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Skin permeation of physostigmine from fatty acids-based formulations: evaluating the choice of solvent

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Abstract

This study was conducted to gain an understanding of the enhancement mechanism of fatty acids in skin permeation of physostigmine (PHY) by using a series of fatty acids and two solvents of opposing lipophilicity (propylene glycol (PG) and mineral oil (MO)). Interaction between fatty acid and drug was proven using NMR and conductivity measurements that showed a dependence on type of solvent used. Permeation flux of physostigmine from mineral oil-based formulations to skin was increased as solubility of physostigmine in mineral oil was enhanced in the presence of fatty acids having a longer chain. Thus, the dominant role of fatty acids in mineral oil was to increase solubility of physostigmine in the formulations that increased the driving force for physostigmine permeation through skin. As for propylene glycol, enhancement caused by fatty acids was attributed to their ability to increase the lipophilicity of formulation and to disrupt the lipid bilayers within the stratum corneum (SC). In conclusion, fatty acids enhancement for drug permeation across the skin was found to be dependent on the solvent used. Among various formulations here, oleic acid in mineral oil yielded fast permeation of PHY with a short lag time, which may be a good vehicle for transdermal delivery of PHY.

Keywords: Fatty acids; Skin permeation; Solvent effect; Physostigmine; Enhancement mechanism

Abbreviations: τ, lag time (h); C10, decanoic acid; C12, lauric acid; C18:1, oleic acid; C18:2, linoleic acid; C2, acetic acid; C3, propionic acid; C8, octanoic acid; C_S, saturated solubility of drug (mg/ml); D, diffusivity; D', normalised diffusivity (s^{-1}); IPM, isopropyl myristate; J_S, steady state flux ($\mu g/cm^2/h$); K, partition coefficient; K', normalised partition coefficient (cm); L, diffusional pathlength (cm); MO, mineral oil; P, permeability coefficient ($cm\,s^{-1}$); PG, propylene glycol; PHY, physostigmine; SC, stratum corneum

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1. Introduction

Physostigmine (PHY) is a competitive inhibitor of the enzyme acetylcholine esterase with the ability to diffuse through the blood brain barrier (Hartvig et al., 1986). Clinically, PHY has been suggested as an alternative that offers protection against organophosphate poisoning-induced brain toxicity (Tuovinen et al., 1999; Solana et al., 1990) as well as the potential benefit of PHY in treating symptoms of Alzheimer's disease (Coelho Filho and Birks, 2001; Krall et al., 1999). The therapeutic use of PHY is limited by biological constraints such as short elimination half-life, narrow effective dose range, poor in vivo stability and low oral bioavailability (Walter et al., 1995). A PHY transdermal delivery system is an attractive option given the potent nature of the drug and the bypass of hepatic "first pass" elimination effect.

Since the skin acts as a natural barrier to foreign substances, most drugs permeate the skin at extremely low rates without any form of penetration enhancement. Fatty acids are well-established permeation enhancers that are used to increase permeation of basic drugs, across the stratum corneum (SC). Aungst et al. (1990) attributed the improvement in drug permeation to several possible mechanisms. They are the reduction of skin resistance as a permeation barrier, increased skin/vehicle partitioning of the drug and increased solvent transport into/across the skin. Most of the skin resistance lies in the highly structured SC, and the most common fatty acid used to reduce this resistance is oleic acid. Golden et al. (1987) have shown that skin permeability changes induced by oleic acid were proportional to physical changes in the SC lipids by using differential scanning calorimetry and Fourier transform infrared spectroscopy.

For basic drugs, the formation of a more lipophilic ion pair between the fatty acid and the drug resulted in increased skin/vehicle partitioning of the drug, which favoured permeation. This was reported by Green and Hadgraft (1987) and Green et al. (1988), who have shown that increased diffusion of β-blockers was due to the increased isopropyl myristate (IPM)/buffer partition coefficient of drug and this increase was attributed to increase in lipophilicity after the formation of ion pairs between the drug and the fatty acid.

Aungst et al. (1986) reported that propylene glycol (PG) as an adjuvant for fatty acids resulted in the highest permeation of naxolone. Thereafter, PG has been widely used as solvent for fatty acids. However, the exact cause for the highest permeation with PG as solvent was not known. Work by Jenner et al. (1995) concluded that the mixtures of propionic acid, oleic acid and PG yielded high permeation of PHY along with skin irritation and the exact enhancement mechanism of fatty acids was not elaborated. Therefore, the objectives of this paper were to understand the enhancement mechanisms of fatty acids in skin permeation of physostigmine and to investigate the permeation enhancement of fatty acids in both polar and non-polar solvents. PG and mineral oil (MO) were used as the polar and non-polar solvent, respectively. The rationale of using a non-polar solvent like MO was to restrict the ionisation degree of fatty acid (i.e. to increase lipophilicity of fatty acids) and to evaluate the importance of the other mechanisms such as lipid disruption and the solvent drag effect. A series of fatty acids with different chain length were used to study the influence of the nature of fatty acid on physostigmine permeation.

