



FORMULATION AND EVALUATION OF BIOPOLYMER BASED TRANSDERMAL DRUG DELIVERY

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ABSTRACT

The aim of the present study was to formulate transdermal films loaded with Ketoprofen (KF). Transdermal films were prepared by using sodium alginate (SA) and xanthan gum (XG) as biopolymers by varying the blend ratios viz., 10:0, 8:2, 6:4, 4:6 and 2:8 (w/w %) through solution casting method. The drug loaded membranes were evaluated for thickness, tensile behaviors, content uniformity, transdermal permeation of KF through rat abdominal skin was determined by Franz diffusion cell. *In vitro* skin permeation profile of optimized formulation was compared with that of KF conventional gel. Carrageenan induced rat paw edema model was used to investigate their *in vivo* performances. Ketoprofen was found to be compatible and stable with the prepared formulation as confirmed by Fourier transform infrared spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC), studies. *In-vitro* release studies reveals effectiveness after 24 h when compared with the conventional gel. The study results suggest that biopolymer based transdermal films are potential vehicles for improved transdermal delivery of KF for effective therapy.

Keywords: Ketoprofen, Sodium alginate, Xanthan gum, Transdermal release, Skin permeation

INTRODUCTION

In recent years transdermal route now ranks with oral treatment as the most successful innovative research area in drug delivery, with around 40% of the drug delivery candidate products under clinical evaluation related to transdermal or dermal system. The transdermal drug delivery systems (TDDS) have been designed as an alternative route for systemic drug delivery. The systemic drug administration though skin holds several advantages such as maintenance constant drug level in blood, decrease of side effects, and improvement of bio availability by circumvention of hepatic first pass metabolism and increase patient compliance. Now a day's skin considered as a safe port for drug administration, to provide continuous drug release into systemic circulation¹. Recently, bio polymers used in the fabrication of transdermal films has received much attention due to their excellent biocompatibility and bio degradation². One of the most promising techniques for enhancement for transdermal permeation of drugs is transdermal patches³. Sodium alginate (SA) is a natural polymer is very promising and has been widely exploited in pharmaceutical industry, because of its tailor-made to suit the demands of applications^{4, 5}. Xanthan gum is a hydrophilic polymer, had been limited for use in thickening, suspending, and emulsifying water-based systems. It is gaining appreciation for the fabrication of pharmaceuticals with uniform drug release characteristics. Drug release property of matrices is preceded by polymer hydration and the rate of drug release from polymer carrier can be tailor-made by selecting a suitable polymer-blends composition and drug concentration^{6, 7}. The effect of hydrophilic plasticizers such as glycerin on physicochemical properties on sodium alginate – xanthan gum (SA/XG) film was evaluated. Ketoprofen (KF) belongs to the group of substituted 2-phenylpropionic acids which has analgesic, anti-inflammatory and antipyretic effects. KF exerts the majority of its analgesic actions through inhibition of the synthesis of prostaglandins by inhibiting the enzyme cyclooxygenase (COX)⁸. KF had the best topical penetration ability when compared to ketorlac, indomethacin and other studies have found that topical KF was effective for the treatment of well localized soft tissue injury, joint pain, in reducing muscle soreness after repetitive muscle contraction⁹. The importance of KF in the therapeutic field has stimulated the development of topical dosage forms to improve its percutaneous absorption through the application site. Moreover topical dosage forms could provide relatively consistent drug levels for prolonged periods and avoid gastric irritation, as well as the other typical side effects of oral NSAID administration¹⁰. Penetration depends on ability of drug to penetrate the stratum corneum, enter the systemic circulation and to achieve the therapeutic effect. A drug

