

IN-VITRO SKIN PERMEATION AND BIOLOGICAL EVALUATION OF LORNOXICAM MONOLITHIC TRANSDERMAL PATCHES

MOHAMED A EI NABARAWI¹, DALIA S SHAKER^{2*}, DALIA A ATTIA³, SHEREEN A HAMED²

¹Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt, ²Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Helwan University, Cairo, Egypt, ³Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt. Email: dalias_shaker@hotmail.com

Received: 11 Jan 2013, Revised and Accepted: 29 Feb 2013

ABSTRACT

Objective: Transdermal patch is a promising approach that allows continuous input of drugs with short biological half-lives. The present study was designed to evaluate the short $t_{1/2}$ - lornoxicam (LX) transdermal patches through in-vitro skin permeation, skin irritation and biological evaluation on rat induced paw edema.

Methods: LX patches were prepared using different polymer blends and plasticizers. The effect of Span80 and Transcutol® as permeation enhancers in absence and presence of oleic acid (OA), isopropyl myristate (IPM), triacetone and castor oil, on transdermal permeation through rat skin, was investigated. The safety of LX patches was evaluated through skin irritation study. The biological evaluation regarding the anti-inflammatory effect of LX patches on rat paw edema was tested.

Results: The following were the principal findings of this research. First, there was very good correlation between LX flux and the presence of IPM, Oleic as well as propylene glycol compared to other oils and triacetone. Second, span80 had significantly improved LX permeation from Eudragit blends (E100). while combining transcutol- castor oil showed no remarkable increase in drug flux. Third, the primary irritancy index (PII) proved the non-irritancy of the drug or any of the film components and showed that the innovated films are safe to be applied to skin for the intended period of time. Finally, LX patches had significantly inhibited the carrageenan induced rat paw edema compared to oral treatment.

Conclusion: This study has supplied us with brightening results concerning the questionable equipotent therapeutic efficacy of transdermal versus oral LX and not irritant to skin.

Keywords: Lornoxicam, Transdermal patches, Inhibition of edema, Irritation test

INTRODUCTION

Transdermal drug delivery systems have been used as safe and effective drug delivery devices since 1981. A lot of progress has been done in the field of transdermal patches. Due to large advantages of the transdermal drug delivery system, this system interests a lot of researchers. Many new researches investigate incorporating newer drugs via this system [1,2]. Lornoxicam (LX) is widely used for the symptomatic treatment of pain and inflammation in patients with rheumatoid arthritis and osteoarthritis. Moreover, it showed great efficacy in various clinical trials in management of pre-operative and post-operative pain associated with gynecological, orthopedic, abdominal, and dental surgeries [3]. LX has spinal nociceptive inhibitor properties in addition to cox-1 and cox-2 inhibition, animal models have shown an analgesic effect 12 times higher than tenoxicam and 3 times higher than piroxicam. LX has also been shown to inhibit the cyclo-oxygenase enzyme 100 times more than tenoxicam and 40 times more than piroxicam [4].

However, LX usefulness is limited due to its short half-life that ranges from 3 to 5 h resulted in repetitive administration [5,6]. Added to that, LX shows a distinct pH-dependent solubility characterized by very poor solubility in acidic conditions present in the stomach [6]. Thus it remains in contact with the stomach wall for a long period which might lead to irritation and ulceration. On the other hand, the transdermal route provides sustained and controlled delivery. It also allows continuous input of drugs with short biological half-lives and can eliminate pulsed entry into systemic circulation, which often causes undesirable side effects.

The main objective of the current research is to develop LX monolithic transdermal patches in order to provide extended drug release over 24 h. This approach aims to avoid LX repetitive administration and avoid all adverse effects associated with oral drug intake as well. Additionally, the irritation that may occur at the site of application of prepared films was studied in order to improve patient compliance. The pharmacodynamics activity of the selected monolithic matrix films in terms of anti-inflammatory effect was

evaluated and compared to oral route. The study revealed that transdermal patches of LX can be suggested to be used especially for the treatment of inflamed area since it displays immediate anti-inflammatory effect compared to oral administration.

MATERIALS AND METHODS

Materials

Lornoxicam was kindly donated by DELTA Pharmaceuticals Company, Cairo, Egypt. Ethyl cellulose, Eudragits (RS100, RSPM, E100) were obtained from Fluka (Switzerland), polyvinyl pyrrolidone (PVP K-25) from Sigma (USA), sodium hydrogen phosphate, potassium dihydrogen phosphate, oleic acid, methanol and chloroform of analytical grade were purchased from Adwic Co. (Egypt). Isopropyl myristate, PEG 400, PG, DBP were obtained from Fluka chemicals, USA. Carrageenan, Triacetone and Span 80 were obtained from Sigma (USA). Transcutol was kindly supplied by Gatte Fossé (Saint Priest, France)

Preparation of transdermal cellulosic and Eudragit films:

Ethyl cellulose was mixed with Eudragit E100 in different ratios (1:1, 1:2, 2:1) also ethyl cellulose was mixed with PVP in different ratios (1:1.6 and 1:2). Eudragit RS100 or RSPM was mixed with PVP in different ratios (1:1.6 and 1:2). These ratios were chosen according to preliminary tests. To the polymer mixtures, blends of five plasticizers namely polyethylene glycol 400 (PEG 400), propylene glycol (PG), dibutyl phthalate (DBP), isopropyl myristate (IPM) and oleic acid (OA) were added. The above mixtures were dissolved in specified amount of chloroform by stirring with a glass rod on a water bath adjusted at 50°C for 30 min. [7,8]

Different concentrations of Lornoxicam (10%, 5%, 2.5%, and 1.25%) w/w were dissolved in specified amount of chloroform. The prepared drug solutions were warmed on a water bath till reached 50°C then added to polymer/ plasticizer mixtures and then stirred for 15 min. The solutions were cooled and poured into plastic molds. The films were left to dry for 24 hr. at room temperature (using funnel inverted method).

