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FORMULATION, OPTIMIZATION AND IN-VIVO ANTI-INFLAMMATORY STUDY OF OPTIMIZED TRANSDERMAL PATCHES

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ABSTRACT In the present study, an attempt was made to prepare, characterize and evaluate of transdermal matrix patches of Diclofenac Sodium for inflammation and pain related disease. Based on results of various evaluation parameters like thickness, strength, elongation, better compatibility and stability the transdermal matrix patches was successfully designed and developed by trial and error method. Formulations were prepared by employing combination of HPMCK15M, PVPK30, PEG-400 and EC among penetration enhancer and patches were evaluated for uniformity of thickness, weight-variation test, folding-endurance, tensile strength, % elongation, % flatness, % moistures absorption, Moistures vapor transmittance rate, assay done. Cellophane membrane employed for the diffusion study. The result revealed that formulations containing enhancer eucalyptus oil, oleic acid and tea tree oil have better anti-inflammatory action as compared to formulation without it because eucalyptus oil and tea tree oil both are natural anti-inflammatory remedies as well as permeation enhancer. The Formulation F 6 show highest percentage inhibition of edema as compared to others formulation.

KEYWORDS : Diclofenac Sodium, Inflammation and Pain, Transdermal matrix patches, HPMCK15M, PVPK30, PEG-400

INTRODUCTION

Transdermal systems by delivering drugs across the skin into systemic circulation resist the alteration in absorption rate[1], metabolism and prevent the gastrointestinal adverse effects occurring due to oral administration of drugs. Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently prescribed for arthritis[2], low back pain and some joint diseases (1). The well reported mechanism of action is reversible inhibition of cyclooxygenase enzyme (COX) and decrease the synthesis of prostaglandins[3]. However, due to inhibition of prostaglandins (PGs) which protects the gastric mucosa, they show side effects including dyspepsia to peptic ulcer and gastrointestinal haemorrhage. NSAIDs are acidic in nature they may produce local irritation and lesions on the gastrointestinal mucosa[4]. Hence, some of the NSAIDs are administered percutaneously and transdermally to achieve local or systemic effect as an alternative to oral and parenteral administration. However, the barrier layer i.e. stratum corneum prevents the penetration of the drugs to lower layers of the skin and/or to enter systemic circulation. In this context, the formulation plays a key role in the penetration and absorption of the active ingredient[5].

MATERIALAND METHOD

Diclofenac was received as a gift sample from Mylan Laboratories Limited Hyderabad, India, HPMC and EC S.D. Fine Chemicals, Mumbai. Methanol, Chloroform and ethanol S.D. Fine Chemicals, Mumbai, were of analytical reagent grade. Oleic acid, Eucalyptus oil,Tea Tree oil & clove oil was procured from Gardens of Aroma Greater Noida, 201308,U.P. All other chemicals used in this study were of analytical grade.

Preparations Of Transdermal Patches

The transdermal patches of composition listed in Table 1 were prepared by solution casting technique employing a glass substrate (Bangles wrapped with aluminium foil)[6]. Membrane type transdermal systems with containing 10 mg Diclofenac Sodium prepared by employing various proportions of $HPMCK_{15}M$, $PVPK_{30,}$ and Ethyl Cellulose. The polymers was accurately weight and dissolved in a suitable solvent mixed until clear solution formed with magnetic stirrer then added drugs and placed for 30 mint in ultra sonicator bath machine (Elmasonic S150) for complete dissolution after that this sonicated solution mixed with PEG400 as a plasticizer[7] .The polymer solution was poured into bangles placed in a suitable level, hard rigid surface and patches were dried at a room temperature in a dust free environment for 24 hrs. An inverted funnel was covered over the bangles to avoid fast evaporation of the solvent. Patches of 3.14 cm^2 were prepared by cutting and packed in an aluminum foil and kept in a desiccator[8-10].

Evaluation Of Transdermal Patches[11-15]

Thickness of patches

The thickness of Patches were measured by digital Vernier calipers with least count 0.001mm at three different sites average of three reading was taken with standard deviation.

Weight variation

The three disks of 3.14 cm^2 were cut and weighed on electronic balance for weight variation test. The test was done to check the uniformity of weight and thus check the batch- to- batch variation.

