ABSTRACT
The objective of this study was to formulate and evaluate in situ topical gels of Fluconazole based temperature induced systems for the treatment of oral candidiasis. Conventional oral formulations like solution, suspension, and ointments have many disadvantages which result into poor bioavailability of drug in the buccal cavity. The poor bioavailability and therapeutic response may be overcome by the use of mucoadhesive in situ gel forming systems that are applied as liquid into the buccal cavity and undergo a sol-gel transition which have good mucoadhesion with buccal mucus layers. Mucoadhesive systems were prepared by using polaxamer 188 combined with carbapol 934 to enhance the gel bioadhesion properties. Increase in the concentration of mucoadhesive agent enhanced the mucoadhesive force significantly. In vitro release of Fluconazole from the mucoadhesive system in simulated salivary fluid was influenced significantly by the properties and concentration of carbapol 934 showed to enhance bioavailability through its longer oral residence time and ability to sustain the release of the drug.

Keywords: Mucoadhesive, Fluconazole, Polaxamer, Carbapol, Thermoreversible in situ gel, Oral drug delivery.

INTRODUCTION
Oral candidiasis is a common opportunistic infection of the oral cavity caused by an overgrowth of Candida species, the commonest being Candida albicans.

The incidence varies depending on age and certain predisposing factors. There are three broad groupings consisting of acute candidiasis, chronic candidiasis, and angular cheilitis. Risk factors include impaired salivary gland function, drugs, dentures, high carbohydrate diet, and extremes of life, smoking, diabetes mellitus, Cushing’s syndrome, malignancies, and immunosuppressive conditions. Management involves taking a history, an examination, and appropriate antifungal treatment with a few requiring samples to be taken for laboratory analysis. In certain high risk groups antifungal prophylaxis reduces the incidence and severity of infections. The prognosis is good in the great majority of cases. Oral candidiasis is an opportunistic infection of the oral cavity. It is common and under diagnosed among the elderly, particularly in those who wear dentures and in many cases is avoidable with a good mouth care regimen. It can also be a mark of systemic disease, such as diabetes mellitus and is a common problem among the immune compromised. Oral candidiasis is caused by an overgrowth or infection of the oral cavity by a yeast-like fungus, candida. [1,2]

The field of in situ forming implants has grown exponentially in recent years. in parallel to the development of new protein therapeutics. It caused by genomic information and the final mapping of the human genome [3]. A dose reduction resulting from the avoidance of peaks and valleys, as well as the enhancement of patient compliance by reducing the frequency application, are further potential benefits from a manufacturing point of view, in situ forming depot systems offer the advantage
that they are relatively simple manufacture from polymers adapted for this approach. Compared to microspheres, which have to be washed and isolated after preparation, operating expenses for the production of in situ forming applications are marginal, thus lowering investment and manufacturing costs injectable in situ forming implants are classified into four categories, according to their mechanism of depot formation:

1. Thermoplastic pastes.
2. In situ cross-linked polymer systems.
3. In situ polymer precipitation, and
4. Thermally induced gelling systems.

**MATERIALS AND METHODS**

Fluconazole and polaxamer were the kind gifts by Burgeon Pharmaceutical Pvt. Ltd., (Kancheepuram, India) and ISP (Hong Kong Ltd., Hyderabad, India), respectively. Carbopol 934 was kind gifts of Dr.Reddy’s laboratory (Hyderabad, India). Benzalkonium chloride was purchased from RFCL Ltd. (New Delhi, India). Mucin type II and Cellulose membrane were purchased from Sigma-Aldrich chemicals pvt. Ltd., New Delhi. Diethanol amine was purchased from Sisco Research Lab. (Mumbai, India). All other reagents were of analytical grade.

**Preparation of formulation**

Optimum formulation of Fluconazole In situ gel was prepared with the ratio of polaxmer 188 and carbopol 934 (15% w/w): (0.2% w/w).

**Step 1:** The method involved slow addition of polaxmer 188 and methyl paraben and solubilization in required quantity of cold distilled water.

