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Preparation and Evaluation of Muco-adhesive Buccal Films of Clotrimazole for Oral Candida Infections

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Mucoadhesive buccal films of clotrimazole for local delivery of the drug to the oral cavity were formulated by the solvent casting technique. A number of different bioadhesive and film- forming polymers were evaluated. Propylene glycol was used as the plasticizer while the solvents depended on the type of polymer chosen. The films were evaluated on the basis of their physical characteristics, bioadhesive performance, release characteristics, surface pH, folding endurance and stretchability. A combination of Carbopol-934P and hydroxypropyl cellulose - M in the ratio of 1:5 and using ethanol (95%) as the solvent was found to give satisfactory results. The film exhibited an *in vitro* adhesion time of 4 hours and maintained the concentration of clotrimazole in the dissolution medium (isotonic phosphate buffer pH 6.6) above the MIC of *Candida albicans* ($T>^{MIC}$, d) for upto 4 hours. A maximum concentration (C_{max}, d) of 21.1 μg/ml was obtained in the dissolution medium after 2 hours (t_{max}, d). The drug released from the formulation was found to be microbiologically active.

LOCAL delivery of drugs to the tissues of the oral cavity has a number of applications including the treatment of toothache[1], periodontal diseases[2], dental caries[3], bacterial[4] and fungal infections[5,6] and aphthous stomatitis[7,8]. The conventional formulations for the local delivery of drugs to the oral cavity are the mouth paints, rinses, troches, pastilles, creams, gels and suspensions.

The aim of the present study is to develop sustained release muco-adhesive polymeric films containing an antifungal agent, clotrimazole. Clotrimazole was amongst the first imidazoles to be synthesized and marketed, and is still widely used as a first line treatment for oral candidiasis[9]. The factors favouring the use of clotrimazole, as against other anti-fungal agents, are its cost effectiveness, low side effects and absence of significant drug interactions. It can be used in patients regardless of liver function and/or underlying clinical disease[10]. Being tasteless, clotrimazole has a better patient compliance as compared to bitter drugs like nystatin.

Initially, placebo films using the polymers, plasticizer and solvents in different combinations, were formulated, Selected films which were complete, homogenous, flexible, non-sticky and smooth were taken up for further studies. The films were loaded with the drug so as to maintain the concentration above 2 μg/ml, the MIC against *Candida albicans*[11,12], for a prolonged period of time.

MATERIALS AND METHODS

Clotrimazole (CLT) was a gift sample from M/s Dee Pharma Ltd., New Delhi. Carbopol - 934 P (CP-934P) and hydroxypropyl cellulose - M (HPC-M)

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were obtained as gift samples from M/s Ranbaxy Labs. Ltd., New Delhi. Carbopol - 974 P (CP-974 P) and hydroxypropyl methyl cellulose - E4M (HPMC - E4M) from M/s Max India Ltd., New Delhi and Eudragit - NE30D (Eu-NE30D) and Eudragit - RLPM (Eu- RLPM) from Rohm Pharma, Germany. All other materials used were of reagent grade. Subculture of *Candida albicans* J 1012 was obtained from Patel Chest Research Institute, University of Delhi.

Formulation of buccal films

Buccal films were prepared by the solvent casting technique[13]. Among the various substrates for film formation including mercury, teflon, glass and aluminium, mercury surface was found to give best results. All further work was done using this substrate. Table 1 shows the composition of different buccal films. The ingredients were accurately weighed and mixed by trituration in a glass pestle and mortar. The mixture was then added gradually to a magnetically stirred solvent system containing the plasticizer. Stirring was continued until a clear solution was obtained. The solution was then transferred quantitatively to glass rings (dia.-5.7 cm) kept on the surface of mercury in petriplates. The petriplates were covered with inverted funnels to allow controlled evaporation of the solvent. These were left undisturbed at room temperature (20-25°) for one to two days depending upon the solvent system used. The films could be retrieved intact by slowly lifting the rings from the mercury substrate. Small patches of 14 mm diameter, 0.2 to 0.3 mm thick and containing 10 mg of CLT per patch, were punched out from the films using a specially fabricated punch.

Study of physical characteristics of the formulations

Bioadhesive strength of the film patches was measured on a modified physical balance using the method described by Gupta *et al*[14]. The method used bovine cheek pouch as the model mucosal membrane and isotonic phosphate buffer pH 6.6 (IPB) as the moistening fluid. The surface of the mucosal membrane was first blotted with a filter paper and then moistened with 25 µl IPB. The weight, in grams, required to detach the film patch from the mucosal surface gave the measure of bioadhesive strength.

