Improved Solubility and Dissolution Rate of Ketoprofen by Beta Cyclodextrin Ternary Complexes Incorporating Hydrophilic Polymers

Mohammed Jafar1*, Sadath Ali2, Hassan Mahmoud Ghonaim1

1Department of Pharmaceutics, College of Clinical Pharmacy, University of Dammam P.O.Box-1982, Dammam-31441, Saudi Arabia.
2School of Pharmacy and Medical Sciences, Singhania University, Pacheri Bari, Jhunjhunu, Rajasthan. India.

Received: 2nd Jan, 2017; Revised: 15th Jan, 2017; Accepted: 6th Feb, 2017; Available Online: 1st March, 2017

ABSTRACT
The aim of the present study was to improve the aqueous solubility and dissolution rate of a BCS Class II drug, ketoprofen by β-cyclodextrin ternary complexes incorporating hydrophilic polymers polyethylene glycol 6000 (PEG6000) and polyvinyl pyrrolidone (PVP). Initially, ketoprofen (KPF) binary complexes with β-Cyclodextrin (βCD) were formulated by physical mixing, co-grinding, and solvent evaporation methods which was followed by ternary complex formulation of selected KPF-βCD binary complex incorporating PEG6000 and PVP. The solvent evaporation method was used in the formulation of ternary complexes of ketoprofen, since in the beginning of this study, it was proved to be the best method comparatively in yielding promising binary complexes of ketoprofen. Ketoprofen formed 1:1:1 stoichiometric binary and ternary inclusion complexes as demonstrated by the A2-type of phase solubility graph. An increase in the stability constant value (Kc) of KPF-βCD complex in the presence of PEG6000 and PVP conceded higher complexation competency. FTIR and SEM studies evidenced the perfect ternary inclusion complex formation. Ternary complexes showed improved dissolution rate compared with Ketoprofen alone and KPF-βCD binary complex. The ternary complex containing 1:1:2 molar ratio of KPF:βCD:PEG6000 exhibited 94.24% drug dissolution in 2 hours, which was significantly high in relation to ternary complexes containing PVPh and it was found to follow first order release mechanism. Complex studied for stability showed no significant change in physical appearance, drug content and drug dissolution characteristic indicating high stability.

Keywords: Ketoprofen; Inclusion complex; Hydrophilic polymers; Stability.

INTRODUCTION
Ketoprofen is a potent and safe nonsteroidal anti-inflammatory drug confered with good analgesic properties1 its very low water solubility (0.13 mg mL−1 at 25°C)2 can give rise to formulation problems and limit its therapeutic applications and bioavailability3. Cyclodextrins (CD’s) are hydrophobic, cyclic, non-reducing oligosaccharide compounds of 6-8 glucopyranan units and have been extensively used to improve the aqueous solubility of poorly soluble drugs. CD’s have the ability to form inclusion complex with many drug molecules wherein the guest drug molecule is entrapped partially or completely within the cyclodextrin cavity thus resulting in an increase in the solubility and dissolution rate of drugs4-5. The inclusion complexes so formed were also shown to improve chemical stability6-7 and bioavailability8,9 of many drug substances. Among the various Cyclodextrins, β-cyclodextrin (βCD) has been the most commonly used inclusion compound owing to its vast availability, low cost, absolute biocompatibility and also wide regulatory acceptance10. Moreover, recent report evidenced that preassociating NSAIDs with βCD could save rats against the damaging GI adverse effects of NSAIDs while enhancing their bioactivity11. Numerous investigations reported that, incorporation of small quantity of hydrophilic excipients such as organic acids, amino acids, basic compounds, and polymers to an aqueous complexation medium usually give rise to an enhancement in the CE of cyclodextrins and ultimately reduce their amounts in pharmaceutical formulations12. The purpose of the current study was to improve the aqueous solubility, dissolution rate and thus bioavailability of ketoprofen. This could be achieved by β-cyclodextrin ternary complexation of ketoprofen employing hydrophilic polymers PEG6000 and PVP.

