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Formulation development of medicated Bigels for management female genital infection

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Abstract
Background: Bigels are innovative semi-solid compositions that have piqued the curiosity of numerous academics and researchers due to their major advantages above conventional gels. The purpose of this study was to develop & evaluate innovative bigels for vaginal drug delivery of an antifungal drug (Clindamycin and terbinafine) by mixing Xanthan gum hydrogel with oil (sesame oil and olive oil) based organogel.
Result: The bigel prepared using olive oil-based organogel and Xanthan gum hydrogel (4%) was shown to be stable for more than 90 days. The gels displayed a fibre-like structure due to the trapping of the organogel inside hydrogel molecules; this trapping was proved to be uniformly done, resulting in formulation stability.
Conclusion: When clindamycin and terbinafine, the preferred therapy for bacterial vaginosis, were loaded in bigels, it exhibited diffusion-mediated drug release. In point of fact, the developed bigels might be employed as delivery vehicles for medications administered vaginally to treat vaginal infections.

Keywords: hydrogel, organogel, vagina, vaginal infection**s,** xanthan gum

1. Introduction
Vaginal drug delivery refers to the delivery of medications into the vaginal canal to produce local or, less typically, systemic pharmacological effects. Because of the rich network of blood arteries, the vagina is an excellent route for medication administration for both local and systemic action [1].
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 [2].
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.
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 [3].
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 [5].

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, and 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 [6].

2. Materials and Methods

2.1 Materials

Xanthan gum, Tween 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 Sesame oil were obtained from the Local Market in standard packs. Clindamycin and Terbinafine was gifted by KLM Laboratories Pvt Ltd, Vadodara, Gujarat, India.

2.2 Methods

2.2.1 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 Xanthan gum in water and diluting it to 100ml at 60-70^{0}C,500 RPM [7]. In a similar way, 4 percent Xanthan gum hydrogel was prepared as shown in table no.1.

For the preparation of surfactant-based organogel, the surfactant (Span 20 and Tween 80) were dispersed in different oil at 60 °C, and in the co-solvent at 500 RPM and subsequently cooled to 25^{0}C as shown in table no.2.[7] Based on the RHLB value of oil, the surfactant mixture of span 20 (83.33%) and tween 80(16.66%) was added in the organogel phase.

The organogel preparation method was modified due to the separation of two phases i.e. organogel and hydrogel. The modified preparation method contains 60% oil dispersed in co-solvent (Propylene glycol and water) to form a stable organogel.

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, 0.5% (Clindamycin) in hydrogel and 0.5% (Terbinafine) in organogel during the time of mixing.

2.3 Evaluation of Bigel

2.3.1 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).
- **Spread ability:** Spread ability of the formulated gels was determined by putting 0.5gm formulated gel inside a circle of 1cm diameter pre-marked 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 [8].

$$S = \frac{MxD}{T}$$

Where,
S-Spread ability, g.cm/s
M-Weight put on the upper glass slide
D-diameter of spreading
T-Time for spreading gel in sec.

2.3.2 Microscopy

The microscopy was performed on a microscope**.**

2.3.3 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 [9].

2.3.4 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 [10].

2.3.5 *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 [9].

2.3.6 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 [10].

2.3.7 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.

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

Table 1: Microbial testing of drugs (Clindamycin and terbinafine)

Species	Candida albicans				
Formulations	C1	C2	C3	C4	C5
Clindamycin	0.5%	1%	-	-	0.5%
Terbinafine	-	-	0.5%	1%	0.5%
Species	Staphylococcus aureus				
Formulations	S1	S2	S3	S4	S5
Clindamycin	0.5%	1%	-	-	0.5%
Terbinafine	-	-	0.5%	1%	0.5%

From the data, it can be concluded that C5 (Clindamycin and Terbinafine) and S5 (Clindamycin and Terbinafine) show the maximum zone of inhibition when compared with the other drug batches (Terbinafine and Clindamycin) alone in two different micro-organisms. So this combination of Terbinafine and Clindamycin is further selected for incorporation into bigel phase.

Table 2: Zone of inhibition of bigel batches

Formulations	Zone of inhibition in mm
C1	5
C2	11
C3	17
C4	25
C5	40
S1	20
S2	35
S3	8
S4	11
S5	43

3.2 Formulation of organogel and hydrogel

Table 3: Formulation of hydrogel and organogel

Ingredients	HG1	HG2	OG1	OG2
Xanthan gum	2g	4g	-	-
Olive oil	-	-	60ml	-
Sesame oil	-	-	-	60ml
Propylene glycol	-	-	20ml	20ml
Surfactant mixture	-	-	5ml	5ml
Water	Upto 100 ml			

*Surfactant mixture was taken, 84% and 16% for span 20 and tween 80 respectively. Propylene glycol, water and tween 80 was prepared and oil and span 80 were prepared. Then these two phases were mixed to form organogel.

The bigel batches were prepared by mixing hydrogel and organogel in a ratio of 60:40 as mentioned below (table 2). The bigels were then evaluated.

