DEVELOPMENT AND CHARACTERIZATION OF CYCLODEXTRIN DERIVATIVES INCLUSION COMPLEX OF EZETIMIBE FOR FAST DISSOLUTION TABLETS

Nishan N. Bobade¹, Ashish K Khrobragade², Shagufta A Khan ³, Pramod G Yeole³

¹Department of Pharmaceutics, Vidya - Bharati College of Pharmacy, C. K. Naidu Road, Camp Amravati, Dist Amravati, State Maharashtra. India 444602.
²Macleods Pharmaceuticals Ltd, Ganesh Industrial Estate, Survey No. 363/1, Kachigam, Daman-396210 India.
³Department of Pharmaceutics, Institute of Pharmaceutical Education and Research (IPER), Borgaon (Meghe), Wardha, 442001, Maharashtra State, India.

ARTICLE INFO
Article history
Received 05/10/2015
Available online
31/10/2015

Keywords
Ezetimibe, Cyclodextrin, Solubility Enhancement, Methyl B-Cyclodextrin.

ABSTRACT
The objective of present research work was to select cyclodextrins derivative for inclusion complex with Ezetimibe and formulate fast dissolution tablets to enhance pharmacokinetic and pharmacodynamic performance of drug. Ezetimibe is a poorly water soluble drug and erratically absorbed in stomach and possess several dissolution related problems thus it has poor bioavailability, attempts have been made to formulate inclusion complexes using various water soluble carriers with objectives. The phase solubility studie was performed with drug and betacyclodextrin, hydroxyl propyl betacyclodextrin and methylated betacyclodextrin. The inclusion complex was prepared with Hydroxy propyl beta-cyclodextrin and Methylated betacyclodextrin by Kneading Method, Ultrasoundification Method and Physical Mixture. The Characterization Of Inclusion Complexes was performed by sophisticated analytical technique FTIR Spectroscopy, X-ray diffraction, DSC. The results of these studies indicated that complex of the EZE molecule into the cyclodextrins cavities Inclusion complexes of Ezetimibe prepared with M-β-CD and HP-β-CD showed improved stability and dissolution behavior as compared to plain drug. Cyclodextrine derivative inclusion complex was evaluate for UV Interference, Drug content,Solubility,Dissolution studies and stability studies. Complexes were stable with non significant changes in drug content, solubility and dissolution rate after three month stability testing under 75±5% RH & 40±2°C. hydroxypropyl cyclodextrin and methylated beta cyclodextrin inclusion complex was incorporated into fast dissolution tablets with various concentration of cross povidone . Fast dissolution tablets for drug were evaluated for different test Uniformity of weight, Hardness, Friability,Thickness,Drug content,Disintegration study,dissolution studies and stability studies. Tablet formulation HP-4 (HPUS 1:1) and Me-4 (MeUS 1:2) showed highest dissolution of all.

Corresponding author
Nishan N. Bobade
Department Of Pharmaceutics,
Vidya-Bharati College of Pharmacy, C.K.Naidu Road,
Camp Amravati, Dist Amravati, State Maharashtra. India 444602
nishan01_vicky@yahoo.co.in

Please cite this article in press as Nishan N. Bobade et al. Development And Characterization of Cyclodextrin Derivatives Inclusion Complex of Ezetimibe For Fast Dissolution Tablets. Indo American Journal of Pharmaceutical Research.2015:5(10).

Copy right © 2015 This is an Open Access article distributed under the terms of the Indo American journal of Pharmaceutical Research, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

www.iajpr.com
INTRODUCTION

Ezetimibe (EZE) is chemically 1-(4-fluorophenyl)-3-(R)-[3-(4-fluorophenyl)-3(S)-hydroxypropyl]-4(S)-(4-hydroxyphenyl)-2-azetidinone. Ezetimibe reduces blood cholesterol by inhibiting the absorption of cholesterol by the small intestine. Ezetimibe had no clinically meaningful effect on the plasma concentrations of the fat-soluble vitamins A, D, and E, and did not impair adrenocortical steroid hormone production. Ezetimibe has a mechanism of action that differs from those of other classes of cholesterol-reducing compounds (HMG-CoA reductase inhibitors, bile acid sequestrants, fibric acid derivatives, and plant stanols) Ezetimibe neither inhibit cholesterol synthesis in the liver nor increase bile acid excretion. Instead, Ezetimibe localizes and appears to act at the brush border of the small intestine and inhibits the absorption of cholesterol, leading to a decrease in the delivery of intestinal cholesterol to the liver. This causes a reduction of hepatic cholesterol stores and an increase in clearance of cholesterol from the blood; this distinct mechanism is complementary to that of HMG-CoA reductase inhibitors. After oral administration, EZE is absorbed and extensively conjugated to a pharmacologically active phenolic glucuronide (EZE-glucuronide). After a single 10-mg dose of EZE to fasted adults, mean EZE peak plasma concentrations (C\text{max}) of 3.4 to 5.5 ng/mL were attained within 4 to 12 hours (T\text{max}). EZE-glucuronide mean C\text{max} values of 45 to 71 ng/mL were achieved between 1 and 2 hours (T\text{max}). The absolute bioavailability of EZE cannot be determined, as the compound is virtually insoluble in aqueous media.

