Innovating the phase-solubility and compatibility study of Anticancer drug complexed with β-cyclodextrin and hp – β-cyclodextrin

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Abstract
Poor aqueous solubility and dissolution rates are critical problems that hinders the formulation, development and delivery of most of BCS class II and class IV drugs. Gefitinib is a cytotoxic chemotherapeutic drug used in treatment of cancer. The objective of the present study was to investigate the drug-cyclodextrin compatibility study by FTIR and DSC study. The phase solubility study revealed formation of 1:1 stoichiometry binary inclusion complex. The complex was prepared by kneading method. FT-IR spectra provided the data indicating that the HP-β-CD was more effective than β-CD. Differential scanning calorimetry thermograms indicated stronger amorphization and entrapment of gefitinib with HP-β-CD.

Keywords: gefitinib, cyclodextrins, complexation, DSC, FTIR.

Introduction
Gefitinib is the first selective inhibitor of epidermal growth factor receptor's (EGFR) tyrosine kinase domain. The chemical name of gefitinib is N-(3-chloro-4-Fluorophenyl)-7-methoxy-6-(3-(4-morpholinyI) propoxy)-4-quinazolinamine. It has poor oral bioavailability of approximately 59% as it is sparingly soluble in water. The objective of present work was to prepare host-guest binary inclusion complex of gefitinib with natural and chemically derived cyclodextrin to overcome its solubility, bioavailability and dissolution problem. The structure of gefitinib is shown in figure 1.

Truncated cone structured cyclodextrin having toroidal molecules with inner cavity having lipophilic and outer cavity hydrophilic surface and form non-covalent host-guest (CD-Drug) inclusion complexes with an appropriate shape/size. Pharmaceutically these cyclodextrin provides the useful complexation characteristics to broad range of drugs. Complexation is widely acceptable and feasible technique in laboratories and industries for modifying the critical issues or limitations associated with delivery of BCS class II and class IV drugs. The structure of β-CD and HP-β-CD is shown in figure 2.
Materials

Gefitinib was obtained as a gift sample from Sky lab Mumbai, India. β- cyclodextrin and Hydroxypropyl-β-Cyclodextrin were received from Roquette Pharma, France as gift samples. All the chemicals used for study were of analytical grade.

Experimental Methods

Phase Solubility Studies

The solubility studies were carried out according to the method reported by Higuchi and Connors. For this an excess amount of drug gefitinib (100 mg) was added to vials containing variable amount/different concentrations of β-CD or HP-β-CD. These vials were shaken for 48 hours. After shaking, the solutions were filtered and their absorbance was noted at 245 nm. The solubility of the Glimepiride in every β-CD or HP-β-CD solution was calculated and phase solubility diagram were plotted. The stability constant was calculated by using Eq. 1.

\[ K_{1:1} = \frac{\text{slope}}{S_0 (1-\text{slope})} \]  

Where, \( S_0 \) is the intrinsic solubility of drug in absence of CDs at 30±1°C

Preparation of Inclusion complex

The binary inclusion complex of gefitinib with cyclodextrins were prepared by physical mixture and kneading method.

Physical mixture

Gefitinib with β-CD/HP-β-CD in 1:1 molar ratio (1:1M) were prepared by mixing in a mortar for about one hour with constant triturations, then passed through sieve No. 80 and stored in desiccator over fused calcium chloride.

Kneading

Gefitinib with β-CD/HP- β-CD in 1:1M ratio were used to prepare kneaded complex. Firstly β-CD/HP- β-CD cyclodextrin was added to the mortar, small quantity of 50% ethanol was added with triturations to get slurry like consistency. Then slowly the drug gefitinib was incorporated into the slurry and triturations was continued for 60 minutes. The slurry was then air dried at 25°C for 24 hours, and passed through sieve No. 80 and stored in desiccators.

Investigation of compatibility study

The compatibility of gefitinib with β-CD/HP- β-CD can be done by various techniques like FTIR, DSC, XRD, NMR, chromatography and electrochemical methods. However, in this study we had used two simple techniques for compatibility study.

