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RESEARCH PAPER

Vehicle Composition Influence on the Microneedle-Enhanced Transdermal Flux of Naltrexone Hydrochloride

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ABSTRACT

Purpose Transdermal delivery of drugs is often limited by formidable barrier properties of stratum corneum (SC). Microneedles (MN) enable creation of transient microchannels in the SC and bypass this barrier. Many reports have focused on the great effectiveness of MN in improving percutaneous flux values of a variety of drugs over a large molecular size spectrum. The objective of the present study is to evaluate the influence of formulation on MN-enhanced transdermal transport of naltrexone hydrochloride (NTX HCl).

Methods A series of *in vitro* experiments employing binary mixtures of propylene glycol (PG) and water as vehicle were used with either MN-treated or untreated skin. A simple model taking into account two parallel flux values through intact skin and microchannels was used to analyze data.

Results Transdermal permeation of NTX HCl from different donor solutions indicated that PG-rich formulations greatly limited MN-enhanced transport but had a much smaller effect on transport through intact skin.

Conclusions Diffusion through the microchannel pathway seems to be donor viscosity-related and follows the relationship predicted by the Stokes-Einstein equation as shown by linear dependence of flux on diffusivity of NTX in donor solutions.

KEY WORDS transdermal drug delivery · microneedles · propylene glycol · viscosity · naltrexone hydrochloride

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INTRODUCTION

SC possesses formidable barrier properties that prevent most xenobiotics from permeating through the skin. Since the pool of molecules possessing necessary physicochemical properties to cross this barrier is limited, the amount of drugs that can be delivered percutaneously at therapeutically relevant rates is also low (1–3). Increasing drug permeation rates across skin represents a great challenge. MN offer a method of substantially increasing the transdermal delivery of drugs by transiently compromising the barrier properties of SC. The use of MN enables the formation of microchannels that extend over upper layers of skin and allow drug molecules to diffuse directly into deeper layers of skin. In general, this process results in up to several orders-of-magnitude enhancement of transdermal transport of drugs (4–7).

Naltrexone is a potent, competitive opioid receptor antagonist. It is used to treat opioid and alcohol addiction (8). This drug undergoes an extensive first-pass effect and is characterized by low and varying bioavailability ranging from 5% to 40% (9,10). The currently marketed oral ReVia® and Depade® are not well-suited for long-term therapy, as they produce gastrointestinal side effects and result in poor patient compliance. On the other hand, injectable Vivitrol® affords effective plasma levels for as long as 30 days but necessitates reporting to a healthcare professional upon administration (11). Also, it cannot be easily discontinued, if needed, without painful surgical removal. It would, therefore, be advantageous to use the transdermal route of delivery for this drug. A transdermal patch would eliminate some of the drawbacks related to either oral or injectable alternatives. Since naltrexone itself does not possess the physicochemical properties that would result in percutaneous transport at therapeutically relevant rates, an enhancement method is needed (12). Previous studies showed that a prodrug strategy resulted in increased

percutaneous transport of NTX; however, observed flux values were still below expectations (13–15). Alternatively, MN were used to improve the delivery of the highly soluble hydrochloride salt form of NTX. Initial *in vitro* and *in vivo* animal studies proved the utility of the MN method of enhancement showing approximately an order-of-magnitude increase in the transdermal flux over that through non-MN-treated skin (7). Subsequently, the first published human MN study demonstrated that skin pretreatment with MN followed by the application of four NTX patches afforded drug plasma levels in the lower end of the therapeutic range targeted (16). The results of these studies validated the interest put into the development of MN techniques for increased transport of drugs across skin. Although a large number of articles focusing on the engineering aspects of MN use and their effectiveness has been published, there is little systematic work done in the area of the formulation influence on the rate of MN-enhanced transdermal delivery of drugs. Recent publications report the efforts to combine liposomes with skin MN pretreatment to further increase drug delivery rates (17,18). Y. Qiu *et al.* studied the combined strategy of using conventional and elastic liposomes with MN skin pretreatment for the delivery of poorly water-soluble docetaxel through porcine skin. The results indicated that this approach afforded superior flux values from both liposomal drug formulations as compared to the saturated docetaxel solution alone. Additionally, the elastic liposomes produced shorter lag times as compared to conventional liposomes. Another study by M.M. Bardan *et al.* focused on the effect of the microneedle length and flexible liposomal formulation on the transport of a model hydrophilic compound, mannitol, through human skin. It was shown that the flexible liposomal formulation delivered a larger fraction of the dose applied in the donor compartment as compared with the aqueous mannitol solution. Moreover, the effect of MN length on flux values was more pronounced in liposomal formulations. These examples illustrate that adequate formulation choice may have a substantial effect on the delivery through MN-enhanced skin.

