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STUDYING *IN VITRO* RELEASE OF DEXCHLORPHENIRAMINE MALEATE FROM AQUEOUS PHASES AND WATER/OIL EMULSIONS (CREAMS) WITH VARIOUS PENETRATION ENHANCERS

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The effect of various penetration enhancers on the permeation of dexchlorpheniramine maleate from a 2 wt% aqueous phase and from water/oil emulsions (creams) was studied using a modified Franz diffusion cell. A polyamide membrane filter (modeling a biomembrane) was impregnated with *n*-octanol, sandwiched between two dialysis membranes, and placed between the source and receiver compartments. The permeation coefficient in the aqueous phase was 270 times greater than that in the cream, while the lag time was 3.5 times longer in the cream. A mixture of isopropyl myristate and ethanol (8 : 2) gave the highest enhancement ratio, while oleic acid acted as a penetration retardant in the cream formulation.

Introduction

Dexchlorpheniramine maleate (CPM) is a cationic amphiphilic amine compound with a hydrophobic ring structure of the molecule, which has a hydrophilic side chain containing a charged cationic amino group (Fig. 1). It is a potent antihistamine used for the treatment of several allergies and skin irritation [1]. When given orally, CPM produces the side effects, which are well known for all antihistamines, such as drowsiness, muscular weakness, and gastrointestinal disturbances. In order to avoid side effects, firstly the latter one that results in only 25 – 45% of the orally administered dose reaching the blood circulation, topical formulations can provide a safe and effective alternative.

Velissaratou and Papaioannou [2] studied the *in vitro* release of CPM suspended in ointment bases and found the release rate to be very small (1.75% after 3 h of experiment). In addition, the rate of CPM release from a water/oil (w/o) type emulsion ointment was almost twice that from an o/w type emulsion (40 and 23%, respectively). The authors explained this difference as being due to phase inversion occurring in the w/o emulsion, which led to a higher drug concentration in the water-soluble phase of the w/o emulsion.

Andronis et al. [3], evaluated a transdermal CPM drug delivery system and found the 0.6 volume fraction of ethanol to give the highest diffusion rate J = 1.591 mg/(cm^2 · h). Tas et al. [4] concluded that percutaneous absorption of CPM across the skin can be achieved through topical application with a gel based on cellulose derivatives. Chang and Bodmeier [5] used CPM as a model drug to study phase transformations. Upon determining the ternary phase diagram of CPM, monoglycerides and water, CPM was found useful in transforming the cubic phase into a lamellar phase and then into an isotropic solution phase, thus producing a low-viscosity monoglyceride delivery system. Attama and Nkemnele [6] used CPM in self-micro-emulsifying drug delivery systems and established that *in vitro* drug release varied depending on the medium and particle size.

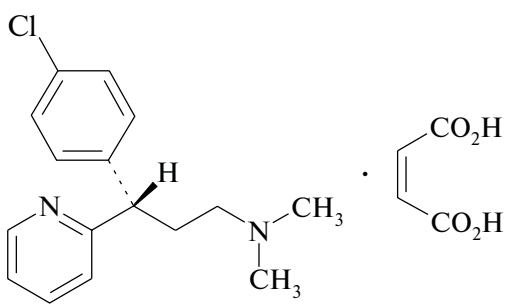

Fig. 1. Chemical structure of dexchlorpheniramine maleate (CPM).

Although the transdermal route has been recognized as a popular alternative to the oral delivery of drugs, the outermost layer of the skin (stratum corneum, SC) is known to represent a major obstacle against the permeation of drug molecules. SC is a multilayer structure organized in such a way that densely packed keratin networks (corneocytes) are embedded in an intercellular lipid-rich matrix [7].

The intercellular lipids of SC have been extensively investigated for composition [8] and structural organization [9]. A layered structure proposed by Elias [10] reflected the organization of lipids in SC. The attempts at making *in vitro* model of SC lipid layer structure were successful only when pH of the lipids was adjusted to that of the skin [11]. Then, free fatty acids are partially saponified and a layered structure is spontaneously formed that can accommodate the remaining lipid components [12]. In this structure, the polar compounds are packed in lamellae with the hydrocarbon chain mirroring each other and the polar groups facing the aqueous layers. Thus, such an intercellular pathway is bicontinuous, consisting of nonpolar and polar diffusion pathways.

