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Research Article

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Preparation and *in vitro* Permeation Studies of Diclofenac potassium *Ficus benghalensis* Fruit Mucilage Transdermal Patches

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ABSTRACT

The main purpose of the present study was to develop transdermal patches (matrix type) of Diclofenac potassium using various ratios of *Ficus benghalensis* fruit mucilage. Physical parameters such as moisture content, moisture uptake, tensile strength, elongation and folding endurance were evaluated. The transdermal patches were prepared using Diclofenac potassium with *Ficus benghalensis* fruit mucilage by the solvent evaporation technique. The interactions between Diclofenac potassium & *Ficus benghalensis* fruit mucilage were performed. The transdermal patches were subjected to various physicochemical parameters viz., mechanical properties, permeation studies and skin irritation studies were performed. 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 key role as permeability enhancers. The patches were found to seemingly free of potentially hazardous skin irritation. The experimental result shows that the release of drug from the patch was delayed in controlled manner as the proportion of *Ficus benghalensis* fruit mucilage increased. It was concluded that Diclofenac potassium can be developed as a transdermal patches with *Ficus benghalensis* fruit mucilage.

Key words: Diclofenac potassium, *Ficus benghalensis* fruit mucilage, transdermal patches, *in vitro* permeation

INTRODUCTION

Transdermal delivery has many advantages over conventional dosage forms as it avoids 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 to systemic circulation.

Diclofenac potassium is a NSAID, which is a commonly prescribed drug for relieving pain and inflammation. It is chemically is 2-[(2, 6-dichlorophenyl) amino] benzeneacetic acid monopotassium salt. Diclofenac potassium is a weak acid (PKa = 4.0±0.2 at 25^{o}C in water). Diclofenac potassium is a faintly yellowish white to light beige, virtually odorless, slightly hygroscopic crystalline powder. It is freely soluble in methanol, ethanol, and practically insoluble in chloroform and in dilute acid. Diclofenac potassium is soluble in water [2].

Diclofenac potassium is 50 mg three times a day [3]. The pharmacokinetics and dosage schedule supports once daily controlled release formulations for Diclofenac potassium for better control of pain and inflammation [4]. The transdermal patches were evaluated *in vitro* and for controlled release. Various experimental reports indicated that Diclofenac potassium as a good candidate for controlled release formulation. In this study, *Ficus benghalensis* (FB) fruit mucilage was used as a matrix polymer for controlling release of Diclofenac potassium.

MATERIALS AND METHODS

Materials

Diclofenac potassium was obtained as a gift sample from Waksman Selman Pharmaceuticals Pvt. Ltd, Anantapur. *Ficus benghalensis* fruits (FB) were procured from plant growing around Anantapur and authenticated by the department of Pharmacognosy, Balaji College of Pharmacy, Anantapur, India and voucher specimen number was obtained (BCP/PCOG/56). The same was preserved in Pharmacognosy department herbarium of Balaji College of Pharmacy, Anantapur. Glycerin, Propylene glycol, Methyl paraben, Propyl paraben and Span-80 procured from S.D. Fine chemicals Mumbai. All the reagents used were of AR grade. The drug samples were characterized and authenticated by means of UV spectrophotometric method along with determination of solubility and pH.

Methods

Extraction of mucilage

The fresh fruits of FB were obtained from plants growing around Anantapur town. 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 pulps of the fruits were crushed and soaked in water for 5–6 h, boiled for 30 min and left to stand for 1 h 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 (in the quantities of three times the volume of filtrate) was added to precipitate the mucilage [4]. The mucilage was separated, dried in an oven at 40°C, collected, ground, passed through a # 80 sieve and stored in a desiccator at 30°C and 45% relative humidity till use.

Preparation of transdermal patches

Various ratios of FB mucilage was taken in a beaker, Propylene glycol (plasticizer), Span-80 (penetration enhancer) Propyl paraben, Methyl paraben (preservatives) and Diclofenac potassium (100 mg) were 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 of the Petri dish. The rate of evaporation was controlled by inverting a funnel over the Petri dish. After 24 h the dried patches were taken out and stored in desiccator [5, 6]. The various formulae were showed in Table 1.

Table 1: Different formulae of transdermal patches

INGREDIENTS	DFB-1	DFB-2	DFB-3	DFB-4	DFB-5
Diclofenac potassium(mg)	150	150	150	150	150
FB fruit mucilage (%)	25	50	75	100	125
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)	0.05	0.05	0.05	0.05	0.05
Propyl paraben(g)	0.05	0.05	0.05	0.05	0.05
Water up to (ml)	20	20	20	20	20

Pre formulation study

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 patches were dissolved in isopropyl alcohol (IPA) 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 at the range of 200–400 nm (Systronics-117, Mumbai).

