AAPS PharmSciTech, Vol. 9, No. 4, December 2008 (© 2008)
DOI: 10.1208/s12249-008-9166-1

Research Article

Design and *In Vitro*/*In Vivo* Evaluation of Novel Mucoadhesive Buccal Discs of an Antifungal Drug: Relationship Between Swelling, Erosion, and Drug Release

Soad A. Yehia,[1] Omaima N. El-Gazayerly,[1] and Emad B. Basalious[1,2]

Received 4 May 2008; accepted 14 November 2008; published online 11 December 2008

Abstract. Two groups of fluconazole mucoadhesive buccal discs were prepared: (a) Fluconazole buccal discs prepared by direct compression containing bioadhesive polymers, namely, Carbopol 974p (Cp), sodium carboxymethyl cellulose (SCMC), or sodium alginate (SALG) in combination with hydroxypropyl methylcellulose (HPMC) or hydroxyethyl cellulose (HEC). (b) Fluconazole buccal discs prepared by freeze drying containing different polymer combinations (SCMC/HPMC, Cp/HPMC, SALG/HPMC, and chitosan/SALG). The prepared discs were evaluated by investigating their release pattern, swelling capacity, mucoadhesion properties, and *in vitro* adhesion time. *In vivo* evaluation of the buccal disc and *in vivo* residence times were also performed. Fluconazole salivary concentration after application of fluconazole buccal systems to four healthy volunteers was determined using microbiological assay and high-performance liquid chromatography. SCMC/HPMC buccal disc prepared by direct compression could be considered comparatively superior mucoadhesive disc regarding its *in vitro* adhesion time, *in vivo* residence time, and *in vitro*/*in vivo* release rates of the drug. Determination of the amount of drug released in saliva after application of the selected fluconazole disc confirmed the ability of the disc to deliver the drug over a period of approximately 5 h and to reduce side effects and possibility of drug interaction encountered during systemic therapy of fluconazole, which would be beneficial in the case of oral candidiasis.

KEY WORDS: buccal; fluconazole; mucoadhesive disc; oral candidiasis; residence time.

INTRODUCTION

Oral candidiasis is an opportunistic fungal infection caused by *Candida albicans*. These yeast infections are usually treated locally by application of gels or suspensions. Release of drugs from these preparations involves initial burst of activity whose level rapidly declines to subtherapeutic concentrations (1). Thus, systemic antifungals such as fluconazole are usually preferred for treating oral candidiasis. The oral dose of fluconazole for the treatment of oral candidiasis (100 mg/day for 1 or 2 weeks) results in notable side effects varying from headache, nausea to liver dysfunction, and hepatic failure. Furthermore, oral fluconazole is reported to interact with a number of medications, including oral hypoglycemics, coumarin-type anticoagulants, cyclosporins, terfenadine, theophylline, phenytoin, rifampin, and astemizole (2). The pathogenic yeasts in oral candidiasis are usually detected in the superficial layers of the oral mucosa. Thus, the effectiveness of the systemic fluconazole may be partially topical through its concentration in oral fluids (3). The reported topical efficacy of fluconazole together with the adverse effects and drug interaction of systemic fluconazole encouraged us to design a buccal disc containing a small dose of fluconazole to increase the contact between the drug and the pathogenic yeast for a long time.

Many conventional formulations such as mouth paints, rinses, troches, lozenges, or oral gels containing different antifungal agents are available for the treatment of oral candidiasis, but these formulations are incapable of maintaining the salivary concentration of drugs for a prolonged period of time due to the flow of saliva and swallowing, so it is a prime candidate for the development of mucoadhesive drug delivery systems, which adhere to the buccal mucosa and remain in place for a considerable period of time. This fact has stimulated researchers, both in academic and in industry, all over the world; so many authors reported (4–6) the development of mucoadhesive drug delivery systems for the local delivery of certain drugs to the buccal cavity for treatment of various diseases. In earlier works, we have designed and formulated mucoadhesive films for local administration of fluconazole in the oral cavity. However, buccal discs offer advantages over adhesive films in terms of stability and easier manufacture on a large scale (24).

The main objective of this work is to formulate a fluconazole mucoadhesive erodible buccal discs using two techniques (freeze drying and direct compression) and containing a small dose of fluconazole for topical treatment of oral candidiasis to ensure satisfactory fluconazole level in the mouth for prolonged duration of time and to reduce side effects and possibility of drug interaction encountered during systemic therapy of fluconazole. The rate of drug release from

[1] Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.
[2] To whom correspondence should be addressed. (e-mail: emad_a65@hotmail.com)

these mucoadhesive discs is explained by correlating the drug release with the swelling rate, erosion of the matrix, and the kinetic of drug release. The prepared formulations were evaluated through *in vitro* and *in vivo* testing of their adhesive and release properties.

MATERIALS AND METHODS

Materials

Fluconazole was kindly supplied by Alkan Pharma, Egypt. Hydroxypropyl methylcellulose (HPMC; Methocel K4M) was obtained from Tama, Tokyo, Japan. Carbopol 974P was purchased from BF. Goodrich, USA. Polycarbophil (Noveon AA-A) was purchased from Goodrich Chemicals, England. Chitosan (85% degree *N*-deacetylation), hydroxyethyl cellulose (HEC), ethyl cellulose (ethoxy content 49%), and carboxymethyl cellulose sodium salt (high viscosity) were purchased from Fluka Chemie GmbH CH-9471 Buchs. Sodium alginate, viscosity of 2% solution=3,500 cps, was purchased from Sigma Co. USA. Glacial acetic acid, potassium dihydrogen phosphate, and disodium hydrogen phosphate were provided by Merck, Darmstadt, Germany. Acetonitrile and methanol (high-performance liquid chromatography [HPLC] grade) were obtained from Sigma Chemical Company, USA). Sabouraud dextrose agar was composed of microbiological peptone 10 g, dextrose 40 g, agar 15 g, and distilled water to 1,000 mL. All other chemicals were of analytical grade and used as received.

Preparation of Fluconazole Buccal Disc

Preparation of Fluconazole Buccal Discs by Direct Compression

Discs were prepared by directly compressing 150 mg of finely powdered mixtures of bioadhesive polymers (Cp, SCMC, or SALG), fluconazole, and HPMC or HEC in the ratios given in Table I at a pressure of 5,000 kg for 15 s using the infrared hydraulic press (Shimadzu, Japan). The discs were prepared using the 13-mm diameter set.

Preparation of Fluconazole Buccal Discs by Freeze Drying

Four types of discs containing different polymer combinations (SCMC 1% *w*/*w*/HPMC 3% *w*/*w*, Cp 1% *w*/*w*/HPMC 3% *w*/*w*, SALG 1% *w*/*w*/HPMC 3%*w*/*w*, and chitosan 2%*w*/*w*/SALG 2%*w*/*w*) were prepared by freeze drying. The weighed quantities of polymers were mixed and gradually added to the required amount of water with constant stirring. For chitosan/SALG disc, the weighed quantity of chitosan was dissolved in the required amount of water containing 1% *v*/*v* acetic acid with stirring. Fluconazole (4% *w*/*w*) dissolved in hot propylene glycol (10% *w*/*w*) was incorporated in the polymeric solution. The medicated gel was left overnight at room temperature to ensure clear, bubble-free gel. The gel (0.5 g) was poured into plastic mold and freeze-dried (Savant Novalyphe-NL500, Savant VLP 200 Valupump, Savant Instrument Inc., Holbrook, NY, England). The formulated discs contained 20 mg fluconazole and had a diameter of 13 mm.

