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Transdermal delivery of glucose through hairless rat skin in vitro: effect of multiple and simple emulsions

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Abstract

Three types of oil–water emulsions (W/O/W, O/W and W/O emulsions) were obtained and evaluated on hairless rat skin biopsies, using Franz diffusion cells. Natural emulsifiers (soybean phospholipids) were used to formulate stable multiple and simple emulsions. The qualitative and quantitative composition of the three emulsions was the same. In order to compare the emulsions that have been prepared with this new utilization of soybean phospholipids as emulsifier for vesicular systems and to achieve the importance of application conditions on the diffusion of glucose, a finite dose in open-cap and an infinite dose with occlusion were evaluated. After 24 h of diffusion, the maximum flux (0.69 ± 0.21 $\mu g/cm^2/h$) for a finite dose was obtained with simple O/W emulsion, with a rank order of emulsions identical when compared to an infinite dose application: O/W > W/O/W > W/O.

Keywords: Finite dose; Glucose; In vitro; Infinite dose; Natural emulsifiers; Soybean phospholipids; Simple emulsion; Skin absorption; W/O/W multiple emulsion

1. Introduction

The particular structure of the skin, especially the barrier properties and the physiology, presents one of the principal difficulties to be overcome in

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order to improve the diffusion of molecules through this biological membrane. Different strategies have been used to this end. From a formulation point of view, intensive efforts have been made in the last two decades to design systems that are able to deliver drugs more efficiently to the target site. Multiple emulsions, which constitute new vesicular systems, are of interest because of their potential for protecting encapsulated substances and their ability to provide sustained release (De Luca et al., 1990, Raynal et al., 1993). Water/oil/water (W/O/W) multiple emulsions are vehicles in which drops of oil in dispersed phase themselves contain even smaller aqueous dispersed droplets, which consist of a liquid identical with the aqueous continuous phase. The potential of using multiple emulsions for the controlled and prolonged release of drugs has been reported by various authors (Yoshika et al., 1982, Fukushima et al., 1983, 1987, Grossiord et al., 1993). The goal of many investigators working in industrial pharmacy or cosmetology is to formulate products with good stability, efficacy, acceptability and tolerance. The aims of the present study were: (1) to formulate multiple and simple emulsions with the same composition as soybean phospholipids, which are known to have an excellent tolerance compared to classical anionic or cationic emulsifiers; (2) to investigate the ex vivo diffusion profiles of glucose, a hydrophilic drug model, from O/W, W/O/W and W/O emulsions with the same composition, after application of a finite dose or an infinite dose on hairless female rat skin biopsies.

2. Materials and methods

2.1. Preparation of emulsions

2.1.1. Materials

All chemicals used for the formulation of the excipients, emulsions and reference vehicle were European Pharmacopoeia grade when available; otherwise they were commercial quality pharmaceutical or cosmetic ingredients. Glucose was obtained from Sigma (France) and used as received. Radiolabeled glucose (D-[3-^{3}H]glucose) with a specific radioactivity of 10–20 Ci/mmol (1 mCi/ml in aqueous solution, Steri-Packaged) was purchased from New England Nuclear (Les Ulis, France).

Soybean phospholipids (Emulmetik 100 and 300) were used as emulsifiers. They were supplied by Lucas Meyer (France). Medium-chain triglycerides were purchased from the Societé des Oléagineux (St Laurent de Blangy, France). Sodium chloride was used in the internal aqueous phase as a stabilizing agent and as a marker.

2.1.2. Methods

2.1.2.1. Emulsion preparation. A two-step process was used for the multiple emulsion preparation (Grossiord et al., 1989). In the first step, the primary W/O emulsion was prepared at high agitation speed (3000 rpm) at room temperature (20°C), adding the aqueous phase containing unlabeled glucose and D-[3-^{3}H]glucose for 30 min. It was then incorporated into an aqueous solution of hydrophilic emulsifier at a low agitation speed (400 rpm) at room temperature (20°C) for 180 min. The two stages of emulsification were carried out using a Rayneri agitator.

