

FORMULATION AND *IN VITRO* & *IN VIVO* EVALUATION OF TRANSDERMAL PATCHES OF LORNOXICAM USING NATURAL PERMEATION ENHANCERS

C SOUJANYA^{1*}, B LAKSHMI SATYA¹, M LOKESH REDDY¹, K MANOGNA¹, P RAVI PRAKASH², A RAMESH³

¹Dept. of Pharmaceutics, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak Dist, AP, India. ²Dept. of Pharmaceutics, CES College of Pharmacy, Kurnool, AP, India. ³Dept. of Pharmacology, Vishnu Institute of Pharmaceutical Education and Research, Narsapur, Medak Dist, AP, India.

Email: soujanyakashinath@gmail.com

Received: 11 Feb 2014 Revised and Accepted: 28 Feb 2014

ABSTRACT

Objective: The study was to develop a transdermal therapeutic system for lornoxicam (LX) using various polymers like Ethyl cellulose (EC) and Hydroxy Propyl Methyl Cellulose (HPMC) and natural permeation enhancers like eugenol and cineole. by solvent casting method.

Methods: The patches were prepared using solvent casting method, and the prepared patches were evaluated for physicochemical properties, *in vitro* drug release, *ex vivo* skin permeation studies and *in vivo* studies. The interactions between LX and polymers were investigated by Fourier Transform Infrared Spectroscopy (FTIR).

Results: The *in vitro* release studies revealed that the release was sustained up to 24h and it follows Zero order kinetics. Finally, the best formulation (L3) selected through *in vitro* study was subjected to *ex vivo* skin permeation using rat skin and *in vivo* studies in rabbits. The patch was also subjected to skin irritation and anti inflammatory activity and found that there was no skin irritation and edema levels were lowered in the albino rats.

Conclusion: As a patient friendly the transdermal patches containing Lornoxicam could be promising in the pasture of controlled drug delivery.

Keywords: Lornoxicam, Skin Permeation studies, Solvent casting method, Anti inflammatory studies, Transdermal patches.

INTRODUCTION

Since last decade, TDDS gained a lot of interest due to its advantages over the conventional dosage forms and oral controlled release delivery, notably avoidance of hepatic first pass effect [1]. Optimization of drug release (flux) through the skin into the systemic circulation and simultaneously minimize the retention and metabolism of the drug in the skin is the major goal of transdermal products [2].

Lornoxicam is a Non-steroidal anti-inflammatory drug and it is an oxamic class with analgesic, anti-inflammatory and antipyretic properties and majorly used in the treatment of inflammatory diseases of the joints, osteoarthritis, surgery, sciatica. The oral administration of LX can produce some side effects related to gastro intestinal tract including dizziness, headache, stomach pain, upset stomach, diarrhea, nausea, vomiting and indigestion [4, 5]. It is absorbed in GIT, bioavailability is 90–92% and it inhibits the prostaglandin synthesis. LX was chosen as a model drug for these studies, it possess near ideal characteristic, that a drug must have in formulating a drug delivery system such as low molecular weight, effective in low plasma concentration as well as high degree of patient compliance and it reduces in parallel both the carrageenan-evoked edema and spinal c-Fos expression[6]. When compared to other standard formulations, the onset of action of pain relief of LX was shorter and more effective [7].

Complete and rapid absorption of Lornoxicam is through the gastrointestinal tract. Peak plasma concentration is attained within 2.5 hrs. Food reduces the absorption of the drug. It has a relatively short plasma half-life (3 to 5 hours). LX gets eliminated in the form of metabolized 5'-hydroxy-Lornoxicam, which does not undergo enterohepatic recirculation [8]. Thus in current research work an attempt was made to develop transdermal patches of Lornoxicam in order to supply local medication to the affected tissues (painful joints), avoid its gastrointestinal side effects and to improve patient compliance by preparing sustained release patches.

