ELSEVIER

International Journal of Pharmaceutics 109 (1994) 251–259

international journal of pharmaceutics

Vehicle influence on in vitro release of metronidazole: role of w/o/w multiple emulsion

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(Received 25 October 1993; Accepted 28 February 1994)

Abstract

Few studies have been made on the topical administration of w/o/w multiple emulsions, although their advantages in the controlled release by oral and parenteral administration have already been shown. The objective of this study was to compare the in vitro release of a hydrosoluble molecule (metronidazole) from w/o/w multiple, w/o and o/w emulsions. In order to avoid any influence of the formulation, all emulsions were prepared according to the same formula. The microscopic aspect, conductivity values and rheological parameters confirmed that three emulsion types were obtained. Metronidazole release was studied on synthetic membranes and on rat skin biopsies. When the synthetic membrane offered negligible resistance to passage of the drug (cellulose membrane), metronidazole release from the w/o/w emulsion was slightly slower compared with the o/w emulsion, while much slower release was observed with the w/o emulsion. When the synthetic membrane was rate-controlling diffusion (silicone), the difference between the emulsions was decreased, although the rank order remained the same. In the case of skin, the absorption of metronidazole is similar from w/o/w and o/w emulsions, however, it is faster from these two emulsions than from the w/o emulsion.

Key words: w/o/w emulsion; o/w emulsion; w/o emulsion; In vitro release; Percutaneous absorption; Metronidazole

1. Introduction

The stratum corneum is the principal barrier for cutaneous penetration and allows only slow absorption for the majority of drugs. In any case, the use of appropriate vehicles allows drug absorption to be increased by changing either the permeability of the stratum corneum (Roberts and Anderson, 1975) or the thermodynamic activity of the drug (Schaefer et al., 1978). In this respect, the best vehicle for topical controlled

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Abbreviations/nomenclature: τ, shear stress; ω, pulsation, angular frequency; N, frequency; G^*, shear modulus; δ, phase angle stress/strain; η, steady flow viscosity; ϵ_c, critical strain; ϕ, volume fraction of the dispersed phase; M, mass of released drug; J, dM/dt, steady-state flux; D, diffusion coefficient; C, drug concentration (mg/ml); h, membrane thickness; P, permeability coefficient.

SSDI 0378-5173(94)00089-N

release would be the one which contributes to a reversible decrease in the stratum corneum resistance and allows the controlled diffusion of molecules into the vehicle itself.

Multiple emulsions have already shown their advantages for controlled release in vitro (Fukushima et al., 1987; Omotosho et al., 1989) and in vivo after oral administration (Mishra et al., 1990; Omotosho et al., 1990; Lin et al., 1992) as well as after parenteral administration (Miyakama et al., 1993). Few studies have been performed on topical administration. In fact, there is only one published report (Kundu, 1990) in which it was shown that multiple emulsions release their contents more slowly than solutions, however, no comparison was made with simple emulsions.

The aim of this work was to compare the release of a hydrosoluble molecule (metronidazole) from a w/o/w multiple emulsion and from simple emulsions (o/w and w/o). In order to avoid any influence of the formulation and to study only the effect of the emulsion type, a w/o/w multiple emulsion as well as simple emulsions (o/w and w/o) were prepared with exactly the same composition. Metronidazole release and percutaneous absorption were investigated on synthetic membranes and on rat skin biopsies, respectively. It was shown that the release obtained with w/o/w multiple emulsions was intermediate between that achieved with o/w and w/o simple emulsions. This same rank order was obtained when the rate of release was controlled by the vehicles as well as when the membrane played the role of limiting step.

2. Materials and methods

2.1. Materials

Metronidazole was purchased from Sigma Chimie S.a.r.l. (France). The following chemicals, obtained from commercial sources, were used in formulation of the emulsions: lipophilic surfactant (Hypermer A60: a modified polyester) and hydrophilic surfactant (Synperonic PE/F127: a block copolymer of ethylene oxide and propylene oxide) were purchased from ICI Surfactants (Clamart, France) and paraffin oil was supplied by Coopération Pharmaceutique (Paris, France). All other chemicals used for analysis were analytical reagent grade or HPLC grade.

