PREPARATION AND CHARACTERISATION OF β-CYCLODEXTRIN NEBIVOLOL INCLUSION COMPLEX

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ABSTRACT: Nebivolol HCl (NEB) is antihypertensive drug having β-adrenergic blocking properties. Although NEB is rapidly absorbed after oral administration, it has low bioavailability (12%), due to its low water solubility. The purpose of present work was to enhance the solubility and dissolution rate of NEB by inclusion complexation technology. Complexation is the association between two or more molecules to form a noncovalent based complex that has higher solubility than the drug itself. From solubility standpoint, complex can be put in to two categories, stacking complexes and inclusion complexes. The aim of this work was to study the influence of β-cyclodextrin (β-CD) on the biopharmaceutical properties of Nebivolol HCl. To this purpose the physicochemical characterization of NEB-β-cyclodextrin binary systems was performed both in solution and solid state. Present study includes deals with the preparation of inclusion complex of NEB β-cyclodextrin as carrier and to evaluate NEB β-cyclodextrin inclusion complex for various parameters viz., % practical yield, drug content, in vitro release study, drug-excipients interaction study, etc.

INTRODUCTION: Nebivolol hydrochloride is a class II drug that selectively blocks β1 receptor with therapeutic applications as antihypertensive and can also used as monotherapy for initial management of uncomplicated hypertension. The main objective of this work was to investigate the possibility of improving the solubility and dissolution rate of NEB by preparing inclusion complexation with β-cyclodextrin by five different methods viz. physical mixture, kneading, microwave irradiation, co-evaporation and freeze drying method. The prepared inclusion complex were evaluated for % practical yield, drug content, invitro dissolution rate studies and interactions between drug and carriers using DSC, XRD, FTIR and SEM studies.1,2 Cyclodextrin are cyclic (α-1, 4)-linked oligosaccharides of α-D-glucopyranose, containing a relatively hydrophobic central cavity and hydrophilic outer surface. Owing to lack of free rotation about the bonds connecting the glucopyranose units, the cyclodextrin are not perfectly cylindrical molecules but the toroidal or cone shaped. Based on this architecture, the primary hydroxyl groups are located on the narrow side of the cone shape, while the secondary hydroxyl groups are located on the wider edge.

During the past two decades, cyclodextrin and their derivatives have been of considerable interest in the pharmaceutical field because of their potential to form complexes with a variety of drug molecules. Cyclodextrin are used to increase the solubility of water insoluble drug through inclusion complexes formulation. The hydrophobic cavity of cyclodextrin is capable of trapping a variety of molecules within to produce inclusion complexes. Many advantages of drugs complex with cyclodextrin have been reported in scientific literature which includes-increased solubility,
enhanced bioavailability, improved stability, masking of bad test or odor, reduced volatility, transformation of liquid or gas into solid form, reduced side effect, and the possibility of a drug release system, etc. The solid state characteristics of the drug after preparation and during storage will depend on the processing variables as well as the characteristics of the system.

When the objective of the formulation is to attain faster dissolution rates, presence of amorphous of the drug and improved dissolution rate would therefore be a result of presence of amorphous high energy forms of the drug as well as the ability of the cyclodextrin to form a soluble complexes with the drug, the performance of the product over the shelf life would depend on the ability of the cyclodextrin to prevent crystallization of the amorphous drug to its stable crystalline forms. However the use of cyclodextrin in the solid oral dosage forms is limited to low dose drugs with large stability constant due to mass limitations of oral dosage.

In this study, an attempt was made to improve the solubility and dissolution rate of Nebivolol by complexing with β-Cyclodextrin, thereby increasing its bioavailability and therapeutics efficiency. The complex of β- cyclodextrin with Nebivolol was prepared by using physical mixture method, kneading method, microwave irradiation method, co-evaporation method and freeze drying method at 1:1, 1:2, 1:3 stoichiometric ratio.

The characterization of drug with β-Cyclodextrin using differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD), FTIR, In vitro aqueous solubility and dissolution rate profile of complexes were performed. Complexation is a unique technique used to increase solubility, dissolution and bioavailability of poorly water-soluble drugs.

