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Development and *in vitro* Evalution of Guar Gum based Fluconazole *in situ* Gel for Oral Thrush

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Abstract
The objective of the present study was to prolong the delivery of the active drug in the oral cavity using a suitable carrier such as *in situ* gel which can effectively deliver the drug for an extended duration of time hence not only reduce the systemic side effects but also improve the therapeutic efficacy, patient compliance. Buccal drug delivery systems are better suitable for antifungal drugs particularly for oral thrush. The viscosity of in situ system was found to be in the range (49 to 75 cps) for the sol, whereas for the gels it was up to (17000 cps). The maximum gel strength and mucoadhesion was found to be up to (98 seconds) and (85.99 dynes/cm^2) respectively. The *in vitro* drug release of both the formulations G13 and G14 released 85.3%, whereas the formulations G3 and G4 released 89.40% and 89.10% respectively at 8th hour. More over to comprehend the drug release mechanism the data were fitted in to korsmeyer -peppas exponential model where the 'n' values were in the range of 0.06 to 0.113. It was understood that G code formulation were following predominantly first order and fickian diffusion mechanism of drug release .Different techniques, FTIR spectroscopy and differential scanning calorimetry (DSC) were used to estimate the incompatibility.

Keywords: Oral thrush, Fluconazole, Guar gum, Carbomer 934, HPMC, *in situ* gel.

INTRODUCTION

Oral thrush is an infection of yeast fungus, *Candida albicans* and saprophytic fungi of the genus *Candida*, which includes eight species of fungi; the most common species is *Candida albicans* in the mucous membranes of the mouth. Oral candidiasis may present in a multiplicity of clinical forms, and the three main variants are the pseudomembranous type, commonly called as thrush, and the erythematous and hyperplastic variants [1]. In the late 1950s there was a steadily increasing number of reports on superficial *Candida* infections associated with the administration of broad-spectrum antibiotics such as tetracycline [2-3]. In subsequent years, the extensive use of steroids, immunosuppressive agents in organ transplant recipients [4-5] myeloablative radiation therapy [6-7], and antineoplastics in patients with hematologic malignancies [8-11] contributed to the increasing morbidity associated with *Candida*. Initiation of the imidazole and azole groups of antifungal drugs during the last two decades has revolutionized the management of fungal infections [12] and currently available antifungal agents for the treatment of oral candidiasis are miconazole, clotrimazole, ketoconazole, fluconazole, and itraconazole [13]. The euphoria surrounding the efficacy of the azoles groups has now been tempered by the awareness of moderate or high-level resistance to fluconazole in some species, such as C. glabrata, C. krusei, and C. albicans [14-15]. To eliminate fungal infection, antifungal agents should be administered either locally or systemically. One way to improve the efficacy in eradicating the infection is to deliver the antifungal drug locally in the oral cavity. The conventional formulations for the local delivery of drugs to the oral cavity are the mouth paints, rinses, troches, creams and suspensions. A major difficulty for the complete eradication of fungal infections of the oral cavity is the dilution and rapid elimination of topically applied drugs due to the flushing action of saliva. Even though the clinical efficacy of systemic antifungal treatment is well established, the potency is decreased by thousand fold when reaches the target site, and also large dose and/or prolonged administration is often necessary to maintain an effective drug concentration. In such condition a safe and effective local route of drug delivery system, which will reduce the dose and increase the concentration of drug in the oral cavity with low systemic concentration is highly desirable [16]. The delivery system in which the drug is incorporated, therefore an important consideration and should be formulated to prolong retention of the drug in the oral cavity. Combination of HPMC, carbomer or sodium alginate in order to increase the mucoadhesive strength and control the drug release [17-18].

MATERIALS AND METHOD

Fluconazole was obtained from (Fourts India, Chennai); Guar gum, carbopol 934, and sodium alginate were purchased from Merk, Mumbai. DMSO and propyl paraben was supplied by Fine Chemicals (India). Gentamycin sulphate injection came from M/S Pharmaceutical and Industrial Laboratories, India. All other chemicals were of reagent grade.

