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Effect of neat and binary vehicle systems on the solubility and cutaneous delivery of piperine

Abdullah Hasan Alomrani ^{a,b,*}, Faisal Ibraheem Alhazza ^a, Khalid Mohammed AlGhamdi ^{c,d}, Gamal Mohamed El Maghraby ^e

^a Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

^b Nanomedicine Unit (NMU-KSU), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

^c Department of Dermatology, College of Medicine, King Saud University, Riyadh, Saudi Arabia

^d Vitiligo Research Chair, College of Medicine, King Saud University, Riyadh, Saudi Arabia

^e Department of Pharmaceutical Technology, College of Pharmacy, University of Tanta, Tanta, Egypt



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ABSTRACT

Vitiligo is a skin disease characterized by depigmentation disorders due to lack of melanin production. Piperine, an alkaloid extracted from black piper, is active in melanocytes proliferation. To achieve this, the drug has to reach the melanocytes which exist in the deep layer of the epidermis. Higher drug concentration can be obtained after application of optimized formulation to skin. Accordingly, the aim of this work is to investigate the effect of vehicles on skin penetration of piperine as the first step in development of optimized formulation. The tested vehicles include ethanol (Eth), propylene glycol (PG), polyethylene glycol 400 (PEG), and oleic acid (OA) and their combinations. Water was used as the control and skin permeation was monitored using rabbit ear model skin. The highest piperine solubility (48.6 mg/ml) and flux (40.8 $\mu\text{g}/\text{cm}^2 \text{ h}$) was achieved by Eth and the lowest piperine flux (1.17 $\mu\text{g}/\text{cm}^2 \text{ h}$) was reported for PEG. PG and OA showed piperine flux values comparable to that of the control. Among different combination systems, Eth-OA (75:25) binary system had the highest piperine flux (59.3 $\mu\text{g}/\text{cm}^2 \text{ h}$) followed by Eth-OA (50:50) (32.3 $\mu\text{g}/\text{cm}^2 \text{ h}$) and PG-OA (90:10) (22.7 $\mu\text{g}/\text{cm}^2 \text{ h}$). The study thus introduced a vehicle system as the first step in the development of topical formulation of piperine.

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1. Introduction

Vitiligo is one of the most common acquired skin pigmentation disorders, affecting 0.5–2% of the population worldwide regardless of age, gender, color or ethnic origin (Ongena et al., 2004; Roy, 2017). The disease is characterized by the development of milky white patches, usually with a typical symmetrical distribution pattern (Ongena et al., 2004). The disease results from reduction or disappearance of melanin. Many authors have confirmed the dis-

appearance of melanocytes (active or inactive) from the epidermis of vitiliginous macules (Nordlund and Ortonne, 2006).

Majority of studies on treatment of vitiligo have focused on symptomatic treatment strategies with the aid of phototherapy in order to stabilize the disease and repigment the achromic patches (Ongena et al., 2004). These strategies comprise photochemotherapy, phototherapy with ultraviolet radiation (broadband and narrowband), corticosteroids, immunomodulators, vitamin D3 analogues, and surgical intervention (Falabella and Barona, 2008; Kostovic and Pasic, 2005). Unfortunately, these standard treatment strategies have been reported to achieve limited success (Nordlund and Ortonne, 2006). Accordingly, treatment regimen that focuses on repopulation of macules with melanocytes has been considered as an effective way to treat vitiligo. Many clinical and experimental trials have examined the use of natural products for vitiligo. Lin et al. (1999) studied the effect of black pepper extract on the proliferation of mouse melanocytes. This extract was shown to stimulate growth activity in cell culture of melanocytes. Black pepper, widely used with spice flavors, is listed by the US Food and Drug Administration (FDA) as Generally Recognized as

* Corresponding author at: Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457 – 11451, Riyadh, Saudi Arabia.

E-mail address: aomrani@ksu.edu.sa (A.H. Alomrani).

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Safe (GRAS). It contains 5–9% of active alkaloid piperine (Bajad et al., 2002). Faas et al. (2008) have noticed that piperine and its synthetic derivatives can stimulate pigmentation in mice skin especially when combined with ultraviolet radiation treatment. Such potential effect of piperine attracted our attention to explore the skin delivery characteristic of piperine using different solvent systems.

Piperine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]piperidine (Fig. 1), has molecular formula $C_{17}H_{19}NO_3$, molecular weight 285.34 Dalton, and pK_a 12 at 18 °C. Piperine is slightly soluble in water (Maryadele and Neil, 2006).

One of the main criteria of successful pharmaceutical formulation is to deliver the therapeutic substance to the target organ at therapeutically acceptable levels with the least harm and/or side effects on the patients (Kreilgaard, 2002). The target site for most dermatological diseases is located in the viable epidermis or upper dermis. For vitiligo disorder, piperine must permeate stratum corneum (SC) and reach basal layer (the location of melanocyte) to exert its action. Therefore, passing the SC is an important aspect of topical drug therapy. Basically, two principal approaches have been introduced to optimize the skin permeability of topically applied drugs. The first approach relies on increasing the thermodynamic activity of the drug in a vehicle which could be achieved by increasing its concentration (Kunst and Lee, 2016). The second approach is based on reducing the barrier function of the skin (Rambharose et al., 2017). Alternative chemicals have augmented the permeation of substances through skin and thus been termed “chemical permeation enhancers”. Among these chemical permeation enhancers, water, ethanol, propylene glycol, polyethylene glycol, and oleic acid were selected to investigate their effects on piperine permeation through the skin. The selection was based on their potential to enhance the permeation of a number of drugs through skin (Lopes et al., 2015) and their frequency of use in skin products.