MATeRIALS:
The Nebivolol supplied by Watson Pharma limited (Mumbai, India), β-Cyclodextrin obtained from Loba Chemie Pvt. Ltd. (Mumbai, India)

METHODS:
Phase solubility studies:

Phase solubility studies were performed according to the method reported by Higuchi and Connors. An excess amount of Nebivolol was added to 20 ml of solution i.e distilled water, 0.1N HCl and phosphate buffer pH7.4 containing various concentrations of β-Cyclodextrin (0, 2, 4, 6, 8 and 10 mM) into the glass vials. The contents were shaken for 48 hours at room temperature on rotary flask shaker.

After equilibrium, the samples were withdrawn, filtered through a Whatman paper and appropriately diluted. A portion of samples were analyzed by UV spectrophotometer at 281 nm. The apparent stability constant was calculated from the initial straight portion of the phase solubility diagram using the following equation:

\[
K_c = \frac{\text{Slope}}{\text{Intercept} \times (1 - \text{slope})}
\]

Preparation of Inclusion complexes:
After determining the optimum proportions of drug with the complexing agent the inclusion complexes were prepared using following methods:

1) Physical mixture method:
The required molar (1:1, 1:2, 1:5) ratio quantities of drug and β-CD were weighed and mixed separately in mortar by vigorous trituration for 1 hrs. The mixtures were then passed through sieve no. 40 and were stored in airtight container until further use.

2) Kneading Method:
In this method, a solution of distilled water and methanol (1:1) was used as moistening agent and was added to accurately weighed molar quantities of each of β-CD. This molar quantities of drug were added with constant grinding. This grinding was continued for 1hour and the consistency of pastes was maintained using the appropriate quantities of moistening agent. These pastes were finally dried in a hot air oven at 40ºC for 24 hours. The dried pastes were then passed through sieve number 40 and the resulting mass was collected and stored in airtight containers until further use.

3) Microwave irradiation method:
The required molar quantities of drug and each of β-CD were triturated in a glass mortar using minimum quantity of solvent mixture (Methanol: Water = 1:1 v/v). This mass was then kept in microwave oven, which was set at power level 2,
and the reaction was allowed to take place for 90 seconds. After this more amount of solvent mixture was added to reaction mixture and then precipitated mass was filtered out. These precipitates were dried in hot air oven at 60°C for 24 hours to finally get a white powder mass of inclusion complexes of drug with cyclodextrins. These inclusion complexes were collected and stored in airtight containers until further use.

4) Co-evaporation method:
This method involves dissolving of the drug and β-CD separately in to two mutually miscible solvents such as methanol and water respectively, mixing of both the solutions to get molecular dispersions of drug and complexing agents and finally evaporating the solvent under vaccum to obtain solid powdered inclusion complex. The resulting mixture is stirred for 24 hours evaporated under vaccum at 45°C. The dried mass was pulverized and passed through a sieve number 40.

5) Freeze drying method:
In order to get a porous, amorphous powder with high degree of interaction between drug and cyclodextrine, freeze drying technique is considered as a suitable. In this technique, the solvent system from the solution is eliminated through a primary freezing and subsequent drying of the solution containing both drug and cyclodextrine at reduced pressure. The required molar quantities of drug was added in aqueous solution of β-CD. The resulting solution were frozen at -20°C overnight and lyophilized in freeze dryer maintained at freezing temperature of -70 to -75°C, vaccum applied in dried chamber of 0.1mbar at ambient temp.(16 to 20°C). The lyophilized powder was passed through a sieve no. 40 and stored in sealed glass vials.

TABLE 1: FORMULATION INGREDIENTS, PREPARATION METHOD OF NEBIVOLOL β-CYCLODEXTRIN INCLUSION COMPLEX

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Composition</th>
<th>Method</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Physical mixture</td>
<td>1:1</td>
</tr>
<tr>
<td>F2</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Physical mixture</td>
<td>1:2</td>
</tr>
<tr>
<td>F3</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Physical mixture</td>
<td>1:5</td>
</tr>
<tr>
<td>F4</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Kneading method</td>
<td>1:1</td>
</tr>
<tr>
<td>F5</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Kneading method</td>
<td>1:2</td>
</tr>
<tr>
<td>F6</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Kneading method</td>
<td>1:5</td>
</tr>
<tr>
<td>F7</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Microwave irradiation method</td>
<td>1:1</td>
</tr>
<tr>
<td>F8</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Microwave irradiation method</td>
<td>1:2</td>
</tr>
<tr>
<td>F9</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Microwave irradiation method</td>
<td>1:5</td>
</tr>
<tr>
<td>F10</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Co-evaporation method</td>
<td>1:1</td>
</tr>
<tr>
<td>F11</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Co-evaporation method</td>
<td>1:2</td>
</tr>
<tr>
<td>F12</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Co-evaporation method</td>
<td>1:5</td>
</tr>
<tr>
<td>F13</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Freeze drying method</td>
<td>1:1</td>
</tr>
<tr>
<td>F14</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Freeze drying method</td>
<td>1:2</td>
</tr>
<tr>
<td>F15</td>
<td>β-cyclodextrin + Nebivolol</td>
<td>Freeze drying method</td>
<td>1:5</td>
</tr>
</tbody>
</table>