2.2. Preparation of emulsions

A w/o/w multiple emulsion and (o/w and w/o) simple emulsions were prepared according to the same formula. This was 20% of paraffin oil, 3.2% of Hypermer A60, 0.8% of Synperonic PE/F127, 0.5% of $MgSO_4 \cdot 7H_2O$, 0.5% of metronidazole and 75.0% of distilled water, by weight.

The w/o/w multiple emulsion was prepared in two steps, as described previously (De Luca et al., 1990). Briefly, in the first step the primary emulsion was formed and in the second step 80% of this emulsion was dispersed in aqueous solution of the hydrophilic emulsifier.

The simple emulsions were made by adding aqueous phase containing $MgSO_4 \cdot 7H_2O$ and metronidazole to the oil phase. The mixture was stirred at 70°C. In the case of the w/o emulsion, the two emulsifiers were introduced into the oil phase and stirring was carried out using a Rayneri agitator (turbo-test type, Bioblock, Vanves, France) at 2000 rpm. In the case of the o/w emulsion, each emulsifier was incorporated into the phase for which it had the greater affinity and stirring was carried out with an Ika agitator (Ultra turrax type, Bioblock, Vanves, France) at 3000 rpm. After cooling, the o/w emulsion was immediately homogenized with a Microfluidizer (Microfluids Corp., Newton, MA).

2.2. Preparation of synthetic membranes and biopsies of skin

Cellulose membranes (thickness, 25 ± 1 μm; MWC 5000) (Dianorm, Munich) were rinsed with distilled water and soaked in the receptor liquid (0.05 M phosphate-buffered saline, pH 7.4).

Silicone membranes (Silastic 500–1; thickness, 130 μm) (Dow Corning Corp., MI, U.S.A.) were cleaned with hot soapy water, then rinsed abundantly with hot water followed by distilled water.

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

2.3. Studies of characteristics of the emulsions

After preparation of the emulsions, microscopic observations were made with an optical microscope (Laboval 4, Bioblock, France) at 1000 × magnification after dilution in the appropriate dispersed phase. Furthermore, the particle size of the o/w emulsion was estimated with a Coulter Nanosizer (model N4 MD, Coulter Electronics Inc., Hialeah, U.S.A.).

Conductivity (Conductivity Meter CDM3, Copenhagen) was measured in order to determine the emulsion type (w/o/w, w/o or o/w).

Since the task of obtaining three different emulsion types with the same composition is difficult, rheological analyses were used to characterize the structure of the emulsions, especially the w/o/w multiple and w/o simple emulsions. Rheological behavior was studied with a CSL100 rheometer (Carri-Med, Rheo, Palaiseau, France) using cone-plate geometry. The emulsions were examined by carrying out oscillatory viscoelastic analysis, as previously described (Grossiord et al., 1993; Terrise et al., 1993). During this assay, the sample was submitted to a sinusoidal shear stress τ of pulsation ω ($\omega = 2\pi N$, where N = frequency). The rheological parameters can be calculated from this analysis, as G^* (the shear modulus) and δ (the phase angle of the stress with respect to the strain).

Since the o/w emulsion was very fluid, it was not possible to apply such an oscillatory test. The value of G^* in Table 1 (see below) was calculated as follows: $G^* = \omega\eta$, where η is the steady-state viscosity, determined by viscosimetric analysis.

2.4. In vitro release and percutaneous absorption

Metronidazole release and its percutaneous absorption was determined with modified Franz diffusion cells (membrane surface area 1.76 cm^2 and cell volume 6.7 cm^3). The membrane was held horizontally, dividing the cell into two compartments: the donor and receptor compartments. The donor compartment was covered with Parafilm® (American National Can, Greenwich, CT) in order to achieve occlusive conditions. The receptor fluid (0.05 M phosphate-buffered saline, pH 7.4; containing 0.01% of $HgCl_2$ as preservative) was constantly stirred with a small magnetic stirring bar in order to ensure its homogeneity.

After application of the preparations (500 mg) serial sampling was performed at specified times by totally removing the receptor fluid and refilling with fresh solution. A 20 μl aliquot of the sample was subsequently analysed by HPLC. All experiments were performed at 37°C.

