

Formulation and Evaluation of Lornoxicam as Dissolving Microneedle Patch

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Abstract

The objective of the study was to develop microneedle (MN) patch, with suitable properties to ensure the delivery of a therapeutic level of lornoxicam (LXM) in a period suitable to replace parenteral administration in patients, especially those who fear needles. The used polymers were cold water-soluble polyvinyl alcohol (PVA) and polyvinylpyrrolidone (PVP) of low molecular weight with PEG 400 as plasticizer and Tween 80 (to enhance the release) using micro molding technique. Patches were studied for needle morphology, drug content, axial fracture force measurement and drug release while the optimized formulas were further subjected to pH measurement, folding endurance, ex vivo permeation study, histopathology study, stability study and compatibility study. The patch with 11:1 ratio of PVA to PVP, 30% solid content, 5% PEG 400 and 3% Tween 80 resulted in axial needle fracture force value of (1.35 N) which is suitable for skin penetration. The release was fast with almost 100% of drug released in 60 minutes. The permeation was enhanced significantly with a steady state flux of about 3.1 times that of the solution. The lag time of MN is shorter in comparison with ordinary patch. Histopathology studies demonstrated the safety of the formulation, both stability studies and compatibility studies showed the suitability of the formulation. The results indicated that LXM microneedle patch could enhance drug permeation while achieving fast and painless administration.

Keywords: Microneedle patch, Polyvinyl alcohol, Polyvinylpyrrolidone, Fracture force, Lornoxicam.

صياغة وتقييم لورنوكسيكام كرقعة مجهرية الإبر قابلة للذوبان باستخدام بوليمرات مختلفة

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الخلاصة

تهدف الدراسة إلى تطوير رقعة إبرية مجهرية ذات خواص مناسبة لضمان دخول لورنوكسيكام (LXM) بمستوى علاجي في فترة مناسبة لاستبدال الإعطاء بالحقن للمرضى، وخاصة أولئك الذين يخشون من زرق الإبر. البوليمرات المستخدمة هي كحول البولي فينيل الذائب بالماء البارد (PVA) والبولي فينيل بيروليدون (PVP) ذو الوزن الجزيئي المنخفض مع PEG 400 (كمادة ملدنة) و Tween 80 (لتعزيز تحرير الدواء) باستخدام تقنية الصب. تمت دراسة شكل الإبر للرقع المحضرة بالإضافة إلى دراسة المحتوى الدوائي، قياس قوة الكسر العامودية و تحرير الدواء في حين أن التركيبة المحسنة خضعت لقياس درجة الحموضة، النفاذية خلال جلد حي، دراسة التشريح المرضي، دراسة الثابتية الدوائية ودراسة التوافق الكيميائي. نتج عن التركيبة التي تحتوي على PVA إلى PVP بنسبة 11:1 أو ذات 30% محتوى صلب، 5% PEG 400 و 3% Tween80 أحدثت قوة كسر مقدارها (1,35 نيوتن) وهو مناسب لاختراق الجلد. كان التحرر الدوائي سريعاً مع تحرر 100% تقريباً من الدواء في 60 دقيقة. تم تعزيز النفاذية بشكل كبير مع تدفق بصورة مستقرة حوالي 3,1 أضعاف محلول الدواء. كان وقت التأخير بعبور الدواء من خلال الجلد أقصر مع الرقعة الإبرية المجهرية. أظهرت دراسات التشريح المرضي سلامة النسيج، وأظهرت كل من دراسات الاستقرار ودراسات التوافق مدى ملاءمة التركيبة. ستعزز الرقع الإبرية الميكروية للورنوكسيكام تغلغل الدواء مع تحقيق إعطاء سريع وغير مؤلم. الكلمات المفتاحية: الرقعة الإبرية المجهرية، الكحول البولي فينيل، الكحول البولي فينيل بيروليدون، قوة الكسر، لورنوكسيكام.

Introduction

The largest organ of the body is the skin making it potentially a highly accessible organ of the human beings. But the stratum corneum (SC) represents the main barrier of the skin and its penetration is very difficult. Invasive methods by employing conventional and hypodermic needles were able to bypass this barrier, but it may cause pain, possibility of contamination, infections and fear. Transdermal drug delivery using patches can overcome the drawbacks of hypodermic needles,

but the conventional patch system has many limitations, mainly limited to drugs that can diffuse through the skin barriers of which the SC represents the main challenge and only drugs with low molecular weight <500 Da and satisfactory lipophilicity can successfully penetrate it to result in successful administration. Improving permeation can be achieved only by reversibly disrupting the molecular construction of these barriers ⁽¹⁾.

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Promising technique to facilitate permeation of drug through the skin barriers are microneedles (MN), which are collections of arrays of micron measurement projections that enhances permeability of the SC by reversibly creating channels of micron size in the skin layers, thereby the skin penetration by a relatively impermeable molecules is enabled. MN devices are minimally invasive, due to avoiding the activation of sensory nerve fibers. The main types that have been developed include, "solid, coated, dissolving and hollow MNs". The type that received major attention was "dissolving MNs" because of its potential advantages that overcomes the other types, which are: higher drug loading capacity contrasting both coated MNs and hallow MNs, as these MNs dissolve with no sharp biohazard waste, scaling up to mass production of "dissolving MNs" is possible as the polymers used are inexpensive and the method of fabrication is based on micro molding ⁽²⁾. Hydrophilic polymers that fit the requirements for fabricating polymeric, dissolvable MNs include PVA and PVP ⁽³⁾. They were chosen due to the complementary properties of each other, as PVA imparts strength while PVP imparts flexibility and increases the solubility of the polymeric mixture ⁽⁴⁾. LXM is a member of the oxycam class of NSAIDs. It is commercially available in both oral and parenteral dosage forms. LXM oral dose ranges from 4-8 mg which is usually well tolerated, but still causes some side effects mainly gastrointestinal side effects. LXM plasma half-life is relatively short about 3 to 5 hr. and has good permeability, which make it a good candidate for transdermal delivery ⁽⁵⁾.