Evaluation of Nebivolol- β cyclodextrin inclusion complex:

1) Physical Appearance:
All the batches of Nebivolol - β-cyclodextrin inclusion complex were evaluated for colour and appearance.

2) Percent Practical Yield (PY):
Percentage practical yield were calculated to know about percent yield or efficiency of any method, thus its help in selection of appropriate method of production. Inclusion complex were collected and weighed to determine practical yield (PY) from the following equation.

PY (%) = Practical Mass (SD) / Theoretical Mass (Drug + Carrier)] ×100

3) Assay or Drug content estimation:
Accurately weighed complexes equivalent to 5mg of Nebivolol were transferred in 25ml volumetric flask. 10ml methanol was added and sonicated for 5min. The volume was made up to 25ml with distilled water. This solution was filtered using whattman filter paper in 25ml volumetric flask, again make up to the volume with distilled water up to 25ml. 1ml solution was withdrawn and diluted upto 10ml distilled water and determined spectro photometrically at 281nm
4) **Solubility Studies:**
To the glass vials containing 5 ml of distilled water, excess quantity of drug and each Inclusion complex type were added separately. These vials were shaken on laboratory shaker for 24 hours. The resulting solutions were filtered, appropriate dilutions were made using distilled water and the absorbances were recorded at 281 nm.

5) **Thin Layer Chromatography:**
The solvent system for running TLC of Nebivolol was Toluene: Methanol: Triethylamine (3.8:1.2:0.2). Each Inclusion complex was subjected to TLC using pure Nebivolol as reference sample. Rf values for each were calculated.

6) **Spectral Analysis:**
1. **UV-Visible Spectrum recording:**
UV spectra of pure Nebivolol powder and its Inclusion complex were recorded in methanol. Also a blank was run using methanol.

2. **IR Spectrum Analysis:**
Fourier transform infrared (FTIR) spectroscopy was employed to characterize any possible interaction between drug and carrier in the solid state on a FTIR spectrophotometer by the conventional KBr pellet method. The spectra were scanned over a frequency range 4000–400 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\).

7) **Differential Scanning Calorimetric Analysis:**
The possibility of any interaction between the drugs and the carriers during different approaches was assessed by carrying out thermal analysis of drug as well as the optimized formulation, using DSC. DSC analysis was performed using Perkin Elmer Analyzer Jade DSC on 4 to 5 mg samples. Samples were heated in an open aluminium pan at a rate of 10°C/min conducted over a temperature range of 50 to 300°C under a nitrogen flow of 20 ml/min.

8) **X–Ray Diffraction Studies:**
To determine the powder characteristics, X-ray powder diffraction studies of drugs alone as well as that of the optimized formulation was performed. X-ray powder diffraction patterns were recorded on Bruner AXS, DH advanced model. The scanning rate employed was 6° min\(^{-1}\) over 10 to 50°C diffraction angle (2θ) range.

9) **Scanning Electron Microscopy:**
Morphology of prepared solid dispersion was examined by scanning electron microscope (Jeol model) operating at 10.0 kV accelerating voltage. For conventional imaging in the SEM, specimens must be electrically conductive, at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge at the surface. Therefore the optimized solid dispersions were carbon coated before being subject to electron scanning. The energy of electron beam was set at 10 kV.

10) **Dissolution Studies:**
Each type of Inclusion complex sample equivalent to 5 mg of Nebivolol was subjected to dissolution test using Dissolution Test Apparatus USP XXII (Type–II). Pure Nebivolol was used as control and was subjected to similar dissolution test using 5 mg of pure drug.

