

Formulation Study of Topically Applied Lotion: *In Vitro* and *In Vivo* Evaluation

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ABSTRACT

Introduction: This article presents the development and evaluation of a new topical formulation of diclofenac diethylamine (DDA) as a locally applied analgesic lotion. **Methods:** To this end, the lotion formulations were formulated with equal volume of varying concentrations (1%, 2%, 3%, 4%; v/v) of permeation enhancers, namely propylene glycol (PG) and turpentine oil (TO). These lotions were subjected to physical studies (pH, viscosity, spreadability, homogeneity, and accelerated stability), *in vitro* permeation, *in vivo* animal studies and sensory perception testing. *In vitro* permeation of DDA from lotion formulations was evaluated across polydimethylsiloxane membrane and rabbit skin using Franz cells. **Results:** It was found that PG and TO content influenced the permeation of DDA across model membranes with the lotion containing 4% v/v PG and TO content showed maximum permeation enhancement of DDA. The flux values for L₄ were 1.20±0.02 µg.cm⁻².min⁻¹ and 0.67 ± 0.02 µg.cm⁻².min⁻¹ for polydimethylsiloxane and rabbit skin, respectively. Flux values were significantly different (*p* < 0.05) from that of the control. The flux enhancement ratio of DDA from L₄ was 31.6-fold and 4.8-fold for polydimethylsiloxane and rabbit skin, respectively. In the *in vivo* animal testing, lotion with 4% v/v enhancer content showed maximum anti-inflammatory and analgesic effect without inducing any irritation. Sensory perception tests involving healthy volunteers rated the formulations between 3 and 4 (values ranging between -4 to +4, indicating a range of very bad to excellent, respectively). **Conclusion:** It was concluded that the DDA lotion containing 4% v/v PG and TO exhibit the best performance overall and that this specific formulation should be the basis for further clinical investigations.

Introduction

Diclofenac is an important member of a class of drugs known as nonsteroidal anti-inflammatory drugs (NSAID) which is widely used for the treatment of musculoskeletal disorders, arthritis, toothache, dysmenorrhea and symptomatic relief of pain and inflammation.¹ Diclofenac diethylamine (DDA) has limited bioavailability (40-60%), a short half-life (2-3 h) and low therapeutic dose requirement (25-50 mg).^{1,2} The dose-dependent gastrointestinal, cardiovascular and renal unwanted effects following oral delivery of NSAID has promoted its transdermal delivery,³ which has several advantages over oral delivery to improve patient compliance.⁴ Despite presenting an attractive route for drug delivery, stratum corneum remains the main

constraint because of its barrier properties.⁵ In recent years, extensive research has been carried out to curtail the barrier properties of stratum corneum which compromises the percutaneous absorption using permeation enhancers.⁶⁻¹⁰ Research has been carried out to improve percutaneous absorption of DDA involving various techniques including gel,¹¹ microemulsion,¹² liposomes,^{13,14} lyotropic liquid crystal⁴ and drug-excipient combination^{2,15} based formulations.

Propylene glycol (PG) has been widely used as solvent and permeation enhancer in various transdermal formulations.^{16,17} Natural products including essential oils¹⁸⁻²⁰ are gaining importance as permeation enhancers in transdermal drug delivery owing to their good safety profile.²¹ Turpentine oil (TO) has been used as

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penetration enhancer for a number of hydrophilic and lipophilic drugs.^{22,23} TO contains terpenes which are less toxic and FDA has classified terpenes as 'generally recognized as safe' (GRAS).²⁴

More recently, we have reported an enhanced drug permeation of DDA from lotion formulation containing oleic acid as permeation enhancer.²⁵ In the present work we investigate percutaneous delivery of a new topical DDA formulation containing PG and TO as permeation enhancers. The formulations were assessed for permeation enhancement capability of combination of PG and TO. These formulations were characterized for its pH, viscosity, spreadability and homogeneity, accelerated stability, *in vitro* skin permeation across two model membranes, namely polydimethylsiloxane membrane and rabbit skin. *In vivo* evaluations included animal models and human volunteer's sensory perception testing.

