

**IN VITRO EVALUATION OF MICONAZOLE MUCOADHESIVE BUCCAL FILMS**BAZIGHA K ABDUL RASOOL^{1*}, SAEED A. KHAN¹¹ Department of Pharmaceutics and Pharmacy Practice, Dubai Pharmacy College, Dubai, UAE
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ABSTRACT

Buccal bioadhesive films, releasing topical drugs in the oral cavity at a slow and predetermined rate, provide distinct advantages over traditional dosage forms for treatment of oral candidiasis. Five different film formulations containing 20 mg of miconazole nitrate, drug solubilizers (propylene glycol 10% w/w, polyethylene glycol 3% w/w, tween₂₀ 6% w/w, and oleic acid 5% w/w) and chitosan as film forming polymer, had been prepared by a casting-solvent evaporation technique and characterized in terms of weight uniformity, film thickness, surface pH, swelling capacity, in vitro drug release and in vitro microbiological effectiveness against *Candida albicans* in comparison to a reference. Thickness of the prepared films ranged from 0.11 to 0.23 mm and the film weight ranged from 152.5 to 188 mg, where the surface pH values of all films were in the range 5.84-6.63 which is favorable for oral mucosa. Optimum release behavior and acceptable elasticity were exhibited by film containing propylene glycol 10% w/w. The swelling percent of the selected film after 6 h reached 32.1%. The drug release mechanism was found to follow Fickian diffusion. Furthermore, the antifungal activity of the selected film was significantly ($p < 0.05$) superior to the reference miconazole oral gel (Daktarin®). Mucoadhesive chitosan buccal film for the topical delivery of miconazole nitrate could be a successful mean for the management of oral candidiasis. These preliminary results indicate that the selected film formulation (MC 0.524 mg/cm², PG 10% w/w and chitosan 2% w/w) can represent a valid mean for the management of oral candidiasis.

Keywords: Buccal, Mucoadhesive, Chitosan, Miconazole nitrate, Oral candidiasis**INTRODUCTION**

Buccal drug delivery has lately become an important route of drug administration. Various bioadhesive mucosal dosage forms have been developed, which included adhesive tablets¹, gels², ointments³, patches⁴ and more recently films⁵. Buccal film may be preferred over adhesive tablet in terms of flexibility and comfort. In addition, they can circumvent the relatively short residence time of oral gels on the mucosa, which is easily washed away and removed by saliva⁶.

A wide range of polymers of synthetic, semi synthetic and natural origin like carbopol, polycarbophil, sodium carboxymethylcellulose (SCMC), xanthan and hydroxyl propyl methylcellulose⁷ have been described for the formulation of bioadhesive systems but none of these polymer possess all the characteristics of an ideal polymer for a bioadhesive drug delivery system. Chitosan unique, non toxicity, antimicrobial characteristic, solubility, polycationic character, physical attributes, biocompatibility and biodegradability make it an attractive biopolymer for many pharmaceutical and biomedical applications⁸.

Chitosan is an excellent polymer to be used for buccal delivery because it can act as an absorption enhancer and it has mucoadhesive property, that allow it to stay in the oral cavity for a few hours and release the drug in unidirectional way towards the mucosa in a sustained release fashion^{9,10}.

Candidiasis in the oral cavity is an opportunistic infectious condition caused by a ubiquitous, saprophytic fungus of the genus *Candida*, the most common of which is *Candida albicans*. Although *C. albicans* is a resident commensal fungus of the normal oral flora, it causes a common infection in people wearing dentures, and severe oropharyngeal candidiasis is reported with increasing frequency in patients immunosuppressed or receiving anticancer radiotherapy¹¹. Oral candidiasis, are a major cause of morbidity and mortality in cancer patients¹². Chronic antimycotic therapy in high doses is undesirable for treatment of oral infections due to potential side effects. Therefore, to minimize these adverse effects and the ominous risk of drug resistance, topical therapy should be considered the first-line candidate for the treatment of oral and pharyngeal candidiasis. The efficacy of antifungal therapy for oral candidiasis is related to the time period and the concentration of drug is above the minimum inhibitory concentration (MIC), which

effect can be achieved locally in the mouth using buccal bioadhesive controlled release devices unlike existing conventional formulations such as gels or suspensions¹³. Miconazole (MC) is one of the first line broad-spectrum antifungal agents that has been extensively used for the prophylaxis and treatment of oral and vaginal candidiasis¹⁴. Presently, for the topical treatment for oral candidiasis, miconazole is available only in the form of oral gel (a common brand is Daktarin® oral gel (Janssen-Cilag, USA) which is required to be taken three to four times a day for 14 days. The release of the drug from such preparations involves initial burst of activity, whose level rapidly declines to subtherapeutic concentrations¹⁵. Therefore, there is a need for the development of MC buccal bioadhesive controlled release formulation. Consequently the main objective of this work is to formulate MC mucoadhesive films for topical treatment of oral candidiasis to ensure satisfactory MC level in the mouth for prolonged duration of time. The prepared formulations were evaluated through in vitro testing of their swelling, release and microbiological properties.