The objective of this study is to prepare dissolvable MN patch for LXM, with better penetration ability by bypassing the SC and to investigate the different materials required to optimize the formula.

Materials

LXM was purchased from Chemshuttle, USA, water soluble PVA from Central drug house (P) Ltd, India, PVP K30 from Urchem, China, Na₂HPO₄ from Thomas Baker, India, Potassium dihydrogen phosphate (KH₂PO₄) from Merck, Germany, Triethanolamine and ethanol from Sigma-Aldrich, Germany, Tween 80 from Riedel-De-Haën, Germany, lastly Blue Silica gel powder from Om Chemicals, India.

Methods

Preparation of lornoxicam microneedle patches

Preparation of LXM MN patches was accomplished by using different ratios of PVP K 30, PVA and 5% W/W polyethylene glycol 400 (PEG 400) by micro molding techniques as shown in table (1). First, LXM was dissolved in certain amount of solvent mixture composed of 75 % phosphate buffer, 20% ethanol and 5% triethanolamine using magnetic stirrer. Then adding and dispersing PVP then PVA in different ratios to the drug solution mentioned above, then placing the whole mixture in a sonicator for 1.5 h. After the whole mixture was dissolved, calculated amount of the dispersion was added to the Polydimethylsiloxane (PMDS) microneedle mold (Micropoint, Singapore) with a patch size of 6.25 cm², array size of 15 X 15, needle height of 500 µm and a needle base of 200 µm. Then the filled mold is sonicated to remove air bubbles and finally the mold is placed in a desiccator under vacuum for one day and then in the oven at 40 °C for 24 hours to ensure drying and needle formation, as shown in figure (1) ⁽⁶⁾. Aluminum foil was used as backing layer for the formulated patches ⁽⁷⁾.

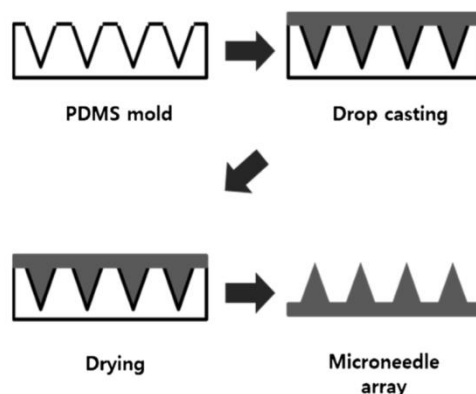


Figure 1. Fabrication process of PVA: PVP microneedle patch ⁽⁶⁾.

Table 1. Composition of prepared micro needle patches using different ratios of PVA and PVP.

Formula No.	Drug (mg)	PVA (g)	PVP (g)	PEG 400 % w/w	Solvent (ml)
F1	40	1	1	5	10
F2	40	1.5	1	5	10
F3	40	1.25	0.75	5	10
F4	40	1.5	0.5	5	10
F5	40	2	0.5	5	10
F6	40	2.5	0.5	5	10
F7	40	3	0.5	5	10
F8	40	3.5	0.5	5	10
F9	40	2.75	0.5	5	10
F10	40	2.75	0.25	5	10

Preparation of lornoxicam ordinary patch

Ordinary patches were made by preparing the same solution mixture of LXM and polymers in same amounts as in preparation of LXN MN. The mixture was casted in petri dish with 8.5 cm diameter. Dried in an oven (Mettler Oven, Germany) at 40°C for 24 h. After complete drying, the patch was cut to smaller patches with same amount of drug and same surface area as MN patches (6.25 cm²) that resulted from MN mold.

Evaluation of LXM MN patch

Microscopic and visual inspection

The prepared Micro needle patches were inspected visually for any defects and by using digital microscope (Depstech, China) to inspect the needles morphology for any defects.

Drug content

The analysis was determined by immersing one patch in 100 ml pH 7.4 phosphate buffer. Then, the sample was diluted and filtered, the absorbance of prepared solution was measured at 376 nm using UV visible spectrophotometer. The percentage drug content was calculated the process was done in triplicate⁽⁸⁾.

In vitro drug release studies

A modified dissolution method was used. MN patches were placed on a mesh wire with sieve opening of approximately 800 micrometers, fastened with an elastic band on the open end of tube with the patch placed in center (9, 10). The tube was immersed in the dissolution jar of the dissolution apparatus (Pharma test, Germany) containing 500 ml of phosphate buffer pH 7.4 which is enough to ensure sink condition according to the used drug loading 8 mg. The apparatus speed was set at 50 rpm⁽¹¹⁾ and temperature was maintained at 35± 0.50 °C⁽¹²⁾. The study continued for 1 hour and samples were withdrawn every 5 minutes. A 5 ml sample was withdrawn from the beaker and replaced with fresh 5 ml of buffer, finally the cumulative drug release percent was calculated. Tween 80 was added in 3%

concentration to the formula that did not achieve 100% drug release during 1h⁽¹³⁾.

Measurement of axial needle fracture force

Axial needle fracture force is defined as “minimum force, applied parallel to the microneedle axis, required to deform or break the microneedle (needle failure)”. Axial needle fracture force was measured using texture analyzer (TA.XT Stable Micro Systems, UK).

The microneedle patch was fixed on a fixed cylindrical platform using double sided adhesive band. The instrument was programmed to axially compress the single microneedle on each occasion by a spherical probe (diameter: 1 inch) travelling at a speed of 0.05 mm/s to a distance of 2 mm⁽¹⁴⁾.

