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Oil–water–oil multiple emulsions for prolonged delivery of hydrocortisone after topical application: comparison with simple emulsions

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Abstract

Multiple emulsions show a high potential for prolonged delivery of drugs. In multiple emulsions, the entrapped substance can be transfered from the internal phase to the external phase through the middle phase, i.e. the membrane phase. These systems could be used for prolonged delivery of drugs. The present study was aimed to assess the suitability of oil–water–oil emulsions as prolonged release topical formulations of hydrocortisone. In order to avoid any influence of the composition of the formulation and to study only the effect of the emulsion type, a multiple emulsion as well as a simple emulsion were prepared with exactly the same composition. The percutaneous absorption of hydrocortisone from these two emulsion types was studied. At a finite dose, the percentage of hydrocortisone absorbed from the simple emulsion was 1.5-fold greater than that observed from the multiple emulsion, and the drug was kept longer in the epidermis and dermis from this galenic form. It was concluded that the multiple oil–water–oil emulsion systems may be used for obtaining a prolonged topical release of hydrocortisone. © 1998 Elsevier Science B.V.

Keywords: Multiple emulsion; Simple emulsion; Hydrocortisone; Percutaneous absorption; Prolonged release

1. Introduction

When a drug is applied to the skin two consecutive physical events may become rate-limiting steps in cutaneous permeation: the release of the active compound from the vehicle and its penetration through the skin barrier. These two processes are closely related, and both are dependent on the physical properties of the drug, vehicle and barrier (Kundu et al., 1993; Nastruzzi et al., 1993). The stratum corneum constitutes the principal barrier for cutaneous penetration and allows only

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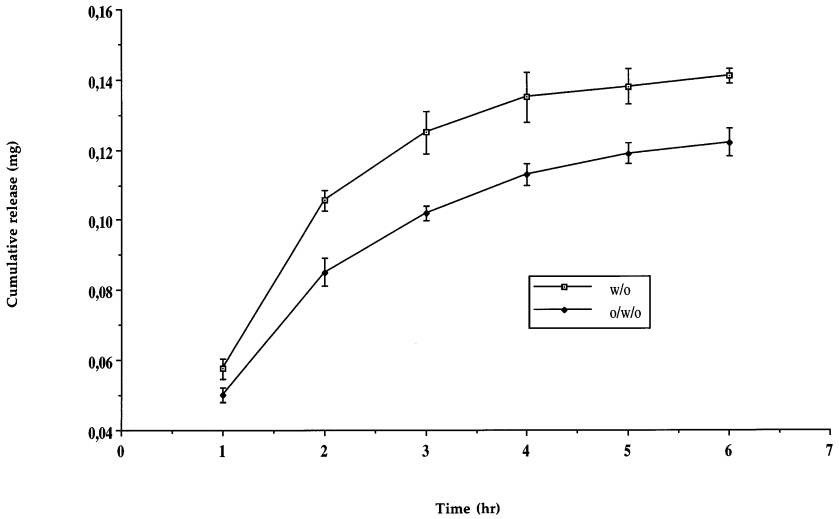

Fig. 1. In vitro release of hydrocortisone from water–oil and oil–water–oil emulsions across a cellulose membrane ($n = 6$).

limited absorption of the majority of drugs. The main characteristic of the drug influencing its release is its partition coefficient. The best vehicle for topical controlled release would be the one which contributes to a reversible decrease in the stratum corneum resistance and allows the controlled diffusion of molecules in the vehicle itself.

The role of the galenic form, particularly the emulsion type, was previously studied. In a multiple emulsion, the entrapped substance can be transfered from the internal phase to the external phase through the middle phase, which is called the membrane phase. The potential for the use of multiple emulsions for the prolonged release of drugs has been reported by various authors (Ferreira et al., 1994; Kampzemann and Sallis, 1995; Mishra and Pandit, 1989; Nakhare and Vyas, 1995).

