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Nortriptyline hydrochloride skin absorption: Development of a transdermal patch

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Abstract

The influence of propylen glycol (PG), ethanol, and oleic acid (OA) on nortriptyline hydrochloride (NTH) penetration through human epidermis was studied in vitro at two different pH values (5.5 and 7.4). The influence of lactic acid and polysorbate 80 was studied for a pH of 5.5. Permeation studies through Heat Separated Epidermis, as well as the enhancing effect of the different vehicles, showed a pH dependency. A pH value of 5.5 in the donor solution decreases significantly the permeability coefficient (K_p) with respect to a pH value of 7.4 ($0.011 \pm 0.004 \cdot 10^{-6}$ versus $0.36 \pm 0.04 \cdot 10^{-6}$ cm/s). The vehicles showed an increasing enhancement effect in the order: polysorbate 80 > ethanol/PG/OA > PG > ethanol > ethanol/lactic acid > lactic acid at pH 5.5 while they reduced the permeation of NTH at pH 7.4. Considering the results obtained at pH 5.5, the maximum enhancement ratios were found for polysorbate 80 and the combination ethanol/PG/OA (10.72 and 3.90). Both vehicles were selected for designing a NTH transdermal delivery system (NTH-TDS) using (hydroxypropyl)methyl-cellulose as polymer. The NTH-TDS based on the combination of ethanol/PG/OA showed an enhancement ratio with respect to control of 2.09 and the addition of polysorbate 80 to the matrix, of 5.82.

Keywords: Percutaneous absorption; Chemical enhancers; Nortriptyline; TDS; Skin

1. Introduction

Nortriptyline hydrochloride (NTH) is a tricyclic antidepressant widely used in the treatment of unipolar depression. Besides that, there is growing evidence of its efficacy for smoking cessation pharmacological therapy [1]. Furthermore, it has been reported that up to 70% of patients who are prescribed oral antidepressants fail to take 25–50% of their prescribed dose [2]. Development of a transdermal drug delivery system (TDS) offers a possible approach to overcome some of the drawbacks of systemic NTH oral therapy as: (a) this route improves compliance of the patient, (b) ensures essentially constant drug input, and (c) bypasses the gastrointestinal tract and the liver as sites of metabolism, that are responsible for the low oral bioavailability of NTH.

The aim of this study was to characterize the diffusion kinetics of NTH and the influence of pH and chemical enhancers in order to get an insight into the possibilities of the transdermal route of administration and to develop a transdermal formulation based on the results obtained.

The pH in the vehicle is very important for drug diffusion through skin in the case of polar molecules because it determines their solubility and partition coefficient and consequently, the penetration of the drug [3]. In the present study, pH 7.4 and 5.5 values have been chosen as representative of physiological values of dermis and skin surface, respectively.

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Nomenclature

5-FU	5-fluorouracil
A	diffusion surface area
AHA	a-hydroxyacid
C	concentration of the permeant
CV	Variation coefficient (%)
D	diffusion coefficient of the permeant in the membrane
D^0	diffusion parameter through the epidermis
EtOH	ethanol
ER	enhancement ratio
ER*	enhancement ratio calculated using flux instead of K_p
HPMC	(hydroxypropyl)methyl-cellulose
HSE	heat separated epidermis
J	flux of the permeant through the HSE (1g/cm^2/h)
K	constant of the Power Law equation
K_p	permeability coefficient (cm^2/s)
L	membrane thickness
LA	lactic acid
n	release exponent of the power law equation
NaCl	sodium chloride
NTH	nortriptyline hydrochloride
OA	oleic acid
P	partition coefficient experimentally determined
P^0	partition parameter through the epidermis
PG	propylen glycol
PS	polysorbate
PVP	polyvinylpyrrolidone
$Q_{(t)}$	absolute cumulative amount of drug released at time t (1g)
Q_{inf}	absolute cumulative amount of drug released at infinite time (1g)
SC	stratum corneum
TDS	transdermal delivery system
t_L	lag time (h)

Chemical enhancers are commonly incorporated in TDS in order to improve the diffusion kinetics of the drugs administered. Many chemicals have been evaluated for this purpose, but none has proven to be ideal and the structure–activity relationship is still unclear in many cases. It is well known that the enhancing properties of chemical enhancers depend on the physicochemical properties of drugs and the combination with the excipients of the formulations [4]. This makes it difficult to choose the most appropriate enhancer for each drug. Ethanol has been widely used in many transdermal formulations, being the solvent of choice in patches. In the present study, 30% (w/w) was chosen as it has been demonstrated that higher concentrations reduce transdermal permeation of drugs due to skin dehydration [5]. PG has been used as an enhancer on its own at 30% [6], and in combination with 1% oleic acid as it has been reported to have a synergistic effect [5]. Lactic acid has been studied as representative of a-hydroxyacids which have also been widely used in cosmetic and dermatological applications [7]. Polysorbate 80 has been chosen as representative of non-ionic surfactants, also widely used in cosmetic and regarded as safe [8].

