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Bioadhesive buccal gels impregnated with fluconazole: formulation, *in vitro* and *ex vivo* characterization

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ABSTRACT

This study describes the formulation of bioadhesive buccal gels for fluconazole delivery via buccal mucosa. In the present study the polymer with well-defined mucoadhesive properties like Carbopol 934 was used. Carbopol-Poloxamer gels of 1% fluconazole was formulated with various absorption enhancers like polyethylene glycol, propylene glycol, glycerol, phosphatidylcholine, mannitol and sodium lauryl sulphate by cold method. The gels were characterised for gelation temperature, bioadhesive force, pH, viscosity, drug release profile, *ex vivo* permeation across goat buccal mucosa and stability profile. The percent drug permeated through the buccal mucosa was in the range of 62-76%. Polyethylene glycol and propylene glycol were found to be better absorption enhancers as compared to others and followed zero order release kinetics.

INTRODUCTION

Candidiasis or thrush is a fungal infection (mycosis) of any of the Candida species, of which *Candida albicans* is the most common. Superficial infections of skin and mucosal membranes by Candida causing local inflammation and discomfort are however common in many human populations. While clearly attributable to the presence of the opportunistic pathogens of the genus Candida, candidiasis describes a number of different disease syndromes that often differ in their causes and outcome. Commonly referred to as a yeast infection, it is also technically known as candidosis, moniliasis, and oidiomycosis (James *et al.*, 2006). Fluconazole is a bis-triazole antifungal drug and structurally related to imidazole derivatives. It is fungistatic in action and exerts its antifungal activity by altering cellular membranes resulting in increased membrane permeability, leakage of essential elements (e.g. amino acid, potassium) and impaired uptake of precursor molecules (e.g. purine and pyrimidine precursor to DNA). Following oral dosing, fluconazole has 90% bioavailability, is almost completely absorbed within two hours and has a half life of 30 h. Like other imidazole- and triazole-class antifungals, fluconazole inhibits the fungal cytochrome P450 enzyme 14α-demethylase. Retentive buccal mucoadhesive formulations have proven to be a feasible alternative to the conventional oral medications as they can be readily attached to the buccal cavity, retained for a longer period of time and removed any time. Buccal adhesive drug delivery systems can be based on matrix tablets, films, patches, layered systems, discs, microspheres, ointments and hydrogel systems.

Bioadhesive formulations designed for buccal application should exhibit suitable rheological and mechanical properties, including pseudoplastic or plastic flow with thixotrophy, ease of application, good spreadability, appropriate hardness, and prolonged residence time in the oral cavity (Salamat-Miller, 2005).

These properties may affect the ultimate performance of the preparations and their acceptance by patients. Absorption of drug via the mucous membranes of the oral cavity can occur in either the sublingual, buccal, or local regions. The local region includes all areas other than the former two regions. In general, the oral mucosa is classified as a somewhat leaky epithelium with a permeability rank order of sublingual, buccal and palatal, based on the thickness and degree of keratinization of the tissues.

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Additionally, drug delivery via this site avoids extensive enzyme degradation and first-pass metabolism seen with oral administration, which is desired outcomes for the delivery of therapeutic proteins and peptides (Shojaei, 1998). Buccal drug delivery has advantages such as the abundant blood supply in the buccal area, bypassing the hepatic first pass effect, excellent accessibility, etc. But the major challenge remains that it is very difficult to apply ointments, solutions, creams and lotions onto the oral mucosa, and have their effects persist for a significant period of time, since they are very easily removed by salivation, temperature, tongue movement and swallowing. Therefore, the new formulations that have suitable adhesion or adhesive time and show controlled release for a period of time are required (Shaikh *et al.*, 2011). The mucoadhesive drug delivery systems utilize the property of bioadhesion of certain water-soluble polymers that become adhesive on hydration and hence can be used for targeting a drug to a particular region of the body for extended periods of time. Bioadhesive formulations designed for buccal application should exhibit suitable rheological and mechanical properties, including pseudoplastic or plastic flow with thixotrophy, ease of application, good spreadability, appropriate hardness, and prolonged residence time in the oral cavity. These properties may affect the ultimate performance of the preparations and their acceptance by patients. In the present study, bioadhesive and gel-forming agents having excellent thick gel barrier formation characteristics with good gelation temperature like Carbopol and Poloxamer were used (Singh *et al.*, 2011).

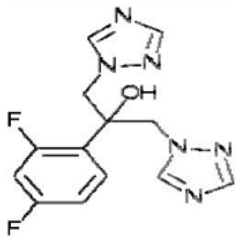

Fig. 1: Chemical Structure of fluconazole.