Cyclodextrins comprise a family of three well-known industrially produced major, and several rare, minor cyclic oligosaccharides. The three major CDs are crystalline, homogeneous, nonhygroscopic substances, which are torus-like macro-rings built up from glucopyranose units.

Among industrially produced, standardized, and available (even in ton amounts) βCD-derivatives, the most important ones are the heterogeneous, amorphous, highly water-soluble methylated βCDs and 2-hydroxypropylated βCDs. A methylated βCD is more hydrophobic than the βCD itself, therefore, it forms a more stable (but soluble) complex with cholesterol. Its affinity to cholesterol is so strong that it extracts cholesterol from the blood cell membranes, resulting in hemolysis already in around 1 mg/cm² concentration.

A particular methylated βCD, the heptakis (2, 6-di-O-methyl-βCD, called DIMEB) is a crystalline product. It is extremely soluble in cold water, but insoluble in hot water; therefore, its purification, and also the isolation of its complexes, is technically very simple.

Up to now, no better solubilizer has been found among the CDs. It is available in more than 95 % isomeric purity for injectable drug formulation, but for widespread industrial application the cheaper randomly methylated βCD (called RAMEB) is produced and marketed.

The objective of the present study was to prepare inclusion complexes of EZE with betacyclodextrin, hydroxyl propyl betacyclodextrin and methylated betacyclodextrin using various methods such as kneading, ultrasonification method and physical mixing to improve its aqueous solubility and dissolution rate. The Characterization Of Inclusion Complexes by sophisticated analytical technique FTIR Spectroscopy, X-ray diffraction, DSC. To prepare fast dissolution tablets which show fastest and highest dissolution to improve bio performance of drug.

MATERIALS AND METHODS

Materials

EZE (molecular weight = 409) was received as a gift sample from Microlab Limited Pondecherry, (India). Hydroxypropyl-β-cyclodextrin (HPb-CD), Methylated β-Cyclodextrin (M-β-CD) and Cyclodextrins (β-CD) were a generous gift from Roquette Frères, France. All chemicals and solvents used in this study were of analytical reagent grade. Freshly distilled water was used throughout the work.

METHODS

STANDARD CALIBRATION CURVE OF EZETIMIBE

Standard calibration curve of Ezetimibe in 0.05 M Acetate buffer pH 4.5

A] Preparation of 0.05 M Acetate buffer pH 4.5

Dissolved 5.4 gm. Of sodium acetate in 50 ml of water add 2.4 ml of glacial acetic acid and dilute with water to 100 ml. Adjusted if pH is necessary

B] Scanning of Ezetimibe in 0.05 M Acetate buffer pH 4.5

Ezetimibe (10g) was accurately weighed and dissolved in sufficient quantity of 0.05 M Acetate buffer pH 4.5 and shaken on mechanical shaker for about 20 min. till a clear solution was obtained. To this sufficient amount of the medium was added to make the volume to 100 ml. The resultant solution was diluted with medium to obtain a concentration of 20μg/ml and scanned between 200-400 nm.

C] Preparation of standard calibration curve of Ezetimibe in 0.05 M Acetate buffer pH 4.5

Drug (10mg) was taken and to this 40 ml 0.05 M Acetate buffer pH 4.5 was added and shaken for about 20 min on mechanical shaker to obtain a clear solution. To this sufficient amount of 0.05 M Acetate buffer pH 4.5 was added to make up the volume up to 100 ml. From above solution various dilutions were prepared to get concentration of 2, 4, 6, 8, and 10 up to 20 mcg/ml. The absorbance of the various dilutions was measured against 0.05 M Acetate buffer pH 4.5 as a blank at 232nm using double beam UV visible spectrophotometer. (UV 2401 PC). The graph of absorbance v/s concentration was plotted and data were subjected to linear regression analysis.
Standard calibration curve of Ezetimibe in Distilled Water

A] Scanning of Ezetimibe in Distilled Water

Ezetimibe (10 mg) was accurately weighed and dissolved in 20 ml of distilled water and shaken on mechanical shaker for about 20 min. till a clear solution was obtained. To this sufficient amount of the distilled water was added to make the volume to 100 ml. The resultant solution was diluted with medium to obtain a concentration of 20μg/ml and scanned between 200-400 nm.

