FORMULATION AND EVALUATION OF BIFONAZOLE EMULGEL FOR ANTIFUNGAL TREATMENT

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Abstract

Every year, fungi cause infections in billions of people, but their impact on the world's disease burden is mainly underestimated. It is important to understand the variables influencing percutaneous absorption when using topical medications. Emulgels are emulsions, either oil-in-water or water-in-oil, that are mixed with a gelling agent to create a gelled consistency. Emulgel serves as a dual control because it has the characteristics of both gel and emulsions. Thus, for making bifonazle more effective this study aims at formulation and evaluation of bifonazole emulgel for antifungal Treatment. The preparation and evaluation of emulgel was performed according to standard methods. In total six formulations of emulgel was created. In that formulations F1 to F4 exhibit while formulations F5 and F6 are described as having "average" homogeneity. The formulations (F1 to F4) generally exhibit favourable physical characteristics, including high washability, consistent appearance, and good homogeneity. The excrudibility of F3 and F4 was excellent and good for F1 and F2 while that of F5 and F6 was average. Then, formulations F1 to F6 exhibit viscosity values ranging from approximately 3025 cps to 3565 cps. These values suggest that all the formulations have a moderately thick consistency. Formulations F1 to F6 have pH values ranging from approximately 6.54 to 6.98. Further the drug content was found to be maximum for F3 which is 99.85±0.15 while for F6 it is 96.65±0.25. Results of % Cumulative drug release of prepared emulgel optimized formulation F3, was found to be 29.95, 39.98, 52.26, 68.85, 85.54, 99.05 after 15, 30, 45, 60, 120 and 240 min respectively. Further the r^2 was estimated by release kinetics data. The r2 value obtained for the first order release kinetics (0.987) is higher than that for the zero order release kinetics (0.863). Thus, it follows first order release kinetic model. Thus, it can be inferred that

Keywords: Emulgel, Fungal infections, Topical treatment Bifonazole

Introduction

Every year, fungi cause infections in billions of people, but their impact on the world's disease burden is mainly underestimated. Millions of people get illnesses that kill at least as many people as malaria or tuberculosis, but the majority are "relatively" mild infections. Due to contemporary medical interventions and immunosuppressive illnesses like AIDS, the incidence of invasive fungal infections is increasing, despite the fact that true mortality rates are unknown due to a lack of reliable epidemiological data. In 2020, there were reported to have been 1.7 million deaths from fungal disease. Other animals can contract a wide variety of fungal infections, some of which can be passed from animals to humans (Pfaller, 1994).
Targeting the infection site, reducing systemic side effects, increasing treatment efficacy, and enhancing patient compliance are just a few of the benefits of topical treatment for fungal infections. Even though these agents have a therapeutic effect when applied topically, their limited drug delivery across the skin leads to an inadequate therapeutic index, and they may cause both local and systemic side effects. Two abnormalities must be addressed for topical drug delivery to be successful: first, the stratum corneum's barrier function, and second, the drug should ideally deposit in the skin with minimal percutaneous absorption. Thus, a number of innovative delivery vehicles have been created to enhance treatment aspects and enable the delivery of antifungal medications (Marchetti *et al*., 2006).

It is important to understand the variables influencing percutaneous absorption when using topical medications. Three pathways exist for molecules to enter the skin: sebaceous follicles, sweat ducts, and intact stratum corneum. Over 99% of the total skin surface that is available for percutaneous drug absorption is found on the stratum corneum (Kyle and Dahl, 2004).

Large volumes of aqueous or hydroalcoholic liquid are trapped in a network of colloidal solid particles to form gels, a relatively new class of dosage forms. These particles can be inorganic, like aluminum salts, or organic, like synthetic or natural polymers. Compared to the ointment or cream base, they have a higher aqueous component that allows for greater drug dissolution and easy drug migration through a vehicle that is essentially a liquid. They are better in terms of patient acceptability and ease of use. Gels have many benefits, but one significant drawback is their inability to deliver hydrophobic medications. In order to get around this restriction, emulgels are made and applied, enabling even a hydrophobic medicinal moiety to benefit from the special qualities of gels (Talat *et al*., 2021).

