Discussion

5. DISCUSSION.

The aim of this work was to develop a tablet for the buccal delivery of the poorly water-soluble drug Posaconazole, for that Solubilization of Posaconazole by complexation with β-Cyclodextrin and then delivery via buccal mucosa using Buccal tablets of Posaconazole to release drug at mucosal site in unidirectional pattern for extended period of time without wash out of drug by saliva. Hydroxypropyl methylcellulose K4M and carbopol 934P were selected as mucoadhesive polymers on the basis of their matrix forming properties and mucoadhesiveness while ethyl cellulose being hydrophobic, as backing material. Ethyl cellulose has recently been reported as excellent backing material, given its low water permeability and moderate flexibility.

6.1. Complexation of Posaconazole with β-Cyclodextrins:-

A. Phase Solubility Analysis of Posaconazole with β-Cyclodextrins:
The observations for phase solubility analysis are shown in Table- 12 and phase solubility diagram in Figure- 19.

This plot indicates linear rise in the solubility of the drug as a function of β-cyclodextrin concentration. Hence, the solubility plot of Posaconazole in the presence of β- cyclodextrin can be classified as A_L type. The linear host – guest complexation plot with slope less than 1 also suggested formation of 1:1 (Posaconazole: β-cyclodextrin) complex with β-cyclodextrin. The apparent solubility constant, K_{1:1}, obtained from the slope of linear portion of phase solubility plot was 6461.8355 M^{-1}. These values suggest good stability of Posaconazole - β-cyclodextrin complexes at 1:1 molar ratios.

B. Preparation of inclusion complexes of Posaconazole with β-cyclodextrin.

Inclusion complexes of Posaconazole were prepared in 1:1M ratios with β-Cyclodextrin using kneading method. The prepared complex was free flowing and off white in colour.

C. Characterization of Complex

FT-IR spectral analysis: The IR spectra of inclusion complexes correspond to superimposition of the spectra of Posaconazole and β-CD, with no significant shift in the major functional group peaks corresponding to Posaconazole. However, there was
Discussion

reduction in intensity of Posaconazole peak in complex which was occurred by cyclodextrin peaks indicating formation of inclusion complex.

**In Vitro Dissolution Studies of Posaconazole and complex:** The Posaconazole complexes with β-CD presented better dissolution performance over pure drug in an *in vitro* test. According to these results, inclusion complexes prepared using with β- Cyclodextrin, at 1:1M ratio showed about 100 % drug release in 80min.

**DSC Study:** - The thermal behavior of the cyclodextrin inclusion complexes was studied using DSC in order to confirm the formation of solid inclusion complexes. When the guest molecules are incorporated in cyclodextrin cavity or in the crystal lattice, their melting, boiling and sublimation points usually shifted to a different temperature or disappear within the temperature range, where the cyclodextrin lattice is decomposed.

DSC graph of Posaconazole reveals sharp melting endotherm with onset at 160 °C. DSC graph of β-Cyclodextrin revealed that endothermic peak at 162°C. In case of Posaconazole- β-Cyclodextrin Complex there was marked reduction in the intensity of the Posaconazole endotherm at around 162.90°C suggesting the successful inclusion of Posaconazole in the β- Cyclodextrin cavity. DSC studies also confirm amorphous state of both drug and cyclodextrin, no interaction between drug and cyclodextrin.

**X-RD Study:** - The X-ray diffractrogram in Fig.23 of Posaconazole- β-Cyclodextrin Complex in comparison with Posaconazole shows fewer and less intense peaks which indicate that complex are markedly less crystalline than Posaconazole.

**6.2. Compatibility Study in Drug and excipients used in Formulations by FT-IR**

In figure- 24 to 28, The IR spectra did not show any significant difference from those obtained for their physical mixtures. These obtained results indicate that there was no positive evidence for the interaction between Posaconazole and the utilized materials more than hydrogen bonding (if any), which may have occurred between donating and accepting groups of both the drug and the utilized buccoadhesive materials. These results clearly indicate the usefulness of the utilized materials for preparation of buccoadhesive tablets of Posaconazole.
6.3. Evaluation for Buccal tablets of Posaconazole:- The prepared buccal tablets were evaluated for thickness, hardness, friability, uniformity of weight, uniformity of drug content, surface pH determination, in vitro bioadhesive strength measurement, Mucoadhesion time measurement, swelling index, in vitro dissolution, in vitro drug permeation study and stability study.