2.5. Solubility of metronidazole in various surfactant solutions at 30°C

The solubility of metronidazole in aqueous solutions of Synperonic PEF/127 at different concentrations was determined by adding an excess amount of the drug to 20 ml of surfactant solution. These mixtures were maintained at 30°C in a water bath and shaken for 5 days. The solutions were then filtered (Millex HV, 0.45 μm, Millipore) and analyzed by HPLC.

2.6. HPLC analysis

The amount of metronidazole in the receptor fluid was determined by reverse-phase HPLC (Waters) with a C-8 column (Lichrosorb RP-8; 4 mm i.d. and 250 mm long). An auto-injector (WISP Model 712, Waters Millipore, Saint Quentin en Yvelines, France), a UV detector (model 484, Waters Millipore, Saint Quentin en Yvelines, France) operated at 320 nm and an integrator (model 745, Waters Millipore, Saint Quentin en Yvelines, France) were used for quantification of drug. The mobile phase consisted of 60% of 0.05 M acetate buffer, pH 4.5 and 40% of methanol at a flow rate of 1 ml/min.

Under these conditions the retention time of metronidazole was 4.5 min. The detection limit was 0.1 μg/ml.

2.7. Statistical analysis

Tests for significant differences between means were made by analysis of variance (ANOVA). Reference to significant difference in the subsequent text denotes that the test was carried out at level $p < 0.05$.

3. Results and discussion

3.1. Characterization of emulsions

The characteristic parameters listed in Table 1 clearly demonstrate that three different emulsion types were obtained. The microscopic aspect of the w/o/w multiple emulsion was characteristic of these systems. The oil globules were multiple and their mean diameter could be estimated at 10 μm, while the mean diameter of the inner aqueous phase globules ranged from 0.5 to 1 μm. On the other hand, the globules of the o/w simple emulsion showed a mean diameter of about 240 nm and those of the w/o emulsion had a mean diameter below 1 μm.

The emulsion type was confirmed by conductivity analysis. Emulsions with aqueous continuous phase (w/o/w multiple and o/w emulsions) yielded conductivity values above those of distilled water (2 μS). On the other hand, the w/o emulsion, which had an oil continuous phase, led to lower conductivity values (0.02 μS). The difference between the conductivity of the w/o/w multiple emulsion and that of the o/w emulsion shows that the efficiency of entrapment of the marker electrolyte ($MgSO_4$) in the internal aqueous phase of the multiple emulsion is close to 100%.

Typical plots of G^*-τ and δ-τ at a fixed frequency ($N = 1$ Hz) of w/o/w multiple emulsion and w/o emulsion are shown in Fig. 1A and B, respectively. At low shear, the graphs demonstrate a plateau region where these parameters are constant. This plateau region represents a structure not disturbed by stress. On the other hand, above a critical strain ϵ_c (defined by the value of δ equal to 45°), the structure is disturbed. The emulsions then become more viscous than elastic and the oscillatory parameters (G^* and δ) vary.

The values of G^* and δ at the plateau region (Table 1) are comparable for w/o/w and w/o emulsions, although beyond ϵ_c, the decrease in G^* and the increase in δ show different profiles for each emulsion.

The critical strain values ϵ_c (Table 1) are consistent with the existence of a dispersed and high volume fraction structure (Princen and Kiss, 1986). This observation is confirmed by the existence of a yield stress, which was determined by steady flow experiments. However, the difference observed between the ϵ_c values of the w/o/w multiple emulsion (0.52) and the w/o emulsion (0.14) is unexpected. In fact, since the volume

Table 1
Characteristics of the w/o/w multiple, w/o and o/w emulsions

Emulsion	Microscopic aspect	Conductivity	Continuous phase	Oscillatory parameters [c]
w/o/w multiple emulsion	multiple globules 10 μm	4.5 μS [b]	aqueous	$G^* = 198$ N m^{-2} $\delta = 21.27°$ $\epsilon_c = 0.52$
o/w simple emulsion	simple globules 0.24 μm [a]	560 μS [b]	aqueous	$G^* = 0.054$
w/o simple emulsion	simple globules < 1 μm	0.02 μS	oil	$G^* = 204$ N m^{-2} $\delta = 28.08°$ $\epsilon_c = 0.14$

[a] Determined by Coulter Nanosizer.
[b] Determined using a CDM3 Conductivity Meter.
[c] G^* and δ values represent the means recorded in the plateau region.

