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Formulation and evaluation of bifonazole organogel for the application of topical drug delivery system

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ABSTRACT

The main aim of the present study was to prepare and evaluate novel in situ gelling topical drug delivery of bifonazole by using sorbitan monostearate. The formulations were prepared and evaluated for rheological study, appearance, texture, viscosity, measurement of pH, gelling capacity, drug content estimation and in vitro diffusion study. Seven formulations were developed using 70 % oil phase and 30 % aqueous phase. It was found that the pH of all the formulations was in the range of to 5.8 -6.6 that suits for the skin pH, indicating skin compatibility. The IR study was performed to confirm interaction of drugs with formulations, it indicated that there was no drug excipient interactions. The optimized formulation BF_4 containing 10% Sorbian monostearate showed prolong release with 83.92% at the end of 8hrs .The developed formulation was stable, non irritant and provided sustained release over 8 hrs.

Key words: Gelling capacity, Bifonazole, Topical drug delivery

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INTRODUCTION

Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders or the cutaneous manifestations of a general disease with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin[1,2]. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solution, organogel and even medicated adhesive systems are in us. Organogels have an organic solvent as the liquid continuous medium. Organogels are formed by specific kind of small organic molecules, which in many solvents very effectively self-assemble into a three dimensional network of nanoscale dimension, thereby turning a liquid into a gel[3,4]. Organogel formation is based on the spontaneous self-association of individual gelator molecules into three-dimensional networks of randomly entangled fiber-like structures, thereby confining the solvent at the microscopic level. Organogels can be distinguished from hydrogels by their predominantly organic continuous phase and can then be further subdivided based on the nature of the gelling molecule: polymeric or low molecular

weight (LMW) organogelators. Polymers immobilize the organic solventby forming a network of either cross-linked or entangled chains for chemical and physical gels, respectively. The latter is possibly further stabilized by weak inter-chain interactions such as hydrogen bonding, van der Waals forces, and π-stacking[5,6]. Despite the large abundance and variety of organogel systems, relatively few have current applications in drug delivery, owing mostly to the lack of information on the biocompatibility and toxicity of organogelator molecules and their degradation products. Bifonazole is a substituted imidazole antifungal agent possesses abroad spectrum of activity in vitro against dermatophytes, moulds, yeasts, dimorphic fungi and some gram positive bacteria. Both noncomparative and comparative clinical trials have clearly demonstrated the efficacy and safety of various formulations of bifonazole 1%(cream,gel,solution and powder) applied once daily in the treatment of superficial fungal infections of the skin such as dermatophytoses, cutaneous candidiasis and pityriasis versicolor. Bifonazole may be useful for treating onchomycoses(in combination cream, bifonazole 1%plus urea40%), otomycoses, erythrasma, sebopsoriasis, seborrhoeic dermatitis and rosacea[7].Thus bifonazole is an effective and well tolerated treatment for superficial fungal infections of the skin. The objective of the present study is for the development of a novel topical vehicle for the delivery of an antifungal agent in terms of effectiveness and elegance as compared to conventional ointment and creams, improved availability at the desired site by use of organogels and Improved patient compliance[8]. The bifonazole organogel was prepared using span 60(sorbitan monostearate) using various concentrations.

MATERIALS AND METHODS

Materials

Bifonazole was received as a gift sample from (Glenmark Pvt.Ltd.) and span 60(Finer chemicals Ltd.).All other excipients were obtained from analytical grade samples.

METHOD OF PREPARATION OF ORGANOGELS

For the preparation of seven formulations of bifonazole ($BF_1$to BF_7) 70 % oil phase and 30 % aqueous phase were used.

Preparation of oil phase: Accurately weighed amount of sorbitan monostearate (Span 60), sorbic acid, Tween 20, isopropyl myristate were taken in a beaker and heated at 60°C in water bath.

Preparation of aqueous phase: Accurately weighed amount of potassium sorbate was added to purified water and heated at 60°C.

