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Preparation and evaluation of bioerodible buccal tablets containing clotrimazole

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Abstract

Buccoadhesive erodible tablets for local delivery of clotrimazole (CLT) to the oral cavity were developed using different bio-adhesive polymers along with soluble excipients like mannitol and polyethylene glycol-6000. An apparatus simulating the in vivo conditions of the mouth was designed in order to assess in vitro, the bio-adhesive performance and release characteristics of these tablets. The in vitro adhesion time and release characteristics were found to be a function of the type of polymer and also the total composition of the tablets. In vivo evaluation of placebo tablets in healthy human volunteers indicated a linear and positive correlation between the in vitro and in vivo adhesion time.

Keywords: Bio-adhesion; Buccal tablet; Candidiasis; Clotrimazole

1. Introduction

Oral candidiasis is an opportunistic infection of the mouth, highly prevalent in a specific group of patients including AIDS patients (Greenspan, 1994). Clotrimazole (CLT) is amongst the first line agents used for the prophylaxis and treatment of this condition (Martin, 1990). As the conventional formulations like mouth paints, rinses, troches, oral gels, etc., have been found to be incapable of maintaining the salivary concentration of drugs for a prolonged period of time (Anders and Merkle, 1989; Collins and Deasy, 1990), it was decided to develop a muco-adhesive buccal tablet which could be stuck on to the inner surface of the cheek and which would maintain the salivary concentration of the drug above the 'minimum inhibitory concentration' (MIC) against *Candida albicans* (2 mcg/ml) (Holt and Newman, 1972; Sawyer et al., 1975) for a prolonged period of time.

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As CLT has very poor aqueous solubility (Hoogerheide and Wyka, 1982), it was our aim to develop an erodible matrix so as to ensure satisfactory drug release in the mouth. Excipients like polyethylene glycol-6000 (PEG 6000) and mannitol were used for the purpose. PEG 6000 has been shown to increase the solubility and release of various poorly soluble drugs (Pedersen and Rassing, 1991; Save and Venkitachalam, 1992). Mannitol has a sweet taste, a good mouth feel, negative heat of solution and dissolution enhancing properties (Czeisler and Perlman, 1991) and hence is a suitable excipient for buccal tablets.

The study also includes the design of a suitable apparatus for the in vitro assessment of the dosage form with respect to drug release characteristics and bio-adhesive performance. Although several apparatuses of varying designs and under varying conditions have been used by different workers (Ishida et al., 1982; Collins and Deasy, 1990; Guo, 1994; Kamath and Park, 1994; Ahuja et al., 1995), no standard in vitro method has yet been developed for these studies. Some of these methods have a poor in vitro/in vivo correlation of the bio-adhesive properties (Bottenberg et al., 1991; Bouckaert et al., 1993).

2. Materials and methods

2.1. Materials

CLT was a gift sample from M/s. DEE Pharma Ltd. Hydroxypropyl cellulose-M (HPC-M) was obtained as a gift sample from M/s. Ranbaxy Labs. Ltd., Hydroxypropyl methyl cellulose-K4M (HPMC -K4M) from M/s. Unichem Labs. Ltd., Sodium carboxymethylcellulose-DVP (SCMC-DVP) from M/s. Max India Ltd., and Guar gum from Dabur Research Foundation. Sodium alginate (CDH), PEG-6000 (S.D. Fine Chemicals) and mannitol (BDH) were obtained from commercial sources. Other solvents and materials used in the study were of reagent grade.

2.2. Formulation of buccal tablets

The tablets were prepared using compositions as given in Table 1. The various components in each formula were mixed by trituration in a glass pestle and mortar. The mixture (200 mg) was then compressed using a 13 mm diameter die on an infra-red hydraulic press (Spectra Lab-SL-89, Bombay) using a compression force of 5 tonnes and a compression time of 15 sec. The tablets were 1.05–1.20 mm thick, depending upon the polymer combination used. Placebo tablets, without the drug were also prepared as above with a total weight of 190 mg.

