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FORMULATION, DEVELOPMENT AND EVALUATION OF SUSTAINED RELEASE FILM-FORMING GEL OF CLOBETASOL PROPIONATE

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Keywords:

Clobetasol propionate; Film-forming gel; Eudragit RS PO; Hydroxypropyl cellulose

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ABSTRACT

The localized treatment of diseases of body tissues requires that the pharmaceutical active be maintained at the site of treatment for an effective period of time. Sweat, clothing, movements and getting washed away easily on contact with water are some of the problems that have limited the effectiveness and residence time of conventional topical formulations for treatment of fungal infections of skin. This necessitates longer treatment duration. Hence, to minimise the duration of therapy and side effects associated with it a composition that adheres to skin surface afflicted and provides localized delivery of an antifungal agent is needed.

The present work aims at designing a dosage form of Clobetasol propionate referred to as a 'film-forming gel' which on application forms a thin, transparent film on skin surface. Eudragit RS PO and hydroxypropyl cellulose were used in combination to provide a matrix film that would permit the release of the antifungal agent for a prolonged time. The formulations were prepared using 3^2 full factorial design. The gel was characterised for pH, viscosity, drug content, effective dosage volume and mechanical properties of the film formed after application; bioadhesion and water vapour permeability. Formulation was also tested for drying time, drug release, skin irritation and stability studies.

Results: All the formulations showed results within acceptable range for various tests. The optimized formulation showed drug release of 98.70% after 24 hours of application.

Conclusion: Such a formulation can be claimed to decrease duration of therapy, will be more accepted by the patients and be a breakthrough in treating fungal infections of the skin.

INTRODUCTION

Topical therapy is an attractive choice for the treatment of the cutaneous infections due to its advantages such astargeting of drugs to the site of infection and reduction of the risk of systemic side effects[1]. Systemic treatment is usually reserved for infections of the nails, extensive cutaneous infections or those which have not responded to topical therapy. Conventional topical formulations are unable to retain the drug over the skin for a prolonged period and hence necessitate longer treatment duration or have to be supplemented by oral therapy. Fungal diseases can be classified into 3 groups: the superficial, subcutaneous, and deep or systemic mycoses. Superficial infections are confined to skin, hair, nails or mucous membranes. The most common fungal skin infections are the dermatophytoses, pityriasis versicolor, and candidiasis. Approximately 90% of fungal skin infections are caused by 'dermatophytes', which are parasitic fungi affecting the skin, hair, nails. One of the leading antifungal agents for topical treatment of fungal infections is Clobetasol. It has been approved by the US Food and Drug Administration in cream, gel, solution and spray dosage forms. Clobetasol is corticosteroid agent widely utilized in the treatment of infections caused by dermatophytes.

For effective local delivery of an antifungal that is applied to the surface of the skin, the agent must be partitioned firstly from the vehicle into the stratum corneum, and then partitioned to the local tissues including the viable epidermis, dermis, subcutaneous tissue and appendages. This is problematic since steroidal compounds are generally hydrophobic and a means is needed to perform this partitioning to deliver therapeutically effective concentrations of active agent in situ. For effective delivery of drugs via dermal route, much effort has been invested in providing chemical enhancers for drug penetration, such as DMSO and azones.

Many of these substances cause irritation and are not desirable due to their toxicity. Hence, there is a need for improved compositions for topical delivery of antifungal agents that would minimize the systemic exposure of the therapeutic agent[1].

The need for multiple applications a day is frequently associated with poor compliance of patients. Thus, prolonging the contact time of active substances to the skin and thereby reducing the application frequency is subject of intensive research[3]. Sustained release delivery systems with features of both semisolid formulations and patches may be employed here. The concept of film forming formulations is very recent. Film forming formulations may be solutions, gels or emulsions. Film forming formulations are defined as non-solid

dosage forms that produce a substantial film in situ after application on the skin or any other body surface. Such compositions can either be liquids or semisolids with a film forming polymer as basic material for the matrix. The formed film is sufficiently substantial to provide a sustained drug release to the skin[4,5]. Very few examples of film forming gel formulations have been reported in literature. BeeGentleTM and GELNIQUE are commercially available film forming gel formulations[6,7].

We hypothesized that incorporation of the drug in a film forming gel would facilitate prolonged contact of the drug on the skin and the film formed on drying would improve its skin retention ability, thereby improving the topical treatment of fungal skin infections. This approach does not only sustain the release of drug and enhance percutaneous absorption, but may even allow for drug targeting to the skin or even its substructure, thereby enhancing drug efficacy and improving patient compliance by reducing application frequency.

In this study a dermal gel containing clobetasol propionate was prepared using the film forming polymer, Eudragit RS PO (Eudragit) and gelling agent, Hydroxypropyl cellulose (HPC). HPC also played the role of a secondary film forming polymer. Triethyl citrate (TEC) was used as a plasticizer.

MATERIALS AND METHODS

Materials:

Clobetasol propionate was received as gift sample from GlaxoSmithKline Pharmaceuticals Ltd., Nashik. Eudragit was received as gift sample from Evonik Degussa India Pvt. Ltd., Mumbai. HPC HF and TEC were purchased from SD Fine Chemicals, Mumbai. Wistar albino rats and were purchased from National Institute of Biosciences, Pune. All other chemicals were of analytical grade and were obtained commercially.

