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Research Article

Micro Beads Loaded Bio Adhesive Vaginal Gel of Voriconazole: Development & Characterization

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ABSTRACT

A micro bead-loaded bio adhesive gel containing the drug voriconazole was developed. Micro beads give extended drug release, and bio adhesive was used to improve the stability of the product. Micro beads were examined for micromeritic properties, swelling index, drug loading, percent yield and encapsulation efficiency. pH, spreadability, extrudability, homogeneity, viscosity, and bio adhesion test of the bio adhesive gel were all evaluated. The swelling index ranged from 55 to 82 %, indicating that micro beads have moderate to good swelling properties. The percent drug loading was calculated to be between 71.37 % 0.07 to 87.59 % 0.91. The percent yield of various formulations F1 - F5 was calculated and found to be between 39.52 % ±0.23 and 72.13 % ±2.42. For the formulation F3, the maximum % yield was found to be 72.13 %. The % encapsulation efficiency was found to be between 50.80 ±52 to 78.420±09, indicating moderate to good efficiency. The pH range of all formulations was within the specified range for the drug delivery system. F1 and F5 formulations had a viscosity range of 25485.50 to 37211.58 cp. The greatest spreadability of the F3 formulation was determined to be 9.09 gm cm/sec.. The viscosity and extrudability have an inverse relationship i.e. the lower the viscosity, the higher the extrudability. Bio adhesion strength was founded within the range of 16.7±0.04 to 21.1±0.13 gm respectively. Highest strength was founded in F3. After 12 hours, the total percent release was measured in SVF (4.5) were 85.16-77.28. The results show that as the polymer concentration is increased, the release of drug from the micro beads laden gel decrease. In comparison to market formulations (Verz 200 tablet), voriconazole micro beads laden gel was found to more effectively limit the growth of Candida albicans in an antifungal investigation.

INTRODUCTION

Voriconazole is an antifungal medication made from a synthetic triazole. Voriconazole inhibits 14-alpha-lanosterol demethylation in fungi, inhibiting the synthesis of ergosterol, an important component of the fungal cell membrane, and causing fungal cell lysis. Adverse effects such as neurotoxicity and hepatotoxicity, as well as drug-drug interactions, may prevent voriconazole (VCZ) from being used systemically. Voriconazole has a very minimal aqueous solubility and its maximum solubility is in acidic conditions [1-5].

The vaginal cavity is an important portion of the female reproductive tract, which has multiple parts. In recent years, the vaginal route has gained popularity as a well-established method for drug delivery. Because of its benefits, such as increased permeability, a larger surface area, the avoidance of the first pass effect, a higher permeability, and low enzymatic activity. The vaginal cavity is a better site for uterine targeting [6]. The vaginal area is well known for being particularly sensitive due to the greater risk of infection. Most vaginal infections, such as vulvovaginal candidiasis, bacterial vaginosis, and trichomonas, are caused by the activities of various types of fungi, bacteria, yeasts, and other microorganisms. It can also spread owing to improper application of gels, creams, and sprays,

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as well as the wearing of tight clothing that is uncomfortable and unsanitary, unsafe sexual activity, and any other activity that can irritate or disrupt the normal vaginal microenvironment [7].

Vulvovaginal candidiasis is defined as a very common situation which can affect about 75% of women at least once in their life period. The major cause for increasing the risk of VVC are pregnancy, sexual activity, recent use of antibiotic, and immune suppression from such conditions, HIV infection, diabetes. Candida albicans is the most common cause of VVC, but other Candida species such as glabrata, parapsilosis, and tropicalis have also been identified as causal factors. Candida is most commonly seen in the rectum, however it can also be found in the vaginal area. The symptoms of VVC are caused by the presence of an excessive amount of yeast and its penetration into the vulvovaginal epithelial cells [8-12].

Now a days for masking the tastes or odours, prolonged the release of drugs, improve bioavailability, impart stability to drug molecules, micro encapsulation is widely used in the pharmaceutical as well as in medical fields as a multi-particulate dosage forms to produce targeted drug delivery. Microencapsulation techniques are commonly used to generate prolonged dosage forms. Micro-beads are monolithic spheres in the size range of about 10-1000um that are distributed throughout the matrix as a molecular dispersion of particle, with molecular dispersion defined as drug particles dispersed in the continuous phase of one or more than one miscible polymer. They are widely used as drug carriers for controlled release [13, 14].

In recent years, a latest type of prolong release form are developed which is known as bio adhesive preparation for the treatment of both systemic and topical diseases. The main application of such type of dosage form is retaining period which is extended for prolong period of time included days and night that's why frequency is automatically reduced.

