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Rabbit Skin and Polydimethylsiloxane as Model Membranes to Evaluate Permeation Kinetics From Topical Formulation

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Abstract.- This study aimed at evaluating influence of turpentine oil (TO) on percutaneous absorption of diclofenac diethylamine (DDA), a potent non-steroidal anti-inflammatory agent, using Franz-type diffusion cell across two model membranes, namely rabbit skin and Polydimethylsiloxane membrane. The flux of DDA across the rabbit skin and polydimethylsiloxane membrane using different ratios (1%, 2%, 3% and 4%) of turpentine oil progressively increased with increasing concentration of turpentine oil content with the formulation containing 4% enhancer showed maximum flux of 40.06 (μg/cm^2/h) and 6.19 (μg/cm^2/h), respectively. The input-rate of active from all solutions has shown a trend of increase with the increase in the concentration of enhancer in the solutions. The vehicle used was predominantly influencing the diffusion of the drug into the skin rather than the partition coefficient throughout the study. Consequently, changes in diffusion and/or partition may occur as a result of quick absorption followed by the depletion of drug in the donor in the presence of turpentine oil inside the skin /or membrane over time which validates our results.

Key words: rabbit skin, turpentine oil, penetration enhancers, transdermal.

INTRODUCTION

Transdermal delivery of Diclofenac may provide better patient compliance over oral administration however; it is not easily absorbed on transdermal application (Kweon *et al*., 2004). Considerable research work has been focused to employ chemical penetration enhancers (Barry and William, 1995), which may increase the permeability of drug through stratum corneum (SC) by increasing drug diffusivity within the membrane. The effect of these compounds on the drug cutaneous absorption has been quantified by permeation parameters, such as drug flux across the skin/membrane and drug uptake (Cordero *et al.*, 1997).

The enhancer solutions are usually in a large molar excess of the drug and their effects should be non-irritating, non-immunogenic. The rapid reversible effect is true if the enhancer permeates the skin to change the solubilizing capacity of the skin for the drug. Lipophilic drugs are related with slow drug absorption leading ultimately to insufficient and variable bioavailability (Amidon *et al*., 1995; Leuner and Dressman, 2000). Terpenes have been used for therapeutic purposes since ancient times. The FDA classified terpenes, obtained from natural sources as very safe and effective penetration enhancers as generally regarded as safe (GRAS) (Vaddi *et al*., 2002). These are considered as the most effective class of sorption promoters for a wide variety of medicaments. Terpenes like menthol, cineole, and limonene have been used for permeation enhancement of both hydrophilic and lipophilic drug (Aqil *et al*., 2007). Terpenes did not cause skin toxicity or if any, only mild irritation (Asbill, 2000; Krishnaiah, 2003) even, did not cause lasting erythema as reported earlier (Okabe, 1990). The essential oils are volatile in nature and widely used as therapeutically; as flavoring agents and as starting materials for the synthesis of other important products (Evans, 2002). Therefore, terpenic oil has been used in the pharmaceutical industry, perfume industry, food

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additives and other chemical industries due to its pleasant fragrance (Mercier *et al.*, 2009).

The aim of this study was to investigate the effect of turpentine oil (TO) as penetration enhancer, when used in different concentrations, on solubility and percutaneous absorption of DDA *in vitro*. Formulations were characterized for pH, viscosity, homogeneity, and drug content determination.

MATERIALS AND METHODS

Turpentine oil (TO), ethyl alcohol, methanol and sodium chloride (Merck, Darmstadt, Germany) were used as received without further purification. Diclofenac diethylamine was a gift from Novartis (Pvt.) Ltd. Jamshoroo, Pakistan. De-ionised water was used throughout the study.

Preparation of diclofenac test solution

Diclofenac diethylamine (2 g) was dissolved in 15 mL of methanol in 100 mL volumetric flask and then four different concentrations (1%, 2%, 3% and 4% v/v) of TO were added into the drug solution individually and the volume was made up to the mark with normal saline (NS). The control solution was prepared without adding any enhancer.

Determination of pH, viscosity and homogeneity

The pH of all solutions was determined by using digital pH-meter.

Viscosity measurements were carried out at room temperature (25±1°C) using a Brookfield viscometer (Model RVTDV II, Brookfield Engineering Laboratories, Inc, Stoughton, MA).