Only films with the highest drug concentration and showing no crystal formation were chosen to ensure saturation of the patch. After drying, the films were cut with a sharp razor into pieces of area 2×3cm² for EC/E100 blends and 3×4cm² for EC/PVP and Eudragits/PVP as well. The films were mounted to Airoplast® (air vented surgical tape) plasters as patch backing, leaving 1.5cm from each side of the film for ease of adherence to skin. Finally a protective peel off plastic strip was applied to the other surface of the film. Finally, the patches were tightly wrapped in aluminum foil and kept at 25° C till further use.

Drug content uniformity

The uniformity of drug distribution was evaluated by determining drug content at different places of the film by spectrophotometric analysis. An area of 1 cm² of the medicated film was dissolved in methanol and the volume was adjusted to 25 ml using volumetric flask and then filtered using 0.45µ Millipore filter, then LX concentration was spectrophotometrically measured at 376 nm. All measurements represented the mean of 6 assays of drug content

In-vitro skin permeation

Preparation of rat skin

Hairs from the abdominal side of male albino rats weighing (150-200 gm) were removed by a commercially available hair remover (Veet®) [9]. The animals were sacrificed. The animal experiments were conducted in full compliance with local, national, ethical, and regulatory principles and local licensing regulations per the spirit of AAALAC International's expectations for animal care and approved by the National Research Institute animal care committee in Egypt. The full thickness skin of the abdomen was surgically removed and the adhering subcutaneous fat was carefully cleaned. Any trace of fat adhering was finally removed by wiping with cotton soaked in isopropyl alcohol [10]. Finally skin was rinsed with distilled water then with saline. Skin was stored at -20 °C soaked in saline and tightly covered with aluminum foil till further use within not more than a week from storage to avoid skin damage or any variation in pore size.

Transdermal permeation studies using Franz - diffusion cell

The excised rat skin was used as the membrane in vertical Franz diffusion cell with a permeation area of 1 cm² and receptor volume of 7 ml. The skin was placed with the stratum corneum facing the donor compartment. The receptor compartment was filled with PBS. The films (area of 1cm² contains 0.63 mg of lornoxicam for blends of EC/E100 and 0.315 mg for blends of EC/PVP and Eudragits/PVP) were tightly adhered to skin then covered with Airoplast® surgical tape as a backing layer. The skin and patch were firmly fastened with a rigid clamp.

The solution in the receptor side was stirred with Teflon-coated magnetic stirrer bars at 1000 rpm for 2 minutes to remove any air bubbles then adjusted at 600 rpm for the rest of the experiment period. During the experiments the receptor phase was maintained at 37° C ± 0.5. Aliquots of 400 µl were collected and immediately replaced with PBS [11].

Sampling was done at designated time intervals for 24h. The drug concentration was determined spectrophotometrically at λ_{\max} 375 nm. Cumulative amounts of permeant were plotted against time. The effect of different plasticizers combinations on LX transdermal permeation from Eudragit films was also investigated; Transcutol/oleic, IPM, Triacetin and castor oil as well as the effect of Span 80.

Data analysis

According to Fick's second law of diffusion. The diffusion flux (J) (µg/cm²/h) of LX was calculated from the slope of the linear portion of the plot of the cumulative amount of lornoxicam permeated per cm² of skin at steady state against the time using linear regression analysis [11].

$$J = C_0KD/L = C_0Kp \quad \text{Eq.1.}$$

Where C₀ is the initial drug concentration in the vehicle, D is the diffusion coefficient corresponding to the diffusivity of the drug in

the membrane, L is the thickness of the membrane and K is the partition coefficient of drug between membrane and vehicle, K_p is the permeability coefficient.

In-vivo Biological evaluation of lornoxicam transdermal patch:

Animals

Male albino rats weighting 200 ± 10 gm. were housed in polypropylene cages (4 per cage). The animals got free access to standard diet and water. They were kept at 25±1°C and 45-55% relative humidity with a 12 hr. light /dark cycle.