Modern TDS consists of self-adhesive matrices [9]. (Hydroxypropyl)methyl-cellulose (HPMC) has been selected because it is an atoxic polymer which provides transparent films with good organoleptic properties. It is also compatible with many cosolvents and enhancers used for transdermal delivery. Besides that, the desired adhesiveness can be achieved by changing its concentration in the matrix.

2. Materials and methods

Nortriptyline (NTH), ethanol, lactic acid (LA), propylene glycol (PG), and (hydroxypropyl)methyl-cellulose (HPMC) were purchased from Sigma Chemical (Madrid, Spain). Oleic acid (OA) and polysorbate 80 were obtained from Fluka (Buchs SG, Switzerland). All other chemicals were of high-performance liquid chromatography (HPLC) analytical grade.

2.1. Determination of NTH

The samples were analyzed by HPLC (Hewlett-Packard 1050. Barcelona, Spain) with ultraviolet detection (k = 254 nm). The column used was a reverse-phase Kromasil C-18 (150 · 3.9 mm, 4 1m, Scharlab S.L. Barcelona, Spain). The mobile phase consisted of an acetonitrile/phosphoric acid solution (1/15 M, pH 3.00) at 50/50 V/V. The flow rate was 1.0 ml min^{-1} and the temperature was 22 °C. The injection volume was 50 1l. The HPLC method was validated for accuracy (9.6 as the highest relative error obtained in the range), precision (2.7% as the highest variation coefficient) and linearity over the concentration range analyzed (r^2 > 0.999). The limit of determination was 50 ng/ml.

2.2. NHT lipophilicity studies

The partition coefficient of NTH was determined using n-octanol as organic solvent and phosphate buffer 1/15 M adjusted to pH 5.5 or 7.4 as aqueous phase. The composition of 1 L phosphate buffer pH 5.5 is: 8.86 g NaH_2PO_4, 0.47 g Na_2HPO_4. The composition of 1 L phosphate buffer pH 7.4 is: 7.84 g NaH_2PO_4, 1.81 g Na_2HPO_4.

Phase volume ratio was 0.5:10. After equilibrating for 24 h in a water bath at 22 °C, the aqueous phase was separated by centrifugation (10,000g for 15 min) and assayed for NTH content.

In addition, heat separated epidermis (HSE)/phosphate buffer 1/15 M partition coefficient was also assessed at the two pH values. These coefficients were obtained by equilibrating 5 ml of NTH buffered solutions with a known mass of fully hydrated epidermis (ranging from 85 to 220 mg). The experimental procedure was similar to that described above.

Six replicates of every condition were carried out. The partition coefficient was calculated as the ratio of organic concentration (estimated by difference between initial and final aqueous concentration) to final aqueous concentration [10].

2.3. NHT solubility studies

The solubility of NTH was determined in the different vehicles assayed. Excess of drug was added to known volumes of the solvent systems, vortex mixed for 2 min, followed by 10 min in ultrasound to solve the drug and then equilibrated at 32 ± 0.5 °C in a laboratory electric oven (Memmert, Schwabach, Western Germany) for more than 24 h under constant agitation at 500 rpm. The contents were then filtered through a 0.45-1m pore cellulose acetate filter. The resulting saturated solvent systems were diluted appropriately and analyzed as described. Three replicates of each system were carried out. Relative thermodynamic activity of NTH was derived from the ratio of the concentration used in the solvent to the corresponding solubility [11].

2.4. NHT in vitro permeation experiments

Permeation experiments were performed on Caucasian abdominal skin samples (from three donor females aged 38–48 years, randomly assigned), obtained from cosmetic surgical corrections performed in the Hospital *9 de Octubre* (Valencia, Spain) and in the Caritaskrankenhaus (Lebach, Germany). Informed consent was previously obtained from the patients. Their identity was masked to the researchers to guarantee their anonymity. Controls have been tested in the skin of the different volunteers used showing very similar values of K_p and flux (data not shown). An average of them has been calculated to compare the enhancement ratio of every vehicle.

Excess fatty and connective tissues were removed and the samples stored in a freezer at −26 °C for less than three months. Epidermal membranes were prepared by a heat-separation technique. After the skin had been immersed in water at 60 °C for 45 s, the epidermis was carefully separated from the underlying dermis.

