

Development of a novel ketoprofen transdermal patch: Effect of almond oil as penetration enhancers on *in-vitro* and *ex-vivo* penetration of ketoprofen through rabbit skin

Abid Hussain, Gul Majid Khan*, Shefaat Ullah Shah, Kifayat Ullah Shah, Nauman Rahim, Abdul Wahab and Asim-Ur-Rehman

Drug Delivery Research Center, Faculty of Pharmacy, Gomal University, Dera Ismail Khan, Pakistan

Abstract: The aim of the study was to formulate and evaluate topically applied ketoprofen gels and patches and to see the effect of naturally occurring almond oil as penetration enhancer on the penetration of ketoprofen through artificial membrane/rabbit skin. Prior to ketoprofen gel and patch formulation, the particle size and particle size determination of ketoprofen was analyzed by Particle size analyzer (Horiba LA300). Ketoprofen gels and patches were formulated and almond oil was added in several concentrations i.e. 0.5%, 1%, 1.5%, 2%, 2.5% and 3%. The formulated gels were evaluated by several parameters like pH, spreadibility, consistency, homogeneity, skin irritation and drug content determination. In vitro drug permeation studies from transdermal gels and patches were carried out across artificial membrane and rabbit skin by using Franz Cell Apparatus (PermeGear, USA). Kinetics model was employed to the release patterns of ketoprofen from gel and patches in order to investigate the drug transport mechanism. The cumulative amount of drug penetrated from different formulations was statistically evaluated by using One-way analysis of variance (ANOVA). Stability study was performed for various batches of ketoprofen transdermal gel. Almond oil as penetration enhancer in various concentrations significantly enhances the penetration of drug from transdermal gels and patch across synthetic membrane/rabbit skin but was most significant when used in 3% concentration.

Keywords: Transdermal patch and gel, Ketoprofen, *In vitro* & *Ex-vivo* penetration, Penetration enhancers

INTRODUCTION

Novel drug delivery systems include transdermal drug delivery systems that in drug therapy break many barriers like need of assistance, uncomfortable administration and intermediate dosing (Rajesh and Pitchaimani, 2006). For local and systemic delivery of drugs, transdermal route of administration is amongst the most potential routes of drug administration (Ramesh *et al.*, 2007). Transdermal drug delivery systems are adventitious over conventional modes of drug delivery in that they avoid hepatic first pass metabolism, potentially decreased side effects and improved patient compliance (Das *et al.*, 2006). Now a days gel preparations are widely used to be applied to the skin and to mucosal surfaces of the body for local effects as well as systemic effects by penetrating the drug into systemic circulation. Normally gels are composed of a liquid phase containing thickening agents to control its flow. The liquid phase of gels permits a free diffusion of molecules through the polymers scaffold therefore the release must be equivalent to that from a simple solution (Shivhere *et al.*, 2009). The lipid layer of stratum corneum of the skin provides the primary barrier function and prevents the penetration of drug molecules. The drug molecules have mainly two entrance path ways through the stratum corneum, one is the passage between the cells that is called the intracellular route and the other one is

the passage across the protein corneocytes which is called the transcellular route. Several physicochemical parameters effect the penetration of drug molecules across the stratum corneum such as partition coefficient, solubility and diffusivity between the protein and the lipids phases. It was suggested that the primary effect of penetration enhancers could be on the stratum corneum lipids, keratin fibrils and partitioning effects (Charles *et al.*, 2000).

MATERIALS AND METHODS

Materials

Ketoprofen (Leads Pharma Islamabad, Pakistan), Carboxy polymethylene (Sigma Chemicals, USA), Triethanolamine, PVP K30 (Merk, Germany), Ethyl cellulose, Polyethylene glycol 400, Potassium dihydrogen phosphate, Sodium hydroxide (Merk, Germany), Ethanol, Almond oil (Sigma Aldrich, Germany), Magnetic stirrer, pH meter, Weighing balance, UV-Visible Spectrophotometer (UVIDEC-1601 Shimadzu, Japan), Franz diffusion cell Apparatus (Perm Gear, USA), Particle size analyzer (Horiba LA 300).

Construction of standard calibration curve of ketoprofen

For the preparation of stock solution, 20 mg of ketoprofen was taken in 100 ml of phosphate buffer (pH 7.4) and was kept in ultra sonifier until the complete dissolution of the drug. From the stock solution suitable dilutions were

Corresponding author: drgulmajeed@yahoo.com

prepared in decreasing order which were analyzed UV Visible spectrophotometer at 258 nm and absorbance values were recorded as given below in table 1. Then a standard calibration curve was constructed using MS Excel as shown in fig 1.

