FORMULATION AND EVALUATION OF CLOTRIMAZOLE LOZENGES

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ABSTRACT

Clotrimazole is formulated as lozenges to provide slow release medicament for the management of oral thrush. Clotrimazole is an azole antifungal that works by preventing the growth of fungus. Many dosage forms like syrups, tablets available in market but still there is a need for new dosage form which acts effectively and locally for paediatrics and people with difficulty in swallowing. So the present investigation has been taken up design prepare and evaluate hard candy lozenges to meet the need of improved bioavailability. The benefits of these prepared lozenges showed increase in bioavailability, reduction in gastric irritation, bypassing of first metabolism and increase in onset of action. The lozenges are prepared using sucrose as base; liquid glucose is used for transparency and smoothness; Hydroxy propyl methyl cellulose K15M (HPMC K15M) are used as polymers. Sodium saccharine are used as artificial sweeteners. Sweeteners along with flavours are used to mask the bitter taste of drug. All the formulations prepared are subjected to various physicochemical parameters like weight variation, hardness, thickness, friability, content uniformity, and moisture content etc. the prepared formulations have a hardness of 8-11 kg/cm2, non-gritty and pleasant mouth feel. Some selected formulations are also tested for drug excipient interactions subjecting to Infrared Spectral analysis, in vitro release rate studies showed that the drug release for lozenges was maximum in formulation F6 (99.52±1.23%) after 25 minutes. The moulded lozenges can provide an attractive alternative formulation in allergic condition.

KEYWORDS: Clotrimazole, antifungal, lozenges, saccharine, K15M, liquid glucose.
INTRODUCTION
The investigation is to design and develop the Lozenges of Clotrimazole for oral thrush. Lozenges are unit dosage forms intended to be sucked in the oral cavity. These are very effective dosage forms for incorporating the drugs that act in the oral cavity and get readily absorbed from the oral cavity. Clotrimazole lozenges are prepared by using different polymer at different concentrations and were evaluated for In vitro drug release and other physical parameters.

MATERIALS AND METHODOLOGY
Materials
Drug: Clotrimazole.
Excipients: Sucrose, Liquid glucose, Xanthum gum,
Sweetener: Sodium saccharine.
Polymer: HPMC K15M
Preservatives: Propyl paraben, Methyl paraben.
Colour: FDA approved food colours.
Flavours: Menthol, Eucalyptus oil

Preformulation studies: Preformulation studies are primarily done to investigate the physicochemical properties of drug and to establish its compatibility with other excipients. Drug-Excipient Compatibility study

Drug-excipient compatibility study: Clotrimazole (DRUG) was mixed with all excipients, used in the formulation in different ratios and subjected to FTIR/Physical observation.

Drug-excipient compatibility study by physical observation: Clotrimazole (DRUG) was mixed in different proportions with all excipients which were used in the formulation, in different ratios and kept at 400C/75% RH conditions for two months. The physical properties (colour change) were monitored regularly. The change in colour of any mixture was considered as incompatibility and the excipient blend was discarded from study.\textsuperscript{[2,4]}

FT-IR: A Fourier Transform Infra-Red Spectrophotometer (FTIR Spectrum BX series 2.19 version) equipped with spectrum v2.19 software was used to study the non-thermal analysis of drug-excipient (binary mixture of drug: excipient 1:1 ratio) compatibility. The
spectrum for each sample was recorded over the 450-4000 cm\(^{-1}\) spectral region with a resolution of 4 cm\(^{-1}\).

**Determination of \(\lambda_{\text{max}}\) using UV Visible Spectrophotometer:** Standard stock solution of Clotrimazole (1 mg/mL) was prepared in methanol. For the selection of analytical wavelength solutions of drug Clotrimazole 100 µg/ml was prepared by appropriate dilution of standard stock solution with distilled water and scanned in the spectrum mode from 200-300 nm. The wavelength with maximum absorption was chosen for further analysis.\(^{[1,2]}\)

**Standard graphs**

**Principle:** The standard curve is based on the spectrophotometry. The maximum absorption of drug Clotrimazole was observed at 229 nm.

**Standard stock solution:** Standard stock solution of pure drug containing 100 mg of Clotrimazole/100 mL is prepared using 6.8 pH phosphate buffer. The working standards were obtained by diluting the stock solution with corresponding buffer. The standard curve for Clotrimazole was prepared in concentration range of 2-25µg/mL at the selected wavelength 229 nm. Their absorptivity values were used to determine the linearity. Solution was scanned and Beer Lamberts law limit was obeyed in the concentration range of 2, 4, 6, 8, 10,12, 20, 25 µg/mL.

