Effect of Oleic Acid on the Permeation Kinetics of Diclofenac Diethylamine

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Summary: In vitro permeation studies were done using modified Franz diffusion cell through rabbit skin and silicone membrane utilizing different ratios of oleic acid with diclofenac diethylamine (DDA) in normal saline and methanol mixture during present study. Solubility studies indicated linear increase in drug solubility with carrier concentration. The enhancing effect of all the enhancer's ratios was found to be significantly greater than that of standard without enhancer (control). 'Benchmark' values with which to compare the performance of the vehicle are the flux values which were statistically no significant difference ($P>0.05$) across rabbit skin and silicone membrane. The input-rate values of all the ratios have shown a constant trend. The vehicles used were predominantly influencing the partition of the drug into the skin rather than the diffusion throughout the study. Consequently, changes in diffusion and/or partition may occur as a result of absorption or depletion of permeation enhancer inside the membrane/or skin over time which validates our results.

Introduction

The Drug-vehicle based enhancement approach is exploited to circumvent the stratum corneum and to increase the flux through skin membrane, is used in transdermal research as better alternative method to enhance permeation of drugs through skin [1]. The use of oleic acid (OA) like other saturated (stearic acid) and unsaturated (linoleic acid etc.) fatty acids for drug permeation enhancement is of interest in the area of topical and percutaneous absorption research and has shown to be effective penetration enhancer for many drugs in earlier studies [2, 3]. As penetration enhancer, oleic acid which increases skin permeability appears to act selectively on the extracellular lipids representing the main regulatory channel for the penetration of small molecules [4]. In vitro studies investigating the mode of action of OA have generated two mechanistic scenarios, which may account for the action of this enhancer; (i) lipid fluidization and (ii) lipid phase separation. In the studies presented earlier, indicated that OA induces lipid disordering only in the superficial layers of the SC; additionally, OA was found to exist in a liquid phase at all levels of the SC [3]. DDA was chosen as a lipophilic model drug which may provide better patient compliance than oral and it was stated in the literature that highly lipophilic drugs with partition coefficients greater than 2 or 3 tend to remain in the stratum corneum for an extended period of time and will not penetrate well into the lower skin layers [5]. The mechanism of penetration enhancement and effect of surfactants is primarily believed to be due to the promotion of membrane-vehicle partitioning tendency of the drug [6, 7].The aim of this study was to investigate the effect of oleic acid as penetration enhancer when used in different concentrations in the solution, on the percutaneous absorption of DDA *in vitro*.

Results and Discussion

Pre-formulation studies and pH determination was shown in Table-1 and 2, respectively. All other physical parameters like viscosity and homogeneity were also given in Table-2.

Table-1: Preformulation study of drug

	Solubility (mg/mL)			Partition co-efficient $K_{o/w}$ [33]	pK_a	M.P° C
	Water	NS	OA			
DDA + 1ml methanol + 1ml Nitric acid will produce red color	42.28 ±0.59	199.23 ±1.39	413.33 ±21.27	4.40	4.07	280

Table-2:Values for evaluation of Physical parameters

Vehicle(OA) Percentage	pH	%Drug Content	Viscosity (dyn.s/cm^2)	Rabbit Skin extraction (mg/mL)	Homogeneity
1	6.2±0.1	98.56	0.91×10^{-4}	1.32	Good
2	6.2±0.1	98.74	0.83×10^{-4}	1.17	Good
3	6.2±0.1	99.13	0.82×10^{-4}	1.05	Good
4	6.2±0.1	98.87	0.82×10^{-4}	1.03	Good

Solubility Studies

The solubility of DDA in distilled water was 42.282±0.588 mg/mL, at 32°C, which is in line with

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values reported in the literature [7]. DDA was ~10 fold more soluble in oleic acid (413.33±21.27 mg/mL) than water. In the present study, co-solvent mixtures of DDA were made from saturated solutions of enhancer in water as OA:water mixture at 20:80; 40:60; 60:40; 80:20 and100:00 ratio (v/v) respectively as given in Table-3 and then degree of saturation (DS) was calculated (i.e. 1.2).

Solubility Enhancement Ratio **(ER_{sol})** of DDA in both solvents have been determined as:

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

where C_t is concentration of DDA in presence of enhancer and C_s is concentration of DDA in absence of enhancer (control) and observed ER_{sol} was 9.77561 for OA. This trend was previously described using the same co-solvent mixture [8].