Drug content

Accurately weighed patches were individually dissolved in minimum quantity of methanol and made volume up to 100 ml with PBS pH 7.4 solutions; 10 ml was transferred to flask and made volume 100 ml. The absorbance was recorded. The blank solution was made in the same manner except the patches without drug were used.

Percentage Moisture content

The films were weighed & placed in desiccators containing calcium chloride at 40^0c in a dryer for at least 24 hrs or more until it gives a constant weight. The %of moisture content was the difference between constant weigh taken and the initial weight and as reported with percentage by weight moisture content.

$$\text{Percentage moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Percentage Moisture absorption/ uptake

The films of which the size 3.14cm^2 were put in a desiccators with silica gel for 24 hrs and weighed the patches were transferred to another desiccators containing saturated solution of KCL (84%RH) after equilibrium was attained. Patches were taken out and weighed. Moisture uptake was calculated with following formula.

$$\text{Percentage moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Swelling index

The patches of 3.14 cm^2 were weighed and added into Petri dish whichcontains10mldoubledistilledwaterandwerepermeatedtoabsorb moisture at a fix time interval check the increase weight of the patches. Continue this process till same weight observed until weight remaining the same over a period of time. Swelling index (%S) was determined by applying the formula.

S (percentage) = Wt – Wo / Wo x 100

Where, S percent swelling, Wt patch weight at time t.

Wo patch weight at time zero.

Folding Endurance
This was obtained by constantly folding one patch at the same place without breaking gave the value of folding endurance. This test performed to check folding ability of transdermal patches also indicate brittleness of patches, more brittle patch when folding endurance value.

Percentage Elongation
A film strip (4 x 1cm) was cut on a glass plate with a sharp blade. The percentage elongation break is to be determined by observing the length just before the breaking point with formula by pointer on the graph paper.
% Elongation = [Final length – Initial length] * 100/Initial length

Tensile Strength
The tensile strength of the patches was found by the apparatus and the design of instrument such that, it had done wooden frame that horizontally placed having fixed scale. On the top of frame two clips were attached to hold patches that under study. From two clips one clips fixed & other moved. Instrument also has pulley to hold weight a patch, weight applied to one end of pulley and other end attached to the fixed clip .During the test wooden platform not dislocate from the original place so platform was fixed carefully to avoid dislocation. Three patches were cut for study having 3.14 cm^2 sizes. Thickness and width of patches were noted at three sizes and calculated average value. Rate of stress changes was maintained constant with the addition of 0.5g per 2 minutes. The elongation was observed and the total weights taken were used for calculation
Formula for tensile strength:
Tensile strength= F/a.b (1+L/l)

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***In-vivo* Anti-inflammatory study of optimized formulation**
Formalin-induced Paw Edema
This model based upon the ability of test drug to inhibit the edema produced in the hind paw of the mice after injection of formalin. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue-mediated response. In the first phase there is release of histamine, 5-HT and kinin, while the second phase is related to the release of prostaglandins[17].

Animals
Healthy young adult albino (100-120 gm) of either sex and of approximate same age were used with pelleted food and water *ad libitum*. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. Animal studies were approved by Institutional Animal Ethics Committee with the Guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Formalin induced paw edema model
In-vivo Anti-inflammatory study of optimized Transdermal patch formulation F6 containing permeation enhancer was conducted by formalin induced paw edema model using 12albino rats and divided into three groups of four animals on each. In all groups, acute inflammation was induced by sub.planter injection of 0.1 ml of freshly prepared 1 % suspension of sterilized formalin in normal saline in left hind paw of the rats. The medicated formulations (0.3g) or base or standard were applied topically to the planter surface of hind paw with gentle rubbing with index finger to each rat of respective group one hour before and one hour after the formalin challenge. The paw edema volume was measured using plethysmometer at every 30 mint intervals for 4 hour after injection of formalin. The average paw edema volume of all the groups were calculated and compared with that of control. The percent inhibition of edema was calculated by using following formula.