The route of penetration was found to proceed predominantly through the intercellular spaces [13]. Penetration enhancers can perturb the polar head groups, the aqueous regions between the polar groups, or the hydrophobic part

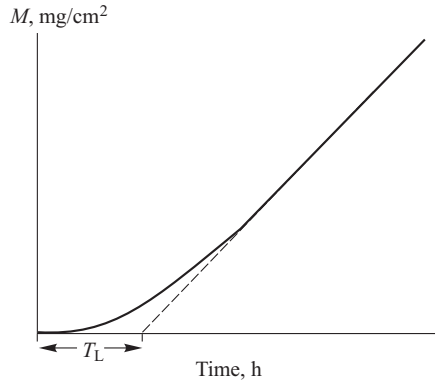

Fig. 2. Schematic diagram of drug absorption in a simple zero-flux case, plotted as the cumulative diffusant mass M (per unit membrane area) versus time t. A steady state is achieved when the plot becomes linear; extrapolation of the linear portion to the time axis gives the lag time T_L.

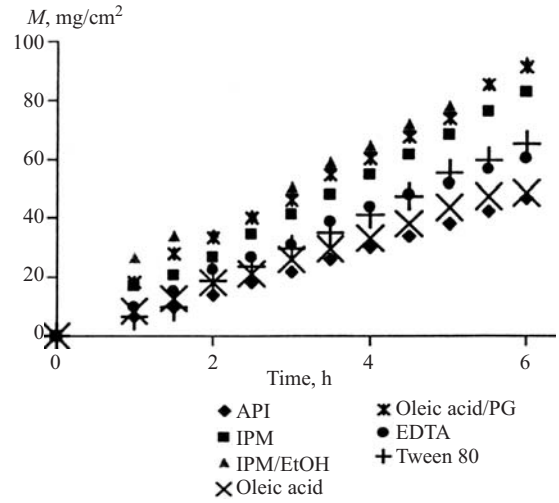

Fig. 3. Plots of M versus time for CPM in the aqueous phases without (API) and with penetration enhancers.

between methyl groups at the end of hydrocarbon tails [14].

The aim of this study was to determine the effect of various penetration enhancers on the permeability of CPM from an aqueous phase and a cream (w/o emulsion) using a modified Franz diffusion cell with a synthetic membrane composed of polyamide filter sandwiched between two layers of dialysis membrane. This design proved to be effective with good correlation with the SC structure [15].

Materials and Methods

Chemicals: Dexchlorpheniramine maleate $C_{16}H_{19}ClN_2 \cdot C_4H_4O_4$ (MW, 390.86) (obtained from Intermed, Italy); beeswax (Kahi & Co.); EDTA (Merck, Barcelona); ethanol (Pharmco, USA); eucerine (Merck); isopropyl myristate (IPM, Industrial Quimicalase, Barcelona); oleic acid (Wood Land Food Industries PTE Ltd.), propylene glycol (PG) (Dow Chemicals Co., Germany); sodium benzoate (Merck); Tween 80 (polysorbate 80) (Croda). All solvents and reactants were of analytical or HPLC grade. Synthetic membranes: dialysis membrane (32/32 A), 0.2 μm pore size; polyamide membrane, 0.2 μm pore size (Sartolon Polyamide, Germany).

Preparation of CPM creams containing various penetration enhancers. Table 1 shows the optimum w/o type emulsion (cream) formula selected from various tried formulations with a penetration enhancer content of 2 wt%. Selected penetration enhancers presented in Tables 2 and 3 were compatible with CPM and auxiliary components. The compatibility tests (not presented here) were performed under stress conditions (40°C) for seven months.

Table 1

Selected Cream Formula (w/o Emulsion)

Material	Function	Content, g	%
Dexchlorpheniramine maleate	Active material	40.0	2.0
Eucerine	Base	1720.0	86.0
Beeswax	Emulsifying agent	60.0	3.0
Sodium benzoate	Preservative	40.0	2.0
Penetration enhancer	Enhancer	40.0	2.0
Distilled water	Solvent	100.0	5.0
Total		2000	100

In vitro release studies. A modified finite-dosing diffusion cell described elsewhere [15] was developed to improve the hydrodynamic conditions of the original Franz diffusion cell. An apparatus for *in vitro* release study was designed so that the agitation element rotated in a plain parallel to the drug-releasing surface. A synthetic membrane was composed of three layers, comprising a polyamide filter membrane sandwiched between two layers of dialysis membrane. The dialysis membrane layers were soaked for half an hour in distilled water for hydration prior to use. The polyamide filter membrane was soaked in *n*-octanol for 24 h to simulate a lipophilic barrier of the skin, and then placed between the hydrated dialysis membranes to prevent *n*-octanol from floating or leaving the filter membrane during the diffusion experiment. The effective surface area of the synthetic membrane was 1 cm^2.