Skin irritation test

The irritancy of the selected films was evaluated in terms of biological investigation, on male albino rats (200 -210 gm.) based upon the method described by Draize et al. [12]. The rats were anesthetized with thiopental (60mg/kg) injection (i.p) then the dorsal side of the rat was shaved with clippers 24 h before the beginning of the experiment. The animals were divided into 4 groups each group consists of 4 rats: Group A served as control, Group B received 0.5ml of a 0.8% V/V aqueous formalin solution as a standard irritant [13]. Group C and D received medicated films for 3 day (A new patch was applied daily). The application sites were examined for edema and erythema after 24 and 72 hr., then graded (0- 4) according to a visual scoring scale always by the same investigator; the final score represents the average of the 24 and 72 h readings. the erythema scale was as [12] : 0, none ; 1,slight; 2, well defined; 3, moderate; and 4, scare formation. The edema scale was: 0, none; 1, slight; 2, well defined; 3 moderate; and 4, severe. The primary irritancy index (PII) was determined for each preparation by adding the edema and erythema scores, the formulations were accordingly classified as non-irritant if PII < 2, irritant if (PII =2-5) and highly irritant if PII=5-8. The data were analyzed statistically by the one-way ANOVA test followed by Duncan multiple comparison test at p<0.05.

Evaluation of the pharmacodynamics activity

The pharmacodynamics activity of the selected monolithic Matrix films in terms of anti-inflammatory effect was evaluated by measuring the change in paw volume with a plethysmometer (Ugo Basile, Italy) using carrageenan induced inflammation edema model according to the method explained by Swingle et al. [14]. The rats were divided into 4 groups, in each group the left hind paw of each rat were marked, just beyond tibiotarsal junction, so that every time the paw is dipped up to the fixed mark to ensure constant paw volume.

Group A served as control group while group B is the oral group (received 0.63 mg lornoxicam dissolved in 5 ml PBS (pH 7.4) by means of oral gavage. Group C and D received medicated films of the selected transdermal formulations after shaving the dorsal side of rats with clippers without damaging the skin. The patch samples were applied to the shaved area. The film was covered with a backing membrane of Airoplast®. A silicon adhesive was used on the edges of the backing membrane to insure tight attachment to the skin. Finally, the patches were covered with (Silk plast® REF.sk-019) adhesive tape 5×5cm. The size of the applied patch was 1cm×1cm for (EC: E100 (1:1) +20%IPM) and 1cm×2cm for (EC: PVP (1:1.6) + 10%oleic). The tested patches were applied 2 hr. prior to the carrageenan injection [15,16]. Acute inflammation was produced by injecting 0.1 ml of 1% w/v carrageenan solution in the sub plantar region of the left hind paw. The paw volume was determined after 0, 1, 2, 4, 6, 8, and 20 hr. The percentage paw swelling was calculated by the following formula [17].

$$\% \text{ paw swelling} = \frac{(b - a) \times 100}{a} \quad \text{Eq.2.}$$

Where, a is the paw volume before induction of edema, b is the paw volume measured hourly after induction of edema. The average % swelling was plotted against time. The AUC (0 - 20h),

E_{max} (maximum anti-inflammatory effect) and T_{max} were calculated [18].The data were analyzed statistically by the one-way ANOVA test followed by Duncan multiple comparison test at p<0.05.

RESULTS AND DISCUSSION

Preparation of transdermal films

Trials revealed that at LX concentration higher than 2.5% w/w and 1.25 w/w % for EC/ E100 and PVP films, respectively, lornoxicam crystals began to appear in the matrix. In other words, the drug reached saturation in the selected matrices.

Drug content uniformity

Measurements showed that the drug content was uniform throughout the patch. It was 0.63 ± 0.2 mg/cm² and 0.315 ± 0.12 mg/cm² in Eudragit and PVP films, respectively. Therefore, the area cut of EC/PVP and Eudragits/ PVP films was twice that of EC/ E100 films to ensure drug content uniformity. The uniformity of film thickness was ensured using a micrometer at three different places (data not shown).

In-vitro skin permeation:

LX transdermal permeation profiles were plotted and the drug flux (J) was calculated (Table 1). The highest flux resulted from Permeation profile of lornoxicam from blends of 1:1 EC/ E100 plasticized with 20% IPM (43.124 ± 3.9 μg/cm²/ hr.) (Fig.1). IPM is an aliphatic ester, which may penetrate between the lipid bilayers of stratum corneum and due to its chain structure, disrupts the order and arrangement of lipid bilayers of stratum corneum and hence improves drug permeation into this layer, this happens because of the lower values of the Hildebrand solubility parameter (δ) of isopropyl myristate (δ=8.02), which is near to that of the human skin (δ=10.5), the enhancement effect of IPM can also be manifested by virtue of its intermediate polar nature, IPM being partitioned into

both the lipid and polar phase of the skin [19,20]. Substituting 20% IPM with the same ratio of PG showed a decrease in LX flux ($J = 39.45 \pm 0.74$ μg/cm²/hr.) (Fig.1). PG may act as penetration enhancer by solvent drag mechanism rather than lipid fluidization; PG may carry the permeant into the tissues [21,22].

Doubling the E100 content in EC/Eudragit films hindered transdermal permeation of LX (Fig.1). The authors attributed the decrease in flux to steric hindrance of drug release. On the other hand, substituting 20% PG with same ratio of Oleic acid decreased LX flux (Table 1, Fig. 2). The presence of double bonds in the structure of oleic acid has been proposed to cause the formation of kinks in the lipid matrix to allow water permeation across the skin [23, 24].