**Step 2:** Required quantities of carbopol 934 were kept overnight for swelling. The polymer solution taken in a beaker with continuous stirring (magnetic stir) until uniform solution obtained.

**Step 3:** After the mixture had been kept at ambient Temperature for 24 hrs. a small amount of triethanolamine was added to adjust the PH 7.

**Step 4:** An appropriate amount of Fluconazole Solubilized in physiologically compatible solvent such as distilled water with continues stirring until uniform Drug solution obtained. Thermo reversible gels were prepared using cold technique.

**Step 5:** Drug solution was added to this preformed gel before invtro studies. [4]

**Evaluation of the formulations**

**Determination of pH**

The pH of the gels was determined using a calibrated pH meter. The readings were taken for average of 3.

**Gelling Capacity**

The gelling capacity of the formed gel was determined using visual inspection and the different grades were allotted as per the gel integrity, weight and rate of formation of gel with respect to time. [5]

**Viscosity Studies**

The rheological studies were carried out using Brookfield programmable DVII+Model pro II type (USA). The viscosity of in situ gels were determined at different angular. calculate the viscosity. Evaluation was conducted in triplicate.

**Spreadability**

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability (S). [6]

\[ S = \frac{ML}{T} \]

Where, M = weight tide to upper slide.
L = length moved on the glass slide.
T = time taken.

**Measurement of Gel Strength:**

A sample of 50 gm of gel was placed in a 100 ml graduated cylinder and gelled in a thermostat at 37ºc. The apparatus for measuring gel strength (weight of apparatus as shown in figure 1, weighing 27 gm) was allowed to penetrate in buccal gel. The gels strength, which means the viscosity of the gels at physiological temperature, was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel. The gels at physiological temperature, was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel.[7]

**Fig 1. Gel strength determination device**
Determination of mucoadhesive Force

As shown in fig 2 The mucoadhesive force of all the optimized batches was determined as follows, a section of mucosa was cut from the chicken cheek portion and instantly fixed with mucosal side out onto each glass vial using rubber band. \[8\] The vial with chicken cheek mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. \[8\] Oral gel was added onto the nasal mucosa of first vial. Before applying the gel, 150μL of simulated saliva solution (2.38 gm Na$_2$HPO$_4$, 0.19 gm KH$_2$PO$_4$ and 8 gm NaCl in 1000 ml of distilled Water. adjusted to pH 7.4) was evenly spread on the surface of the test memberane. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given. Then weight was kept rising in the pan until vials get detached.

Mucoadhesive force was the minimum weight required to detach two vials. The cheek mucosa was changed for each measurement.

\[
\text{Detachment stress (dynes/cm²) = } \frac{mg}{A}
\]
Where, \(m\) is the weight added to the balance in grams;
\(g\) is the acceleration due to gravity taken as 980 cm/s²;
\(A\) is the area of tissue exposed, i.e 8.14cm².

All the above mentioned experiments were carried out in triplicates.

Fig 2. Mucoadhesive Force measuring device

(A) Modified Balance, (B) Weights (C) Glass vial (D) Cicloprox olamine in situ gel (E) Chicken cheek Mucous membrane (F) Height adjustable pan.

Chicken cheek mucosa

Buccal cavity of Isolation of chicken cheek mucosa from the chicken – the cheek of a healthy chicken was obtained from the local slaughter house. It was cleaned and the mucosa was re-moved from the buccal cavity. The mucosa was stored in normal saline with few drops of gentamycin sulphate injection, to avoid bacterial growth. After the removals of blood from the mucosal surface it becomes ready for use. \[9\]