Materials and methods

Materials

Propylene glycol (Merck, Germany), ethanol (Merck, Germany), sodium acetate (Merck, Germany), isopropyl alcohol (Fluka, Switzerland), Carbomer 980 (Fisher, Germany); γ -carrageenan No. 2249 (Fluka Biochemika, Switzerland), turpentine oil (MS Traders, China), diclofenac diethylamine (Novartis, Pakistan) were used as received with minimum purity of 99%. Polydimethylsiloxane membrane with 400 μm -thickness was purchased from Samco, USA.

Preparation of lotion formulations

All lotion formulations were prepared by mixing the ingredients as given in the Table 1. Essentially, 2 g of DDA was dissolved in 20 mL of ethanol and this solution was added to the 20 mL of phosphate buffered saline containing 980 mg of carbomer. These were

mixed for 30 minutes until a clear solution was obtained. To these solutions, permeation enhancers, PG and TO, were added in varying concentration. Finally, the volume was made up to 100 mL by adding ethanol. An enhancer free lotion was also prepared as a control.

Diclofenac diethylamine quantification

HPLC analysis was performed as reported previously.²⁵ The amount of drug was quantified using a Waters UV/Vis HPLC system installed with a symmetry C18 reverse phase column (5 μm , 4.6 \times 25cm) (Waters, UK) with UV detection set at 276 nm. The samples were injected with a rheodyne injector having a 20 μL loop volume. The elution was carried out at ambient temperature and an isocratic mobile phase composed of methanol and 0.1M sodium acetate (70:30 v/v) with a flow rate of 0.8 mL/min was used for separation. The mobile phase was prepared on daily bases and it was filtered and then degassed prior to use. The method was validated as per ICH guidelines with precision (less than 1% RSD) and % accuracy (% RSD 0.865). The limit of detection was 225.2 ng/mL and the limit of quantitation was 350.7 ng/mL for DDA. DDA solutions of known concentrations were used to obtain a standard calibration curve.

In vitro characterization of lotion formulation

pH and rheological measurements

Lotion pH was recorded with a digital pH meter (Mettler & Toledo, Giessen, Germany) by inserting probe into the lotion formulation and allowing it to equilibrate for 1 minute. Viscosity measurements were conducted using a Model RVTDV II Brookfield viscometer (Stoughton, MA). A C-50 spindle was employed with a rotation rate of 220 rpm. The gap value was set to 0.3 mm. Temperature was set at 25°C \pm 2 and these experiments were conducted in triplicate to obtain statistically significant data.

Table 1. DDA containing lotion formulations

Formulation Codes	DDA (g)	Carbomer 980 (mg)	Phosphate buffered saline (mL)	PG (% v/v of total lotion)	TO (% v/v of total lotion)	Ethanol (q.s. for 100 mL lotion)
Control (L _c)	2	1.5	20	0	0	Q.S
L ₁	2	1.5	20	1	1	Q.S
L ₂	2	1.5	20	2	2	Q.S
L ₃	2	1.5	20	3	3	Q.S
L ₄	2	1.5	20	4	4	Q.S

Spreadability and homogeneity determination

The spreadability of each lotion was determined by the wooden block and glass slide method previously detailed somewhere else.²⁶ Essentially, a 5mL volume (100 mg) of lotion was added to a dedicated pan and the time taken for a movable upper slide to separate completely from the fixed slides was noted. Spreadability was determined according to the formula:

$$S = M \times \frac{L}{t}$$

Where:

S = Spreadability expressed in mg.cm.sec⁻¹.

M = Weight/Volumes tide to upper slide (mg)

L = Length of glass slide

t = Time taken to separate the slide completely from each other

Experiments were repeated three times to obtain a statistically significant data.

