Improvement in Drug Release and Pharmacokinetic Parameters of Ketoprofen Crystals

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ABSTRACT: Purpose: The aim of present work was to improve the in-vitro drug release from crystal formulation with the improvement in pharmacokinetic parameters like Cmax, Tmax, and AUC of Ketoprofen by crystal engineering approach. Methods: Ketoprofen crystals were prepared by conventional solvent evaporation process in presence of saccharin sodium excipient at room temperature. Control batch was also prepared by the same technique with devoid of excipient. The prepared crystals were subjected for in-vitro and in-vivo study and the results obtained were compared with the pure drug. Results: In-vitro dissolution study shows the improvement in 2.05 fold drug release rate from Ketoprofen treated crystals compared to the pure drug. In-vivo analysis was carried out by using albino wistar rats which shows the improvement in 2.13 fold higher plasma concentration compared to the pure drug. The results revealed that one group of rats treated with pure drug i.e., Ketoprofen suspension (STANDARD) while the other group of rats treated with Ketoprofen loaded crystals (TEST), the plasma concentration-time profile depicted Cmax of 27900.37 ng/mL and 46155.29 ng/mL while Tmax of 120 minutes and 30 minutes, and AUC found was 221146.22 ng*h/mL and 471504.04 ng*h/mL respectively. Plasma concentration-time profile represented that Ketoprofen loaded crystals (TEST) gave higher plasma concentration (2.13 fold) compared to Ketoprofen pure drug (STANDARD). Conclusions: The crystal engineering approach can be used to improve the poor aqueous solubility and ultimately used to improve plasma concentration of BCS Class-II drugs. © 2020 iGlobal Research and Publishing Foundation. All rights reserved.


INTRODUCTION
Ketoprofen is having poor water solubility, BCS class II drug, and is of propionic acid derivative drug which is used as a potent non-steroidal anti-inflammatory drug (NSAID) and is used for the symptomatic treatment of osteoarthritis, spondylitis, rheumatoid arthritis, and is widely used clinically as antipyretic, and anti-inflammatory agent. On the basis of powder rheology, it is classified as a powder with poor flow property and compressibility [1-5] which is not processed by directly compressible technique. Moreover, Ketoprofen is a BCS Class II drug which has poor aqueous solubility [6, 7] and high permeability with analgesic activity which is processed by crystal engineering approach and prepared Ketoprofen loaded crystals shows improvement in the aqueous solubility [8], in-vitro drug release rate and ultimately it improves the pharmacokinetic parameters of Ketoprofen.

Ketoprofen metabolized in the liver by way of conjugation with CYP3A4 and CYP2C9, gluconic acid, reduction of its keto group and hydroxylation of the benzoyl ring system [9-12]. Primarily, Ketoprofen is used as an anti-inflammatory, antipyretic and analgesic drug which decreases the prostaglandin precursor synthesis which act by reversibly inhibiting both cyclooxygenase enzymes i.e., COX-1 and COX-2 [11, 13]. Ketoprofen is well absorbed after oral
administration and peak plasma level of the conventional capsules can be attained within 0.5–2 hours or 6–7 hours upon administration of extended release capsules [14-21]. The peak plasma level of Ketoprofen delayed by food material [22]. Ketoprofen is rapidly and extensively metabolized in the liver and mainly excreted in urine. Half-life of conventional capsules is about 2-4 hours and extended release capsules is about 4-5 hours [22].

Ketoprofen is mainly used as an anti-inflammatory drug in arthritis, toothache and in the skeletal muscle pain in form of topical patches [23-25]. Ketoprofen ointment, spray, cream, liquid or gel along with other appropriate agents may use in the treatment of nerve pain such as neuralgia, raticulopathy, inflammatory conditions, sciatica etc. as an anti-inflammatory agent in the treatment of pain.

MATERIALS AND METHODS

1. Materials
Ketoprofen (KETO) was gifted by Emcure Pharmaceuticals Limited, Pune. Potassium dihydrogen phosphate (KH2PO4) and Disodium hydrogen orthophosphate dihydrate (Na2HPO4) were purchased from SDFCL, Mumbai. Sodium Hydroxide (NaOH) and Sodium acetate trihydrate were procured from Rankem, New Delhi, India. Saccharin sodium dihydrate (SAC-Na) was gifted by Pure Chem. Pvt. Ltd., Ankleshwar, Gujarat. All other solvents, excipients and chemicals used were of analytical and HPLC grade (Merck Pvt. Ltd., Mumbai, India).

