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Development and Evaluation of Aceclofenac Loaded Transdermal Film

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Abstract: The purpose of this research study was to formulate transdermal film loaded with Aceclofenac (ACF) by solution casting method using chemically modified locust bean gum (MLBG) and sodium alginate (SA) in various proportions and examines the influences of various process parameters like drug: polymer ratio, concentration of plasticizer (glycerol) and permeation enhancer (menthol) on physicochemical properties of drug loaded transdermal film and drug release potential. *An in-vitro* skin permeation study of optimized formulation was studied. Carrageenen induced rat paw edema model was used to investigate their *in vivo* performance. The drug content uniformity of the prepared films lies in the range of 1.90 to1.95 mg/cm^2. Increased ratio of MLBG and decreased ratio of SA showed decreased tensile strength with increased % elongation. The thickness of the films varied from 0.29 to 0.38 mm due to increased MLGB and decreased ratio of SA. Folding endurance decreases as the MLGB concentration increases with decreased SA ratio. ACF was found to be compatible and stable with the prepared formulation as confirmed by Fourier transform infrared (FTIR) and Differential Scanning Calorimetry (DSC) studies. *In-vitro* skin permeation studies showed a controlled release for 24h. Skin irritation test reveals no signs of erythema, edema or ulceration. The study results suggest that polymer based prepared transdermal films are potential vehicle to achieve controlled transdermal delivery of ACF for effective therapy and showed good skin tolerability.

Keywords: Aceclofenac, MLBG, Sodium alginate, Menthol, Glycerine.

INTRODUCTION

The goal of controlled release dosage form is to establish relatively constant plasma drug concentrations, avoiding the peak and valleys associated with conventional dosage forms. Controlled delivery of drugs by the transdermal route provides unique opportunity in order to maintain constant plasma levels of drug [1]. The skin is the most expansive and readily accessible organ of the human body. It has been used as an administration site of drugs with local action on it and on muscle beneath, and it is now recognized that other drugs with systemic action can also be introduced through the skin. Transdermal drug delivery systems (TDDS) provides a variety of advantages inherent in the transdermal route, including elimination of gastrointestinal absorption problems and hepatic first pass effect, reduction of dosage and dose interval, predictable and extended duration of activity, improved patient compliance, and quick termination by simple removal of the system from the skin surface, and possible self administration. The successful introduction of several TDDS has greatly expanded the search for drugs suitable for delivery via the transdermal route [2,3].

Transdermal patches have the aim to transport drugs through the skin into the blood circle. One of the

proposed advantages of transdermal delivery is the possibility to attain sustained and constant drug levels [4]. Therapeutically these dosage forms provide constant plasma drug levels duplicating the benefits of intravenous infusion constantly. Locust bean gum (LBG) is a galactomannan vegetable gum extracted from the seeds of carob tree.It is used as a thickener and gelling agent in food technology. LBG is a poly disperse consisting of non ionic molecules made up of about 2000 residues.LBG insoluble in ethanol,has lower viscosity than gaur gum and soluble in hot water.It is a non ionic gum and not affected by ionic strength or pH, but will degrade at extremes at higher temperature [5].

Aceclofenac is a Aceclofenac is an Non-steroidal anti-inflammatory drug (NSAID) used for relief of pain and inflammation in osteoarthritis, rheumatoid arthritis and ankylosing spondylitis.Aceclofenac is a phenylaceticacid derivative and it is a inhibitor of prostoglandin synthesis. Aceclofenac is readily absorbed from the gastrointestinal tract, peak plasma concentration occur about 4h after a dose, 99% bound to plasma protein. Unfortunately, the systemic administration of this drug, similar to other NSAIDs, presents gastrointestinal side effects that could be avoided by using a topical application [6-9].

The common method to improve drug permeation through the skin is to use penetration enhancers. Penetration enhancers can change the structure of skin lipids and alter the skin barrier function. These compounds, even if they increase the transdermal flux of several drugs. The occurrence of systemic side-effects with some of these formulations is indicative of absorption through the skin. A number of drugs have been applied to the skin for systemic treatment [10-12]. Sodium alginate is the sodium salt of alginic acid. Occurs as white to yellowish brown filamentous, grainy, granular or powdered forms.it is used as Stabilizer, thickener, gelling agent, emulsifier. Dissolves slowly in water, forming a viscous solution; insoluble in ethanol and ether [13].A thorough literature search that revealed a lack of information on combination of modified locust bean gum (MLBG) and sodium alginate based transdermal films for the transdermal drug delivery using solution casting method. This study had the aim of developing and optimizing a transdermal film and compared with a conventional gel, with the main objective of releasing ACF locally, so that the patient can easily apply it. Evaluate the transdermal film loaded with ACF with the purpose of prolonging its use.