Each formulated lotion was evaluated for homogeneity by naked eye examination. This involved a subjective assessment of appearance including the presence of any aggregates.

Accelerated stability studies

All the formulated lotions were subjected to a 6 month-long protocol of accelerated stability testing conducted at a temperature of 40 ± 2 °C, 75% relative humidity. The accelerated stability testing was performed in accordance to the ICH guidelines. At 12 h, 1 day, 7 days, 1 month, 3 months and 6 months, each formulation was examined for changes in appearance, pH, viscosity and drug content. These experiments were performed in triplicate, too (n=3).

Permeation studies

White New Zealand male rabbits weighing between 3-4 kg were used for the preparation of skin. The skin samples were excised from the abdomen region. Hairs were clipped short and adhering subcutaneous fat was removed carefully from the isolated full-thickness skin. Then, the skin was cut into samples that were just larger than the surface area of the Franz diffusion cells. To remove extraneous debris and any leachable enzyme, the dermal side of the skin was kept in contact with a normal saline solution for 1 hour prior to start of diffusion experiments. For the polydimethylsiloxane membrane studies, pieces were cut out to a size suitable for mounting in Franz cells and then soaked overnight in PBS (pH 7.4). This procedure was performed in order to allow the removal of excipients present within the membrane upon purchase.²⁷

Permeation experiments were performed using Franz cells manufactured 'in house', exhibiting a diffusional area of 0.85cm² and a receptor cell volume of 4.5 mL.

Subsequently, the test membrane (either rabbit skin or polydimethylsiloxane) was inserted as a barrier between the donor and receiver cells. Silicone grease was applied in order to create a good seal between the barrier and the two Franz compartments. To start each permeation experiment, 1 mL volume of each lotion formulation was deposited in the donor cell while receptor compartment was filled with PBS maintained at pH 7.4 which is close to the pH of blood.²⁸ The diffusion cells were placed on a stirring bed (Variomag, US) immersed in a water bath at 37 ± 5°C to maintain a temperature of ~32°C at the membrane surface. At scheduled times, a 0.5 mL aliquot of receiver fluid was withdrawn and the receiver phase was replenished with 0.5 mL of fresh pre-thermostated PBS. Withdrawn aliquots were assayed immediately by HPLC for DDA quantification. Sink conditions existed throughout. Since skin exhibits big sample-to-sample permeability differences,²⁹ so each experiment consisted of 5 replicate runs (n=5).

In vivo characterization

The in vivo research consisted of three separate types of studies. These studies were conducted under conditions that had been regulated and approved by the Animals Ethics Committee of Bahauddin Zakariya University (Pakistan).

Each DDA-containing formulation was evaluated for its anti-inflammatory potency by means of the carrageenan-induced rat paw edema assay.³⁰ The assay was run on male Wistar rats (150 ± 5g) purchased from the Institute of Biotechnology of Bahauddin Zakariya University (Multan, Pakistan). These rats were randomly divided into five groups with three rats in each. The rats were allowed free access to food and water. The protocol involved injecting a 0.1 mL volume of 1% w/v carrageenan suspension in Normal saline into the sub-plantar tissue of each animal's right hind paw. This was immediately followed by applying 1mL of the DDA-containing lotion over a 2 cm² area in the injection site. The control group was provided with lotion without enhancer. After 3 h, the extent of tissue inflammation was quantified by simply measuring the linear paw circumference.³¹

In the next set of in vivo studies, each analgesic-containing lotion was evaluated for its antinociception effect by running a modified version of the established hot water-tail flick test³² on male Wistar rats (≤ 450 g weight). To this end, a 1 mL aliquot of test formulation was applied to each animal's abdomen. The animal was placed in a dedicated cloth restrainer that was specially designed for this version of the flick test.³³ At 30, 45 and 60 min after lotion administration, the animal's tail (2–5cm long) was immersed in water maintained at 53 ± 1 °C. The reaction time was the time taken for the rat to flick its tail. In practice, the first reading was ignored and the reaction time was considered as the mean of the

subsequent two readings. Each analgesic formulation was tested on 3 rats in each group.

