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Research Article

Formulation of antifungal Bigels containing Terbinafine for topical application

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ABSTRACT

The Vaginal formulations have traditionally included solutions, suppositories, gels, foams, & tablets. Bigels are unique semi-solid formulations that have piqued the curiosity of numerous academics due to their major advantages above conventional gels. The purpose of this study was to develop and characterise novel bigels for drug delivery applications by mixing Hydroxypropyl methylcellulose hydrogel with sorbitan monostearate oil (coconut and olive) based organogel. The microscopy revealed the presence of both aqueous & oil phases as bigel. The microstructures and physicochemical characteristics of the bigel were tested using microscopy, viscosity measurement, mechanical analysis, and differential scanning calorimetry analysis. It was observed that the bigel was made with coconut oil-based organogel and HPMC hydrogel was stable for more than 90 days. The in-vitro release demonstrates that bigel can be effective for a longer period of time, up to 6 hours, whereas hydrogel alone exhibits release in 2 hours. So, based on the findings, it can be assumed that terbinafine is released in 2 hours from the hydrogel phase and slowly released in the remaining 4 hours from the organogel phase. Terbinafine, the drug of preference for the treatment of bacterial vaginosis, demonstrated diffusion-mediated drug release when placed into bigels. In general, the produced bigels might be employed as delivery vehicles for drugs delivered vaginally. The drug-loaded gels demonstrated effective antibacterial activity against Candida species.

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INTRODUCTION

The vaginal canal is a muscular tube that is surrounded by nerves & mucous membranes. It links the uterus & cervix to the exterior of the body, enabling menstruating and pregnancy feasible. Because of the rich network of blood arteries, the vagina is an excellent route for medication administration for both local and systemic action. (Mercer, B.M, 1995)

Vaginal drug delivery refers to the delivery of medications into the vaginal canal to produce local or, less typically, systemic pharmacological effects. The optimum vaginal formulation must possess the following characteristics: It should not interfere with coitus, it must be colourless & odourless, & it should be used for atleast a couple of hrs before the intercourse, it should not cause leakage, messiness, or a sense of vaginal fullness, it should not produce local pain and it should be applied with or without an applicator. (Coggins C., 1998).

Vaginal infections are caused by microorganisms. It irritates & infects the vaginal cavity. Infections develop as a result of an excess of bacteria & yeast that dwell in the vagina. As vaginal formulations, traditionally, solutions, suppositories, gels, foams, and tablets have been employed. For the management of vaginal infections, the current treatment options available are oral and topical treatment. (Kimberly A. 2002).

Oral treatment includes *Metronidazole, Fluconazole, Tinidazole, Sacnidazole, Clindamycin, and Itraconazole* are examples of antifungal medications. Fluconazole induces headaches, nausea, and vomiting. It may raise

the chance of miscarriage during pregnancy, and high dosages may cause birth abnormalities. Oral therapy is not advised during pregnancy. As a result, when compared to topical therapy, oral medication is less effective. (Henzel MR, Tsina, Weinstein L, IW, 1994)

Topical treatment includes *suppositories & pessaries* that are simple to manufacture & administer; nevertheless, the vaginal residence period of such formulations is limited and poor necessitating recurrent treatment in many circumstances. *Creams* are difficult to administer, unpleasant, and occasionally embarrassing because they drip and spill into the undergarments. Furthermore, owing to non-uniform distribution and leakage, creams may not offer an accurate dosage[4]. Vaginal gel formulations, on the other hand, are helpful when a limited duration of action is required. Its acceptability, practicality, & non-toxic, non-irritant behaviour for vaginal mucosa are all essential attributes of vaginal gels. Gels give a localized action with minimal adverse effects, are non-greasy, and allow medications to penetrate easily. The rheological attributes of gels & its water holding capacity give the benefit of hydration and lubrication, which is important in some pathological circumstances for example if the vaginal mucosa is dry. (Joshi M, 2014)

These traditional vaginal delivery methods are partially efficient; nonetheless, they have certain drawbacks that must be overcome in order to administer anti-fungal medicine effectively. To overcome the limitations of gels namely (hydrogel, organogel, emulgel) a newer approach has been introduced i.e. Bigels.