The surface pH of the films was determined in order to investigate the possibility of any side effects, *in vivo* due to film pH. The method used was similar to that described by Bottenberg *et al*[3]. The film patches were first allowed to swell by keeping them in contact with 0.5 ml of distilled water (pH 6.5 ± 0.05) for 1 hour in specially fabricated glass tubes. The surface pH was then noted by bringing a combined glass electrode near the surface of the films and allowing it to equilibrate for 1 min.

A self designed and locally fabricated apparatus was used for the determination of 'Percentage elongation at break' which gives the mechanical property of the films. A small film strip (approx. 2 cm x 1 cm) was pulled at a rate of about 5 mm/min, till it broke. The initial and final length of the strip was noted and the percentage elongation of the film was calculated as:

$$\% \text{ elongation at break} = \frac{\text{Increase in length}}{\text{Initial length}} \times 100$$

Folding endurance of the film was determined by repeatedly folding a small strip of film (approx. 2 cm x 2 cm) at the same place till it broke. The number of times, the film could be folded at the same place, without breaking, gave the value of folding endurance.

In vitro release studies and determination of duration of bioadhesive / erosion

These studies were carried out on a self designed continuous flow-through apparatus[15]. The apparatus was based on the modification of a flow-through diffusion cell[16]. The lower side of the upper compartment was completely closed to which was tied mucosal membrane from a bovine cheek pouch.

Table 1: Composition of different buccal films

Composition	Formula Code						
	F-1	F-2	F-3	F-4	F-5	F-6	F-7
Clotrimazole (mg)	165.7	165.7	165.7	165.7	165.7	165.7	165.7
CP-934P (mg)	75.0	-	-	50.0	-	100.0	100.0
CP - 974 (mg)	-	100.0	-	-	75.0	-	-
HPC-M (mg)	-	-	300.0	250.0	250.0	-	-
HPMC-E4M (mg)	-	-	-	-	-	100.0	-
Eu-NE30D (ml)	0.6	0.6	-	-	-	-	-
Eu-RLPM (mg)	-	-	-	-	-	-	300.0
Propylene Glycol (ml)	0.05	0.05	0.1	0.1	0.1	0.125	0.1
Ethanol (95%) (ml)	12.0	12.0	12.0	12.0	12.0	-	-
Methanol: dichloro methane (3:2) (ml)	-	-	-	-	-	12.0	-
Acetone: isopropanol (2:3) (ml)	-	-	-	-	-	-	12.0

The buccal films were stuck on the mucosal surface using 25 µl IPB and a weight of 10 g for 30 s.

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 at a rate of 300 rpm. The two chambers were closed tightly and IPB was pumped through the apparatus at a flow rate of 0.65 ml/min using a pump and a flow regulator. The flow rate chosen corresponded to the mean resting saliva flow rate[17]. The assembly was maintained at 37°.

The duration of bioadhesive or erosion was determined by measuring the time taken for the dislodgement or complete erosion of the patches, respectively, which ever was earlier. For an *in vitro* release study, fractional samples from the outflow were collected at 5, 15, 30, 45, 60, 90, 120, 150 and 180 min or till 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 ($AUC_{to-tn,d}$) was calculated by the trapezoidal rule.

Analytical method

A colorimetric method[18] was used for the estimation of CLT in the dissolution medium. The method is based on the development of a bright yellow color 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

Table 2: Certain important parameters of buccal films, *in vitro*

Formula Code	Bioadhesive strength (g)	Adhesion time(min)	Surface pH	Folding endurance	% Elongation at break
F-1	6.66 (0.28)	29.0 (5.0)*	6.41 (0.02)	>200 (0)	60.82 (4.42)
F-2	6.33 (0.28)	19.6 (3.0)*	5.93 (0.02)	>200 (0)	79.54 (3.93)
F-3	10.00 (0.5)	129.3 (4.7)	7.05 (0)	>200 (0)	46.82 (5.58)
F-4	13.50 (0.5)	223.0 (9.1)	6.08 (0.02)	>200 (0)	15.72 (1.81)
F-5	12.83 (0.57)	168.3 (4.7)	5.93 (0.07)	>200 (0)	13.73 (2.52)
F-6	8.5 (0)	142.2 (6.8)	7.0 (0.02)	136 (12.5)	22.44 (1.90)
F-7	4.0 (0.5)	9.3 (3.5)*	9.3 (0.05)	68.3 (9.0)	14.78 (1.54)

* Dislodged before complete erosion

The ± S.D. values are given in parenthesis

Table 3: $C_{max,d}$, $t_{max,d}$, $T^{>MIC}$,d and $AUC_{to-tn,d}$ values of selected buccal films, *in vitro*