Evaluation of the selected formula Ex vivo permeation study

A modified drug permeation apparatus similar to that used in the release study with same parameters was utilized (figure 2). The volume of dissolution media was 750 ml to ensure sink condition for the specified drug loading. A fresh abdominal rat skin was used in this study, the area was shaved using an electric clipper, after sacrificing the animal the skin was extracted and the subcutaneous layer was removed and kept in saline phosphate buffer pH 7.4 at low temperature (4°C) and used the next day with SC facing the donor chamber, while the other end faces the receptor chamber. Applicator device (MPatch Mini Microneedle Applicator from Micropoint, Singapore) was used to ensure the insertion of MN patch, the applicator speed was 1 (mm/ sec)⁽⁹⁾. 5 mL samples were withdrawn at different time intervals and replenished by equal volume of buffer. The withdrawn samples were analyzed for LXM. The study continued for 6 hours for 22 mg drug loading⁽¹⁵⁾.

The microneedle patch LXN MN was compared to LXM solution (22mg of LXM in 10 ml composed of phosphate buffer pH7.4 with 5% triethanolamine) and LXM ordinary patch with the same composition of polymers and amount of LXM as that of MN patch.

DD solver was used in determining release kinetics of the final selected MN patch. f2 similarity factor function of DD solver was used to determine the degree of similarity between the permeation profiles of selected MN formula with the above-mentioned comparators ⁽¹⁶⁾.

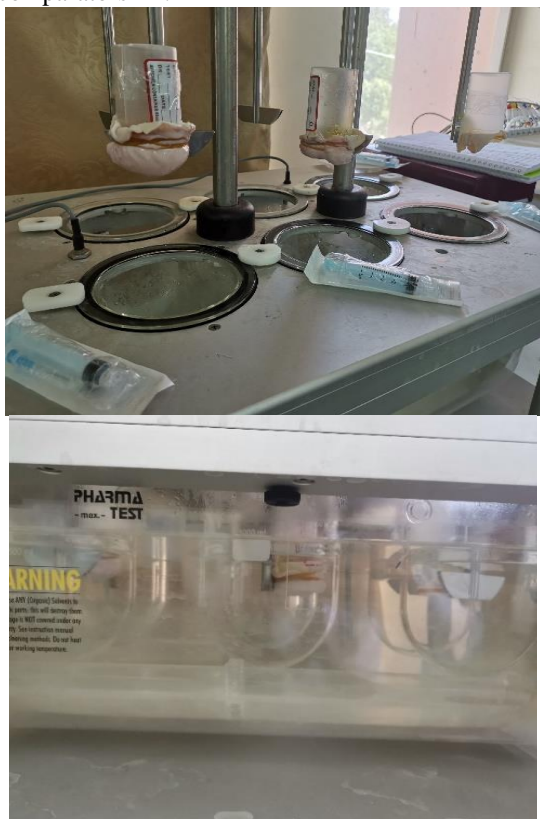


Figure 2. Modified drug permeation apparatus.

Kinetics of Drug Permeation

Permeation kinetics were examined using DD solver to fit the permeation data utilizing four popular release models such as zero-order, first-order, Higuchi and Korsmeyer Peppas equations and n value, which have been described in the literature ⁽¹⁶⁾.

Histopathology study

Histopathology study was done on the abdominal skin used in the permeation study of the selected MN patch loaded with drug compared with unexposed abdominal rat skin to determine the pathological changes. Both tissues were fixed in 10% formalin, routinely processed, and embedded in paraffin. Paraffin sections were cut on glass slides and stained with hematoxylin and eosin to examine the morphological changes to the tissue via blinded study ⁽⁷⁾.

pH Measurement

It was determined by immersing the patch for 30 min with 100 ml of de-ionized water, then the electrode of a pH-meter was placed on the insert surface and the pH was recorded. The process was done in triplicate ⁽¹⁷⁾.

Weight and thickness uniformity

Five patches were selected randomly from each batch and weighed individually using a digital balance; their thickness was measured using a digital Vernier (Neiko, USA), the mean weight, thickness and standard deviation were calculated ⁽¹⁸⁾.

Folding test

Folding endurance is “the number of folds that can be achieved without breaking of the patch when repeated folding at the same position is done”. Folding endurance of three patches of the selected formula was carried out ⁽⁸⁾.

Compatibility study using FTIR

LXM FTIR spectrum was obtained by using the pressed-disk technique. A small amount of drug was ground with potassium bromide powder then the mixture was pressed into a disk. The FTIR spectroscopy was used to investigate the prepared disc at wave number range of 4000-400 cm^{-1} . The FT-IR technique was performed on both the drug alone and the formulated MN to investigate any chemical interaction ⁽⁷⁾.

Stability study

Stability studies of the optimized formulation was carried out by storing the replicates of the patches at 40 ± 0.58 °C and $75 \pm 5\%$ relative humidity for 3 months. Samples were withdrawn every two weeks to measure percent drug content as stability indicating parameter ⁽¹⁹⁾.

Statistical Data Analysis


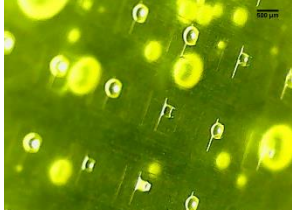

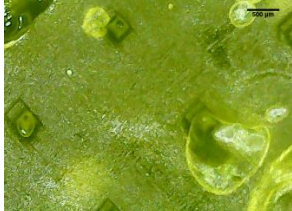
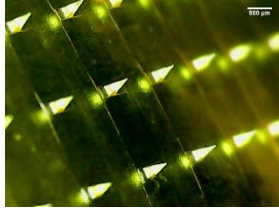
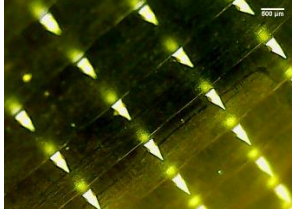

