*Original Article*

# Development and Characterization of Paracetamol Complexes with Hydroxypropyl-β-Cyclodextrin

Sushma Talegaonkar*, Azhar Yakoob Khan, Roop Kishan Khar, Farhan Jalees Ahmad and Zeenat I. Khan

*Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, New Delhi, 110062, India.*

## Abstract

Paracetamol is a sparingly soluble bitter tasting drug. It is widely used as an analgesic and antipyretic. Complexation of drug with different cyclodextrins (α, β and HP-β-CD) was attempted to improve solubility of Paracetamol. During the drug excipient interaction studies, α, β cyclodextrins elicited analytical interference and showed considerable absorbance at λmax (243.5 nm) of Paracetamol while the ones constituting of hydroxypropyl-beta-cyclodextrin (HP-β-CD) did not show any such interference. Therefore, the present study is concentrated on exploring HP-β-CD as complexing agent.  Phase solubility studies showed that complexation of Paracetamol/HP-β-CD at molar ratio 1:1 and showed $A_L$ type solubility curve. Complexation was done by various methods like physical mixing, kneading and freeze drying and resulting drug complexes were characterized by Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FTIR). The thermograms obtained showed an endothermic peak for Paracetamol, for physical mixture to some extent for kneaded mixture, but was completely eliminated for freeze dried product (Inclusion complex). Similar results were obtained during IR studies. Therefore, solid inclusion complex of paracetamol prepared by freeze drying method was found to be an ideal complex. The solubility of paracetamol, was significantly increased (six folds of normal solubility) by complexation with HP-β-CD.

**Keywords:** Complexation; Freeze-drying; Hydroxypropyl-beta-cyclodextrin (HP-β-CD); Kneading; Paracetamol; Partition coefficient; Solubility.

## Introduction

Cyclodextrins have been reported in a number of studies in the pharmaceutical field to form inclusion complexes with several pharmaceutical drugs. Cyclodextrins have been extensively used for improving solubility (1-3) stability (4, 5) and bioavailability (6, 7) of drugs. In recent years, hydroxypropyl-β-cyclodextrin has gained appreciable acceptance among the various types of cyclodextrins. Large number of inclusion complexes of HP-β-CD with a wide range of drugs have been published and patented all over the world. Various methods for inclusion complexes like grinding, kneading, freeze drying, spray drying, slow evaporation etc have been widely reported. Freeze drying and spray drying techniques are most efficient techniques but of these the freeze drying method has not been much explored.

In previous studies, inclusion complexes of Paracetamol with β-cyclodextrin have been prepared and characterized to improve the

* Corresponding author:
  E-mail: Stalegaonkar@hotmail.com

solubility and dissolution of paracetamol (8, 9). But the low aqueous solubility of β-CD (About 1.8% w/v at 25 °C) limits its use nowadays and other more soluble cyclodextrin derivatives have been utilized. Hydroxypropyl-β-cyclodextrin (HP-β-CD) owing to its high solubility, is one of the most frequently used cyclodextrin derivative today (about 50% w/v at 25 °C). Good oral and parenteral tolerability are its further advantages (1).

The present study attempted at increasing the solubility of the paracetamol by using different cyclodextrins. Amongst these, complexes of HP-β-CD with Paracetamol was found to be most suitable as it did not exhibit any analytical interference/ absorbance and hence it was zeroed on for further investigations.

**Experimental**

**Materials**

Paracetamol was obtained from Orient Pharma and chemicals, New Delhi and HydroxyPropyl-Beta-Cyclodextrin was a gift sample from Ranbaxy laboratories. All other chemicals were of analytical grade.

**Methods**
*Phase solubility studies*

The stability constant for the inclusion complex formed between paracetamol and HP-β-CD was determined using the phase solubility method which was carried out according to Higuchi and Connors (10). Briefly, an excess amount of paracetamol was added to the aqueous solutions (10 ml) of cyclodextrin of varying concentration (5-25 mM). Fresh distilled water was used as medium and flasks were protected from light, sealed and were placed in a waterbath shaker for 72 h. After equilibrium was reached the sample of each flask was filtered through millipore filter (0.45 µm). The filtered solution was then diluted and concentration of paracetamol was analysed by U.V spectrophotometer (Shimadzu Double Beam Spectrophotometer 1601) at 243.5 nm. The work was performed at 35±2 °C in triplicate upto a level when the last reading showed the same amount of absorption, indicating the maximum solubility (Figure 1).
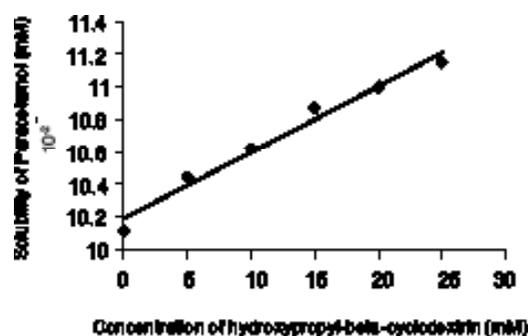


**Figure 1.** Phase solubility diagram of paracetamol with HP-β-CD.

*Preparation of the physical mixture/grinding*

Paracetamol and HP-β-CD (Molar ratio 1:1) were weighed accurately, pulverized and then mixed thoroughly (30 min) by light trituration in a glass mortar until a homogenous mixture was obtained. The resultant mixture was stored in a dessicator.