Accordingly, the main objective of this study is to investigate the effect of these vehicles in neat and combined form on the cutaneous delivery of piperine. This can be considered as the first step in the development of optimized topical formulation of piperine.

2. Materials and methods

2.1. Materials

Piperine was purchased from Sigma-Aldrich Company, Steinheim, Germany. Oleic acid (OA) was obtained from LOBA Chemi PVT. Ltd., India. Propylene glycol (PG) was from WINLAB, UK. Polyethylene glycol 400 (PEG) was imported from Fluka AG, Germany. Methanol, acetonitrile, and ethanol 99% (Eth) were obtained from

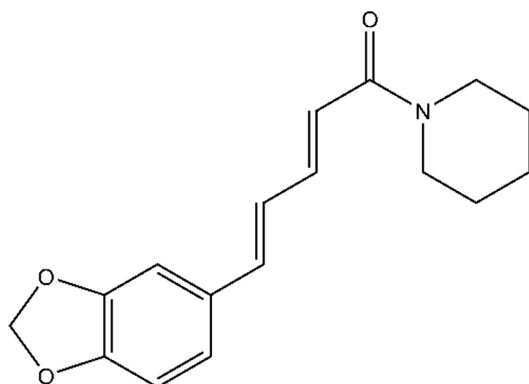


Fig. 1. Piperine chemical structure.

BDH laboratory supplies, Poole, England. All other reagents and chemicals were of analytical grade.

2.2. High pressure liquid chromatography (HPLC)

Piperine was analyzed using HPLC method of assay. The HPLC system consists of a Waters Model 1515 HPLC pump, a Waters autosampler Model 717 plus, and a Waters 2487 dual absorbance UV detector (Waters Inc., Bedford, MA, USA) governed by a computer running Empower software (version 1154). The detector wavelength was set at 309 nm. Separation was achieved by isocratic elution with a mobile phase of acetonitrile and water (52:48) adjusted to pH 3.5 with glacial acetic acid. This was pumped at a flow-rate of 1.2 ml/min at ambient temperature through a C_{18} analytical, μ -Bondapack column (150 mm length \times 4.6 mm i.d., 10 μ m particle size) (Badran et al., 2015).

2.3. Equilibrium solubility

Piperine solubility was conducted by adding excess amounts of piperine to the vehicle systems (water, ethanol, oleic acid, polyethylene glycol and propylene glycol alone and/or combined as ethanol-oleic acid, ethanol-propylene glycol, and propylene glycol-oleic acid) followed by equilibration in a shaking water bath (Julabo – SW 22) for 7 days; it was maintained at 32 °C (mimic skin temperature) with shaking rate of 80 rpm. The excess powder was then removed by centrifugation at 8000 rpm for 10 min before determining the drug content by HPLC after suitable dilution.

2.4. Preparation of piperine in vehicle

Saturated solution of piperine was prepared using different vehicle systems (water, ethanol, oleic acid, polyethylene glycol and propylene glycol alone and/or combined as ethanol-oleic acid, ethanol-propylene glycol, and propylene glycol-oleic acid). The saturated solutions of piperine were equilibrated by continuous shaking in water bath (Julabo – SW 22) adjusted at 32 °C (mimic skin temperature) for 7 days at 80 rpm. Excess powder was added to maintain saturation.

2.5. In vitro drug release

The in vitro release experiments were conducted using the FDC-6 Transdermal Diffusion Cell Drive Console (Logan Instrument Corp., Somerset, NJ, USA). The artificial membrane (Cellulose Tubing, Spectrum Medical Industries, USA, with cut off of 8000–12,000) was soaked in the receptor fluid for one hour before the test was performed to hydrate the membrane. The membrane was mounted between the donor and receptor compartments of the diffusion cells. These cells have a diffusional area of 1.7 cm^2 with each receptor compartment having a capacity of 12 ml. The receptor fluid was a 30% v/v Eth in water (Fang et al., 2008). This is believed to maintain sink conditions. The heater was adjusted to maintain the surface temperature of the membrane at 32 ± 1 °C to mimic skin permeation experimental conditions. The tested systems (1 ml) were loaded into the donor compartment before occluding the donor compartments using a parafilm. Receptor samples (5–10 ml) were collected at predetermined time intervals (1, 2, 3, 4, 6, 10 and 24 h) and fresh receptor fluid was used to compensate the collected samples. Piperine content in each sample was determined by HPLC.