B] Preparation of standard calibration curve of drug in distilled water

Drug (10 mg) was taken and to this 10 ml methanol (as co solvent) and 90 ml distilled water was added and shaken for about 20 min on ultrasonification obtained a clear solution. To this sufficient amount of medium was added to make up the volume up to 1000 ml. From above solution various dilutions were prepared to get concentrations of 2, 4, 8, 10, and 12 up to 20 mcg/ml. The absorbance of the various dilutions were measured against distilled water as a blank at 232 nm using double beam UV visible spectrophotometer. The graph of absorbance v/s concentration was plotted and data were subjected to linear regression analysis.

Phase solubility Study

Phase solubility studies were performed according to the method reported by Higuchi and Connors. An excess amount of Ezetimibe (10mg) was added to the 10 ml of distilled water containing increasing concentration of β-CD, M-β-CD and HP-β-CD solution at various concentrations (0.001-0.01 M) in 10 ml screw capped bottles. The contents were stirred for 72 hours at 37°C on rotary flask shaker. After equilibrium, the samples were filtered through Whatman filter paper no. 42 and analyzed spectrophotometrically for drug content at the wavelength of 233 nm using Double Beam UV Spectrophotometer Model No. UV 2401 PC Shimadzu Corporation, Koyto, Japan. Solubility studies were performed in triplicate.

The apparent stability constant was calculated from the initial straight portion of the phase solubility diagram using the following equation

\[
K_{1:1} = \frac{\text{Slope}}{S_o (1 - \text{Slope})} \quad M^{-1}
\]

Where, \(S_o\) = is solubility of drug without cyclodextrin

\(M\) = means molar concentration

\(K\) = means apparent stability constant

Slope is calculated from regression equation

Preparation of Inclusion Complexes

The complexes of M-β-CD and HPb-CD with EZE were prepared in the molar ratio of 1:1, 2:1 (on the basis of phase solubility study) by different methods like Kneading Method, Ultrasonification Method and Physical Mixture. For ease in discussion, the samples are designated with different abbreviations shown in Table 1-3. Physical mixture Physical mixture (PM) of CDs and EZE were prepared by simply mixing powders with a spatula for 15 min.

Kneading Method

Ezetimibe and the various cyclodextrins were weighed in different ratio as shown in Table 1 and transferred to mortar and kneaded for 45 min. using alcohol-water mixture in ratio 1:1, sufficient solvent was added to maintained paste like consistency. The resulting paste was then dried in oven at 50°C for 24 hours. The dried complexes were grounded in mortar for 2 min and passed through sieve no. 100. The prepared complexes were stored in glass vials and used for further studies.

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Composition</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ezetimibe:HP-β-CD(HP KN)</td>
<td>1:1 (M)</td>
</tr>
<tr>
<td>2</td>
<td>Ezetimibe:M-β-CD(MeKN)</td>
<td>1:2 (M)</td>
</tr>
</tbody>
</table>

**Table 1: Composition of inclusion complexes.**

M’ represents molar ratio.

Ultrasonification Method

Ezetimibe and the various cyclodextrins were weighed in different ratio as shown in Table 2 and transferred to beaker using alcohol-water mixture in ratio 1:1 and 2:1 sufficient solvent was added to maintain paste like consistency. The resulting paste was then ultrasonificated for 6 hours. Throughout ultrasonification paste like consistency was maintained using alcohol-water. Then it was dried in oven at 50°C for 24 hours. The dried complexes were passed through sieve no. 100. The prepared complexes were stored in glass vials and used for further studies.
4.4.3: Physical Mixture
Physical mixtures were prepared by simply blending Ezetimibe and CDs with 1:1 molar ratio uniformly in a mortar.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Composition</th>
<th>Ratio (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ezetimibe:HP-β-CD (PMHP)</td>
<td>1:1 (M)</td>
</tr>
<tr>
<td>2</td>
<td>Ezetimibe:M-β-CD (PMM)</td>
<td>1:2 (M)</td>
</tr>
</tbody>
</table>

‘M’ represents molar ratio.

Characterizations of Complexes

Fourier Transform Infrared (FTIR) Spectroscopic Analysis
The FTIR spectra of pure drug, pure hydroxylpropyl betacyclodextrin, pure methylated betacyclodextrin, physical mixtures and inclusion complexes were taken by preparing KBr pellets using a FTIR Spectrophotometer Model - 8400S Shimadzu Corporation, Koyto, Japan.