Lastly, each formulation was assessed for irritancy by conducting modified Draize skin irritation tests³⁴ on male White New Zealand rabbits (3-4 kg) obtained from Novartis (Jamshoro, Pakistan). For this purpose, a dorsal area on each restrained animal was shaved and then tape stripped three times to detach several upper layers of the stratum corneum. A 0.5mL aliquot of each test lotion was used in these areas which were then covered with a plastic patch. After 4 h, the patch was removed and the rabbits were observed over 14 days for signs of erythema, edema and ulceration. On days 1, 3, 7 and 14, visually-apparent cutaneous changes were assigned scores ranging between 0 and 4 with higher numbers signifying greater skin damage. Each DDA formulation was tested on 3 rabbits.

Sensory perception test

Sensory perception test involved 11 untrained Caucasian volunteers, both male and female, ranging between 20 to 24 years old. This study was ethically approved by the Human Volunteers Ethics Committee of Bahauddin Zakariya University (Pakistan). A small amount of test formulation was applied to a 12 cm² area on the back of each volunteer's hand and left on for 10 min. Each volunteer rated the test lotion's effects in terms of five different subjective sensory categories. The categories were: ease of application, skin sensation immediately after application, long-term skin sensation, skin 'shine' (i.e. visual appearance) and perception of induced skin softness. The rating scale used consisted of nine integer values ranging between -4 to +4, indicating very bad to excellent, respectively. In addition, skin treatment sites were visually examined for signs of cutaneous irritancy. A confidence level of 95% was considered as significant.

Results

In vitro characterization

All the lotion formulations were clear, transparent and homogeneous solutions upon preparation which exhibited a pH of 6.3 with no significant difference with all the formulated lotions. However, increasing PG and TO content in the formulated lotions decreased the viscosity from 89×10^{-4} dynes.s.cm⁻² for L₁; 83×10^{-4} dynes.s.cm⁻² for L₂; 78×10^{-4} dynes.s.cm⁻² for L₃ and 71×10^{-4} dynes.s.cm⁻² for L₄. A similar viscosity trend was observed in case of spreadability of formulated lotions where spreadability was decreased upon subsequent increase in the PG and TO content i.e. 3.02 ± 0.12 mg.cm.s⁻¹ for L₁, 2.14 ± 0.17 mg.cm.s⁻¹ for L₂, 2.12 ± 0.21 mg.cm.s⁻¹ for L₃ and 2.01 ± 0.09 mg.cm.s⁻¹ for L₄. Statistical analysis revealed that there was a significant difference between L₁ and L₄ spreadability. Overall, an

increase in PG and TO content in the lotion formulation decreased the viscosity and spreadability.

During the six-month accelerated stability testing, none of the formulations showed changes in the appearance, color and transparency. Furthermore, there was an insignificant difference among all the formulated lotions in terms of pH, viscosity, spreadability and drug content over the course of accelerated stability testing period suggesting that the formulated lotions were fairly stable.

In vitro permeation studies

Fig. 1 and 2 display the cumulative amount of DDA permeation through polydimethylsiloxane membrane and rabbit skin as a function of time, respectively. The steady-state flux was determined from the slope of linear portion of cumulative amount of drug permeation versus time plot. Permeability coefficients were calculated by applying Fick's laws of diffusion. Flux enhancement ratio (ER) was calculated based on the proportion of flux in the presence and absence of enhancer in the lotion formulation.