Preparation of dermal gel:

The polymeric solutions of Eudragit RS PO and Hydroxypropyl cellulose were prepared in ethanol using dispersion method. Eudragit RS PO was sprinkled over 10 mL of ethanol containing triethyl citrate (7.5 % w/w of Eudragit RS PO). Hydroxypropyl cellulose was sprinkled over 10 mL of ethanol separately. Both solutions were allowed to swell for 24 hours to produce clear solutions. The polymeric solutions were mixed properly with continuous stirring. Accurately weighed quantity (0.025g) of the Clobetasol propionate was dissolved in 5 mL ethanol. The drug solution and polymeric dispersion were mixed properly with continuous stirring and volume was made up to the mark using ethanol[4,8-11].

3^2 factorial design was followed for the development of the formulations. In this design, 2 factors were evaluated each at 3 levels and experimental trials were performed at all 9 possible combinations as reflected in table I.

Table I: Composition of formulations as per 32 factorial design

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ingredient	**%**								
Clobetasol propionate (w/v)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Eudragit RS PO (w/v)	10	12.5	15	10	12.5	15	10	12.5	15
HPC (w/v)	4	4	4	6	6	6	8	8	8
Triethyl citrate (w/w)	0.37	0.93	1.5	0.37	0.93	1.5	0.37	0.93	1.5
Ethanol (v/v)	100	100	100	100	100	100	100	100	100

Evaluation of formulations:

The formulations were tested clarity, pH, and viscosity. Clarity was checked visually and pH of the formulations was checked using digital pH meter 335 [9,12]. The rheological properties of gels were determined by the Brookfield viscometer; type DV-II + PRO using spindle SC4-18.Viscosity values of the formulations were recorded at varying shear rates [9].

Bioadhesion/Peel test:

The bioadhesive properties of the various formulations were evaluated using laboratory-assembled apparatus. The apparatus consisted of a modified double beam physical balance in which the left pan was replaced with a brass wire, to which a polypropylene disc of 2 cm height, 3.8 cm diameter and 2 cm thickness was hanged. Another polypropylene disc of 2 cm height and 1.5 cm diameter was placed right below the suspended disc upon the base of the balance. The right pan was replaced by the lighter pan so that, left pan weighs 5.25 g more than right pan. The lower polypropylene block was intended to hold the rat skin and placed in a beaker containing phosphate buffer solution pH 5.8 at 37 $\pm 2^0$C and enough of the film forming gel to make a thin layer over the skin. 1 mL gel was applied to the lower surface of the upper polypropylene cylinder. Then the gel was allowed to dry for up to 5 minutes. The weights on the right hand side were slowly added in increments of 0.5g till the film gets separated from the rat skin surface. The weight required for complete detachment is noted. (W1) (W1-5.25g) gives force required for detachment expressed in weight (g). The procedure was repeated 3 times and an average was computed. Using this technique, bioadhesion was evaluated through a measurement of the maximum force required to separate the film formed from the biological surface. This force could be correlated to the force required to detach the attached film from the skin in vivo[11,13].

Drug content:

10 mg equivalent of gel was taken in a 100 mL volumetric flask containing 10 mL methanol and volume was made up to the mark with methanol to get a concentration of 100µg/mL. An aliquot of 0.5 mL was transferred to a 10 mL volumetric flask and volume was made up with methanol. The absorbance of prepared solution was measured at λ_{max} by using UV visible spectrophotometer [9].

Drying time:

For the assessment of the drying time the formulation was applied to the inner sides of the forearm of a volunteer, who participated in the study on informed consent basis. After 2 minutes a glass slide was placed on the film without pressure. If no remains of liquid were visible on the glass slide after removal, the film was considered dry. If remains of liquid were visible on the glass slide the experiment was repeated until the film was found to be completely dry [4].

Effective dosage volume:

The calculation of an effective dosage volume of the formulation to be applied per cm^2 area of skin is necessary for ensuring that the drug is available at the site of fungal infection in concentrations above its minimum inhibitory concentration (MIC). Pre-experiments were carried out to determine dose of drug to be delivered per cm^2. For the calculation of an effective dosage volume, the formulation was applied to the inner sides of the forearm of a volunteer, who participated in the study on informed consent basis. Varying volumes of formulation (0.1 to 2 mL) were applied to an area of 2 cm^2 on the inner sides of the forearm of the volunteer. The volume that did not flow away from the application site was noted. The amount of drug in this volume was calculated.

Integrity of formulation on skin:

The formulation was applied to the forearm of a volunteer as described for the assessment of the drying time. The dry film was then worn overnight by the test subject. After 24 hours the test area was examined visually for completeness of the film, appearance of cracks / flaking [4].

Properties of film:

For the assessment of properties of the film, films were produced with a solvent evaporation technique by pouring 1 mL of the preparations into a stainless steel mould lined by Teflon (6 cm x 10 cm). The films were left to dry for 72 hours at room temperature (three hours ventilated in the open air to allow the evaporation of ethanol.

The stickiness of the outer surface was tested by pressing cotton wool on the dry film under low pressure. Depending on the quantity of cotton fibres that were retained by the film the stickiness was rated high (dense accumulation of fibres on the film), medium (thin fibre layer on the film) or low (occasional or no adherence of fibres).