with log P (lipid/water partition coefficient) of ≤ 2 considered as potential candidate for transdermal delivery¹¹. There has been increased interest during recent years in the use of chemical enhancer that could modify drug permeation through skin¹². Many of the chemical enhancers may be harmful, especially in chronic applications, many of them were irritant in nature. It is desirable to develop topical delivery systems that do not require the use of chemical enhancers to facilitate drug permeation through skin. In the present study we made an attempt by using menthol as a penetration enhancer. Because menthol is considered to have good permeation enhancing agent by acting as a lipid disrupting agent that increases the fluidity of stratum corneum lipid by increasing the formation of capillary channels¹³. Transdermal films with varied ratios nonirritating and pharmaceutically acceptable biopolymers SA and XG combination containing the drug KF with permeation enhancer (menthol). The prepared films were compared with the marketed conventional gel. Furthermore, pharmacodynamic study of films was evaluated for anti-inflammatory activity on carrageenan induced rat paw edema model. The purpose was to provide the delivery of drug at a controlled rate across intact skin to achieve a therapeutically effective drug level for a longer duration of time from transdermal films.

Experimental

Materials

Ketoprofen (KF) was obtained from M/s Torrent Pharmaceuticals, Ahmedabad, India, as gift sample. It is White or off-white, odorless, nonhygroscopic and fine to granular powder. Freely soluble in ethyl alcohol, chloroform, acetone, and ether and soluble in phosphate buffer pH 7.4, but practically insoluble in water. The plasma elimination half life is 1.5 to 4h. Ketoprofen is one of the most powerful inhibitors of cyclooxygenase at concentrations well within the range of therapeutic plasma concentrations. It produces reversible COX inhibition by competing with the substrate, arachidonic acid, for the active site of the enzyme. Xanthan gum (XG) was procured from M/s Sigma Aldrich, USA. It is a white or yellowish white, free-flowing powder; soluble in hot and cold water; practically insoluble in organic solvents. Sodium alginate (SA) was procured from Loba Chemie Pvt Ltd, Mumbai, India. It is a white to yellowish brown filamentous, grainy, granular or powdered forms of the sodium salt of alginic acid. It is used as gelling agent, emulsifier, Stabilizer and thickener to increase viscosity in food industry, also used in indigestion tablets and the preparation of dental impressions. Xanthan gum and Sodium alginate were used without further purification. Glycerin, Menthol was obtained from Sd fine Chemicals, and all other chemicals were of analytical grade.

Preparation of drug loaded SA/XG films

Drug containing films were prepared by solution casting method. In brief, the required amounts of a mixture of SA/XG (Table 1) were weighed and prepared polymeric solution using quantity sufficient water, kept aside for 2h after stirring. Accurately weighed KF (2.5 mg/mm²) and menthol (3% w/w) was dissolved in ethanol (6mL) by stirring for 10 min. The above mixture mixed with different

concentrations of glycerin (1–5% w/w) and prepared polymeric solutions for 30 min.

Finally mixed soft mass was poured on to cleaned specially designed glass molds with the plastic transparent sheet and kept in a vacuum drier until to get the dried membrane. The cast polymer films with different formulations were then peeled off covered with aluminum foils and stored in a desiccator until further study.

Table 1: Formulation chart for preparation of KF films and flatness of the films

Formulation	Drug (%)	Sodium alginate (%)	Xanthan gum (%)	Glycerin (%)	Menthol (%)	Flatness (%)
F ₁	2.5	96.0	-	0.5	1.0	100
F ₂	2.5	74.5	20	1.0	2.0	99
F ₃	2.5	52.5	40	2.0	3.0	98
F ₄	2.5	41.5	50	2.5	3.5	90
F ₅	2.5	30.5	60	3.0	4.0	88
F ₆	2.5	9.5	80	3.5	4.5	82
F ₇	2.5	-	100	4.0	5.0	80

Measurements

The thickness of the dry films was measured using microprocessor coating thickness gauge (Quint sonic, Mumbai, India). The dry films (2.5 cm x 2.5 cm) were cut and placed on a control plate and the thickness of the film was measured. Mechanical properties, such as tensile strength and percentage elongation at break of SA/XG blends were measured as per ASTM D 638 using Universal Testing Machine (UTM) H 50 K M, 50K N Hounsfield, UK. A minimum of three samples were tested for each formulation and the average value was recorded.