2.3. Design of the in vitro test apparatus

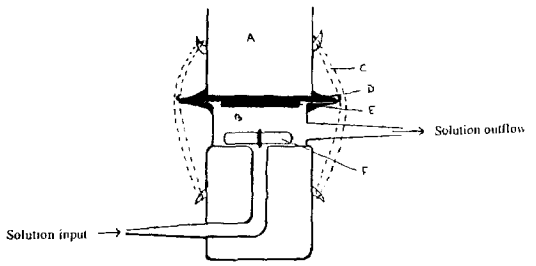
The apparatus was based on a modification of a flow-through diffusion cell (Reifenrath et al., 1994). The lower side of the upper compartment was completely closed. The lower chamber of the apparatus had a small volume compartment (1.5 ml) and the liquid in it was stirred using a teflon coated magnetic needle (length — 10 mm) and a magnet rotating at 300 rpm. The two chambers could be closed tightly by securing springs over the hooks, made on the sides of both the chambers, so that there was no leakage from the apparatus (Fig. 1).

Isotonic phosphate buffer (IPB) pH 6.6, simulating the salivary pH, was continuously pumped through the apparatus at a flow rate of 0.65 ml/min using a small pump and flow regulator.

Table 1
Composition of various buccoadhesive tablets

Ingredient	Weight (in mg) of:				
	X–1	X–2	X–3	X–4	X–5
Clotrimazole	10	10	10	10	10
Polymer	20	60	100	100	140
Mannitol	90	90	90	50	30
PEG-6000	80	40	—	40	20

Formula code (X) polymer: A, sodium carboxymethyl cellulose — DVP; B, hydroxypropyl cellulose — M; C, hydroxypropyl methylcellulose-K4M; D, guar gum; E, sodium alginate.



A - Upper compartment
B - Reservoir chamber
C - Spring
D - Mucosal membrane
E - Adhesive tablet/film
F - Magnetic needle

Fig. 1. Apparatus for the determination of adhesion time and in vitro release studies.

The flow rate chosen corresponded to the mean resting saliva flow rate (Schneyer and Levin, 1955). The whole assembly was maintained at 37°C.

2.4. *In vitro drug release studies and determination of duration of bio-adhesion/erosion*

Fresh bovine cheek pouch was obtained. The mucosal membrane was separated by removing the underlying fat and loose tissues. The membrane was washed with distilled water and then with IPB pH 6.6 at 37°C. A piece of the membrane was tied to the lower surface of the upper compartment with the mucosal side towards the lower compartment. The buccal tablets were stuck on the mucosal surface using 25 μl of IPB pH 6.6 and a weight of 10 g for 30 sec.

The duration of bio-adhesion or erosion was determined by measuring the time taken for the complete erosion or dislodgement of the tablets, whichever was earlier.

For the in vitro release study, fractional samples from the outflow were collected at 5, 15, 30, 45, 60, 75 and 90 min or until the formulations completely eroded or dislodged. The samples were filtered through a Whatman filter paper and analyzed spectrophotometrically.

Graphs were plotted between the concentration of CLT in the dissolution medium and time. The maximal CLT concentration attained (C_{max},d), the time to reach the maximal concentration (t_{max},d) and the time period for which the concentration remained above the minimum inhibitory concentration for *Candida albicans* ($T^{>MIC}$,d), were determined from the concentration — time graphs. The area under the curve ($AUC_{to\text{-}tn}$,d) was calculated by the trapezoidal rule.

2.5. *Analytical method*

A colorimetric method (Hoogerheide and Wyka, 1982) was used for the estimation of CLT in the dissolution medium. The method is based on the development of a bright yellow color that is produced when the drug is heated with perchloric acid. To rule out interference in the analytical method due to polymers, 1 ml of the sample solution was extracted with 3 x 3 ml of ether. The combined extract was evaporated to dryness and to the residue was added 4 ml of perchloric acid. This was heated in a boiling water bath for 5 min and after cooling the solution to room temperature, the absorbance was determined at 436 nm within 2 h.

