International Journal of Pharmaceutics, 90 (1993) 43–50

IJP 02952

Comparative study on the release kinetics of methyl-nicotinate from topic formulations

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(Received 27 April 1992)
(Accepted 11 June 1992)

Key words: Methyl nicotinate; Topical formulation; In vitro release; Synthetic membrane; Percutaneous absorption; Cellulitis

Summary

An in vitro method for the determination of methyl nicotinate (MN) delivery from topical dosage forms, using a Franz diffusion cell system and artificial membranes, is described. Four different MN containing formulations for the topical treatment of the so-called 'cellulitis' were designed. The vehicle influence on MN release has been studied utilizing a composite membrane system especially designed in order to reproduce the barrier properties of the skin stratum corneum (SC). The artificial membranes composed of silicone and cellulose esters were previously tested with MN aqueous solutions. Single and composite membrane permeability coefficients were measured and compared with those of excised human skin. It is suggested that human skin permeability of MN may be predicted by using a particular multilaminated membrane system. The MN permeability coefficients of the different topical dosage forms were determined employing the designed synthetic membrane system. Among the different vehicles, MN retains the highest penetration rate when incorporated into the hydrophilic gel, indicating that polymers based hydrophilic gels can be efficiently employed for design, production and in vitro tests of topical forms suitable for the in vivo treatment of dermatological diseases.

Introduction

An essential prerequisite to design topical formulations with high drug penetration rates through the skin is the selection of an appropriate in vitro test system able to predict the in vivo percutaneous absorption of selected drugs. In vitro experiments carried out with natural samples obtained from animal or human skin have shown that the stratum corneum (SC) represents a limiting step for the percutaneous absorption of drugs (Schalla and Schaefer, 1982). Despite recently published papers (Reifenrath et al., 1991) demonstrating good correlation between data obtained utilizing diffusion cells with excised human skin and those determined in vivo, problems associated with skin utilization are still present, including intrinsic biological variability, availability and pre-treatment requirements needed due to skin use (Yeung et al., 1987).

With the aim of finding alternative experimental models, overcoming the disadvantages of the use of skin, different synthetic membranes have

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been utilized to study the percutaneous absorption of topically applied drugs (Shah et al., 1989; Hatanaka et al., 1992).

In the present study, we have utilized a multilaminated membrane system which mimics the human skin barrier properties. It consists of a hydrophilic membrane composed of cellulose acetate and nitrate esters sandwiched between two lipophilic polydimethylsiloxane (silicone) membranes.

The purpose of the present investigation was to develop a simple, easily reliable and reproducible in vitro system for studying the diffusion and penetration kinetics of active compounds, incorporated into different vehicles, designed for cosmetic use, treatment of skin and subcutaneous tissue diseases.

As model drug, we utilized methylnicotinate (MN), which is largely utilized for the treatment of liposclerosis (the so-called 'cellulitis'). Cellulitis, indeed, represents, a dermo-cosmetic pathology which is still largely unresolved due to the wide variety of clinical aspects, pathogenic modalities and variability of individual responses. Among the possible administration routes for drugs acting on cellulitis, topical treatment is usually preferred for its localized action, and reduction of adverse aspects of systematically administered drugs, such as side and toxic effects. However, topical administration is often limited and scarcely effective, due to poor skin penetration of drugs as well as unpredictable drug concentrations at the different skin layer levels (Barry, 1991). In this respect the finding of vehicles showing an improvement in bioavailability of MN after its topical application could be of great interest to improve its pharmaceutical activity.

Materials and Methods

Permeation experiments

Synthetic membranes Different commercially available synthetic membranes were utilized to study the in vitro diffusion of MN from different topical dosage forms as follows. Silicone-based membranes (polydimethylsiloxane): (a) Silastic® 501.1 (170 μm thickness), Dacron® reinforced and (b) Silastic® 500.3 (250 μm thickness) (Dow Corning Corp., Midland, MI, U.S.A.); cellulose ester-based membranes: (c) cellulose acetate (0.2 μm pore size) (Schleicher and Schuell, GmbH, Dassel, Germany); (d) cellulose mixed esters (150 μm thickness and 0.45 μm pore size) (Millipore Filter Corp., Bedford, MA, U.S.A.) and (e) cellulose mixed esters (0.6 μm pore size) (Schleicher and Schuell, GmbH, Dassel, Germany).

