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FORMULATION AND CHARACTERIZATION OF BUTENAFINE HYDROCHLORIDE FILM FORMING GEL FOR TREATMENT OF FUNGAL INFECTIONS

Madakam. Durga Bhavani*, Purushothaman M

KLR Pharmacy College, Paloncha, Bhadradri Kothgudadm, Telangana- 507511, India.

ABSTRACT

The skin, the body's largest organ and its primary interface with the outside world, makes up over 10% of the entire body. Butenafine is a popular antifungal agent for treating fungal infections on the skin. It is available as creams, gels, solutions, and sprays. Among other uses, Butenafine is an ally amine antifungal agent commonly used to treat dermatophyte infections. Aspergillus, Cryptococcus, and some species of Candida are also reported to have good activity against it in vitro. As a result of the inhibition of squalene epoxidase in the biosynthesis of fungal ergosterol, butenafine induces the accumulation of intracellular squalene and death of cells. Film-forming polymers may be used to form a matrix in either liquids or semisolids. There is sufficient substance in the formed film to ensure sustained release of the drug.

Key words: Formulation, Characterization, Butenafine Hydrochloride, Film Forming Gel

INTRODUCTION

Research in the pharmaceutical industry faces a major challenge in developing new technologies that give formulations unique characteristics that overcome the therapeutic limitations associated with traditional dosage forms, such as adjusting release profiles, allowing multiple active ingredients to be carried simultaneously, improving patient compliance and availability. The purpose of the topical administration of drugs through the skin is for the treatment of skin diseases on a topical basis or for transdermal absorption of drugs into the systemic circulation. It is important to point out that topical delivery is a good alternative both to oral delivery and hypodermic injections, as it offers a large and varied surface as well as the convenience of self-administration [1]. These products also leave a sticky and greasy feeling after application, which leads to a low level of patient compliance after application. Thus, it is essential to develop a dosage form that permits less frequent dosing by maintaining close skin contact for a longer period of time, thus improving patient compliance. A multi-step daily treatment regimen, especially for chronic skin diseases like eczema and psoriasis, often results in poor patient compliance with prescribed dosing regimens. Therefore, it is of continuing interest for dermatological therapy to develop sustained delivery systems for topical drugs that allow less frequent dosing to be possible.

Semisolid dosage forms such as gels contain both solid and liquid components. There may be a liquid component that is hydrophobic or hydrophilic, immobilized within an interconnected three-dimensional network of solids [2].

Anti-Fungal importance as film forming gel

Topical anti-infective and hormonal medications were said to have systemic advantages in the 1940s. Modern transdermal patches first became available in the latter part of the 1970s. Mycoses can be separated into three distinct subtypes: superficial, subcutaneous, and deep.

Corresponding Author: - **Durga Bhavani Email: durgabhavanimadakam1@gmail.com**

A superficial infection is one that does not involve the deeper layers of the body and can affect the skin, hair, nails, or mucous membranes. Fungi are a common cause of skin infections, and the most common types are dermatophytoses, Candida infections, and pityriasis versicolor. Almost all skin diseases can be treated topically, which means that the treatment can reach the desired area directly. By using a topical treatment, systemic side effects may be avoided, or at least attenuated. If you are experiencing a serious skin condition or it is impossible to treat with topical therapy, then you may need more intensive, systemic treatment. Tinea corporis (body ringworm) is pruritic, annular, erythematous, and scaling. It resembles eczema or psoriasis but is solitary. Tinea cruris causes a well-defined, scaling rash in the groins [3].

Figure 1: Schematic representation of permeability of drug through skin to the blood stream

Based on our hypothesis, incorporating the drug into a film forming gel would facilitate prolonged contact between the drug and the skin, and the film that will be formed upon drying will increase the drug's ability to remain on the skin, thereby improving the effectiveness of treatment for fungal skin infections. Medical staff and patients alike have found the buccal route to be particularly popular among those who take the drug. It should be noted that the mucosa is relatively permeable and has a good blood supply [4].