The simple W/O emulsion was prepared by adding the aqueous phase containing unlabeled glucose and D-[3-^{3}H]glucose to oily phase. Emulsification was carried out using a Rayneri agitator.

The simple O/W emulsion was prepared by adding the oily phase to the aqueous phase containing unlabeled glucose and D-[3-^{3}H]glucose. Emulsification was made with an Ultra Turrax T-25 type IKA agitator (Bioblock, France).

Table 1
General formula of the emulsion formulations: W/O/W multiple emulsion, W/O and O/W simple emulsions

Excipient	Emulsion composition (%, w/w)
Emulmetik 100 and 300	9.6
NaCl	0.16
MCT	20
Distilled water	70.24

Table 2
Characteristics of the emulsions

	Emulsion type		
	W/O/W	O/W	W/O
Macroscopic aspect	Homogeneous, creamy, white–yellowish	Homogeneous fluid, white–yellowish	Homogeneous, high viscosity, white
Mean diameter (μm)	16	2	1–2
Centrifugation (3000 rpm/15 min)	Stable	Stable	Stable
Conductivity (μS)	110 [a]	80 [a]	0.2

[a] Conductivity of emulsifier solutions = 100.

The set of two emulsifiers was incorporated in the oily phase for the W/O emulsion. For the O/W emulsion, each emulsifier was incorporated in the phase with greater affinity. A general formula for each of the various emulsion formulations is given in Table 1.

2.1.2.2. Emulsion evaluation. The conductivity of the emulsions was measured with a Conductivity Meter CDM3 (Copenhagen) in order to discriminate the emulsion type. An optical microscope at 1000 × magnification after diluting the emulsion was used for the microscopic observations (Laboval 4; Bioblock, France).

2.2. In vitro percutaneous absorption studies

2.2.1. Preparation of the skin

Hairless female rats (200–250 g) were obtained from IFFA-CREDO (Les Oncins, France). A week before the experiments the animals were housed under standard conditions of temperature and relative humidity, and supplied with normal pellet diet and water ad libitum. The animals were euthanized by ether inhalation, then the whole thickness abdominal skin was excised and mounted on the diffusion cell without any treatment.

2.2.2. Diffusion experiments

Franz diffusion cells (Crown Glass, Sommerville, NJ) with an effective diffusion area of 1.76 cm^2 and an acceptor compartment of 6.5 ml were used ($n = 6$ per vehicle tested). The receptor fluid was an isotonic phosphate buffer solution (pH 7.4) completed with bovine serum albumin 15 g/l (Sigma, France). Throughout the experiment, the receptor chamber content was continuously agitated by a small magnetic stirrer. The temperature of the skin was maintained at 32°C by a water circulating system regulated at 37°C.

2.2.3. Application of the formulations

Finite doses of the vehicles (approximately 13 mg/cm^2) accurately measured by weight were dispensed onto the horny layer surface using a spatula. Infinite doses (approximately 130 mg/cm^2) were applied using a spatula. Occlusive conditions were obtained for the infinite dose by covering the cell with a layer of Parafilm. Otherwise, for the finite doses, the donor chamber was open to the atmosphere. In both cases the exact time of application was noted and considered as time zero for each cell.

2.2.4. Determination of the diffusion

At 2, 4, 6, 8, 10 and 24 h, the receptor fluid was collected and replaced with fresh temperature-equilibrated receptor medium. The total samples were placed in plastic scintillation vials and 15 ml of liquid scintillation cocktail (Picofluor 40; Packard, Rungis, France) were added. The radioactivity in the vial was counted for a 10-min period on a Packard Tricarb 4435 (Packard, Rungis, France) scintillation counter.

At the end of the 24-h period, the skin surface was washed five times alternately with 100 μl of distillated water or ethanol. The washing fluids

were pooled and an aliquot (1 ml) was counted after addition of 15 ml of Picofluor 40. The cells were then dismantled and the dermis separated from the epidermis with the help of forceps. The epidermis and the dermis were placed in contact with 1 ml of Soluene 350 (Packard, Rungis, France) in glass scintillation vials overnight at 40°C. After complete digestion of the tissues, 15 ml of liquid scintillation cocktail (Ionic-Fluor; Packard, Rungis, France) were added and the radioactivity was counted.