METERIALS AND METHODS

Aceclofenac was obtained from M/s Amoli Organics, Mumbai, India as a gift sample. It is white, odorless, nonhygroscopic, and fine to granular powder. Freely soluble in ethyl alcohol, chloroform, acetone, and ether and soluble in phosphate buffer pH 7.4, but practically insoluble in water. The plasma elimination half life is 4h. LBG was procured from M/s Sisco research laboratories, Mumbai, is a tasteless and odorless, white to slightly off white, granular powder. soluble in hot water, glacial acetic acid, ethanol, methanol and propylene glycol, slightly soluble in acetone depending on the degree of substitutions, practically insoluble in cold water. Glycerol and Menthol were procured from SD Fine chemical Ltd, Bangalore, and all other chemicals were of analytical grade.

Fabrication of Tansdermal Films

MLBG was prepared by the method reported by Manjil patel *et al* [14]. Powdered LBG was placed in a porcelain bowl and subjected to heating in hot air oven at103° C, after cooling, clear solution was obtained and stored in airtight container at 25° C.

Drug containig transdermal films were prepared by solution casting method. Required amounts of blend of MLBG and SA (Table 1) were weighed and dispersed in a specified volume of water, allowed to swell for 2 h. Accurately weighed ACF (2.5 mg /mm^2) and menthol (1– 5 % w/w) was dissolved in ethanol (8 ml) by stirring for 20 min.The above mixture mixed with different concentrations of Glycerine (1– 5 % w/w) and prepare polymeric solutions for 30 min. Finally mixed soft mass was pourd on to specially designed cleaned glass moulds with the plastic transparent sheet and kept in a vaccum drier until to get the dried film. The cast polymeric film with different formulations were then peeled off covered with aluminum foils and stored in a desiccator until further study. Conventional gel having the same quantity of (2.5%w/w) ACF.

Evaluation of films

Mechanical properties, such as tensile strength and percentage elongation at break of MLBG blends were measured as per ASTM D 638 Universal Testing Machine (H 50 K M, 50K N Hounsfield, UK). The thickness of the dry films was measured using microprocessor coating thickness gauze (Quint sonic, Mumbai, India). The dry films (2.5cm x 2.5cm) were cut and placed on a control plate and the thickness of the film was measured. A minimum of three samples were tested for each formulation and the average value was recorded.

Drug content in films

Accurately weighed films were cut rectangularly (2.5cm^2) and conventional gel (2.5 gm /ml) was allowed to dissolve in 100 ml phosphate buffer pH 7.4 seperately. Then the above formulations

were sonicated in ultra sonicator for 60 min and appropriately diluted.The concentration of ACF in the receptor phase was determined by HPLC method [15].The HPLC system consists of HPLC shimadzu (Tokyo, Japan) LC – 6A model fitted with a µ- bond pack C_{18} (4.6 x 250 mm) column, flow rate of 1 ml / min, mobile phase consist of acetonitrile and 10 mM phosphate buffer (30:70 v/v), pH – 7.4), wavelength at 276 nm at ambient temparature. The samples were centrifuged at 6000 rpm for 10 min.To remove any particles and supernatant was injected to HPLC, standard solution was prepared by dissolving 20 mg of ACF in phosphate buffer pH – 7.4. Final concentrations of the solution will be in the range of 3 µg/ml. The average drug content of trhee replicate samples was measured.

Folding endurance test

Folding endurance test was determined by repeatedly folding the film at the same place until it broke. The number of times the film could be folded at the same place without breaking was the floding endurance value.

Percentage moisture loss

The percentage moisture loss was carried out to check the integrity of the film at dry condition. This was carried out in the following manner. The films were weighed accurately and kept in the desiccator containing anhydrous calcium chloride. After 3 days, the films were taken out and weighed. The moisture loss was calculated using the formulaed.

Percenage moisture loss=

$$\frac{\text{initial weight- final weight x 100}}{\text{initial weight x 100}} \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Percentage moisture uptake

Films were placed on open 5 ml glass vials containing 3.5 g silica gel beads and held in place with a screw lid having a 0.80 cm diameter of test area (0.50 cm^2). The dessicators containing vials kept in chambers with 75 % RH (saturated Nacl solution) and 95 % relative humidity (RH) (water) were kept at 37°Cfor 7 days. Moisture uptake by the films was measured by weighing the dried film at 100°C for 24 h.