Diffusion cell The scheme of the diffusion cell used in all the synthetic membrane permeation studies is shown in Fig. 1. A standard glass Franz diffusion cell with a 1 cm diameter orifice (0.78 cm^2 area) was utilized. The upper part of the chamber was sealed to avoid evaporation. The receptor phase was stirred by means of a constantly spinning bar magnet and thermostated at 37°C.

Receptor phase An isotonic solution of 60 mM phosphate buffer, pH 7.4, in 0.9% (w/v) NaCl was used. This solution was always degassed before using.

Procedure The studies were carried on using a glass diffusion cell assembly. The cell body was filled to overflowing, in order to avoid air bubble formation, with the degassed receptor phase. 1 ml of MN aqueous solution or 1 g of the form to be analyzed were placed into the donor cell compartment and tamped down on the membrane, previously moistened with the receptor phase.

At predetermined time intervals between 1 and 8 h, samples (0.15 ml) of receptor phase solution were withdrawn and the MN concentration in the receptor phase was measured using HPLC.

The volume of each sample removed was replaced with an equal volume of simple receptor phase. The calculated MN concentrations were plotted as a function of time and the permeability coefficients were computed from the linear portion of the accumulation curve, and expressed both as experimentally observed fluxes (J_o), and as normalized fluxes J_n ($J_n = J_o/C$, where C is the concentration of MN in the analyzed form, expressed in mg/ml). All the obtained permeation rates were determined six to eight times in independent experiments and the mean values ± S.D. were calculated.