The cosmetic attractiveness of the film was assessed by visual examination of the dry films. Transparent films with a low skin fixation had a high attractiveness as they were almost invisible. Opaque films and films with a medium skin fixation were considered less attractive as they exhibited an increased visibility and a slight wrinkling of the skin. Whitish films and films causing heavy wrinkling of the skin due to strong skin fixation displayed only a low attractiveness.

The mechanical properties of the film were tested. The films were cut into size of 10 x 40 mm and the thickness of the film using a digital vernier calliper. Each film was measured at five positions (central and the four corners) and the mean thickness was calculated. Folding endurance was measured manually for the prepared films. A strip of film (10 x 40 mm) was cut and repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance.18

Films were evaluated for tensile strength and % elongation using an apparatus assembled in the laboratory. Films of dimension 10 x 40 mm were attached to a support that was inextensible but flexible and this support was in turn held between two clamps separated by a distance of 3 cm. Clamps were designed to secure the patch without crushing it during the test. These were supported on a metal base. One of the clamps was fixed; the other one was movable and weights could be added to the movable clamp. During measurement, the films were pulled by the movable clamp with the addition of weights. The strength and elongation were measured when the films broke and tensile strength and % elongation were calculated using the following formulae [15-18].

$$\text{Tensile strength} = \frac{\text{Tensile load at break}}{\text{Cross sectional area}}$$

$$\% \text{ Elongation} = \frac{\text{Maximum length recorded at break-Original length}}{\text{Original length}} \times 100$$

Weight variation test:

For each formulation, three film samples (10 x 40 mm) were used. Each film sample was weighed individually and the average weight was calculated.

Drug content of films [19]:

Prepared film was put into 100 mL phosphate buffer solution pH 5.8 and stirred vigorously for 2 hours. Then the whole solution was sonicated for 15 minutes. The above solution was filtered and drug was estimated spectrophotometrically at λ max

Water vapour permeability [4]:

The water vapour permeability (WVP) was investigated according to a method modified from the British Pharmacopoeia. Films were produced with a solvent evaporation technique as described earlier. Circular samples with a diameter of 2.0 cm were cut from the dry film sheets with the help of a scalpel. For the sample preparation 10 ml glass vials with an opening of 1.2 cm diameter (A = 1.13 cm^2) were filled with approximately

8 g of distilled water, covered with the circular film samples and the vial was sealed tightly with an aluminium foil. To start the experiment, the top of the vial cap was opened and the weight of the vial was determined with an analytical scale. The vials (three replicates per formulation) were then placed into a desiccator containing a desiccant to create a climate of low relative humidity (approximately 0%). They were kept at a determined temperature (37°C) for 72 hours and weighed. From the weight loss of the vials W (g) the WVP was calculated as the amount of water that had permeated through the film in relation to the surface area (A cm^2) and the time (t, 24 hours) using the following formula:

WVP = W/ (A*t) (g cm-2 24 hrs-1)

***In-vitro* Drug Release Study (Diffusion study): [20]**

Laboratory-assembled apparatus resembling a Franz diffusion cell was used to determine the release profile of drug from film forming gel. The cell consisted of two chambers, the donor and the receptor compartment between which a diffusion membrane (egg membrane) was mounted. The donor compartment, with inner diameter 24 mm, was open i.e. exposed to the atmosphere at one end and the receptor compartment was such that it permitted sampling. The diffusion medium used was phosphate buffer solution pH 5.8 (PBS). 1 mL of the drug containing film forming gel was placed in the donor compartment separated from the receptor compartment by the egg membrane. The egg membrane was previously soaked for 24 hr. in PBS. The donor and receptor compartments were held together using a clamp. The position of the donor compartment was adjusted so that egg membrane just touches the diffusion medium. The whole assembly was fixed on a magnetic stirrer. The receptor compartment with 100 mL PBS was placed on a thermostatically controlled magnetic stirrer. It was

maintained at 37 ± 0.5 ^{o}C and stirred constantly at 50 rpm. Samples of 1 mL were collected at predetermined time intervals and analysed for drug content by UV Spectrophotometer at λ_{max}against blank. The receptor phase was replenished with an equal volume of phosphate buffer at each time of sample withdrawal.

Optimization study[5]:

Optimization of the formulations was studied by 3^2 full factorial design. The amounts of eudragit RS PO (X1) and hydroxypropyl cellulose (X2) were selected as independent variables and the dependent variables were % drug release and antifungal activity. The data obtained were treated using Design expert version 8.0.4.1 software and analysed statistically using analysis of variance (ANOVA). The data were also subjected to 3-D response surface methodology to study the effect of eudragit RS PO and hydroxypropyl cellulose on the dependent variables.

Evaluation of Optimized formulation:

The batch which was selected from the solutions obtained by optimization study was further evaluated for skin irritation, best fit kinetic model and stability study.

Skin irritation study[22-26]:

The protocol was approved by Institutional Animal Ethics Committee. The rats (n=9) were randomly divided into 3 equal groups for application of standard irritant, optimized formulation or test and control (no application). Hairs were removed by hair removal cream (Anne French) from an area (2 cm^2) on the dorsal side of the albino rats to make a hairless area. A 0.8% v/v aqueous solution of formalin was applied as a standard irritant to rats chosen randomly for standard irritant application (n=3) on the following day. The optimized formulation was applied to group 2 of rats (n = 3) for assessing any kind of irritation at specified sites. Formulation was removed after 24 h and skin was examined for any sign of erythema and oedema. The administration sites were assessed for signs of skin irritation, and this test procedure was repeated for another 6 days. The resulting reactions were compared against control group (n=3) and scored according to table II.

