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## Development and Evaluation of Mucoadhesive Films of Miconazole Nitrate

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The aim of the study was to develop and evaluate mucoadhesive films of miconazole nitrate for the treatment of oral candidosis. Films were prepared by casting procedure using various polymer combinations and were evaluated for their *in vitro* bioadhesive performance and release characteristics. The *in vitro* adhesion time and release behaviour were found to be a function of the type of polymer used. The formulation containing Carbopol 934P and Hydroxy propyl cellulose-M combination was found to give the best results. Placebo films prepared on aluminium foil showed adequate comfort, non-irritancy and taste compliance, etc. when tested on healthy human volunteers.

Oral candidosis is one of the most common pathological conditions affecting the oral mucosa<sup>1-4</sup>. An increasing spectrum of antifungal agents, including imidazoles, are available for treatment and suppression of this common infection, as lozenges, mouthwashes, troches and oral gels. However, these have the disadvantage of an initial burst of activity followed by a rapid decrease in concentration to below therapeutic levels and are incapable of maintaining the salivary concentration for a prolonged period<sup>5-6</sup>. In order to prolong the residence time of the drug in the oral cavity, mucoadhesive films with slow release properties were developed<sup>7-10</sup>. Lot of work has been done on the formulation of tablets containing miconazole<sup>11-14</sup> but films containing this drug have not been prepared.

These films contained mucoadhesive hydrocolloid polymers along with the drug dissolved in suitable solvent systems. The films were characterised on the basis of their uniformity, homogeneity, flexibility and clarity. The final optimized film maintained the M.I.C. value against *Candida albicans* (5-10 µg/ml) for about 6 h.

### MATERIALS AND METHODS

Miconazole nitrate was obtained as a gift sample

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from Gufic India Ltd., Mumbai. Hydroxy propyl methyl cellulose-E4M (HPMC E4M), hydroxy propyl cellulose-M, (HPC-M) and Carbopol-934P were obtained as gift samples from Ranbaxy Labs. Ltd., Gurgaon. Hydroxy propyl methyl cellulose-10 cps (HPMC 10 cps) and hydroxy propyl cellulose-L (HPC-L) were gifted by Max India Ltd., New Delhi. Hydroxy propyl methyl cellulose-E 15 (HPMC E 15) was obtained as a gift sample from Panacea Biotec Ltd., New Delhi. Other materials used in the study were of analytical grade and procured from commercial sources.

### Polymer interference study:

To eliminate the possibility of polymers interfering with the analysis of drug, 0.05% w/v polymer solutions were prepared and analyzed colorimetrically at 618 nm.

### Preparation of films:

The films were prepared using various polymers and the compositions are given in Table 1. Carbopol 934P was dispersed by continuous stirring for 2 h in 8 ml dichloromethane. The other polymers were separately dispersed in 4 ml of isopropanol and the two polymer solutions were mixed by stirring for about 2 h. Propylene glycol (0.15 ml) was added to the polymer solution and was stirred for about 4 h. The polymer solutions contain-

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TABLE 1 : COMPOSITIONS OF VARIOUS FORMULATIONS\*

S. No.	INGREDIENTS	QUANTITY (in mg)				
		X-1	X-2	X-3	X-4	X-5
1.	Carbopol 934p	120	120	60	80	100
2.	Hydroxy propyl methyl cellulose E 15	150	—	—	—	—
3.	Hydroxy propyl methyl cellulose E 4M	—	100	—	—	—
4.	Hydroxy propyl cellulose-L	—	—	240	—	—
5.	Hydroxy propyl cellulose-M	—	—	—	300	—
6.	Hydroxy propyl methyl cellulose 10 cps	—	—	—	—	250
7.	Miconazole nitrate	208.8	208.8	208.8	208.8	208.8
8.	Propylene glycol	0.15	0.15	0.15	0.15	0.15
9.	Ethanol	—	—	—	12 ml	—
10.	Isopropanol Dichloromethane (1:2)	12 ml	12 ml	12 ml	—	12 ml

\*The table shows the compositions of different mucoadhesive formulation; X-1 : CP 934P and HPMC E15; X-2: CP 934P and HPMC E4M; X-3: CP 934P and HPC-M; X-4: CP 934P and HPC-L; X-5: CP 934P and HPMC 10cps

ing the drug were poured into glass rings which were kept on two different substrates namely mercury and aluminium. The solutions were covered with inverted funnels, the ends of which were plugged with cotton wool to allow uniform evaporation of the solvents. After drying at room temperature for 24 h, the films were retrieved intact by slowly lifting the ring from the surface of the substrate. Small patches of 14 mm diameter were punched out from the films. Each patch contained 10 mg of miconazole nitrate.

