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COMPARATIVE EVALUATION OF *IN VITRO* DICLOFENAC SODIUM PERMEABILITY ACROSS EXCISED MOUSE SKIN FROM DIFFERENT COMMON PHARMACEUTICAL VEHICLES

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ABSTRACT: The comparative *in vitro* skin permeability of diclofenac sodium through excised mouse skin from different common pharmaceutical vehicles like castor oil, olive oil, arachis oil, liquid paraffin, white soft paraffin melt, 3% sodium carboxymethyl cellulose (CMC) aqueous gel and isopropyl myristate (IPM), excluding other excipients was evaluated. Significantly ($p < 0.05$) higher flux of diclofenac sodium (0.26 ± 0.01 mg/cm^2/hr) was provided by isopropyl myristate as vehicle than other vehicles used. Maximum permeability coefficient and diffusion coefficient were also observed for isopropyl myristate and minimum in case of white soft paraffin melt. But, isopropyl myristate had minimal lag time (0.60 ± 0.05 hr); while, maximum lag time (0.86 ± 0.05 hr) was observed for white soft paraffin melt. We have also investigated the *in-vitro* skin permeability of diclofenac sodium through excised mouse skin from liquid paraffin and olive oil with addition of 0.1 % menthol and dried *Aloe vera* juice as permeation enhancers. Flux, permeability coefficient, diffusion coefficient and enhancement ratio were better in the case of liquid paraffin with 0.1 % menthol as permeation enhancer than others. We believe this investigation is considered to be useful in preformulation step to predict the best vehicle in further experiment and also provides valuable data that can be utilized for designing and development of various topical formulations of diclofenac sodium.

KEY WORDS: Vehicles; Diclofenac sodium; *In vitro*; Skin permeation; Permeation enhancer.

INTRODUCTION

During the past few decades', skin has been shown to be a suitable delivery route for drugs formulated topically. Topical administration facilitates safe and effective delivery of drug molecules with lower doses when compared with that of oral dosage forms; hence, it is hypothesized that the topical delivery limits the systemic absorption of drugs. Another potential advantage of this type of drug delivery is the optimization of drug concentration at the desirable sites, reducing the chances of side-effects.[1] Therapeutic efficacy of any topical formulation depends on its ability to deliver drugs to their sites of action from the skin surface for either local or systemic purposes. [2-3] The rate-limiting step for topical delivery is the passage or diffusion through skin. Since the uppermost layer of skin, the stratum corneum represents an efficient protective barrier to the transport of most drug molecules. The stratum corneum (horny layer) has a thickness of about 15-20 μ, and is a layer of compressed, overlapping keratinized cells that form a tough, flexible and coherent membrane. This layer contains dead cells

with keratin filaments in a matrix of proteins with lipids and water-soluble substances. The thickness and penetration properties of stratum corneum depend upon its hydration, which normally contains around 20% water.[4] The efficacy of topically applied drugs is often limited by poor skin penetration.[5] However, drug molecules encounter various types of resistance such as membrane and diffusion resistance when they permeate through biological membranes.

In spite of the barrier function of the skin, topical drug delivery provides a convenient route of administration for a variety of clinical indications. The first challenge of developing an effective topical drug delivery system ultimately involves ensuring adequate drug permeability through the skin. Moreover, it is also important to ensure that the drug is not unduly metabolized and delivered according to the desired pharmacokinetics and pharmacodynamics.[6] Several factors are known to influence the rate and extent of drug absorption through skin like mode of application, skin condition, vehicle used, concentration and physicochemical properties of drug molecules.[7] Among them, vehicles play an important role in drug permeation through skin barrier as vehicles used in topical formulations decide the consistency, physical properties and also affects the drug release from topical formulations.[8] Therefore, like percutaneous absorption enhancer, the selection of appropriate vehicle is an important key issue in increasing the efficacy of a topically applied formulation. Hereby, *in vitro* skin permeation models have been useful as basic systems for comparing effectiveness of various vehicles and excipients on drug availability since physicochemical properties of vehicles strongly influence drug release topically.[9]

