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DESIGN, DEVELOPMENT AND ASSESSMENT OF IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF AN ACECLOFENAC TOPICAL EMULGEL USING LEMONGRASS OIL

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ABSTRACT

Aceclofenac is a hydrophobic BCS class II non-steroidal anti-inflammatory drug having potent analgesic, anti-inflammatory and antipyretic activity. This research is based on designing and developing a topical emulgel of aceclofenac using two different gelling polymers and lemongrass oil as a permeation enhancer. The research shows the impact of lemongrass oil as a permeation enhancer and analgesic. Six different emulgel formulations were prepared using different concentrations of gelling polymers and oil. Evaluation were carried out for various parameters like physical test, viscosity, pH, Spreadability, drug content, in-vitro drug release, in-vivo anti-inflammatory activity and skin irritation study. Carbopol 934 (2% gel) was the best polymer to formulate emulgel with 92.5 ± 0.76% of drug release than xanthan gum and combination gel formulations. Thus, the study reveals that this aceclofenac emulgel prepared using Carbopol gel and lemongrass oil improves the permeation through skin and it does not have any side effects or allergic symptoms like redness and swelling.

KEYWORDS: Emulgel, Lemongrass oil, Gelling polymers, Carbopol 934.

1. INTRODUCTION

Aceclofenac is a new non-steroidal anti-inflammatory drug having remarkable anti-inflammatory, analgesic and antipyretic activity.[1] Some controlled clinical preliminaries have shown that aceclofenac is viable and very much endured in patients with Osteoarthritis,

rheumatoid joint pain (RA), and spondylitis (anky-losing). Aceclofenac is a favoured COX-2 inhibitor having calming and pain-relieving possibilities.[2][3][4] Lemongrass (Cymbopogon) having potent effect as analgesic. According to a 2005 study by Dr. Sue Chao, lemongrass oil is one of the top six essential oils with anti-inflammatory properties.[5]

Gels have a major limitation in the delivery of poorly water - soluble drugs; therefore, emulgels are prepared to overcome this constraint. Emulgels are dosage forms that combine gels and emulsions. In recent years, new polymers with complex functions as emulsifying agent and thickening agents are considerably used.[6][7]

The purpose of this study was formulation of emulgel of aceclofenac (hydrophobic drug) using Carbopol 934 and xanthan gum as a gelling agents and lemongrass oil as an oil phase and also as a permeation enhancer. As a result, lemongrass oil can be utilized as an oil phase in the formulation of emulgel for topical drug administration of aceclofenac which improve the absorption and bioavailability.

2. MATERIALS AND METHODS

Materials

Aceclofenac was purchase from Dhamtech Pharma and Consultants, Mumbai. Carbopol 934 and xanthan gum was obtained from SD chemicals Mumbai. All other chemicals used were of pharmaceutical grade and were used without further modification.

3. METHODOLOGY

3.1. Melting point determination

Melting point was determined by use of a melting point apparatus.[8]

3.2. UV- Visible spectrophotometer study[9]

Calibration curve of aceclofenac in phosphate buffer pH 6.8

Weighed 5 mg of aceclofenac and dissolved in 50 ml of phosphate buffer pH 6.8 (100 µg/ml). From this solution 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml, 3 ml, 3.5 ml, 4 ml was pipette out and diluted up to 10 ml using phosphate buffer Ph 6.8 solution to obtain a working standard solution of 5- 40 µg/ml. The prepared concentrations were analysed in UV- Visible spectrophotometer (Shimadzu japan) at 275nm.

3.3. Fourier Transform Infra-Red Spectroscopy

FTIR emission spectrometer (Shimadzu, Japan) was used to record the FTIR spectrum of the drugs from 400 to 4000 cm−1 to evaluate the molecular states of the pure drug and the formulations and so know about any changes in the chemical structure of the drug. The sample was pressed to an appropriate size disc for measurement after being grounded with KBr.