In vitro permeation studies were carried out using static Franz diffusion cells FDC (type 4G-O1-00-20, Perme Gear, Riegelsville, PA, USA) or glass Franz type cells with an available diffusion area of 0.78 cm^2 and 6 ml of receptor cell volume placed in heating/stirring module. The skin pieces were mounted over the diffusion cells with the stratum corneum side in contact with the donor compartment and the dermal side over a dialysis membrane circle situated on the receptor compartment; they were equilibrated for 1 h, and then air bubbles were removed off the diffusion cell. The receptor phase consisted of phosphate buffer solution 1/15 M of pH 5.5 or 7.4, according to the pH of the donor solution. This procedure is in accordance with Wagner et al., as they demonstrated that the pH of the skin can be shifted in every direction [3,12]. In previous experiments carried out with pH 7.4 in the receptor phase and 5.5 in the donor phase, both the dialysis membrane and the skin were pushed to the donor compartment and a precipitation of NTH in the receptor phase occurred. To overcome this problem, pH 5.5 was used in the receptor phase. By doing so, sink conditions during pH 5.5 assays were maintained during the whole experimental time. Subsequently, 1 ml of the different solvent systems was randomly applied to the stratum corneum side in each of the donor compartments and covered with parafilm to prevent evaporation. Samples (200 1l) were withdrawn at specified intervals from the receptor compartment followed by replacement with fresh receptor solution. At the end of the experiments, phenol red 1% solution (200 1l) was added in order to test the integrity of skin samples.

The cumulative amount of drug permeated through the skin was plotted as a function of time. The permeability coefficients, K_p, and lag time, t_L, were calculated following the Scheuplein diffusion model as described below. This author demonstrated [13] the following relationship as representative of the process:

$$Q_{(t)} = A \cdot P \cdot L \cdot C \cdot D \cdot \left(\frac{t}{L^2} - \frac{1}{6} - \frac{2}{p^2} \cdot \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \cdot \exp\left(\frac{-D \cdot n^2 \cdot p^2 \cdot t}{L^2} \right) \right) \quad (1)$$

The symbols used stand for: $Q_{(t)}$ is the quantity which passes through the membrane and reaches the receptor solution at a given time, t; A represents the actual diffusion surface area (1.767 or 0.78 cm^2, depending on the FDC design). According to Fick's theory and the results obtained by Netzlaff and Chilcott, the use of FDC with different areas of diffusion does not have an influence on the permeation of drug [14]. P represents the partition coefficient of the permeant between the membrane and the donor vehicle; L is the membrane thickness; D is the diffusion coefficient of the permeant in the membrane; and C is the concentration of the permeant (0.5 or 20 mg ml^{-1}, depending on the pH). To determine K_p and t_L directly, Eq. (1) was transformed by substituting $P \cdot D/L$ by K_p and $L^2/6D$ by t_L. The fitting equation was:

$$Q_{(t)} = A \cdot K_p \cdot C \cdot \left(t - t_L - \frac{12 \cdot t_L}{p^2} \cdot \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \cdot \exp\left(\frac{-n^2 \cdot p^2 \cdot t}{6 \cdot t_L} \right) \right) \quad (2)$$

Data from experiments were fitted to diffusion Eq. (2) [15] by means of the computer program Sigma plot 8.0. An equal weighting scheme was applied. Exemplarily calculations with Eq. (1) have shown that the results obtained with the Eq. (2) are very similar. Starting parameters of the fit were obtained by linear regression from the steady state part of the diffusion experiment.

The diffusion parameter D^0 and the partition parameter P^0 were then calculated taking into account the equivalences (Eq. (2a))

$$D^0 = 1/6 \cdot t_L \quad \text{and} \quad P^0 = K_p/D^0: \qquad (2a)$$

The mean of four replicates was used as representative of the NTH penetration in the different conditions.

The flux was calculated using the following expression:

$$J = K_p \cdot C_0 \cdot A$$

In which J stands for flux, C_0 for the concentration in the donor phase during the experiment, and A for diffusion area. In infinite dose experiments, it is assumed that, for steady state conditions, the concentration in the donor does not change more than 10% and therefore, it can be considered to be constant.

The enhancement ratio (ER) was calculated following Williams and Barry [14] as the ratio of K_p values in the presence and absence of enhancer (Eq. (3))

$$\text{ER} = \frac{K_p \text{ with enhancer}}{K_p \text{ without enhancer}}: \qquad (3)$$

2.5. Preparation of NHT film formulations

Two different films were assayed. A mixture of PG, EtOH, and water (30:30:35, w/w) was prepared. In the case of film A, 1% water was added. In film B, 1% polysorbate 80 was included. 500 mg of NTH was dissolved in the respective solutions under stirring, with a speed of 500 rpm. Oleic acid was incorporated at a concentration of 1% for both cases and the mixture was stirred overnight. HPMC (2%) was weighted and slowly incorporated to the mixture increasing the stirring speed to 800 rpm. The amount of polymer remained constant because it is well known that the change in the polymer content in the film can modify the diffusive mobility of the drug in the polymeric network [16]. Once it was fully hydrated and gel consistency was obtained, 20 g of the mixture was dried on a 95 mm diameter Petri-dish at 50 °C for 6 h.

2.6. Physico-chemical characteristics of the prepared films

2.6.1. Film thickness

The thickness of the films was measured at six different points using a micrometer, tactile probe 10B (Heidenhein, Traunreut, Germany), with an accuracy of ±1 μm. Mean values were calculated and are reported.

2.6.2. Film NTH content uniformity

Three circles of the prepared films were cut with a 9 mm diameter punch. The circles were weighted and dissolved in a known volume of phosphate buffer 1/15 M pH 5.5. The content of NTH was analyzed by HPLC as described.