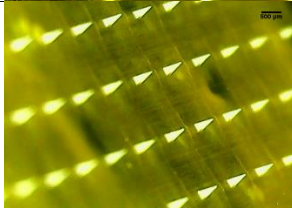

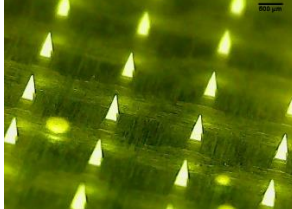
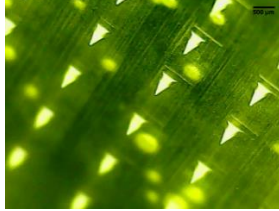
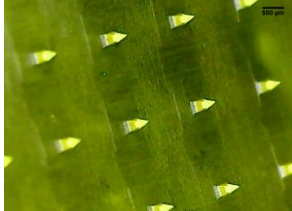
The results of the experiments were given as a mean of triplicate samples \pm (SD) and were analyzed according to the one way analysis of variance (ANOVA) to determine if the changes in the applied factors are statistically significant at level of ($P \leq 0.05$) and non-significant at level of ($P > 0.05$).

Results and Discussion

Microscopic and visual inspection

Inspection was done visually and by digital microscope Depstech 1600X, images are illustrated in table (2). Formulas F7 and F8 failed from visual inspection due to the high viscosity of the polymer matrix created a challenge to fill the mold micro cavities by vacuum application ⁽²⁰⁾. The other formulas show clear needles. The pyramidal shape of MN donates a superior mechanical strength, thus increasing the chances of effective skin penetration ⁽²¹⁾.

Table 2. Microscopic images of all prepared microneedle patches.

Formula code	Microscopic images	Formula code	Microscopic images
F1		F7	
F2		F8	
F3		F9	
F4		F10	
F5		F10TW3%	
F6		F9 TW3%	

Drug content

The mean percent drug content of MN is illustrated in table (4). The results demonstrated drug content of the formulations was within the limits of USP38/NF33 (2015) (22).

Table 4. The Percent of Drug Content of MN patches (mean \pm SD) n=3

Formula code	Drug content %
F4	99.51 \pm 0.038
F5	98.67 \pm 0.125
F6	98.58 \pm 0.121
F9	97.33 \pm 0.172
F10	98.12 \pm 0.233
F9 3% Tween 80	98.17 \pm 0.221
F10 3% Tween 80	98.92 \pm 0.156

In vitro drug release studies

In vitro cumulative percent of LXM release at different sampling time is shown in the figures (3). Formulas with successful MN formation were chosen to study the effect of PVP: PVA ratio on drug release. The release of (F4, F5 and F6) was 100% in 60 min, so they were categorized as fast release formulations, and this is believed to be the result of a high PVP: PVA ratio. It is shown that increasing the PVP concentration, reduces the time needed for dissolution and generated holes in the MNs with water resulting in an enhanced dissolution rate. The actions of PVP was attributed for its both hygroscopicity and moisture adsorption property, which resulted in the hydration and the burst effect made the release of drug from patches containing higher amount of PVP faster (4, 23). While F10 and F9 showed 78% and 83% respectively, during 1 hour of release time, due to a lower PVP: PVA ratio. Tween 80 was chosen as the surfactant of choice due to its availability and studies identifying its effects to be greater than Tween 60 and 20 in improving solubility (24). Tween 80 was added for both F10 and F9 at 3% W/W concentration to improve their drug release. Fast release with 100% release was achieved with 3% Tween 80 addition for both formulas as shown in figure (4). The improvement in dissolution rate caused by Tween 80 was due to the reduction in the interfacial tension which increased the wetting of the polymer to a higher degree thus increasing the erosion of the polymer (25).

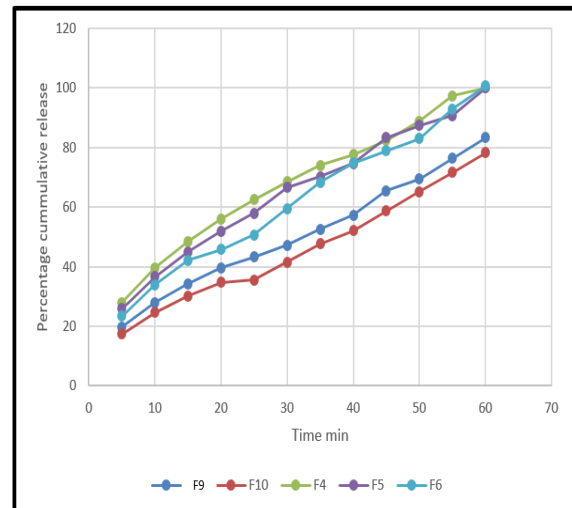


Figure 3. In vitro release studies of LXM MN formulations in phosphate buffer pH 7.4 and at a temperature of 35 ± 0.50 °C.

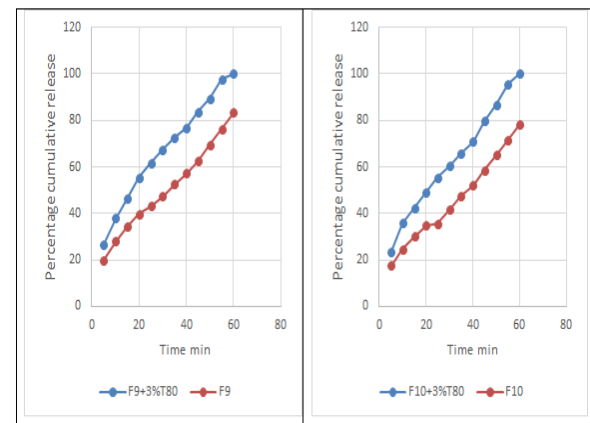


Figure 4. In vitro release studies of F10 and F9 with and without 3% Tween 80 in phosphate buffer pH 7.4 and at a temperature of 35 ± 0.50 °C.