Percentage moisture uptake =

$$\frac{\text{final weight – initial weight x 100}}{\text{final weight}} \quad \ldots\ldots\ldots\ldots..(2)$$

Flatness

An ideal transdermal patch should possess smooth surface and should not constrict over time after application to the skin. Therefore, the flatness of the films was studied by cutting them into strips and placing the strips on the square glass molds and then measuring their lengths. Percent flatness was determined as follows:

Percentage flatness=

$L_1 - L_2 / L_1 \times 100$……..... (3)

Where L_1 and L_2 are initial and final lengths respectivey.

FTIR- Analysis

In order to evaluate the integrity and compatibility of the drug with the carrier polymer in the polymer-drug matrix formulations, FTIR spectra of the pure drug, optimized formulation, were obtained by FTIR spectrophotometer using Jassco - 4100, Japan.

Differential Scanning Calorimetry (DSC)

All dynamic DSC studies were carried out using DuPont thermal analyzer with 2010 DSC194 module. The instrument was calibrated using high purity indium metal as standard. The DSC scans of the samples were recorded in the temperature range ambient to 180 ^{0}C under nitrogen gas purge at a heating rate of 10°C/ min.

Drug Diffusion Studies

Drug diffusion studies were carried out using a bi chambered glass diffusion tube having donor receptor compartment. A specimen dimension of film formulation (2 cm^2) was fixed to the hydrated cellophane membrane at one end of the open glass tube and placed in the donor compartment containing pH 7.4 phosphate buffer. The entire surface of the membrane was in contact with the receptor compartment containing 100 ml of pH 7.4 phosphate buffer. The content of the receptor compartment was agitated by a magnetic stirrer at 80 rpm. The temperature of the system was maintained at 37 ± 1°C. Samples of 1 ml were withdrawn from receptor compartment at regular intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8 h) and replaced by equal volumes of fresh receptor medium. The drug concentration was determined by HPLC.

Stability of the transdermal film and prepared ACF gel

Formulation F3 (2.5 cm^2) and conventional gel were subjected for stability studies at 25° C/60% RH, 30°C/65% RH and 40°C/75% RH for 90 days.The

above formulations were evaluated for drug content periodically.

In Vitro Skin Permeation Studies

In vitro skin permeation studies were performed on a Franz diffusion cell with an effective diffusional area of 2.5 cm^2 and 12 ml of receiver chamber capacity using rat abdominal skin. The animal study protocol was reviewed and approved by the Animal Ethics Committee at the Department of Pharmaceutics, Bharathi College of Pharmacy, Mandya, India. Male wistar rats weighing 145-156 g were used to excise full thickness skin. Rats were anaesthetized by ether and then hair of abdominal skin was removed by using electric clipper. Special care was taken while removing hairs, so as not to destroy the stratum corneum. The cleaned skin was washed with distilled water and stored in the deep freezer at –21°C until further use. The skin was brought to room temperature and mounted between the donor and receiver compartment of the Franz diffusion cell, where the stratum corneum side faced the donor compartment and the dermal side faced the receiver compartment. Initially the donor compartment was empty and the receiver chamber was filled with ethanolic phosphate-buffered saline (PBS) pH 7.4 (30:70% v/v) [16]. The receiver fluid was stirred with a magnetic rotor at a speed of 250 rpm, to maintain the hydro dynamics of receiver fluid and the temperature maintained at 32°C ± 1°C. All the ethanolic PBS was replaced after every 30 minutes to stabilize the skin. It was found that the receiver fluid showed negligible absorbance after 5h and beyond, indicating complete stabilization of the skin. After complete stabilization of the skin, 2.5cm^2 of the optimized film of ACF (F3) was placed into each donor compartment and sealed with paraffin film to provide occlusive conditions. Samples (1.0 ml) were withdrawn at regular intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8h), filtered through a 0.45-membrane filter.The volume of release media was maintained by adding equal volume of fresh media after every sampling. Concentration of the ACF in the sample was measured by HPLC.

