Formulation, in vitro and in vivo characterization of transdermal patches of etoricoxib

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ABSTRACT

The study was to develop a transdermal therapeutic system for Etoricoxib (EX) using various polymers like Hydroxy Propyl Methyl Cellulose (HPMC), Ethyl Cellulose (EC) and Polyvinyl Pyrrolidone (PVP) and plasticizers by solvent casting technique. The prepared patches were evaluated for physicochemical properties, in vitro release, in vitro skin permeation and in vivo release. The interactions between EX and polymers were investigated by Fourier Transform Infrared (FTIR) Spectroscopy. The in vitro release studies revealed that the release was sustained up to 24h and it follows zero order kinetics. Finally, the patch formulation containing E4 (0.75% HPMC, 0.25% PVP) selected through our in vitro study was characterized for in vitro skin permeation using various biological membranes like rat skin and Guinea pig skin. The patch exhibited no skin irritation and better Pharmacodynamic activity when compared with a commercially available Diclofenac patch with all the tested in vivo parameters. As a patient friendly and once a day dosing therapeutic system, the transdermal patches incorporating EX could be promising in the pastures of Controlled drug delivery.

Keywords: Etoricoxib; skin permeation studies; solvent casting technique; transdermal patches.

INTRODUCTION

Etoricoxib [5-chloro-2-[6-methylpyridin-3-yl]-3-[4-methylsulfonylphenyl] pyridine] is a novel, selective second-generation cyclooxygenase-2 inhibitor administered orally as an analgesic and anti-inflammatory drug that is used for the treatment of osteoarthritis, rheumatoid arthritis and gouty arthritis (Shahi SR, 2009). The oral administration of EX can produce some side effects related to gastrointestinal tract included nausea, dyspepsia, diarrhoea and upper abdominal pain (Hunt RH, 2003). In order to avoid the irritation of gastrointestinal tract one promising method is to administer the drug via skin (McNeill, 1992).

Topical application allows localized drug delivery to the site of interest. The therapeutic effect of the drug enhances thus minimize systemic side effects. Topical application of drug bypasses systemic deactivation or degradation and avoids gastrointestinal incompatibility (Apostolos G, 2004, Modamio P, 2000) and ease of drug input termination in problematic cases as well as maintaining suitable plasma concentration for longer duration through a non-invasive zero order delivery are the well documented advantages of this route of administration (Williams AC, 1992).

Here we investigated the release of EX, an anti-inflammatory drug from patches containing Hydroxy propyl methyl cellulose, Ethyl cellulose and poly vinyl pyrrolidone with the following objectives to overcome gastrointestinal incompatibility, to avoid hepatic first pass metabolism, reduce the frequency of administration, minimize systemic toxicity, to obtain greater therapeutic efficacy and to improve patient compliance.

Materials

EX was a gift sample obtained from Sun Pharmaceuticals Ltd (India). The polymers such as hydroxy propyl methyl cellulose (15 cps), Ethyl cellulose (20 cps) and Poly vinyl pyrrolidone (15 cps) obtained from S.d. fine Chemicals. All other chemicals were of analytical grade.

Determination of partition coefficient

The partition coefficient of EX was carried out in n-octanol/phosphate buffer pH 7.4 (Couarraze G, 1996). The two phases taken in separating funnel were shaken together initially to ensure mutual saturation. An accurately weighed quantity of EX was dissolved in 10ml of the n-octanol phase and shaken manually against 10 ml aqueous phase in a sealed container at regular intervals upto 24h. The separated n-octanol...
phase was assayed by UV spectroscopy to determine its residual concentration and hence the amount partitioned into the aqueous phase at 235nm. The partition coefficient was expressed as the concentration of drug in the n-octanol phase (% w/v) divided by the concentration in the aqueous phase (Marin EL, 1998).

Drug-Polymer Interaction Study

The Drug and polymer interaction between EX and polymers used in the films were studied by using Fourier Transform Infrared (FTIR) Spectroscopy (Perkin Elmer FT-IR, Perkin Elmer Inst. USA by KBr pellet method).

Casting of Patches

Transdermal patches were fabricated using different polymers containing EX by Solvent Casting technique (Jamakandhi VG, 1996; Liang Fang, 2009). Adhesive patches containing EX were prepared by dissolving polymers in suitable solvents namely dichloromethane and ethanol. Propylene glycol (30%v/v) of polymer composition was used as a permeation enhancer. The solution was poured into a glass ring which is covered with funnel. The solvent was allowed to evaporate at ambient conditions for 24 h. The patches were then covered with backing membrane cut into appropriate sizes, packed in aluminum foil and stored in desiccators for further studies. The composition of different formulations prepared using varying amounts of the polymers were listed in Table 1.