Increasing oleic acid concentration from 10 % to 20 % enhanced LX flux from patches composed of EC/ E100 as well as EC / PVP in a ratio 1: 1.6 (Table 1) demonstrating that the mechanism by which fatty acids increase skin permeability appeared to involve disruption of the densely packed lipids that fill the extracellular spaces of the stratum corneum. Despite the fact that the authors found that 20 % OA softened the PVP patches and consequently this concentration was excluded, OA was found to be the best skin permeation enhancer with PVP (Fig. 3).

Increasing of PVP ratio resulted in relatively higher permeation (Fig. 4), the improvement of skin flux with increased PVP ratio may be attributed to its anti-nucleating effect that converted the crystalline drug into amorphous state which generally possesses a high-energy state with improved solubility. The enhancement of solubility of drug increases thermodynamic activity that facilitates the permeation rate of drug through the skin [25].

Table 1: The composition of transdermal patches and their permeation parameters through rat skin

Composition	J (μg/cm ² /h)	K _p cm ² /hr × 10 ⁻³	Q ₂₄ μg/cm ²	R ²
EC:E100(1:1) 20%PG	39.45±0.74	62.3±1.2	621±7	0.9886
EC:E100(1:2) 20%PG	27.9±2.3	45±4	511±37.6	0.9977
EC:E100(1:1) 20% oleic	17.8±11.62	18.4±2.3	437±14.73	0.9928
EC:E100(1:2) 20% oleic	17.97±0.463	26±5	433±13.5	0.9991
EC:E100(1:1) 20% IPM	43.124±3.9	68.9 ±6.6	632±5.43	0.9983
EC:E100(1:2) 20% IPM	29.83±2.6	47.6±4.4	585±22.7	0.9985
EC:PVP(1:1.6) 10% oleic	21.7±0.35	71±7	287.5±10.6	0.997
EC:PVP(1:1.6) 10% PEG	8.816±2.7	27.9±8.2	100±12.5	0.996
RS100:PVP(1:2) 10%oleic	8.461±2.07	26.9±5	240±10.89	0.978
RS100:PVP(1:1.6) 10%IPM	3.73±0.47	12±1.4	110.8±13.1	0.9802
RS100:PVP(1:2) 10%IPM	6.41±1.1	21.5±2	197.5±18.17	0.9992
RSPM:PVP(1:1.6) 10%oleic	4.4 ±2.5	16 ±8	106.7±11.71	0.9965
RSPMPVP(1:2) 10%oleic	10.83±0.057	46±1.1	285±7.1	0.9966
RPM:PVP(1:1.6) 10%IPM	6.96±7.2	23±3	132.7±2.52	0.9971
RSPM:PVP(1:2) 10%IPM	10.37±0.45	23±1.6	254.3±16.6	0.9967
RSPM:PVP(1:2) span80	14.5 ±0.25	46 ± 7	210.5±4.9	0.9965
RPM:PVP(1:2) 10% Transcutol	7.4 ±2.75	20.5 ± 7	116.5±9.2	0.9959
RSPM:PVP(1:2) 5%Transcutol+ 5%castor oil	4 ± 1.23	18 ± 4	122.7±24.1	0.9987
RS100:PVP(1:2) 5%Transcutol + 5% castor oil	8.6 ± 1.38	22 ± 3	165.5±24.8	0.9992
RSPM:PVP(1:2) 5% Transcutol + 5%oleic	9.54±5.4	30.5±1.7	182±52.9	0.9984
RSPM:PVP(1:2) 5%Transcutol+5%Triacetin	2.99± 0.22	13 ±7	115.5±6.4	0.9976
RSPM:PVP(1:1.6) 5%Transcutol+ 10%IPM	4±2.5	16±8	106.7±11.7	0.9943
RSPM:PVP(1:2) 5%oleic+ 5%IPM	6.01±3.5	22±1.1	185.3±60.3	0.9982

Effect of using other plasticizers and plasticizers combinations

The effect of Span80 and Transcutol® as permeation enhancers in absence and presence of OA, IPM, triacetin and castor oil, on transdermal permeation through rat skin, was investigated. The high flux was only related to span 80 (Fig. 5), a non-ionic surfactant with HLB 4.3 reported to be potent penetration enhancer [26]. The penetration enhancement of non-ionic surfactants is likely to be a combination of the molecule's ability to penetrate into the lipid region and fluidize the lipid bilayers. The results of this study showed that the

more lipophilic the surfactant the higher the penetration rate. The 10% Transcutol® (diethylene glycol mono ethyl ether) showed lower enhancement effect than 10 % span 80 (Table 1). The results were in agreement with literature related to the mechanism of action of Transcutol® on skin. It is known to act by swelling of stratum corneum intercellular lipids without alteration of their multiple bilayer structure [27].

The increase in fluxes by changing in Eudragit type from RSPM to RS100 (Fig. 5) was related to polymers structure. Both polymers

have nearly the same structure but the crystalline nature of RS100 and the presence of 5% Talc in RSPM amorphous powder seemed to affect the drug permeation.

