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Formulation study of a transdermal delivery system of primaquine

P. Mayorga[a,b], F. Puisieux[a], G. Couarraze[a,*]

[a]*Laboratoire de Physico-Chimie, Pharmacotechnie et Biopharmacie, URA CNRS 1218, Université Paris-Sud., 5, Rue Jean Baptiste Clément, 92296 Chatenay-Malabry Cedex, France*

[b]*Laboratório de Desenvolvimento Galênico, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Rio Grande do Sul, Brazil*

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Abstract

A potential transdermal application of an anti-malarial drug, primaquine, was investigated. In-vitro percutaneous absorption through hairless rat skin using either the salt or free base form of this drug was studied. Investigations were performed in order to choose an adequate vehicle for transdermal delivery of the free base form. The vehicles studied were Mygliol® 840 (M), Labrafac Hydrophile® (LH), Transcutol® (T), propylene glycol (PG), oleic acid (OA) and a mixture LH/T 50:50. Finally, transdermal release of primaquine from different matrix transdermal therapeutic systems (TTS) was compared. In this optimization we studied the influence of polymer type (Eudragit® RL 100 or ethyl cellulose), adhesive layer and drug concentration in the polymeric matrix on the release profiles. Primaquine free base was found to be very suitable for transdermal delivery. Mygliol® 840 showed the best enhancement factor of the percutaneous flux of primaquine. The optimized TTS, which was an ethyl cellulose-based formulation with Mygliol® 840 as vehicle, showed a percutaneous flux of 180 $\mu g\,cm^{-2}\,h^{-1}$.

Keywords: Primaquine; Percutaneous absorption; Transdermal drug delivery system; Partition coefficient; Enhancing effect

1. Introduction

Malaria, one of the most important diseases in the tropics and subtropics, remains an important cause of mortality in these regions. It is caused by protozoan parasites of the genus *Plasmodium*, by four species pathogenic to man: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. *P. falciparum* (the most dangerous malaria) and *P. vivax* represent 95% of human malaria.

It is possible to identify three stages in the life-cycle of the parasite. In man, the first stage is represented by the tissular or hepatic form called exo-erythrocytic. In the case of *P. vivax* and *P.*

* Corresponding author.

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ovale, this form is responsible for late relapses of the disease. Later, an erythrocytic schizogony takes place into the red blood cells and is responsible for the clinical symptoms of the disease. After differentiation of the parasite during the erythrocytic cycle, the sexual forms of the parasite, called gametocytes, are generated. These sexual forms are not pathogenic but assure parasite transmission because they develop into the infecting form, sporozoites, in the mosquito vector. This process is called sporogonie and is the third stage in the life-cycle of the parasite.

Theoretically, the goal of the chemotherapy of malaria is to find a drug that shows both antirelapse and blood schizontocide activity, with minimal side-effects. So far, there is no single drug that shows both functions (Winstanley and Breckenridge, 1987).

There are many drugs, as well as drug combinations, in current use as blood schizontocides. Some examples of this group are chloroquine, amodiaquine, proguanil, quinine, pyrimethamine, thrimethroprim, sulfadoxine and mefloquine. However, there are today only two drugs in clinical use as tissue schizontocides, two 8-aminoquinolines, primaquine and, to a lesser extent, quinocide (Nodiff et al., 1991)

At present, primaquine is the only 8-aminoquinoline which is widely employed as an antimalarial drug. Moreover, it is the only compound available for clinical use that acts in the liver on the hipnozoites of *P. vivax* and *P. ovale* which may persist after suppressive treatment with chloroquine. It is also effective against the primary exo-erythrocytic stage (causal prophylactic) as well as being a gametocytocidal and sporonticidal agent (Nodiff et al., 1991; Bhat et al., 1984).