In the first series of experiments, a fresh drug solution (10 ml) prepared by dissolving 2 g of CPM and 2 g of a penetration enhancer in 100 ml of distilled water was added to the donor compartment. In the second series of experiments, the donor compartment was filled with a 2 wt% CPM w/o emulsion (cream) containing 2 wt% of a penetration enhancer. The source compartment was sealed with waterproof parafilm and maintained at ambient temperature (25 ± 1°C), while the receiver compartment was maintained at 32°C by circulating water bath. In order to keep sink condition in the receiver media, it was mixed continuously during diffusion experiments with a magnetic stirrer bar operating at 500 rpm. Periodic sampling from the receiver phase was performed with 30 min intervals over six hours.

The amount of CPM released from various cream formulations was analyzed using HPLC. The HPLC system

Table 2

Summary of CPM Diffusion Parameters in Aqueous Phases without (API) and with Penetration Enhancers

Additive	Slope	Intercept	Q, mg	M, mg/cm^2	T_L	D	P	K	ER
API	8.1778	–2.4366	36.8989	46.7074	0.3	1.4×10^{-4}	0.41	45.69	1.00
IPM/EtOH	14.431	6.0846	73.8285	93.4538	0.42	1×10^{-4}	0.72	114.09	1.76
Oleic acid/PG	14.47	3.622	72.2254	91.4246	0.25	1.7×10^{-4}	0.72	67.91	1.76
IPM	13.895	–0.5313	65.7084	83.1752	0.04	11.2×10^{-4}	0.69	9.96	1.68
EDTA	9.7484	3.1142	47.5528	60.1934	0.32	1.3×10^{-4}	0.49	58.39	1.20
Tween 80	8.8422	–5.3031	56.1699	71.1011	0.6	7.1×10^{-5}	0.44	99.43	1.07
Oleic acid	8.1673	1.5904	38.7693	49.075	0.19	2.2×10^{-4}	0.41	29.82	1.00

consisted of an S_5P column (125 × 4.6 mm i.d.) with phenyl groups chemically bonded to porous silica gel particles (5 – 10 μm). The mobile phase was a solution of potassium dihydrogen phosphate, triethylamine hydrochloride, and sodium lauryl sulfate in 60 : 40 mixture with methanol. The flow rate was 1.0 ml/min. CPM was detected by a spectrophotometric detector tuned to λ = 214 nm.

Calculation of penetration data. There is little evidence to support specialized active transport systems involved in skin permeation; therefore, diffusion through the horny layer is controlled by simple passive process. The steady state permeation flux per unit area (J) is described using Fick's first law as

$$J = KD(C_{app} - C_{rec})/h, \quad (1)$$

where K is the partition coefficient between SC and the applied formulation, D is the diffusion coefficient, C_{app} is the applied concentration of the permeant in the vehicle, C_{rec} is the permeant concentration in the receptor phase, h is the thickness of the skin barrier. The permeation coefficient of the skin barriers P is defined as

$$P = KD/h. \quad (2)$$

In many instances we can assume that $C_{rec} < C_{app}$ and, hence, Eq. (1) can be simplified to

$$J = PC_{app}. \quad (3)$$

Figure 2 shows a schematic diagram of the cumulative mass M of a diffusant passing per unit area through a membrane, which is measured as a function of the time. For a sufficiently long time, a steady state flux is established and the plot approaches a straight line with constant slope dM/dt given by the formula

$$dM/dt = DC_{app}K/h. \quad (4)$$

The steady state can be expressed mathematically as

$$y = ax + b, \quad (5)$$

where y represents $M(t)$, x is the time, a is the slope of the steady state line, and b is the intercept with the y axis. The lag time T_L is defined as

$$T_L = h^2/6D \quad (6)$$

and can be determined from Eq. (5) for $y = 0$ as

$$T_L = -b/a. \quad (7)$$

The ratio of the permeation coefficients determined with (P_{PE}) and without (P) the use of a penetration enhancer is called the enhancement ratio (ER):

$$ER = P_{PE}/P. \quad (8)$$

The greater the ER value, the higher the effect provided by the given penetration enhancer.