Powder X-ray diffraction (PXRD) analysis
The physical state of drug in the various preparations was evaluated by powder X-ray diffraction study. Powder X-ray diffraction patterns of all samples were determined using X-Ray Diffractometer PANanalytical Spectris, Pvt. Ltd., Singapore using copper target, a voltage of 40 Kv and a current of 30 mA. The scanning was done over 2θ range of 5º to 60º.

Differential Scanning Calorimetry (DSC) Analysis
DSC scans of all powdered samples were recorded using DSC Model No. Mettler DSC 30S, Mettler Toledo India Pvt. Ltd., Switzerland, using crucible Al 40µL, at of 10ºC/min heating rate, under nitrogen environment. The temperature range used was 0 – 400ºC.

Drug Content
The percent drug content of each inclusion complexes were determined using powder equivalent to 10 mg Ezetimibe and was dissolved in 20 ml 0.05 M acetate buffer pH 4.5using the mechanical shaker for 20 min. and to the solution obtained 0.05 M Acetate buffer pH 4.5 was added and volume was made to 50 ml. The solution was then filtered through whatman filter paper no.42 and required dilutions were being made and absorbance was taken at 232nm. using Double Beam UV Spectrophotometer Model No. UV 2401 PC Shimadzu Corporation, Koyto, Japan.

Solubility Studies

The solubility of Ezetimibe as bulk drug and its inclusion complexes were determined in 0.05 M acetate buffer pH4.5 and distilled water. Inclusion complexes equivalent to 10 mg of drug was taken and to this 10 ml of the respective medium was added in 100 ml stoppered volumetric flasks and shaken for 24 hours at room temperature (25 ºC) on a mechanical shaker. After 24 hours samples were filtered through Whatman filter paper No. 42 and aliquots were suitably diluted for estimation.

Dissolution Studies
Dissolution Studies of drug in powder form, its complexes with β-CD, M-β-CD and HPb-CD were performed to evaluate drug release behaviour. Dissolution studies were carried out using USP dissolution apparatus type II, Dissolution test Apparatus Model No. DA-3 Veego Scientific Devices, Mumbai with 500 ml of 0.05 M Acetate buffer pH 4.5 as dissolution medium at 37 ºC ± 0.5 ºC and 50 rpm for 4 hours. Ten ml of aliquots was withdrawn at predetermined time interval for every 5 min. up to 1 hr. and was replaced by same volume of fresh medium. The sample were analyzed for drug content using double beam UV spectrophotometer at 232 nm against blank using 0.05 M acetate buffer buffer ph 4.5. The dissolution was carried out in duplicate for each formulated batch. The cumulative % drug release was calculated using the equation generated from the standard calibration curve.

Formulation Studies
After selection of optimized inclusion complexes conventional tablets were prepared using different grades of super disintegrant.
Method

All ingredients were passed through sieve No.100, and blended in glass mortar uniformly. After sufficient mixing of all components, magnesium stearate was added and again mixed for additional 2-3 minutes. The tablets were compressed using 8 mm concave punch on a single stroke punching machine Model No. H/416/95 Cadmach Machinery Pvt. Ltd., Ahamadabad. The tablets were studied in triplicates for release profile of drug using the same methodology as described in in vitro dissolution studies.

STABILITY STUDIES OF OPTIMIZED TABLET FORMULATIONS

The stability study of optimized formulation (FP10) and (FM10) was carried out at accelerated condition of 40 ± 2°C and 75% ± 5%R.H. for a period of three months.

Method

The tablets were individually wrapped using aluminum foil and packed inambered coloured screw capped bottle and kept at above specified conditions for a period of three months. After each month tablet sample was analyzed for the physical parameters such as physical appearance, thickness, hardness, drug content and in vitro drug release. The method adopted was same as described earlier. The dissolution profiles were compared with the tablet at zero time.

STATISTICAL ANALYSIS

All values were expressed as mean ± S.D. The data were analysed by one way analysis of variance (ANOVA) followed by Dunnett test or by Unpaired Student ‘t’ test. A value of P < 0.01 was considered as significant. Graph Pad Insat Demo Version was used for analysis of data.

Marketed formulation was considered as control and compared with optimised batches (HP-4 and Me-4) by applying one way ANOVA followed by Dunnett test.