Despite its activity, primaquine shows a wide range of side-effects, including gastrointestinal disturbances, development of methaemoglobinaemia and haemolytic anaemia, which are dose-dependent (Clyde, 1981; Winstanley and Breckenridge, 1987). Thus, its prophylactic and therapeutical applications are greatly limited. In addition, primaquine has a short apparent plasma elimination half-time (5.6 h) and is subject to first-pass metabolism (Bhatia et al., 1986).

To improve the treatment efficacy and reduce the side-effects of this drug some possible ways of modifying its distribution have been investigated, including liposomes (Pirson et al., 1982), nanoparticles (Rodrigues et al., 1994) and a transdermal therapeutic system (Thassu and Vyas, 1993).

As a result of its pharmacokinetic profile and toxicological behaviour, primaquine seems to be a good candidate for transdermal delivery. Moreover, the prolonged action desired and the possibility of photo-induced reactions with melanin, resulting in phototoxicity (Kristensen et al., 1994), suggest that an occlusive transdermal system (TTS) is a suitable dosage form for this purpose.

In order to obtain a suitable transdermal therapeutic system for the treatment of malaria, the aim of this work was to investigate the availability of primaquine for percutaneous absorption, using a Franz-type diffusion cell.

Initially, the intrinsic skin permeability of both primaquine diphosphate and the free base was evaluated because of their different degrees of lipophilicity. Then, the percutaneous absorption of primaquine (free base) from different vehicles was investigated to obtain a maximal percutaneous flux. Finally, in a TTS formulation, the influence of the type of polymer (a cellulose derivative and an acrylate–methacrylate copolymer), adhesive layer as well as the concentration of drug within the polymeric matrix was evaluated.

2. Materials and methods

2.1. Materials

2.1.1. Drug

Primaquine diphosphate was purchased from Sigma Chimie (France) and primaquine free base was obtained in our laboratory by extraction with organic solvent.

2.1.2. Vehicles

The studied vehicles were a monoethyl ether of diethylene glycol (Transcutol®, T), a mixture of glycolysed ethoxylated C_8/C_{10} glycerides (Labrafac® Hydrophile, LH) both provided by

Table 1
Composition (per cm^2) of TTS formulations used in release kinetics

Formulation	Matrix[a]		Adhesive layer	
	Polymer (mg)	Drug (mg)	Adhesive (mg)	Drug (mg)
1a	Eudragit® (52)	10.4	26	2.6
1b	EC (52)	10.4	26	2.6
2a	Eudragit® (52)	10.4	13	5.2
2b	EC (52)	10.4	13	5.2
3a	Eudragit® (26)	10.4	13	5.2
3b	EC (26)	10.4	13	5.2

[a]EC = ethyl cellulose.

Gattefossé SA (France), propylene glycol (PG), oleic acid (OA) purchased from Prolabo (France), and a mixture of propylene glycol dicaprylate/caprate (Mygliol® 840, M) provided by Hüls (Germany). Polyethyleneglycol 400 (PEG) was purchased from Aldrich-Chemie (Germany).

2.1.3. Polymers

Acrylate–methacrylate copolymer, Eudragit® RL 100 (Röhm Pharma, Germany) and ethyl cellulose (Dow Chemical Company, Netherlands) were used with glycerol triacetate and diethyl phthalate (Prolabo, France), respectively, as plasticizers. Pressure-sensitive adhesive acrylic resin, Durotak® 280 2287, was obtained from National Starch & Chemical (France).

2.2. Methods

2.2.1. Extraction of the basic form of primaquine

Primaquine diphosphate (3.0 g) was dissolved in about 100 ml of distilled water and the pH was increased to 11 with ammonia solution (Merck, Germany). Then, the free base was extracted twice with 100 ml of chloroform. The organic phase was twice washed with both distilled and saturated solution of sodium chloride. Anhydrous sodium sulphate was added to the organic solution, in order to eliminate residual water, and filtered on sintered glass before evaporation of the solvent in a rotary evaporator. The compound obtained was identified by its infra-red and nuclear magnetic resonance spectra. The efficiency of extraction was $79 \pm 10\%$.