Several *in vitro* skin permeation studies have done to investigate the vehicle effect on various topical drug deliveries.[7; 10-14] In the present study, diclofenac sodium was selected as the model drug. Diclofenac sodium is a non-steriodal anti-inflammatory drug (NSAID) and widely used clinically, because of its strong analgesic and anti-pyretic effect.[15] It has a short half-life (2 hrs). Diclofenac sodium causes gastrointestinal disturbances, peptic ulceration with bleeding, if present in large doses in gastrointestinal tract (GIT).[16] It has been generally administered orally and rectally but, not topically due to its poor absorptivity across the skin.[17] Some works were also done to understand the effect of vehicle on *in vitro* skin permeation of diclofenac sodium from different topical formulations like gels, creams, emulsions etc.[17-19] But, the effect of vehicles on skin permeation can be well understood by keeping other factors constant. Therefore, the *in vitro* skin permeation of diclofenac sodium from different vehicles only can be investigated to reveal the actual effect of vehicle on skin permeation. Only Takahashi et al has done an experiment to understand the effect of some vehicles like liquid paraffin, castor oil and olive oil on *in vitro* transport of diclofenac sodium across skin barrier using some oleaginous vehicles separately with the drug alone.[17] The poor flux of diclofenac sodium was observed from these oleaginous vehicles separately. In this study, we have evaluated *in vitro* skin permeability of diclofenac sodium from several common pharmaceutical vehicles which have been frequently used as vehicle in various pharmaceutical and cosmetic preparations like castor oil, olive oil, arachis oil, liquid paraffin, white soft paraffin melt, 3% sodium carboxymethyl cellulose (CMC) aqueous gel and isopropyl myristate (IPM), excluding other excipients. Different dispersions containing diclofenac sodium prepared only by using above vehicles without or with selected permeation enhancers were evaluated. 0.1% menthol and dried *Aloe vera* juice ware used as permeation enhancers. The *in vitro* skin permeation flux, lag time, diffusion coefficient and permeation coefficient of diclofenac sodium through excised mouse skin from various dispersions using different pharmaceutical vehicles without or with permeation enhancer were investigated. We believe this investigation provides valuable data that can be utilized for designing and development of various topical formulations.

MATERIALS AND METHODS

Materials

Diclofenac sodium was gift sample from Techno Remedies, Kolkata, India. Liquid paraffin (Nice Chemicals Pvt Ltd, India), white soft paraffin (Loba chemie Pvt Ltd, India), sodium carboxymethyl cellulose (Loba chemie Pvt Ltd, India), isopropyl myristate (Merck, India), arachis oil (B D & Co, India), olive oil (RP Oomerbhoy Pvt Ltd, India), castor oil (BD Pharmaceutical Works, India), menthol (Qualigens Fine Chemicals, India), disodium hydrogen phosphate (S.D. Fine Chemicals Limited, India), potassium dihydrogen phosphate (S.D. Fine Chemicals Limited, India) and sodium chloride (S.D. Fine Chemicals Limited, India) were purchased commercially. All other chemicals and reagents used were of analytical grade. Stock solutions were prepared fresh when required.

METHODS

Preparation of test formulations

Diclofenac sodium dispersions using different common pharmaceutical vehicles: 10 mg of diclofenac sodium was suspended in 10 ml of castor oil, olive oil, arachis oil, liquid paraffin, white soft paraffin melt, 3% sodium carboxy methylcellulose (NaCMC) aqueous gel and isopropyl myristate (IPM), separately (Table 1).

Diclofenac sodium dispersions using different common pharmaceutical vehicles with permeation enhancers: 10 mg of diclofenac sodium was suspended in 10 ml of olive oil and liquid paraffin, as made above. 0.1% w/v menthol and 1 gm of dried *Aloe vera* juice were added as permeation enhancers in each formulation (Table 1).

Preparation of dried *Aloe vera* juice

Dried *Aloe vera* juice was prepared from the full size mature leaves of *Aloe vera*. They were cut from the plant and the green rind was removed. From the cut leaf bases the yellow juice was allowed to drain into a container and dried at room temperature for 3 days. This dried *Aloe vera* juice was used for experiments.

Determination of partition coefficient

The partition studies were conducted by shake flask method. n-octanol was saturated with phosphate buffer saline (PBS), pH 7.4 for 24 hours before the experiment. Accurately weighed amount about, 1000 mg of diclofenac sodium was added to each 20 mL of vehicle and water mixture (1:1 ratio). The solution was kept for 24 hours shaking at room temperature on rotary shaker. Two phases were separated and filtered through 0.45 µ membrane filter. The concentration of the drug in the aqueous phase was analyzed spectrophotometrically; concentration of the drug in vehicle was calculated from the difference between the initial amount and amount in aqueous phase. The partition coefficient was calculated using following equation:

$$\text{Partition coefficient (P)} = \frac{[\text{Drug}]_{\text{vehicle}}}{[\text{Drug}]_{\text{Aqueous}}} \quad \ldots\ldots\ldots\ldots (1)$$

Solubility study

Excess amount of diclofenac sodium was added to the test vehicles. The mixture was then allowed to stand at 37 ± 0.5 °C for 24 hours under agitation and filtered through membrane filter with a pore size 0.45 µ. The concentration of diclofenac sodium was measured by UV-Visible Spectrophotometer (Shimadzu UV- Visible Spectrophotometer, UV-1601).