Bigels are made by combining hydrogel & organogel in specific ratios. By developing the organogels to a bigel, the release of drug rate from the organogels can be multiplied several times. They may be regarded as emulsions with both internal and exterior immobilized phases. The immobility of the exterior phase interrupts any mobility of the continuous phase, hence eliminating the possibility of continuous phase coagulation. If the exterior component of a bigels is externally cross-linked, a permanent bigel is formed. Physical bigels are created if physical cross-linking is prominent in the exterior phase. (A. Shakeel et al, 2019)

MATERIALS AND METHODS

Materials

Hydroxy Propyl Methyl Cellulose (HPMC), Span 80, Span 20 and other materials required for preparing reagents were purchased from S.D Fine Chem Ltd., Mumbai, India. Food grade Olive oil and Coconut oil were obtained from the Local Market in standard packs. Terbinafine was gifted by KLM Laboratories Pvt Ltd, Vadodara, Gujarat, India.

Methods

Preparation of Bigels

The polar phase (hydrogels) and nonpolar phase (organogel) were prepared individually. For the hydrogel preparation, 2 percent w/w gel was made by dissolving 2 g of HPMC K-100 in water and diluting it to 100ml at 60-70^{0}C,500 RPM. In a similar way, 4 percent HPMC K-100 hydrogel was prepared as shown in table no.1. (G. Haering & P.L. Luisi, 1986)

For the preparation of surfactant-based organogel, the surfactant (Span 80 and Span) 20 were dispersed in different oil (Olive oil and Coconut oil) at 60 °C, 500 RPM and subsequently cooled to 25^{0}C as shown in table no.2.[7]Based on the RHLB value of coconut oil and olive oil (i.e. 8 and 7 respectively), the surfactant mixture of span 20(90%) and span 80(10%) was added in the organogel phase. (G. Haering & P.L. Luisi, 1986).

For the preparation of bigel, the nonpolar phase (organogel) was gently added into the polar phase (hydrogel), with an overhead stirrer (60-70^{0}C, 500 RPM.). The stirring was repeated till the uniform & homogeneous mixture was formed[7]. The drug was incorporated in both the phases, 0.2% in hydrogel and 0.8% in organogel during the time of mixing. (G. Haering & P.L. Luisi, 1986).

Evaluation of Bigel

Physiochemical properties

At various time intervals, the pH, spreadability, colour, odour, and appearance of the gels were evaluated.

Viscosity: The rheological characteristics of the bigel as a function of time have been investigated using the Brookfield viscometer (Version DVELV).

Spreadability: Spreadability of the formulated gels was determined by putting 0.5gm formulated gel inside a circle of 1cm diameter premarked on a glass plate. On the top of this glass plate, a similar glass plate was placed. The 1000 g weight was kept on the upper glass plate for 5 min. The increased diameter caused by the spreading of the gel was measured.(Ansari et al, 2013).

Microscopy

The microscopy was performed on a Scanning Electron microscope.

Stability studies

For three months, the stability study was carried out in accordance with ICH recommendations. The goal of the stability studies is to offer information on how the API changes over time as a result of environmental factors like humidity, temperature, and light. The study was conducted at 25ºC±2ºC (60%RH) & 45ºC±2ºC (75%RH). All of the prepared mixtures were crimped into an aluminum collapsible tube. The packaged bigels were then stored under the different temperature and environmental conditions listed above. Following the completion of the trial, the bigels were analyzed for percent drug content, viscosity, spreadability and pH.

Thermal properties

The thermal properties (Tm) of the produced bigels were obtained using the drop-ball method with the EI melting point apparatus-931. A differential scanning

calorimeter (DSC 200F3 Maia) was used to examine the thermal profiles of the bigels. Bigels were precisely weighed and wrapped in aluminum pans with punctured covers. The analysis was carried out in a nitrogen atmosphere with a flow rate of 40 ml/min. Scanning at a frequency of 5.0 °C/min inside the temperature range of 0 to 300°C yielded the heating and cooling DSC profiles. (D.K Pradhan et al, 2013).