Physico Chemical Evaluation of the Prepared Patches

Thickness and Drug content

The thickness of the patch at three different points was determined using thickness gauge and the patches. Films of specified area were cut and weighed accurately (Mundada AS, 2009). Pieces were taken into a 100 ml volumetric flask containing phosphate buffer (pH 7.4), and the flask was sonicated for 8 h. And the solution concentration is found in respective nm (Agrawal SS, 1996).

Folding endurance test

Folding endurance test was carried out by folding the patch at the same point a number of times till it broke, (Ubaidulla U, 2007). The test was carried out to check the efficiency of the plasticizer and the strength of the film prepared using varying ratios of the polymers. The test was carried out in triplicate.

Moisture Uptake

Accurately weighed films of each formulation (n = 3) were kept in a desiccator which is maintained at 79.5% relative humidity (saturated solution of aluminium chloride) at room temperature and weighed after 3 days, (Panner Selvam P, 2010). The percentage of moisture uptake was calculated as the difference between final and initial weight with respect to initial weight.

Moisture Loss

Accurately weighed films of each formulation (n = 3) were kept in a desiccator and exposed to an atmosphere of 98% relative humidity (containing anhydrous calcium chloride) at room temperature and weighed after 3 days, (Kusum Devi V, 2003). The percentage of moisture loss was calculated as the difference between initial and final weight with respect to initial weight.

Tensile strength and Percentage elongation at break

Mechanical properties of the film were evaluated using an “Instron Tensile Strength tester” (Series IX Automated Material Testing System). A film strips with the dimension (15 cm x 7.5 cm) and free from air bubbles (or) physical imperfections was prepared and cut into a Dumbell shape, before fed into the equipment. This test was carried out with 50% humidity at 20°C, (Ghosal SK, 2002). The crosshead speed employed were 100mm/min, with the full-scale load range of 500 Kgf. The force and percentage elongation were measured, when the films were broken. Measurements were run in three replicates for each formulation.

Water Vapor Transmission Rate

Transmission cell of equal diameter were used for water vapor transmission studies (Patel HJ, 2009). These cells were thoroughly washed and dried in an oven. About 1 gm of calcium chloride anhydrous was placed in cell and the patch was fixed over the rim with the aid of the solvent. They were accurately weighed and placed in a desiccator containing potassium chloride saturated solution to maintain 84% RH humidity. The cells placed in desiccator were removed and weighed after 1, 2, 3, 4, 5, 6 and 7th day (Siva Kumar T, 2010).

\[ W \ V \ T = \frac{W L}{S} \]

Where, W is transmitted water vapor in mg, L is patch thickness in mm, S is exposed surface area in cm².

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Table 2: Physico Chemical Evaluation of the Prepared Patches

<table>
<thead>
<tr>
<th>F. Code</th>
<th>Thickness (mm) ± SD</th>
<th>Drug content (%)</th>
<th>Folding Endurance ± SD*</th>
<th>Tensile Strength Kgf/cm² &amp; Percentage Elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>0.29 ± 0.01</td>
<td>97.32</td>
<td>282 ± 1.0</td>
<td>360.25 &amp; 0.321</td>
</tr>
<tr>
<td>E2</td>
<td>0.26 ± 0.02</td>
<td>97.53</td>
<td>250 ± 1.4</td>
<td>325.61 &amp; 0.350</td>
</tr>
<tr>
<td>E3</td>
<td>0.24 ± 0.02</td>
<td>98.15</td>
<td>269 ± 2.0</td>
<td>333.34 &amp; 0.311</td>
</tr>
<tr>
<td>E4</td>
<td>0.25 ± 0.01</td>
<td>99.10</td>
<td>285 ± 0.3</td>
<td>392.25 &amp; 0.361</td>
</tr>
<tr>
<td>E5</td>
<td>0.32 ± 0.03</td>
<td>98.23</td>
<td>264 ± 2.0</td>
<td>304.82 &amp; 0.289</td>
</tr>
<tr>
<td>E6</td>
<td>0.30 ± 0.04</td>
<td>97.65</td>
<td>270 ± 1.5</td>
<td>314.18 &amp; 0.290</td>
</tr>
</tbody>
</table>