3.4. Solubility study of Aceclofenac

To find out suitable oil phase and surfactants for formulation solubility of drug sample in different oils and surfactants were determined. Excess amount of an Aceclofenac was dissolved in 5 ml of selected oil and surfactant till saturation. Preliminary mixing was carried out over magnetic stirrer for few minutes. Then this samples were kept in a mechanical shaker for 72 hrs at 37^{o}C. The supernatant was separated, membrane filtered, and after appropriate dilution with phosphate buffer pH 6.8, by analysing samples at 275nm spectrophotometrically the solubility was determined.[10]

3.5. Preparation of emulgel[11]

Table 1: Composition of different formulation batches (%w/w)

Ingredients	F1	F2	F3	F4	F5	F6
Aceclofenac	1.5	1.5	1.5	1.5	1.5	1.5
Lemongrass Oil	10	20	10	20	10	20
Span 80	1	1	1	1	1	1
Tween 80	0.5	0.5	0.5	0.5	0.5	0.5
Ethanol	2.5	2.5	2.5	2.5	2.5	2.5
Methyl paraben	0.03	0.03	0.03	0.03	0.03	0.03
Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Carbopol 934	1	2	-	-	0.5	1
Xanthan gum	-	-	1	2	0.5	1

Step 1: Preparation of gel base

Here gel base was prepared by use of two different polymers. i.e. Carbopol 934 and xanthan gum. The gelling polymers were dissolved in 100 ml of purified water with persistent mixing on mechanical stirrer. The pH was adjusted by using TEA to 6-6.5.

Step 2: Preparation of emulsion.

The emulsion was prepared by using following method.

Oil phase: oil phase was prepared by dissolving span 80 in lemongrass oil.

Water phase: The aq. Phase was prepared by dissolving aceclofenac in ethanol, tween 80 and methyl paraben in water and all these three solutions was mixed together at constant stirring to prepared aqueous phase.

Then both the aqueous phases and oil phase were heated up to 70-80^{o}C. Then oil phase is added into aq. Phase with constant stirring on mechanical stirrer until cooled to room temp.

Step 3: Incorporation of gel base into emulsion to form an emulgel

The gel base and emulsion were mixed in 1:1 ratio to prepared the emulgel by constant stirring on mechanical stirrer.

4. CHARACTERIZATION OF EMULGEL

4.1. Physical examination

The emulgel formulations were inspected visually for their appearance, colour and consistency.

4.2. Viscosity

The viscosity of the formulated batches was determined using a Brookfield viscometer. (DV-E) at 25^{o}C.[12]

4.3. Determination of pH

The pH of emulgel was determined using digital pH meter at room temp. The calibration of pH meter was done with buffered solution before each use.[13]

4.4. Spreadability Study

Spreadability was determined by using the apparatus suggested by Multimer et al (1956)[14]

Spreadability measured by formula.

$S = M \times L / T$

Where, S= Spreadability, M= Weight tied to the upper glass slide, L= Length of the glass slide

T= Time in seconds

4.5. Drug Content

Accurately weighed 1 gm of emulgel was dissolved in 100 ml of phosphate buffer pH 6.8. in volumetric flask. This volumetric flask was sonicated for 2 hrs. The solution was filtered

through Whatman filter paper. The absorbance was measured spectrophotometrically after appropriate dilutions i.e. 2 ml diluted to 10 ml.[15]

The drug content was determined using following formula.

% Drug Content: Ca × Va / Wa

Where: Ca = Total concentration of drug loaded, Va = Volume of solution, Wa = Theoretical amt. of drug.

4.6. In vitro drug release study

The diffusion studies were carried out using Franz diffusion cell. The formulation about 1gm equivalent to 5mg is applied on dialysis membrane. The membrane was placed between donor and receptor compartment of cell. The receptor compartment was filled with 20 ml of phosphate buffer pH 6.8 at maintained temp 37°C and stirred on magnetic stirrer. The 1ml sample was withdrawn at suitable time intervals and replaced with equal amt of fresh media. Samples analysed for drug content using UV visible spectrophotometer at particular wavelength after appropriate dilutions and the release profile were evaluated by zero order, first order, Higuchi, Korsmeyer-Peppas model, respectively.[16]

4.7. Skin irritation study

The test was carried out on rat skin. The hairs were removed from rat skin at 4 cm2 area. Then emulgel sample 0.5% was applied over hair free skin. This skin surface was observed for redness, swelling after 24, 48 and 72 hrs from application of formulation.[17][18]