Table II: Score rating for skin irritation study

Sr. no.	Score	Rating
1.	0	Nil
2.	0-2	Mild
3.	2-4	Moderate
4.	4-6	Severe
5.	6 and above	Very severe

Best fit kinetic model[5]:

To examine the drug release kinetics, the release data of optimized formulation was fitted to models representing zero order, first order, Higuchi's square root of time kinetics and KorsemeyerPeppas kinetics. The coefficient of determination (r^2) values were calculated from the plots of %CDR vs. t for zero order, log %CDR remaining vs. t for first order and %CDR vs. t1/2 for Higuchi model, where %CDR is the amount of drug released at time t, log %CDR is the amount of drug remaining after time t. The best fit kinetic model was determined from r^2 values.

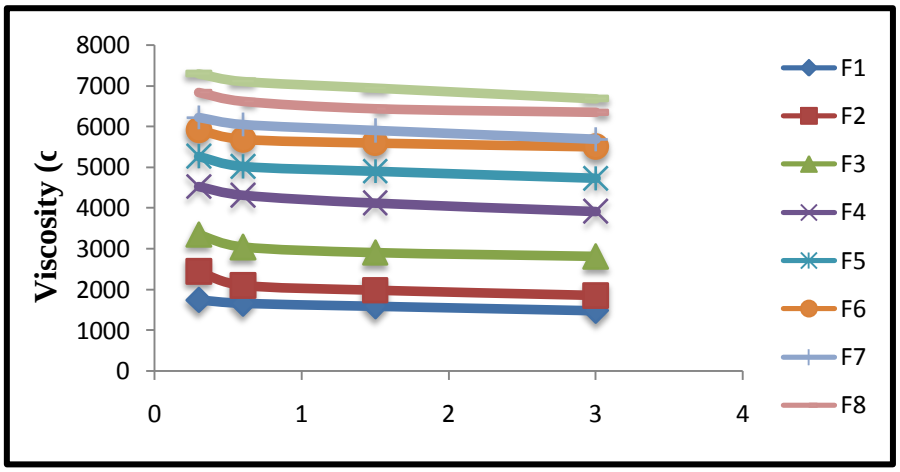
Stability study [27]:

The formulations were evaluated mainly for their physical characteristics at the predetermined intervals of 1 month and up to 3 months. Physical appearance/clarity, pH, viscosity, drug content and antifungal activity were evaluated.

RESULTS AND DISCUSSION

All formulations were found to be clear on visual inspection. The pH of the formulations was found to be between 5.68 and 5.86. Ideally, the dermal gel should possess pH in the range of 5-6, so as to minimize discomfort or irritation due to acidic pH and microbial growth due to basic pH. Hence, the formulations displayed pH values within acceptable range. The viscosity profile of formulations F1 to F9 has been shown in figure I.

Figure I: Viscosity profile of formulations



Viscosity v/s rpm plots for all formulations shows decrease in viscosity as shear rate (rpm) was increased. Concentration of hydroxypropyl cellulose and Eudragit RS PO was a major factor affecting viscosity of formulations. Formulations exhibited considerable increase in viscosity when concentration of hydroxypropyl cellulose increased over the range of 4% w/v to 8% w/v.

Bioadhesion/Peel test:

Bioadhesion of the film formed on drying must be sufficient to ensure that it remains adherent to the skin for duration of 24 hours. The results for bioadhesion test have been given below.

Table no. III Results for Bioadhesion/Peel test

Sr. no.	Formulation code	Observed value (±S.D.) (N)
1.	F1	0.3139±0
2.	F2	0.6049±0.003
3.	F3	0.8731±0
4.	F4	0.327±0.003
5.	F5	0.6066±0.003
6.	F6	0.8747±0.003
7.	F7	0.3368±0.003
8.	F8	0.6115±0.003
9.	F9	0.8764±0.003

Drug Content:

The Drug content of formulations is shown in table no. IV.

Table no. IV Drug content of dermal gel

Sr. no.	Formulation code	Drug content (%) (±S.D.)
1.	F1	98.49 ±0.08
2.	F2	97.96 ±0.11
3.	F3	99.39 ±0.07
4.	F4	100.9 ±0.08
5.	F5	99.09 ±0.07
6.	F6	98.94 ±0.1
7.	F7	99.24 ±0.13
8.	F8	101.12 ±0.12
9.	F9	98.72 ±0.18

The percentage drug content of all prepared dermal formulations was found to be in the range of 97-102 %. Therefore uniformity of content was maintained in all formulations.

Drying time:

The drying time or film formation time has been tabulated in table no.V .

Table no. V Drying time of dermal gel

Sr. no.	Formulation code	Drying time
1.	F1	2 min 37 sec ±5 sec
2.	F2	2 min 52 sec ± 5 sec
3.	F3	3 min 2 sec ± 5 sec
4.	F4	3 min 15 sec ± 5 sec
5.	F5	3 min 37 sec ± 5 sec
6.	F6	3 min 52 sec ± 5 sec
7.	F7	4 min 11 sec ± 5 sec
8.	F8	4 min 43 sec ± 5 sec
9.	F9	5 min 5 sec ± 5 sec

Ideally, the dermal gel should dry to form a thin, invisible film on the surface of skin at the application site within 5 minutes, so as to minimize discomfort to patient.