*Average of three determinants for each parameter

Table 3: Physico Chemical Evaluation of the Prepared Patches

<table>
<thead>
<tr>
<th>F. Code</th>
<th>Moisture uptake ± SD*</th>
<th>Moisture Loss ± SD*</th>
<th>Water Vapor Transmission Rate (mg/cm²/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>5.13 ± 0.05</td>
<td>4.97 ± 0.05</td>
<td>5.05 X10⁻⁵</td>
</tr>
<tr>
<td>E2</td>
<td>6.61 ± 0.01</td>
<td>5.13 ± 0.01</td>
<td>6.27 X10⁻⁵</td>
</tr>
<tr>
<td>E3</td>
<td>2.13 ± 0.03</td>
<td>3.88 ± 0.01</td>
<td>4.69 X10⁻⁵</td>
</tr>
<tr>
<td>E4</td>
<td>6.37 ± 0.05</td>
<td>8.64 ± 0.01</td>
<td>8.50 X10⁻⁵</td>
</tr>
<tr>
<td>E5</td>
<td>1.94 ± 0.01</td>
<td>2.03 ± 0.05</td>
<td>7.32 X10⁻⁵</td>
</tr>
<tr>
<td>E6</td>
<td>2.17 ± 0.15</td>
<td>2.54 ± 0.10</td>
<td>5.19 X10⁻⁵</td>
</tr>
</tbody>
</table>

*Average of three determinants for each parameter

**In vitro Drug Release Studies**

The in vitro release studies were carried out in Franz diffusion cell. The receptor compartment was maintained at 37 ± 1°C by means of a water bath, circulator, and a jacket surrounding the cell during the drug release study. The receptor cell was filled with freshly prepared isotonic phosphate buffer of pH 7.4. The receptor phase was stirred with small magnetic beads at 60 rpm by means of Teflon coated magnetic stirrer. The Semi-permeable membrane was placed between the donor and receptor compartment. The prepared patch was placed on one side of the semi-permeable membrane (Ji-Hui Zhao, 2007). Aliquots of 1mL were removed from the receptor compartment by means of a syringe and replaced immediately with the same volume of buffer solution. Test samples were taken from the medium at predetermined time intervals over a period of 24 hours and the samples were analyzed for EX content by UV spectrophotometer at 235nm (Vlassios Andronis, 1995).

**Skin permeation studies**

Ethical clearance for the handling of experimental animals was obtained from the Institutional animal ethical committee (IAEC) formed for this purpose. The approval number was 661/05/c/CPCEA AWD dt 07.06.2005/KMCP/IIECA/018/08dt 19.01.2008.

Male guinea pig skin (approximate 1mm in thickness) and rat skin was carefully excised (Liang Fang, 2009). After removing the hypodermal adipose tissue, the skin was used as a barrier membrane for the in vitro transdermal penetration (Ke GM, 2005). The permeation of F4 was performed in Franz-type glass single-diffusion cells with 5 cm² penetration area and a 100.0 ml receptor volume (Meyer and Zschemisch W, 2002). The receptor phase was magnetically stirred at 37 ± 0.5°C. The samples were analyzed at 235nm against blank by UV spectrophotometer.

**In vivo Studies**

*Primary Skin Irritation Test*

The dorsal part of rabbit was shaved carefully, and patch was applied on that skin. Conditions of the skin were observed at 24, 48 and 72 h after application, for signs of irritation and are evaluated most often by modification described by Draize which is based on scoring method (Draize, 1944).

*In vivo drug release*

Four Male Rabbits of 10-12 weeks old weighing 2-3kg were selected. They were kept with husk beeding and were fed with standard rodent pellet diet and water (Saisivam S, 1991). Light and dark cycle with 12 hrs light and 12hrs dark was maintained. The temperature, relative humidity conditions were 28°C ± 2°C and 60±15% respectively. Animal hair at the site of patch application was clipped the night before the experiment. Before applying the patches, the skin was gently wiped with warm water followed by an alcohol swab and patted dry. The animal dose of EX was calculated according to the body weight as 4.225mg. Patch samples were applied on the shaved site in the dorsal surface. At specific intervals of time, the films were removed carefully and analyzed for the remaining drug content subtracted from the initial content (Jayaprakash P, 2010) in the film by UV Spectrophotometer at 235nm.
Pharmacodynamic Study