Table 1: Concentration versus Absorbance of Ketoprofen in phosphate buffer pH 7.4

S. No	Concentration	Absorbance
1	0.025 mg/ml	1.620
2	0.0125 mg/ml	0.830
3	0.0062 mg/ml	0.411
4	0.0031 mg/ml	0.203
5	0.0015 mg/ml	0.107

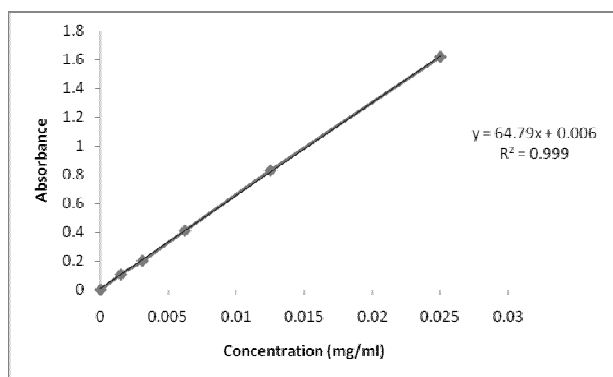


Fig. 1: Standard Curve of Ketoprofen

Particle size and particle size distribution analysis

For the particle size and particle size distribution analysis of ketoprofen, particle size analyzer (Horiba LA 300) was used. Distilled water was used as circulating solvent. The particle size and particle size distribution analysis was performed by setting the instrument ultrasonic circulation for 5 min at a refractive index of 1.18-0.00i.

Ketoprofen gel formulation and preparation

Several types of Ketoprofen 1% (w/v) gel was formulated with /without penetration enhancers. The penetration enhancer (Almond oil) was added to ketoprofen gel at several different concentrations i.e. 0.5%, 1%, 1.5%, 2%, 2.5% and 3% to see their enhancement effect on drug

penetration across artificial/rabbit skin. For ketoprofen gel formulation 1 gm of carboxy polymethylene was dispensed in 50 ml distilled water and a homogeneous dispersion was obtained by stirring it with the help of a magnetic stirrer. In second step 1 gm ketoprofen was taken and dispensed in 10 ml of ethanol and this solution was drop wise added to carboxy polyethylene solution with continuous stirring. Lastly to this solution triethanolamine and remaining distilled water was added to make the final volume 100 ml. This final solution was continuously stirred until the formation of transparent gel.

Ketoprofen gel evaluation

Ketoprofen gel was evaluated for the following parameters.

pH of transdermal gel

The pH of different gel preparations were evaluated using digital pH meter (Shivhere *et al.*, 2009).

Spreadability

Wooden block and glass slide apparatus was used for the spreadability of the gels. About 20 gm of gel was added to the pan and the time for upper slide (movable) was noted until it separates completely from the fixed slides. The spreadability of the gels was calculated by the following equation:

$$S = ML/T$$

Where (S) is spreadability, (M) is weight tied to upper slide, (L) length of glass slide and (T) is time taken to separate the time taken to separate the slide completely from each other (Shivhere *et al.*, 2009).

Consistency

Consistency of the prepared gels were measured by a method in which a cone attached to a holding rod from a fix distance of 10 cm was dropped in such a way that it must fall in the center of a gel holding cup fully filled with gel. The penetration of the cone in respected gel was measured. After 10 sec the distance travelled by cone inside the gel was noted (Shivhere *et al.*, 2009).

Homogeneity

Gels were evaluated for homogeneity on the basis visual inspection. The gels were filled in narrow transparent

Table 2: Formulation of 1% (w/v) ketoprofen transdermal gel with/without penetration enhancer

S. No	Ketoprofen	CPM	TEA	Ethanol	Almond oil (Enhancer)	Distilled water
1	1 gm	1 gm	1 ml	10 ml	-	QS to 100 ml
2	1 gm	1 gm	1 ml	10 ml	0.5%	QS to 100 ml
3	1 gm	1 gm	1 ml	10 ml	1%	QS to 100 ml
4	1 gm	1 gm	1 ml	10 ml	1.5%	QS to 100 ml
5	1 gm	1 gm	1 ml	10 ml	2%	QS to 100 ml
6	1 gm	1 gm	1 ml	10 ml	2.5%	QS to 100 ml
7	1 gm	1 gm	1 ml	10 ml	3%	QS to 100 ml

glass containers and were inspected for the appearance of any particles or aggregates (Shivhere *et al.*, 2009).

