DEVELOPMENT AND EVALUATION OF PIROXICAM LOADED BIOPOLYMER BASED TRANSDERMAL FILM

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ABSTRACT
The aim of the present study was to formulate biopolymer based transdermal film loaded with Piroxicam (PX). Transdermal films were prepared by using sodium locust bean gum (LBG) and Sodium alginate (SA) as biopolymers by varying the blend ratios by solution casting method. The drug loaded membranes were evaluated for thickness, tensile behaviours, content uniformity; transdermal permeation of PX through rat abdominal skin was determined by Franz diffusion cell. In vitro skin permeation profile of optimized formulation was compared with that of PX conventional gel. Carrageen induced rat paw edema model was used to investigate the in vivo performances. Menthol (3%) and glycerin (3%) are used as permeation enhancer and plasticizer, respectively. PX was found to be compatible and stable with the prepared formulation as confirmed by Fourier transform infrared spectroscopy (FTIR) and Differential Scanning Calorimetric (DSC), studies. In vitro release studies reveals effectiveness after 24 h when compared with the conventional gel. The film does not show any signs of edema, erythema or ulceration. From the in vitro skin permeation and anti inflammatory activity data it can be concluded that the developed optimized formulation (F3) has good potential to achieve the transdermal drug delivery of PX for effective therapy.

Keywords: Piroxicam; Locust bean gum; Sodium alginate; Transdermal film; Anti Inflammatory activity.

INTRODUCTION
In recent years topical drug delivery is well-known for the treatment of local skin disorders, but skin as a route for systemic drug delivery is a more recent development. Very few transdermal products have been approved to date, largely because of the complexities involved in achieving a consistent delivery rate. There are many variables that influence the absorption of drugs across the skin and into the general circulation, including the biological properties of the skin, chemical properties of the drug, and the interactions between skin and drug delivery system. Systematic studies have led to complications of permeability data for a range of drugs through skin, both stratum corneum and dermis. These studies reflect the large variability and slowness of the process for most drugs. Consequently, only a few drug candidates are currently available for transdermal drug delivery. There are efforts to improve the process which involve conditioning the skin. 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 skin considered as a safe port for drug administration, to provide continuous drug release into systemic circulation. Recently, bio polymers used in the fabrication of transdermal films has received much attention due to their excellent biocompatibility and bio degradation. One of the most promising techniques for enhancement for transdermal permeation of drugs is transdermal patches. 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. Locust bean gum (LBG) 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. The effect of hydrophilic plasticizers such as glycerin on physicochemical properties on SA/LBG film was evaluated. PX belongs to the group of substituted 2-phenylpropionic acids which has analgesic, anti-inflammatory and antipyretic effects. PX exerts the majority of its analgesic actions through inhibition of the synthesis of prostaglandins by inhibiting the enzyme cyclooxygenase (COX). PX had the best topical penetraion ability when compared to ketorolac; indomethacin and other studies have found that topical PX was effective for the treatment of well localized soft tissue injury, joint pain, in reducing muscle soreness after repetitive muscle contraction. The importance of PX 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 administration. Penetration depends on ability of drug to penetrate the stratum corneum, enter the systemic circulation and to achieve the therapeutic effect. There has been increased interest during recent years in the use of chemical enhancer that could modify drug permeation through skin. 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 channels. Transdermal films with varied ratios no irritating and pharmaceutically acceptable biopolymers’ and LBG combination containing the drug PX with permeation enhancer (menthol). The prepared films were compared with the marketed conventional gel. Furthermore, films were evaluated for anti-inflammatory activity on carrageen an 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.
MATERIALS AND METHODS
Piroxicam was a gift sample from IPCA Mumbai, India. Sodium Alginate, Locust bean gum and Menthol were procured from Loba’ Chemical, Mumbai, India. Solvents and chemicals were of analytical grade.

I. Preformulation Studies

a. Determination of Melting Point
Melting point of drug sample was performed by using Thieles tube method. A fine powder of Piroxicam was filled in a capillary tube, previously sealed at one end and the capillary tube was tied to the bottom of the thermometer. The thermometer and capillary tube were immersed in to the liquid paraffin taken in the tube. Bottom of the tube was heated gently by means of burner. When the sample starts to melt the reading was recorded.

b. Solubility studies
The solubility was done by adding the solute in small incremental amounts to the fixed volume of solvents, after each addition, the system was vigorously shaken and examined visually for the un-dissolved solute particles. When some amount of the solute remains undissolved, the total amount added up to the point served as a good and rapid estimate of solubility.

