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Preparation And Evaluation Of Bifonazole Hydrogel For Topical Application

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ABSTRACT

The present work aimed to prepare and evaluate the topical hydrogel formulation of an anti-fungal drug Bifonazole. Total 10 batches of Bifonazole (1% w/w) hydrogel formulations were prepared by using two different polymers i.e., HPMC K100M and chitosan. SLS, PEG 400 and Oleic acid were used as permeation enhancers. Methyl paraben and propyl paraben were used as preservative. Prepared hydrogel was evaluated for pH, viscosity, rheology, spreadability, drug content, in-vitro diffusion studies, ex-vivo skin permeation studies, release kinetics studies and short-term stability studies. All batches of gel formulations showed uniform homogeneity and spreadability. The physical appearance of the gel formulations was white translucent in nature. pH of the gel formulation was suitable for topical application. Formulation batches containing combination of polymers showed significantly increased viscosity when compared to control formulation. The highest drug permeability was achieved when permeation enhancers were used in the formulation. Release profile was increased with increase in permeation enhancer concentration. Highest percentage of drug release and permeability was achieved from formulation F8. The in-vitro release profile of drug from all gel formulations followed zero order kinetics and showed Super case II transport mechanism. Short term stability studies showed that physico-chemically stable throughout the stability period. In conclusion, permeation enhancer-based hydrogel formulation of Bifonazole was successfully formulated to improve the drug release and skin permeability.

1. Introduction

In recent times, the therapeutic efficacy of developing completely controlled drug administration, in which drug synthesis can be regulated in a specific controlled manner, has become more widely recognized. Skin has long been used as a route of entrance further into body for medicines to be delivered systemically. Because of the skin's barrier qualities, penetration beyond the epithelial boundary is slow, reducing transdermal bioavailability (Shinde *et al.*, 2010).

Transdermal medication administration is becoming increasingly popular in modern medicine. This treatment is utilized for non-ionized drugs with a low dose. Because the skin seems to be a semi-permeable membrane, even small amount of any drug molecule can flow through it without being detected. Therefore, passive diffusion alone isn't enough for many medications. Because ionic medications have a high predisposition towards breaking through the epidermis barrier, they can't be administered transdermally unless a different energy source is used to push the drug through. Ultrasound (phonophoresis) as well as electrical (iontophoresis) energy, are used to give this external energy. The use of topical agents frequently necessitates the use of modulators or chemical penetration enhancers, which can be irritating or sensitizers (Rai *et al.,* 2005; Kassan *et al.,* 1996).

Due to their swelling characteristics, adhesiveness, and biocompatibility, hydrogels have recently gained popularity. Drugs can be incorporated into hydrogels and their release kinetics can be controlled. After adhering to any kind of an epidermis or mucous membrane through hydration and contact, it produces a complex molecular system with uniform drug distribution. It's a revolutionary topical pharmaceutical delivery technology for epidermis, ocular mucosal, nasal cavity, and other places (Xia *et al.*, 2015).

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Skin infections produced by fungi are referred to as fungus skin infections. Skin problems produced by fungi that feed on keratin, a protein found in the skin, hair, & nails, are known as fungus skin infections. Rashes with a range of looks, including such itchy, red, scaly, and dry skin, are signs and symptoms of a fungal infection. Antifungal medications are often used to treat infections caused (Pawar et al., 2015). Bifonazole (BFZ) is an azole antifungal that is used to treat tinea, Athlete's foot (tinea pedis), and body ringworm. Despite its limited absorption rate after systemic treatment using BFZ gel, bifonazole has been utilized to treat Athlete's foot by using it topically once a day for 2-3 weeks (Sahoo *et al.,* 2013; Karpe *et al.,* 2013)

Materials And Methods

Materials

Bifonazole, HPMC K30, Chitosan, Sodium lauryl sulphate (SLS) and PEG 400 were obtained from Yarrow Chem products, Mumbai, India. Triethanolamine and oleic acid (Himedia, Mumbai, India), Methylparaben and Propylparaben (Sigma-Aldrich, USA) were the other materials used in the study. The chemicals and reagents were mostly of good analytical quality.