Measurement of axial needle fracture force

Interspacing between MN plays an important role in the force of insertion and when it is increased, the force is decreased which indicates a higher stress on the MNs at small interspacing and lower stress at wider interspacing. There is a reduction in the resistance to MN insertion in a form of stress on the tips which signifies that the MNs face less resistance when the needles are spaced at a greater area (26). So the mold used in this study was selected with an interspacing of 1500 μ m to reduce the required force of insertion.

The accepted axial needle fracture force value is 0.0856 N/MN array for interspacing of 600 μ m and above when the velocity of insertion is 1 mm/s

(which is the velocity of the applicator used in this study) ⁽²⁶⁾. For 15 array MN patch, the calculated insertion force for this research MN patch is 1.284 Newton (N). Therefore; all the patches with axial fracture force below 1.284 N was considered as failed formulations. The results of the fracture force are shown in table (3). Fracture force test showed that when PVP concentration increased, the elasticity increased while fracture force reduced, thus the applied force in N required to break the needles decrease ⁽²⁷⁾. PVA polymer enforces hardness and rigidity, thus increasing PVA concentration results in a harder needles and higher force should be applied (N) to break the needles ⁽²⁸⁾. The results show only F10 has exceeded this insertion force and with an axial fracture force of 1.72 N. as it has the highest ratio of PVA: PVP (11:1) among other formulas.

Tween 80 was added to F9 and F10 at 3% (W/W) concentration to improve their release profile. The addition of Tween 80 increased the flexibility of the polymers thus reducing the mechanical strength of the formed MN patches ⁽²⁹⁾, this was shown with both F10 and F9 as the fracture force was 1.35 N and 0.82 N respectively. The selected formula was F10 + 3% Tween 80 (F10%+3%T80) as its fracture force exceeds the required insertion force.

Table 3. Peak force and the time required for fracture of some of LXN MN formulas using Texture Analyzer.

Formula	Peak Force "Hardness" (N)	Time (S)
F1	0.52	2.10
F2	0.69	2.52
F3	0.71	2.78
F4	0.96	2.50
F5	1.03	3.00
F6	1.20	4.90
F9	1.23	4.33
F10	1.72	6.39
F9 + 3%(w/w)Tween 80	0.82	2.52
F10 + 3%(w/w)Tween 80	1.35	5.00

Ex vivo permeation study

Microneedle patches of F10+3%T80 showed the greatest cumulative amount of drug

permeated which reached 67% during 6 hr., while ordinary patch only 24% of LXN permeated, while LXM solution permeated only 19% in the permeation study. The study states flux rate (ssfr), which was calculated as the slope from the straight portion of permeation graph, for the three arms showed the superiority of MN patch with ssfr of 3.98 $\mu\text{g}/\text{cm}^2/\text{min}$, while ordinary patch 1.46 $\mu\text{g}/\text{cm}^2/\text{min}$ and the LXM solution resulted in 1.27 $\mu\text{g}/\text{cm}^2/\text{min}$. the overall improvement with respect to flux rate was obtained with LXM MN as it increased by 3.1 folds when MN patch is compared to LXM solution. Also the lag time for MN patch was the shortest with only 10 minutes, while ordinary patch lag time was 50 minutes, and LXM solution had initially faster drug permeation when compared to ordinary patch as the drug was already in solution overcoming the time required to dissolve the polymer matrix which is essential for solubilizing the drug. But the ordinary patch had a higher cumulative amount of drug permeated, this is probably due to the surfactant and solubilizing nature of both PVA and PVP which aided in higher amount of drug to be permeated ⁽³⁰⁾. The MN patches improved permeation due to the pores created by the needles breaching the SC allowing the drug to permeate at a higher rate, the created holes are estimated to remain open during the time of application of the MN patch by the action of high polymer concentration in the holes preventing their closure, this is true when the polymer matrix act as the backing layer ⁽³¹⁾. In addition, the hydration of the drug reservoir in the polymer matrix was enhanced by the fluid from the skin entering form channels created by MN which improves drug diffusion ⁽²⁶⁾.

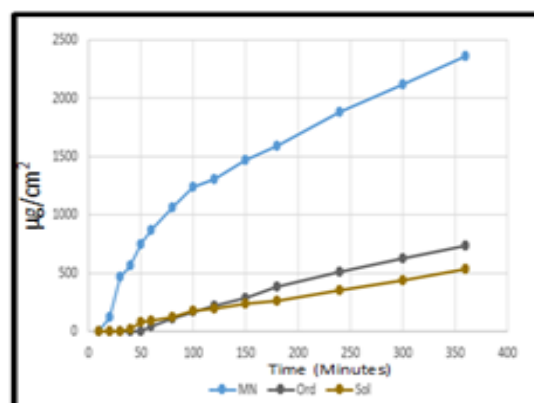


Figure 5. Collective Ex vivo permeation study of MN F10+3%T80, ordinary patch F10+3%T80 and LXM solution in phosphate buffer pH 7.4 and at a temperature of 35± 0.50 °C.

Kinetics of permeation

By using DD Solver Excel add-in program the kinetics of drug release was deduced by calculating the R² value of zero order, first order and

Higuchi models also the n value in Korsmeyer Peppas models. The R2 was the highest for Higuchi model with a value of 0.991, while the results were 0.955 and 0.681 for first order and zero order respectively. The n value from Korsmeyer Peppas model was 0.531 which indicated an anomalous (non-Fickian) transport that implies the release

mechanism was governed by both diffusion and relaxation or erosion⁽³²⁾.