MATERIALS AND METHODS

Lornoxicam was a gift sample obtained from Hetero Pharmaceuticals Ltd (India). The polymers such as HPMC, Ethyl cellulose (20 cps) obtained from S.D Fine Chemicals. All other chemicals were of analytical grade.

Drug-Polymer Interaction Study

The drug and polymer interaction used in patches were studied by using Fourier Transform Infrared spectroscopy (FTIR) by KBr pellet method.

Preparation of patches by solvent casting method

Transdermal patches were fabricated using different polymers containing LX by solvent casting method. Adhesive patches containing LX were prepared by dissolving polymers in suitable solvents namely dichloromethane and methanol. Eugenol and cineole of polymer composition were used as permeation enhancers, Poly ethylene glycol (PEG 400) is used as plasticizer. The solution was poured into a Petri plate and covered with a funnel in inverted position. 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. The composition of different formulations prepared using various amounts of polymers were listed in Table 1.

Physicochemical evaluation of the prepared patches.

Physical Appearance

All the prepared formulations were visually inspected for color, clarity, flexibility and smoothness [9].

Thickness and Drug content

The thickness of the patch at three different patches was determined by using digital caliper and the average of the three readings was calculated [10]. Films of specified surface area were cut into pieces

and weighed accurately, these pieces are transferred into volumetric flask containing phosphate buffer pH 7.4 and the flask was sonicated for 8-12 h, the contents of flask was filtered and the solution was examined for the drug content at 376 nm, against the reference solution containing placebo patches [11].

Folding endurance test

Folding endurance test was conducted by folding the patch at the same point n number of times till the patch is broken. This test is conducted to know the efficiency of plasticizer and the strength of the prepared patch using various ratios of polymers. This test is carried out thrice for reproducibility [10, 12, and 13].

Table 1: Composition of Transdermal patches using LX

Formulation	L1	L2	L3	L4
Lornoxicam (mg)	8	8	8	8
HPMC (mg)	300	-	150	150
Ethyl Cellulose(mg)	-	300	150	150
Eugenol (ml)	0.25	-	0.25	-
Cineole (ml)	-	0.25	-	0.25
PEG 400	Q.S	Q.S	Q.S	Q.S
Solvent	Q.S	Q.S	Q.S	Q.S

Moisture Loss

Accurately weighed films of each formulation were kept in desiccator and exposed to an atmosphere of 98% relative humidity (anhydrous Calcium carbonate) at room temperature and weighed after 3 days. An average of three readings was recorded and the percentage moisture uptake was calculated using [15].

$$\% \text{Moisture Uptake} = \frac{(\text{Final Weight} - \text{Initial Weight})}{\text{Initial Weight}} \times 100$$

Tensile Strength

Tensile strength of the film was determined with Universal strength testing machine (Hounsfield, Slinfold, Horsham, U.K.). The sensitivity of the machine was 1 g. It consisted of two load cell grips. The lower one was fixed and upper one was movable. The test film of size (4 × 1 cm²) was fixed between these cell grips and force was gradually applied till the film broke [12,16]. The tensile strength of the film was taken directly from the dial reading in kg. Tensile strength is expressed as follows:

$$\text{Tensile Strength} = \frac{(\text{Tensile Load at break})}{(\text{Cross sectional area})} \times 100$$

Water Vapour Transmission Rate

Glass vials of 5 ml capacity were washed thoroughly and dried to a constant weight in an oven. About 1 g of fused calcium chloride was taken in the vials and the polymer films of 2 cm² were fixed over the brim with the help of an adhesive tape. Then the vials were weighed and stored in a humidity chamber of 80-90 % RH condition for a period of 24 h [17,18]. The vials were removed and weighed at 24 h time intervals to note down the weight gain.

$$\text{WVT} = \frac{W}{L \times S}$$

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

In Vitro Drug Release Studies

The *in vitro* release studies were performed using Franz diffusion apparatus, the synthetic cellophane membrane was placed between the donor and receptor compartment [17]. The receptor compartment was filled with 100 ml phosphate buffer pH 7.4; the temperature of the system was maintained at 37 ± 1°C. The assembly was set on a magnetic stirrer and continuous, constant stirring was maintained in the receptor compartment using a magnetic bead at 50 rpm. Aliquots of 2 ml were taken at regular interval of time for a period of 24 hours using a syringe and replaced immediately with

Moisture uptake

Accurately weighed patches of each formulation were kept at room temperature and at a relative humidity of 75% (a saturated solution of sodium chloride) in desiccator.