Fourier transforms infrared radiation measurement (FTIR)

The FTIR spectra of pure drug gefitinib, β-CD/HP- β-CD, physical mixture and the kneaded complex were obtained from Fourier Transmission Infrared Spectrophotometer (Shimadzu Model 8400S). The samples were prepared by the potassium bromide disc method and scanned in the range of 6000–4000cm⁻¹. Dry KBr (approx. 100 mg) was finely ground in an agate mortar and samples of pure drug; pure CDs and their inclusion complexes (approx 1 mg) were mixed gently. Pellets were prepared by using manual press. The smoothing of the spectra and the baseline correction were applied. The FTIR spectra of the inclusion complexes were compared with their pure CD and drug. The formation of inclusion complex was identified by the shift and intensity changes on the peaks of inclusion complex.
compared to those of the individual components. A blank KBr disc was used as background (Riekes et al., 2010).

**Differential Scanning Calorimetry (DSC)**

DSC studies were performed to confirm the formation of inclusion complex. The DSC studies of pure drug, CDs, physical mixtures and complexes were performed using a Shimadzu DSC-60 Systems (Shimadzu, Kyoto, Japan) equipped with a computerized data station TA-50WS/PC. The thermal behavior of samples were studied by heating in a sealed flat bottom aluminum pan, using an empty pan sealed as reference, over the temperature range of 50–350°C, at a rate of 10°C/min and under a constant flow of dry nitrogen. Gefitinib (approx., 5mg), β-cyclodextrin and hydroxypropyl-β-cyclodextrin each were then formulated and measured. Change in the temperatures (melting), taken as onset temperature was used as an indication of complex formation.

**Results and Discussion**

**Calibration curve of gefitinib**

The calibration curve of gefitinib was prepared in methanol by using UV Spectrophotometer. The prepared solutions of concentration ranging 10-50 μg/ml were scanned at λmax 245 nm and the absorbance was measured. The correlation coefficient (R2) was found to be 0.996. Thus, it follows Beer’s Lambert law in concentration range. A good linearity was observed between drug Concentration and absorbance as the correlation coefficient calculated was found to be very near to one.

**Table 1: Absorbance of gefitinib solution at 245 nm**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration (μg/ml)</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>10</td>
<td>0.14</td>
</tr>
<tr>
<td>2.</td>
<td>20</td>
<td>0.251</td>
</tr>
<tr>
<td>3.</td>
<td>30</td>
<td>0.413</td>
</tr>
<tr>
<td>4.</td>
<td>40</td>
<td>0.535</td>
</tr>
<tr>
<td>5.</td>
<td>50</td>
<td>0.637</td>
</tr>
</tbody>
</table>

**Figure 3: Standard calibration curve of gefitinib**

Phase solubility study is the preliminary study that helps to evaluate the affinity between cyclodextrins and drug molecule in water. This process is used to determine the exact molar ratios in which the drug-cyclodextrin inclusion complex is to be made. The solubility diagram is a useful tool for determining the solubilizing ability and stability constant of the inclusion complexes. The phase solubility diagrams of gefitinib with β-CD and HP-β-CD in aqueous medium (distill water) at room temperature is shown in Figure 5F-2. The values of coefficient of determination (R2) for the phase solubility diagrams with β-CD and HP-β-CD were 0.998 and 0.919 respectively; therefore, the diagrams were classified as AL-type curves. The slope was found to be less than 1 indicating that the complexes are of the first order with respect to the CDs (i.e. 1:1 stoichiometry).

AL profiles indicate a linear increase in solubility as a function of solubilizer concentration. The solubility of BM was found to increases linearly with the increase in molar concentration of Epi-β-CD. The apparent stability constant (Ks) of the inclusion complex was calculated from the linear fit of the curve. These calculated values of stability constant suggest the formation of favorable and true complex. The formation of non-covalent inclusion complex led to increase in solubility maximum with HP-β-CD and minimum with β-CD. Moreover, the Theoretical molar ratio (1:1) was chosen to prepare the binary complex with kneading method.

