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ORIGINAL ARTICLE

The effect of permeation enhancers on the viscosity and the release profile of transdermal hydroxypropyl methylcellulose gel formulations containing diltiazem HCl

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Abstract

Background and Method: The objective of this study was to characterize the release of Diltiazem HCl (DTM HCl) from hydroxypropyl methylcellulose gels containing the following permeation enhancers at a 0.5% (w/w): sodium lauryl sulfate, dimethyl sulfoxide (DMSO), polysorbate 80, propylene glycol, *N*-methylpyrrolidone (NMP), fatty acids (oleic acid, caprylic acid, and myristic acid), and isopropyl myristate (IPM). The enhancers' effects on the gel's viscosity were also investigated. *Results*: The novel findings of this study were the following: (i) polysorbate 80 was used for the first time as an enhancer with a hydrophilic compound in a hydrophilic carrier and it rendered the highest permeation flux (57.1 ± 0.9 μg/cm^2/h) compared with the rest of the enhancers, (ii) myristic acid (a 14-carbon-chain fatty acid) rendered the highest permeation flux (18.4 ± 0.49 μg/cm^2/h) among all fatty acids because of a decrease in the gel's viscosity, (iii) NMP (46.5 ± 0.7 μg/cm^2/h) and IPM (15.3 ± 0.41 μg/cm^2/h) increased the permeation flux from the second day onward. Both enhancers increased the gel's viscosity, (iv) sodium lauryl sulfate decreased the viscosity of the gel and the drug's permeation flux (8.1 ± 0.21 μg/cm^2/h) because of its binding with the drug, (v) propylene glycol decreased the permeation flux (10.2 ± 0.32 μg/cm^2/h) by increasing the gel viscosity, and (vi) DMSO increased the permeation flux (13.8 ± 0.4 μg/cm^2/h) without altering the viscosity. *Conclusion*: These findings indicate that to formulate DTM HCl into a hydroxypropyl methylcellulose gel the enhancers of choice should be polysorbate 80, myristic acid, DMSO, NMP, and IPM or combinations thereof.

Key words: *Diltiazem HCl; drug binding; gel formulation; permeation enhancers; transdermal delivery; viscosity*

Introduction

Diltiazem (DTM) is an optically active compound; the (+)-*cis* DTM with configuration (2*S*, 3*S*) is used in therapy for the treatment of high blood pressure, specific types of angina (i.e., chest pain), and occasionally arrhythmias and migraines. DTM is a calcium antagonist that acts by relaxing the blood vessels and improves the blood flow[1].

The importance of the transdermal delivery of DTM HCl rests on the fact that the oral delivery of DTM HCl presents many challenges[2]. First, since DTM HCl has a very short half-life (3–4.5 hours) it needs to be administered often and hence very strong compliance from the patients is required during therapy[3]. Also, DTM HCl is subject to an extensive first-pass metabolism, resulting in an absolute bioavailability of about 40%[3,4]; transdermal delivery overcomes these obstacles. The ease of use and the minimized side effects are also some of the advantages of transdermal delivery[5]. Additionally, the application of a patch or a gel does not involve disruption of the skin and is therefore painless.

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The skin is a particularly efficient barrier protecting the body from excessive water loss as well as xenobiotics' ingress[6,7]. The stratum corneum (SC) layer, the outer layer of the skin, is the main barrier and the hardest layer to permeate and therefore is considered the rate-controlling barrier for the diffusion of any substance[8,9]. SC is composed of dead, flattened cells (corneocytes) surrounded by a complex mixture of intercellular lipids; the corneocytes contain the protein keratin the main role of which is to keep the skin hydrated[6]. On an average, the SC is comprised of 10–15 layers of dead cells and is approximately 10 μm thick although this number can vary considerably depending on the area of the body[9].

In this study, DTM HCl was formulated for the first time in a gel for the purpose of transdermal delivery with the use of nonionic hydroxypropyl methylcellulose (HPMC) polymer. The goals of the study were the following: (i) to evaluate the potential for transdermal delivery of DTM HCl through this formulation, (ii) to understand the effect certain enhancers have on the transdermal delivery of DTM HCl and to identify the ones with the optimum results, (iii) to test whether any enhancers interact with the drug, (iv) to record any changes affecting the viscosity of the gel upon the addition of the enhancers in it.

To facilitate and expedite permeation through the skin, several enhancers were tested for their potential to improve the release profile of DTM HCl from the HPMC gel. The following enhancers were added at a 0.5% (w/w): sodium lauryl sulfate (SLS), Dimethyl sulfoxide (DMSO), oleic acid, caprylic acid, myristic acid, polysorbate 80 (or Tween 80), propylene glycol (PG), isopropyl myristate (IPM), and *N*-methylpyrrolidone (NMP).

Viscosity studies were also conducted. For every enhancer included in this study the viscosity of the gel was measured. This was done to evaluate whether the enhancers had any possible effects on the viscosity of the gel; any change on the viscosity of the gel might have a direct effect on the release profile of the drug.

Physicochemical as well as solubility studies were conducted. The partition, permeability, and diffusion coefficients, as well as the lag time and log *P* were calculated.

Materials and methods

Materials

The quality of all the chemicals used in this experiment was of analytical grade or higher. DTM HCl was purchased from Haauri Pharmaceuticals Inc. (Edison, NJ, USA), HPMC was purchased from Dow Chemical Company (Midland, MI, USA), *n*-octanol was purchased from Spectrum Chemical Company (Gardena, CA, USA), cellulose membrane [Spectra/Por Dialysis Membrane, Molecular Weight cutoff (MWCO): 1,000 Da, wet in 0.1% preservative sodium azide, flat width: 38 mm, diameter: 24 mm] was purchased from Spectrum Laboratories Inc. (Irving, TX, USA). SLS, caprylic acid, myristic acid, isopropyl myristate, DMSO, oleic acid, and NMP were purchased from Sigma Chemical Company (St. Louis, MO, USA). Polysorbate 80 and propylene glycol were purchased from VWR (West Chester, PA, USA). Human cadaver skin was purchased from the NY Firefighter Skin Bank (New York, NY, USA). High-performance liquid chromatography (HPLC) grade water was purchased from EM Science (Gibbstown, NJ, USA). HPLC grade Acetonitrile and phosphoric acid were purchased from J.T. Baker (Philipsburg, NJ, USA).