2.6. Skin permeation studies

The ear skin of the rabbit (healthy male rabbits weighing about 2 kg) was used as a skin model for in vitro skin permeation of

piperine (Nicoli et al., 2008). The inner part of the ear was peeled carefully from cartilage of the ear, cleaned and directly applied to the Franz Diffusion Cell (FDC-6). The full thickness of the rabbit ear skin was mounted between the donor and receptor compartment with the stratum corneum side facing the donor compartment. All formulations used for permeation study were in solution forms. The receptor fluid was similar to that used in release studies. The formulations (1 ml) were loaded in the donor compartments. Samples (5–10 ml) were collected at predetermined time intervals (1, 2, 3, 4, 6, 8, 10, 12, and 24 h). Fresh receptor fluid was used to compensate the collected samples. Piperine content in each sample was determined by HPLC. The cumulative amount of piperine permeated was plotted as a function of time to produce the permeation profiles. These profiles were used to calculate the transdermal piperine flux (J), which was obtained from the slope of the regression line fitted to the linear portion of the profile. The lag time of the flux at steady state was calculated from the intercept. Lag time was taken from the x-axis interception of the extrapolated line of the steady state flux (Scheuplein, 1978). The permeability coefficient (K_p) was calculated according to the following equation (Scheuplein, 1978):

$$J_{ss} = K_p C$$

$$K_p = J_{ss}/C$$

where J_{ss} is the piperine flux at steady state and C is piperine concentration in the donor compartment.

The ratio of piperine steady state flux of the investigated vehicle to that of water was used to represent the Enhancement Factor (ER) of the vehicle.

2.7. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) with Tukey's multiple-comparison. Statistical differences yielding $P \leq .05$ were considered significant.

3. Results

3.1. Effect of single vehicle system on piperine solubility and flux

The solubility of piperine in different pure vehicles is shown in Table 1. The solubility of the drug depends on the type of vehicle with water showing the least capacity to solubilize the drug. Eth was able to dissolve the greatest amount of piperine when compared with the other vehicles ($p < .05$). The vehicles' capacities to dissolve piperine were ranked as Eth > PEG > PG > OA \gg water (Table 1).

The transdermal permeation profile of piperine after application of saturated drug solution in different pure vehicles is shown in Fig. 2. The calculated permeation parameters are shown in Table 1. These results revealed that the values of piperine flux using water, OA, and PG were comparable (4.83, 5.59, and 5.52 $\mu\text{g}/\text{cm}^2 \text{ h}$,

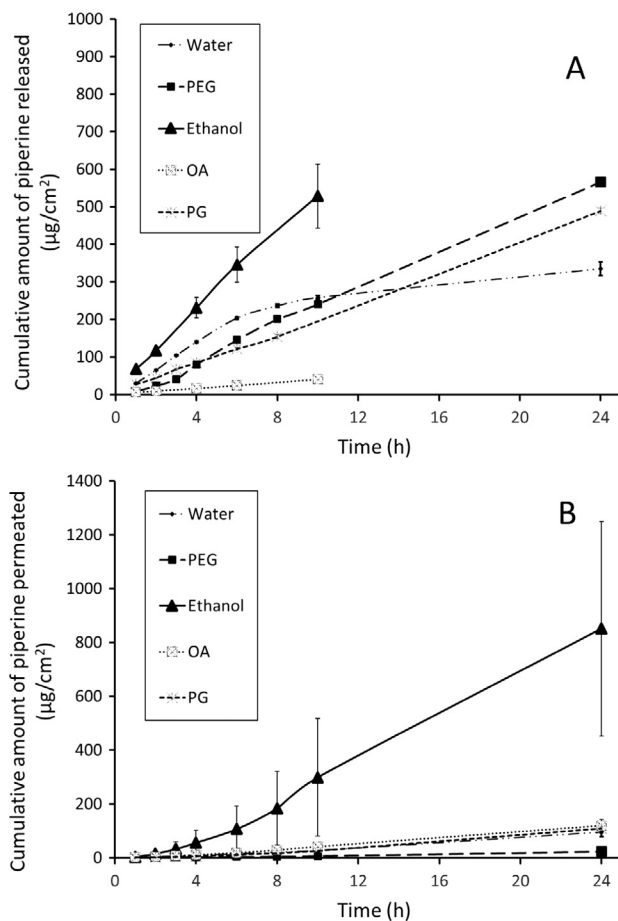


Fig. 2. Release (A) and permeation (B) profiles of piperine dissolved in different solvents ($n = 3$).

respectively). Pure ethanol recorded the highest value of piperine flux through skin (40.8 $\mu\text{g}/\text{cm}^2 \text{ h}$) when compared with other vehicles ($p < .05$). PEG recorded the lowest piperine flux of 1.17 $\mu\text{g}/\text{cm}^2 \text{ h}$ compared to the rest of the investigated vehicles (Table 1 and Fig. 2). The Lag time of piperine did not differ significantly by changing the vehicle ($P > .05$, Table 1).

The permeability coefficient (K_p) of piperine varied according to the nature of the vehicle. The highest value of K_p was recorded with water and the lowest one was recorded with PEG (Table 1).

The Higuchi's theory (Higuchi and Higuchi, 1960) declared that the thermodynamic activity of a compound in solution form is considered a significant factor that influences the release and flux of the compound from the Vehicle. Thermodynamic activity is directly proportional to the concentration of a compound under the condition that the effects of other factors is negligible. Accordingly, the maximum thermodynamic activity of a compound could

Table 1

Effect of different types of solvents on the solubility, flux, release and skin permeation parameters of piperine. Results are presented as mean (SD) of three replicates.