All solutions were tested for homogeneity by visual inspection after they have been set in the container. They were tested for their appearance and presence of any aggregates/ precipitates.

Drug content determination

Drug content of test solutions were determined by taking accurately 10 mL solution (200 mg of DDA) in a 100 mL conical flask shaker well, filtered and drug the estimated by UV-spectrophotometric at 276nm against NS as blank.

Solubility studies

The solubility of the DDA in the vehicle and vehicle combinations were determined by adding excess amount of DDA to each solution and stirred with a magnetic bar for 48 hours in a water bath maintained at 32±1ºC. The solutions were centrifuged for 30 min at 4000 rpm. The supernatant was then diluted and assayed by UV-spectrophotometric at 276 nm. Experiments were performed in triplicate (n=3) and mean values with standard deviation (±SD) and coefficient of variation were calculated.

Solvent uptake and skin extraction measurements

The uptake of the selected vehicles into polydimethylsiloxane membrane and rabbit skin was evaluated in this study. The uptake of vehicles was experimentally determined by cutting polydimethylsiloxane membrane and rabbit skin to an appropriate size (~1cm^2) and weighed. They were then placed in a sample bottle containing the vehicle and soaked for 24 h. The membranes were blotted dry with tissue paper and re-weighed. The experiments were performed in triplicate, at room temperature. The amount of solvent taken up by the membrane was expressed as a weight percent. The solvent uptake was calculated by the following equation:

$$\%\, Solvent\ uptake = \frac{Skin\ weight\ after\ treatment - dry\ skin\ weight}{dry\ skin\ weight} \times 100$$

The experiments were performed at 32±1ºC in triplicate.

Diffusion studies through rabbit skin and polydimethylsiloxane membrane

Diffusion studies across rabbit skin and polydimethylsiloxane membrane were performed using Franz diffusion cells (FDC, made of Germany at SOP, London) that have a receptor phase volume of ~4.5 mL and a diffusion area of ~0.85cm^2. The full thickness rabbit skin was taken from the abdominal surface and hairs were carefully cut as short as possible using scissors, without damaging or scratching the skin surface.

Rabbit skin and sheets of polydimethyl-siloxane membrane were cut according to the diameter of the diffusion cell and was placed in a normal saline solution before mounting on to the

diffusion cell (Shah *et al*., 2012). Both rabbit skin and polydimethylsiloxane membrane were soaked overnight in the receptor solution *i.e.* NS. The skin/ or membrane was then sandwiched between the donor (upper) and the receptor (lower) chambers of the diffusion cells and held together with a clamp using Silicone grease (Dow, USA) to produce a leak-proof seal between the membrane and the two compartments of the diffusion cell. The receptor chamber was filled with NS and making sure all air under the skin/membrane was removed. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor chamber was continuously stirred using a Teflon coated magnetic stirring bar (400 rpm) and the temperature of the receptor chamber was kept at 32±1°C by a water jacket. 1.0 mL of each DDA containing solution was topically applied to the surface of the skin/membrane in the donor compartment having 0.85 cm² diffusion area and then covered with Parafilm (American National Can™, Chicago, USA). All diffusion and permeation experiments were conducted under occlusive conditions. Samples (500 µL) from the reception solution were drawn at regular time intervals (15, 30, 45, 60, 90, 120, 150, 180, 240, 300,…1440 min with the help of 1 mL syringe (Sun, Korea) from the sampling port and were immediately replaced by 500 µL fresh pre-thermostatic NS. Sink conditions existed throughout the experiment. The analytically determined assay values in the FDC samples were correspondingly corrected for the replenishments.

The sample taken from the receptor cell was run on U.V-spectrophotometer (Agilent 2005; software version 2005) at 276 nm. All experiments were performed at 32±1°C in ±SD (n=5) and sampling time was 0-3 h for silicone membrane while for rabbit skin studies sampling time was 0-24h.

The factor of difference value (FoD)

The flux (J) values calculated from the present permeation study of saturated formulations of DDA has been compared (rabbit skin permeability data vs polydimethylsiloxane membrane data) by means of the factor of difference value (FoD) described by the following Dick and Scott equation;

$$FoD = \frac{J_{RS}}{J_{SM}}$$

Where J_{RS} and J_{SM} denotes maximum flux value (J) through rabbit skin (RS) and polydimethylsiloxane membrane (SM). This study suggested that the artificial membrane model represents a significant prediction for the human skin behaviour if its associated FoD value is less than 3 (Dick and Scott, 1992).

