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DEVELOPMENT AND CHARACTERIZATION OF CICLOPIROX OLAMINE LOADED BUCCOADHESIVE FILM FOR TREATMENT OF ORAL CANDIDIASIS

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ABSTRACT:
Buccal bioadhesive films, releasing drug in the oral cavity at a slow and predetermined rate, provide distinct advantages over traditional dosage forms. The study was aimed to prepare and evaluate buccal bioadhesive films of Ciclopirox Olamine (CPO) for oral candidiasis. The film was designed to release the drug at a concentration above the minimum inhibitory concentration for a prolonged period of time so as to reduce the frequency of administration of the available conventional dosage forms and increase patient compliance. The different proportions of various bioadhesive polymers were used for the preparation of films. The films were prepared by solvent casting method and evaluated for various physicochemical properties, bioadhesion, *in vitro* drug release and effectiveness against *Candida albicans. In vitro* drug release from the film was determined using a modified Franz diffusion cell while bioadhesiveness was evaluated with an in house developed device. Films containing HPMC K4MC (3%) /Na CMC (1%) was found to be the best with moderate swelling along with favorable bioadhesion force, residence time and in vitro drug release. The microbiological studies revealed that drug released from the film could inhibit the growth of *C. albicans* for 8 h. The drug release mechanism was found to follow anomalous diffusion. The results of short term stability study revealed stable characteristics of CPO buccal film.

Keywords: Bioadhesion, Ciclopirox olamine, HPMC K4M, NaCMC

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INTRODUCTION

Candidiasis in the oral cavity is an opportunistic infections condition caused by a ubiquitous, saprophytic fungus of the genus Candida, the most common of which is Candida albicans. C. albicans is a resident commensal fungus of the normal oral flora. It can infect when predisposing factors such as antibiotic therapy, corticosteroid therapy, xerostomia (dry mouth), diabetes mellitus, chemo/radiation therapy, and immunosuppression are present. Recently the advent of the human immunodeficiency virus infection has resulted in a resurgence of oral Candida infections. General debilitation, poor oral or dental hygiene, and ill-fitted dentures are some of the other predisposing factors responsible for the cause of oral candidiasis. Fungal opportunistic infections, including oral candidiasis, are a major cause of morbidity and mortality in cancer patients[1-4]. 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 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[5].

Recent years have seen an increasing interest in the development of novel buccal bioadhesive dosage forms. These are useful for both for systemic delivery of drugs,

as well as for local targeting of drugs to a particular region in the body[6, 7]. A wide range of polymers of synthetic, semi synthetic and natural origin like carbopol, polycarbophil, sodium carboxymethylcellulose (SCMC), hydroxypropylmethyl cellulose, chitosan and xanthan gum have been described for the formulation of bioadhesive systems.Therefore, the present study was aimed to design and develop buccal bioadhesive films of CPO which would maintain the salivary concentration of the drug above the MIC against *C. albicans* for a prolonged period of time.

MATERIALS AND METHODS

Materials

Ciclopirox Olamine was kindly gifted by Cipla Ltd (Mumbai, India). Carbopol 974P (CP) and Eudragit RLPO (EUDGT) were kindly gifted by Corel Pharmaceutical Ltd. (Ahmedabad, India). Hudroxy propyl cellulose (HPMC K4M), Hydroxy ethyl cellulose (HEC), sodium alginate (Na CMC) and Polycarbophil (PCF) were gifted by Suvik Pharmaceuticals Pvt Ltd. (Gandhinagar, India).

Preparation of CPO buccal film using different bioadhesive polymers

Distilled water (DW) used in the preparation of polymeric gel was degassed under vacuum to avoid the formation of air bubbles. Weighed amount of HPMC K4M was added to one-third portion of the required DW and kept undisturbed until a clear solution was formed. Then it was stirred for 1 h. CPO was dissolved in a minimum volume of ethanol and added to NaCMC contained in a dry beaker. The remaining two-third portion of DW was added to the above mixture with stirring to form a homogeneous dispersion. The HPMC K4M solution and required volume of PEG 400 were added to the dispersion of NaCMC and stirred well. The resulted gel was kept overnight undisturbed under refrigeration to ensure the formation of clear, bubble-free gel which was finally poured on a borosilicate glass petridish, allowed to settle and dried under convective flow of hot air at a temperature of 45-50°C for 24 h till a flexible film was formed. After drying, the films were cut into smaller pieces of 2×2 cm sizes, wrapped in aluminum foil and stored in glass containers were preconditioned at room temperature and relative humidity of 60%[8].