2. Materials and methods

2.1. Materials

Acetic (C2), propionic (C3), octanoic (C8), decanoic (C10), lauric (C12), oleic (C18:1) and linoleic (C18:2) acids, purity >95%, and deuterated chloroform were purchased from Sigma–Aldrich, Germany and Malaysia. Oleic acid is an 18-carbon fatty acid with one double bond while linoleic acid is an 18-carbon fatty acid with two double bonds. Therefore they were denoted by C18:1 and C18:2, respectively. PHY free base (or eserine free base) was purchased from Sigma Chemical, USA. Propylene glycol (PG, USP grade), isopropyl myristate, acetonitrile and salts used to prepare the phosphate-buffered saline (PBS, pH 7.4) were obtained from Merck, Germany. White MO was purchased from Sino Chemicals Co, Singapore. The viscosity of MO at 50 °C is 1.78cSt. Scotch® Magic™ Tape 810 from 3 M, USA was used for tape stripping.

2.2. Skin preparation

Porcine lower abdominal skin was obtained from the animal holding unit in National University of Sin-

gapore, cleaned briefly, wrapped between aluminium foils and stored at −24 °C prior to use for up to six months. Full thickness skin without subcutaneous fat was used for permeation studies. The thickness of skin used ranged from 0.75 to 0.95 mm.

2.3. Formulation preparation

In this study, two types of solvents were used (PG and MO). Each solvent was used to dissolve fatty acids and PHY, forming two series of 0.5 M fatty acid containing formulations. The solubility of PHY was determined by dissolving excess drug in each formulation. The mixture was vortex mixed at high speed and allowed to equilibrate at room temperature for 2 h in presence of excess drug. The saturated solution was then filtered with a 0.2 μm PVDF syringe filter (Whatman, UK) and the concentration of PHY was measured by a modified HPLC assay (Rubnov et al., 1999) after appropriate dilution.

The HPLC system consisted of a 2690 Separation Module and a 996 PDA detector (Waters, Milford, MA, USA). An Inertsil® C8 reverse phase analytical column (4.6 mm × 150 mm i.d., 5 μm) protected by an Inersil® C8-RP guard column (4.6 mm × 12.5 mm i.d., 5 μm) (GL Sciences Inc, Japan) was used at a flow rate of 1 mL/min. The mobile phase composed of an aqueous solution of 20 mM ammonium acetate and acetonitrile with the volume ratio of 75:25. The column and sample temperatures were set at 35 and 10 °C, respectively. The wavelength was set at 248 nm. The calibration curve was constructed to determine PHY concentration in the range from 0.02 to 0.20 mg/mL and the R^2 values were at least 0.999.

2.4. Apparent partition coefficient of PHY between IPM and vehicle

Ten milligrams of PHY were dissolved in 5 mL of PG-based formulation containing 0.5 M of fatty acid, and equal volume of IPM was added. The mixture was vigorously shaken for 3–5 min and allowed to equilibrate at 32 °C for two days. The concentration of PHY in both phases was determined by HPLC. The apparent partition coefficient was calculated as the concentration of drug in IPM over the concentration of drug in the vehicle (Aungst et al., 1986). Apparent partition coefficients between IPM and MO were not determined as they were miscible.

2.5. ^1H NMR spectroscopy

The ^1H NMR spectra of PHY, C2, C18:1 and their mixture were measured in $CDCl_3$ in 5 mm tubes with a Bruker Avance 400 spectrometer (Bruker, USA). Chemical shifts were recorded as units relative to tetramethylsilane (internal standard).

2.6. Conductivity measurement

The conductivities of the solvent (PG and MO), fatty acid containing solvent (0.5 M of fatty acid in PG and MO) and fatty acid-drug mixtures (molar ratio of acid:drug = 50:1) were measured to evaluate the formation of ion pairs between physostigmine and fatty acid in PG and MO. The electric conductivity of these solutions was measured at room temperature with a conductivity meter (AR20, Accumet Research, UK). Electric conductivity, κ, was obtained by direct reading of the conductivity meter and given by:

$$\kappa = \left(\frac{d}{a}\right) G$$

where (d/a) is the cell constant, set at 1 cm^{-1} during measurement and G denotes the electrical conductance in Ω^{-1}. The unit of electric conductivity is S cm^{-1}.

2.7. Permeation study and calculation of permeation parameters

Franz type vertical diffusion cells were used for diffusion studies of PHY through the skin. The SC was arranged to face the donor solution and the available skin area for permeation was approximately 1.77 cm^2. 0.02% NaN_3 in PBS was used as receptor fluid. The skin was equilibrated for 12 h before the receptor medium was replaced and 1 mL of saturated drug solution was then added to the donor compartment. The receptor compartment was stirred magnetically at 600 rpm at 37 °C. Aliquots of 1 mL were withdrawn periodically and replaced with equal volume of fresh receptor fluid for 48 h. For permeation study across tape stripped skin, the thickness of SC was reduced by peeling with 20 strips of Scotch® Magic™Tape 810 after equilibration. According to the results reported by Pellett et al. (1997), tape stripping with 20 strips reduced the thickness of SC greatly. Cumulative amount

of the drug permeated through the skin was plotted as a function of time. For all the formulations used, the percentage of drug that permeated the skin was 10% or less and also well below the solubility of PHY in the receptor medium. Thus, sink condition in receptor compartment can be safely assumed. Steady state flux (J_s) and lag time (τ) were obtained from the slope and x-intercept of the steady state portion of the release profile. Permeability coefficient (P) was calculated from the following equation (Vaddi et al., 2002):

$$P = \frac{J_s}{C_s} \quad (1)$$

where C_s is the solubility of the drug in the formulation.