In-vitro drug release

The in vitro release patterns of drugs from bigels were investigated using a two-compartment modified Franz's diffusion cell. Simulated Vaginal Fluid (SVF) was used for the release trials. One gram of each sample was precisely weighed and deposited on the donor compartment (goat vaginal membrane). The donor compartment was immersed in a SVF containing receptor compartment while being stirred at 100 rpm (37 °C). Specimens were taken at regular intervals and examined spectroscopically with a UV-vis spectrophotometer. The CPDR (cumulative percentage of drug release) was computed. (A. Cunha et al, 2017)

Antimicrobial testing

The agar well diffusion technique is frequently used to assess the antibacterial activity of drugs. The agar plate surface is colonized in the same way that the disk-diffusion technique is, by spreading a volume of microbial inoculum across the whole agar surface. Using a sterile cork borer or tip, a hole with a width of 6 mm is aseptically punched, and a volume (20–100 L) of the terbinafine is put into the well. Then, test micro-organisms i.e. Candida albicans were incubated under appropriate conditions. The antimicrobial agent spreads in the agar medium, inhibiting the development of the tested microbial strain. (D.K Pradhan et al, 2013)

Inversion test

The most common diagnostic test of gelation is to turn a beaker containing the sample upside down and then note whether the sample flows under its own weight. It was performed for bigels.

RESULT

The organo-gel and hydro-gel were prepared individually as shown below (Table 1).

Table 1: Formulation of Hydrogel and Organogel

Ingredients	HG1	HG2	OG1	OG2
HPMC	2g	4g	-	-
Olive oil	-	-	90ml	-
Coconut oil	-	-	-	90ml
Surfactant mixture	-	-	10ml	10ml
Water	upto 100 ml	upto 100 ml	-	-
Total	100ml			

**Surfactant mixture: Span20 and Span80 in a ratio of 9:1*

The bigel batches were prepared by mixing hydrogel and organo-gel in a ratio of 60:40 as mentioned below (table 2).

Table 2: Formulation of bigel

Formulation Batches	F1	F2	F3	F4
HPMC hydrogel (2%) (HG1)	12gm	-	12gm	-
HPMC hydrogel (4%) (HG2)	-	12gm	-	12gm
Olive oil organogel (OG1)	8gm	-	8gm	-
Coconut oil organogel (OG1)	-	8gm	-	8gm
Total	20gm			

The bigels were then evaluated.

Inversion test

An inversion test was performed for all 4 batches of bigel.

Table 3. Inversion test of bigels

Formulation	Time
F1	37 mins
F2	68 mins
F3	44 mins
F4	133 mins

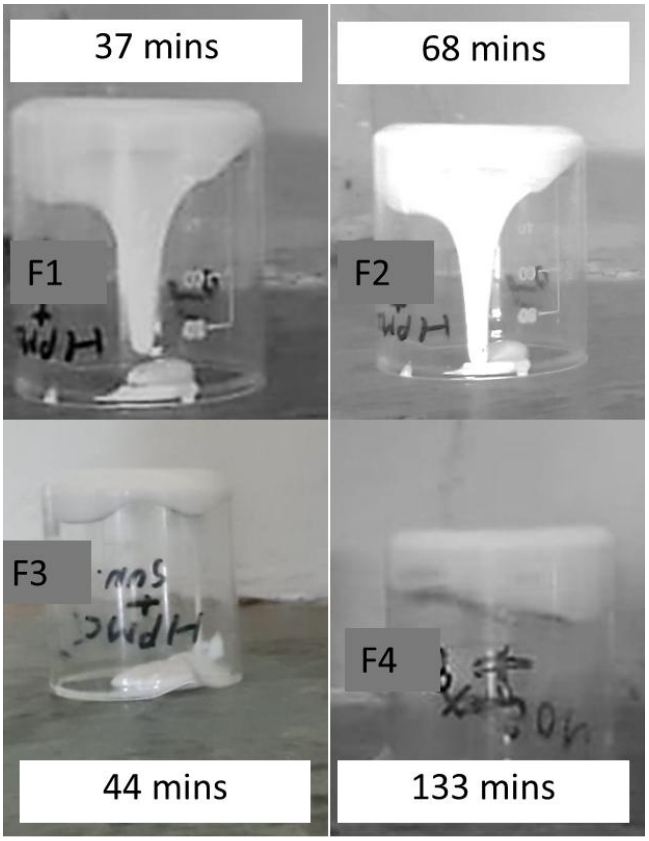

Fig. 1: Inversion Test for Bigel Batches

The results are shown in the table. Based on the findings, it can be inferred that F2 and F4 show good results for inversion tests, indicating that they do not flow by their own weight against gravity.

