FABRICATION AND *IN- VITRO* PERMEATION STUDIES OF INDOMETHACIN-*FICUS CARICA* FRUIT MUCILAGE PATCHES

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ABSTRACT: The main objective of the present study was to develop matrix-moderated transdermal systems of Indomethacin using various proportions of *Ficus carica* fruit mucilage. Physical evaluation was performed such as moisture content, moisture uptake, tensile strength, flatness and folding endurance. *In-vitro* permeation studies were performed in a Keshary-Chien diffusion cell. The matrix-type transdermal systems were prepared using Indomethacin with *Ficus carica* fruit mucilage by the solvent evaporation technique. The interactions between Indomethacin and *Ficus carica* fruit mucilage were performed. The transdermal patches were subjected to various physicochemical parameters viz., mechanical properties, permeation studies and skin irritation studies. The prepared patches possessed satisfactory pre-formulary and formulary characteristics. *In vitro* permeation studies were performed using a Keshary-Chien diffusion cell across hairless Albino rat skin. The non-ionic surfactants Span 80, Glycerin, Propylene glycol in the formulation played a role as permeability enhancer. The patches were seemingly free of potentially hazardous skin irritation. The experimental results shows that the release of drug from the patch delayed in controlled manner as the proportion of *Ficus carica* increased. It was concluded that Indomethacin can be developed as a transdermal patches with *Ficus carica* fruit mucilage
Key words: Indomethacin, *Ficus carica* fruit mucilage, transdermal patches, *in-vitro* permeation, skin irritation.

INTRODUCTION

Transdermal delivery has many advantages over conventional modes of drug administration, it devoid of hepatic first pass metabolism and improves patient compliance. Intensive research has shown that transdermal route is a potential mode of delivery of lipophilic drugs in systemic circulation.

Indomethacin is an arylacetic acid derivative, which has excellent anti-inflammatory, analgesic and antipyretic efficacy. Indomethacin has been widely used for treating conditions such as chronic rheumatoid arthritis, osteoarthritis, spondylosis deformans, acute gout and periarthritis, humeroscapularis (Rusu D *et al*., 1998). The drug has a biological half-life of about 5 to 10 hours and a plasma clearance of 1 to 2.5ml/kg/min; make Indomethacin a suitable candidate for administration by transdermal route (Claas SA and Glasser SP 2005).

The transdermal patches were evaluated *in-vitro* and for controlled release. Various experimental reports indicated that Indomethacin is a good candidate for controlled release formulation. In this study, *Ficus carica* fruit mucilage was used as a matrix polymer for controlling release of Indomethacin.

MATERIALS AND METHODS

Materials

Indomethacin was obtained as a gift sample from Waksman Selman Pvt. Ltd, Anantapur, India. *Ficus carica* fruits were obtained from the main market of Anantapur and authenticated by the Botany department of Sri Krishnadevaraya University, Anantapur. Glycerin, Propylene glycol, Methyl paraben, Propyl paraben, Span-80 procured from S.D. Fine chemicals Mumbai. All the reagents used were of AR grade. The drug samples were characterized by means of UV spectrophotometric method along with determination of solubility and pH for their authentication.

Methods

Extraction of mucilage

The fresh ripen fruits of *Ficus carica* were obtained from main market of Anantapur, India. The fruits were thoroughly washed with water to remove dirt and debris then cut it into two pieces. The seeds which were present inside the fruit were removed. The pulp of the fruits were crushed and soaked in water for 5–6 hours, boiled for 30 min and left to stand for 1 hour to allow complete release of the mucilage into the water. The mucilage was extracted using a multi layer muslin cloth bag to remove the marc from the solution. Acetone (three times the volume of filtrate) was added to precipitate the mucilage (Baveja SK *et al.,* 1988). The mucilage was separated, dried in an oven at 40°C, collected, ground, passed through a # 80 sieve and stored in desiccator at 30°C and 45% relative humidity before use.

Preparation of transdermal films

Various proportions of *Ficus carica* mucilage was taken in a beaker, add Propylene glycol as plasticizer, Span-80 as penetration enhancer, Propyl paraben and Methyl paraben as preservatives and finally Indomethacin(100 mg) was added with continuous stirring using teflon-coated magnetic bead placed in magnetic stirrer for 30 min at 500 rpm. The above mixture was poured within the glass bangles (6.1 cm diameter) placed on mercury surface in a petri dish. The rate of evaporation was controlled by inverting a funnel over the petri dish. After 24 hours the dried films were taken out and stored in desiccator (Cleary GW 1993; Mukherjee B *et al.,* 2005). The composition of various formulae were shown in Table 1.