MATERIALS AND METHODS
Materials
Ketoprofen was obtained as a gift sample from Shreya labs Pvt.Ltd, Aurangabad. βCD and polymers (PEG6000 and PVP) and all other reagents used were of analytical grade and were purchased from S.D Fine chem. Ltd Mumbai, India

Methods
Phase solubility study
Solubility determination of KPF was performed as per the reported method13. Excess quantity of KPF (50 mg) was transferred to 20 ml aqueous βCD solution of increasing

*Author for Correspondence: mjmar@uod.edu.sa
concentration (3mM to 15mM) in 50 ml Stoppard conical flasks. After shaking the contents of the flask at 37°C for 72 hours on a mechanical shaker, the undissolved KPF was filtered through a 0.45mm filter and the solutions after appropriate dilutions were assayed for KPF content at 259nm spectrophotometrically. Phase solubility studies of KPF were also conducted with the incorporation of polymers (PEG6000, PVP) at a concentration of 0.5% w/v to the solutions containing βCD. The blank trials were run simultaneously in the same concentrations of βCD in distilled water in order to cancel out any absorbance if showed by Cyclodextrin molecules. The above solubility experiments were repeated for two more times to get accuracy in the results. The apparent stability constants (K_{1:1}) were computed from phase solubility diagrams using the below equation:

\[
K_{1:1} = \frac{S_{t}}{S_{s}} (1 - \text{Slope})
\]

Where \( S_{s} \) is the intrinsic solubility of pure ketoprofen.

**Formulation of Ketoprofen solid complexes**

Binary complexes of KPF with βCD at 1:0.5, 1:1, and 1:1.5 molar ratios respectively were prepared by the following methods.

**Physical mixture method (PM)**

The physical mixtures were formulated by triturating the calculated quantity of KPF and βCD in a glass mortar. The physical mixtures thus obtained were screened through 44-mesh sieve and then stored in a dessicator until utilized for further investigations.

**Co-grinding method (GM)**

The weighed quantity of KPF and βCD were transferred to a clean glass mortar and kneaded together with 2ml of dimethyl formamide. The damp mass thus obtained was passed through 44-mesh sieve and the granules obtained were dried at 60°C under vacuum, until a constant weight was obtained. The granules after drying sufficiently were stored in a dessicator until used for further studies.

**Solvent evaporation method (SM)**

The calculated amount of KPF and βCD were dissolved in small volume of dimethyl formamide in a china dish and the solution was allowed to stand overnight. The solvent was removed at 60°C under vacuum until binary complex was completely dried. The dried mass was crushed and screened through 44-mesh sieve and the powder obtained was stored in a dessicator until used in further studies.

**Formulation of Ketoprofen ternary complexes**

Ternary complexes of KPF were prepared using KPF:βCD: hydrophilic polymers (PEG6000, PVP) at 1:0.5:1, 1:0.5:2, 1:1:1 and 1:1:2 molar ratios respectively by solvent evaporation method (as described above).

**Drug content estimation**

Formulation equal to 100 mg of KPF was dissolved in 100ml of methanol in a volumetric flask. From this stock solution 10µg/ml of KPF solutions were prepared using methanol and the contents were assayed for KPF by UV-visible spectroscopy at 259 nm using methanol as a blank.

**Fourier Transform Infrared Spectroscopy**

Fourier transform Infrared (FTIR) spectra of KPF, and its ternary inclusion complex were obtained using Perkin elmer spectrophotometer by KBr pellet method.

**Scanning Electron Microscopy**

The surface morphology of the KPF and its ternary inclusion complex were studied by scanning electron microscopy (SEM). Samples were fixed on the brass stub using double-sided tape and made electrically conductive by coating with a thin layer of gold by sputter coater (Ion sputter). Photographs were taken at an electric voltage of 20 kV.

**Wetability study**

Small amounts of Pure ketoprofen and its solid binary and ternary inclusion complexes were placed at the bottom of a sintered glass funnel (33 mm internal diameter). The funnels were pushed into glass beakers containing 0.1N Hydrochloric acid such that the surface of a solution in a beaker touches the base of the funnel. A small amount of methylene blue powder was spread across the surface of the drug or its complex in a funnel. The time taken to wet the methylene blue powder was recorded. The procedure was repeated thrice for each sample and the mean wetting time was calculated.

**In vitro dissolution study**

In vitro dissolution studies for ketoprofen and its solid binary and ternary complexes was carried out using USPXXI Type-1 dissolution test apparatus (Electrolab, India) by the powder dispersion method. Sample equal to 50 mg of KPF was used in each test. The dissolution tests were performed using 900 ml of 0.1N HCl at 37 ± 0.5°C with paddle rotation maintained at 50 rpm. The release of KPF from each sample was determined by withdrawing 5 ml samples at preset time intervals, and were filtered, appropriately diluted and analysed spectrophotometrically at 259nm 5 ml of fresh medium was transferred to maintain sink conditions.

