INTRODUCTION

The mucosa is considered as a potential site for drug administration. Transmucosal routes of drug delivery (i.e., the mucosal linings of the nasal, rectal, vaginal, ocular and oral cavity) offer distinct advantages over peroral administration for systemic drug delivery. These advantages include possible bypass of the first pass effect, avoidance of presystemic elimination of gastro intestinal tract (GIT). Buccal delivery of drugs provides an attractive alternative to the oral route of drug administration, particularly in overcoming deficiencies associated with the latter mode of administration problems such as high first pass metabolism; drug degradation in gastro intestinal environment can be circumvented by administering a drug via buccal route [1,2]. Moreover, buccal drug absorption can be terminated promptly in case of toxicity by removing the dosage form from the buccal cavity. It is also possible to administer the drug to patients who cannot be dosed orally to prevent accidental swallowing. Therefore, mucoadhesive dosage forms were suggested for oral drug delivery, which includes adhesive tablets, adhesive gels, and adhesive patches.

Amphotericin B is a anti-fungal antibiotic used for serious systemic fungal infections, but not for less severe infections like throat infections, vaginal infections, oral thrush etc. It is only effective treatment for some infections like protozoal infections. Amphotericin B shows strong affinity for ergosterol in the fungal cell membrane. It binds to ergosterol molecule and repositions them. It leads to formation of 6-membered polar pore, through which small ions (K⁺&Mg²⁺) diffuse freely and ultimately cell death. From the technological point of view, an ideal buccal dosage form must have three properties; it must maintain its position in the mouth for a few hours, release the drug in controlled fashion, and provide drug release in a unidirectional way towards mucosa.
Thus in this research work a novel mucoadhesive bilayer buccal tablet formulation is tried which enhances the oral bioavailability of the poorly water soluble Amphotericin B. The objectives of the present work include development and characterization of mucoadhesive bilayer buccal tablets containing amphotericin B, improve oral bioavailability by buccal cavity and to it is also possible for this formulation to avoid hepatic first pass metabolism and to reduce the dose required to produce same pharmacological effect where by dose related side effects can be reduced [3,4,5,6,7].

MATERIALS AND METHODS
Amphotericin B (SRL chemicals, Tirupati), Hydroxy propyl methyl cellulose, Ethyl cellulose (Oxford laboratory agent), Sodium lauryl sulphate (Merck), Micro crystalline cellulose (SDFCL Ltd), Di sodium hydrogen phthalate(SDFCL Ltd), Potassium dihydrogen phosphate (SDFCL Ltd), Magnesium sterase (Himedia). All the other solvents, reagents used were of pharmacopoeial and analytical grade.

Drug excipient compatibility studies
I.R spectroscopy can be used to investigate and predict any physiochemical interaction between different excipients. A physical mixture of drug, polymer and other excipients were prepared and mixed with suitable quantity of potassium bromide. This mixture was compressed to form a transparent pellet using a hydraulic press at 15 tons pressure [8,9]. It was scanned from 4000 to 400 cm-1 in a FTIR spectrophotometer (FTIR 4100, Jasco). The IR spectrum of the physical mixture was compared with those of pure drug and polymer and peak matching was done to detect any appearance or disappearance of peaks and the results were shown in fig no: 2,3.

Preparation of Mucoadhesive bilayer buccal tablets of amphotericin B
Bilayer buccal tablets containing Amphotericin B were prepared by direct compression method. All the ingredients including drug, polymer and and excipients were weighed accurately and transferred into a glass motor and pestle and triturated for 30 minutes to obtain an homogenous mixture. The powder mixture equivalent to 150 mg was directly compressed using an 11 mm diameter die in a single stroke multitstation tablet machine. The upper punch was raised and the backing layer of ethyl cellulose was placed on the above compact. Then two layers were compressed into an mucoadhesive buccal tablet with a total weight of 200 mg/ tablet [10,11].Formulation chart of mucoadhesive bilayer buccal tablets were given in table no 1.