2.6.3. In vitro drug release study

The release of drug from film preparations was examined using the static FDC described above. The receptor compartment was filled with 12 or 6 ml (as appropriate) phosphate buffer solution 1/15 M pH 5.5 free of air bubbles and a 15 mm-diameter circle of dialysis membrane was sandwiched between donor and receptor compartment. 100 μl of phosphate buffer 1/15 M pH 5.5 was placed over the membrane in order to moisture it and assure the adhesion of the film. Finally, a 13 mm diameter film circle was placed on the donor compartment and covered with parafilm. The FDC were placed in a 32 °C tempered oven and stirred at 500 rpm. Samples of 400 μl were collected after 1, 2, 6, 18, 24, 30 h. The same volume of fresh buffer was replaced in the receptor after sampling. At the end of the study, the remaining film at the donor compartment was dissolved in a known volume of fresh buffer and analyzed for NHT content in order to establish the mass balance. Concentration of NTH in the samples was quantified by HPLC as described.

Cumulative amounts of NTH versus time were plotted and the release characterized by fitting the Power law equation (Eq. (4)) to data [17]. The non-linear regression program SigmaPlot 8.0 was used.

$$Q_t/Q_{inf} = k \cdot t^n; \qquad (4)$$

where Q_t and Q_{inf} are the absolute cumulative amounts of drug released at time t and infinite time, respectively, k is a constant that includes structural and geometric characteristics of the device, and n is the release exponent, indicative of the mechanism of drug release.

2.6.4. In vitro skin penetration study

The penetration profile of NHT from film preparations was examined using the static FDC described above (Section 2.6.3). The experimental conditions were as indicated, except for the type of membrane that was HSE prepared as explained in Section 2.4.

2.7. Statistical analysis

Homogeneity was confirmed by Barlett test. One-way ANOVA, followed by the Tukey's multiple comparison test when appropriate was performed on the permeability coefficients (K_p) to establish differences between the calculated means. The Student t test was used to compare values obtained at the two pH.

3. Results and discussion

3.1. Lipophilicity of NTH

Classical partition coefficients obtained using *n*-octanol as the organic solvent show a pH dependency as predicted by the basic nature of NTH with a pK_a of 9.23. The obtained P value was 48.56 ± 0.98 for pH 5.5 and 787.13 ± 147.59 for pH 7.4. The non-ionized fraction varies from 0.0186% to 1.479% for 5.5 and 7.4, respectively. Theoretical background concerning transdermal penetration would recommend the use of pH 7.4 as vehicle as the drug shows an optimal lipophilicity to diffuse through the stratum corneum (SC).

When experiments using HSE as the organic phase were carried out, the results showed differences not as high as in *n*-octanol assays. P at pH 5.5 was calculated as 12.8 ± 1.8 and 19.2 ± 0.7 for the pH value of 7.4. This result agrees with Sznitowska et al. studies [18] which concluded that partition coefficients of polar compounds do not match their *n*-octanol/water partition coefficients. Nevertheless, as expected, P is higher for pH 7.4 than 5.5 in both systems.

3.2. Solubility of NTH

Saturation solubility of NTH increased among the different vehicles assayed (Table 1), the differences being statistically significant ($p < 0.05$). Differences are also important between both pH-conditions due to changes in NTH ionization. In fact, both PG and ethanol enhance better the ability to dissolve at acidic pH than a neutral one, being more sensitive the ethanol to this pH change. Surprisingly, the enhancement of solubility produced by the mixture ethanol/PG/OA is not affected by pH.

As the concentration of NTH was constant in the different solvents used at every pH in in vitro permeation studies, the relative thermodynamic activity varied (Table 1). Note that it depends on the pH of the vehicle for PG and ethanol, while it is maintained for both controls and the mixture ethanol/PG/OA.

3.3. In vitro permeation studies

The most representative in vitro skin permeation profiles of NTH are illustrated in Fig. 1. NTH skin permeation parameters and enhancement ratios calculated are listed in Table 2.

The results presented here do not show a relationship between permeability coefficient values and relative thermodynamic activity of NTH in these vehicles. Taking this result into account, it could be concluded that the relative thermodynamic activity of the drug in these solvent systems does not explain the permeation behaviour of NTH. The critical parameter seems to be the pH of the vehicle.

3.3.1. Influence of pH

As expected, the pH of donor and receptor fluid in the Franz cell highly determines the ability of penetration of a polar molecule as NTH (see Table 2, control). In fact, for control at pH 7.4, the estimated K_p was 33 times higher than for pH 5.5 control. This can be explained on the basis of the ionization of the compound which varies in the range from 1.479% to 0.0186% of unionized form for 7.4 and 5.5, respectively, and produces a significant change in the lipophilicity of the substance. Similar results were obtained by Nicoli et al. for Oxybutynin hydrochloride [19]. This can also be related to the partition coefficient calculations. P is higher for the pH value of 7.4 in *n*-octanol/water and HSE assays, so that, it was expected to have a better permeability at this pH value. This is in agreement with the classical concept that considers the SC as the main barrier to diffusion through the skin [20].