Evaluation of Fluconazole Mucoadhesive Buccal Discs

Disc Friability

A sample of ten whole discs was selected. The sample was accurately weighed and placed in the drum of tablet friability apparatus (digital test apparatus, Model DFI-1, Veego, Bombay, India). The samples underwent 25 rpm, for 4 min, and were then reweighed. This process was repeated for all formulations and the percentage friability was calculated using the following equation (7):

$$F = \frac{W_1 - W_2}{W_2} \times 100 \quad (1)$$

where F represents the percentage weight loss and W_1 and W_2 are the initial and final discs weights, respectively. If obviously cracked, cleaved, or broken discs are present in the disc sample after tumbling, the sample fails the test. A maximum weight loss of not more than 1% of the weight of the discs being tested is considered acceptable. This procedure was used to determine friability of discs prepared by direct compression.

Disc Thickness

The thickness of the buccal discs was determined using a vernier caliper (For-bro Engineers, Mumbai, India). The thickness of five discs was measured and the average thickness was determined.

Drug Content Uniformity

Five discs were dissolved in 50 mL phosphate buffer (pH 6.8), then filtered through cellulose acetate membrane (0.45 μm). The amount of drug was determined spectrophotometrically at λ_{max} 260.8.

Weight Uniformity

Five discs were randomly selected and accurately weighed using an electronic balance (Sartorius GmbH, Gottingen, Germany). The results are expressed as the mean values of five determinations.

In Vitro Release of Fluconazole from Different Buccal Discs

The release of fluconazole from the prepared bioadhesive discs into phosphate buffer pH 6.8 at 37±0.5°C was performed where each bioadhesive disc was adhered to the side wall of a vessel (100 mL beaker) using cyanoacrylate (8). Adequate sink conditions were provided by placing 50 mL of phosphate buffer pH 6.8 in each vessel. Each covered vessel was fitted with a magnetic stirrer rotating at a rate of approximately 100 rpm. Aliquots of 3 mL were withdrawn at different time intervals, filtered through cellulose acetate membrane (0.45 μm), and the content of fluconazole was determined spectrophotometrically at a wavelength of 260.8 nm, as mentioned before. At each time of withdrawal, 3 mL of fresh corresponding medium was replaced into the dissolution vessel. The release studies were conducted in

Table I. Composition of Fluconazole Buccal Discs Prepared by Direct Compression

		Polymer composition (mg)					Excipients (mg)	
Formulae	Fluconazole (mg)	Cp	SCMC	SALG	HPMC	HEC	PEG	Mannitol
F1	20	25	–	–	75	–	10	20
F2	20	50	–	–	50	–	10	20
F3	20	75	–	–	25	–	10	20
F4	20	25	–	–	–	75	10	20
F5	20	50	–	–	–	50	10	20
F6	20	75	–	–	–	25	10	20
F7	20	–	25	–	75	–	10	20
F8	20	–	50	–	50	–	10	20
F9	20	–	75	–	25	–	10	20
F10	20	–	–	25	75	–	10	20
F11	20	–	–	50	50	–	10	20
F12	20	–	–	75	25	–	10	20
F13	20	20	–	–	60	–	30	20
F14	20	40	–	–	40	–	30	20
F15	20	60	–	–	20	–	30	20
F16	20	20	–	–	–	60	30	20
F17	20	40	–	–	–	40	30	20
F18	20	60	–	–	–	20	30	20
F19	20	–	20	–	60	–	30	20
F20	20	–	40	–	40	–	30	20
F21	20	–	60	–	20	–	30	20
F22	20	–	–	20	60	–	30	20
F23	20	–	–	40	40	–	30	20
F24	20	–	–	60	20	–	30	20

triplicates and the mean values were plotted *versus* time. The absorbance of the polymeric additives was negligible and did not interfere with λ_{max} of the drug.

The effect of drug/polymer ratio (1:4 and 1:5) and polymer/polymer ratio (1:3, 1:1 and 3:1) on the fluconazole release properties from the buccal discs prepared by direct compression and had different polymer combinations (Cp/HPMC, Cp/HEC, SCMC/HPMC, and SALG/HPMC) was studied through full factorial design using the computer software StatView 4.57 followed by post hoc multiple comparisons using Fisher's PLSD test. Differences between formulations were considered to be significant at $p<0.05$. The design of the experiment is shown in Table II.

Kinetic Analysis of the In Vitro Release Data

The release data were kinetically analyzed using the Korsmeyer–Peppas model. The release exponent (n) describing the mechanism of drug release from the matrices was calculated by regression analysis using Eq. 2 (9):

$$M_t/M_\infty = Kt^n \tag{2}$$

where $M/M\infty$ is the fraction of drug released (using values of $M/M\infty$ within the range 0.10–0.60) at time t and K is a constant incorporating the structural and geometric characteristics of the release device. A value of $n=0.45$ indicates case I (Fickian) diffusion, $0.45<n<0.89$ indicates anomalous (non-Fickian) diffusion, and $n=0.89$ indicates case II transport.

In order to characterize drug release, the mean dissolution time (MDT) was calculated according to Eq. 3 (10) using the n and K values derived from Eq. 2:

$$MDT = \left(\frac{n}{n+1}\right)K^{-\left(\frac{1}{n}\right)}. \tag{3}$$

Furthermore, the contribution of Fickian (diffusional) release and the case II erosional release over the first 60% of

Table II. Full Factorial Experimental Design to Study the Effect of Polymer/Polymer Ratio and Drug/Polymer Ratio on the Release and Mucoadhesion Properties of Buccal Discs Containing Different Polymer Combinations

		Drug/polymer ratio	
Polymer combination	Polymer/polymer ratio	1:5	1:4
Cp/HPMC	1:3	F1	F13
	1:1	F2	F14
	3:1	F3	F15
Cp/HEC	1:3	F4	F16
	1:1	F5	F17
	3:1	F6	F18
SCMC/HPMC	1:3	F7	F19
	1:1	F8	F20
	3:1	F9	F21
SALG/HPMC	1:3	F10	F22
	1:1	F11	F23
	3:1	F12	F24

the release curves can be quantified according to the heuristic model (Eq. 4) (11):

$$Mt/M\infty = K_1 t^m + K_2 t^{2m} \quad (4)$$

where the first term of the right-hand side is the Fickian contribution and the second term being the case II erosional contribution. The coefficient m is the purely Fickian diffusion exponent for a device of any geometrical shape which exhibits controlled release. The Fickian kinetic constant (k_1) and the relaxational/erosional kinetic constant (k_2) could be used to calculate the Fickian release fraction (F) according to Eq. 5 (12):

$$F = \frac{1}{1 + (K_2/K_1)t^m}. \quad (5)$$

Determination of the Swelling Index of the Fluconazole Buccal Discs in Distilled Water

The discs were coated on the lower side with ethyl cellulose (to avoid sticking to the dish), then weighed (W_1), and placed separately in Petri dishes containing 25 mL of distilled water. The dishes were stored at room temperature. After specified time intervals, the discs were removed and the excess water on their surface was carefully removed using filter paper. The swollen discs were weighed (W_2) and the percentage of swelling was calculated using Eq. 6 (13):

$$Swelling\ index = \frac{W_2 - W_1}{W_1} \times 100. \quad (6)$$

This procedure was used to evaluate the swelling percent of the buccal discs prepared by freeze drying (after 15, 30, 45, 60, 75, 90, 105, 120, 135, and 150 min) and the discs prepared by direct compression (after 30, 60, 90, 120, 150, 160, 210, and 240 min).