Microscopy

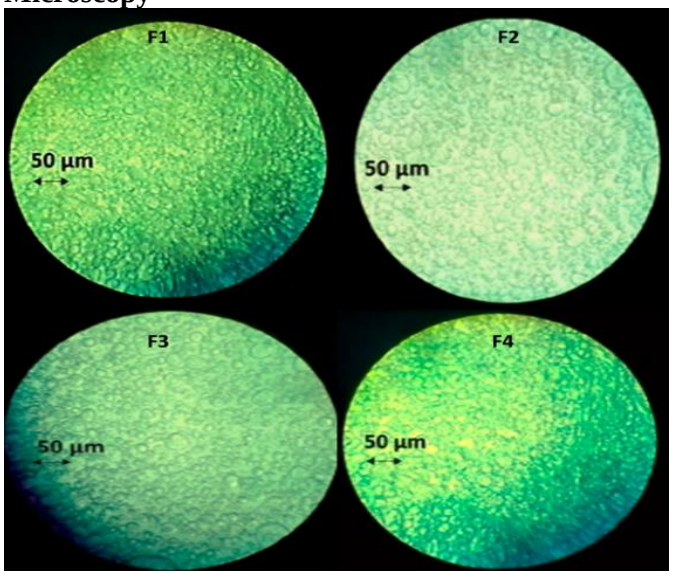

Fig.2 Microscopy of Bigel batches F1, F2, F3, F4

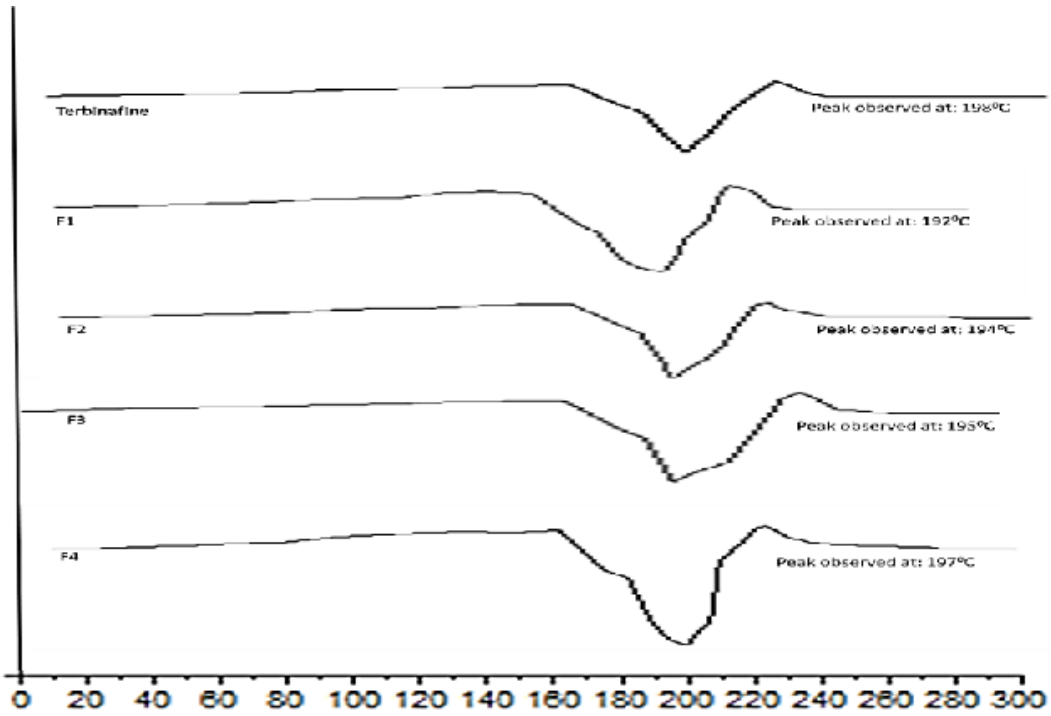

Fig. 3 DSC of Terbinafine and Bigels

The uniform structural characteristics of bigel shown by SEM are owing to improved gel homogeneity**.**

Thermal Properties

From the data obtained through DSC, it was observed the peak obtained for the drug was at 198^0C and for F1, F2, F3, F4 was at 192^0C, 194^0C 195^0C and 197^0C respectively.

