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Characterization of "Novel Anti Fungal Drug Candidate Gel Formulation"

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ABSTRACT

Voriconazole is a triazole antifungal medication that is generally used to treat serious, invasive fungal infections. These are generally seen in patients who are immunocompromised, and include invasive candidiasis, invasive aspergillosis, and certain emerging fungal infections. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. Carbopol based gel formulations with Voriconazole were made. The formulation study was aimed to keep all other ingredients constant and only change in Carbopol 940 concentrations. Gel formulations were characterized for Physical Evaluation, Rheological Studies, Sensitivity Test, Skin Irritation Test, *In-vitro* drug diffusion through animal membrane for the optimized formulation and *in-vitro* antifungal activity. The results were found satisfactory for all the parameters studied.

INTRODUCTION

Voriconazole is a triazole antifungal medication that is generally used to treat serious, invasive fungal infections. These are generally seen in patients who are immunocompromised, and include invasive candidiasis, invasive aspergillosis, and certain emerging fungal infections[1].

Delivering medicine to the general circulation through the skin is seen as a desirable alternative to taking it by mouth. Patients often forget to take their medicine, and even the most faithfully compliant get tired of swallowing pills, especially if they must take several each day. Additionally, bypassing the gastrointestinal (GI) tract would obviate the GI irritation that frequently occurs and avoid partial first-pass inactivation by the liver. Further, steady absorption of drug over hours or days is usually preferable to the blood level spikes and troughs produced by oral dosageforms[2].

There are various skin infections caused by fungus. Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Antifungal compounds works by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effect on host. Voriconazole is an imidazole antifungal derivative and used for the treatment of local and systemic fungal infection. A wide variety of vehicles ranging from solid to semisolids and liquid preparations are available for topical treatment of dermatological disease as well as skin care. Topical drug administration is a localised drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical route[3].

There are various medicated products that are applied to the skin. Such products are referred as topical or dermatological products. There are various Hydrophilic polymers such as Carbopol 940, hydroxyl propyl methyl cellulose (HPMC), Sodium alginate that are used in topical gel delivery system[4]. Based on molecular fraction these polymers are used concentration between 1-5 % in topical formulation.

Brief Information on Gel

Gels for dermatological use have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removed, emollient, non-staining, compatible with several

excipients and water soluble or miscile[5-6]. The USP defines gel as a semisolid system consisting of dispersion made up of either small inorganic particles or large organic molecules enclosing an interpenetrated by liquid. The inorganic particles form a three dimensional structure. Gels consist of two phase system in which inorganic particles are not dissolved but merely dispersed throughout the continuous phase and large organic particles are dissolved into the continuous phase[7]. Fungal infections are very common and can be topical as well as systemic. Treatment of fungal infection includes medicines like Voriconazole, fluconazole, ketoconazole, clotrimazole and grisofulvin[8].

MATERIALS AND METHODS

Voriconazole, Carbopol 940, Benzyl alcohol, Oleic acid, Glycerin, Triethanolamine

Preparation of Gel Base:

Purified water was taken and Carbopol 940 was added and allowed to soak for 24 hours. To this, required amount of drug (1 gm) was dispersed in water and then Carbopol 940 was then neutralized with sufficient quantity of triethanolamine. Glycerin as a moistening agent and oleic acid as a penetration enhancer and benzyl alcohol as a preservative were added slowly under continuous stirring until the homogenous gel was formed. Formulation of various batches is shown in the Table 1.

Table 1: Formulation Table for Voriconazole gel preparation

Sl.No	Ingredients	Role	VGF1	VGF2	VGF3
01	Voriconazole	Active	1 gm	1 gm	1 gm
02	Carbopol 940	Gelling Agent	1 gm	2 gm	3 gm
03	Benzyl alcohol	Preservative	2 mL	2mL	2 mL
04	Oleic acid	Permeation Enhancer	1 mL	1 mL	1 mL
05	Glycerin	Humectant	20 mL	20 mL	20 mL
06	Triethanolamine	pH adjusting agent	3 mL	3 mL	3 mL
07	Water	Base/Vehicle	Q.S.	Q.S.	Q.S.

Evaluation:

1) **Physical Evaluation**[9]: The gel formulations of Voriconazole were evaluated for organoleptic characteristics, Color, Odor, Phase separation, Occlusiveness, and Washability etc.

2) **Rheological Studies:** The viscosity of the different gel formulae was determined at 25°C using rotational Brookfield viscometer of cone and plate structure with spindle CPE-41 and CP-52[10]. The apparent viscosity was determined at shear rate 40 sec^{-1}. The flow index was determined by linear regression of the logarithmic form of the following equation:

$$\tau = k\ \gamma\ n \text{.....Equation (1)}$$

Where "τ" is the shear stress, "γ" is the shear rate, k is the consistency index, and n is the flow index. When the flow is Newtonian n=1, if n>1 or n<1, shear thickening or shear thinning is indicated, respectively. Evaluation was conducted in triplicate.