The addition of OA to Transcutol® produced slight increase in skin permeation ($J = 9.54 \pm 5.4 \mu\text{g}/\text{cm}^2/\text{hr.}$) compared to 10 % Transcutol® ($J = 7.4 \pm 2.75 \mu\text{g}/\text{cm}^2/\text{hr.}$) (Fig. 6). In contrast, the addition of 5 % triacetine to 5 % Transcutol® decreased the permeation of LX ($J = 2.99 \pm 0.22 \mu\text{g}/\text{cm}^2/\text{hr.}$) while combining 10 % IPM with Transcutol® produced no synergistic effect on the

permeation of lornoxicam ($J = 4 \pm 2.5 \mu\text{g}/\text{cm}^2/\text{hr.}$) (Table 1). On the other hand, Karande and Mitragotri [28]. reported that the skin permeability of clobopride from a binary mixture of Transcutol® and IPM was 80- fold higher as compared to that of IPM alone.

The results in this study revealed that the addition of 5% oleic acid to 5% IPM decreased the permeation of the drug ($J = 6.01 \pm 3.5 \mu\text{g}/\text{cm}^2/\text{hr.}$) than that produced from using each plasticizer alone. In conclusion, IPM , PG and Span80 appeared to be the enhancers of choice in LX transdermal delivery systems.

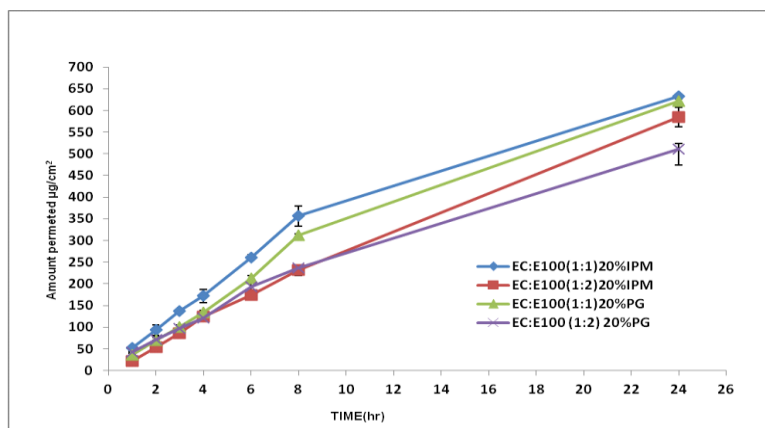


Fig. 1: Permeation profile of lornoxicam from blends of EC with E100 plasticized with 20% IPM and 20 % PG

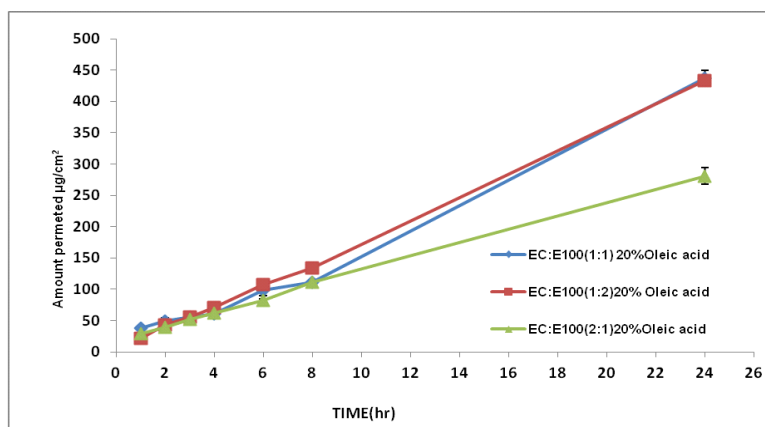


Fig. 2: Permeation profile of lornoxicam from bends of EC with E100 plasticized with 20% oleic.

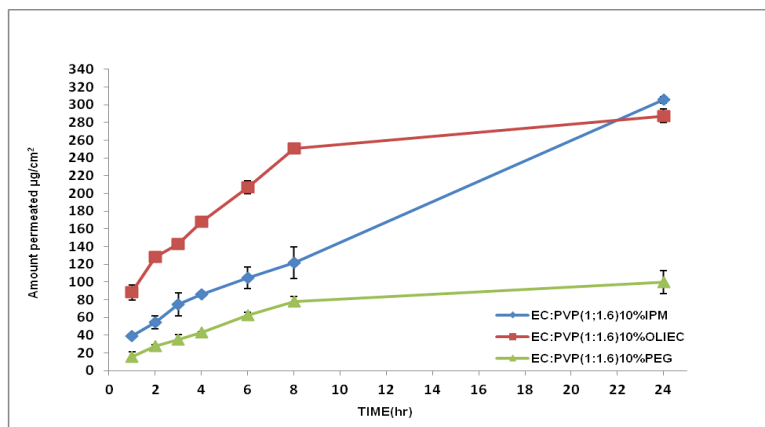


Fig. 3: Permeation profile of lornoxicam from blends of EC with PVP plasticized with 10% oleic, 10% IPM and 10%PEG

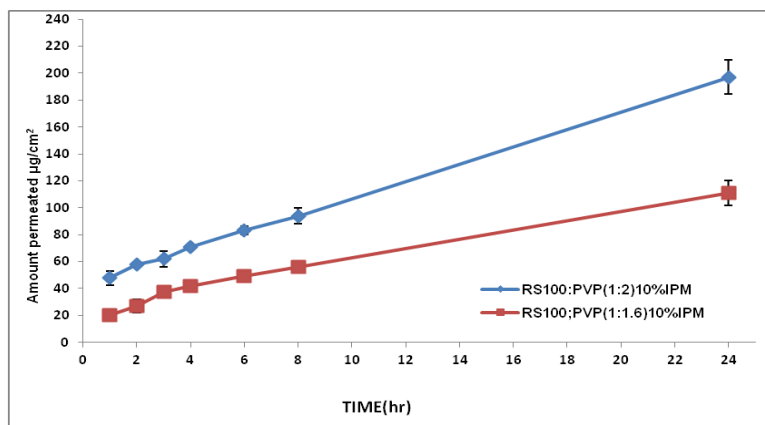


Fig. 4: Permeation profile of lornoxicam from bends of RS100 with PVP plasticized with 10% IPM.