**Stability Study**

Stability Study for selected binary and ternary complexes of KPF was carried out by storing 1gm of each selected complex in an ambered coloured screw capped glass bottles at controlled and accelerated temperatures and relative humidities for a period of 3 months as per the reported method. The complexes were evaluated for physical appearance, drug content and in-vitro dissolution at the end of three months.

**RESULTS AND DISCUSSION**

**Phase solubility study**

Solubility curves obtained for solid inclusion complexes were classified according to Higuchi and Connors classification as A1 type curves, demonstrating a linear increase of drug solubility indicative of the formation of soluable complexes. The ratio between the slopes of the phase solubility curves of the ternary and binary complexes, assumed as an index of the relative solubilizing efficacy, it was 1.14 for KPF-βCD-PEG6000 ternary complex describing the higher effectiveness of the ternary complex in comparison to binary complexes. The stability constant values obtained for binary complexes of KPF are 134.3M⁻¹, 151.6M⁻¹ and 138.5M⁻¹ for βCD,
PEG6000, and PVP. The higher constant value which was observed with KPF demonstrates KPF interacts more strongly with PEG6000. The stability constant values obtained for ternary complexes of KPF are 182.3 M$^{-1}$, and 164.6 M$^{-1}$ for βCD-PEG6000 and βCD-PVP respectively. The higher solubility observed in ternary inclusion complexes in relation to their binary complexes could be due to synergistic effect of added polymers, higher constant that was observed with βCD-PEG6000 demonstrates KPF interacts more strongly with βCD in presence of PEG6000. Thus PEG6000 independently and in combination with βCD showed highest complex formation/phase solubilization of KPF as compared to PVP.

**Drug content estimation**

The estimated drug content was in the range of 89.02%±1.092 to 98.78%±1.008 for binary complexes and

![Figure 1: Phase solubility curves of a) KPF-βCD b) KPF-βCD-PVP c) and KPF-βCD-PEG6000 complexes.](image1)

![Figure 2: FTIR Spectrum of (a) Ketoprofen (b) KPF-βCD-PEG6000 ternary complex.](image2)
Figure 3: SEM Images of (a) Ketoprofen (b) KPF-βCD-PEG6000 ternary complex.

Figure 4: Cumulative % drug dissolved vs time profile of ketoprofen ternary complexes.

*KPF= Ketoprofen, F1 to F4= KPF-βCD-PEG6000 ternary complexes, F5 to F8= KPF-βCD-PVP ternary complexes.
96.07±0.494 to 98.67±1.037 for ternary complexes with low SD and CV values. These values indicate that there was no significant loss of ketoprofen throughout the formulation of its binary/ternary complexes. The coefficient of variation (CV) was found to be slightly higher than 1 in all the prepared complexes, which indicates that the methods employed in the preparation of ketoprofen binary and ternary complexes were acceptable.

**Fourier Transform Infrared Spectroscopy**

The FTIR spectrum of ketoprofen and ternary complex exhibited characteristic absorption bands as given below. There is a significant change in the nature and positions of the peaks. Many of the peaks of IR spectrum of pure drug are missing in the spectrum of ternary complex. Fourier transform infrared spectroscopy was performed on ketoprofen showed prominent peaks at 3435, 3050, 2960 to 2977, 840, for hydrogen bonding, aromatic C-H stretch, aliphatic ring stretch, aromatic substitution respectively. The group frequencies of drug confirmed to the respective structure. Ternary inclusion complex of ketoprofen exhibited the peaks at 2960 to 2973, 1778 and 1126 to 1089, for ring structure, oxygen linkage, and C-H stretch respectively. In the ternary complex spectra the peak for NH groups are appeared as broad bands. Similarly, the position of peaks for C-H stretching involving asymmetric and symmetric stretching for CH3 and CH2 groups are totally changed. The overall observation of the spectra for ternary complex revealed that there is a change in the position of peaks for functional groups as well as different types of bonds. This type of changes in the nature and position of the peaks is possible only when inclusion complexes are formed (Fig. 2a&b).

**Scanning Electron Microscopy**

From the SEM images as seen in Fig. 3a, pure KPF particles appeared as crystalline structure. Microscopic observation of ternary inclusion complex (KPF-βCD-PEG6000) (Fig. 3b) showed a small and irregular piece and like inclusion of material in the cavity. Pandya P, et al., have reported that a modification in the shape of drug particles was indicative of a new solid state18. Thus, changes in the morphology of complex as compared to drug showed interaction between KPF and a complexing agent. These results are consistent with the above FTIR results.