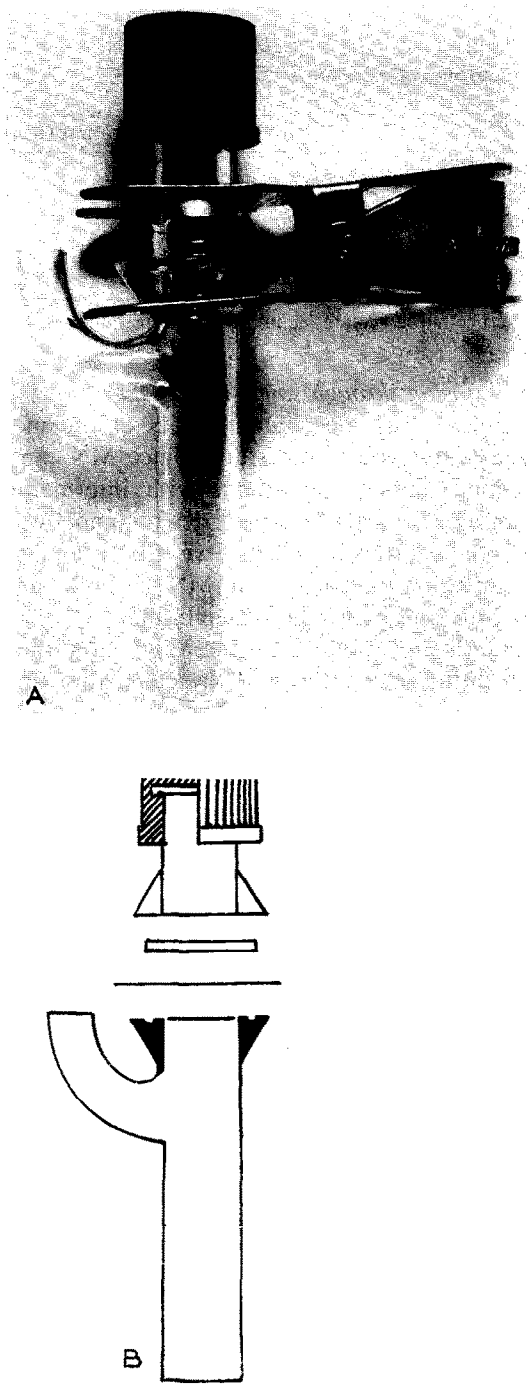

Fig. 1. (A) Glass diffusion cell employed to determine MN permeability coefficients. The upper compartment contains the donor phase, the lower contains the phosphate buffer receptor phase. (B) Scheme of Franz diffusion cell system.

Chromatographic analysis

A 30 μl sample of receptor phase was injected into the liquid chromatograph and quantitated by using a MN standard of known concentration.

The RP-HPLC analyses were performed with a Bruker apparatus (Bremer, Germany) consisting of a three-plunger alternative pump, a variable-wavelength UV detector, operating at 265 nm, and a Rheodyne injection valve with a 100 μl loop. A Vydac C18 stainless-steel column (25 × 0.46 cm) packed with 5 μm particles was eluted at room temperature with a mobile phase consisting of acetonitrile/0.1 M ammonium acetate buffer (pH 7.4) (20:80 v/v), the flow rate being 1.0 ml/min. Under these conditions MN showed a retention time of 7.5 min.

Preparation of topical dosage forms

w/o emulsion The components of the formulation were divided into those that were oil-soluble and those that were water-soluble. Both were then dissolved in their respective solvents by heating the solutions to about 75–80°C. When solubilization was complete the oil phase was slowly added to the water phase, under vigorous stirring by using a turbine mixer, until 40°C. MN (5 mg/ml) was then added and the emulsion was cooled to room temperature.

o/w emulsion and o/w liquid crystals (LC) emulsion The procedure for the preparation of these forms is similar to that above, except for the mixing modalities of the oily and aqueous phases, which in this case was made in a single step at 70°C.

Hydrophilic gel For the preparation of the carboxyvinyl polymer (Carbopol 940, Biochim, Milano) based gel, Carbopol was placed in a water/propylene glycol solution (100:4 w/w) and left to swell at room temperature to obtain a homogeneous and liquified mixture.

After overnight incubation, triethanolamine (to neutralize the solution) and MN (5 mg/ml), were added. All the dosage forms described here were stored at 4°C until use to minimize possible degradations of MN.

Results and Discussion

The present experiments were performed utilizing an in vitro test based on a percutaneous absorption glass cell (Franz diffusion cell) (Franz, 1975), assembled with single or multiple synthetic membranes (see Materials and Methods).

For the calculation of the permeability coefficients, the following procedure was used in every case. The amount of MN penetrated through the membrane(s) per unit area was plotted vs time and the slopes, which represent the steady-state fluxes, were calculated by linear regression.

It should be stressed that the calculated regression coefficients were never less than 0.97. The slopes were then substituted into the following equation for the determination of J_n (permeability coefficient).

$$J_n = J_o/C$$

TABLE 1

Characteristics of membranes

Membranes	Pore size (μm)	Thickness (μm)
Silastic 500.3	–	250
Silastic 501.1	–	170
Cellulose acetate	0.20	150
Cellulose mixed esters [a]	0.45	150
Cellulose mixed esters [a]	0.60	150

[a] Mixed cellulose acetate/cellulose nitrate membranes.

MN diffusion through single membranes

Table 1 lists the characteristics of the membranes employed in the study. Since MN can be classified among compounds with intermediate lipophilic/hydrophilic properties (octanol/water partition coefficient = 0.36), we firstly analyzed the diffusion coefficient of MN in both single lipophilic and hydrophilic membranes.