2.2.2. Determination of partition coefficient

Octanol/water partition coefficients were determined for test compounds (salt and basic forms of primaquine) as follows. About 50 mg of primaquine diphosphate were added to 1.0 ml of distilled water. After addition of an equal volume of octanol the tube was vortexed for 1 min at high speed and then centrifuged for 15 min at 3000 rpm (Beckman L755). A sample of 100 μl was taken from both the oil and aqueous phase to drug assay. The octanol and water employed were previously saturated by high-speed mixing of both solvents. The same methodology was employed to determine the partition coefficient of the free base, except that the drug was initially dissolved in octanol and after added to distilled water. The partition coefficients were then calculated as the ratio of the tested compound concentration in oil to that in the aqueous phase.

2.2.3. Preparation of TTS formulations

The matricial TTS formulations were obtained by casting 14 ml of a mixed isopropanol/acetone solution of the matrix components in PTFE (Teflon®) moulds and drying at 25°C for up to 5 days. Afterwards, the adhesive layer was applied as an ethanol/acetone solution of the acrylic adhesive (Durotak®), loaded with primaquine (free base) and dried at 25°C for 5 days. Mygliol® 840 was added to all formulations at 20% of dry polymer. The ratio of plasticizers to Eudragit® and ethyl cellulose was 10% of glycerol triacetate and 20% of diethyl phthalate, respectively (based on dry polymer). Table 1 summarizes the composition per cm^2 of the TTS formulations prepared.

2.2.4. Skin permeation kinetics

To investigate the intrinsic permeability of the salt and free base forms of primaquine and to optimize a TTS formulation, including the choice of vehicle, a two-compartment Franz-type diffusion cell was employed. The skin permeation kinetics were determined at 37°C. The receptor compartment contained 8.0 ml of a phosphate buffer solution (pH 7.4) of Tween® 80 (5% w/v). A mixture of the antibiotics penicillin and streptomycin was added to the solution. The donor sample was either a volatile deposit, a solution or a TTS formulation as described below. The skin samples of 2.54 cm^2 were obtained from the abdomens of 5–6 week-old male hairless rats (Iffa Credo, France). The skin fragments placed on the cell were equilibrated with the receptor phase for 12 h before beginning the experiment. At this moment the receptor phase was renewed and the donor samples were deposited. At regular intervals up to 24 h, the whole receptor phase was taken for drug assay. For each system, five or six experiments were performed.

2.2.5. Skin permeability characterization of salt and free base forms of primaquine

To compare the skin penetration of the two forms of the drug, two different sample conditions in permeation kinetics were established. First, a volatile sample was deposited in the donor compartment of diffusion cells, either a methanolic solution of salt form (200 μl $\approx$ 1.0 mg of primaquine) or an acetonic solution of free base form (100 μl $\approx$ 2.0 mg of primaquine). Second, skin permeation was performed but using a hydrophilic vehicle (1.0 ml) in the donor compartment. The vehicle contained 20.0 mg of either salt or free base form in a polyethylene glycol 400/aqueous solution (40/60 v/v).

2.2.6. Skin permeation kinetics from vehicles

To make a choice between different vehicles, skin permeation kinetics were performed. The deposited samples were 1.0 ml of each vehicle (M, LH, T, PG, OA and mixture LH/T 50:50) containing 70 mg of primaquine (free base).

2.2.7. Skin permeation kinetics from TTS formulations

Three experiments were performed with TTS formulations prepared with each polymer, Eudragit® RL 100 and ethyl cellulose. The parameters studied were type of polymer, drug concentration (free base) in the polymeric matrix and the amount of adhesive layer applied. The release kinetics through hairless rat skin were determined using the same conditions as described above for skin permeation kinetics except that the receptor phase samples were removed at regular intervals during 48 h.