The basic rational for the use of multiple emulsions as a mean for controlled delivery of drugs is that the drug, contained in the inner phase, is forced to diffuse through several phases prior to being released into the body fluids of patients (Laugel et al., 1996). For example, the release and percutaneous absorption of metronidazole and glucose from a water–oil–water emulsion was found to be intermediate between oil–water and water–oil emulsions following application at an infinite dose (Ferreira et al., 1994).

The present study was to assess the hydrocortisone liberation from different vehicles and the distribution of drug within the skin (dermis and epidermis) after percutaneous absorption. In the first part, we studied the hydrocortisone liberation from vehicles across a synthetic membrane and skin biopsies: hydrocortisone from oil–water–oil and water–oil emulsions applied at finite and infinite doses. When a finite dose is applied, the physicochemical and thermodynamic conditions of the freshly applied emulsion change radically (Shah et al., 1989). On the contrary, when an infinite dose is applied the formulation does not involve at the skin surface and the thermodynamic parameters are not altered. In order to avoid any influence of the composition of the formulation and to study only the effect of the emulsion type, a multiple emulsion as well as a simple emulsion were prepared with exactly the same qualitative and quantitative composition. In the second part, we compared the distribution of the drug in the different compartments of the skin. The skin permeation of hydrocortisone from each vehicle has been measured in vitro using excised human skin. This experiment was carried out to show a possible reservoir effect of multiple emulsions.

Table 1
Distribution of hydrocortisone into hairless rat skin after application

Type of emulsion	Surface wash	Epidermis	Dermis	Receptor fluid	Total yield (%)
Water–oil	87.5 ± 0.8	3.5 ± 0.2	2.3 ± 0.6	6.8 ± 0.3	100.1 ± 1.8
Oil–water–oil	87.5 ± 1.8	4.4 ± 0.3	4.9 ± 0.7	4.4 ± 0.3	101.2 ± 2.2

Values are given as mean (%) $\pm$ S.D., ($n = 6$).

2. Experimental

2.1. Preparation of emulsions

2.1.1. Materials

Hydrocortisone was purchased from Sigma (France).

The lipophilic (HLB = 4,6) and hydrophilic (HLB = 22) emulsifiers were non-ionic surfactants.

Oil phase: liquid paraffin (Cooperative pharmaceutique française, Melun, France), microcrystalline wax (Laserson, Etampes, France), lipophilic emulsifier (glycerol sorbitan fatty acid ester—ICI, Clamart, France) and hydroxyoctacosanyl hydroxystearate (ICI).

Aqueous phase: hydrophilic emulsifier (copolymer of ethylene and propylene oxides—ICI) and deionized distilled water.

All other chemicals used for analysis (methanol, acetic acid) were analytical HPLC grade obtained from Prolabo (Paris, France).

2.1.2. Manufacturing procedure

An oil–water–oil multiple emulsion and a water–oil emulsion were manufactured according to the same quantitative and qualitative formula. The oil–water–oil multiple emulsion was prepared in two steps, as described previously (De Luca et al., 1991; Matsumoto et al., 1976).

For the simple emulsion, the two emulsifiers (lipophilic and hydrophilic) were incorporated into the liquid paraffin and shaken until the solution was limpid. The hydrophilic emulsifier was only added to the oily phase to keep the same composition for the two types of formulations. The aqueous phase was incorporated into the oil phase by agitation for 15 min at 2000 rpm at 70°C. The temperature was allowed to fall to room temperature (25°C).

For the multiple emulsion, the first emulsification step provides an oil–water primary emulsion. The hydrophilic emulsifier was dissolved in the water. This phase and the liquid paraffin were homogenized at 1500 rpm for 1 h at 70°C, then the temperature was decreased to room temperature (25°C). The second emulsification step provides an oil–water–oil emulsion. The newly prepared oil–water emulsion was introduced into the outer oily phase containing the lipophilic emulsifier under agitation (1100 rpm) at 70°C for 20 min. The agitation was then kept for 20 min at 25°C.

Hydrocortisone at a concentration of 0.5% (w/w) was introduced by dispersion into the simple emulsion (water–oil) and in the primary emulsion (oil–water) for the multiple emulsion.

For each emulsion, the agitator consisted of a micro-vortex with a centrifugal turbine of 30 mm diameter.