EVALUATION

Physicochemical evaluation[9]

Weight Uniformity

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

Film Thickness

The thickness of the prepared films was determined by means of micrometer. The thickness of six films was measured and the average thickness was determined.

Folding Endurance

Folding endurance was determined by repeatedly folding the film at the same place till it broke or folded up to 300 times.

Drug Content Uniformity

Uniformity of drug content was determined according to the following procedure. Ten randomly selected films of each formulation batch were weighed accurately and dissolved in 10 ml of ethanol. Of this CPO solution, 0.5 ml was transferred into a 100 ml volumetric flask containing 20 ml of PEG-400, and stirred continuously for 8 h on a magnetic stirrer. The volume was made up to 100 ml with phosphate buffer (pH 6.8) and the absorbances were measured in UV/Vis spectrophotometer at 302 nm (Jasco 7800, UV/Vis Spectrophotometer, Tokyo, Japan). Concentrations of CPO were calculated from a standard calibration curve of CPO in phosphate buffer (pH 6.8) containing 20% PEG-400 without interferences of excipients.

Swelling Index

The films were coated on the lower side with ethyl cellulose (to avoid sticking to the dish) then weighed (W1) and placed separately in petri dishes containing 25 ml of distilled water. The dishes were stored at room temperature. After 5, 10, 15, 20, 30, 45 and 60 minutes, the films were removed and the excess water on their surface was carefully removed using filter paper. The swollen discs were weighed (W2) and the percentage of swelling was calculated by the following formula[10]

Swelling index =W2 - W1/*W*1* 100

Microenvironment pH

The microenvironment pH of the prepared buccal bioadhesive CPO films was 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, and the pH was measured at time intervals of 2, 4, and 6 h by placing the electrode in contact with the microenvironment of the swollen films. The average pH of five determinations was reported.

Mechanical Characterization of the Films

Mechanical parameters, tensile strength and elongation at break were calculated from the load time profiles of the films using INSTRON® tensile tester. Upper and lower grips of the sample with a gauge length of 5×1 cm, were attached to the crosshead and the base plate respectively in such a way that the former was located exactly 5 cm above the latter. The crosshead was moved upwards at a speed of 1 cm/s. The force and elongation were measured when the film broke[11]. Results were reported as the mean (±SD) of five replicates.

Ex-vivo mucoadhesion time

Several types of mucosa including rat intestine, pig oral, bovine sublingual, cow vaginal mucosa have been used as model biological tissues for the evaluation of mucoadhesion[12, 13]. A modified self developed force detachment devise was used to measure the minimum detachment force (Figure 1). A piece of rat intestine (2.0 cm x 1.0 cm) removed from newly sacrificed rat was adhered to a piece of glass, which was fixed on a plank and the plank was assembled with a little crown block. After hydrating the rat intestine with distilled water, the CPO buccal film was brought into contact with the rat intestine by applying little force or minute. After the initial contact, the tablet was encircled by a thread which fastened a light plastic beaker through the crown block. Next, water was dropped into the beaker at a speed of 3.0 ml/min using peristaltic pump until the CPO buccal film and rat intestine were pulled apart by the gravity of water. The beaker containing water was weighed and the minimum detachment force was calculated accordingly. The experiments were performed in triplicate and average values with standard deviation (SD) were reported. The Protocol (KBIPER/2012/31) was approved by Institutional Animal Ethics Committee (K.B Institute of Pharmaceutical Education and Research) under CPCSEA before carrying out this experiment.

