Enhancement of solubility and dissolution of poorly soluble drug: Ketoprofen as a model drug

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ABSTRACT
The objective of the study was to formulate micro particles of a poorly soluble drug Ketoprofen with the aid of Chitosan in presence of salting out agents. Dissolution Studies were carried out in 0.1N HCl using USP type II dissolution apparatus. The effect of Chitosan in presence of salting out agent predominated drug dissolution rate with the increasing concentrations of chitosan. A decrease in dissolution rate can be explained based on the viscous gel formation by chitosan at higher concentration (above 0.4%). The fine and fluffy physical state of optimized formulation along with their porous and rough surface as supported by SEM photomicrographs might have contributed to the enhanced solubility and dissolution rate of ketoprofen. Accelerated stability studies were performed as per ICH guidelines.

Keywords: Microparticles, high dispersion homogenizer, salting out agents, Carrageenan-induced paw edema, ICH guidelines.

INTRODUCTION
Among various drug delivery systems, oral drug delivery is the most common, convenient, safer and favoured route due to the low cost of drug treatment and patient compliance. Oral drug absorption is governed by factors viz. solubility, dissolution and permeability and their enhancement is one of the most challenging aspects of drug delivery system. Poor solubility manifests many in vivo limitations like incomplete release, poor bioavailability, food effects and higher inter-subject variability. However many methods are available to improve bioavailability by increasing dissolution rate like, formulation of solid dispersions, solid solutions [1],[2], micronization[3], nanosuspension[4], cocrystal [5], molecular encapsulation with cyclodextrin[6], co solvency[7], hydrotrophy, spray drying[8], solubilisation with surfactant [9], microemulsion, salt formation [10] , polymorphism [11] and combinations of techniques.
A number of natural polysaccharides aid in the improvement of the solubility and dissolution rate [12], [13]. One such polymer is chitosan, which is a linear hydrophilic [14] cationic copolymer obtained from N-deacetylation of chitin. Properties such as biodegradability, low toxicity and good biocompatibility make it suitable for use in pharmaceutical formulations. It has been used to enhance the dissolution of the poorly soluble drugs [15], [16], [17], [18].

Non steroidal anti-inflammatory drugs (NSAIDs) are considered to be the first-line symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Ketoprofen is a BCS class II NSAID with analgesic properties [19]. However its low aqueous solubility limits its therapeutic applications and bioavailability. Therefore, ketoprofen was selected as a model drug and the studies aimed at improving its solubility, dissolution rate and its bioavailability in suitable animal model.

**EXPERIMENTAL SECTION**

**Materials:** Ketoprofen obtained as a gift sample from Ranbaxy, India. Chitosan and Carrageenan obtained from Sigma Aldrich, USA. Sodium citrate and Sodium sulphate obtained from SRL Pvt. Ltd., India. Solvents like acetic acid, acetonitrile are of HPLC grade.

**Methods: Preparation of Ketoprofen microparticles**

Accurately weighed amount of ketoprofen was dispersed in chitosan solution in glacial acetic acid using high dispersion homogenizer [13]. This dispersion was added to distilled water with [20] or without salting out agent (Table 1) to precipitate chitosan on drug crystals [21]. The suspension was then centrifuged at 4 °C, 20000 rpm for 20 min. The obtained pellets were freeze dried.

**Table: 1 Composition of different formulations**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>A4</th>
<th>A15</th>
<th>T2</th>
<th>S2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug (mg)</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Chitosan (%)</td>
<td>0.4</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>1% acetic acid (ml)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>3% Sodium citrate solution (ml)</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3% Sodium potassium tartrate solution (ml)</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>3% Sodium sulphate solution (ml)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
</tr>
<tr>
<td>Distilled water (ml)</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Characterization of formulation:**

**Solubility of ketoprofen and formulations:**

Solubility was determined by taking excess quantity of ketoprofen (pure drug) and prepared formulations in fixed volumes of solvent (distilled water/0.1 N HCl). The resulting solution was placed on water bath shaker for 24 hrs. Supernatant was filtered and the concentration of drug in the saturated solution was determined spectrophotometrically (Shimadzu, UV-1601PC) at 259 nm [22].