The kinetics of the swelling was calculated according to Eq. 7:

$$M_t = kt^n \quad (7)$$

where M_t represents the amount of liquid transferred at time t and k is the swelling constant which depends on the amount of liquid transferred after infinite time, the porosity of matrix, and diffusivity. The exponent n indicates the mechanism of swelling (14).

Determination of in vitro Adhesion Time of Fluconazole Buccal Discs

The *in vitro* adhesion time of fluconazole discs was evaluated by assessing the time for these discs to detach from a chicken pouch membrane in a well-stirred beaker (8,15). The chicken pouch membranes were fixed on the side of the beaker with cyanoacrylate glue. The films were attached to the membrane by applying light force with finger tip for 60 s. The beaker was then filled with 500 mL phosphate buffer pH 6.8 at 37°C and magnetically stirred at an approximate rate of 150 rpm to simulate buccal and saliva movement (15). The time necessary for complete erosion or detachment of the discs from the chicken pouch membrane was taken as an indication of the *in vitro* adhesion time.

In Vivo Evaluation of Mucoadhesive Performance of Fluconazole Buccal Discs

The adhesion properties of buccal discs were tested in three healthy volunteers aged 28–32 years. The volunteers were instructed to press the discs against the gingival mucosa above the canine tooth for 60 s (16). The discs were observed for 8 h. Then, the discs which remained in the buccal cavity were removed and dissolved in 50 mL phosphate buffer pH 6.8. Three milliliter sample was withdrawn, filtered through cellulose acetate membrane of 0.45 μm pore size, and assayed spectrophotometrically at 260.8 nm for the amount of drug remaining in the discs.

The volunteers were asked to record the residence time (time of complete erosion or detachment of the disc from the buccal mucus membrane) and to monitor for fragment loss, irritation, bad taste, swelling, dry mouth, or increase in salivary flow.

In Vivo Evaluation of the Selected Fluconazole Buccal Discs

Based on the *in vitro* evaluation testing, the prepared discs were selected for *in vivo* evaluation by applying the discs to four healthy volunteers, two males and two females. The study was approved by the Cairo University Protection of Human Subjects Committee and the protocol complies with the declarations of Helsinki and Tokyo for humans. The volunteers were instructed to brush their teeth and place the disc on the buccal mucosa between the cheek and gingival in the region of the upper canine with slight pressure for 60 s. The volunteers were not allowed to drink water or to eat food for half an hour before the study. Drinking was allowed *ad libitum* starting from 30 min after application of the disc and fasting was strictly observed throughout the experiment. However, no drinking was allowed for 10 min before the collection of salivary samples (17). The volunteers were instructed not to touch the disc with the tongue for 10 min before collection of samples to avoid abnormally high drug levels (5). Blank saliva samples were taken before disc application. At fixed time intervals, saliva samples were collected, centrifuged at 3,000 rpm for 15 s (Remi Laboratory Centrifuge R32 A, Bombay, India), and the supernatant was kept frozen until analysis, using microbiological or HPLC methods.

Microbiological Analysis of Fluconazole from the Selected Discs Using Candida albicans. Sterile melted sabouraud dextrose agar was cooled to 45°C and inoculated with 20 μL of adjusted *Candida albicans* suspension (10^5 cfu/mL) per 20 mL agar. The inoculated agar was mixed well, poured into sterile Petri dishes, and left to solidify. Before use, the cooled plates were dried at 37°C for 10 min to remove excess surface moisture. Cups of 10 mm in diameter were produced in the inoculated agar with a cork borer, then the collected saliva samples were placed in the cups produced in the inoculated sabouraud dextrose agar. The plates were incubated at 37°C for 18 h. Inhibition zones of the fungal growth surrounding the cups were measured in two diameters and the

concentration of fluconazole in saliva samples was computed through a standard calibration curve obtained by plotting log concentration of the standards (in micrograms per milliliter) against the average zone diameter (in millimeters) and joining these points with the best-fit straight line.

The following parameters were calculated from the measured salivary fluconazole concentration:

C_{max}	the peak salivary concentration
T_{max}	the time of the peak salivary concentration
$T^{>MIC}$	the time period above the minimum inhibitory concentration (MIC) value for fluconazole against Candida albicans used (6 μg/mL)
AUC_{0-t}	the area under the salivary concentration time curve up to the last measured time point (t) and calculated by trapezoidal rule

HPLC Analysis of Fluconazole from the Selected Discs. A modified HPLC method of Koks *et al.* (18) for the determination of fluconazole in saliva was used. The method involved precipitation of saliva protein by addition of 0.5 mL acetonitrile. After vortexing for 30 s and centrifugation for 10 min, at 6,000 rpm, the upper layer was transferred to another tube, filtered through 0.45 μm cellulose acetate membrane. Fifty microliters were injected into the HPLC column (XTerra RP18 5 μm, 4.6×250 mm, Ireland) for analysis using mobile phase composed of acetate buffer: methanol (70:30% *v*/*v*). The acetate buffer was adjusted to pH 5 with glacial acetic acid. The mobile phase flow rate was 1.5 mL/min and the detection wavelength was 261 nm.

RESULTS AND DISCUSSION

Physical Characterization of Fluconazole Mucoadhesive Buccal Discs

Friability test was applied only to fluconazole buccal discs prepared by direct compression. Several preliminary experiments were done to prepare fluconazole buccal discs