Finally, drug was dispersed in oily phase and aqueous phase was slowly added in oil phase with stirring under mechanical stirrer. Complete homogenization was achieved by tissue homogenizer. The compositions of different formulations are summarized in **Table 1**

Table 1: Sorbitan Monostearate Organogels formulations from BF_1 to BF_7

Components	Content	Formulations						
		BF_1	BF_2	BF_3	BF_4	BF_5	BF_6	BF_7
Drug	Bifonazole (%)	1	1	1	1	1	1	1
Oil phase	Span 60 (%)	7	8	9	10	11	12	13
	Tween 20 (%)	2	2	2	2	2	2	2
	Sorbic acid (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Vitamin E (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Isopropyl myristate up to (%)	100	100	100	100	100	100	100
Aqueous phase	Potassium sorbate (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Purified water (ml)	30	30	30	30	30	30	30

EVALUATION OF IN SITU GEL

Drug excipient studies (FTIR Study):

The FTIR allows identification of functional groups in various chemicals as well as incompatibilities between the drug and excipients. The FTIR study of bifonazole(BF) was carried out by KBr pellet method. FTIR spectrum was taken for pure BF and also physical mixture of excipients with drug. In this method 3mg of sample and 300mg of potassium bromide was finely ground using mortar and pestle. A small amount of mixture was placed under hydraulic press compressed at10kg/cm to form a transparent pellet. The pellet was kept in the sample holder and scanned from 4000cm to 500cm $^{-1}$in Shimadzu FTIR spectrophotometer.

Melting Point
The melting point of bifonazole was determined by capillary method.

Psychorheological Characterization
The formulated gels were inspected visually for colour, presence of any clog and sudden viscosity changes. To evaluate the feel, the formulations were applied on the skin and the feel was experienced psychorheologically.

Texture evaluation
Texture of the in situ gel in terms of stickiness and grittiness was evaluated by mildly rubbing the gel between two fingers.

Viscosity
Viscosity of all the batches of in situ gels were measured using Brookfield DV-E viscometer. The samples were taken in 100ml beaker and viscosity was measured using spindle no.6at the rotation of 2 rpm shear rate at room temperature.

Drug content
Drug content for Bifonazole organogel was done by the assay method. First the prepared organogel (100mg API) was crushed and added to 100ml of methanol (Stock solution). After 30 minutes the solution was filtered. From the stock solution 4ml was withdrawn and diluted upto 50ml getting desired concentration 8µg/ml. From the desired concentration the drug content of formulations were calculated using calibrated standard curve equation y=0.027x-0.004(R^2 Value=0.998) at λ_{max} 254 nm.

pH Determination
The pH of formulated organogels was determined using pH meter. 2 g of gel was stirred in distilled water to get an uniform suspension.Volume was made upto 40 ml. The electrode was immersed in organogel - distilled water suspensions and readings were recorded on pH meter.

Spreadability
Spreadability of formulations was determined by an apparatus suggested by Multimer *et al*. which was fabricated in laboratory and used for study. The apparatus consists of a wooden block, with a fixed glass slide and movable glass slide with one end tied to weight pan rolled on the pulley, which was in horizontal level with fixed slide.An excess of gel sample 2.5 g was placed between two glass slides and a 1000g weight was placed on slides for 5 minutes to compress the sample to a uniform thickness. Weight (60g) was added to the pan. The time (seconds) required to separate the two slides was taken as a measure of spreadability.

It was calculated using the formula,

S = m.l / t

Where, S - Spreadability in g.cm / sec
m - Weight tied to upper slide
l - Length of glass slide
t - Time in seconds

Length of glass slide was 11.3 cm and weight tied to upper slide was (60g) throughout the experiment.

In vitro diffusion study
The pattern of release of drug from formulations, in vitro diffusion studies were carried out using Keshary Chien diffusion cell. The developed formulations were subjected to in vitro diffusion study through dialysis membrane (HIMEDIA); with molecular weight cut off 12000 -14000 KD, using modified Keshry Chien diffusion cell. Pieces of dialysis membrane were soaked in medium used for diffusion study for 24 hrs before mounting on a diffusion cell. The receptor compartment was filled with saline phosphate buffer Ph 7.4 containing SLS(Sodium Lauryl Phosphate). The whole assembly was maintained at 37° ± 1° C and receptor solution was stirred with a magnetic stirrer at 600 rpm throughout the experiment. Care was taken that no air bubbles were trapped under the membrane. Aliquots (5 ml) were withdrawn at regular interval of 1 hr. for a period of 8 hr. and replaced with equal volume of

fresh medium equilibrated at 37° ± 1° C. All the samples were suitably diluted with medium and analyzed spectrophotometrically at 254 nm for bifonazole content. The cumulative % drug release was calculated for each time interval.

