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Evaluation of the transepidermal permeation of diethylene glycol monoethyl ether and skin water loss

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Abstract

The rate of Transcutol® (TC)-water interchange, was studied using whole and stripped hairless rat skin in a modified vertical Franz diffusion cell. The presence of stratum corneum limited the passage of the two solvents, but after stripping of the skin, the rate of TC transfer increased and the water flow rose until the vapour pressure at the skin surface and in the ambient air was similar. The results suggest that TC increased the donor hydration by increasing the outflow of water, with a change in the donor composition. These two opposite fluxes and the alteration of the bulk constitution of the skin and of the donor compartment throughout the experiment, are important factors to be considered for the permeation studies with this solvent. © 1997 Elsevier Science B.V.

Keywords: Skin absorption; Penetration enhancer; Epidermal water loss; Diethylene glycol monoethyl ether

1. Introduction

Permeation enhancers increase drug absorption through the skin and mucosal barriers, but the mechanisms by which these agents act are unclear. Their success rate varies from drug to drug and this is why many investigations have focused attention on their mechanism of action. Among known permeation enhancers, diethylene glycol monoethyl ether (Transcutol®, TC) has shown a clear influence on the percutaneous absorption rate of many drugs, mainly in combination with other enhancers (Rieg-Falson et al., 1989; Touitou et al., 1991; Watkinson et al., 1991). It has been shown that the permeation of some drugs is improved if they are dissolved in TC; sometimes a binary solvent system including water is used to obtain a more favourable partition coefficient between the skin and the solvent (Ritschel and Hussain, 1988; Bialik et al., 1993). That is why the transport of water from the skin or from the

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receptor to the donor solution, could influence the permeation of a drug. Firstly, because it is generally accepted that substances that increase hydration will, in most cases, promote drug absorption (Barry, 1991); humectants such as glycerol or propylene glycol can absorb water from the skin, thus increasing the water content of the stratum corneum and consequently decreasing its diffusional resistance. Secondly, because a change in the donor composition can influence drug solubility and the thermodynamic activity of the drug (De Carvalho, 1995; De Carvalho et al., 1993, 1996). Furthermore, it has been shown (e.g. Watkinson et al., 1995) that a vehicle may alter the degree of absorption of a substance due to its own penetration. TC can influence the partitioning behaviour of a drug or of a co-enhancer because it penetrates into the skin, where it changes the bulk constitution of the tissue. The penetration of the solvent can temporarily alter the barrier properties by the replacement of water molecules in the stratum corneum with those of TC (Zatz and Dalvi, 1983; Barry, 1987). The presence of TC in the interfacial region tends to minimise the free energy barrier of the partition step, promoting in this way drug absorption (Rieg-Falson et al., 1989; Watkinson et al., 1991; Hadgraft and Walters, 1994). Moreover, a high penetration rate of the solvent into or through the skin would increase drug penetration, through a solvent drag mechanism of permeation enhancement (Yamada et al., 1987; Aungst et al., 1990). Therefore, this work was focused on assessing the ex vivo kinetics of solvent exchange (TC-water) through whole skin, but as the stratum corneum constitutes the major barrier to permeation of substances and water evaporation (Van der Valk and Maibach, 1990), the study was also performed using stripped skin.

2. Materials and methods

2.1. Materials

The diethylene glycol monoethyl ether (Transcutol®) was kindly provided by Gattefossé S.A., France; penicillin/streptomycin (50 000 U/ml:25 mg/ml) was purchased from bioMérieux, France; sodium chloride (SigmaUltra > 99.5%) and potassium dichromate (ACS reagent) from Sigma, France; sulphuric acid 95–97% (Fluka, Switzerland); 8 week old hairless male rats were supplied by IffaCredo, France.

2.2. Analytical assay for TC

A spectrophotometric method was used to determine polyols after dichromate oxidation (modified method from Sargent and Rieman, 1956). The oxidizing reagent was 0.1 N potassium dichromate in concentrated sulphuric acid. A 2-ml volume of reagent was added to 1 ml of the sample solution. The mixture was heated in a boiling-water bath for 15 min, then cooled and the absorbance measured at 610 nm against a blank solution which was prepared by adding 1 ml of saline solution to 2 ml of the oxidizing reagent.

2.3. Permeation experiments

The permeation of TC was investigated using whole and stripped dorsal rat skin (the stratum corneum was stripped 10 times using a commercial adhesive tape). The skin was clamped into position between the donor and receptor compartments of a static vertical diffusion cell based on the Franz design (area = 2.54 cm^2). The receptor medium consisted of 9 ml of 0.9% (w/v) saline solution with penicillin/streptomycin (50 U/ml:25 μg/ml). This was maintained at 37°C by a circulating water pump, and constantly stirred with a teflon-coated magnetic bar. The skin was hydrated overnight with the receptor medium before a known volume of TC (1 ml) was applied to the donor compartment. Samples of 200 ml were taken at appropriate time intervals from the receptor compartment over a 48 h period (replacing them with an equivalent volume of fresh solution) and analysed for diethylene glycol content. At the end of the experiment, the residual amount of TC in the donor compartment was quantified using the same analytical method. The results were corrected for dilution effect.