**Wetability Study**

The mean wetting time of pure ketoprofen was 36.33 (±1.527 SD) seconds, KPF-βCD binary complex (1:1 molar ratio) gave the mean wetting time of 23 (±1 SD) seconds, Whereas the mean wetting times of KPF-βCD-PEG6000 & KPF-βCD-PVP (1:1 molar ratios respectively) ternary complexes were 12.66 (±0.289 SD) seconds and 16.33 (±0.577 SD) seconds respectively. These findings are in accordance with the above phase solubility results.

In vitro dissolution studies

The binary complexes of KPF and their corresponding physical mixtures (PM’s) and also ternary complexes were

<table>
<thead>
<tr>
<th>Complex</th>
<th>DE10 (%)</th>
<th>DE30 (%)</th>
<th>DE60 (%)</th>
<th>DP50</th>
<th>T30 (min)</th>
<th>T50 (min)</th>
<th>MDT30</th>
<th>First order rates K1 x 10^2 (min^-1)</th>
<th>Hix.Crow K1IC x 10^2 (mg/L min^-1)</th>
<th>R</th>
<th>K1</th>
<th>R</th>
<th>K1IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>22.52</td>
<td>27.26</td>
<td>43.26</td>
<td>13.079</td>
<td>76.7</td>
<td>149.1</td>
<td>11.43</td>
<td>0.9438</td>
<td>-0.0047</td>
<td>0.8810</td>
<td>-0.0014</td>
<td>0.9411</td>
<td>0.8819</td>
</tr>
<tr>
<td>F2</td>
<td>19.07</td>
<td>37.99</td>
<td>42.93</td>
<td>29.098</td>
<td>31.2</td>
<td>60.70</td>
<td>9.21</td>
<td>0.9411</td>
<td>-0.0114</td>
<td>0.8819</td>
<td>0.0032</td>
<td>0.9312</td>
<td>0.8874</td>
</tr>
<tr>
<td>F3</td>
<td>22.49</td>
<td>38.14</td>
<td>43.09</td>
<td>13.698</td>
<td>73.4</td>
<td>142.6</td>
<td>10.56</td>
<td>0.9539</td>
<td>-0.0120</td>
<td>0.7930</td>
<td>0.0033</td>
<td>0.9311</td>
<td>0.8819</td>
</tr>
<tr>
<td>F4</td>
<td>24.60</td>
<td>39.77</td>
<td>44.68</td>
<td>30.289</td>
<td>29.7</td>
<td>57.8</td>
<td>9.17</td>
<td>0.9417</td>
<td>-0.0038</td>
<td>0.8721</td>
<td>0.0012</td>
<td>0.9417</td>
<td>0.8721</td>
</tr>
<tr>
<td>F5</td>
<td>22.07</td>
<td>40.99</td>
<td>48.93</td>
<td>29.082</td>
<td>31.2</td>
<td>60.70</td>
<td>9.21</td>
<td>0.9311</td>
<td>-0.0114</td>
<td>0.8819</td>
<td>0.0032</td>
<td>0.9311</td>
<td>0.8819</td>
</tr>
<tr>
<td>F6</td>
<td>23.46</td>
<td>28.18</td>
<td>42.14</td>
<td>12.019</td>
<td>74.7</td>
<td>136.1</td>
<td>12.45</td>
<td>0.9264</td>
<td>-0.0212</td>
<td>0.8270</td>
<td>0.0053</td>
<td>0.9431</td>
<td>0.8519</td>
</tr>
<tr>
<td>F7</td>
<td>23.85</td>
<td>36.94</td>
<td>43.62</td>
<td>58.693</td>
<td>17.25</td>
<td>30.22</td>
<td>8.56</td>
<td>0.9431</td>
<td>-0.0114</td>
<td>0.8519</td>
<td>0.0032</td>
<td>0.9431</td>
<td>0.8519</td>
</tr>
<tr>
<td>F8</td>
<td>22.07</td>
<td>38.99</td>
<td>43.89</td>
<td>29.099</td>
<td>31.2</td>
<td>60.70</td>
<td>9.21</td>
<td>0.9431</td>
<td>-0.0114</td>
<td>0.8519</td>
<td>0.0032</td>
<td>0.9431</td>
<td>0.8519</td>
</tr>
</tbody>
</table>

*Where, DE=dissolution efficiency after 10, 30 and 60 min, DP= percent of drug dissolved after 30 min (DP). T50 T50= Time necessary to dissolve 30% and 50% of drug. R= coefficient of correlation; K1, K1IC= release rate constants for First order and Hixon crowell’s model respectively.