DISCUSSION AND CONCLUSION

The procured sample of ezetimibe was tested for its identification. The drug sample showed compliance with the data given in Merk-Index and Martindale, which reflects its quality and purity. The carrier such as betacyclodextrin, hydroxypropylbetacyclodextrin, methylated betacyclodextrin, and all excipients provided by the supplier confirmed by their identification test official in USP 24, IP and EP. All the excipients showed results in compliance with standard specifications.
Standard calibration curve of ezetimibe

From the scanning of drug in 0.05 M Acetate buffer pH 4.5 and distilled water it was concluded that the drug had λmax of 231.6nm and 232.4nm respectively. From the standard calibration curve of drug, it was concluded that drug obeys Beer-Lamberts law in concentration range of 0-20 mcg/mL. The linear equation were obtained as

1) 0.05 M Acetate buffer pH 4.5  \[ Y = 0.0221x \quad R^2 = 0.9972 \]
2) Distilled water  \[ Y = 0.0314 \quad R^2 = 0.9984 \]

Correlation coefficient values indicated the linear correlation between concentration and absorbance.

The dissolution data of plain drug in 0.05 M Acetate buffer pH 4.5 showed that the highest release of drug is less than 64.54 % and thus it can be conclude that ezetimibe is poorly soluble drug and also possess several dissolution related problem that might be a reason for its poor bioavailability. Therefore, inclusion complexes of drug were prepared by using different polymers. The inclusion complexes were prepared by kneading method and ultrasonification method as these are easiest to perform and most preferred method.

![Standard calibration curve of Ezetimibe in 0.05 M Acetate buffer pH 4.5 at 232 nm.](image1)

![Standard calibration curve of Ezetimibe in distilled water at 232 nm.](image2)

Phase solubility study

The phase solubility method is useful for investigating an inclusion complexation of drug with cyclodextrin and its derivatives in distilled water because it gives not only the solubilising ability of host molecules but also the stability constant of complexes with the help of phase solubility curve.

From the phase solubility study Fig 3. And Table 6. It was observed that β-CD showed B3 type phase solubility curve indicating limited solubility.

From (0.01 to 0.05M) β-CD concentration, the solubility of ezetimibe was suddenly increased linearly due to the formation of soluble complexes. As the ascending portion of the phase solubility diagram may be considered as A1 type phase solubility diagram, it is possible to determine the complex stoichiometry.

As the slope value is less than 1 in the distilled water it could be considered as formation of 1:1 complex on a molar basis. This fact also reported by Pascal et al.
At the β-CD concentration value of 0.05 M, the solubility limit of this complexes is reached and so further addition of β-CD results in precipitation of the complexes. From 0.06 M β-CD concentration, ezetimibe solubility decreased to reach a plateau. These observations suggest that β-CD concentration above 0.06 M forms another complex with a different stoichiometry (probably 1:2) and shown lowest solubility as per Pascal et al. The stability constant (Ks) of the 1:1 complex of drug with betacyclodextrin was calculated by the ascending part of the diagram in Bs type solubility diagram and it was found to be .114.05

From these it could be concluded that β-CD is not the proper carrier for increasing solubility. Hence β-CD was not taken for further study.

From the phase solubility study it was observed that HP-β-CD and M-β-CD showed AL type phase solubility curve indicated improved solubility. This fact is well supported by Challa et al. Solubility of Ezetimibe increased in all media in a linear fashion with increased concentration of HP-β-CD and M-β-CD and showed AL type phase solubility curve indicating that soluble complexes were formed and no precipitation was observed.

\[(M\text{-}\beta-\text{CD}, \text{distilled water}) \quad Y = 0.0022X + 0.00003 \quad R^2 = 0.995\]
\[(\text{HP}\text{-}\beta-\text{CD}, \text{distilled water}) \quad Y = 0.002X \quad R^2 = 0.990\]

The stability constant (Ks) of the 1:1 complex of drug with hydroxypropyl-betacyclodextrin and 1:2 complex of methylated betacyclodextrin was calculated from slope of straight line in AL type solubility diagram and was found to be following 185.367 and 211.478 M\(^{-1}\) respectively.

Ks values obtained are adequate for the formation of inclusion complexes which may contribute improving the bioavailability of poorly water soluble drugs.

![Phase Solubility Diagram of Ezetimibe with β-CD](image)

**Table 6 : Summary of Ezetimibe cyclodextrin phase solubility studies.**

<table>
<thead>
<tr>
<th>Cyclodextrin/medium</th>
<th>Type of phase solubility diagram</th>
<th>Stability constant M(^{-1})</th>
<th>Increased solubility St/ So</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betacyclodextrin in distilled water</td>
<td>Bs</td>
<td>114.05</td>
<td>11.95</td>
</tr>
<tr>
<td>Hydroxypropylbetacyclodextrin in distilled water</td>
<td>AL</td>
<td>185.367</td>
<td>20.22</td>
</tr>
<tr>
<td>Methylated betacyclodextrin in distilled water</td>
<td>AL</td>
<td>211.478</td>
<td>24.38</td>
</tr>
</tbody>
</table>

Where,  
St- highest solubility of Ezetimibe with carrier  
So - solubility of Ezetimibe without carrier  
M- moles
Characterization of inclusion complexes
The optimized inclusion complexes were further characterized by FTIR and X-RD, DSC.