Carrageenan induced edema model

The sustaining action and anti-inflammatory of the best formulation was evaluated by the Carrageenan induced hind paw edema method in rats. Six male albino rats weighing approximately 200-250g were taken (Rama Rao P, 1998; Winter CA, 1965). One day before the experiment, the left hind thigh of each animal was shaved without damaging the skin. The patch samples were applied to the shaved area in the left hind thigh. The first group (control) received orally 0.5ml of normal saline solution. The second (standard) group received Diclofenac sodium patches. The third groups received E4 formulation respectively. One hour before patch application 0.1 ml of 1% Carrageenan in isotonic saline was injected subplantarily into left hind paw. The volume of the left hind paw was measured using a displacement of plethysmometer.

Stability evaluation

Stability studies were performed for 3 months using EX prepared patches, (Alfrd Martin, 1991, Panchagula R, 2005). Prepared patches were kept in refrigerator, stability chamber and incubator for maintaining the temperature of 4°C, 40°C and 60°C respectively. At specific interval of time 15, 30, 45, 60, 75, 90th day the patches were allowed to determine the drug content by UV Spectrophotometer at 235nm.

RESULTS AND DISCUSSION

Transdermal drug delivery system containing EX was prepared by using different polymeric ratios of hydroxypropyl methyl cellulose, poly vinyl pyrrolidone and hydrophobic poly mer of ethyl cellulose in combination or individual.

The observed partition coefficients of EX were determined using n-octanol/water at 37°C the results indicates that the drug had sufficient lipophilicity and therefore it is suitable for transdermal delivery. Compatibility between a drug and polymer is a factor in determining the effectiveness of polymeric delivery systems. Herein, to consider compatibility between polymer and drug, FTIR studies were carried out on pure drug and Mixture of Polymers. These results suggest that there is no interaction between the drug and polymers used in the present study.

EX transdermal patches developed by Solvent evaporation technique using EX and in combination with HPMC, EC and PVP (Table 1) were evaluated for thickness, weight variation, content uniformity, tensile strength, moisture uptake, moisture loss and water vapor transmission rate. Thickness of the patches varied from 0.25 ± 0.01 to 0.32 ± 0.04 mm (Table 2 and 3). The results indicate that the process adopted for casting the films in this investigation is capable of giving uniform drug content and minimum intra batch variability. Folding endurance test results indicated that the patches would maintain the integrity with general skin folding when applied. The percentage moisture uptake in the formulation E5 (0.75% EC, 0.25% PVP) has shown the lowest value of moisture absorption 1.943 ± 0.01 which may be due to the hydrophobic nature of the polymer used in it. The formulation E4 (0.75% HPMC, 0.25%PVP) shows higher value of moisture loss 8.64 ± 0.01 and formulation E5 (0.75% EC, 0.25% PVP) shows low value of 2.034 ± 0.05 which is due to its hydrophilic nature and hydrophobic nature respectively. Formulation E5 (0.75% EC, 0.25% PVP) shows low value of 304.82 & 0.289 and formulations E4 (0.75% HPMC, 0.25%PVP) have shown highest value of 392.25 & 0.361 for tensile strength and percentage elongation respectively. The formulation E4 (0.75% HPMC, 0.25%PVP) has shown maximum water vapor transmission of 8.500 X10^-6 may be due to the presence of high hydrophilic nature of the polymer.

In vitro Drug Release Studies

Table 4 lists the cumulative percentage drug release of various formulations. The cumulative percentage of drug released in 24 h was found to be highest (98.56%) from formulation E4. Figure1 exhibits the dissolution profile obtained for formulation E4. The Higuchi’s plot has shown the regression value of 0.984, which indicates that diffusion mechanism influencing the drug release. In order to confirm this fact, Peppa’s plot was drawn which has shown slope value of 0.598, which confirms that the diffusion mechanism involved in the drug release was of non – fickian diffusion type. Hence formulation E4 was selected as the optimized formulation by virtue of its drug release kinetics.

