degree of ethoxylation (Walters et al., 1981, 1983).

In contrast, a recent study involving several series of chemical enhancers has demonstrated that the potencies of the enhancers are essentially independent of the polar functional group, except for the n-alkyl-azacycloheptanones, which were four times more potent than the other chemical permeation enhancers (Warner et al., 1998).

Polysorbates are an important series of surfactants in pharmaceutical formulations. Cappel and Kreuter compared the enhancement potential of several polysorbates in transdermal penetration of methanol and octanol. Positive effects were observed only for methanol, for which the more lipophilic polysorbates, 21 and 81, alter the barrier properties of the skin to a greater extent than the hydrophilic analogs (Cappel and Kreuter, 1991).

The objective of our study was to add new experimental data regarding the influence of the polar functional group on the effects that non-ionic surfactants have on skin permeability. Sorbitan monolaurate (Span®20) was selected for this study because of its C12 alkyl chain and relatively high lipophilicity. Its ethoxylate derivative polysorbate 20 (Tween®20) and Azone®, both with the same C12 alkyl chain, were used for comparative purposes. Tween®20 has been reported to increase the skin penetration of several compounds (Shahi and Zatz, 1978; Sarpotdar and Zatz, 1986a,b) but not to have effects on others (Aungst et al., 1986). The effects of Azone® as an enhancer of different types of compounds are well known (Allan, 1995). In our study the relative potency of the three molecules as enhancers in the permeability of a series of compounds with lipophilicities ranging from log Poct = −0.95 to log Poct = 2.33 was evaluated. The influence of the enhancer concentration was also studied. For this purpose the epidermis of Wistar rat was pretreated with ethanolic solutions (1 and 5%, w/v) of each enhancer.

2. Materials and methods

2.1. Permeants

5-Fluorouracil (5-FU) (log Poct = −0.95; Leo et al., 1971), antipyrine (log Poct = 0.23; Hansch and Anderson, 1967), 2-phenyl ethanol (log Poct = 1.34; López et al., 1998) and 4-phenyl butanol (log Poct = 2.33; López et al., 1998) were all purchased from Sigma at >99% purity. The compounds were prepared as saturated solutions buffered to pH 6.2, with an excess of compound added to maintain saturation for the duration of the experiments.

2.2. Skin pretreatment with enhancers

Span®20 and Tween®20 were purchased from Sigma (>99% purity). Azone® was a gift from Whitby Research (USA). Enhancers were used as ethanolic solutions prepared at 1 and 5% (w/v). The epidermal membranes were pretreated with either 400 μl of phosphate-buffered saline (pH 6.2) as control I or 400 μl of ethanol as control II or the same amount of the enhancer solution at one of the two concentrations assayed (1 or 5%, w/v) overnight.

2.3. In vitro diffusion experiments

All the permeation experiments were performed on Wistar rat skin (aged 20–25 days), obtained from our laboratory colony. Epidermal membranes were prepared by a heat-separation technique as previously reported (Díez-Sales et al., 1993). Diffusion experiments were performed in a six-cell battery system with the membrane placed in a vertical position as previously reported (Borrás-Blasco et al., 1997).

In all cases Eq. (1) was used to fit the experimental data (Scheuplein, 1967).

$$Q_{(t)} = APhC\left[D\frac{t}{h^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right)\right] \quad (1)$$

where $Q_{(t)}$ is the quantity of penetrant which passes through the membrane and reaches the receptor solution at a given time, t; A represents the diffusional surface area (4.52 cm^2); P, the partition coefficient of the permeant between the membrane and the donor vehicle; h, the membrane thickness; D, the diffusion coefficient of the permeant in the membrane, and C is the concentration of the penetrant in the donor solution. The terms Ph and D/h^2 were replaced in Eq. (1) by P_1 and P_2 respectively, and calculated by fitting the theoretical equation to individual in-vitro permeation data sets using a computerized non-linear least-squares method (Multi) (Yamaoka et al., 1981). The permeability coefficients, K_p ($= P_1P_2$), were calculated and used as representative permeation parameters.