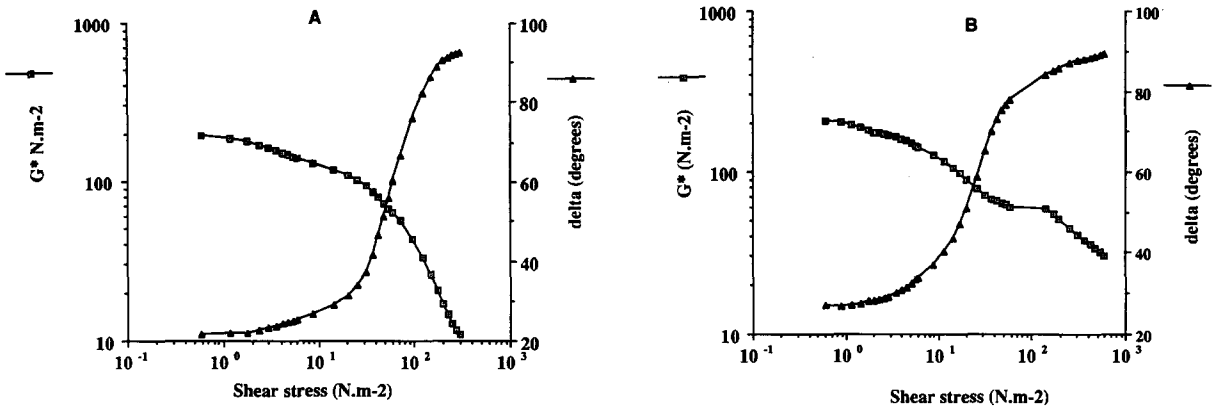

Fig. 1. Evolution of G^* and δ at a frequency fixed at 1 Hz as a function of shear stress scanning for (A) the w/o/w multiple emulsion and (B) the w/o emulsion.

fractions of the dispersed phase were close to each other ($\phi \cong 0.76$), they would be expected to have very similar critical strain values. This difference could be attributed to a small change in the volume fraction of the multiple emulsion. This phenomenon can be explained by the breakdown of multiple droplets. Such breakdown would induce a decrease in the volume fraction and an increase in the ϵ_c values.

It can seem very surprising to obtain, using the same formula and according to different manufacturing procedures, either a w/o/w emulsion or a o/w or w/o emulsion. This original result can be explained by the fact that multiple emulsions are situated between w/o and o/w emulsions. In addition, a multiple emulsion is one in which o/w and w/o emulsions can exist simultaneously. An HLB value of about 9 facilitated the preparation of these emulsions.

Having the same formula for three emulsions allowed us to study the influence of the emulsion type alone on the in vitro release and percutaneous absorption of metronidazole.

3.2. *In vitro release of metronidazole*

The in vitro release of metronidazole studied a with cellulose membrane was non-linear and very rapid in the case of w/o/w and o/w emulsions; about 50% of the dose applied was released within 1 h. In contrast, the release from the w/o emulsion was 20-fold lower and linear as a function of time; only about 1,5% of the dose applied was released within 1 h (Fig. 2). At 5 h, the cumulative amount of metronidazole released from the o/w emulsion was significantly greater than that from the w/o/w emulsion.

These results show, on the one hand, that the release of metronidazole depends markedly on the emulsion type and, on the other, that the cellulose membrane presents only negligible resistance to the diffusion of metronidazole. It is expected, therefore, that a plot of the cumulative