Moisture up take

Films were placed on open 5mL glass vials containing 3.5g silica gel beads and held in place with a screw lid having a 0.80cm diameter of test area (0.50cm²). The desiccators containing vials kept in chambers with 75% RH (saturated NaCl solution) and 95% RH (water) were kept at 37°C for 7 days. Moisture uptake by the films was measured by weighing the dried film at 100°C for 24h.

Drug contents in films

Accurately weighed films were randomly cut rectangular (2.5 cm²) were dissolved in 50mL phosphate buffer (pH 7.4). Then it was sonicated in ultra sonicator for 30min and appropriately diluted. The concentration of KF in the receptor phase was determined by HPLC method¹⁴. The HPLC system consists of HPLC-shimadzu (Tokyo, Japan) LC-6A model fitted with a μ -bond pack C₁₈ (4.6x250mm) column, flow rate of 1mL/min, mobile phase consisted of acetonitrile and 10mM phosphate buffer (30:70v/v), (pH-7.4), wavelength at 263nm at ambient temperature. The samples were centrifuged at 5000rpm for 15min. In order to eliminate any particles and the supernatant was injected to HPLC, standard solution was prepared by dissolving 20mg of KF in Phosphate buffer (pH 7.4). Final concentration of the solution will be in the range of 1.00-5.00 μ g/mL. The average drug content of three replicate samples was measured.

FTIR analysis

FTIR spectra of the pure drug, optimized formulation, stratum corneum before and after treatment with menthol were obtained by FTIR spectrophotometer (Jasco - 4100, Japan).

Differential scanning calorimetry (DSC)

All dynamic DSC studies were carried out using DuPont thermal analyzer with 2010 DSC Q 200, module. The instrument was calibrated using high purity indium metal as standard and the scans of the samples were recorded in the temperature range ambient to 150 °C under nitrogen gas purge at a heating rate of 10 °C/ min.

Drug diffusion studies

Drug diffusion studies were carried out in an open glass diffusion tube. A specimen dimension of films (2.5 cm²) was fixed to the hydrated cellophane membrane at one end of the open glass tube and placed in the receptor compartment containing buffer solution. The assembly was placed on a magnetic stirrer and stirred at 100 rpm. The temperature of the system was maintained at 37°C \pm 1°C. A known amount of receptor medium (buffer) was withdrawn at regular intervals of time and sink condition was maintained by replacing equal volume of fresh saline. The drug concentration was determined by HPLC.

Stability of the transdermal films and prepared KF gel

Formulation F₃ (2.5 cm²) and conventional gel were subjected for stability studies at 25 °C/60% RH, 30 °C/65% RH, 40 °C/75% RH for 90 days and the above formulations were evaluated for drug content periodically.

In vitro skin permeation studies

In vitro skin permeation studies were performed on a Franz diffusion cell with an effective diffusion area of 2.5 cm² and 16 ml of receiver chamber capacity using rat abdominal skin. The animal study protocol was reviewed and approved by the Animal Ethics Committee at the Department of Pharmaceutics, Bharathi College of Pharmacy, Mandya, India. Male albino rats weighing 128-130 g were used to excise full thickness skin. Rats were anaesthetized by ether and then hair of abdominal skin was removed by using electric clipper. Special care was taken while removing hairs, not to destroy the stratum corneum. The cleaned skin was washed with distilled water and stored in the deep freezer at -21°C until further use. The skin was brought to room temperature and mounted between the donor and receiver compartment of the Franz diffusion cell, where the stratum corneum side faced the donor compartment and the dermal side faced the receiver compartment. Initially the donor compartment was empty and the receiver chamber was filled with ethanolic phosphate-buffered saline (PBS) pH 7.4 (30:70% v/v). The receiver fluid was stirred with a magnetic rotor at a speed of 300 rpm, to maintain the hydro dynamics of receiver fluid and the temperature maintained at 32 °C \pm 1°C. All the ethanolic PBS was replaced every 30 minutes to stabilize the skin. It was found that the receiver fluid showed negligible absorbance after 5 hours and beyond, indicating complete stabilization of the skin. After complete stabilization of the skin, 2.5 cm² of the optimized film was placed into each donor compartment and sealed with paraffin film to provide occlusive conditions. Samples (0.5mL) were withdrawn at regular intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8 hours), filtered through a 0.45-membrane filter. The volume of release media was maintained by adding equal volume of fresh media after every sampling. Concentration of the KF in the sample was measured by HPLC.