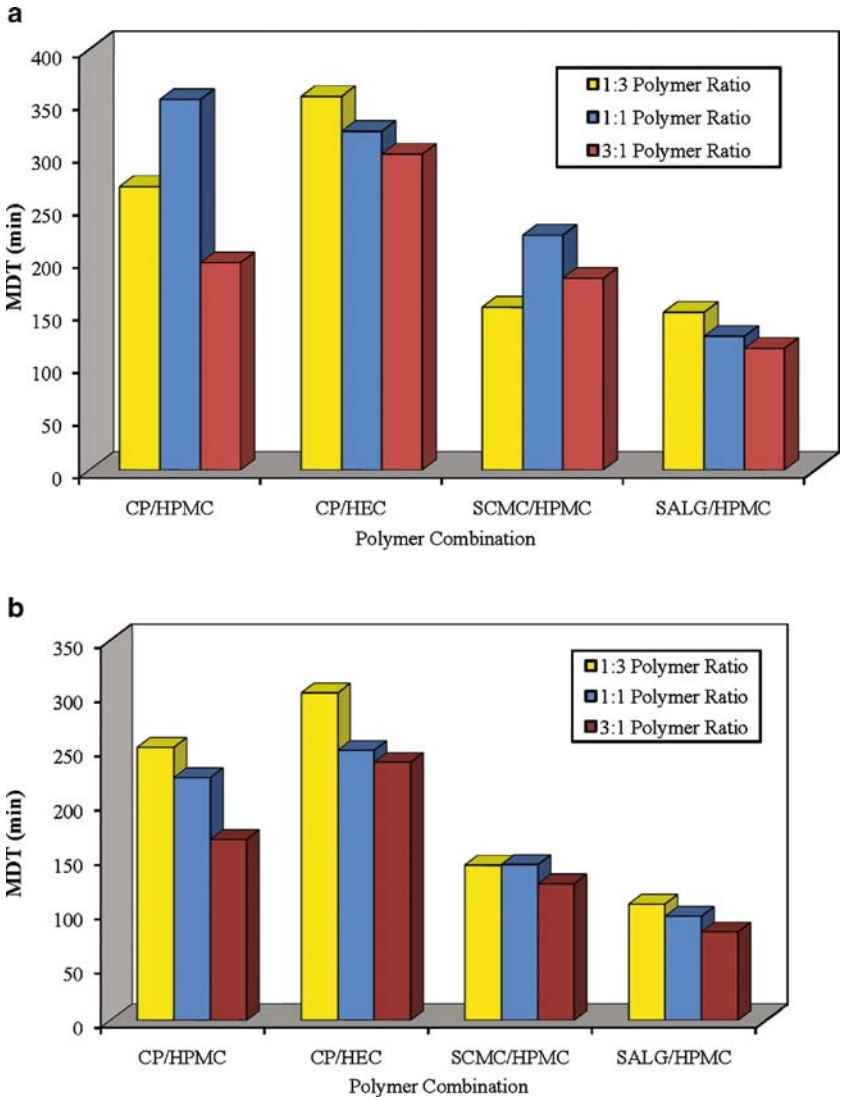

Fig. 1. MDT of fluconazole to be released from buccal discs containing different polymer combinations and different polymer/polymer ratios. **a** 1:5 drug/polymer ratio, **b** 1:4 drug/polymer ratio

containing several combinations of two polymers. Cp/HPMC, Cp/HEC, SCMC/HPMC, SCMC/HEC, SALG/HPMC, and SALG/HEC were used to prepare buccal discs in ratios of 1:3, 1:1, and 3:1. The friability test was conducted for all prepared formulae. All discs showed friability values well below the 1% tolerance limit set by the British Pharmacopoeia for pharmaceutical tablets (19) except for the discs prepared with SCMC/HEC and SALG/HEC in polymer ratios of 1:3, 1:1, and 3:1. These formulae, having friability values above the 1% tolerance limit, were excluded.

Disc thickness ranges of the discs prepared by direct compression and discs prepared by freeze drying were 1.85–1.95 and 3.75–3.89 mm, respectively.

Disc weight ranges of the discs prepared by direct compression and discs prepared by freeze drying were 147–152 and 83–91 mg, respectively.

The fluconazole content was determined for each buccal disc. It was found that the fluconazole content in all buccal discs was in the range from 19.2 to 21.2 mg.

In Vitro Release of Fluconazole from Different Discs

In Vitro Release of Fluconazole from Discs Prepared by Direct Compression

The release of fluconazole from the different hydrophilic polymer combinations prepared by direct compression (Cp/HPMC, Cp/HEC, SCMC/HPMC, and SALG/HPMC in ratios of 1:3, 1:1, and 3:1) and containing drug/polymer in ratios of 1:5 or 1:4 was studied. Excipients like polyethylene glycol (PEG 6000) and mannitol were used to develop an erodible buccal disc to ensure drug release in the mouth. It was reported that PEG 6000 could increase the release of drugs from the matrix and that mannitol had a sweet taste, a good mouth feel, negative heat of solution, and dissolution-enhancing properties (16).

The effect of drug/polymer ratio and polymer/polymer ratio on the fluconazole release properties from the buccal discs prepared by direct compression with different polymer combinations (Cp/HPMC, Cp/HEC, SCMC/HPMC and SALG/HPMC) was studied through full factorial design. The design of the experiment is shown in Table II. The MDT of the drug to be released has been suggested as the most reasonable parameter to explain the effect of formulation variables on the release behavior (20).

Statistical analysis of the results revealed that although there is a significant difference ($p<0.05$) between the different levels of the factors: polymer combination, polymer/polymer ratio, and drug/polymer ratio; no significant difference ($p>0.05$) was obtained between two of the tested levels of polymer/polymer ratio (1:3 and 1:1) (results are not shown).

As shown in Fig. 1a,b, the values of MDT are in the order of Cp/HEC>Cp/HPMC>SCMC/HPMC>SALG/HPMC. Thus, the highest fluconazole release rate was obtained from buccal discs formulated from the SALG/HPMC polymer combination.

For most of the tested formulations, the values of n were >0.45 and <0.89, indicating anomalous (non-Fickian) diffusion where drug release is controlled by a combination of diffusion

Table III. Swelling Kinetic Parameters and Kinetic Analysis of the Release Data of Fluconazole From Discs Prepared by Direct Compression