c. Determination of partition co-efficient
The partition co-efficient study was performed using n-octanol as oil phase and phosphate buffer pH 7.4 as aqueous phase. The two phases were mixed in an equal quantity and were saturated with each other on a mechanical water bath shaker at 32°C for 24h. The saturated phases were separated by centrifugation at 2000 rpm on a Remi Centrifuge. Standard plots of drug were prepared from both the phosphate buffer and octanol. Equal volumes (10ml each) of the two phases were taken in triplicate in conical flask and to each 100mg of weighed amount of drug were added. The flasks were shaken at 320C for 6h to achieve a complete partitioning at 100rpm. The two phases were separated by centrifugation at 100rpm for 5min and they were then analyzed for respective drug contents. The partition co-efficient of drug Ko/w was calculated using the following formula.

\[ Ko/w = \frac{\text{Concentration in octanol}}{\text{Concentration in phosphate buffer pH 7.4}} \]

II. Evaluation of Transdermal Films

a. Preparation of transdermal films
The Transdermal films were prepared by using solvent casting technique. The bottom of the mold was wrapped with aluminium foil which was used as backing membrane. Drug containing films were prepared by solution casting method. In brief, the required amounts of a mixture of LBG (Table 1) were weighed and prepared polymeric solution using quantity sufficient water, kept aside for 2h after stirring. Accurately weighed PX (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 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

d. 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 PX in the receptor phase was determined by HPLC method. The HPLC system consists of HPLC-shimadzu (Tokyo, Japan) LC-6A model fitted with a - 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 PX in Phosphate buffer (pH 7.4). Final concentration of the solution will be in the range of 1.00-5.00 g/ml. The average drug content of three replicate samples was measured. The folding endurance was measured manually for the prepared films. A strip of film 2x2cm was cut evenly and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gave the exact value of folding endurance.

e. Scanning Electron Microscopy (SEM)
The surface characteristics of the prepared films were determined by scanning electron microscopy (model-LV 5600, Jeol, USA) and photomicrographs were recorded, by suitable magnification at room temperature.

f. 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 105°C under nitrogen gas purge at a heating rate of 100°C/ min.

g. Fourier transforms infrared spectroscopy (FTIR)
FTIR spectra of pure drug and drug loaded films were obtained using KBr pellet method (Pressure of 6000 kg/cm2). Spectral measurements were obtained by powder diffuse reflectance on a
FTIR spectrophotometer (Shimadzu, Model 8400S, Japan) in the wave number region of 400-4000 cm-1 to study drug excipients interactions if any.

h. 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 ± 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.

i. Stability of the transdermal films and prepared PX 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.

j. 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. Male albino rats weighing 110-125 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 ethanol 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 320°C ± 10°C. All the ethanol PBS was replaced every 30 minutes to stabilize the skin. It was found that the receiver fluid showed negligible absorbance after 5 h 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 (0.5 ml) 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 PX in the sample was measured by HPLC.

k. Anti inflammatory studies

The Anti inflammatory test was carried out on male albino rats weighing 110 to125 g. The animals were kept under standard laboratory conditions, with temperature of 25°C ± 10°C and relative humidity of 60% ± 5%. The animals were housed in cages, 5/cage, with free access to a standard laboratory diet. The anti-inflammatory activity of PX from film formulation F3 was evaluated by the carrageenan-induced hind paw edema method in albino rats 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 PX transdermal film.

Permeation data analysis

Results are given mean ± standard deviation (S.D). The cumulative amount of drug permeated through the skin (mg/cm2) was plotted as a function of time (t) for each formulation. Drug flux (permeation rate) at steady state (Jss) was calculated by dividing the slope of the linear portion of the graph by the area of the diffusion cell. The permeability coefficient (Kp) was calculated by dividing Jss by the initial concentration of the drug in the donor cell (Co).

\[ Kp = \frac{Jss}{Co} \]  

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

\[ Er = \frac{Jss \text{ of formulation}}{Jss \text{ of control}} \]

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

RESULTS AND DISCUSSION

I. Pref ormulation studies

a. Determination of Melting Point

Melting point of PX was found to be 203°C to 197°C.

b. Solubility studies

PX is freely soluble in phosphate buffer pH 7.4, ethyl alcohol, methyl alcohol, chloroform, acetone, dichloro methane, and ether insoluble in water.

c. Determination of partition co-efficient

The partition co-efficient studies were performed in triplicate. The value of partition co-efficient (P) value was experimentally found to be 0.832.