Anti inflammatory studies

The Anti inflammatory test was carried out on male albino rats weighing 128 to 130 g. The animals were kept under standard laboratory conditions, with temperature of $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and relative humidity of $60\% \pm 5\%$. The animals were housed in cages, 5 per cage, with free access to a standard laboratory diet. The anti-inflammatory activity of KF from film formulation F_3 was evaluated by the carrageenan-induced hind paw edema method in albino rats¹⁵ and compared with conventional gel. The transdermal film was applied to the shaved abdominal skin of male rats. Just before administration of transdermal film, 1% carrageenan-saline solution (0.1 ml) was injected into each hind paw of rats. The thickness of paw edema induced by carrageenan was measured by using a standard screw gauge during 8 h after application of KF transdermal film.

Permeation data analysis

Results are given mean \pm standard deviation (S.D). The cumulative amount of drug permeated through the skin (mg/cm^2) was plotted as a function of time (t) for each formulation. Drug flux (permeation rate) at steady state (J_{ss}) was calculated by dividing the slope of the linear portion of the graph by the area of the diffusion cell. The permeability coefficient (K_p) was calculated by dividing J_{ss} by the initial concentration of the drug in the donor cell (C_0)

$$K_p = J_{ss} / C_0 \dots\dots 1$$

Enhancement ratio (E_r) was calculated by dividing the flux of the respective formulation by the flux of the control formulation:

$$E_r = J_{ss} \text{ of formulation} / J_{ss} \text{ of control} \dots\dots 2$$

The results were analyzed statistically using Student's t- test and significance was determined at 95% confident limit ($P < 0.05$).

RESULTS AND DISCUSSION

Preparation of SA/XG films containing drugs

Seven film formulations of films were prepared using solution casting method and dried. Films consist of glycerine as a plasticizer and menthol as permeation enhancer. Drug loaded films were light yellow opaque in colour. All surface of the film was smooth, with

elegant appearance, good physical properties. Flatness of the films was observed better when the amount of SA $> 50\%$ in the formulated films, might be SA having α - L-gulonic acid, which is interact with XG produces good flatness to the film¹⁶. Thus these formulations can maintain a smooth and uniform surface when applied on skin

Mechanical properties

Thickness of the prepared films was in the range of 123 to 136 μm is shown in Table 2. Thickness, tensile strength and % elongation of the films increasing by increased ratio of XG and plasticizer in the films. Added glycerin alters the physical and mechanical properties by enhancing the mobility of polymers chains of SA, XG by hydrogen bonding¹⁶. However it was found that 2% of glycerin gives the best plasticizer effect for KF loaded film.

Moisture uptake

Low moisture uptake was found in films with less percent of plasticizer, after stored in the above conditions. Films with low percent of plasticizer showed a lower capacity to absorb water compared to those with plasticizer. As the ratio of plasticizer and RH increases, moisture uptake was increased. This effect was more pronounced on films containing more amount of plasticizer and more amount of plasticizer showed a significant increases in moisture up take at increased RH.

FTIR Analysis

The FTIR spectra of pure drug and formulation F_3 presented in Fig 1. The characteristic IR absorption peaks of Ketoprofen, at 2978, 2938 cm^{-1} (C-H stretching of CH_3 group (asymmetric) masked by O-H stretching), 2876 cm^{-1} (C-H stretching of CH_3 group (symmetric) masked by O-H stretching), 1693 cm^{-1} (C=O stretching of acid), 1599 cm^{-1} C=C stretching of aromatic ring, 1442 cm^{-1} (C-H deformation of CH_3 group (asymmetrical)), 1369 cm^{-1} (C-H deformation of CH_3 group- symmetrical) and 825 - 640 cm^{-1} C-H deformation of aromatic ring. The FTIR spectra of the pure drug, formulation F_3 indicated that characteristic peaks of KF were not altered without any changes in their positions, after successful encapsulation indicating, there is no chemical interaction occurred between the drug and the polymers used.