The permeability coefficient is directly proportional to the partition coefficient and the diffusion coefficient. Results (Table 2) show a linear relationship between K_p and P^0 ($r > 0.86$).

Table 1
Solubility of NTH, and relative thermodynamic activity for solvent systems

Vehicle	Solubility (mg ml^{-1}) (Mean ± SD)	Experimental concentration (mg ml^{-1})	Thermodynamic activity
Phosphate buffer pH 5.5			
pH 5.5 (Control)	27.80 ± 1.74	20.00	0.72
pH 5.5 + PG	366.80 ± 1.90	20.00	0.05
pH 5.5 + EtOH	311.10 ± 3.23	20.00	0.06
pH 5.5 + PG/EtOH/OA	193.90 ± 3.02	20.00	0.10
pH 5.5 + lactic acid	49.260 ± 2.12	20.00	0.40
pH 5.5 + lactic acid/EtOH	289.30 ± 4.32	20.00	0.07
Polysorbate 80	52.910 ± 1.02	20.00	0.38
Phosphate buffer pH 7.4			
pH 7.4 (Control)	0.72 ± 0.002	0.50	0.69
pH 7.4 + PG	135.80 ± 4.58	0.50	$3.6 \cdot 10^{-3}$
pH 7.4 + EtOH	270.00 ± 6.57	0.50	$1.8 \cdot 10^{-3}$
pH 7.4 + PG/EtOH/OA	192.50 ± 4.96	0.50	$2.6 \cdot 10^{-3}$

The data are means of three determinations. Standard deviation is also indicated.

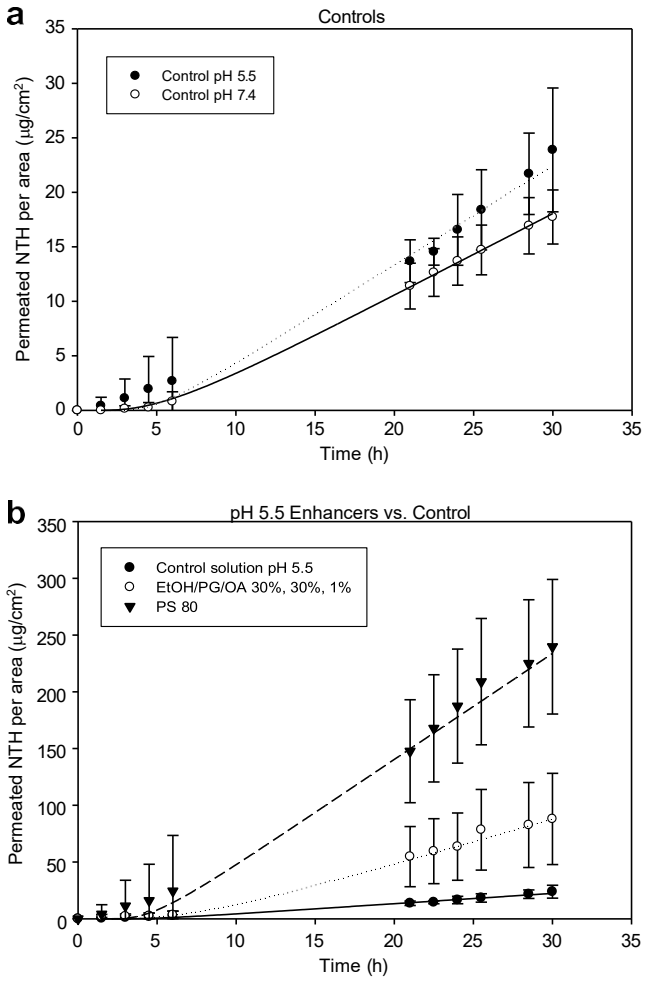

Fig. 1. Permeation profiles (mean ± SD) of NTH across human epidermis from different solvent systems at different pHs (a) and pH 5.5 with penetration enhancers (EtOH, ethanol, PG, propylen glycol, OA, oleic acid, PS 80, polysorbate 80) (b).

3.3.2. Influence of chemical enhancers

The effect of the different enhancers tested depends on the pH of the donor-solution as well as the thermodynamic activity and the interactions of the enhancers with the skin, which modify its properties. Permeability coefficients, flux, lag time, and enhancement ratios obtained in the different conditions are summarized in Table 2.

Considering experiments carried out at pH 5.5, it can be observed that all enhancers and combination of them produced an increase of the NTH K_p if compared with pH 5.5 control. A different picture is obtained if the chemical enhancement of penetration is calculated comparing K_p obtained in the presence and absence of these same substances at pH 7.4, as data suggest that they act to reduce K_p. Furthermore, if the estimation of ER takes into account the solubility between different media, i.e. calculating the ratio between pH 5.5 as control and pH 7.4+enhancers or the ratio of fluxes, similar conclusions can be achieved. An analysis of the different types of enhancers [5] is needed in order to explain the results.