The phrase "bio adhesion" can be described as a process in which interfacial forces hold two materials together for an extended length of time, with at least one of the materials having a biological nature. The word "bio adhesion" is often employed in the context of drug delivery. A drug carrier system is connected to a specific biological site in this situation. A biological surface might be made of epithelial tissue or the mucous coat on the surface of a tissue. The term mucoadhesion refers to the adherence of the mucous coat, which includes the mucosal linings of the rectal, vaginal, nasal, oesophageal, ocular, and oral cavities [15]. The terms bio adhesive and muco adhesive are now often used in the pharmaceutical field as novel drug delivery technologies, owing to the pioneering work of many research groups in the United States, Japan, and Europe in the mid-1980s. The concept of "sticking" dosage forms to the place of application and/or drug absorption emerged at that time and has sparked interest among researchers all over the world. Bio adhesion has several major advantages, including:

(i) Extending the residence time at the site of drug absorption (e.g., minimising the dosing frequency for bio adhesive controlled release formulations) and,
(ii) To make more contact with the mucosal epithelial barrier that lies beneath (e.g., enhancement of the epithelial transport of usually poorly absorbed drugs, such as proteins and peptides).

The drug delivery systems (DDS) intimate proximity to the absorptive mucosa should result in a steeper concentration gradient, increasing the absorption rate [16].

MATERIALS AND METHODS

Materials

Voriconazole and carbopol 934P were purchased from Balaji Drugs, Mumbai, India. Triethanolamine was purchased from Merck Specialist Pvt. Ltd, Mumbai. All the other chemicals and reagents used in this study were of analytical grade. Fungal stain *candida albican* was procured from Microbial Type Culture Collection and Gene Bank (MTCC),CSIR-Institute of Microbial Technology, Chandigarh, INDIA

Methods

Preparation of Micro Beads by Ionotropic Gelation Method

In the case of ionotropic gelation method, sodium alginate is a common polymer. Sodium alginate and Sodium Carboxy methyl cellulose (SCMC) were added in different proportion (Table 1) for the preparation of voriconazole micro beads. After dispersing the sodium alginate and SCMC in 100 mL of water the drug was added to it and mixed properly to form a thick solution which can be filled in a syringe having the needle size of 24.

Table 1: Different drug polymer ratio used for the preparation of micro beads

S.NO.	Formulation Code	Drug: Polymer ratio	Solvent (% w/v)
1	F1	Drug: sodium alginate: SCMC (1:1:1)	10
2	F2	Drug: sodium alginate: SCMC(1:2:1)	10
3	F3	Drug: sodium alginate: SCMC(1:3:1)	10
4	F4	Drug: sodium alginate: SCMC (1:1:2)	10
5	F5	Drug: sodium alginate: SCMC (1:1:3)	10

The filled solution was then poured drop by drop from the syringe into a 10% calcium chloride solution that had been previously made by mixing calcium chloride with enough water to make spherical micro beads. The droplets must retain for about 20 min in calcium chloride 10% solution for stabilization and to complete the curing reaction for production of spherical rigid micro beads. After sometime micro beads were filtered through watman filter paper and collected in blotting paper to overcome the sticking problem and then air dried at room temperature for overnight and stored in a dry and cool place or desiccator [17].

Preparation of the Simulated Vaginal Fluid (SVF 4.5):

Simulated vaginal fluid (SVF) was prepared by mixing (3.51 g/l) NaCl, (1.40 g/l) KOH, (0.222 g/l.) Ca(OH)2,(0.018 g/l) bovine serum albumin, (2 g/l) lactic acid, (1 g/l) acetic acid, (0.16 g/l) glycerol, (0.4 g/l) urea, (5 g/l) glucose and adjusted the pH up to 4.5 by using 0.1M HCl [18].

Preparation of Calibration Curve of Voriconazole in SVF(4.5):

Voriconazole was weighed accurately (100mg) transferred into a 250ml standard volumetric flask, then 100 ml SVF(4.5) was added to it and shakes for few minutes to prepare 1000microgram/ml. (solution A). Accurately pipetted out 1 ml of solution A into a 10 ml standard flask and made up to the volume using SVF(4.5) to get a concentration of 100 µg /ml (solution B). Accurately pipette out 0.5ml, 1.0ml, 1.5ml, 2.0ml, 2.5ml and 3.0ml of solution B into six separate 10ml standard flask and made up to the volume with SVF(4.5) to get a concentration of 5, 10, 15, 20, 25, 30µg/ml (solution C). One of the above solutions was scanned in UV range using SVF(4.5) as blank and wavelength of maximum absorption was found to be about 256nm. The absorption solutions of different concentration were measured at 256nm. Calibration curve was plotted between absorbance v/s concentration [19]. Voriconazole showed linearity range from 5-30µg/ml at the selected wavelength. From the prepared dilutions a standard curve is plotted by determining the concentration using 256nm wavelength (Fig.1).