Enhancement ratios (ER) were calculated from the following equation (Goodman and Barry, 1988).

$$ER = \frac{\text{Permeability coefficient after enhancer pretreatment}}{\text{Permeability coefficient from control I}} \quad (2)$$

The values reported are mean ratios from four replicates.

2.4. Analytical procedure

The concentration of permeants in the receptor samples was determined by HPLC using a Perkin-Elmer liquid chromatograph which included an Isocratic Series 10 Pump, an LC sample processor, ISS 200 model automatic injector, an LC 95 UV detector set at 254 nm and a 1020 model integrator. An analytical Novapack C-18 column was employed. The mobile phases were composed of mixtures of acetonitrile and phosphate buffer solution (pH 6.2) in variable proportions, depending on the permeant, and were delivered at a flow rate of 1 ml min^{-1} at room temperature.

Calibration curves covering the entire range of concentrations assayed for the compounds were prepared in triplicate. The accuracy of the method was evaluated by calculating the relative error, which was always less than 9%, and precision was evaluated by calculating the variation coefficient, which was lower than 10% and is considered acceptable (Karnes and March, 1993).

2.5. Statistical analysis

Homogeneity was confirmed by Barlett's test. A logarithmic transformation of permeability coefficients was used in some cases in order to obtain homogeneous variability. An ANOVA was used prior to the Sheffee test; $P < 0.05$ was considered significant.

3. Results and discussion

Tables 1 and 2 give the permeability coefficients (K_p) and the amounts accumulated in the receptor compartment at 32 h (Q_{32}) of the compounds assayed without (control I) and with pretreatment of the skin with ethanol (control II) or an ethanolic solution of Span®20, Tween®20 or Azone®, at selected concentrations for membrane pretreatment (1 and 5%, w/v).

As can be observed, pretreatment of the skin with ethanol almost never produced an increase in the amount of compound penetrated (Q_{32}). The exception was the most hydrophilic compound

(5-FU), whose mobility through the membrane seems to be affected by the solvent used for applying the enhancer on the skin. There were no significant differences between the permeability values of antipyrine, 2-phenyl ethanol and 4-phenyl butanol when the membranes were pretreated with buffered solution pH 6.2 (control I) and those determined when the membrane had been previously pretreated with ethanol (control II). Consequently, for these compounds increases in the permeability values when the membrane was treated with the enhancers with respect to control I could be attributed to a direct effect of the enhancer on the skin.

Table 1
The effect of skin pretreatment with ethanol (control II), Span®20 (1 and 5%, w/v), Tween®20 (1 and 5%, w/v) and Azone® (1 and 5%, w/v) on the permeation parameters of 5-fluorouracil and antipyrine ($n = 4$, mean ± S.D.)[a]

Pretreatment	Concentration, % (w/v)	5-Fluorouracil		Antypirine	
		$K_p \times 10^3$ (cm h^{-1})	Q_{32} (mg)	$K_p \times 10^3$ (cm h^{-1})	Q_{32} (mg)
None (control I)		0.68 ± 0.01	1.04 ± 0.05	0.53 ± 0.08	34.34 ± 4.39
Ethanol (control II)		0.82 ± 0.04	1.32 ± 0.13	0.481 ± 0.07 (NS)[c]	39.00 ± 5.83 (NS)[c]
Span®20	1	1.19 ± 0.20	1.45 ± 0.30	1.45 ± 0.16	112.48 ± 8.29
Span®20	5	5.29 ± 0.50	7.59 ± 0.55	6.36 ± 0.75	351.39 ± 43.49
Tween®20	1	0.62 ± 0.03 (NS)[b]	0.86 ± 0.07 (NS)[b]	0.46 ± 0.06 (NS)[c]	39.96 ± 7.95 (NS)[c]
Tween®20	5	1.48 ± 0.17	2.39 ± 0.38	0.51 ± 0.01 (NS)[c]	40.83 ± 2.22 (NS)[c]
Azone®	1	4.58 ± 0.29	5.74 ± 0.25	1.10 ± 0.09	112.74 ± 10.57
Azone®	5	132.99 ± 11.61	191.62 ± 12.57	24.55 ± 3.30	2088.30 ± 254.99

[a] The results of the statistical analysis are also included.
[b] NS, not statistically different from control I and control II ($P > 0.05$).
[c] NS, not statistically different from control I ($P > 0.05$).