#### ***In vitro* test apparatus:**

The apparatus was based on the modification of a flow device cell and a dissolution apparatus. The apparatus consisted of a flow controlling device, flask, and a flow through cell<sup>15</sup>. The flow through cell was constructed of glass and was closed at both ends. A small inlet tube of 0.5 cm diameter was attached to one end of the cell and another outlet tube of the same diameter was attached to the opposite end. In the middle of the base there was cavity for the placement of mucosal membrane (lower chamber). A portion of the top of the cell was removable in order to keep the mucosal membrane and to place a mucoadhesive formulation on top of it

(upper chamber). The drug release occurred from only one side of the formulation (flat surface and perimetric edges).

Isotonic Phosphate Buffer (IPB) (pH 6.6) simulating the salivary pH was continuously pumped at a flow rate of 0.65 ml/min using a small pump and flow regulators. The flow rate chosen corresponded to the mean saliva flow rate. The whole assembly was maintained in a hot air oven maintained at 37°.

#### ***In vitro* drug release was studied and was determined duration of bioadhesion/erosion :**

A strip of the mucosal membrane was washed with IPB pH 6.6 and kept in the central cavity of the flow through cell and the membrane was stabilised with IPB in order to remove soluble components. After stabilisation the tablet/film was stuck on the mucosal membrane using 25 µl of IPB and a weight of 10 g which was removed after 30 s. The two chambers were secured with the help of springs. The flow through cell was fixed at an angle of 40° in such a manner, that the tablet/film attached to the membrane was in a position just below the flow of dissolution fluid. IPB pH 6.6 was taken in a flask and a flow controlling device was adjusted to give a flow rate of 0.65 ml/min. Four millilitre samples were collected at fixed

TABLE 2 : IMPORTANT PARAMETERS OF MUCOADHESIVE FILMS

Formula Code	Bioadhesive Strength (g) ( $\pm$ S.D.)	Adhesion Time (min.) ( $\pm$ S.D.)	Surface pH ( $\pm$ S.D.)	Folding Endurance ( $\pm$ S.D.)	% Elongation at Break ( $\pm$ S.D.)
X-1	10.66 ( $\pm$ 0.68)	271 ( $\pm$ 0.12)	6.51 ( $\pm$ 0.076)	252.66 ( $\pm$ S.D.)	20.92 ( $\pm$ 0.15)
X-2	11.46 ( $\pm$ 0.157)	298.33 ( $\pm$ 0.312)	6.84 ( $\pm$ 0.066)	> 250	28.96 ( $\pm$ 0.183)
X-3	15.18 ( $\pm$ 0.121)	303.33 ( $\pm$ 0.23)	6.64 ( $\pm$ 0.016)	> 250	39.42 ( $\pm$ 0.24)
X-4	17.91 ( $\pm$ 0.101)	296.66 ( $\pm$ 0.14)	6.33 ( $\pm$ 0.019)	> 250	38.72 ( $\pm$ 0.34)
X-5	8.68 ( $\pm$ 0.015)	191.66 ( $\pm$ 0.11)	6.23 ( $\pm$ 0.019)	> 250	19.65 ( $\pm$ 0.234)

\*The table lists the various physical evaluation parameters of all the mucoadhesive films. Experiments were carried out in triplicate; X-1 : CP 934P and HPMC E15; X-2: CP 934P and HPMC E4M; X-3: CP 934P and HPC-M; X-4: CP 934P and HPC-L; X-5: CP 934P and HPMC 10cps

time intervals. Each sample was filtered through Whatman filter paper (No. 42). To 2.0 ml of the sample, 10 ml chloroform was added for the extraction of the drug. To this, 10 ml of the colouring reagent was added<sup>16</sup>. The chloroform layer was separated using a separating funnel and analysed colorimetrically. Graphs were plotted between the concentration of miconazole nitrate in the sample and time. Maximum concentration of miconazole nitrate attained [ $C_{max}$ ], time to reach the maximum concentration [ $t_{max}$ ], time period for which the concentration remained above the minimum inhibitory concentration for *Candida albicans* (5-10  $\mu$ g/ml) [ $T^{MIC}$ ] and area under the concentration time curve upto the last sampling time [ $AUC_{0-t_n}$ ], were calculated using the trapezoidal rule.