METHODS

Formulations were prepared with various ratio of polymers was soaked in sufficient quantity of deionised water and kept overnight for swelling and propyl paraben solution was added to the above polymeric mixture. An appropriate amount of fluconazole was solubilized in suitable solvent with 14 mmol

Table 1. Composition of ion induced formulation

Ingredient	*G1 (gm)*	*G2 (gm)*	*G3 (gm)*	*G4 (gm)*	*G5 (gm)*	*G6 (gm)*	*G7 (gm)*	*G8 (gm)*	*G9 (gm)*	*G10 (gm)*	*G11 (gm)*	*G12 (gm)*	*G13 (gm)*	*G-14 (gm)*	*G-15 (gm)*
Fluconazole	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Guar Gum	0.1	0.2	0.3	0.4	0	0	0	0	0.2	0.3	0.4	0.2	0.3	0.4	1
HPMC	0	0	0	0	0.1	0.2	0.3	0.4	0.3	0.4	0.5	0.3	0.4	0.5	1
Sod.Alginate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0	0	0	0.5	0.5	0.5	0.5
Propyl Paraben	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
DMSO	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Deionized Water	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml	Upto 50ml

GG - Guar gum, SG- Sodium alginate, HPMC- Hydroxyl propyl methyl cellulose

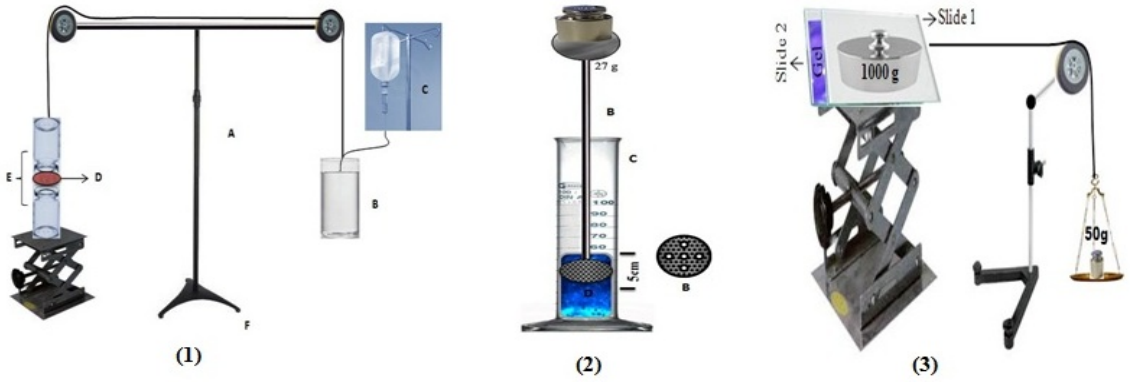

Figure 1. Assembly of mucoadhesive force measuring device, Figure 2. Assembly of Gel Strength measuring device, Figure 3. Assembly of spredability measuring device.

of calcium chloride and 0.1 % of sodium citrate with continuous stirring until uniform solution was obtained. Finally a small amount of triethanolamine was added to adjust pH 7. The detailed composition of prepared formulation is depicted in Table 1.

EVALUATION OF *IN SITU* GEL

Gelling capacity

The gelling capacity of the prepared formulation is determined by placing a drop of the formulation in a vial containing 2.0 ml of freshly prepared simulated tear fluid and visually observed. The time taken for its gelling is noted [19-20].

Determination of Mucoadhesive Force

The experimental technique used for determining the mucoadhesive force has been derived from a previously published method [21-22]. The experimental setup is presented in Figure 1. The mucoadhesive force of the formulations was determined as follows; a section of membrane was cut from the chicken and instantly fixed with mucosal side out onto each glass vial (E) using rubber band. The vial with chicken mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan (A). Fluconazole was added onto the mucosa of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then, the switch (C) of the infusion apparatus was opened to make the water drop into the glass vial (B) with a constant flow rate of 5 ml/min. The weight of the water in the glass vial (B) kept increasing until the gel and the mucosal tissue were detached. Mucoadhesive force, the detachment stress (dynes/cm^2), was determined from the minimal weights that detached the gel. The chicken membrane pieces were changed for each measurement. All measurements were performed in triplicate (n = 3).