% Edema inhibition = (1-Vt/Vc) 100
Where, Vt = Mean edema volume of test, Vc = Mean edema volume of control
Eight groups of animals four each:
- Group I -Received transdermal patch base
- Group II –Received Diclofenac Sodium transdermal patch
- Group III- Received Diclofenac Sodium transdermal patch with permeation enhancer (FE formulation)
- Group III- Received diclofenac Sodium marketed preparation

Skin Irritancy Study
Skin irritation study was performed on healthy rats weighing between 200-250 g. the hairs of albino rats were withdraw from dorsal side by clipping skin portion 1 day prior of the experiment. The experimental rats were distributed into 4 groups (n=2), group I acts as control, group II with patch FE formulation, group III received a blank transdermal patch and group IV received a 0.8% (v/v) formalin solution as irritant. Rats backside skin are a ware moved 24 hours before experimental study.

RESULTS AND DISCUSSION

Table 1: Composition of Transdermal patches

Formulation Code	Drug (mg)	HPMCK15M (mg)	PVP K30 (mg)	EC (mg)	PEG-400 *(ml)	Solvent (M:DCM) (1:1) (ml)	Natural Penetration Enhancer
F1	10	50	250	100	0.2	4	-
F2	10	50	250	100	0.2	4	2:04 (oleic acid &Eucalyptus oil)
F3	10	50	250	100	0.2	4	2:04 (oleic acid &Tea Tree oil)
F4	10	50	250	100	0.2	4	2:4 (oleic acid &clove oil)
F5	10	50	250	100	0.2	4	2:02:02 (oleic acid, Eucalyptus oil & clove oil)
F6	10	50	250	100	0.2	4	2:2:2 (oleic acid, Eucalyptus oil & Tea Tree oil)
F7	10	50	250	100	0.2	4	2:2:2 (oleic acid, Tea Tree oil& clove oil)

HPMC: Hydroxypropyl methyl cellulose, PVP: Polyvinyl pyrolidone, PEG: Polyethylene glycol, DMSO: Dimethyl sulphoxide, *M: Methanol *DCM: Dichloromethan

Table 2: Physicochemical Evaluation data of Diclofenac Sodium Transdermal Patches

Formulation Code	Thickness (mm)	Weight variation (mg)	% Drug Content Diclofenac Sodium	Folding endurance	Tensile strength Kg/mm2
F1	0.31± 0.09	0.159± 0.01	98.15± 2.02	58± 02.04	3.21± 0.81
F2	0.30± 0.02	0.151± 0.005	98.4± 2.42	57.7± 12.0	2.89± 0.70
F3	0.32± 0.004	0.150± 0.021	97.42± 2.17	58± 08.20	3.12± 0.70

F4	0.31± 0.09	0.158± 0.011	98.73± 1.43	59± 14.13	3.30± 1.70
F6	0.32± 0.29	0.154± 0.017	98.34± 2.02	58± 22.03	3.34± 1.80
F6	0.31± 0.013	0.156± 0.014	98.91± 1.42	58± 11.42	3.33± 1.83
F7	0.32± 0.012	0.157± 0.015	98.33± 2.02	59± 59.41	3.34± 1.76

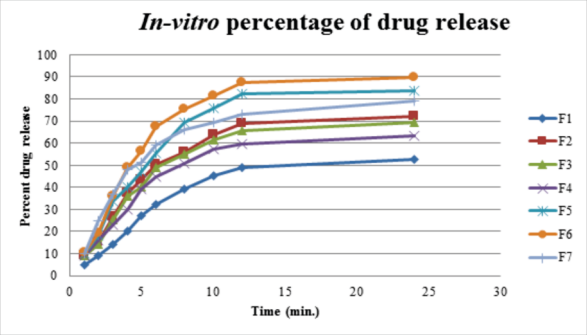

Figure 1: In-vitro percentage of drug release

Drug release kinetic modeling of optimized formula

On comparison of kinetic modeling and release profile data it was evident that Transdermal Patch containing Diclofenac Sodium was found to release the drug in accordance to Hixson rate order kinetics, the regression coefficient was not found to be exactly near to 1, which could be due to influence of some other factors.

Table 3: R2 value of optimized formulation Tf6

Model Name	Zero order	Fist order	Higuchi model	Hixson	Cross peppas	Best fit model
R2 value of F6 for Diclofenac Sodium	0.9924	0.979	0.9092	0.9884	0.959	First order Hixson model

***In-Vivo* Anti-Inflammatory Study of Optimized Formulation**

Percentage inhibition of edema by Diclofenac transdermal patch without containing permeation enhancer (F1formulation) in rat's left hind paw was observed to be- 34.69% (at 1 hr.) and 39.88% (at 2 hr.), where as in case of Diclofenac Sodium patch containing permeation enhancer (F6 formulation) was observed to be- 44.32% (at 1 hr.) and 49.89% (at 2 hr.). All the results were compared with standard Diclofenac Sodium marked preparation. % Inhibition (Mean±SEM). The result revealed that formulations containing natural enhancer eucalyptus oil, oleic acid and tea tree oil has better anti-inflammatory action as compared to formulation without it. The Formulation F 6 show highest percentage inhibition of edema as compared to others formulation (Table. & fig.).