For all the type of scintillation cocktails, specific quenching curves were established and the integrated software of the counter converted counts per minute (cpm) to disintegrations per minute (dpm).

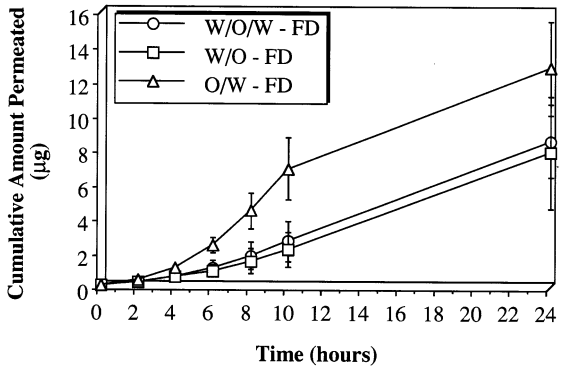

Fig. 1. Penetration profiles of glucose at 1% through hairless rat skin from three emulsion types: W/O/W, W/O and O/W. Finite dose (FD) in open application: 11 mg of vehicle per cm^2 for a diffusional area of 1.76 cm^2.

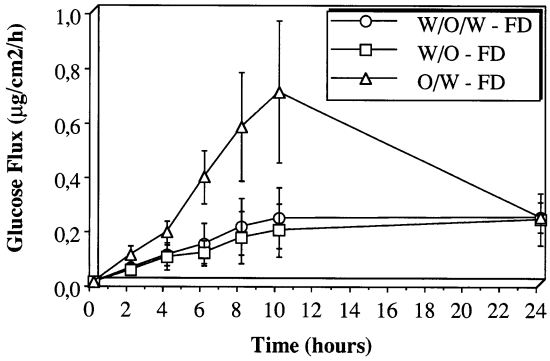

Fig. 2. Flux of glucose ($\mu g/cm^2/h$) at 1% through hairless rat skin from three emulsion types: W/O/W, W/O and O/W. Finite dose (FD) in open application: 11 mg of vehicle per cm^2 for a diffusional area of 1.76 cm^2.

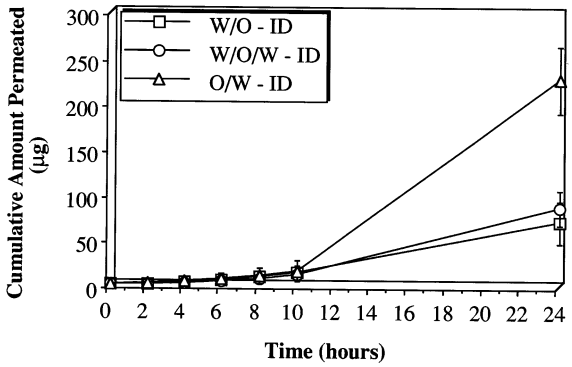

Fig. 3. Penetration profiles of glucose at 1% through hairless rat skin from three emulsion types: W/O/W, W/O and O/W. Infinite dose (ID) under occlusion: 130 mg of vehicle per cm^2 for a diffusional area of 1.76 cm^2.

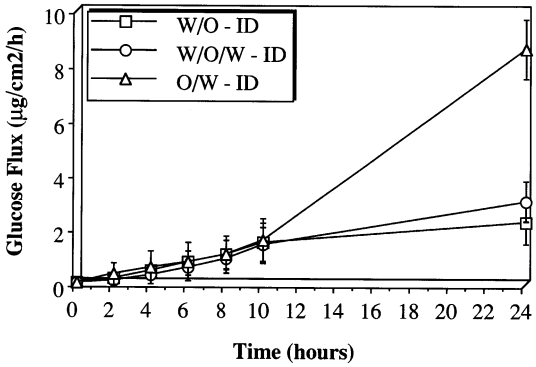

Fig. 4. Flux of glucose ($\mu g/cm^2/h$) at 1% through hairless rat skin from three emulsion types: W/O/W, W/O and O/W. Infinite dose (ID) under occlusion: 130 mg of vehicle per cm^2 for a diffusional area of 1.76 cm^2.