Formulae	K	n	R^2	K_1	K_2	Main transport mechanism	Swelling exponent (n)	Kinetic constant (k)	R^2	Swelling rate (%/min$^{1/2}$)	R^2
1	0.0142	0.6519	0.9899	0.0243	0.0005	Fickian	0.3266	39.28	0.9498	15.40	0.9508
2	0.0263	0.5249	0.9809	0.0264	0.0002	Fickian	0.2250	63.40	0.9908	17.16	0.9411
3	0.0383	0.5125	0.9332	0.0253	0.0013	Fickian	0.2480	63.62	0.9407	21.09	0.9642
4	0.0199	0.5687	0.9837	0.0210	0.0005	Fickian	0.3949	50.54	0.9497	30.55	0.9847
5	0.0256	0.5369	0.9588	0.0214	0.0007	Fickian	0.3431	58.36	0.9867	27.14	0.9832
6	0.0254	0.5443	0.9854	0.0212	0.0008	Fickian	0.1992	98.53	0.9716	23.84	0.9319
7	0.0097	0.7911	0.9926	0.0237	0.0014	Fickian	0.2644	83.71	0.9896	27.30	0.9556
8	0.0056	0.8370	0.9865	0.0061	0.0020	Fickian	0.4102	53.01	0.9713	34.49	0.9853
9	0.0100	0.7619	0.9843	0.0131	0.0020	Fickian	0.2609	122.44	0.9802	40.46	0.9660
10	0.0156	0.7061	0.9934	0.0202	0.0019	Fickian	0.3324	35.11	0.9726	15.74	0.9716
11	0.0118	0.7833	0.9973	0.0207	0.0022	Fickian	0.2406	62.08	0.9903	18.04	0.9438
12	0.0102	0.8272	0.9971	0.0139	0.0032	Fickian	0.1636	95.01	0.9739	19.26	0.9075
13	0.0294	0.5359	0.9928	0.0312	0.0003	Fickian	0.5285	18.56	0.9814	21.39	0.9893
14	0.0349	0.5172	0.9840	0.0227	0.0013	Fickian	0.4144	25.56	0.9686	15.63	0.9780
15	0.0157	0.6910	0.9428	0.0207	0.0016	Fickian	0.4833	19.49	0.9976	17.92	0.9992
16	0.0335	0.4987	0.9862	0.0262	0.0005	Fickian	0.5230	31.13	0.9732	34.36	0.9894
17	0.0146	0.6560	0.9817	0.0186	0.0010	Fickian	0.4483	40.69	0.9793	30.51	0.9909
18	0.0187	0.6152	0.9831	0.0264	0.0006	Fickian	0.3160	61.11	0.9900	22.18	0.9650
19	0.0145	0.7263	0.9824	0.0173	0.0023	Fickian	0.2906	93.88	0.9541	35.00	0.9618
20	0.0114	0.7716	0.9820	0.0140	0.0025	Fickian	0.2533	160.77	0.9731	54.25	0.9610
21	0.0110	0.7986	0.9490	0.0227	0.0021	Fickian	0.2321	187.56	0.9307	57.63	0.9510
22	0.0152	0.7591	0.9884	0.0223	0.0028	Fickian	0.4491	34.34	0.9273	29.06	0.9952
23	0.0064	0.9568	0.9948	0.0212	0.0030	Fickian	0.1525	160.57	0.9967	34.90	0.9424
24	0.0129	0.8389	0.9766	0.0205	0.0041	Fickian	0.1344	177.17	0.9405	36.24	0.9290

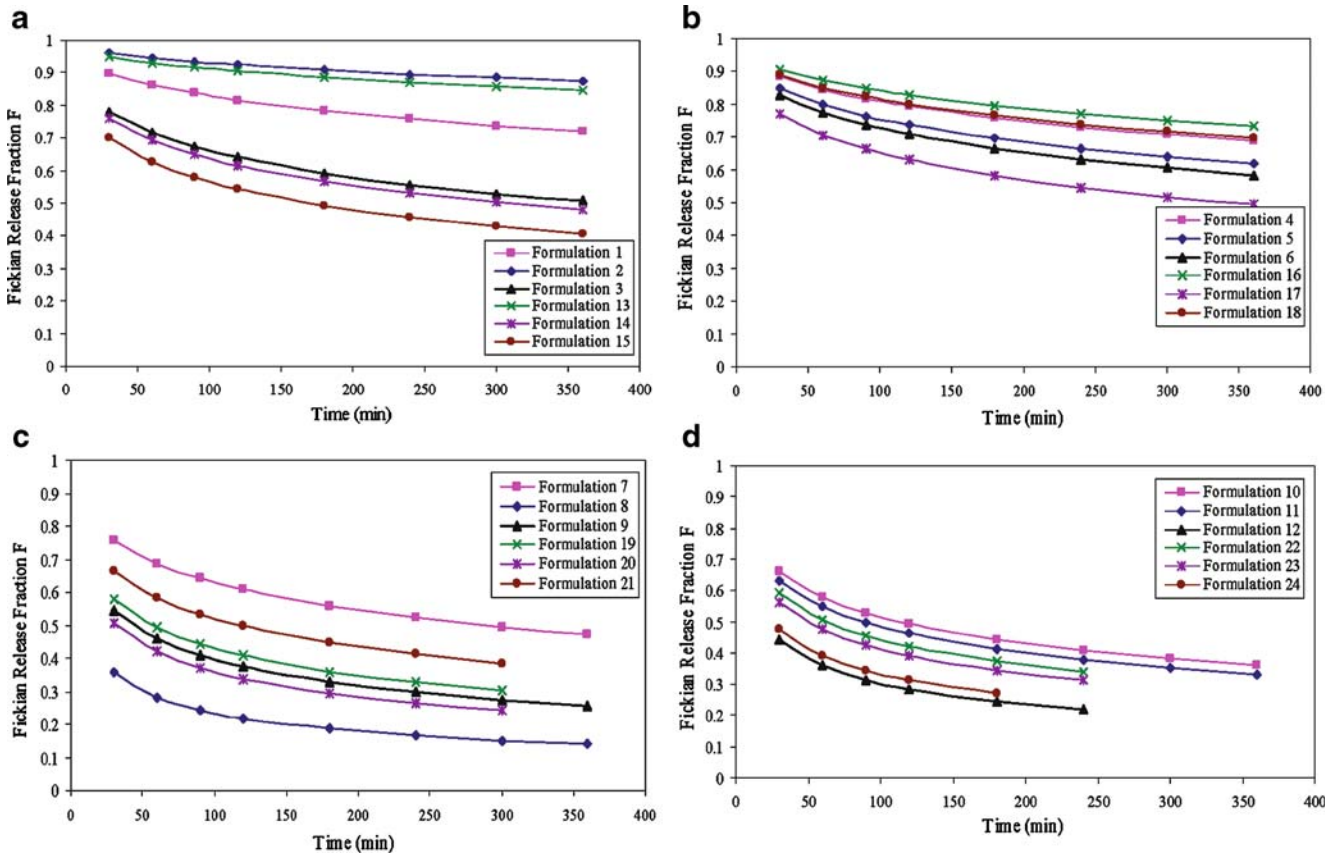

Fig. 2. The Fickian release fractions obtained from fluconazole: **a** Cp/HMPC, **b** Cp/HEC, **c** SCMC/HPMC, and **d** SALG/HPMC buccal discs prepared by direct compression

and polymer relaxation. The last finding was verified by the larger values of k_1 which indicated that the mechanism of drug release was mainly controlled by drug diffusion (Table III). The swelling exponents (n) for all discs were ≤0.5, indicating a diffusion-controlled swelling in which the rate of diffusion of the liquid was much less compared with the rate of relaxation of the polymer segment (Table III).

The Mechanism of Fluconazole Release from Cp/HPMC Buccal Discs. Figure 2a shows the fraction contribution of Fickian diffusion of fluconazole release from Cp/HPMC buccal discs. It is clear that, in the case of formulations 3, 14, and 15, Fickian diffusion predominated for the first release period followed by gradual polymer relaxation. However, with the other formulations (1, 2, and 13), the Fickian contribution was dominant for the entire release time period.