2.4. Water transfer

This experiment was carried out by taking samples from the donor compartment at various time intervals and quantifying the water content by the Karl Fischer technique. The results were corrected for dilution effect. The total volume of liquid in the two compartments (donor and receptor) at the end of the experiment was also measured by volumetry.

3. Results and discussion

The influence of vehicles on drug permeation has been widely studied. Some authors such as Watkinson et al. (1995), have shown that interactions were possible between the vehicle and a membrane and that such interactions may involve the inflow of solvent into the membrane, increasing or decreasing the partition coefficient of a solute. Scheuplein and Blank (1971) had previously found that some solvents can increase the permeability of the stratum corneum because of their ability to displace water from the tissue and to form a large percentage of the membrane substance. The replacement of water molecules by another solvent naturally influences drug partitioning behaviour (Zatz and Dalvi, 1983).

The results of this paper show that TC passes through the whole skin at a constant rate of 0.58 mg cm^{-2} h^{-1} (0–32 h), and that this rate is limited by the presence of the stratum corneum (Fig. 1). The stripping of the stratum corneum resulted in an initial burst in diffusion followed by a rate very similar to that of whole skin from 3 to 24 h and a 2.53-fold increase in the rate of permeation from 24 to 48 h. The amount of TC found in the receptor compartment at the end of the experiment showed a transfer of about 5% of the initial volume for the whole skin and about 20% for the stripped skin (Fig. 2). The difference between the donor and receptor percentages gave an approximation of the amount in the skin. This quantity was low for the whole skin ($\approx 2\%$) and greater when the stratum corneum was removed ($\approx 35\%$). In both cases, the continuous flux of TC across the skin suggests that TC can promote the

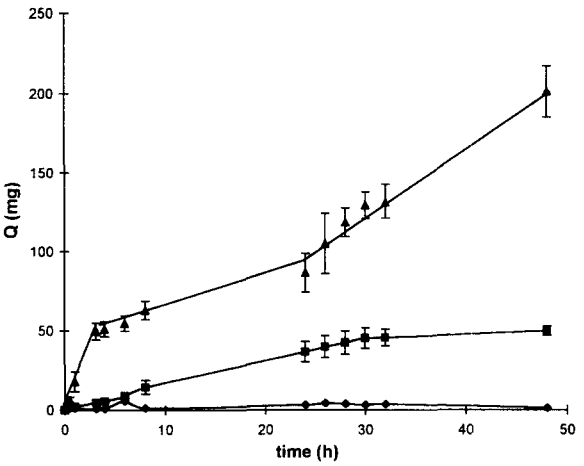

Fig. 1. Quantity Q (mg) of TC diffusing through hairless rat skin (area = 2.54 cm^2). Whole skin ($n = 6$) ■; stripped skin ($n = 4$) ▲. The control ◆ was a similar permeation experiment with an empty donor compartment. Mean ± S.D.

absorption of a drug, by improving its partitioning into the skin; however, if the drug has a greater affinity for the intracutaneous components than for TC, the formation of an intracutaneous drug deposit is probable, as shown the studies of Ritschel and Hussain (1988); Panchagnula and Ritschel (1991) and Touitou et al. (1991).

On the other hand, we found that TC promotes to some extent, the transport of water from the receptor to the donor compartment. This transference of water must be taken into account, because

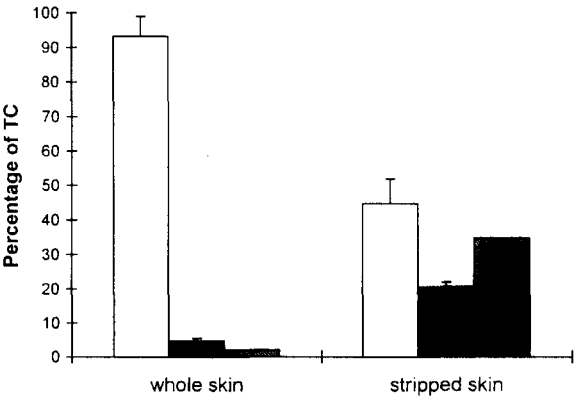

Fig. 2. Distribution of TC at the end of the permeation experiment (48 h) for whole and stripped skin. Donor □; receptor ■; skin ■. The percentage is related to 1 ml of TC in the donor compartment at $t = 0$. Mean ± S.D.