The characteristic peak of ezetimibe such as
FTIR study
The characteristic peak of ezetimibe such as OH-Streching 3442 cm\(^{-1}\) & 3300 cm\(^{-1}\), Asymmetric C-H Streching 2937 cm\(^{-1}\), Symmetric C-H Streching 2817 cm\(^{-1}\), O stretching 1716.53 cm\(^{-1}\), Aromatic C-H Streching 3024.18 cm\(^{-1}\) and C=C 1622 cm\(^{-1}\). The result of FTIR Was Showed In Fig.4.

Were found to be retained in all inclusion complexes showing only architectural fitting of the drug molecule into the cyclodextrins without any chemical interaction.

X-Ray Diffractometry
Characteristic peaks of ezetimibe lies between 20:6 to 30. Broadening of some of these characteristic peaks and complete disruption of remaining in all the complexes shows change in solid characteristic of the drug with subsequent reduction in the crystallinity. Thus X-RD confirms formation of complexes. The result of X-Ray Diffractometry Was Showed In Fig.5.
DSC

The endothermic peak with Tmax 163.0-166.0°C corresponds to the melting of the ezetimibe. In physical mixtures of ezetimibe with cyclodextrin as well as inclusion complexes, there is a decrease in the melting point. However, in the case of physical mixture with HP-β-CD, the change is slight. Whereas, maximum decrease in melting point (160-164°C) was formed with drug-methylated βCD complex prepared by ultrasonification, which confirms maximum interaction and complexation with this derivative of cyclodextrin. The result of DSC was showed in Fig. 6.

---

**Fig. 6  DSC Spectra of Drug- Cyclodextrins Inclusion Complexes**

A) Ezetimibe

B) Methylated Cyclodextrin 1:2 US

C) Methylated Cyclodextrin (1:2 KN)

D) Hydroxy Propyl BCD (1:1 US)

E) Hydroxy Propyl BCD (1:1 KN)

F) Methylated Cyclodextrin (1:2 PM)

G) Hydroxy Propyl BCD (1:1 PM)
UV Interference

In order to study the possibility of any drug polymer interaction, the scanning of the various inclusion complexes were carried out in 0.05 M acetate buffer pH 4.5 and scanning results indicated that there was no interference or shifting of \( \lambda_{\text{max}} \) of ezetimibe which reflects no drug polymer interaction.

**Drug content**

Drug content of all inclusion complexes were in the range of 74.66-85.97%. This indicates the proper loading of drug in inclusion complexes and effectiveness of kneading method and ultrasonification.

**Solubility and Dissolution**

The solubility of all inclusion complexes was studied in distilled water and 0.05 M acetate buffer pH 4.5. The data indicated that solubility increased in all cases but highest increase was found in inclusion complexes prepared by ultrasonification with HP-\( \beta \)-CD prepared in 1:1M (HPUS) and in methylated betacyclodextrin prepared in 1:2 ratio (MeUS).

The mean dissolution curve of Ezetimibe from various binary systems with CD’s is present in Fig.7-9. It is evident that all system with CD’s exhibited better dissolution properties than pure drug alone. Significant difference in term of dissolution was found in all Ezetimibe M-\( \beta \)-CD system and the one with HP-\( \beta \)-CD reflecting greater solubility. The greater ability of M-\( \beta \)-CD in Ezetimibe amorphization could explain the better dissolution properties of the drug. As for the influence of the preparation method, an analog trend was observed with both CD’s; the greatest improvement of drug dissolution was obtained with ultrasonification product, followed in order by kneading and finally by physical mixture. The increased dissolution rate (physical mixture) is attributable both to improvement in drug wettability and to formation of readily soluble complexes in dissolution medium. Further improvement obtained with kneading and ultrasonification could be explain by both the more intimate contact between drug and carrier and the decrease of drug crystallinity, as well as a phenomenon of at least partial drug inclusion complexation. On the contrary, the influence of the preparation method was clearly more marked in case of product with M-\( \beta \)-CD, where kneaded and ultrasonification product showed an increase in dissolution efficiency of 99 or 138%, in comparison to corresponding physical mixture. The best performance of these product seemed to confirm that drug inclusion complexation occurred substantially only in such systems, thus allowing to obtain the highest dissolution improvement.