**Percentage yield and Drug content**

The practical yield was measured and percentage yield of microparticles was calculated. Drug content of prepared crystals were determined in distilled water.

**Particle size**

The mean particle size (d50, d90 and d75) of pure drug and prepared microparticles were determined by using scatteroscope after dispersing in distilled water.
Infrared spectroscopy (IR)
IR spectroscopy was conducted using a Shimadzu FTIR 8300 Spectrophotometer (Shimadzu, Tokyo, Japan) and the spectrum was recorded in the wavelength region of 4000–400 cm\(^{-1}\).

Differential scanning calorimeter (DSC)
Thermal analysis was carried out using Shimadzu DSC-60 calorimeter. Samples were crimped in aluminium DSC pans under a nitrogen flow (30 ml/min) and then analysed, at a heating rate of 5°C/min from 25 to 250°C.

X ray diffraction (XRD)
Powder XRD patterns were recorded using Jeol Powder PW 1729 powder X-ray diffractometer, using Ni-filtered Cu ka radiation, a voltage of 40 kV and a current of 25 mA. The scanning rate employed was 1°/min over the angle 2θ range of 0-50°.

Scanning Electron Microscopy (SEM)
The surface morphology of the ketoprofen and prepared optimised crystals were studied by SEM (JEOL, JSM 50A, Tokyo, Japan). The accelerating voltage was 20 kV.

In vitro dissolution studies
The in vitro dissolution rate of ketoprofen from prepared formulations were determined using USP type II dissolution apparatus (Electrolab TDT-08L, Mumbai, India) using 0.1N HCl agitated at 50 rpm. A sample volume of 5ml was withdrawn at each sampling point, filtered and analysed spectrophotometrically, subsequently replacing with the fresh dissolution medium. All dissolution tests were performed in triplicates [6].

Stability studies
The optimized formulations were stored for one month in a stability chamber (Thermolab, Mumbai, India) maintained at 40±2°C and 75±5% RH. For comparison with samples stored at ambient condition, control samples were stored at room temperature for one month and evaluated for the drug content and physical change.

In vivo studies
The preclinical study protocol was approved by the Institutional Animal Ethical Committee, Kasturba Medical College, Manipal (Approval No. IAEC/KMC/19/2010-2011).The preclinical studies (anti-inflammatory, analgesic and pharmacokinetic studies) were carried out in Wistar rats and Swiss albino mice.

Anti inflammatory study
The anti-inflammatory effect after oral administration of the pure drug (6 mg/kg) and optimized formulation was measured by Carrageenan-induced paw edema in rats. The overnight fasted rats were divided into three groups with six rats each (n = 6). The first group was administered without drug and was used as a negative control; the second group was given pure drug, while the remaining group was treated with the prepared optimised formulation (A15). After 30 min of drug administration, edema was induced on the right hind foot of the rat by a subplantar injection of 0.1 ml of 1.0% carrageenan dissolved in saline [23] The paw volumes were measured with a plethysmometer, prior and after administration of carrageenan. The percent inhibition of edema was calculated.
Analgesic activity (Writhing test) [23]
The animals were divided into three groups with six mice each in group. The first group was administered without drug and was used as a negative control; the second group was given pure drug, while the remaining group was treated with the optimized formulation A15. Drug was administered orally to mice at scheduled intervals 1 hour before testing. Then the mice were injected intraperitoneally with 0.6% acetic acid at a dose of 10 ml/kg body weight. After the injection of acetic acid, the number of writhing responses was counted for 20 min. The analgesic activity was evaluated in terms of the percentage of writh inhibition.

Pharmacokinetic Study
The pharmacokinetic studies were carried out in Wistar rats. The overnight fasted animals were divided into 2 groups with six rats each in group (n=6) and treated orally. The first group was given pure ketoprofen (6 mg/kg) in 0.5% sodium CMC, while the second group was treated with the prepared optimised formulation containing equivalent amount of Ketoprofen (6 mg/kg) in 0.5% CMC. Then blood samples were collected at predetermined intervals of post-dose into centrifuge tubes from the orbital sinus. The plasma was separated immediately by centrifugation stored until analysis.