For Cp/HPMC having a drug/polymer ratio of 1:5, formula 3 had the highest swelling rate, (21.09%/min$^{1/2}$) followed by formula 2 (17.16%/min$^{1/2}$), and finally, formula 1 (15.4%/min$^{1/2}$). Thus, formula 3 had the ability to hydrate more rapidly than the other two formulae. The resulting drug diffusional path length for formula 3 was, therefore, the longest. It would follow that the drug release rate from formula 3 would be the slowest. However, the drug dissolution rates (MDT) showed that formula 3 had the highest drug release rate (Fig. 1). It is obvious, therefore, that Fickian drug diffusion through the gradually expanding hydrated matrix with increasing diffusional path length was not the only mechanism accounting for drug release. The fact that formula 3 had the fastest swelling rate but did not yield the slowest release rate could be explained by polymer relaxation/erosion. Erosion increased the drug release rate, thus compensating, to some extent, for the high swelling capacity and the consequent slowing of drug diffusion by the

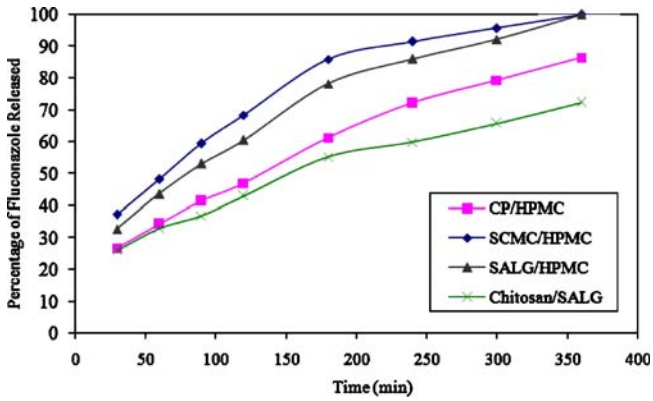

Fig. 3. Release profiles of fluconazole from the buccal discs prepared by freeze drying

Table IV. Swelling Kinetic Parameters and Kinetic Analysis of the Release Data of Fluconazole From Discs Prepared by Freeze Drying

Formulae	K	n	R^2	K_1	K_2	Main transport mechanism	Swelling exponent (n)	Kinetic constant (k)	R^2	Swelling rate (%/min$^{1/2}$)	R^2
Chitosan/SALG	0.0611	0.4136	0.9801	0.0445	−0.0003	Fickian	0.3524	37.32	0.9486	12.86	0.9670
SCMC/HPMC	0.0828	0.4381	0.9936	0.0749	−0.0011	Fickian	0.2205	83.48	0.9801	17.86	0.9332
SALG/HPMC	0.0714	0.4453	0.9988	0.0613	−0.0004	Fickian	0.2649	50.01	0.9910	12.66	0.9468
Cp/HPMC	0.0548	0.4550	0.9842	0.0436	0.0001	Fickian	0.0564	1776.20	0.9778	204.79	0.8199

increasing diffusional path length (20). This is consistent with kinetic analysis of the release data which showed that drug release from formula 3 was initiated by Fickian diffusion followed by gradual polymer relaxation.

For Cp/HPMC having a drug/polymer ratio of 1:4, formula 13 had the highest swelling rate (21.39%/min$^{1/2}$), followed by formula 15 (17.92%/min$^{1/2}$), and finally, formula 14 (15.63%/min$^{1/2}$). Thus, formula 13 had the ability to swell more rapidly than the other two formulae, resulting in long drug diffusional path length and the consequent reduction of drug release rate. This is in agreement with kinetic analysis of the release data which showed that, for drug release from formula 13, the diffusional contribution was dominant for the entire release time period.

The Mechanism of Fluconazole Release from Cp/HEC Buccal Discs. As shown in Table III, formulae 16 had the highest swelling rate (34.36%/min$^{1/2}$), followed by formulae 17, 4, and 5 (30.51, 30.55, and 27.14%/min$^{1/2}$, respectively), and finally, formulae 6 and 18 (23.22 and 22.18%/min$^{1/2}$, respectively). Thus, the swelling percent of Cp/HEC discs increased with increasing content of HEC relative to Carbopol in the discs. It was reported that HEC matrices formed a viscous gel layer immediately after coming in contact with the release medium and this gel layer was durable and resistant to erosion (21). This is consistent with kinetic analysis of the release data which showed that, for drug release from Cp/HEC discs, the diffusional contribution was dominant for nearly the entire release time period (Fig. 2b).

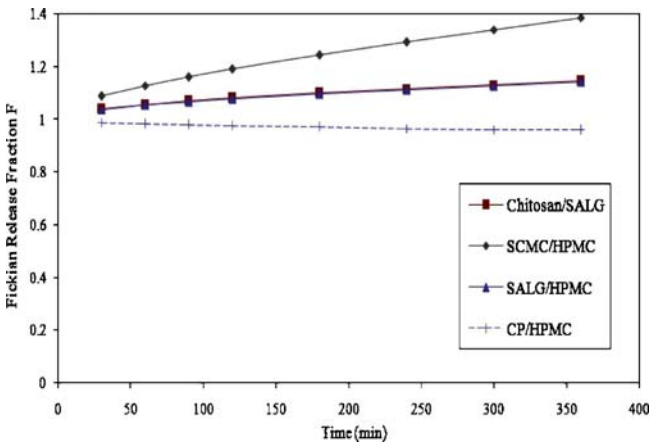

Fig. 4. The Fickian release fractions obtained from fluconazole buccal discs prepared by freeze drying

The Mechanism of Fluconazole Release from SCMC/HPMC and SALG/HPMC Buccal Discs. The swelling of SCMC/HPMC and SALG/HPMC discs increased with increasing SCMC or SALG content relative to HPMC in the discs (Table III). However, the MDT (Fig. 1) showed that the drug release rate increased with increasing SCMC or SALG content in the formulations of these discs. It is obvious, therefore, that Fickian drug diffusion through the gradually expanding hydrated matrix with increasing diffusional path length was not the only mechanism accounting for drug release. Erosion increased the drug release rate, thus compensating, to some extent, for the high swelling capacity and the consequent slowing of drug diffusion by the increasing diffusional path length (20). It was reported that SCMC matrices were usually fragmented in water giving stable colloidal dispersion due to their hydrophilic and fast hydration properties (22). This is consistent with kinetic

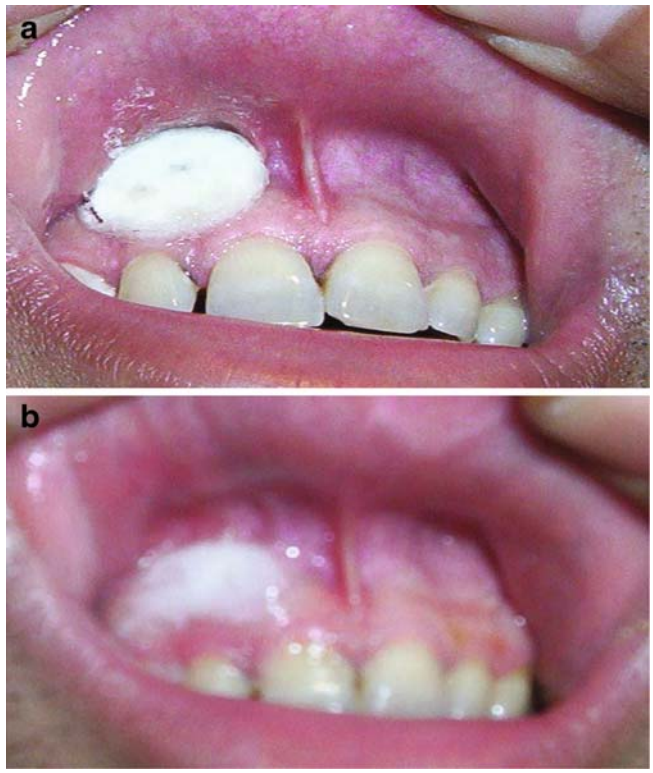

Fig. 5. *In vivo* mucoadhesion behavior of the selected SCMC/HPMC buccal disc **a** just applied and **b** after 4 h