Quantification of diltiazem HCl

DTM HCl was quantified by validated UV–Vis spectrophotometry and HPLC methods. In the case of UV–Vis spectrophotometry, the instrument used was Carry 50 scan (Analytical Instruments, Varian, Palo Alto, CA, USA). One diffusion cell was used in each experiment as a blank (no drug was contained in the gel). The collected samples were filtered before being assayed (using syringe micro-filters Acrodisc, 0.45 μm; Pall, Ann Arbor, MI, USA) in order to remove any possible skin tissue traces that might have been released in the media. The standard curve was obtained by plotting the known concentration of DTM HCl solutions in phosphate buffer saline (PBS) against the absorbance; the linear equation obtained was: $y = 33.891^{*} x - 0.0084$ with an $R^2 = 0.99982$. Good linearity was obtained at a 250 nm wavelength between 6 and 34.4 μg/mL ($R^2 = 0.9992$). The intra- and inter-day precision values were <1.78% for all samples analyzed. The accuracy, determined from recovery studies, was between 98.5% and 101.3%.

HPLC analysis was also performed (in conjunction with UV–Vis spectroscopy studies) using a modified method[10]. The HPLC system consisted of a Waters 717 plus Autosampler, SPD-10AA Shimadzu UV-Vis detector, Shimadzu HPLC Pump Model LC-10AD, Perkin Elmer Nelson 900 series Interface, and the data handling system Perkin Elmer Software TotalChrom® navigator (PE Nelson—Version 6.2.1). Separation was achieved on a C18 column (Hypersil ODS, 150 × 4.6 mm, 5 μm particle size) eluted with a mobile phase consisting of Acetonitrile and 6.25 mM pH 3.0 phosphate buffer (40:60) and delivered at a flow rate of 0.75 mL/min. The detection wavelength was 254 nm. The Autosampler's temperature was maintained at 25°C. The injection volume was 50 μL. The retention time was 5.8 minutes. Peak area was used to determine DTM HCl concentration. Calibration curves were linear in the

range 0.1–100 μg/mL (R^2 > 0.99). The CV (%) for inter-day assays [lower limit of quantification (LLOQ)] at 0.100 and 100 μg/mL were 2.6 and 0.57, respectively.

Permeation studies

Permeation studies were carried out in vitro with modified jacketed Franz diffusion cells[11]. The system was maintained at 37°C by means of a water bath circulator[12–15]. The radius of the cells was 15 mm, the effective diffusion area was 7.07 cm^2 (~7cm^2) and the receptor volume was 13 mL. The receptor was filled with PBS of pH 7.4 and stirred to prevent any diffusion-layer effects. The amount of DTM HCl was quantified by collecting 0.5 mL at designated time intervals[16]. The volume of the receptor fluid withdrawn was replaced each time with PBS. The concentration of the samples was assayed with UV–Vis Spectrophotometer and HPLC as previously described. Semi-synthetic cellulose membrane [molecular weight (MW) cutoff: 1000 Da][17] and human cadaver skin (epidermis layer of average thickness 0.45 mm) were placed between the donor and the receptor compartment as diffusion barriers[18].

The studies with cellulose membrane were conducted in order to determine whether any interactions were taking place between the drug and the enhancers. It is easier to assess this possibility with cellulose membrane studies than with human cadaver skin because in the case of human cadaver skin the enhancers may interact with the SC layer. In vitro human cadaver skin studies were also performed for the assessment of each enhancer's ability to improve the flux of DTM HCl.

The cellulose membrane was prepared by soaking it for one hour in distilled water in order to remove any traces of preservative contained in the membrane. The human cadaver skin used for the experiments came from the dorsal portion. Prior to the experiments, the skin was maintained at a temperature of –80°C. The pieces of skin were thawed overnight at 4°C and then soaked in PBS (pH 7.4) at a temperature of 37°C for 5 minutes. The subcutaneous tissue was surgically removed and the dermis was wiped with isopropyl alcohol to remove residual adhering fat. The epidermis was separated from the dermis with the help of a scalpel and tweezers after treatment with 2 M sodium bromide solution in water for 8 hours[19,20]. All the skin pieces were carefully inspected with a magnifying glass before being used to ensure that no damage had occurred during the separation.

Preparation of gels and modified phosphate buffer saline buffer

For the preparation of 100 mL of the nonionic hydrophilic polymer HPMC gel, four steps were followed: (i) Initially the drug was dissolved in 30 mL of water, (ii) In a separate beaker, 0.5 g of HPMC were added to 50 mL of water at 80°C and stirred. When the mixture was uniform, 20 mL of water at ambient temperature was added to it to rapidly reduce the temperature at 60°C, (iii) Then, the 30 mL solution of DTM HCl was added to the gel and the temperature was maintained at 52–58°C, (iv) Finally, the enhancers were added at a concentration of 0.5% (w/w) of the solids' content.

It was very important for this study to get comparable results for all the enhancers by using them all at the same percentage and to evaluate their effects without anyone of them damaging the skin. For that reason all the enhancers were used at a very low percentage because some of the substances may damage the skin at higher concentrations. For example, for DMSO the most effective concentration is 60% (v/v), but at this concentration severe skin reactions have been observed[21,22].

Another parameter taken into account for this study was the possibility of micelles formation, which could significantly affect the release profile of the drug. In order to eliminate such possibility all the enhancers were added at a concentration at least ten times lower than their reported Critical Micelle Concentration (CMC)[23–25]. Therefore, neither one of the enhancers could form micelles at the added concentration.

A modified PBS solution (pH 7.4) was used as the receptor medium. When PBS was prepared according to the USP, DTM HCl reacted with NaOH and a precipitate was observed. The modified PBS contained monobasic potassium phosphate and dibasic potassium phosphate, which were added in a solution of sodium chloride in water. The solubility (S_{PBS}) of DTM HCl in the modified PBS solution at pH 7.4 was experimentally determined to be 349.9 mg/mL.