Histopathological study

Histopathology of the abdominal tissue (Figure 6) indicated the absence of tissue damage and toxicity. Both LXM loaded microneedle patch treated and untreated abdominal skin tissues showed no effect on the microscopic structure of the skin. No cell necrosis was detected after application of LXM MN patch and saline buffer pH 7.4 as comparator.

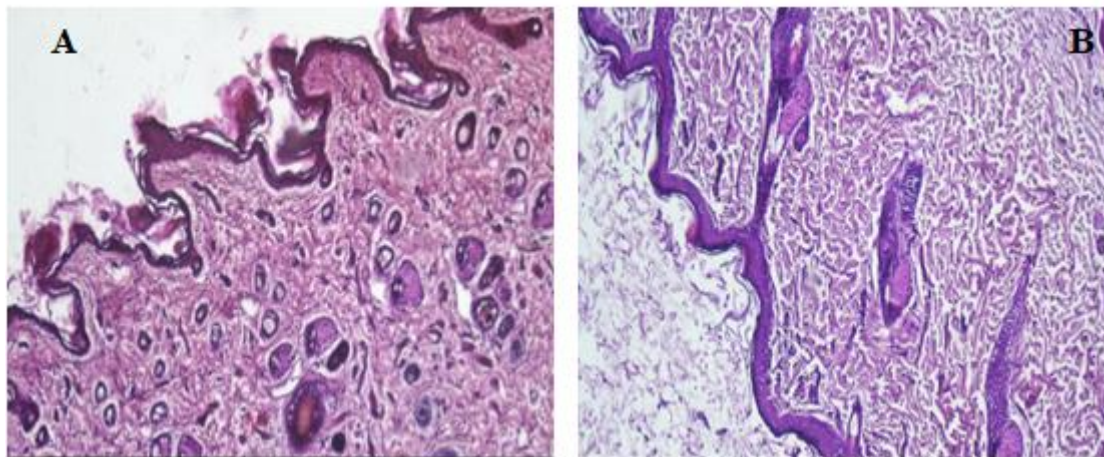


Figure 6. Histopathological evaluation of sections of rat abdominal tissues. a. Rat abdominal tissue incubated in the diffusion chamber with LXM loaded microneedle patch showing the created channels, b. Rat abdominal tissue incubated in phosphate buffer pH 7.4.

pH Measurement

The pH of the selected formula was 7.7 ± 0.2 , this result may be due to the effect of triethanolamine which has a pH adjusting activity, and has the ability to increase and maintain pH level about 7.8 which is known to have no skin irritating effects⁽³³⁾.

Weight and thickness uniformity

Five patches were selected randomly from each batch; the thickness of each patch was measured at five positions using a digital vernier caliper⁽¹²⁾. The mean thickness and standard deviation were calculated ($1 \text{ mm} \pm 3\%$). Batches with a variation of more than $\pm 5\%$ from the mean were rejected⁽³⁴⁾. The mean weight for tested patches was $0.913 \pm 0.043\text{g}$.

Folding test

The selected formula F10 + 3% T 80 withstood breakage for 210 ± 2 folds. Which indicates its high suitability for application and strength.

Compatibility study using FTIR

The FTIR spectrum of LXM and the selected formula (F10+3%T80) is shown in the figure (7). LXM peaks at 3468.18 cm^{-1} indicates O-H stretching, intense bands at 3064 cm^{-1} indicates N-H stretching, 1594 cm^{-1} indicates C=O stretching of amide, 1037 cm^{-1} indicates S=O stretching. These peaks were also observed in FTIR spectrum of the final formula, suggesting that there was no interaction with drug and polymer and all bands were compared with reference⁽⁷⁾.