Table V. *In Vivo* Evaluation of Fluconazole Mucoadhesive Buccal Discs Prepared by Direct Compression and by Freeze Drying

Polymer combination	Formula	*In vivo* residence time (h±SD)	Percent release after 8 h (%±SD)	Formula	*In vivo* residence time (h±SD)	Percent release after 5 h (%±SD)
SCMC/HPMC	F7	>8	15.5±5.96	F19	6.5±0.70	87.3±3.18
	F8	>8	25.8±10.08	F20	5.2±0.35	Complete release
	F9	>8	26.2±4.24	F21	4.75±0.35	Complete release
SALG/HPMC	F10	>8	42.8±4.17	F22	5.5±0.70	Complete release
	F11	>8	53.1±5.44	F23	4.75±0.70	Complete release
	F12	>8	49.5±8.34	F24	4.25±0.35	Complete release
Discs prepared by freeze drying						
SCMC/HPMC		>8	94.3			
SALG/HPMC		>8	82.5			

analysis of the release data which showed that drug release from all formulation of these discs was initiated by Fickian diffusion followed by polymer relaxation except formula 7 in which Fickian diffusion predominated for nearly all the release period (Fig. 2c,d).

In Vitro Release of Fluconazole from Discs Prepared by Freeze Drying

As shown in Fig. 3, the values of the percentage released of fluconazole from Cp/HPMC, SCMC/HPMC, SALG/HPMC, and chitosan/SALG discs were 86.47%, 100%, 100%, and 72.32%, respectively, after 360 min.

All these systems were characterized by the initial burst release of the drug where 37.29%, 32.72%, 26.69%, and 26.02% of fluconazole were released from SCMC/HPMC, SALG/HPMC, Cp/HPMC, and chitosan/SALG discs, respectively. This could be due to the high rate of liquid penetration and the rapid gelation of the freeze-dried polymers. Nagai and Konishi (23) reported that the rate of gelation of freeze-dried HPC/Cp was faster than of physical mixture HPC/Cp and that the viscosity of the gel layer of freeze-dried HPC/Cp is decreased because of the dispersion of Cp in small particles by freeze drying.

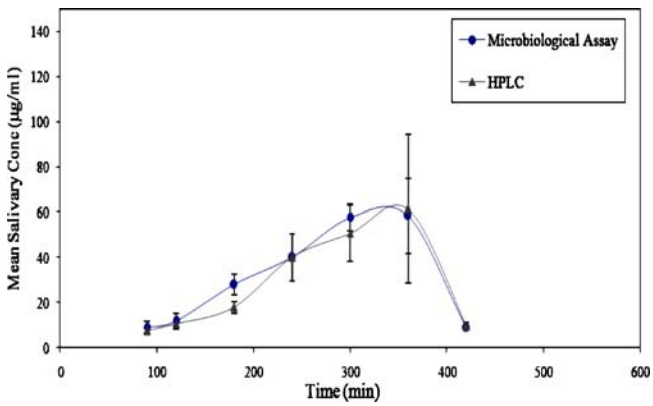

Fig. 6. Mean salivary concentrations of fluconazole following the application of its buccal disc of formula 19 using microbiological assay and HPLC

The Mechanism of Fluconazole Release from Discs Prepared by Freeze Drying

As shown in Table IV, the values of n were around 0.45, indicating Fickian diffusion. SCMC/HPMC, SALG/HPMC, and Cp/HPMC discs were characterized by smaller swelling rates (17.86, 12.66, and 12.86%/min$^{1/2}$, respectively) compared to the same discs prepared by direct compression. The low swelling index and the consequent short diffusional path length of these discs account for the high diffusion coefficient of the drug and the relatively high release rate. The high swelling rate of chitosan/SALG (204.79%/min$^{1/2}$) and the consequent long diffusional path length account for the low diffusion coefficient of the drug and the low release rate. This is in agreement with the kinetic analysis of the *in vitro* release data which showed that the drug release from these formulations followed pure Fickian diffusion for the entire release period (Fig. 4).

In Vitro Adhesion Time of Fluconazole Mucoadhesive Buccal Discs

In Vitro Adhesion Time of Fluconazole Mucoadhesive Buccal Discs Prepared by Direct Compression

Cp/HPMC and Cp/HEC buccal discs showed the longest adhesion time (>12 h). SCMC/HPMC and SALG/HPMC buccal discs showed short adhesion time ranging from 3.5±0.70 h for formula (F24) to 9.5±0.70 h for formula (F7). The results revealed that decreasing the drug polymer ratio from

Table VI. *In Vivo* Parameters of Fluconazole Following the Application of the Selected SCMC/HPMC Mucoadhesive Buccal Films Using Microbiological Assay and HPLC

In vivo parameters	Microbiological assay (±SE)	HPLC (±SE)
C_{max} (µg/mL)	72.70 (±11.23)	83.14 (±26.85)
T_{max} (min)	330.00 (±17.32)	330.00 (±17.32)
$T^{>MIC}$ (min)	307.50 (±15.00)	330.00 (±0.00)
$AUC_{0-t\ min}$ (µg/h mL)	201.93 (±27.25)	187.24 (±43.64)

1:5 to 1:4 remarkably decreased the *in vitro* adhesion times for SCMC/HPMC and SALG/HPMC buccal discs.

In Vitro Adhesion Time of Fluconazole Mucoadhesive Buccal Discs Prepared by Freeze Drying

The *in vitro* adhesion times of SALG/HPMC, SCMC/HPMC, Cp/HPMC, and chitosan/SALG mucoadhesive discs prepared by freeze drying were 8, 10, >12, and >12 h, respectively.

In Vivo Residence Time of Fluconazole Mucoadhesive Buccal Discs

In Vivo Residence Time of Fluconazole Mucoadhesive Buccal Discs Prepared by Direct Compression

The study of *in vivo* residence time in three healthy volunteers was done only for discs having *in vitro* adhesion time ≤10 h; SALG/HPMC and SCMC/HPMC buccal discs prepared by direct compression and freeze drying. Figure 5 shows the *in vivo* mucoadhesion behavior of the SCMC/HPMC disc. It is clear that the mucoadhesive discs were readily retained on the buccal mucosa. All discs eroded completely without observing any signs of local irritation. Table V shows the *in vivo* residence time of the buccal discs prepared by direct compression and freeze drying. It is obvious that the formulae having drug/polymer ratios of 1:5 showed greater residence time (>8 h) than formulae having drug/polymer ratios of 1:4 (4.25–6.5 h). The formulae having drug/polymer ratios 1:5 showed the lowest percent drug released after 8 h (15.5% for F7 to 53.1% for F11). However, the formulae having drug/polymer ratios of 1:4 showed higher percent drug released after 5 h (87.3% for F19 and complete drug release for F20–F24).