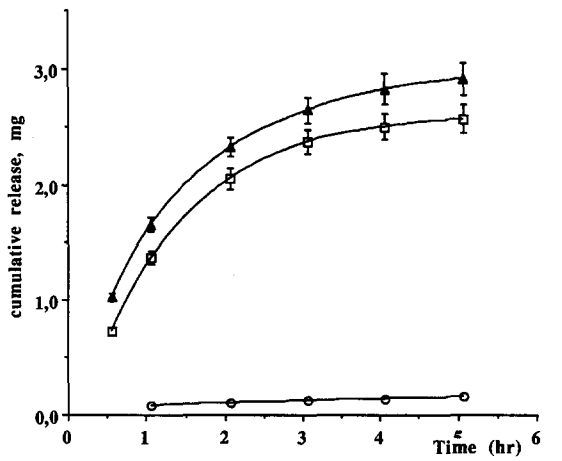

Fig. 2. In vitro release of metronidazole from w/o/w (□), o/w (▲) and w/o (○) emulsions across cellulose membrane. Values are the means ($n = 4$) ± SD.

Table 2
Solubility of metronidazole in various emulsifier (Synperonic PEF/127) solutions at °C

Synperonic PEF/127			
0% (aqueous)	1%	2%	4%
11.24 mg/ml	12.15 mg/ml	12.22 mg/ml	12.36 mg/ml

amount vs the square root of time would be linear. However, this was not observed in the case of w/o/w and o/w emulsions. The release process described by Higuchi (1962) (Bottari et al., 1974), when the membrane offers little resistance to drug penetration, is based on a series of assumptions. Among these is that the percentage of drug released should be not too large (< 30%). In fact, metronidazole is released too rapidly from the w/o/w and o/w emulsions to allow this relationship to hold.

For the o/w emulsion, partitioning of metronidazole favouring the external aqueous phase would be expected. As a rule, if the free metronidazole concentration in the aqueous phase were greater than that in the micelles, this should increase the rate of release from the o/w emulsion. In fact, we found that the solubility of metronidazole in various hydrophilic surfactant solutions (Synperonic PE F/127) was similar (Table 2). Takashima et al. (1983) showed that the rate of release of fluocinolone from creams increased when the free drug concentration increased in the aqueous phase of o/w emulsions.

In contrast, for the w/o emulsion, the favourable partitioning towards the internal aqueous phase would render the drug almost unvailable in the external oil phase. As the drug concentration in the oil phase is depleted, it is compensated by the partitioning of metronidazole from the aqueous phase. On other hand, since the cellulose membrane is saturated with the receptor phase, the metronidazole in the oil phase must partition into this aqueous receptor phase. This process therefore becomes the rate-controlling factor for diffusion.

Although the release of metronidazole from the w/o/w multiple emulsion was slower than that obtained with the o/w emulsion, this difference was much smaller than expected. This phenomenon can be explained by the transport of water or other products present in the receptor compartment to the donor compartment. This event could result in a greater extent of diffusion of the water and consequently of the metronidazole across the oil phase from the internal aqueous compartment to the external aqueous phase of the w/o/w emulsion due to the increased difference in osmolarity between the two phases. A large decrease in the viscosity of this emulsion was observed 1 h after emulsion application, nevertheless, the microscopic aspect of the w/o/w multiple emulsion after the permeation test remained unchanged.

The in vitro release of metronidazole with a silicone membrane was studied for 24 h (Fig. 3). Comparison with the patterns of metronidazole diffusion across the cellulose membrane revealed several differences, although the rank order of the emulsions was the same. Diffusion from the o/w emulsion was 1.8-fold faster that from the w/o emulsion, whereas diffusion from the w/o/w emulsion was intermediate and significantly different from the two simple emulsions. After 24 h only 1.74, 2.17 and 2.96% of the dose applied was released from the w/o, w/o/w and o/w emulsions, respectively, and no exhaustion of the donor phase was observed. The plot of the rate of release of metronidazole from these emulsions

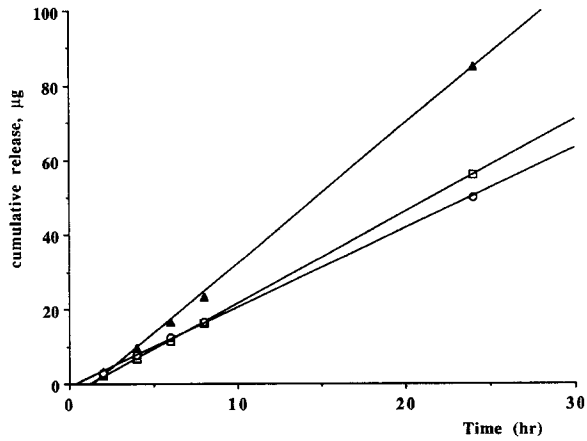

Fig. 3. In vitro release of metronidazole from w/o/w (□), o/w (▲) and w/o (○) emulsions across silicone membrane. Values are the means ($n = 5$) ± SD.

was linear as a function of time and the rates were slower than those obtained with the cellulose membrane. There was a poor correlation between the quantity released and square root of time. This suggests that the membrane is the rate-controlling barrier.