FTIR Spectra

Fig. 1 showed no significant differences between pure DDA and OA. The main peak remained unchanged and only some peak ratios differed slightly. FTIR spectra of DDA and OA showed characteristic broad peak of DDA in the range of 3500 to 2500 cm−1 because of hydrogen bonding. The characteristic peaks of DDA at 1698 and 2920 cm−1 were because of carbonyl and hydroxyl stretching, respectively. FTIR studies are in good agreement with the literature [9], suggesting the drug stability.

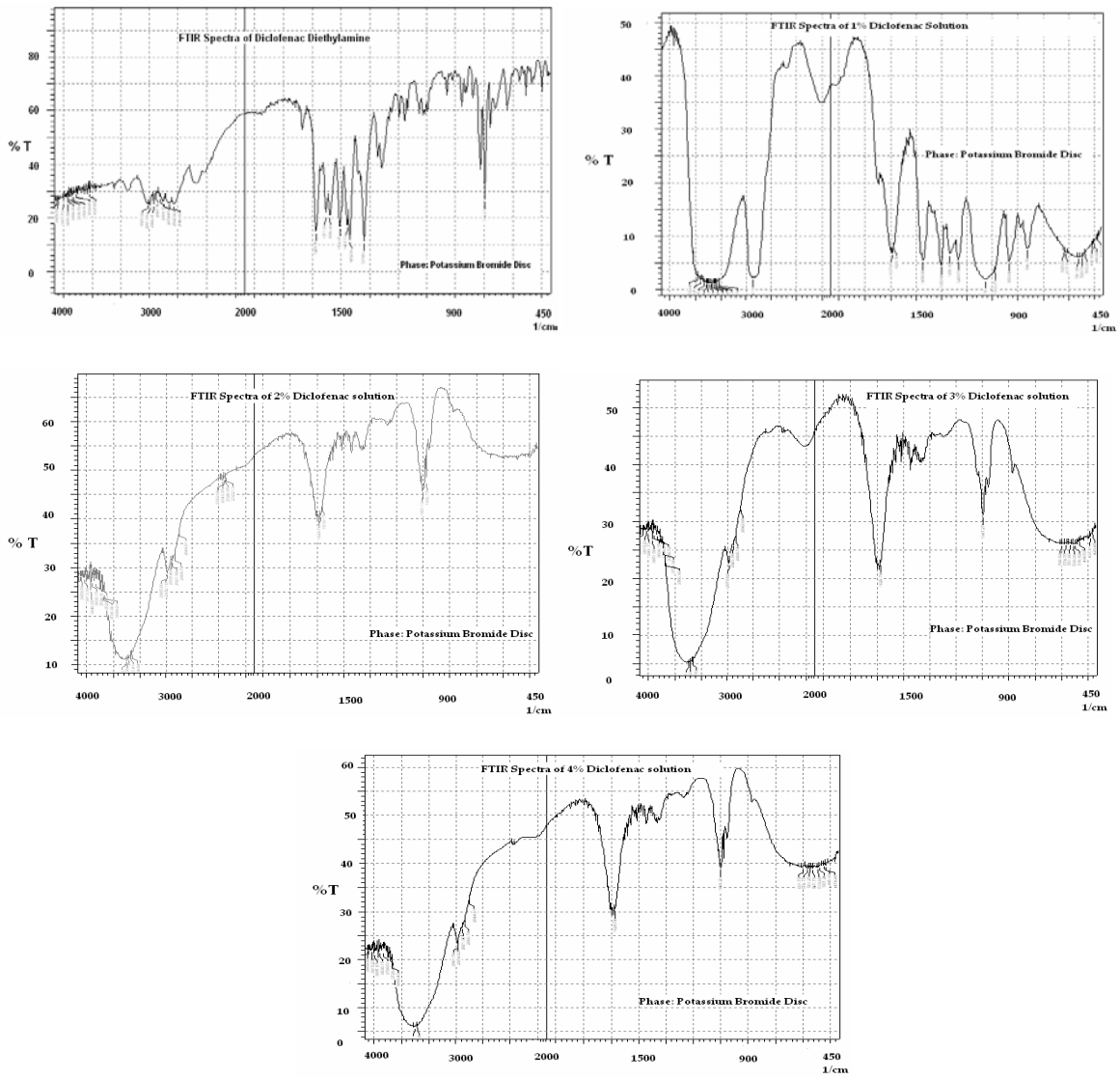


Fig. 1: FTIR Spectra of Pure DDA and different %ages of Diclofenac solution using OA as permeation enhancer.