2.2. Control of the emulsions

After preparation of the multiple emulsions, microscopic observations were made with an optical microscope (Laboval 4; Bioblock, France) at $1000\times$ magnification after dilution in the oil phase to check the multiplicity. Moreover, the stability and the multiplicity of the multiple emulsions have been controlled microscopically during all the experiments.

2.3. Solubility of hydrocortisone

The solubility of hydrocortisone in an aqueous phosphate buffer at pH 7.4 was determined by adding an excess amount of the drug to 20 ml of this solution. This mixture was stirred with a magnetic bar. The solution was then filtered

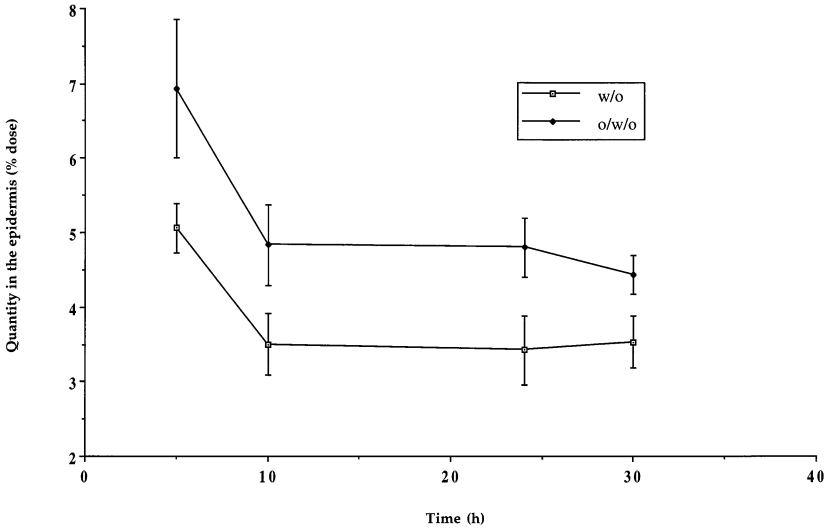

Fig. 2. Hydrocortisone quantity in the epidermis absorbed from water–oil and oil–water–oil emulsions.

(Millex HV, 0.22 μm, Millipore) and analyzed by HPLC.

2.4. Preparation of synthetic membranes and skin biopsies

Prior to their use, cellulose membranes (thickness: $25 + 1$ μm, HWC 5000, relative permeability: 0.8, Dianorm, Munich) were rinsed with distilled water and soaked in the receptor liquid (0.05 M phosphate buffer at pH 7.4).

Abdominal skin biopsies were excised from male hairless rats (350–400 g, Iffa Credo, I'Arbresle, France). Animals were sacrificed by ether inhalation, and abdominal skin was used immediately after removal of the subcutaneous fat.

2.5. In-vitro release and percutaneous absorption experiments

Hydrocortisone release across synthetic membrane and percutaneous absorption were determined with Franz diffusion cells (membrane surface area 1.76 cm^2 and cell volume 6.7 cm^3). The diffusion membrane was placed horizontally, dividing the cell into two compartiments: the donor and receptor compartments. The receptor fluid (0.05 M phosphate buffer at pH 7.4) was constantly stirred with a small magnetic stirrer bar in order to ensure its homogeneity.

Experiments were carried out at finite dose and infinite dose conditions. For the infinite dose, 500 mg of preparations were applied and the donor compartment was then covered with Parafilm (American National Can, Greenwich, CT) in order to achieve occlusive conditions. For the finite dose, 15 mg of the emulsions were applied to the skin and the donor compartment was left open so that the volatile components could escape, in order to mimic the used conditions.

Serial sampling was performed, at specified times, by totally removing the receptor fluid and refilling with fresh solution. The amount of hydrocortisone was determined by HPLC (high performance liquid chromatography).