Table 2: Mechanical properties of the prepared films

Formulation	Thickness of film (μm) Mean \pm S.D*	Tensile Strength (MPa/ mm^2) Mean \pm S.D*	%Elongation Mean \pm S.D*	Moisture uptake (75 %) Mean \pm S.D* $\times 10^{-3}$	Moisture uptake (90%) Mean \pm S.D* $\times 10^{-3}$	Drug content (mg/cm^2) Mean \pm S.D*
F ₁	123 \pm 1.23	2.44 \pm 0.011	21.30 \pm 0.85	1.72 \pm 0.015	1.87 \pm 0.021	2.37 \pm 0.014
F ₂	124 \pm 1.42	2.52 \pm 0.014	21.69 \pm 0.12	1.86 \pm 0.021	1.92 \pm 0.026	2.40 \pm 0.156
F ₃	128 \pm 1.33	2.66 \pm 0.023	19.45 \pm 0.38	2.39 \pm 0.018	2.45 \pm 0.030	2.48 \pm 0.186
F ₄	131 \pm 1.25	2.84 \pm 0.024	24.41 \pm 0.58	2.49 \pm 0.029	2.58 \pm 0.020	2.41 \pm 0.014
F ₅	132 \pm 1.21	3.03 \pm 0.152	29.84 \pm 0.36	2.68 \pm 0.036	2.85 \pm 0.014	2.40 \pm 0.014
F ₆	135 \pm 1.18	3.18 \pm 0.120	35.36 \pm 0.45	2.72 \pm 0.039	2.95 \pm 0.020	2.38 \pm 0.311
F ₇	136 \pm 1.69	3.25 \pm 0.035	39.26 \pm 0.69	2.83 \pm 0.065	2.98 \pm 0.011	2.39 \pm 0.212

* n=3

Differential scanning calorimetry (DSC)

To understand the compatible state of the drug, DSC studies were carried out on pure drug and drug loaded film, the thermo grams are shown in Fig 2. KF exhibits a sharp endothermic peak at 93.02°C , but formulation F_3 exhibited a endothermic peak at 94.46°C (with glycerin). This result clearly indicated that the drug was distributed in the film without any thermal degradation. But formulation F_3 exhibited the endothermic peak at 88.59°C (without glycerin). These thermal changes of the drug in the film might be due to glycerin involve in recrystallization of drug, so that a phase transition of the drug in the film was occurred after heat induction¹⁹.

Stability studies

The optimized formulation F_3 and conventional gel was subjected for stability studies and estimated drug content at the end of 90 days

(8th hour). However no significance change in drug content from formulation F_3 and conventional gel after the study period, indicating drug was stable.

Diffusion studies

Diffusion studies were carried out in an open glass diffusion tube, using hydrated cellophane as a diffusion membrane. Diffusion studies for all the films were carried out for 8 h in normal saline. From the diffusion studies, it was observed that, there was no significant diffusion of drug from KF films at gastric pH. At the end of 8th h, drug diffuses from formulation F_3 (92.8) was maximum than F_1 (87.1%), F_2 (86.3%), F_4 (84.8%), F_5 (82.2%), F_6 (80.31%), F_7 (78.8%) and conventional gel (88.3%) shown in Fig 3. From the figure, it was clear that maximum amount of KF was diffuses from the formulation (F_3). From the above results, it can be concluded that drug diffusion from the films was controlled due to increased

amounts of XG showed higher swellability of the film and leached plasticizer from the film could reduce tortuosity of aqueous pore channels of the films, respectively¹⁸. In order to understand mechanism of drug release, *in vitro* release data were treated to

kinetic models and linearity was observed with respect to Higuchi equation. The correlation coefficient obtained from Higuchi plot was found to be in the range of 0.907 to 0.9917. This indicates that mechanism of drug release was diffusion type.