In vitro release kinetics

Diffusion Medium

The diffusion medium used was phosphate buffer pH 7.4 Assembly of diffusion cell- For in – vitro diffusion studies the oral diffusion cell was designed as per the dimension given. The diffusion cells were placed on the magnetic stirrers. The outlet of the reservoir maintained at 37±0.5°C and was connected to water jacket of diffusion cell using rubber latex tubes. The receptor compartment was filled with fluid. Then the prepared chicken cheek mucosa was mounted on the cell carefully so as to avoid the entrapment of air bubble under the mucosa. Intimate contact of mucosa was ensured with receptor fluid by placing it tightly with clamp. The speed of the sitting was kept content throughout the experiment. With the help of pipette 1ml of sample was withdrawn at a time intervals of one hour from sampling port of receptor compartment and same volume was the replaced with receptor fluid solution in order to maintain sink condition. The samples were appropriately diluted and the absorbance was measured at 244 nm for miconazole gel and for fluconazole gel 266.16 nm using Shimadzu 1700 UV-VIC Spectrophotometer. Here the surface area of the chicken mucosa exposed to the receptor fluid is 6.15 cm² \[10\]

Release kinetics

To analyze the in vitro release data various kinetic models were used to describe the release kinetics. The zero order rate Eq. (1) describes the systems where the drug release rate is independent of its concentration (Hadjiioannou 1993). The first order Eq. (2) describes the release from system where release rate is concentration dependent (Bourne, 2002). Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Eq. (3). The Hixson-Crowell cube root law Eq. (4) describes the release from systems where there is a change in surface area and diameter of particles or tablets (Hixson and Crowell, 1931).

\[
C = k^0t \quad (1)
\]
Where, \(k^0\) is zero-order rate constant expressed in units of concentration/time and \(t\) is the time.

\[
\log C = \log C_0 - \frac{kt}{2.303} \quad (2)
\]
Where, \(C_0\) is the initial concentration of drug and \(k\) is first order constant.

\[
Q = Kt^{1/2} \quad (3)
\]
Where, \(K\) is the constant reflecting the design variables of the system.

\[
Q_0^{1/3} - Q_t^{1/3} = K t \quad (4)
\]
Where, Qt is the amount of drug released in time t, Q0 is the initial amount of the drug in tablet and \( K_{HC} \) is the rate constant for Hixson-Crowell rate equation. [11]

The following plots were made:
1. Cumulative % drug release vs. time (zero order kinetic model);
2. Log cumulative of % drug remaining vs. time (first order kinetic model);
3. Cumulative % drug release vs. square root of time (higuchi model);
4. Log cumulative % drug release vs. log time (korsmeyer model) and
5. Cube root of drug % remaining in matrix vs. time (hixson-crowell cube root law).

And \( R^2 \) values are tabulated in table no.20 and graphs are shown in graphs no’s.

RESULTS AND DISCUSSION

Determination of pH
The pH of the gel was determined using a calibrated pH meter. Average pH was found to be 6.1.

Gelling Capacity
The two main prerequisites of an in situ gelling system are viscosity and gelling capacity. All the optimized formulation was found to be good gelling capacity. The formulation F4 exhibited good gelling immediately remains for after 8 hour’s. In comparison with F4 the formulation F2, F1 showed (moderate) gelling capacity remains few hour. In comparison with F1 and F2 the formulation F3, F4 found to be good gelling (stiff) capacity remain for extended period of time.

Gel Strength
All three formulations (F2, F3 and F4) exhibited good gel strength. This may be due to increase concentration of Polaxamer and carbopol 934.

Mucoadhesive Force
The mucoadhesive force is an important physicochemical parameters for prolonging buccal retention time and there by better therapeutic effects. Detachment stress of F4 was found to more in comparison with F1 formulation. The formulation F2, F3, F4 showed more mucoadhesive force than F1. This may be due to increased concentration of Polaxamer and Carbopol 934 in the formulation.

Spreadability
F4 showed good spread ability as compared with the any other formulation. Comparing F3 and F4 showed good spread ability by comparing with F1 and F2.

Gelling temperature
Gelling temperature of the formulation-1 which I prepared by thermo reverse gelation was found to be 37°C.

In vitro Release Studies
The in vitro dissolution profile of drug from in situ gels containing different composition of \((a: c:b, a:c:b, a:b: c)\). The formulation f3 and f4 containing the three polymer ratio \((0.3:0.3:0.2, 0.4:0.4:0.2)\). which showed the release profile up to 8 hours with 66.316% release and 7 hours with 60.00%. In comparison the gels containing \((0.2:0.2:0.2),(0.2:0.3:0.2)\) Polymers in situ system \((F1,F2)\) were showed 74.241 and 91.579% of release profile within 8 hours.