Vehicle	Solubility* (mg/ml)	Flux ($\mu\text{g}/\text{cm}^2 \text{ h}$)	Lag time (h)	K_p ** (cm/h)	EF***	Release ($\mu\text{g}/\text{cm}^2 \text{ h}$)
Water	0.017 (0.002)	4.83 (0.90)	4.1 (0.1)	284.12	1	38.35 (6.7)
PEG	22.6 (0.03)	1.17 (0.72)	3.3 (3.4)	0.052	0.24	21.56 (1.9)
PG	12.7 (2.07)	5.52 (0.86)	3.5 (0.4)	0.44	1.1	20.52 (1.3)
OA	6.1 (0.54)	5.59 (1.13)	2.7 (0.3)	0.91	1.1	5.89 (1.3)
Eth	48.6 (3.31)	40.8 (15.9)	3.6 (2.3)	0.84	8.5	59.97 (25.5)

* Solubility of piperine in different solvents at 32 °C.

** K_p : Permeability coefficient calculated from the mean value of flux and saturation concentration of piperine for each vehicle system.

*** EF: Enhancement Factor obtained by relating the steady state flux of piperine dissolved in an investigated vehicle to that of water.

be achieved when the compound has reached its saturated concentration in a solution. Furthermore, at a constant drug concentration, the thermodynamic activity of a drug reduces as its solubility in a vehicle system increases (Levang et al., 1999).

Therefore, the study of the release rate of piperine from the investigated Vehicles through a semipermeable membrane was conducted. The results of the release test showed that Eth and water recorded the highest release rates of piperine, 59.9 $\mu\text{g}/\text{cm}^2 \text{ h}$ and 38.3 $\mu\text{g}/\text{cm}^2 \text{ h}$, respectively, compared with the other Vehicles. PG and PEG recorded 20.5 $\mu\text{g}/\text{cm}^2 \text{ h}$ and 21.5 $\mu\text{g}/\text{cm}^2 \text{ h}$, respectively. The lowest piperine release rate was observed with the OA Vehicle, 5.89 $\mu\text{g}/\text{cm}^2 \text{ h}$, Table 1.

3.2. Effect of binary vehicle system on piperine solubility and flux

Binary systems of PG-OA, PG-Eth, and Eth-OA were prepared at different ratios to explore the impact of solvents combination and their ratio on the solubility and permeability of piperine through skin. Table 2 presents the results of piperine solubility and skin permeation parameters obtained from different binary systems.

The presented data of PG-OA binary system (Table 2) indicated that the solubility of piperine is significantly increased by mixing these two solvents together as compared to the solubility results of each solvent alone (Table 1). PG-OA (50:50) ratio showed the maximum amount of piperine dissolved (20.1 mg/ml), and the solubility of piperine tends to decrease beyond this ratio. For Eth-PG binary system, the solubility data in Table 2 showed no further enhancement in piperine flux when PG was added to Eth as compared to neat Eth. On the other hand, mixing Eth with OA brought significant changes in piperine solubility and flux through skin. It is clear that the solubility of piperine is significantly increased by mixing Eth and OA together. Eth-OA (25:75) and Eth-OA (50:50) ratios showed the maximum amount of piperine dissolved (43.9 and 43.7 mg/m, respectively), and the solubility of piperine decreased beyond these ratios (Table 2).

Regarding piperine flux, it is obvious that the mixing of OA with PG resulted in significant enhancement in permeation rate of piperine through skin (8.9–22.7 $\mu\text{g}/\text{cm}^2 \text{ h}$) ($p < .05$) as compared to that of neat OA (5.59 $\mu\text{g}/\text{cm}^2 \text{ h}$) or PG (5.52 $\mu\text{g}/\text{cm}^2 \text{ h}$) (Tables 1 and 2, and Fig. 3). The maximum flux of piperine was recorded for PG-OA (90:10) binary system (22.7 $\mu\text{g}/\text{cm}^2 \text{ h}$), followed by PG-OA (75:25), 19.9 $\mu\text{g}/\text{cm}^2 \text{ h}$, PG-OA (50:50), 14.7 $\mu\text{g}/\text{cm}^2 \text{ h}$, and PG-OA (25:75), 8.9 $\mu\text{g}/\text{cm}^2 \text{ h}$. Plotting piperine flux against concentration of OA in PG-OA binary system revealed a line with negative slope, which means that piperine flux exhibited its maximum value at low concentration of OA (Fig. 3A). Further increase in the concentration of OA resulted in negative impact on the flux of piperine from PG-OA binary system. The permeability coefficient (K_p) of piperine increased with PG-OA binary system. The maximum K_p value of piperine was recorded for binary system of PG-