Bio Analytical Studies
Analysis of drug in rat plasma [24], [25]
A sensitive high performance liquid chromatographic (HPLC) method was used to analyze the ketoprofen in plasma. The response factor (peak area ratio of drug peak area to the internal standard peak area) of the standard solution and the sample were calculated and the concentration of the ketoprofen present in the plasma samples was calculated from the calibration curve. The data was analyzed using PK Solutions 2.0™. Non-compartmental pharmacokinetic data analysis software is used to calculate the pharmacokinetic parameters.

RESULTS AND DISCUSSION

Solubility Studies:
The increase in saturation solubility from 0.1965± 0.03 mg/ml to 0.7801± 0.01 mg/ml in water and 0.0983± 0.07mg/ml to 0.2935mg/ml in 0.1N HCl was observed with chitosan and salting out agents. The increase in solubility of ketoprofen was presumably contributed to the degree of drug amorphization and the particle size reduction [9], [26]. At higher chitosan concentrations (>0.4%), the solubility of ketoprofen was lower than assumed. This might be due to increase in the viscosity of polymer around the drug.

Characterization:
Percentage yield, drug content and particle size distribution:
The percentage yield varied from 71.32 to 88.68 % and drug content ranged from 73.62 to 99.51 % among the different batches of formulation prepared. The particle size of all the prepared formulation was considerably reduced during the preparation process in comparison to the pure drug. This might be due to high attrition during the homogenization process. Among the use of different salting out agents, similar results were observed. Tartaric acid salt showed better percentage yield, drug content but higher particle size distribution than citrate and sulphate salts.

Differential Scanning Calorimetry (DSC)
It was observed that there was a slight decrease in the melting point of drug when prepared in the formulation in comparison with pure ketoprofen (97.5°C ) (fig.1).Formulation A15, T-2 and
S-2 showed melting point at 93.77 °C, 93.86 °C and 94.39 °C respectively. This reduction in melting point can account for increased solubility and reduced crystallinity of Ketoprofen.

**Infrared spectroscopy**
Formulations exhibited identical IR spectra in comparison to the pure drug indicating that there are not any changes at the molecular level. Major peaks were observed at 1697, 1651, 1448, 1369, 1282 cm⁻¹.

**X-ray diffraction:**
The X-ray diffraction pattern of the pure drug exhibits its characteristic diffraction peaks at various diffraction angles indicating the presence of crystallinity. The diffraction pattern of the optimised formulation showed broadening, and reduction of major ketoprofen diffraction peaks indicating that partial amorphous form existed. These results could explain the observed enhancement of solubility and rapid dissolution of ketoprofen. Thus XRD data supports the DSC studies which indicated the decreased crystallinity of drug in the prepared formulation by exhibiting lower values of melting points.

**Scanning Electron Microscopy (SEM)**
Pure drug ketoprofen appeared as bigger size plate-like crystals with smooth surface (fig.2). In contrast optimised formulation (A15) was present in the form of fine porous powder (fig.3) with rough surface which might have led in the enhanced dissolution rate.
In vitro dissolution studies (figure 4)
The pure drug showed a release of 47.12 ± 1.08 % at the end of 2 hr. All prepared formulations showed improved release when compared to that of pure drug. Formulations were prepared by increasing the concentration of chitosan to improve release. A4 formulation containing 0.4% chitosan showed a release of 69.27±1.93% at the end of 2 hr. Further increase in Chitosan concentration caused decrease in release rate owing to the viscous gel formation.

Further, the formulations were developed by replacing distilled water with sodium citrate solutions (1, 3 and 5%), which acts as an efficient salting-out agent for chitosan [20]. Highest drug release was observed with A15 crystals with 0.4% chitosan and 3% sodium citrate solution. Formulation A15 showed 96.31±1.72% release in 2 hr with initial drug release 84.45±1.67% in 45 min. chitosan was able to increase the dissolution rate at lower concentrations when associated with sodium citrate. This could be due to efficient adsorption of chitosan on drug particles in the presence of sodium citrate. The reaction of chitosan with multivalent anions like sodium citrate allows the formation of bridges between the polymeric chains and results in cross-linking (by electrostatic interaction) between the chitosan molecules, which might have eventually resulted in efficient adsorption of chitosan on drug particles. Hence 3% of sodium citrate was considered to be suitable concentration for the formulation.