Table 2
The effect of skin pretreatment with ethanol (control II), Span®20 (1 and 5%, w/v), Tween®20 (1 and 5%, w/v) and Azone® (1 and 5%, w/v) on the permeation parameters of 2-phenyl ethanol and 4-phenyl butanol ($n = 4$, mean ± S.D.)[a]

Pretreatment	Concentration, % (w/v)	2-Phenyl ethanol		4-Phenyl butanol	
		$K_p \times 10^3$ (cm h^{-1})	Q_{32} (mg)	$K_p \times 10^3$ (cm h^{-1})	Q_{32} (mg)
None (control I)		108.74 ± 12.65	285.77 ± 33.78	155.06 ± 28.97	54.57 ± 10.69
Ethanol (control II)		109.38 ± 13.47 (NS)[b]	274.13 ± 16.12 (NS)	164.13 ± 11.44 (NS)[b]	60.84 ± 9.38 (NS)[a]
Span®20	1	172.11 ± 26.88	446.83 ± 49.63	200.45 ± 19.91 (NS)[b]	71.16 ± 6.25 (NS)[a]
Span®20	5	208.02 ± 17.53	533.94 ± 32.44	272.42 ± 38.88	94.90 ± 14.12
Tween®20	1	118.52 ± 17.31 (NS)[b]	281.84 ± 23.23 (NS)	194.54 ± 38.03 (NS)[b]	65.04 ± 11.47 (NS)[b]
Tween®20	5	126.34 ± 23.56 (NS)[b]	279.98 ± 22.07 (NS)	145.52 ± 11.09 (NS)[b]	51.17 ± 4.92 (NS)[b]
Azone®	1	133.46 ± 32.21	471.79 ± 78.73	290.61 ± 56.22	103.82 ± 17.53
Azone®	5	210.78 ± 8.09	571.71 ± 5.255	301.54 ± 31.60	110.41 ± 14.36

[a] The results of the statistical analysis are also included.
[b] NS, not statistically different from control I ($P > 0.05$).

For 5-FU (log Poct = −0.95) only the pretreatment of the skin with a 1% ethanolic solution of Tween®20 did not produce an increase in its permeability values. In all the other cases the permeability parameters (K_p and Q_{32}) showed statistically significant differences with respect to control I and control II ($P < 0.05$). The increment in these values calculated for 5-FU depended, for all the enhancers assayed, on the concentration of pretreatment of the membrane and was always greater for 5% than for 1% w/v. Pretreatment of the membrane with 5% ethanolic solution of Azone® dramatically increased the amount penetrated and the K_p value of 5-FU ($ER = 194.71$).

Although in the case of antipyrine (log Poct = 0.23), pretreatment of the skin with Tween®20 (1 and 5%, w/v) produced a small increase in the amount of compound penetrated, it was not statistically significant. Span®20 and Azone® produced a significant increase in the amount of compound penetrated (Q_{32}) and in the K_p value regardless of the concentration of enhancer employed. Moreover, when the skin was pretreated with 5% ethanolic solutions of these enhancers, the permeability parameters were also significantly higher than those obtained when pretreatment was with 1% ethanolic solutions of enhancers ($P < 0.05$).

In the case of 2-phenyl ethanol (log Poct = 1.34) when pretreatment of the skin was carried out with 1 and 5% of Span®20 and Azone® respectively, the permeation parameters obtained showed a significant increase over control I. However, neither of the enhancers yielded significantly different permeability values with either of the two conditions of skin treatment. For this compound the increase that Tween®20 produced in the values of the permeation parameters was not statistically different from control I.