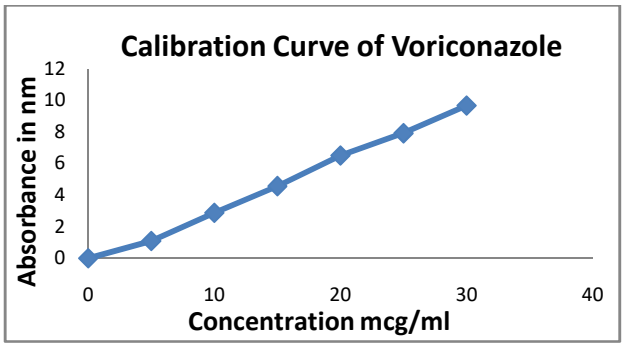

Figure 1: Calibration Curve of Voriconazole in Simulated Veginal Fluid (SVF) 4.5

Characterization of Micro Beads

Micromeritic Properties

The maximum angle achievable between the surface of a pile of micro beads and the horizontal plane is called angle of repose. It was decided to use a fixed funnel approach. Pouring the samples in bulk into a graduated cylinder was used to assess apparent bulk density. A graduated cylinder containing a known mass of powder was placed on a mechanical tapper equipment to estimate the tapped density. Samples were tapped until there was no further volume reduction. Carr's index was calculated [9].

Particle Size Studies

Optical microscopy was used to determine the particle size of micro beads. A calibrated microscope was used to count around 100 microspheres [13].

Swelling Index

The swelling index of prepared micro beads was determined using solvents such as SVF (4.5). Micro beads were accurately weighed and poured into several beakers in which SVF (4.5) had already been present for 12 hours. The micro beds were then filtered at various

intervals, weighed, and the swelling study calculated using the formula below.

$$\text{Swelling index} = \frac{\text{Wt} - \text{Wo}}{\text{Wo}} \times 100$$

Where, Wt = weight of micro beads observed at 8th hour, and Wo = initial weight of micro beads.

Determination of Drug Loading, % Yield and Encapsulation Efficiency

Weigh micro beads 50 mg equivalent to drug was dissolve in 50ml of methanol until get dissolved completely, then filtered in a beaker and make suitable dilution to check the absorbance at 256nm. Drug loading, encapsulation efficiency and % yield was measured by using following formula [20].

$$\%\ \text{Drug Loading} = \frac{\text{weight of drug}}{\text{weight of micro beads}} \times 100$$

$$\text{Encapsulation efficiency} = \frac{\text{actual drug content}}{\text{theoretical drug content}} \times 100$$

$$\%\ \text{Yield} = \frac{\text{M}}{\text{Mo}} \times 100$$

Where,
M = weight of micro beads,
Mo = total expected weight of drug and polymer.

Compatibility Study by FT-IR Analysis

Accurately weight amount of drug and polymers were taken and mixed with KBr in a uniform way then this mixture was compressed with a pressure to form a semi transparent pellet and recorded the spectrum of the pellet from 450-4000cm^{-1} [21].

Morphological Analysis by SEM

SEM analysis was used to determine the morphological properties of the micro beads and to confirm their spherical shape. SEM investigation was performed at room temperature using a scanning electron microscope model Zeiss Evo 40, India.

Preparation of Bio Adhesive Gel (1%)

As a bio adhesive polymer, carbopol 934P was used to produce a bio adhesive gel. Accurately weighed carbopol 934P (1gm) was dispersed in distilled water (88 gm) containing glycerol (10g) and stirred the mixture with homogeniser until thickening occurred. Benzyl alcohol was added as a preservative. When the thick mixture was formed it will sonicate for 20 min to reduce the insoluble particles to get solubilised. The pH was maintained by adding triethanolamine (50 percent W/W) drop by drop to neutralise the acid. Continue to mix the solution until you have a translucent gel [22-24].

Incorporation of Micros Beads in Bio Adhesive Gel

Micro beads obtained from ionotropic gelation method were mixed properly into bio adhesive gel of carbopol 934P at 25 rpm for 2 min by an electrical mixture.

Characterization of Bio Adhesive Gel

Spreadability:

Spreadability is determined by the formulation's slip and drag characteristics. A glass slide that is 20cm in length and is mounted on a table. On the glass, 2g of gel is applied. The gel was then sandwiched between this slide and a glass slide with the same dimensions. For 5 minutes, a 500g weight was placed on top of the two slides to eliminate air and establish a homogenous gel coating between the slides. The top glass plate was then pulled at a distance of 7cm with 50g of weight connected to the upper slide. The spreadability improves as the time it takes the slides to move the specified distance of 7cm decreases [25].

Spreadability is calculated from the following formula:

$$S = \frac{M \times L}{T}$$

Where,
S= spreadability(g.cm/ sec)
M= wt on upper slide(g)
L= Length moved by the glass slide (cm)
T= Time taken to separate the slides completely from each other (sec)

Oraganoleptic Characteristics

Each formulation of micro bead loaded gel was tested for color, odor, and feel upon application (stiffness, grittiness, greasiness and tackiness).