Formula Code	$C_{max,d}$ (μg/ml)	$t_{max,d}$ (min)	$T^{>MIC}$,d (min)	$AUC_{to-tn,d}$ ($\mu g.min.ml^{-1}$)
F-1	10.9	30.0	29.0	266.0
F-2	11.9	30.0	29.0	269.5
F-3	17.0	45.0	148.5	1550.25
F-4	21.1	120.0	239.0	3683.5
F-5	15.3	90.0	178.0	1968.5
F-6	13.2	60.0	148.5	1520.5
F-7	07.9	15.0	13.0	78.5

extracted with 3x3 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 RT, the absorbance was determined at 436 nm within 2 hours.

Microbiological evaluation of optimized buccal film

Antifungal efficacy of CLT released from the optimized buccal film was determined by performing the disc agar diffusion assay[19] on aliquots of the dissolution samples obtained during *in vitro* release studies. Sabouraud agar plates inocculated with *Candida albicans* J1012 were used for the study. 0.1 ml of the samples were carefully pipetted into uniformly spaced 7.0 mm diameter wells. These were allowed to prediffuse for 2 hours at room temperature and then incubated in the inverted position at 37° in a B.O.D. incubator, for 18 to 24 hours. The diameter (mm) of growth inhibition surrounding each agar well was measured and the concentration of CLT was determined from the calibration curve constructed under identical conditions.

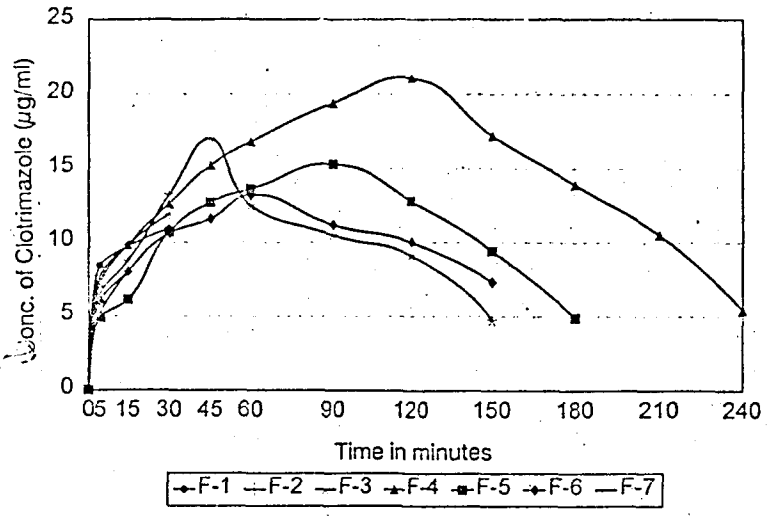

Fig. 1: Drug release from different buccal films, *in vitro*

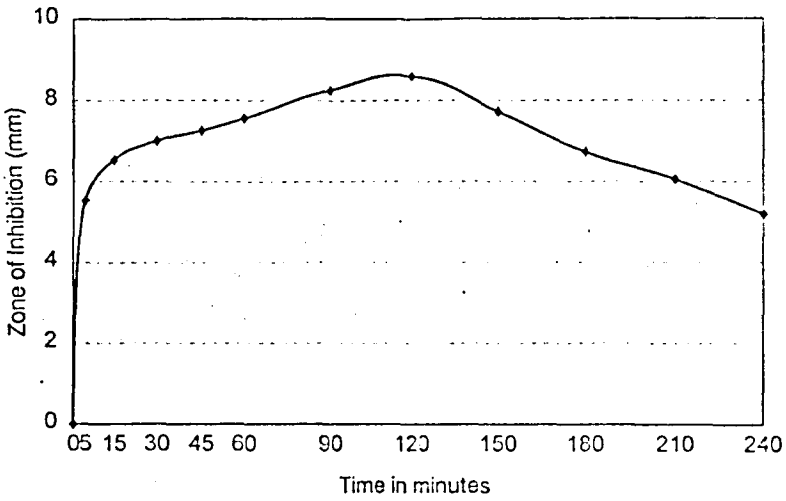

Fig. 2: Antifungal activity of drug released from optimized buccal film

Stability Studies

Stability studies on the optimized formulations were carried out to determine the effect of the presence of formulation additives on the stability of the drug and also to determine the physical stability of the formulations under accelerated storage conditions to temperature. The muco-adhesive buccal patches were stored in closed glass petriplates lined internally with aluminium foil. These were placed in hot air ovens maintained at 37°, 45° and 60°. Samples were withdrawn at 0, 15, 30 and 60 days and were analyzed for active drug content, bioadhesive strength, adhesion time, elongation at break, folding endurance and weight gain/loss.