It is widely accepted that the SC presents a rate limiting barrier to transdermal drug delivery and the diffusional pathlength (l) is taken to be the pathlength of the drug across the SC. Since most molecules permeate through the SC mainly by a tortuous intercellular route, the thickness of the skin is not equal to the diffusional pathlength (Williams and Barry, 1991). However, it is difficult to determine the exact pathlength of the drug in various vehicles. Therefore, diffusivity (D) and partition coefficient (K) were normalised as D' and K' respectively (Williams and Barry, 1991), which can be obtained from the following equations:

$$D' = \frac{D}{l^2} = \frac{1}{6\tau} \quad (2)$$

$$K' = Kl = 6P\tau \quad (3)$$

2.8. Statistical analysis

Statistical analysis of the data was performed using SigmaStat 2.03 (SPSS Inc, USA). Comparison between groups was analysed by one way ANOVA with Tukey post-hoc test. For comparison between two groups of data, significance was determined by t-test. Data were considered significant at $p < 0.05$.

3. Results and discussion

3.1. ^1H NMR spectra

The ^{1}H NMR spectra of C2, C18:1, PHY and their mixtures are shown in Fig. 1. A chemical shift of the peak at 4.15 ppm in the spectrum of PHY, belonging to the hydrogen atom sandwiched between the two tertiary amines (Fig. 2), was observed in the presence of acids. This peak shift indicated an increase in electron density around the hydrogen atom as a result of the interaction between the tertiary amine of PHY and the carboxylic group of the acid. This interaction was dependent on the chain length of the acid. In the presence of C2, the extent of shift was 0.5 ppm while in the presence of C18:1, the extent of shift was 0.3 ppm. Though the difference between the two shifts is only 0.2 ppm, it was significant, considering the high sensitivity of NMR spectroscopy. Since the acidity of C2 is higher than that of C18:1, the complex of PHY and C2 is expected to be stronger as reflected by greater chemical shift. Due to the complexation of PHY and the fatty acid, the permeation of PHY across the skin may be affected by the permeability of the fatty acid.

3.2. Conductivity measurement

The conductivity of a solution containing charged species is largely dependent on the population of ions present and their mobility. An increase in conductivity after addition of PHY to the fatty acid containing solvent implies an increase in the population of ions present, which provides some evidence of ion pairing between PHY and fatty acid. Fig. 3 illustrated the changes in conductivity after the addition of PHY to the fatty acid loaded solvent. The conductivities of blank PG and MO were low before loading of fatty acids. With the exception of C10 in PG, loading of fatty acids into both PG and MO resulted in a small increase in solution conductivity. The pH values of fatty acids were measured in PG and the results showed that pH decreased with reducing chain length of the acid. This indicates that the degree of ionisation of fatty acids increased with decreasing chain length. However, the conductivity measurements did not show a large difference in conductivity between C2 and C18:2. This may be attributed to the viscous nature of PG that could restrict the mobility of ions and thus smaller conductivity difference.

The addition of PHY to fatty acid loaded PG caused a huge increase in solution conductivity and an increase in pH, suggesting the formation of ion pairs. Such formation enhanced ionisation of both PHY and fatty acids in PG and thus resulted in much higher conductivity as compared to fatty acids loaded PG

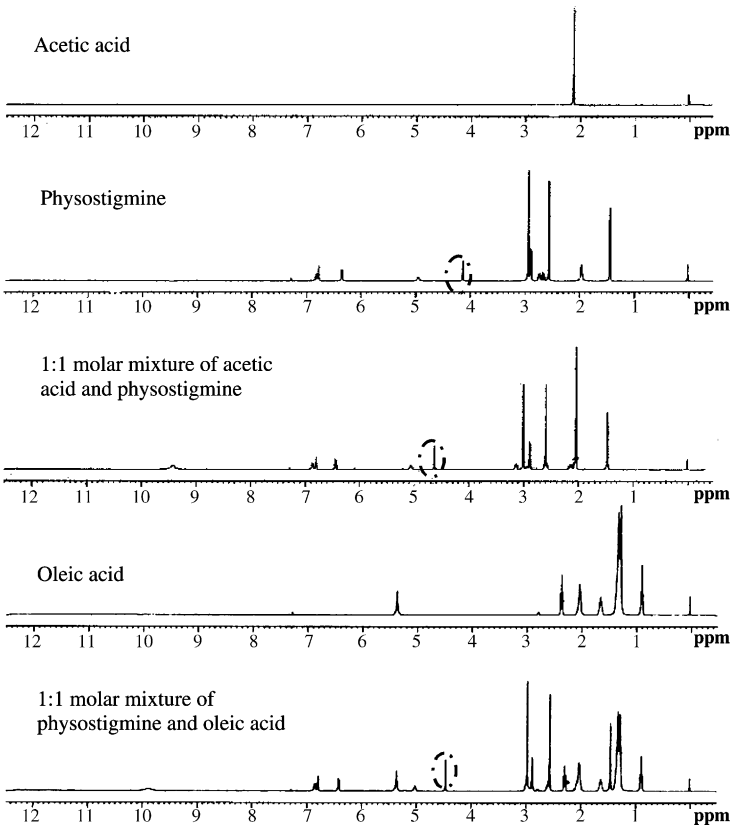

Fig. 1. ^{1}H NMR spectra of C2, C18:1, PHY and their mixtures.

and PHY in PG. The formation of the ion pairs also improved the solubility of PHY in PG. In addition, conductivity measurements showed a decrease in conductivity with increase in chain length of fatty acid (Fig. 3). As chain length of the acid increased, degree of ionisation of the carboxyl group decreased and thus the population of ions reduced. This resulted in greater conductivity. On the other hand, the ion pairs between PHY and C18:1 were more bulky than those between PHY and C2, and thus reduced mobility. Reduced