Table 1: Different formulae of transdermal patches

Ingredients	IFC-1	IFC-2	IFC-3	IFC-4	IFC-5
Indomethacin (mg)	100	100	100	100	100
Ficus carica fruit mucilage (%)	5	10	15	20	25
Glycerin(ml)	0.3	0.3	0.3	0.3	0.3
Propylene Glycol(ml)	0.18	0.18	0.18	0.18	0.18
Span-80 (ml)	0.06	0.06	0.06	0.06	0.06
Methyl paraben(g)	0.025	0.025	0.025	0.025	0.025
Propyl paraben(g)	0.015	0.015	0.015	0.015	0.015
Water up to (ml)	20	20	20	20	20

Evaluation of Transdermal Films

Thickness

The thickness of the patch was determined using Digital caliper (BAKER-EC 10, Hyderabad, India). The mean thickness was measured at five different points of the film. Figure-1

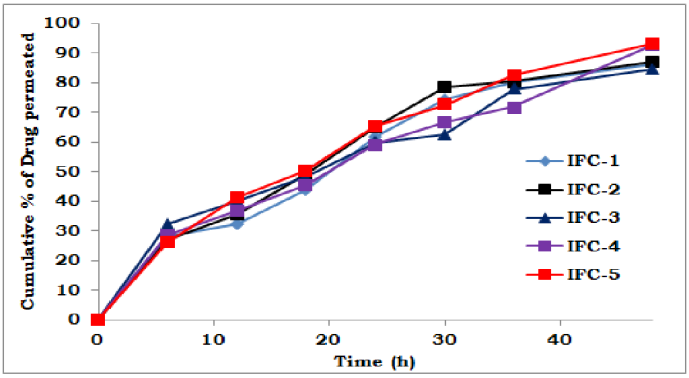

Figure. 1: Zero order plots of formulated transdermal patches

Determination of tensile strength

Tensile strength was determined by using computerized Precisa bottom-loading balance, with necessary modifications. A 1 X 1cm patch was taken and subjected to studies.

Flatness and elongation brake

Longitudinal strips were cut out from the prepared transdermal patches. The flatness was determined at various points by using vernier calipers (Arora P, Mukherjee B *et al.,* 2002). The percentage elongation brake was determined by noting the length just before the break point and substituted in the eq.1.

Elongation (%) = $L_1 - L_2$ X 100/ L_2 (1)

Where

L_1 = final length of each strip
L_2 = initial length of each strip.

Folding endurance

Folding endurance of patches was determined by repeatedly folding a small strip of film (2 X 2 cm) at the same place till it broke. The number of times the film could be folded at the same place without breaking was the folding endurance value (Tanwar YS *et al.,* 2007).

Moisture content

The strips were then weighed individually and kept in a desiccator containing activated silica at 30°C for 12 hours. The films were reweighed individually until a constant weight was obtained (Gupta R and Mukherjee B 2003). Percentage of moisture content was then calculated based on the change in the weight with respect to the initial weight of the film. The prepared patches were cut into 20 × 50 mm strips. The film was weighed and kept in a desiccator containing calcium chloride at 30°C and dried for at least 12 hours. The film was weighed until it showed a constant weight. The moisture content was the difference between the constant weight taken and the initial weight.

Moisture uptake

The physicochemical studies like moisture content and moisture uptake provide the information regarding the stability of the formulation. The moisture content was determined by keeping the drug matrix patches in a desiccator containing activated silica until they showed constant weight. The percentage moisture content was calculated from the weight differences relative to the final weight. The water absorption capacities of various films were determined at 75% and 93% relative humidity (RH). Films were cut into 25 × 60 mm strips. A strip was weighed and kept in a desiccator at 40°C for 24 hours, removed and exposed to RH conditions of 75% (containing saturated solution of sodium chloride) and 93% (containing saturated solution of ammonium hydrogen phosphate) in different desiccators at room temperature. Then the films were measured periodically to constant weights. The water absorption capacity of the films (in weight %) was calculated in terms of percentage increase in the weight of film over the initial weight of the specimen.