Washability

Taking 1 gm of micro bead loaded gel and placed it on skin to spread properly. After 1 min the gel formulations were washed with tap water and result was visually examined.

Phase Separation
Phase separation was examined during the preparation of formulations. After formulating the gel it was placed in a beaker and again examine in every 1 hr by visual method.

Determination of pH
Digital pH meter was used to examine the pH of each formulation. 1 gm of micro bead loaded gel was transferred in a beaker containing 20 ml of water and electrode was dipped in the beaker to note the reading. This procedure was done for at least 3 times for each formulation.

Extrudability Study
Filling the gel in a collapsible tube from the end and crimping the tube using a crimping machine to prevent rollback was used to test extrudability of formulations. The gel was allowed to extrude until the pressure in the tube was relieved by removing the cap.

Homogeneity and Grittiness
Developed formulations were tested for homogeneity as a small quantity of gels pressed between two thumbs and the index finger in order to notice the consistency of gel that any coarse particles being attached or detached on the finger.

Viscosity Measurement
The viscosity of the formulations was determined using brookfield Rheometer at RT. The formulations were rotated at 12 to 2 rpm with spindle. At each RPM, the corresponding reading of viscosity and torque was noted. Experiments were carried out in triplicate.

Bio Adhesion Measurement
The *In-Vitro* technique was used to examine the bio adhesion of the produced micro beads laden gel. An agar plate in SVF (4.5) was prepared for assessing bio adhesion, and a 50 mg test sample was placed in the centre of the plate. After 5 minutes, the agar plate was linked to a USP disintegration test apparatus, which permits it to move up and down in SVF (4.5) at 37±1°C. The sample on the agar plate was dipped in the solution until it reached the lowest point and then removed from the solution until it reached the highest point. The retention period of the test sample on the agar plate was visually evaluated [26, 27].

***In-Vitro* Drug Diffusion Study**
The franz diffusion cell was used to determine *in vitro* drug diffusion from voriconazole micro beads laden gel. Before testing, a semipermeable cellophane membrane was soaked overnight in SVF(4.5). Between the empty donor and receptor compartments containing 100ml of SVF(4.5), a precise amount of formulation was applied. 5 ml of sample was taken out of the receptor compartment every hour and replaced with an equal amount of fresh medium solution until 12 hours had passed. Make the appropriate dilutions, and use a UV spectrophotometer set to 256nm to get the spectrum data of the samples.

Antifungal Activity
The Agar cup method was used to test the antifungal properties of the prepared formulations against the Candida albican strain. A cup with a diameter of 10mm was made aseptically using Sabouraud dextrose agar media. The fungal suspension strain that had been examined was then disseminated throughout the agar surface. Then incubated for 24 hrs at room temperature for allowing the growth of micro organisms. The prepared formulations F1, F2, F3, F5, Placebo along with the marketed formulation (Verz Tablet 200 mg) were introduced in each cup after calculated volume & solubilised in SVF 4.5. Finally, the zone of inhibition of each cup was observed, the radius of the zone of inhibition was computed, and the results were compared to the marketed formulation (Vorier, Tablet 200 mg Zydus Oncoscience) [28, 29].

Mathematical Modeling
The *In-Vitro* data was used and analysed by various conventional mathematical models to establish the release pattern from designed micro beads laden gel (Zero order, First order, Higuchi model and Korsmeyer- Peppas model). From the curve fitting of release data, the values of R (correlation coefficient), k (release constant), and n (diffusion exponent) were used to establish a suitable release model for the formulation.

RESULT AND DISCUSSION
Five formulations (F1-F5) of micro beads loaded bio adhesive gel of voriconazole were formulated because they are soluble in aqueous media and have release rate controlling ability, non-toxicity, non-irritancy, stability at vaginal pH, and compatibility with the drug [30]. Carbopol 934 was chosen as the polymer for the gel preparation because carbopols have demonstrated outstanding bioadhesive qualities on the mucosal surface [31] and have been used in

the development of several vaginal delivery systems [32-34], and it produces a transparent gel. The evaluation parameters were done like micromeritics parameters, swelling index, % drug content, drug loading, encapsulation efficiency and % yield for micro beads where as for bio adhesive gel it was subjected to spreadability, viscosity, extrudability, bio adhesion measurement, *In-Vitro* drug diffusion and anti fungal study.

Characterization of Micro Beads

Micromeritic Properties

The angle of repose (in degrees) and compressibility index of micro beads were calculated to investigate their flow properties (CI, %). Table 2 displays the gathered data as well as related parameters. The micro beads' flow properties ranged from 25.20±0.25 to 38.5°±0.26, showing that they had good flow properties. The CI value was determined to be between 9.87±0.01 to 22.94±0.02 percent, indicating good to exceptional flow characteristics. Table 2, shows that the particle size of the produced micro beads ranged between 565.680±017 and 743.602±1.34m. The size of microspheres grew larger as the polymer content rose.