Fig. 2 and Table 2, respectively, show the in vitro diffusion kinetics and the calculated fluxes

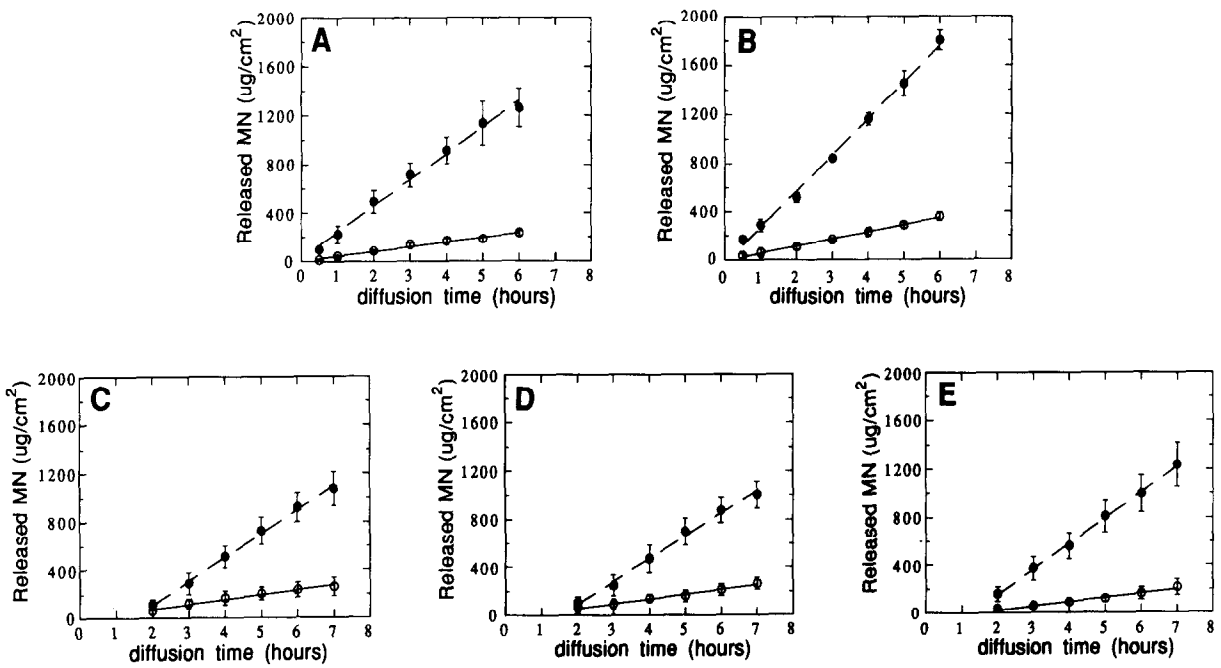

Fig. 2. In vitro diffusion kinetics of 1 mg/ml (○) or 5 mg/ml (●) MN aqueous solutions through the indicated single synthetic membranes. The results reported represent the means ± S.D. of six independent experiments. (A) Silastic 500.3; (B) Silastic 501.1; (C) cellulose acetate, 0.2 μm pores; (D) celluose mixed esters, 0.45 μm pores; (E) celluose mixed esters, 0.6 μm pores.

TABLE 2

In vitro permeability coefficients of aqueous solutions of methyl nicotinate, determined utilizing the indicated single membrane systems

Membranes	J_s (μg/cm^2 per h)	C (mg/ml)	J_n (cm/h) ($\times 10^3$)	Log J_n
Silastic 500.3	38.7	1	38.7	1.58
	216.0	5	43.2	1.63
Silastic 500.1	58.6	1	58.6	1.76
	297.0	5	59.4	1.77
Cellulose acetate	39.8	1	39.8	1.59
0.2 μm pores	197.8	5	39.6	1.59
Cellulose mixed esters	39.9	1	39.9	1.60
0.45 μm pores	189.1	5	37.8	1.57
Cellulose mixed esters	35.9	1	35.9	1.55
0.6 μm pores	214.9	5	42.9	1.63
Human skin [a]	3.1	1	3.1	0.49
	17.0	5	3.4	0.53

[a] MN fluxes through the skin were taken from (Dal Pozzo et al., 1991). The reported results represent the average of six independent experiments.