$$AUC_{0-t_n} = \frac{[C_0 + C_1] [t_1 - t_0]}{2} + \frac{[C_1 + C_2] [t_2 - t_1]}{2} + \dots + \frac{[C_{n-1} + C_n] [t_n - t_{n-1}]}{2}$$

The duration of bioadhesion or erosion was determined by measuring the time required for the formulation to erode completely or the time for which the formulation was maintained at its position without dislodging.

#### Measurement of bioadhesive strength:

Bioadhesive strength was determined using a modified double beam balance as described by Gupta *et al.*<sup>17</sup>

#### Measurement of percentage elongation:

A simple apparatus was used for the determination of longitudinal strain/percentage elongation at break as described by Khanna *et al.*<sup>19</sup>. The parameters Longitudinal strain (LS) (increase in length/initial length) and percentage elongation at break (LS x 100) were calculated.

#### Measurement of folding endurance:

A small strip of film (2 cm x 2 cm) was cut evenly and repeatedly folded at the same place till it broke. The number of time, the film could be folded at the same place, without breaking, gave the value of folding endurance.

#### Measurement of surface pH :

An acidic or alkaline formulation is bound to cause irritation on the mucosal membrane and hence this parameter assumes significance while developing a mucoadhesive formulation. The surface pH was determined by the method similar to that used by Bottenberg *et al.*<sup>18</sup> A combined glass electrode was used for this purpose. The films were kept in contact with 0.5 ml of distilled water for 1 h. pH was noted by bringing the electrode near the surface of the formulations and allowing it to equilibrate for 1 min.

TABLE 3 : *IN VIVO* EVALUATION CRITERIA

Score	Irritancy	Taste	Comfort	Dry Mouth	Salivation	Heaviness at the Place of attachment
0	None	Normal	Very Comfortable	None	None	None
1.	Slight	Unpleasant (but acceptable)	Comfortable	Slight	Slight	Slight
2.	Moderate	Very unpleasant	Slightly uncomfortable	Moderate	Moderate	Moderate
3.	Severe	Pleasant	Moderately uncomfortable	Severe	Severe	Severe
4.	—	Very pleasant	Severely uncomfortable	—	—	—

Score criteria for irritancy, taste, comfort, dry mouth, salivation and heaviness at the place of attachment.

***In vivo* evaluation of placebo mucoadhesive films:**

*In vivo* evaluation of placebo mucoadhesive films was carried out. The aim of the study was to determine the time of erosion/adhesion of selected mucoadhesive films, to investigate the acceptability of different polymers for use in mucoadhesive films and to determine any irritation or side effects produced by the formulations.

The study was conducted in five healthy human volunteers (aged 21 to 28 y). Informed consent was obtained from the volunteers before the study. Mucoadhesive films (prepared using aluminium foil as the substrate) X-1, X-2, X-3, X-4 and X-5, without the drug were used in the study. Food was prohibited from 0.5 h before till the end of the study. Water was provided as and when required.

The volunteers were given different coded films, along with written instruction sheets. They were instructed to press the films against the palate for about 30 s without moistening the films before application. The volunteers were asked to record the time of film insertion and the time and circumstances of the end of adhesion (erosion or dislodgement of the films). The volunteers were asked to complete a questionnaire after trial period for scoring irritancy, comfort, taste, dry mouth, salivation and heaviness at the place of attachment produced by formulations (Table 3). The results of the study are presented in Tables 3 and 4.

**Data treatment :**

Experiments in triplicate were carried out for all the studies performed. Analysis of the data generated was done to obtain the arithmetic mean and standard deviation at 5% level of significance.

**RESULTS**

The polymer combinations which exhibited satisfactory film forming properties were selected and taken up for further studies. The films so selected were loaded with the drug and their bioadhesive strength, duration of bioadhesion/erosion, release capability, surface pH, folding endurance and percentage elongation at break were evaluated.