Measurement of Gel Strength

A sample of 50 gm of gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37 $^{\circ}$C. The apparatus for measuring gel strength (weighing 27 gm) was allowed to penetrate in gel shown in Figure 2. The gel strength, which means the viscosity of the gels at physiological stimuli was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel [21].

Viscosity Studies

The rheological studies were carried out using Brookfield programmable DVII+ Model pro II type (USA). The viscosity of *in situ* gel and the solution were determined at different angular velocities and average of two reading was used to calculate the viscosity.

Diffusion across the chicken cheek mucosa

Chicken cheek mucosa [23] was isolated from a healthy chicken which was obtained from the local slaughter house

and was cleaned to remove blood cells. It was stored in normal saline with few drops of gentamycin sulphate injection, to avoid bacterial growth. The diffusion medium used was phosphate buffer (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8 g NaCl in 1000 ml of distilled water adjusted to pH 7.4).The oral diffusion cell was designed as per the dimension given. The diffusion cells were placed on the magnetic stirrers. The outlet of the reservoir maintained at 37 ± 0.5°C and was connected to water jacket of diffusion cell using rubber latex tubes. The receptor compartment was filled with fluid. Then the prepared chicken cheek mucosa was mounted on the cell carefully so as to avoid the entrapment of air bubble under the mucosa. Intimate contact of mucosa was ensured with receptor fluid by placing it tightly with clamp. The speed of the stirring was kept constant throughout the experiment with the help of micropipette. Aliquots of samples were withdrawn at time intervals of one hour from sampling port of receptor compartment and same volume was replaced with receptor fluid solution in order to maintain sink condition. The samples were withdrawn and drug content was determined as per the above procedure.

Content Uniformity

The formed gel (1g) was completely crushed with the help of glass road followed by vigorous shaking until the formed gel gets completely dispersed to give clear solution [24]. Final volume was adjusted to 100 ml with simulated saliva pH 7.4.Obtained solution was filtered through 0.45 micron filter membrane and the drug concentration was determined by UV Visible spectrophotometer at 260 nm. (Shimadzu UV1700, Japan)

RESULT AND DISCUSSION

Physicochemical properties such as viscosity, pH, appearance and clarity test were performed and the results are recorded in Table 2. Aqueous solutions of varying concentration containing polymers in various combinations such as GG: SG, HPMC: SG, GG: HPMC and GG: HPMC: SG were prepared and evaluated for gelling capacity and viscosity in order to identify the composition suitable for as *in situ* gelling systems. Many experiments were conducted by varying the concentration of these polymers in order to identify the optimum concentration required for the gel forming solution. The *in situ* system containing GG in the range of 0.1 to 0.4, HPMC in the range of 0.1 to 0.5 and SG (0.5 gm) were utilized in the G code formulation. A constant ratio of primary polymers SG without compromising the gelling capacity and rheological properties of the delivery system may be achieved by the addition of viscosity enhancing polymers such as guar gum and HPMC. This also helped the gels for its adhesion property to the mucous membrane and subsequent prolonged release.

The two main fundamentals of an *in situ* gelling system are viscosity and gelling capacity. To instill easily at the affected site the formulation must possess optimum viscosity. Further, the formulation should undergo rapid sol to gel transition upon contact at the affected site. Hence, the viscosity of sols and gels of various 'G' code formulations was determined at various shear rates and it was found to be shear thinning systems as shown in Figure 4-5.