Drug content determination of film

Four pieces of 1 cm^2 each (1 X 1 cm) were cut from different parts of the prepared transdermal patch. Each was taken in separate stoppered conical flasks containing 100 ml of suitable dissolution medium (0.1N HCL: CH_3OH mixture) and stirred vigorously for 6 hours using magnetic stirrer. The above solutions were filtered and suitable dilutions were made. Absorbance was observed using UV-Visible spectrophotometer (Systronics 117, Mumbai, India) at their respective wavelengths, against a blank solution which was prepared by the same protocol but not containing drug.

In-Vitro skin permeation studies with polymeric matrices

The transdermal patches were subjected to *in-vitro* evaluation across rat dorsal skin. After removal of epidermal hair, skin was cleaned and any adhering subcutaneous tissue and blood vessels were removed. The skin was mounted overnight (12 hours) on receptor phase to remove any water-soluble (UV absorbing) material. The *in-vitro* skin permeation of Indomethacin from various transdermal patches was studied using locally fabricated Keshary-Chien type of diffusion cell (Cleary GW 1993). The diffusion cell consists of two parts. The upper part is the donor compartment and contains the active ingredient and the carrier adhesive/patch; the bottom part contains the receptor solution, the water jacket for temperature control and the sampling port. The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm^2 and 17.5 ml respectively. The temperature was maintained at 37±2°C. The receptor compartment contained 17.5 ml of phosphate buffer saline (PBS) IP (pH 7.4) stirred by magnetic stirrer. The permeability studies were carried out across both rat and cadaver skin. Samples (1.0 ml) were withdrawn and replaced with the same volume of fresh receptor solution, through the sampling port of the diffusion cell at predetermined time intervals till 48 hours. Absorbance of the withdrawn samples were measured at 238 nm. The experiments were done in triplicates, simultaneously blanks were also run and the average values reported.

Evaluation of skin irritation potential of polymeric matrices

The primary skin irritation studies were carried out using modified Draize test (Draize JH., 1994). The hair of rabbits were removed by shaving from the dorsal area on both sides 24 hours before test, one side of the back of each rabbit i.e. untreated skin area serves as the control for the test. Medicated patch was secured on experimental side using adhesive tape and the non-medicated patch was adhered on the control side of six rabbits (Kligman AM and Christopher E., 1963). These patches were covered with occlusive covering to approximate the condition of use. The medicated patches were changed after 48 hours and the fresh patches were secured at the same site. However the patches on the control side were not changed. The patches were secured on the back for seven days. After removal of patch after a week each of the areas were examined for any sign of erythema or edema.

Drug- Polymer Interaction studies

Interaction studies were conducted on the medicated TDDS formulations by comparing them with the pure drug and placebo formulations on the basis of assay, UV, FTIR and DSC analyses.

Assay

The TDDS was dissolved in isopropyl alcohol and the drug content was determined by UV spectrophotometry.

UV Analysis

The medicated and blank formulations were filtered through Whatman filter paper no. 42 and scanned spectrophotometrically in the range of 200–400 nm.

Fourier Transform Infra-Red analysis

The FTIR absorption spectra of the pure, medicated and blank formulations were taken in the range of 400–4000 cm^{-1} using the potassium bromide disc method (Hitachi-270-30 IR spectrophotometer, Japan).

Differential Scanning Calorimetry

DSC of pure drug and in combination with polymer used were studied (Perkin Elmer, USA) at scanning rate of 10^0C/ min between 50 to 300^0C.

Stability studies

Stability studies were conducted according to the International Conference on Harmonization (ICH) guidelines by storing the TDDS samples at 40± 0.5 °C and 75 ± 5% RH for 3 months (Remunan C *et al.,* 1992).