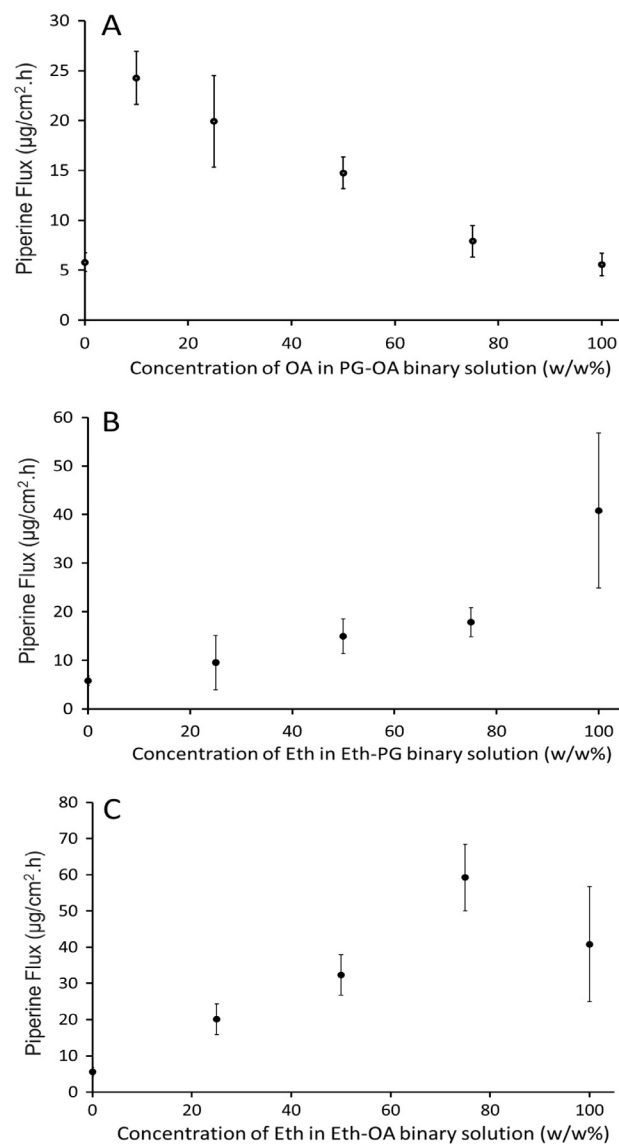


Fig. 3. Piperine flux values change with changing vehicles concentrations in binary systems; (A) OA in PG-OA; (B) Eth in Eth-PG; (C) Eth in Eth-OA (n = 3).

OA (90:10); beyond this concentration, the K_p value of piperine tends to decrease.

The flux of piperine in case of Eth-OA and Eth-PG systems had Eth concentration dependent effect (Table 2, Fig. 3B and C). This effect varied according to the vehicle system used. In case of Eth-

Table 2

Effect of different ratios of binary vehicle systems on the solubility and skin permeation parameters of piperine. Results are presented as mean (SD) of three replicates.

Vehicle system	Solubility (mg/ml)	Flux ($\mu\text{g}/\text{cm}^2 \text{ h}$)	Lag time (h)	K_p^* (cm/h)	EF ^{**}
PG-OA (90:10)	9.4 (0.9)	22.7 (6.4)	1.8 (0.4)	2.42	4.7
PG-OA (75:25)	8.6 (0.2)	19.9 (4.5)	0.8 (0.1)	2.32	4.1
PG-OA (50:50)	20.1 (0.6)	14.7 (1.6)	0.4 (0.3)	0.73	3.1
PG-OA (25:75)	16.2 (0.4)	8.9 (1.7)	0.3 (0.1)	0.55	1.8
Eth-PG (75:25)	37.2 (0.3)	17.9 (2.9)	2.8 (0.4)	0.48	3.69
Eth-PG (50:50)	44.2 (3.2)	14.9 (3.6)	5.1 (0.4)	0.34	3.09
Eth-PG (25:75)	24.5 (0.4)	9.5 (5.6)	3.0 (1.9)	0.39	1.98
Eth-OA (25:75)	43.9 (1.5)	20.1 (4.2)	0.5 (0.2)	0.46	4.2
Eth-OA (50:50)	43.7 (0.5)	32.3 (5.6)	0.5 (0.5)	0.74	6.7
Eth-OA (75:25)	35.4 (0.2)	59.3 (9.2)	0.6 (0.5)	1.67	12.3

* K_p : Permeability coefficient calculated from the mean value of flux and saturation concentration of piperine for each vehicle system.

** EF: Enhancement Factor.

OA binary system, the maximum piperine flux was achieved with Eth-OA (75:25) ratio. The flux of piperine at the Eth-OA (75:25) binary system represented a 1.45-fold of piperine flux as compared to pure Eth. In contrast, the magnitude of piperine flux when Eth is being mixed with PG at different ratios did not exceed what was observed with neat Eth (Fig. 3B).

The binary systems of Eth-OA and PG-OA recorded the lowest Lag time for piperine flux (Table 2). The presence of Eth and OA together in a vehicle system at different ratios significantly ($p < .05$) reduced the Lag time when compared with that of neat solvent (Tables 1 and 2). The maximum recorded flux of piperine was observed in a binary system of Eth-OA (75:25) (Table 2).

3.3. Piperine solubility in vehicles vs piperine flux

The impact of drug solubility on its flux through biological membrane has been discussed (Mustapha et al., 2011). It was concluded that the ability of a vehicle to deliver a drug across biological membrane (such as skin) is influenced by the extent of drug solubility in the vehicle. According to the data generated from the current study and as shown in Fig. 4, a moderate correlation ($R^2 = 0.4$) was shown between piperine solubility and its flux. This indicated that the solubility of the drug has a low impact on its flux through biological membrane.

4. Discussion

The solubility of piperine in different vehicles has been determined at 32 °C. This temperature was chosen to mimic the skin permeation experimental conditions. The recorded solubility data reflected the lipophilic nature of the drug with the lowest solubility being recorded in water and the highest solubility being noticed in Eth. Combination of vehicles showed synergistic increase in drug solubility due to the co-solvent effect.