PG is a very common, generally recognized as safe (GRAS), non-irritating cosolvent widely used in topical formulations (19). It is fully miscible with water and as such is often used to increase the solubility of poorly water-soluble compounds in formulations. Its effect on the transdermal delivery of drugs has been the subject of many studies. In the ideal case when the vehicle does not affect the integrity and barrier properties of the SC, flux values from saturated solutions of a given drug should be the same as suggested by Higuchi (20). Therefore, the presence of PG in an aqueous formulation of a saturated drug solution should not translate into a different flux. However, it rarely is the case. B.W. Barry examined the PG-human skin interactions and implied that this cosolvent can act as a skin permeation enhancer by solvating keratin and occupying hydrogen-bonding sites as well as promoting drug partitioning into the skin (21). Similarly, Michael Goodman *et al.* suggested that the effect of PG on human skin may be related to the solvation effects on keratin in the corneocytes (22). Later, N.A. Megrab, A.C. Williams and B.W. Barry showed that saturated PG-rich donor solutions afforded higher flux values of estradiol through human epidermal membranes as compared to their water-rich counterparts (23). Authors explained the outcome in terms of increased solubility of estradiol in SC which was not offset by somewhat decreased apparent diffusivity of the drug in the barrier. On the other hand, L. Trottet *et al.* studied the depletion of PG from topical drug-containing formulations *in vitro*. It was found that the permeation enhancement effect on human cadaver skin showed time-dependence that correlated with PG levels in the formulation (24). In another study, Christophe Herkenne *et al.* investigated the effect of PG on ibuprofen absorption into human skin *in vivo*. Authors found that PG enters SC, increasing ibuprofen's solubility in the barrier, but does not affect the drug's diffusivity in the barrier (25). Moreover, R.M. Watkinson *et al.* studied the influence of the PG concentration on the skin permeation of ibuprofen *in vitro*. The experiments were analyzed in terms of thermodynamic (partitioning) and kinetic (diffusivity) parameters. In accordance with the above studies, it was concluded that the effect of PG on the human epidermis had little effect on the drug diffusivity in the barrier but had a substantial influence on the partitioning from the vehicle into the SC (26). In other words, it seems that the predominant effect of the PG on the human skin is reflected in the increased solubility of lipophilic drugs in the PG-altered SC, but it has little effect, if any, on the drug diffusivity in this rate-limiting environment. These are a few examples illustrating that PG affects and modifies dermal permeation rates of drugs through intact skin.

No published studies to date have focused on the investigation of the PG effect on the MN-enhanced transdermal delivery of drugs. Since PG is ubiquitous in the area of topical formulation, understanding its influence on transport could provide some insights into formulation optimization for this new enhanced route of delivery. The present study is an evaluation of the effect of different PG-water donor solution compositions on the rate of delivery of NTX HCl through MN-treated skin.

MATERIALS AND METHODS

Chemicals

NTX hydrochloride was purchased from Mallinckrodt (St. Louis, MO, USA). Water was purified using NANOpure

Diamond™, Barnstead water filtration system. 1,2-Propanediol, Hanks' balanced salts modified powder and sodium bicarbonate were purchased from Sigma (St. Louis, MO). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), gentamicin sulfate, trifluroacetic acid (TFA), triethylamine (TEA), 1-heptane sulfonic acid sodium salt, and acetonitrile (ACN) were obtained from Fisher Scientific (Fairlawn, NJ). Reagent-grade chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Donor Solution Preparation

Adequate volumes of deionized water and PG (total 5 ml) were pipetted into a disposable plastic beaker and stirred magnetically until well mixed. Seven donor solution PG-water binary mixtures with the following compositions were prepared: 0–100, 10–90, 25–75, 40–60, 75–25, 90–10 and 0–100% (*v*/*v*). To each of these donor solutions NTX HCl was added to obtain the concentration of 110 mg/ml. The solutions were placed in glass vials, closed tightly with a plastic cap, sonicated for 15 min and left in the shaking water bath at 32°C overnight before use to ensure all the drug is dissolved.

NTX HCl Solubility

The apparent solubilities of NTX HCl in different composition PG-water binary mixtures were obtained by equilibrating excesses of NTX HCl with these mixtures. Each slurry was continuously shaken in a tightly closed glass vial in a shaking water bath at 32°C. Samples were taken at 24 h, 48 h, 72 h and 96 h, filtered using a 32°C prewarmed syringe and VWR syringe filter with a 0.2 μm membrane, and diluted with the adequate amount of acetonitrile-water 70:30 (*v*/*v*) to be analyzed by HPLC. Long equilibration time proved to be necessary for PG-rich donor solutions for which the attainment of equilibrium was slow. The solubility determination was repeated twice for each sample ($n=2$).

Viscosity Measurements

A Brookfield DV-III LV programmable cone/plate rheometer with a CPE-40 spindle was used for measuring viscosity of the donor solutions. The sample volume was 0.5 ml. All determinations were carried out at 32°C using a circulating water bath TC-102. Each donor viscosity measurement was taken three times at low, medium and high instrument torque value in the range of 10–100% ($n=3$). Results are reported as mean and standard deviation.

In Vitro Diffusion Studies

Full-thickness Yucatan minipig skin (approximately 1.8 mm thick) was harvested from the dorsal region of a euthanized 6-month-old animal. Animal studies were approved by the University of Kentucky IACUC. Subcutaneous fat from skin samples was removed with a scalpel and sparse hair shaved. Such skin samples were immediately placed in a plastic bag and frozen until use (−20°C). Before the permeation study, skin samples were allowed to thaw for about 30 min. Skin used for MN treatment was placed on a wafer of polydimethylsiloxane polymer, which mimicked the naturally occurring mechanical support of underlying tissue because of its comparable structural elasticity. MN which were obtained from Dr. Prausnitz's laboratory were fabricated by laser cutting of stainless steel sheets as reported previously (27). MN were solid metal, two-dimensional "in-plane" rows each containing five MN oriented with their axis parallel to the steel sheet. This design is characterized by the following MN dimensions: 750 μm long, 200 μm wide and 75 μm thick. The insertion of MN into skin was carried out manually by applying gentle finger pressure followed by their instantaneous removal. The diffusion area of skin (0.95 cm^2) was pierced 20 times with a row containing five MN (i.e., to make a total of 100 individual and non-overlapping piercings) before mounting the skin in the diffusion cell. If any damage to MN was observed, a new array was used. Untreated skin samples were placed directly into the diffusion cells. Three to four cells were used per donor solution composition per case (untreated or MN-treated skin) ($n=3$–4). A PermeGear flow-through (In-Line, Riegelsville, PA) diffusion cell system was used for the *in vitro* diffusion studies. Isotonic HEPES-buffered Hanks' balanced salts solution (pH 7.4) was used as the receiver solution. The flow rate of the receiver fluid was set at 1.5 ml/h to maintain sink conditions. Skin samples in the diffusion cells were kept at 32°C using a circulating water bath. The diffusion experiments were initiated by charging the donor compartment with 0.3 ml of the donor solution. Samples were collected from the receiver compartment in 6-h increments over 48 h. All samples were stored at 4°C until processed by HPLC. The permeation data were plotted as the cumulative amount of NTX HCl collected in the receiver compartment as a function of time. The flux value for a given run was calculated from Fick's first law of diffusion:

$$\frac{1}{A}\left(\frac{dM}{dt}\right) = J_{SS} = P^{*}\Delta C \tag{1}$$

where J_{ss} is the steady-state flux, M is the cumulative amount of NTX HCl permeating the skin [nmol], A is the area of the skin [0.95 cm^2], P is the effective permeability coefficient [cm h^{-1}], and ΔC is the concentration difference [nmol cm^{-3}] of the drug in donor and receiver solutions. Since sink conditions prevail under constant flow of the receiver solution, ΔC is well-approximated by the drug donor concentration alone.

Quantitative Analysis

Quantitative analysis of NTX HCl concentrations by HPLC was carried out using a modification of the assay described by Hussain *et al.* (28). The HPLC system consisted of a Waters 717 plus autosampler, a Waters 600 quaternary pump, and a Waters 2487 dual wavelength absorbance detector with Waters Empower™ software. A Brownlee (Wellesley, MA, USA) C-8 reversed phase Spheri-5 μm column (220× 4.6 mm) with a C-8 reversed phase guard column of the same type (15×3.2 mm) by Perkin Elmer® was used with the UV detector set at a wavelength of 215 nm or 278 nm. The mobile phase consisted of 70:30(*v*/*v*) ACN:(0.1% TFA with 0.065% 1-octane sulfonic acid sodium salt, adjusted to pH 3.0 with TEA aqueous phase). Samples were run at a flow rate of 1.5 ml/min with a run time of 4 min. The injection volume used was 100 μl. The retention time of NTX HCl was 2.3± 0.1 min. Samples were analyzed within a linear range of the NTX HCl standard curve from 100 to 10,000 ng/ml. The standard solutions exhibited excellent linearity over the entire concentration range employed in the assays.

Statistical Analysis

The statistical analysis of data was carried out with one-way ANOVA using SIGMA-STAT (SPSS, Inc., Chicago, IL, USA) software.

RESULTS AND DISCUSSION

Parameters in Classical Transdermal Transport

The description of percutaneous transport across intact skin usually comprises several assumptions about the nature of the barrier and the nature of the drug penetrating this barrier. Often, the barrier properties of the skin can be well-described by accounting for the barrier properties of SC alone. Despite its heterogeneous nature, the SC is frequently assumed to be a thin layer of skin that is lipoidal in nature. Current marketable drugs that are passively delivered through the skin into systemic circulation are uncharged, small and moderately lipophilic compounds (3). It is believed that these drugs traverse the SC mainly through continuous intercellular lipids, which becomes the rate-limiting step in transport across skin. Hence, the transdermal delivery of fairly lipophilic drugs through intact skin is usually described by the following equation:

$$J_{ISP} = P_{ISP} * \Delta C = K_{SC/veh} * \frac{D_{SC}}{h_{SC}} * \Delta C \quad (2)$$

where J_{ISP} is flux, P_{ISP} is permeability coefficient, $K_{SC/veh}$ is partition coefficient between SC lipid domain and vehicle, D_{SC} is the diffusivity of the drug in SC, h_{SC} is effective thickness of the SC, and ΔC is the drug concentration difference across SC. A good understanding of the above parameters and the role they play in transdermal delivery permits recognition of potential drug candidates and optimization of delivery systems.

Many studies have found that the partitioning between SC lipids and aqueous vehicle, although relatively hard to be determined directly, can be estimated by use of the octanol-water partition coefficient $K_{oct/w}$. The character of the octanol microenvironment, although isotropic in nature, was found to be similar to the microenvironment of anisotropic SC lipids, and they can be correlated through a linear free-energy relationship (29,30). On the other hand, the diffusivity of a drug molecule in SC lipids can be linked to its size through a free volume theory. Habitually, molecular volume is substituted with molecular weight for simplicity and without large loss of prediction accuracy. This relationship predicts a sharp drop in the drug diffusivity within the SC's lipid bilayers with increasing molecular size (31,32). Also, the effective thickness of the SC differs from the real SC thickness. The microstructure of the SC shows keratinocytes being surrounded by a continuous phase of lipid bilayers in a brick-and-mortar-like arrangement (33–35). The actual pathway along which a drug molecule diffuses using intercellular lipid domain across the SC is longer than just its thickness (33,36–39). Lastly, transdermal flux is affected by the concentration (more precisely, activity) gradient across the SC. When sink conditions are maintained on the receiver side of skin, then the concentration gradient is simply the drug concentration in the donor solution. Therefore, the upper limit of the transdermal delivery rate can be attained by using saturated donor solutions. Moreover, having the same drug saturated in different solvents corresponds to the same (maximum) activity of the drug in those solvents (20). Thus, transdermal flux values obtained from such donor solutions should be the same. This is the basis for a generalization that a certain percent solubility of a drug in different solvents will provide approximately the same percutaneous flux. This is expected to be true only if transport across skin is occurring through the intercellular lipid domain of SC and there is no effect of the solvent used on the barrier properties of SC. In general, parameters such as molecular weight below 500 Da, logP around 2 and low melting point are often evoked as favorable physicochemical characteristics of a drug intended for transdermal delivery (40–42).