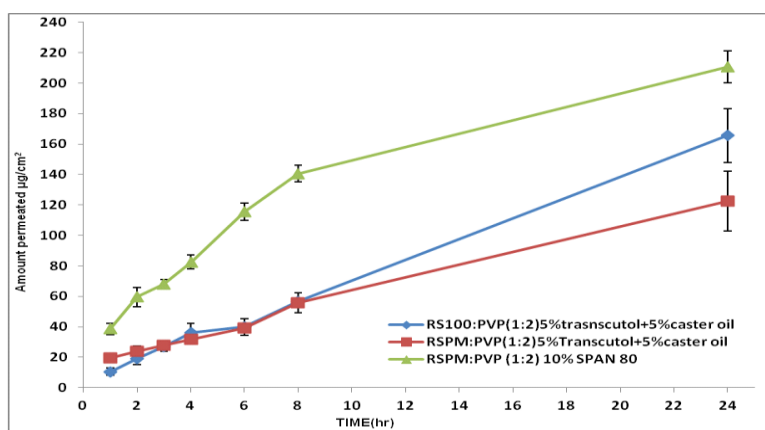


Fig. 5: Permeation profile of lornoxicam from bends of RSPM or RS 100 with PVP plasticized with 10% Span 80, 5% Transcutol and 5% castor oil.

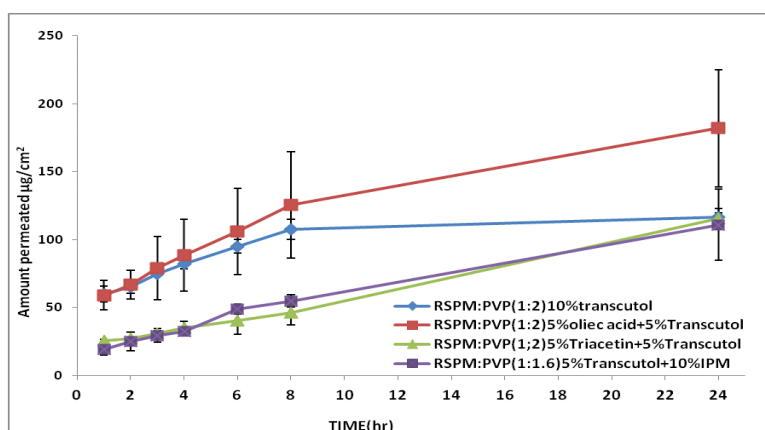


Fig. 6: Permeation profile of lornoxicam from bends of RSPM with PVP plasticized with Transcutol combined with other plasticizers.

In-vivo Biological evaluation of lornoxicam transdermal patch

Skin irritation test: According to Draize et al. [12], all the tested transdermal films were considered to be negative (non-irritant) [PII < 2]. Statistical analysis using the one-way ANOVA followed by Duncan multiple comparison test at $p < 0.05$ showed that, compared to the control, the formalin solution was found to be significantly irritant [PII = 6.75 ± 1.32] ($p < 0.05$) while the selected transdermal preparations were non-irritant ($p > 0.05$); and the PII was (0.25 ± 0.5) and (0.5 ± 0.58) for EC:E100 (1:1) 20% IPM and EC:PVP (1:1.6) 10% oleic transdermal patches, respectively. The irritation indices proved the non-irritancy of the drug or any of the film components and showed that the innovated films are safe to be applied to the skin for the intended period of time.

Evaluation of the pharmacodynamics activity

The paw of mice and rats are very sensitive to carrageenan when it's injected in the sub plantar region of the left hind paw it causes swelling – redness and edema of the paw. LX transdermal patches have been proved to decrease the swelling of the injected paw according to equation 2. The pharmacological efficacy was expressed in terms of the decrease in the percentage swelling according to Figure (7) and the area under the percentage swelling versus time curve (AUC_{0-20h}) was calculated and listed in Table 2 also the maximum anti-inflammatory effect (E_{max}) and the time taken to reach that maximum effect was (T_{max}) were calculated and listed in Table 3.