At the end of the experiments, the hydrocortisone remaining on the surface of the skin was determined by washing twice times with 200 μl of ethanol and three times with 200 μl of water and removing the residue with a cotton swab. The washing solvent, pipette tips and cotton swab were added to a bottle containing 30 ml of ethanol. The concentration of hydrocortisone was

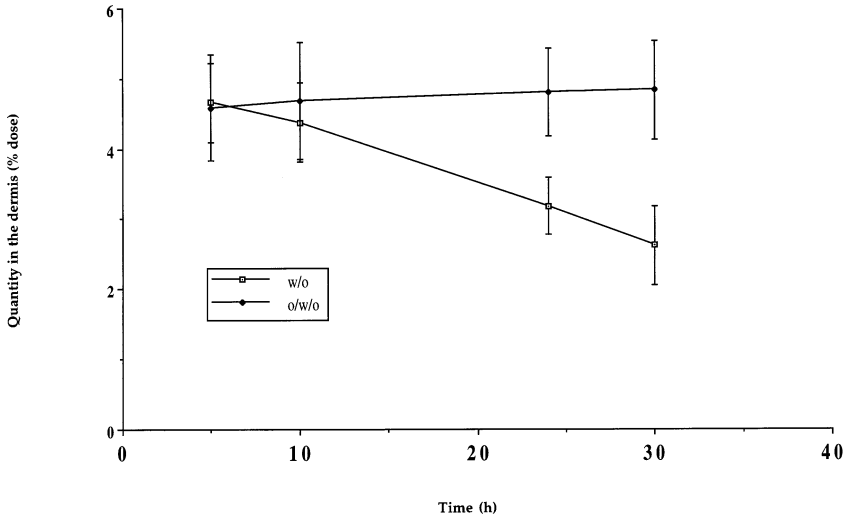

Fig. 3. Hydrocortisone quantity in the dermis absorbed from water–oil and oil–water–oil emulsions.

measured by RP-HPLC. The epidermis was mechanically separated from the dermis with spring nippes. The epidermis and the dermis were digested with 1 and 2 ml of sodium hydroxyle (0.5 M), respectively, overnight at 60°C. The amount of hydrocortisone in the filtered solutions was determined by HPLC.

2.6. HPLC analysis

The amount of hydrocortisone in the receptor fluid was determined by reversed phase HPLC with a C_{18} column (Spherisorb, 5 μm, 250 × 4 mm ID, Prolabo, Nogent-sur-Marne, France). A UV detector (Shimadzu SPD-2A UV spectrophotometer, Touzart et Matignon, Vitry-sur-Seine, France) operating at 254 nm and an integrator (Hewlett-Packard 3395) were used for quantification of the drug. The mobile phase consisted of 50% methanol and 50% acetate buffer 0.15 M at pH 2. It was filtered through a 0.22 μm Millipore® filter under vacuum. The flow rate was 1 ml/min. The limit of detection was about 1.6×10^{-6} g/l.

For the determination in the multiple emulsions, samples were diluted with the paraffin oil (1:1) prior to injection.

3. Results and discussion

3.1. Solubility of hydrocortisone in the receptor fluid

The structure of hydrocortisone does not confer on it a good solubility neither in hydrophilic or lipophilic media. The determination of hydrocortisone solubility was carried out to verify that the solubility of hydrocortisone in the receptor medium did not constitute a limiting factor in the absorption process. We found that the hydrocortisone was soluble in the receptor fluid at about 0.6 g/l. If the total quantity of applicated hydrocortisone at an infinite dose was absorbed, the concentration in the receptor medium should be 0.4 g/l.

The partitioning coefficient of hydrocortisone was then determined. The composition of the liquid media was chosen to meet the oil–water–oil emulsion with a relative phase volume and identical concentrations of surfactants: the aqueous layer consisted of water and hydrophilic emulsifier. The organic layer was liquid paraffin containing the lipophilic surfactant. After 24 h of strong contact, the partitionning coefficient was calculated through the chromatographic measure-