METHODOLOGY

A. Preformulation Studies

- **Determination of Solubility of Butenafine**

Solubility of the Butenafine λ max was tested in various solvents according to the standard procedure

- **Determination of λ max Butenafine**

In phosphate buffer pH 7.4 Stock solution was prepared by dissolving 100mg of pure drug in 100ml of methanol, this was designated as stock solution A (1mg/ml). 10ml of the stock solution was taken in 100ml volumetric flask and made up to 100 ml with Phosphate buffer pH 7.4. This was designated as stock solution B (100 mcg/ml). This stock solution B is further diluted to get 10mcg/ml. The above solution was scanned between 200-400 nm after suitable dilution

Standard curve of Butenafine

From the above stock solution B aliquots of dilutions are made with Phosphate buffer pH 7.4 to get concentration of 2, 4, 6, 8, 10, 12, 14, 16 and 18mcg/ml.

The absorbance of these solutions was measured at 241 nm and a graph was drawn with concentration on X axis and absorbance on Y axis.

B. Formulation of film forming gel

The dispersion was kept undisturbed for one hour. Butenafine was dissolved in about 3 ml of ethanol and mixed. The gel was prepared using high speed homogenizer rotated at 1500 rpm. HPMC K100M was dispersed in water taken in a beaker. The dispersion was kept undisturbed for one hour. Butenafine was dissolved in about 3 ml of ethanol and mixed thoroughly with the HPMC dispersion until a uniform dispersion is formed. Then film forming polymer was dissolved in sufficient quantity of ethanol previously mixed with Polyethylene glycol and the solution was mixed with the dispersion of HPMC and Butenafine until a uniform dispersion was obtained. Mixture of Menthol and Camphor (1:1) was dissolved in propylene glycol and added to the dispersion and it was thoroughly mixed for about two hours to obtain a smooth uniform dispersion. The formulated gel was packed in a collapsible tube [5].

C. Evaluation of Film Forming Gel

- **Properties of Gel**

pH

The pH of the gel was discovered using automated pH meter. Standardized by using buffer solution (pH7) before use. The pH measurement of each of the formulation was done in triplicate form and mean values were calculated[45].

Viscosity

The viscosity of the formulated batches was determined using a Brookfield Viscometer with spindle 04. The average of three readings was taken in 10 minutes was noted as the viscosity of gel[59].

Spreadability

100 mg of the sample was kept at the centre of a glass slide. The slide was covered with another slide and the slides were pressed between fingers until no more expansion of the circle formed by the gel between the slides is observed. The diameter of the circle formed by the gel is measured in centimetres.

Drying time

For the assessment of the drying time the formulation was applied to the glass slide. After 2 minutes another 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.

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.

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.

Bio adhesion test

A uniform film was formed on a glass plate and dried for 24 hr at room temperature. The film was divided into equal areas of 1 mm by cutting with scalpel (0.37 mm blade thickness) both in parallel and perpendicular direction. Total number of squares of the film that adhered on the tape was determined and percentage peel off was determined by the formula [6].

$$Percentage\ peel\ of\ film = \frac{Initial\ squares\ of\ film - Final\ squares\ of\ film}{Initial\ squares\ of\ film} \times 100$$

Film thickness

The films were cut into size of 10 x 40 mm and the thickness of the film using a digital vernier caliper. Each film was measured at five positions (central and the four corners) and the mean thickness was calculated.

Film stickness

Low pressure cotton wool is used to press the dry film in order to determine the stickiness of it. The stickiness is rated depending on how much of the cotton fiber is retained by the film. The stickiness is rated high if there is a thick accumulation of fibers on the film, medium if there is a thin fiber layer on the film and poor if fiber adherence occurs rarely or never. This parameter of assessment is important, as the developed formulation is supposed to be non-sticky to prevent sticking to the clothing of the patients.

Folding endurance

The number of times the film could be folded at the same place without breaking/cracking gave the value of folding endurance.

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 film

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 [7].

Water vapour permeability

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 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 3 ml of distilled water, covered with the circular film samples. 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[59]. 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:

$$\mathbf{WVP = W/ (A*t)\ (g\ cm^{-2}\ 24\ hrs)}$$

***In vitro* drug diffusion study**

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 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°C stirred constantly at 50 rpm. Samples of 1 ml were collected at predetermined time intervals and analyzed 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 [8].