As representative of a-hydroxyacids (AHAs), lactic acid (1% w/w) shows a slight enhancement compared to control, whereas the combination of it with ethanol (1:30%, w/w) results in a small decrease in the K_p and flux. Although the mechanism of action of AHAs is still unknown, Kraeling and Bronaugh suggested that they reduce the SC cohesion by interference with ionic bonding [5]. On this basis, NTH, a tertiary amine, could form an acid–base complex with lactic acid reducing the effective concentration of the AHA for the enhancer effect. It would also represent an increase in the molecular size of the drug, explaining the decrease in D^0 and the corresponding increase in lag time. Copov´ı et al. [21] studied the influence of a-hydroxyacids on permeation of drugs with different lipophilicity. They demonstrated that for the most hydrophilic compounds, their K_p increased as a function of LA concentration, whereas K_p tended to decrease as the lipophilicity of the drugs increased, independently of LA concentration. According to this study, for the log P of NTH, a moderate enhancement would have been expected. Our results confirm this tendency. On the other hand, lactic acid and its combination with ethanol slightly change the NTH diffusion coefficient through skin but retard the lag time.

Among the group of alcohols and glycols, two substances have been employed: ethanol (30%, w/w) and PG (30%, w/w). Ethanol increases twice the K_p and maintains a similar lag time if data obtained at pH 5.5 are used. The effect is masked if data obtained at pH 7.4 are compared, as a reduction in K_p about four times can be seen, maintaining the lag time as well. PG's enhancement is higher at pH 5.5 whereas no significant influence is observed for the 7.4-pH value; on the other hand, it increases significantly the lag time at pH 5.5 and reduces it at pH 7.4. In order to explain these results, the scientific literature refers to three main hypotheses about their mechanism of action. The first one is their ability of increasing drug solubility in the vehicle, which has also been proven for NTH (see Table 2). Second, they can permeate through the skin altering the solubility properties of the tissue and improving consequently the drug partitioning into the membrane [22] in a reversible way [23]; this effect is of relevance when polar compounds (as NTH) are under study. The third hypothesis suggests that a volatile solvent might extract some of the lipid fraction of the SC if it is used for a long time [5]. Having PG at a higher boiling point than ethanol (421 versus 78.5 °C), a slower evaporation of it can be predicted, while the evaporation of the PG/water and Ethanol/water-mixtures is very fast. Consequently, a good enhancer ability of both vehicles should be predicted if the third mechanism is considered. Thus, every mechanism is in agreement with our results. Although many authors have published enhancing results for ethanol and PG [11], less information is available concerning a decrease in the permeation efficacy of drugs. Gao et al. [24] found that ethanol and PG alone decreased 5-FU and tamoxifen transdermal permeation coefficients from a isotonic NaCl solution (0.9%).

Table 2
NTH epidermis permeation kinetic parameters from solvent systems: flux, permeability coefficients (K_p), and lag time (t_L) estimated[a], and enhancement ratios (ER) calculated

Vehicle	Flux (1g cm^{-2} h^{-1}) (Mean ± SD)	$K_p \cdot 10^6$ (cm s^{-1}) (Mean ± SD)	Lag time (t_L, h)	P^0 ($\cdot 10^3$) (Mean ± SD)	D^0 (h^{-1})	ER	ER*
Phosphate buffer pH 5.5							
Control	0.65 ± 0.06	0.011 ± 0.004	4.91 ± 0.97	1.662	0.034	–	–
PG	2.59 ± 0.82	0.036[a] ± 0.011	9.81 ± 6.94	7.628	0.017	3.27	3.92
EtOH	1.69 ± 1.05	0.023[a] ± 0.006	6.23 ± 1.54	3.095	0.027	2.09	2.56
EtOH/PG/OA	3.13 ± 1.60	0.043[a] ± 0.022	2.48 ± 1.41	0.230	0.067	3.90	4.74
Lactic acid	0.90 ± 0.02	0.016[b] ± 0.003	18.66 ± 2.57	6.448	0.009	1.45	1.36
Lactic acid/ EtOH	0.62 ± 0.07	0.011[b] ± 0.001	16.60 ± 6.02	394.4	0.010	1.00	0.93
Polysorbate 80	8.53 ± 2.07	0.118[a] ± 0.028	2.85 ± 2.88	7.260	0.058	10.72	12.92
Phosphate buffer pH 7.4							
Control	0.66 ± 0.08	0.36 ± 0.04	3.42 ± 0.70	25.96	0.570	–	–
PG	0.64 ± 0.19	0.35[b] ± 0.10	7.21 ± 0.62	1.058	1.190	0.97	0.97
EtOH	0.14 ± 0.01	0.09[a] ± 0.01	6.23 ± 1.54	12.11	0.026	0.25	0.21
EtOH/PG/OA	0.08 ± 0.02	0.04[a] ± 0.01	5.84 ± 7.39	4.734	0.028	0.11	0.12
Patch (pH 5.5)							
A	5.80 ± 2.40	0.023 ± 0.091	6.12 ± 0.65	0.003	0.027	2.09	8.92
B	14.52 ± 1.80	0.064 ± 0.008	5.63 ± 0.66	0.008	0.029	5.82	27.86

The data are means of four determinations. Standard deviations are indicated.
ER* = flux/flux control = flux/0.66.
[a] Significant differences with respect to control solution (Tuckey test, $a = 0.05$).
[b] Non-significant differences with respect to control solution (Tuckey test, $a = 0.05$).