Swelling index was done for the formulation F1 to F5 respectively and found to be in the range of 55-82%. The maximum swelling index was found to be 82% for the formulation F3. Table 2 shows that the % swelling index of micro beads containing drug was highest in the eighth hour.

Drug Loading, % Yield & Encapsulation Efficiency

The percent drug loading was done for the formulation F1 to F5 respectively and it was founded within the range of 71.37%±0.07 to 87.59%±0.91. F5 shows the maximum drug loading that is 87.59 as compare to other formulations which shows that F5 possess highest incorporated amount of drug within the micro beads shown in table 3. The percent yield of different formulation F1 to F5 were calculated and it was found to be in the range of 39.52%±0.23 to 72.13 %±2.42. The maximum % yield was found to be 72.13% for the formulation F3. Which shows good % yield . The percent encapsulation efficiency was calculate and founded within the range of 50.8±0.52 to 78.42±0.09 for the formulation F1 to F5 respectively. The maximum % encapsulation efficiency was founded as 78.42% for formulation F3 which indicates if the polymer concentration increases the efficiency of encapsulating the drug was also increased. The all data for all formulation are given in Table 3.

Compatibility Study by FT-IR Analysis

The drug and drug-excipient interactions reveal that there were no notable shifts in the absorption bands(peaks) and that all of the drug's characteristic peaks are present in the combination of drug and excipient spectra, showing that the drug and excipient used in the formulation are compatible (Fig. 2).

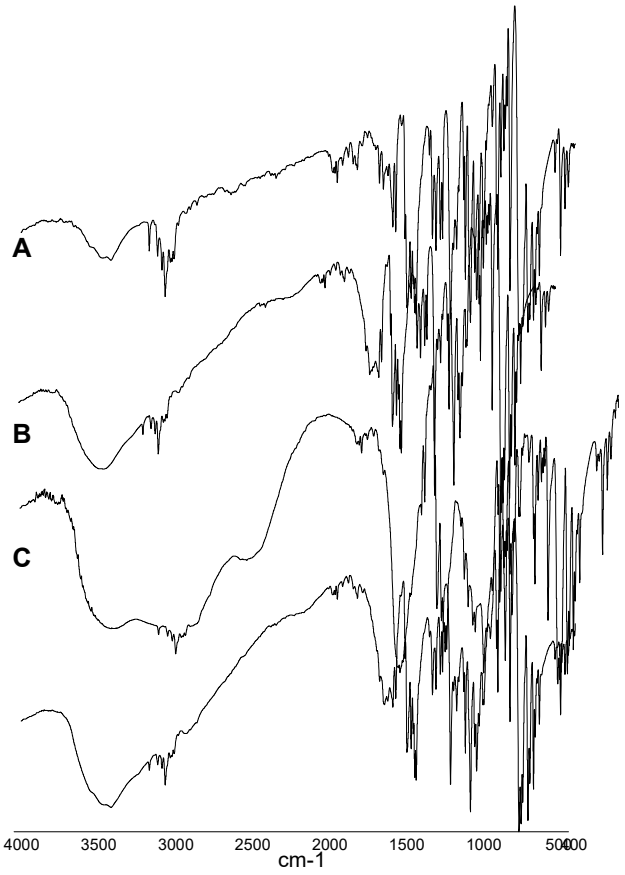

Figure 2: (A) FTIR Spectra of Voriconazole (B) FTIR Spectra of Drug and Sodium alginate (C) FTIR Spectra of Drug and SCMC (D) IR Spectra of Drug and Carbopol 934

Morphological Analysis by SEM

Scanning electron microscopy (SEM) was used to examine the shape and surface features of the micro beads in Fig. 3. SEM was used to see if tiny beads were present. For all of the formulations, non-aggregated micro beads with a spherical shape were achieved. Furthermore, F3 had a smooth surface, indicating that voriconazole was evenly distributed throughout the carrier.