2. Crystal preparation by conventional solvent evaporation technique
Ketoprofen loaded crystals were prepared by solvent evaporation technique using Saccharin sodium dihydrate excipient in molar proportion of (1M:1M) which formed hydrogen bonding with Ketoprofen. This bond lead to improve physicochemical properties of Ketoprofen like aqueous solubility [8], in-vitro dissolution rate and ultimately improve pharmacokinetic parameters like Cmax, Tmax and AUC by in-vivo study which was investigated and presented in this work. A batch of control crystals was also formulated without adding excipient by keeping same other experimental parameters [26].

3. Drug release study of Ketoprofen crystals, control batch and pure drug
As per IP-X, In-vitro dissolution measurements were carried out in USP dissolution test apparatus (Electrolab Dissolution Tester TDT-06P, USP). The dissolution profile of Ketoprofen pure drug, control batch and Ketoprofen loaded crystals were studied in 900 mL of Phosphate buffer pH 7.5. 100 mg Ketoprofen pure drug and its control batch were filled in capsule separately while in case of drug-loaded crystals, 100 mg equivalent to Ketoprofen drug was filled and placed in a dissolution flask containing 900 mL of the dissolution medium, thermostated at 37 ± 0.5 °C, with paddle (USP Type II) with rotation speed of 50 RPM for one hour.

After each time interval i.e., 5, 10, 15, 20, 30, 40, 50 and 60 minutes, the samples (5 mL) were withdrawn and replaced immediately with fresh dissolution medium. The samples were filtered and one milliliter of the filtrate was diluted with respective buffer solution till the absorbance was measured in the range of 0.2 - 0.8. All the samples were assayed similarly by measuring the absorbance spectrophotometrically at 260 nm wavelength for dissolved drug. The dissolution experiments were conducted in triplicate and the mean of the absorbance was calculated. After one hour of dissolution, the amount (%) of the Ketoprofen drug dissolved were calculated graphically and used as comparison parameter in dissolution studies.

4. Pharmacokinetic study of Ketoprofen crystals compared with pure drug
Wistar albino rats (weighing approximately 210 ± 30 g) of either sex were used for the pharmacokinetic study of Ketoprofen pure drug and Ketoprofen loaded crystals. The animals were maintained in temperature and humidity controlled room with a 12:12 h light:dark cycle and were supplied with food and water ad libitum. The animal requirement for pharmacokinetic study of Ketoprofen was approved by the Institutional Animal Ethics Committee (IAEC) with protocol number IAEC/DPS/SU/1522 and all experiments were conducted as per the norms of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India [6, 7].

The overnight fasted rats (~12 h; n=4) with free access to water were used for the experiment of Ketoprofen pure drug and Ketoprofen loaded crystals. Two groups of rats were used among which one group was administered orally with aqueous suspension of Ketoprofen pure drug (STANDARD group) and another group was administered with Ketoprofen loaded crystals (TEST group) [27-30].

Samples of the Ketoprofen pure drug (9 mg/kg) (obtained by converting human dose to rat dose [30, 31]) and Ketoprofen loaded crystals (9 mg/kg equivalent to Ketoprofen) were accurately weighed and separately dispersed into distilled water (3 mL) by mixing homogeneously for 30 s prior to dosing. Each formulation was administered separately to rats by oral gavage using an animal feeding needle.
4.1 Collection of blood samples
Under ether anesthesia, blood samples (0.5 mL) were collected via the retro-orbital plexus at pre-dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 12 and 24 hours, after oral administration, into polypropylene tubes containing K3EDTA solution as anticoagulant tubes. The plasma was harvested by centrifuging the blood at 15000 rpm for 10 min at 4 °C temperature using a cooling centrifuge (REMI, Mumbai, India) and stored frozen in Deep freeze (Remi Quick Freezer, Mumbai, India) at -80 ± 10 °C until analysis. Both group animals were allowed to feed 2 h post-dose.

An aliquot of 300 µL of thawed plasma samples was processed as described in the following sample preparation sections.

4.2 Preparation of calibration curve of Ketoprofen in rat plasma (Spiked CC Standards)
Drug free rat plasma having K3EDTA as an anticoagulant was procured from Department of Pharmaceutical Sciences, Saurashtra University, Rajkot. CC standards were prepared by spiking the respective CC spiking solutions in drug free K3EDTA rat plasma as described in Table 1.

4.3 Quality control (QC) spiking solutions
QC spiking solutions were prepared in Methanol using Drug Stock Solution (2 mg/mL) by serial dilution as described in Table 2.