The stability constant for gefitinib with HP-β-CD was found to be 897 M⁻¹and 354 M⁻¹ with gefitinib-β-CD.
Drug-Excipient compatibility studies

To study the compatibility of drug with various polymers, IR spectra of the drug and formulations were carried out. The obtained spectrum shows compatibility between the drug and formulation components.

FTIR

The FTIR spectrum of the physical mixtures (PM) in all the cases (G-β-CD, G-HP-β-CD) showed approximate superimposition of the individual patterns of both CDs and gefitinib, indicating that the physical mixture did not lead to complexation. However, the FTIR spectra of kneaded inclusion complex were slightly similar to the corresponding cyclodextrins due to the coincidental absorption of both the host and guest molecules in the same spectral region. The FTIR spectrum of β-CD showed prominent absorption peaks at 3385 cm⁻¹ (for O–H stretching vibrations), 2920 cm⁻¹ (for C-H stretching vibrations), 1425 cm⁻¹ (-OH stretching), 1650 cm⁻¹ (for H–O–H bending), 1171 cm⁻¹ (for C–O stretching vibration), 960–520 cm⁻¹ (cyclodextrin ring) and 1028cm⁻¹ (C–O–C stretching vibration). The FTIR spectrum of HP-β-CD showed absorption peaks in the range of 3300 – 3500 cm⁻¹ (O-H stretching), 1630 cm⁻¹ (-C=C stretching vibration), 1164 cm⁻¹ (C-H stretching), 1085 cm⁻¹ (C-O stretching), in the range of 2800-3000 cm⁻¹ (-CH & CH2). There were certain specific and obvious changes in the spectrum after formation of complex by kneading method. The small shifts in characteristic peaks of gefitinib confirmed the presence of drug in the kneaded inclusion complexes. The complexation was most efficient with HP-β-CD as compared to β-CD. FTIR technique involves the study of the different functional groups of guest and host molecules by analyzing the significant changes in the shape and position of the absorbance bands. Data obtained from FTIR spectrophotometric study clearly indicates insignificant changes in spectra obtained from complex of β-cyclodextrin and HP- β-CD with gefitinib.
In order to investigate the effect of heat on the physical and chemical nature of the samples (pure drug G, CDs and inclusion complexes) thermal analysis (DSC) was performed. It is known that when guest molecules are embedded in the cavity of CD or in the crystal lattice, their melting, sublimating and/or boiling points generally shift to a different temperature or disappear in the case of CD decomposition (18). The difference in energy input required to maintain the sample and reference at exactly the same temperature is plotted as a function of the sample temperature.
The pure drug gefitinib showed a sharp endothermic peak at 195°C, which represents its melting point. The thermogram of β-CD and HP-β-CD showed a broad band at 110°C and 270°C. In the Physical mixtures of gefitinib with β-CD and HP-β-CD, the phase transition thermal profile of gefitinib remained recognizable with the dislocation, reduction, and broadening of the drug fusion peak, with concomitant shift towards lower temperature. However, in case of kneaded complex of gefitinib with HP-β-CD, no characteristic peak corresponding to drug fusion was observed. The disappearance of the gefitinib peak may be related to any chemical or physical interaction between the drug and CD with the possible formation of an amorphous system. The complete disappearance of the fusion indicates the formation of true inclusion complex. The DSC curves of pure drug, CDs, physical mixtures and inclusion complexes are shown in figure 6.

Conclusion

Compatibility study performed by FTIR and differential scanning calorimetry (DSC) indicated the formation of inclusion complexes in the solid state by natural and chemically derived cyclodextrin. The cyclodextrin derivative HP-β-CD formed amorphous complexes with gefitinib and provided greater improvement in the solubility and dissolution of the drug. Moreover, linear relationship was obtained in phase solubility study. In future, other sophisticated techniques like NMR and Mass will be used to characterize these complexes.

References