*Preparation of inclusion complexes*
*Kneading method*

Equimolar quantities of the paracetamol and HP-β-CD were mixed in a mortar and pestle for 10 min which was then kneaded with a mixture of water and ethanol (1:1) for 60 min. Kneading was done till the product started drying on the walls of mortar. The product was further dried in an oven at 40 °C for 30 min until a constant weight was obtained. The resultant product was sieved and stored in a dessicator.

*Freeze-drying (Lyophilization)*

Equimolar quantities of paracetamol and HP-β-CD were taken separately in 20 ml of water and mixed thoroughly. The resultant solution was frozen in a deep freezer (New Brunswick Scientific, Germany) at -70 °C for about 6 h. The frozen mixture was then freeze dried in the freeze dryer (Heto drywinner with rotary vane pump, Germany) for 8 h at -110 °C to -120 °C under vacuum. The resultant product was sieved and stored in a dessicator.

*Characterization of solid inclusion complexes*
*Fourier Transform Infrared Spectroscopy (FTIR)*

The FTIR spectra of all samples were

recorded on Perkin Elmer instrument using KBr disc method. 10 mg of dried sample was mixed with 40 mg of KBr by using a clean glass pestle and mortar. The mixed homogenized powder was compressed in an IR pellet machine to get the pellets which were used immediately for recording the spectra from 400 to 4000 cm$^{-1}$.

### Differential Scanning Calorimetry (DSC)

DSC was performed by using Perkin Elmer Pyris-6DSC system using 3 mg sample in crimped aluminium pans at a heating rate of 10°C/min in the range of 50-250°C.

### Partition Coefficient Determination

The partition coefficient of the drug and complexes between octanol/water was determined at ambient temperature (30±2°C). Ten milliliters each of octanol and distilled water were taken in a glass stoppered flasks, to which 10 mg of accurately weighed drug was added and the mixture was then shaken with the help of mechanical shaker for 24 h at room temperature. The mixture was then transferred to a separating funnel and allowed to equilibrate for 6 h. The aqueous and octanol phase were separated and filtered and drug content in aqueous phase was analyzed by UV spectoophotometer at 243.5 nm.

The partition coefficient was calculated.

### Solubility

The equilibrium solubility of paracetamol and complex was determined in distilled water (10). An excess amount of the drug was shaken in separate flasks with distilled water and different pH buffers using a mechanical shaker at room temperature (25±2°C) for 24 h. The sample was then filtered and analyzed by UV spectoophotometerat 243.5 nm.

### Statistical Analysis

Graph Pad InStat version 3.05 was used for statistical analysis of partition coefficient results. An unpaired, two tailed, t-test was used for analyzing the significance at p<0.05.

## Results and Discussion

Phase solubility study of Paracetamol with HP-β-CD was carried out in distilled water at temperature 35±2°C (Figure 1). The solubility of paracetamol increases linearly with increasing concentrations of HP-β-CD. Thus, showing a typical A$_L$-type phase solubility curve. According to Higuchi and Connors (10), these A$_L$ type curves indicate the formation of complex between the



**Figure 2.** IR spectra of paracetamol, hydroxypropyl-beta-cyclodextrin (HP-β-CD) and their various combination forms. (A) paracetamol; (B) Hydroxypropyl-beta-cyclodextrin (HP-β-CD); (C) Physical mixture of paracetamol / HP-β-CD (1:1); (D) Kneaded mixture of paracetamol / HP-β-CD (1:1); (E) Freeze-dried complex of paracetamol / HP-β-CD (1:1).
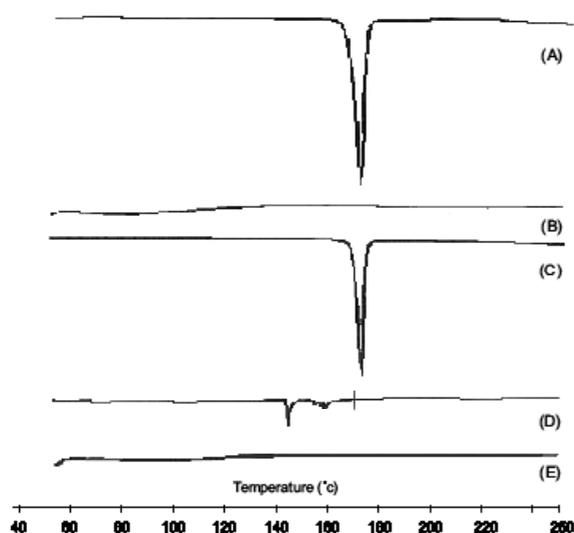


**Figure 3.** DSC thermograms of paracetamol, hydroxypropyl-beta-cyclodextrin (HP-β-CD) and their various combination forms. (A) paracetamol; (B) Hydroxypropyl-beta-cyclodextrin (HP-β-CD); (C) Physical mixture of paracetamol / HP-β-CD (1:1); (D) Kneaded mixture of paracetamol / HP-β-CD (1:1); (E) Freeze-dried complex of paracetamol / HP-β-CD (1:1).

substrate (paracetamol) and the ligand (HP-β-CD) and a first order dependency of the interactions on the cyclodextrin concentration. The regressed curve has a slope value 0.0407, intercept $10.189 \times 10^{-2}$ mM and correlation coefficient of 0.9768.