4.8. In vivo anti-inflammatory studies

The anti-inflammatory efficacy of the optimized formulation was evaluated using male wistar rat (200-300 gm) after permission approved recommended by the IAEC of YSPM YTC SATARA (organization) in its meeting of project proposal No. YSPM/YTC/PHARMA/ 11/2021-2022. The rats were divided into three groups of 6 rats in each group, namely standard/ positive control (Acent gel), negative control and test group. Oedema was induced on the left hind paw of the rats by sub-plantar injection of 0.1 ml of freshly prepared 1% w/v solution of carrageenan lambda. The test formulation and Acent gel (positive control) were applied 30 min before carrageenan administration. The volume of the paw was measured at 0, 30, 60, 120, 180, 240 and 300 min using a modified plethysmometer by mercury displacement method. Increase in the paw volume in the test group was compared with control group.[19][20][21]

4.9. Stability Studies

The optimized formulations were stored in a stability chamber at accelerated stability conditions of 40^0C temp. and 75% RH for three months. The formulation was analysed for organoleptic properties, pH, Spreadability and drug content at one-three months duration.[22]

5. RESULT AND DISCUSSION

5.1. Melting point

The melting point of aceclofenac was found to be 149^{o}C. The reported melting point of drug was 149-150^{o}C.

5.2.Drug Excipient Interaction Study: (FTIR)

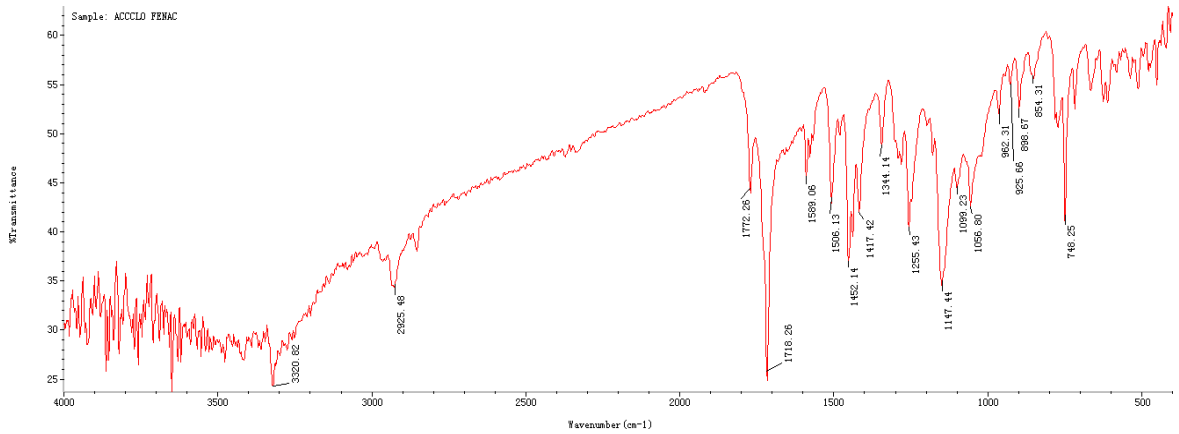

Fig. 1: FTIR Spectrum of Aceclofenac.

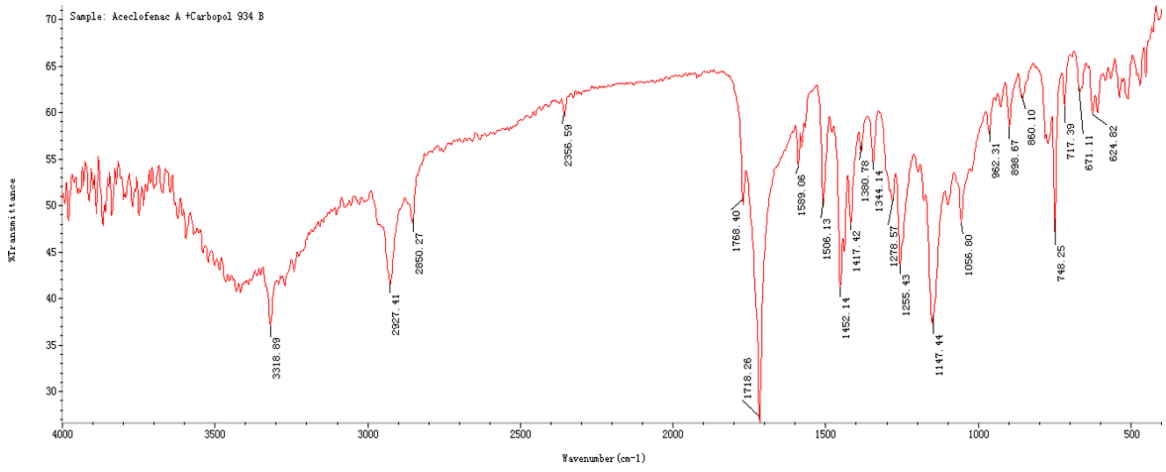

Fig. 2: FTIR spectrum of optimized Aceclofenac emulgel F2.