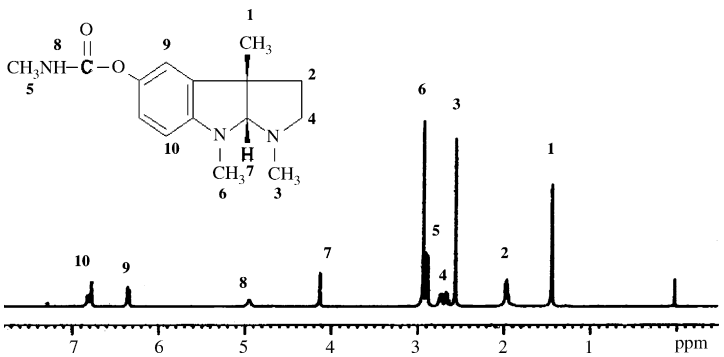

Fig. 2. Analysis of PHY ^{1}H NMR spectrum.

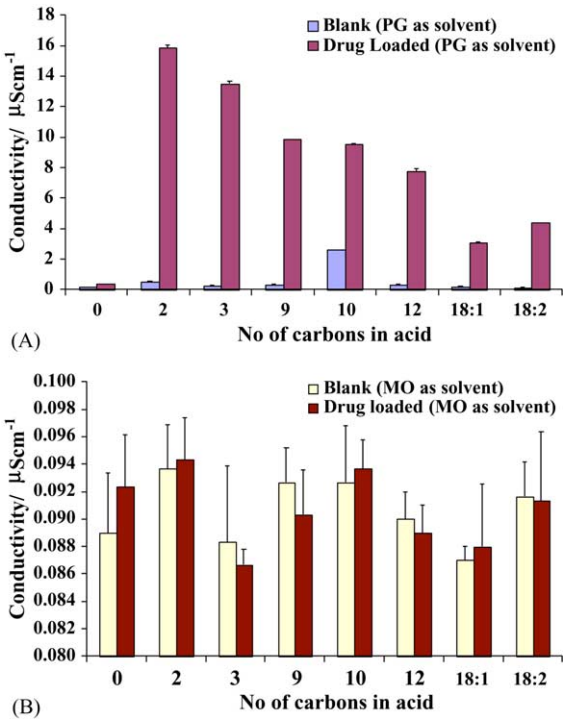

Fig. 3. Conductivity measurements of solvent and 0.5 M fatty acid-containing solvent before and after the addition of physostigmine (A) PG used as solvent (B) MO used as solvent. ($n = 3$, mean ± S.D.).

mobility also led to lower conductivity. In contrast, no significant increase ($p > 0.05$) in conductivity was observed after addition of PHY to fatty acid loaded MO. This indicated that ion pairs of PHY and fatty acid were unlikely to exist in MO. These results combined with those obtained from NMR spectroscopy in the earlier section provided strong evidence of ion pairing of PHY and fatty acid in PG but not in MO. Thus, complexes of PHY and fatty acid in PG will be referred to as ion pairs in the following sections and ion pairs formed were weaker with increasing chain length of fatty acids. Although ion pairs were not formed in MO, the authors believed that complexes of PHY and fatty acids existed because of the basic nature of PHY and evidence derived from NMR spectra.

3.3. Apparent partition coefficients

Partitioning of PHY between IPM and PG was studied to mimic the in vitro partitioning of drug between the drug vehicle and SC lipids. The apparent partition coefficient of PHY between IPM and MO could not be determined as both were completely miscible. Since apparent partition coefficient is a ratio of the drug solubility in the two phases, there would be greater partitioning of PHY into the IPM phase for MO-based formulations as the drug has a much lower solubility in MO than in PG. Since the molar ratio of the fatty acids to PHY used in the partitioning study was 70:1 and the interactions between PHY and fatty acids were stronger than those between PHY and IPM, it was postulated that most drug molecules would exist as PHY/acid ion pairs in the presence of the fatty acids and drug partitioning into IPM phase was in the form of PHY/acid ion pairs.

Partitioning of PHY into IPM from PG-based vehicles was dependent on the chain length of the fatty acid (Table 1). In order to explain the trend in partition coefficient, two factors have to be considered, the formation of ion pairs with reduced polarity and the increase in lipophilicity of fatty acid with increasing chain length. A strong ion pair with its high polarity has lower lipophilicity than a weaker one. Partitioning into IPM phase was enhanced with increasing chain length of the acid as weaker ion pairs were formed with relatively higher lipophilicity.

The partitioning of PHY into IPM was reduced in the presence of short chain fatty acids, as a strong PHY/acid ion pair was formed and thus lower solubility in IPM. Although C10 and C12 were lipophilic in nature, apparent partition coefficients of PHY in the presence of C10 and C12 were low. Here, the effect of formation of charged ion pairs outweighed the effect of higher lipophilicity with increasing chain length of fatty acids. However, for longer chain acids such

Table 1
Apparent partition coefficients of PHY between IPM and PG ($n = 3$, mean ± S.D.)