Emulgels are emulsions, either oil-in-water or water-in-oil, that are mixed with a gelling agent to create a gelled consistency. The drug particles in the emulsion are trapped in the internal phase, travel through the external phase to the skin, and then slowly absorb as a controlled release drug delivery system. The internal phase of the skin serves as a reservoir for the drug, which is delivered to the external phase of the skin in a controlled manner. Gel's crosslinked network allows it to capture tiny drug particles and release them gradually. Its mucoadhesive quality lengthens the time that a medication is in contact with the skin. Emulgel serves as a dual control because it has the characteristics of both gel and emulsions (Vanpariya *et al*., 2021; Tanaji, 2018).

The way that bifonazole functions is by preventing the synthesis of ergosterol, a material that is vital to the structure of fungal cell membranes. The fungal cytochrome p450 enzyme, also referred to as lanosterol 14-alpha demethylase, is destabilized by it. This is essential to the fungus's cell-membrane structure. Its inhibition causes lysis of the cells. The disruption of ergosterol production results in holes and disruption of the cell membrane. Fungi's cell membranes are essential to their survival. They stop the contents of the cells from leaking out and prevent undesirable materials from entering the cells. Essential components of the fungal cells may seep out as bifonazole creates holes in the cell membranes. As a result, the fungus die (Berg *et al*., 1984; Hector *et al*., 1987).

Thus, for making bifonazle more effective this study aims at formulation and evaluation of bifonazole emulgel for antifungal Treatment.

Materials and Methods

Procurement of drug

Bifonazole was obtained as gift sample from pharmaceutical industry.

Chemicals and reagents

The Carbomer 941, Liquid paraffin, Span 20, Tween 20, Propylene glycol, water etc were obtained from S.D Fine chemicals, Mumbai Pvt Ltd.

Formulation development of emulsion

Preparation of emulsion

The general method was employed for preparation of an emulsion was as follows: The oil phase was prepared by dissolving Span 20 in liquid paraffin in the different ration while the

aqueous phase was prepared by dissolving Tween 20 in purified water (Vats *et al.,* 2014). 1 gram of Bifonazole was dissolved in 5 ml of ethanol, while 0.15 g of methylparaben and 0.05 g of propylparaben were dissolved in 5 gm of propylene glycol and both were mixed with aqueous phase. Both the oily and aqueous phases were separately heated to 70-80°C. Then, the oil phase was added to the aqueous phase with continuous stirring at 500 rpm until cooled to room temperature.

Preparation of carbopol gel

Fifty (50) grams of the carbopol gel was prepared by dispersing 1 gram of carbopol powder in 50 ml purified water with aid of moderate speed stirrer (50 rpm), and then the pH was adjusted to 6.5-6.8 using 0.5 N of sodium hydroxide (Kute and Saudagar, 2013).

Formulation of Bifonazole emulgel

Six formulations of Bifonazole were prepared by dispersing the obtained emulsions with the gel in 1:1 ratio with gentle stirring until get homogenous emulgel.

Table 1: Different formulations of Bifonazole emulgel (% w/w)

Formulation	Bifonazole (mg)	Carbomer 941	Liquid paraffin	Span 20	Tween 20	Propylene glycol	water
F1	500	0.5	5	2	5	5	100
F2	500	0.5	5	2	10	5	100
F3	500	1.0	10	4	5	5	100
F4	500	1.0	10	4	10	5	100
F5	500	1.5	5	2	5	5	100
F6	500	1.5	5	2	10	5	100

Evaluation of emulgel

Physical characteristic

The **physical** characteristic was checked for emulgel formulations (colour, clogging, homogeneity and texture) and observations were noted (Asija *et al.,* 2015).

Determination of pH

The pH of the emulgel was determined by digital pH meter (Bhatt *et al.,* 2013). One gram of gel was dissolved in 25 ml of distilled water and the electrode was then dipped in to gel formulation for 30 min until constant reading obtained. And constant reading was noted. The measurements of pH of each formulation were replicated two times.

Washability

Formulations were applied on the skin and then ease and extent of washing with water were checked manually and observations were noted (Singla *et al.,* 2012).

Extrudability study

The emulgel formulations were filled into collapsible metal tubes or aluminium collapsible tubes (Magdy, 2004). The tubes were pressed to extrude the material and the extrudability of the formulation was checked.