***In vitro* drug release and duration of bioadhesion:**

The results of *in vitro* release studies from the films prepared using different polymers are shown in Fig. 1. The studies show that high concentrations of miconazole nitrate could be achieved using these films. It is evident that among the polymers studied, films containing hydroxy propyl cellulose-M (HPC-M) and carbopol 934P (CP 934P), code X-3, released the drug in a sustained manner. The concentration of the drug released from these films was well above the MIC for the entire period of study. Cmax was highest for this formulation, i.e. 90 µg/ml. This was followed by films made from CP 934P and HPMC-E4M, code X-2 and films made from CP 934P and HPMC 10 cps, code X-5. In case of formulation X-2,

TABLE 4 : VOLUNTEERS RESPONSE TO *IN VIVO* EVALUATION OF MUCOADHESIVE FILMS

Formula Code	Mean		Mean Score* ( $\pm$ S.D.)				
	Adhesion time ( $\pm$ S.D.)	Irritancy	Taste	Comfort	Dry Mouth	Salivation	Heaviness at the place of attachment
X-1	235 ( $\pm$ 5.0)	0 ( $\pm$ 0)	(0 $\pm$ 0)	1.33 ( $\pm$ 0.57)	0.33 ( $\pm$ 0.57)	0 ( $\pm$ 0)	0 ( $\pm$ 0)
X-2	278.33 ( $\pm$ 5.56)	0 ( $\pm$ 0)	(0 $\pm$ 0)	1.33 ( $\pm$ 0.57)	0 ( $\pm$ 0)	0 ( $\pm$ 0)	0 ( $\pm$ 0)
X-3	265 ( $\pm$ 4.04)	0 ( $\pm$ 0)	0.33 ( $\pm$ 0.57)	1.33 ( $\pm$ 0.57)	1 ( $\pm$ 1) ( $\pm$ 0)	0	0 ( $\pm$ 0)
X-4	269 ( $\pm$ 2.08)	0 ( $\pm$ 0)	0.66 ( $\pm$ 0.57)	1 ( $\pm$ 0)	0.66 ( $\pm$ 0.57)	0 ( $\pm$ 0)	0 ( $\pm$ 0)
X-5	170.66 ( $\pm$ 7.08)	0 ( $\pm$ 0)	0.33 ( $\pm$ 0.57)	1 ( $\pm$ 0)	0.66 ( $\pm$ 0.57)	1.33 ( $\pm$ 0.57)	0 ( $\pm$ 0)

\*Response to placebo mucoadhesive films in terms of score criteria starting from a minimum 0 to a maximum 4 (ref. Table 3)

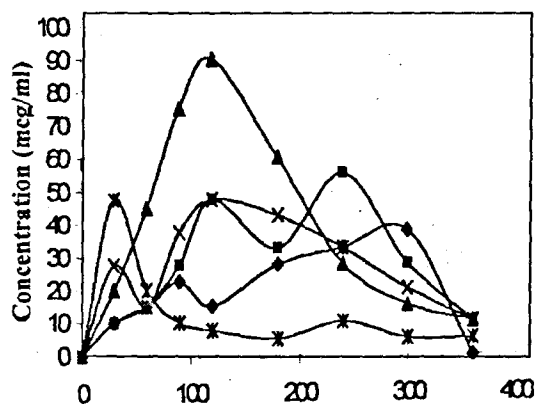


Fig. 1 : *In vitro* release curves for different mucoadhesive films

Time(min)

◆ HPMCE15    ■ HPMCE4M    ▲ HPC-M  
 ✕ HPC-L      \* HPMC-10cps

very little release occurred till 1h and a maximum concentration of 55  $\mu$ g/ml was obtained at 4 h. From films with code X-5, immediate release occurred and maximum concentration of 55  $\mu$ g/ml was obtained in 20 min. The films with code X-1 exhibited very little release with a maximum concentration of 37.5  $\mu$ g/ml in 5 h. The concentration was below the MIC till 20 min. The films with code X-4 release the drug in a sustained manner but the maximum concentration was less, i.e. 47.5  $\mu$ g/ml as com-

pared to that obtained with formulation with code X-3. The optimized formulation was chosen on the basis of values of Cmax and AUC0-6h.