Results and Discussion

Figure 3 shows experimental data obtained for the diffusion of CPM through the synthetic membrane for 2 wt% CPM aqueous phases of seven compositions. The cumulative amount M of CPM penetrated per unit area of a synthetic membrane (mg/cm^2) is plotted as a function of the time (h). From the steady state attained for each curve, the slope and intercept were determined using Eq. (5). The aqueous phase compositions are presented in Table 2 together with calculated values of the lag time (T_L), diffusion coefficient (D) permeation coefficient (P), partition

Table 3

Summary of CPM Diffusion Parameters in Cream Phases without (API) and with Penetration Enhancers

Additive	Slope	Intercept	Q, μg	M, μg/cm^2	T_L	D	P	K	ER
API	30.732	–33.295	119.9677	151.8578	1.08	4×10^{-5}	1.5×10^{-3}	0.62	1.00
IPM/EtOH	50.829	43.396	278.122	352.0532	0.85	5×10^{-5}	2.5×10^{-3}	0.81	1.67
Oleic acid/PG	40.887	18.488	211.4824	267.6992	0.45	9×10^{-5}	2.0×10^{-3}	0.35	1.33
EDTA	35.184	–26.224	148.2566	187.6665	0.75	6×10^{-5}	1.8×10^{-3}	0.49	1.20
IPM	33.771	–13.295	147.9447	187.2818	0.39	11×10^{-5}	1.7×10^{-3}	0.25	1.13
Tween 80	32.039	–19.413	139.3535	176.3968	0.61	7×10^{-5}	1.6×10^{-3}	0.36	1.07
Oleic acid	18.689	–7.2499	84.0775	106.4272	0.39	11×10^{-5}	9×10^{-4}	0.14	0.60

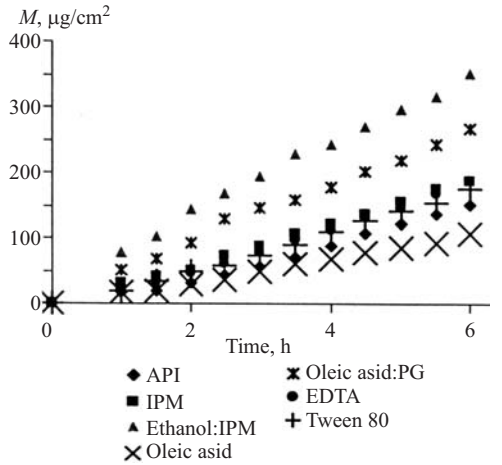

Fig. 4. Plots of *M* versus time for CPM in the cream phases without (API) and with penetration enhancers.

coefficient (*K*), and enhancement ratio (*ER*). The *ER* was found to increase in the following order:

(IPM/EtOH) = (oleic acid/PG) > IPM > EDTA > Tween 80 > oleic acid.

The T_L values were found to increase in the following order:

Tween 80 > (IPM/EtOH) > EDTA > (oleic acid/PG) > oleic acid > IPM.

Figure 4 shows the results obtained for the diffusion of CPM from 2 wt% creams with various penetration enhancers. The cumulative amount of CPM penetrated per unit area for 6 h has the maximum value for IPM/EtOH (similarly to the aqueous phase). Note that, in the presence of oleic acid, the cumulative penetration is lower than that for CPM alone. The calculated diffusion parameters are presented in Table 3. The ER values for the cream formulations were found to increase in the following order:

(IPM/EtOH) > (oleic acid/PG) > EDTA > IPM > Tween 80 > oleic acid

(oleic acid acts as a penetration retardant). The T_L values increased in the following order:

(IPM/EtOH) > EDTA > Tween 80 > (oleic acid/PG) > oleic acid = IPM.

A comparison of the diffusion parameters of CPM in creams to those in aqueous phases shows that T_L values are about 3.5 times greater in creams than in solutions. This is expected, since CPM has first to diffuse through the cream base before saturating the membrane and reaching the receiver compartment. The permeation coefficient in aqueous phases is about 270 times that in creams. The average steady state flux in creams is about 30.7 μg/(cm^2 · h). The T_L values are reduced significantly in both aqueous phase and cream in the presence of IPM, which is probably due to a decrease in the interfacial tension between the oil phase (*n*-octanol) and water containing CPM; in addition, IPM can create holes in the intact membrane, thus increasing *P* and the flux. The ER values are 1.68 in the aqueous phase and 1.13 in the cream. When IPM was mixed with EtOH in 8 : 2 ratio, the ER values increased to 1.76 in the aqueous phase and to 1.67 in the cream. Here, ethanol plays the role of a co-solvent for IPM, promoting its activity in *n*-octanol and increasing the CPM permeability. The large values of T_L are probably due to initial saturation of the membrane with ethanol that causes precipitation and delay of CPM penetration.