**Test Parameters used for Dissolution Test**
- **Speed of Paddle:** 50 rpm.
- **Temperature of Dissolution Medium:** 37°C ± 0.5°C
- **Apparatus Type:** USP XXII (paddle)

**Procedure:**
Inclusion complex equivalent to 5 mg of Nebivolol were placed in various dissolution medium (0.1N HCl and phosphate buffer pH7.4)and apparatus was run. 5 ml aliquots were withdrawn at time intervals of 5min., 10min., 15min., 20min., 25min., 30min., 35min., 40min., and 50min.55min. 60min. and 5ml of fresh dissolution medium was added after each with drawl. Each sample was filtered through Whatman filter paper (No 41) and absorbances were measured at 281 nm using UV visible double beam Spectrophotometer (Shimadzu, UV-1800).

**RESULT AND CONCLUSION:**
In the present investigation, an attempt was made to improve the solubility and dissolution rate of a drug Nebivolol by complexation method using β-cyclodextrin as carrier. Inclusion complex was prepared by Physical mixture,Kneading method, co-evaporation method, Microwave irradiation method and freeze drying method. The prepared
Inclusion complex of Nebivolol were prepared by different method using carrier as β-cyclodextrin. In the present work, total 15 formulations were prepared and their complete composition is shown in Table. The entire Inclusion complexes prepared were found to be fine and free flowing powders.

The results of percent practical yield studies are shown in Table 5. The % Practical yield of the prepared Inclusion complex was found to be in the range of 83.40 – 99.01 %. The actual drug content of all the 15 formulations is shown in Table 6. The drug content of the prepared Inclusion complex...
was found to be in the range of 78.85 – 100.04 % indicating the application of the present methods for the preparation of inclusion complex with high content uniformity. The maximum % drug content was found to be 100.04% in F15.

**TABLE 6: DISSOLUTION PROFILE OF PURE DRUG, OPTIMIZED BATCH F15 AND MARKETED PREPARATION IN 0.1N HCL**

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>% Cumulative drug release ± S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug</td>
<td>Marketed product</td>
</tr>
<tr>
<td>5</td>
<td>4.95±0.96</td>
</tr>
<tr>
<td>10</td>
<td>5.85±0.89</td>
</tr>
<tr>
<td>15</td>
<td>9.67±1.04</td>
</tr>
<tr>
<td>20</td>
<td>13.5±0.90</td>
</tr>
<tr>
<td>25</td>
<td>16.67±0.97</td>
</tr>
<tr>
<td>30</td>
<td>18.22±0.88</td>
</tr>
<tr>
<td>35</td>
<td>21.82±0.96</td>
</tr>
<tr>
<td>40</td>
<td>23.17±1.21</td>
</tr>
<tr>
<td>45</td>
<td>25.87±0.91</td>
</tr>
<tr>
<td>50</td>
<td>26.32±0.90</td>
</tr>
<tr>
<td>55</td>
<td>27±1.12</td>
</tr>
<tr>
<td>60</td>
<td>27.45±1.01</td>
</tr>
</tbody>
</table>

Drug release from Inclusion complex was faster than pure drug. Cumulative percent drug released after 60 minutes was 72.67 for F15, while it was 27.45% in 60 minutes for pure drug Nebivolol. *In vitro* release study revealed that there was a marked increase in the dissolution rate of Nebivolol from all inclusion complexes when compared to pure Nebivolol itself. From the in-vitro drug release profile, it can be seen that formulation F-15 containing β-cyclodextrin (1:5 ratio of drug: β-cyclodextrin) show higher dissolution rate i.e. 72.67% compared with other formulations. In all above formulations, the drug Nebivolol is in the free state and available for absorption.

**TABLE 4: STANDARD CALIBRATION DATA OF NEBIVOLOL IN METHANOL**

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Absorbance at 281nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.105</td>
</tr>
<tr>
<td>10</td>
<td>0.221</td>
</tr>
<tr>
<td>15</td>
<td>0.321</td>
</tr>
<tr>
<td>20</td>
<td>0.434</td>
</tr>
<tr>
<td>25</td>
<td>0.533</td>
</tr>
<tr>
<td>30</td>
<td>0.649</td>
</tr>
<tr>
<td>35</td>
<td>0.766</td>
</tr>
<tr>
<td>40</td>
<td>0.839</td>
</tr>
</tbody>
</table>

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**FIGURE 3: COMPARISION OF OPTIMIZED BATCH WITH PURE DRUG AND MARKETED FORMULATION OF NEBIVOLOL IN 0.1N HCL**

The polymer taken in this study is β-cyclodextrin which contains number of secondary hydroxyl group and primary ─OH group. These two functionalities present in the molecules have shown a broad peak around 3400cm-1 corresponding to primary and secondary ─OH groups. C─H absorption is notice at 2921.63 and 2379.73 cm-1. Strong absorption peak is also notice at 1027.87 cm-1. These data are in conformity with structure of the β-cyclodextrin. When these two constituents, Drug and polymer β-cyclodextrin used for the required formulation. The IR spectrum of which has shown the presence of all the functionalities present in the drug as well as β-cyclodextrin suggests these during the preparation of formulation chemical reaction between two has not taken place.