Pachagnula et al. [25] found also a decrease in the transdermal permeability of naloxone using a PG/water (33.5/66.5) mixture, an EtOH/water (66.5/33.5) mixture, and a PG/EtOH (66.5/33.5) mixture, with a receptor solution of pH 7.4 but an enhancement for other combinations of the same solvents.

Concerning fatty acids, oleic acid was selected as model. Its lipophilicity forced the addition of ethanol (30%) and PG (30%) to properly dissolve it in the phosphate buffer at 1% (w/w) concentration. On the other hand, oleic acid and PG have been reported to have a synergistic action [5,24]. At pH 5.5, the combination of the three enhancers showed the highest enhancement ratio with respect to each of the components alone, and with respect to pH 5.5 control. The lag time was also significantly reduced, being approximately half of that obtained for the control. This result could be interpreted on the basis of a better diffusion of NTH through the SC in the presence of OA, which effect overcomes the action of PG and ethanol by its own (both of them increase the lag time). In fact, it has been demonstrated that the enhancer interacts and modifies the lipid domains of the SC, thus facilitating permeation through the membrane [26]. Nevertheless, when results obtained at pH 7.4 are analyzed, the mixture ethanol/PG/OA reduces NTH K_p with respect to 7.4 control and increases the lag time. This effect can be due to the higher ionization of oleic acid at this pH value, that would make it less efficient in interacting with the SC phospholipids, so that the effect of ethanol would be predominant in this case.

As surfactant, polysorbate 80 (1%) was used. It produced the highest enhancement ratio and a considerable reduction of the lag time. Considering its mechanism of action, it has been postulated that it solves the SC lipids allowing a better diffusion. In agreement with this hypothesis and our results, it has been reported to accelerate lorazepam and diazepam permeation through rat skin [27,28]. Nevertheless, there are some reports that demonstrate no enhancement effect on other drugs, as nicardipine or ketorolac, also polar drugs [29].

As a higher amount can be solved for the different vehicles at pH 5.5, the amount of NTH permeated is always higher, as can be seen by the fluxes (Table 2). Taking into account that the solubility of NTH at pH 7.4 is enhanced by the addition of cosolvents, higher concentrations of NTH could be used in the donor to obtain an increased flux. Even though, it can be calculated that the permeation is more effective for pH 5.5 condition.

Although it is difficult to separate the influence of the pH and the thermodynamic activities, it can also be seen that the higher activity formulations have a higher flux for both pHs.

3.4. Film characterization

Both films were chosen from a series of trials using different polymers on the base of the chemical compatibility among all the components, the organoleptic properties and adhesiveness appearance. The presence of polysorbate 80 provides a clearer and less fatty appearance to the film but there is a contraction of the surface after drying and therefore increases the thickness of the resulting membrane. As a result of this process, while film A showed a

thickness of 0.4566 ± 0.0176, the corresponding thickness of film B was 0.9329 ± 0.0148 mm. After evaporation of the solvents, the quantity of NTH was 66 ± 1.1 (1g/mg) for film A and 68 ± 2.8 (1g/mg) for film B. Note that dispersion is very low (CV 1.6% and 4.1%, respectively), meaning that both films have a uniform drying process and a uniform distribution of NTH.

The in vitro release profile is shown in Fig. 2. The amount of NTH that is released to the receptor compartment is 57.69% of the dose applied in the donor compartment for the film A and 51.01% for the film B. The mass balance (sum of cumulative amounts released and amount recovered from donor) was 96.11% of the total and 98.06%, for films A and B, respectively. This means that there is no loss of drug in the system. The comparison between the two films suggests that the polysorbate 80 is able to retain in the matrix a slight amount of the total NHT incorporated in the matrix. The non-linear regression fitting to the in vitro drug release data estimated a rate of 43.55% $(\pm 2.63)h^{-0.14}$ ($r^2 > 0.999$) for the film A and 17.31% $(\pm 1.01)h^{-0.16}$ for the film B ($r^2 > 0.999$), in agreement with the hypothesis of NHT micellisation that would slow the process of release.