**Saturation solubility studies:** A saturated solution of Clotrimazole was made by adding an excess drug to 6.8 pH phosphate buffer and 0.1 N HCl. Then they were placed on a mechanical shaker for agitation for 48 hrs. Then the suspension was filtered through whatmann filter paper, filtrate was collected and the drug content was estimated using UV-visible spectrophotometer at 229 nm.\(^{[3]}\)

**Method of preparation:** Heating and congealing method

- Sucrose is accurately weighed and is dissolved in one third amount of water by heating on cookers until all sugar granules are dissolved.
- Liquid glucose is added when cooking temperatures reaches 110°C.
- Heating is continued until final temperature is 141-156°C.
- Mixture is cooled to 135°C and colour is added.
- Further cooling is carried out mixed until temperature reaches 40°C.
Flavour, drug, and polymer is added and mixed for 4-6 minutes and poured in lubricated moulds.[3,4]

**Evaluation of lozenges:** The prepared Clotrimazole lozenges were studied for their physicochemical properties like weight variation, hardness, thickness, friability, drug content, drug release studies and release kinetics.

**Weight variation test:** Twenty lozenges were taken and their weight was determined individually and collectively on a digital weighing balance. The average weight of one tablet was determined from the collective weight. The weight variation test would be a satisfactory method of determining the drug content uniformity. The percent deviation was calculated using the following formula.

\[ \% \text{Deviation} = \frac{\text{Individual weight} - \text{Average weight}}{\text{Average weight}} \times 100 \]

**Lozenge hardness:** Hardness of lozenge is defined as the force applied across the diameter of the tablet in order to break the tablet. The resistance of the tablet to chipping, abrasion or breakage under condition of storage transformation and handling before usage depends on its hardness. For each formulation, the hardness of 6 tablets was determined using Monsanto hardness tester and the average was calculated and presented with standard deviation.

**Lozenge thickness:** The thickness and diameter of the lozenge from production run is carefully controlled. Thickness can vary with no change in weight due to difference in the density of granulation and the pressure applied to the tablets, as well as the speed of the tablet compression machine. Hence this parameter is essential for consumer acceptance, tablet uniformity and packaging. The thickness and diameter of the tablets was determined using Digital Vernier callipers. Ten tablets from each formulation were used and average values were calculated. The average thickness for tablets is calculated and presented with standard deviation.[5,6]

**Friability:** Tablet hardness is not an absolute indicator of the strength because some formulations when compressed into very hard tablets lose their crown positions. Therefore, another measure of the tablet strength, its friability is often measured. Tablet strength is measured by using Roche friabilator. Test subjects to number of tablets to the combined effect of shock, abrasion utilizing a plastic chamber which revolves at a speed of 25 rpm for 4 minutes, dropping the tablets to a distance of 6 inches in each revolution. A sample of pre
weighed tablets was placed in Roche friabilator which was then operated for 100 revolutions. The tablets where then dedusted and reweighed.

Percent friability (%F) was calculated as

\[ \text{Friability} \% = \frac{(\text{Initial weight of 10 Tablets} - \text{Final weight of 10 tablets}) \times 100}{\text{Initial weight of 10 tablets}} \]

\[ F(\%) = \frac{[W_0 - W]}{W_0} \times 100 \]

Where, \( W_0 \) is the initial weight of the tablets before the test and \( W \) is the final weight of the tablets after test.

**Determination of drug content:** Twenty lozenges were finely powdered; quantities of the powder equivalent to 60 mg of Clotrimazole (Drug) where accurately weighed, transferred to a 100 mL volumetric flask containing 50 mL of 6.8 phosphate buffer and allowed to stand for 30 minutes with intermittent sonication to ensure complete solubility of the drug. The mixture was made up to volume with distilled water. The solution was suitably diluted and the absorption was determined by UV-Visible spectrophotometer at \( \lambda_{\text{max}} \) 229nm. The drug concentration was calculated from the standard curve.\(^{[2,3]}\)

**Moisture content:** By Gravimetric method 1 g sample is weighed and placed in vacuum oven at 60-70°C for 12-16 hrs. Final weight is subtracted from initial and the difference in moisture content is calculated.

\[ \% \text{Moisture Content} = \frac{(\text{Initial wt} - \text{Final wt})}{\text{Initial wt}} \times 100 \]

**In vitro drug release studies**

**Dissolution conditions**

**Apparatus:** USP II (Paddle) apparatus

**Dissolution medium:** 250 ml of pH 6.8 phosphate buffer

**Temperature:** 37±0.5 °C

**Rotating speed of the paddle:** 50 rpm

**Sample time intervals:** 5, 10, 15, 20, 25, 30 minutes

**Detection:** UV-Visible spectrophotometer at \( \lambda_{\text{max}} \) 229 nm

The samples were withdrawn at pre-determined time points, diluted appropriately and analysed spectrophotometrically at 229 nm. The cumulative percentage standard deviation was calculated and the results are presented in the table.
**Release kinetics:** Data of in vitro release was fit into different equations to explain the release kinetics of Clotrimazole lozenges. The kinetic equations used were zero-order and first-order equations.