The water absorption capacity of patches was determined after 24 h by periodical weighing until a constant weight was obtained. An average of three readings was recorded and the percentage moisture uptake was calculated using [14].

$$\% \text{Moisture Uptake} = \frac{(\text{Final Weight} - \text{Initial Weight})}{\text{Initial Weight}} \times 100$$

fresh buffer. The test samples were analyzed using UV spectrophotometer at 376 nm against blank.

Skin Permeation studies

Ethical clearance for handling of experimental animals was obtained from the institutional animal ethical committee (IAEC) formed for this purpose. The approval number was 1358/ac/10/CPCSEA. Albino rat skin was carefully excised. After removing the hypodermal adipose tissue the skin was used as a barrier membrane for the studies. The best formulation from *in vitro* studies was selected for this study, the rat skin was used as membrane between donor and receptor compartment. The receptor compartment was filled with 100ml buffer and was stirred using a magnetic stirrer at 37 ± 1°C. The samples were analyzed using UV spectrophotometer at 376 nm against a blank.

In vivo drug release studies

Four male rabbits of 10-12 weeks old weighing 2-3 kg were selected. They were kept with husk bedding and were fed with standard rodent pellet diet and water [19]. Light and dark cycle with 12 hrs light and 12 hrs dark was maintained. The temperature and 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. 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 drug content subtracted from the initial content [20] in the film by UV spectrophotometer at 376nm.

Pharmacodynamics Study

Carrageenan induced edema model

The anti-inflammatory activity [21] was performed for the best formulation by Carrageenan induced hind paw edema method in rats. Six male albino rats were selected weighing approximately 200-250g. One day before the experiment the left hind thigh of each animal was shaved without damaging the skin. The patch samples were applied on the shaved area. The first group (control) received orally 0.5 ml of normal saline solution, the second group (standard) received diclofenac sodium patches, and the third group received best 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 plethysmometer.

$$\% \text{Edema Inhibition} = (1 - V_t \div V_c) \times 100$$

Where, V_t - Mean edema volume of test; V_c - Mean edema volume of control

Skin Irritation Studies [22]

In the present study 10 rats (approved by Institutional Animal Ethical Committee,) of either sex weighing between (400-500g) was used. Animals were divided in to 2 groups of 5 animals each. Hairs were depleted from the back of rats with the help of clippers and area 4 cm² was marked on both the sides. One side served as control while the other as test and animals were used after 24 hrs. After hair depletion, Patch was applied once a day for 7 days and observed for any sensitivity and the reaction if any was graded as.

Score	Erythema scale
A	No reaction
B	Slight, patchy erythema
C	Slight but confluent or moderate but patchy erythema
D	Moderate erythema
E	Severe erythema with or without edema

RESULTS & DISCUSSION

Transdermal drug delivery system containing Lornoxicam was prepared by using different polymeric ratios of HPMC, EC in

combination and individual, and also effect of permeation enhancers was observed and found that eugenol showed better results compared to cineole.

Physicochemical parameters

Compatibility between drug and polymer was determined by FTIR studies, pure drug and mixture of polymers were sent for studies and the results proved that there were no interactions between drug and polymer.