In vitro release of CPO buccal film

The release of CPO from the prepared bioadhesive films into phosphate buffer pH 6.8 at 37± 0.5°C was performed using a special modified Levy method[14]. Each bioadhesive film was adhered to the side wall of a vessel (100 ml beaker) using cyanoacrylate[15]. 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 150 rpm. After time intervals each of 5, 15, 30, 60, 90, 120, 180, 240 and 300 mins, 3 ml sample was withdrawn, filtered through a millipore filter of 0.45 µm pore size and assayed spectrophotometrically at λmax 302 nm. Immediately after each sample withdrawal, a similar volume of phosphate buffer pH 6.8 was added to the release medium to maintain the volume in the vessel constant. The absorbance of the polymeric additives was negligible and did not interfere with λmax of the drug.

Table 1: Formulation of CPO loaded buccal film

Batch No	Polymer	Ciclopirox (mg)	Aspartame (mg)	Plasticizer PEG 400 (%)
CO1	HEC (2%)	10	2	1.5
CO2	HEC(2%)/PCF(0.6%)	10	2	1.5
CO3	HPMC K4M (3%)/NaCMC (1%)	10	2	1.5
CO4	EUDGT RL PO (20%)/PCF (0.6%)	10	2	1.5

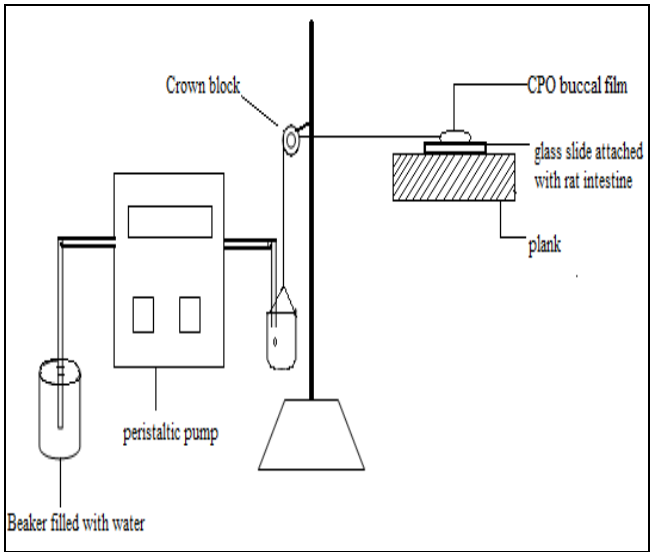

Fig. 1 A modified self developed force detachment device

Antifungal efficacy of CPO buccal film

Preparation of the Agar Plates: The agar plates used in this study were prepared by dissolving SDA 65 g in 1 L of distilled water and sterilized by autoclaving (at 15 lb pressure and 121°C) for 15 min. The agar solution was poured into sterile Petri dishes. The agar plates are then allowed to cool and solidify at room temperature; then they were inoculated (cultured) with *C. albicans, C.glabrata and C.tropicalis* by using a sterile swab[16].

Agar diffusion assay of CPO buccal film: Antifungal efficacy of the selected buccal bioadhesive film of CPO (Batch CO3) was determined by subjecting the aliquots of in-vitro drug release studies to disc agar diffusion assay. Aliquots of in-vitro drug release samples were collected at 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 h. A 0.1 ml of each sample was carefully pipetted into uniformly spaced 7 mm diameter wells of the agar plates. These plates were allowed to prediffuse for 2 h at room temperature and then incubated for 24 h. The diameter (millimeter) of the growth inhibition zone surrounding each agar well inoculated with *C. albicans.* The mean of three determinations of each sample was determined[17].

Short term stability study: The CPO buccal films were charged for the accelerated stability studies as per ICH guidelines (40±2°C and 75±5% RH) for a period of 3 months in stability chambers (Model-TH 90 S, Thermolab, India). They were placed in flint vials and hermetically sealed with rubber plugs and aluminum caps. The samples were taken out at 30, 60 and 90 days and evaluated for the various parameters[18].