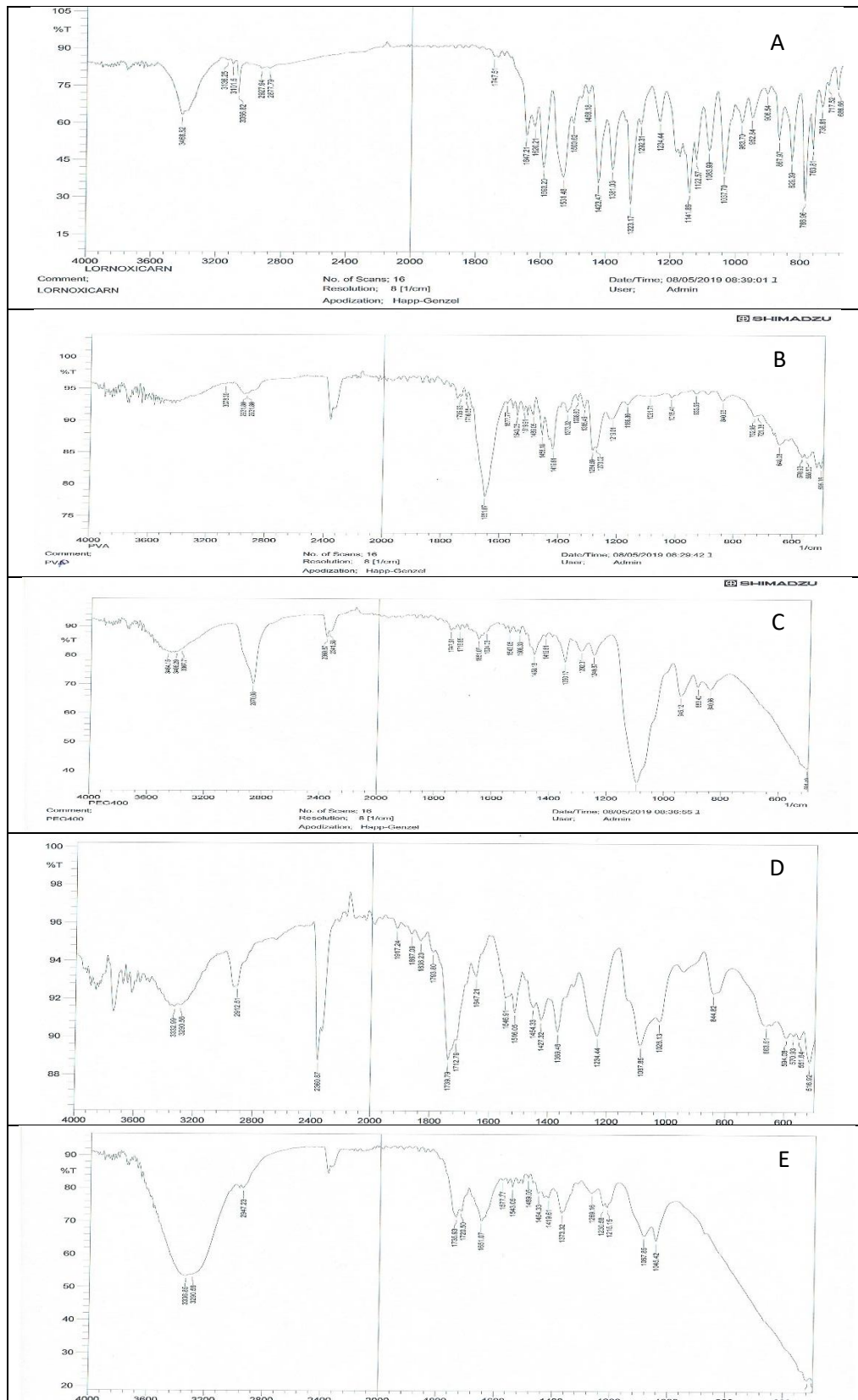


Figure 7. Compatibility FTIR, IR spectrum of A: Lornoxicam, B: PVP, C: PEG 400, D: PVA and E selected formula.

Stability test

Effect of storage condition on stability indicating parameters, folding endurance and percent drug content are presented in table (5). Insignificant decrease was observed in percent drug content and folding endurance, indicating the stability of the developed formulation .

Table 5. Stability data of optimized microneedle patch (F10 3% T80) stored at 40 ± 0.58 °C and $75 \pm 5\%$ relative humidity.

Time of Sample	Folding Endurance	Drug Content%
At the time of preparation	211	98.33
After two weeks	211	98.08
After four weeks	211	97.82
After Six weeks	209	97.14
After Eight weeks	208	96.62
After Ten weeks	207	95.92
After Twelve weeks	205	95.54

Conclusion

Based on the results obtained from this study, it is concluded that LXM MN patch was successfully prepared using PVP and PVA. All the formulations were found to have the desired amount of drug content. The appropriate ratio of PVA to PVP will result in the desired fraction force with rapid dissolution and good permeability profile. The optimized formula F10 3% Tween 80 showed drug release of 100% within 1 h. Ex-vivo studies on rat abdominal skin showed permeation of 67% within 6h. the selected formula exhibited diffusion by Highuchi model. The mechanism of release of the selected formula showed both diffusion and erosion mechanism. The optimized formula was found to be safe, since no histopathological changes were observed after incubation with the formulas for 24 hrs.

References

- Chandrashekar NS and Shobha Rani RH. Physicochemical and Pharmacokinetic Parameters in Drug Selection And Loading for Transdermal Drug Delivery. Indian Journal of Pharmaceutical Sciences. 2008; 70(1): 94-96.
- KimYC, ParkJH, PrausnitzMR. Microneedles For Drug And Vaccine Delivery. Adv Drug Deliv Rev. 2012;64(14):1547-68.
- Lee IC, He JS, Tsai MT, Lin KC. Fabrication of a Novel Partially Dissolving Polymer Microneedle Patch for Transdermal Drug Delivery. Journal of Materials Chemistry B. 2015;3(2):276-85.
- Shim WS, Hwang YM, Park SG, Lee CK, Kang NG. Role of Polyvinylpyrrolidone in Dissolving Microneedle for Efficient Transdermal Drug Delivery: In Vitro and Clinical Studies. Bulletin of the Korean Chemical Society. 2018; 39(6):789-93.
- Zhang Y, Zhong D, Si D, Guo Y, Chen X, Zhou H. Lornoxicam Pharmacokinetics in Relation to Cytochrome P450 2C9 Genotype. British Journal of clinical pharmacology. 2005; 59(1):14-7.
- Park Y, Kim KS, Chung M, Sung JH, Kim B. Fabrication and Characterization of Dissolving Microneedle Arrays for Improving Skin Permeability of Cosmetic Ingredients. Journal of Industrial and Engineering Chemistry. 2016; 25;39:121-6.
- Baviskar DT, Parik VB, Jain DJ. Development of Matrix-type Transdermal Delivery of Lornoxicam: in Vitro Evaluation and Pharmacodynamic and Pharmacokinetic Studies in Albino Rats. PDA Journal of Pharmaceutical Science and Technology. 2013; 1;67(1):9-22.
- Soujanya C, Satya BL, Reddy ML, Manogna K, Prakash PR, Ramesh A. Formulation and in Vitro and in Vivo Evaluation of Transdermal Patches of Lornoxicam Using Natural Permeation Enhancers. Int J Pharm Pharm Sci. 2014;6(4):282-6.
- Khalid W, Jaafar J, Mowafaq M. Study of Formulation Variables of Mefenamic Acid Using Peppermint Oil and Liquid Paraffin. Journal of Science. 2015;5(11): 2277 – 3290.
- Rajab NA, Rassol AA, Assaf SM, Sallam AS. Preparation and Evaluation of Fentanyl Transdermal Patches Using Lidocaine as a Model Drug and Azelaic Acid as a Penetration Enhancer (Doctoral dissertation, Ph. D. Thesis, Faculty of Pharmacy-University of Baghdad).
- Habib F, Abdel Azeem M, Fetih G, Safwat M. Mucoadhesive Buccal Patches of Lornoxicam: Development and In-Vitro Characterization. Bull. Pharm. Sci. Assiut Univ. 2010 1;33:59-68.
- Chaisson D. Dissolution Performance Testing Of Transdermal Systems. Dissolution Technologies. 1995;2(1):7-11.
- Som I, Bhatia K, Yasir M. Status of Surfactants as Penetration Enhancers in Transdermal Drug Delivery. Journal of pharmacy and bioallied sciences. 2012 ;4(1):2.
- Aggarwal P, Johnston CR. Geometrical Effects in Mechanical Characterizing of Microneedle for Biomedical Applications. Sensors and Actuators B: Chemical. 2004 13;102(2):226-34.
- Ammar HO, Ghorab M, Mahmoud AA, Makram TS, Noshi SH. Topical Liquid Crystalline Gel Containing Lornoxicam/Cyclodextrin Complex. Journal of