Skin irritation test

Human volunteers were used for Skin irritation tests. For each gel formulations three volunteers were selected with good healthy skin having no known allergic conditions. To each volunteer 1 gm of each prepared gel was applied to an area of 2 square inch of the skin near the wrist and these volunteers were observed for irritations and lesions (Shivhere *et al.*, 2009).

Drug content

The drug content of prepared gels was determined by a method in which 100 mg gel was taken and was dispensed in 100 ml phosphate buffer pH 7.4. A mechanical shaker was used to make sure the complete solubility of gel in the buffer and the process was carried out for 3 hours. The prepared solution was filtered through a membrane filter (0.45µm pore size) and was analyzed for drug content at 258 nm (Shivhere *et al.*, 2009).

Stability study

Different batches of transdermal ketoprofen gel with different penetration enhancers were selected for stability study which was conducted at accelerated climatic conditions. In accelerated stability study of 6 months, minimum three points including the initial time and the final time were analyzed by using UV-visible spectrophotometer. The conditions for accelerated stability study were comprised of temperature $40 \pm 2^\circ\text{C}$ and relative humidity $75 \pm 5\%$ RH for 6 months in stability chamber.

Transdermal patch synthesis

Ketoprofen transdermal matrix patches were prepared by dispensing the drug in rate controlling polymer either lipophilic or hydrophilic or combination of both. For transdermal ketoprofen patch, the initial step was the development of a backing membrane which is used as a supporting material for the drug matrix layer. Backing membrane was prepared by dispensing 4 gm PVA in 100 ml distilled water and was heated up to 80°C until 25% of the solution was evaporated. This solution was stirred continuously with the help of a magnetic stirrer. From this

resultant solution 15 ml was taken and was poured in petri dish having an area of 62 cm^2 . The Petri dish containing this solution was dried in open air covered with invert funnel for 24 hrs and was stored safely for further studies. In another step a weighed amount of ketoprofen, polymer (Ethocel) and plasticizer (PG) was dispensed in 100 ml of Ethanol + chloroform solution (ratio 1:1) taken in a conical flask and was mixed gently until homogenous solution was formed. This solution was then poured into the petri dish containing backing membrane and the solvent was evaporated in open air covered with an inverted funnel. The prepared dry film in petri dish was collected and was cut into small matrix patch having diameter of 1.5 cm^2 . The developed patches were covered with aluminum foil and were stored for ex vivo studies. The quantity of contents per unit patch is given below in table 3.

Rabbit skin preparation

Rabbit skin was used for ex-vivo studies of ketoprofen gel and patch. For rabbit skin a healthy albino rabbit weighing approximately 1.25 kg was selected. The rabbit was given anesthesia with chloroform. The hair from the dorsal region was shaved carefully with special razor and was cleaned from stitched hairs with wet cotton. The rabbit was scarified and the hairless clean skin was excised with the help of surgical blades. The subcutaneous fats from the skin were removed with the help of scalpel and the epidermis was removed by immersing the skin in hot water maintained at 60°C for 1 to 2 min and then teasing the epidermis from the dermis carefully. The obtained skin was cleaned with distilled water and was stored in suitable conditions for further use.

Permeation studies

For the penetration studies of ketoprofen gels and patches across artificial membrane/rabbit skin Franz cell apparatus (Perm Gear, USA) was used. First of all the artificial membrane/rabbit skin was fixed between the donor and the receptor compartments of Franz cell apparatus. Phosphate buffer having pH 7.4 was used as receptor medium. A 5 ml of the buffer was filled in each of the receptor compartments of Franz cell. The diffusion area of artificial membrane/rabbit skin was 0.75 cm^2 . The

Table 3: Formulation of ketoprofen transdermal patch with/without penetration enhancer

S. No	Ketoprofen	Eudragit	Ethocel	PG	Almond oil (Enhancer)	Ethanol + Chloroform (1:1 ratio)
1	50 mg	100 mg	100 mg	60 mg	-	QS to 100 ml
2	50 mg	100 mg	100 mg	60 mg	0.5%	QS to 100 ml
3	50 mg	100 mg	100 mg	60 mg	1%	QS to 100 ml
4	50 mg	100 mg	100 mg	60 mg	1.5%	QS to 100 ml
5	50 mg	100 mg	100 mg	60 mg	2%	QS to 100 ml
6	50 mg	100 mg	100 mg	60 mg	2.5%	QS to 100 ml
7	50 mg	100 mg	100 mg	60 mg	3%	QS to 100 ml