Formulation of Bifonazole Hydrogel

Bifonazole 1 % w/w hydrogel compositions were made with two distinct polymers i.e., HPMC K100M and chitosan. SLS, PEG 400 and Oleic acid were used as permeation enhancers. Methyl paraben (MP) and propyl paraben (PP) were used as preservative. Briefly, polymer was slowly dispersed into small quantity of distilled water and allowed to swell for overnight. Bifonazole 1% w/w solution was prepared by dissolving drug into oleic acid (0.5%) solution. The drug solution was slowly poured into the gel base and mixed well for content uniformity. Through careful blending was done followed by incorporation of enhancers to the base. Finally, preservative was added and mixed thoroughly. Then the final volume was made with the previously boiled and cooled water[8]. The formulation chart of Bifonazole hydrogel is mentioned in table 1.

Evaluation of Bifonazole hydrogel

Determination of pH

The pH levels within gels have been measured using a calibrated pH meter. (Techno-scientific products Mumbai). All values have been calculated using the average of the three samples.

Viscosity and rheological studies

The viscosity and rheological behavior of Bifonazole hydrogel were determined employing Brookfield digital viscometer (Model RV DV2T, USA) with spindle no 6. The thickness of the gel was measured at various angular velocities at a temp of 25°C. The angular velocity was changed between 5 and 25 rpm throughout a typical run (Chen *et al.,* 2015).

Spreadability

Excess sample was placed between two glass slides and cramped to uniform thickness by placing1000 gm weight for 5 minutes to assess spreadability. 50gm weights were placed in the pan. The amount of time required to detach two slides was noted down, including the time it has taken for upper glass slides and move across the lower plate, was used to calculate spreadability (S).[9]

Drug content estimation

The UV technique was used to determine the drug concentration. 100 cc of pH 6.8 PBS with Tween 80 was used to dissolve 1 gramme of gel (1 percent). After passing 1 mL of solution into a 10 mL volumetric flask, the final volume was determined using the same solutions. Finally, the produced sample's absorbance was measured at 254 nm (Shimadzu-1800, Japan). The drug content was determined as a percentage (Mundada *et al*., 2013).

In-vitro drug diffusion profile

A Franz diffusion cell with cellophane dialysis membrane with 8000 Dalton molecular weight was used for the *in-vitro* drug diffusion tests. The water-jacketed recipient compartment had two arms, one for sampling and another for the thermometer, and carried a total of 25 ml. The donor chamber had a 2-centimeter inner diameter. The donor compartment was created to only come into touch with the receptor compartment's diffusion medium. Phosphate buffer (PBS) containing Tween 80 (1%) was used as the receptor media (buffer solution) in the receptor chamber, which was kept at 37°C±1. The donor chamber has been loaded with 1 g of 1 percent BFZ (w/w) hydrogel. Aliquots (1 ml) of both the sample have been pipetted out now and reconstituted with an equivalent amount of new diffusion media after 1, 2, 4, 6, 8, 10, and 12 hours. After being filtered using Whatman No. 2 filter paper at 254 nm, the solution was analyzed using a UV spectrophotometer (Shimadzu-1800, Japan) (Nava *et al*., 2011).

Ex-vivo skin permeation studies

Franz diffusion cells with a 2 cm2 high diffusion surface are used in *ex-vivo* skin permeation study. Both donor and receptor compartments of Franz diffusion cells' Stratum corneum have been divided, with a dissected skin sample from a rat (dorsal side) towards the donor compartment. After that, 1 gram of BFZ (10 mg) hydrogel was deposited in the donor chamber. Fresh pH 6.8 phosphate buffer (PBS) with Tween 80 (1%) was added to the receptor chamber and kept at 37°C±1 with constant stirring (100 rpm). 1 ml of a receptor chamber sample was withdrawn & reconstituted with the same volume of the pure medium at predetermined intervals (1, 2, 4, 6, 8, 10, and 12 hours). All materials were filtered via Whatman filter paper before being analyzed with a UV spectrophotometer (Sabale *et al*., 2012)

Drug release kinetics studies

The Higuchi equation, zero order kinetics, and the first order kinetics were used to examine release of the drug from a Bifonazole hydrogel. This release mechanism was identified by Korsmeyer Peppas' model (Jalalil *et al.,* 2008).

