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 behavior, 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, biopolymers used in the fabrication of transdermal films has received much attention due to their excellent bio-compatibility and bio degradation. One of the most promising techniques for enhancement for transdermal permeation of drugs is transdermal patches5. 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 applications4. 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-blend composition and drug concentration6. 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 anti-inflammatory and antipritypic effects. KF exerts the majority of its analgesic actions through inhibition of the synthesis of prostaglandins by inhibiting the enzyme cyclooxygenase (COX)7. 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 insoluble 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, granular, 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 5d 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 desicator until further study.

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

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug (%)</th>
<th>Sodium alginate (%)</th>
<th>Xanthan gum (%)</th>
<th>Glycerin (%)</th>
<th>Menthol (%)</th>
<th>Flatness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>2.5</td>
<td>96.0</td>
<td>-</td>
<td>0.5</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>F2</td>
<td>2.5</td>
<td>74.5</td>
<td>-</td>
<td>2.0</td>
<td>3.0</td>
<td>98</td>
</tr>
<tr>
<td>F3</td>
<td>2.5</td>
<td>52.5</td>
<td>40</td>
<td>2.5</td>
<td>3.5</td>
<td>90</td>
</tr>
<tr>
<td>F4</td>
<td>2.5</td>
<td>30.5</td>
<td>50</td>
<td>2.5</td>
<td>3.5</td>
<td>88</td>
</tr>
<tr>
<td>F5</td>
<td>2.5</td>
<td>9.5</td>
<td>60</td>
<td>3.0</td>
<td>4.0</td>
<td>82</td>
</tr>
<tr>
<td>F6</td>
<td>2.5</td>
<td>-</td>
<td>80</td>
<td>3.5</td>
<td>4.5</td>
<td>82</td>
</tr>
<tr>
<td>F7</td>
<td>2.5</td>
<td>100</td>
<td>-</td>
<td>4.0</td>
<td>5.0</td>
<td>80</td>
</tr>
</tbody>
</table>

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.

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, Bharati Vidyapeeth 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 ± 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 (0.5 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 KA in the film 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°C ± 1°C and relative humidity of 60% ± 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 F1 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 KF 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 (Kps) was calculated by dividing Jss by the initial concentration of the drug in the donor cell (C0).

\[
K_p = \frac{J_{ss}}{C_0} \ldots \ldots 1
\]

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

\[
Er = \frac{J_{ss \text{ of formulation}}}{J_{ss \text{ of control}}} \ldots \ldots 2
\]

The results were analyzed statistically using Student’s t-test and significance was determined at 95% confidence 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-guluronic acid, which is interact with XG produces good flatness to the film. 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 bonding. However it was found that 2% of glycerine gives the best plasticizer effect for KF loading 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 an significant increases in moisture up take at increased RH.

FTIR Analysis

The FTIR spectra of pure drug and formulation F2 presented in Fig 1. The characteristic IR absorption peaks of Ketoprofen, at 2978, 2938 cm⁻¹ (C-H stretching of CH3 group (asymmetric) masked by O-H stretching), 2876 cm⁻¹ (C-H stretching of CH3 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 CH3 group (asymmetric)), 1369 cm⁻¹ (C-H deformation of CH3 group- symmetrical) and 825 - 640 cm⁻¹ C-H deformation of aromatic ring. The FTIR spectra of the pure drug, formulation F2 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

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Thickness of film (µm)</th>
<th>Mean ± S.D*</th>
<th>Tensile strength (MPa/mmol)</th>
<th>Mean ± S.D*</th>
<th>% Elongation</th>
<th>Moisture uptake (75%)</th>
<th>Mean ± S.D* x 10⁻¹</th>
<th>Moisture uptake (90%)</th>
<th>Mean ± S.D* x 10⁻¹</th>
<th>Drug content (mg/cm²)</th>
<th>Mean ± S.D*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>123±1.23</td>
<td>2.44±0.011</td>
<td>21.30±0.85</td>
<td>1.72±0.015</td>
<td>1.87±0.021</td>
<td>3.97±0.014</td>
<td>2.62±0.026</td>
<td>2.85±0.004</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>124±1.42</td>
<td>2.52±0.014</td>
<td>21.69±0.12</td>
<td>1.86±0.021</td>
<td>1.92±0.026</td>
<td>3.97±0.014</td>
<td>2.65±0.026</td>
<td>2.48±0.014</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>128±1.33</td>
<td>2.66±0.023</td>
<td>19.45±0.38</td>
<td>2.39±0.018</td>
<td>2.45±0.030</td>
<td>3.97±0.014</td>
<td>2.62±0.026</td>
<td>2.41±0.014</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
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</tr>
<tr>
<td>F4</td>
<td>131±1.25</td>
<td>2.84±0.024</td>
<td>24.41±0.58</td>
<td>2.49±0.029</td>
<td>2.58±0.020</td>
<td>3.97±0.014</td>
<td>2.62±0.026</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
<td></td>
</tr>
<tr>
<td>F5</td>
<td>132±1.21</td>
<td>3.03±0.152</td>
<td>29.84±0.36</td>
<td>2.68±0.036</td>
<td>2.85±0.004</td>
<td>3.97±0.014</td>
<td>2.62±0.026</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
<td></td>
</tr>
<tr>
<td>F6</td>
<td>135±1.18</td>
<td>3.18±0.120</td>
<td>35.36±0.45</td>
<td>2.72±0.039</td>
<td>2.95±0.020</td>
<td>3.97±0.014</td>
<td>2.62±0.026</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>136±1.69</td>
<td>3.25±0.035</td>
<td>39.26±0.69</td>
<td>2.83±0.065</td>
<td>2.98±0.011</td>
<td>3.97±0.014</td>
<td>2.62±0.026</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
<td>2.40±0.014</td>
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</tr>
</tbody>
</table>

* 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 thermograms are shown in Fig 2. KF exhibits a sharp endothermic peak at 93.02°C, but formulation F1 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 F2 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 induction.

Stability studies

The optimized formulation F3 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 F3 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 F1 (92.8) was maximum than F1 (87.1%), F2 (86.3%), F3 (84.8%), F4 (82.2%), F5 (80.3%), F6 (78.0%) and conventional gel (80.3%) shown in Fig 3. From the figure, it was clear that maximum amount of KF was diffuses from the formulation F1. 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, 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 0.907 to 0.9917. This indicates that mechanism of drug release was diffusion type.

**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 F1 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 (F7) 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 F7. 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. 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/w to 5% and plasticizer from 2.5% to 4% w/w in formulation F3 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 F2 (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 though the skin.
**CONCLUSION**

On the basis of good mechanical properties, better compatibility and stability of drug with polymer, highest drug permeation, we selected film formulation F3 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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