5.3.Calibration curve of aceclofenac in phosphate buffer pH 6.8.

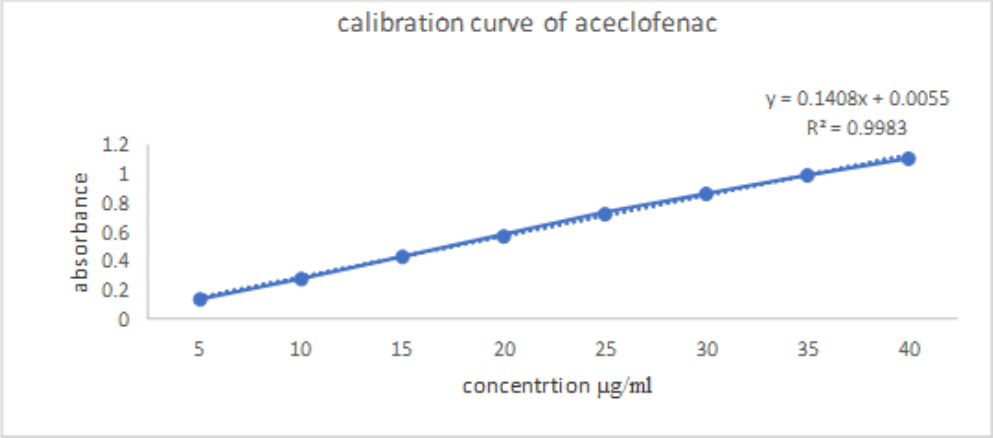

Fig. 3: Calibration curve of aceclofenac in phosphate buffer pH 6.8.

5.3. Solubility study of aceclofenac

Aceclofenac has physicochemical features that it could be useful for topical delivery of drug. Lemongrass oil (10.366mg/ml) had the highest Aceclofenac solubility among the selected oils that were examine, hence it was chosen as the oil phase. Tween 80 (246.62±16.08mg/ml) and span 80 (151± 18.3mg/ml) shows reasonable solubility for the aceclofenac among the surfactants.

Table 2: Solubility study of drug (aceclofenac) in different oils and surfactants.

Vehicles		**Solubility of Aceclofenac (mg/ml)**
Oils	Lemongrass oil	10.366±15.7
	Oleic acid	8.560±14.02
Surfactants	Tween 80	246.62±16.08
	Tween-20	190.12±17.12
	Span 80	151± 18.3
	Span 20	125.65±16.3

5.5. Physical appearance

All emulgel formulations were white/buff, thick, creamy preparations with smooth uniform texture and a glossy appearance.

5.6. pH

The pH value of all emulgel formulations were varied from the range 5.9 to 6.4 which complies with the normal skin pH range.

Table 3: Rheological studies, Spreadability and drug content of formulation F1-F6.

Formulation	Spindle No.	RPM	Viscosity (cPs)	Spreadability (gm.cm/sec)	Drug content (%)
F1	64	10	12179	0.96	90.75
F2	64	10	12890	0.97	96.20
F3	64	10	11908	0.85	85.8
F4	64	10	10620	0.81	95.5
F5	64	10	11250	0.83	92.04
F6	64	10	12008	0.90	95.75

5.7. Rheological Properties

The viscosities of emulgel of aceclofenac at 10 rpm is given in table 2. Formulation containing CBP (F2) exhibit high viscosity than other formulations. Due to the different type of gelling agent which results in changing the structure, consistency and low hygroscopicity of Xanthan gum and mixture of polymers[(CBP: XG) (1:1)],[(CBP: XG) (1:2)] shows low viscosity as compared to CBP 934.

5.8. Spreadability

Emulgels prepared with low concentration of Carbopol and xanthan gum having more Spreadability values. With increasing concentration of Carbopol and xanthan gum stiff and semi stiff formulations were made. The Spreadability of formulation reduces as the gelling polymers concentration increases in the formulation.

5.9. Drug Content

The percentage aceclofenac content in the six emulgel formulations was between 85.8% to 96.20%. Highest release was found in formulation F2 with 96.20% of drug content.