2.2.8. Drug assay

To assay primaquine, a high performance liquid chromatograph was employed (Waters 501). The chromatograph was equipped with an automatic sampler injector (Waters 712 WISP), a variable wavelength UV detector (Waters 484) and a reversed phase column (C18, 3.9 mm × 300 mm, particle size, 4 μm). The mobile phase was acetonitrile:methanol:phosphate buffer (3.7 g/l K_2HPO_4, 3.5 g/l KH_2PO_4), 30:35:35. An ion pairing agent, *N,N*-dimethyloctylamine, was used (0.5 ml/l). The UV detection was at either 254 or 356 nm depending on vehicle interference, with a flow rate of 0.8 ml/min.

3. Results and discussion

3.1. Partition coefficient

Despite several advantages over conventional routes of drug administration, transdermal delivery of pharmaceutically active agents is greatly limited. Regardless of the different mechanisms implicated in percutaneous absorption, the relative impermeability of the stratum corneum with its intercellular lipid multilayers provides the principal resistance to skin penetration, especially for hydrophilic compounds (Coderch et al., 1994; Ho et al., 1994). In contrast, for highly lipophilic drugs, the diffusion through the hydrophilic domain of viable skin (viable epidermis and dermis) can represent the rate-limiting step of skin absorption (Díez-Sales et al., 1993).

Table 2
Primaquine skin permeation parameters in intrinsic permeability studies: steady-state flux (J) and lag time

Sample deposited	Flux ± SD[a] ($\mu g\ cm^{-2}\ h^{-1}$)		Lag time (h)	
	Free base	Salt	Free base	Salt
Volatile	24.2 ± 9.5	0.32 ± 0.32	1.41	16.17
Aqueous	105.6 ± 8.6	0.45 ± 0.30	5.83	23.35

[a] $n \geq 4$.

In percutaneous absorption it is generally accepted that to first cross the lipid-rich stratum corneum and then the more hydrophilic region (the viable skin), the penetrant must have balanced lipophilic–hydrophilic properties (Guy and Hadgraft, 1989; Calpena et al., 1994). Determination of the octanol/water partition coefficient is a useful approach to evaluate the lipophilicity of a drug and therefore its suitability for transdermal delivery (Takahashi et al., 1993).

In our study, the partition coefficient values (K_p) determined for the salt and basic forms of primaquine were 0.019 ± 5 ($\log K_p = -1.7$) and 88.23 ± 9.1 ($\log K_p = 1.95$), respectively.

Studies on groups of chemically related compounds have implied that flux measurements across skin show a characteristic parabolic shape with an optimal value for the partition coefficient. In a comparison of percutaneous absorption of structurally related phenol and steroid analogs, a parabolic dependence was observed, in vitro and in vivo, of penetrant permeation on $\log K_p$, with maximum corresponding to $\log K_p = 2–3$ (Surber et al., 1993). Many authors have reported a similar relationship between permeability of biological barriers and lipophilicity of a drug, with a maximum occurring in the same range of $\log K_p$ (Lee et al., 1994; Rim et al., 1986).

The large difference in lipophilicity between the two compounds suggests that the basic form of primaquine could be an excellent candidate for transdermal delivery. This observation is in accordance with our results obtained from intrinsic skin permeability determinations for the salt and basic forms of primaquine.

3.2. Comparison of skin permeation of different forms of primaquine

In this comparison, the skin penetration kinetics of tested compounds after vehicle evaporation showed that the percutaneous flux values (J) were much higher for the basic form and the lag time values observed were smaller than the values observed for the salt form. Similar results were obtained when absorption kinetics were determined using an aqueous/PEG vehicle. The flux of penetrants through skin as well as the lag time values determined in these two experiments are summarized in Table 2.

In many dermal absorption studies a volatile solvent such as acetone has been used to deliver the chemical to the skin. This approach gives useful but limited information because of a possible influence of solvent on the early phase of the absorption process before its evaporation.

The steady-state flux obtained with the free base was 75-fold and 230-fold greater than the values yielded by the salt form from volatile and hydrophilic solvents, respectively. These observations confirmed the basic form as a very good candidate for transdermal delivery. In contrast, Thassu and Vyas (1993) observed a high percutaneous flux value with the salt form of primaquine through human cadaver skin from an aqueous saturated mixture of the same composition (PEG 400/water, 40/60).