For the most lipophilic compound assayed in this work, 4-phenyl butanol (log Poct = 2.33), and in both conditions of pretreatment of the skin with Azone® (1 and 5%) there was a significant increase in permeability values over those of control I. However, no differences were observed between the two conditions of Azone® skin treatment (1 and 5%). In the case of Span®20 only the pretreatment of the skin with high concentration solution (5%) gave a significant increase in the 4-phenyl butanol permeation parameters. No significant effects were observed with Tween®20 for this compound.

Therefore, from the results it can be deduced that the influence of the enhancer concentration depends on the lipophilicity of the penetrant. In fact, for the most hydrophilic compound (5-FU) the highest concentration of enhancers used in the skin treatment produced the major enhancement. However, for the most lipophilic compound assayed (4-phenyl butanol) there were no statistically significant differences between the enhancement produced at the different concentrations of enhancers.

In order to quantify and compare the potency of the three molecules as enhancers in relation to the lipophilicity of the penetrants, we estimated the enhancement ratio (ER) calculated from the permeability coefficients for all the permeants used in this study. These data are given in Figs. 1 and 2. Differences in the enhancers' behavior in the two conditions assayed, and depending on the lipophilicity of the penetrants were observed. To explain these differences it is necessary to consider the different molecular mechanisms involved in the diffusion through the stratum corneum of hydrophilic and lipophilic molecules (Blank et al., 1967) and also the main mechanisms of action of percutaneous enhancers that have been described (Barry, 1987).

If we take Azone®, the well known enhancer, as a reference, it is interesting to note that the greatest enhancer effect is observed for the penetrant with the lowest lipophilicity (5-FU), and there is a linear relationship between the lipophilicity of the penetrant and the efficacy of the enhancer. This linear relationship between the log Poct and the efficacy (represented by the calculated log ER) has a correlation coefficient of $r^2 = 0.9103$ (Azone® 1%) and $r^2 = 0.687$ (Azone® 5%). These values are consistent with those previously reported for Azone® and other Azone® analogs (Phillips and Bozena, 1995). The inverse dependence between the lipophilicity of the penetrant and the efficacy of Azone® could be explained by the fact that Azone® not only has a well-known fluidifying action on the skin lipids effected by

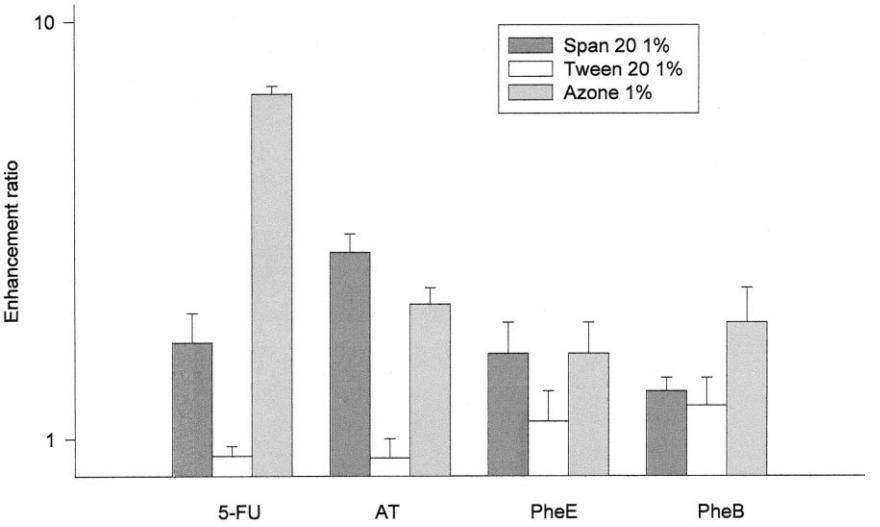

Fig. 1. The penetration enhancing activity of Span®20, Tween®20 and Azone® for a skin treatment concentration of 1%, w/v expressed as enhancement ratios. Each experiment was the mean of four replicates. Abbreviations are as follows: AT, antipyrine; 5-FU, 5-fluorouracil; PheB, phenyl butanol; PheE, phenyl ethanol.

disrupting their highly ordered structure between corneocytes, but also affects the hydration of the stratum corneum (Sugibayashi et al., 1992; Díez-Sales et al., 1996). The latter effect allows the polar molecule to partition across the potential barriers more easily. As the concentration of Azone® increases, the water content of skin could increase, and this would explain the greater enhancer effect on 5-FU.