Effective dosage volume:

This test was carried out to define the volume of formulation that would cover a unit area of skin to deliver an effective dose. As part of a pre-experiment it was found that a concentration of 0.49 ±0.2 mg/cm^2 was made available by a quantity of commercial cream required to cover an area of 1 cm^2 completely. Thus, a volume of formulation that could deliver equivalent amount of drug was needed to be calculated. 1 mL of formulation covered an area of 2 cm^2. This volume contained 10 mg of drug. That means the formulation delivered 0.5 mg/cm^2 which is effective as stated in literature (0.5 to 25 mg/cm^2) and comparable to commercial cream.

Integrity of formulation on skin:

The integrity of the formulations on the skin in the form of a thin, almost invisible film was evaluated and the results of the test have been tabulated below.

Table no.VI Results for Integrity of film after 24 hours

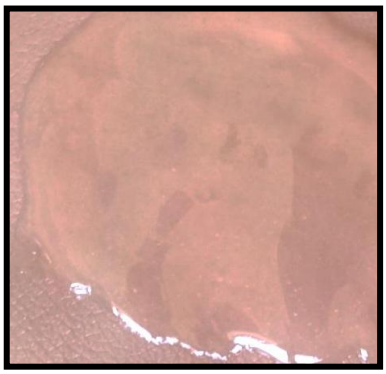
Sr. no.	Formulation code	Integrity of film after 24 hours
1.	F1	Partly missing
2.	F2	Good
3.	F3	Partly missing
4.	F4	Partial flakes formed
5.	F5	Good
6.	F6	Flaky
7.	F7	Flaky and partly missing
8.	F8	Good
9.	F9	Flaky

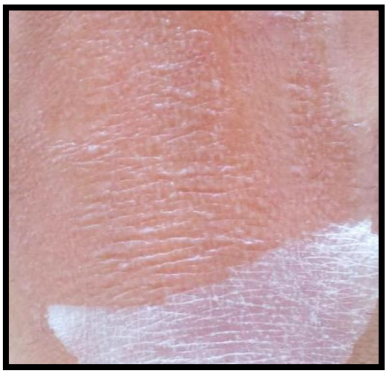
Films formed from formulations F1 and F2 were flaky due to brittleness of the film and parts of the film were missing. F3 formed non-flaky film and parts of the film were missing. Formulations F4, F5 and F6 formed films that were flexible, soft to touch and completely present after 24 hours. F7, F8, F9 formulations formed films that were flaky and ruptured in some parts.

The integrity of film formed using formulation F5 has been shown in figure no. II.

Figure no. II Integrity of film on skin;

A: On application **B: After 24 hours (with a peeled portion)**





Properties of film:

Outward stickiness:

The results for outward stickiness of the formulations have been tabulated in table no. VII

Table no. VII Results for Outward stickiness

Sr. no.	Formulation code	Observation
1.	F1	Low
2.	F2	Low
3.	F3	Medium
4.	F4	Low
5.	F5	Low
6.	F6	Medium
7.	F7	Low
8.	F8	Low
9.	F9	Medium

Cosmetic attractiveness:

Results for cosmetic attractiveness of the film formed after drying have been given in table no. VIII.

Table no. VII Results for Cosmetic attractiveness

Sr. no.	Formulation code	Observation
1.	F1	Medium
2.	F2	Medium
3.	F3	Medium
4.	F4	High
5.	F5	High
6.	F6	High
7.	F7	Low
8.	F8	Low
9.	F9	Low

Mechanical properties of the film:

Film thickness:

The results (table no. IX) indicate that as the concentration of Eudragit RS PO increased there was an increase in the thickness of the film.

Table no. IX Results for Film thickness

Sr. no.	Formulation code	Observed value (±S.D.) (mm)
1.	F1	0.492 ±0.001
2.	F2	0.534 ±0.002
3.	F3	0.632 ±0.001
4.	F4	0.492 ±0.001
5.	F5	0.535 ±0.001
6.	F6	0.636 ±0.001
7.	F7	0.496 ±0.001
8.	F8	0.533 ±0.001
9.	F9	0.636 ±0.001

Folding endurance:

Folding endurance measures the ability of the film to withstand rupture, higher the folding endurance lower will be the chances of film rupture. The folding endurance values were found to be between 57 and 64 folds which are considered satisfactory.

Table no. X Results for Folding endurance

Sr. no.	Formulation code	Observed value (±S.D.)
1.	F1	63.3 ±0.58
2.	F2	58.3 ±0.58
3.	F3	58 ±1
4.	F4	63.6 ±0.56
5.	F5	58.3 ±0.58
6.	F6	57.6 ±0.56
7.	F7	62.3 ±1.15
8.	F8	57.3 ±1.15
9.	F9	57.6 ±0.56

Tensile strength and % Elongation:

Mechanical properties such as tensile strength and % elongation at break are determined to characterise polymeric films for their abrasion resistance and flexibility respectively. Tensile strength results have been tabulated in table no. XI. Table no. XII shows the results for % elongation measurements.