| Table 1. Formulation Chart for mucoadhesive bilayer buccal tablets of Amphotericin B |
|----------------------------------------|--------|--------|--------|--------|--------|
| Ingredients              | F1 (mg)| F2 (mg)| F3 (mg)| F4 (mg)| F5 (mg)|
| Amphotericin B            | 10     | 10     | 10     | 10     | 10     |
| Hydroxy propyl methyl cellulose | 80     | 85     | 90     | 95     | 100    |
| Sodium lauryl sulphate   | 4      | 4      | 4      | 4      | 4      |
| Micro crystalline cellulose | 64     | 59     | 54     | 49     | 44     |
| Magnesium sterase        | 2      | 2      | 2      | 2      | 2      |
| Ethyl cellulose          | 50     | 50     | 50     | 50     | 50     |
| Total weight (mg)        | 200    | 200    | 200    | 200    | 200    |

Fig 1. Formulated mucoadhesive bilayer buccal tablets of Amphotericin B
EVALUATION OF MUCOADHESIVE BUCCAL TABLETS
The prepared batches of tablets were evaluated for hardness, friability, weight variation, drug content uniformity, swelling index, surface pH, mean mucoadhesive strength, in vitro drug release.

Hardness test
The crushing strength (kg/cm$^2$) of tablets were determined by using Mansanto hardness tester [9].

Friability test
It is determined by weighing 20 tablets after dusting, placing them in the friabilator and rotating the plastic cylinder at 25 rpm for 4 min. After dusting, the total remaining weight of tablets was noted and the percent friability was calculated (%loss in weight) [10].

Weight variation
The weight (mg) of each tablet of 20 individual tablets was determined by dusting each tablet off and placing it in an electronic balance. The weight data from the tablets were analyzed for sample mean and percent deviation from the mean [10].

Uniformity of drug content
Five tablets were powdered in a glass mortar and the powder equivalent to 12.5 mg is placed in a stoppord 100 ml conical flask. The drug extracted with 40 ml methanol with vigourous shaking on a mechanical gyratory shaker (100 rpm) for 1 hr then heated on water bath with occasional shaking for 30 min and filtered into 50 ml volumetric flask through whattman filter paper and more methanol is passed through the filter to produce 50 ml. Aliquots of solution are filtered through 0.25 µm membrane filter disc and analysed for drug content by measuring the absorbance at 416 nm against solvent blank [11].

Surface pH
For the determination of surface pH of the buccal tablets, a combined glass electrode is used. The tablet is allowed to swell by keeping it in contact with 1 ml distilled water in a petri dish for 2 hrs at room temperature. The pH was identified by bringing the electrode into contact with tablet surface and allowing the surface to equilibrating for 1 min.

Swelling index
The swelling index of the mucoadhesive bilayer buccal tablet was evaluated by using phosphate buffer pH 6.8. The initial weight of the tablet was determined ($w_1$). Then the tablet is placed in the 6 ml of phosphate buffer of pH 6.8 in a petri plate. It is then kept in incubator at 37 +1°C and the tablets were removed at different time intervals and tablet is reweighed (336,39,40). Swelling index can be calculated using the formula,

\[
\text{Swelling index} = 100 \left( \frac{w_2 - w_1}{w_1} \right)
\]

Mucoadhesion strength
Mucoadhesion strength of the tablet was measured by using modern physical balance. The apparatus consists of a double beam physical balance in which left pan was removed. To left arm of balance a thick thread of suitable length was hanged. To bottom side of thread a glass stopper with uniform surface was tied. A clean glass mortar was placed below hanging glass stopper. In this mortar a 500 ml clean glass beaker placed within which another 50 ml glass beaker placed at inverted position and weighed to 50 g for preventing floating. The balance was adjusted such that right hand side was exactly 5 g heavier than the left side.

Method
The bovine cheek pouch was washed and tied tightly with mucosal side upward using thread over the base of inverted 50 ml glass beaker. The beaker suitably weighed was lowered into 500 ml beaker, which was filled with isotonic phosphate buffer (pH 6.8) kept at 37°C such that buffer reaches the surface of mucosal membrane and keeps it moist. This was kept below left hand side balance. The buccal tablet was then struck to glass stopper through its backing membrane using an adhesive. The 5 g on right side is removed this cause the application of 5 g of pressure on bilayer tablet overlying moist mucosa. The balance was kept in position for 3 min and then slowly weights were increased on the right pan till tablet seperates from mucosal membrane. The total weight on the right pan minus 5 g gives the force required to separate tablet from mucosa [12].