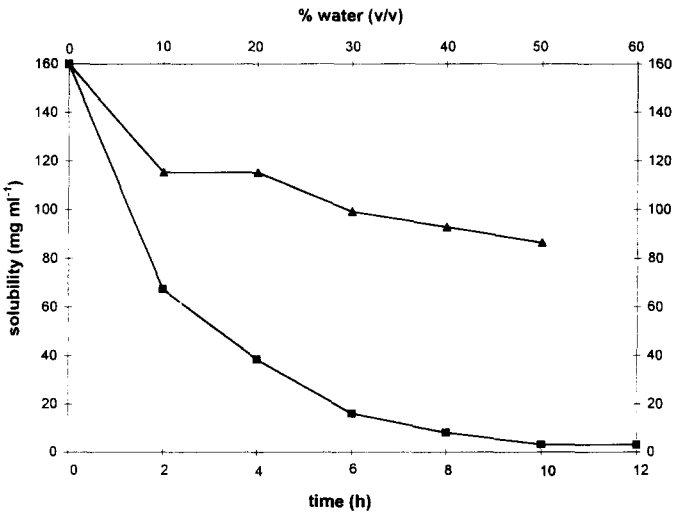

Fig. 3. Estradiol solubility in TC versus time (▲) and versus water percent (■). From: De Carvalho et al. (1996) [with permission of the authors].

it implies a change in the donor composition, which can influence drug solubility. In this respect, De Carvalho (1995) and De Carvalho et al. (1993, 1996) have shown that there is a change in the donor phase composition during the permeation of β-estradiol through whole hairless rat skin, when TC is used as solvent. Effectively, as shown Fig. 3, the continuous enrichment in water content of the donor solution during permeation produces a decrease of drug solubility in this phase, which drops from 164 mg ml^{-1} to 83 mg ml^{-1} in 12 h, when the content of water reaches 8%. Our results showed that the transference of water in the case of whole skin, occurs at a constant rate of 1.82 mg cm^{-2} h^{-1} (Fig. 4), founding about 150 μl of water in the donor phase at the end of the experiment, as determined by the K. Fischer technique and by measuring the final donor volume (Table 1). In the absence of the stratum corneum, there was a greater increase in the amount of water in the donor phase, where the volume of water reached 840 μl (K. Fischer technique) and 1.525 ml (direct measurement). The significant difference between the two mea-

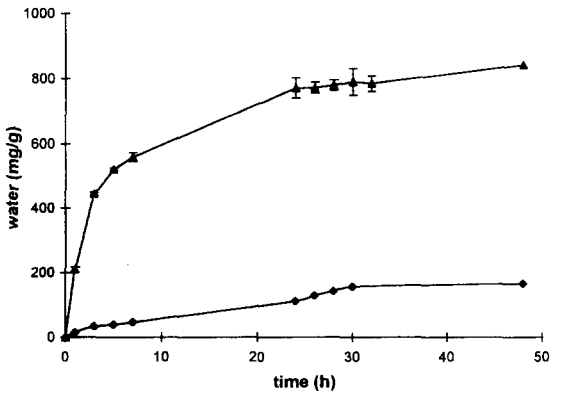

Fig. 4. Water concentration (mg/g) in the donor phase during the permeation experiment. Whole skin ($n=6$) ◆ (S.D. smaller than symbols); stripped skin ($n=4$) ▲. Mean ± S.D.

Table 1
Amount of water (ml) in the donor and receptor compartments at the end of the experiment (mean ± S.D.)

	Donor		Receptor
	K.F.[1]	Vol.[2]	Vol.[2]
Whole skin ($n=6$)	0.156 ± 0.017	0.15 ± 0.08	8.53 ± 0.16
Stripped skin ($n=4$)	0.840 ± 0.015	1.525 ± 0.28	7.3 ± 0.26
Control whole skin ($n=4$)	≈0.001	<0.01	8.9
Control stripped skin ($n=4$)	n.d.	0.4	8.4

[1]K.F., Karl Fischer technique.
[2]Vol., volumetric determination.
n.d., not determined.