2.6. *Measurement of bio-adhesive strength*

Bio-adhesive strength of the tablets was measured on a modified physical balance using the method described by Gupta et al. (1992). The method used bovine cheek pouch as the model mucosal membrane and IPB pH 6.6 as the moistening fluid. The surface of the mucosal membrane was first blotted with a filter paper and then moistened with 25 μl of IPB pH 6.6. The weight, in g, required to detach the tablets from the mucosal surface gave the measure of bio-adhesive strength.

2.7. *Surface pH of the tablets*

The surface pH of the tablets was determined in order to investigate the possibility of any side effects, in vivo. As an acidic or alkaline pH may cause irritation to the buccal mucosa, it was our attempt to keep the surface pH as close to neutral as possible. The method used was similar to that

described by Bottenberg et al. (1991). The tablets were first allowed to swell by keeping them in contact with 1.0 ml of distilled water (pH 6.5 ± 0.05) for 2 h in specially fabricated glass tubes. The surface pH was then noted by bringing a combined glass electrode near the surface of the tablet and allowing it to equilibrate for 1 min.

2.8. *In vivo evaluation of placebo tablets*

A preliminary in vivo evaluation of a few placebo tablets was carried out at an early stage during the development of buccoadhesive erodible carriers. The aims of the study were: (i) to determine the time of erosion/adhesion of selected formulations; compare it with the results obtained during in vivo studies and hence, to standardize the designed apparatus for measurement of duration of adhesion/erosion, (ii) to investigate the acceptability of different polymers for use in bio-adhesive buccal formulations and (iii) to determine any irritation or side effects produced by the formulations.

Twelve healthy human volunteers (aged 22–33 years) participated in the study. Informed consent was obtained from the volunteers before the study. Formulations A,B,C,D,E — 2 and 3, without the drug, were used in the study. The volunteers were given different coded tablets on different occasions, along with written instruction sheets. They were instructed to press the tablets against the cheek for about 30 sec without moistening the tablets before application. The volunteers were asked to record the time of tablet insertion and the time and circumstances of the end of adhesion (erosion or dislodgement of the tablets). They were also instructed to report any irritation or side effects produced by the formulations.

3. Results

3.1. *In vitro drug release studies and duration of bio-adhesion*

The in vitro drug release from tablets prepared using different bio-adhesive polymers is shown in Figs. 2–6. At lower concentrations e.g., at concentration levels 1 and 2 of A, B, C, D and E, the muco-adhesive polymers did not seem to play a major role in determining the time of erosion or adhesion. At higher concentration of polymers, the release of the drug as well as the adhesion time were found to be dependent on the type of polymers as well as the total composition of the tablet. The mechanism of drug release seems to be tablet erosion as observed visually during the release study. However, at higher concentration, the formulations containing guar gum exhibited swelling which was predominant over erosion. Formulation D-5 formed a swollen matrix which did not erode in 3 h. The maximum drug concentration achieved in the dissolution medium using this formulation was much less as compared with other formulations. The polymers did not interfere with the analytical method.

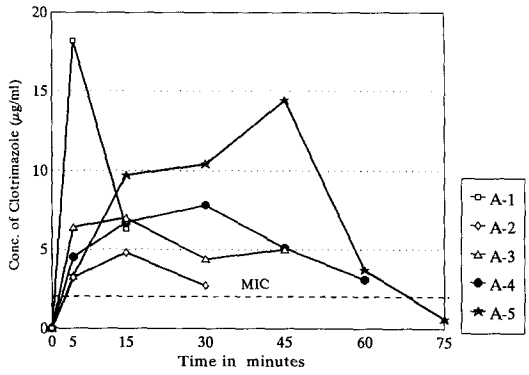


Fig. 2. Drug release from tablets containing SCMC.