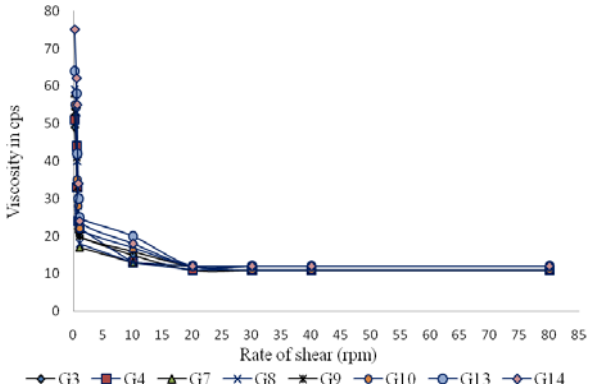

Figure 4 . Showing the viscosity of solution

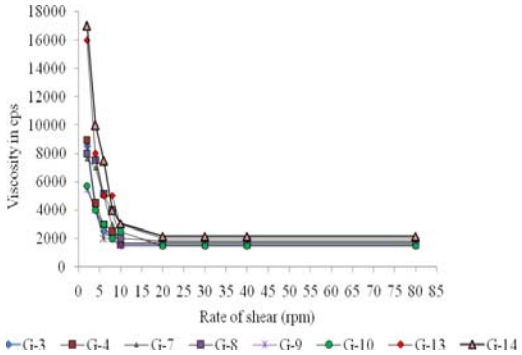

Figure 5. Showing the viscosity of in situ gel

The formulation G15 having higher concentration polymers is a poor candidate for *in situ* gel formulation since it is exhibited higher viscosity and hence was not pourable.

The prepared formulations were found to have good gelation capacity. The formulations G7 (0.3: 0.5), G8 (0.4: 0.5) containing HPMC: SG exhibited good gelation immediately after addition in to the simulated saliva solution and remained for about 8 hours. The formulations G3, G4, G9 and G10 showed moderate gelation capacity and remained for few hours. The formulations G13 and G14 (Guar gum / HPMC / Sodium alginate) (0.3/0.4/0.5) (0.4/0.5/0.5) were found excellent gelation capacity and remained for extended period of time as shown in Table 3.

Table 2. Physicochemical Evaluation of Solution

Tests	*G3*	*G4*	*G7*	*G8*	*G9*	*G10*	*G13*	*G14*
Visual appearance	***	**	***	***	***	**	***	***
Clarity	***	**	***	***	***	**	***	***
pH	6.8	6.9	6.8	7.0	6.9	6.8	6.9	6.9
Viscosity (cps)	49	51	58	59	53	55	64	75

Table 3. Characteristics of Various Fluconazole *in situ* Gel Formulations*(n = 3±SD).

Formula code	*Viscosity (cps)*	*% drug content (w/w)*	*mucoadhesive force (dynes/cm^2)*	*Spreadability (gm.cm/sec)*	*Gel strength (sec)*	*GT (sec.)*	*Gelation Capacity*	*GT*
G3	8500	92.55	55.40 ± 1.23	20.33	75	95	**	95
G4	8900	92.33	55.43 ± 2.01	20.41	81	94	**	94
G7	7600	89.00	78.55 ± 1.44	17.25	82	79	***	79
G8	7950	89.10	78.92 ± 1.54	17.42	81	80	***	80
G9	5450	88.15	77.50 ± 1.96	20.11	76	90	**	90
G10	5700	88.95	77.10 ± 2.13	20.45	78	95	**	95
G13	16000	90.25	82.80 ± 1.87	28.66	95	83	***	83
G14	17000	90.23	85.99 ± 1.24	28.86	98	82	***	82

*** Exhibited good gelation capacity remained for about 8 hours
*** Exhibited excellent gelation capacity remained for extended period of time

The values of spreadability indicate that the gel is easily spreadable by small amount of shear. The spreadability of formulation G13 and G14 (28.66 and 28.86 gm.cm/sec) was found to be more as compared to other optimized formulations as shown in Table 3. This indicates spreadability of *in situ* system containing fluconozole gel having three polymers was good as compared with two polymer combination.