Stability studies

According to ICH requirements, six months of stability testing was performed on the optimized batches of Bifonazole hydrogel. The *in-vitro* release profile led to the selection of Formulation F8. The selected combinations were packed in wide-mouth glass jars having tightly sealed mouths and coated in aluminum foil. Stability tests were conducted for six months at 25°C/60% RH and 40°C/75% RH. The hydrogels' pH, drug content, and drug release were all investigated (Mao *et al.,* 2019). The t-test was used to analyze the data. The $p<0.05$ significance criterion was used to determine whether the results were statistically significant.

Bifonazole hydrogel were determined using a Brookfield digital viscometer with spindle no 6. Formulations F7, F8, F9, and F10, which contained a mixture of HPMC K100M (1%) and chitosan (0.5%), were viscouser than formulations F3, F4, F5, and F6, which contained a mixture of HPMC K100M (0.5%) and chitosan (0.5%). (1 percent). As that the concentration of Hydroxypropyl Methylcellulose in the gel rises, the consistency of the gel grows. The polymer mixture had a viscosity that was significantly higher than the controls, F1 and F2. The formulations' rheological studies were examined by graphing shear rate vs viscosity on a graph (figure 1). According to a rheological investigation, the viscosity of the formulations reduces as the sharing rate increases, indicating pseudoplastic and non-Newtonian flow

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Bifonazole (%w/w)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
HPMC K100M (%)	1.5	--	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0
Chitosan (%)	--	1.5	1.0	1.0	1.0	1.0	0.5	0.5	0.5	0.5
Sodium lauryl sulphate (SLS) (%)	--	--	1.0	2.0	--	--	1.0	2.0	--	--
PEG 400 (%)	--	--	--	--	1.0	2.0	--	--	1.0	2.0
Oleic acid (%)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methylparaben (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propylparaben (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Triethanolamine	quantity sufficient									
Distilled Water Q.S(ml)	100	100	100	100	100	100	100	100	100	100

Table 1: Formulation design of Bifonazole hydrogel

Results And Discussion

Fungal infection of skin is now-a-days one of the common skin related problems. Solid, semisolid, & liquid dosage forms are among the therapeutic alternatives available to doctors. Clear transparent gels have been used as a topical preparation for cosmetics and pharmaceuticals for a long time. The skin, readily distributed organs for systemic application on the human body, is the most common route for transdermal medication delivery. A BFZ hydrogel formulation for transdermal medicine administration was created as a result. HPMC K100M and chitosan as a gel foundation were used to create a bifonazole hydrogel. The hydrogels' pH, viscosity, spreadability, & drug content, as well as drug release behaviors, ex-vivo skin permeation experiments, *in-vitro* release kinetic analyses, including short-term stability tests, were all investigated.

Determination of pH

A validated pH meter measures the pH of the Bifonazole Hydrogels. A total of three samples were collected for the readings. At 25°C±1°C temperature, the pH of the produced hydrogel ranged from 6.5±0.81 to 7.2±0.87. As a result, the pH range obtained is physiologically appropriate for topical treatments. Table 2 shows the pH results.

Viscosity determination studies

The firmness of gel compositions is often reflected in their viscosity. The viscosity and rheological parameters of properties (shear thinning). Because topical formulations should be applied topically in a thin layer, sample uniformity is critical. As a result, non-Newtonian formulations are favored since they have the least amount of flow resistance when applied at high shear rates.