Table-3: Solubility of Diclofenacdiethylamine in Oleic Acid/water vehicles.

% Oleic Acid in water (v/v)	Solubility (mg/mL)±SD(n=3)
0	42.28±0.59
20	197.31±12.61
40	274.23±8.38
60	328.08±1.92
80	376.80±4.00
100	413.33±21.27

Solvent Uptakeand Skin Extraction Measurements

The uptake was observed for normal saline which confirms the idea introduced above as the lipophilic solvents have solubility parameters closest to that of the membrane. The solubility parameter of silicone membrane is reported in the literature to be 7.5 $(cal/cm^3)^{1/2}$ by Cross *et al.* [10]. The DDA concentration determination within rabbit skin (Table-2) confirmed the deposition of the excess of DDA in the skin which was 1.32, 1.17, 1.05 and 1.03 (w/v).

FoD of Formulations of DDA Across Rabbit Skin vs Silicone Membrane

In this study, FoD value for saturated solution of OA was 1.51, 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 hours study.

I-Kinetics of Permeation Studies through Rabbit Skin

The effect of oleic acid in amount of 1%, 2%, 3% and 4% (v/v) in the Diclofenac [11, 12] solutions on the permeability rate through rabbit skin was shown in Table-4 and Fig. 2 which explained all the permeation parameters with associated standard deviations(± SD). The OA might affect fluidity of SC structure and DDA could be permeated better through the rabbit skin. This famous penetration enhancer 'OA' penetrated into the SC, decompressing it and reduced it's resistance to drug penetration [3] which explained our results. Fig. 3 explained the enhancing ratio ER (J) and ER (D) and ER (J) was observed in the order as 1% <2% <3% <4% while same trend was also observed in ER (D) which is comparable with the earlier work. The input rate obtained is given in Table-5 which is almost 9-12 folds higher than for control.

II-Kinetics of Permeation Studies through Silicone Membrane

The permeation of Diclofenac solutions through silicone membrane, using OA of varying concentrations (1%, 2%, 3% & 4% v/v) was evaluated and enlisted in Table-6. There is no significant difference (P >0.05) between all permeation parameters of the solutions, these values almost behaving as increase with the increase in the concentration of enhancer solution from 1% to 4%.Fig. 4 explains the enhancing ratio ER (J) & ER (D) and the values were observed in the order as 1% <2% <3% <4% which is comparable with the earlier work [13]. The input rate obtained is given in Table 5 which is almost 2-4 folds higher than for control. The minimum standard deviation values assured that the process used for preparing the solution system is capable of giving reproducible results which is further confirmed by earlier studies data [14].

Table-4: Permeation kinetics of Diclofenac-Diethylamine in the presence of oleic acid through Rabbit Skin (n=5) at 37ºC±2.

Vehicle (OA) % age	Flux* (µg/cm²/min) ± SD×10⁻³	D** (cm².min⁻¹) x 10⁻² ± SDx10⁻⁴	K_p*** (cm.min⁻¹) x10⁻⁸ ± SDx10⁻⁹	K**** x10⁻⁴ ±SDx10⁻⁹	ER
1	0.7725 ± 2.306	14.24 ± 12.33	9.34 ±27.89	0.58 ±49.78	2.00
2	0.862 ± 10.472	11.28 ± 39.91	10.43 ±126.7	0.82 ±382.0	2.23
3	1.035 ± 28.74	5.65 ± 52.14	12.52 ±3.47	1.96 ±0.225	2.68
4	1.095 ± 39.43	6.00 ± 23.65	13.25 ±4.77	1.95 ±8.26	2.83
Control	0.105 ± 0.0005	118.13 ±23.41	0.053 ±0.27	0.039 ±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).

Table-5: Input-rate of DDA in different concentrations of vehicle's solutions across rabbit skin and silicone membrane (n=5) at 37°C±2.