The prepared patches were evaluated for physico chemical parameters shown in table 2&3, from the results it is clearly evident that the prepared patches were transparent, uniform, and flexible. The thickness of the patches varied from 0.29±0.03 to 0.24±0.01 mm. All the prepared patches showed the content uniformity in the range of 99.75 to 96.32, folding endurance test results indicated that the patches can maintain the integrity with general skin folding. The tensile strength of the formulations was in the range of 398.52 to 352.65 the strength obtained may be due to the nature of polymers used in the patch. The percentage moisture uptake was in the range of 6.69±0.03 to 4.21±0.01 this may be due to hydrophilic and hydrophobic nature of the polymer. The moisture loss was in the range 7.32±0.05 to 4.25±0.03. Formulation with HPMC has shown highest water vapour transmission this may be due to hydrophilic nature of the polymer 8.52x10⁻⁶.

Table 2: Physico chemical evaluation of prepared patches

F. Code	Thickness (mm)± SD	Drug Content (%)	Folding Endurance ± SD*	Tensile Strength Kgf/cm ²
L1	0.29±0.03	97.45	274±1.2	352.65
L2	0.24±0.01	96.32	265±0.5	374.25
L3	0.26±0.02	99.75	289±0.3	398.52
L4	0.28±0.04	98.64	281±0.8	387.54

*Average of three determinants for each parameter

Table 3: Physico chemical evaluation of prepared patches

F. Code	Moisture Uptake± SD*	Moisture Loss± SD*	Water Vapour Transmission rate (mg/cm ² /hr)
L1	6.69±0.03	7.32±0.05	8.52x10 ⁻⁶
L2	4.21±0.01	5.26±0.04	4.65x10 ⁻⁵
L3	5.21±0.02	4.25±0.03	5.23x10 ⁻⁶
L4	5.34±0.03	4.54±0.04	5.59x10 ⁻⁵

*Average of three determinants for each parameter

Table 4: Drug release parameters of prepared formulations

S. No.	Drug Release Studies	Drug Release (%) at 12 th hour	Drug Release (%) at 24 th hour
<i>In vitro</i> Permeation Studies			
1	L1	98.12	--
2	L2	41.12	79.25
3	L3	52.36	98.76
4	L4	46.25	82.15
Skin Permeation Studies			
5	L3	45.26	94.28
In vivo Drug release			
6	L3	38.12	90.52

Release studies

The *in vitro* release studies were carried out in a cellophane membrane to select the appropriate formulation, release profiles of the patches are given in table 4 Fig. 1, the results depicted that the patch with hydrophilic polymers released the 90% of the drug with in 12 hrs. The patch formulated with HPMC and EC as polymers and eugenol (L3) as the permeation enhancer has shown highest release (98.76%) compared to that of cineole (L4) as the permeation

enhancer. The Higuchi's plot has shown regression value of 0.983, which indicates that diffusion mechanism is influencing for the drug release. The formulation L3 was selected for the skin permeation studies using rat skin as the biological membrane at 24th hour the release rate was 94.28% the results are listed in table 4. The *In vivo* release study revealed that the release pattern of the formulation L3 was reproducible in the biological environment; the drug release pattern is shown in Table 4 and Figure 1. At the end of the 24th hour the drug release was 90.52%.

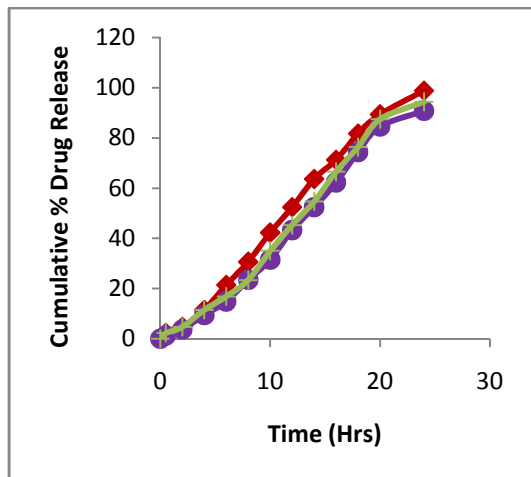


Fig. 1: In vitro, skin permeation and in vivo drug release profile of formulation L3

Anti inflammatory and Skin irritation studies

Percentage increase in paw volume (inflammation) and percentage inhibition of inflammation in groups treated with test and marketed

products and the results are given in the Tables 5, 6 Fig. 2. In control group which received carrageenan alone, a rapid and continuous increase in paw volume (i.e. inflammation) was observed and the inflammation was sustained during the entire period of 6 hours of study. In the groups which received test products, the percentage increase in paw volume was low when compared to the control group, indicating that the test and marketed product possess good anti-inflammatory activity.