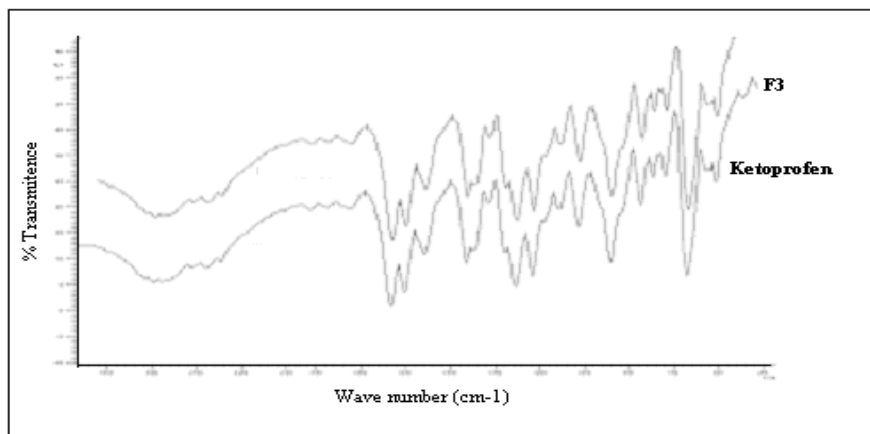


Fig. 1: FTIR spectra of pure drug, optimized formulation

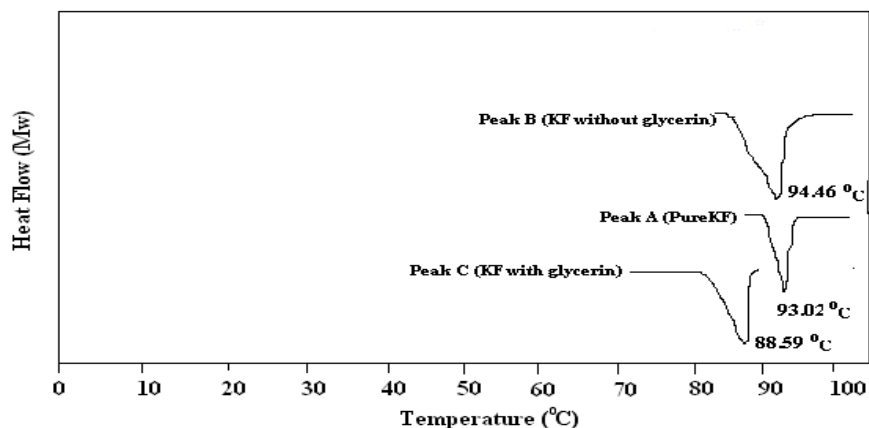


Fig. 2: DSC thermogram of Ketoprofen and Ketoprofen patch

***In vitro* skin permeation studies**

In vitro skin permeation studies were performed to compare the release of drug from 7 different film formulations (F₃-F₇) and conventional gel, all having the same quantity of (2.5% w/w) KF. As expected the flux of KF from films was found significantly higher (P < 0.05) than the flux of KF from conventional gel presented in Table 3 and fig. 3. *In vitro* skin permeation was highest and lowest in formulation F₃ and F₇ respectively, presented in Fig. 3. The formulations F₄ showed an intermediate skin permeation profile. Increasing the concentration (3 to 5% w/w) of penetration enhancer showed a significant difference (P < 0.05) in the flux of KF. The highest flux and enhancement ratio for KF from the film (F₃) containing menthol was found to be 0.261 ± 0.01 mg/cm²/h & 8.70 mg/cm²/h respectively. The skin permeation profile of film F₃ was significantly different (P < 0.05), when compared with that of F₄. Thus, menthol is expected to be a moderate skin permeation enhancer. In contrast, menthol enhanced the skin permeation of the drug by increasing both the skin concentration and the diffusion rate in skin because menthol contains functional group of hydrogen bonding. KF is lipophilic drug and menthol is a lipophilic terpene found to be more effective because menthol found to enhance the penetration of drug by both lipid and pore pathway^{20, 21}. Increase in the concentration of penetration enhancer from 1% wt/wt to 3%