Microbial testing

Microbial assay for all four batches of bigels was performed by using the agar diffusion method and Candida albicans as test micro-organisms. The results are shown in the table no.4

Table 4: Zone of Inhibition of Bigel Batches

Formulations	*Zone of inhibition in mm*
F1	14.9
F2	15.0
F3	14.8
F4	15.1

From the data, it can be concluded that Terbinafine shows good efficiency against Candida species.

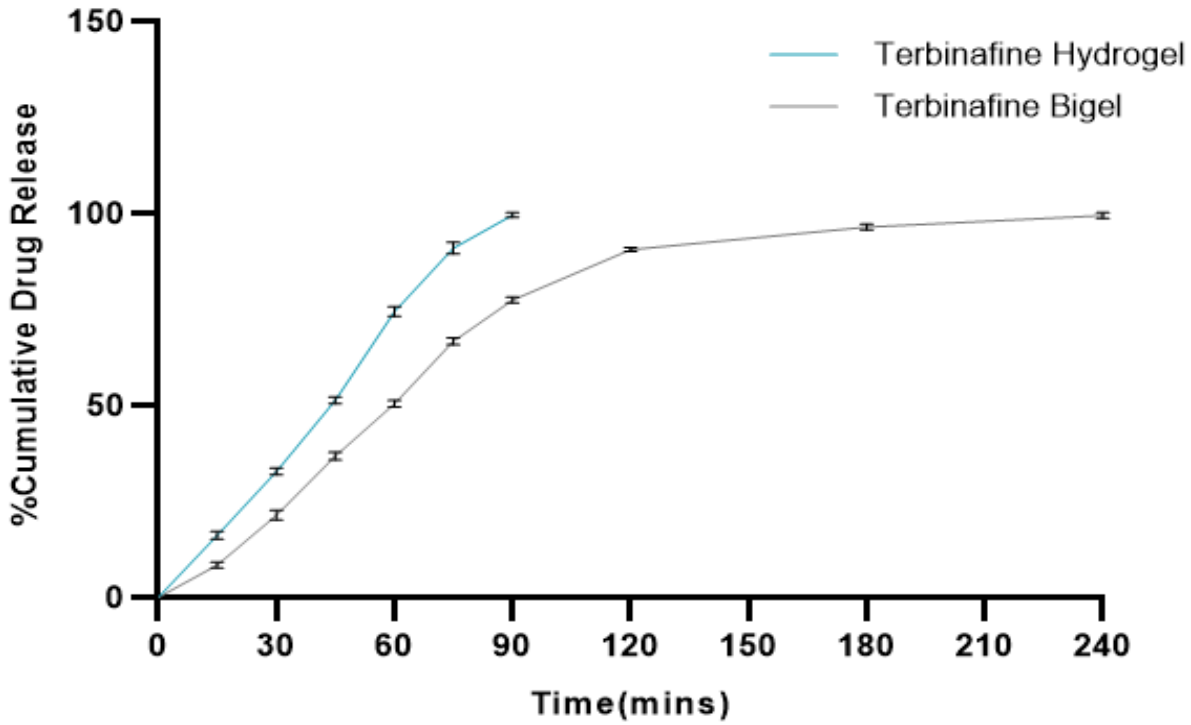

Fig. 4: Percentage Cumulative Drug Release of Hydrogel and Bigel (F4)

In-vitro drug release

Based on the data plot, it can be concluded that the drug is initially released from the hydrogel phase and then slowly released from the organogel phase. From the statistical analysis, the Higuchi equation depicts diffusion-mediated drug release (r^2-0.99).