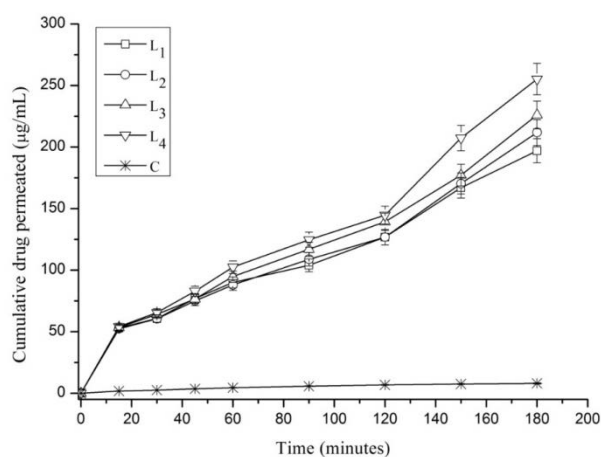


Fig. 1. Cumulative drug permeated through polydimethylsiloxane membrane (n=5).

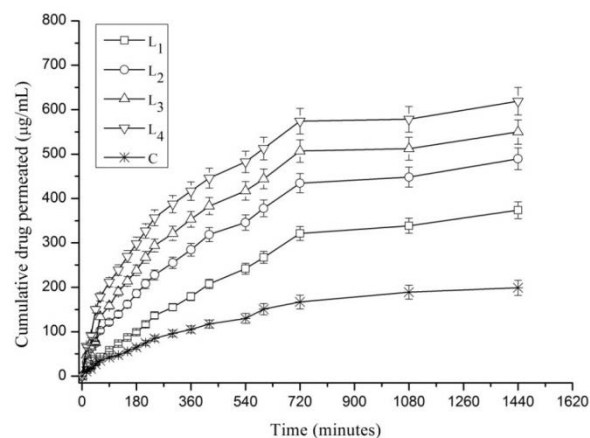


Fig. 2. Cumulative drug permeated through rabbit skin (n=5)

Permeation parameters of DDA across polydimethylsiloxane membrane and rabbit skin are summarized in Tables 2 and 3. In case of polydimethylsiloxane membrane, flux values were $0.86 \pm 0.02 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ for L₁, $0.95 \pm 0.02 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ for L₂, $1.01 \pm 0.01 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ for L₃ and $1.20 \pm 0.02 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ for L₄. The corresponding flux enhancement ratio (ER) was 22.6-fold for L₁, 25.0-fold for L₂, 26.6-fold for L₃ and 31.6-fold for L₄. The permeability coefficient was found to be 21.58×10^{-4} (cm.min⁻¹) for L₁, 21.58×10^{-4} (cm.min⁻¹) for L₂, 21.58×10^{-4} (cm.min⁻¹) for L₃ and 21.58×10^{-4} (cm.min⁻¹) for L₄.

In case of the rabbit skin, flux values were $0.43 \pm 0.02 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ for L₁, $0.55 \pm 0.02 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ for L₂, $0.62 \pm 0.01 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ for L₃ and $0.67 \pm 0.02 \mu\text{g}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ for L₄. The corresponding flux enhancement ratio

(ER) was 3.1-fold for L₁, 3.9-fold for L₂, 4.4-fold for L₃ and 4.8-fold for L₄. The permeability coefficient was found to be 20.53×10^{-8} for L₁, 26.07×10^{-8} for L₂, 29.88×10^{-8} for L₃ and 31.78×10^{-8} for L₄.

In vivo studies

Fig. 3 shows the data obtained from the carrageenan challenge anti-inflammatory tests. It can be seen that application of each of the DDA-containing formulations significantly ($p < 0.05$) reduced tissue inflammation in the rat model. Another noteworthy point from statistical analysis is that while anti-inflammatory effect of L₁ was significantly different from L₂, L₃ and L₄, the latter three formulations did not differ significantly from each other in anti-inflammatory potency which can be explained on the basis of permeability coefficient which was insignificantly different for L₂, L₃ and L₄.