It was established that the thermodynamic activity of a compound is directly proportional to its concentration. Accordingly, the maximum thermodynamic activity of a compound could be achieved at its saturated concentration in a solution (Kunst and Lee, 2016). To investigate the effect of vehicle on skin permeation of drug, the thermodynamic activity was kept constant by employing saturated solution of the drug in each vehicle. This ensures that any difference in transdermal flux will depend on the nature of vehicle.

The transdermal permeation parameters depended on the type of vehicle. For majority of the investigated solvents, no correlation

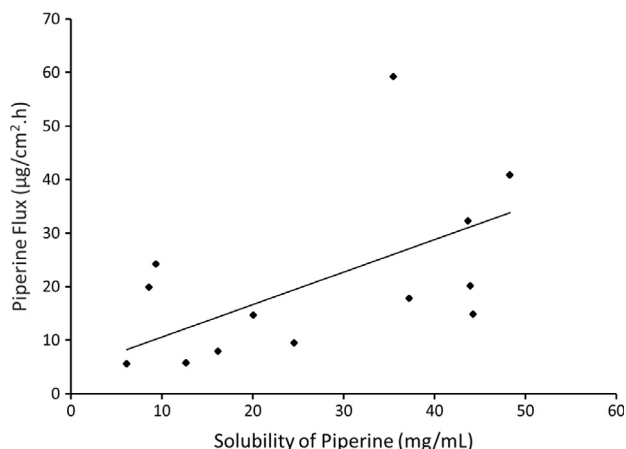


Fig. 4. Correlation between piperine flux and its solubility in different vehicles at different ratios ($n = 3$), $R^2 = 0.4$.

was observed between the solubility and flux of piperine; however, an inverse relationship was observed in case of PEG. Despite the high piperine solubility in PEG (22.6 mg/ml), this vehicle showed the lowest value of piperine steady state flux ($1.17 \mu\text{g}/\text{cm}^2 \text{ h}$) ($p < .05$). This finding is in agreement with Kaushik et al. (2010) who suggested that PEG works, most likely, as a permeation retardant instead of working as a permeation promoter. It was proposed that the highest permeation of a drug is more likely obtained with vehicles that the drug has the least solubility on them, taking into consideration the fact that the vehicle system does not alter the membrane integrity (Gao and Singh, 1998).

Comparing the transdermal flux with the release rate, there was no correlation. For example, comparable flux was recorded from water and PG, but the release rate of piperine from water was greater than that of PG. The high release value of piperine from water could be attributed to the partitioning effect in which piperine prefers to leave water compartment and migrates to receptor compartment (30% ethanolic solution). Moreover, the low solubility of piperine in water supported this suggestion.

The results of piperine flux using PG as a vehicle in this study is in agreement with results of other studies using aspirin (Levang et al., 1999) and nicardipine (Krishnaiah et al., 2002).

The flux and release results of piperine using OA as vehicle (Table 1) revealed that OA has the ability to deliver almost all the librated amount of piperine across the skin, which reflects the power of OA to facilitate piperine permeation through the skin. The low release rate value of piperine from OA when compared with that of water could be attributed to the solubility reason. Piperine has affinity towards OA 360 times more than water as it is reflected from piperine solubility data.

In case of Eth, the enhancement factor (ER) of Eth recorded the highest value (8.5), whereas PEG recorded the lowest value (0.24) when compared with control (water). Such finding of Eth is in agreement with other studies (Krishnaiah et al., 2002; Levang et al., 1999; Panchagnula et al., 2001).

The enhancement of drug permeation through skin in the presence of Eth has been attributed to a number of phenomena, including increase in the thermodynamic activity of the drug due to evaporation of Eth, a phenomenon known as push effect (Kadir et al., 1987; Panchagnula et al., 2001), disruption of the SC integrity by extracting the intracellular lipids of SC, increasing the fluidity of the lipid bilayer, and dragging effect “pull effect” (Panchagnula et al., 2001).

It was stated that K_p of drug through membrane is a parameter influenced by the flux of the drug through the membrane and its solubility in vehicle (Krishnaiah et al., 2002; Ree et al., 2007). The permeability coefficient of drug is directly proportional to the drug's flux and inversely proportional to its solubility. Accordingly, the high K_p value of water could be attributed to the low solubility of piperine in water.

The earlier discussed results revealed that Eth was the best vehicle for piperine with respect to transdermal delivery, whereas PEG was the worst one. However, Eth alone is not recommended to be applied topically due to its side effect on skin. Moreover, further enhancement in piperine permeation could be achieved when another vehicle is combined with Eth. Increasing the skin partitioning for one chemical permeation enhancer by another enhancer was proposed as a mechanism illustrating the effectiveness of using combination of chemical enhancers in the skin formulations. Therefore, the effect of combined vehicles (mixture of two vehicles at different ratios) on the solubility and skin permeation of piperine was investigated.

The flux of piperine in case of Eth-OA and Eth-PG systems had Eth concentration dependent effect. This effect varied according to the vehicle system used. The flux of piperine at the Eth-OA (75:25) binary system represented a 1.45-fold of piperine flux as

compared to pure Eth. In contrast, the flux of piperine using Eth-PG at different ratios did not exceed what was observed with neat Eth. This is in disagreement with other studies (Panchagnula et al., 2001; Levang et al., 1999). However, these studies showed that the maximum flux of aspirin and naloxone was achieved with Eth-PG system containing 80 and 66% of Eth, respectively, and no more increase in their flux beyond these concentrations.