When we compare the effects of Azone® with those of Span®20 it can be observed that the latter produces an enhancement of skin penetration for

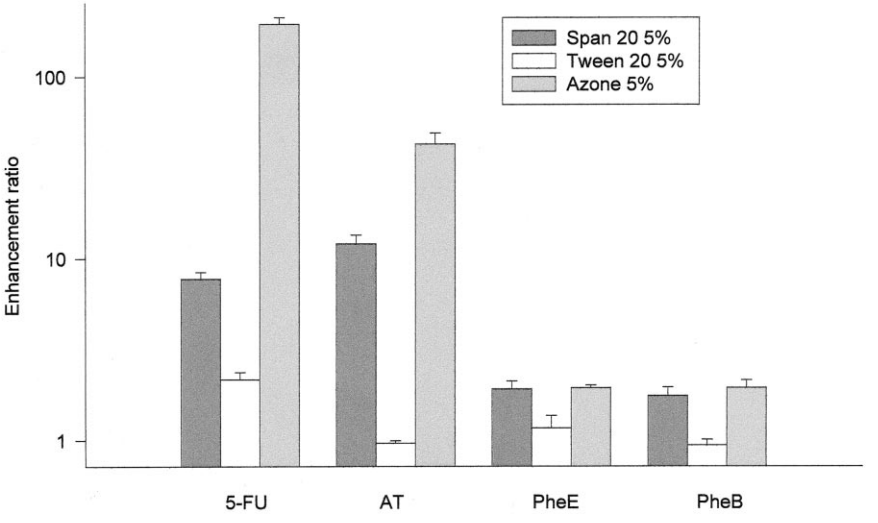

Fig. 2. The penetration enhancing activity of Span®20, Tween®20 and Azone® for a skin treatment concentration of 5%, w/v expressed as enhancement ratios. Each experiment was the mean of four replicates. Abbreviations are as follows: AT, antipyrine; 5-FU, 5-fluorouracil; PheB, phenyl butanol; PheE, phenyl ethanol.

all of the compounds, but in contrast with what occurs with Azone®, there is no linear relationship between enhancement with Span®20 and the lipophilicity of the penetrants. The greater enhancer effect was, in fact, observed for antipyrine. It is interesting to note that for the more lipophilic compounds (2-phenyl ethanol and 4-phenyl butanol) the enhancement ratios of Span®20 and Azone® were similar. Because lipophilic molecules diffuse through the stratum corneum by solubilizing in the continuous intercellular lipid phase of the stratum corneum (lipophilic pathway), it may be that Span®20, like Azone®, affects the intercellular lipids by making them more fluid and thus enhances the diffusivities of these compounds with the same potency as Azone®. However, the fact that Span®20 does not have an additional skin hydration effect of makes the enhancement for the more hydrophilic compounds lower for Span®20 than for Azone®.

Finally, it can be observed that Tween®20 acts as a skin penetration enhancer only for the most hydrophilic compound (5-FU), for which the presence on the skin of the highest concentration of surfactant (5%) resulted in a twofold increase in its permeability coefficient ($ER = 2.16$). If we compare the enhancement ratios for 5-FU calculated in the same skin treatment conditions for Span®20 ($ER = 7.74$) and for Azone® ($ER = 194.71$), clear differences can be observed.

As the three molecules assayed as enhancers have the optimum C12 alkyl chain for biological activity, differences in behavior could be attributed to the nature of the head groups. This observation contrasts with those of Kushla and Zatz, who worked with three series of cationic surfactants and found that the nature of the surfactant head group has little influence on cutaneous barrier impairment (Kushla and Zatz, 1991).