Table 4: Evaluation parameters of ketoprofen transdermal gel

S. No	Parameters	Ketoprofen gel (Blank)	Ketoprofen gel with almond oil at different concentrations					
			0.5%	1%	1.5%	2%	2.5%	3%
1	pH	6.9	6.8	6.9	6.9	6.9	6.8	6.9
2	Spreadibility (g.cm/sec)	5.7	5.4	5.5	5.6	5.4	5.5	5.4
3	Consistency (60 sec)	6mm	6mm	6mm	6mm	6mm	6mm	6mm
4	Homogeneity	good	good	good	good	good	good	good
5	Skin irritation test	No	No	No	No	No	No	No
6	Drug content (%)	98.8	99.2	98.5	98.8	99.3	99.1	99.4

upper donor compartments were filled with ketoprofen gels with/without penetration enhancers and the temperature was maintained at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with the help of circulating water. From each receptor compartment of Franz cell, samples of 2 ml from each compartment were withdrawn at specific time intervals according to standard operating procedures. The receptor phase was immediately replaced with fresh receptor solvent stored at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. The collected samples were filtered through membrane filter ($0.45\mu\text{m}$) and were analyzed by UV spectrophotometer at a detection wave length of 258 nm. The amount of drug permeated through the artificial membrane/rabbit skin was calculated from regression line equation. Similar method was adopted in order to study ketoprofen permeation from transdermal patch in which the developed patch was fixed on the rabbit skin in such a way that the drug matrix layer of the patch was facing the epidermis layer of the skin and then was fixed in between the donor and the receptor compartments.

Drug release kinetics

The mechanism by which ketoprofen was transdermally penetrated through artificial/rabbit skin can be determined by putting the penetration data in Korsmeyer Pappas equation:

$$M_t / M_{\infty} = K_5 t^n$$

Where M_t / M_{∞} is the fractional drug release into the receptor solvent, k is drug delivery related constant and (n) is a diffusion coefficient which indicates the transport mechanism of drug. If the value of (n) is equal to 0.5 then the drug is transported by a quasi-Fickian diffusion mechanism, if the value of (n) is greater than 0.5 then anomalous or non-Fickian diffusion mechanism is involved and when the value of (n) equals to 1 then it indicates zero order release mechanism (Khan and Zhu, 1999).

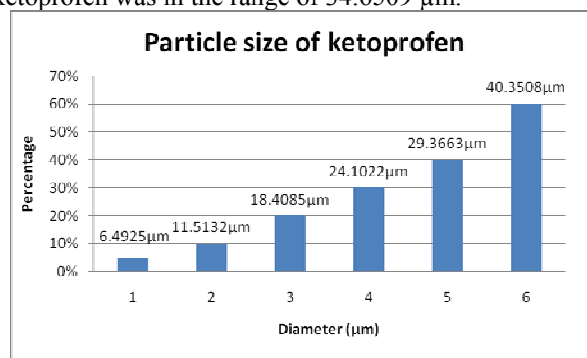
Data analysis

The cumulative amount from different formulations was evaluated statistically by using one way analysis of variance (ANOVA) and the difference between the cumulative amount of drug penetrated from different formulations were considered statistically significant with a P -value ≤ 0.05 .

RESULTS

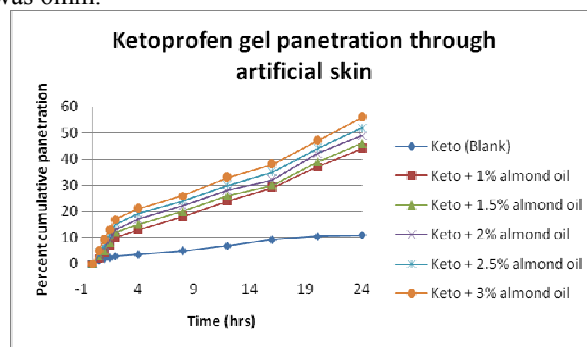
Particle size and particle size distribution analysis

Fig. 2 shows the particle size and particle size distribution of Ketoprofen. It could be seen that a large fraction of the drug particles (60%) were having diameter of $40.3508\mu\text{m}$ and the smallest fraction of particles were having diameter of $6.4925\mu\text{m}$. The median particles size of ketoprofen was in the range of $34.6509\mu\text{m}$.