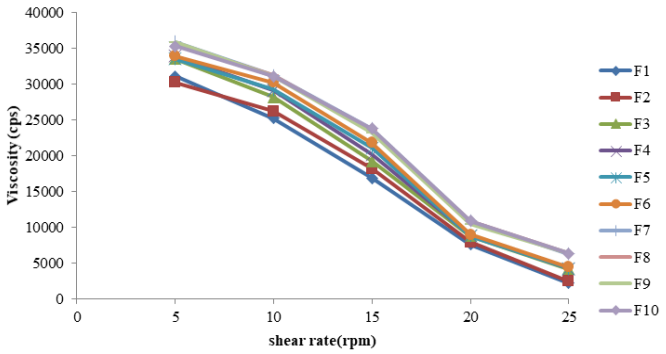

Figure 1: Rheological profile of Bifonazole hydrogel formulations (F1-F10)

Determination of spreadability

The spreadability of various formulations is shown in Table 2. Spreadability rises as HPMC K100M concentrations fall, and vice versa, according to this study. Control compositions F1 as well as F2 were found being more spreadable than other formulations. All gel compositions demonstrated good spreadability, as evidenced by the data.

Drug content estimation

The drug content estimation was done by using UV spectrophotometer (Shimadzu UV-1800). Drug content data of BFZ hydrogel was obtained between 97.81±0.45 to 99.16±0.62 % w/w. This indicates that the BFZ was thoroughly mixed throughout the formulations as evident from the low standard deviation value. The result of drug content was shown in table 2.

Formulation	pH	Spreadability (gm.cm/sec)	Drug content (% w/w)
F1	6.8±0.25	9.8±0.25	98.81± 0.58
F2	6.9±0.29	9.9±0.29	97.84± 0.29
F3	6.5±0.81	7.5±0.81	97.81± 0.45
F4	7.1±0.55	7.1±0.55	98.90± 0.34
F5	6.6±0.26	7.6±0.26	98.02± 0.38
F6	6.9±0.74	7.3±0.53	97.95± 0.63
F7	7.2±0.87	5.9±0.28	98.44± 0.26
F8	7.0±0.35	5.5±0.46	99.16±0.62
F9	6.6±0.69	6.1±0.33	97.90± 0.41
F10	7.1±0.56	5.8±0.59	99.03± 0.28

Table 2: Results of pH, spreadability and drug content of Bifonazole hydrogel

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

In-vitro release studies were performed to predict whether a delivery mechanism would function in the best-case scenario and to demonstrate its in-vivo performance. Because the amount of medicine available for absorption is determined by drug release. A Franz diffusion unit towards a cellophane membrane was used to conduct the drug release studies in vitro (molecular weight of 8000 Dalton). Figure 2 compares the percent release of several enhancers to control formulations (F1 and F2) after 12 hours. The application of a penetration enhancer is one way to improve topical drug delivery. Several compounds have been studied in this area. The most often utilized excipient in topically administered dosage forms has been propylene glycol. The effect of PEG 400 and SLS (1 and 2%, respectively) concentrations on Bifonazole permeability was investigated in this study. Formulation F8, which contains 2% SLS, achieved maximum flow (98.65%) in just 6 hours, and Formulation F10, which contains 2% PEG 400, achieved maximum flow (99.41%) in just 8 hours. Formulations F3 and F5, which contained 1% SLS and 1% PEG 400, respectively, enhanced the release of the drug as compared to control formulations. The F1 as well as F2 control formulations produced 78.39 percent and 67.02 percent of the BFZ, respectively, after 12 hours. It's possible that the deficiency of permeability enhancers in these formulations is to blame for the delayed drug release. There are two types of penetration enhancers within hydrogel formulations F3-F10 (SLS and PEG 400). Each enhancer was used in two concentrations, with higher concentrations resulting in higher release rates. Compared to the formulations containing the highest amount of HPMC K100M, a hydrogel formulation including a high proportion of chitosan exhibited lower drug release. The amount of medication discharged from batch to batch of hydrogel formulations ranged from F8>F10>F4>F6>F3>F5>F7>F9>F1>F2 a few milligrams to hundreds of milligrams. The mean standard deviation was calculated to be between 0.21±0.76.