Statistical Analysis: The results obtained were subjected to statistical analysis using a computer program Sigma Stat® for one-way analysis of variance (ANOVA; $p<0.05$).

Result and discussion

Preliminary study: The buccal film was formulated using various mucoadhesive polymers initially to select the appropriate polymers suitable for film either alone or in combinations. The film was evaluated in regards of film thickness, swelling and morphological appearance. Table 2 depicts the thickness and swelling of films of preliminary batches.

Final formulation composition of CPO buccal film: Based on the results of preliminary study of mucoshesive film, final batches of CPO buccal film were prepared using optimized polymers with their appropriate concentration. The plasticizer (PEG-400) was optimized at 1.5% w/w of polymer based on film flexibility and appearance. The values of physicochemical properties of CPO loaded buccal film are presented in table 3. The thickness of the prepared films ranged from 0.5 to 0.99 mm. The surface pH values of all films were in the range 4.5-6.5.

Table 2: Results of preliminary batches

Film Type	Film Thickness (mm)	Swelling % after 10 min.
SOD ALG/CP	1.02 ± 0.30	222.0
SOD ALG	1.09 ± 0.18	83.7
SOD ALG/PCF	1.06 ±0.32	349.2
SOD ALG/NaCMC	1.08 ± 0.21	206.9
HEC/CP	0.75 ± 0.21	118.5
HEC	0.50 ± 0.08	152.0
HEC/PCF	0.78 ± 0.20	136.7
HEC/NaCMC	0.70 ± 0.10	208.4
HPMC/CP	0.86 ± 0.22	76.8
HPMC	0.98 ± 0.23	101.1
HPMC/PCF	0.70 ± 0.10	88.2
HPMC/NaCMC	0.098 ± 0.29	146.9
EUDGT/CP	0.91 ± 0,32	104.0
EUDGT	0.88 ± 0.11	222.0
EUDGT/PCF	0.99 ± 0.31	109.7
EUDGT/NaCMC	0.91 ± 0.23	119.0

The degree of swelling of bioadhesive polymers is an important factor affecting adhesion. Adhesion occurs shortly after the beginning of swelling but the bond formed is not very strong[11]. Uptake of water results in relaxation of the originally stretched, entangled or twisted polymer chains, resulting in exposure of all polymer bioadhesive sites for bonding to occur. As the swelling of the polymer increased it leads to initiate diffusion and formation of adhesive bonds resulting in faster initiation of bioadhesion[19]. Table 3 shows the percentage of swelling of CPO buccal films containing different film forming materials alone or in combination. It was found that Eudragit (CO4) film had the least swelling capacity and HEC (CO1) films had the highest swelling capacity.

The work of adhesion usually gives more useful information concerning bioadhesion than does maximum detachment force alone. Thus, the work of adhesion of CPO buccal film was measured using a previously designed and validated apparatus in our laboratory. Maximum bioadhesion was obtained by batch CO2 comprised of HEC and Polycarbophil. The mutual action of both polymer aided the bioadhesion power of polymer. In the trial batches, least bioadhesion was found with film prepared by Eudragit RLPO.

Figure 2 presents the release profiles of CPO from different mucoadhesive films. It could be seen that addition of bioadhesive polymers predominately decreased the release rates from the different mucoadhesive films. These polymers exhibit high swelling resulting in an increase in diffusion path length of drug and the consequent reduction of drug release[20]

In addition, the thick gel layer formed on the swollen film surface is capable of preventing matrix disintegration and controlling additional water penetration[21]. Eudragit films produced sustained release in all formulations. This may be due to the water insolubility of Eudragit and the consequent lower dissolution and slower erosion of films. Satisfactory dissolution pattern was obtained by batch CO3 comprised of HPMC K4M/NaCMC.

Dissolution data of the optimized formulation CO3 were fitted to various mathematical models (zero order, first order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas, and Weibull) in order to describe the kinetics of drug release. Drug release from optimized formulation (CO3) fitted well into Korsmeyer-Peppas kinetics with least sum of square of residuals (SSR= 7.72), Fischer's ratio (F= 1.93) and maximum R^2 value 0.999 with anamolous diffusion with release exponent value (n) of 0.81.