Table 2. Permeation profile of DDA across polydimethylsiloxane membrane

Formulated Lotion	Flux ($\mu\text{g}/\text{cm}^2/\text{min}$)	Lag Time (t_{lag}) (min)	Permeability Coefficient ($10^{-4} \times K_p$)(cm/min)	ER
L _c	0.038 ± 0.006	47.23 ± 9.98	0.95 ± 0.14	-
L ₁	0.86 ± 0.02	60.54 ± 2.00	21.58 ± 0.58	22.6
L ₂	0.95 ± 0.02	51.83 ± 2.38	23.65 ± 0.49	25.0
L ₃	1.01 ± 0.009	50.84 ± 1.22	25.32 ± 0.22	26.6
L ₄	1.20 ± 0.02	42.09 ± 2.37	29.91 ± 0.48	31.6

Results are presented as mean \pm SD ($n = 5$).

Table 3. Permeation profile of DDA across rabbit skin

Formulated Lotion	Flux ($\mu\text{g}/\text{cm}^2/\text{min}$)	Lag Time (t_{lag}) (min)	Permeability Coefficient ($10^{-8} \times K_p$)(cm/min)	ER
L _c	0.14 ± 0.001	37.53 ± 1.81	0.053 ± 0.003	-
L ₁	0.43 ± 0.02	19.28 ± 0.67	20.34 ± 0.05	3.1
L ₂	0.55 ± 0.09	67.31 ± 2.60	26.07 ± 0.01	3.9
L ₃	0.62 ± 0.08	97.01 ± 2.30	29.85 ± 0.01	4.4
L ₄	0.67 ± 0.01	142.73 ± 1.10	31.78 ± 0.02	4.8

Results are presented as mean \pm SD ($n = 5$).

Fig. 4 displays the data derived from the hot tail anti-nociception studies. The graph clearly indicates that the reaction time measured following treatment with a DDA-containing lotion was always significantly longer than the reaction time measured following treatment with the L_c . Furthermore, the extent of induced anti-nociception followed the trend; $L_4 > L_3 > L_2 > L_1$, indicating that PG and TO content influenced antinociception potency of DDA by enhancing its permeation.

With respect to the Draize irritation tests, results indicated that application of all lotion formulation were invariably associated with no skin irritation throughout the entire 14-day period. With respect to the L_4 formulation, all tested rabbits showed some mild erythema (score of 1) by day 14 although not at the earlier observation times (data not shown).

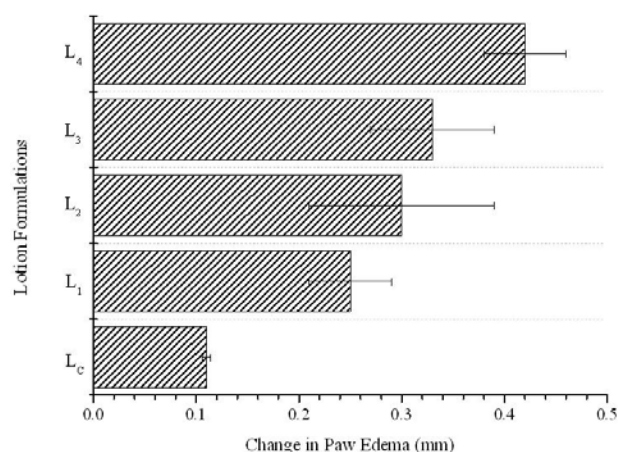


Fig. 3. Bar graphs showing the *in vivo* edema reduction induced by each DDA formulation in carrageenan-challenged rabbits. Error bars represent SD values, with $n=3$.