Statistical analysis

Data analysis was carried out using Microsoft Excel version 2007. Statistical significance was determined between the sample means of the treatment groups using the one-way ANOVA and a post hoc Turkey multiple test was applied where the difference in mean was significant. A probability of $p<0.05$ was considered statistically significant. All results were presented as the mean ± SD, unless otherwise stated. The minimum standard deviation values assured that the process used for preparing the delivery system is capable of giving reproducible results which is further confirmed by earlier studies data (Jayaprakash *et al*., 2010).

RESULTS AND DISCUSSION

Solubility studies

The solubility of diclofenac diethylamine (DDA) in the TO was determined as given in Table I and III showed the solubility expressed in mg/mL, and the standard deviation (±SD) associated with each experiment. Diclofenac diethylamine is ~2.5 fold more soluble in TO than water. The solubility of DDA in distilled water is 42.282±0.588 mg/mL, at 32±1ºC, which is in line with values reported in the literature (Roy *et al*., 1996).

In the present study, we made co-solvent mixtures of DDA from saturated solutions of enhancer in water as TO: water mixture at 20:80; 40:60; 60:40; 80:20 and 100:00 ratio (v/v) respectively as given in Table III. The degree of saturation (DS) was calculated by dividing the amount dissolved in the mixture by the solubility at equilibrium in the same co-solvent mixture which was 1.1 for TO. Each data point represents the mean ± SD (*n*=3). Solubility Enhancement Ratio (ER_{sol})

of DDA in the solvent have been determined as:

$$ER_{sol} = C_t / C_s$$

Where $\mathbf{C_t}$ is concentration of DDA in the presence of enhancer and $\mathbf{C_s}$ is concentration of DDA in absence of enhancer (control), the ER_{sol} for TO was 2.485.

Table I.- Pre-formulation study of drug.

Solubility (mg/ml)			Partition co-efficient $K_{o/w}$ (Guy 2003)	pK_a	M.P°C
Water	NS	TO			
42.28 ±0.59	199.23 ±1.39	105.06 ±2.42	4.40	4.07	280

Table II.- Values for evaluation of physical parameters.

Vehicle (TO) % age	pH	%Drug content	Viscosity (dynes'/ cm^2)	Rabbit skin extraction (mg/ml)	Homogeneity
1	6.3±0.1	98.78	90×10^{-4} ±0.02	1.42	Good
2	6.3±0.1	98.73	91×10^{-4} ±0.04	1.23	Good
3	6.3±0.1	99.21	91×10^{-4} ±0.03	1.04	Good
4	6.3±0.1	98.94	91×10^{-4} ±0.02	1.02	Good

Table III.- Solubility of Diclofenac diethylamine in turpentine oil/water vehicles.

% Turpentine oil in water (v/v)	Solubility (mg/mL)±SD (n=3)
0	42.28±0.59
20	47.69±0.96
40	69.16±2.00
60	81.98±5.63
80	96.73±0.96
100	105.06±2.42

Solvent uptake

The solubility parameter of polydimethyl-siloxane membrane is reported in the literature to be 7.5 $(cal/cm^3)^{1/2}$ by Cross *et al.* (2003) while for TO was 2.1%. For the vehicles with low log P values, it would be difficult to overcome poor solubility and it is unlikely that a high flux will be achieved even if the solvent flux is high. Table II showed the observed values of skin extraction measurements.

I-kinetics of permeation studies through rabbit skin

The effect of TO in amount of 1%, 2%, 3% and 4% (v/v) on the permeability rate of DDA through rabbit skin is shown in Table IV and Figure 1. The enhancer solutions might affect stratum corneum structure and drugs could be permeated better through the rabbit skin. The enhancing ratio (ER) values was observed in the order as 1% <2% <3% <4% which was comparable with the earlier work (Dey *et al.*, 2009). The input rate obtained is given in Table V which is almost 2-5 folds higher than for control.