Vehicle (OA) %age	Rabbit Skin (µg/min)	Silicone Membrane (µg/min)
1	0.121	0.631
2	0.135	0.655
3	0.163	0.713
4	0.172	0.764
Control	0.061	0.031

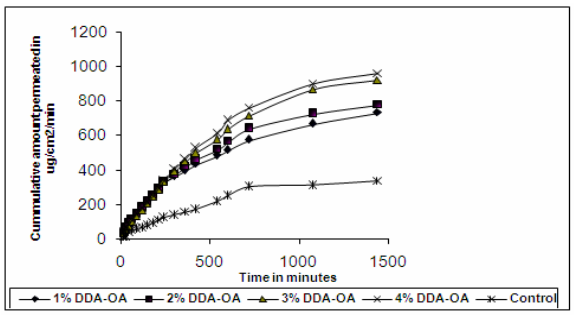


Fig. 2: Permeation of Diclofenacsolution through rabbit skin (n=5) at 37°C±2.

Table-6: Permeation kinetics of Diclofenac-Diethyl-amine in the presence of oleic acid across silicone membrane (n=5) at 37°C±2.

Vehicle (OA) % age	Flux* (µg/cm²/min) ± SD×10⁻³	D** (cm².min⁻¹) x 10⁻⁴ ± SDx10⁻⁵	K_p*** (cm.min⁻¹) x10⁻⁴ ± SDx10⁻⁷	K**** x10⁻⁴ ± SDx10⁻⁵	ER
1	0.803 ±12.67	7.554 ± 8.73	0.803 ±0.127	9.468 ±11.97	21.2
2	0.835 ±14.49	6.553 ±3.66	0.4174 ±7.24	5.624 ±4.06	22.0
3	0.908 ±3.11	6.559 ± 0.85	0.302 ±1.04	4.060 ±5.03	24.0
4	0.973 ±12.17	5.962 ±1.56	0.243 ±3.04	3.594 ±1.31	25.7
Control	0.037 ± 0.0005	6.09 ±23.41	0.945 ±0.27	14.69 ±0.009	-

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

Discussion

It is not explicit for sometime increasing the lipid solubility, the partition coefficient (K) between a lipid and water, has been the standard working paradigm for increasing permeation of the skin and the permeability coefficient (k_p = distance/time) has been the quantitative measure of the results. The shorter chain and more water soluble alcohols exhibiting lower (K) values gave the greater flux values (J = amount/area×time; the more clinically relevant measure of permeation) and D values, regardless of whether they were applied neat or in an aqueous vehicle as in this study while K_p showed opposite trends for the solutions [15].

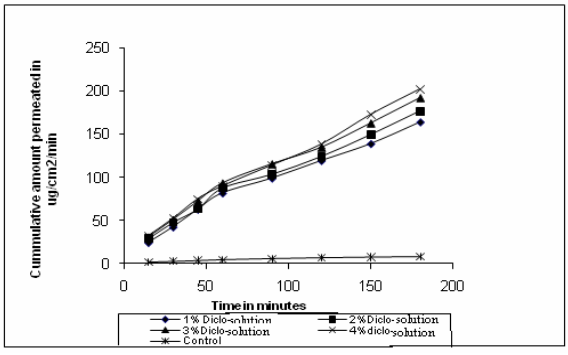


Fig. 3: Permeation of Diclofenacsolution through Silicone membrane (n=5) at 37ºC±2**.**

The permeation rates of the drug calculated from the permeation profiles of each ratios are shown and among these tested, the ratio which was composed of 2% DDA, 4% (v/v) of Oleic acid showed the highest permeation rate (1.095±39.43µg/cm²/min). The quantity of OA in solution affected the skin permeation rate of DDA significantly (Fig. 2). As the amount of OA was decreased from 4% (v/v) to 1% (v/v) the skin permeation rate of DDA also decreased which 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 in the enhancers' mixture [4]. The reported data in this study (Fig. 4, 5) showed that K is increasing and D is decreasing from 1% (v/v) to 4% (v/v), hence permeation through rabbit skin is partitioning; although diffusion is occurring in the skin as the earlier studies confirmed the deposition of DDA into the skin [16].