Optimization of formulation has been done by using $3^2$ full factorial designs after evaluating the preliminary data obtained from seven batches of formulation ($T_1$ to $T_7$). Polynomial equations were derived for $t_{50\%}$, $t_{70\%}$ and swelling index (after 6 hours) values by backward stepwise linear regression analysis using PCP Disso 2000 V3 software. Validity of derived equations was verified by preparing two check point formulations of intermediate concentrations ($C_1$ and $C_2$).

Physicochemical Properties: - It could be observed that all the prepared tablets fulfill the IP requirements for Physicochemical Properties. The flow pattern for trial formulations powder beds was found to be passable while increasing the amount of lubricants in factorial formulations gives excellent flow pattern for power beds of factorial design batches. The hardness of prepared buccal tablets was found to be in the range of 4.14 to 4.71 kg/cm$^2$. Thickness was in the range of 2.8 to 3.3 mm. The friability of all tablets was less than 1% i.e. in the range of 0.26 to 0.68 %. The percentage deviation from mean weights of all the batches of tablets was found to be within the prescribed limits as per IP. The low values in standard deviation indicates uniform drug content in all the batches prepared as observed from data table given in table 14-16.

Mucoadhesion time:-

Mucoadhesion time of tablets increases with increase in polymer content. Mucoadhesion test was performed using sheep buccal tissue. The time for tablet to detach from buccal tissue was recorded as mucoadhesion time. Formulations containing polymer HPMC alone like $T_1$, $T_2$ exhibited less mucoadhesion time (1 to 3 hrs) but the formulations containing carbopol alone and along with HPMC like $T_3$, $T_4$, $T_5$, $T_6$, $T_7$ and all the factorial formulations i.e. $F_1$ to $F_9$ exhibited mucoadhesion time more than 12 hours.
Discussion

Bioadhesive Strength Measurement:-

Bioadhesion strength measurement of tablets indicated that the bioadhesive strength was proportional to carbopol content. The mean bioadhesive strength values after 3 min of contact time was 0.2934 N for formulation F1. The values of bioadhesive strength Table-22., Fig-33-34. were decreased in the following order: T7>F9>T6>F6>C2>F3>T5>C1>F8>F5>T3>F2>F7>F4>F1>T2>T1. Therefore, increasing carbopol concentration increases the bioadhesion. This increase in the bioadhesion could be due to the formation of secondary mucoadhesive bonds with mucin because of rapid swelling and interpenetration of the polymer chains in the interfacial region, while other polymers undergo only superficial bioadhesion. The peak detachment force was considered to be dependent on the formation of hydrogen bonds between the functional groups of the bioadhesive and the mucus. HPMC alone had poor adhesive properties, but when used in combination with Carbopol, its overall adhesion was increased. Very strong bioadhesion could damage the epithelial lining of buccal mucosa.

Swelling Index:-

In-vitro water uptake studies are of great significance as variation in water content causes a significant variation in mechanical properties of formulations. The capacity of the formulation to take up water is an important intrinsic parameter of the polymeric system in consideration to the release of the drug on the mucosal surface. Water absorbing capacity of system (SI after 6 hours.) decreased in the following order T7>T6>T5>F9>F8>C2>F6>F5>F4>T4>F3>T2>C1>F2>F1>T3>T1; with decreasing concentration of Carbopol.

The Surface pH:-

The surface pH of all formulations was found to be within ±1 units of neutral pH hence these formulations should not cause any irritation in buccal cavity.

In vitro drug release study:-

This study was performed using USP TDT 06 (paddle) dissolution test apparatus at 50 rpm using 900 ml of pH 6.8 Phosphate buffer + 0.5% w/v SLS solution maintained at 37 ± 0.5°C as dissolution medium. The results were shown in tables- 23 to 28. From above data, it is evident that as the proportion of polymers in the formulation increases, cumulative percent drug released was found to be reduced.
**Discussion**

Among the seven trial batches, formulation T\textsubscript{1} to T\textsubscript{4} have released 87 to 100\% drug in 10 hours, whereas T\textsubscript{5} to T\textsubscript{7} formulations have released 70 to 75\% drug in 10 hours. A higher diffusive flux develops as a consequence of the higher Solubilization rate operated by \(\beta\)-CD, which increases the amount of mobile species. Both these effects result in an enhanced release rate of drug.

It indicates that controlled release of drug can be obtained with increased in amount of polymers (HPMC K4M and carbopol 934P).

In seven trail formulations, T\textsubscript{5} formulation has shown promising dissolution parameters (\(t\textsubscript{50\%}=6.3\) hours, \(t\textsubscript{70\%}=8.9\) hours) and good mucoadhesion time (> 12 hours).