Anti- fungal activity

An agar diffusion method was used for the determination of antifungal activity of formulations. Standard Petri dishes (7.5 cm diameter) containing medium to a depth of 0.5 cm were used. The sterility of the lots was controlled before use. Inoculum was prepared by suspending 1-2 colonies of *Candida albicans* (NCIM no. 3102) from 24 hr. cultures in Sabouraud's medium into tubes containing 10 mL of sterile saline. The tubes were diluted with saline. The inoculum (0.5 mL) was spread over the surface of agar and the plates were dried at 35°C for 15 min prior to placing the formulation. Bores of 0.5 cm diameter were prepared and 20µl samples of formulation (1% w/v) were added in the bores. After incubation at 35°C for 2 days, the zone of inhibition around the bores was measured [9].

RESULTS

A. Preformulation study

Solubility studies:

The solubility of Butenafine was determined in various solvents and it was observed that the solubility of drug is better in Methanol when compared to other solvents.

Determination of wavelength

With the help of a UV spectrophotometer and with dilutions of medication (10µg/ml) in phosphate buffer pH 7.4, the absorbance of Butenafine in the UV range of 200-400 was determined. At 240nm, the maximum absorbance was determined and thus the absorption maximum of the drug was determined. The results were shown in figure 2.

Preparation of calibration curve

To determine the calibration curve for Butenafine drug, 100mg of the pure drug were weighed accurately and made up to 100ml in methanol, which was Stock-A. 10ml of Stock-A was then taken out of Stock-A and made up to 100ml in phosphate buffer 7.4, which was Stock-B.

There are several dilutions that have been made using this method, including 2, 4, 6, 8, 10. The regression values were also calculated to be 0.9998.

B. FORMULATION OF BUTENAFINE FILM FORMING GEL

The film forming gel formulation was prepared by using HPMC 100M as gelling agent and Eudragit RS100 and Eudragit RL100 as film forming polymer. The gels were prepared in ethanol by dispersion method. Ten different batches of gels were prepared by varying the concentration of film forming polymer. All the prepared formulations were subjected to characterization of gel and film and *in vitro* drug diffusion studies to find out the best formulation.

C. Evaluation of film forming gel

pH, Spreadability, Drying time

The pH value of the Film Forming gel formulation. The pH values were found to be in the range of 4.5 to 6.8. According to the spreadability test, the gels spread between a diameter of 4.13 cm and a diameter of 5.77 cm depending on the gel's spreadability. Drying time or film formation time is listed. In order to minimize discomfort to patients, film forming gel should dry quickly on the skin to form a thin film. A satisfactory range of results was achieved with all formulations.

Drug content of gel, Viscosity

Table 6 shows that the drug content in Film Forming gel formulations is 89.19% - 97.45%, indicating that the drug is distributed evenly in the formulation. In order to evaluate the viscosity of developed formulations, Brookfield viscometers were used. The viscosity of formulations F1 to F10 is shown below the table

Evaluation of film properties

Weight variation test, Film thickness, Film stickness

A weight variation test was conducted on the films. There was a weight difference between 0.0312g and 0.052g between the films. There was a range of thicknesses possible with the formulations, from 0.023mm to 0.061mm. Due to the increased amount of polymer used, film thickness increased as polymer concentration increased. The results for outward stickiness of all formulations are shown.

Drug content of film, Folding endurance, Bioadhesion test

All formulations were uniformly tested for content uniformity, and the results are shown in table.11. Spectrophotometric analysis was performed on each formulation to determine its drug content. Butenafine was found in the film in concentrations ranging from 88.87% to 96.92%. In terms of folding endurance, the film's ability to resist rupture is measured. A film with a higher folding endurance will be less likely to rupture. There was a range of folding endurance values between 11 and 20. The bioadhesion of the film formed after drying must be sufficient to ensure it adheres to the skin for 24 hours. Table shows the results of the bioadhesion test.