MATERIALS AND METHODS

Materials

Fluconazole was kindly provided by Cadila Pharmaceuticals Ltd., India. Carbopol 934, polyethylene glycol, propylene glycol, glycerol, mannitol and sodium lauryl sulphate were purchased from Lobo Chemie Pvt. Ltd., India. Poloxamer 188, phosphatidyl choline were procured from Himedia Lab Pvt. Ltd., India. All other chemicals used in the study were of analytical grade and were used as received.

Methods

Drug excipient interaction studies

Drug-excipient interaction studies were determined by FTIR spectroscopy. Fluconazole powder was separately mixed with various excipients in the ratio of 20:80. The resultant physical mixture was kept in sealed glass vials and placed at different temperature conditions for 3 weeks. Two evaluation parameters were employed to study the interaction between the drug and excipients. The contents of each vial were observed for any change in their physical characteristics and for their characteristic peaks by FTIR Spectrophotometer (Shimadzu 8400S). The results are depicted in **Table 1**. Physical changes of drug excipient mixtures in solid state at different condition are recorded in Table 2. The FTIR data showed that fluconazole and excipients did not react with each other and retained their action at room temperature.

Table 1: Position of characteristic absorptions at definite wave number

Wave Number (cm^{-1})	Characteristic absorptions
1800-1600	C=N
3000-2850	C-H
3150-3028	Aromatic C-H
1446	Benzene
3550-3200	O-H intermolecular hydrogen bond
1400-1000	C-F

Table. 2: Physical changes in drug excipient mixture.

S. No.	Drug:Excipient (20:80)	Physical changes after 3 weeks		
		At room temp	At 50°C	At 4°C
1	Fluconazole: Poloxamer 188	No change	Poloxamer 188 melted	No change
2	Fluconazole: Carbopol 934	No change	No change	Carbopol 934 absorbed moisture

Formulation of buccal bioadhesive gel

Carbopol-poloxamer gels of 1% fluconazole with different absorption enhancers like polyethylene glycol, propylene glycol, glycerol, phosphatidyl choline, mannitol and sodium lauryl sulphate were prepared by cold method (Table 3). Poloxamer 188 (2%) was added to minimum amount of water with gentle stirring at 5°C (Shin *et al.*, 2000). Subsequently, enhancer and drug were added with required quantity of ethanol. In a separate beaker, Carbopol 934 (5%) was stirred with water and added to the prepared solution of Poloxamer 188 and continuously stirred for 1h. The preparation was then brought to 25 ml with distilled water and stored at room temperature.

Table. 3: Composition of fluconazole buccal bioadhesive gels.

Formulation	Absorption Enhancer
S1	Polyethylene glycol
S2	Propylene glycol
S3	Phosphatidylcholine
S4	Mannitol
S5	Glycerol
S6	Sodium lauryl sulphate

Each formulation contained Carbopol 934 – 5%, Poloxamer 188 – 2% and Fluconazole - 1%;

Characterization of buccal bioadhesive gel

Measurement of gelation temperature

Two gm of fluconazole gel was placed in a 100 ml transparent beaker over a modified low-temperature thermostat water bath. Poloxamer gel was heated at the rate of 5°C/5 min with continuous stirring at a rate of 50 rpm (Choi, 1998; Yong, 2001). When the magnetic bar stopped moving due to gelation, the temperature was noted, which indicated the gelation temperature. The gelation temperature of the various formulations is presented in Table 4.

Table 4: Characterization of fluconazole gel.

Formulation	Gelation temperature (°C)	pH at 6 h	Bioadhesive force (gm force)
S1	35	6.52	48.40
S2	35	6.75	43.65
S3	35	6.42	43.40
S4	40	7.19	37.22
S5	45	7.12	35.00
S6	35	7.68	46.10

DETERMINATION OF BIOADHESIVE FORCE

The bioadhesive force of poloxamer gel was determined by using measuring device fabricated in house (Figure 2). A section of goat buccal tissue was secured with mucosal side out onto glass vial (C) using a rubber band. The vials with the buccal tissue were stored at 36.5 °C for 10 min. One vial with a section of tissue (E) was connected to the balance (A) and the other vial was placed on a height-adjustable pan (F). One gm Fluconazole gel (D) was applied onto the buccal tissue on the vial. Subsequently the height of the vial was adjusted so that the poloxamer gel was placed between the mucosal tissues of the two vials. The weights (B) were raised until the two vials remained attached. Bioadhesive force, the detachment (gm force), was determined from the minimal weights that detached the two vials. The buccal tissue pieces were changed for each measurement (Choi, 1998; Yong, 2001). The observations are presented in Table 4.