For the determination of active drug content, a stability indicating two phase titrimetric assay method[18] was used. For determining the weight gain/loss, six film patches were individually weighed and the average weight determined. Any gain/loss in the weight from the initial value was then calculated. All other parameters were determined by the methods described earlier.

RESULTS AND DISCUSSION

Bioadhesive performance and physical properties

Table 2 gives the values of bioadhesive strength, adhesion time, surface pH, folding endurance and percentage elongation of different buccal films. The surface pH of all the formulations was within ± 1.5 units of the neutral pH and hence, these formulations should not cause of the buccal mucosa.

Films containing Eu-NE30D (F-1 and F-2) and Eu-RLPM (F - 7) exhibited good physical and mechanical properties but these had very short adhesion time. Films containing Eu-NE30D dislodged due to excessive swelling of the polymer while films containing Eu- RLPM had poor bioadhesive strength and dislodged due to the agitation conditions in the apparatus. In the concentration used, the Eudragit polymers were found to mask the bioadhesive property of Carbopol resins. These might be useful, in lower concentrations, for increasing the mechanical strength of buccal films.

Films containing HPC-M alone (F-3) and HPMC-E4M in combination with CP-934P (F-6), although maintaining satisfactory concentration of CLT in the

Table 4: Effect of storage at elevated temperatures on the properties of buccal films at the end of two months

Properties	Storage Conditions			
	Initial values	37°C	45°C	60°C
Drug content, %	100.26 (0.4)	100.03 (0.4)	99.56 (0.4)	98.90 (0.34)
Bioadhesive strength, g	13.33 (0.28)	13.33 (0.28)	13.16 (0.28)	12.50 (0.50)
Adhesion time, min	223.00 (9.16)	222.00 (10.8)	219.00 (7.5)	205.60 (6.0)
Elongation at break, %	15.81 (1.1)	16.24 (1.09)	15.48 (2.25)	11.85 (1.40)
Folding endurance	>200 (0)	>200 (0)	>200 (0)	151.60 (13.0)
Weight gain/ loss, % w/w	—	-0.17	-0.17	-1.20

Each value represents a mean of three readings.

The ±S.D. values are given in parenthesis

dissolution medium had a short adhesion time. The films eroded completely in less than 2.5 hours. Both the films formulated with HPC-M along with CP-934 (F- 4) or CP-974 (F-5) exhibited satisfactory bioadhesive and physical properties.

In vitro drug release studies

Fig. 1 shows the drug release from different buccal films, *in vitro*. Although a number of films exhibited satisfactory drug release, the best results were obtained with film (F-4) containing CP-934P and HPC-M in the ratio of 1:5 and using propylene glycol as the plasticizer and ethanol (95%) as the solvent. Table 3 gives the Cmax,d, tmax,d, $T^{>MIC}$,d and $AUC_{to\text{-}tn}$,d values of different buccal films obtained *in vitro*, during the release study. Films containing HPC-M alongwith CP-934P (F-4) or CP-974 (F-5) were both satisfactory in their release profile but since F-4 exhibited a greater bioadhesive strength, adhesion time, $T^{>MIC}$,d and $AUC_{to\text{-}tn}$,d it was taken as the optimized formulation. The bioadhesive strength of this formulation was the maximum among all the formulations and the values for surface pH, folding endurance and percentage elongation at break were also satisfactory. As can be seen from the table, the selected film (F-4) maintained the concentration of CLT in the dissolution medium above the MIC of *Candida albicans* for upto 4 hours. A maximum concentration of 21.1 μg/ml was obtained in the dissolution medium after 2 hours.

Microbiological evaluation

Fig. 2 shows the microbiological efficacy of the aliquot samples against *C. albicans* J1012. The drug released from the optimized buccal film was able to inhibit the growth of *C. albicans* J1012 for upto 4 hours. A maximum growth inhibition zone of 8.6 mm corresponding to a concentration of 20.18 μg/ml of CLT was obtained with the aliquot from the 2 hour dissolution sample.

Stability Studies

The Stability studies of the optimized buccal films revealed that no significant changes in the physical

parameters of the formulations occured at storage temperatures of 37° and 45° (Table 4). However, significant changes in the parameters occured at 60° which could be attributed to a loss of moisture from the formulations at this temperature. No significant reduction in the content of active drug occured over a period of two months. As indicated, storage temperature not exceeding 45° are essential to ensure the stability of these formulations.

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