**6.4. Factorial Design**

Based on the composition of T\textsubscript{5} formulation, we have fixed the constraints for the level of independent variables (\(X\textsubscript{1}\) and \(X\textsubscript{2}\)) i.e. 10 to 30 mg for HPMC K4M (\(X\textsubscript{1}\)) and 5 to 15 mg for carbopol 934P (\(X\textsubscript{2}\)) in designing formulation of \(3^2\) full factorial design.

In this \(3^2\) full factorial design, two factors (proportion of two polymers) are evaluated, each at three levels and experiments are performed on all nine possible combinations. Dissolution parameters i.e. \(t\textsubscript{50\%}\), \(t\textsubscript{70\%}\) and swelling index values were selected as dependent variables. Formulation code of the nine batches of factorial formulations along with dissolution parameter values (\(t\textsubscript{50\%}\), \(t\textsubscript{70\%}\), swelling index) values were selected as dependent variables. Formulation code of the nine batches of factorial formulations along with dissolution parameter values (\(t\textsubscript{50\%}\), \(t\textsubscript{70\%}\) swilling index) and cumulative percent drug released in 10 hours. From the data in the above table, it is evident that formulation F\textsubscript{1} has shown highly satisfactory values for dissolution parameters (\(t\textsubscript{50\%}=3.9\) hours; \(t\textsubscript{70\%} = 5.4\) hours and swelling index =22.74 hours) and has released approximately 99.41\% drug in 10 hours. Hence, formulation F\textsubscript{1} may be considered as the optimized buccal tablet containing Posaconazole inclusion complex with \(\beta\)-CD for improved bioavailability.
Discussion

Drug Release Kinetics

In-vitro drug release data of all the buccal tablet formulations was subjected to goodness of fit test by linear regression analysis according to zero order equations, Higuchi’s and Korsmeyer-Peppas models to ascertain the mechanism of drug release.

From the above data, it can be seen that except formulation T1, T2 and T3 all the trial formulations containing combination of polymers HPMC and carbopol have displayed zero order release kinetics (‘r’ values in the range of 0.996 to 0.911 ). From Higuchi’s and Peppas data, it is evident that the drug is released by non-Fickian diffusion mechanism except formulation containing HPMC and carbopol alone.

The values of ‘r’ for Higuchi’s equation of factorial formulations range from 0.971 to 0.992. This data reveals that drug release follows non-Fickian diffusion mechanism. This is because as the proportion of polymers in the matrix increased there was an increase in the amount of water uptake and proportionally greater swelling leading to a thicker gel layer. Zero-order release from swellable hydrophilic matrices occurs as a result of constant diffusional pathlengths. When the thickness of the gelled layer and thus the diffusional pathlengths remain constant, zero-order release can be expected, as seen for formulations.

This analysis highlights that the introduction of β-CD in surface eroding controlled release tablets supplies an additive mean to tailor the release by modulating the dissolution rate of the drug in the swollen layer as well as the erosion rate of the matrix.

Development of Polynomial Equations

Factorial formulations F1 to F9, polynomial equations for three dependent variables (t50%, t70% and swelling index) have been derived using ‘PCP Disso 2000 V3 software’. Polynomial equation for table-32 full factorial designs is:

\[ Y = b_0 + b_1 X_1 + b_2 X_2 + b_{12} X_1 X_2 + b_{11} X_1^2 + b_{22} X_2^2 \]  

.....1
**Discussion**

Where Y is dependent variable, \(b_0\) arithmetic mean response of nine batches, and \(b_1\) estimated coefficient for factor \(X_1\). The main effects \((X_1\) and \(X_2\)) represent the average result of changing one factor at a time from its low to high value. The interaction term \((X_1, X_2)\) shows how the response changes when two factors are simultaneously changed. The polynomial terms \((X_1^2\) and \(X_2^2)\) are included to investigate non-linearity.