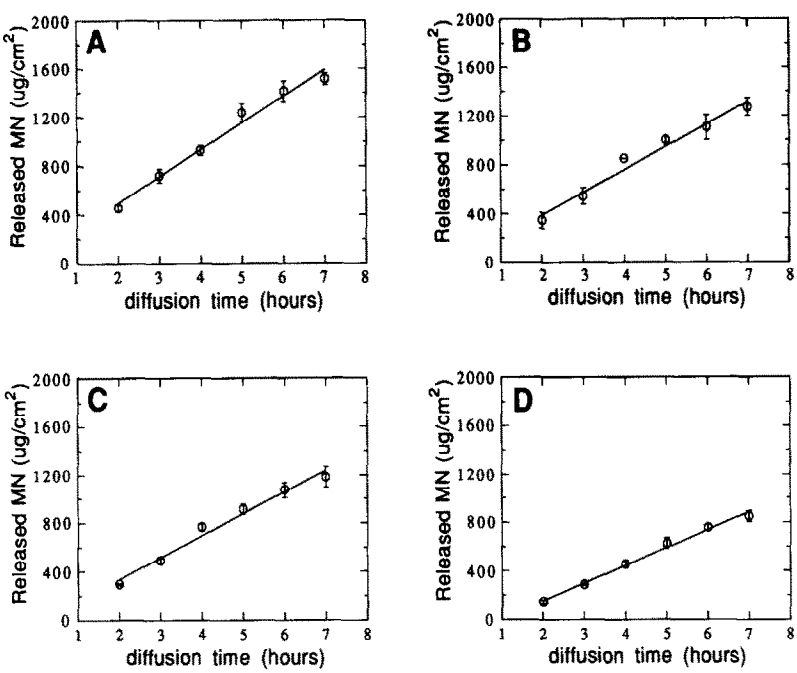

Fig. 3. In vitro diffusion kinetics of 1 mg/ml MN aqueous solutions through the indicated synthetic multilaminated membrane systems. The results reported represent the means ± S.D. of six independent experiments. (A) Silastic® 500.3/cellulose acetate, 0.2 μm pores; (B) Silastic® 500.3/cellulose mixed esters, 0.45 μm pores; (C) Silastic® 500.3/cellulose mixed esters, 0.6 μm pores; (D) Silastic® 500.3/cellulose mixed esters, 0.45 μm pores/Silastic® 500.3.