Preparation of excised mouse skin

Swiss albino mouse weighing 80-100 gms were selected for permeation studies. The animals were sacrificed using anesthetic ether. Hairs from the abdominal region were carefully removed with fine forceps. A full thickness of skin was taken out and then trimmed to remove the fatty material. Finally, the epidermal skin was taken and examined microscopically to ensure the integrity of the stratum corneum. The thickness of the excised skin was measured with digital micrometer.[19] The thickness was found to be $2.25 \times 10^{-2} \pm 0.03$ cm, after 3 measurements.

***In vitro* skin permeation experiments**

In vitro skin permeation studies were carried out using Franz diffusion cell. The cell consists of two chambers, the donor and the receptor compartment with a diffusion area of 3.14 cm^2. The donor compartment was open at the top and was exposed to atmosphere. The excised mouse skin was mounted between the compartments of the diffusion cell with stratum corneum facing the donor compartment and clamped into position. Magnetic stirrer bars were added to the receptor chambers and filled with the receptor phase. Phosphate buffer saline (PBS) pH 7.4 was used as receptor medium. The entire setup was placed over magnetic stirrer and the temperature was maintained at 37 ± 0.5° C. Samples of 1 mL was collected from receptor compartment at predetermined intervals over study period and replaced with same amount of fresh buffer. The amount of permeated drug was measured using UV-Visible spectrophotometer (Shimadzu UV-Visible Spectrophotometer, UV-1601) by measuring absorbance at 276 nm.

Determination of drug content in the samples

Phosphate buffer solution (PBS) at pH 7.4 was decided to be used as the receptor phase in permeation experiments of diclofenac sodium through mouse skin. Maximum wavelength (λ_{max}) obtained by scanning all samples from 200 to 400 nm and this was 276 nm. The samples were filtered through 0.45 µ filter paper, diluted suitably. Absorbance values of different samples were measured from at 276 nm (λ_{max}) using a UV- Visible Spectrophotometer (Shimadzu UV-Visible Spectrophotometer, UV-1601).

Statistical Analysis

The permeation flux values obtained for various vehicle systems were tested for significant differences using a one-way analysis of variance (ANOVA) using a Dunnett's test as post hoc analysis in this study. The statistical analysis was conducted using SPSS software version 17.0 (SPSS Inc., Chicago). Each value represents the mean ± SD, (n = 3).

RESULT AND DISCUSSION

Skin permeation of drug depends on both the solubility and the partition characteristics. Table 2 shows the solubility of diclofenac sodium in different common pharmaceutical liquid vehicles. The solubility of diclofenac sodium was measured at 37 ± 0.5°C.

Diclofenac sodium was found to have the maximum solubility (1.65 ± 0.07 % w/v) in castor oil (V 3) and the minimum solubility (0.17 ± 0.03 % w/v)

in isopropyl myristate (V 7). In the rest of vehicles like olive oil, arachis oil and liquid paraffin, the solubility of diclofenac sodium was between 0.23 ± 0.02 % w/v to 0.74 ± 0.08 % w/v. The partition coefficient of diclofenac sodium between vehicle (oil phase) and phosphate buffer saline, pH 7.4 (aqueous phase) was also found to be maximum (12.02 ± 1.34) with castor oil (V 3), while the minimum partition coefficient (0.72 ± 0.09) was observed with isopropyl myristate (V 7). Figure 1 presents the permeation profile of diclofenac sodium (permeated amount of diclofenac sodium / cm^2 skin surface vs. time) through excised mouse skin from different common pharmaceutical vehicles only. Vehicles used for permeation were olive oil (V 1), arachis oil (V 2), castor oil (V 3), liquid paraffin (V 4), white soft paraffin melt (V 5), 3% sodium carboxymethyl cellulose (NaCMC) aqueous gel (V 6) and isopropyl myristate (IPM) (V 7).