MATERIALS AND METHODS**Materials**

Miconazole was kindly supplied by JULPHAR (U.A.E). Chitosan (medium molecular weight), oleic acid (OA), potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate and phosphate buffer saline (PBS) tablets (pH 7.4) were all purchased from Sigma-Aldrich (Germany). Propylene glycol (PG), polyethylene glycol (PEG400) and tween 20 were obtained from BDH chemical Ltd. (Pool, UK). Glacial acetic acid was obtained from Fisons Scientific Equipment (Loughborough, UK). Sabouraud dextrose agar was from Himedia, SDA. Double distilled, de-ionised water was used throughout.

Methods**Preparation of miconazole-chitosan films**

Chitosan buccal films were prepared by casting method. Chitosan 2% w/w was dissolved in acetic acid solution 1% w/v at room temperature. The mixture was stirred using magnetic stirrer for 1 hr until viscous gel like solution was formed. Miconazole nitrate (20 mg) was then incorporated into the polymeric solution. The viscous solution was left overnight at room temperature to ensure clear,

bubble-free gel. Drug solubilizing agents: propylene glycol (PG), polyethylene glycol (PEG400), tween 20, and oleic acid (OA), were mixed with the prepared gels at different concentrations (w/w) of the total dry weight of chitosan. The bubble free liquid then spread on a clean dry glass plate in a dust free atmosphere and dried at 50°C for 24 hr. The dry films obtained were peeled off, cut into circles of 13 mm diameter, packed in aluminium foil and stored in a well closed container at room temperature until evaluation. Table 1 represents the prepared formulae of miconazole buccal films.

Evaluation of miconazole films

Weight uniformity

For determination of film weight uniformity, six films were weighed individually and the average weight was determined.

Film thickness

The thickness of the prepared films was determined by means of micrometer (Mitutoyo Co., Kanagawa, Japan). The thickness of six films was measured and the average thickness was determined.

Film surface pH

The surface pH values of the film formulations were determined to evaluate the possible irritation effects on the mucosa. The films were left to swell in 5 ml of distilled water (pH 6.8) in small beakers. The pH was measured at time intervals of 2, 4, and 6 h by placing the electrode in contact with the surface of the swollen films. The average pH of three determinations was reported.

Determination of the swelling index¹⁶

The swelling index of the prepared buccal films was determined by weighing five films and recording their weights before placing them separately in weighed beakers. The total weight was recorded (W1). Five milliliters of phosphate buffer (pH 6.8) was added to each beaker and then placed in an incubator at 37± 0.5 °C. After 6 h excess water was carefully removed, and the swollen films were weighed (W2). The experiment was repeated three times, and the average W1 and W2 were reported. The swelling index was determined from the formula:

$$\text{Swelling index \%} = \frac{W_2 - W_1}{W_1} \times 100$$

In vitro drug release study

MC release from the prepared buccal films was determined by introducing single film in modified Franz diffusion cell (3.14 cm² permeation area). The receptor compartment was filled with 17 mL of phosphate buffer (pH 6.8) containing 20% PEG-400, maintained at 37± 0.5 °C and continuously stirred at 50 rpm. Samples of 3 mL were withdrawn at predetermined time intervals of over 4 h and replaced with equal volumes of the dissolution medium equilibrated at the same temperature. Drug concentration of the withdrawn samples was analyzed after filtration (0.45 µm Millipore filter) by UV spectrophotometer at 272 nm (Cintra 5, GBC Scientific equipment, Australia). All experiments were carried out in triplicate. Sink conditions were maintained throughout the study. The release data were kinetically analyzed using different kinetic models to determine the mechanism of drug release from the different mucoadhesive systems.