Table 4: Formulation of bigel

Formulation Batches	F1	F2	F3	F4
Xanthan gum hydrogel (2%) (HG1)	12 g	12g	-	-
Xanthan gum hydrogel (4%) (HG2)	-	-	12g	12g
Olive oil organogel (OG1)	8g	-	8 g	-
Sesame oil organogel (OG2)	-	8 g	-	8 g
Total	20gm			

3.3 Spread ability

Table 5: Spread ability of bigel formulations

Formulations	Spread ability(g.cm/s)
F1	14.69
F2	15.12
F3	9.80
F4	10.50

From the results it can be concluded that there are no considerable differences between formulations regarding the spreadability. To achieve adequate efficacy and user acceptance of bigel, spreadability is an important property to evaluate in bigel formulations.

3.4 Gel sol transition temperature

Table 6: Gel to sol transition temperature of bigel formulations

Formulations	Gel-sol transition temperature
F1	$40^{O}C$
F2	$45^{O}C$
F3	$55^{O}C$
F4	$50^{O}C$

Gel-sol transition is a function of temperature and thermal stability. Also these, bigels show higher values of change in enthalpy, associated with the evaporation of the water. The thermal properties of the other organogel phase after it is incorporated into a bigel system remained the same as the organogel phase. So the prepared Bigel batches are stable even after applying heat and the kinetics of gel remain the same even after heating.

3.5 Inversion test
An inversion test was performed for all 4 batches of bigel. The study was performed upto 360 mins.

Table 7: Inversion test of bigels

Formulation	Time
F1	37 mins
F2	45 mins
F3	360 mins
F4	267 mins

The results are shown in the table. No 3. Based on the findings, it can be inferred that F3 and F4 show results for inversion tests, indicating that they do not flow by their own weight against gravity.

3.6 *In vitro* drug release

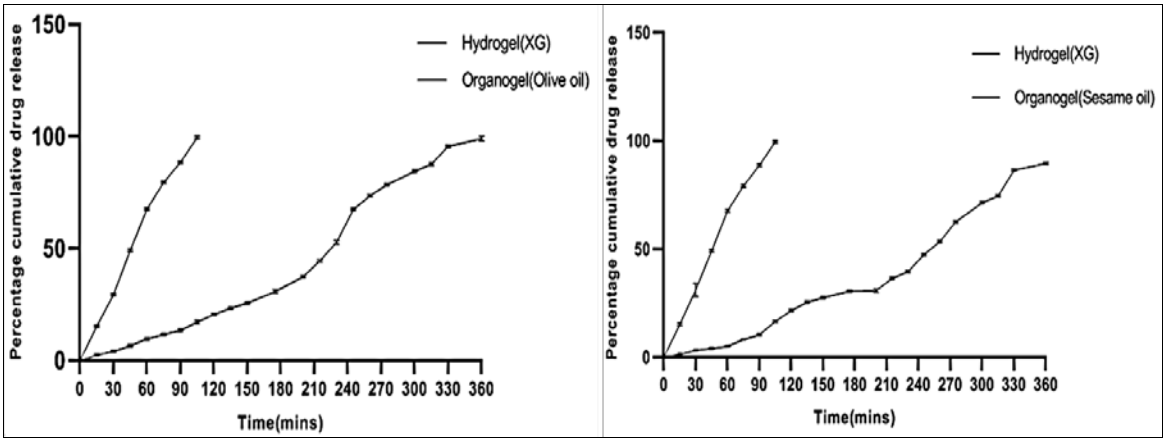

Fig 1: Percentage cumulative drug release of hydrogel and bigel

Based on the data plot, it can be concluded that the clindamycin is initially released from the hydrogel phase and then terbinafine is slowly released from the organogel phase. From the graph, it can be concluded that Olive oil has shown to have better drug release when compared with others and it can be confirmed by using f2 similarity index that there is a significant difference observed between the formulations. Further confirmation is done by using statistical analysis. From the statistical analysis, the Higuchi model depicts diffusion-mediated drug release (r^2-0.99).

3.7 Statistical data analysis

ANOVA test was used to test statistically significant differences within the bigel formulations. A p-value ≤ 0.05 was considered significant. By using ANOVA, it can be concluded that the formulated bigels have significant differences where the p-value was found to be ≤ 0.001. Student's t test was used to test statistically significant differences between the efficacy of the individual bigel formulations and control. Using a student t-test, comparing the mean of the release of the drugs in each of the bigel with a hypothetical mean of 100 it showed that F3 formulated bigel is significantly different when compared with other batches.

3.8 Thermal Properties

Thermal properties was studied for the optimized bigel batch.