The procedure that was followed in order to decide the composition of the modified buffer was as follows:

As is well known, a 0.9% (w/v) normal saline solution contains 0.16 mol of NaCl. This parameter must be taken into account when preparing an isotonic buffer. The phosphoric acid has three p*K*a values: 2.15, 6.86, and 12.32[26]. For the preparation of pH 7.4 PBS, the proportions of monosodium phosphate and its conjugate base, that is, disodium phosphate, can be calculated from the Henderson–Hasselbach equation. The p*K*a will be 6.86 since it is the one closest to the desired pH value:

$$\begin{aligned} \mathrm{pH} &= \mathrm{p}K\mathrm{a} + \log([\mathrm{Base}]/[\mathrm{Acid}]) \\ &\rightarrow ([\mathrm{Base}]/[\mathrm{Acid}]) = 3.467. \end{aligned} \quad (1)$$

The MW and NaCl equivalent weight (E) values[27] for KH_2PO_4 and K_2HPO_4 are:

KH_2PO_4 : MW
=136.07 g/mol, E value: 0.05916503 mEq/L.

K_2HPO_4 : MW
=174.168 g/mol, E value: 0.08374159 mEq/L.

For NaCl the MW = 58.443g/mol, mass of NaCl equivalent to the required 0.16 moles is

$$m = n \times \text{MW} = 9.35088 \text{ g}.$$

For the preparation of 0.1M buffer→ [Base] + [Acid] = 0.1 and from Equation (1)

KH_2PO_4 :[Acid] = 0.0229 moles = 3.116 g and K_2HPO_4 :[Base] = 0.0711 moles = 12.366 g.

After multiplication with the E values, the equivalent-to-NaCl gram for each substance is:

KH_2PO_4 : 0.18435 g equivalent to NaCl and K_2HPO_4 : 1.0355 g equivalent to NaCl

Therefore, in order to have an isotonic solution, we need additional NaCl calculated as shown below:

9.35088 g, NaCl needed − 0.18435 g KH_2PO_4 equivalent to NaCl − 1.124482 g K_2HPO_4 equivalent to NaCl = 8.131 g additional NaCl needed.

So, for the preparation of 1L of modified phosphate buffer we will need:

KH_2PO_4 : 3.116 g, K_2HPO_4 : 12.366 g, NaCl = 8.131 g.

The pH was verified with a Fisher Scientific Accumet pH-meter 10 (Pittsburgh, PA, USA).

Determination of partition coefficient

The partition coefficient was determined for both water and PBS as a solvent. In both cases the solvent was saturated with *n*-octanol. When water was used as solvent, 10 mL of drug in water solution and 10 mL of *n*-octanol were mixed in borosilicate separating funnels and covered with teflon cap liners. The funnels were shaken vigorously for 24 hours at 37°C and they were then left to settle for another day. The layers were then separated and centrifuged for 10 minutes at 16,000 × *g* to ensure further separation of any possible traces of one solvent into the other. The concentration of the drug in the aqueous layer was determined with UV–Vis spectrophotometer and then the amount of DTM HCl in the aqueous layer and the nonaqueous layer were mathematically determined. The same procedure was followed when the modified PBS solution was used as the aqueous layer.

Measurements and calculations of flux and other coefficients

The lag time for the gel formulation can be calculated from Figure 1 (where the cumulative mass is plotted against time) by extrapolating the tangent of the curve to the *X*-axis; this line intersects with the time axis at some point where the amount of drug is zero; this time is called lag time (t_{lag})[28]. The diffusion D_s and permeability coefficients K_p were obtained as shown below and are presented in Table 1:

$$D_s = h^2 / 6t_{lag} \text{ and } K_p = D_s K_{o/w} / h.$$

where h is the epidermis thickness.

The cumulative amount of the drug permeating through the cellulose membrane or the epidermis layer was plotted against time. The steady-state flux was calculated from the slope of the linear region of the plot (Figures 1–5).

Measurement of gel viscosity

The viscosity of the HPMC gel with and without enhancers was measured with a Brookfield viscometer (High Shear CAP-1000+; Middleboro, MA, USA) at ambient temperature and at different shear rates. Viscosity measurements were repeated six times for each formulation and the average values were recorded. The viscosities were recorded for shear rate equal to 100/s.

Statistics

Each experiment was repeated at least six times. The mean values and standard deviations are presented in each table. Student's *t*-test was used to compare the parameters with the level of significance set at $P < 0.05$.