FTIR 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)

The DSC of the pure drug and drug-polymer blend was studied at a scanning rate of $10^{o}C$/ min between 50 to $300^{o}C$ (Perkin Elmer, USA)

Evaluation of formulated 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.

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.

Elongation brake

Longitudinal strips were cut out from the prepared transdermal patches. The flatness was determined at various points by using vernier caliper [7]. The percentage elongation brake was determined by noting the length just before the break point and substituted in the following equation.

$$\text{Elongation (\%)} = L_1 - L_2 \text{ X } 100 / L_2$$

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 [8].

Moisture content

The strips were then weighed individually and kept in a desiccator containing activated silica at 30°C for 12 h. The films were reweighed individually until a constant weight was obtained [9]. 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 h. 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 properties 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 h, 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 medium (0.1-N HCL: CH_3OH mixture) and stirred vigorously for 6 h using magnetic stirrer. The above solutions were filtered and suitable dilutions were made. Absorbance was observed using UV-Visible spectrophotometer (Systronics-117, Mumbai) 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/blood vessels were removed. The skin was mounted overnight (12 h) on receptor phase to remove any water-soluble (UV absorbing) material. The *in vitro* skin permeation of Diclofenac potassium from various transdermal patches was studied using locally fabricated Keshary-Chien type of diffusion cell [10]. 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

surrounded by the water jacket for temperature control and the sampling port [11]. 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 [13]. 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 h [14]. Absorbance of the withdrawn samples was measured at 277 nm. The experiments were done in triplicates, simultaneously blanks were also run and the average values reported. The *in vitro* permeation data follows zero order release and showed in Fig.1.

Evaluation of skin irritation potential of patches

The primary skin irritation studies were carried out using modified Draize test [16]. The hair of rabbits were removed by shaving from the dorsal area on both sides 24 h 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 [17]. These patches were covered with an occlusive till use. The medicated patches were changed after 48 h 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.

Accelerated Stability studies

Stability studies were conducted according to the International Conference on Harmonization (ICH) guidelines for the selected formulation (DFB-5) at 40 ± 0.5°C and 75 ± 5 % RH for 3 months using programmable environmental test chamber (Remi, India). The samples were evaluated for physicochemical parameters like thickness, flatness, folding endurance, tensile strength, moisture content, moisture uptake, drug content as well as drug release. The drug solvent was determined by withdrawing the sample at 0, 30, 60 and 90 days and analyzed for the drug content by HPLC (Waters, USA). The chromatographic conditions were as follows: column Lichrospher RP- 5µm (125×4 mm); mobile phase: methanol: 0.01M disodium hydrogen phosphate (60:40); flow rate: 1.5 ml min^{-1}; injection volume: 20 µL; detector: UV at 277 nm; Retention time: 5.5 min. The results were represented in Table 6.

RESULTS AND DISCUSSION

The UV, FTIR and DSC analysis revealed that there were no incompatibilities between Diclofenac potassium and FB fruit mucilage. The thickness of formulated matrix transdermal patches was ranged from 576±12.9 to 585±15.5µm. Tensile strength indicates the strength of film and the risk of film cracking. But, no sign of cracking in prepared transdermal films was observed, which might be attributed to the addition of the plasticizer, Propylene glycol. The results of tensile strength were shown in Table 2. Tensile strength of formulated patches was ranged from 0.322±0.02 to 00.331±0.01N/mm^2. The elongation of formulated matrix transdermal patches were ranged from 11.34±0.24 to 20.18±0.15%. 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 would maintain their integrity with general skin folding when used. The results of folding endurance were shown in Table 2. It was found to be high in patches containing higher amount of the FB fruit mucilage and it was ranged from 81±1.9 to 91±1.8. The weight of the tablets was within the Pharmacopoeial limits and ranged from 1.54±0.02 to 1.59±0.01g. The results of the moisture content studies for different formulations are shown in Table 3. The moisture content varied to a small extent in all the trials. 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 moisture content was ranged from 2.27±0.13 to 2.55±0.12%. The results of moisture uptake studies for different formulations are shown in Table 3. The moisture uptake of the transdermal formulations was also low, which could protect the formulations from microbial contamination and also reduce bulkiness of films. At RH 75% the moisture content was ranged from 3.15±0.51 to 5.55±0.41% and at RH 93% it ranged from 3.28±0.23 to 55.69±0.22%. The drug content in formulated films was ranged from 99.3±4.84 to 100.2±3.15 %.