Table 2: Stability data of ketoprofen ternary complexes.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Physical appearance</th>
<th>%Drug content</th>
<th>Cumulative% Drug dissolved (120th minute)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial 25c, 60c, 75c RH 3M</td>
<td>25c, 60c, 75c RH 3M</td>
<td>Initial 25c, 60c, 75c RH 3M</td>
</tr>
<tr>
<td>F2</td>
<td>White powder</td>
<td>White powder</td>
<td>98</td>
</tr>
<tr>
<td>F3</td>
<td>White powder</td>
<td>White powder</td>
<td>97</td>
</tr>
<tr>
<td>F4</td>
<td>White powder</td>
<td>White powder</td>
<td>99</td>
</tr>
<tr>
<td>F8</td>
<td>White powder</td>
<td>White powder</td>
<td>98</td>
</tr>
</tbody>
</table>
evaluated for dissolution characteristics and compared with the pure KPF. The dissolution data obtained for KPF and its binary and ternary complexes was evaluated statistically. The In-vitro dissolution results were assessed on the basis of cumulative percentage drug release, dissolution efficiency and correlation coefficient (r). The percentage of KPF dissolved at 30th min (DP30) and dissolution efficiency at 10th min (DE10), at 30th min (DE30) and at 60th min (DE60); and the characteristic time for 30% and 50% dissolution of KPF (T30 and T50 min respectively) were computed for all binary and ternary complexes. The dissolution efficiency of all formulations was computed as per the method reported by Khan19.

Binary complexes

All binary complexes showed improved rate and extent of drug dissolution and their dissolution efficiency values were higher than the PMs and pure KPF. The value of T30 of all solid binary complexes was much smaller than pure KPF. Improved drug dissolution rate and dissolution efficiency observed in the binary complexes was due to the formation of solid inclusion complexes with good interaction of KPF and βCD during the formulation process. Increasing the concentration of βCD in the formulation increases the rate of drug dissolution from the formulations. This could be due to high wettability of the formulation with the dissolution media.

Ternary complexes

In vitro dissolution data of KPF and its solid ternary complexes are showed in Fig.4. The percent drug dissolved from the solid ternary complexes prepared with PEG6000 after 120 minutes was 90.31 (GI 1), and 92.73 (GI 2), similarly, the percent drug dissolved from the solid ternary complexes prepared with PVP after 120 minutes was 96.00 (GA-1), and 98.85 (GA-2), respectively. It was observed that the drug dissolution was constantly increased with increasing concentration of hydrophilic polymers in the ternary complexes. The various dissolution attributes of KPF ternary complexes are summarized in Table 1. Dissolution profiles of pure KPF, and all its binary and ternary complexes were assessed in relation to Hixson-Crowell’s cube root law and first order kinetics. The correlation coefficient (r) values of the first order kinetics were found to be slightly higher to the 'r' values of Hixson-Crowell’s cube root model. Hence the release pattern in KPF and all its solid binary and ternary complexes were found to follow imperatively first order kinetics in relation to Hixson-Crowell’s cube root law.

Stability Study

There was no significant change in the physical appearance, drug content and cumulative percent drug dissolution in the KPF complexes. Stability results clearly indicates that the complexes were sufficiently stable under controlled and accelerated conditions (Table 2).

CONCLUSION

The present investigation revealed that the wettability and solubility of Ketoprofen was found to be higher in ternary complexes (KPF-βCD-PEG6000) as compared to its binary complexes (KPF-βCD) and other ternary complexes prepared by PVP. SEM and FTIR characterization showed that the shape of KPF was changed due to the formation of ternary complex. These changes in characteristics of KPF led to the enhancement in solubility as well as dissolution rate of the drug. Nevertheless, further pharmaceutical dosage form formulation of the KPF-βCD-PEG6000 ternary complex and its pharmacological evaluation would yield much satisfactory results.

ACKNOWLEDGEMENT

We are thankful to Shreya Labs Pvt.Ltd. Aurangabad, Maharashtra, India, for providing ketoprofen gift sample. We also extend our sincere thanks to the University of Dammam, Dammam. Saudi Arabia, for providing sample characterization facility and also E-Library (free E-Journal access) facility for this research work.

REFERENCES