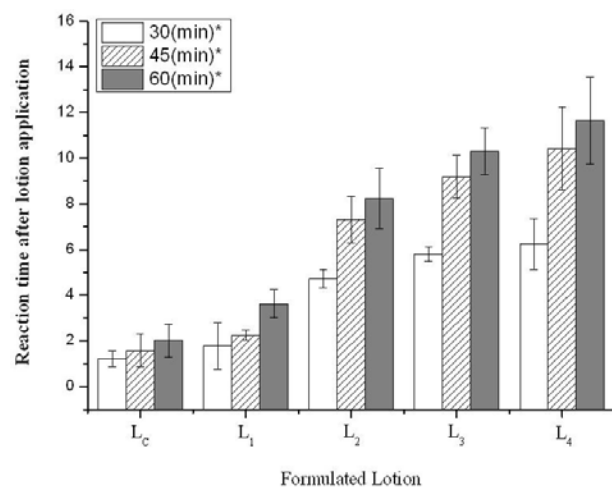


Fig. 4. Bar graph diagram showing the *in vivo* tail flick response times (antinociception) associated with each DDA formulation at 30, 45 and 60 mins after lotion application. Error bars represent SD values, with $n=3$.

Sensory perception data

The volunteers rated all DDA containing lotions as scoring between 3 and 4 in terms of all categories: ease of application, skin sensation immediately after application, long-term skin sensation, skin 'shine' and induced skin softness. No lotion caused any observable cutaneous irritation (Data not shown).

Discussion

This article presents an alternative route for administration of DDA which is a potent anti-inflammatory drug from NSAID class. DDA undergoes extensive hepatic metabolism after oral administration and maximum achieved bioavailability is 50% which is insufficient to produce therapeutic effects for prolonged periods of time.² Therefore, transdermal route of DDA delivery is attractive in terms of avoidance of hepatic first pass effect and the drug reaching to the blood without being metabolized by the liver. In this study, lotion formulation of DDA has been formulated with various concentrations of permeation enhancers, namely propylene glycol and turpentine oil. As far as we could ascertain, there is no report published which describes the lotion formulation of DDA containing PG and TO in combination. There are various mechanisms associated with the permeation enhancement of drug by a permeation enhancer. They can increase the thermodynamic activity, skin/vehicle partition coefficient, and solubility power of the skin to the drug. They can also reversibly reduce the impermeability of skin.³⁵

In vitro permeation profile is an important tool that predicts how drug will behave *in vivo*. *In vitro* permeation of DDA containing lotions were performed using two model membranes, namely polydimethylsiloxane and rabbit skin. In case of polydimethylsiloxane membrane, a non-classical behavior of DDA permeation was achieved i.e. initial burst diffusion of DDA from the lotion formulation followed by steady state behavior towards the end of the experiment. Similar effect was observed previously when a different combination of enhancer system was used to study the permeation of DDA.²⁵ This effect was attributed to the polydimethylsiloxane membrane material undergoing perturbation due to interaction between polydimethylsiloxane membrane and vehicle system; consequently, increasing the diffusion coefficient of the drug. Therefore, it was decided to select a period of 15 to 180 minute in order to calculate the steady-state flux. The cumulative amount of drug permeated as a function of time revealed that increasing enhancer concentration in the lotion markedly increased the permeation of DDA as compared to that in the control. Moreover, there was no significant difference

observed in permeation of DDA among all the formulated lotions suggesting a concentration independent increase in the permeability of DDA in case of polydimethylsiloxane membrane. The flux and permeability coefficient values were significantly different from those of the control. Furthermore, a gradual increase in the flux rates and permeability coefficient values was observed with increasing concentration of PG and TO. Lag time (t_{lag}) is the time taken by the drug to reach its steady-state, so data revealed that L₄ has the lowest t_{lag} and DDA permeation has reached to its steady-state quicker than the other formulations containing lower or no enhancer content. This may be explained on the basis that the diffusion of drug across polydimethylsiloxane membrane was faster in the presence of enhancers. Therefore, the drug permeated through the membrane in less time as the concentration of enhancers increased in the formulation which led to the decrease in the lag times.