Parameters in MN-Enhanced Transdermal Transport

Transdermal transport characteristics change dramatically when MN are used as an enhancement method. MN create transient aqueous channels through the upper skin layers,

thus providing a new route of transport. This new pathway bypasses the SC barrier and continues through microchannels directly into deeper layers of skin. Transport across microchannels, therefore, is not expected to be SC-limited. A diffusing drug molecule needs to traverse several layers in series before it reaches the receiver solution (*in vitro* experiments) or the microvasculature bed (*in vivo* experiments). Although this scenario is analogous to delivery across intact skin, one important difference needs to be taken into account. In intact skin, the diffusional resistance of the SC is typically orders of magnitude larger and overrides that coming from vehicle, epidermis and dermis (1,43,44). In the current study, MN-enhanced delivery was limited to the use of MN in skin pretreatment and subsequent application of the drug-containing formulation on the treated area resulting in zero-order drug delivery. Other applications of MN, such as hollow MN with drug, coating MN with drug, using self-dissolving drug-containing MN, etc., were not considered in this study. In the case of the microchannel pathway, the resistance from SC is eliminated and diffusional resistances from the microchannel and the dermis beneath the microchannel become important. Also, transport is not dependent on partitioning between two distinct hydrophilic and lipophilic phases. The relative contributions of individual layer resistances to the total resistance will determine a new rate-determining step in skin transport. The individual layer resistance depends on drug diffusivity in that layer as well as on its thickness. G. Flynn reported that drug diffusivities in viable tissue (epidermis and dermis) are typically no more than 10 times lower than in bulk water and are without great molecular selectivity (1), while Kretsos *et al.* calculated the free drug diffusivities in the viable tissue to be about 3.7-fold lower than their diffusivities in water (44). The thickness of the viable tissue used in *in vitro* diffusion experiments can be controlled by using either different length MN or different thicknesses of skin. In the present work, the thickness of the microchannel layer was about half the thickness of the underlying dermis layer (0.6 mm and 1.2 mm, respectively). Hence, it is anticipated that the dermis layer would present a higher resistance to a diffusing drug molecule as compared to the microchannel itself. Nevertheless, the difference between these two layers is not expected to be anywhere close to that between SC and viable tissue seen in delivery through untreated skin. Another crucial difference in transport via the microchannel pathway is the absence of a lipoidal layer that separates vehicle from viable tissue. By creating microchannels in the skin and bypassing the SC, an aqueous pathway is formed for transport which connects an aqueous vehicle through interstitial fluid to the aqueous milieu of viable tissue. As expected, the partition coefficients between aqueous vehicle and dermis for a variety of drugs are close to 1 (1 and authors unpublished data). Moreover, aqueous vehicles are miscible with interstitial fluid within the microchannels. These conditions sharply contrast those met in the delivery across intact skin. An equation that can describe the steady-state flux through the microchannel pathway is as follows:

$$J_{MCP} = P_{MCP} * \Delta C = \left(\frac{1}{R_{MC} + R_{VT}}\right) * \Delta C = \left(\frac{1}{\frac{h_{MC}}{D_{MC}} + \frac{h_{VT}}{D_{VT}}}\right) * \Delta C \tag{3}$$

where J_{MCP} is flux, D_{MC} is diffusivity of the drug in the microchannel, D_{VT} is diffusivity of the drug in the viable tissue, h_{MC} is thickness of the microchannel, h_{VT} is thickness of the underlying viable tissue, and ΔC is the drug concentration difference across skin. The terms in this equation no longer relate to SC as it is not involved in the transport. Instead, two individual resistance terms from the microchannel and the underlying viable tissue are included. One important realization is that, contrary to intact skin, transport through microchannels no longer revolves around SC-oriented optimization of the physicochemical parameters of a drug molecule. A logP value around 2 provided favorable drug partitioning into the lipid domain of the SC lipids, and a small molecular volume assured that diffusivity of the drug across SC would be sufficiently high to provide desired transdermal flux. These physicochemical parameters of the drug molecule seem to be no longer critical.

Drug diffusivity in the bulk solution doesn't follow the relationship described by the free volume theory used for SC. Rather, it obeys the Stokes-Einstein equation:

$$D = \frac{kT}{6\pi\eta r} \tag{4}$$

where k is the Boltzmann constant, T is the absolute temperature, η is the viscosity and r is the hydrodynamic radius of the solute. Although this equation predicts the decrease of diffusivity with increasing molecular size of the drug molecule, the magnitude of this effect is much smaller. It has been shown by McAllister *et al.* that MN-enhanced transdermal flux values for a variety of compounds of different sizes correlated well with prediction based on the Stokes-Einstein Eq. 5. It suggests that diffusivity of drug molecules in the microchannels will resemble that in the bulk solution rather than in the SC lipid bilayers. The thicknesses of layers that a diffusing molecule has to traverse in series in an *in vitro* diffusion experiment depend on several factors. In contrast to the SC, the effective thicknesses of the microchannel layer and underlying dermis layer are expected to resemble closely their real thicknesses. Dermis, for the purpose of diffusion studies, is a highly aqueous, gel-like phase with a meshwork of collagen fibers surrounded by a proteoglycan matrix. Molecular motion in this milieu is not restricted to a large extent, and

drug molecules can diffuse relatively unhindered along the concentration gradient.

Simple Model

Upon piercing the skin with MN, a new route of delivery is created. This new microchannel pathway is in parallel with the intact skin pathway, which was originally the only route available before the MN treatment. Assuming independence of these two routes, percutaneous flux can be described according to this equation:

$$J_{TOT} = J_{MCP} + J_{ISP} = (f_{MCP} * P_{MCP} + f_{ISP} * P_{ISP})\Delta C \quad (5)$$

where J_{TOT} is the total flux through MN-treated skin, J_{MCP} is the microchannel pathway flux, J_{ISP} is the intact skin pathway flux, f_{MCP} is the fractional area of the microchannel pathway, P_{MCP} is the apparent permeability coefficient of the microchannel pathway, f_{ISP} is the fractional area of the intact skin pathway, P_{ISP} is the apparent permeability coefficient of the intact skin pathway and ΔC is the drug concentration difference across skin. In other words, the flux values coming from two independent parallel routes are additive. The relative importance of each pathway for a given drug molecule depends on that pathway's distinct features, reflected in the respective permeability coefficient and the fractional area of skin each pathway covers. A MN treatment produces numerous microchannels; however, the fractional area occupied by them is typically very low. For example, McAllister *et al.* estimated that in their *in vitro* experimental set-up the fractional area of microchannel pathway was on the order of 0.001 or 0.1% (5). Consequently, the fractional area of intact skin pathway decreases only by 0.1% to 99.9% of the original value. This allows a simplification to be introduced. Since $f_{ISP} \approx 1$ (100%), the $f_{ISP} * P_{ISP}$ term can be well-approximated by the value of P_{ISP} alone. Additionally, from a practical point of view, the exact knowledge of f_{MCP} is not necessary as long as it remains very small.