Table 2: Result of Micromeritic properties ± SD (n =3)

Formulation code	Bulk density (gm/cc)	Tapped density (gm/cc)	Carr's compressibility index%	Angle of repose θ	Swelling Index (8 hr)	Particle Size in µm
F1	0.585±0.001	0.65±0.02	10.00±1.00	25.2 ±0.25	55%	565.68±0.017
F2	0.900±0.01	1.01±0.02	10.89±0.02	38.5±0.26	68%	676.03 ±0.02
F3	0.575±0.002	0.64±0.02	9.87±0.01	27.1±0.15	82%	743.60±1.34
F4	0.393±0.001	0.51±0.03	22.94±0.02	29.5±0.15	63%	691±0.019
F5	0.565±0.001	0.62±0.05	10.03±0.01	37.5±0.20	74%	720.76±0.01

Table 3: Drug Loading, % Yield & Encapsulation Efficiency ± SD (n= 3)

Formulation code	Drug Loading	% Yield	Encapsulation Efficiency
F1	71.37%±0.07	39.52%±0.23	50.8±0.52
F2	75.87%±1.03	43.93 %±0.92	66.03±1.72
F3	86.38%±0.78	72.13 %±2.42	78.42±0.09
F4	79.74%±0.55	56.04 %±1.71	56.62±1.33
F5	87.59%±0.91	62.71 %±0.02	69.12±1.88

Table 4: Results of physical evaluation

Formulation code	Colour	Odour	Washability	Homogeneity	Grittiness	Phase Separation
F1	Transparent	Odourless	Washable	Yes	No	No
F2	Transparent	Odourless	Washable	No	No	No
F3	Transparent	Odourless	Washable	Yes	No	No
F4	White Transparent	Odourless	Washable	Yes	No	No
F5	Slightly Transparent	Odourless	Washable	Slightly homogeneous	No	No

Table 5: Extrudability, Viscosity, Spreadibility & pH of bioadhesive gel

Formulation code	Extrudability (gm/cm^2)	viscosity(cp) (Average)	Spreadibility (g.cm/sec)	pH
F1	14.61±0.10	25485.50	6.42±0.38	6.49 ±0.81
F2	12.87±0.12	27005.53	8.02±0.320	7.15 ±0.05
F3	10.10±0.61	35748.50	9.09±0.29	7.21 ±0.53
F4	9.03±0.42	29806.60	7.81±0.07	6.70 ±0.15
F5	8.70±0.09	37211.58	8.96±0.10	6.83 ±0.65

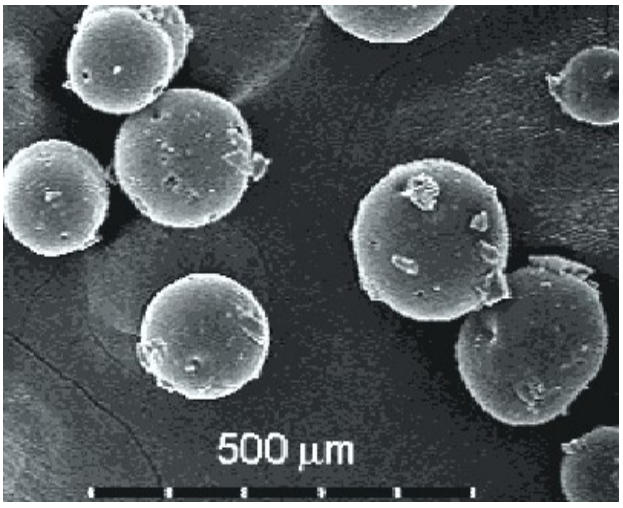

Figure 3: Scanning Electron Microscopy of F3

Characterization of Bio Adhesive Gel
Evaluation Parameters of Bio Adhesive Gel

The pH was measured with pH meter and all the pH range of the formulation were within range i.e 6.49 ±0.81 to 7.21 ±0.53 which is the required range for the drug delivery system.

The viscosity of bioadhesive gel was determined by using a brookfield rheometer at different RPM. i.e., (12, 10, 8, 6, 4, and 2) and the rate of shearing is directly proportional to shearing stress and as the shear rate increase the viscosity flow increase which shows that the formulation is non- Newtonian in nature and having Pseudo plastic flow and viscosity range were found to be 25485.50 to 37211.58 cp of F1 & F5 formulation.

The spreadability was ranged from 6.42±0.38 to 9.09±0.29 gm cm/sec and maximum spreadability was found to be 9.09 gm cm/sec of F3 formulation. The viscosity and extrudability have an inverse relationship, meaning that the lower the viscosity, the higher the extrudability. All five formulations were found to have an extrudability range of 8.700 ±09 to 14.610 ±010.

Bio Adhesion Measurement

Bio adhesion strength was founded within the range of 16.7±0.04 to 21.1±0.13 gm respectively as shown in Table 6.

Table 6 : Bio adhesion strength of formulation F1-F5

Formulation code	Bio adhesion strength (gm)
F1	18.5±0.02
F2	19.2±0.06
F3	21.1±0.13
F4	16.7±0.04
F5	20.8±0.12

Highest strength was founded in F3. The presence of carbopol 934, whose bio adhesive ability is maximum at vaginal pH, can be ascribed to the improved bio adhesivity of voriconazole micro beads laden gel. Additionally, increasing the concentrations of sodium alginate and sodium CMC greatly improved the mucoadhesive force.