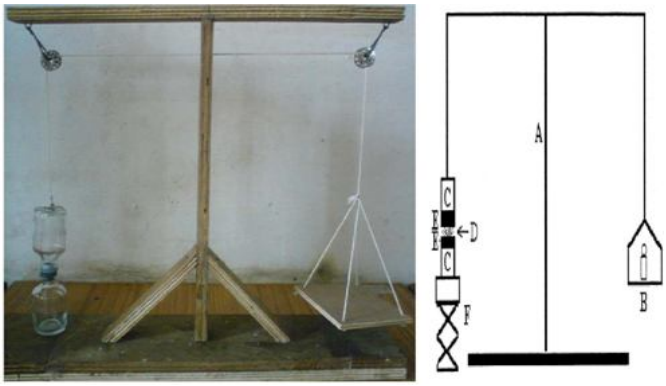

Fig. 2: Fabricated bioadhesive force-measuring device; modified balance (A), weights (B), glass vial (C) gel (D), buccal tissue (E), height-adjustable pan (F).

pH of bioadhesive gel

One gm gel was placed in a glass beaker with phosphate buffer pH 6.8 (15mL) and allowed to swell. Thereafter, surface pH measurements were recorded at predetermined intervals of 0.30, 1.0, 1.30, 2, 3, 4, 5 and 6 h. The results are presented in Table 4.

Viscosity studies

The viscosity of gels was measured using Brookfield DV-I+ Viscometer (Version 5.1). The measurements were performed with a T shape spindle (LV Spindle No. S63). Viscosity parameters were measured at different rpm with 1-minute equilibration time at each rpm. Samples were applied to the spindle using a spatula to ensure that formulation shearing did not occur and the viscometer was set at room temperature. The viscosity of various gels with different absorption enhancers is depicted in Table 5. The viscosity of sample was determined by multiplying the observed reading by the shear rate.

Viscosity (cps) = (300 / N) × observed reading

Where N = rpm

Table 5: Viscosity studies of bioadhesive gel

S. No.	rpm	Viscosity (cps)					
		S1	S2	S3	S4	S5	S6
1	20	270	252	204	122.7	126.6	261
2	50	516	327	270	168.9	180.9	294
3	100	616	576	456	234	242.7	474

Drug release study

The drug release study of the fluconazole bioadhesive gel was carried out with modified Franz diffusion cell. Specially designed diffusion tubes with internal diameter of 2cm having cellophane membrane at one end were used. Two gm gel was placed inside the tube. This assembly was immersed in a beaker containing 20 mL of Phosphate buffer (pH 6.8) placed over a thermostatically controlled magnetic stirrer set at 37±1°C. The contents in the beaker were stirred with the help of a teflon coated bead at 300 rpm. The samples (2mL) were withdrawn at predetermined intervals of 0.30, 1, 2, 3, 4, 5 and 6 h and replaced with phosphate buffer (pH 6.8) to maintain the sink conditions. The drug content in the sample was quantitated spectrophotometrically (Figure 3).

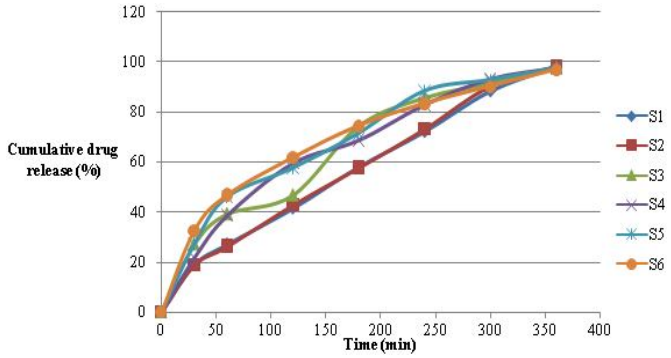

Fig. 3: Drug release study of fluconazole gel in phosphate buffer (pH 6.8).