The inflammation due to carrageenan was markedly inhibited by the test and marketed products. The percentage inhibition was much higher in the initial time points. A comparison of the percentage inhibition of inflammation (i.e., anti-inflammatory activity) of test and marketed products is made in Table 6. Comparable anti-inflammatory activity was found with the test product with that of marketed product these results indicated the rapid onset of action and higher activity during the initial periods due to their enhanced dissolution and absorption rates. The anti inflammatory activity of the test sample was because of the drug as well as may be because of eugenol which is having analgesic activity.

Following seven days application of the patch, the results of skin irritation test were tabulated in Table 7. The results indicated that the control preparation (which did not contain any drug), test (L4) and marketed product did not cause any skin reaction. It can be assured that lornoxicam and the excipients did not cause any skin irritation and can be used in the formulation and are safe for skin application.

Table 5: Mean Paw edema volume of the albino rats

Time(hrs)	Control	Test	Standard
0	0.224	0.204	0.224
1	0.258	0.163	0.192
2	0.279	0.103	0.112
3	0.332	0.097	0.106
4	0.364	0.096	0.099
5	0.392	0.099	0.103
6	0.428	0.105	0.109

Table 6: % Inhibition of the edema in albino rats

Time(hrs)	Test	Standard
0	21.38	24.36
1	25.58	37.59
2	59.85	63.08
3	68.07	70.72
4	72.66	73.41
5	73.72	74.54
6	74.53	75.56

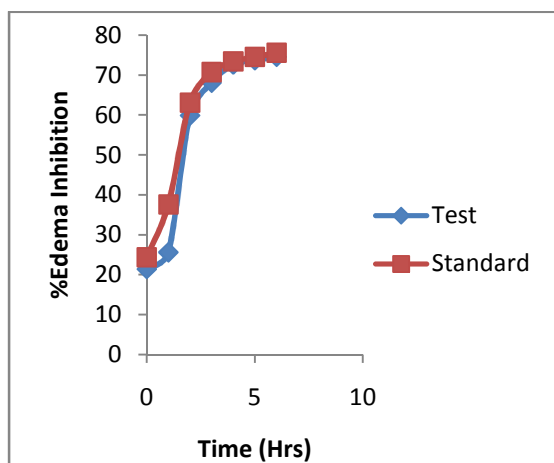


Fig. 2: Comparison of % edema inhibition of formulation L3 with standard

Table 7: Skin irritation studies of formulation L3 and Standard

S. No.	Treatm ent	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	Control	A	A	A	A	A	A	A
2	Test	A	A	A	A	A	A	A
3	Standar d	A	A	A	A	A	A	A

A - No reaction, B - Slight, patchy erythema, C - Slight but confluent or moderate but patchy erythema, D - Moderate erythema, E - Severe erythema with or without edema.

CONCLUSION

Lornoxicam is NSAID of oxicam class used as anti inflammatory, analgesic and anti pyretic also used for arthritis. To overcome side effects of oral therapy and to have benefits of topical therapy transdermal patches of lornoxicam were prepared. From the results it can be concluded that the combination of HPMC & EC using eugenol (L3) as the permeation enhancer showed best result among all the formulation. There was good correlation between in vitro and

in vivo which reveals the ability of formulation to release the drug through all biological membranes. Formulation L3 has shown comparable anti inflammatory activity as that of standard drug there was no skin irritation found. In view of all the above results reported in the present study, it can be proposed that lornoxicam is suitable candidate as transdermal drug delivery system.

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