**Fig. 2:** Particle size and Particle size distribution of Ketoprofen

Ketoprofen gel evaluation

Ketoprofen gel formulations with/without various concentrations of penetration enhancers were prepared and evaluated for different parameters. The results of these parameters are given in table 4. The pH values of all the gel formulations were ranging from 6.8 to 6.9; the drug content were ranging from 98.5 to 99.4; and the spreadibility values range from 5.4 to 5.7; the consistency was 6mm.

**Fig. 3:** Release profiles of ketoprofen from transdermal gel across artificial skin with different concentrations of almond oil as penetration enhancer.

Penetration studies from gel

In order to check the penetration mechanism of ketoprofen from gel, Korsmeyer Pappas kinetic model was employed to the gel formulations with/without penetration enhancers. In Korsmeyer Pappas equation an (n) value represents the mechanism of drug release/penetration. The (n) values of ketoprofen gel without penetration enhancer and with 0.5%, 1%, 1.5%, 2%, 2.5% and 3% penetration enhancer were 0.634, 0.728, 0.521, 0.721, 0.552 and 0.131, respectively. The percent penetration values of ketoprofen from transdermal gel with/without penetration through artificial skin after 24 hrs are shown fig 3; while the percent cumulative release of drug from ketoprofen gel with/without penetration enhancers through rabbit skin could be seen from fig 4.

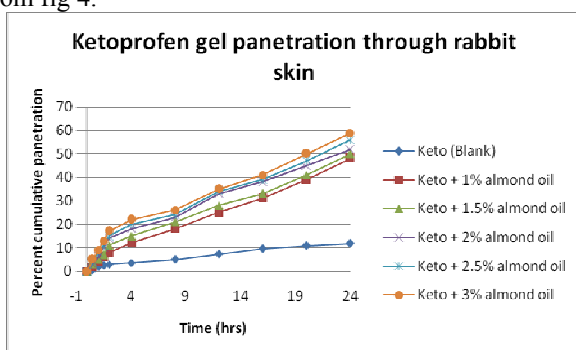


Fig. 4: Release profiles of ketoprofen from transdermal gel across rabbit skin with different concentrations of almond oil as penetration enhancer.

Penetration studies from patch

The *in vitro* permeation profiles of ketoprofen with/without penetration enhancers from transdermal patch is shown in fig 5. As shown in fig, the maximum penetration (85%) of ketoprofen from transdermal patch through rabbit skin is shown from the patch with 3% of almond oil as penetration enhancer; while decreasing the concentration of almond oil the percent cumulative penetration of ketoprofen decreases.

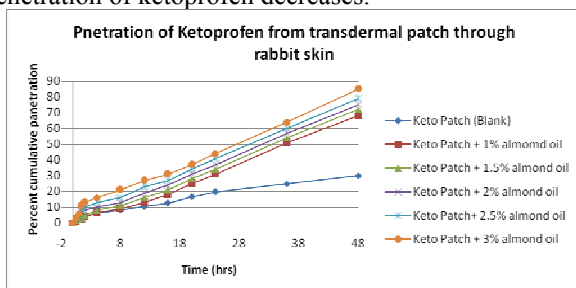


Fig. 5: Release profiles of ketoprofen from transdermal patch across rabbit skin with different concentrations of almond oil as penetration enhancer.

DISCUSSIONS

The aim of the present study was to develop transdermal gel and patch of ketoprofen containing almond oil as

natural penetration enhancer to improve and enhance the penetration of ketoprofen through natural and artificial skin. The particle size of a drug plays a key role in transdermal drug deliveries; drugs with smaller particle size are more suitable for transdermal delivery. For this purpose the first step of the study was to investigate particle size and particle size distribution analysis of ketoprofen by using Particle size analyzer (Horiba LA300).

Almond oil is a natural penetration enhancer which enhances the penetration of drug by certain modification in the lipid layer of stratum corneum and thus it could enhance the transdermal penetration. Ketoprofen gel formulations with/without various concentrations of penetration enhancers were prepared and evaluated for different parameters. The pH values of all the gel formulations were ranging from 6.8 to 6.9 which are all in normal range. The drug content of all the formulated gels were ranging from 98.5 to 99.4 which shows a good content uniformity. The spreadability values of all developed gels ranges from 5.4 to 5.7 which indicate that the gels can be spared easily on skin surface with a little stress. The consistency of all the gels was 6mm. The homogeneity of all the formulations was good and there were no visual clots or any other particles in the gels and the gels were transparent. As the pH of all the developed gel formulations was in good range so there was no skin irritation or edema found.