4.4 Equipment conditions
4.4.1 Chromatographic conditions
The concentration of Ketoprofen in rat plasma of both groups were determined by HPLC (LC20AD, Shimadzu Corporation, Japan) analysis consisted of binary gradient system with Photon Diode Array (PDA) Detector and Lab Solution Software 5.72 B (Shimadzu Corporation, Japan). HPLC system equipped with binary pump (LC-20AD) along with an auto sampler (SIL-HTC) was used to inject 10 µL plasma of the samples, processed as described in the extraction procedure section, onto a chromatographic column Gemini C18, 150 mm x 4.6 mm, 5 µm. The chromatographic conditions for Ketoprofen is described in Table 3.

<table>
<thead>
<tr>
<th>Spiking Solution ID</th>
<th>Spiking Solution Conc. (µg/mL)</th>
<th>Spiking Volume (mL)</th>
<th>Plasma Volume (mL)</th>
<th>Total Volume (mL)</th>
<th>Spiked Conc. (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>SS STD1</td>
<td>1000</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>SS STD2</td>
<td>500</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>SS STD3</td>
<td>250</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>12.5</td>
</tr>
<tr>
<td>SS STD4</td>
<td>100</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>SS STD5</td>
<td>50</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>SS STD6</td>
<td>30</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>1.5</td>
</tr>
<tr>
<td>SS STD7</td>
<td>16</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>0.8</td>
</tr>
<tr>
<td>SS STD8</td>
<td>8</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>0.4</td>
</tr>
<tr>
<td>SS STD9</td>
<td>4</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>0.2</td>
</tr>
<tr>
<td>SS STD10</td>
<td>2</td>
<td>0.5</td>
<td>9.5</td>
<td>10</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 1. Spiked CC standards in rat plasma for Ketoprofen

<table>
<thead>
<tr>
<th>Stock Dil. Conc. (µg/mL)</th>
<th>Volume Taken (mL)</th>
<th>Volume of Methanol (mL)</th>
<th>Total Volume (mL)</th>
<th>Spiking Solution Conc. (µg/mL)</th>
<th>Spiking Solution ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>2.25</td>
<td>2.75</td>
<td>5</td>
<td>900</td>
<td>SS HQC</td>
</tr>
<tr>
<td>900</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>300</td>
<td>SS MQC</td>
</tr>
<tr>
<td>300</td>
<td>0.4</td>
<td>4.6</td>
<td>5</td>
<td>24</td>
<td>SS LQC</td>
</tr>
</tbody>
</table>

Table 2. Quality control (QC) spiking solutions for Ketoprofen
Data from the plasma samples of both groups were used to plot curves for the estimation of concentration of Ketoprofen in plasma with time and also used for the comparison of pharmacokinetic parameters of Ketoprofen loaded crystals (Test) with Ketoprofen pure drug (Standard).

Table 3. Chromatographic conditions for Ketoprofen

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Gemini C 18, 150 mm x 4.6 mm, 5 μm</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>Formic acid in water, 0.1 %v/v : Methanol, 35 : 65 %v/v</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.00 mL/min</td>
</tr>
<tr>
<td>Column oven temperature</td>
<td>40 ± 0.3 °C</td>
</tr>
<tr>
<td>Auto sampler temperature</td>
<td>15 ± 3 °C</td>
</tr>
<tr>
<td>Volume of injection</td>
<td>10.0 µL</td>
</tr>
<tr>
<td>Detector</td>
<td>PDA detector</td>
</tr>
<tr>
<td>Retention time</td>
<td>Analyte at about 5.13 minutes</td>
</tr>
<tr>
<td></td>
<td>ISTD at about 3.50 minutes</td>
</tr>
<tr>
<td>Run time</td>
<td>8.0 minutes</td>
</tr>
</tbody>
</table>

4.5 Extraction procedure

4.5.1 Procedure for extracted sample preparation

Note: 500 µL of respective spiking solution was spiked into tube containing 9500 µL of rat plasma & vortex to mix.

Step 1. Retrieve the required number of CC standards and QC samples from the deep freezer, thaw them at R.T. and vortex the tubes to mix. Transfer 0.3 mL of sample into pre-labelled tube.

Step 2. Add 50 µL of ISTD (Internal Standard) dilution to all the samples except STD Blank and vortex for about 15 seconds.

Step 3. Add 50 µL of 0.1 N Hydrochloric acid in water solution to all the samples and vortex for about 15 seconds.