Permeation Data Analysis

The cumulative amount of drug permeated through the skin (mg/cm^2) was plotted as a function of time (t) for each formulation. Drug flux (permeation rate) at steady state (J_{ss}) was calculated by dividing the slope of the linear portion of the graph by the area of the diffusion cell. The permeability coefficient (K_p) was calculated by dividing J_{ss} by the initial concentration of drug in the donor cell (C_0),

$$K_p = J_{ss}/C_0 \quad \text{..... (4)}$$

Enhancement ratio (Er) was calculated by dividing the J_{ss} of the respective formulation by the J_{ss} of the control formulation,

$$E_r = \frac{J_{ss} \text{ of formulation}}{J_{ss} \text{ of control}} \quad \text{.....(5)}$$

Anti inflammatory studies

The Anti inflammatory test was carried out on male albino rats weighing 142 to151 g. The animals were kept under standard laboratory conditions, with temperature of 25^0 C ± 1^0 C and relative humidity of 60% ± 5%. The animals were housed in cages, 5 per cage, with free access to a standard laboratory diet. The anti-inflammatory and sustaining action of the optimized formulation F3 was evaluated by the carrageenaninduced hind paw edema method on Wistar rats. The anti-inflammatory activity of ACF transdermal films were evaluated with rat paw edema test. The transdermal film was applied to the shaved abdominal skin of male rats. Just before administration of transdermal film, 1% carrageenan-saline solution (0.1 ml) was injected into each hind paw of rats. The thickness of paw edema induced by carrageenan was measured by using a standard screw gauge during 8 h after application of ACF transdermal film.

Statistical Analysis

Results are given as mean ± standard deviation (S.D). The cumulative amount (mg/cm^2) of KF permeation through rat skin was plotted as a function of time (h). The slope of the linear portion of the plot is presented as the flux (mg/cm^2/h). The results were analyzed statistically using Student's *t*- test or one way analysis of variance (ANOVA).Significance was determined at $P < 0.05$.

Table 1: Compositions of various film formulations

Formulation	Drug (%)	MLBG (%)	SA (%)	Glycerine (%)	Menthol (%)
F1	2.5	60	36.0	1.0	1.0
F2	2.5	64	32.0	2.0	2.0
F3	2.5	68	25.5	3.0	3.0
F4	2.5	72	19.5	4.0	4.0
F5	2.5	75	14.0	5.0	5.0

Table 2: Physico chemical evaluation of drug loaded films

Formulation	Tensile strength (Mpa/mm^2)*	Drug content (mg/cm^2)*	Thickness (µm)*	Folding endurance*	Moisture uptake (75%)*	Moisture uptake (95%)*	Elongation (%)*
F1	2.425	2.38	133	154	1.44	1.8	27.34
F2	2.721	2.39	136	151	1.71	1.98	28.75
F3	2.917	2.43	138	149	2.32	2.68	29.54
F4	3.012	2.32	139	147	2.65	2.95	30.12
F5	3.370	2.34	141	145	2.85	3.12	31.43

* n =3

Table 3: Stability studies of F3 and conventional gel formulations

Sampling intervals (days)	40 ºC/75% RH*	40 ºC/75 % RH*
15	98.94	98.94
45	98.89	98.82
60	98.76	98.71
90	98.71	98.66

* n =3

Table 4: Permeability parameters of all formulations

Formulation	Menthol (% w/w)	$J_{SS} \pm$ SD ($mg/cm^2/h$) *	Permeability coefficient*	* E_r	Irritation score*
Gel (control)	3.0	0.224	0.209	1.0	4.10
Gel (control)	-	0.043	0.133	1.0	0.87
F1	1.0	0.165	0.163	3.8	1.98
F2	2.0	0.196	0.185	4.5	2.89
F3	3.0	0.232	0.213	5.3	4.13
F4	4.0	0.205	0.195	4.8	3.21
F5	5.0	0.189	0.179	4.3	2.65

* n =3

RESULT AND DISCUSSION

Evidence have shown in the recent years that polymer Modified locust bean gum[15] used to prepare transdermal film had the physical properties and behaviour suitable to fabricate flexible, desired thickness, biocompatible transdermal film for the transdermal drug delivery. In the present study, solution casting method was employed using blend of inert MLBG and SA (polymer), Glycerine (plasticizer), and menthol (permeation enhancer) using non toxic solvent to disperse the drug in transdermal film formulations. The present method is quite different from reported by somashekhar *et al* [16], because, research works have been failed to succeed in formulating transdermal films by blend of MLBG and SA solution via casting method. In the present study, examines influences of various process parameters on physicochemical properties and drug release potential from transdermal film have been studied. The physico chemical evaluations of the transdermal films have shown different physical characterstics, which change according to the presence of polymer nature and the percent of polymer. All the film formulations show better results for physicochemical evaluation presented in Table 2.