Amount of diclofenac sodium permeated through excised mouse skin over 6 hours period were plotted against the function of time. The slope and intercept of the linear portion of plots were derived by regression. The flux (J, mg/cm^2/hr) was calculated as the slope is divided by the skin surface area. The lag time (L_t, hr) was determined by extrapolation of the linear portion of the plot on the abscissa.[21] It has shown that the linearity of such a plot was not achieved until lag time (L_t) of 2 hours.[22]

The permeability coefficient (P) was calculated using relation derived from Fick's 1 st law of diffusion, which is expressed by equation no 2: [23]

$$P = J / C \qquad \text{……..(2)}$$

where, J is the steady state flux and C is the initial drug concentration.

The diffusion coefficient (DC) was calculated using the relation derived from Fick's 2 nd law of diffusion, which is described in equation no 3:[24]

$$DC = h^2 / 6 L_t \qquad \text{……..(3)}$$

where , h is the thickness of the skin and L_t is the lag time.

Table 3 presents permeation parameters of diclofenac sodium through excised mouse skin from different common pharmaceutical vehicles only. The result reveals the maximum flux, permeability coefficient and diffusion coefficient with isopropyl myristate (V 7) and minimum in case of white soft paraffin melt (V 5). The permeation of diclofenac sodium from isopropyl myristate (V 7) had minimal lag time (0.60 ± 0.05 hr); while, maximum lag time (0.86 ± 0.05 hr) was observed for white soft paraffin melt (V 5). On comparing the flux of diclofenac sodium permeation from various common vehicles, it was observed that significantly ($p < 0.05$) higher flux was provided by isopropyl myristate (V 7). The maximum skin permeability of diclofenac sodium from isopropyl myristate (V 7) and minimum in case of white soft paraffin melt (V 5) could be attributed to lower solubility and partioning of diclofenac sodium in these two vehicles. Isopropyl myristate is an aliphatic ester, which is widely used as a safe penetration enhancer in various dermatological formulations. The mechanism of action of higher skin permeability of drugs from isopropyl myristate is not precisely understood, but it seems that isopropyl myristate penetrates between the lipid bilayers of stratum corneum and due to its' chain structure, disrupts the order and arrangement of lipid bilayers of stratum corneum and hence improves drug permeation into this layer.[4, 25]

The partitioning phenomenon is an equilibrium process described by the apparent oil / aqueous partition coefficient. Only the fraction of the total drug concentration, which is present in aqueous phase, F, could be absorbed.

$$F = 1 + \alpha/1 + K_\alpha \qquad \text{……..(4)}$$

where, K_α is the apparent oil (here vehicle) / aqueous partition coefficient and α is the ratio of volume of oil (vehicle) phase to that of the aqueous phase (V_o/V_w). The equation suggests that the fraction of drug available for absorption is controlled by partition coefficient (K_α) and the ratio of volume of two phases (α). Since V_w is a physiologic parameter, it is usually constant and therefore, the value of is the ratio of volume of oil (vehicle) phase to that of the aqueous phase (α) is to be determined solely by the value of volume of oil (vehicle) phase (V_o).[26] The rate of drug absorption can be expressed by equation no 5:[26]

$$d(c)/ dt = K_a . F. (Dt) \qquad \text{…(5)}$$

where, Dt is the total drug concentration in both, oil (vehicle) phase and aqueous phase. K_a is the absorption rate constant.

The above discussion indicates that the rate of absorption of drug from vehicles would depend on the fraction of the total drug concentration, which is present in aqueous phase (F). It would be appropriate to mention here that the total drug concentration, present in aqueous phase (F) in turn depends on the apparent oil (vehicle) / aqueous partition coefficient. Therefore, our skin permeation results validates that the higher permeability of diclofenac sodium through excised mouse skin from vehicles with lower partition coefficient and oppositely, lower permeability of diclofenac sodium from vehicles with higher partition coefficient. Thus, the results of permeation experiments correlate well with partition characteristics of diclofenac sodium.