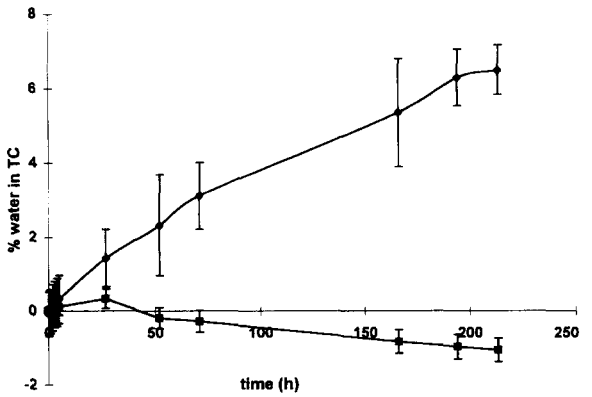

Fig. 5. TC kinetics hydration at two relative humidities: 98% ◆ and 50% ■. Mean ± S.D.

surements can be accounted for by the presence of condensation water on the glass wall of the donor compartment (a closed chamber) which was not taken into account by the K. Fischer technique. In other words, the Karl Fischer technique measured only water mixed with TC whereas the direct measurement took into account all the liquid in the compartment. Anyway, the mass balance of the total water recovered from the donor and receptor compartments showed that the excess of water in the donor solution came from the receptor medium. The high outflow of water during the first hours with stripped skin is due, besides the absence of the skin barrier, to the high water content of the skin, which on one side is in contact with water and can hence be regarded as being exposed to 100% RH throughout the experiment. A control experiment without solvent in the donor compartment, showed that the quantity of water that back diffused was of about 1 μl and 400 μl for whole and stripped skin, respectively. The main driving force for transepidermal water transfer seems to be the vapour pressure gradient between the skin and ambient air, as well as the presence of a hygroscopic substance such as TC that absorbs water. Fig. 5 gives the kinetics of hydration of TC at two relative humidities (RH), 98% and 50% and it is clear that when TC is exposed to a high RH, it is capable of absorbing a large quantity of water, even if this quantity was quite minor than in the permeation experiment; on the contrary, at a low RH we found that TC lost humidity.

Effectively, the outward flow of water causes a rise in the vapour pressure of the donor compartment with a decrease in the vapour pressure difference between the skin surface and the ambient air. Therefore, the driving force causing water loss is decreased (Grice et al., 1972). On the other hand, if one considers that TC behaves as a solute, it could induce a reduction of the solvent vapour pressure which according to Raoult's law, depends solely on the amount of solute. In dilute solutions, that is without solute-solute interactions, there is a decrease in the number of solvent molecules passing into the vapour phase and this is due to a dilution effect in which the solute molecules, in this case TC, replace those of the solvent (water) with the result that there are less solvent molecules available for evaporation. These mechanisms may explain the decrease in water outflow after 8 h. Furthermore, the increase in the flux of TC through stripped skin after 24 h could be attributed to the high ambient humidity in the donor compartment, which contributes to the improvement of permeability of the skin by increasing the water content of the surface (McCallion and Li Wan Po, 1994). The presence of the stratum corneum limits the water-TC interchange, whereas its absence allows an almost free flow between the compartments. In the two cases, stripped skin and whole skin, there is an inflow of TC $(J_T(x)A)$ and an outflow of water $(J_w(x)A)$ (Fig. 6). The diffusion of molecules of both solvents through the system occurs with a tendency to reach at $t = \infty$, a uniform distribution in all the three compartments (donor, skin, receptor).

In summary, the action of TC as a penetration enhancer could be attributed, on one hand, to its permeation into the skin and its effect on the partition behaviour of a drug, and on the other hand, to the hydration of TC which can absorb water not only from the air, but also from the skin (change of vehicle composition) improving the skin penetration of certain drugs, maximizing its thermodynamic activity due to a change of drug solubility (Ritschel and Hussain, 1988; Bialik et al., 1993).

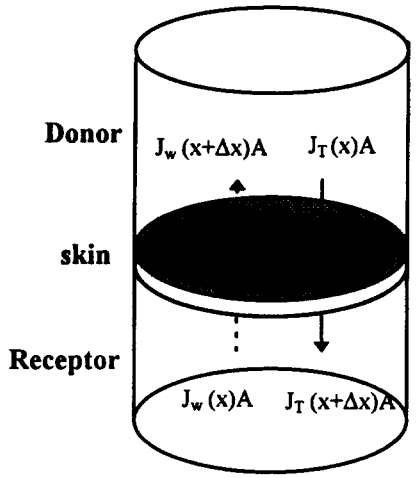

Fig. 6. Transport of matter between the two compartments separated by the skin of area (A) and a thickness of $[(x + \Delta x) - x]$, where $J_w(x)A$ and $J_T(x)A$ are, respectively, the fluxes of water and TC.

4. Conclusions

The results showed that an interchange TC-water is established between the donor and the receptor compartment. TC passes through the skin, reaching the receptor compartment at a constant rate, limited by the presence of the stratum corneum. An opposite water flux from the receptor to the donor compartment is improved by the hygroscopic properties of TC, which takes water not only from the environment, but from the skin. Stripping of the skin, resulted in an increase of TC and water fluxes. The results presented suggest that these two opposite fluxes will affect the permeation rate of a given drug by changing its partitioning into the skin as well as its thermodynamic activity in the donor solution.

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