In vitro microbiological study

a) Isolation and identification of *Candida albicans*

A strain of *Candida albicans* was isolated from infected patients (vaginal swab) in Dubai Specialized Medical Center and Medical Research Labs and identified using germ tube test (identification

test for *Candida albicans*). The isolated fungi were subcultured on sabouraud dextrose agar.

b) Preparation of sabouraud dextrose agar

Sabouraud dextrose agar medium was prepared by dissolving 65 gm of sabouraud dextrose powder in one liter of distilled water. Then it was heated to boiling and sterilized by autoclave for 15 minutes at 121°C and 15 p.s.i. The sterilized medium was poured in disposable petridishes and cooled for culturing of *Candida albicans*.

c) Microbiological effect of MC from the selected films using *Candida albicans*

The effectiveness of the selected formulae (F2) in comparison to F1 (without solubilizer) and the reference (miconazole oral gel 20 mg/g, Daktarin®, Janssen-Cilag, USA against *Candida albicans* was studied. The study was performed by placing 0.01 g of the reference Daktarin® gel which contains 0.2 mg of miconazole on the sabouraud dextrose agar previously cultured with *Candida albicans*. A 0.382 cm² of F1 and the selected formula F2 were placed on the same media. Samples were incubated at 37 °C for 48 hours. The zones of growth inhibition were measured as a mean ± SD, n=3.

Statistical analysis

The results were statistically analyzed by using t –test and given as a mean ±S.D. P values <0.05 were considered as significant.

RESULTS AND DISCUSSION

Film properties

MC-chitosan films were homogenous, clear and flexible [figure1]. The additions of enhancers to the prepared films increased their flexibility and enhanced their moisture uptake.

Also the prepared formulations provide an acceptable pH range [Table 2] that is compatible with normal buccal mucosa pH (6.78 ± 0.04) in healthy people¹⁷ consequently these films can be considered non irritant to the buccal cavity.

Maximum swelling capacity was obtained with films containing PG 10% (F2), as shown in [Table 2]. This may be due to the fact that, PG can absorb moisture from environment because of its humectants ability¹⁸ resulting in increase of film moisture uptake. The degree of swelling of the bioadhesive polymers is an important factor affecting film bioadhesion. The faster the swelling of the polymer is the faster the initiation of drug diffusion and formation of adhesive bonds resulting in faster initiation of bioadhesion.

Release study

The addition of solubilizers was notably increased MC release from film formulations in comparison to F1 (no solubilizer). In particular, propylene glycol 10% w/w significantly increases (P<0.05) MC in vitro release in comparison to other formulas and the reference miconazole oral gel (Daktarin®), as shown in figure 2. This phenomenon can be explained by the humectant nature of the chemical enhancer. The greater amount of water absorbed into the film by propylene glycol [Table 2] would contribute to the more rapid release of drug from the film. The reference was not able to modulate miconazole release because approximately 80% of the drug was delivered from the gel within only 30 min; the remaining part of the drug was delivered in about 4 h of dissolution. When miconazole was incorporated in chitosan-based films, the drug delivery was delayed because the percent released at 30 min was only 15–35%; after 4 h, F2 released 96% of miconazole, while other film formulations do not exceed 80%.