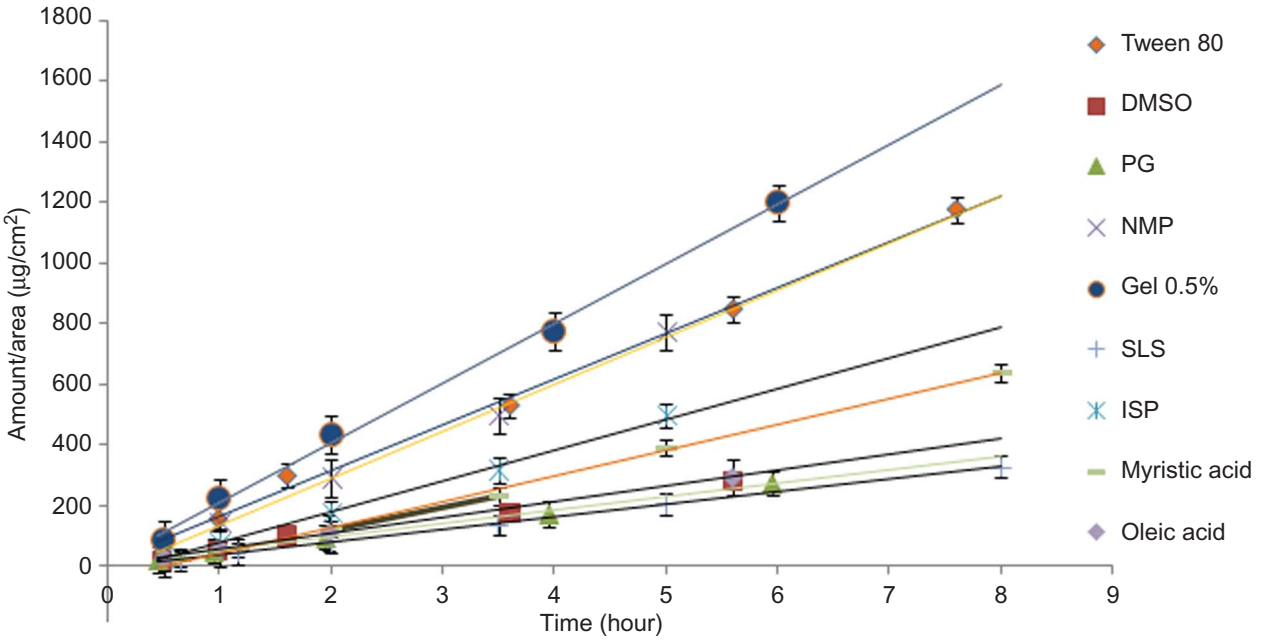

Figure 1. Release profile of DTM HCL from gel formulation with enhancers through cellulose membrane.

Table 1. Experimentally determined physicochemical properties and coefficients.

Media/vehicle	Diffusion barrier	Parameter	Value
In water at 37°C	N/A	Solubility	557.1 mg/mL
In water at 37°C	N/A	$K_{o/w}$	4.4
In water at 37°C	N/A	log *P*	0.64
In PBS pH 7.4 at 37°C	N/A	Solubility	349.9 mg/mL
In PBS pH 7.4 at 37°C	N/A	$K_{o/w}$	5.6
In PBS pH 7.4 at 37°C	N/A	log *P*	0.75
GEL 0.5% at 37°C	Human cadaver skin	t_{lag}	0.12 hour (~7.2 minute)
GEL 0.5% at 37°C	Human cadaver skin	Skin diffusion coefficient D_s	1.7×10^{-7}cm^2/h
GEL 0.5% at 37°C	Human cadaver skin	Permeability coefficient K_p	2.1×10^{-2}cm/h

Results and discussion

Physicochemical properties evaluated

The apparent partition coefficients for DTM HCl in water and in PBS pH 7.4 were determined and are presented in Table 1. Also, Table 1 shows the values of other experimentally determined coefficients and physicochemical parameters such as the solubility of DTM HCl in water and in PBS, the log *P* and lag time, and the diffusion and permeability coefficients.

As can be seen from Table 1, DTM HCl is a hydrophilic drug with high solubility in water and in PBS solution of pH 7.4. According to Guy et al.[6], a good range of the log *P* value for transdermal delivery is $-1 < \log P < 2$. Other sources[29] report as the optimum range $-1.5 < \log P < 3.5$ in percutaneous delivery and the highest acceptable value of log *P* to be 3–4 in transdermal delivery. Therefore, DTM HCl with a log *P* value of 0.75 in PBS and 0.64 in water is a good candidate for transdermal delivery.

Viscosity studies

The viscosity of the HPMC gel without and with permeation enhancers was measured at different shear rates. The viscosity values presented in Table 2 were recorded at a shear rate of 100/s. IPM, PG, and NMP increased the viscosity of the gel. The viscosities of these enhancers before they were added into the gel were: NMP: 17 ± 1 poise, PG: 0.486 ± 0.02, IPM: 57 ± 3 poise. Oleic acid, Tween 80, Caprylic acid, and DMSO did not alter the gel viscosity in a statistically significant manner as their initial viscosities were very similar to the 0.5% (w/v) HPMC