2.3. Data analysis

All data were expressed as appropriate mean ± standard deviation ($n = 6$).

The statistical comparisons between the different results were made using a variance analysis; the level of significance was taken at $p = 0.05$.

3. Results

3.1. Characterization of emulsions

Three emulsions types were developed (W/O/W, W/O, O/W) with the same amounts of each

Table 3
Distribution of glucose at 1% (expressed as a percentage of the applied dose (a) and as μg (b)) after 24 h of diffusion through hairless rat skin in vitro: finite dose (FD) of investigated formulation in open application (11 mg of vehicle per cm^2 with a diffusional area of 1.76 cm^2)

Emulsion FD	Receptor phase	Epidermis	Dermis	Surface wash
(a) % $\pm$ S.D. of applied dose ($n = 6$)				
O/W	6.83 $\pm$ 2.55	8.65 $\pm$ 3.28	8.70 $\pm$ 2.04	67.96 $\pm$ 5.64
W/O/W	4.28 $\pm$ 1.13	8.33 $\pm$ 2.35	4.77 $\pm$ 0.38	74.87 $\pm$ 4.73
W/O	3.88 $\pm$ 1.90	13.71 $\pm$ 3.92	4.99 $\pm$ 1.26	75.14 $\pm$ 3.85
(b) μg $\pm$ S.D. of glucose permeated ($n = 6$)				
O/W	12.73 $\pm$ 2.70	18.62 $\pm$ 6.81	18.78 $\pm$ 4.28	147.67 $\pm$ 18.48
W/O/W	8.49 $\pm$ 2.14	17.66 $\pm$ 5.09	10.09 $\pm$ 0.81	158.69 $\pm$ 14.64
W/O	7.82 $\pm$ 3.27	30.58 $\pm$ 8.15	11.13 $\pm$ 2.55	168.43 $\pm$ 12.79

constituent using natural emulsifiers (soybean phospholipids), but with different experimental conditions. The general formula of each emulsion formulation is presented in Table 1.

The microscopic aspect of the W/O/W multiple emulsion was characteristic of these systems. Their mean diameter was estimated at 16 μm. The globules of the O/W and W/O simple emulsions showed a mean diameter below 2 μm and 1–2 μm, respectively. Table 2 presents characteristic values of the emulsions and the emulsifier solutions.

3.2. Percutaneous absorption

The ex vivo percutaneous absorption of glucose was studied with two different application techniques: a finite dose (FD) with an open-cap application and an infinite dose (ID) under occlusion.

For the FD study, the total amount of glucose permeated in terms of cumulative percentage of the applied dose after an open application of O/W, W/O/W and W/O emulsions were 6.83 $\pm$ 2.55% (12.73 $\pm$ 2.70 μg), 4.28 $\pm$ 1.13% (8.49 $\pm$ 2.14 μg) and 3.88 $\pm$ 1.90% (7.82 $\pm$ 3.27 μg), respectively. Figs. 1 and 2 show the cumulated amount penetrated (μg) and the flux (absorbed amount per cm^2 per hour) of glucose in the receptor phase plotted as a function of time (h). A significant difference was observed between the simple O/W emulsion and the other vehicles tested (W/O/W multiple emulsion and simple W/O emulsion). The glucose flux (μg/cm^2/h) obtained for the simple emulsion with aqueous external phase was rapid, reaching a maximum after 10 h and then slowly decreasing (Fig. 2).