Spreadability

Two glass slides of standard dimensions (6×2) were selected. The emulgel formulation whose spreadability had to be determined was placed over one of the slides. The second slide was placed over the slide in such a way that the formulation was sandwiched between them across a length of 6 cms along the slide. 100 grams of weight was placed up on the upper slide so that the emulgel formulation between the two slides was traced uniformly to form a thin layer.

The weight was removed and the excess of the emulgel formulation adhering to the slides was scrapped off. The lower slide was fixed on the board of the apparatus and one end of the upper slide was tied to a string to which 20 gram load could be applied 50 with the help of a simple pulley. The time taken for the upper slide to travel the distance of 6 cms and separate away from lower slide under the direction of the weight was noted. The experiment was repeated and the average of 6 such determinations was calculated for each emulgel formulation.

Viscosity

The measurement of viscosity of the prepared gel was done using Brookfield digital Viscometer (Kokane and Naik, 2013). The viscosity was measured using spindle no. 6 at 10 rpm and 25^0C. The sufficient quantity of gel was filled in appropriate wide mouth container. The gel was filled in the wide mouth container in such way that it should sufficiently allow to dip the spindle of the Viscometer. Samples of the gels were allowed to settle over 30 min at the constant temperature (25 $\pm/1^0$C) before the measurements.

Drug content

1 gm. of the prepared gel was mixed with 100 ml. of ethanol. Aliquots of different concentrations were prepared by suitable dilutions after filtering the stock solution and the absorbance was measured at 272 nm. Drug content was calculated by linear regression analysis of the calibration curve.

***In-vitro* drug release studies using the prehydrated cellophane membrane**

Preparation of cellophane membrane for the diffusion studies:

The cellophane membrane approximately 25 cm x 2cm was taken and washed in the running water (Ayub *et al.,* 2007). It was then soaked in distilled water for 24 hours, before used for diffusion studies to remove glycerin present on it and was mounted on the diffusion cell for further studies.

Diffusion Studies:

The *in-vitro* diffusion of drug from the different gel preparations were studied using the classical standard cylindrical tube fabricated in the laboratory; a simple modification of the cell is a glass tube of 15 mm internal diameter and 100 mm height. The diffusion cell membrane was applied with 1 gram of the formulation and was tied securely to one end of the tube, the other end kept open to ambient conditions which acted as donor compartment. The cell was inverted and immersed slightly in 250 ml of beaker containing neutralizing 7.4 pH phosphate buffer, freshly prepared as a receptor base and the system was maintained for 2 hrs at 37± 0.5^{o} C. The media was stirred using magnetic stirrer. Aliquots, each of 5 ml volume were withdrawn periodically at predetermined time interval of upto 12 hrs and replaced by an equal volume of the receptor medium. The aliquots were suitably diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 272 nm using neutralizing 7.4 pH phosphate buffer as blank.

Data analysis via drug release kinetics study

The results of in-vitro release profile obtained for all the formulations were plotted in kinetic models as follows,

1. Cumulative of drug released versus time (Zero order kinetic model).
2. Log cumulative percent drug remaining to be absorbed versus time (First order model).
3. Cumulative amount of drug release versus square root of time (Higuchi model).
4. Log cumulative drug released versus log time (Korsmeyer-Peppas model).

Results and Discussion

In total total six formulations of emulgel was created. In that formulations F1 to F4 exhibit good homogeneity, indicating that the ingredients are well mixed and distributed uniformly within the formulations. This is a positive characteristic, ensuring that the active ingredients and excipients are evenly distributed throughout the product, leading to consistent performance.

Formulations F5 and F6 are described as having "average" homogeneity. This suggests that there may be some variations or uneven distribution of components within these formulations. Achieving good homogeneity is crucial to ensure consistent efficacy and safety of the product.

The formulations (F1 to F4) generally exhibit favorable physical characteristics, including high washability, consistent appearance, and good homogeneity. However, formulations F5 and F6 raise concerns due to the presence of clogging and average homogeneity. Addressing these issues may be necessary to improve the overall quality and user experience of these formulations.

The excrudibility of F3 and F4 was excellent and good for F1 and F2 while that of F5 and F6 was average. Formulations F1 to F6 exhibit viscosity values ranging from approximately 3025 cps to 3565 cps. These values suggest that all the formulations have a moderately thick consistency. The viscosity values suggest that all the formulations have a similar consistency, falling within a moderately thick range. This consistency is likely suitable for emulgel formulations, as it allows for easy application without being too runny or too thick.