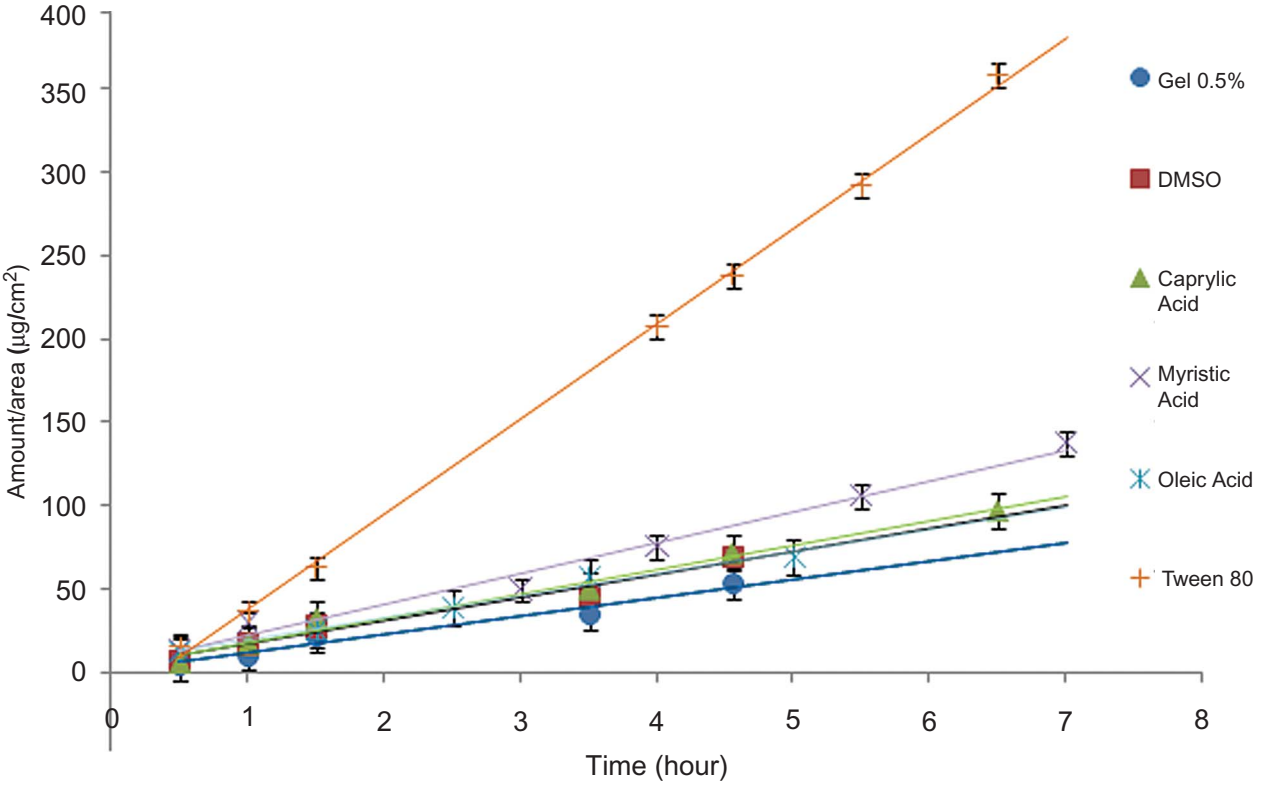

Figure 2. Enhancers that improved the release profile of DTM HCL through human cadaver skin.

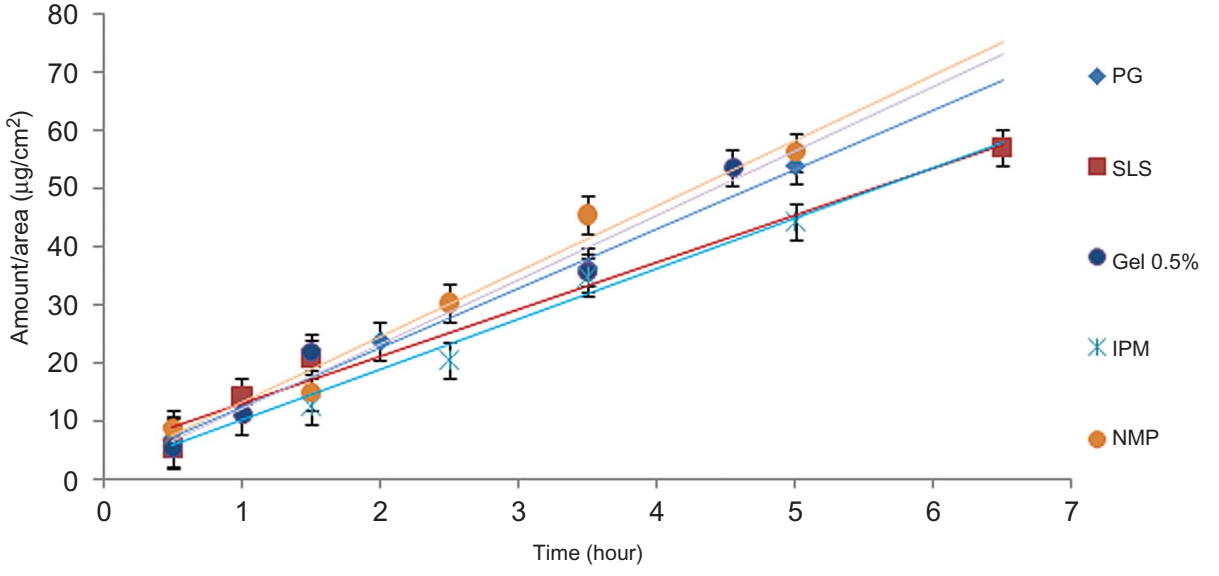

Figure 3. Enhancers that did not improve the release profile of DTM HCL through human cadaver skin up to 8 hours.

gel viscosity. The only enhancers that decreased the gel viscosity were myristic acid and SLS. These two special cases will be discussed later in more details in the section 'Discussion of viscosity and flux combined results'.

Influence of permeation enhancers on the flux of DTM HCl

Cellulose membrane studies

The cellulose membrane was one of the diffusion barriers used to study the effect of the absorption enhancers on

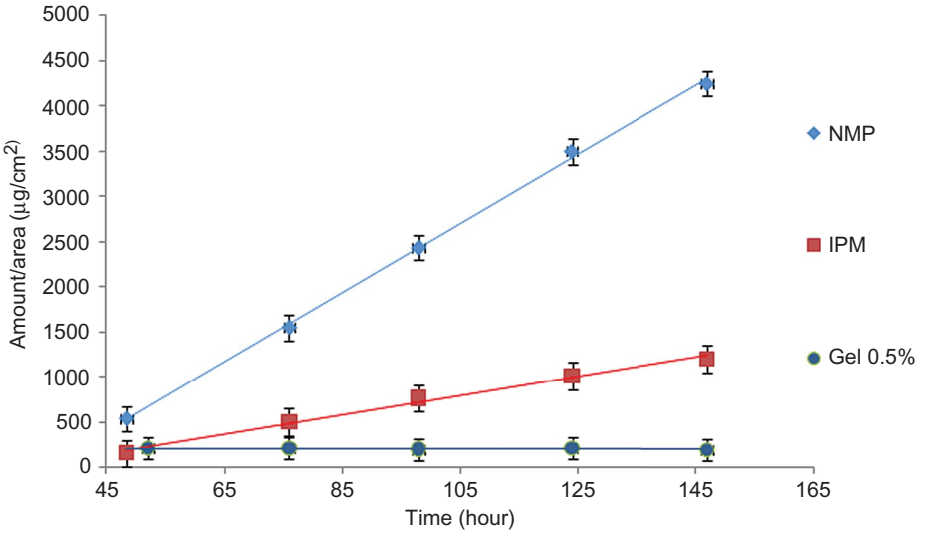

Figure 4. Effect of ISP and NMP on the release profile of DTM HCL after 48 hours through human cadaver skin.

the release rate of DTM HCl. When cellulose membrane was used as the diffusion barrier, it was noticed that all the enhancers rendered a reduced flux compared to the flux obtained with the 0.5% (w/v) gel formulation without enhancers (Table 3 and Figure 1).