***In-Vitro* Drug Diffusion Study**

In vitro diffusion study on all five formulations of voriconazole micro beads loaded in bio adhesive gel were carried out in SVF 4.5 using cellophane membrane and modified apparatus. The cumulative percent release after 12 hrs was found to be 85.16-77.28. From results it was observed that as we increase the polymer concentration, release of drugs from micro beads loaded gel decreases. Result revealed that F3 formulation was better than among other formulations.

In Vitro Release Kinetics

The drug release data was fitted into multiple kinetic models to get the release constant and regression coefficients (r2). The F3 formulation had the best fit with the Korsmeyer Peppas model, with a regression coefficient of 0.9980. This shows that the amount of drug released was dependant on the drug load in the matrix. The value of the diffusion exponent (n) obtained was 1.074, which is greater than 1, indicating that the drug release mechanism in question is the Super Case II transport mechanism.

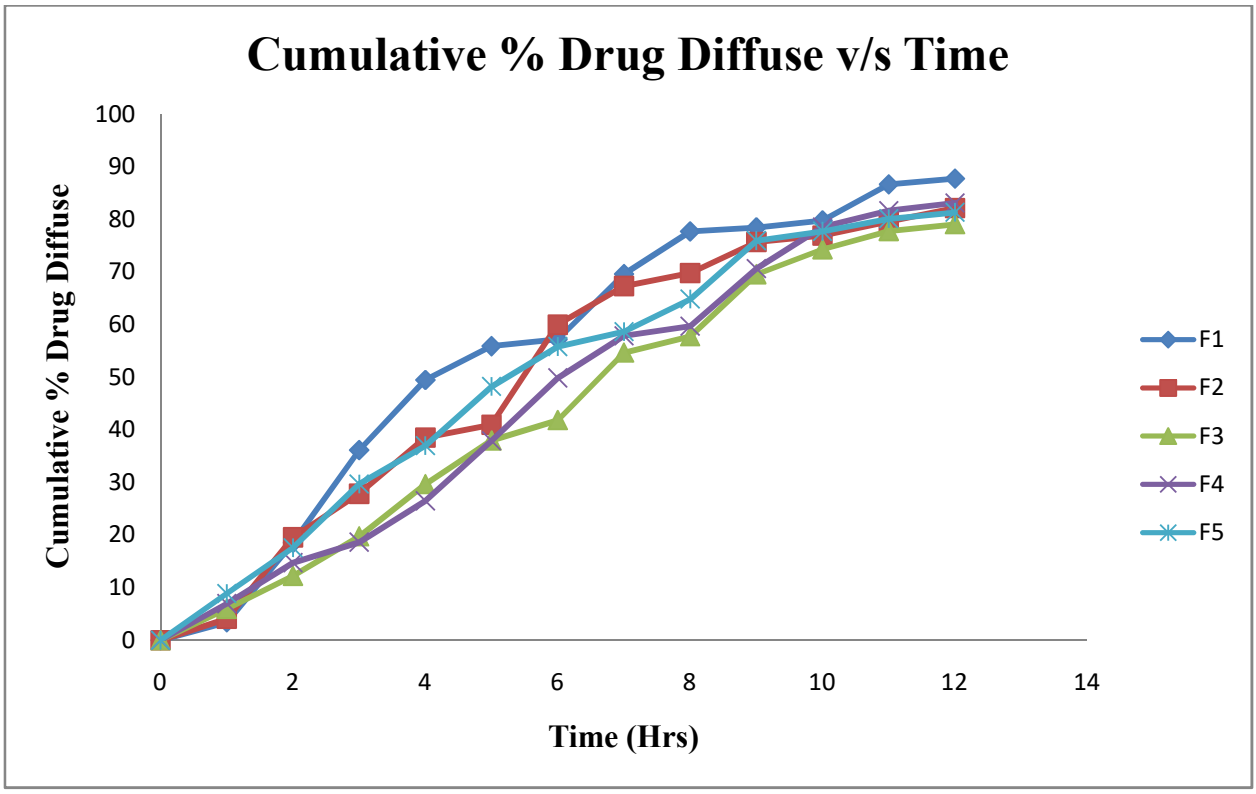

Figure 4: *In-Vitro* drug diffusion profile of Formulation F1 to F5

Antifungal Study

Antifungal activity was performed for formulations F1, F2, F3, F5, Placebo, Market Preparation (Verz 200 tablet). F3 shows the best zone of inhibition i.e. 21.3±0.16 in mm. The voriconazole micro beads laden gel was able to suppress the growth of C. Albicans for more than 12 hours in an antifungal study using saboured culture. To conclusion, the generated voriconazole micro beads loaded gel has acceptable antifungal activity against the selected test organism (Candida albicans) and is superior to the commercial formulation.