Water permeability

Table presents the results of water vapour permeability tests. 0.015 to 0.036 g/cm/h was observed as a range of water vapour permeability for the film. In films with a water vapour permeability exceeding 0.05 g/cm/h, the films can be considered non-occlusive since water vapour can pass through them.

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Table 1: Formulation details of Butenafine film forming gel

INGREDIENTS	FORMULATION CODE									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Butenafine Hydrochloride %w/v	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
HPMC K100	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Eudragit RL100	2.5	5	7.5	10	12.5	-	-	-	-	-
Eudragit RS100	-	-	-	-	-	5	7.5	10	12.5	15
PEG 400	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Propylene glycol	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Menthol: Camphor	1	1	1	1	1	1	1	1	1	1
Ethanol (100ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Table 2: Calibration curve of Butenafine

S.No	Concentration (µg/ml)	Absorbance (nm)
1	2	0.115
2	4	0.211
3	6	0.295
4	8	0.375
5	10	0.456

Table 3: pH, Spreadability and drying time of developed film forming gel

Formulation code	pH	Spreadability diameter (cm)	Drying time
F1	5.6±0.019	5.13±0.05	5mins 28secs±12secs
F2	5.4±0.10	4.13±0.05	5mins 45 secs±41secs
F3	5.25±0.009	4.62±0.04	6mins 05 secs±45secs
F4	5.8±0.012	4.73±0.25	4mins 15 secs±14secs
F5	5.5±0.10	5.53±0.21	4mins 49 secs±41secs
F6	5.71±0.009	5.63±0.05	5mins 15 secs±14secs
F7	5.19±0.09	5.22±0.25	5mins 51 secs±40secs
F8	5.3±0.06	4.87±0.25	6mins 25 secs±41secs
F9	5.7±0.1	4.95±0.15	4mins 21 secs±40secs
F10	5.9±0.05	5.77±0.25	4mins 15 secs±22secs

Table 4: Drug content and Viscosity of developed film forming gel

Formulation code	Drug content	Viscosity
F1	91.25%	11500
F2	94.59%	11650
F3	96.36%	11800
F4	93.74%	11960
F5	97.21%	12200
F6	97.45%	11700
F7	90.49%	11940
F8	96.58%	12300
F9	89.19%	12600
F10	98.25%	12900

Table 5: Weight variation of Film, Film Thickness and Film stickness

Formulation code	Weight variation (g)	Film Thickness (mm)	Film stickness
F1	0.0429±0.03	0.022±0.01	Fair
F2	0.0443±0.03	0.038±0.02	Good
F3	0.0448±0.04	0.042±0.01	Fair
F4	0.0492±0.01	0.053±0.01	Good
F5	0.0523±0.03	0.065±0.01	Good

F6	0.0482±0.03	0.028±0.01	Good
F7	0.0356±0.03	0.037±0.01	Good
F8	0.0405±0.03	0.046±0.01	Fair
F9	0.0446±0.03	0.059±0.01	Good
F10	0.0312±0.03	0.061±0.01	Good

Table 6: Drug content of film, Folding endurance, Bioadhesion test

Formulation code	Drug content	Folding endurance	%Peel off
F1	90.85%	14	4
F2	93.86%	11	4
F3	96.03%	15	7
F4	92.65%	17	9
F5	96.76%	20	9
F6	94.84%	19	4
F7	89.78%	17	4
F8	96.24%	19	9
F9	88.87%	15	0
F10	96.92%	21	0

Table 7: Water vapour permeability of developed films

S.no	Formulation code	Water vapour permeability (g/cm/h)
1	F1	0.031
2	F2	0.036
3	F3	0.025
4	F4	0.019
5	F5	0.016
6	F6	0.035
7	F7	0.015
8	F8	0.033
9	F9	0.028
10	F10	0.015