Cellulose membrane is a very simple construction as opposed to the SC layer, which is very complex. The presence of lipids and keratin in the skin significantly affect the permeation of any substance due to interactions taking place. Because cellulose membrane is lacking this sort of complexity, it was used to eliminate the factor 'drug–skin components interactions' in order to investigate whether any other phenomena were occurring. Therefore, having excluded any possible skin interactions, it might be assumed that the observed reduction on the permeation rate of DTM HCl through cellulose membrane in the presence of enhancers may be due to the binding of the drug with the enhancers or/and due to changes in the viscosity of the gel.

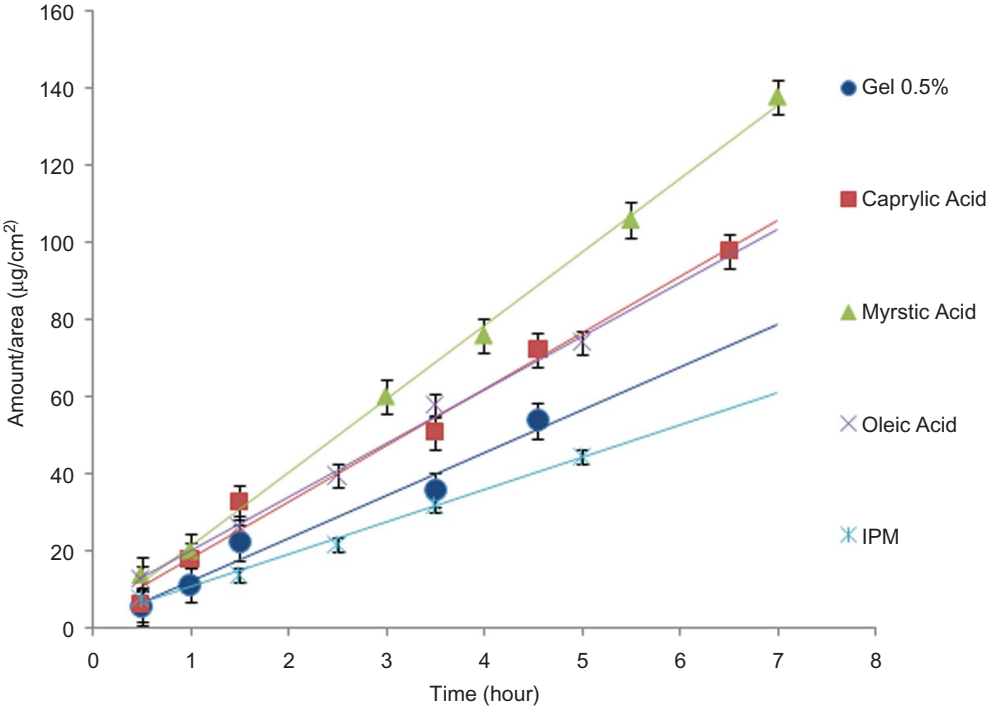

Figure 5. Effect of fatty acids on the release profile of DTM HCL through human cadaver skin.

Table 2. Viscosity measurements for HPMC gels with and without enhancers.

Vehicle	Enhancer	Viscosity[a] (poise)	Shear rate (1/s)
0.5% (w/v) HPMC gel	None	0.2676 ± 0.105	100
0.5% (w/v) HPMC gel	Isopropyl myristate	0.3225 ± 0.109	100
0.5% (w/v) HPMC gel	Oleic acid	0.2714 ± 0.075	100
0.5% (w/v) HPMC gel	Tween 80	0.2775 ± 0.060	100
0.5% (w/v) HPMC gel	Caprylic acid	0.2634 ± 0.098	100
0.5% (w/v) HPMC gel	Myristic acid	0.2223 ± 0.066	100
0.5% (w/v) HPMC gel	Sodium lauryl sulfate	0.2391 ± 0.068	100
0.5% (w/v) HPMC gel	*N*-Methyl pyrrolidone	0.2966 ± 0.072	100
0.5% (w/v) HPMC gel	Dimethyl sulfoxide	0.2575 ± 0.067	100
0.5% (w/v) HPMC gel	Propylene glycol	0.3075 ± 0.108	100

[a]**M**ean of six samples.

Table 3. Flux of DTM HCl through cellulose membrane as the diffusion barrier.

Type of formulation	Name of permeation enhancer	Flux IN μg/cm^2/h
Gel 0.5% DTM HCl (HPMC)	None	196.7 ± 10.9
Gel 0.5% DTM HCl (HPMC)	NMP	155.5 ± 12.2
Gel 0.5% DTM HCl (HPMC)	Polysorbate 80	151.7 ± 12.8
Gel 0.5% DTM HCl (HPMC)	ISP	102.1 ± 11.1
Gel 0.5% DTM HCl (HPMC)	Caprylic acid	92.4 ± 11.01
Gel 0.5% DTM HCl (HPMC)	SLS	49.4 ± 7.8
Gel 0.5% DTM HCl (HPMC)	DMSO	48.9 ± 3.5
Gel 0.5% DTM HCl (HPMC)	Propylene glycol	44.6 ± 3.25
Gel 0.5% DTM HCl (HPMC)	Oleic acid	41.4 ± 2.97
Gel 0.5% DTM HCl (HPMC)	Myristic acid	21.3 ± 1.8

The expectation that the drug may be binding to some enhancers is based on the following argument: DTM HCl is an acidic salt with a p*K*a 7.7. Therefore, according to the Henderson–Hasselbach equation, when DTM HCl permeates the skin, it is nonionized (skin pH = 5.5), whereas in the PBS solution DTM HCl is 50% ionized (PBS pH = 7.4). When DTM HCl is in the ionized form (cationic drug) it may bind to anionic substances and therefore reduce the flux. The flux may also be reduced due to an increase in the gel's viscosity in the presence of some enhancers. To better understand and explain any interactions taking place, studies with human cadaver skin as well as viscosity studies were conducted.

Human cadaver skin studies

In vitro studies with human cadaver skin rendered different results. First of all, the release rate through human cadaver skin was significantly smaller than the one observed with cellulose as shown in Table 4. For the gel formulation without enhancers, the observed permeation rate was 11.1 ± 0.37μg/cm^2/h. Second, every enhancer altered the release rate of DTM HCl in a different way.