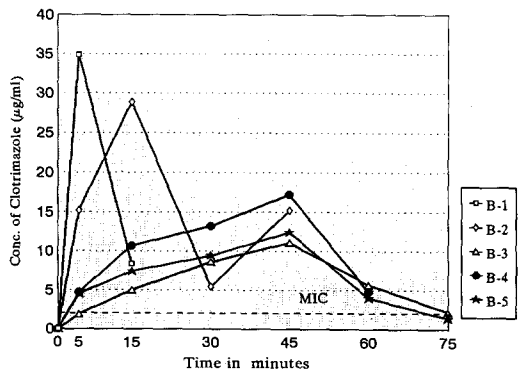


Fig. 3. Drug release from tablets containing HPC-M.

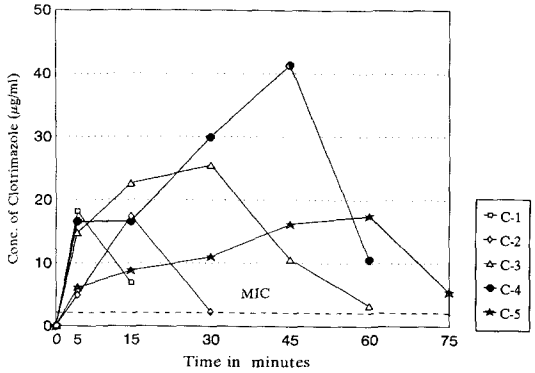


Fig. 4. Drug release from tablets containing HPMC-K4M.

Table 2 gives the C_{max}.,d, t_{max}.,d, $T^{>MIC}$,d and $AUC_{to\text{-}tn}$,d values of the best formulations with each polymer. The formulation chosen is one having the highest value of $AUC_{to\text{-}tn}$,d.

3.2. Bio-adhesive strength in vitro

The bio-adhesive strength of the formulations containing the same polymer was found to be a function of the concentration of the polymer. Among the formulations containing different polymers, those containing SCMC exhibited maximum bio-adhesive strength followed by those containing HPMC-K4M, HPC-M and sodium alginate which exhibited almost similar bio-adhesive strength. The results are in agreement with earlier studies (Smart et al., 1984; Lehr et al., 1992) for bio-adhesive performances of different polymers. As none of the formulations dislodged before complete erosion, the bio-adhesive strength exhibited by all the formulations is satisfactory for maintaining them at the buccal site. This aspect was further confirmed by simultaneously carrying out in vivo evaluation of selected placebo tablets in healthy human volunteers.

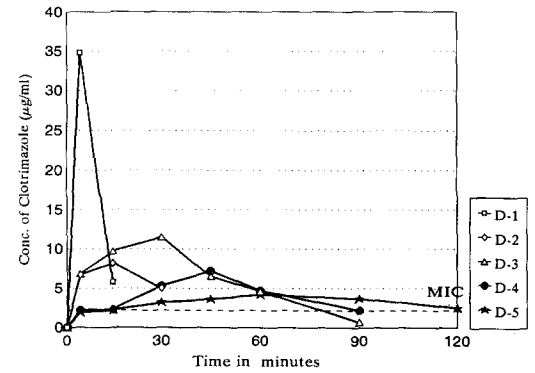


Fig. 5. Drug release from tablets containing Guar gum.

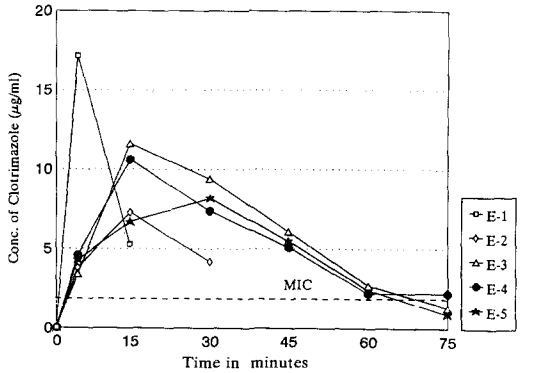


Fig. 6. Drug release from tablets containing sodium alginate.