The apparent stability constant Kc was calculated according to the method of Higuchi and Connors (10) by using the following formula.

Kc = Slope / Intercept (1-Slope)

where Kc is stability constant of inclusion complex.

The stability constant (Kc) for paracetamol was found to be $416.4$ $M^{-1}$. Therefore, it can be concluded that Paracetamol will form a stable complex of drug with HP-β-CD in 1:1 molar ratio.

There are several methods for the preparation of the cyclodextrin-guest complexes depending on the physical properties of the guest molecules, including physical mixing, kneading and freeze drying. Lyophilization was used in this study because it is suitable for substances like paracetamol which are sparingly water soluble or for drugs which decompose on drying (11). For each method, complexes were prepared in a molar ratio of 1:1.

Complexation was done by physical mixing, kneading and freeze drying methods (1:1 molar ratio) and the resulting drug complexes were characterized.

FTIR spectrum of paracetamol (Figure 2) showed absorption bands for hydroxyl groups at 3324 and 3162 $cm^{-1}$, unsaturation (1653, 1610 $cm^{-1}$) and aromatic ring (1562, 1505, 836 $cm^{-1}$). HP-β-CD spectrum exhibited absorption bands of hydroxyl group at 3405 $cm^{-1}$ and vibration bands of C-O and O-H groups at 1083 $cm^{-1}$ and 1032 $cm^{-1}$ respectively. The FTIR spectrum of the complexes showed only one broad absorption band for hydroxyl group at 3384 $cm^{-1}$ which is due to complexation of the drug with HP-β-CD (Intermolecular hydrogen bonding of paracetamol with HP-β-CD).

Although the results obtained by the solubility studies indicate the formation of a true complex of paracetamol-HP-β-CD, they do not perclude the possibility that the product is simply a physical mixture. Thus, the thermal behaviour of cyclodextrin inclusion complex was studied by DSC in order to confirm the formation of the above mentioned complex. When the guest molecules are incorporated in the cyclodextrin cavity or in the crystal lattice their melting point and boiling point usually shift to different temperatures or disappears within the temperature range when cyclodextrin lattice is decomposed. This is one of the best method for detecting drug-cyclodextrin complexation.

The DSC thermogram of paracetamol, HP-β-CD and complexes prepared by different methods are shown in Figure 3. The DSC thermograms of paracetamol show one sharp characteristic endothermic peak at $171.46 \pm 0.82$ °C and $\Delta H = 274.86 \pm 3.8$ J/g which is indicative of its melting temperature. In case of HP-β-CD owing to its amorphous nature, a broad endothermic peak was observed at about 60 °C. The physical mixture of paracetamol with HP-β-CD shows an endothermic peak at $171.41 \pm 0.67$ °C while the solid inclusion complex formed by kneading method showed a new endotherm with decrease in enthalpy ($\Delta H = 21.62 \pm 6.3$ J/g) which is indicative of fusion of paracetamol with HP-β-CD. In contrast to this, a complete disappearance of the endothermic peak of paracetamol in the freeze dried complex is attributed to formation of a true inclusion complex. This is in agreement with the previous findings suggesting the incorporation of the drug molecule into the crystal lattice of HP-β-CD. Therefore, complex prepared from this method was adopted for further study.

Solubility of paracetamol in distilled water was found to be $13.69 \pm 1.2$ mg/ml at room temperature. The complexation of paracetamol with HP-β-CD resulted in more than six fold ($79.30 \pm 4.2$ mg/ml) increase in solubility. This high increase in the solubility can be attributed to amorphous nature of the complex being formed with HP-β-CD.

The partition coefficient of paracetamol was found to be $2.70 \pm 0.14$ while for the freeze-dried complex it was estimated as $2.23 \pm 0.08$. The statistical analysis of the data revealed a p value of 0.0072, showing a significant difference in partition coefficient. This difference can be attributed to the enhanced hydrophilicity, which was primarily due to amorphous nature of the

paracetamol/HP-β-CD complex.

## Conclusion

Amongst the various cyclodextrins explored for the study, hydroxypropyl-β-cyclodextrin yielded most favorable results in terms of the drug-cyclodextrin compatibility. While eliciting an augmentation in the solubility, it also showed an appreciable hydrophilicity. Moreover, the freeze drying technique offers itself as a potential tool for the complexation of sparingly soluble drugs.

## Acknowledgements

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