Formulations F1 to F6 have pH values ranging from approximately 6.54 to 6.98. All these pH values fall within a slightly acidic to near-neutral range. The viscosity and pH values of the emulgel formulations (F1 to F6) suggest that they have suitable physical properties for topical applications.

Further the drug content was found to be maximum for F3 which is 99.85±0.15 while for F6 it is 96.65±0.25. Results of % Cumulative drug release of prepared emulgel optimized formulation formulation F3, was found to be 29.95, 39.98, 52.26, 68.85, 85.54, 99.05 after 15, 30, 45, 60, 120 and 240 min respectively. These results indicate the progressive release of the drug from the emulgel formulation over time. The cumulative drug release represents the total amount of drug released at each time point relative to the total drug content in the formulation.

Further the r^2 was estimated by release kinetics data. The r2 value obtained for the first order release kinetics (0.987) is higher than that for the zero order release kinetics (0.863). A higher r2 value suggests a better fit of the data to the first order release kinetic model compared to the zero order model.

Table 2: Physical characteristic

Formulation	Washability	Observation	Clogging	Homogeneity
F1	+++	white cream	Absent	Good
F2	+++	white cream	Absent	Good
F3	+++	white cream	Absent	Good
F4	+++	white cream	Absent	Good
F5	+++	white cream	Present	Average
F6	+++	white cream	Present	Average

Table 3: Extrudability and Spreadability study

Formulation	Extrudability	Spreadability (gcm/sec)
F1	++	13.25±1.15
F2	++	12.65±0.95
F3	+++	12.98±1.25
F4	+++	11.65±1.62
F5	+	9.65±1.78
F6	+	8.85±1.13

Table 4: Viscosity and pH

Formulation	Viscosity (cps)	pH
F1	3565±15	6.98±0.05
F2	3285±13	6.75±0.03
F3	3165±16	6.95±0.04
F4	3295±12	6.54±0.06
F5	3145±09	6.74±0.08

F6	3025±11	6.82±0.02

Table 5: Results of drug content of emulgel

Formulation	% Drug content
F1	98.85±0.15
F2	98.12±0.32
F3	99.85±0.15
F4	97.65±0.36
F5	98.12±0.11
F6	96.65±0.25

Table 6 % Cumulative drug release of formulation F1-F6

S. No.	Time (min)	% Cumulative drug release						Marketed Formulation
		F1	F2	F3	F4	F5	F6	
1	15	18.85	22.32	31.15	22.15	23.52	20.14	33.32
2	30	29.98	35.65	38.85	30.52	36.65	33.36	49.98
3	45	36.65	45.65	50.23	42.25	41.12	45.47	96.65
4	60	49.98	55.62	65.58	53.32	58.85	53.32	-
5	120	63.32	69.98	83.32	81.14	76.65	69.98	-
6	240	75.65	86.65	98.78	88.98	83.32	75.45	-

Table 7: *In vitro* drug release data for optimized formulation F3

S. No.	Time (min)	Square Root of Time	Log Time	Cumulative* Percentage Drug Release ± SD	Log Cumulative Percentage Drug Release	Cumulative Percent Drug Remaining	Log cumulative Percent Drug Remaining
1	15	3.873	1.176	31.15	1.493	68.85	1.838
2	30	5.477	1.477	38.85	1.589	61.15	1.786
3	45	6.708	1.653	50.23	1.701	49.77	1.697
4	60	7.746	1.778	65.58	1.817	34.42	1.537
5	120	10.954	2.079	83.32	1.921	16.68	1.222
6	240	15.492	2.380	98.78	1.995	1.22	0.086

Conclusion

Based on the results above, we can say that Bifonazole, which was made with varying amounts of Carbomer 941, Liquid paraffin, Span 20, Tween 20, Propylene glycol, had acceptable diffusion, rheological properties, and physical appearance. The results of this study unequivocally demonstrated that, when compared to synthetic formulations, the emulgel formulations of bifonazole made with carbomer 941. The most promising method has all ideal characteristics. Further this emulgel can be evaluated for in vivo studies and clinical trials.

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