A) **Zero – Order release kinetics**

It defines a linear relationship between the fractions of drug released versus time

\[ Q = kt \]

Where, \( Q \) is the fraction of drug released at time \( t \)

\( K \) is the zero order release rate constant

A plot of the fraction of drug released against time will be linear if the release obeys zero order release kinetics.

B) **First – Order release kinetics**

Wagner assuming that the exposed surface area of a formulation decreased exponentially with time during dissolution process suggested that drug release from slowest release formulation could be described adequately by apparent first - order kinetics. The equation used to describe first – order release kinetics is

\[ \ln (1-Q) = -kt \]

Where, \( Q \) is the fraction of drug released at time \( t \) and \( K \) is the first order release rate constant.

Thus, a plot of the logarithm of the fraction of drug remained against time will be linear if the release obeys first order release kinetics.\(^{[6,7]}\)

**In vivo evaluation of Taste and Mouth feel**

Single blind study was designed for the taste masking test and disintegration time in the buccal cavity. Six volunteers participated in the test. They were asked to rate the bitter taste of the ten formulations using a scale of 0-3 (indicated as -, +, ++, +++) when the score was - or +, the taste was considered as acceptable. If the score was higher than +, the bitterness of the formulation was not acceptable. The same human volunteers who participated in taste evaluation test were asked to give their opinion about the feeling of smoothness or grittiness of the lozenge soon after the lozenge got completely dissolved in the oral cavity.

- = No bitterness, + = slight bitter, ++ = moderate bitter, +++ = strong bitter
Results: FTIR studies
The compatibility between the drugs, polymer and excipients was compared by FTIR spectroscopy. The IR spectra were evaluated for the presence of principal peaks of drug, shifting and masking of the drug peaks due to presence of polymers and other excipients. The FTIR spectra of drugs and optimized formulation of lozenges are shown in figure.

Fig. 1: FTIR Spectrum of clotrimazole.

Fig. 2: FTIR Spectrum of Clotrimazole and Excipient.

Fig. 3: FTIR Spectrum of optimized formulation of lozenges.
The results summarized are follows

**Table 1: Fourier transforms infrared spectroscopy studies were carried out for pure drug along with drug and excipient combination.**

<table>
<thead>
<tr>
<th>FTIR Spectra</th>
<th>Peak of Functional Groups [Wavelength (cm⁻¹)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OH</td>
</tr>
<tr>
<td>CLM</td>
<td>3753</td>
</tr>
<tr>
<td>CLM + Excipients</td>
<td>3745</td>
</tr>
<tr>
<td>Optimized Formulation</td>
<td>3769</td>
</tr>
</tbody>
</table>

The peaks are not affected and prominently observed in IR spectra of drugs and excipient, which indicates that there were no interactions between drugs and excipients.

**Saturation solubility studies:** Saturation solubility studies of Clotrimazole and excipients were carried out and the results were tabulated. The studies were replicated in triplicate (n=3), and mean was calculated.

The solubility of Clotrimazole was found to be 22 µg/mL and 34 µg/mL in 6.8 pH phosphate buffer and 0.1 N HCl respectively. An order of solubility in 6.8 pH phosphate buffer < 0.1 N HCl.

**Table 2: Saturation solubility of clotrimazole.**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Initial Volume</th>
<th>concentration</th>
<th>Dilution factor</th>
<th>Amount percent(µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.8PH phosphate buffer</td>
<td>10 mL</td>
<td>2.2</td>
<td>1000</td>
<td>22 µg/mL</td>
</tr>
<tr>
<td>0.1 N HCl</td>
<td>10 mL</td>
<td>3.4</td>
<td>1000</td>
<td>34 µg/mL</td>
</tr>
</tbody>
</table>

**Standard graph of clotrimazole:** Standard stock solution of pure drug containing 100 mg of Clotrimazole/100 mL is prepared using 6.8 pH phosphate buffer. The working standards were obtained by diluting the stock solution with corresponding buffer. The standard curve for Clotrimazole was prepared in concentration range of 2-25µg/mL at the selected wavelength 229 nm. Their absorptivity values were used to determine the linearity. solution was scanned and Beer Lamberts law limit was obeyed in the concentration range of 2, 4, 6, 8, 10, 12, 20, 25 µg/mL.
Table 3: Standard graph of clotrimazole at $\lambda_{\text{max}}$ at 229nm.