Oleic acid is a popular penetration enhancer. However, in the system under consideration, oleic acid (present in an amount of 2 wt%) did not lead to an increase in the ER for CPM in the aqueous phase and even acted as a penetration retardant in the cream. This is probably due to the formation of a retarding layer on the membrane (because of a low solubility in the aqueous phase), which decreases the permeation coefficient for CPM. However, when oleic acid with low concentration (0.2 wt%) was used in the presence of PG (1.8 wt%), the latter acted as a solubilizer for oleic acid in *n*-octanol, and the ER values for CPM increased to 1.76 in the aqueous phase and to 1.33 in the cream. The enhancement of diffusion is due to the effect of oleic acid that can disrupt the packed structure of the lipid bilayer, thus decreasing the resistance of the membrane.

With 2 wt% EDTA, the ER values increased to 1.2 for CPM in both the aqueous phase and the cream. EDTA acts as a chelating agent that can remove divalent ions from the absorption pathways, which can result in enhancement of the CPM transfer. EDTA is used in topical dosage forms as a synergistic antioxidant in concentrations within 0.01 – 0.02%; therefore its effect as penetration enhancer will remain negligible.

The effect of the nonionic surfactant Tween 80 present in an amount of 2 wt% on the CPM permeation coefficient was not significant (neither in aqueous phases nor in the creams). Tween 80 only reduced T_L values for CPM in the cream to 0.61 h instead of 1.08 h for CPM alone.

In conclusion, we have studied the influence of selected penetration enhancers added to 2 wt% CPM in the aqueous phase and w/o emulsion (cream). A modified Franz diffusion cell was used for *in vitro* drug release monitoring. Typical penetration profiles obtained over a period of 6 h showed the permeation coefficient in aqueous phase to be about 270 times that in the cream with an average steady state flux of 30.7 μg/(cm^2 · h) from the cream compared to 8.2 mg/(cm^2 · h) from the aqueous phase. In the aqueous phase, the IPM/EtOH (8 : 2) and oleic acid/PG (1 : 9), gave the highest ER values, IPM was next, EDTA showed a moderate effect, while Tween 80 and oleic acid did not have any significant effect on the permeation coefficient. In the cream formulations, IPM/EtOH had the maximum ER value, while oleic acid/PG was next, followed by EDTA, IPM and Tween 80, whereas oleic acid alone acted as a penetration retardant (reducing ER to 0.6).

The results obtained in this study may be useful for the formulation of topical CPM preparations. The permeability of CPM through a synthetic membrane depended on the vehicle and the penetration enhancers used.

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МОДЕЛЬНОЕ (*in vitro*) ИССЛЕДОВАНИЕ ПРОНИКНОВЕНИЯ ДЕКСХЛОРФЕНИРАМИНА ИЗ ВОДНОЙ ФАЗЫ И ЭМУЛЬСИИ (КРЕМА) ЧЕРЕЗ БИОМЕМБРАНУ В ПРИСУТСТВИИ УСИЛИТЕЛЕЙ ПРОНИЦАЕМОСТИ

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Исследовано проникновение дексхлорфенирамина (ДХФА) из водной фазы (2% раствор) и вода/масло (в/м) эмульсии (крема) через биомембрану в диффузионной ячейке Франца в присутствии различных добавок, стимулирующих проникновение биологически активных соединений. Биомембрана (полиамидная пленка, моделирующая кожу) была заключена между двумя диализными мембранами и помещена между отделениями диффузионной ячейки. Установлено, что коэффициент проникновения (КП) ДХФА из водной фазы в 270 раз выше, чем из крема, а период задержки проникновения из крема в 3,5 раза дольше, чем в случае раствора. Смесь изопропилмиристата и этанола (8 : 2) в наибольшей степени стимулирует проникновение ДХФА (максимальный КП), а олеиновая кислота, наоборот, задерживает проникновение ДХФА из крема.