Table 8: Results of antifungal activity

S.No	Formulation code	*Aspergilus spp*	
		Zone of inhibition (mm) ± SD	% Efficacy
1	**STANDARD VALUE**	**21**	**100**
2	**F1**	19.57±0.11	92.20
3	**F2**	17.70±0.81	79.23
4	**F3**	15.17±0.11	71.25
5	**F4**	18.57±0.11	85.25
6	**F5**	19.67±0.11	95.25
7	**F6**	15.76±0.11	75.25
8	**F7**	14.71±0.11	71.21
9	**F8**	13.17±0.11	67.25
10	**F9**	09.57±0.11	51.25
11	**F10**	20.57±0.11	99.45
12	**ETHANOL (CONTROL)**	1.25±0.25	5.26
13	**MARKET CREAM**	**16.28±0.25**	**76.36**

Figure 1. Schematic representation of permeability of drug through skin to the blood stream

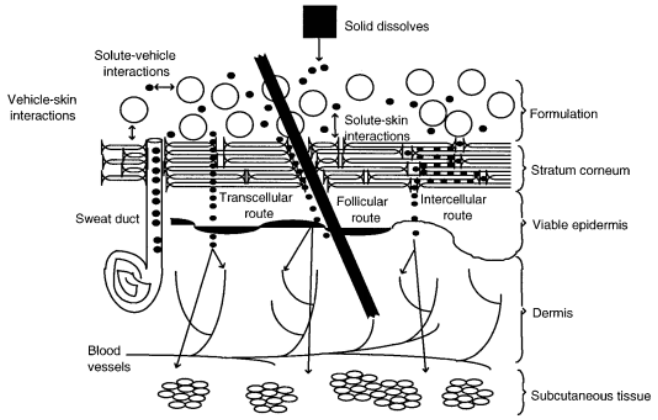


Figure 2. Determination of λ max of butenafine

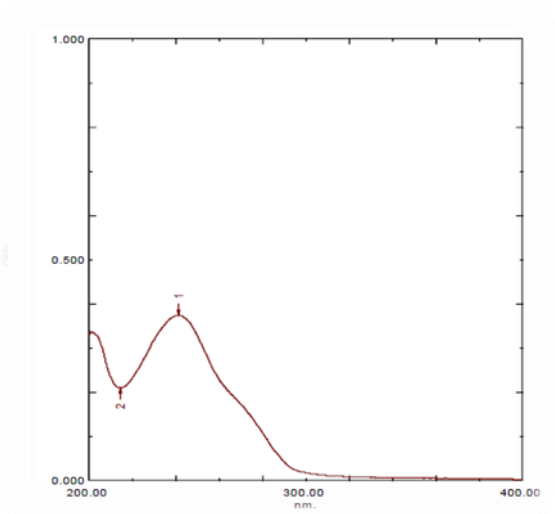


Figure 3. Calibration curve of Butenafine

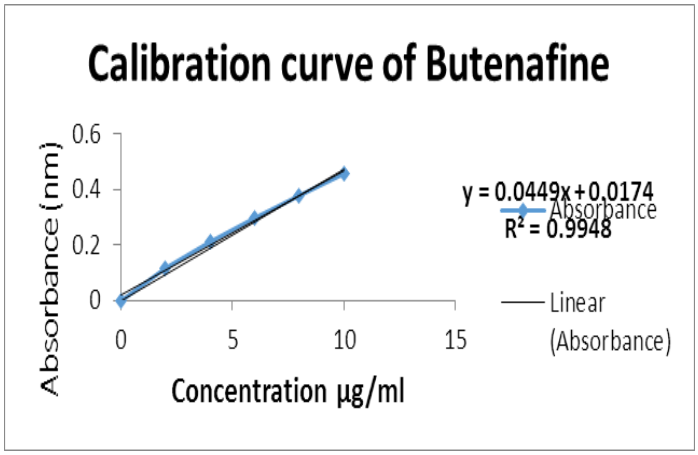


Figure 4. In vitro drug diffusion study (F1-F5)