We have also investigated the *in-vitro* skin permeability of diclofenac sodium through excised mouse skin from liquid paraffin and olive oil with addition of 0.1 % menthol and dried *Aloe vera* juice as permeation enhancers (V 8 to V 11). Figure 2 presents the permeation profile of diclofenac sodium (permeated amount of diclofenac sodium / cm^2 skin

surface vs. time) and Table 4 presents permeation parameters of diclofenac sodium through excised mouse skin from liquid paraffin and olive oil with addition of 0.1 % menthol and dried *Aloe vera* juice. The result reveals the maximum flux, permeability coefficient and diffusion coefficient for liquid paraffin with 0.1 % menthol (V 8) and minimum for olive oil with dried *Aloe vera* juice (V 11). Among them, the permeation of diclofenac sodium from liquid paraffin with 0.1 % menthol (V 8) had minimal lag time than others (0.36 ± 0.02 hr). While, the lag time for others (V 9, V 10 and V 11) was between 0.55 ± 0.08 to 0.69 ± 0.03 hr. On comparing the flux of diclofenac sodium through excised mouse skin from liquid paraffin and olive oil with addition of 0.1 % menthol and dried *Aloe vera* juice, it was observed that significantly ($p < 0.05$) higher flux of diclofenac sodium was provided by liquid paraffin with 0.1 % menthol (V 8) than liquid paraffin and olive oil with dried *Aloe vera* juice (V 10 and V 11). Again, significantly ($p < 0.05$) lower flux of diclofenac sodium was provided by olive oil with dried *Aloe vera* juice (V 11) than other three (V 8, V 9 and V 10).

On comparing flux of diclofenac sodium through excised mouse skin from liquid paraffin without and with 0.1 % menthol and dried *Aloe vera* juice as permeation enhancers, it is seen that significantly ($p < 0.05$) lower flux of diclofenac sodium was provided by liquid paraffin without any permeation enhancer (V 4) than liquid paraffin with 0.1 % menthol (V 8) and with dried *Aloe vera* juice (V 10) (Figure 3). Similarly, significantly ($p < 0.05$) lower flux of diclofenac sodium was provided by olive oil without any permeation enhancer (V 1) than olive oil with 0.1 % menthol (V 9) and with dried *Aloe vera* juice (V 11) (Figure 4).

Permeation enhancers are the substances that facilitate the absorption of penetrant through the skin by temporarily diminishing the impermeability of the skin. They may act by one or more of three main mechanisms to increase the skin permeability: [21,27] (i) improved partition of the drug or solvent into stratum corneum, (ii) disruption of highly ordered structure of stratum corneum lipid, (iii) interaction with intracellular proteins. In the present investigation, we have used 0.1 % menthol and dried *Aloe vera* juice as permeation enhancers. Menthol is a terpene derivative and has long been used permeation enhancer in a variety of formulations.[28-29] Menthol preferentially distributes into the intercellular spaces of stratum corneum and possibly causes the reversible disruption of lipid domains.[30] The mechanism of menthol as an enhancer may be the permeation of drugs by both the lipid and pore pathways, thus enhancing the permeation of drugs.[31] On the other hand, there are some reported evidences to suggest that *Aloe vera* has skin penetration enhancement properties.[32-33] One proposed mechanism for penetration enhancement effect of *Aloe vera* juice is that the more lipophilic components of the *Aloe vera* may penetrate the stratum corneum more readily and modulate it in some way.[33]

In the last part of this study attempts were made to compare the "enhancement ratio (ER)" of permeation enhancers used. Enhancement ratio (ER) is a ratio of permeability coefficient following the use of permeation enhancer, divided by the permeability coefficient before the use of penetration enhancer.[4] The enhancement ratio (ER) of various concentrations of permeation enhancers used in this study is summarized in Table 5. The greater the enhancement ratio (ER), the greater the permeation enhancement ability of permeation enhancer used. This finding suggests that the addition of 0.1 % menthol as a permeation enhancer to liquid paraffin and olive oil appears to provide the greatest permeability rate of diclofenac sodium through excised mouse skin than dried *Aloe vera* juice.

CONCLUSION

The comparative evaluation of *in vitro* skin permeability of diclofenac sodium through excised mouse skin from several common vehicles, which have been frequently used as vehicle in various pharmaceutical and cosmetic preparations is considered to be useful in preformulation step to predict the best vehicle in further experiments as the choice of an appropriate vehicle is particularly crucial in the development of topical formulations for better permeability of drug through skin. According to our results, isopropyl myristate, which is itself a permeation enhancer, may be a good vehicle candidate for the topical delivery of diclofenac sodium, giving considerably higher diclofenac sodium permeability than the other vehicles used. The effect of 0.1 % menthol and dried *Aloe vera* juice as permeation enhancers with liquid paraffin and olive oil on *in vitro* skin permeability of diclofenac sodium through excised mouse skin show that flux through the skin was better in the case of liquid paraffin with 0.1 % menthol as a permeation enhancer. We believe this investigation provides valuable data that can be utilized for designing and development of various topical formulations of diclofenac sodium.