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Concentration (µg/mL)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blank</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.067</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.107</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.145</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.184</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.235</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>0.361</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>0.492</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>0.565</td>
</tr>
</tbody>
</table>

Fig. 4: Standard graph of Clotrimazole in 6.8 pH phosphate buffer.

Evaluation of physical parameters of hard candy lozenges

All the prepared formulations were tested for physical parameters like weight variation, hardness, thickness, friability, content uniformity, and moisture content found to be within the Pharmacopoeia limits. The results of the tests were tabulated.

Table 4: Characterization of clotrimazole lozenges.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Weight (mg)</th>
<th>Hardness (kg/cm$^2$)</th>
<th>Thickness (mm)</th>
<th>Friability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>3000.5±1.58</td>
<td>11.60±0.47</td>
<td>7.41±0.05</td>
<td>0.53±0.02</td>
</tr>
<tr>
<td>F2</td>
<td>2999.8±1.97</td>
<td>10.81±0.92</td>
<td>7.28±0.07</td>
<td>0.48±0.08</td>
</tr>
<tr>
<td>F3</td>
<td>2997.4±1.56</td>
<td>11.33±0.70</td>
<td>7.32±0.05</td>
<td>0.45±0.07</td>
</tr>
<tr>
<td>F4</td>
<td>2999.9±2.21</td>
<td>10.41±0.46</td>
<td>7.35±0.04</td>
<td>0.56±0.03</td>
</tr>
<tr>
<td>F5</td>
<td>2999.7±2.50</td>
<td>10.52±0.49</td>
<td>7.37±0.05</td>
<td>0.41±0.07</td>
</tr>
<tr>
<td>F6</td>
<td>3000.7±3.60</td>
<td>10.40±0.52</td>
<td>7.38±0.06</td>
<td>0.53±0.11</td>
</tr>
<tr>
<td>F7</td>
<td>2998.6±2.31</td>
<td>10.39±0.29</td>
<td>7.38±0.05</td>
<td>0.57±0.12</td>
</tr>
<tr>
<td>F8</td>
<td>3000.3±2.30</td>
<td>10.57±0.39</td>
<td>7.41±0.02</td>
<td>0.45±0.02</td>
</tr>
<tr>
<td>F9</td>
<td>3000.5±2.61</td>
<td>11.17±0.66</td>
<td>7.31±0.04</td>
<td>0.42±0.08</td>
</tr>
<tr>
<td>F10</td>
<td>2999.3±2.65</td>
<td>10.78±0.58</td>
<td>7.28±0.06</td>
<td>0.54±0.21</td>
</tr>
</tbody>
</table>

a) Results are mean of 20 observations ± SD
b) Results are mean of 10 observations ± SD
The weight variation in all formulations was found to be in the range of 2999.4±1.56 mg to 3000.7±3.60 mg, which is within the pharmacopoeia limit. Hardness of all lozenges was maintained between 10.39±0.29 and 11.60±0.47 kg/cm². The thickness varies between 7.28 and 7.41 mm. Friability of all lozenges was maintained between 0.41±0.07 and 0.57±0.12 %. The weight variation, hardness, thickness and friability of optimized formulation (F6) were 3000.7±3.60 mg, 10.40±0.52 Kg/cm², 7.38±0.05 mm and 0.53±0.11 % which was within acceptable limits.

Table 5: Characterization of clotrimazole lozenges.

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Content uniformity (%)</th>
<th>Moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>99.13±1.77</td>
<td>0.86±0.08</td>
</tr>
<tr>
<td>F2</td>
<td>98.85±1.79</td>
<td>0.84±0.06</td>
</tr>
<tr>
<td>F3</td>
<td>98.48±1.60</td>
<td>0.87±0.05</td>
</tr>
<tr>
<td>F4</td>
<td>98.59±1.66</td>
<td>0.92±0.05</td>
</tr>
<tr>
<td>F5</td>
<td>99.13±1.77</td>
<td>0.83±0.02</td>
</tr>
<tr>
<td>F6</td>
<td>99.43±1.65</td>
<td>0.82±0.05</td>
</tr>
<tr>
<td>F7</td>
<td>98.87±1.95</td>
<td>0.87±0.07</td>
</tr>
<tr>
<td>F8</td>
<td>99.43±2.06</td>
<td>0.85±0.05</td>
</tr>
<tr>
<td>F9</td>
<td>99.26±1.82</td>
<td>0.87±0.07</td>
</tr>
<tr>
<td>F10</td>
<td>99.16±1.71</td>
<td>0.84±0.06</td>
</tr>
</tbody>
</table>

Result are mean of 3 observations ± SD

Assay was performed and percent content uniformity of all the lozenges was found to be between 98.48±1.60 and 99.43±2.06% of Clotrimazole which was within the acceptable limits. Moisture content was found to be between 0.82±0.06 and 0.92±0.05 % which was within the acceptable limits.