Solvent used	Fatty acid present[a]	$K_{IPM/PG}$
PG	Blank/control	0.134 ± 0.004
	C2	0.005 ± 0.001
	C3	0.005 ± 0.000
	C8	0.018 ± 0.003
	C10	0.029 ± 0.001
	C12	0.051 ± 0.000
	C18:1	0.225 ± 0.035
	C18:2	0.219 ± 0.015

[a] Concentration of the fatty acid in PG was 0.5 M.

as C18:1 and C18:2, the partition coefficients of PHY were much higher than the control, indicating that the increase in lipophilicity dominated despite formation of charged ion pairs. The number of double bonds present in the chain of fatty acid did not alter partitioning of PHY greatly as there were no significant differences ($p > 0.05$) in apparent partition coefficient of PHY between C18:1 and C18:2.

3.4. Permeation profiles and parameters through full skin

Fatty acids increased the solubility of PHY (C_s) in both solvents (Table 2). Since PHY dissolved in blank PG readily, further increase in solubility after addition of fatty acids was less pronounced than in MO-based formulations. Fig. 4 shows drug permeation profiles through the full skin in MO-based formulations. Permeation studies of PHY in C2/MO and C3/MO were not performed as a separate sticky phase was observed after the addition of PHY. For the saturated fatty acids, the permeation flux of PHY increased with increasing chain length of the fatty acid. Permeation flux of PHY in C18:1/MO was high as well, attributed to the long and kinked alkyl chain of C18:1 (Francoeur et al., 1990). The presence of an additional C=C as in the case of C18:2/MO yielded lower permeation flux than C18:1/MO (Fig. 4). In addition, a lag phase was observed in the permeation profiles, ranging from 4.8 to 13.0 h with C18:1/MO having the shortest lag time.

As for PG-based formulations (Fig. 5), there was no drug permeation from blank PG across the skin throughout the experiments. The addition of short chain fatty acids such as C2, C3, C8, C10 and C12 to PG did not improve the permeation. However, C18:1 enhanced the drug permeation greatly while further increase in number of double bonds (C18:2) lowered the permeation flux. The lower permeation flux was attributed to the presence of an additional double bond in the acid that resulted in a more "kinked" chain. This may favour the retention of the PHY/C18:2 complexes in the SC lipids.

The calculated partition coefficients (K') based on permeation data were found to be dependent on the chain length of the fatty acids (Table 2) for MO-based formulations. From Table 2, PHY molecules in blank MO had the greatest partition coefficient. The K' values were lowered after the addition of acids. There was no distinct relationship between the chain length of the fatty acid used and the K' values. However, in the presence of C18:2, the K' value of PHY was greatly reduced as compared with the rest of fatty acids. Since C18:1 and C18:2 had comparable lipophilicity, the sharp drop in K' value may be attributed to the presence of an additional double bond in the alkyl chain. This may favour the retention of the complexes in the SC lipids and thus reduce the partitioning of PHY from the SC into the aqueous epidermis.

The permeation flux for MO-based formulations was found to be more dependent on the drug solubility

Table 2
Summary of PHY permeation parameters through the porcine skin ($n = 3$, mean ± S.D.)

Solvent used	Fatty acid used	J ($\mu g\,cm^{-2}\,h^{-1}$)	τ (h)	C_s ($mg\,ml^{-1}$)	$P \times 10^7$ ($cm\,s^{-1}$)	$D' \times 10^4$ (s^{-1})	$K' \times 10^3$ (cm)
MO	Blank	2.5 ± 0.7	9.1 ± 0.0	1.7	4.04 ± 1.1	0.051 ± 0.000	79.6 ± 0.1
	C8	3.2 ± 0.6	13.0 ± 0.4	5.1	1.74 ± 0.3	0.036 ± 0.001	48.8 ± 0.3
	C10	6.6 ± 2.3	8.4 ± 0.7	10.8	1.70 ± 0.6	0.055 ± 0.004	31.0 ± 0.9
	C12	14.0 ± 3.0	8.4 ± 0.3	15.3	2.54 ± 0.5	0.055 ± 0.002	46.4 ± 0.4
	C18:1	13.9 ± 7.1	4.8 ± 1.2	15.9	2.43 ± 1.2	0.101 ± 0.028	37.0 ± 3.3
	C18:2	3.9 ± 1.1	9.2 ± 0.2	15.7	0.68 ± 0.2	0.050 ± 0.001	13.9 ± 0.1
PG	Blank[a]	–	–	71.0	–	–	–
	C8[a]	–	–	116.0	–	–	–
	C10[a]	–	–	80.7	–	–	–
	C12	0.2 ± 0.0	9.4 ± 0.3	88.7	0.01 ± 0.00	0.049 ± 0.001	0.1 ± 0.0
	C18:1	19.7 ± 8.2	8.8 ± 1.4	83.9	0.65 ± 0.27	0.053 ± 0.008	12.5 ± 0.8
	C18:2	2.4 ± 0.9	24.2 ± 0.5	85.3	0.08 ± 0.03	0.019 ± 0.000	4.0 ± 0.0

[a] Drug release was not observed during the course of 34 h.