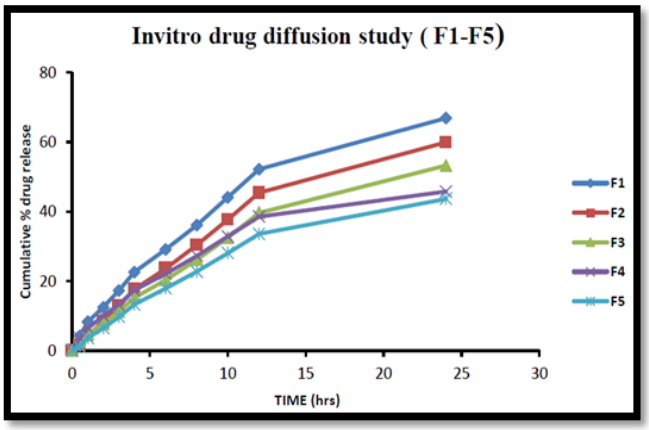
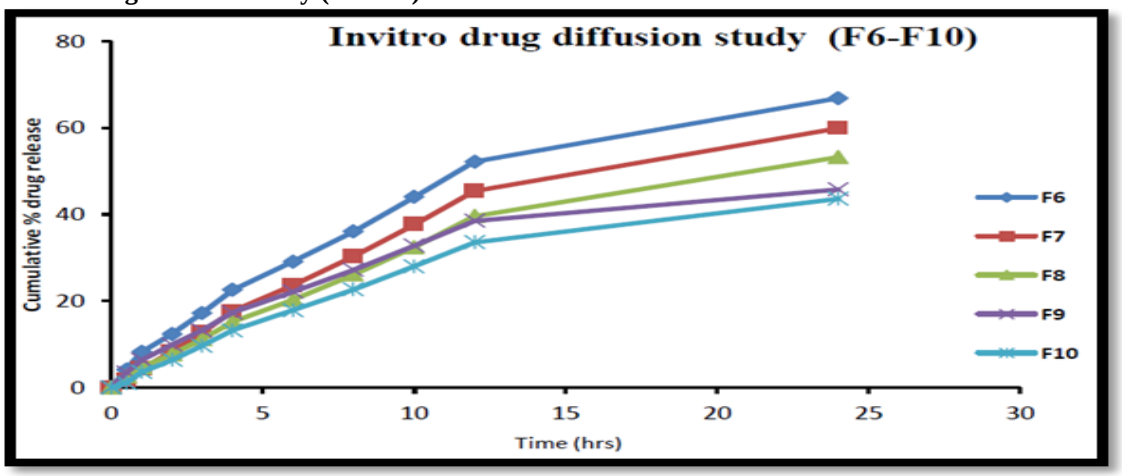


Figure 5. In vitro drug diffusion study (F6-F10)



In vitro drug – diffusion study

In these studies, we found that the Film Forming gel releases drug over a period of 12 hours. Tables 15 and 16 show the results of an in vitro drug diffusion study of a topical gel that had been prepared. The graphical representation of the in vitro drug diffusion study of topical gel is shown in figures 4 and 5. Using formulations F1 to F5, Eudragit RL100 increases the percentage of drug release while increasing the polymer concentration. In contrast, formulations containing Eudragit RS 100 reduce the percentage of drug release while increasing the concentration of polymer and sustain the release of drug for a longer period of time. Among the formulations tested, formulation F10 containing 15% Eudragit RS100 was chosen as the best formulation for long-term drug release.

Butenafine has been shown to retain its antifungal efficacy when formulated as a film-forming dermal gel and to be active against selected strains of microorganisms. In a study conducted on butenafine for the treatment of Aspergillus spp. the zone of inhibition was found to be 21 mm in diameter for standard. It was found that the zone of inhibition for F10 formulation was 20.57 mm and 99.45%. As a control, ethanol's intrinsic antifungal activity was also determined by calculating its zone of inhibition.

CONCLUSION

A synthetic benzylamine antifungal agent known as butenafine hydrochloride is used to treat fungal infections was selected for the formulation of film forming gel (Transdermal delivery system) as it complies with physiochemical properties required to permeate through skin. By developing transdermal delivery of Butenafine, the present study contributed to improving patient compliance through better patient compliance. Butenafine release was largely controlled by diffusion with an optimized formulation showing a sustained drug release profile. A highly innovative method of administering Butenafine to treat fungal infections, it can be easily applied on the skin.

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