Permeation profiles of NTH through human epidermis from the films assayed are shown in Fig. 3. Table 2 shows the flux, permeation coefficient, lag time, ER and ER* of both. Being the diffusion area and concentration of NHT in the films similar, the increased thickness of the film B with respect to film A produced that the amount of drug in the donor compartment was different (for film B it was 7.3 mg whereas for A it was 4.3 mg). Regarding the K_p results, it can be concluded that there is an enhancement of the permeation with respect to control pH 5.5 solution for both films, but not if compared to the combination of enhancers in solution, where a decrease was found. This feature can be easily explained because the mobility of the drug and enhancers is reduced in the polymeric network. It brings about the interactions between them and the SC more limited making the diffusion process more difficult.

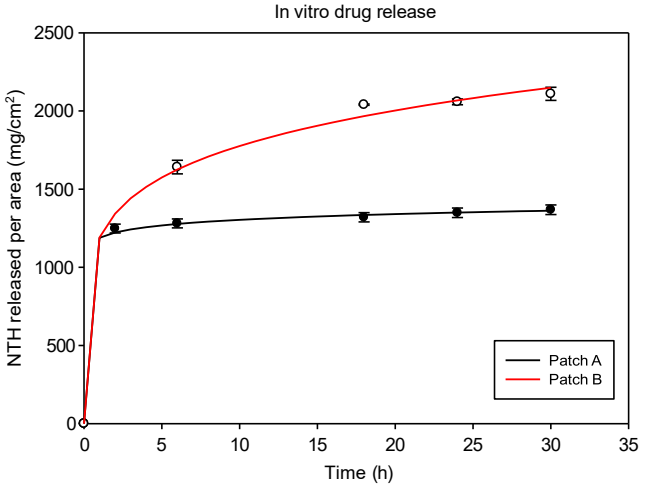

Fig. 2. In vitro drug release of NTH from the patches in static Franz diffusion cells.

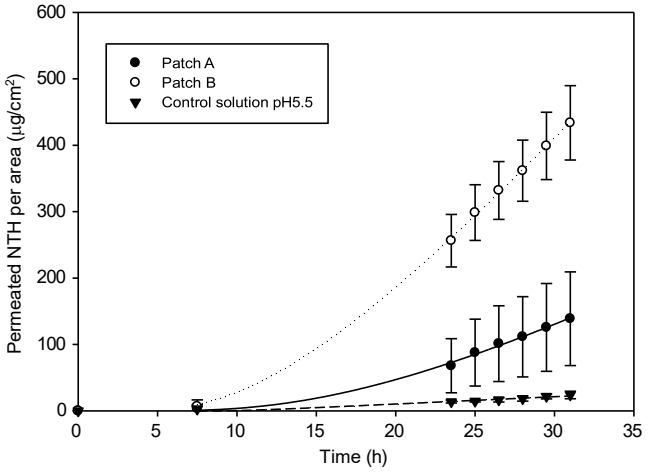

Fig. 3. Permeation profiles of NTH across human epidermis from Patch A, Patch B, and control solution pH 5.5.

These profiles and the in vitro drug release can be compared in order to understand the drug release mechanism. For both films, after 2 h, the released amount of NTH was higher than the amount permeated through skin after 30 h. Taking this into account, it could be concluded that the element of control of NTH diffusion through skin from the film would be the SC barrier. Nevertheless, as can be observed in Table 2, the lag time obtained for both films is similar to the control solution pH 5.5, but longer than that corresponding to the enhancer solution. On the other hand, the $K_p$values of the patches are about half of the corresponding enhancer solution. Both facts indicate that release from the patches is controlling the rate diffusion through the epidermis. This would allow a better design of the TDS.

The recommended daily oral dose of NTH is 100 mg in the smoke cessation therapy [30]. The reported oral bioavailability of this drug is between 30% and 50% [31]. This means that 30–50 mg NTH is needed per day. Considering the flux obtained for the patch B, this amount could be reached by means of a 100 cm^2 (10 · 10 cm) patch. Since the content of the drug can be increased in the matrix, a higher flux would be expected, enabling the use of a smaller patch with the same composition.

4. Conclusions

The results presented in this work show that NTH permeates through the skin by passive diffusion. pH-influence is remarkable for this molecule. Lactic acid and its combination with ethanol slightly change the NTH diffusion coefficient through skin but retard the lag time. Ethanol, PG, and a combination of ethanol/PG/OA significantly increase the K_p of NTH through the skin for a pH value of 5.5, whereas it decreases for a pH value of 7.4. Polysor-

bate 80 highly increases the permeation of NTH for a pH value of 5.5.

The best permeation coefficient is provided by the control 7.4, but the solubility of NTH in that medium is not high enough to develop a transdermal formulation containing the needed amount of drug per day (100 mg/day for oral administration), with a bioavailability of 30% which would lead to a transdermal administration of 30 mg/day. Taking into account that the physiologic pH of the outermost human skin is 5.5, dosage forms of this pH would be less irritant and more convenient for long term treatments. So that, vehicles containing the combination of ethanol/PG/OA at pH 5.5 or polysorbate 80 1% seem to be good candidates for the development of transdermal patches of NTH. The films assayed in this work showed promising results. They can be improved and many other characterization assays are to be done.

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