In Vivo Residence Time of Fluconazole Mucoadhesive Buccal Discs Prepared by Freeze Drying

The buccal discs prepared by freeze drying (SALG/HPMC and SCMC/HPMC) had high *in vivo* residence time (>8 h). The percent drug released after 8 h for SALG/HPMC and SCMC/HPMC buccal discs were 94.3% and 82.5%, respectively.

SCMC/HPMC buccal disc prepared by direct compression (formulation 19) could be considered a comparatively superior mucoadhesive disc regarding its *in vitro* adhesion time, *in vivo* residence time, and *in vitro*/*in vivo* release rates.

In Vivo Evaluation of the Selected Fluconazole Mucoadhesive Buccal Discs

Figure 6 shows the mean salivary concentration of fluconazole following the application of its mucoadhesive buccal disc (formulation 19) to four volunteers using microbiological assay and HPLC. Fluconazole concentrations in saliva samples were analyzed using HPLC to validate the microbiological assay. The high correlation coefficient (r= 0.9819) revealed that both assays were well-correlated. Table VI shows the *in vivo* parameters of fluconazole from the selected buccal disc. Analysis of variance test revealed a significant difference ($p<0.05$) between $T^{>MIC}$ obtained using microbiological assay and HPLC. However, there was no significant difference between T_{max}, C_{max}, and AUC obtained using microbiological assay and HPLC ($p>0.05$).

CONCLUSIONS

Mucoadhesive erodible buccal discs containing a small dose of fluconazole could be satisfactory to ensure optimum fluconazole levels in the mouth cavity for prolonged duration of time (>300 min). The use of fluconazole mucoadhesive discs would be beneficial for topical treatment of oral candidiasis.

REFERENCES

1. R. Khanna, S. P. Agarwal, and A. Ahuja. Mucoadhesive buccal drug delivery: a potential alternative to conventional therapy. *Ind. J. Pharm. Sci.* **60**:1–11 (1998).
2. Martindale; The Complete Drug Reference, 34th Edition. In S. C. Sweetman (ed.), The Pharmaceutical Press London 372 (2005).
3. C. Koks, P. Meenhorst, M. Hillebrand, and A. Bult. Pharmacokinetics of fluconazole in saliva and plasma after administration of an oral suspension and capsules. *Antimicrob. Agents Chemother.* **40**:1935–1937 (1996).
4. A. E. Collins, and P. B. Deasy. Bioadhesive lozenge for the improved delivery of cetylpyredinium chloride. *J. Pharm. Sci.* **79**2:116–119 (1990).
5. S. Bouckaert, R. A. Lefebvre, and J. P. Remon. *In vitro/in vivo* correlation of the bioadhesive properties of a buccal bioadhesive miconazole slow-release tablet. *Pharm. Res.* **10**6:853–856 (1993).
6. T. S. Owens, R. J. Dansereau, and A. Sakr. Development and evaluation of extended release bioadhesive sodium fluoride tablets. *Int. J. Pharm.* **288**1:109–122 (2005).
7. M. L. Vueba, L. A. E. B. deCarvalhob, F. Veigaa, J. J. Sousaa, and M. E. Pinaa. Influence of cellulose ether polymers on ketoprofen release from hydrophilic matrix tablets. *Eur. J. Pharm. Biopharm.* **58**:51–59 (2004).
8. M. S. El-Samaligy, S. A. Yahia, and E. B. Basalious. Formulation and evaluation of diclofenac sodium buccoadhesive discs. *Int. J. Pharm.* **286**:27–39 (2004).
9. R. W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, and N. A. Peppas. Mechanisms of solute release from porous hydrophillic polymers. *Int. J. Pharm.* **15**:25–35 (1983).
10. J. E. Mockel, and B. C. Lippold. Zero-order drug release from hydrocolloid matrices. *Pharm. Res.* **90**:1066–1070 (1993).
11. N. A. Peppas, and J. J. Sahlin. A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. *Int. J. Pharm.* **57**:169–172 (1989).
12. M. Ozyazıcı, E. H. Gokce, and G. Ertan. Release and diffusional modeling of metronidazole lipid matrices. *Eur. J. Pharm. Biopharm.* **63**:331–339 (2006).
13. B. Parodi, E. Russo, G. Caviglioli, S. Cafaggi, and G. Bignardi. Development and characterization of a buccoadhesive dosage form of oxycodone hydrochloride. *Drug Dev. Ind. Pharm.* **22**:445–450 (1996).
14. K. Nam, J. Watanabe, and K. Ishihara. Modeling of swelling and drug release behavior of spontaneously forming hydrogels composed of phospholipid polymers. *Int. J. Pharm.* **275**1–2:259–269 (2004).
15. R. Y. Han, J. Y. Fang, K. C. Sung, and O. Y. Hu. Mucoadhesive buccal disks for novel nalbuphine prodrug controlled delivery: effect of formulation variables on drug release and mucoadhesive performance. *Int. J. Pharm.* **177**2:201–209 (1999).
16. T. Save, M. U. Stah, A. R. Ghamande, and P. Venkitachalam. Comparative study of buccoadhesive formulations and sublin-

gual capsules of nifedipine. *J. Pharm. Pharmacol.* **46**3:192–195 (1994).
17. J. Ali, R. Khar, A. Ahuja, and R. Kalra. Buccoadhesive erodible disk for treatment of oro-dental infections: design and characterization. *Int. J. Pharm.* **238**:93–103 (2002).
18. C. H. W. Koks, H. Rosing, P. L. Meenhorst, A. Bult, and J. H. Beijnen. High-performance liquid chromatographic determination of the antifungal drug fluconazole in plasma and saliva of human immunodeficiency virus-infected patients. *J. Chromatogr. B.* **663**:345–351 (1995).
19. The British Pharmacopeia, British Pharmacopoeia Commission, HMSO, London, 2007: Electronic version.
20. J. Sujja-areevath, D. L. Munday, P. J. Cox, and K. A. Khan. Relationship between swelling, erosion and drug release in hydrophillic natural gum mini-matrix formulations. *Eur. J. Pharm. Sci.* **6**:207–217 (1998).
21. D. S. Roy, and B. D. Rohera. Comparative evaluation of rate of hydration and matrix erosion of HEC and HPC and study of drug release from their matrices. *Eur. J. Pharm. Sci.* **16**:193–199 (2002).
22. L. Perioli, V. Ambrogi, D. Rubini, S. Giovagnoli, M. Ricci, P. Blasi, and C. Rossi. Novel mucoadhesive buccal formulation containing metronidazole for the treatment of periodontal disease. *J. Control. Rel.* **95**:521–533 (2004).
23. T. Nagai, and R. Konishi. Buccal/gingival drug delivery systems. *J. Control. Rel.* **6**1:353–360 (1987).
24. S. A. Yehia, O. N. El-Gazayerly, E. B. Basalious, and M. S. El-Din. Preparation and evaluation of fluconazole mucoadhesive buccal films. *AAPS J.* **8**S2 (2006) Abstract T3172.