Invitro chicken cheek diffusion studies were carried out for different formulations. The % drug release values for the formulations are tabulated.

- F-1 shows 74.241% drug release in 8 hrs
- F-2 shows 91.579% drug release in 8 hrs
- F-3 shows 66.316% drug release in 8 hrs
- F-4 shows 59.21% drug release in 8 hrs
- F-5 shows 66.316% drug release in 8 hrs

The release data analysis was carried out using the various kinetic models i.e using cumulative % drug release vs. time (zero order kinetic model); log cumulative % drug remaining vs. time (first order kinetic model); cumulative % drug release vs. square root of time (higuchi model); log cumulative % drug release vs. log time (korsmeyer model) and cube root of drug % remaining in matrix vs. time (hixson-crowell cube root law).

The R2 values are tabulated in table no.20, 21. Different plots using the various models are represented.

For formulation F-1 the R2 values for Zero order plot is 0.995, First order plot is 0.974, Higuchi model plot is 0.982, Korsmeyer model plot is 0.965 and Hixon-crowell model plot is 0.990. The best linearity was found in zero order release model.

For formulation F-2 the R2 values for Zero order plot is 0.983, First order plot is 0.867, Higuchi model plot is 0.947, Korsmeyer model plot is 0.966 and Hixon-crowell model plot is 0.938. The best linearity was found in zero order release model.

For formulation F-3 the R2 values for Zero order plot is 0.997, First order plot is 0.960, Higuchi model plot is 0.973, Korsmeyer model plot is 0.994 and Hixon-crowell model plot is 0.936. The best linearity was found in Zero order release model.
For formulation F-4 the $R^2$ values for Zero order plot is 0.997, First order plot is 0.980, Higuchi model plot is 0.973, Korsmeyer model plot is 0.987 and Hixon-crowell model plot is 0.991. The best linearity was found in Zero order release model.

Table 1. Composition of Different formulations

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Ingredients (W/W)%</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
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<tbody>
<tr>
<td>1.</td>
<td>Fluconazole</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>2.</td>
<td>Polaxamer 188</td>
<td>18</td>
<td>19</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>3.</td>
<td>Carbopol 934 (c)</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
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<tr>
<td>4.</td>
<td>Alcohol</td>
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<td>2</td>
<td>2</td>
<td>2</td>
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<tr>
<td>5.</td>
<td>Methyl paraben</td>
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<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>6.</td>
<td>Deionized water</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
<td>q.s</td>
</tr>
<tr>
<td>7.</td>
<td>Gelling capacity</td>
<td>*</td>
<td>**</td>
<td>***</td>
<td>***</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of optimized gel

<table>
<thead>
<tr>
<th>Formulation</th>
<th>pH</th>
<th>Viscosity (cps)</th>
<th>Mucoadhesive force (dynes/cm²)</th>
<th>Gel strength (sec)</th>
<th>Spreadability gms/sec</th>
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<tbody>
<tr>
<td>F1</td>
<td>6.1</td>
<td>24,000</td>
<td>13678</td>
<td>99</td>
<td>24.0</td>
</tr>
<tr>
<td>F2</td>
<td>6.0</td>
<td>27,500</td>
<td>17844</td>
<td>110</td>
<td>27.5</td>
</tr>
<tr>
<td>F3</td>
<td>6.0</td>
<td>31,000</td>
<td>18,594</td>
<td>113</td>
<td>29.2</td>
</tr>
<tr>
<td>F4</td>
<td>6.1</td>
<td>33.750</td>
<td>18,760</td>
<td>115</td>
<td>30.5</td>
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</table>