Table 4: Mean Percentage inhibition of edema

Group	Percentage inhibition of edema			
	1 hr	2hr	3hr	4hr
Control	-	-	-	-
F1	34.69+0.16	39.88+0.70	43.12+0.24	48.03+0.93
F6	44.72+0.16	49.87+0.23	53.74+0.02	58.35+0.80
Standard Drug	43.02+0.16	48.00+0.13	51.11+0.05	55.85+0.90

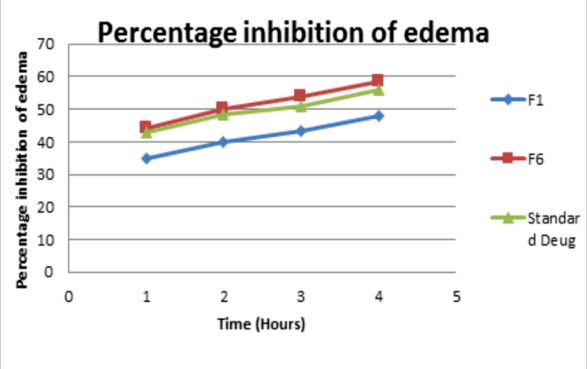

Figure 2: Percentage inhibition of edema

Skin Irritation Test

The skin irritation test was conducted for a period of seven days and the results are tabulated in Table. The results indicated that the control preparation, F6 formulation containing both drug and permeation enhancer and marketed products did not cause any skin reaction. It can be assured that drug, permeation enhancer and other excipients did not cause any skin irritation and can be used in the gel formulation.

Table 5: Data From The Skin Irritation Study For Prepared Formulations

Normal	F6:Drug	Blank film	Formalin
-	-	-	++**
-	-	-	++**

Erythema
-Nil
+Mild
++Severe
+++Verysevere

Edema
- Nil
*Mild
** Severe
*** Verysevere

Figure 3: Skin irritation study for prepared formulations

A=skin of rabbit at 0 day,
B= Application of transdermal Patch F-6 formulation
C= skin of test group rabbit after 7 days of patch application

CONCLUSION

In the present study, an attempt was made to prepare, characterize and evaluate of transdermal matrix patches of Diclofenac Sodium for inflammation and pain related disease. Based on results of various evaluation parameters The Tensile-strength of film found to be in range of 2.89±0.80 to 3.35 ±1.84 Kg/mm^2. Flatness of all prepared patches was found to be 100%. Folding-Endurance of patch found to be in range of 57±22.03 to 59±14.13. The drug contents found in between 97.41±2.17 to 98.5±2.42. The % moisture absorption for all batches found to be in range of 2.53±0.77 to 3.53±0.98 %. The % moisture uptake for all batches found to be in range of 4.25±2.7 to 5.25±1.25 %. % Elongation for all formulation was in the range of 32.98±4.18 to 36.94±4.71 %. The In vitro diffusion studies were performed in phosphate-buffer pH-7.4 for 24hours. The batch F6 was optimised batch of Diclofenac Sodium transdermal patches prepared by using $HPMCK_{15}M$, $PVPK_{30}$,PEG-400 and EC with natural permeation enhancer oleic acid, eucalyptus oil and tea tree oil showed good physical properties and ideal release kinetics. The release kinetics studies revealed that the drug release from formulations F6 followed hixson order kinetics. Moisture content of patches depends on the concentration of PVP and EC. When concentration increases then moisture content also increases. All the formulation shows good folding endurance.

The skin irritation test was conducted for a period of seven days and the results are tabulated in Table 16. The results indicated that the control preparation, F6 formulation containing both drug and permeation enhancer and marketed products did not cause any skin reaction. It can be assured that both drug ,permeation enhancer and other excipients did not cause any skin irritation and can be used in the gel formulation

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