Gel strength is important because strong gels will support a much higher pressure than weak gels before they are washed out of the targeted site. The formulations G13 and G14 (83 and 82 sec) exhibited good gel strength among all optimized G code formulation as shown in Table 3.which may be due to increase in concentration of viscosity enhancer (GG and HPMC) and ion induced polymer SG. The formulations G13and G14 showed gelation within 83 seconds and 82 seconds and remained for extended period of time. These may be due to primary polymer combination with secondary polymer (GG, HPMC).

The mucoadhesive force is an important physic-chemical parameter for topical application in buccal cavity. The formulation G13 and G14 (82.80, 85.99 dynes/cm^2) showed maximum mucoadhesive force than all optimized formulations as shown in Table 3. These may be due to increase in concentration of GG and HPMC in the formulations. The results also indicated that the presence of secondary polymers GG and HPMC significantly increased the viscosity as well as mucoadhesive properties.

The *in vitro* diffusion profile of fluconazole from *in situ* gels from formulations G3, G4, G7, G8, G9, G10, G13, and G14 were conducted in diffusion medium pH 7.4. The formulations G3 and G4 containing guar gum and sodium alginate in the ratio of 0.3:0.5 and 0.4:0.5 showed 89.40% and 89.1% respectively at 8 hours. Whereas formulations G7 and G8 containing HPMC and SG in the ratio of 0.3:0.5 and 0.4:0.5 released 55.8% and 54.6% respectively up to 8 hours. Formulations G9 and G10 containing GG and HPMC in the ratio of 0.2:0.3 and 0.3:0.4 released 70.4% and 68.8% respectively up to 8 hours. The formulations G13, and G14 which were made with combination of three polymers namely GG, SG and HPMC in the ratio of 0.3:0.4:0.5 and 0.4:0.5:0.5 released 85.3% respectively up to 8 hours as shown in Figures 6. From the *in situ* gel, as the value of 'r' for first order kinetics ranged from 0.8142 to 0.9229 and also found to be higher than that of zero order which ranged from 0.714 to 0.8433. The 'r' values for Hixoncrowell ranged from 0.923 to 0.95 and that of Higuchi kinetics ranged from 0.841 to 0.978. In addition to comprehend the drug release mechanism the data were fitted in to korsmeyer -peppas exponential model where the 'n' values were in the range of 0.06 to 0.113. It was understood that G code formulation were following predominantly first order and fickian diffusion mechanism of drug release.

Table 4. Release kinetics of G Code formulation

Order of process	*G3*	*G4*	*G7*	*G8*	*G9*	*G10*	*G13*	*G14*
Zero order	R^2 0.972	0.968	0.984	0.982	0.968	0.966	0.959	0.963
	M 8.336	8.381	5.836	5.754	6.692	6.39	8.154	8.209
	C 2.254	3.254	-5.018	-5.981	1.78	0.445	3.8	2.927
First order	R^2 0.950	0.95	0.991	0.989	0.975	0.958	0.948	0.954
	M 0.094	-0.097	-0.037	-0.036	0.052	-0.047	-0.088	-0.087
	C 2.069	2.065	2.02	2.025	1.991	1.998	2.037	2.043
Higuchi	R^2 0.972	0.968	0.984	0.982	0.968	0.966	0.959	0.963
	M 8.336	8.381	5.836	5.754	6.692	6.39	8.154	8.209
	C 2.254	3.254	5.018	5.981	1.78	0.445	3.8	2.927
korsmeyer	R^2 0.961	0.959	0.843	0.835	0.95	0.946	0.951	0.95
	M 0.063	0.061	0.104	0.113	0.064	0.066	0.06	0.062
	C 1.373	1.394	0.853	0.767	1.273	1.221	1.394	1.377

Table 5 .FTIR spectra

Compound	*C-H cm^{-1} stretching*	*C-H cm^{-1} bending*	*C-N cm^{-1} vibration Ar-tertiary*	*C-F cm^{-1} stretching*	*C-N cm^{-1} Ar-tertiary*	*Tertiary cm^{-1} alcohol*	*OH cm^{-1} bending*
Fluconazole	2950	1460	1380	1280	1360	1350	-
GG + SG + HPMC	2950	1460	-	-	-	1310	1100
GG + SG + HPMC + Fulconazole	2950	1460	1380	1300	1380	1350	1100