Five transdermal film formulations (F1 –F5) were prepared using solution casting method and dried. The prepared films consist of glyceine as a plasicizer and menthol as permeatiom enhancer.Dru loaded films were light pink opaque in colour. The

surface of the prepared films was smooth, with elegant appearanceand good physical prpoerties.Flatness of the films was observed better when amount of > 25 % in the formulated films, might be SA having α –L – guloronic acid, which is interact with MLGB (modified non starch poly saccaride) produces flatness to the films. Thus these films can maintain a smooth and uniform surface when applied on skin.

Incorporation of drug into different ratios of MLBG and SA blend affects the physical appearance of the film was observed. In the present study blend of MLGB and SA, plasticizer and permeation enahancer (68: 25.5: 03: 03) suitable to produce solid, discrete film having a sufficient mechanical strength. Resultant films did not have any surface irregularities. It was found that the higher the ratio of drug used (3, 4 and 5 % w/w) resulted films were unsuitable for pharmaceutical uses. SEM photographs also indicated the presence of the drug crystals on the surface of the films. Because surface accumulated drug resulting in burst release and impossible to control the drug release from the film during diffusion.

In the present study, optimized ratio of 68 %of MLBG and 25.5 % w/w of SA was used to produce film. It was found that higher ratio of MLGB (> 68 % w/w) and SA (> 26 % w/w) or decreased ratio of MLGB (< 68 % w/w) and SA (< 25 % w/w), the produced films were not suitable and impossible to distinguish as individual film. In order to avoid the formation of irregular shaped films, an optimum of MLGB (> 68 % w/w) and SA (> 26 % w/w) ratio was used to prepare sterdy films. An attempt was made to prepare transdermal film with out the addition of plasticizer and as it resulted in formation of hard film which is not suitable for pharmaceutical purpose. To obtain optimal concentrations of glycerine as plasticizer, concentarions ranging from 1 to 5 % w/w of the total formulations were investigated. In the present study, optimum concentration, 3% w/w of glycerine was used to produce better films.In order to obtain optimal concentrations of permeation enhancer various concentrations of menthol ranging from 1.0 to 5.0 % w/w of the total formulations were investgated. In the present study, optimum concentration, 3.0 % w/w of menthol was used as a permeation enhancer.

The drug content uniformity of the prepared films lies in the range of 1.90 to 1.95 mg/cm^2. The results of drug content uniformity of the prepared films have shown that the process used to prepare the films in this study was capable of giving films with uniform drug content with minimum intra batch variability.These values were in the expected range as per Indian Pharmacopoeia (IP) standards (1-9 mg/cm^2) [17]. The thickness of the films varied from 133 to 141μm as shown in Table 2.Thickness, Tensile strength, percent elongation and folding endurance of the films increasing by increased ratio of MLBG and plasticizer ratio in the films. Added plasticizer alters the physical and mechanical properties by enhancing the mobility of polymers chains of MLBG, SA by hydrogen bonding [18]. However it was found that 3% w/w/glycerin gives the best plasticizing effect for ACF loaded film.

Low moisture uptake was found in films with less percent of plasticizer, after stored in the above conditions. Prepared films with low percent of plasticizer showed a lower capacity to absorb moisture.As the ratio and RH increases, moisture uptake of the films increased.This effect was more pronounced on films containing more amount of plasticizer and more amount of plasticizer showed an significant increases in moisture uptake at increased RH. The prepared films possess smooth surface, free from constriction after application into skin.

SEM photomicrographs showed that the film (formulation F3) had a smooth surface with eligant appearance (Fig.1.a) and sufficient mechanical strength; when they cured at 24 h at 100° C. SEM photomicrographs of the films reveal the uniform distribution of the drug in the film (F3). When the films were cured more than the 100^o C at 24 h , surface inward dents with fine pores (Fig.1.b) and shrinkage were observed (collapse of the wall of the films), which might be due to drop in residual moisture content from films. Drug crystals were observed on the surface of the film as a result of their migration along with water to the surface during drying.This result clearly indicates that influence of moisture content on surface morphology of the film.