Others authors (Laughlin, 1978) hypothesize that surfactants with hydrophilic head groups should more effectively enhance the percutaneous penetration of polar molecules, while those of lesser hydrophilicity should be less effective. The results obtained in the present work contrast with Laughlin's hypothesis because Tween®20 (log Poct = 3.72; calculated by ACD software) which is more hydrophilic than Span®20 (log Poct = 4.26; calculated by ACD software) is less effective in enhancing 5-FU skin penetration. This could be attributed to the large volume associated with the Tween®20 hydrophilic head group consisting of several polyoxyethylene chains (20 U). This structure could impede penetration into the tails of the lipid bilayers on the stratum corneum. Consequently, its small effect on the skin penetration of 5-FU cannot be attributed to the mechanism of disruption and fluidization of the alkyl chain in the core of the lipid bilayers that has been reported for Azone® and has also been discussed in our study for Span®20. It may be that Tween®20 exerts its effects by allowing the polar molecule to partition across the barrier more easily. This could be due to the increase in the water content of the stratum corneum. However, because the experiments were conducted in an aqueous medium it could be that at the highest concentration (5%) Tween®20 forms large micelles that have the potential to extract lipids from the skin, thus modifying the composition of the membrane and favoring permeation by hydrophilic molecules (Ridout et al., 1991).

In conclusion, the nature of the enhancer head group seems to exert an important influence on cutaneous barrier impairment. The enhancer concentration should also be taken into account, even though the influence of the concentration seems to depend on the lipophilicity of the penetrant assayed.

Finally, Span®20 can be considered as suitable as Azone® in enhancing percutaneous penetration of compounds with intermediate lipophilicity.

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References

Allan, G., 1995. Azone®. In: Smith, E.W., Maibach, H.I. (Eds.), Percutaneous Penetration Enhancers. CRC Press, Boca Raton, FL.

Aungst, B.J., Rogers, N.J., Shefter, E., 1986. Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides. Int. J. Pharm. 33, 225–234.

Barry, B.W., 1983. Dermatological Formulations. Marcel Dekker, New York.

Barry, B.W., 1987. Mode of action of penetration enhancers in human skin. J. Control. Release 6, 85–97.

Blank, I.H., Scheuplein, R.J., MacFarlane, D.J., 1967. Mechanism of percutaneous absorption. 3. The effect of temperature on the transport of non-electrolytes across the skin. J. Invest. Dermatol. 49 (6), 582–589.

Borrás-Blasco, J., López, A., Morant, M.J., Díez-Sales, O., Herráez-Domínguez, M., 1997. Influence of sodium lauryl sulphate on the in vitro percutaneous absorption of compounds with different lipophilicity. Eur. J. Pharm. 5, 15–22.

Cappel, M.J., Kreuter, J., 1991. Effect of non-ionic surfactants on transdermal drug delivery. Int. J. Pharm. 69, 155–167.

Díez-Sales, O., Perez-Sayas, E., Martín-Villodre, A., Herráez-Domínguez, M., 1993. The prediction of percutaneous absorption: I. Influence of the dermis on in vitro permeation models. Int. J. Pharm. 100, 1–7.

Díez-Sales, O., Watkinson, A.C., Herráez-Domínguez, M., Javaloyes, C., Hadgraft, J., 1996. A mechanistic investigation of the in vitro human skin permeation enhancing effect of Azone. Int. J. Pharm. 129, 33–40.

Florence, T., Tucker, I.G., Walters, K.A., 1984. Interactions of non-ionic alkyl and aryl ethers with membranes and other biological systems. In: Rosen, M.J. (Ed.), Structure/Performance Relationships in Surfactants. ACS Symp. Ser. 253, 189–207

Goodman, M., Barry, B.W., 1988. Action of penetration enhancers on human skin as assessed by the permeation of model drugs 5-fluorouracil and estradiol. I. Infinite dose technique. J. Invest. Dermatol. 91, 323–327.