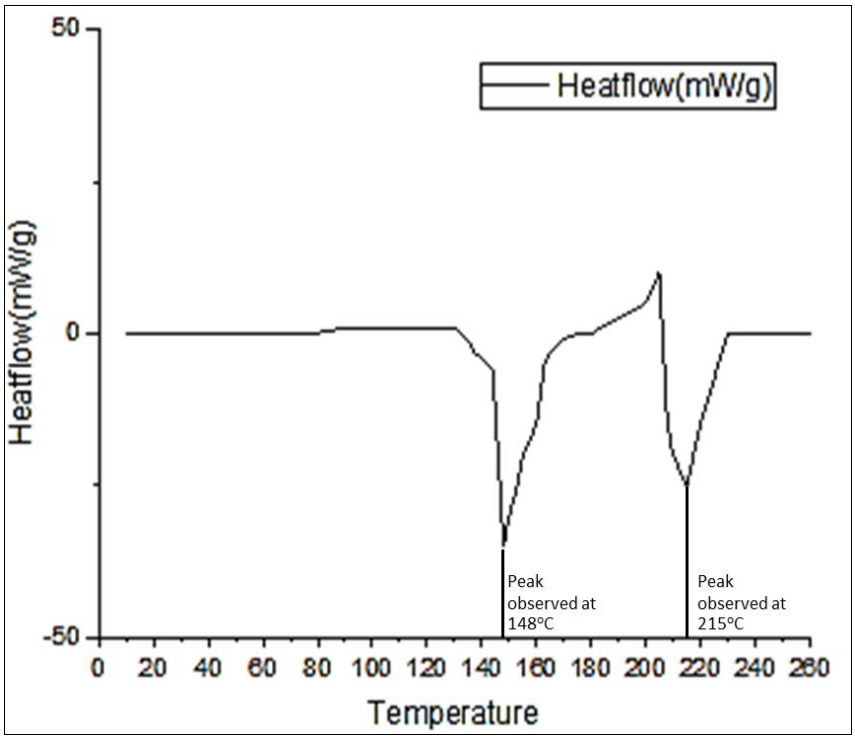

Fig 2: DSC thermogram of Clindamycin and Terbinafine

From the graph, it can be concluded that the presence of water which vaporizes when heated, is indicated by the endothermic peak, whereas the exothermic peak shows a rearrangement of molecules to create crystals. According to the results, the bigel produced is thermoplastic and exists in a semi-crystalline condition. There was no incompatibility found.

3.9 Microscopy

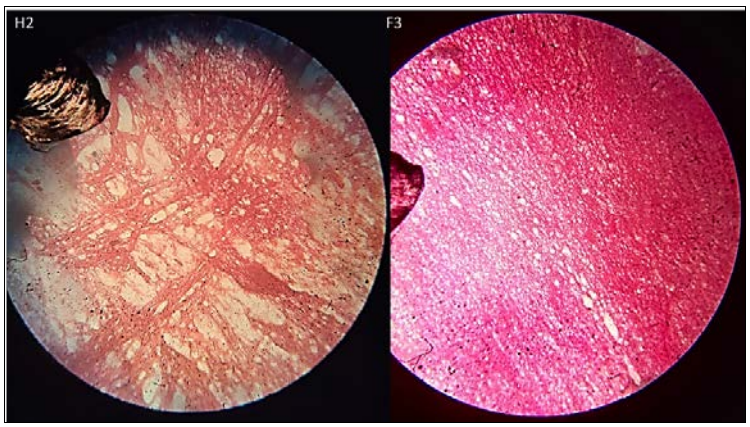

Fig 3: Microscopy of Xanthan gum (4%) and Bigel (F3)

Microscopy reveals that the organogel phase is entrapped into the hydrogel phase forming an integrated 3D network that is responsible for forming a stable bigel.

3.10 Stability of the hydrogel, organogel and bigel with physiochemical properties

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

Table 8: Physiochemical studies and stability

Formulation Batches	Physiochemical properties	Storage conditions		
		Initial	25º ±2ºC/ 65 ±5%RH	40º ±2ºC/75 ±5%RH
		0 Month	3 months	3 months
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
F3	Colour	Opaque	Opaque	Opaque
	Appearance	Homogenous	Homogenous	Homogenous
	pH	7.31	7.25	7.18
	Viscosity	4708	4698	4620
	Spreadability	14.29	14.08	13.95
	Drug content	99.99	99.98	99.98
	Inversion test	Pass(upto 360 mins)	Pass(upto 350 mins)	Pass(upto 345 mins)

After performing all 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

The microbial data suggests that the combination of drugs (Clindamycin and terbinafine) shows good antimicrobial/antifungal activity showing a maximum zone of inhibition. 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 assumed that clindamycin is released from the hydrogel phase in 2 hours and terbinafine is 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 olive oil-based organogel and Xanthan gum hydrogel (F3) was found to be stable for more than 90 days. 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 and clindamycin) is not decomposed even after formulating in bigel and the terbinafine bigel was also found to be stable.

Conclusion

Clindamycin when given alone is was more effective in S.aureus species and terbinafine when given alone it was more effective against C.albicans. So the suggested treatment can be combination of this two drugs which is effective against both the species. This suggested treatment can be used to treat polymicrobial vaginal infection. In

general, the bigels formed might be used as delivery vehicles for drugs administered vaginally.

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Conflict of interest
There is no conflict of interest.

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Ethics approval
None to declare.

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