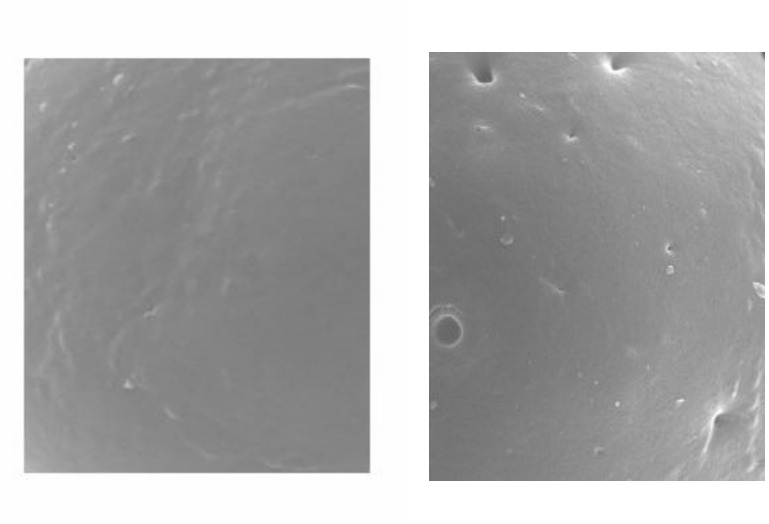

Fig.1.a **Fig.1.b**

Figure 1.SEM images of formulation F3 had a smooth surface with eligant appearance (Fig.1.a). and surface inward dents with fine pores (Fig.1.b)

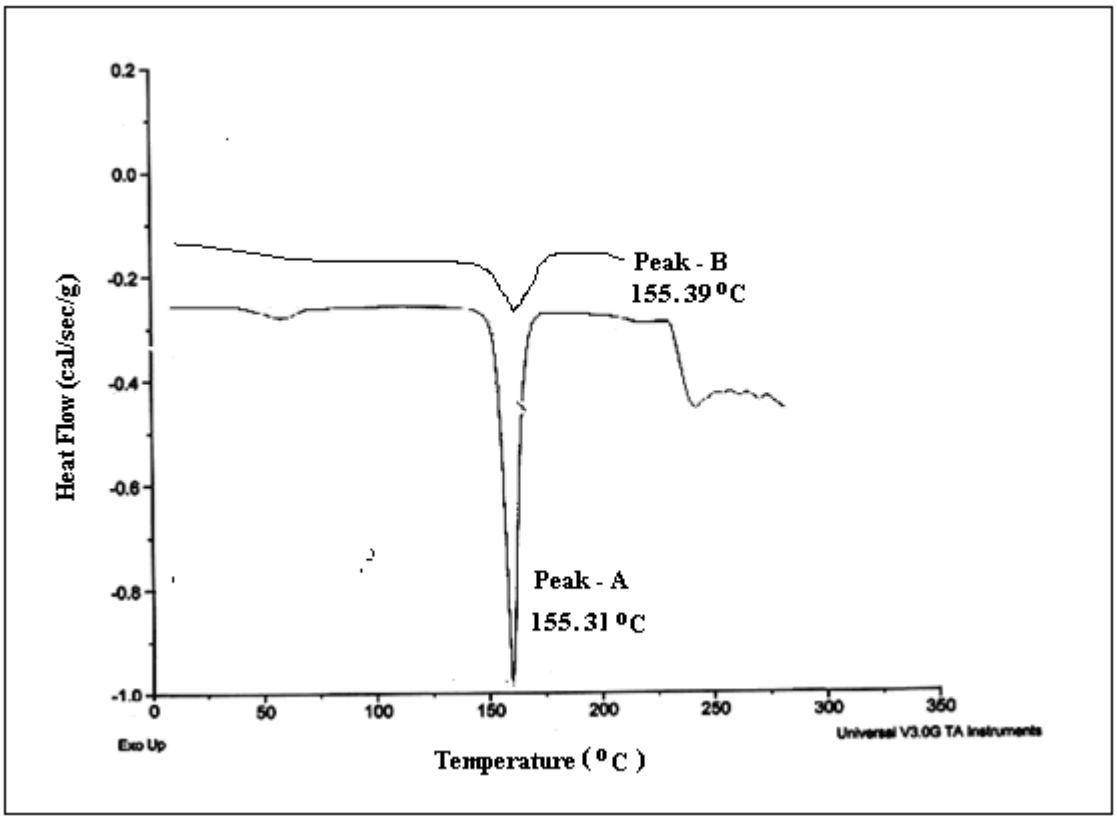

Figure 2: DSC thermograms of pure drug (peak A) and drug loaded film (peak B)