3) **Sensitivity Test:**

A drop of diluted suspension of the tested gel (1:1) and another drop of saline (control) were put on two corresponding spots of the arms of three human volunteers. After ten minutes, the spot was investigated for any erythema, wheel or any allergic reaction.

4) **Skin Irritation Test**[11 & 12]**:**

As the formulation was intended for dermal application, skin irritancy should be tested. Skin irritation tests were conducted in rabbits to determine irritancy after single application of Voriconazole gel. The back of rabbits after depilation was used in this experiment. About 0.5 g of Voriconazole gel was applied on two different rabbits and then the applied area was covered with gauze and adhesive bandage. The formulation was removed after 24 h and the exposed skin was graded for formation of edema and erythema. Scoring was repeated 72 h later. Based on the scoring, the formulation was graded as 'nonirritant', 'irritant,' and 'highly irritant.'

The total scores for irritation test were calculated using the following equation:

$$\text{Average irritation scores} = \frac{(\text{Erythema reaction scores} + \text{Edemareaction scores})}{\text{Time interval } (h)}$$

5) *Ex-Vivo* [*In-Vitro*] Diffusion Study[13]:

The abdominal skin of Albino mice, weighing 20 – 25 gm of 8 – 10 weeks old was shaved using razor and cleaned the skin with hot water cotton swab. 5 gm of gel was applied uniformly to skin. The skin was mounted between the compartments of the Frantz diffusion cell with stratum corneum facing the donor compartment. Reservoir compartment was filled with 100 mL Phosphate buffer of pH 6.8. The study was carried out at 37 ± 1°C and speed was adjusted until the vortex touches the skin and it carried out for 4½ h. 5 ml of sample was withdrawn from reservoir compartment at 30 min interval and the drug content was measured. Each time the reservoir compartment was replenished with the 5 mL volume of Phosphate buffer pH 6.8 solution to maintain constant volume.

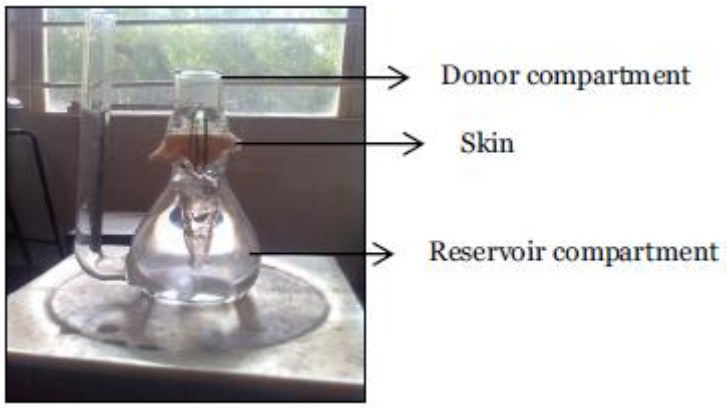


Figure No. 1: Franz diffusion cell with skin mounted between compartments

6) *In Vitro* Antifungal activity[14]

Weighed 16.25 gm of sabouraud dextrose agar was transferred in a 500 ml conical flask and 250 ml of purified water and some amount of heat is applied to dissolve it completely. After sterilizing for 15 min at 121°C at 15 lb pressure in autoclave for about 20 min, cooled it at room temperature. The fungal strain (*Candida albicans*) was dispersed in the medium and then the medium was poured it into three petridishes allowed it cool at room temperature until it solidifies and then three cups are bored in each petridish with the help of sterile steel bore of 6 mm. Then calculated concentration of the standard drug (Voriconazole), gel formulation (F1) and placebo gel were placed in the bores and incubated the petriplates for 72 h at 37°C in incubators. The zone of inhibition was observed and calculated.

RESULTS AND DISCUSSION

1) **Physical Evaluation:**All the three gel formulations of Voriconazole were evaluated for organoleptic characteristics, Color, Odor, Phase separation, Occlusiveness, and Washability etc. and found acceptable with respect to the evaluated physical evaluation. The results are given in Table 2.

Table No. 2: Physical Evaluation of Voriconazole Gel Formulations.

S. No.	Formulation Code	Color	Odor	Phase Separation	Wash-ability	Occlusiveness
1	VGF1	White to off white	Odorless	No	Washable	No
2	VGF2	White to off white	Odorless	No	Washable	No
3	VGF3	White to off white	Odorless	No	Washable	No

2) **Rheological Studies:** The rheological behavior of the prepared formulae showed shear-thinning flow indicating structural breakdown of the existing intermolecular interactions between polymeric chains. The different rheological parameters are given in Table 3.