![Figure 1: In-Vitro drug release study of Formulation E4](image)

Skin Permeation Studies

The in vitro skin permeation of E4 was performed using various biological membranes such as Guinea pig skin and Rat skin showed drug diffusion of 24 h up to the extent of 89.63% and 93.61% respectively. Table 4 lists the in vitro skin permeation of various biological membranes. The drug diffusion of E4 from Guinea pig skin at all the concentrations tested was compared with Rat skin. As the Porcine ear has more fat deposition and thickness, it might have hampered the drug release.
Table 4: Drug Release Parameters of Prepared Formulations

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Drug Release Studies</th>
<th>Drug release (%) at 24&lt;sup&gt;th&lt;/sup&gt; hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>In-vitro Drug release study</td>
<td>E1: 83.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E2: 81.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E3: 85.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E4: 98.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E5: 79.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E6: 77.46</td>
</tr>
<tr>
<td>2</td>
<td>In-Vitro Skin permeation study (E4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guinea pig skin</td>
<td>89.63</td>
</tr>
<tr>
<td></td>
<td>Rat skin</td>
<td>93.61</td>
</tr>
<tr>
<td>3</td>
<td>In-vivo drug release (E4)</td>
<td>91.14</td>
</tr>
</tbody>
</table>

Table 5: Anti Inflammatory Activity Data for formulation E4

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Paw volume (ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Standard</td>
</tr>
<tr>
<td>Before 30</td>
<td>0.245±0.013</td>
<td>0.249±0.018</td>
</tr>
<tr>
<td>0</td>
<td>0.680 ± 0.33</td>
<td>0.611±0.025</td>
</tr>
<tr>
<td>30</td>
<td>0.700 ± 0.013</td>
<td>0.558±0.025</td>
</tr>
<tr>
<td>60</td>
<td>0.728 ± 0.023</td>
<td>0.503±0.02</td>
</tr>
<tr>
<td>120</td>
<td>0.749 ± 0.019</td>
<td>0.465±0.023</td>
</tr>
<tr>
<td>240</td>
<td>0.766 ± 0.017</td>
<td>0.398±0.014</td>
</tr>
</tbody>
</table>

through the membrane. Figure 2 exhibits the in vitro skin permeation of E4.

**In vivo Studies**

**Primary Skin irritation test**

The skin irritation test of the optimized formulation E4 showed no skin irritation. The skin irritation study results indicate that the polymeric patches are compatible with the skin and hence can be used for transdermal application.

Figure 2: Skin permeation drug release of Formulation E4

**In Vivo Drug Release Study**

In vivo study was carried out in rabbit revealed that the consistence in vitro release pattern of the formulation E4 was reproducible even in biological environment. Table 4 lists the in vivo drug release of formulation E4. At the end of 24<sup>th</sup> hour the in vivo drug release showed 91.14% release. The correlation between the results obtained by the in vitro and in vivo techniques was very good. To ensure the correlation between the in vitro and in vivo release pattern, the regression analysis was carried out. Figure 3 exhibits the correlation between the in vitro and in vivo release pattern. They are well correlated, so the release pattern has followed the predicted zero order kinetics in biological systems also.

Figure 3: In-vitro-In-vivo correlation graph for Formulation E4

**Pharmacodynamic Study**

Foot edema induced by carrageen was effectively suppressed by EX patch. The result obtained in this study showed that the percentage paw edema inhibition was 46.64% for animal treated with EX patch and 48.04% for animal treated with Diclofenac sodium patch. Table 5 lists the Anti Inflammatory Activity data for formulation E4.
**Stability Studies**

The formulation E4 was subjected to accelerated stability testing at 40°C, 40°C and 60°C for 90 days. The formulation was found to be stable with respect to EX assay when analyzed by HPLC. Less degradation and good physical appearance was observed on performing the stability studies and period of expiry was found to be 197 days at 25°C could be assigned to the TDDS.

**CONCLUSION**

The transdermal formulation of EX in combination with HPMC and PVP produces smooth and flexible film, which was found to control and prolong the drug release till 24 hours. The formulation E4 (0.75% HPMC, 0.25% PVP) has shown optimum release in concentration independent manner. While release kinetics (Hiuchi’s plot) of drug release is the zero-order process, suggesting that the release of drug from the adhesive is associated with the diffusion process. Good correlation is observed between in vitro and ex vivo profile, which reveals the ability of the formulation to reproduce the in vitro release pattern through various biological membrane. Patches containing formulation E4 show promise for in vivo and pharmacodynamic performance evaluation in a suitable animal model. The shelf life of the formulation E4 was found to be 197 days. In view of the overall results reported in this study, it can be proposed that EX is suitable candidate for the design of transdermal drug delivery system.

**Acknowledgment**

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