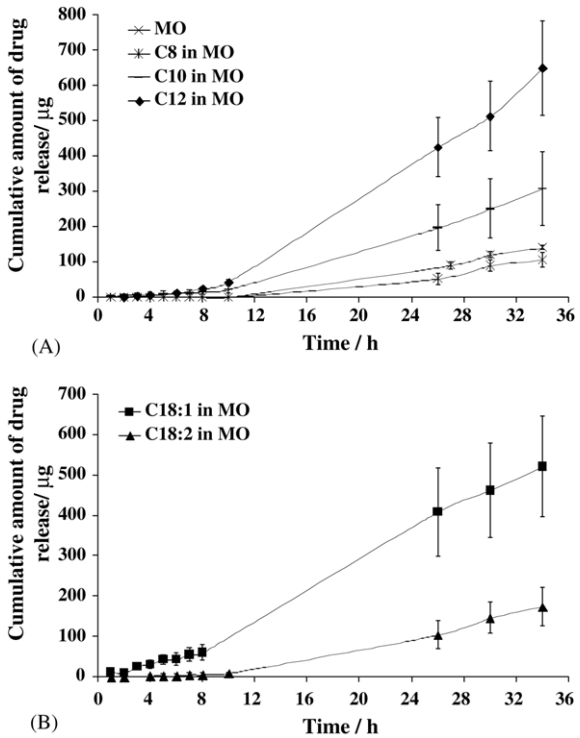

Fig. 4. Cumulative PHY permeated through an area of 1.77 cm^2 of the porcine skin for 34 h in MO-based formulations with (A) saturated fatty acids (B) unsaturated fatty acids. Saturated PHY solutions were used. ($n = 3$, mean ± S.D.).

rather than the permeability coefficient. Generally, there was an increase in the permeation flux with an increase in the drug solubility (C_s), which increased the driving force for drug diffusion. That was why PHY in blank MO had the lowest permeation flux despite having the highest permeability.

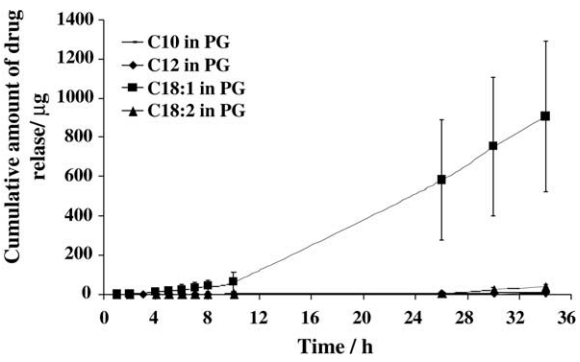

Fig. 5. Cumulative PHY permeated through an area of 1.77 cm^2 of the porcine skin for 34 h in PG-based formulations. Saturated PHY solutions were used. ($n = 3$, mean ± S.D.).

For PG-based formulations, there was no drug permeation from blank PG and formulations with short chain fatty acids. Thus the values of D' and K' cannot be determined. This is because short chain fatty acids formed strong ion pairs with PHY, which affected the partitioning into SC. The change in K' values was similar to that of the apparent partition coefficients ($K_{IPM/PG}$). In the presence of the same fatty acid, the partition coefficient of PHY (K') was much lower in PG-based formulations than in MO-based formulation, indicating that the use of MO as solvent had improved the partitioning of the drug into the skin. In addition, it appeared that the permeation flux of PHY in PG-formulations was not dependent on the drug solubility as the drug had similar solubilities in PG, regardless of the fatty acid present. Yet, the permeation flux was improved greatly when the permeability value was the highest as in the case of C18:1/PG. Thus the permeation of PHY in PG-based formulations was greatly dependent on the ability of the formulation to increase the permeability of the drug.

3.5. Permeation profiles and parameters through the tape stripped skin

Permeation of PHY was carried out with tape stripped skin to evaluate the barrier effect of the SC in both MO- and PG-based formulations. The thickness of SC was greatly reduced after 20 strips with adhesive tape, allowing drug permeation to occur at a much higher rate as shown in Fig. 6. For MO-based formulations, negligible lag time was observed and permeation flux increased with the increasing chain length of fatty acid. Both C12 and C18:1 yielded a high permeation flux of PHY. There was no lag time for PG-based formulations as well. The permeation flux of PHY in C8/PG was lower than that in the blank PG. The reduced permeation of PHY was attributed to the formation of ion pairs, as demonstrated by its lower $K_{IPM/PG}$ value than that of PHY in blank PG. The increased chain length of fatty acid yielded the formation of a weaker ion pair of lower polarity, leading to higher permeation flux of PHY.

Interestingly, two distinctive trends in permeability coefficient were observed when different solvents were employed (Table 3). When MO was used as solvent,

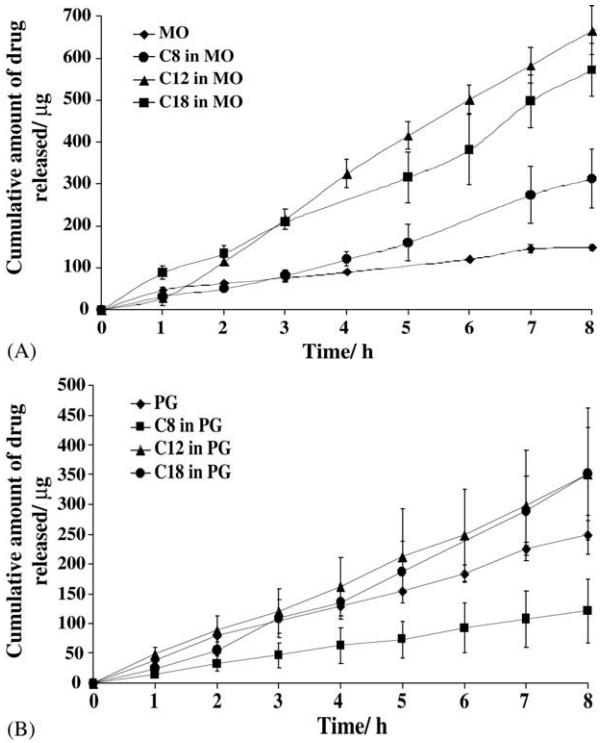

Fig. 6. Cumulative PHY permeated through an area of 1.77 cm^2 of the tape stripped porcine skin for 8 h in (A) MO-based formulations (B) PG-based formulations. Saturated PHY solutions were used. ($n = 3$, mean ± S.D.).