In vitro dissolution procedure
Dissolution rate of the tablets prepared was studied using dissolution test apparatus USP II employing a paddle stirrer at 50 rpm & at 37°C ± 1°C. Phosphate buffer of pH 6.8 (500ml) was used as a dissolution
fluid. Samples of 5 ml each, were withdrawn at 0, 0.25, 0.5, 1, 2, 4, 6, 8 hrs and the samples were suitably diluted with the dissolution fluid and assayed for Amphotericin B at 416nm and using the corresponding dissolution fluid as blank. And the cumulative amount of drug release is calculated using standard calibration curve. Each sample withdrawn was replaced with an equal amount of drug free dissolution fluid [12,13]. And graphical representation of drug release profile shown in fig.

Kinetic Modeling of Drug Dissolution Profiles
The dissolution profile of most satisfactory formulation was fitted to zero order, first order, and Korsmeyer-Peppas models to ascertain the kinetic modeling of the drug release. The methods were adopted for deciding the most appropriate model [14,15].

a. Zero order kinetics
The zero order rate equation describe the systems where the drug release rate is independent of its concentration. A plot of % cumulative drug release vs. time is linear.

\[ C = K_0t \]

Where,
\[ K_0 = \text{Zero order rate constant} \]
\[ t = \text{Time} \]

b. First order kinetics
The first order equation describes the system where the drug release rate is dependent of its concentration. A plot of log % drug remaining vs time is linear.

\[ \log C = \log C_0 - Kt / 2.303 \]

Where,
\[ C_0 = \text{Initial concentration of drug} \]
\[ K = \text{First order rate constant} \]

c. Korsmeyer-Peppas model
Korsmeyer et al., (1983) derived a simple relationship which described drug release from polymeric system

\[ M_t / M_\infty = Kt^n \]

Where,
\[ M_t / M_\infty = \text{Fraction of drug released at time 't'} \]
\[ K = \text{Release rate constant and} \]
\[ n = \text{Release exponent}. \]

In this model, the value of \( n \) characterizes the release mechanism of drug. 0.45 ≤ \( n \) corresponds to a Fickian diffusion mechanism, 0.45 < \( n < 0.89 \) to Non-Fickian transport (the rates of solvent penetration and drug release are in the same range), \( n = 0.89 \) to Case II (relational) transport, and \( n > 0.89 \) to super case II transport.

RESULTS AND DISCUSSION

Compatibility studies
An I.R study was carried out to check the compatibility between the drug and selected excipients. The spectra obtained for I.R study at wavelength from 4000 cm\(^{-1}\) to 400 cm\(^{-1}\) are shown in the figure. After interaction through the above spectra it was confirmed that there are no major shifting, loss or appearance of functional peaks between the spectra of drug, HPMC, ethyl cellulose, micro crystalline cellulose, sodium lauryl sulphate, magnesium stearate, physical mixture of drug + mixture. From the I.R studies it was concluded that, the selected polymer and other excipients are compatible with the selected drug Amphotericin B.

Evaluation parameters of mucoadhesive bilayer buccal tablets
Post compression parameters like mean hardness, mean weight variation, friability, surface PH, swelling index, mucoadhesive strength were carried out for mucoadhesive bilayer buccal tablets. F5 formulation had better results when compare to the other F1, F2, F3, and F4 formulations. The results were listed in the below Table 2 & 3.

In vitro drug dissolution study
The in vitro drug dissolution studies for bilayer tablet were carried out in phosphate buffer of 7.4. After in-vitro drug dissolution studies it is clear that F5 formulation showed better release when compared to F1, F2, F3, F4. In case of F1, F2, F3, and F4 formulations the % cumulative drug release were found to be 33.69%, 76.96%, 86.57 % and 91.3 % by the end of 8 hours, whereas F5 formulation has shown 96.19 % of drug release. Cumulative % of drug release for various formulations were given in Table no 4.