Rheological Properties

The Rheology of the optimized formulation (BF_4) was studied using Brookfield Viscometer. For Nonionic Surfactant Organogel spindle no. 6 was selected and speeds of 0.3, 0.5, 0.6, 1.0, 1.5 and 2 rpm were selected. Different torque values at respective spindle speeds were obtained for an ascending and descending curve. Rate of shear and shearing stress were calculated by using following formula.

$$Shear\ rate\ (\gamma) = \frac{2\ \omega\ R_c^2\ R_b^2}{X\ (R_c^2\ R_b^2)} \quad (\sec^{-1})$$

Where,

ω = Angular velocity of spindle (rad^{-1})

$[\frac{2\pi}{60}$ * spindle speed (rpm)]

R_c = Radius of container (cm)

R_b = Radius of spindle (cm)

X = Radius at which shear rate is being calculated (cm)

$$\text{Shear stress}\,(\sigma) = \frac{M}{2\ \pi\ R_b^2\ L} \quad \text{dyne/cm}^2$$

Where,

M = Measured Torque

$$Torque\ input = \frac{Torque\ *Full\ scale\ torqu\ (7187.0)\ dynes/cm}{100}$$

L = Effective length of spindle (cm)

Rheogram was obtained by plotting rate of shear, (γ) on Y-axis versus calculated value of shear stress, (σ) on X-axis.

In vitro antifungal activity

The in vitro antifungal activity of bifonazole of the optimized formulation (BF_4) was carried out using *Candida albicans* as a representative fungi, adopting the cup – plate method. Commercial clotrimazole ointment was taken as a reference standard. Clotrimazole is a well known effective antifungal drug and it is available as a topical formulation. Suspension of *Candida albicans* was inoculated in sabouraud dextrose agar medium and then poured into the sterile petridish and allowed to solidify. Wells were done in plate using borer and the formulations were poured into wells. These plates were incubated at 37º C for 24 hours. The standard and test were tested at a concentration of 0.1mg/ml. The mean zone of inhibition was calculated using Hiantibiotic Zone scale for each plate and this value was taken as an indicator for the antifungal activity.

Stability studies

a)Freeze-thaw and thermal cycling test:

The selected organogel was exposed to different temperature conditions in a cyclic pattern that simulate the changes likely to be encountered during its use or in distribution process. During the course of study the selected formulations were subjected to refrigerated temperature (2 - 8° C) for two days, followed by 40° C for two days, one cycle. Such types of three cycles in twelve days were completed and major changes were observed.

b)The selected organogel was also subjected to the following condition of temperature and relative humidity during stability studies.

1. 25°C ± 2°C at 75 ± 5% RH
2. 40°C ± 2°C at 75 ± 5% RH

Formulation was evaluated for various parameters after every month for three months. The parameters of the organogels studied were Drug content , Viscosity and In vitro diffusion study.

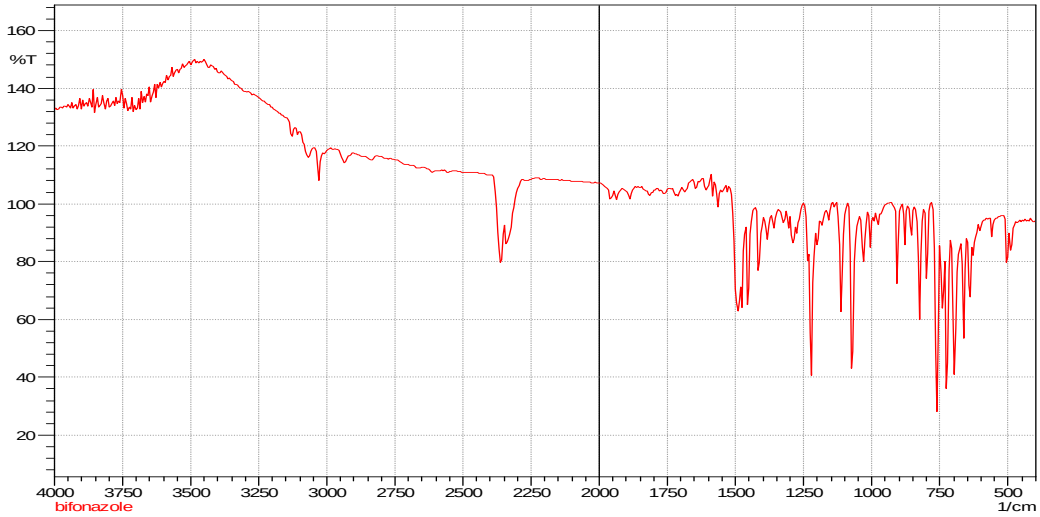
RESULTS AND DISCUSSION

FTIR studies:

The study of the FTIR spectra of bifonazole demonstrated that the characteristic absorption peaks for the aromatic C-H stretching at 3025 cm $^{-1}$, aromatic C-C stretching at 1487 cm $^{-1}$, aliphatic C-H stretching at 2950 and 2830 cm $^{-1}$ and C-H deformation at 1452 cm^{-1}. This further confirms the purity of bifonazole.