In addition, these results are in accordance with the hypothesis of the existence of polar and non-polar pathways in passive transdermal penetration of hydrophilic and lipophilic drugs, respectively (Berner and Cooper, 1987).

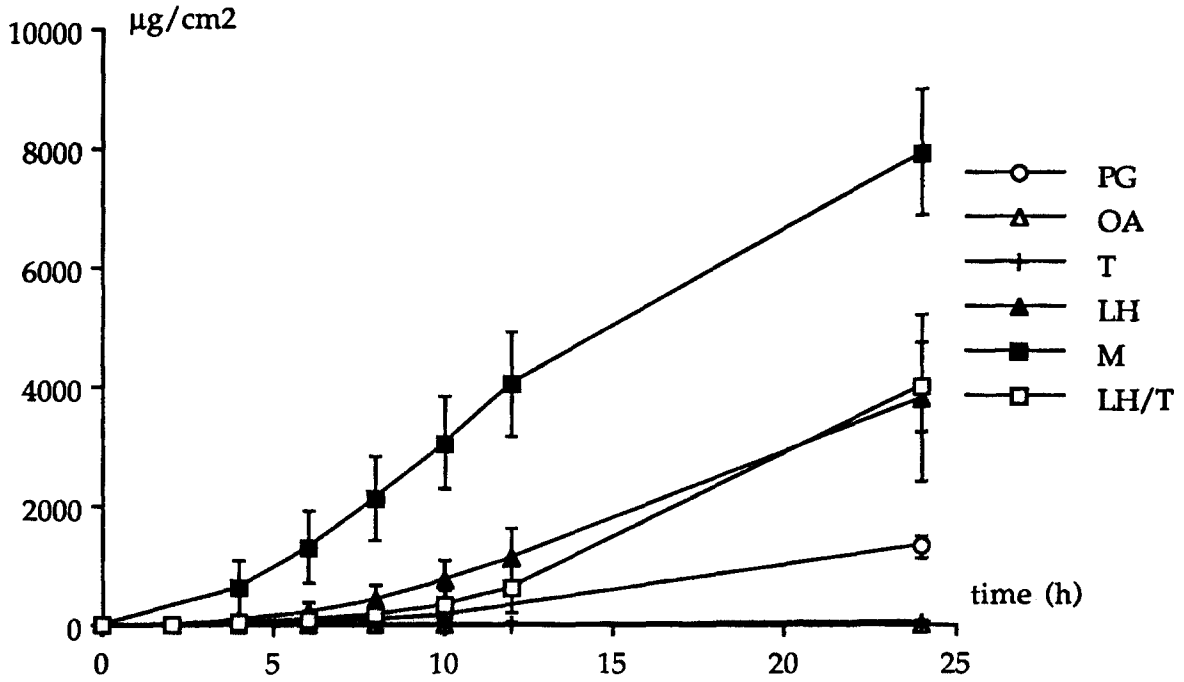

Fig. 1. Cumulative amount of primaquine in percutaneous absorption from different vehicles (propylene glycol, PG; oleic acid, OA; Transcutol®, T; Labrafac® Hydrophile, LH; Mygliol® 840, M; Transcutol®:Labrafac® Hydrophile 50:50, LH/T). Error bars ± SD.

For primaquine free base, the presence of PEG 400 increased the flux values about four-fold. This suggests that the use of a vehicle can provide an optimization of the percutaneous absorption of the selected basic form.

For the salt derivative, a large variability of flux value was observed. Liu et al. (1993) studied the variation of in vitro skin permeation for ionic and neutral permeants through human cadaver skin. The authors observed that the flux data for the ionic permeants were highly variable, in contrast to the neutral compounds. They suggest that the distribution profile and other descriptive parameters, such as median, mode or geometric mean, may be used to represent the permeation data for ionic penetrants. However, the reason behind the non-normal distribution of data for ionic permeants is unclear.