the permeability coefficient decreased with increasing chain length of the fatty acid present. The increase in chain length of the fatty acid increased both the overall size and lipophilicity of drug complex. This reduced both the solubility and mobility of complex in the aqueous epidermis, resulting in lower diffusivity. The decreased permeability coefficient might be mainly attributed to drop in diffusivity. Although the permeability coefficient of PHY in MO was high, its flux was very low due to insufficient driving force derived from its low solubility in MO. As for PG-based formulations, the permeability coefficients were much lower than that of MO-based formulations. As the chain length of fatty acid increased, partition coefficient of PHY increased due to the existence of a weaker ion pair. The partitioning through the SC was more rate-limiting than diffusion of the drug complex through aqueous epidermis for the PG-based formulations. The permeability coefficient of PHY in C8/PG was lower than that of the drug itself due to reduction in partition coefficient after the formation of ion pair. The flux was well related to the permeability coefficient because the solubilities of PHY in the formulations were similar.

Table 3
Summary of PHY permeation parameters through the tape stripped porcine skin ($n = 3$, mean ± S.D.)

Solvent used	Fatty acid used	J ($\mu g\,cm^{-2}\,h^{-1}$)	$P \times 10^7$ ($cm\,s^{-1}$)	ER[a]
MO	Blank	8.55 ± 0.93	13.82 ± 1.50	3.4
	C8	23.73 ± 5.28	12.83 ± 2.85	7.4
	C12	47.96 ± 2.72	8.73 ± 0.49	3.4
	C18:1	39.80 ± 5.48	6.96 ± 0.96	2.9
PG	Blank	12.07 ± 3.94	0.47 ± 0.15	–
	C8	7.95 ± 3.34	0.19 ± 0.08	–
	C12	24.22 ± 7.95	0.76 ± 0.25	114.3
	C18:1	26.30 ± 5.68	0.87 ± 0.19	1.3

[a] ER, enhancement ratio = permeability coefficient with tape stripping/permeability coefficient without tape stripping.

The tape stripped permeation studies also reiterated that the SC was a major barrier in PHY permeation and revealed the extent of the barrier in different formulations. For MO-based formulations, the reduced SC layer increased the permeability of PHY by 2.9–7.4 times (Table 3), depending on the nature of fatty acid used. As for PG-based formulations, the permeability was enhanced by 114 times in the case of C12 and mere 1.3 times for C18:1. It was interesting to note that the enhancement ratios, for C18:1 in PG and MO, were quite comparable and lower than the rest of the acids. This suggested that the lipid structure within the SC posed as a lesser barrier to the PHY/C18:1 complex, regardless of the solvent used. The findings from the tape stripped permeation studies also indicated that in general, the SC was a greater barrier for PHY/acid complexes in PG than in MO.

3.6. Elucidation of enhancement mechanisms of fatty acids

It was clear that the use of different solvents for fatty acids has resulted in entirely different permeation profiles and trends. There are several proposed mechanisms, by which fatty acids can improve drug permeation. To date, no study has clearly related the contribution of each mechanism to the final drug permeation. The relative contribution of each possible enhancement mechanism to the final permeation of

the drug is expected to be dependent on the solvent used as well as the nature of drug to be delivered. Two distinctive solvents for the fatty acids were used in this study. By comparing the behaviour of the drug complexes in these two solvents, it is possible to evaluate the contributions of each possible mechanism and to elucidate the probable permeation pathways.

Drug permeability across the skin is a function of the initial partition into the SC, diffusion across the SC, second partitioning out of the SC into epidermis and the final diffusion across the epidermis. The partitioning processes were estimated by the K' values while the diffusion component was estimated by the D' values. The first step of drug permeation across the skin is the partitioning of the drug between the drug vehicle and the SC. One of the proposed enhancement mechanisms of fatty acids was by increasing the partitioning of the drug into the SC with the formation of lipophilic complexes. The formation of such complexes altered the partitioning between the formulation and the SC, as exemplified by the apparent partition coefficients ($K_{IPM/PG}$) values and calculated K' values. Previous studies (Green and Hadgraft, 1987; Green et al., 1988) have proposed ionic pairing as a means to improve the permeation of hydrophilic drugs. Results by our group showed that the resultant complexes are not always more lipophilic than the drug molecule itself. For short chain fatty acids, the formation of complexes actually lowered the partitioning into the SC. The choice of the solvent also played a role in partitioning. Complexes in MO-based formulations partitioned into the SC more readily than those in PG-based formulations as illustrated by the higher K' values for MO-based formulations.

The lipid bilayers in the SC pose a major barrier to most permeating substances. Another possible enhancement mechanism of fatty acids was to increase permeation by disrupting the lipids bilayers in the SC. A previous study by our group (Wang et al., 2004) has shown that the interaction between the fatty acids and the lipids in the SC was dependent on the solvent used. It was vital that the fatty acids were able to partition into the SC lipid domains. MO allowed short chain fatty acids to disrupt the lipid bilayers in the SC while competing with the partitioning of the acids when long chain fatty acids were used. On the other hand, short chain fatty acids did not cause any lipid disruption in the presence of PG. Long chain fatty acids were able to exert greater lipid disruption in the SC when used along with PG than with MO, as there was no competition between PG and the acids during partitioning into the SC.