Table 2: Result of mechanical properties of formulated transdermal patches

Parameter	Thickness (µm)	Tensile strength (N/mm^2)	Elongation (%)	Folding endurance
DFB-1	585±15.5	0.322±0.02	11.34±0.24	81±1.9
DFB-2	580±24.5	0.326±0.01	12.38±0.18	82±0.9
DFB-3	583±15.5	0.328±0.02	13.15±0.16	84±1.8
DFB-4	576±12.9	0.329±0.01	15.23±0.22	88±1.6
DFB-5	580±18.9	0.331±0.01	20.18±0.15	91±1.8
All values mentioned as mean ±S.D; 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%	
DFB-1	1.59±0.01	2.41±0.11	5.55±0.41	5.69±0.22	99.8±2.88
DFB-2	1.58±0.01	2.55±0.12	4.11±0.41	4.85±0.25	99.5±2.26
DFB-3	1.58±0.02	2.27±0.13	3.18±0.59	4.58±0.40	100.2±3.15
DFB-4	1.55±0.01	2.28±0.04	4.22±0.52	4.55±0.22	99.3±4.84
DFB-5	1.54±0.02	2.29±0.21	3.15±0.51	3.28±0.23	100.1±6.69
All values mentioned as mean ±S.D; Number of trials (n) = 3					

In vitro skin permeation studies with polymeric matrices

The results of skin irritation studies showed negligible erythema with prepared films when compared with control. The absence of edema indicates that the polymeric patches are compatible with the skin and hence can be used for transdermal application. The patches did not show any visible erythema or edema with medicated and non-medicated patches. The values were shown in table 4.

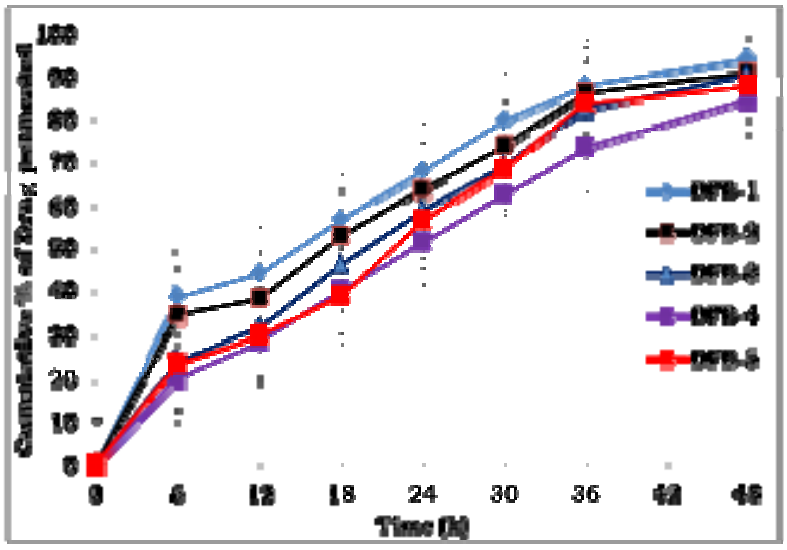

Fig. 1: Zero order plots of DFB formulations

Table 4: Results of skin irritation test

Formulation	Visual observation	
	Erythema	Edema
Normal	0.00±0.00	0.00±0.00
Adhesive tape(USP)	1.31±0.25	1.62±0.29
DFB-5 (Diclofenac potassium-patch)	1.55±0.30*	1.25±0.19
Blank	1.50±0.16	1.19±0.40
Formalin (0.8% v/v)	3.76±0.11	3.37±0.30

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

Table 5: Accelerated stability study of selected DFB-5patches

Parameter	Before *stability studies*	After *stability studies(90 days)*
Thickness (µm)	580±18.9	580±18.7
Elongation (%)	20.18±0.15	21.05±0.11
Folding endurance	91±1.8	90±1.2
Tensile strength (N/mm^2)	0.331±0.01	0.332±0.01
Moisture content (%)	2.29±0.21	2.31±0.22
Moisture uptake (RH 75%) %	3.15±0.51	3.16±0.55
Moisture uptake (RH 93%) %	3.28±0.23	3.29±0.22
Drug content (%)	100.1±6.69	100.0±6.65

All Values mentioned as mean ±S.D; Number of trials (n) =3

CONCLUSION

This study revealed that *Ficus benghalensis* 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 FB fruit mucilage can be used as a matrix former in transdermal delivery systems.

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