For the ID application under occlusion, the total amount of glucose permeated after 24 h (cumulative percentage of the applied dose) for the three vehicles O/W, W/O/W and W/O emulsions were 10.04 $\pm$ 1.55% (226.60 $\pm$ 36.69 μg), 3.99 $\pm$ 1.06% (85.57 $\pm$ 19.10 μg) and 3.21 $\pm$ 1.02% (69.01 $\pm$ 23.44 μg), respectively. Figs. 3 and 4 show the time course of cumulated amount penetrated (μg) and the flux (absorbed amount per cm^2 per hour) of glucose in the receptor phase. No significant difference was observed for the three emulsions during the first 10 h of diffusion. There was a significant difference between 10 and 24 h for the simple O/W emulsion and the other vehicles (W/O/W and W/O emulsions), illustrating the importance of the aqueous external phase in the enhancement of percutaneous absorption of glucose.

The distribution profiles of glucose for the two types of application (FD and ID) in each compartment represented by the receptor phase, the epidermis, the dermis and the washing solution at the end of the experiment (percentage of the applied dose and μg) are given in Tables 3 and 4. In the dermis, the difference between O/W simple emulsion compared to W/O/W multiple emulsion and W/O simple emulsion according to glucose concentration was significant.

Table 4
Distribution of glucose at 1% (expressed as a percentage of the applied dose (a) and as μg (b)) after 24 h of diffusion through hairless rat skin in vitro: infinite dose (ID) of investigated formulation with occlusion (130 mg of vehicle per cm^2 with a diffusional area of 1.76 cm^2)

Emulsion ID	Receptor phase	Epidermis	Dermis	Surface wash
(a) % ± S.D. of applied dose ($n = 6$)				
O/W	10.04 ± 1.55	0.37 ± 0.13	2.39 ± 1.01	85.90 ± 9.87
W/O/W	3.99 ± 1.06	0.35 ± 0.29	2.07 ± 0.61	89.56 ± 6.85
W/O	3.21 ± 1.02	0.28 ± 0.11	0.83 ± 27	93.29 ± 6.73
(b) μg ± S.D. of glucose permeated ($n = 6$)				
O/W	226.60 ± 36.69	8.57 ± 3.05	54.53 ± 23.33	1954.64 ± 219.86
W/O/W	85.57 ± 19.10	8.04 ± 6.62	47.50 ± 13.93	2053.20 ± 161.43
W/O	69.01 ± 23.44	6.34 ± 2.64	19.12 ± 6.18	2135.71 ± 156.77

4. Discussion

4.1. Finite dose study

The percutaneous absorption of W/O and W/O/W emulsions are similar in terms of cumulative amount of glucose in the receptor phase. The maximum flux after 10 h for O/W emulsion is probably due to the location of the drug in the external phase of the vehicle. This glucose location in the external phase, therefore available for diffusion through the skin, could explain the results obtained with the simple O/W emulsion. The partitioning of drug in the skin is facilitated by the diffusion of water in the substrate, indicating the importance of hydration for the skin absorption of molecules. In contrast, the diffusion of glucose after application of W/O/W and W/O emulsions is slower. This result is in agreement with a better encapsulation of glucose in the aqueous disperse phase of the emulsion, unavailable for the diffusion.

The distribution profiles of glucose are different in the epidermis and dermis: the greater amount of glucose in the epidermis in the case of vehicle with oily external phase is probably due to the skin surface which is a lipophilic substrate. In contrast, the amount of drug in the dermis is significant when the O/W emulsion is used as vehicle. The W/O/W multiple emulsion diffusion is comparable to that obtained with W/O emulsion if we consider the cumulative amount of drug in the receptor phase and the dermis. In fact, after the application of W/O/W multiple emulsion, the nature of the vehicle in the first step is probably of W/O vehicle type due to the evaporation of the external aqueous phase. The difference observed in the epidermis can be attributed to the nature of the residue after evaporation of water.

The total diffusion of glucose is greater with the O/W emulsion vehicle. This is due to a good bioavailability of the emulsion and the nature of the residue (vesicular form?).

4.2. Infinite dose study

There is no statistical difference between the three emulsions during the first 10 h of diffusion. The diffusion of glucose is more significant for the O/W emulsion after 10 h because of its bioavailability and the hydration of the skin by the water of the vehicle external aqueous phase.