Hansch, C., Anderson, S.J., 1967. The effect of intramolecular hydrophobic bonding on partition coefficients. J. Org. Chem. 32, 2583–2585.

Karnes, H.T., March, C., 1993. Precision, accuracy, and data acceptance criteria in biopharmaceutical analysis. Pharm. Res. 10, 1420–1426.

Kushla, G.P., Zatz, J.L., 1991. Correlation of water and lidocaine flux enhancement by cationic surfactants in vitro. J. Pharm. Sci. 80 (11), 1079–1083.

Laughlin, R.G., 1978. Relative hydrophilicities among surfactants hydrophilic groups. In: Brown, G.H. (Ed.), Advances in Liquid Crystals. Academic Press, New York.

Leo, A., Hansch, C., Elkins, D., 1971. Partition coefficients and their uses. Chem. Rev. 71, 525–553.

López, A., Faus, V., Díez-Sales, O., Herráez, M., 1998. Skin permeation model of phenyl alcohols: comparison of experimental conditions. Int. J. Pharm. 173, 183–191.

Phillips, C.A., Bozena, B.M., 1995. Transdermal delivery of drugs with differing lipophilicities using Azone analogs as dermal penetration enhancers. 84 (12), 1427–1433.

Ridout, G., Hinz, R.S., Hostynek, J.J., Reddy, A.K., Wiersema, R.J., Hodson, C.D., Lorence, C.R., Guy, R.H., 1991. The effects of zwitterionic surfactants on skin barrier function. Fundam. Appl. Toxicol. 16, 41.

Sarpotdar, P.P., Zatz, J.L., 1986a. Evaluation of penetration enhancement of lidocaine by non-ionic surfactants through hairless mouse skin in vitro. J. Pharm. Sci. 75, 176–181.

Sarpotdar, P.P., Zatz, J.L., 1986b. Percutaneous absoption enhancement by non-ionic surfactants. Drug. Dev. Ind. Pharm. 12, 1625–1647.

Scheuplein, R.J., 1967. Mechanism of percutaneous absorption II. Transient diffusion and the relative importance of various routes of skin penetration. J. Invest. Dermatol. 48, 79–88.

Shahi, V., Zatz, J.L., 1978. Effect on formulation factors on penetration of hydrocortisone through mouse skin. J. Pharm. Sci. 67, 789–792.

Sugibayashi, K., Nakayama, S., Seki, T., Hosoya, K., Morimoto, Y., 1992. Mechanism of skin penetration-enhancing effect by Laurocapram. J. Pharm. Sci. 81, 58–64.

Walters, K.A., Dugard, P.H., Florence, A.T., 1981. Non-ionic surfactants and gastric mucosal transport of paraquat. J. Pharm. Pharmacol. 33, 207–213.

Walters, K.A., Florence, A.T., Dugard, P.H., 1983. Interaction of polyoxethylene alkyl ethers with cholesterol monolayers. Colloid Interface Sci. 89, 584–587.

Walters, K.A., 1990. Surfactants and percutaneous absorption. In: Scott, R.C., Guy, R.H., Hadgraft, J. (Eds.), Prediction of Percutaneous Penetration. IBC Technical Services, London, pp. 148–162.

Walters, K.A., Hadgraft, J., 1993. Pharmaceutical Skin Penetration Enhancement. Marcel Dekker, New York.

Warner, K.S., Suhonen, T.M., Li, S.K., Ghanem, A.H., Higuchi, W.I., 1998. Importance of alkyl chain length in the mechanism of action of various skin permeation. In: American Association of Pharmaceutical Scientists (Eds.), PharmSci (Suppl.), S-525.

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T., 1981. A pharmacokinetic analysis program (multi) for microcomputer. J. Pharm. Dyn. 4, 879–885.

Zaslavsky, B.Y., Ossipov, N.N., Krivitch, V.S., Baholdina, L.P., Rogozhin, S.V., 1978. Action of surface-active-substances on biological membranes. II. Hemolytic activity of non-ionic surfactants. Biochim. Biophys. 507, 1–7.