Table No. 3: Details of the Rheological Properties of Voriconazole Topical Gels

Sl. No.	Formulation Codes	Coefficient of determination (R^2)	Flow Index (n)	Viscosity (centipoise)(η)	Flow Behavior
1	VGF1	0.9291	0.2350	1918	Shear Thinning
2	VGF2	0.9315	0.2156	2159	Shear Thinning
3	VGF3	0.9487	0.1354	2458	Shear Thinning

3) **Sensitivity Test:** The formulated Voriconazole gel formulations of all three caused no irritation or sensitivity to the skin when subjected to sensitivity test.

4) **Skin Irritancy Study:** This test is one of the important test parameter which needs to be evaluated for the topical application dosage from. Average response scores of skin irritation for single application. From the results it indicates that all the gel formulations have low skin irritation. The details of the index is shown in Table 4.

Table No. 4: The Skin irritation Index of Voriconazole Formulated Gels

Formulation	Primary Irritation Index		
	24 hrs.	48 hrs.	72 hrs.
VGF1	0.051	0.0973	0.092
VGF2	0.048	0.0922	0.089
VGF3	0.050	0.0944	0.097

5) ***Ex-Vivo* [*In-Vitro*] Diffusion Study:** The release studies clearly reveal that the drug Voriconazole is released to a lesser extent from the animal skin [mice] when compared to the cell membrane. The results clearly indicates that the higher the concentration of Carbopol, lesser is the release as the higher concentration of polymers might be retarding the release of the drug while the drug is released to a greater extent in the cell membrane. Though the VGF1 was able to give good release over a period of time when tested in the cell membrane, however, the same showed lesser release because of skin thickness and also the polymer concentration. The % drug diffusion is shown in the Table 5 and also the graphical representation of % drug diffusion is shown in the Figure No. 2.

Table No. 5: The % drug diffused across the mice skin for all 3 formulations

Sl. No.	Time Points [in Minutes]	Formulation Codes & % Drug Diffused		
		VGF1	VGF2	VGF3
1	30	14	11	11
2	60	22	19	17
3	90	34	31	28
4	120	45	42	34
5	150	54	48	41
6	180	66	55	49
7	210	72	61	56
8	240	79	67	61

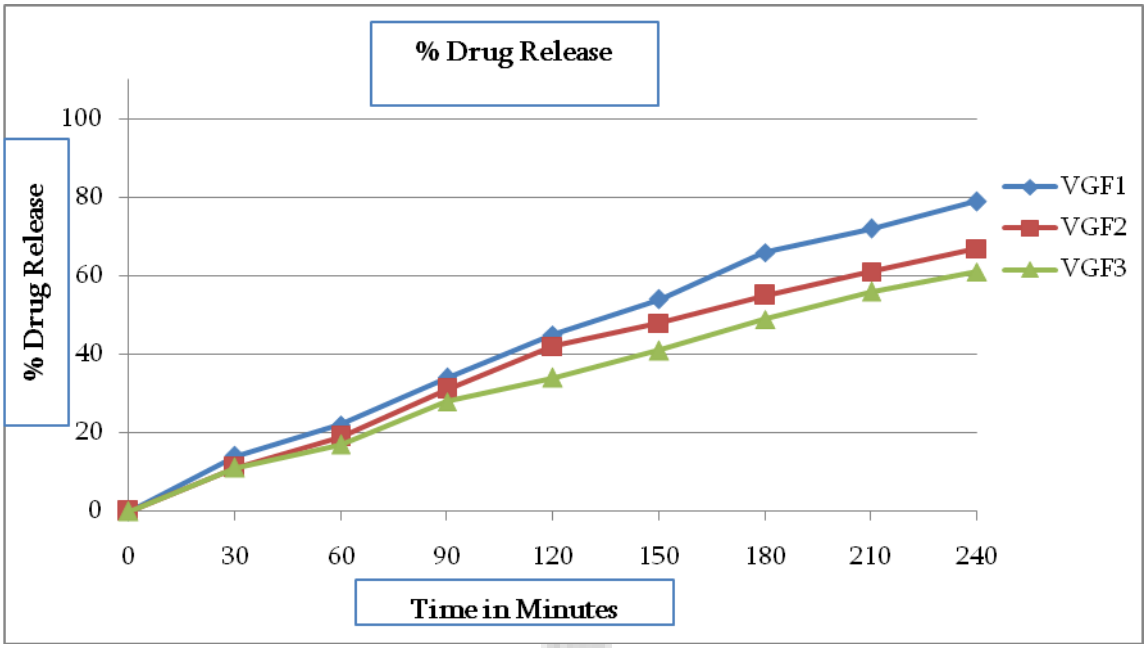


Figure No. 2: Graphical representation of % drug diffused of all 3 formulations across mice skin