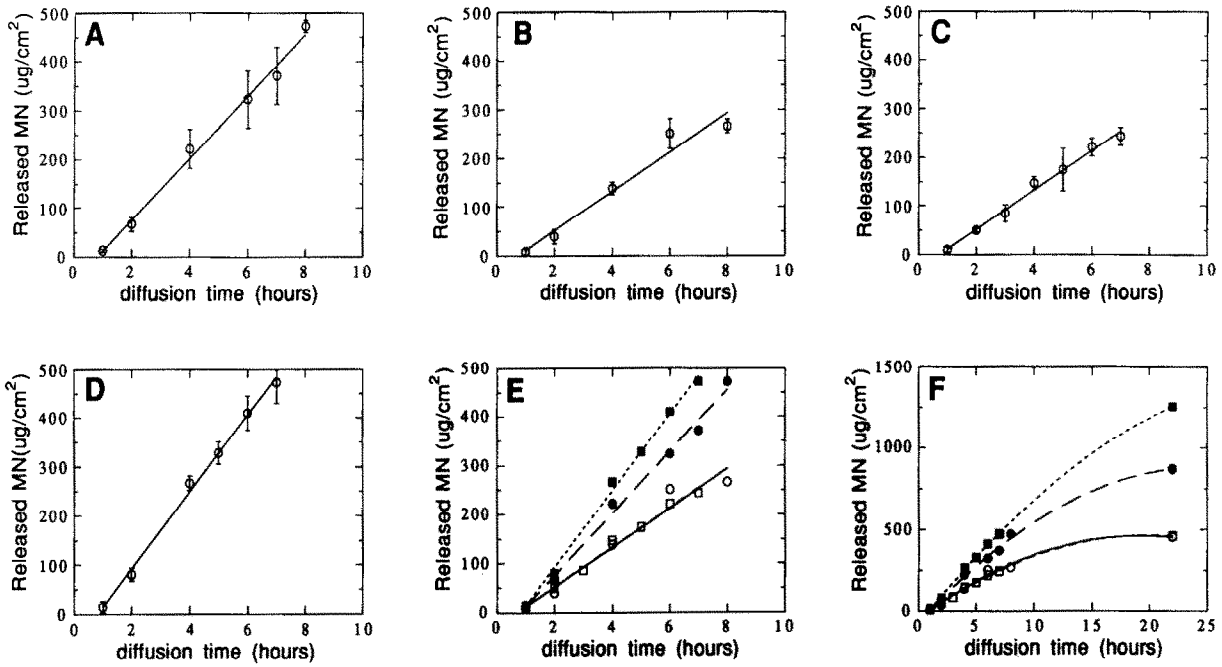

Fig. 4. In vitro diffusion kinetics of methyl nicotinate incorporated in the reported topical dosage forms. The experiments were carried out with a multilaminated system (SCS), composed of a cellulose mixed ester membrane, sandwiched between two Silastic® membranes. The results reported represent the means ± S.D. of eight independent experiments. (A) Water in oil emulsion (w/o); (B) oil in water emulsion (o/w); (C) liquid crystal water in oil emulsion (LC); (D) hydrophilic gel. Panels E and F display the different MN diffusion kinetics, performed for 8 (E) or 22 (F) h. [(●) water in oil emulsion; (○) oil in water emulsion; (□) liquid crystal water in oil emulsion; (■) hydrophilic gel)].

for MN aqueous solutions, determined by using single silicone-based (lipophilic, Fig. 2A, B) and cellulose ester-based (hydrophilic, Fig. 2C–E) synthetic membranes. The different membranes were tested with both 1 and 5 mg/ml MN solutions in order to evaluate the precise linear relationship between the J_s value and MN concentration in the donor phase. The lowest J_n values were determined with Silastic 500.3 and cellulose mixed esters (pore size 0.45 μm). However, the J_n values determined were in every case quite different from that obtained with human excised skin, reported for the purpose of comparison in Table 2.

TABLE 3

In vitro permeability coefficients of aqueous solutions of methyl nicotinate, determined utilizing the indicated multiple membrane systems

Membranes	J_s (μg/cm^2 per h)	C (mg/ml)	J_n (cm/h) ($\times 10^3$)	Log J_n
S500.3/AC 0.2	218.7	5	43.7	1.64
S500.3/MixC 0.45	184.8	5	36.9	1.56
S500.3/MixC 0.6	181.0	5	36.2	1.55
S500.3/MixC 0.45/S500.3	145.6	5	29.1	1.46

S500.3: Silastic® 500.3; AC 0.2: cellulose acetate, 0.2 μm pores; MixC 0.45: cellulose mixed esters, 0.45 μm pores; MixC 0.6: cellulose mixed esters, 0.6 μm pores. The reported results represent the average of six independent experiments.