Table no. XI Results for Tensile strength

Sr. no.	Formulation code	Observed value (±S.D.) (N/m^2)
1.	F1	0.6622 ±0.04
2.	F2	0.6622 ±0.04
3.	F3	0.6131 ±0
4.	F4	0.7031 ±0.03
5.	F5	0.7358 ±0
6.	F6	0.7194 ±0.03
7.	F7	0.7194 ±0.03
8.	F8	0.7194 ±0.03
9.	F9	0.7848 ±0

Table no. XII Results for % Elongation

Sr. no.	Formulation code	Observed value (±S.D.) (%)
1.	F1	4.1667 ±0.14
2.	F2	4.3333 ±0.14
3.	F3	4.6667 ±0.14
4.	F4	4.1667 ±0. 14
5.	F5	4.8333 ±0. 14
6.	F6	5.333 ±0. 14
7.	F7	4.6667 ±0. 14
8.	F8	5.25 ±0.25
9.	F9	5 ±0.25

Weight variation test

Films were tested for weight variation. Results have been tabulated below in table no. XIII.

Table no. XIII Results for Weight variation test

Sr. no.	Formulation code	Observed value (±S.D.)
1.	F1	43.95 ±0.02
2.	F2	42.80 ±0.03
3.	F3	47.87 ±0.05
4.	F4	43.58 ±0.05
5.	F5	46.05 ±0.06
6.	F6	47.31 ±0.03
7.	F7	42.54 ±0.04
8.	F8	47.19 ±0.08
9.	F9	48.73 ±0.03

Drug content of film

Results for drug content of film have been given below in table no. XIV.

Table no. XIV Results for Drug content of film

Sr. no.	Formulation code	Observed value (±S.D.)
1.	F1	94.96 ±0.02
2.	F2	96.91 ±0.08
3.	F3	98.79 ±0.09
4.	F4	100.37 ±0.03
5.	F5	98.95 ± 0.03
6.	F6	98.79 ±0.02
7.	F7	98.49 ±0.03
8.	F8	100.75 ±0.02
9.	F9	98.27 ±0.03

Water vapour permeability

Results for WVP have been tabulated in table no. XV.

Table no. XV Results for WVP determination

Sr. no.	Formulation code	Observed value (±S.D.) (g cm^{-2} $24h^{-1}$)
1.	F1	0.012 ±0.006
2.	F2	0.014 ±0.004
3.	F3	0.017 ±0.003
4.	F4	0.016 ±0.007
5.	F5	0.017 ±0.009
6.	F6	0.014 ±0.005
7.	F7	0.015 ±0.003
8.	F8	0.02 ±0.009
9.	F9	0.018 ±0.013

According to the British Pharmacopoeia a material can be considered permeable to water vapour when the WVP exceeds 0.05 g cm^{-2} $24h^{-1}$. The films displayed such WVP values that show permeability above the limit set in the Pharmacopoeia and can therefore be considered non-occlusive.

***In-vitro* drug release study**

The *In-vitro* drug release study of formulations is shown in table XVI.

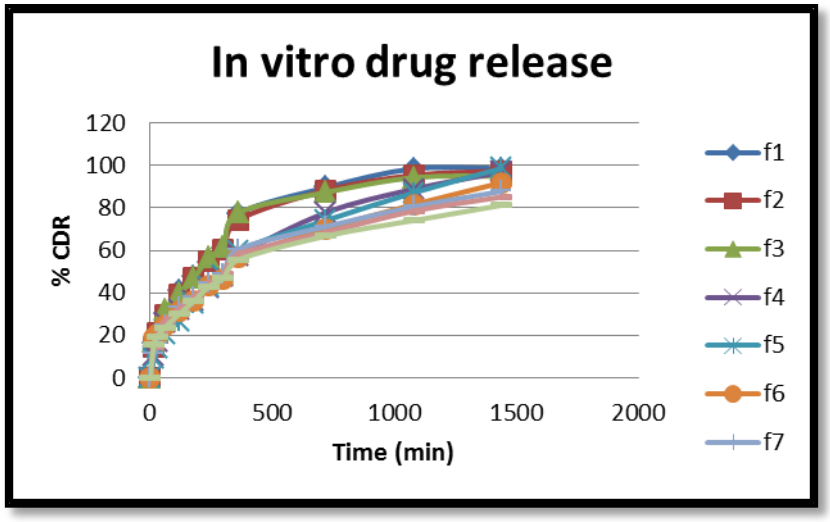
Table no XVI. Cumulative Drug release of formulations

	Cumulative Drug Release (%) (±S.D.)								
Time (hr.)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
0.25	16.18±0.02	14.22±0.03	16.56±0.03	09.91±0.01	08.90±0.04	18.57±0.03	11.48±0.05	14.26 ±0.04	15.89±0.01
0.5	20.29±0.02	21.05±0.02	21.57±0.02	16.69±0.42	13.53±0.03	20.62±0.07	16.37±0.02	21.34±0.05	19.39±0.01
1	30.04±0.07	29.42±0.05	31.75±0.01	25.68±0.05	20.63±0.08	24.56± 0.01	24.55±0.03	27.11 ±0.02	23.71±0.01
2	40.79±0.03	39.40±0.03	39.58±0.02	31.68±0.05	26.60±0.05	31.14±0.02	33.24±0.04	32.69 ±0.04	30.10±0.03
3	47.32±0.02	46.76±0.03	46.96±0.03	35.94±0.05	34.89±0.06	36.30±0.04	37.66±0.04	38.10 ±0.02	36.21±0.04
4	54.54±0.06	54.67±0.04	56.85±0.04	42.32±0.03	42.77±0.04	43.55±0.09	44.44±0.06	44.32 ±0.05	42.97±0.05
5	60.91±0.02	60.40±0.07	61.73±0.05	47.80±0.06	48.50±0.02	46.53±0.25	49.03±0.02	48.29 ±0.05	46.76±0.06
6	77.26±0.02	74.34±0.05	77.64±0.05	57.40±0.05	59.70±0.05	56.14±0.03	60.42±0.04	58.25 ±0.02	55.56±0.09
12	89.61±0.03	88.29±0.04	87.37 ±0.03	77.79±0.03	74.13±0.03	70.18±0.04	71.23±0.01	98.36±0.02	66.78±0.07
18	98.48±0.03	95.41±0.05	94.33±0.09	88.89±0.02	87.18±0.05	81.63±0.01	80.60±0.01	78.56±0.07	74.10±0.02
24	98.56±0.03	97.41±0.05	95.05±0.12	96.90±0.36	98.70±0.05	91.92±0.1	87.82±0.01	85.20±0.004	81.28±0.03