Dissolution data of inclusion complexes also indicated that there is increase in dissolution as compared to pure drug and maximum increase was observed in case of inclusion complexes (1:1HPUS) and(1:2 MeUS). But data of solubility and dissolution studies indicate that there was a increase in both the solubility and dissolution as compared to (1:1 HPKN) and (1:2 MeKN). The batch (1:1 HPUS) and (1:2 MeUS) was considered as optimized batch since it showed significant difference(P<0.01) in both solubility and dissolution characteristic as compared to batch 1:1HPKN and 1:2 MeKN.

The results of solubility and dissolution are as shown in Table No 7, Fig.7 -9. For HP-\( \beta \)-CD and M-\( \beta \)-CD complexes respectively.

**Table 7: Solubility of Ezetimibe from various inclusion complexes.**

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Inclusion complexes</th>
<th>Solubility in distilled water (mg/100ml)</th>
<th>Solubility in 0.05 M buffer pH 4.5(mg/10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ezetimibe</td>
<td>0.8±0.002</td>
<td>2.714±0.00001</td>
</tr>
<tr>
<td>2</td>
<td>1:1HPPM</td>
<td>1.592±0.0001</td>
<td>3.167±0.007</td>
</tr>
<tr>
<td>3</td>
<td>1:2 MePM</td>
<td>2.229±0.0002</td>
<td>3.167±0.0006</td>
</tr>
<tr>
<td>4</td>
<td>1:1 HPKN</td>
<td>5.095±0.098</td>
<td>4.072±0.0001</td>
</tr>
<tr>
<td>5</td>
<td>1:2 MeKN</td>
<td>6.687±0.026</td>
<td>4.971±0.0003</td>
</tr>
<tr>
<td>6</td>
<td>1:1 HPUS</td>
<td>7.643±0.037</td>
<td>6.334±0.0001</td>
</tr>
<tr>
<td>7</td>
<td>1:2 MeUS</td>
<td>8.914±0.98</td>
<td>8.597±0.0008</td>
</tr>
</tbody>
</table>

* Represents mean ± S.D. (n= 3).
Stability studies

A result of stability study indicated that the inclusion complex (HPUS) and (MeUS) was stable and there was no significant changes (P>0.05) observed in the drug content.

Tablet formulation of selected inclusion complexes

The drug, inclusion complex, and polymer were evaluated for the physical parameters. The values of bulk density, tapped density, compressibility index and Hausner ratio were calculated (Table 8). The physical parameters of inclusion complexes as well as the excipients concluded that these were considerably good to formulate the tablet using direct compression technique.
Table 8: Physical properties of drug and excipients.

<table>
<thead>
<tr>
<th>Material</th>
<th>Bulk density (g/cm³)</th>
<th>Tapped density (g/cm³)</th>
<th>Compressibility Index (%)</th>
<th>Hausner ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ezetimibe</td>
<td>0.2192</td>
<td>0.2941</td>
<td>25.43</td>
<td>1.34</td>
</tr>
<tr>
<td>Cross Povidone</td>
<td>0.328</td>
<td>0.396</td>
<td>17.10</td>
<td>1.20</td>
</tr>
<tr>
<td>Microcryatalline cellulose</td>
<td>0.260</td>
<td>0.431</td>
<td>39.58</td>
<td>1.65</td>
</tr>
<tr>
<td>Inclusion complex (HPUS)</td>
<td>0.595</td>
<td>0.694</td>
<td>14.28</td>
<td>1.16</td>
</tr>
<tr>
<td>Inclusion complex (MeUS)</td>
<td>0.367</td>
<td>0.462</td>
<td>20.58</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Interparticulate interactions that influence the bulking properties of a powder interfere with the powder flow. A comparison of the bulk density and tapped density can give a measure of the relative importance of these interactions in a given powder, such a comparison is often used as an index of the ability of the powder to flow, indication of the ease with which a material can be induced to flow is given by application of a compressibility index. The value for bulk density, tapped density and compressibility index and Hausner ratio reflects free flowing characteristics of inclusion complex HPUS and MeUS as compared to plain drug.

Evaluation of ezetimibe tablets

The ezetimibe tablets were prepared by direct compression technique using 8 mm concave punch. The prepared tablets were physically characterized for the physical parameters of tablets of each batch. Results reflected that all batches had good physical characteristics i.e. weight variation, thickness, hardness, and friability and disintegration time values well within permissible range. The physical parameters of prepared tablets are as shown in Table 9.