In order to prove this hypothesis, metronidazole release from aqueous solution at 0.5% was studied (Fig. 4). The rate of release obtained with the aqueous solution (1.209 $\mu g/cm^2$ per h) was slower than that observed with the o/w emulsion (2.119 $\mu g/cm^2$ per h). This result confirms the validity of the above hypothesis. The fact that the emulsion released the metronidazole faster than the aqueous solution could be explained by the following: the favourable partitioning of metronidazole toward the aqueous phase of the o/w emulsion can increase its thermodynamic activity in this phase and/or the surfactants in the o/w emulsion may improve wetting of the membrane surface and consequently increase the area of contact between the donor phase and the membrane.

The in vitro percutaneous absorption profiles of metronidazole are shown in Fig. 5. The absorption profiles of metronidazole across the skin show a longer lag time than that observed with the silicone membrane. This observation demonstrates the resistance of the stratum corneum to the passage of the drug, on the one hand, and emphasizes the difference between the emulsions, on the other. The o/w emulsion demonstrated a greater lag time than that for the w/o/w and w/o emulsions. In the o/w emulsion the drug is free and partitioning can take place immediately with the skin, while in the other two cases the drug must equilibrate at different interfaces before being free to be pass into the skin.

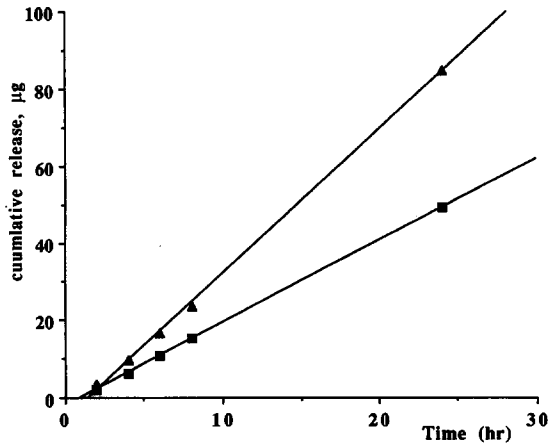

Fig. 4. In vitro release of metronidazole from (▲) an o/w emulsion and (■) an aqueous solution (0.5%) across silicone membrane. Values are the means ($n = 5$)±SD.

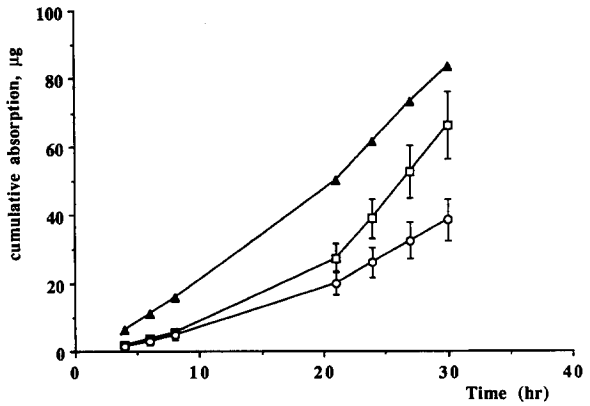

Fig. 5. Percutaneous absorption profiles of metronidazole from w/o/w (□), o/w (▲) and w/o (○) emulsions. Values are the means ($n = 5$)±SD. The SD values for the o/w emulsions are not represented for purposes of clarity.