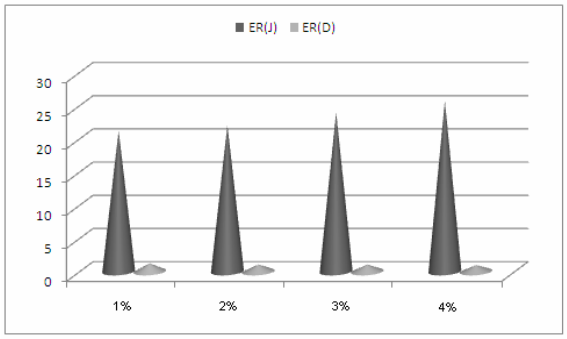


Fig. 4: Enhancing Ratio(ER) w.r.t. Flux (J) and Diffusion co-efficient (D) of DDA in Diclofenac solution throughSilicone membrane (n=5) at 37 °C±2.

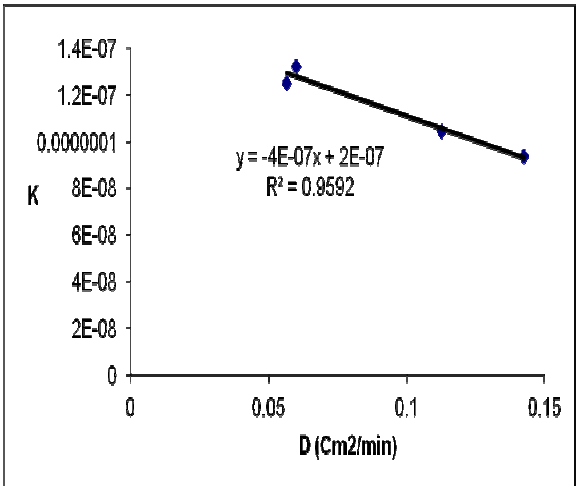


Fig. 5: Correlation b/w partition co-efficient (K) and diffusion co-efficient (D) through rabbit skin.

It was also found that the permeation of the DDA in solution was significantly influenced by the presence of ethanol. The literature supported our data that skin permeation rate of DDA was increased by 7-9 folds [17]. It is possible that in the presence of

alcohol, the size of internal phase of the solution may be decreased, making the surface area of the droplet increased significantly and the influence of alcohol in solutions upon the transport behavior of several permeants across the skin has been evaluated earlier [18, 19]. 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 [20]. As the %age (v/v) of oleic acid was increased, the number of internal phase (aqueous and lipid channels) was increased which increased the permeation rate of drug [4] as in 4% solution flux value was 1.095±39.43 (µg/cm^2/min) and 0.973±12.17 (µg/cm^2/min) in rabbit skin and silicone membrane respectively whereas in 1% solution it was 0.7725±2.306 (µg/cm^2/min) and 0.803±12.67 (µg/cm^2/min) respectively (Fig. 2, 3). Solution ratios used in this study enter the SC, change its solution properties by altering the chemical environment and thus dissolve the barrier capacity of the cutaneous layer [21]. Input Rate of the solutions of was 0.1613µg/min. The lyophilic domain of the solution can interact with the stratum corneum. DDA dissolved in the lipid domain of the solution can directly partition into the lipids of the stratum corneum or the lipid vesicle themselves can intercalate between the lipid chains of the stratum corneum, thereby destabilizing its bilayer structure. In effect, these interactions will lead to increase the permeability of the lipid pathway to DDA. Consequently, the OA influences the penetration in accordance with earlier studies [22, 23]. The lag time always played a significant role in the percutaneous absorption of the drug and was calculated from x-intercept of the slope at the steady state. As OA partition into and interact with SC constituents to make a temporary, reversible increase in skin permeability and after passing definite time, the equilibrium will be created; it is more important that the lag time must be in an agreeable range if topical solutions possess lag time because they have less resident time on skin [24].