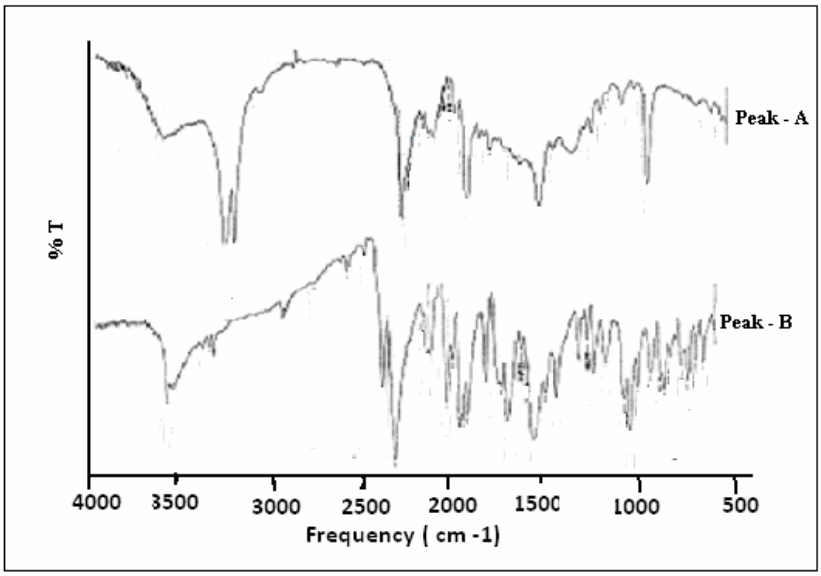

Figure 3: FTIR spectra of pure drug (peak A) and drug loaded transdermal film (peak B)

Differential Scanning Calorimetry (DSC)

To understand the compatible state of the drug, DSC studies were performed on pure drug, drug loaded film and film witout drug, and the thermograms are shown in fig.2. ACF exhibits a sharp endothermic endothermic peak at 155.31 ºC (with glycerin).This result clearly indicated that the drug was distributed in the film. Corresponding to the melting point of the ACF and a identical peak was observed at 155.39 º C in the formulation F3 (without glycerin). These thermal changes of the drug in the film might be due to glycerin involved in recrystallization of drug. A phase transition of the drug in the film was occurred after heat induction [6].

FTIR Spectrophotometry

From the FTIR studies, the characteristic IR absorption peaks for importatnt functional groups of pure drug (ACF), drug containg film and film witout drug were identified. From the figure 3, it was observed that similar characteristic IR absorption peaks of ACF were not altered in drug containg film without any change in their position, indicating no chemical interaction occurred between the drug and the polymer used. Gowda *et al* [19] compared IR spectra at 1287.57 cm^{-1} due to C-N stretching, 1415.65 cm^{-1} due to C=C aromatic stretching, 3271.57 cm^{-1} due to N – H stretching and 1253.64 cm^{-1} due to C-O stretching. A comparison and interpretation of this region in our spectra agrees with their conclusions.

Stability studies

The optimized formulation F3 and conventional gel was subjected for stability studies and estimated drug content at the end of 90 days (8h).However no significant change in drug content from formulation F3 and conventional gel after the study period.

Diffusion studies

From the drug diffusion studies, at the end of 24 h, formulation F3 shown diffuses maximum percentage of drug 97.94 % when compared to F1(95.32), F2(93.56), F4(90.89), F5(88.32), conventional gel with (3% w/w of menthol and glycerol (96.32 %) and conventional gel without menthol and glycerol (16.43%) respectively. It was observed that an insignificant amount of drug was diffused in the first two h from all the formulations at gastric pH. Maximum amount of ACF was diffuses from the formulation F3.The effect of polymer content on drug diffusion from the formulations as a function of time was found to be significantly different ($P< 0.00$, single factor anova). From the above, it can be conluded that drug diffusion from the films was controlled, due to increased amounts of MLBG showed higher swellability of the film and leached plasticizer from the film could reduce tortousity of aqueous pore channels of the films, respectively. In order to understand mechanism of drug release, diffusion data were trated to kinetic models and linearity eas observed with respect to Higuchi equation. The correlation coefficient obtained from Higuchi plot was found to be in the range of 0.9934 to 0.9987. This indicated that mechanism of drug release was diffusion type. In comparing the drug diffusion profile, drug diffusion is directly proportional to the amount of MLBG and glycerin present in the formulations.