3.3. Skin permeation of free base from vehicles

In transdermal delivery, the goal of dosage design is to maximize the flux through the skin into the systemic circulation. Drug, vehicle and skin parameters play an important role in optimizing drug penetration through the skin.

A useful strategy for improving percutaneous flux seems to be the choice of a vehicle appropriate for the drug being used for the transdermal route (Hilton et al., 1994; Bonina and Montenegro, 1994; Goto et al., 1993). A major research objective is to identify chemicals that significantly enhance drug penetration. These chemicals should ideally be safe and non-toxic, pharmacologically inert, non-irritating and non-allergen (Shah, 1994).

In the present study we investigated the influence of low toxicity vehicles, propylene glycol, oleic acid, Transcutol®, Labrafac® and Mygliol®, on the penetration of primaquine free base through hairless rat skin. The profiles of skin penetration of primaquine free base from these

Table 3
Study of vehicles. Primaquine skin permeation parameters: steady-state flux (J), lag time and enhancement factor for vehicle (propylene glycol, PG; oleic acid, OA; Transcutol®, T; Labrafac® Hydrophile, LH; Mygliol® 840, M; Transcutol®:Labrafac® Hydrophile 50:50, LH/T)

Vehicle	Flux ± SD[a] ($\mu g\,cm^{-2}\,h^{-1}$)	Lag time (h)	Enhancement (J_v/J_a)[b]
M	459.6 ± 74.7	3.3 ± 1.2	19
LH	205.7 ± 70.7	6.0 ± 0.5	8.5
T/LH	230.9 ± 39.4	7.5 ± 0.6	9.5
PG	100.1 ± 5.5	7.1 ± 0.3	4
T	2.8 ± 1.2	5.5 ± 0.9	0.1
OA	1.3 ± 1.0		0.05

[a] $n \geq 4$.
[b] J_v = flux from vehicle; J_a = flux from volatile sample.

vehicles are showed in Fig. 1. Skin permeation parameters, steady-state flux, lag time and enhancement factor are summarized in Table 3. The enhancement factor was calculated as the ratio of flux from each vehicle to that determined in intrinsic permeability experiments from a volatile sample.

Mygliol® and Labrafac® were the vehicles that showed highest flux values through the skin. Mygliol® showed the highest enhancement factor, 19-fold, with a lag time of about 3 h and was selected as an adequate vehicle for TTS formulations. The enhancement factor of Mygliol® was about five-fold higher than the value observed with propylene glycol, which showed a percutaneous steady-state flux of 100 $\mu g\,cm^{-2}\,h^{-1}$. It has been observed that ester derivatives of propylene glycol (Mygliol® and propylene glycol dipelargonate) can improve the percutaneous flux of drugs in relation to propylene glycol (Blanchon, 1991; Bonina et al., 1993). In accordance with these results, optimal percutaneous absorption is obtained with vehicles for which the polarity is similar to the polarity of the drug (Sloan et al., 1986). In contrast, the percutaneous flux obtained with oleic acid, which also has a similar theoretical solubility value, is not in accordance with this observation.

Transcutol® showed a very low flux value but displayed a synergistic effect when associated with Labrafac® (Table 3). It is well known that a combination of lipophilic and hydrophilic vehicles can improve the percutaneous flux compared to that obtained from each one individually. Rojas et al. (1991) observed the same synergistic effect of these vehicles in the percutaneous absorption of morphine.