Step 4. Add 1.2 mL of Ethyl acetate, cap the tubes and vortex all the samples on cyclo mixer, for 10 minutes.

Step 5. Extracted samples were centrifuged at 10000 RPM, at 10 ± 2 °C for 10 minutes.

Step 6. 1.0 mL of supernatant was transferred into pre-labelled tubes and evaporated to dryness under vacuum by using an vacuum oven set at 40 ± 5 °C.

Step 7. After drying, samples were reconstituted with 100 µL of reconstitution solution and vortexed for about 30 seconds.

Step 8. Reconstituted samples were transfer into pre-labeled auto sampler vials, arrange them in the auto sampler and inject by using HPLC-PDA.

4.5.2 Aqueous sample preparation

1. Take 350 µL of reconstitution solution in pre-labelled tubes.
2. Add 500 µL of ISTD dilution, vortex to mix.
3. Add 150 µL of respective spiking solution and vortex to mix.
4. Appropriate volume of samples was transfer into pre-labelled auto sampler vials, and injected by HPLC-PDA.

4.5.3 Post spiked sample preparation for recovery experiment for drug and ISTD

Step 1. Retrieve the required number of STD BL from the deep freezer, thaw them at R.T. and vortex the tubes to mix. Transfer 0.3 mL of sample into pre-labelled tube.

Step 2. Add 50 µL of Methanol to all the samples, vortex for about 15 seconds.

Step 3. Add 50 µL of 0.1 N Hydrochloric acid in water solution to all the samples and vortex for about 15 seconds.

Step 4. Add 1.2 mL of Ethyl acetate to all samples and vortex for about 15 seconds.

Step 5. Centrifuge the samples at 10000 RPM, at 10 ± 2 °C for 10 minutes.

Step 6. 1.0 mL of supernatant was transferred into pre-labelled tubes and evaporated to dryness under vacuum by using the vacuum oven set at 40 ± 5 °C.

Step 7. After drying, samples were reconstituted with 100 µL of respective aqueous solution (Vial) and vortexed for about 30 seconds.

Step 8. Transfer samples into pre-labeled auto sampler vials, arrange them in the auto sampler and inject by using HPLC-PDA.

5. Data processing

The chromatograms were acquired by using Lab Solution Software 5.72 B supplied by Shimadzu. The calibration curve was plotted as the peak area ratio (Drug/ISTD) on Y-axis Vs the nominal concentration of Drug on the X-axis. The
concentrations of the unknown samples were calculated by using linear regression equation with $1/C^2$ weighting factor.

6. Pharmacokinetic parameters
Pharmacokinetic parameters $C_{\text{max}}$ and $T_{\text{max}}$ were calculated using plasma concentration versus time profile. The area under the plasma concentration versus time curve from zero time to the last experiment point, (AUC$_{0\rightarrow\infty}$) was calculated by trapezoidal method.

6.1 Trapezoidal method
The plot of plasma concentration time profile is divided into geometric figures whose area can be determined individually using appropriate geometric formula for each figure. The area under the curve of plasma concentration time plot was obtained by adding the area of each segment represented by the geometric figure. This plot resulted into one triangle and remaining trapezoids [32]. The following relationship was used to calculate each geometric figure.

\[
\text{AUC} = \left[ (0.5) \left( c_1 + c_2 \right) (t_2 - t_1) + \ldots \right] \\
\text{Area of triangle} = (0.5) \text{(height)} \times \text{(base)} \\
\text{Area of trapezoid} = 0.5 \left( \text{sum of two parallel sides} \right) \times \left( \text{base}_2 - \text{base}_1 \right) \\
\text{AUC} = \text{Area of triangle} + \text{Total area of trapezoids}
\]

RESULTS AND DISCUSSION

In-Vitro drug release study

In-Vitro drug release profile of Ketoprofen pure drug, its control batch and treated crystals were studied and results obtained are illustrated in the following Figure. In-vitro dissolution profile (Figure 1) depicted that treated crystal formulation with 1:1 molar proportion showed greater drug release (57.88%) with 2.05 fold while in case of 1:2 molar ratio of KETO:SAC-Na (52.54%) with 1.86 fold drug release compared to Ketoprofen pure drug within one hour. Results also revealed that the treated crystals showed drastic increase in dissolution rate compared to control batch (48.77%) as well as pure drug (28.27%) within one hour.

From the above results, it was concluded that the solubility and dissolution rate of treated crystals with molar ratio 1:1 of KETO:SAC-Na was better than pure drug, control batch and other molar ratio 1:2 of KETO:SAC-Na. Therefore, molar ratio of 1:1 was selected as optimized ratio for the in-vivo pharmacokinetic study.