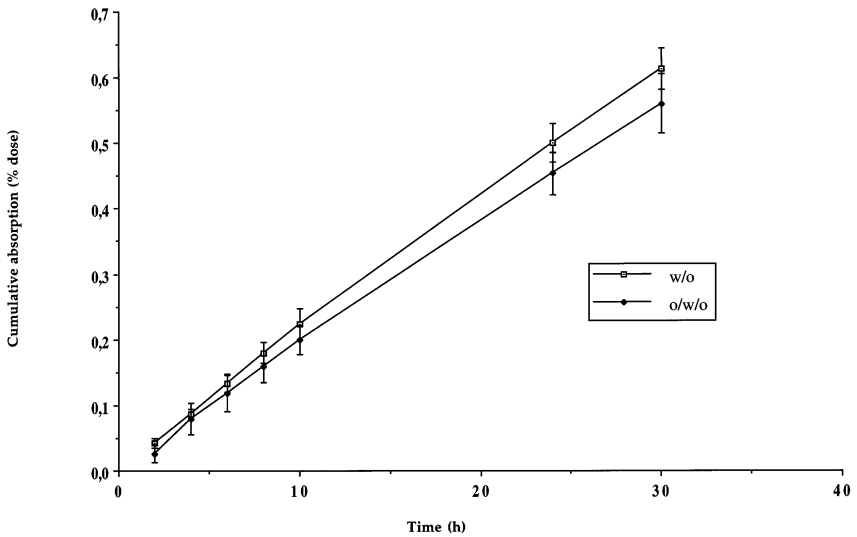

Fig. 4. Percutaneous absorption profiles of hydrocortisone from water–oil and oil–water–oil emulsions at infinite dose.

ment of hydrocortisone in both the layers. The value of 15.38 (± 0.05, $n = 3$) allows us to conclude that the hydrocortisone remained preferentially in the oily phase.

After manufacturing the multiple emulsion, the yield of entrapment of hydrocortisone was calculating according to the following equation:

$$P_0 = \left(\frac{Q_W - Q_0}{Q_W} \right)$$

where Q_W is the amount of hydrocortisone introduced in the primary emulsion, and Q_0 is the amount of tracer present in the external aqueous phase immediately after manufacture. Q_0 was determined chromatographically according to the technique previously described.

The entrapment of the hydrocortisone was 85.3% (± 0.15, $n = 3$) in the internal oily phase. This value allows us to consider that hydrocortisone will remain in the inner oily phase of the oil–water–oil emulsion and would not significantly diffuse into the external oily phase of the multiple emulsion. Anyhow, all the experiments were carried out within a short period (15 days maximum) after manufacturing the emulsions.

3.2. In vitro hydrocortisone release from the galenic form

The in-vitro release of hydrocortisone studied for an infinite dose with a cellulose membrane showed, as expected, an exponential profile. The release was slower from the multiple emulsion than from the simple emulsion (Fig. 1), according to the concentration in the receptor fluid. These results show, on the one hand, that the release of hydrocortisone depends markedly on the emulsion type and, on the other, that the cellulose membrane presents only negligible resistance to the diffusion of the hormone. At 6 h after application, the quantity of hydrocortisone released from the simple emulsion was significantly 20% greater that the one from multiple emulsion. However the total amount released for the two types of emulsions was only about 10% of the total applied dose. This last result was mainly due to the presence of an oily continuous phase. As reported by some published works, the ability of the emulsion to be retained and its substantivity were improved in systems with an oily external phase (Kundu et al., 1993) This phase slowed the release of the