Table 4. Flux of DTM HCl through human cadaver skin as the diffusion barrier.

Gel 0.5% DTM HCl (HPMC) containing as enhancer	Flux in μg/cm^2/h at initial times (first 6 hours)	Flux in μg/cm^2/h at later times (48 hours and onward)
None	11.1 ± 0.37	Steady state
SLS	8.1 ± 0.21	Steady state
Propylene glycol	10.2 ± 0.32	Steady state
DMSO	13.8 ± 0.4	Steady state
Polysorbate 80	57.1 ± 0.9	Steady state
Caprylic acid	14.6 ± 0.43	Steady state
Myristic acid	18.4 ± 0.49	Steady state
Oleic acid	13.1 ± 0.33	Steady state
IPM	8.7 ± 0.22	15.3 ± 0.41
NMP	11.3 ± 0.34	46.5 ± 0.7

According to Figure 2, DMSO, caprylic acid, myristic acid, oleic acid, and polysorbate 80 resulted in permeation enhancement within the first 6 hours when studied in vitro. IPM and NMP also increased the flux, but this effect manifested itself from the second day onward (Figure 4). Finally, both SLS and PG decreased the release of DTM HCl (Table 4 and Figure 3).

It should be stressed that polysorbate 80 showed greater increase in the release rate of DTM HCl than the rest of the enhancers; specifically, it increased the flux almost 5 times compared to the flux of gel without enhancers. This finding is of great importance because Tween 80 (polysorbate 80) has never been studied before for its potential as a permeation enhancer for hydrophilic, freely soluble drugs released from a hydrophilic vehicle. Polysorbate 80 has been employed in previous studies for its ability to increase the solubility of lipophilic compounds[30]. However, the drug used in this study, DTM HCl, is a freely soluble drug and therefore Tween 80 is exclusively studied for its potential as a permeation enhancer in transdermal delivery; as mentioned previously, the results were very satisfying.

The flux of NMP after 48 hours was also very high (almost 4 times higher than the flux of the gel formulation without enhancers). At initial times, NMP did not alter the release profile of DTM HCl in as statistically significant manner. After 48 hours however, NMP caused a delayed increase in the flux, which may be of great benefit in transdermal delivery; for example, NMP may be combined with another enhancer of immediate action and render an extended release profile or it may be used alone in cases where enhancement in the flux may be required from the second day of application and thereafter.

IPM rendered similar results. Although, IPM decreased the release of DTM HCl during the first 6 hours compared to the formulation in which no enhancers were included (Figure 3), 48 hours later it rendered a higher flux than the HPMC gel formulation without enhancers (Figure 4). The delayed increase in the flux noted with IPM was much smaller than the one noted with NMP (Figure 4). Currently, there are no similar reports in the literature with NMP or IPM.

Fatty acids were studied separately. The fatty acids chosen for this study had a maximum of 18 carbons in their chain and different degrees of saturation. Also, one fatty acid ester was included. Studies with fatty acids indicated that the number of carbons (chain length) was critical for the permeation rate of DTM HCl from a gel vehicle through human cadaver skin (Figure 5). Specifically, the flux increased by increasing the number of carbons, reached the maximum for substances containing 14 carbons and decreased again for substances containing chains with more than 14 carbons.

The maximum effect on the flux was observed with the saturated myristic acid (14 carbons chain). Other studies conducted in the past involving saturated fatty acids report a maximum flux with 12 carbons or less in the fatty acid chain[31]. Myristic acid has never been reported before as the optimum choice as an enhancer among other fatty acids for any drug.

Oleic acid had the longest chain of all the other fatty acids and one unsaturated bond. Oleic acid rendered a flux lower than the flux observed with the shorter chain saturated acids, that is, caprylic and myristic.

Discussion of viscosity and flux combined results

Fatty acids

Fatty acids can be used for both hydrophilic and lipophilic substances, but the flux appears to improve with hydrophilic rather than with lipophilic permeants. Thermal analyses have indicated that the fatty acids interact with the lipid domain within the SC; a new structure was identified in the intercellular lipids suggesting that a novel lipid domain was induced in the lipids[32]. The formation of these pools results in permeability defects within the lipid bilayer. Therefore, it may be assumed that the permeation of hydrophilic substances may be facilitated through these 'defective' areas.

In general, the fatty acids did not alter the viscosity of the gel. The only two exceptions were myristic acid and IPM. Myristic acid is a crystalline waxy powder whose melting point is 53–56°C [data obtained from the Material Safety Data Sheet (MSDS)]. When myristic acid was added to the gel during the fourth step of preparation, as described previously, the temperature of the solution was 52–58°C. As a result, myristic acid turned into its liquid form. The viscosity of liquid myristic acid at that temperature was determined to be 0.10 ± 0.08 poise [lower than the 0.5% (w/v) HPMC gel's viscosity]. Therefore, when myristic acid was introduced into the gel solution, it lowered the gel's final viscosity. Apparently, the increased flux observed when myristic acid was used as an enhancer can be partly attributed to the disruption of the lipid bilayer—as described above—and partly to the reduction of the gel's viscosity.

In the case of IPM, the delayed increase of the flux may be explained with findings from the viscosity studies as well. In Table 2, it can be seen that IPM significantly increased the viscosity of the HPMC gel. Therefore, the delayed increase in the flux resulted from a delayed release of the enhancer from a more viscous carrier.

N-Methyl-pyrrolidone

According to Table 2, NMP also increased the viscosity of the gel significantly. However, NMP rendered a much better release profile than IPM not only within the first 6

hours but also later on; specifically, no reduction in the flux was observed during the first 6 hours (as it was observed with IPM) and second, the increase in the flux was significantly higher from the second day and on. NMP is a polar aprotic solvent and a reasonable assumption would be that it has a more intense effect on the tissues than IPM.

Also, NMP increased the gel's final viscosity less than IPM did. As a result, the drug could diffuse easier through the less viscous vehicle containing NMP, than through the more viscous vehicle containing IPM. Therefore, NMP, by both being a 'stronger' solvent than IPM and rendering a less viscous final mixture, resulted in a better release profile than IPM.