The W/O/W multiple emulsion and the W/O simple emulsion are similar in terms of percutaneous absorption, indicating the encapsulation efficiency of glucose. The amount of glucose in the receptor phase after an infinite dose is more significant because of the applied dose and occlusion which enhance the diffusion of drug through the skin (Blank, 1985).

These results demonstrate that it is possible to obtain stable multiple W/O/W emulsions and simple (W/O and O/W) emulsions containing a water-soluble drug, especially glucose, with the same

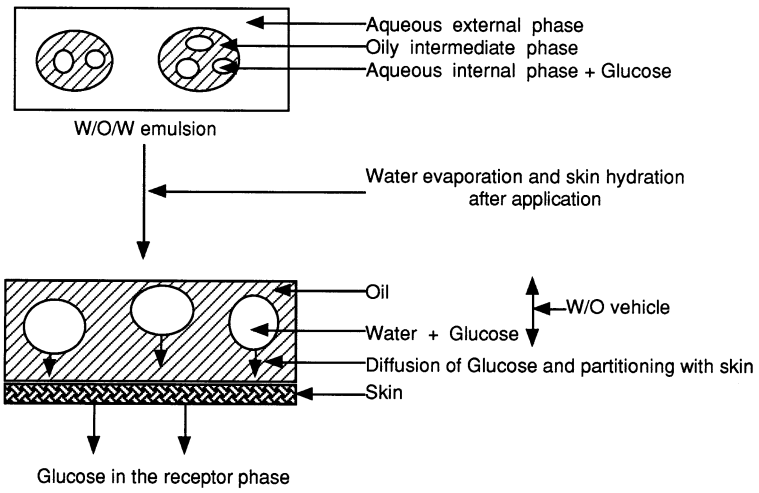

Fig. 5. Schematic representation of W/O/W emulsion after application on the skin.

general formula (Table 1), using natural products as emulsifiers and medium-chain triglycerides as the oily phase of the emulsion.

The ex vivo percutaneous absorption of glucose shows that the emulsion type is a significant criterion to consider in the cutaneous pharmacokinetics of molecules tested. In the present study, the best vehicle for the diffusion of glucose through the skin was the O/W emulsion. The location of glucose in the external aqueous phase is available for the partitioning and subsequently the diffusion of drug through the skin (Figs. 5–7). The rank order obtained for the three emulsions tested was: O/W > W/O/W > W/O.

These results are in good agreement with the results obtained by Ferreira et al. (1994, 1995a,b). In their study, using two models of hydrophilic molecule (metronidazole and glucose), they formulated three emulsion types (W/O/W, W/O and O/W) containing glucose according to the same general formula (35% paraffin oil, 2.8% Hypermer A60, 1.2% Synpéronic PE F/127, 0.5% glucose and 60.5% distilled water, by weight). In the case of synthetic membranes that offer negligible resis-

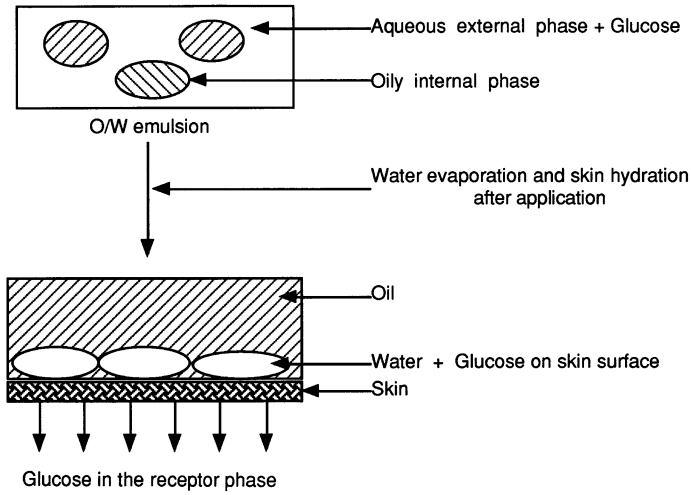

Fig. 6. Schematic representation of O/W emulsion after application on the skin.