Table 4 shows the antifungal activity of the aliquot sample against *C. albicans*, *C. glabrata* and *C. tropicalis*. The drug released from the selected film CO3 was able to inhibit the growth of *C. albicans* for 8 h. A maximum growth inhibition zone of 47± 0.5 mm was obtained with the aliquot from 8 h dissolution sample.

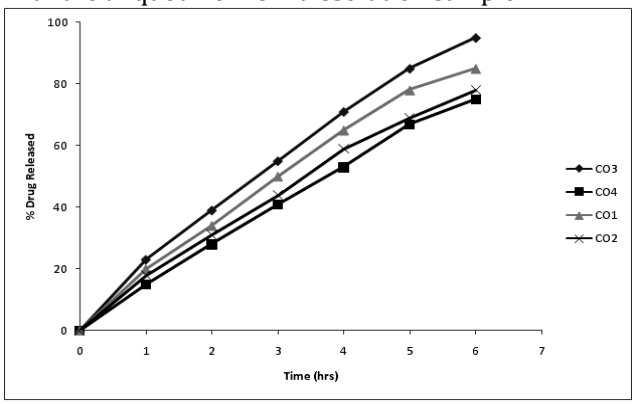

Fig. 2: In vitro dissolution profiled of CPO buccal film

The optimized formulations subjected to short term stability study were evaluated for physical appearance, bioadhesion, drug content, drug release pattern, % swelling and anti fungal activity. There was no alteration in physical appearance of batch CO3. There was no significant change in drug release profiles before and after stability study period. (f2 = 78.38±0.017, t test, P value= 0.029). Table 5 depicts the results of various characteristics parameters of batch CO3 before and after 1, 2 and 3 months. The results were found to be within the official limits

CONCLUSION

It can be concluded from the study that combination of HPMC K4M with NaCMC yielded a suitable design of buccal film of CPO with satisfactory bioadhesion, swelling and drug release. The short term stability study revealed stable characteristics of CPO buccal film. Present study showed the in-vitro efficacy of buccal bioadhesive films of CPO against various fungi strains for prolonged period of time.

Table 3: Evaluation of final CPO buccal films

Batch No	% Drug Content	Bioadhesion (N)	Folding endurance (times)	Tensile strength (gm)	Percent elongation at break (%)	Swelling % after 10 min	Thickness
CO1	99.73±0.07	0.59 ± 1.11	124	24	7	150.2	0.50 ± 0.08
CO2	99.12 ± 0.04	0.66 ± 0.75	131	29	8	133.4	0.78 ± 0.20
CO3	98.92 ± 0.2	0.63 ± 1.20	139	33	8	142.6	0.98 ± 0.29
CO4	99.23 ± 0.15	0.54 ± 2.25	142	36	9	107.5	0.99 ± 0.31

Table 4: Antifungal activity of CPO buccal film against various fungal strains

Antifungal Organisms	Diameter of Zone of Inhibition (mm) CPO buccal film	Diameter of Zone of Inhibition (mm) Distilled water
C.albicans	47 ± 0.5	4 ± 1
C.glabrata	44 ± 0.8	3 ± 0.5
C.tropicalis	43 ± 0.7	3 ± 1

Table5: Results of short term stability study of CPO buccal film

Characteristics	Batch CO3			
	0 month	1 month	2 month	3 month
Bioadhesion	0.63 ± 1.20	0.61 ± 1.50	0.65 ± 0.95	0.62 ± 1.30
Drug Content (%)	98.92 ± 0.2	98.77 ± 0.4	99.10 ± 0.4	98.72 ± 0.02
$T_{90\,\%}$ (hrs)	5.93	6.05	5.98	6.10
% Swelling	146.9	144.2	145.5	146.3
pH	6.8	6.8	6.7	6.8
Zone Inhibition (mm) (*C.albicans*)	47 ± 0.6	47 ± 0.4	46 ± 0.8	47 ± 0.5

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