It can be deduced from *in vitro* diffusion study that formulations F1 to F3 did not sustain the drug release over 24 hours. This may be attributed to low levels of drug release modulating polymers and low viscosity of theformulations. [29] Formulations F4 and F5 sustained drug release over 24 hours. On the other hand, formulations F6 to F9 did not completely release the drug. Drug release was found to be sustained at intermediate levels of hydrophobic polymer, Eudragit and hydrophilic polymer, HPC.

Of the nine formulations, maximum release was found to be for formulation F5 after 24 hours. 98.70% of the drug in the formulation was available for antifungal activity. The composite film had hydrophobic and hydrophilic portions which provide competition for drug release as both the polymers have different release properties. Therefore, as the polymer ratio varies, competition to release drug also varies. Formulation F5 showed steady state release up to 24 hours which also indicates that this formulation would show better contact with biological membrane. *In-vitro* drug release profile of formulations has been shown in figure III.

Figure III: *In-vitro* drug release profile of formulations F1 to F9



Optimization: [5]

From design expert version 8.0.4.1 thirty nine solutions were found. The batch with Eudragit 12.5 % w/v and HPC 6 % w/v with desirability 1 was found to be optimum. From this data formulation F5 was selected as the optimum formulation. The figures below show the effect of concentration of Eudragit RS PO and Hydroxypropyl cellulose on drug release and antifungal activity. It is shown that both the independent variables have a significant effect on the dependent variables and drug release and antifungal activity decrease as concentration of polymers increases.

Figure IV: Surface response plot showing effect of Eudragit RS PO and Hydroxypropyl cellulose on Drug release

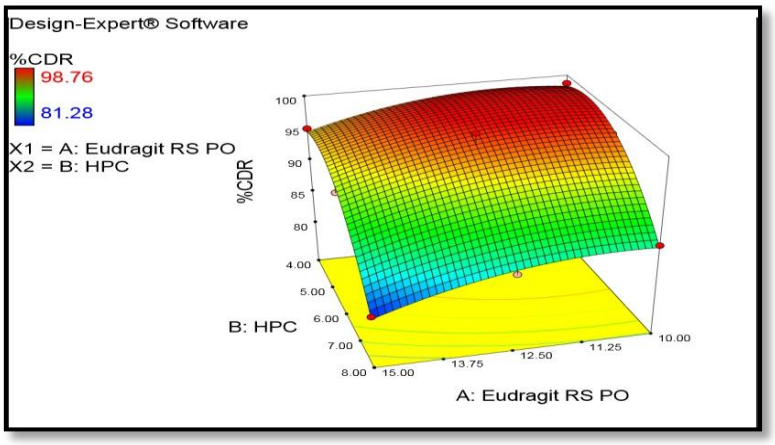
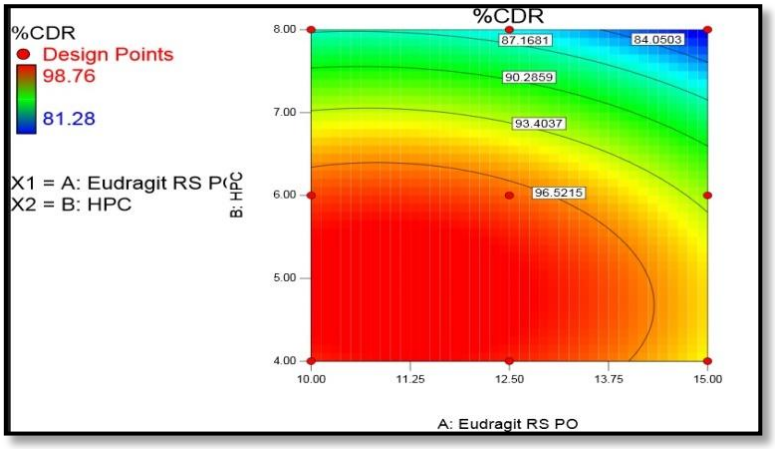


Figure: V Contour plot showing effect of Eudragit RS PO and Hydroxypropyl cellulose on Drug release



In the figures above, it can be seen that as the concentration of polymers increases the drug release go on decreasing. Hence it can be concluded that the two factors: X1 and X2 have a combined effect on drug release.