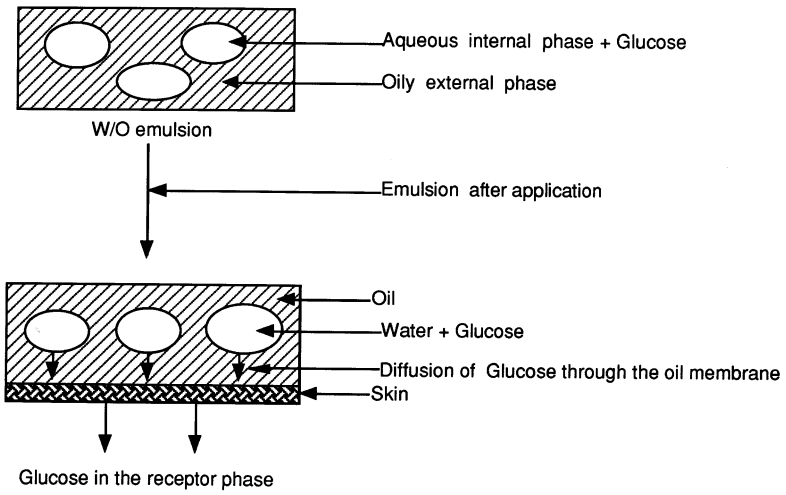

Fig. 7. Schematic representation of W/O emulsion after application on the skin.

tance compared to the skin, the release rates of metronidazole and glucose were higher and the rank order was O/W > W/O/W > W/O. After the skin diffusion experiments, the rank order observed was the same when the flux values from emulsions with an aqueous continuous phase (W/O/W and O/W emulsions) were compared to those obtained with the W/O emulsion. According to percutaneous absorption studies, when a model of hydrophilic drug with intermediate polarity (metronidazole) was tested, its absorption was similar for the three emulsions. However, when a model of hydrophilic drug with high polarity (glucose) was studied, its absorption was greater from the O/W emulsion than from the two other emulsions.

This study demonstrates, with regard to the results of Ferreira et al., that after using different compounds (natural emulsifiers and oily phase) for the formulation of three emulsion types (W/O/W, W/O and O/W emulsion) with the same composition, the rank order obtained for a model of hydrophilic drug according to percutaneous absorption studies was the same (O/W > W/O/W > W/O). This suggests the importance of the vehicle, especially the nature of the external phase in case of emulsion on the diffusion of molecules through the skin (Youenang Piemi et al., 1994). These results clarified that, for a hydrophilic drug (glucose), the nature of the vehicle (W/O/W, W/O, O/W emulsion) and subsequently the nature of the residue plays a significant role in the skin bioavailability.

5. Conclusion

A new utilization of natural products, especially soybean phospholipids used as emulsifier for the formulation of vesicular systems, has been described in this study. Their high tolerance is a good point contributing to the development of topical or systemic applications. For delivering drugs through the skin or in other sites, multiple emulsions have great promise: first, because of the potential of these systems to protect the drug in the internal phase of the emulsion, and, second, the possibility to control the release rate of drugs. Our experimental results indicate that, on the one hand, using multiple and simple emulsions with the same composition formulated with natural emulsifiers such as soybean phospholipids and synthetic emulsifiers (Ferreira et al., 1995a,b) respectively, the results were identical, and that, on the other hand, the pharmacokinetic profiles of multiple W/O/W emulsion and simple W/O emulsion are the same, whatever the applied dose of emulsion. It is important to recall that the difference between these two types of drug delivery systems (W/O/W and W/O emulsion), favorable

to W/O/W system compared to oily simple emulsion, are the cosmetic qualities of multiple emulsion. This vehicle with an aqueous external phase is an emulsion with better cosmetic acceptability than a simple emulsion with oily external phase. The cosmetic characteristics of W/O/W multiple emulsion are comparable to those obtained with simple O/W emulsion which improve the skin diffusion of a hydrophilic drug model (glucose). These vesicular systems, which are multiple emulsions obtained with natural emulsifiers (soybean phospholipids), require further study in order to evaluate the role of emulsion type on the percutaneous absorption of molecules with different physicochemical properties.

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