II. Evaluation

a. Preparation of LBG/SA films containing drug

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.

b. Measurements of Mechanical properties

Thickness of the prepared films was in the range of 168 to 175 m is presented in Table 2.
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 is increased. This effect was more pronounced on films containing more amount of plasticizer and more amount of plasticizer showed an significant increases in moisture up take at increased RH.

b. Diffusion studies
Diffusion studies were carried out in an open glass diffusion tube, using hydrated cellulose 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 PX films at gastric pH. At the end of 12th h, drug diffuses from formulation F3 (91.28%) was maximum than F1 (85.74%), F2 (88.24%), F4 (87.66%), F5 (86.66%), F6 (83.12%), F7 (82.10%) and conventional gel (89.32%).

i. Stability studies
The optimized formulation F3 and conventional gel was subjected for stability studies and estimated drug content at the end of 90 days (8thh). However no significance change in drug content from formulation F3 and conventional gel after the study period, indicating drug was stable.

j. In vitro skin permeation studies
In vitro skin permeation studies were performed to compare the release of drug from 7 different film formulations (F1- F7) and conventional gel, all having the same quantity of (2.5% w/w) PX. As expected the flux of PX from films was found significantly higher (P <0.05) than the flux of PX from conventional gel presented in Table 3. In vitro skin permeation was highest in formulation F3 and lowest in formulation F7.

k. Anti inflammatory studies
The anti-inflammatory efficacy of formulations in mice paw oedema induced by the Carrageenan was tested and the results were compared with that of the conventional gel without menthol. The paw oedema volume was measured for 12h period, after the Carrageenan injection.

DISCUSSION
The results of partition co-efficient obtained also indicate that the drug possesses sufficient lipophilicity, which fulfil the requirements of formulating the selected drug into a transdermal film. The biphasic nature of drug mimics the biphasic nature of skin, thus ensuring easy penetration through the skin. 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 > 60% in the formulated films, might be SA having α-L-guloronic acid, which is interact with LBG produces good flatness to the film21. Thus these formulations can maintain a smooth and uniform surface when applied on skin.

Thickness, tensile strength and % elongation of the films increasing by increased ratio of LBG and plasticizer in the films. Added glycerin alters the physical and mechanical properties by enhancing the mobility of polymers chains of LBG, SA, by hydrogen bonding. However it was found that 3% of glycerin gives the best plasticizer effect for PX loaded film.

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 an significant increases in moisture up take at increased RH.

Photomicrograph reveals the absence of crystals of the drug on the surface of film, indicating uniform distribution of the drug within the film.

PX exhibits a sharp endothermic peak at 200°C, but formulation F3 exhibited a endothermic peak at 201°C (with glycerin). This result clearly indicated that the drug was distributed in the film without any thermal degradation.

The FTIR spectra of the pure drug, formulation F3 indicated that characteristic peaks of PX 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.

Diffusion study result shows that, it was clear that maximum amount of PX was diffuses from the formulation (F3) and it can be concluded that drug diffusion from the films was controlled due to increased amounts of LBG showed higher swell ability of the film and leached plasticizer from the film could reduce tortuosity of aqueous pore channels of the films, respectively. 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 0.9934 to 0.9956. This indicates that mechanism of drug release was diffusion type.

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 PX. The highest flux and enhancement ratio for PX from the film (F3) containing menthol was found to be 0.247 ± 0.012mg/cm²/h & 8.2 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. PX is lipophilicity drug and menthol is a lipophilically terpene found to be more effective because menthol found to enhance the penetration of drug by both lipid and pore pathway. 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 3.5 % to 5% %w/w and plasticizer from 3.5 % to 5% w/w in formulation F4 and F7 showed decreased enhancement ratio. Because increased ratio of LBG/SA in the films showed higher swell ability 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.

The inhibition of oedema was observed and the comparative reduction in paw oedema volume, according to the mode of administration was recorded with respect to the control group. A significant inhibition of inflammation was found with conventional gel formulation (without menthol). 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 as compared to gel formulation without penetration enhancer 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 PX.
though the skin. The anti inflammatory studies were performed
to confirm the safety of optimized formulation F3. Literature survey
reported21.22 that a value between0 to 9 indicates that the applied
formulation is generally not an irritant to human skin. The
enhancement ratio was found to formulation F3 8.3. From this it was
concluded that optimized formulation F3 was safe to be used for
transdermal drug delivery.
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 have great
potential for transdermal drug delivery. 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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films: effect of plasticizers on film properties, drug permeation and drug release

### Table 1: Formulation chart of Piroxicam transdermal films

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Piroxicam (%w/w)</th>
<th>Locust bean gum (%w/w)</th>
<th>Sodium alginate (%w/w)</th>
<th>Glycerin (%w/w)</th>
<th>Menthol (%w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>2.5</td>
<td>4.0</td>
<td>91.5</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>F2</td>
<td>2.5</td>
<td>8.0</td>
<td>85.5</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>F3</td>
<td>2.5</td>
<td>12.0</td>
<td>80.5</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>F4</td>
<td>2.5</td>
<td>16.0</td>
<td>75.5</td>
<td>3.5</td>
<td>3.5</td>
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<tr>
<td>F5</td>
<td>2.5</td>
<td>20.0</td>
<td>68.5</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>F6</td>
<td>2.5</td>
<td>24.0</td>
<td>63.5</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>F7</td>
<td>2.5</td>
<td>28.0</td>
<td>59.5</td>
<td>5.0</td>
<td>5.0</td>
</tr>
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</table>