**Bioadhesive strength *in vitro*:**

The bioadhesive strength of the formulation was found to be dependent upon the type of polymer used. Among the formulations, X-1, X-2, X-3, X-4 and X-5, those containing HPC-L and CP-934P (X-4), exhibited maximum bioadhesive strength followed by those containing HPC-M and CP-934P, HPMC-E4M and CP-934P, HPMC-E15 and CP-934P and HPMC-10cps and CP-934P. (Table 2, Fig. 2).

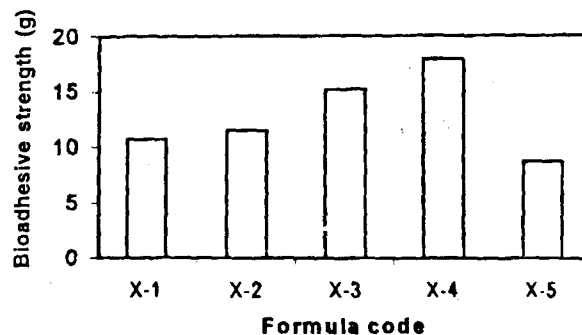


Fig 2 : Bioadhesive strength of mucoadhesive films

X-1: CP 934P and HPMC E15; X-2: CP 934P and HPMC E4M;  
 X-3: CP 934P and HPC-M; X-4: CP 934P and HPC-L;  
 X-5: CP 934P and HPMC 10cps

The adhesion time for all the formulations ranged from 190 to 300 min. Minimum adhesion time was observed for films containing HPMC-10 cps and CP-934P (X-5) and the maximum adhesion time was observed for films containing HPC-M and CP-934P (X-3). None of the formulations dislodged due to excessive or low hydration before complete erosion. On the basis of these results, X-3 was evolved as the optimized formula.

#### Folding endurance:

The values of folding endurance for all the formulations are given in Table 2. All the films showed a folding endurance of more than 200. Since the folding endurance was measured manually, the films were folded 250 times and if the films did not show any cracks, it was taken as the end point.

#### Surface pH:

The surface pH of the films was found to be well within 1.5 units of neutral pH. The values of surface pH of all the formulations are given in Table 2. Since the pH was very close to the neutral, it was assumed that these formulations should not cause any irritation in the oral cavity.

#### Per cent elongation at break:

The values of percent elongation for all the formulations are given in Table 2. The percent elongation was maximum for films containing HPC-M and CP-934P (39.42%) and minimum for films containing HPMC-10 cps and CP-934P (19.65%), thus indicating that all the films had good tensile strength.

#### *In vivo* evaluation of placebo mucoadhesive films:

All the mucoadhesive films eroded completely and none had to be removed due to irritation. The films did not cause any discomfort to the volunteers and their taste was acceptable. No side effects like taste alteration, heaviness at the place of attachment, dry mouth or severe salivation were observed with the films.

#### DISCUSSION

The present study was aimed to develop a new mucoadhesive system for the release of miconazole nitrate. The drug is established for the treatment of oral candidosis and is widely used as a first-line treatment. The system was developed using mucoadhesive polymers. This delivery system was designed to ensure inti-

mate contact of the drug with the oral mucosa for prolonged period of time. Initially placebo films were prepared in order to determine the best combination of polymers, plasticizer and solvents required for the formation of stable films. The combinations which yielded complete, homogenous, flexible, smooth and non-sticky films were selected and loaded with drug. All the films showed satisfactory drug release, bioadhesive performance and surface pH. Since films containing CP-934P and HPC-M exhibited greater drug release and good physical and mechanical properties. These films were selected as the optimized formulation. This formulation contained 60 mg of CP 934P, 240 mg of HPC-M and 0.15 ml of propylene glycol. The films were 0.21 mm thick and of 14 mm diameter, containing 10 mg of miconazole nitrate. These films exhibited a  $C_{max}$  of 90  $\mu\text{g/ml}$  and  $AUC_{0-6h}$  of 1481.75  $\mu\text{g}\cdot\text{min/ml}$ . Although the concentration of miconazole nitrate was maintained above the MIC for a period of 6h, the time of erosion and hence  $T^{MIC}$  was about 5 h. *In vivo* evaluation of placebo mucoadhesive films in healthy human volunteers did not cause any irritation or discomfort to the volunteers and the taste was acceptable.

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