The formulated product is just take physical mixture none of the functional groups absorption is not affected. The formulation done by taking drug and the polymer in 1:2 ratio. Hence this formulated is a physical mixture of all the two constituents but not the reaction product of any constituents.

Comparing the FTIR data of formed optimized complex with Nebivolol. It was found that the peak observed for complexes (Figure4C) is identical with each other at near about same characteristics.
peaks. Therefore from IR data it was confirmed that there was absence of undesired interaction or chemical interaction with excipient (i.e no change in chemical nature of drug molecule). The drug remained stable in complex formed. There was H-H bond formation take place due to interaction between Nebivolol and β-cyclodextrin 1 and 2 alcoholic group in tern resulted in to complex formation leading to solubility enhancement.

The drug Nebivolol subjected for DSC study, it started melting at 224.4°C and completed at 233.88°C, suggesting that these narrow range of melting is due to the present of single compound in the pure form. The carbohydrate polymer β-cyclodextrin is taken for DSC measurement this polymer has given rang to wide range to melting process started at 110°C to 160°C.

This is the characteristics behavior of the carbohydrates. When the formulation obtained by the drug and β-cyclodextrin for DSC study two melting ranges are obtained in one peak, melting process started at 90.4°C and reach the peak at 130.93°C in an another peak process started at 210°C reach the peak at 226.02°C, suggesting that, when these two molecules (Drug and β-cyclodextrin) used for the formulation product obtained is nothing but a physical mixture of the two. In all above formulations, the drug Nebivolol is in the free state and available for absorption.

For conventional imaging in the SEM, specimens must be electrically conductive, at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge at the surface. Therefore the optimized inclusion complex were carbon coated before being subject to electron scanning. The energy of electron beam was set at 10 kV. Nebivolol existed as needle shape crystals (Figure 6a), whereas β-Cyclodextrin seen as clusters present in compact form consist of irregular size crystals (Figure 6b).

The complex appeared in the form of irregular shape reduced size particles in which the original morphology of both component disappeared and tiny particles of irregular size were present (Figure 6d). Therefore, the reduced particle size increased the surface area and close contact between the hydrophilic carriers and Nebivolol might be responsible for solubility enhancement.
CONCLUSION: The data obtained from the study of Nebivolol β-cyclodextrin inclusion complex in which the β-cyclodextrins used as a complexing agent. The following points can be concluded: The dissolution rate of Aspirin from inclusion complex i.e., F1-F15 was significantly higher than that of pure drug, inclusion complex prepared by Freeze drying method showed faster drug release than the other method, IR studies indicated that no chemical interaction between drug and polymer took place during preparation of inclusion complex of Nebivolol and β-cyclodextrin, DSC studies indicated that Nebivolol was homogeneously distributed within the carrier in an amorphous state and no drug crystallized out of the dispersion suggesting that drug and polymer exist in the form of a mixture rather than the reaction product.

In-vitro drug release of Nebivolol β-cyclodextrin inclusion complex of 1:5 (F15) showed higher drug release, the IR study showed that there was no chemical interaction between Nebivolol and polymer β-cyclodextrin. From overall formulation, F-15 is the best formulation containing 1:5 ratio of β-cyclodextrin and Nebivolol.

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REFERENCES:

1. Olga Hilas, Danielle Ezzo, Nebivolol (Bystolic), a Novel Beta Blocker for Hypertension, Drug Forecast, 2009; 34: 4
4. Szejtli Jozsef, Past present and future of cyclodextrin research, Pure applied chemistry, 2004; 76, 1829-1833
5. Martin E. M., Cyclodextrin and their uses: A Review , process biochemistry, 2003; 30; 5-7
7. Patil J.S., Kadam D.V., Inclusion complex system a novel technique to improve the solubility and bioavailability of

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