In Vitro Skin Permeation Studies

In vitro skin permeation studies were performed to compare the release profile of drug from five formulations (F1-F5) and conventional gel with (3% w/w of menthol and glycerol) and conventional gel without menthol and glycerol , all having the same quantity (2.5 % w/w) of ACF. Steady state flux (J_{ss}), pearmeability coefficient (K_p) and enhancement ratio (E_r) of ACF from films significantly higher ($P < 0.05$) than the flux of ACF from conventional gel presented in Table 4. *In vitro* skin permeation was highest in formulation F3 and lowest for F5. Increased penetration enhancer from 1 to 3 % w/w, showed increased enhancement ratio and flux. The highest flux and enhancement ratio for ACF from the film (F3) containing 3 % w/w menthol was found to be 0.232 mg/cm^2/h and 5.3 mg/cm^2/h, respectively. The skin permeation profile of film F3 was significantly different ($p < 0.05$), when compared with that of F4.Thus, menthol is expected to be a moderate skin permeation enhancer.In contrast, menthol enhanced the skin permeation of the drug by incraesing both skin concentration and the diffusion rate in skin due to presence of hydrogen bonding in menthol as a functional group. ACF is lipophillic drug and menthol is a lipophillic terpene found to be more effective because menthol found to enhance the penetration of drug by both lipid and pore pathway [20]. The formulations F4 showed an intermediate skin permeation profile even it contain more amount of menthol and glycerol, but formulation F5 shows burst permeation profile due to more amount of glycerol. Increased ratio of MLGB in the films showed higher swellability of the film, plasticizer was leached from the film could reduce tortuosity of aqueous pore channels of the films. So drug was permeated from film at a controlled rate across intact skin to achieve a therapeutically effective drug level for a longer duration. When enhancement ratio < 1.0 indicates that enhancer has no permeation enhancing activity.

Anti inflammatory studies

Based on higher drug permeation, formulation F3 was selected for the anti inflammatory effects and compare with conventional gel. A significant inhibition ($p < 0.05$) of inflammation was found with the film formulation F3 containing 3 % w/w menthol in comparison to the conventional gel without menthol. The percent inhibition after 24h was found to be more F3 (87.65 %) as compared to gel formulation without penetration enhancer (24.12 %) and with 3 % w/w menthol (85.32 %) presented in figure 4. The difference between formulation F3 and conventional gel percent inhibition was significant (< 0.05). The enhanced anti inflammatory effects of formulatios F3 could be due to the enhanced permeation of ACF through the skin. The Anti inflammatory study was performed to confirm the safety of the optimized formulation F3. Literature survey reported [21] that a value between 0 and 9 indicates that the applied formulation is generally not an irritant to human skin. The mean skin irritation score for formulation F3 was 4.13. From this it was concluded that the optimized formulation F3 was safe to be used for transdermal drug delivery.

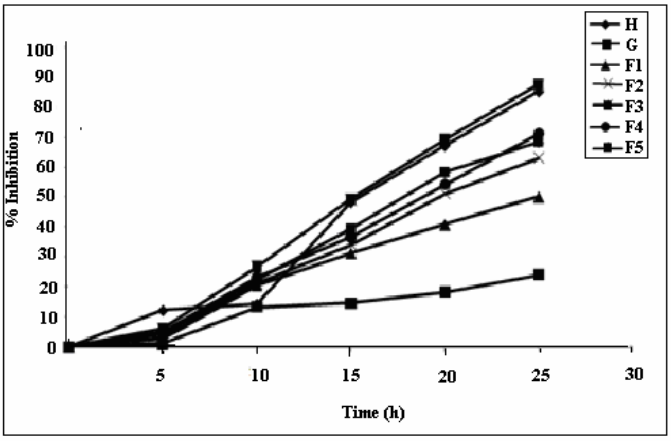

Figure 4: Compare the % inhibition of films formulations with gel

Gel with menthol H — Gel without menthol —
Formulation F1 — F2 — F3 — F4 — F5 —

CONCLUSIONS

Used method was quite easy, economical and does not imply use of toxic solvents.On the basis of drug content, mechanical properties and highest drug permeation; we selected optimized formulation F3 for anti inflammatory activity. FTIR, DSC and stability studies conformed that the drug was stable and compatible. The optimized formulation F3was non irritant and safe to be used for transdermal drug delivery. The *in vivo* studies revealed a significant increase in anti inflammatory effects as compared with conventional gel with and without menthol.From *in vitro and in vivo* data it can be concluded that the developed film formulation F3 have great potential for transdermal drug delivery. But additional evaluation parametrs should be conducted before the film formulations are used on humans.

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