5.10. In vitro drug release

The in-vitro drug release study was recorded from 0.5 to 8 hrs. All the formulations of Aceclofenac emulgel showed drug diffusion within the ranges of 73.76% to 92.58% at the end of 8 hrs. Formulation F2 containing 2% Carbopol 934 and 20 ml of lemongrass oil had highest drug release of 92.5 ± 0.76% at 8 hrs. On other hand formulation F6 containing 1% Carbopol 934 and 1% Xanthan gum and 20 ml of lemongrass oil had the lowest drug release of 73.76 ± 0. 78% at 8 hrs. The concentrations of both Carbopol 934 and xanthan gum contribute to release of aceclofenac.

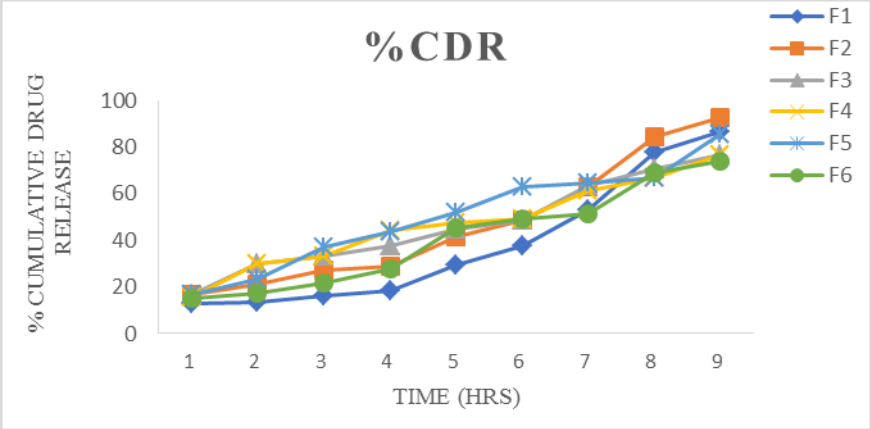

Fig 4: In vitro % cumulative drug release of formulation F1-F6.

5.11. Drug release kinetic study

The release kinetics were studied according to zero order, first order, higuchi, korsmeyer peppas model. The regression equation of optimized formulation F2 were find out 0.9509 at zero order, 0.9886 at first order, 0.9509 at higuchi-peppas model. The release data were best fitted to first order model since it had highest values of R^2.

5.12. Skin irritation study

The rats were explored with optimized formulation. During their time of exposure, all rats were examined for swelling and redness. No allergic symptoms appeared on rats up to 48 hrs.

5.13. In vivo anti- inflammatory studies

After carrageenan injection, the paw volume in all animals increased progressively, which indicate inflammatory reaction and reached maximum at three hours. It was observed that at 1, 2, 3,4 hrs. Inhibition by Acent gel and optimized formulation F2 at 2 hr and 3 hr after carrageenan injection was found be statistically significant ($p<0.05$). Formulation F2 containing aceclofenac and lemongrass oil, exhibited relatively more pronounced anti-inflammatory activity. Mechanically being a penetration enhancer, Lemongrass oil is believed to have increased the penetrability of aceclofenac through skin layers producing much larger concentration of aceclofenac at the site of action.

5.14. Stability studies

A three-month stability data of optimized formulation is summarized in table 4.

Table 4: A three-month stability study of optimized formulation F2.

Parameters	pH	Spreadability (gm.cm/sec)	Drug content (%)
Initially	6.4	0.97	96.20
1 month	6.4	0.96	96.10
2 months	6.4	0.97	96.20
3 months	6.4	0.97	96.20

6. CONCLUSION

On basis of previous finding the following could be concluded that the nature and concentration of polymers used in the preparation of gels and their concentrations showed an effect on release of aceclofenac from emulgels base. Aceclofenac emulgels were prepared using Carbopol 934 and xanthan gum as gelling polymers with the aid of lemongrass oil as oily phase and permeation enhancer. Carbopol gel 2% was the best polymer to formulate emulgel with 92.587% CDR than xanthan gum and gel combination formulations. Thus, the study reveals that this aceclofenac emulgel prepared using Carbopol gel and lemongrass oil improves the permeation through skin and it does not have any side effects or allergic symptoms like redness and swelling also the formulation F2 containing aceclofenac and lemongrass oil, exhibited relatively more pronounced anti-inflammatory activity.

7. ACKNOWLEDGEMENT

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