The transdermal flux obtained from binary systems of PG-OA (90:10) and Eth-OA (75:25) revealed that the optimum permeation enhancement activity can be recorded for OA at its low concentration in a binary system. The positive impact of low percentage of OA on the drug permeation through skin was observed in other studies (Gao and Singh, 1998).

The mechanism of individual or combined skin permeation enhancers has been described in many reports. It was suggested that OA may work on the nonpolar route of the SC. The molecular configuration of OA (cis-configuration) may affect the tightness and packing of the lipids within SC. The entrance of OA into the SC may perturb the crystalline packing of the SC lipids; such perturbation enhances the lipid fluidity and membrane permeability and forms channels with weak resistance to drug migration across the SC (Yamane et al., 1995; Williams and Barry, 2004). This phenomenon has been supported by the DSC results, in which T_m of the SC lipids has reduced in the presence of OA (Walker and Hadgraft, 1991; Yamane et al., 1995).

The mechanisms by which Eth enhances the permeation of a drug through skin are suggested by a number of authors. These mechanisms include the ability of Eth to solubilize low aqueous soluble drug; such action leads to maintain drug in solution and prevents depletion of soluble drug from donor compartment (Kurihara-Bergstrom et al., 1990). Eth can undergo rapid permeation through SC fluidizing the lipids of the membrane (Mollgaard and Hoelgaard, 1983). Eth, being a volatile vehicle, may evaporate from the donor compartment causing alteration in thermodynamic activity of drug in solution. It is well established that the thermodynamic activity plays a significant role in drug release and partitioning into skin membrane (Kadir et al., 1987).

The presence of two skin permeation enhancers in a system may cause a synergistic effect on drug permeation through skin. It was suggested that PG-OA binary system acts through synergistic effect in which PG interacts with the head group region of the SC composition. Such interaction facilitates partitioning of OA into lipid alkyl domain within the SC (Larrucea et al., 2001). Another report suggested that co-vehicle, such as PG appears to change proteins domain within SC by vehicle effect. Therefore, the presence of chemical permeation enhancer, that works on lipid domain in SC, with PG may get a synergistic effect in which PG reduces the resistance of proteinaceous horny cells and the other chemical enhancer reduces the integrity of the intercellular lipid bilayers (Goodman et al., 1989). The absence of synergistic permeation effect of Eth-PG binary system could be attributed to the similarity of the mechanism of permeation enhancement action. It was believed that PG effect is comparable to what Eth performs (Williams and Barry, 2004).

5. Conclusion

In conclusion, piperine flux and solubility are dramatically changed according to the vehicles systems. The vehicle systems of neat solvents exhibited differences in piperine solubility and flux through skin. Eth was superior with respect to the solubilizing capacity and transdermal delivery efficiency. Neat PG and OA showed only a trend of increased transdermal flux when compared with that obtained from aqueous solution. In contrast, PEG showed a penetration retarding effect. Combination of OA with either Eth

or PG resulted in synergistic skin penetration enhancing effect for piperine. This study can be considered as the base for development of transdermal delivery formulation for piperine.