Table 9: Characteristics of tablets of Ezetimibe inclusion complex (US).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Thickness* (mm)</th>
<th>Hardness* (Kg/cm²)</th>
<th>%Friability*</th>
<th>Drug content* (%)</th>
<th>Disintegration* (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-1</td>
<td>1.7±0.03</td>
<td>5±0.068</td>
<td>0.439±0.015</td>
<td>99.78±0.20</td>
<td>3.78±2.31</td>
</tr>
<tr>
<td>HP-2</td>
<td>2.0±0.35</td>
<td>4.8±0.115</td>
<td>0.55±0.036</td>
<td>99.83±0.24</td>
<td>3.61±1.15</td>
</tr>
<tr>
<td>HP-3</td>
<td>2.2±0.126</td>
<td>4.8±0.058</td>
<td>0.457±0.019</td>
<td>97.58±0.28</td>
<td>3.33±0.58</td>
</tr>
<tr>
<td>HP-4</td>
<td>2.0±0.6</td>
<td>4.8±0.058</td>
<td>0.621±0.010</td>
<td>98.67±0.28</td>
<td>2.28±0.15</td>
</tr>
<tr>
<td>HP-5</td>
<td>2.0±0.25</td>
<td>4.7±0.21</td>
<td>0.702±0.019</td>
<td>99.51±0.14</td>
<td>3.77±0.58</td>
</tr>
<tr>
<td>Me-1</td>
<td>2.0±0.56</td>
<td>5±0</td>
<td>0.521±0.01</td>
<td>99.52±0.4</td>
<td>3.68±1</td>
</tr>
<tr>
<td>Me-2</td>
<td>2.0±0.33</td>
<td>4.8±0.058</td>
<td>0.344±0.009</td>
<td>98.2±0.08</td>
<td>2.17±0.18</td>
</tr>
<tr>
<td>Me-3</td>
<td>2.3±0.68</td>
<td>4.5±0.058</td>
<td>0.427±0.007</td>
<td>98.05±0.047</td>
<td>2.18±1.0</td>
</tr>
<tr>
<td>Me-4</td>
<td>2.2±0.019</td>
<td>5±0.258</td>
<td>0.459±0.469</td>
<td>97.07±0.02</td>
<td>3.46±1.05</td>
</tr>
<tr>
<td>Me-5</td>
<td>1.8±0.037</td>
<td>5±0.171</td>
<td>0.218±0.003</td>
<td>99.7±0.173</td>
<td>1.26±0.15</td>
</tr>
</tbody>
</table>

*Represents ± S.D (n =3).

Dissolution studies

In vitro dissolution indicated that the release of ezetimibe varied according to the type and ratio of the different cyclodextrins. In the inclusion complexes HPUSHP the batches HP-1 to HP-5 and MeUS , batches Me-1 to Me-5  were prepared using Cross- Povidone as distintegrant in different concentration. All the formulation showed release of Ezetimibe more than 90% but more and faster release of the drug was found in batch HP-4 that is 99.76 % as compared to other batches and marketed formulation within 15 min and in batch Me-4 that is 99.48 % as compared to other batches and marketed formulation within 10 min . The results of dissolution are as shown in Fig.10 .
Statistical Analysis

Table 10: Statistical treatment to dissolution data of optimised inclusion complexes.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Formulation comparison</th>
<th>P value</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Marketed Vs HP-4</td>
<td>P &lt; 0.01</td>
<td>Significant difference</td>
</tr>
<tr>
<td>2</td>
<td>Marketed Vs Me-4</td>
<td>P &lt; 0.01</td>
<td>Significant difference</td>
</tr>
</tbody>
</table>

Marketed formulation was considered as control and compared with optimised batches (HP-4 and Me-4) by applying one way ANOVA followed by Dunnett test.

Stability study

Results of stability study indicated that there were no significant changes in physical properties and dissolution profile.

CONCLUSION

Inclusion complexes of Ezetimibe prepared with M-β-CD and HP-β-CD showed improved stability and dissolution behavior as compared to plain drug. Amongst all complexes prepared with M-β-CD, complexes MeUS (1:2M) prepared by ultrasonification showed significant increased in solubility and dissolution. Complexes were stable with non significant increases in drug content, solubility and dissolution rate after three month stability testing under 75±5 % RH & 40±2°C. Tablet formulation HP-4 (containing HPUS 1:1) and Me-4 (containing MeUS 1:2) showed fastest and highest dissolution of all. Hence the bioperformance of ezetimibe can be improved by using methylated and hydroxylpropyl betacyclodextrin as carrier. Also, effective and stable tablet formulation could be developed using cross povidone as super disintegrant.

ACKNOWLEDGMENT

We wish thank to AICTE Delhi to provide PG scholarship for M.Pharm dissertation work.

Conflict of interests

No conflict of interests
REFERENCE
1. www.drugs.com/ezetimibe.html