The major peaks of sorbitan monostearate was found to at 1486, 3020, 2951 cm^{-1}. In the formulation of organogel (BF_4) peak at 3020 cm^{-1} was due to presence of sorbitan monostearate, peak at 3025cm^{-1} and 1487 cm^{-1} was due to the presence of drug bifonazole in the formulation. So from the study it can be concluded that the major peaks of drug (3025cm^{-1}, 1487 cm^{-1}) remains intact and no interaction was found between the drug and sorbitan monostearate. Hence polymer mixture reveals that here is no incompatibility was observed between bifonazole

Fig. 1- FTIR spectra of bifonazole



Melting Point

The melting point was found to be in the range of 142-145°C. The reported melting point is in the range of 142-143°C.

Psychorheological Characterization

The psychorheological characterization like colour, clogging, sudden viscosity change and feel of organogels are depicted in Table no.2.

Table 2:Psychorheological characterization of organogels.

Sr. No	Parameters	Sorbitan Monosterate Organogels
1.	Colour	White
2.	Clogging	----
3.	Sudden Viscosity change	No change
4.	Feel	Smooth

Viscosity

Viscosity is one important parameter which provides important information during the optimization of the organogel. The results of evaluation of bifonazole organogel batches are shown in Table-3.The batch BF_1 was found to be low viscosity. The batch BF_4 has met the specifications and the optimized batch**.**

Drug content

From the content uniformity test by assay method it was found that the percentage of drug content (%D.C) of BF_1 to BF_7 formulations were within the limit and the values were given in the Table no. 3.

pH Determination

The pH of the organogels of batches of BF_1 to BF_7 formulations were shown in Table no.3.pH of all batches were found to be in the range 5.82 to 6.53.

Spreadability

The spreadability of the organogels of batches of BF_1 to BF_7 formulations were shown in Table no.3. Spreadability of all batches were found to be in the range of 32.28 to 52.15.

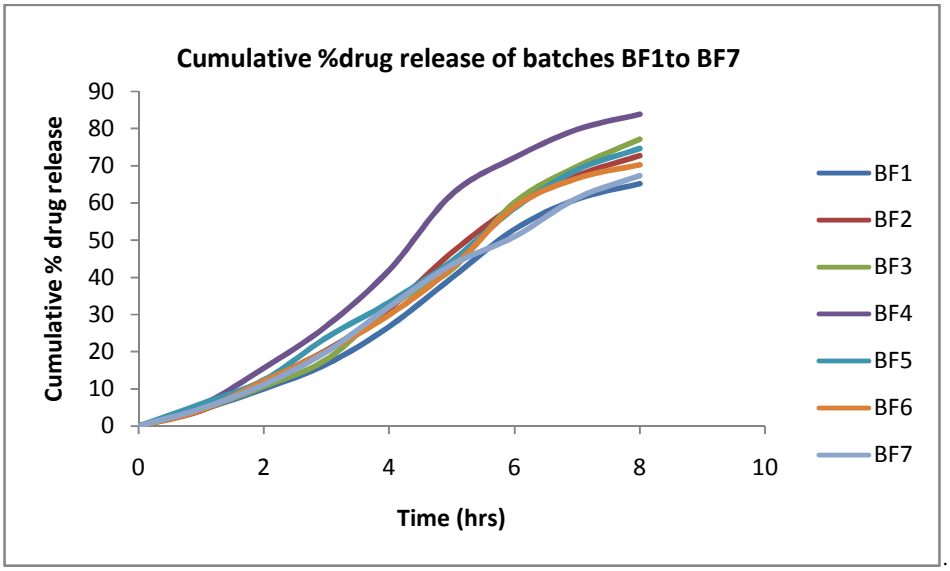
Table no 3 Viscosity, Drug content, pH and Spreadability of batches from BF_1 to BF_7

Formulation Codes/batches	Viscosity (centipoise)	Drug Content	pH	Spreadability
BF_1	20500	98.89	5.93	52.15
BF_2	24150	99.12	5.82	48.42
BF_3	29300	99.24	6.46	45.19
BF_4	32500	100.11	6.53	42.37
BF_5	35400	98.43	6.30	39.88
BF_6	42000	97,87	6.12	35.68
BF_7	49500	98.65	6.31	32.28