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References

- Badran, M., Alhazza, F.I., Alomrani, A.H., 2015. Development of piperine loaded deformable liposomes - a new vesicular carrier of piperine: characterization and ex vivo skin penetration studies. *Lat. Am. J. Pharm.* 34, 244–252.
- Bajad, S., Johri, R.K., Singh, K., Singh, J., Bedi, K.L., 2002. Simple high-performance liquid chromatography method for the simultaneous determination of ketoconazole and piperine in rat plasma and hepatocyte culture. *J. Chromatogr. A* 949, 43–47.
- Faas, L., Venkatasamy, R.C., Hider, A.R., Young, S.A., 2008. In vivo evaluation of piperine and synthetic analogues as potential treatments for vitiligo using a sparsely pigmented mouse model. *Br. J. Dermatol.* 158, 941–950.
- Falabella, R., Barona, M.L., 2008. Update on skin repigmentation therapies in vitiligo. *Pigment Cell Melanoma Res.* 22, 42–65.
- Fang, J.Y., Fang, C.L., Liu, C.H., Su, Y.H., 2008. Lipid nanoparticles as vehicles for topical psoralen delivery: solid lipid nanoparticles (SLN) versus nanostructured lipid carriers (NLC). *Eur. J. Pharm. Biopharm.* 70, 633–640.
- Gao, S., Singh, J., 1998. Effect of oleic acid/ethanol and oleic acid/propylene glycol on the in vitro percutaneous absorption of 5-fluorouracil and tamoxifen and the macroscopic barrier property of porcine epidermis. *Int. J. Pharm.* 165, 45–55.
- Goodman, M., Brian, W., Barry, B.W., 1989. Lipid-protein-partitioning (LPP) theory of skin enhancer activity: finite dose technique. *Int. J. Pharm.* 57, 29–40.
- Higuchi, W.I., Higuchi, T., 1960. Theoretical analysis of diffusional movements through heterogeneous barriers. *J. Pharm. Sci.* 49, 598–606.
- Kadir, R., Stempler, D., Liron, Z., Cohen, S., 1987. Delivery of theophylline into excised human skin from alkanolic acid solutions: a “push-pull” mechanism. *J. Pharm. Sci.* 76, 774–779.
- Kaushik, D., Costache, A., Michniak-Kohn, B., 2010. Percutaneous penetration modifiers and formulation effects. *Int. J. Pharm.* 386, 42–51.
- Kostovic, K., Pasic, A., 2005. New treatment modalities for vitiligo focus on topical immunomodulators. *Drugs* 65, 447–459.
- Kreilgaard, M., 2002. Influence of microemulsions on cutaneous drug delivery. *Adv. Drug Deliv. Rev.* 54 (Suppl. 1), S77–S98.
- Krishnaiah, Y., Satyanarayana, V., Karthikeyan, R.S., 2002. Effect of the solvent system on the in vitro permeability of nifedipine hydrochloride through excised rat epidermis. *J. Pharm. Pharm. Sci.* 5, 124–130.
- Kunst, A., Lee, G., 2016. Release and skin permeation of scopolamine from thin polymer films in relation to thermodynamic activity. *J. Pharm. Sci.* 105, 1496–1500.
- Kurihara-Bergstrom, T., Knutson, K., Noble, L.J., Goates, C.Y., 1990. Percutaneous absorption enhancement of an ionic molecule by ethanol-water systems in human skin. *Pharm. Res.* 7, 762–766.
- Larrucea, E., Arellano, A., Santoyo, S., Ygartua, P., 2001. Combined effect of oleic acid and propylene glycol on the percutaneous penetration of tenoxicam and its retention in the skin. *Eur. J. Pharm. Biopharm.* 52, 113–119.
- Levang, A.K., Zhao, K., Singh, J., 1999. Effect of ethanol: propylene glycol on the in vitro percutaneous absorption of aspirin, biophysical changes and macroscopic barrier properties of the skin. *Int. J. Pharm.* 181 (255), 263.
- Lin, Z., Hoult, R., Bennett, D., Raman, A., 1999. Stimulation of mouse melanocyte proliferation by Piper nigrum fruit extract and its main alkaloid, piperine. *Planta Med.* 65, 600–603.
- Lopes, L.B., Garcia, M.T., Bentley, M.V., 2015. Chemical penetration enhancers. *Ther. Deliv.* 6, 1053–1061.
- Maryadele, J., Neil, O., 2006. Merck Index an Encyclopedia of Chemicals, Drugs and Biologicals, 14 ed. Merck Research Laboratories Division of Merck and Co. Inc., p. 7469.
- Mollgaard, B., Hoelgaard, A., 1983. Vehicle effect on topical drug delivery. I. Influence of glycols and drug concentration on skin transport. *Acta Pharm. Suec.* 20, 433–442.
- Mustapha, R.B., Lafforgue, C., Fenina, N., Marty, J.P., 2011. Influence of drug concentration on the diffusion parameters of caffeine. *Indian J. Pharmacol.* 43 (2), 157–162.
- Nicoli, S., Padula, C., Aversa, V., Vietti, B., Wertz, P.W., Millet, A., Falson, F., Govoni, P., Santi, P., 2008. Characterization of rabbit ear skin as a skin model for in vitro transdermal permeation experiments: histology, lipid composition and permeability. *Skin Pharmacol. Physiol.* 21, 218–226.
- Nordlund, J., Ortonne, J., 2006. Genetic hypomelanoses: acquired de-pigmentation. In: Nordlund, J., Boissy, R.E., Hearing, V.J. (Eds.), *The Pigmentary System: Physiology and Pathophysiology*. second ed. Blackwell Publishing, Oxford, pp. 551–598.

- Ongenaes, K., Geel, N., Schepper, S., Haeghen, Y., Naeyaert, J., 2004. Management of vitiligo patients and attitude of dermatologists towards vitiligo. *Eur. J. Dermatol.* 14, 177–181.
- Panchagnula, R., Salve, P.S., Thomas, N.S., Jain, A.K., Ramarao, P., 2001. Transdermal delivery of naloxone: effect of water, propylene glycol, ethanol and their binary combinations on permeation through rat skin. *Int. J. Pharm.* 219, 95–105.
- Rambharose, S., Kalhapure, R.S., Jadhav, M., Govender, T., 2017. Exploring unsaturated fatty acid cholesteryl esters as transdermal permeation enhancers. *Drug Deliv. Transl. Res.* 7, 333–345.
- Ree, Y.S., Huh, J.Y., Park, C.W., Nam, T.Y., Yoon, K.R., Chi, S.C., Park, E.S., 2007. Effects of vehicles and enhancers on transdermal delivery of clebopride. *Arch. Pharm. Res.* 30, 1155–1161.
- Roy, A.K., 2017. Vitiligo: a white patch that affects the soul. *Pigment. Disorders* 4, 254.
- Scheuplein, R., 1978. Site variations in diffusion and permeability. *Physiol. Pathophysiol. Skin* 5, 1731–1752.
- Walker, M., Hadgraft, J., 1991. Oleic acid a membrane fluidizer for fluid within the membrane. *Int. J. Pharm.* 71, R1–R4.
- Williams, A.C., Barry, B.W., 2004. Penetration enhancer. *Adv. Drug Deliv. Rev.* 56, 603–618.
- Yamane, M.A., Williams, A.C., Barry, B.W., 1995. Effects of terpenes and oleic acid as skin penetration enhancers towards 5-fluorouracil as assessed with time, permeation, partitioning and differential scanning calorimetry. *Int. J. Pharm.* 166, 237–251.