The equation derived for \(t_{50}\) is:

\[
Y_1 = 5.9556 + 1.000 X_1 + 0.6667 X_2
\]  
\[\ldots 2\]

The equation derived for \(t_{70}\) is:

\[
Y_2 = 8.3333 + 1.35 X_1 + 0.7667 X_2
\]  
\[\ldots 3\]

The equation derived for swelling index is:

\[
Y_3 = 62.82 + 26.8650X_1 + 9.36 X_2
\]  
\[\ldots 4\]

Validity of the above equations was verified by designing two check point formulations \((C_1 \text{ and } C_2)\) and studying the drug release profiles. The dissolution parameters predicted from the equations derived and those observed from experimental results are summarized in the table below:
**Discussion**

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Predicted values (hours)</th>
<th>Observed values (hours)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>t(_{50})%</strong></td>
<td><strong>t(_{70})%</strong></td>
<td><strong>Swelling index (after 6 hours)</strong></td>
<td><strong>t(_{50})%</strong></td>
</tr>
<tr>
<td>C(_1)</td>
<td>5.12</td>
<td>7.27</td>
<td>44.70</td>
<td>5.4</td>
</tr>
<tr>
<td>C(_2)</td>
<td>6.78</td>
<td>9.39</td>
<td>80.93</td>
<td>6.9</td>
</tr>
</tbody>
</table>

The closeness of predicted and observed values for \(t_{50}\)%, \(t_{70}\)% and swelling index values indicates validity of derived equations for the dependent variables.

The response surface and contour plot reveal that it varies in a somewhat linear fashion with the amount of two polymer(s). However, the steeper ascent in the response surface with CP than with HPMC is clearly discernible, indicating that the effect of CP is comparatively more pronounced than that of HPMC.

**6.5. In-vitro Drug permeation study of formulation F\(_1\)**

Based on the results of factorial design of all formulations, the F\(_1\) formulation was selected for in vitro drug permeation studies. The oral mucosa of sheep resembles that of humans more closely in terms of structure and composition and therefore sheep buccal mucosa was selected for drug permeation studies. The results of drug permeation from buccal tablets through the sheep buccal mucosa reveal that Posaconazole was released from the formulation and permeated through the sheep buccal membrane and could possibly permeate through the human buccal membrane. The drug permeation was slow and steady and 68.47 ± 2.11% of Posaconazole could permeate through the buccal membrane in 10 hours with average flux of 139.28 µg/cm\(^2\)/min.

The results, reported show that Posaconazole permeation through mucosa was quite good and increased in the presence of β-Cyclodextrin. This effect, in principle, can be attributed to both an increase of driving force for permeation due to the increase of Posaconazole apparent solubility in the presence of β-CD as well as to enhancing effect of β-CD. Thus β-Cyclodextrin has been suggested to act as penetration enhancers. They enhance the permeation of the drug by carrying the drug.
Discussion

through the aqueous barrier towards the surface of the membrane, where the drug passes from the complex into the membrane. Addition of β-CD to the matrix increased the flux by increasing the solubility of Posaconazole, thus improving the diffusible form of the drug species at the tablet membrane interface. Though the complex did not penetrate the membrane, the drug in the complex was in rapid dynamic equilibrium with the “free” drug, thus continuously supplying the drug molecules to the membrane surface in a diffusible form.

The role of dissolution enhancement in increasing the rate of delivery is more relevant when the tablet is employed as transmucosal system since, differently from solution conditions; a very limited contribution to delivery derives from matrix erosion.

6.6. Potential of Buccoadhesive tablets containing Posaconazole inclusion complex with β-CD

A successful design of a buccal delivery system should guarantee both an intimate contact with the mucosa for an adequate time interval and proper release rates. Actually, before a drug passes through the mucosal barrier and reaches blood circulation, it should dissolve in the medium penetrating inside the buccal tablet. This step is generally critical for lipophilic drugs that, although being well absorbed, exhibit a slow dissolution rate in aqueous media. Buccoadhesive tablets containing Posaconazole inclusion complex with β-CD could therefore be of interest as a transmucosal delivery system due to their recognized bioadhesive properties and the possibility of improving release features of drugs poorly soluble in aqueous media, which has been illustrated above. However, the incorporation of Posaconazole and β-CD a binary systems in the tablets for such an application should not impair the overall mucoadhesive properties of the system which is the interdiffusion of polymer chains and mucus components at interface. An overall evaluation of the mucoadhesive behavior of tablets shows good bioadhesive properties. Although containing considerable amounts of β-CD, and are suitable for transmucosal applications.

The feasibility of a buccal delivery for Posaconazole was preliminary assessed by measuring in vitro permeation of Posaconazole through sheep buccal mucosa.
Discussion

Stability Studies

Stability study was performed on the promising formulation F₁ by storing the samples at 45±1°C for 3 months (90 days). The samples were tested for any changes in physical appearance and drug content at weekly intervals. In vitro drug release studies were performed at the end of 3 months storage. These results indicate that there were no significant changes in drug content and dissolution profile of the formulation F₁ during storage at 45°C for 3 months.