Ex vivo permeation of drug through the buccal mucosa

Freshly excised goat buccal tissue was used for the *ex vivo* permeation studies within 2 h of removal (Patel *et al.*, 2007). The underlying tissues were removed from the mucosa with surgical scissors, making sure that the basal membrane was retained. The prepared buccal mucosa was washed and examined for integrity, and then stored at 4°C for 24 h in phosphate-buffered saline pH 6.8, before being used for the permeation experiments. The permeation experiments were performed using modified Franz diffusion cells. Specially designed diffusion tubes (internal diameter - 2cm) were used having goat buccal mucosa at one end. Two gm gel was placed inside the tube. This assembly was immersed in a beaker containing 20mL of Phosphate buffer (pH 6.8) and was placed over a thermostatically controlled magnetic stirrer at 37±1°C. The contents in the beaker were stirred with the help of a teflon coated bead at 600 rpm. Aliquots of

samples (2mL) were withdrawn at predetermined intervals of 0.30, 1, 2, 3, 4, 5 and 6 h and replaced with Phosphate buffer (pH 6.8) to maintain the sink conditions. The drug content was analysed in the samples spectrophotometrically (Table 6).

Table 6: Permeation of drug through the buccal mucosa.

Formulation	Drug permeated (%)
S1	76.23
S2	75.26
S3	75.12
S4	68.22
S5	66.25
S6	62.41

Kinetic analysis of drug release data

The release data of all the batches was fitted to zero-order, first-order, and Higuchi equations to ascertain the kinetic model of drug release (Table 7).

Table. 7: Kinetic assessments of drug release data of fluconazole bioadhesive gel.

Formulation	Zero order	First order	Higuchi
S1	0.9975	0.8301	0.9877
S2	0.9946	0.8505	0.9932
S3	0.9565	0.9378	0.9781
S4	0.9500	0.9386	0.9881
S5	0.9636	0.9557	0.9847
S6	0.9523	0.9281	0.9723

Table. 8: Stability study of fluconazole bioadhesive gel after 45 days.

Formulation	Storage temperature (^{o}C)	Stability study
S1	4	No change in colour
	25	No change in colour
	50	Dry and colour change to light yellow
S2	4	No change in colour
	25	No change in colour
	50	Dry and colour change to light yellow
S3	4	No change in colour
	25	No change in colour
	50	Dry and colour change to brown
S4	4	No change in colour
	25	No change in colour
	50	Dry and colour change to light yellow
S5	4	No change in colour
	25	No change in colour
	50	Dry and colour change to light yellow
S6	4	No change in colour, becomes hard
	25	No change in colour
	50	Dry and colour change to light yellow, more viscous

Stability studies

Shelf life as a function of time and storage temperature was evaluated by visual inspection of the bioadhesive gels at different time periods. Stability was monitored at 4°C, 25°C and 50°C (Table 8).

RESULT AND DISCUSSION

Measurement of gelation temperature

Gelation temperature is the temperature at which liquid phase makes transition to gel phase. Gelation temperature range suitable for bioadhesive gel would be 30–36°C. If the gelation temperature of bioadhesive gel is lower than 30 °C, gelation would occur at room temperature leading to difficulty in administration. However, if the gelation temperature is higher than 36 °C, the gel would remain in the liquid form at physiological temperature, resulting in leakage from the buccal mucosa. Poloxamer 188 was selected due to its thermosensitive gelling properties while solutions of poloxamer 188 alone did not gel at the desirable range. The results indicated that Poloxamer 188 alone could not provide the suitable gelation temperature. However, formulations having Poloxamer 188 mixtures with carbopol 934 gelled at physiological temperature. Poloxamer molecules exhibit a well-arranged zigzag configuration. With increasing temperature, the zigzag configuration of Poloxamer may be transformed into a closely-packed meander configuration, forming a viscous gel. The gelation temperatures of poloxamer gels were affected by the compositions and concentration of poloxamers and carbopol 934. Table 4 depicts the gelation temperature of various formulations.

Determination of bioadhesive force

Bioadhesive force means the force with which bioadhesive gel binds to buccal mucosal membranes. Since the buccal mucosal membranes consist of oligosaccharide chains, the polymers with hydrophilic groups can bind strongly to oligosaccharide chains, resulting in strong bioadhesive force. The stronger the bioadhesive forces are, the more it can prevent the gel from flushing out from the buccal environment and check the pathway for the first pass effect. But, if the bioadhesive force is too excessive, the gel can damage the mucous membranes. Therefore, bioadhesive gel must have an optimum bioadhesive force. These results suggested that the poloxamers with hydrophilic Carbopol bioadhesive polymer could bind to oligosaccharide chains, resulting in moderate bioadhesive forces. Carbopol 934, which enhanced gel strength, also efficiently increased the bioadhesive force. The results are presented in Tab 4.

pH of bioadhesive gel

Surface pH evaluation of oral mucosal dosage forms is an important aspect for characterisation, since an acidic or alkaline pH may cause irritation to the oral mucosa. It was therefore necessary to determine if any extreme surface pH changes occurred with the buccal bioadhesive gel during the drug release period investigated. The pH of the gel remained fairly constant at a pH of approximately 6.7–7.0 over the 6-h test period, confirming that the pH of the gel was within the neutral conditions of the saliva (pH 5.8–7.1) and that no extremes in pH occurred throughout the evaluation period. These results suggested that the polymeric blend identified was suitable for oral application owing to the acceptable pH measurements shown in Table 4.