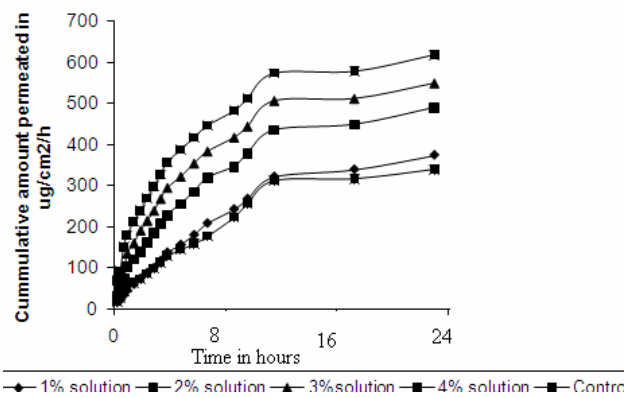


Fig. 1. Permeation of diclofenac solution through rabbit skin (n=5).

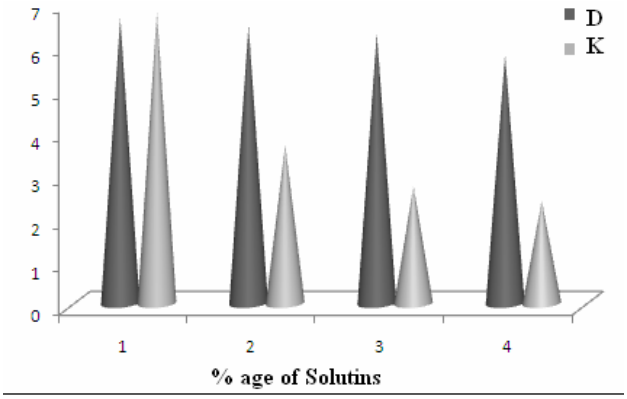


Fig. 2. Correlation b/w partition co-efficient (K) and diffusion co-efficient (D) through rabbit skin.

Table IV.- Permeation kinetics of diclofenac diethylamine in the presence of turpentine oil through rabbit skin (n=5).

Vehicle (TO) % age	Flux* (µg/cm²/h) ± SD×10⁻³	D** × 10⁻² (cm².h⁻¹) ± SD×10⁻⁴	K_p*** (cm.h⁻¹) ± SD×10⁻⁴	K****×10⁻⁵ ±SD×10⁻⁹	ER
1	25.64 ± 2.30	6.59 ± 12.33	5.05 ±27.89	6.74 ±49.78	1.27
2	32.87 ± 10.47	6.43 ± 19.91	2.68 ±126.7	3.67 ±382.0	1.62
3	37.63 ± 8.74	6.26 ± 12.14	1.93 ±3.47	2.71 ±0.22	1.86
4	40.06 ± 9.43	5.75 ± 13.65	1.54 ±4.77	2.36 ±8.26	1.98
Control	20.25 ± 1.05	3.65 ±13.41	2.07 ±0.27	12.49 ±0.009	-

*One-way ANOVA confirmed no significant difference. **One way ANOVA confirmed no significant difference (P<0.05).
One way ANOVA confirmed significant difference (P>0.05).*One-way ANOVA suggests significant difference (P<0.05).

Table V.- Input-rate of DDA in different concentrations of vehicle's solutions across rabbit skin and silicone membrane (n=5).

Vehicle (TO) %age	Rabbit skin (µg/h)	Silicone membrane (µg/h)
1	7.26	37.86
2	8.11	39.31
3	9.78	42.78
4	10.32	45.84
Control	3.66	1.86

II-kinetics of permeation studies through silicone membrane

The permeation of DDA through silicone membrane, using TO of varying concentrations (1%, 2%, 3% and 4% v/v) was evaluated and enlisted in Table VI. The flux values with associated standard deviations (±SD), the permeation parameters t_{lag}, D, k_p and K are also illustrated in Figure 3. It can be seen that although there is no significant difference (P >0.05) between permeation of DDA from all the solutions carrying various enhancer concentrations yet a trend of linear increase in the permeation rate with increasing TO concentration is observable.

The values of enhancing ratio (ER) were observed in the order of 1% <2% <3% <4% which is comparable to the earlier studies (Dey *et al.*, 2009). The input rate is almost 18-23 folds higher than for control (Table V).