In-Vivo pharmacokinetic study
Preparation of calibration curve of Ketoprofen with internal standard Carbamazepine (CBZ) in rat plasma (Spiked CC Standards)
Peak of Ketoprofen with internal standard Carbamazepine in rat plasma by HPLC method is illustrated in Figure 2 and calibration curve of Ibuprofen in rat plasma by HPLC method is illustrated in Figure 3.

Comparison of mean plasma concentration - time profile of Ketoprofen drug with its treated crystals in rat plasma
In-vivo absorption study was carried out to assess the solubility and dissolution enhancement of Ketoprofen in treated crystal formulation. Enhancement of In-vitro dissolution of drug from treated crystals could increase the GI absorption of drug after oral administration.

![Figure 2](https://example.com/Figure2.png)

**Figure 2** Peak of Ketoprofen with internal standard carbamazepine (CBZ) in rat plasma by HPLC method

![Figure 3](https://example.com/Figure3.png)

**Figure 3** Calibration curve of Ketoprofen in rat plasma by HPLC method
The plasma concentration - time profiles of Ketoprofen in albino rats (Wistar strain) following oral administration of suspension of Ketoprofen pure drug (STANDARD) and treated crystal formulation (TEST) are revealed and depicted in Figure 4.

**Table 4. Comparison of pharmacokinetic parameters of pure Ketoprofen drug (STANDARD) with treated crystals (TEST)**

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Ketoprofen Pure Drug (STANDARD)</th>
<th>Treated Crystals (TEST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL) ± SD*</td>
<td>27900.37 ng/mL ± 3263.74</td>
<td>46155.29 ng/mL ± 3345.93</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h) ± SD*</td>
<td>2 h ± 0.05</td>
<td>0.5 h ± 0.02</td>
</tr>
<tr>
<td>AUC$_{0\rightarrow24}$ (ng<em>h/mL) ± SD</em></td>
<td>221146.22 ng*h/mL ± 97589.60</td>
<td>471504.04 ng*h/mL ± 76540.95</td>
</tr>
</tbody>
</table>

* Results are expressed as mean ± SD (n = 4)

By examining the results obtained from the above individual analysis, it was revealed that the rats treated with pure drug i.e., Ketoprofen suspension (STANDARD) and Ketoprofen loaded crystals (TEST), the plasma concentration-time profile observed $C_{\text{max}}$ of 27900.37 ng/mL and 46155.29 ng/mL while $T_{\text{max}}$ of 120 minutes and 30 minutes, respectively as shown in Figure 4.

Plasma concentration-time profile represented that Ketoprofen loaded crystals (1M:1M) (TEST) gave higher plasma concentration (2.13 fold) compared to Ketoprofen pure drug (STANDARD). It was observed that Ketoprofen loaded crystals increased the rate of dissolution by way of hydrogen bonding with Saccharin sodium excipient. Hence, there was a drastic increment in the absorption of Ketoprofen from its crystal formulation which also enhanced the pharmacokinetic parameters and bioavailability of the drug.

Consequently, the pharmacokinetic parameters illustrated in Table 4 explained that Ketoprofen loaded crystal formulation exhibited better dissolution of drug in stomach. Hence, better absorption was achieved compared to pure drug. As shown in Table 4, $T_{\text{max}}$ for pure drug was achieved in 2 hours whereas for treated crystals, it was only 0.5 hour. The plasma drug concentration of treated crystals was 46155.29 ng/mL (TEST) which was again much higher compared to the plasma drug concentration of pure drug (STANDARD) i.e., 27900.37 ng/mL.

**CONCLUSION**

The aim of present work was to study the improvement in the in-vitro drug release and pharmacokinetic parameters of Ketoprofen treated crystals compared to the pure/plain drug as in the previous study it was found the improvement in aqueous solubility, drug release rate and mechanical properties which lead to the improvement in phiscochemical, mechanical and pharmacotechnical parameters of Ketoprofen, BCS Class II drug having poor flow property. In the present study, it was found that the Ketoprofen loaded crystals could drastically increase the dissolution rate and ultimately improve the pharmacokinetic parameters like $C_{\text{max}}$, $T_{\text{max}}$ and AUC compared to the pure Ketoprofen drug.

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**DATA AVAILABILITY**

Not declared.

**CONFLICTS OF INTEREST**

The authors declare no conflict of interest in this research article.
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