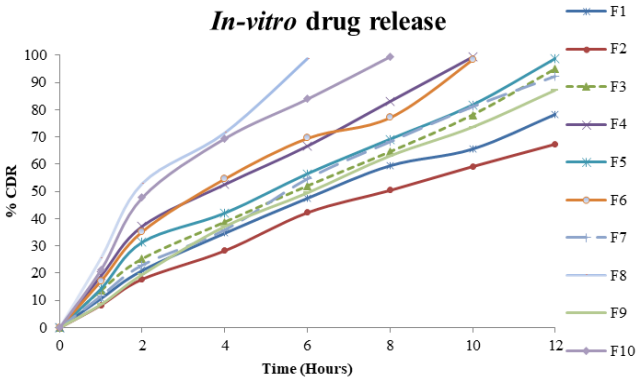

Figure 2: In-vitro drug release profile Bifonazole hydrogel formulations

***Ex-vivo* skin permeation study**

Figure 3 shows the BFZ permeation profiles of the hydrogel compositions that were created. The amount of Bifonazole delivered via hydrogel formulation F8 was 826.04 g, much more than the subsequent groups of hydrogel formulations. The quantity of medication that penetrated from the control formulations, F1 and F2 (without permeation enhancers), was significantly lower. Permeation enhancers' high permeability allows them to easily penetrate the skin and circumvent the barrier function by compressing through stratum corneum's intracellular lipid, leading to better Bifonazole permeation via Bifonazole hydrogel. Also, underneath the influence of the osmotic, hydrogels transfer from the dry stratum corneum to a deep hydrated layer, as well as the occurrence of permeation enhancers within hydrogel aids in the solubilization of the lipid layer within stratum corneum, allowing for a high permeability of enhancer-containing hydrogel. The flow coefficient values increased as penetration enhancers were added, showing that BFZ penetration enhanced. It's expected that increasing permeation enhancer concentrations may lead to continuing solubilized complexes with the medication, as well as signs of greater drug penetration. In general, increasing overall concentrations of penetration enhancers in gels is thought to promote drug penetration.

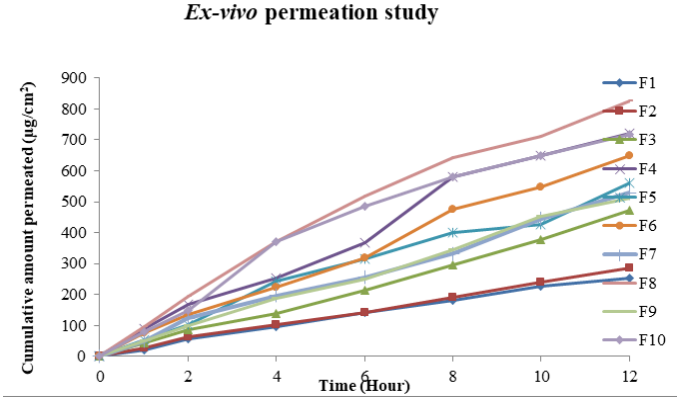

Figure 3: Ex-vivo permeation profile Bifonazole hydrogel formulations

Formulation	Flux value (J) (µg/cm²/hr)	Permeability coefficient (K_p) (cm/h)
F1	21.46	0.0215
F2	23.25	0.0233
F3	38.25	0.0383
F4	51.67	0.0517
F5	45.08	0.0451
F6	53.71	0.0537
F7	42.53	0.0425
F8	68.69	0.0687
F9	42.85	0.0429
F10	61.39	0.0613

Table 3: Permeability parameters of different batches of Bifonazole hydrogel

Peppas model to corroborate the diffusion mechanism. The gel formulations F1-F10 demonstrated strong linearity (r2 = 0.545-0.789) and slopes (n) ranging from 1.117 to 1.495, indicating that diffusion-controlled drug release. All the developed hydrogel formulations utilised a Super Case II process because their 'n' values were greater than 0.89.