These absorption profiles indicate the same rank order as that observed with the silicone membrane when the steady-state rates of permeation between the two simple emulsions are compared. The fluxes observed with the silicone membrane and the skin are significantly faster from the o/w and w/o/w emulsions than from the w/o emulsion. In the case of skin, however, the steady-state flux from the w/o/w multiple emulsion was not significantly different from that observed with the o/w emulsion (Table 3). The absorption of metronidazole from the w/o/w emulsion was significantly lower in extent than that from the o/w emulsion only during the first 8 h after application of the preparations. In fact, the lipids in the stratum corneum could break down the multiple globules and/or increase the diffusion of metronidazole across the oil membrane.

It is interesting to note than the profiles of release of metronidazole with the three mem-

Table 3
Permeability coefficients (P) and steady-state flux (J)[a] of metronidazole from emulsions across silicone membrane and hairless rat skin

Emulsions	Silicone membrane		Skin	
	J (μg/cm^2 per h)	P (cm/h) ($\times 10^{-4}$)	J (μg/cm^2 per h)	P (cm/h) ($\times 10^{-4}$)
w/o/w multiple emulsion	1.494 (0.245) [b]	2.738	2.409 (0.348)	4.907
o/w emulsion	2.119 (0.238)	3.939	2.112 (1.034)	3.826
w/o emulsion	1.204 (0.028)	2.317	1.168 (0.165)	2.246

[a] Values of steady-state flux are the means of five determinations.
[b] Values in parentheses represent the SD.

branes present the same rank order. However, the vehicle governs the release of the drug in the case of the cellulose membrane, whereas the membranes are the rate-controlling barriers when the silicone membrane and skin biopsies are used. In order to gain some insight into the principles governing these diffusion models, the release of metronidazole from the w/o emulsion across the cellulose membrane was studied more quantitatively. The rate (flux) was calculated as follows (Shah et al., 1989):

$$J = \mathrm{d}M/\mathrm{d}t = D \cdot C/h$$

where M is the mass of drug released, D denotes the diffusion coefficient, C is the concentration of the drug and h represents the thickness of the membrane. The steady-state flux value was evaluated from the linear portion of the plots of M vs t.

The flux calculated from the slope of the linear portion ($r = 0.99$) of this line was 10.212 μg/cm^2 per h. This value is about 8-fold greater than that obtained with the skin or silicone membrane (Table 3). This result confirmed the above hypothesis about the diffusion models.

When the membrane presents a negligible resistance to diffusion (cellulose), the release of metronidazole from the w/o/w emulsion is slightly slower compared with the o/w emulsion, while much slower release is observed with the w/o emulsion. The rank order is the same when the membrane is the rate-controlling barrier (silicone), however, the difference observed between the flux values from the w/o/w or o/w emulsions and those from the w/o emulsion was decreased. In the case of skin, the rank order is the same only when the flux values from emulsions with an aqueous continuous phase are compared with those obtained with the w/o emulsion.

The faster absorption observed in the case of emulsions with an aqueous continuous phase (w/o/w multiple and o/w emulsions) can be attributed to favourable partitioning or to an increase in the diffusion coefficient. The structure of the w/o/w multiple emulsion does not appear to be a determining factor of this increase in absorption. However, the difference observed between the w/o/w and o/w emulsions can be attributed to the slow diffusion of drug across the oil membrane of multiple globule, since this membrane acts as a barrier to drug release. The w/o/w emulsion containing drug in the internal phase showed prolongation of diffusion due to the oil membrane playing the role of a barrier for the release of the internal phase to the external phase (Fredo-Kumbaradzi et al., 1991).

4. Conclusion

The main interest and motivation of the first part of this work was in obtaining three types of emulsions with the same formula (invariant composition). This has been possible due to the na-

ture of emulsifiers and the HLB value, as well as to the specific conditions of fabrication for each emulsion type.

In the second part of this work, the release of a hydrophilic drug incorporated into these emulsions was studied. When the membrane presents a negligible resistance to diffusion (cellulose), the release of metronidazole from the w/o/w emulsion is slightly slower compared with the o/w emulsion, while much slower release is observed with the w/o emulsion. The rank order is the same when the membrane is the rate-controlling barrier (silicone), however, in case of skin, the rank order is the same only when the flux values from emulsions with an aqueous continuous phase (w/o/w multiple and o/w emulsions) are compared those obtained with the w/o emulsion.

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