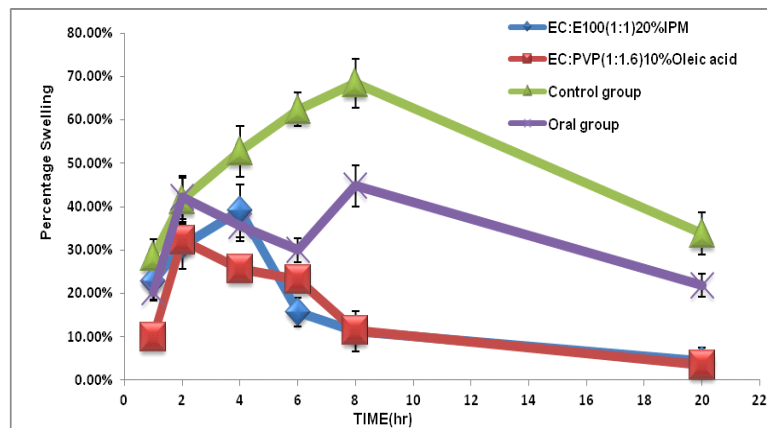


Fig. 7: Anti-inflammatory effect of lornoxicam transdermal patches in rat paw edema model induced by carrageenan.

Table 2: Anti-inflammatory effect of Lornoxicam in male albino rats expressed in terms of AUC

RAT	AUC _(0-20h)			
	Group A	Group B	Transdermal Group C	Transdermal Group D
1	9.17%	6.11%	2.10%	2.31%
2	9.81%	6.69%	3.47%	2.63%
3	10.29%	6.66%	2.80%	2.52%
4	10.29%	6.49%	2.54%	2.66%
Mean	9.89%	6.49%	2.73%	2.53%
SD	0.53	0.27	0.573	0.159

Table 3: Maximum anti-inflammatory effect (less percentage swelling volume) in male albino rats using the carrageenan induced paw edema model.

RAT	E-MAX			
	Group A	Group B	Transdermal Group C	Transdermal Group D
1	0.60%	0.29%	0.07%	0.12%
2	0.70%	0.33%	0.18%	0.12%
3	0.72%	0.28%	0.10%	0.10%
4	0.72%	0.3%	0.10%	0.12%
Mean	0.68%	0.30%	0.11%	0.12%
SD	0.057	0.022	0.047	0.01

From the examination of % swelling curve Figure (7) we noticed that the control group showed a continues increase in paw swelling edema all over the experiment time while both the oral and transdermal groups showed percent swelling lower than that of the control group. For oral group there was a decrease in the percentage swelling started nearly around 4 h then increased again after 6 h.

While for transdermal patches (groups C and D) showed a reasonably gradual decrease in the percentage swelling till the end of the experiment. The paw volume nearly returned to normal.

Statistical analysis using the one-way ANOVA indicated that there was a significant difference between groups. While Duncan multiple comparison test at ($p < 0.05$) revealed that there was a significant difference between each two groups at each time interval through the whole experiment time intervals. The anti-inflammatory effect expressed in terms of AUC_(0-20h) manifested very promising results, Table 2. The AUC of all transdermal groups was far lower than the oral formula and control group. The statistical analysis of the data of AUC_(0-20h) using one-way ANOVA test followed by Duncan Multiple comparison test at $P < 0.05$ showed a significant difference ($p < 0.05$) between control group and both transdermal and oral groups. Also there was a significant difference ($p < 0.05$) between oral and transdermal groups. In - between the transdermal groups there was no significant difference $p > 0.05$. Table 3 illustrated the E_{max} (maximum anti-inflammatory effect) which was tabulated according to T_{max}.

Statistical analysis of the data showed a significant difference ($p < 0.05$) between the control group and all other groups regarding E_{max}. Moreover, there was a significant difference ($p < 0.05$) between group B (oral group) and both group C and D (transdermal groups). in contrast, there was no significant difference ($p > 0.05$) between the transdermal groups using one way ANOVA followed by Duncan Multiple comparison test at $p < 0.05$ [29].

CONCLUSION

A transdermal patch comprising of EC: E100 (1:1) +20%IPM) or EC: PVP (1:1.6) + 10%oleic was deemed promising as a successful delivery system of Lornoxicam. This study has supplied us with brightening results concerning the questionable equipotent therapeutic efficacy of transdermal versus oral lornoxicam.

REFERENCES

1. Transdermal patches: a recent approach to new drug delivery system. Dhiman S, Singh T G , Rehni AK. Int J Pharm Pharm Sci. 2011; 3 Suppl 5:26-34.
2. Ansari K, Singhai AK, Saraogi GK. Recent advancement in transdermal drug delivery system. Int J Pharm Pharm Sci. 2011; 3 Suppl 5: 52-59.
3. Homdrum EM, Likar R, Nell G . A novel effective tool for pain treatment. Eur Surg 2006; 38,3: 42-52.
4. Tamaze SA, Inan N, Goktug A, Erdogan I, Aunag ME, Ceyhan A. The Analgesic effect of 8 and 16 mg lornoxicam administrated