TABLE 4

Composition of the utilized topical forms containing methyl nicotinate

Oil phase		Water phase	
Component	Concentration (%, w/w)	Component	Concentration (%, w/w)
(A) w/o emulsion			
Arlacel 582			
(EO glyceryl-sorbitan			
isostearate)	7.00	propyl glycol	4.00
Vaseline	4.00	$MgSO_4$	0.50
Vaseline oil	11.00	EDTA	0.10
Isopropyl miristate	11.00	water	q.s. to 100.00
(B) o/w emulsion			
Ameroxol (20)			
(20 mol EO oleic alcohol)	2.00	propyl glycol	4.00
ST55 (2)	3.00	EDTA	0.10
(2 mol EO stearyl alcohol)			
Vaseline oil	15.00	water	q.s. to 100.00
Cetyl stearyl alcohol	5.00		
Silicol 200			
(silicone oil)	1.00		
(C) o/w emulsion ("Liquid Crystals")			
Ameroxol (20)	2.00	EDTA	0.10
ST55 (2)	3.00	water	q.s. to 100.00
Ambra Wax	4.00		
Vaseline oil	15.00		
Cetyl stearyl alcohol	5.00		
(D) Hydrophilic gel			
		Carbopol 940	
		(carboxy vinyl polymer)	1.00
		Propyl glycol	4.00
		Triethanolamine	1.00
		EDTA	0.10
		Water	q.s. to 100.00

MN diffusion through coupled membranes

In consideration of the significant physiological and structural differences between the highly complex human skin and synthetic membranes, the use of multilaminated sheets can be proposed in order to realize an in vitro model mimicking

TABLE 5

In vitro permeability coefficients of methyl nicotinate incorporated in different topical forms, determined utilizing the multimembrane system Silastic / cellulose / Silastic (SCS)

Formulation	J_o (mg/cm^2 per h)	C (mg/ml)	J_n (cm/h) ($\times 10^3$)	Log J_n
w/o emulsion	63.4	5	12.7	1.10
o/w emulsion	40.3	5	8.1	0.90
o/w LC emulsion	40.6	5	8.1	0.90
Hydrophilic gel	78.4	5	15.7	1.20

The data represent the mean values of eight independent experiments. The multimembrane system utilized was composed of a cellulose mixed ester membrane (pores 0.45 μm) sandwiched between two Silastic® membranes. The reported results represent the average of six independent experiments.

the properties of skin. The multimembranes utilized are constituted of alternate layers of hydrophilic and hydrophobic polymers. In this way, an in vitro permeation model is provided, more strictly resembling the behaviour of human skin and in particular the high complexity of SC. Accordingly, for the determination of the permeability coefficients of MN, Silastic® sheeting was used as the hydrophobic layer and cellulose ester membranes as the hydrophilic layer.

In Fig. 3 and Table 3 are shown the in vitro permeability coefficients of MN solubilized in water. The data refer to experiments performed using both types of membranes, combined to form different multimembrane systems with alternate hydrophilic and lipophilic layers.

It should be noted that the use of more than three membrane layers, even leading to a J_n value to a certain extent closer to that of human skin, could affect the procedural complexity, without significantly increasing the experimental validity of the system. In this respect, the in vitro experimental system proposed here represents an appropriate and predictive model for the dermal absorption of MN, closely reproducing the behaviour of the human skin.

Comparative analysis of MN release from topical forms

Table 4 details the compositions of the MN-containing formulations. The calculated permeability coefficients for MN incorporated into the different topical forms are reported in Table 5. The normalized fluxes (J_n) were 12.7, 8.1, 8.1 and 15.7 $\times 10^3$ cm/h for the w/o, o/w, LC o/w emulsions and hydrophilic gel, respectively. The highest diffusion coefficient for MN was obtained when the drug was incorporated in the hydrophilic Carbopol-based gel. This indicates that this kind of vehicle could be efficiently employed for topical delivery of MN.

In conclusion, the present results show that this experimental model could be proposed: (a) in preformulatory studies aiming at the selection of topical dosage forms with high diffusion and skin penetration for MN or other drugs; (b) to ensure batch-to-batch uniformity; and (c) to perform pre-marketing quality controls for a large variety of dermatologic and cosmetic products, such as creams, gels and ointments. Moreover, the use of synthetic membranes can efficiently overcome drawbacks associated with the use of human skin, such as variability, availability and in addition risks related to possible skin contamination by HIV (Shah et al., 1989) or other infective microorganisms.

Acknowledgements

This investigation has been supported by AIRC and by Regione Emilia Romagna.

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