Viscosity studies of bioadhesive gel

The viscosity of the various formulations is presented in Table 5. The highest viscosity value was observed in the S1 formulation which contained Poloxamer and Carbopol with polyethylene glycol at room temperature. Significant changes in

the viscosity of formulations were observed with different absorption enhancers.

Drug release study

The release of fluconazole from the prepared carbopol-poloxamer gels was studied through cellophane membrane at 37±1°C in phosphate buffer (6.8 pH). The release profile of fluconazole from various gels is illustrated in Figure 3. The cumulative percent drug release from the formulations S1, S2, S3, S4, S5 and S6 was found to be 98.22, 98.13, 97.74, 97.61, 97.41 and 96.86 % respectively. Carbopol 934 being a hydrophilic polymer, absorbs water, thereby promoting the dissolution, and hence the release, of the drug. Moreover, the hydrophilic polymers leaches out and, hence, create more pores and channels for the drug to diffuse out of the gel. Carbopol 934 controlled the drug release in increasing concentration. This could have been due to the extensive swelling of the polymers, which created a thick gel barrier, making drug diffusion in controlled manner. It was apparent that S1, S2 and S3 gel were better than other formulations.

Permeation of drug through the buccal mucosa in vitro

Effects of various permeation enhancers on the permeation of fluconazole through the goat buccal mucosa were investigated. The enhancers such as polyethylene glycol, propylene glycol, phosphatidyl choline, mannitol, glycerol and sodium lauryl sulphate were used. Permeation enhancer efficacy was evaluated by the determination of % drug permeation of the formulation. The effect of the different enhancers is presented in Table 6. The glycols, such as polyethylene glycol and propylene glycol, increased the permeation rate of the drug more significantly.

Kinetic analysis of drug release data

Kinetic assessments of drug release data of fluconazole bioadhesive gel presented in Table 7, indicate that the fluconazole gel showed the controlled drug release.

SUMMARY AND CONCLUSION

Polymer employed in the present study for buccal bioadhesive gel was Carbopol 934 having well defined mucoadhesive property. Carbopol 934 being a hydrophilic polymer, absorbs water, thereby promoting the dissolution, and hence the release, of the drug. Moreover, the hydrophilic polymers would leach out and, hence, create more pores and channels for the drug to diffuse out of the gel. Carbopol 934 controlled the drug release in increasing concentration. This could have been due to the extensive swelling of the polymers, which created a thick gel barrier, making drug diffusion in controlled manner and increase drug concentrations at increasing time intervals. Poloxamer 188 has amphiphilic structure, and surfactant properties that make them useful to increase the water solubility of hydrophobic, oily substances or otherwise increase the miscibility of two substances with different hydrophobicities. In the present study buccal bioadhesive gels for fluconazole were prepared. Carbopol-poloxamer gels of 1% fluconazole containing different absorption enhancers were prepared by cold method. The bioadhesive gel was characterized for gelation temperature, bioadhesive force, pH, viscosity, *in vitro* drug release profile and *in vitro* permeation. Kinetic analysis of drug release data and stability study of gel in different ambient conditions was also determined. The cumulative percent drug release from the formulations S1, S2, S3, S4, S5 and S6 was found to be 98.22, 99.13, 97.74, 97.61, 97.41 and 97.86 respectively, and percent drug permeated through buccal mucosa was found to be 76.23, 75.26, 75.12, 68.22, 66.25 and 62.41 respectively. On the basis of various *in vitro* and permeability studies, it was concluded that S1, S2, S3 formulations were comparatively better than other formulations and followed the zero order release kinetics. Mucoadhesive semi-solid formulations overcome the problem of scarce bioavailability of the conventional topical formulations by allowing the application of the drug at the pathological site, and increasing the contact time between formulation and mucosa. In this respect, semi-solid formulations like bioadhesive gel designed in the present study possess high biocompatibility and bioadhesivity that should allow adhesion to the mucosa in oral cavity.

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