3.4. Skin permeation of free base from TTS

After vehicle choice, the last part of this study was an optimization of a matrix type TTS formulation of primaquine. Thus, the parameters evaluated were polymer type, adhesive layer and drug concentration within polymeric devices. The compositions of the TTS formulations studied are summarized in Table 1. Percutaneous kinetic parameters, percutaneous flux and cumulative amount of penetrant per cm^2 at 48 h, are shown

Table 4
Permeation parameters in percutaneous release of primaquine from TTS formulations (the composition of TTS formulations 1a, 1b, 2a, 2b, 3a and 3b are summarized in Table 1)

Formulation	Flux ± SD[a] ($\mu g\,cm^{-2}\,h^{-1}$)	Qt 48 h ± SD[a] ($mg\,cm^{-2}$)
1a	15.0 ± 9.1	0.51 ± 0.22
1b	12.3 ± 2.0	0.58 ± 0.10
2a	75.2 ± 6.7	2.97 ± 0.4
2b	83.2 ± 13.2	2.99 ± 0.47
3a	93.0 ± 15.7	4.29 ± 0.63
3b	181.0 ± 25.5	5.87 ± 0.84

[a] $n \geq 5$.

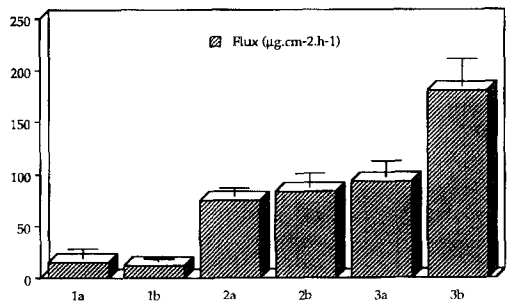

Fig. 2. Percutaneous flux values in release kinetics from TTS formulations (the composition of TTS formulations 1a, 1b, 2a, 2b, 3a and 3b are sumarized in Table 1). Error bars ± SD.

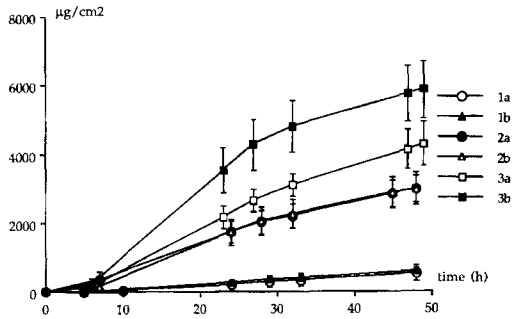

Fig. 3. Transdermal delivery profile of primaquine from TTS formulations (the composition of TTS formulations 1a, 1b, 2a, 2b, 3a and 3b are sumarized in Table 1). Error bars ± SD.

in Table 4. Percutaneous flux values are plotted as a histogram for comparison (Fig. 2).

For both polymers, Eudragit® and ethyl cellulose, it was observed that a decrease of adhesive layer and increase of drug concentration could improve the percutaneous flux values five- to six-fold. This observation suggests that a difference in diffusion behaviour through the polymeric matrix and adhesive layer causes a reduction in the permeating flux, limiting drug release from these devices. In addition, it is well known that an increase of priming dose (drug concentration in the adhesive layer) can improve the percutaneous flux of a drug from TTS formulations.

Improvement of flux values was also observed with increasing drug concentration in the polymeric matrix. The most efficient formulation was the ethyl cellulose TTS which showed a flux value of 181.0 ± 25.5 $\mu g\,cm^{-2}\,h^{-1}$. Only in this case was the difference between the flux values observed with different polymer type matrices significant (Student's t-test, $p \geq 0.05$). For the two last formulations (3a and 3b) the amounts of drug released through the skin at 48 h were 4.29 ± 0.63 and 5.87 ± 0.84 mg for Eudragit® and ethyl cellulose matrix, respectively. Nevertheless, the ethyl cellulose matrix showed a decline of steady-state flux at about 30 h (Fig. 3). This suggests that the performance of this formulation (3b) can be improved by modification of its composition.

In conclusion, we have shown in this study the suitability of primaquine for transdermal absorption. Furthermore, we have established that an ethyl cellulose-based TTS formulation is a viable dosage form that would confer the advantages of transdermal delivery in primaquine administration. Moreover, despite the preliminary optimization undertaken in this work, it is necessary to study many parameters related to a final dosage form, such as mechanical properties, chemical stability, skin irritancy as well as its pharmacokinetics.

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