The samples of ketoprofen gel placed in climatic chamber for 6 months revealed that there was no considerable change in the different parameters of the gel and were declared stable after 6 months of accelerated stability climatic conditions. The physical appearance of gel was clear, the drug content, colour variation, viscosity, fluidity, homogeneity, consistency, spreadability and pH remained the nearly same which could lead to conclude that the different batches of ketoprofen transdermal gel were physically and chemically stable (ICH Harmonized Tripartite Guidelines, 2003). The 'p' value for all the batches were ranging from 0.1 to 0.3 (range NMT 0.05) which were within the limits.

In order to check the penetration mechanism of ketoprofen from gel, Korsmeyer Pappas kinetic model was employed to the gel formulations with/without penetration enhancers. In Korsmeyer Pappas equation an (n) value represents the mechanism of drug release/penetration. The (n) values of ketoprofen gel without penetration enhancer and with 0.5%, 1%, 1.5%, 2%, 2.5% and 3% penetration enhancer were 0.634, 0.728, 0.521, 0.721, 0.552 and 0.131, respectively, which show that the formulations follow both non-Fickian (anomalous) and super case II transport (Khan and Zhu, 1999). The percent penetration values of ketoprofen from transdermal gel with/without penetration through artificial

skin after 24 hrs are given in fig 3. It could be seen that ketoprofen gel without penetration enhancers has the smallest percent penetration value of 10.9% across artificial skin. With the addition of penetration enhancers to the gel formulation the penetration of ketoprofen from gel increases. The greatest value of percent cumulative release of ketoprofen was 56% from the gel formulation containing 3% almond oil as penetration enhancer. The penetration effect of almond oil was increased by increasing its concentration in ketoprofen gel formulation which could be clearly seen from the figure. Almond oil is natural oil and has been successfully investigated for its penetration enhancer effect in veterinary animals as well as humans and small laboratory animals such as rats, pigs and rabbits (Beatrice *et al.*, 2001).

The percent cumulative release of drug from ketoprofen gel with/without penetration enhancers through rabbit skin could be seen from fig 4. It could be observed that the cumulative percent penetration of ketoprofen through rabbit skin was higher as compared to that from artificial skin. The reason could be that natural skin i.e. rabbit skin has a large number of hair follicles and it has been suggested that the permeation enhancement effect may occur within the hair follicles, so the more the hair follicles the more will be the penetration effect (Beatrice *et al.*, 2001). It could be noted that the thickness of artificial skin was 2.60 mm while that of rabbit skin was 1.56 mm, so more drug can be penetrated through a thinner skin as compared to a thicker one. Hence, more of the drug was observed to be penetrated through rabbit skin as compared to artificial skin.

Patches are widely used for transdermal delivery of drugs. The *in vitro* permeation profiles of Ketoprofen from transdermal patch, with/without penetration enhancers, is shown in fig 5. As shown in fig, the maximum penetration (85%) of ketoprofen from transdermal patch through rabbit skin is shown from the patch with 3% of almond oil as penetration enhancer while by decreasing the concentration of almond oil the percent cumulative penetration of ketoprofen decreases. It is clear from the figure that almond oil could successfully increase the penetration of ketoprofen from transdermal patches. In ketoprofen patch formulations polymer Ethocel was used as a rate controlling polymer which successfully extends the release of ketoprofen up to 48 hrs (Khan and Zhu, 2001). These permeation studies showed that highest amount of drug penetrated from the gel across the rabbit skin was directly proportional to the amount of penetration enhancer used i.e. maximum drug was penetrated across the skin with 3% almond oil as penetration enhancer. In fact, the permeability of ketoprofen containing 3% almond oil is significantly (One way ANOVA $P < 0.05$) greater than the other concentrations studied. Similarly in case of ketoprofen penetration from transdermal patch, the amount of drug

penetrated was highest from formulations with 3% olive oil and were significantly greater than the other formulations (One way ANOVA $P < 0.05$).

CONCLUSION

In this study the effect of different concentrations of almond oil as penetration enhancer on ketoprofen penetration from transdermal gels and patches through artificial membrane and rabbit skin were studied. The different parameters used for evaluation of prepared gels suggest that the formulation was suitable to develop topically applied gels and patches of ketoprofen. From the study it was concluded that almond oil in different concentrations can be used to enhance the penetration of NSAID's like ketoprofen from topically applied gels as well as transdermal matrix patches.

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