Antifungal Study:

In the antifungal study, the fungi used were *Candida albicans*. The studies were carried for the optimized formulation [VGF1] and zone of inhibition observed at F1 is 6.2 mm^2, placebo gel as 0 mm^2 and the pure drug, Voriconazole possess a zone of inhibition 7.4 mm^2. The study indicated that the results are satisfactory. The details of the zone inhibition are shown in the Table 6. The graphical representation of antifungal activity is shown in the Figure No. 3. The photographic images of VGF1, Placebo and pure drug substances are shown in the Figure No. 4, 5 & 6 respectively.

Table No. 6: Reported Zone of Inhibition in mm^2 for Placebo, Pure Drug & Optimized Formulation

Sl. No.	Formulation	Zone of Inhibition [mm^2]
1	Placebo gel	0
2	Pure Drug [Voriconazole]	7.4
3	VGF1	6.2

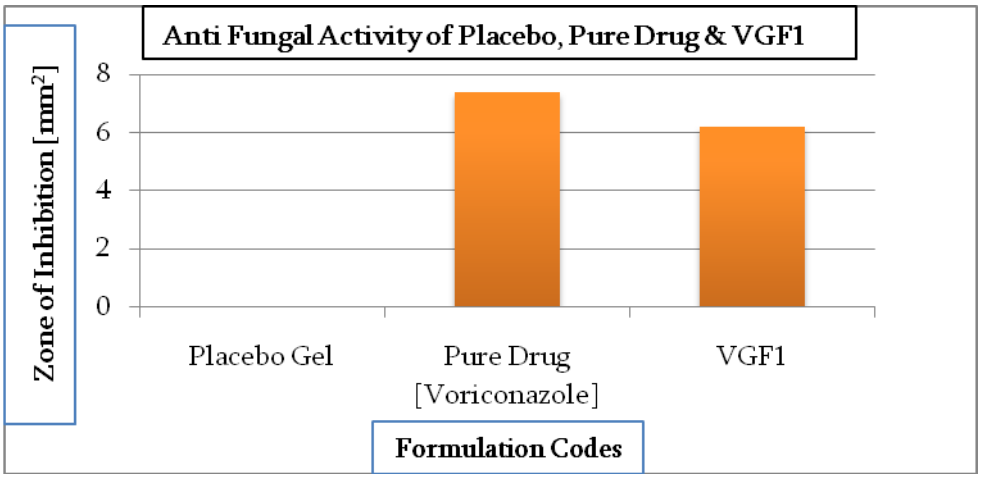
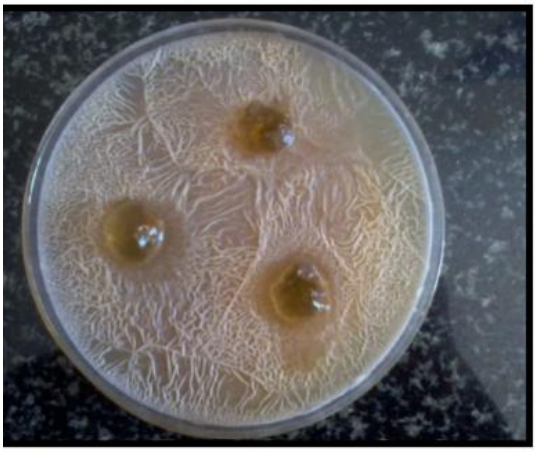


Figure No. 3: The graphical representation of antifungal activity of Placebo, Pure Drug & VGF1



Zone of inhibition of F1 gel formulation

Figure No. 4: VGF1 Formulation Zone Inhibition

Figure No. 5: Zone Inhibition Placebo Gel Formulation

Figure No. 6: Zone Inhibition of Pure Drug [Voriconazole]

CONCLUSION

The physical assessment of various formulations was successfully carried out. The appearance of formulations was translucent to off white. The gel formulation showed no sensitivity and no skin irritation. The rheological properties were found satisfactory. The viscosities of Carbopol gels ranged from 1900 to 2500 centipoises (cP). It can be concluded that gel formulations showed acceptable physical properties and drug diffusion study. Among all the three gel formulations, Carbopol 940 having 1 % concentration showed the promising results with respect to % drug diffused. Being optimized formula, the VGF1 was subjected to % drug diffusion and antifungal activity which showed promising results. Further, in the Carbopol gel formulations, the % drug diffusion was decreased with increase in Carbopol concentration because of viscosity increases with increase in polymer concentration. From the above results it can be concluded that the Voriconazole Gel formulation VGF1 was suitable for topical application.

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