GG - Guar gum, SG- Sodium alginate, HPMC- Hydroxyl propyl methyl cellulose

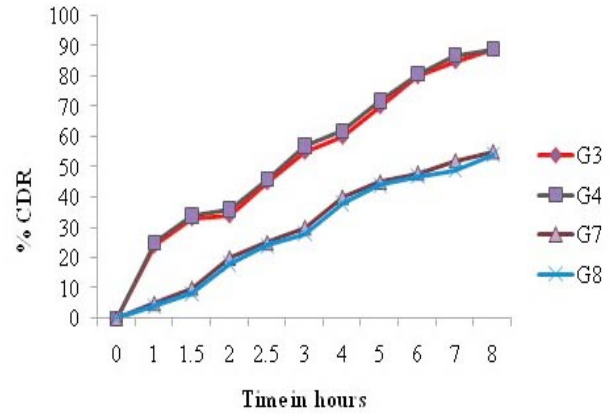

Figure 6. Showing the Diffusion of optimized formulation

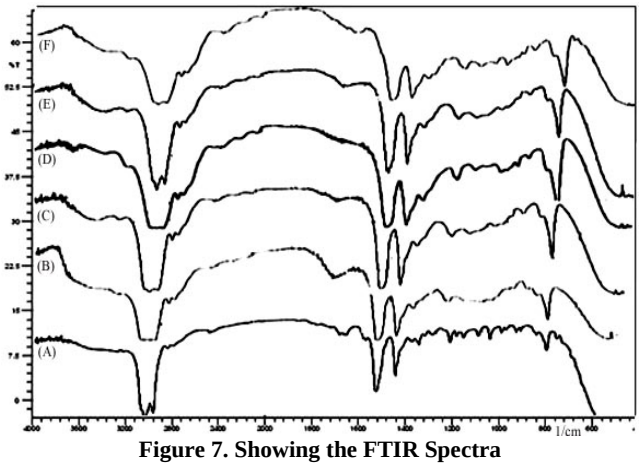

Figure 7. Showing the FTIR Spectra

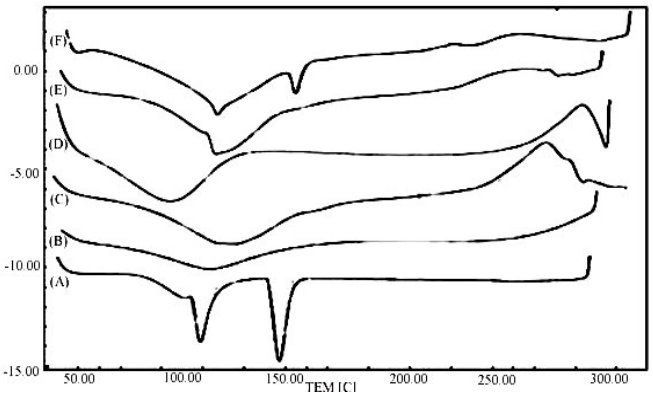

Figure 8. Showing the DSC Spectra

FTIR spectra of physical mixture formed the polymerized active constituent .It was observed that there were no changes in these main peaks in the IR spectra of a mixture of drug and polymers (Table 5). The FTIR study revealed no physical or chemical interactions of fluconazole with uar gum, sodium alginate and HPMC as evident from Figures 7.

The DSC thermogram studies of Fluconazole showed a sharp endothermic peak at 145.85°C and another peak at 107.30°C which may be related to moisture. The DSC curve of the sodium alginate polymer showed a broad endothermic peak at 119.29 °C and 257.73°C. The DSC curve of the HPMC showed at 90.64°C.combination of three polymer endothermic peak at 112.20°C. Physical mixture of polymer combination and drug showed a peak at 106.37°C and 142.36°C. From the results of DSC study it can be concluded that there was no significant Drug- excipient interaction as evident from Figures 8**.**

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