*In Vitro* drug release Data and Profiles for candy based lozenges

Fig. 5: Graphical presentation of cumulative percent of clotrimazole release from lozenges.
*Results mean of 3 observations ± SD

Formulation F1, F2, and F3 of Clotrimazole (Hard candy lozenges without polymer) containing varying concentration of sucrose and liquid glucose recorded the drug release of 98.20±0.24%, 95.80±2.3%, 96.50±2.1% respectively at the end of 15 minutes and for F4 and F5 (Hard candy lozenges with polymer) recorded the drug release of 88.11±1.11% and 97.52±1.31% at the end of 25 minutes and 30 minutes.

Fig. 6: graphical representation of cumulative percent of clotrimazole release from lozenges.

Formulation F6, F7, F8 (Hard lozenges with polymer) containing varying concentration recorded the drug release of 99.52±1.23%, 90.2±0.76%, 97.9±0.53% respectively at the end of 30 and 25 minutes and for F9 it was 94.6±2.43% at the end of 20 minutes and F10 (with sodium saccharin) it was 92.5±0.45% at the end of 15 minutes. The percentage drug release of optimized formulations (F6) was 99.52±1.23% which showed highest percent of drug release compared to other formulations.

**Release kinetics**

The mechanics and kinetics of drug release of Clotrimazole is determined by the application of zero order, first order, Higuchi and Korsemeyer-peppas kinetics as shown in table. Optimized formulation (F6) follows the first order release as their R2 values are 0.6665.
Fig. 7: Zero order release kinetics of formulation of F6.

Fig. 8: First order release kinetics of formulation of F6.

Fig. 9: Higuchi diffusion kinetics of formulation F6
In vivo taste evaluation of clotrimazole

Taste evaluation was performed on six healthy human volunteers and the results were reported in the table. The study was approved by Institutional Human Ethics Committee (Approval No. IHEC/VGOPC/078/2018).

Table 6: Comparative evaluation of taste and mouth feel for optimized formulation

<table>
<thead>
<tr>
<th>Volunteers</th>
<th>Taste</th>
<th>Mouth feel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>No grittiness, good mouth feel</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

➢ = No bitterness, + = slight bitter

DISCUSSION

The study was designed to formulate and evaluate Clotrimazole lozenges for treatment of oral thrush. The suggested ratio of the sugar and liquid ratio is 60:40 for attaining transparency and smoothness. This is due to prevention of sugar crystallization by liquid glucose.

But in the present investigation sufficient transparency was attained within the ratio of 55:35. This suggests that even low concentration of liquid glucose has the ability to retain the capacity to prevent crystallization of sugar.
The lozenges of Clotrimazole were prepared by using different polymers of different concentrations by heat congealing method (F1-F10), among the all formulations F6 (HPMC K15M) showed the highest percentage of drug release, drug content. Hence it was considered as the optimized formulation among all the formulations. The stability studies were performed there is no change in drug content, friability, weight variation. FT-IR studies were performed and from the FT-IR spectra it was evident that there were no interactions between the drug and the excipients being used.

This difference in the concentration of liquid glucose to attain the smoothness and transparency may be due to the type of apparatus used in the cooking process as follows: (Catena, 1997)
- 20% - Open kettle
- 30% - Batch vacuum cookers
- 35% - Semi continuous
- 40% - Continuous- cookers

This is also due to the increasing amount of mechanical action or turbulence to which candy is suspected after cooking.
- More agitation, more requirement of liquid glucose.
- Other mechanism to control the crystallization are:
  - High molecular weight sugar in the liquid glucose.
  - Low cooking temperatures.
  - Minimum mixing during cooking

**CONCLUSION**

The formulation was developed using artificial sugar (sodium saccharine) could not tolerate high temperatures and it was having less viscosity compared to the sucrose so it’s not suitable for making lozenges.

The formulation was prepared with using fructose as sugar base and its give better transparency and hardness.

Finally, it can be concluded that considering the ease of preparation, attractiveness and drug release characteristics, hard candy lozenges are alternatives for drug delivery from Clotrimazole for its antifungal activity.
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REFERENCE