Stability Studies

Temperature, humidity, and other external conditions all have an impact on product quality; hence stability testing is useful. Throughout preservation, shipment, and handling, medications as well as their formulations are subjected to a variety of circumstances. In bifonazole hydrogel formulations kept at 25°C±2°C/60 percent RH and 40°C±2°C/75 percent RH for 1, 2, 3, and 6 months, pH, assays, as well as in medication dispersion were investigated. Based on the in-vitro release profile, Formulation F8 was chosen. The appearance as well as pH of the hydrogel did not alter much. The data showed that the drug content and release behaviour did not change significantly ($p>0.05$) after six months of storage. As a result, the BFZ hydrogel's physicochemical stability was maintained during the stability period. The stability testing results are shown in Table 5

Formulation	Release Kinetics Models					Best Fit Model	Drug release mechanism
	Zero order	First order	Higuchi	Korsmeyer-Peppas			
	r^2	r^2	r^2	r^2	n		
F1	0.987	0.787	0.941	0.748	1.248	Zero order	Super case 2
F2	0.958	0.826	0.895	0.782	1.182	Zero order	Super case 2
F3	0.995	0.595	0.912	0.717	1.117	Zero order	Super case 2
F4	0.980	0.805	0.963	0.636	1.236	Zero order	Super case 2
F5	0.991	0.856	0.937	0.697	1.297	Zero order	Super case 2
F6	0.974	0.834	0.912	0.681	1.181	Zero order	Super case 2
F7	0.953	0.794	0.893	0.755	1.255	Zero order	Super case 2
F8	0.991	0.693	0.974	0.545	1.145	Zero order	Super case 2
F9	0.995	0.852	0.983	0.789	1.341	Zero order	Super case 2
F10	0.993	0.841	0.942	0.598	1.495	Zero order	Super case 2

Table 4: Results of kinetic model fitted for Bifonazole hydrogel formulations

Duration (Month)	25°C/60% RH			40°C/75% RH		
	pH	% Drug content	% CDR	pH	% Drug content	% CDR
Initial	7.0±**0.35**	99.16±**0.62**	98.65±0.74	7.0±**0.35**	99.16±**0.62**	98.65±0.74
1	7.0±**0.48**	99.13±**0.40**	98.61±0.49	7.0±**0.21**	99.10±**0.78**	98.24±0.82
2	6.9±**0.27**	99.05±**0.69**	98.54±0.82	6.9±**0.16**	99.02±**0.50**	97.96±0.33
3	6.9±**0.13**	98.97±**0.82**	98.09±0.56	6.9±**0.90**	98.95±**0.36**	97.41±0.52
6	6.8±**0.59**	98.86±**0.71**	97.15±0.20	6.8±**0.37**	98.83±**0.47**	97.05±0.49
p=value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
	non-significant			non-significant		

Table 5: Results of stability studies of Bifonazole hydrogel formulation (F8)

Release kinetics investigation:

Table 4 illustrates the findings of release kinetics investigations that fit numerous kinetics models using cumulative releasing data. When compared to first order (r2 = 0.595-0.856) and the Higuchi model (r2 = 0.893-0.983), the in-vitro release rate of pharmaceuticals among all gel formulations may be represented by zero order kinetics. As a result, all formulations were assumed to follow a zero-order release pattern. To confirm the diffusion mechanism, the drug release data was subjected into the Korsmeyer-

Conclusion

In this study, multiple types of penetration enhancers have been used to make BFZ hydrogel. Permeation enhancer

levels were varied, and the effect upon drug release activity were measured. pH, viscosity, drug content, in-vitro drug release studies, ex-vivo skin penetration, as well as release kinetics investigations were all performed on the generated hydrogel. The highest penetration of Bifonazole was achieved when permeable enhancers were used in the formulation. Total drug release behaviour enhanced whenever the proportion of permeability enhancers was increased. All gel preparations' in-vitro drug release profiles follow zero order kinetics, indicating a Super case II process. Finally, a permeation enhancer based Bifonazole hydrogel formulations was effectively produced to boost release of the drug and permeability.

Conflict of Interest: NIL

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