3.3. Surface pH

The surface pH of all the formulations was found to be within ± 1.5 units of the neutral pH and hence, these formulations should not cause any irritation in the buccal cavity.

3.4. In vivo performance of placebo tablets

Table 3 gives the in vitro adhesion time and in vivo performance of selected formulations. All the tablets eroded completely in vivo and none dislodged before complete erosion. The formulations were generally acceptable and no irritation of the buccal mucosa was observed.

3.5. In vitro — in vivo correlation

The scatter diagram (Fig. 7) shows a positive correlation between the mean adhesion time in vitro and in vivo. The value of correlation coefficient was found to be 0.989 which is statistically significant at the 5% confidence level.

Table 2
$C_{max.}$,d, $t_{max.}$,d, $T^{>MIC}$,d and $AUC_{to\text{-}tn}$,d values of selected buccal formulations

Formulation code	$C_{max.}$,d, (mcg/ml)	$t_{max.}$,d, (min)	$T^{>MIC}$,d (min)	$AUC_{to\text{-}tn}$,d (mcg min.ml^{-1})
A–5	14.4	60.0	64.5	578.00
B–4	17.2	45.0	58.0	642.20
C–4	41.3	30.0	59.0	1300.00
D–3	11.5	30.0	79.0	556.25
E–3	11.6	15.0	64.5	453.25

Table 3
In vitro and in vivo performance of selected buccal formulations

Formula code	In vitro performance		In vivo performance		Irritation (yes/no)
	Mean adhesion time (min)	Erosion/dislodgement	Mean adhesion time (min)	Erosion/dislodgement	
A–2	28.0	Erosion	32.6	Erosion	No
A–3	54.3	Erosion	56.6	Erosion	No
B–2	24.3	Erosion	23.6	Erosion	No
B–3	68.3	Erosion	65.3	Erosion	No
C–2	24.3	Erosion	24.3	Erosion	No
C–3	52.6	Erosion	52.0	Erosion	No
D–2	23.6	Erosion	23.3	Erosion	No
D–3	76.3	Erosion	66.3	Erosion	No
E–2	28.6	Erosion	29.3	Erosion	No
E–3	69.3	Erosion	66.0	Erosion	No

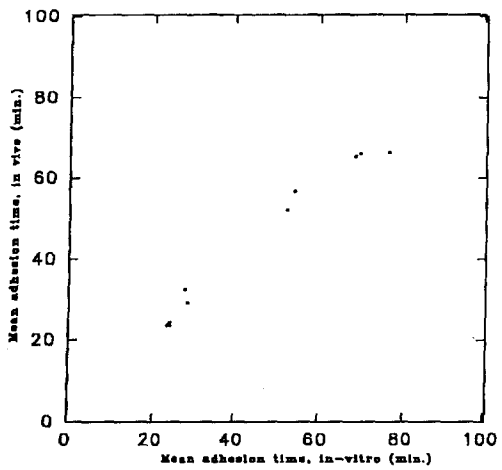


Fig. 7. Scatter diagram showing correlation between the mean adhesion time, in vitro and in vivo.

4. Discussion

Buccal formulations of CLT in the form of muco-adhesive, erodible tablets were developed to a satisfactory level in terms of drug release, bio-adhesive performance and surface pH, using different bio-adhesive polymers. Although significant concentration of clotrimazole could be achieved in the dissolution medium, the time of erosion and hence the $T^{>MIC}$,d for the tablets was less than what would be ideal. However, results obtained from this study would be helpful for the further development of muco-adhesive buccal tablets.

In vivo evaluation of the selected formulations showed that the tested polymers have low irritation potential and hence could be used for the development of buccal formulations.

Since a good correlation was observed between the mean adhesion time in vitro and in vivo, it could be concluded that the designed apparatus sufficiently mimics the in vivo conditions for the determination of adhesion time of different formulations.

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