Stability of the hydrogel, organogel and bigel with physiochemical properties

The physicochemical properties of organogel, hydrogel and bigel were evaluated.

Table 5: Physiochemical studies

Formulation Batches	Physiochemical properties	Storage conditions		
HG 1		Initial	$25^{o}\pm2^{o}$C/65 ±5%RH	$40^{o}\pm2^{o}$C/75 ±5%RH
		0 Month	3 months	3 months
	Colour	Transparent	Transparent	Transparent
	Appearance	Homogenous	Homogenous	Homogenous
	pH	7.06	6.97	6.89
	Viscosity	4093	4001	3978
	Spreadability	10.00	10.21	10.38
	Drug content	99.99	99.99	99.94
HG 2	Colour	Transparent	Transparent	Transparent
	Appearance	Homogenous	Homogenous	Homogenous
	pH	7.13	7.09	7.11
	Viscosity	4572	4545	4510
	Spreadability	7.59	7.49	7.43
	Drug content	99.97	99.97	99.95
OG 1	Colour	Yellowish	Yellowish	Yellowish
	Appearance	Homogenous	Homogenous	Homogenous
	pH	6.9	6.81	6.79
	Viscosity	1569	1555	1521
	Spreadability	10.58	10.	10.58
	Drug content	99.99	99.99	99.97
OG 2	Colour	Yellowish	Yellowish	Yellowish
	Appearance	Homogenous	Homogenous	Homogenous
	pH	7.1	6.98	6.87
	Viscosity	1613	1613	1613
	Spreadability	15.87	15.0	11.03
	Drug content	99.97	99.97	99.95
F1	Colour	Opaque	Opaque	Opaque
	Appearance	Homogenous	Homogenous	Homogenous
	pH	6.98	6.75	6.59
	Viscosity	5328	5298	5295
	Spreadability	12.38	12.07	12.25
	Drug content	99.99	99.99	99.99
F2	Colour	Milky white	Milky white	Milky white
	Appearance	Homogenous	Homogenous	Homogenous
	pH	6.83	6.75	6.56
	Viscosity	5537	5439	5408
	Spreadability	12.89	12.58	12.39
	Drug content	99.99	99.99	99.99
F3	Colour	Opaque	Opaque	Opaque
	Appearance	Homogenous	Homogenous	Homogenous
	pH	7.31	7.25	7.18
	Viscosity	5898	5865	5795
	Spreadability	14.29	14.08	13.95
	Drug content	99.99	99.98	99.98
F4	Colour	Milky white	Milky white	Milky white
	Appearance	Homogenous	Homogenous	Homogenous
	pH	7.21	7.14	7.07
	Viscosity	6053	5975	5928
	Spreadability	16.28	16.05	15.98
	Drug content	99.99	99.99	99.99

After performing all physicochemical tests like colour, appearance, pH, viscosity, etc, the bigels were found to be stable, no significant difference was observed and no physical separation was observed after 90 days.

DISCUSSION

A light and scanning electron microscopy analysis of the gels revealed that the gels exhibited fibre-like structures due to the trapping of the organogel inside hydrogel molecules; this entrapment was demonstrated to be uniformly accomplished, resulting in formulation stability and the DSC study reveals that the drug (terbinafine) is not decomposed even after formulating in bigel and the terbinafine bigel was also found to be stable. The microbial data suggests that the drug shows good antimicrobial/antifungal activity. The in-vitro release demonstrates that bigel can be effective for up to 6 hours, whereas hydrogel alone exhibits release in 2 hours. Based on the data, it can be inferred that terbinafine is released from the hydrogel phase in 2 hours and slowly released from the organogel phase in the remaining 4 hours. The Higuchi model suggests that the release is diffusion mediated. The optimized bigel had good viscosity and it also passes the inversion test. The bigel produced using coconut oil-based organogel and HPMC hydrogel (F4) was found to be stable for more than 90 days.

CONCLUSION

When terbinafine, the preferred therapy for bacterial vaginosis, was introduced in bigels, it showed diffusion-mediated drug release. In general, the bigels formed might be used as delivery vehicles for drugs administered vaginally.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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NA.

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