Fig. 1: Miconazole - chitosan film

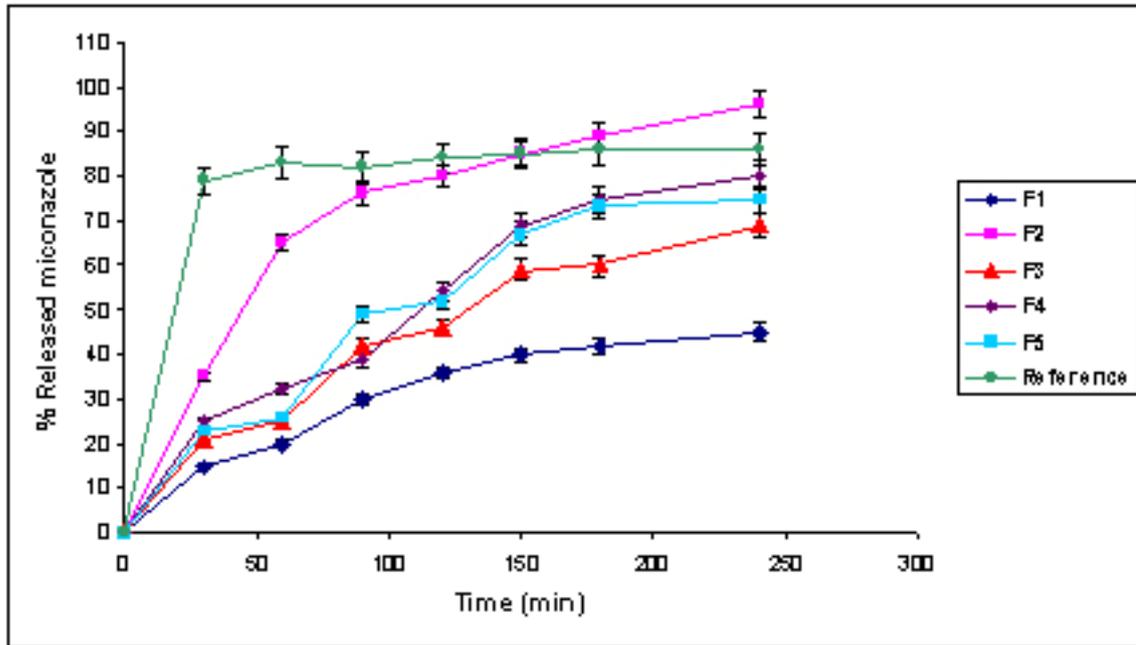


Fig. 2: In vitro release profiles (USP phosphate buffer, pH 6.8) of miconazole from buccal films (n=3)

Kinetic analysis of miconazole in vitro release data

As the regression analysis of the obtained results for two kinetic models including zero order and Higuchi's model showed that Higuchi's model gave the highest value of R² with significant difference (p < 0.05).

Higuchi's model, where the cumulative amount of the released drug per unit area is proportional to the square root of time, is more suitable model to describe the release kinetics of miconazole from the film preparations examined in the present study¹⁹.

$$Q = K t^{1/2}$$

Where; Q is amount of drug released per unit area of the matrix, t is time and K is release rate constant.

In vitro microbiological study

Results indicated that Candida albicans was more susceptible to the selected formulae (F2) as well as to the brand (Daktarin® oral gel, U.K). Inhibition growth zones of Candida albicans for all the samples were measured. Results indicated that, significant antifungal activity (p < 0.05) was observed for F2 in comparison with F1 and the reference [Table 3]. The results of the microbiological study confirm the considerable role of PG, solubilizing agent, in enhancement of MC release and antifungal activity.

Table 1: Composition of MC-chitosan buccal film formulations

Formulation code	Formulae contents					
	MC (mg)	Chitosan (% w/w)	PG (% w/w)	PEG ₄₀₀ (% w/w)	OA (% w/w)	Tween 20 (% w/w)
Blank	-	2.0	-	-	-	-
F1	20	2.0	-	-	-	-
F2	20	2.0	10.0	-	-	-
F3	20	2.0	-	3.0	-	-
F4	20	2.0	-	-	5.0	-
F5	20	2.0	-	-	-	6.0

Table 2: Thickness, weight, surface pH and swelling capacity of MC-chitosan films

Film code	Film thickness (mm) ± SD ^a	Film weight (mg) ± SD ^a	Film surface pH (mean ± SD ^b)	Swelling capacity (%)
Blank	0.11 ± 0.02	152.5 ± 4.9	6.16 ± 0.12	1.16%
F1	0.13 ± 0.01	172.5 ± 4.9	6.49 ± 0.12	1.18%
F2	0.18 ± 0.05	180 ± 7.07	6.62 ± 0.16	*32.1%
F3	0.23 ± 0.02	179 ± 14.5	6.53 ± 0.11	9.98%
F4	0.18 ± 0.02	188 ± 2.80	6.64 ± 0.19	6.69%
F5	0.17 ± 0.09	178 ± 18.5	6.61 ± 0.09	8.39%

* indicates significant effect (P < 0.05)

^a n=6; standard deviation for six determinations

^b n=3; standard deviation for three determinations

Table 3: Release rate constants, correlation coefficient (R²) and diameter of zone of inhibition of the film formulations

Film code	Release rate constant (%/min ^{-1/2})	R ²	Diameter of zone of inhibition (mm) (mean± SD ^a)
F1	3.065 ± 0.88	0.978	22.0 ± 3.2 mm
F2	*6.967 ± 1.38	0.975	*32.7 ± 0.9 mm
F3	4.377 ± 0.98	0.968	-
F4	5.102 ± 0.45	0.955	-
F5	4.969 ± 0.42	0.948	-
Reference	6.121 ± 0.69	0.921	21.7 ± 1.8 mm

^an =3; standard deviation for three determinations

* indicates significant effect (P < 0.05)

Table 3 indicates the release rate constants of MC from the prepared film formulations. The maximum release rate constant was obtained for F2 where PG 10% had been incorporated.

The amount of miconazole released from chitosan films, for all the tested time intervals, was found to be greater than the reported miconazole MIC (6.7mg/l) against *Candida albicans*²⁰.

CONCLUSION

Chitosan film formulation, MC 0.524 mg/cm², PG 10% w/w and chitosan 2% w/w, can be considered as a successful candidate for miconazole buccal film since it showed significant enhancement of miconazole in vitro release and antifungal activity against *Candida albicans* in comparison to the reference (Daktarin® oral gel).

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