In the experiments involving MN, it is sometimes assumed that intact skin is impermeable to drug, and flux is ascribed to the microchannel pathway exclusively. This premise may or may not be sound depending on the drug being used and particular experimental conditions. In the present work, this assumption was not made. Additional experiments with untreated skin were carried out to determine the value of J_{ISP} (P_{ISP}) independently, which allowed calculation of J_{MCP} ($f_{MCP} * P_{MCP}$) from J_{TOT} (P_{TOT}).

MN-enhanced transport permits the use of ionized drug molecules and increases the maximum achievable driving force for diffusion through the use of highly water-soluble, charged molecules. The question whether charged molecules can permeate through SC becomes important because the majority of skin surface area after MN treatment is still intact SC, and this pathway may contribute to the total flux. The area of transdermal research dealing with transport of charged molecules across SC is not extensively developed and less understood. There are reports that indicate that the transport of small charged molecules is possible through a polar route in SC (45,46). Transport via this polar route is not expected to obey the relationships discussed earlier for classical transdermal delivery. In most cases, the treatment of ionic equilibria in percutaneous transport evokes the pH-partition theory which states that the neutral form of a drug molecule traverses membranes far more readily than its ionized counterpart (31). Therefore, the correction introduced to solutions containing partly ionized drug is to multiply the total drug concentration by the fraction of uncharged drug. While this approach works, it is important to realize that although the SC permeability of charged species is low, their solubility might be much higher. Consequently, while working with saturated donor solutions at different pHs, the experimental results may be somewhat counterintuitive as evoked by Hadgraft showing higher transdermal flux of ibuprofen in its charged form through intact skin as compared to its uncharged form. These results suggest that, in this particular case, lower permeability of the charged species is more than compensated for by its increased solubility (47). Another relevant question is how the diffusivity of a molecule in bulk solution changes as its charge varies. It has been shown that the ionization of molecules typically doesn't change their diffusivity much, if at all (43). In the case of small molecules, the diffusivity of charged species may drop slightly due to solvation effects and the resulting increase in the hydrodynamic radius. Those effects are not expected to play an important role with regard to larger molecules. Therefore, as long as the microchannel environment can be properly described by bulk solvent properties, one would expect that diffusional resistance of the microchannel should be similar for both charged and uncharged species.

In Vitro Experiment

The aim of this study was to investigate the influence of PG-water donor solution composition on the MN-enhanced transdermal flux of naltrexone hydrochloride *in vitro*. In order to do so, a series of seven donor solutions containing equal concentrations of NTX HCl 110 mg/ml were prepared. This subsaturation concentration was chosen to avoid potential problems related with the use of saturated solutions with excess solid, such as obstruction of microchannel openings or deposition of solid drug into the microchannels. These donor solutions consisted of binary mixtures of PG and water. One set of *in vitro* experiments aimed at evaluating transdermal NTX HCl flux with the use of MN; the other employed untreated (no MN) skin.

Naltrexone pK_a values at 32°C calculated with the help of the Van't Hoff's equation are 8.20 and 9.63 (48). The first pK_a corresponds to the dissociation of the protonated aliphatic nitrogen proton and the second to the dissociation of the phenolic proton. The pH of a 110 mg/ml aqueous solution of NTX HCl was found to be 4.95. Hence, in this solution NTX is expected to be positively charged.

Fig. 1 shows a representative diffusion profile of NTX HCl through MN-treated skin and untreated skin. The steady state is reached quickly for MN-treated skin but less rapidly for untreated skin. This is consistent with a previous report which showed decreased lag times upon pretreatment with MN (7). Transdermal flux values were obtained from the slope of the linear, terminal portion of the graph. Individual flux values obtained from each donor solution composition were collected and plotted against the increasing concentration of PG in the donor solution. Fig. 2 shows the summary of data derived from two sets of experiments. The first set of experiments evaluated the permeation of NTX HCl through MN-enhanced skin. Transdermal flux values varied greatly with the highest flux of 30.9±4.4 μg cm^{-2} h^{-1} obtained from a pure aqueous solution and the lowest flux 0.8±0.3 μg cm^{-2} h^{-1} corresponding to a pure PG solution. The gradual drop in the transdermal flux was a non-linear function of the PG content in the donor solution with the greatest effect seen in water-rich solutions. The magnitude of the flux difference obtained from MN-treated skin is as much as ≈40 fold and indicates how great the influence of the formulation on this route of delivery can be. Additionally, the same set of experiments was repeated with the intact, non-MN-treated skin. The results revealed that for all donor solutions tested the flux values were much lower, ranging from 0.3±0.02 to 3.4±1.0 μg cm^{-2} h^{-1}. Initially, the increase in the PG content in the donor solution resulted in a slight rise in the flux, but between 10% and 40% PG-water (*v*/*v*) the flux values leveled off and finally dropped from PG-rich solutions. The maximum flux was measured from a 25% (*v*/*v*) PG-water donor solution. Although there is a qualitative trend in the data showing that water-rich donor solutions provide higher flux over PG-rich donor solutions, this trend doesn't mimic the one seen with MN-treated skin. Hence, the effect of donor solution composition is different and had a much more pronounced effect on MN-treated skin as compared to the untreated skin.