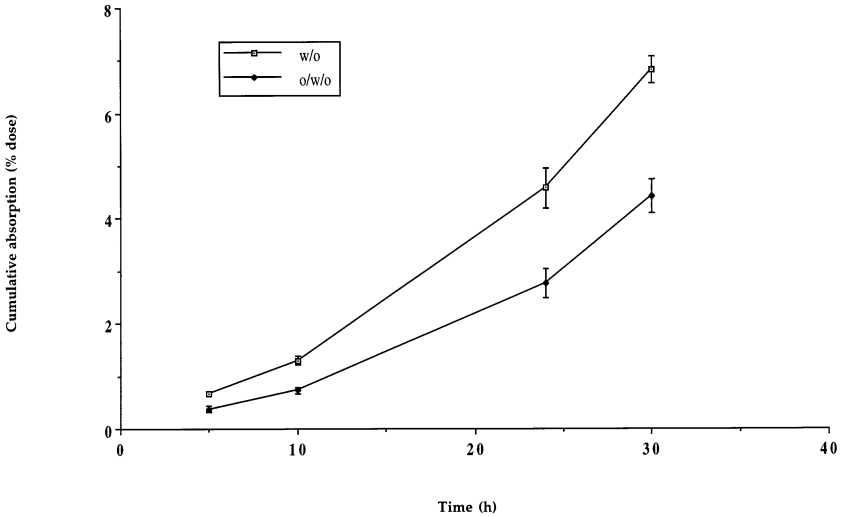

Fig. 5. Percutaneous absorption profiles of hydrocortisone from water–oil and oil–water–oil emulsions at finite dose.

incorporated substance in comparison with an aqueous continuous phase.

3.3. Distribution of hydrocortisone in the skin

The two types of emulsions (simple and multiple) containing 0.5% of hydrocortisone were compared. The measurements were carried out for a period of 30 h or less after the application, because these experimental conditions whould be too far removed from the therapeutical administration (one or two applications per day).

The in-vitro uptake of hydrocortisone at a finite dose into the skin from the emulsion at the end of the 30 h test period is presented in Table 1. It was interesting to study the distribution of hydrocortisone within the skin (epidermis and dermis) in order to verify whether the absorbed quantities of hydrocortisone correlated with the sum of the drug delivered in the receptor fluid and the drug retention on the skin. Upon combining the quantity found in the receptor, in the surface, in the epidermis and in the dermis, complete recovery mass balance (100%) for hydrocortisone was found for the two emulsions studied. The sink conditions were respected at a finite dose and the solubility was not a limiting parameter for the in-vitro release and in-vitro percutaneous distribution studies.

Table 2
Relative distribution of hydrocortisone into hairless rat skin after application

Type of emulsion	Epidermis	Dermis	Receptor fluid
Water–oil	27.7 ± 1.0	18.3 ± 07	54.0 ± 1.3
Oil–water–oil	32.1 ± 0.7	35.8 ± 1.0	32.1 ± 0.3

Values are given as mean (%) $\pm$ S.D., ($n = 6$).

Liquid chromatographic quantitative determinations of active principle in each level of skin samples showed the reservoir effect of this galenic form. The quantities of hydrocortisone recovered in both epidermis and dermis at $t = 30$ h are higher with multiple emulsion than simple emulsion. In the epidermis (Fig. 2), the quantity kept from the multiple emulsion was higher, and decreased more slowly. For both emulsions, the equilibrium in the epidermis was reached about 10 h after application. More spectacular were the differences observed in the dermis (Fig. 3). The hydrocortisone was clearly kept in the dermis from the multiple emulsion, whereas the hydrocortisone quantity from the simple emulsion decreased rapidly with the time.

The total absorption was expressed as total hydrocortisone quantities detected according to time in the receptor fluid (Fig. 4). The results after

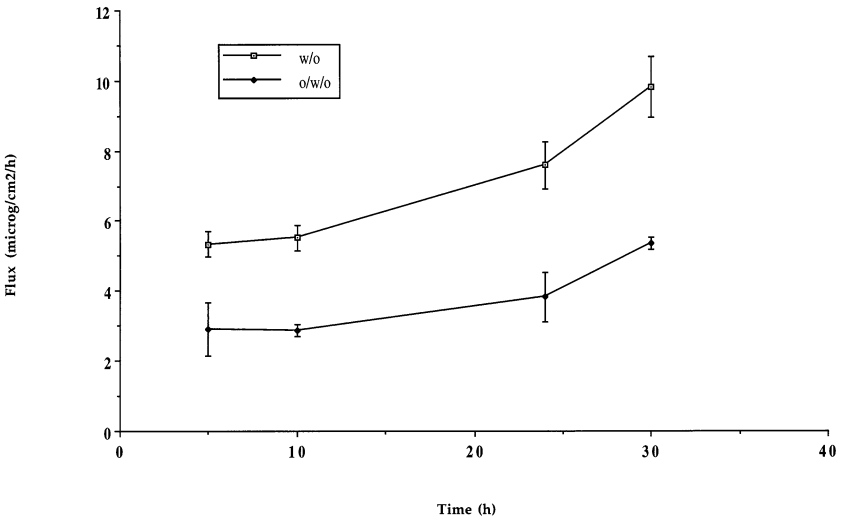

Fig. 6. Hydrocortisone flux across biopsie skin from water–oil and oil–water–oil emulsions as a function of time.