In vitro diffusion study

In vitro diffusion studies were performed in phosphate buffer pH 7.4 containing SLS on the above promising formulation BF4 gives maximum amount of drug release comparing to other formulations. The percentage of drug release of BF4 is best giving 83.92% which contains 10% Sorbian monostearate.BF1,BF2,BF3,BF5,BF6 and BF7 formulations drug release 65.24%,72.79%,77.23%,74.69%,70.36%and 67.39% respectively because the formulations contain 7%,8%,9%,11%,12%and 13% respectively. The dissolution profiles of the above formulations are depicted in figure no.2

Fig.2-Comparative cumulative %drug release of sorbitan monostearate organogel formulations from batches BF1 to BF7



Rheological Properties

The rheological properties like shear rate and shear stress of optimized organogel formulation BF_4 with different rpm of spindle of viscometer is given in Table no.4.

Table 4-Shear rate and shear stress of optimized formulation BF_4

RPM		BF_4	
		Shear rate(sec^{-1})	Shear stress ($dynes/cm^2$)
ASCENDING	0.3	0.0299	3.219
	0.5	0.0498	3.403
	0.6	0.0598	3.565
	1.0	0.0997	3.858
	1.5	0.1495	4.186
	2.0	0.1993	4.321
DESCENDING	1.5	0.1495	4.275
	1.0	0.0997	3.998
	0.6	0.0598	3.476
	0.5	0.0498	3.219
	0.3	0.0299	2.989

In vitro antifungal activity

The antifungal activity of the optimized gel was quite effective for inhibition of fungi with desired concentration. The optimized gel was compared with control giving more efficacy than control which was depicted in fig.3.The zone of inhibition was compared with reference standard *candida albicans* which was given in Table no.5.The zone of inhibition mean for optimized formulation was found to be 23.5mm comparing with clotrimazole 18mm.Hence the optimized formulation BF_4 is most effective than clotrimazole.

Fig. 3. The zones of inhibition shown by the gels and standard

A. Control **B. BF_4**

A. Control for candida albicans
B. BF4 (optimized formulation) most effective than control.

Table. 5: Antimicrobial activity of gels in comparison to reference standard using *Candida albicans*

Zone of inhibition (mm)					
Formulation	1	2	3	4	**Mean**
BF4	22	23	24	25	23.5
Clotrimazole	15	18	19	20	18

Stability studies

From freeze-thaw and thermal cycling test it was concluded that there was no phase separation observed in all the batches of bifonazole formulations. The stability study of optimized formulation BF_4 was stored in 25° C ± 2° C at 75 ± 5 % RH for three months and40° C ± 2° C at 75 ± 5 % RH for three months. The variations of drug

content,viscosity, spreadability and %cumulative drug release were within the limit which was depicted in Table no.6.

Table 6 : Stability Study of Organogel BF_4

Formulation		Storage condition							
BF4		25° C ± 2° C at 75 ± 5 % RH				40° C ± 2° C at 75 ± 5 % RH			
		Months				Months			
Sr. No.	Parameters	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
1.	Drug content (%)	100.11 ± 0.042	99.28 ± 0.026	99.56 ± 0.017	98.85 ± 0.028	100.11± 0.042	99.59 ± 0.012	98.85 ± 0.024	98.97 ± 0.014
2.	Viscosity (Poise)	325	324	321	320	325	323	321	320
3.	Spreadability (g.cm/sec)	42.37± 0.892	43.19 ± 0.776	44.03 ± 0.884	43.82 ± 0.995	42.37 ± 0.892	42.85 ± 0.652	42.92 ± 0.540	43.06 ± 0.755
4.	Diffusion study Cumulative % drug release	83.92± 0.026	83.27 ± 0.034	82.69 ± 0.040	81.96 ± 0.037	83.92 ± 0.026	83.31 ± 0.021	82.76 ± 0.047	82.03 ± 0.066

CONCLUSION

A organogel based drug delivery system can be designed for controlled release of Bifonazole using Sorbitan monostearate. It was evident from the results that rate of drug release can be controlled through Sorbitan monostearate from the organogel. From the developed formulations the release of Bifonazole was best in BF_4 formulation higher cumulative amount of drug permeation, higher anti-fungal activity as compared to other formulations.. From the FTIR study, it was confirmed that the drug & excipients in the formulations were compatible with each other.

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