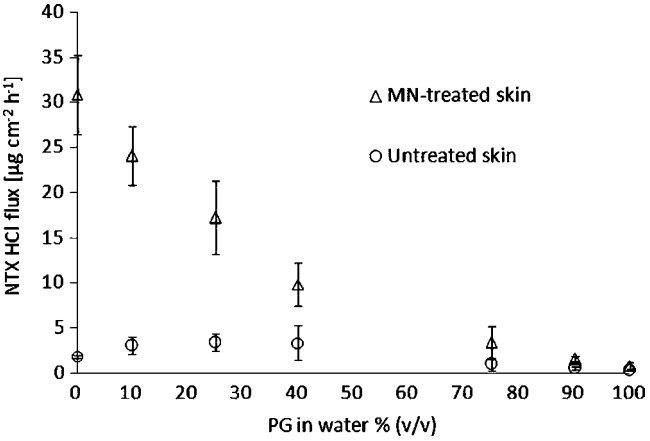

Fig. 2 Steady-state flux values of NTX HCl permeating through untreated and MN-treated skin as a function of the donor solution composition. The concentration of NTX HCl is constant 110 mg/ml for all donors, while the amount of PG increases to the right of the graph ($n = 3$–4).

The same data can be presented in terms of the flux enhancement calculated as the flux through MN-treated skin over that through untreated skin. Fig. 3 shows such enhancement as a function of the donor solution composition. Clearly, the enhancement obtained by piercing the skin with MN is not the same for all formulations and equals ≈17 for the pure aqueous donor but drops to only ≈3 for PG-rich donors. Therefore, it seems that the flux enhancement, although providing a quick measure of the efficacy of skin MN-treatment, may be of limited practical use as it varies with formulation.

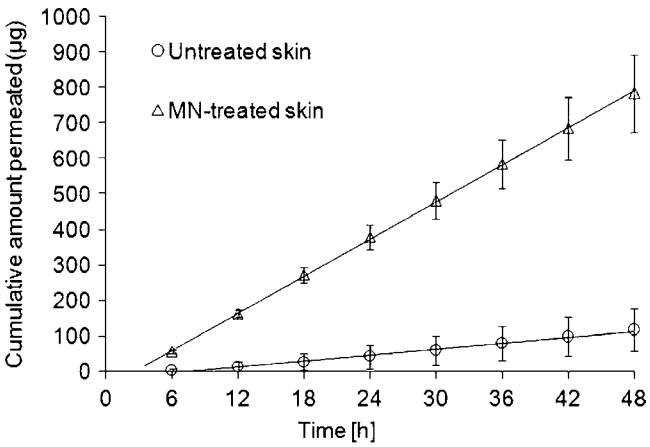

Fig. 1 Representative diffusion profile of NTX HCl through MN-treated and intact skin (PG-water 25:75 *v*/*v*) ($n = 3$).

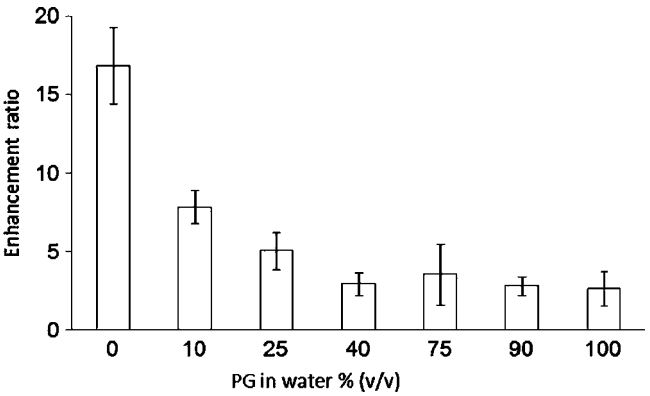

Fig. 3 NTX HCl flux enhancement as a result of MN-treatment of the skin ($n = 3$–4).

Table I Viscosities of NTX HCl (110 mg/ml) in PG-water Binary Mixtures ($n = 3$)

NTX HCl 110mg/ml in PG-water %(*v/v*)	0–100	10–90	25–75	40–60	75–25	90–10	100–0
Viscosity [cP] ± SD	1.03 ± 0.01	1.48 ± 0.03	2.47 ± 0.01	4.01 ± 0.01	14.40 ± 0.00	28.43 ± 0.12	46.33 ± 1.01

In order to examine in more detail the possible reason for seeing a non-linear rise in the MN-enhanced flux with increasing donor solution water content, the viscosities of PG-water binary mixtures were measured and are presented in Table I. The donor solutions' viscosities seem to be inversely related to the flux obtained from experiments with MN-enhanced skin. Such a relationship may be described by the Stokes-Einstein equation (Eq. 4). To better visualize the interrelation between flux and donor viscosity, the original data is plotted as NTX HCl flux *versus* its calculated diffusivity in the donor solution. The diffusivity of NTX in water was calculated using the Stokes-Einstein equation using an experimentally determined viscosity value of 1.03 cP and assuming the hydrodynamic molecular radius is 4.6 Å yielding a value of 4.7 10^{-6} cm^2 s^{-1} (49). Other diffusivity values of NTX in PG-containing donors were obtained by employing the experimentally determined viscosity of the relevant donor solution. In Fig. 4, two data sets correspond to the experiments performed with MN-enhanced and untreated skin, and the third set results from the treatment of data according to Eq. 5. The experiments carried out with MN-treated skin provided transdermal flux corresponding to J_{MCP} and J_{ISP}, while those with untreated skin produced flux corresponding to J_{ISP} alone. By simple subtraction, the value of the microchannel pathway flux is obtained. It is worth noting that in Fig. 4 the data points corresponding to PG-rich solutions are situated to the left of the graph.