Table 3. Drug release studies of different formulations

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>% Drug Release Formulation-1</th>
<th>% Drug Release Formulation-2</th>
<th>% Drug Release Formulation-3</th>
<th>% Drug Release Formulation-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>1</td>
<td>14.211</td>
<td>25.263</td>
<td>12.632</td>
<td>11.053</td>
</tr>
<tr>
<td>2</td>
<td>20.526</td>
<td>34.737</td>
<td>22.105</td>
<td>15.789</td>
</tr>
<tr>
<td>3</td>
<td>33.158</td>
<td>44.211</td>
<td>28.421</td>
<td>23.684</td>
</tr>
<tr>
<td>4</td>
<td>42.632</td>
<td>50.526</td>
<td>34.737</td>
<td>31.579</td>
</tr>
<tr>
<td>5</td>
<td>50.526</td>
<td>56.842</td>
<td>42.632</td>
<td>38.684</td>
</tr>
<tr>
<td>6</td>
<td>58.421</td>
<td>75.789</td>
<td>50.526</td>
<td>44.211</td>
</tr>
<tr>
<td>7</td>
<td>69.74</td>
<td>86.842</td>
<td>60.000</td>
<td>52.105</td>
</tr>
<tr>
<td>8</td>
<td>74.241</td>
<td>91.579</td>
<td>66.316</td>
<td>59.211</td>
</tr>
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</table>

Table 4. Release parameters of different formulations

<table>
<thead>
<tr>
<th></th>
<th>Zero order model</th>
<th>First order model</th>
<th>Higuchi model</th>
<th>Korsmeyer model</th>
<th>Hixon-Crowell model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation – 1</td>
<td>$R^2$ 0.995</td>
<td>0.974</td>
<td>0.982</td>
<td>0.965</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>m 9.267</td>
<td>0.082</td>
<td>35.90</td>
<td>0.633</td>
<td>-0.216</td>
</tr>
<tr>
<td></td>
<td>c 4.708</td>
<td>2.060</td>
<td>-26.27</td>
<td>1.363</td>
<td>4.697</td>
</tr>
<tr>
<td>Formulation – 2</td>
<td>$R^2$ 0.983</td>
<td>0.867</td>
<td>0.947</td>
<td>0.966</td>
<td>0.938</td>
</tr>
<tr>
<td></td>
<td>m 0.082</td>
<td>0.160</td>
<td>0.634</td>
<td>0.051</td>
<td>-0.315</td>
</tr>
<tr>
<td></td>
<td>c 4.708</td>
<td>2.060</td>
<td>-20.45</td>
<td>1.374</td>
<td>4.736</td>
</tr>
<tr>
<td>Formulation – 3</td>
<td>$R^2$ 0.997</td>
<td>0.960</td>
<td>0.973</td>
<td>0.994</td>
<td>0.936</td>
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<tr>
<td></td>
<td>m 7.908</td>
<td>0.063</td>
<td>30.51</td>
<td>0.788</td>
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</tr>
<tr>
<td></td>
<td>c 5.020</td>
<td>2.042</td>
<td>-21.62</td>
<td>1.092</td>
<td>4.650</td>
</tr>
<tr>
<td>Formulation – 4</td>
<td>$R^2$ 0.997</td>
<td>0.968</td>
<td>0.969</td>
<td>0.981</td>
<td>0.991</td>
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<tr>
<td></td>
<td>m 7.375</td>
<td>0.059</td>
<td>28.34</td>
<td>0.655</td>
<td>-0.146</td>
</tr>
<tr>
<td></td>
<td>c 9.046</td>
<td>2.007</td>
<td>-15.33</td>
<td>1.200</td>
<td>4.661</td>
</tr>
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</table>
CONCLUSION
The present work was carried out to develop a Novel in situ gum based buccal drug delivery system by using thermo reverse gelation technique. The methodology adopted for preparation of in situ gel solution was very simple and cost effective. It is newer approach to improve easy buccal instillation residence time and prolong drug release. From the study conducted, the following conclusion were drawn the gels which was prepared by using the technique thermo reverse gelation with fluconazole shown good antifungal activity by in vitro and showed sustained release and in vivo by comparing with the standard co-trimoxazole gel. The In situ systems showed increased residence time and prolonged drug release for over 8 hrs.

REFERENCES