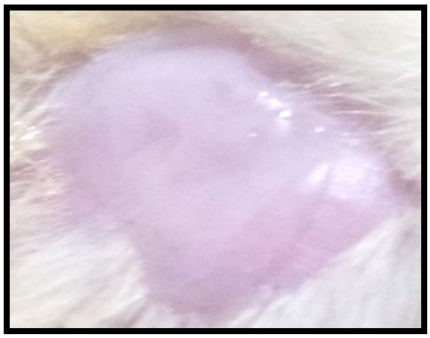
Evaluation of Optimized batch:

Skin irritation study

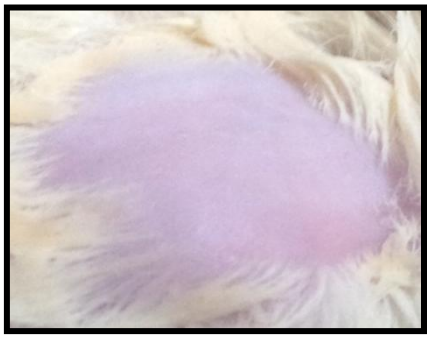
Skin irritation study on rats showed that after application of the optimized formulation there was no evidence of irritation (erythema and oedema). Hence, the optimized formulation F5 was found to be safe.

Figure: VI Photographs of skin irritation test;

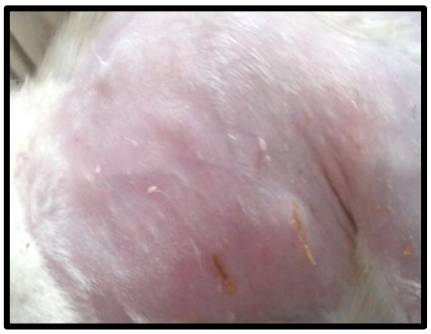
A] On application of film



B] After removal of film



C] Standard irritant



D] Negative Control

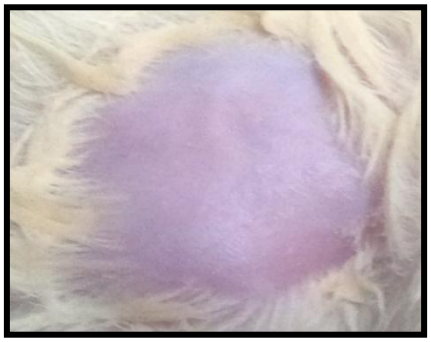


Table no. XVII Evaluation table for Skin irritation test according to Draize scoring method

Rat No.	Control group		Formulation group		Formalin group	
	Erythema	Oedema	Erythema	Oedema	Erythema	Oedema
1.	0	0	0	0	4	2
2.	0	0	0	0	4	2
3.	0	0	0	0	4	2

Erythema scale: **0**- none; **1**-slight; **2**- well defined; **3**-moderate; and **4**- scar formation

Oedema scale: **0**- none; **1**- slight; **2**- well defined; **3**- moderate; and **4**- severe.

Best fit kinetic model for optimized formulation:

The best fit model for the optimized formulation with highest R^2 value and least slope value was the first order model. R^2 value for Higuchi model of optimized formulation shows that drug release occurs via diffusion.

Table no. XVIII R^2 and slope values for optimized formulation F5

Sr. no.	Model	R^2	Slope
1.	Zero order	0.913	0.055
2.	First order	0.990	-0.002
3.	Higuchi	0.977	2.491
4.	Korsemeyer-Peppas	0.980	0.562

Stability studies

The optimized formulation was evaluated after storage at room temperature and after accelerated stability studies at elevated temperature (40°C/75% RH) in stability chamber.Results have been given in table no. XIX and table no. XX.

The results of stability studies show that the formulation was stable at room temperature and also at elevated temperature conditions. A slight increase in pH and viscosity and a slight decrease in drug content and antifungal activity were observed however, these were not significant so as to affect the quality and safety of the formulation after storage.

Table no. XIX Stability studies data for F5 formulation at room temperature

Sr. no.	Observation		Before study	During study
				3rd month
1.	**Clarity**		Clear	Clear
2.	**pH**		5.83 ±0.01	5.86 ±0.01
3.	**Viscosity (rpm)**	**0.3**	5183.39	5191.23
		0.6	5013.67	5024.9
		1.5	4836.93	4844.3
		3	4742.87	4751.43
4.	**Drug content**		98.70 ±0.13	98.62 ±0.06

Table no. XX Accelerated Stability studies data for F5 formulation

Sr. no.	Observation		Before study	During study
				3rd month
1.	**Clarity**		Clear	Clear
2.	**pH**		5.83 ±0.01	5.87±0.01
3.	**Viscosity (rpm)**	**0.3**	5183.39	5194.333
		0.6	5013.67	5027.333
		1.5	4836.93	4851.533
		3	4742.87	4755.267
4.	**Drug content**		98.70 ±0.13	98.57 ±0.08

CONCLUSION

Film forming gel of Clobetasol propionate was prepared using Eudragit RS PO and hydroxypropyl cellulose. The concentrations of both the polymers were optimized by 3^2 full factorial design to obtain optimum drug release. Thus, desirable goals could be achieved by systematic formulation approach. The film forming dermal gel prepared in this study fulfils all necessary parameters required for topical use. This novel dosage form will improve both the accuracy and the positioning of a delivered dose.

The optimized formulation with better bioadhesive property may improve the bioavaibility of topical administration of clobetasol in gel form and can be alternative to the conventionally administered topical formulations.

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