The third possible enhancement mechanism for fatty acids was that it might enhance the permeation of the bulk solvent into the SC. Thus, if the drug has a high affinity with the solvent, the increased formulation uptake by the SC should carry a greater amount of drug into the SC. PG together with fatty acids increased the formulation uptake by the SC (Wang et al., 2004). It was found that there was a limited amount of formulation that could be incorporated into intercellular lipids. The maximum uptake by the SC for MO-based formulations, excluding C2/MO and C3/MO, was around 15% of the initial SC weight. As for PG-based formulations with fatty acids, the formulation uptake ranged from 34 to 57%. The high formulation uptake was attributed to uptake by the abundant corneocytes in the SC. Unlike PG-based formulations, MO-based formulations were likely to face difficulties entering the abundant corneocytes in SC, leading to low uptake. Since PHY had a high affinity with PG as shown by the high solubility, solvent drag mechanism may play an important role in enhancing drug permeation in PG-based formulations. This mechanism was not likely to be significant for MO-based formulations as solubility of PHY in blank MO was low and MO did not permeate the SC in large amounts.

Based on the above discussion, it was concluded that the main role of fatty acids in MO-based formulations was to increase the solubility of PHY and thus driving force for PHY permeation through the skin. This implied that the improved permeation rate in the presence of fatty acids was not mainly due to the lipid disruption capabilities of the fatty acids, as maximum permeation did not correspond with maximum disruption of the lipid bilayers. Thus, lipid bilayers were not limiting the permeation of the PHY/acid complexes in the presence of MO. This was reasonable as the MO-based formulations can partition well into the SC and the drug complex can diffuse across the lipid bilayers well when carried in the lipophilic MO. Rather, drug permeation for MO-based formulations was limited by the amount of drug complexes that can partition into the SC, which was dependent on the drug solubility. The low formulation uptake by the SC and the ease in entering the lipid domains further suggested that drug

permeation for MO-based formulations might occur predominantly by the intercellular route within the SC.

As for PG-based formulations, the SC was a major barrier in drug permeation. The hydrophilic PG did not enhance the partitioning of the drug into the SC. The role of the fatty acids in PG-based formulations was to increase the lipophilicity of the drug by forming ion pairs. However, increase in lipophilicity was only achieved with long chain acids such as C18:1. The SC lipids were a barrier for PG-based formulations and the lipophilicity of the ion pairs must be sufficient for them to partition into and diffuse across the SC. This was why the permeation flux of C8 was much lower than that of C12 and C18:1 even when the thickness of the SC was reduced. C18:1 was able to disrupt the lipid bilayers significantly besides increasing the lipophilicity of the drug as seen from the FTIR studies conducted in a previous study (Wang et al., 2004). The disruption of the lipid bilayers was vital to the permeation of the drug complex in PG-based formulations as PG was unable to act as a carrier for drug complex in lipid domains of the SC. Based on the high formulation uptake by the corneocytes and the low partitioning into the lipid domains of the SC, it was postulated that drug permeation for PG-based formulations was *via* the transcellular pathway.

Aungst et al. (1986) reported that the enhancement effects of fatty acids were dependent on the vehicle and the greatest effect was observed when PG was used instead of isopropanol, PEG 400, mineral oil and isopropyl myristate. Based on the findings from this study, a plausible explanation is that drugs in PG-based formulations permeate through the SC via the transcellular pathway while drugs in MO-based formulations permeate through the intercellular pathway. Between these two pathways, the transcellular pathway provides a greater permeation capacity and thus PG as solvent allows greater drug permeation. However, this is valid only if the fatty acid present is sufficiently lipophilic to cross the lipid bilayers. In the case of shorter chain acids such as C8 and C10, the reverse is true. MO serves as a better solvent as it is able to carry the complexes across the SC.

Although fatty acids were extensively used as permeation enhancers, the choice of an optimum fatty acid will depend on the drug used as well as the solvent (Elyan et al., 1996; Stott et al., 2001; Aungst, 1989; Tanojo et al., 1997; Tanojo and Junginger, 1999; Kandimalla et al., 1999). In this study, PHY had the greatest permeation in C12/MO while there was little permeation in C12/PG. The quest for the optimum fatty acid is further complicated by formation of complexes between the fatty acids and the drugs. In the event of complexation, the eventual lipophilicity of the complexes affects its skin permeation, especially through the SC. Based on the results obtained, C18:1 in MO provided the best permeation profile of PHY, delivering 13.9 $\mu g/cm^2$ h with a lag time of 4.8 h. The volume percentage of fatty acid used in our study (16% oleic acid) was lower than that in formulations used by Jenner et al. (1995). Furthermore, MO is commonly used in the cosmetic industry.

4. Conclusion

This study has shed new insights into the use of fatty acids as permeation enhancers in transdermal drug delivery. Possible enhancement mechanisms of fatty acids and PHY permeation pathways were discussed for both MO and PG-based formulations. Short chain fatty acids were able to act as permeation enhancers when carried in a lipophilic solvent like MO. However, there was no drug permeation observed when short chain fatty acids were used in PG. As for long chain fatty acids, both MO and PG yielded high drug permeation. In particular, C18:1/MO yielded fast permeation of PHY with a short lag time, which may be a good vehicle for transdermal delivery of PHY.

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