Table 1: Various vehicles and permeation enhancers used in *in-vitro* skin permeation study of diclofenac sodium.

Vehicle code	Vehicles	Permeation enhancers
V 1	Olive oil (10 mL)	-
V 2	Arachis oil (10 mL)	-
V 3	Castor oil (10 mL)	-
V 4	Liquid paraffin (10 mL)	-
V 5	White soft paraffin melt (10 mL)	-
V 6	Sodium carboxymethyl cellulose aqueous gel (10 mL)	-
V 7	Isopropyl myristate (10 mL)	-
V 8	Liquid paraffin (10 mL)	Menthol (0.1%)
V 9	Olive oil (10 mL)	Menthol (0.1%)
V 10	Liquid paraffin (10 mL)	Dried *Aloe vera* juice (1 gm)
V 11	Olive oil (10 mL)	Dried *Aloe vera* juice (1 gm)

Table 2: Solubility and partition characteristics of diclofenac sodium in different vehicles.

Vehicle code	Vehicles	Solubility (% w/v)	Partition coefficient*
V 1	Olive oil	0.31 ± 0.03	1.63 ± 0.13
V 2	Arachis oil	0.74 ± 0.08	6.56 ± 0.38
V 3	Castor oil	1.65 ± 0.07	12.02 ± 1.34
V 4	Liquid paraffin	0.23 ± 0.02	1.23 ± 0.10
V 7	Isopropyl myristate	0.17 ± 0.03	0.72 ± 0.09

*Partition coefficient between vehicles and phosphate buffer saline (PBS), pH 7.4.
Values are mean ± SD, (n=3).

Table 3: Permeation parameters of diclofenac sodium through excised mouse skin from different common pharmaceutical vehicles.

Vehicle Code	Flux* ($mg/cm^2/hr$)	Lag time* (hr)	Permeability coefficient* (cm/hr)	Diffusion coefficient* (cm^2/hr)
V 1	0.20 ± 0.01	0.73 ± 0.03	1.77 ± 0.04	1.15 ± 0.05
V 2	0.18 ± 0.01	0.76 ± 0.02	1.64 ± 0.05	1.09 ± 0.04
V 3	0.15 ± 0.01	0.83 ± 0.02	1.44 ± 0.08	1.00 ± 0.04
V 4	0.21 ± 0.01	0.68 ± 0.07	1.85 ± 0.10	1.24 ± 0.14
V 5	0.13 ± 0.02	0.86 ± 0.05	1.20 ± 0.05	0.97 ± 0.06
V 6	0.14 ± 0.01	0.83 ± 0.02	1.33 ± 0.04	1.00 ± 0.04
V 7	0.26 ± 0.01[a]	0.60 ± 0.05	2.41 ± 0.03	1.39 ± 0.04

*Values are mean ± SD, (n=3).
[a] Statistically significant ($p < 0.05$) compared with all vehicles determined by one way ANOVA followed by Dunnett's test.

Table 4: Permeation parameters of diclofenac sodium through excised mouse skin from liquid paraffin and olive oil using menthol and dried *Aloe vera* juice as permeation enhancers.

Vehicle Code	Flux* ($mg/cm^2/hr$)	Lag time* (hr)	Permeability coefficient* (cm/hr)	Diffusion coefficient* (cm^2/hr)
V 8	0.28 ± 0.02[a]	0.36 ± 0.02	2.56 ± 0.16	2.30 ± 0.17
V 9	0.27 ± 0.01	0.55 ± 0.08	2.41 ± 0.06	1.55 ± 0.26
V 10	0.25 ± 0.01	0.62 ± 0.03	2.35 ± 0.07	1.35 ± 0.07
V 11	0.23 ± 0.01[b]	0.69 ± 0.03	2.09 ± 0.05	1.22 ± 0.06