application of an infinite dose of 500 mg did not show significant difference between the vehicles: 30 h after application, the percentage of hydrocortisone found in the receptor fluid was about equal for both the simple and the multiple emulsions. These quantities are clearly lower for the experiments carried out with skin samples than those obtained with cellulose membrane. This observation can be explained by the polar structure of cellulose and the lipophilic barrier role of the skin. The controlled release of hydrocortisone from the multiple emulsion was not demonstrated when an infinite dose of the drug was applied on the skin.

Then, the further experiments were conducted with a finite application of 15 mg. For this one, the percentages of hydrocortisone found in the receptor as a function of time are presented in Fig. 5. The total absorption range appeared quite low (4–6%) but these values are usually obtained with lipophilic drug substance (Table 1). At 30 h after application, the amount of hydrocortisone recovered from the skin surface in the washing liquids represents 87.5% of the applied dose for both emulsions. However, the percentage of total absorbed hydrocortisone found in the receptor fluid from the water–oil emulsion (6.5%) was a significant 1.5-fold greater that the one measured from the oil–water–oil emulsion (4.4%).

Our results showed that the cumulative absorption of hydrocortisone is the same for both the studied emulsions: the sums of epidermis, dermis and receptor fluid amounts are similar and high i.e. 10% of the total applied dose. However the distribution of this 'active part', within the layers of the skin is markedely different (Table 2). The multiple emulsion favoured the retention of hydrocortisone in the dermis, improving the concentration of the drug within the site to be treated. In that point of view, the multiple emulsion can be attempted to prolong the therapeutic local effect. This observation must be supported by further works with various drugs characterised by different polarity.

The hydrocortisone absorption flux profiles exhibited two drug release phases, the initial slow release, followed by a rapid release phase thereafter (Fig. 6). It is interesting to notice that the hydrocortisone flux seemed to increase with time for the two emulsion types studied. This profile reveals that the release is not due to a pure phenomenon only. A hypothesis for the presence of two phenomena can be drawn: (a) a dynamical one, i.e. diffusion, almost identical for both the emulsions because its related to remanence (this phenomenon depends on the oil continuous phase) and (b) a statistical one, i.e. accumulation in sebaceous glands. These two phenomena oc-

cured simultaneously in the first part of flux profile.

During the second stage, the increase in flux is related to the diffusion of accumulated hydrocortisone. Moreover, the hydrocortisone flux is significantly lower after a topical administration of the oil–water–oil type multiple emulsion compared with the water–oil emulsion.

The slow release rates of hydrocortisone from the oil–water–oil emulsions may implay an interfacial barrier prolonged release in which the drug contained in the internal phase would be released at a rate governed by its ability to partition into and diffuse through the interfacial film as well as through the water membrane separating the two oil phases.

This result is indicative of a delay in the release of hydrocortisone within the multiple emulsion, either, some differences in kinetics of diffusion between the emulsions which is attributed to both the evaporation rate of the volatile components and the structure remaining on the skin after application (Langlois and Friberg, 1993), or accumulation of hydrocortisone in the sebaceous glands which is higher when the multiple emulsion is applied.

4. Conclusion

The percutaneous absorption of hydrocortisone from two emulsion types obtained with the same composition was studied.

The evidence presented in this report led us to conclude that the multiple emulsion oil–water–oil system can be utilized as a potential prolonged release dosage form of hydrocortisone. The topical use of the oil–water–oil multiple emulsion with hydrocortisone tends to decrease the systemic effect of this drug, because it was kept in the epidermis and dermis. In the next study, these results should be confirmed with in-vivo or in-vitro experiments with the human skin.

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