- before shock wave lithotripsy: Endo urology and stones. *J Urology* 2008; 72,2: 282-5.
5. Balfour JA, Fitton A, Barradell LB. Lornoxicam. A review of its pharmacology and therapeutic potential in the management of inflammatory conditions. *Drugs* 1996; 51: 639-657.
 6. Skjodt NM, Davies NM. Clinical pharmacokinetics of lornoxicam a short half life oxicam. *Clin Pharmacokinetics* 1998; 34: 421-8.
 7. Mukherjee B, Mahapatra S, Gupta R, Patra B, Tiwari A, Arora P. A comparison between povidone - ethyl cellulose and povidone - eudragit transdermal dexamethasone matrix patches based on in vitro skin permeation. *Eur J Pharm* 2005;59: 475-483.
 8. PenzesT, Blazso G, Aigner Z, Falkay G, Erös I. Topical absorption of piroxicam from organogels in-vitro and in-vivo correlations. *Int J Pharm* 2005; 298: 47 – 54.
 9. Krishnaiah YS, Satyanarayana V, Bhaskar P. Influence of menthol an pressure-senstive adhesives on the in vivo performance of membrane-moderate transdermal therapeutic system of nicardipine hydrochloride in human volunteers. *Eur J Pharm Biopharm* 2003; 55: 329-337.
 10. Gonzalez N, Sumano H. Design of two liquid ibuprofen-poloxamer- limonene or menthol preparations for dermal administration. *Drug Delivery* 2007; 14: 287-293.
 11. Watkison A C, Brain K R. Basic Mathematical Principles in Skin Permeation in: *Dermatological and Transdermal Formulations*, Walters KA, Ed., Marcel Dekker, Inc, New York, 2002, p. 20-28.
 12. Draize J, Woodward G, Calvery H. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J Pharmacol Exp Ther* 1944; 82: 377-379.
 13. Mutalki S, Udupa N. Pharmaceutical evaluation of membrane moderated transdermal system of glipizid. *Clin Exp Pharmacol Physiol* 2006; 33: 17-27.
 14. Swingle KF, Grant TJ, Jacques LW, Kvam DC. Interaction of anti-inflammatory drugs in carrageenan-induced foot edema of the rat. *J Pharmacol Exp Ther* 1969; 172: 423 - 425.
 15. Chang AhY, Choi JK, Choi YK, Moi Ki H, Ho Bae. J. A novel transdermal patch incorporating meloxicam: In-vitro and in-vivo characterization. *Int J Pharm* 2010; 385: 12-19.
 16. Marzok MA, Kassem AA, Samy AM, Amer IR. In-vitro, thermodynamics and pharmacodynamic studies of Aceclofenac Transdermal Eudragit patches. *Drug Invention Today* 2009; 1,1: 16-22.
 17. Charoo AN, Shamsher AA, Kohli K, Pillai K, Rahman Z. Improvement in bioavailability of transdermally applied flurbiprofen using tusli (*Ocimum sanctum*) and turpentine oil. *Colloidal and Surfaces B: Biointerfaces* 2008; 65: 300-307.
 18. Bryant CC, Roberts KW, Byun J S, Evans CJ Morphine analgesic tolerance in 129P3/J and 129S6/SvEv mice. *Pharmacol Biochem Behav* 2006; 85: 769 -779.
 19. Panigrahi L, Pattnaik S, Saroj K. The Effect of pH and organic esters penetration enhancers on skin permeation kinetics of Terbutaline Sulphate from pseudolatex-type transdermal delivery systems through mouse and human cadaver skins. *AAPS Pharm Sci Tech* 2005; 6, 2: E167-E173.
 20. Mortazavi SA, Aboofazeli R. An investigation into the effect of various penetration enhancers on percutaneous absorption of piroxicam. *IJPR* 2003; 2: 135 - 140.
 21. Babu RJ, Singh M, Kanikkannan N. Fatty alcohols and Fatty Acids in: *Percutaneous Penetration enhancers.*, Smith EW, Maibach H I., Ed., Taylor & Francis, New York, 2006, p. 137-154.
 22. Yokomizo Y. Effect of phosphatidyl glycerol on the in vitro percutaneous drug penetration through the dorsal skin of guinea pigs, and analysis of the molecular mechanism, using (ATR-FTIR) spectroscopy. *Int J Pharm* 1997; 147: 219-231.
 23. Williams A.C., Barry B.W. Chemical Permeation Enhancement, in: *Enhancement in Drug Delivery*, Touitou E., and Barry W.B. Ed. Taylor & Francis, New York, 2006, p. 233-351.
 24. Touitou E, Godin B, Karl Y, Bujanover S, Becker Y. Oleic acid a skin penetration enhancer affects langerhans cells and corneocytes. *J control Rel* 2002; 80: 1-7.
 25. Rama Rao P, Reddy MN, Ramakrishna S, Diwan PV. Comparative in-vivo evaluation of propranolol hydrochloride after oral and transdermal administration in rabbits. *Eur J Pharm Biopharm* 2003; 56: 81-85.
 26. Kim JH, Lee CH, Choi HK. Transdermal delivery of physostigmine : effects of enhancers and pressure-sensitive adhesives. *Drug Dev Ind Pharm* 2002; 28, 7: 833- 839.
 27. Godwin DA, Kim NH, Felton L A. Influence of Transcutol CG on the skin accumulation and transdermal permeation of ultraviolet absorbers. *Eur. J. Pharm. Biopharm* 2002; 53, 1: 23 – 27.
 28. Karande P, Mitragotri S. Enhancement of transdermal drug delivery via synergistic action of chemicals. *Biochimica et Biophysica Acta* 2009; 1788, 3: 2362-2373.
 29. *Pharmaceutical statistics: practical and clinical applications*, Bolton S. Ed., Taylor & Francis, New York, 2003.