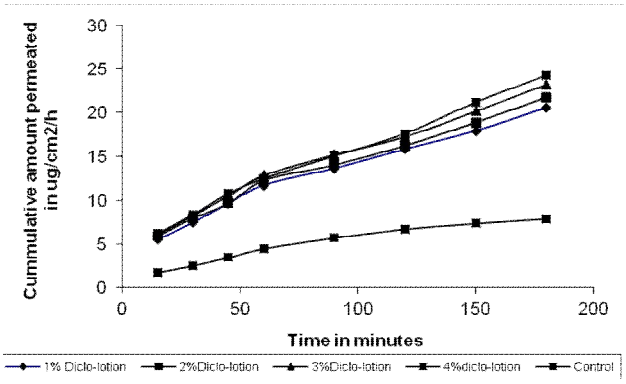


Fig. 3. Permeation of diclofenac solution through silicone membrane (n=5).

Table VI.- Permeation kinetics of diclofenac diethylamine in the presence of turpentine oil across silicone membrane (n=5).

Vehicle (TO) % age	Flux* (µg/cm²/h) ± SD×10⁻²	D** (cm².h⁻¹) ± SD×10⁻⁵	K_p*** ×10⁴ (cm.h⁻¹) ± SDx10⁻⁷	K****×10⁻⁷ ± SD×10⁻⁵	ER
1	5.05 ±2.67	3.49 ± 8.73	0.12 ±0.12	3.87 ±11.97	2.31
2	5.36 ±4.49	9.61 ±3.66	0.15 ±7.24	1.45 ±4.06	2.45
3	5.79 ±3.11	11.98 ± 0.85	0.17 ±1.04	1.32 ±5.03	2.65
4	6.19 ±2.17	16.55 ±1.56	0.19 ±3.04	1.01 ±1.31	2.82
Control	2.19 ± 0.05	7.33 ±23.41	0.11 ±0.27	0.014 ±0.009	-

*One-way ANOVA confirmed no significant difference. **One way ANOVA confirmed significant difference (P<0.05). ***One way ANOVA confirmed no significant difference (P<0.05). ****One-way ANOVA suggests significant difference (P<0.05).

FoD of formulations of DDA across rabbit skin vs silicone membrane

In this study, FoD value for TO was 0.43 (Table VII), showing that the flux values determined by using silicone membrane (SM) were in the same order of magnitude as that of flux values calculated with rabbit skin for permeation for 3 h study.

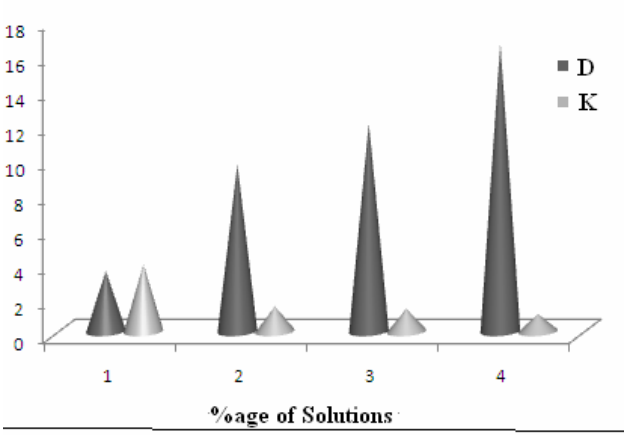


Fig. 4. Correlation b/w partition co-efficient (K) and diffusion co-efficient (D) through silicone membrane.

Table VII.- The factor of difference value (FoD) in the presence of saturated enhancer's solution across rabbit skin and silicone membrane (n=5)

Vehicles	J_{RS} (µg/cm^2/min)	J_{SM} (µg/cm^2/min)	FoD
TO	0.138	0.059	0.43

DISCUSSION

The skin permeation rates of the DDA from solutions containing varying amount of enhancer were evaluated and among these, DDA solution containing 4% (v/v) TO showed the highest permeation rate (6.191 and 40.067 µg/cm^2/h for polydimethylsiloxane and rabbit skin, respectively). The concentration of enhancer in solutions affected the skin permeation rate of DDA significantly. It is interesting to observe that the concentration of enhancer in solutions was decreased from 4% (v/v) to 1% (v/v), the skin permeation rate of DDA also decreased *i.e.* it may be due to thermodynamic activity of drug in the solution as DDA is poorly water soluble (42.282± 0.588 mg/mL) and yet solubilized in the enhancers' mixture (Kweon *et al.*, 2004). The reported data in this study (Fig. 2) showed that D and K is decreasing from 1% (v/v) to 4% (v/v), hence permeation through rabbit skin is diffusional although partitioning is occurring in the skin as the earlier studies confirmed the deposition of DDA into the skin (Green *et al.*, 1988).