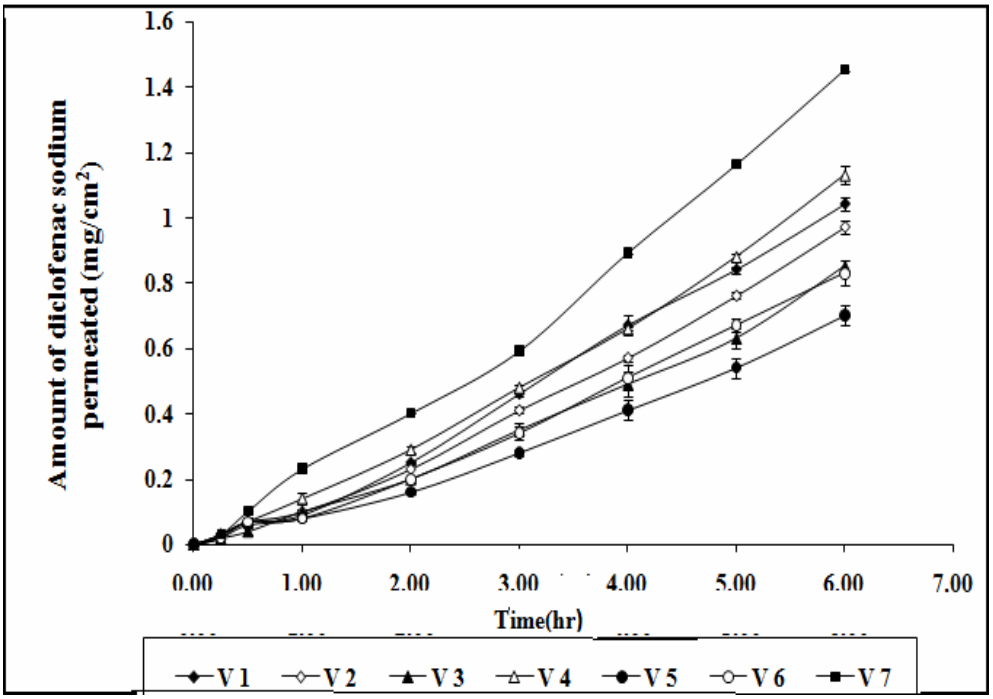
*Values are mean ± SD, (n=3).
[a] Statistically significant ($p < 0.05$) compared with V 10 and V 11.
[b] Statistically significant ($p < 0.05$) compared with V 8, V 9 and V 10. Statistical significance was determined by one way ANOVA followed by Dunnett's test.

Table 5: Permeation enhancement ratio of menthol (0.1%) and dried *Aloe vera* juice incorporated as permeation enhancers in liquid paraffin and olive oil.

Vehicle code	Permeation enhancement ratio
V 8	1.38
V 9	1.36
V 10	1.27
V 11	1.18

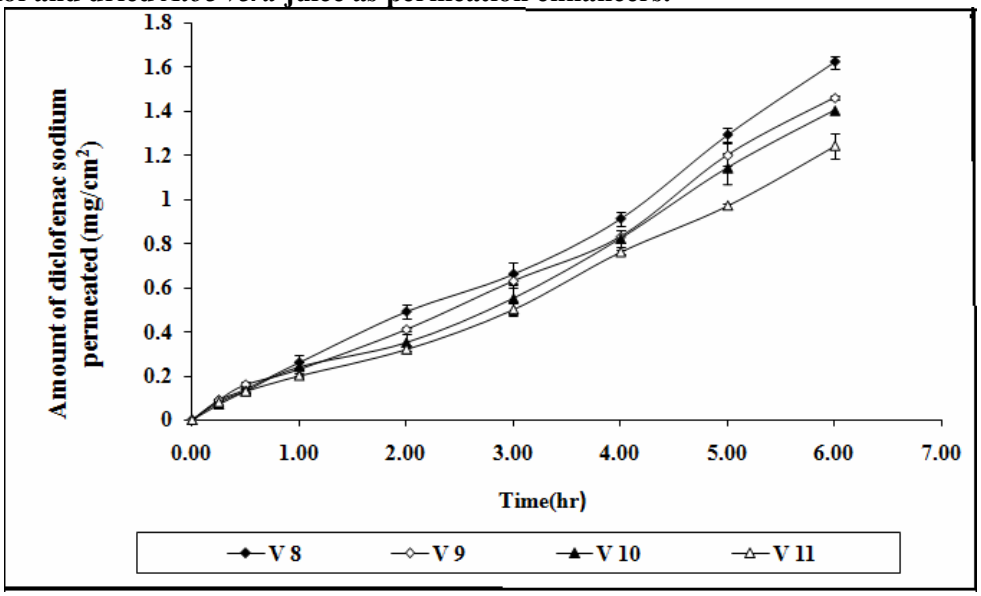
Enhancement ratio (ER) = permeability coefficient with permeation enhancer / permeability coefficient without permeation enhancer. (V 8, liquid paraffin + menthol; V 9, olive oil + menthol; V 10, liquid paraffin + dried *Aloe vera* juice; V 11, olive oil + dried *Aloe vera* juice.)