### Table 2: Mechanical properties of the prepared films

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Thickness of film (μm) Mean±S.D</th>
<th>Tensile Strength (MPa/mm2) Mean±S.D</th>
<th>%Elongation Mean±S.D</th>
<th>Moisture uptake (75 %) Mean±S.D*×10²</th>
<th>Moisture uptake(95 %) Mean±S.D*×10²</th>
<th>Drug content (mg/cm²) Mean±S.D</th>
<th>Folding endurance (mg/cm²) Mean±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.12±0.01</td>
<td>2.41±0.02</td>
<td>21.30±0.52</td>
<td>1.48±0.14</td>
<td>1.61±0.34</td>
<td>2.55±0.10</td>
<td>235.33±5.85</td>
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<tr>
<td>F2</td>
<td>0.13±0.02</td>
<td>2.52±0.02</td>
<td>21.88±0.22</td>
<td>1.53±0.10</td>
<td>1.59±0.42</td>
<td>2.4±0.10</td>
<td>248.57±7.01</td>
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<tr>
<td>F3</td>
<td>0.13±0.01</td>
<td>2.56±0.03</td>
<td>22.41±0.32</td>
<td>1.62±0.15</td>
<td>1.65±0.65</td>
<td>2.44±0.12</td>
<td>263.65±4.01</td>
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<tr>
<td>F4</td>
<td>0.14±0.02</td>
<td>2.79±0.01</td>
<td>23.35±0.12</td>
<td>1.72±0.12</td>
<td>1.77±0.35</td>
<td>2.41±0.14</td>
<td>265.32±5.02</td>
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<tr>
<td>F5</td>
<td>0.14±0.01</td>
<td>3.01±0.15</td>
<td>25.35±0.25</td>
<td>1.82±0.40</td>
<td>1.87±0.32</td>
<td>2.40±0.12</td>
<td>268.4±6.34</td>
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<tr>
<td>F6</td>
<td>0.15±0.01</td>
<td>3.15±0.11</td>
<td>28.36±0.12</td>
<td>1.85±0.16</td>
<td>1.89±0.24</td>
<td>2.36±0.12</td>
<td>275.2±5.42</td>
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<tr>
<td>F7</td>
<td>0.16±0.01</td>
<td>3.21±0.02</td>
<td>33.12±0.21</td>
<td>1.88±0.35</td>
<td>1.94±0.24</td>
<td>2.38±0.14</td>
<td>273.01±6.57</td>
</tr>
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</table>

### Table 3: In vitro skin Permeability parameters of different formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Menthol(%)</th>
<th>Jss (mg/cm²/h)</th>
<th>Permeability coefficient(Kp)</th>
<th>Er (mg/cm²/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel control</td>
<td>-</td>
<td>0.027 ± 0.032</td>
<td>0.118 ± 0.078</td>
<td>1.0 ± 0.11</td>
</tr>
<tr>
<td>F1</td>
<td>1.0</td>
<td>0.152 ± 0.012</td>
<td>0.150 ± 0.0065</td>
<td>5.2 ± 0.12</td>
</tr>
<tr>
<td>F2</td>
<td>2.0</td>
<td>0.221 ± 0.024</td>
<td>0.210 ± 0.054</td>
<td>7.5 ± 0.15</td>
</tr>
<tr>
<td>F3</td>
<td>3.0</td>
<td>0.247 ± 0.012</td>
<td>0.218 ± 0.031</td>
<td>8.2 ± 0.22</td>
</tr>
<tr>
<td>F4</td>
<td>3.5</td>
<td>0.224 ± 0.021</td>
<td>0.211 ± 0.052</td>
<td>7.4 ± 0.34</td>
</tr>
<tr>
<td>F5</td>
<td>4.0</td>
<td>0.205 ± 0.055</td>
<td>0.190 ± 0.046</td>
<td>6.9 ± 0.10</td>
</tr>
<tr>
<td>F6</td>
<td>4.5</td>
<td>0.195 ± 0.043</td>
<td>0.178 ± 0.022</td>
<td>6.5 ± 0.09</td>
</tr>
<tr>
<td>F7</td>
<td>5.0</td>
<td>0.172 ± 0.011</td>
<td>0.160 ± 0.010</td>
<td>5.6 ± 0.18</td>
</tr>
</tbody>
</table>

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