It was also found that the permeation of the DDA through rabbit skin was significantly influenced by the content of ethanol and enhancers' mixture due to increasing solubility of DDA. The literature supported our data that skin permeation rate of DDA was increased by 9.7-folds (Walker and Hadgraft, 1991). It has been reported that alcohol may alter or form additional pore/polar pathways in the stratum corneum as a result of combination of changes in protein conformation, reorganization within the lipid polar head region or lipid extraction and also induced the reduction in the barrier property of SC (Bommannan *et al.*, 1990). As the percentage (v/v) of TO was increased, the number of internal phase of skin/membrane was increased; which increased the permeation rate of drug (Kweon *et al.*, 2004). It was observed that flux in 4% solution was 6.191±0.003 (µg/cm^2/h) and 40.067±0.054 (µg/cm^2/h) in silicone membrane and rabbit skin, respectively whereas in 1% solution flux was 5.057±0.031 (µg/cm^2/h) and 25.643±0.002 (µg/cm^2/h) respectively. Solvent used in this study enter the SC, change its solution properties by altering the chemical environment and thus reduced the barrier capacity of the cutaneous layer (Barry, 2001; Shahzad *et al.*, 2012).

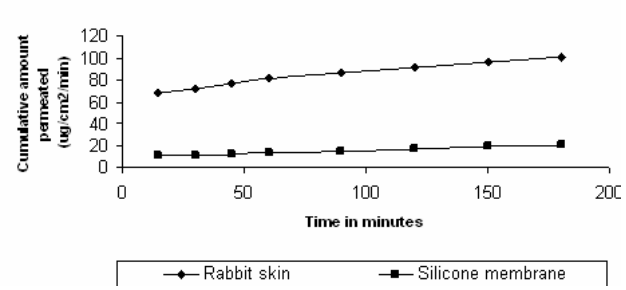
On the other hand, there is a general experience that hydration of the skin plays an important role in the percutaneous uptake of DDA. When the aqueous fluid of the sample enters the polar pathways, it will increase the interlamellar volume of stratum corneum lipid bilayers, resulting in the disruption of the interfacial structure. Since some lipid chains are covalently attached to corneocytes, hydration of these proteins will also lead to the disorder of lipid bilayers (Idson, 1978; Bouwstra *et al.*, 2003; Wang *et al.*, 2006). 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. The greater drug penetration enhancing activity of solution may be attributed to the combined effects of both lipophilic and lipophobic domains of solutions (Yamada *et al.*, 1987; Obata *et al.*, 1991).

Since terpenes are relatively safe compounds, their incorporation in low concentrations into topical formulations could be recommended (Khan *et al.*,

2012; Mohammad-Samani *et al*., 2010; Nokhodchi *et al*., 2007; Ota *et al*., 2003). However, further elucidation of the mechanism of action of permeation enhancement is yet to be explored and further similar studies have made (Shah *et al*., 2012) to determine its enhancing properties for more lipophilic model drugs in efficacious topical formulations.

Comparison of formulations of DDA across rabbit skin vs polydimethylsiloxane membrane

In this study, FoD value was 0.43 (Table VII), showing that the flux values determined by using polydimethylsiloxane membrane (SM) were in the same order of magnitude as that of flux values calculated with rabbit skin as illustrated in Figure 5 for permeation study after 3 hours using TO (Cilurzo *et al*., 2007). Moreover, the FoD values did not appear to be related to any physicochemical properties of the enhancer solutions as reported in the above results.



One way ANOVA confirmed significant difference ($P<0.05$) and F value is 206.84

Fig. 5. The factor of difference value (FoD) in the saturated enhancer's solution (TO) across rabbit skin and silicone membrane (n=5).

CONCLUSIONS

The current research showed a significant increase in the permeation of DDA through the rabbit skin as a function of increasing concentration of TO in solutions. This increase in the permeation rate was mainly attributed to the increase in the drug diffusion into and through the skin. Silicone membrane did not show any significant enhancement as compared to the rabbit skin which might be explained on the basis of structural dissimilarity between silicone membrane and rabbit skin. It can be concluded that 4% TO concentration in the formulation enhanced the drug permeation to maximum. It is difficult to suggest if this is true for the other drugs but it is envisaged that compounds having physicochemical properties similar to the DDA could produce the similar results.

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Declaration of interest

The authors report no conflict of interest.

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