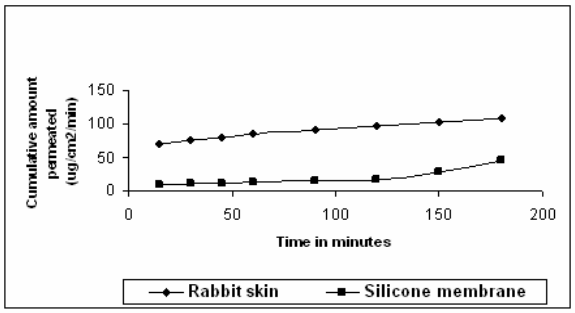
In summary, we utilized Drug-vehicle based enhancement approach to evaluate the enhancing effect of OA through silicone membrane/or rabbit skin, however, the earlier scientists [25] presented results to demonstrate that topical application of OA induces stratum corneum lipid structure disorder *in vivo.* OA may enhance percutaneous penetration mainly through a dual mechanism involving stratum corneum lipid bilayer perturbation and lacunae formation as earlier studied by Jiang [26]. The results revealed that lipophilic enhancers were more effective than lipophobic ones [27] and in simple diffusion experiments, this is very difficult to reveal possible interactions and it could be irrational to try and do so.

Comparison of Saturated Solution Across Rabbit Skin vs Silicone Membrane

In this study, FoD value obtained was 1.51 (Table-7), 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 as illustrated in Fig. 6-8 for permeation study after 3 hours. Thus, considering all this discussion together with the FoD (Table-7), this animal model (Rabbit skin) and silicone model membrane can be regarded as predictive of human skin permeability [28].

Table-7: The factor of difference value (FoD) in the presence of saturated enhancer's solution across rabbit skin and silicone membrane (n=5) at 37ºC±2.

Vehicles	J_{RS} (µg/cm^2/min)	J_{SM} (µg/cm^2/min)	FoD
OA	0.137	0.205	1.51



One way ANOVA confirmed significant difference (P<0.05) and F value is 16.49

Fig. 6: The factor of difference value (FoD) in the saturated enhancer's solution (OA) across rabbit skin and silicone membrane (n=5) at 37ºC±2.

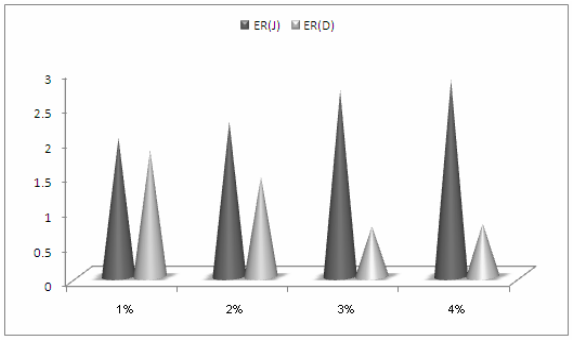


Fig. 7: Enhancing Ratio(ER) w.r.t. Flux (J) and Diffusion co-efficient (D) of DDA in Diclofenac solution through rabbit skin (n=5) at 37ºC±2.

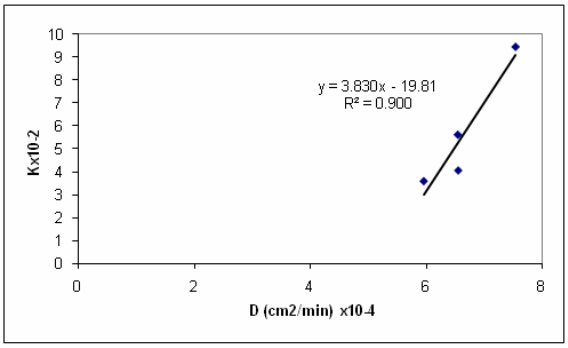


Fig. 8: Correlation b/w partition co-efficient (K) and diffusion co-efficient (D) through Silicone membrane**.**

Experimental

Materials and Methods

Oleic acid, Ethyl alcohol, Methanol and Sodium chloride were all purchased from Merck Chemical Co., Germany. Reference standard Diclofenac Diethylamine powder was a gift from Novartis (Pvt.) Ltd. Jamshoroo, Pakistan.

Preparation of Diclofenac Solution

2 g Diclofenac Diethylamine was dissolved in 15 mL of ethanol in 100 mL volumetric flask and then added various concentrations (i.e. 1%, 2%, 3% and 4% v/v) of oleic acid into the drug solution and the volume was made up to the mark with normal saline (NS).

Control Solution

2 g Diclofenac Diethylamine was dissolved in 15 mL of ethanol in 100 mL volumetric flask and the volume was made up to the mark with NS. This was used as reference control solution without any enhancer.

In-Vitro studies

It is a stage of development during which characterizes the physico-chemical properties of the drug solution and its interaction with various components.

pH

The pH of all solutions was determined by using digital pH-meter (Mettler & Toledo Germany).