w/w, resulted increases in the enhancement ratio and the flux. But even after increasing the penetration enhancer from 4% w/wt to 5% and plasticizer from 2.5% to 4% w/w in formulation F₄ and F₇ showed decreased enhancement ratio. Because increased ratio of XG in the films showed higher swellability of the film, plasticizer leaches from the film could reduce tortuosity of aqueous pore channels of the films. So that delivery of drug at a controlled rate across intact skin to achieve a therapeutically effective drug level for a longer duration of time from transdermal films. When enhancement ratio < 1.0 indicates that enhancer has no permeation enhancing activity.

Anti inflammatory studies

Based on higher drug permeation, formulation F₃ was selected for the *in vivo* anti inflammatory effects and compare with conventional gel. A significant inhibition (p < 0.05) of inflammation was found with the film formulation F₃ containing 3 % w/w menthol in comparison to the conventional gel without menthol. The percent inhibition value after 24h was found to be more F₃ (86.3%) as compared to gel formulation without penetration enhancer (39.8%) (Presented in Fig.3), and the difference between formulation F₃ and conventional gel percent inhibition was significant (p < 0.05). The enhanced anti inflammatory effects of formulation F₃ could be due to the enhanced permeation of KF through the skin.

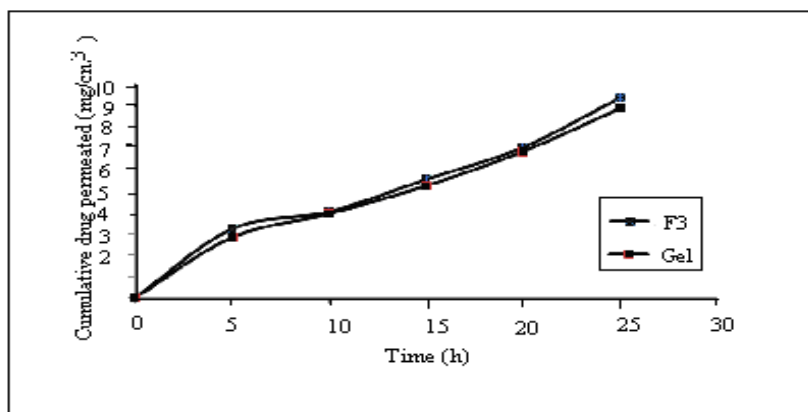


Fig. 3: Comparative in vitro skin permeation profile of KF from Film F₃ and conventional gel formulation

Table 3: Permeability Parameters of Different Formulations

Formulation	Menthol (%)	$J_{ss} \pm SD$ (mg/cm ² /h)	Permeability coefficient (Kp)	E_r
Gel (control)	-	0.030 ± 0.087	0.123 ± 0.096	1.0
F ₁	1.0	0.162 ± 0.096	0.153 ± 0.130	5.4
F ₂	2.0	0.239 ± 0.085	0.212 ± 0.100	7.9
F ₃	3.0	0.261 ± 0.001	0.221 ± 0.126	8.7
F ₄	3.5	0.229 ± 0.031	0.209 ± 0.121	7.6
F ₅	4.0	0.202 ± 0.041	0.192 ± 0.112	6.7
F ₆	4.5	0.194 ± 0.023	0.181 ± 0.014	6.4
F ₇	5.0	0.172 ± 0.013	0.163 ± 0.013	5.7

*(n = 3)

CONCLUSION

On the basis of good mechanical properties, better compatibility and stability of drug with polymer, highest drug permeation, we selected film formulation F₃ (3% Menthol) for use in *In vivo* studies. The *in vivo* studies revealed a significant increase in anti-inflammatory effects as compared with conventional gel without menthol. From *in vitro* and *in vivo* data it can be concluded that the developed film formulation F₃ has the best effective combination of polymer to achieve therapeutic plasma concentration. But additional experiments should be carried out before the film formulations are used on humans.

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