Fluxes and permeability coefficients were measured for all the DDA containing lotions across rabbit skin. The drug permeation was more or less linear till the 700 minutes, after that it reached to the steady-state region where drug permeation rate was constant over the time period from 700 to 1440 minutes. This phenomenon was also observed in previous studies²⁵ when oleic acid was used in combination of TO to improve the permeation parameters of DDA. Therefore, the time period after 700 minutes was deliberately ignored in order to calculate the steady-state flux. It was noteworthy that the permeation rate was ceased after approximately 700 minutes which could be attributed to the precipitation of DDA on the surface of rabbit skin which reduced the effective diffusion area; consequently, sinking the permeation of DDA. There was a gradual increase in flux rate with increasing content of PG and TO in the lotions while a remarkable improvement was observed in the permeability coefficient for all lotion formulations in comparison with that of control. Statistical analysis revealed a significant difference ($P < 0.05$) in permeability coefficients for all the formulated lotions as compared to control. The enhancement ratio on the basis of flux was highest for the L₄ (4.8-folds) and lowest for the L₁ (3.1-folds) which was related to the enhancer concentration in DDA containing lotions. It was interesting to notice that the lag time increased with the increase in enhancer concentration which might be attributed to the impact of enhancer on the apparent permeability of the DDA. The contrasting lag times for DDA permeation through polydimethylsiloxane membrane and rabbit skin could be due to the structural differences between both membranes and how the permeation enhancer interacts with the membrane. It can be explained by the fact that TO can penetrate rapidly and deposit in the skin owing to its physicochemical properties; thus, causing a delayed permeation which

consequently enhanced lag times with higher concentrations in the case of DDA permeation across rabbit skin. Additionally, the enhancing effect of PG is exerted by enhancing the drug partitioning into the stratum corneum. To do this, PG has to partition into the SC where it accumulates into the intercellular and protein regions of SC, thus changing its solubility power with subsequent increased drug partitioning into the SC.^{36,37}

It is well established that hydration of the skin plays an important role in the percutaneous uptake of DDA. When the aqueous fluid of the sample enters the polar pathways, it will increase the interlamellar volume of stratum corneum lipid bilayers, resulting in the disruption of the interfacial structure. Since some lipid chains are covalently attached to corneocyte, hydration of these proteins will also lead to the disorder of lipid bilayers.³⁸⁻⁴⁰ Similarly, swelling of the intercellular proteins may also disturb the lipid bilayer; a lipophilic drug like DDA can then permeate more easily through the lipid pathway of the stratum corneum. Overall, the incorporation of combination of PG and TO in the formulations significantly improved the drug permeation across two model membranes investigated in this study. The increase in the permeation rate with increase in the PG and TO content was in agreement with the previously published report where TO alone enhanced the DDA permeation across the skin.⁴¹

In terms of formulation characterization, all the formulations were suitable in terms of their physical properties and were fairly stable over the 6-month stability testing period. L₄ lotion produced maximum anti-inflammatory and anti-nociception effects in the carrageenan challenge anti-inflammatory tests and hot-tail flick test, respectively. This can be related to the enhanced drug permeation into the skin; thus, proving to be the ideal formulation for reducing the inflammation. An ideal topical formulation should not produce any kind of irritation or allergic reaction to the skin. Draize skin irritation testing of the formulated lotions revealed no irritation caused by the lotions during the study which in turn reflects the suitability of lotion formulations. This was further confirmed through the sensory perception testing involving healthy volunteers.

Conclusion

Based on the results from this study, it is possible to conclude that PG and TO has effectively improved the permeability of DDA. For all the formulations studied, the best effective *in vitro* permeation and *in vivo* performance was achieved when the highest PG and TO concentrations were used in the formulation; L₄ in this case. It is envisaged that this particular formulation should be the basis of further studies in the clinically relevant environments.

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Ethical issues

This study was ethically approved by the Human Volunteers Ethics Committee of Bahauddin Zakariya University (Pakistan).

Competing interests

Authors declared no competing interests.

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