Viscosity

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

Homogeneity

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

The volumetric flask containing solution (10 mL) was shaken well and filtered and estimated spectrophotometrically at 276nm using normal saline (NS) as blank.

Fourier Transforms Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) of DDA and the all solutions were obtained using FTIR Spectrophotometer [Model: 8400 S; Shimadzu Scientific Instruments (SSI), Kyoto, Japan] (phase: KBr discs) to investigate any interaction between enhancers and the drug. The spectra were scanned over the wave number range from 4000 to 400 cm^{-1}.

Solubility Studies

The drug must be able to diffuse through the lipid-aqueous bilayer of the skin for effective topical absorption [29]. For this reason the solubility of the permeants in the vehicle and vehicle combinations were determined by adding excess amount of DDA to each solvent or co-solvent mixture [30] and stirred with a magnetic bar for 48 hours (to attain equilibrium) in a water bath maintained at 37ºC±2. Solutions were centrifuged for 30 minutes at 4000 rpm. The supernatant was then diluted and assayed by UV-spectrophotometer at the wavelength of 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/ or Skin Extraction Measurements

The uptake of the selected vehicles into silicone membrane and rabbit skin was evaluated in this study. The uptake of vehicles was experimentally

determined by cutting Silicone 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 hours. 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 is expressed in the following equation as:

The experiments were performed at 37ºC±2 in triplicate.

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 silicone membrane data) by means of the factor of difference value (FoD) described by the following Dick and Scott equation;

$$FoD = \frac{J^{RS}}{J^{SM}}$$

$$\% \text{ Solvent Uptake} = \frac{\text{Skin weight after treatment - Dry Skin weight}}{\text{Dry Skin weight}} \times 100$$

where J_{RS} and J_{SM} denotes maximum flux value (J) through rabbit skin (RS) and silicone 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 [31].

Diffusion Studies through Rabbit Skin and Silicone Membrane

Diffusion studies across rabbit skin and silicone membrane were performed using Franz-type diffusion cells (made of Germany at SOP, London) that have a receptor phase 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/or sheets of silicone membrane were cut according to the diameter of the diffusion cell. The skin was placed in a normal saline solution before mounting on to the diffusion cell [32]. Both rabbit skin and silicone membrane were soaked overnight in the receptor solution i.e. NS. The skin/ or membrane was then placed between the two compartments of the diffusion cells using Silicone grease (Dow, USA) to produce a leak-proof seal between the membrane and the two compartments of the diffusion cell. The receptor compartment was filled with NS and each solution (1 mL) was placed in the donor compartment. To remove air bubbles and prevent the buildup of air pockets in the receptor phase, NS was degassed in an ultrasonic bath. To prevent evaporation from the receptor compartment, the cell arm was covered with a glass lid. Uniform mixing of the receptor solution was obtained with a magnetic stirrer that was placed in the receptor compartment. The diffusion cells were placed on a stirring bed immersed in a water bath at 35°C±2. After one hour interval, the receptor solution was completely removed and refilled with fresh pre-thermostated NS. Sink conditions were met in all cases. From the side arm of the receptor compartment, 0.5 mL of the sample was drawn each time at an interval with the help of 1 mL syringe (Sun, Korea) and correcting the receptor half cell volume with pre-thermostated NS. The sample taken from the receptor cell was run on U.V. spectrophotometer (Agilent2005; software version 2005) at the wavelength of 276 nm. The diffusion experiments were performed under occluded conditions by covering the donor compartment with Para film. All experiments were performed at 37°C±2 in ±SD (n=5) and sampling time was 0-3 hours with predetermined intervals for silicone membrane while 24 hours for rabbit skin studies.

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. A probability of $p<0.05$ was considered statistically significant. All results are presented as the mean ± SD, unless otherwise stated.

Conclusion

we could conclude that the enhanced permeation of DDA may be by the partitioning of the drug into the stratum corneum and also by modifying intercellular lipids, disrupting their highly ordered structure and thus increasing the diffusion of DDA through the membrane with increased solubility and it is important to observe the increased amounts of DDA in the skin may also be retention of the drug by the skin. The benefit of penetration enhancement in this study was counterbalanced by the fact that at this

range of concentration, the use of OA cannot harm the skin.

Declaration of Interest

The authors report no conflicts of interest.

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