Kinetics of Drug Analysis
The cumulative amount of drug released per each tablet through the buccal membrane was plotted against time was not optimally fit to Zero-order kinetics (\( R^2 = 0.869 \)). However, the release profile of the formulated tablets followed First-order kinetics (\( R^2 = 0.921 \)), which indicates that the release of drug from the F5 formulation was governed by a diffusion mechanism.
Kinetic modeling of drug release
The kinetic profile of F5 formulation was shown in the figures 4 (a,b,c)

Table 2. Post compression parameters for mucoadhesive bilayer buccal tablet of Amphotericin B

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Mean hardness (kg/cm²)</th>
<th>Mean weight variation (mg)</th>
<th>Friability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>5.19±0.01</td>
<td>179.5±1.05</td>
<td>0.96±0.01</td>
</tr>
<tr>
<td>F2</td>
<td>5.47±0.02</td>
<td>182.4±1.00</td>
<td>0.88±0.03</td>
</tr>
<tr>
<td>F3</td>
<td>4.29±0.20</td>
<td>184.7±0.51</td>
<td>0.71±0.13</td>
</tr>
<tr>
<td>F4</td>
<td>4.20±0.10</td>
<td>187.5±0.60</td>
<td>0.36±0.12</td>
</tr>
<tr>
<td>F5</td>
<td>4.03±0.19</td>
<td>198.5±0.55</td>
<td>0.26±0.14</td>
</tr>
</tbody>
</table>

Table 3. Post compression parameters for mucoadhesive bilayer buccal tablet of Amphotericin B

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Mean % Drug Content</th>
<th>Mean surface pH</th>
<th>Mean swelling index (after 9 hrs)</th>
<th>Mean mucoadhesive strength (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>87.61±1.216</td>
<td>6.20±0.025</td>
<td>26.57±0.653</td>
<td>3.64±0.166</td>
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<tr>
<td>F2</td>
<td>91.54±0.515</td>
<td>5.83±0.020</td>
<td>40.80±0.463</td>
<td>5.20±0.010</td>
</tr>
<tr>
<td>F3</td>
<td>95.64±2.756</td>
<td>6.10±0.100</td>
<td>48.23±0.166</td>
<td>5.70±0.138</td>
</tr>
<tr>
<td>F4</td>
<td>97.21±0.953</td>
<td>5.87±0.036</td>
<td>54.42±0.707</td>
<td>5.80±0.050</td>
</tr>
<tr>
<td>F5</td>
<td>98.89±0.720</td>
<td>6.13±0.015</td>
<td>63.41±0.151</td>
<td>6.24±0.030</td>
</tr>
</tbody>
</table>

Table 4. Cumulative % of drug release for F1,F2,F3,F4 and F5 formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>% Cumulative drug release</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>F1</td>
<td>4.84±0.66</td>
</tr>
<tr>
<td>F2</td>
<td>4.84±0.44</td>
</tr>
<tr>
<td>F3</td>
<td>4.84±0.30</td>
</tr>
<tr>
<td>F4</td>
<td>9.65±0.60</td>
</tr>
<tr>
<td>F5</td>
<td>14.46±0.45</td>
</tr>
</tbody>
</table>
The cumulative amount of drug released was plotted against time was not optimally fit to Zero-order kinetics ($R^2 = 0.869$). However, the release profile of the formulated mucoadhesive bilayer tablets followed First-order kinetics ($R^2 = 0.921$) and Korsmeyer Peppas equation ($R^2 = 0.951$), which indicates that the release of drug from the F5 formulation was governed by a diffusion mechanism.
CONCLUSION
The results of present study indicate that mucoadhesive bilayer buccal tablets of amphotericin B with controlled release can be prepared by direct compression method using HPMC as mucoadhesive polymer and ethyl cellulose as backing layer. Based on the high drug release, mucoadhesion strength, dissolution studies and the higher bioavailability F5 formulation containing HPMC (100 mg), ethyl cellulose (50 mg), magnesium stearate (2 mg), sodium lauryl sulphate (4 mg), microcrystalline cellulose (44 mg) was selected as a best formulation.

REFERENCES
10. Amish VP, Markand M, Viral HS, Umesh U. Formulation and In vitro evaluation of mucoadhesive bilayered buccal tablets of Rosuvastatin calcium. IUPSR, 2012, 3(8), 2733-2740.