- Inclusion Phenomena And Macrocyclic Chemistry. 2012 1;73(1-4):161-75.
16. Zhang Y, Huo M, Zhou J, Zou A, Li W, Yao C, Xie S. DDSolver: An Add-In Program For Modeling And Comparison Of Drug Dissolution Profiles. *The AAPS Journal*. 2010 1;12(3):263-71.
 17. Al-Saedi ZHF, Alzhrani RM, Boddu SHS. Formulation and in Vitro Evaluation of Cyclosporine-A Inserts Prepared Using Hydroxypropyl Methylcellulose for Treating Dry Eye Disease. *J Ocul Pharmacol Ther*. 2016;32(7):1-12.
 18. Adhyapak A, Desai BG. Preparation And In Vitro Characterization Of The Transdermal Drug Delivery System Containing Tamoxifen Citrate For Breast Cancer. *Asian Journal of Pharmaceutics (AJP): Free full text articles from Asian J Pharm*. 2014 23;5(1).
 19. Singh, S. Drug Stability Testing And Shelf Life Determination According To International Guidelines. *Pharm. Tech*. 1999, 23 (6), 68-88.
 20. Yang S, Feng Y, Zhang L, Chen N, Yuan W, Jin T. A Scalable Fabrication Process Of Polymer Microneedles. *International Journal Of Nanomedicine*. 2012;7:1415.
 21. Lee JW, Park JH, Prausnitz MR. Dissolving Microneedles For Transdermal Drug Delivery. *Biomaterials*. 2008 1;29(13):2113-24.
 22. Monographs GO. USP38-NF33. United States Pharmacopeia and National Formulary (USP 38-NF 33 S1) Baltimore, MD.
 23. Arora P, Mukherjee B. Design, Development, Physicochemical, And In Vitro And In Vivo Evaluation of Transdermal Patches Containing Diclofenac Diethylammonium Salt. *Journal Of Pharmaceutical Sciences*. 2002 ;91(9):2076-89.
 24. Akbari J, Saeedi M, Morteza-Semnani K, Kelidari HR, Moghanlou FS, Zareh G, Rostamkalei S. The Effect of Tween 20, 60, And 80 on Dissolution Behavior of Sprionolactone In Solid Dispersions Prepared By PEG 6000. *Advanced Pharmaceutical Bulletin*. 2015 ;5(3):435.
 25. Sonjoy, M.J., Thimmadetty, G.N., Ratan, B.H. and Kilarimath, 2011. Formulation And Evaluation of Carvedilol Transdermal Patches. *Int. Res. J. Pharm.*, 2: 237-248.
 26. Olatunji O, Das DB, Garland MJ, Belaid LU, Donnelly RF. Influence of Array Interspacing on The Force Required For Successful Microneedle Skin Penetration: Theoretical And Practical Approaches. *Journal of Pharmaceutical Sciences*. 2013 1;102(4):1209-21.
 27. Baccaro S, Pajewski LA, Scoccia G, Volpe R, Rosiak JM. Mechanical Properties Of Polyvinylpyrrolidone (PVP) Hydrogels Undergoing Radiation. *Nuclear Instruments And Methods In Physics Research Section B: Beam Interactions With Materials And Atoms*. 1995 2;105(1-4):100-2.
 28. Wu CB. WP Enhanced PVA/LRSS Composite. In *Advanced Materials Research 2014 (Vol. 1015, pp. 291-294)*. Trans Tech Publications.
 29. Ali S, Shabbir M, Shahid N, Amin U, Hamid I, Raza M. Effect of Polysorbate 80 Through Rabbit's Skin Using Transdermal Patch Loaded With Bisoprolol Fumarate As Model Drug. *Pakistan J. Zool*. 2016 1;48(1):227-34.
 30. Amodwala S, Kumar P, Thakkar HP. Statistically Optimized Fast Dissolving Microneedle Transdermal Patch of Meloxicam: A Patient Friendly Approach to Manage Arthritis. *European Journal of Pharmaceutical Sciences*. 2017 15;104:114-23.
 31. Lee JW, Choi SO, Felner EI, Prausnitz MR. Dissolving Microneedle Patch For Transdermal Delivery of Human Growth Hormone. *Small*. 2011 ;7(4):531-9.
 32. Padmaa M, Preethy AN, Setty CM, Peter GV. Release Kinetics Concepts and Applications. *International Journal of Pharmacy Research and Technology*, 2018 (8).
 33. Green JM, Cahill WR. Enhancing The Biological Activity Of Nicosulfuron With pH Adjusters. *Weed Technology*. 2003 Jun;17(2):338-45
 34. Ali MM, Rajab NA. Preparation and Evaluation of Domperidone Transdermal Patches. *World J Pharm Res*. 2014;3(7):50-78.