RESULTS

The thicknesses of formulated matrix transdermal patches were ranged from 630±35.6 to 690±25.6 µm. The tensile strength of formulated patches was ranged from 0.285 ± 0.25 to 0.326 ± 0.10 N/mm^2 and these values were represented in Table 2. The elongation of formulated matrix transdermal patches were ranged from 15.33±0.89 to 26.23±0.84 %. The folding endurance of formulated patches was ranged from 98±1.8 to 124±0.9 and these values were represented in Table 2. The weight of the formulated patches was ranged from 1.561±0.51 to 1.597±0.01g. The moisture content was ranged from 2.645±0.35 to 2.854±0.56%. The moisture uptake at relative humidity of 75% was ranged from 2.210± 0.96 to 4.125± 0.52% and at relative humidity of 93% was ranged from 3.906 ± 0.59 to 6.145 ± 0.01. The drug content in formulated films was ranged from 97.4± 0.02 to 100.7± 0.45%. All these parameters were shown in Table 3. The optimized formulation (IFC-5), adhesive tape used (USP) and blank (formulation without drug) were evaluated for skin erythema and edema on rabbits back by comparing with a Formalin (0.8%v/v) solution. The optimized formulation IFC-5 (Indomethacin-patch) scored 1.52±0.35 for erythema and 1.24±0.17 for edema whereas blank scored 1.51±0.14 for erythema and 1.18±0.42 for edema. The skin irritation test values were represented in Table 4. In the present work, stability study was carried out for selected formulation (IFC-5) at 40± 0.5°C and 75±5% RH for 3 months using programmable environmental test chamber (Remi, India).

Table 2: Result of mechanical properties of formulated transdermal patches

Parameter	Thickness (µm)	Tensile strength (N/mm^2)	Elongation (%)	Folding endurance
IFC-1	630±35.6	0.294 ± 0.14	15.33±0.89	98± 1.8
IFC-2	650±62.5	0.285 ± 0.25	18.22±0.23	124±0.9
IFC-3	685±55.8	0.311 ± 0.05	22.66±0.36	115±1.2
IFC-4	690±25.6	0.325 ± 0.12	24.95±0.39	99±1.5
IFC-5	635±29.6	0.326 ± 0.10	26.23±0.84	119±1.4
Number of trials (n) = 3				

Table 3: Result of mean weights, moisture content, moisture uptake and dug content of formulated transdermal patches

Formulation	Weights (g)	Moisture content (%)	Moisture uptake (%)		Drug Content (%)
			RH 75%	RH 93%	
IFC-1	1.561±0.51	2.848±0.12	3.206± 0.37	6.145 ± 0.01	97.4± 0.02
IFC-2	1.584±0.12	2.851±0.23	4.125± 0.52	5.249 ± 0.12	98.3± 0.19
IFC-3	1.564±0.14	2.645±0.35	3.130± 0.73	3.936 ± 0.49	99.7± 0.23
IFC-4	1.566±0.34	2.758±0.35	2.210± 0.96	5.219 ± 0.20	100.2± 0.22
IFC-5	1.597±0.01	2.854±0.56	3.206± 0.37	3.906 ± 0.59	100.7± 0.45
Number of trials (n) = 3					

Table 4: Results of skin irritation test of formulated transdermal patches.

Formulation	Visual observation	
	Erythema	Edema
Normal	0.00±0.00	0.00±0.00
Adhesive tape(USP)	1.31±0.21	1.60±0.25
IFC-5 (Indomethacin-patch)	1.52±0.35	1.24±0.17
Blank	1.51±0.14	1.18±0.42
Formalin (0.8% v/v)	3.75±0.18	3.39±0.36

Visual observation values are expressed as Mean ±SEM, n=6;
* Significant compared to formalin ($p<0.05$);
IFC-5=Indomethacin *Ficus carica* fruit mucilage patch;
Blank= Patch without drug

DISCUSSION

The formulated patches showed uniformity in thickness. The prepared patches did not show any signs of cracking, which might be attributed to the addition of the plasticizer, propylene glycol. The folding endurance measures the ability of patch to withstand rupture. The folding endurance was measured manually and results indicated that the patches would not break and maintain their integrity with general skin folding when used. The moisture content of the prepared transdermal film was low, which could help the formulations remain stable and from being a completely dried and reduce brittleness during storage.

The patches did not show any visible erythema or edema with the formulation or the base used. After the accelerated stability studies the patches were evaluated for physicochemical parameters like thickness, flatness, folding endurance, tensile strength, moisture content and moisture uptake, drug content as well as drug release. The absence of edema indicates that the polymeric patches are compatible with the skin and hence can be used for transdermal application. The drug permeation from prepared patches was sustained within the therapeutic range. The stability study indicates that the formulation is quite stable at accelerated conditions.

CONCLUSION

This investigation revealed that *Ficus carica* fruit mucilage appears to be suitable for use as a matrix former in the manufacturing of transdermal patches because of its satisfactory physical and mechanical properties. The *in-vitro* permeation data revealed that dried *Ficus carica* fruit mucilage can be used as a matrix former in transdermal delivery systems.

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