As mentioned previously, apart from the delayed increase in the flux, NMP exhibited a sustained release as well (instead of reaching a steady state). The sustained release may be explained as follows: NMP is more effective with hydrophilic than with lipophilic substances[33] and it 'partitions well in the SC where it alters the solvent nature of the tissue'[34]. The later statement means that NMP possibly generates a reservoir/pool within the tissue. It is expected that such a reservoir formation containing the hydrophilic drug DTM HCl may offer potential for sustained release of the permeant over an extended period of time. This observation along with the increase in the gel viscosity may explain the behavior NMP exhibited in this study at later times, that is, after 48 hours NMP extended the release of the drug instead of reaching a steady state.

Dimethyl sulfoxide

Figure 2 indicates that DMSO was one of the effective enhancers. DMSO is also a powerful aprotic solvent with a complex mechanism of action. It denatures proteins and when applied on the human skin changes the intercellular keratin from α-helical to a β-sheet conformation[35,36]. Thermal analysis has shown that DMSO also interacts with the polar group of bilayer lipids. As a result, the packing geometry of lipids is altered.

According to the discussion above, one might expect a much better enhancement effect on the release rate. However, this is not the case, because in this study the concentration of DMSO was only 0.5% (w/v) in order to avoid damage of the skin, since high concentrations of this chemical can cause severe irritation and delamination of the SC by denaturing proteins[33]. DMSO did not alter the viscosity of the gel.

Propylene glycol

Whenever PG was used as a 'stand-alone' permeation enhancer in other studies[33, 37], only a very mild enhancement was observed. In this study, PG decreased the release rate. PG is known to act mainly by modifying the solubility of the drug in the formulation[38,39]. As mentioned previously, DTM HCl is already a very soluble drug, and therefore no significant improvement in the release profile was expected in the first place; a decrease, however, was not foreseen either. Viscosity studies helped shed light on this aspect: PG, being a viscous liquid itself, increased the HPMC gel's viscosity and, therefore, negatively affected the release profile of DTM HCl.

Ionic surfactant sodium lauryl sulfate

Surfactants are usually added to the formulation to increase the solubility of lipophilic active ingredients and hence they have the potential of solubilizing lipids within the SC as well.

SLS is an anionic surfactant and can potentially damage the skin. It is a powerful irritant and can increase transepidermal water loss in humans. In addition, it causes swelling of the SC and interacts with the intercellular keratin[40]. Anionic surfactants in particular can swell the SC (probably by uncoiling the keratin fibers and altering the α-helices to a β-sheet conformation). They also modify the absorption of water on the SC and cause the skin to become brittle (possibly due to the extraction of natural moisturizing factor); generally, they are considered irritants[40].

Obviously, SLS's action is mainly due to the damage of the skin. As expected, SLS in this study could not destroy the epidermis because it was used at a low percentage (0.5%, w/w). Also, since DTM HCl is a cationic drug, it may bind to anionic surfactants such as SLS, therefore resulting in a decreased flux across the skin. In vitro studies with cellulose membrane as well as through human cadaver skin indicated a flux reduction with SLS; such an observation may lead to the assumption that either binding is taking place between the drug and SLS or the viscosity increases or both things happen at the same time. Viscosity studies indicate that SLS resulted in a decrease in the gel viscosity; normally reduction in viscosity should lead to a flux increase. The fact that the flux decreased despite lowering the gel viscosity confirms that SLS really binds to the cationic DTM HCl.

Nonionic surfactant polysorbate 80 (Tween 80)

Polysorbate 80 is a nonionic surfactant. Nonionic surfactants are generally safe and very few incidents of chronic toxicity are known so far[23]. Surfactants generally interact with skin constituents in many ways. For example, they can create a network with skin proteins; this may explain the inactivation of enzymes located in the SC as well as the fact that surfactants partition strongly with the upper epidermis layer. Surfactants extract lipids from the SC and can disrupt the lipid

bilayer packing in the tissue[23]. The mechanism of action appears to benefit hydrophilic compounds very strongly and therefore it seems very reasonable that polysorbate 80 demonstrated a significant flux enhancement even though it was used only at a very small percentage [0.5% (w/w)].

Conclusions

In this study the drug DTM HCl was formulated for the first time in an HPMC gel intended for transdermal delivery. Several enhancers were evaluated in order to investigate any effect they might have on the release rate of DTM HCl from a transdermal HPMC gel formulation. Among all the enhancers, the effective ones were DMSO, caprylic acid, myristic acid, oleic acid, and polysorbate 80 as they all increased the flux within the first 6 hours as compared to the gel formulation without any enhancers.

Additionally, NMP and IPM also increased the flux as compared to the gel formulation without any enhancers. Specifically, it was observed that NMP and IPM improved the release of DTM HCl from the gel, but not immediately; the improved release profile was observed with a 48-hours delay. This delayed enhancement was caused by an increase in the HPMC gel's viscosity. No similar findings have been reported so far.

Polysorbate 80 also yielded very interesting results; although it was added at a very low concentration [0.5% (w/w) of solids content], it gave a flux of 57.1 ± 0.9 μg/cm^2/h for a drug concentration as low as 0.5% (w/v). It should be noted that in this study Tween 80 was studied for the first time as an enhancer for hydrophilic drugs released from a hydrophilic carrier.

Fatty acids were another chemical group that was studied extensively here. In this study the selected group included fatty acids with a maximum of 18 carbons in their chain (saturated and unsaturated) and an ester. Myristic acid yielded the best release profile compared to the other fatty acids. This is the first report in the literature of myristic acid yielding the optimum flux for a drug as compared to other fatty acids.

The permeation studies through cellulose membrane and human cadaver skin were combined with viscosity measurements. The viscosity studies revealed additional interesting and novel findings about the interaction of the enhancers with DTM HCl and their effect on the viscosity of the gel; specifically, it was shown that drug-enhancer binding as well as viscosity changes caused by enhancers are a significant part of the enhancers' mechanism of action and should be taken into consideration.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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