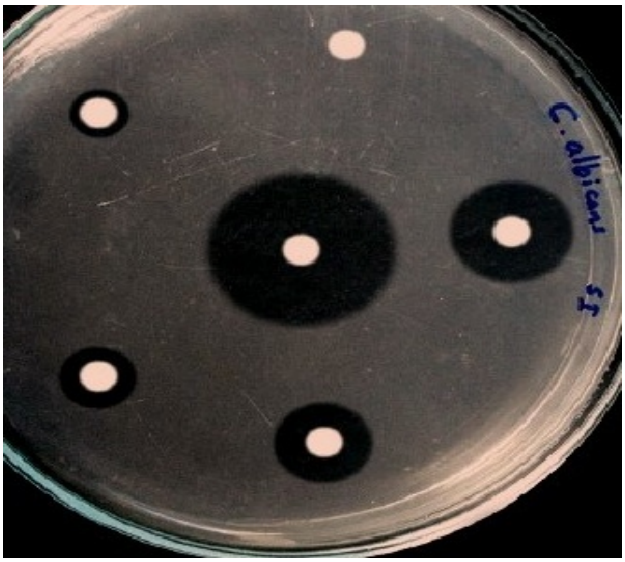

Figure 5: Comparative anti fungal study of (a) Optimized microbead gel formulation F3 (b) Marketed gel formulation (c) Placebo (d)Formulation F1 (e) Formulation F2 (f)Formulation F5.

Discussion

Using an ionotropic gelation technique and a variety of polymer drug ratios, five formulations of voriconazole micro beads loaded in bio adhesive gel were effectively formulated. This method is very common and was able to produce discrete, free flowing micro beads of voriconazole is an antifungal drug. Bio adhesive gel was prepared and act as a base for incorporating of micro beads in to the vagina. Different evaluation parameters were examined for voriconazole, micro beads and bio adhesive gel respectively.The FTIR spectroscopy was done for ensuring the compatibility of drug with other polymers (sodium alginate, carbopol 934P) used for the preparation of formulation. FTIR spectra of drug and polymer, as well as a physical mixing of drug and polymer, revealed that all of the drug's unique peaks are also present in the combination spectra, confirming drug and polymer compatibility. Different parameters such as micromeritic characteristics, swelling index, % drug loading, percent yield, and encapsulation efficiency were calculated to characterize micro beads. SEM study of the morphology of micro beads was performed for the optimized formulation F3. In summary, the results show that the majority of the parameters are proportional to the polymer concentration.

For the formulations F1 - F5, the bio adhesive gel was evaluated by observing various properties. The physical evaluation, pH, viscosity, Spreadibility, homogeneity, extrudability was done for all five formulations (F1-F5).All parameters observed within range.

In vitro diffusion study on all five formulations of voriconazole micro beads loaded in bio adhesive gel were carried out in SVF 4.5 using cellophane membrane and modified apparatus. F3 formulation consider optimized preparation among all formulations. From results it was observed that polymer concentration is inversely proportional to the release of drugs from micro beads loaded gel. In order to drug kinetic study mechanism, F3 formulation was best fitted with Korsmeyer Peppas model & diffusion exponent (*n*) value indicates that the drug release mechanism involved is Super case II transport mechanism of drug release. Highest bio adhesive strength was founded in F3 among all formulations. The increased bio adhesivity of voriconazole micro beads loaded gel can be attributed to the presence of carbopol 934 as its bio adhesive potential is highest at vaginal pH. Also increase in the concentration of sodium alginate and sodium CMC enhanced the mucoadhesive force significantly. Antifungal activity revealed that F3 shows best zone of inhibition as compared to market formulation (Verz 200 tablet). Antifungal study shows that the voriconazole micro beads loaded gel was capable to control the growth of *C. Albicans* efficiently.

CONCLUSION

Voriconazole is an antifungal drug. The voriconazole micro beads were loaded in a bio adhesive gel utilising the ionotropic gelation method, which is highly common, and the gel was made with the bio adhesive polymer carbopol 934. The bio adhesive gel served as a base for placing the micro beads into the vaginal cavity. This provides micro beads their stability. Such a microbead-loaded gel could be used to provide pharmacological ingredients via topical,

vaginal, and rectal routes. Micro beads and a bio adhesive gel were successfully prepared using carbopol 934P. These FTIR analyses revealed that there was no interaction between the chosen drug and the drug placed in micro beads. SEM analysis revealed that the structure of micro beads is spherical and free flowing particles.Bio adhesion measurement estimated the strength of gel for imparting the retention time. Antifungal activity was done, F3 formulation showed better antifungal activity than marketed preparation. Among all Five Formulations F3 showed best results.The in-vitro diffusion studies revealed that as we increase the polymer concentration the release of drug from micro beads loaded gel was decreased.

As a result, we can conclude that our formulation may be a promising alternative for the treatment of vulvovaginal candidiasis. In the future, pre-clinical and clinical research will be required.

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