It can be noted that J_{ISP} doesn't show linear dependence on diffusivity of the donor solution. This is not surprising, as in the presence of SC one would expect that it will provide a major diffusional resistance. If the drug molecule traverses SC by partitioning into intercellular lipids and diffusing along its tortuous route, then in the ideal case, the transdermal flux should correlate with percent saturation of the donor solution. The apparent solubility of NTX HCl in donor solutions is summarized in Table II. There is a qualitative agreement between percent saturation in donor solution and observed flux. Furthermore, PG is known to be a weak permeation enhancer and may affect SC characteristics, adding additional complexity to the interpretation of data from intact skin experiments.

On the other hand, J_{MCP} correlates with diffusivity of the donor solution. The fact that a very good linear correlation ($R^2 = 0.99$) was observed for microchannel flux is interesting because it indicates that although the diffusivity calculations in Fig. 4 account for the changes of the donor solution exclusively, it seems that they reflect drug diffusivity changes in the whole microchannel pathway. A possible explanation for this is that PG diffuses out of donor solution into microchannels and dermis, affecting their diffusional properties. Initially, a low-resistance, water-based milieu of microchannels and viable tissue would be affected by the presence of PG, which is expected to increase the diffusional resistance of those layers. If, again, this effect would be similar to that which would be expected in the bulk solution (and can be described by Eq. 4), then this correlation would not be surprising. Considering the above, it seems that it is not reasonable to assume that by varying the propylene glycol-water composition of the donor solution, only the properties of the vehicle and microchannel but not dermis are affected. In such case, in water-rich solutions, transport through the microchannel pathway would likely be controlled by diffusion through viable tissue underlying the microchannel. Therefore, transdermal flux would not be expected to correlate with viscosity of the donor solution until the viscosity of the donor solution would be high enough to produce microchannel resistance that would overcome the resistance of the underlying dermis. The resulting profile in Fig. 2 should, in consequence, reveal insensitivity of transdermal flux to the changes in composition of water-rich donors, but their dependence on the donor composition in the PG-rich region. The profile obtained in the current study clearly showed that this is not the case. Conversely, the observed profile can be explained by the influence of propylene glycol on the diffusional properties of both microchannel

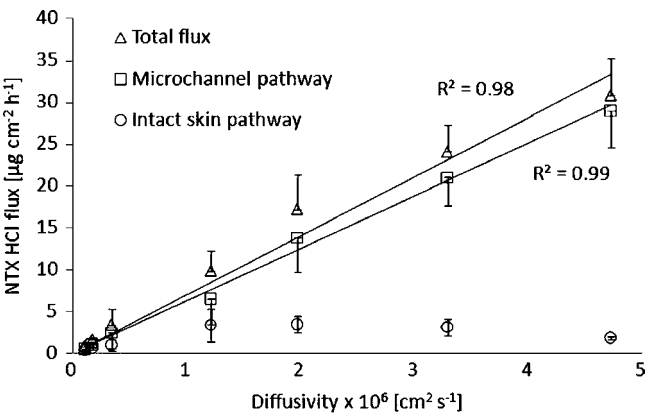

Fig. 4 Steady-state flux values of NTX HCl for intact skin pathway, microchannel pathway, and the sum of both plotted against calculated diffusivity of NTX in the donor solution. Low diffusivity on the left of the graph corresponds to the PG-rich donors ($n = 3$–4).

Table II Apparent NTX HCl Solubility and Corresponding Viscosity in Binary Mixtures of PG-water ($n=2$ for Solubility Determinations and $n=3$ for Viscosity Determinations)

PG-water %(*v/v*)	0–100	10–90	25–75	40–60	75–25	90–10	100–0
NTX HCl solubility [mg/ml] ± SD	121 ± 1	148 ± 24	159 ± 1	189 ± 22	222 ± 12	301 ± 3	130 ± 5
Viscosity [cP] ± SD	1.09 ± 0.00	1.70 ± 0.02	2.94 ± 0.01	5.20 ± 0.01	20.97 ± 0.06	61.00 ± 0.53	51.57 ± 0.25

and underlying dermis through the relationship predicted by the Stokes-Einstein equation.

The J_{TOT} obtained from MN-treated skin experiments also seems to follow the linear correlation with NTX donor solution diffusivity calculated from viscosity determinations ($R^2=0.98$). The likely reason for this is that the contribution of J_{ISP} to the J_{TOT} in water-rich donors is minor. As shown in Fig. 5, the contribution of J_{ISP} to the total flux is of most importance in the PG-rich donors, where it accounts for around 40% of J_{TOT} but diminishes to less than 10% in water-rich donors. Fig. 4 obscures the region corresponding to the PG-rich solutions which could reveal difference between behavior of J_{MCP} and J_{TOT} as their values diverge in this area. In order to have a closer look at the difference between J_{TOT} and J_{MCP}, the original data set was transformed by normalization of transdermal flux on the basis of viscosity. Since it was observed that viscosity of donor solutions seems to affect transdermal flux in MN-treated skin in accordance to the Stokes-Einstein equation, viscosity influence on flux can be cancelled out by multiplying the observed fluxes by individual viscosities of donor solutions according to this equation:

$$J' = J^{*}\eta \tag{6}$$

where $\mathcal{J}$ is the viscosity-normalized flux, $\mathcal{J}$ is the flux and η is the viscosity of the donor solution. Such viscosity-normalized flux values are presented in Fig. 6. The viscosity-normalized flux values correspond to a donor viscosity of 1 cP, which is close to the value obtained for aqueous solutions. It can be noted that the viscosity-normalized flux values remain relatively constant both for the microchannel pathway alone and for the microchannel and intact skin pathways combined (MN-treated skin total flux) irrespective of the donor composition used. Statistical analysis (ANOVA) revealed no statistically significant difference between viscosity-normalized flux values within either of the two data sets at $\alpha=0.05$. Because of large uncertainty in viscosity-normalized flux values in PG-rich donor solutions, it is impossible to discriminate between $\mathcal{J}_{TOT}$ and $\mathcal{J}_{MCP}$. Nevertheless, Fig. 6 suggests that while $\mathcal{J}_{MCP}$ flux shows no trend as donor solution composition is varied, the $\mathcal{J}_{TOT}$ shows a slightly increasing trend with increasing PG content in the donors. This observation is in accordance with the expectation that only the microchannel pathway resistance closely correlates with donor solution composition. Total flux, besides $\mathcal{J}_{MCP}$, also incorporates $\mathcal{J}_{ISP}$, which doesn't depend greatly on the viscosity of the donor solution. Overall, the behavior of viscosity-normalized $\mathcal{J}_{MCP}$ indicates that accounting for viscosity of the donor solution can very well predict the microchannel pathway transdermal flux. It is important to note that in the experimental set-up used in the present work, the concentration of NTX HCl in all donor solutions was maintained at a constant value, and therefore the relationship shown here between flux and viscosity is exactly the same for corresponding permeability coefficients and viscosity.

Since from the practical point of view one is often interested in the maximum flux attainable, it is interesting to look at the apparent solubility of NTX HCl in different

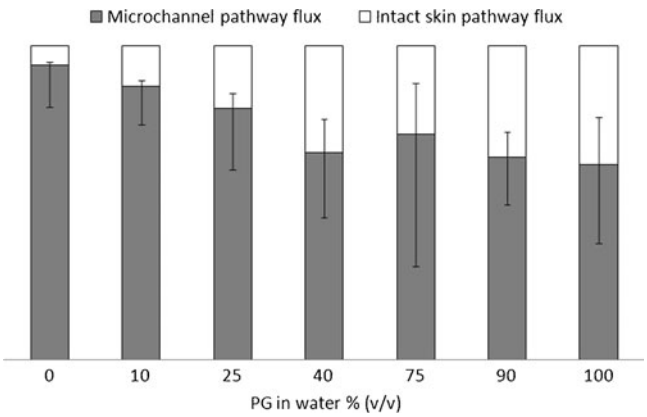

Fig. 5 Relative contribution of microchannel pathway flux and intact skin pathway flux to the total transdermal flux of NTX HCl ($n=3$–4).

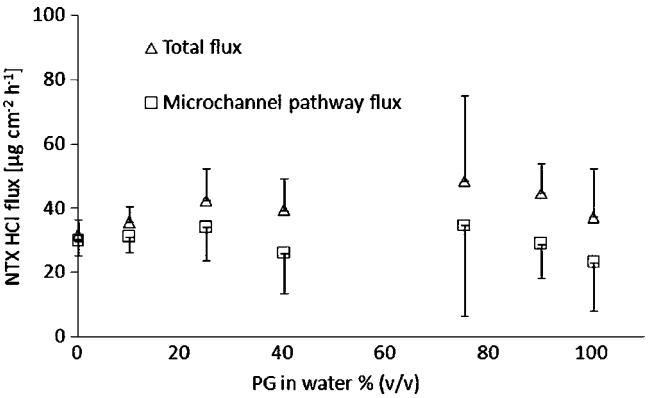

Fig. 6 Viscosity-normalized flux of NTX HCl through MN-treated skin ($n=3$–4).

donor solutions and their viscosities. As reported in Table II, the addition of PG to an aqueous donor gradually increases NTX HCl solubility up to the donor solution composition of PG-water 90-10 after which a sharp drop is observed. Simultaneously, higher NTX HCl concentration causes elevated viscosity of the donors. The data show that increased solubility is more than compensated for by elevated viscosity, and therefore saturated and PG-containing solutions of NTX HCl do not deliver the drug at higher rates through MN-treated skin as compared to purely aqueous, saturated donor solution (data not shown).

Overall, it is possible that factors other than the viscosity of the donor solution can influence MN-enhanced percutaneous flux. One example is the case of drugs with lipid-binding and protein-binding potential in dermis. Low binding to immobile cell-membrane dermal lipids as well as low-mobility albumin would be expected to elongate lag times, but not to affect steady-state flux values. However, in the case of drugs extensivly bound to albumin, the diffusivity of drug-albumin complex would need to be considered and could translate into lower-than-expected permeation rates. Other parameters of interest include, but are not limited to, the osmotic pressure gradient or the pH gradient present within the skin during diffusion experiments. These gradients can add more complexity to the analysis of the data by the potential creation of convectional flux. In this work, all the flux values were analyzed solely in terms of diffusion. At present, no alternative hypothesis was tested to investigate the influence of other factors.

CONCLUSIONS

In conclusion, this work investigated the influence of donor solution composition on the rate of transdermal transport of NTX HCl through MN-enhanced skin. It was found that flux correlated well with changes in drug diffusivity caused by donor viscosity variations. Also, it is likely that the microchannel pathway flux rather than total flux (combined microchannel pathway and intact skin pathway flux) correlates with viscosity changes of donor solution through the Stokes-Einstein equation. Moreover, this work indicates that percutaneous flux of charged species across intact skin may become important when large skin area is exposed to the drug formulation. In the broader context, the findings of this work may help improve the design of delivery systems for MN-enhanced skin. In classical percutaneous delivery, the vehicle plays a critical role in the appearance and delivery rates of a drug. Excipients impart the desired rheological character to the dosage form. Also, they determine physical properties of the vehicle and its ability to modify stratum corneum, the rate-limiting barrier to transport of most drugs. PG, PEGs (polyethylene glycol), and other viscous cosolvents are common excipients used in topical dosage forms. Changes in the physicochemical characteristics may promote drug partitioning from a topically applied formulation into the barrier as well as diffusion of drug molecules through the barrier. Based on the results from current work, it seems reasonable to state that when maximum flux through MN-treated skin is sought, then the viscosity of the formulation should be kept as low as possible.

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