Figure 1: Permeation profile of diclofenac sodium through excised mouse skin from different common pharmaceutical vehicles.



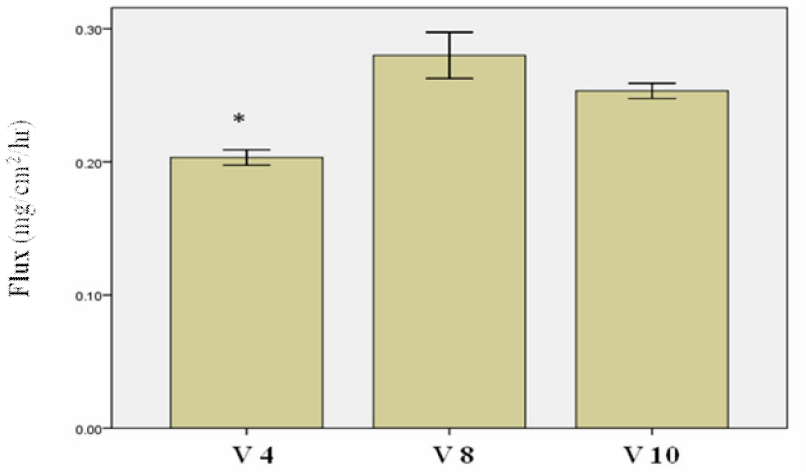
Each value represents mean ± SD, (n=3). Vehicles used for permeation were olive oil (V 1), arachis oil (V 2), castor oil (V 3), liquid paraffin (V 4), white soft paraffin melt (V 5), 3% sodium carboxymethyl cellulose (NaCMC) aqueous gel (V 6) and isopropyl myristate (IPM) (V 7).

Figure 2: Permeation profile of diclofenac sodium through excised mouse skin from liquid paraffin and olive oil using menthol and dried *Aloe vera* juice as permeation enhancers.



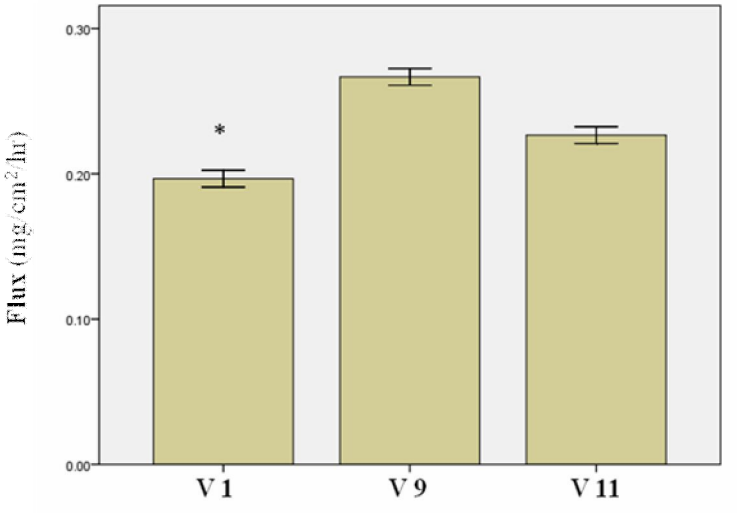
Each value represents mean ± SD, (n=3). (V 8, liquid paraffin + menthol; V 9, olive oil + menthol; V 10, liquid paraffin + dried *Aloe vera* juice; V 11, olive oil + dried *Aloe vera* juice.)

Figure 3: Comparison of diclofenac sodium flux (mg/cm^2/hr) through excised mouse skin from liquid paraffin with and without permeation enhancers.



Each value represents mean ± SD, (n=3).
* Statistically significant ($p < 0.05$) compared with all vehicles determined by one way ANOVA followed by Dunnett's test. (V 4, liquid paraffin; V 8, liquid paraffin + menthol; V 10, liquid paraffin + dried *Aloe vera* juice)

Figure 4: Comparison of diclofenac sodium flux (mg/cm^2/hr) through excised mouse skin from olive oil with and without permeation enhancers.



Each value represents mean ± SD, (n=3).
* Statistically significant ($p < 0.05$) compared with all vehicles determined by one way ANOVA followed by Dunnett's test. (V 1, olive oil; V 9, olive oil + menthol; V 11, olive oil + dried *Aloe vera* juice.)

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