Sodium potassium tartrate and sodium sulphate were also studied to salt out chitosan at same concentration as that of sodium citrate. Results revealed that effect of tartrate and sulphate concentration on the drug release was similar to citrate. At 3% concentration both salts showed maximum release (T2 & S2).

Stability studies
The results of accelerated stability studies indicated that A15 formulation did not show any physical changes during the study period and the drug content was found more than 97.5% at the end of 1 month in accelerated condition (taking initial drug content as 100%).

Preclinical studies
Anti-inflammatory activity
The increased paw edema volume before and after drug administration was calculated and % increase in paw edema at each time point was calculated (Table 2). A15 showed better anti-inflammatory effect when compared with that of pure ketoprofen.
Table 2: Anti-inflammatory study results of ketoprofen and A15

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose</th>
<th>% increase in paw volume (1h)</th>
<th>% increase in paw volume (3h)</th>
<th>% increase in paw volume (5h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>0.25% CMC</td>
<td>38.23 ± 9.57</td>
<td>64.23 ± 10.67</td>
<td>55.92 ± 8.22</td>
</tr>
<tr>
<td>II</td>
<td>Ketoprofen</td>
<td>6 mg/kg</td>
<td>7.73 ± 1.98*</td>
<td>18.18 ± 2.46*</td>
<td>13.81 ± 1.85*</td>
</tr>
<tr>
<td>III</td>
<td>A15</td>
<td>6 mg/kg</td>
<td>8.81 ± 1.10*</td>
<td>16.14 ± 3.96*</td>
<td>10.40 ± 1.12*</td>
</tr>
</tbody>
</table>

n = 6; mean ± SEM, Results were analyzed by one-way ANOVA followed by post hoc Dunnett’s test [ p < 0.05 compared to control was considered significant].

Analgesic activity
Intraperitoneal administration of a solution of 0.6% acetic acid induced a muscular constriction causing a concavity of the abdominal flank, and although comparatively rare, the behaviour was easily recognized. The first group was administered without drug, the second group was given pure drug, while the remaining group was treated with the optimized formulation A15. Figure 5 clearly indicates the more rapid and effective analgesic activity of A15 formulation than that of pure ketoprofen. The improved pharmacological response of A15 formulation may be attributed to improved solubility and dissolution rate of ketoprofen, which in turn improved its rate of absorption.

Pharmacokinetic study
The reverse phase HPLC method was selected for the estimation of ketoprofen in rat plasma. Using UV detector, the protein precipitation extraction was followed in the present study because of its simplicity, selectivity and sensitivity. Glibencamide was selected as an internal standard (IS). The peaks were well resolved with the retention time of 6.36 min for ketoprofen and 11.03 min for glibencamide respectively (Fig.6), linear relationship (r² = 0.9991) was observed. The drug concentration in the plasma was quantified.
The pharmacokinetic parameters (Table 3) were calculated from the plasma concentration–time curves. Optimized formulation A15 showed better $t_{\text{max}}$ and $C_{\text{max}}$ greater AUC value indicating improved bioavailability. Hence the pharmacokinetic study indicates higher absorption and therefore higher bioavailability of drug from A15 formulation in comparison with pure drug. This could be due to improved solubility and dissolution rate of drug from prepared crystals.

**Table 3: Pharmacokinetic parameters from the plasma concentration-time curves of ketoprofen (pure drug) and optimized formulation (A15)**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pure drug*</th>
<th>A15*</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>2.4±1.1</td>
<td>8.6±2.8</td>
</tr>
<tr>
<td>$t_{\text{max}}$ (hr)</td>
<td>0.8±0.2</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>4.65±0.95</td>
<td>4.23±0.8</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (µg/ml)</td>
<td>8.6±2.9</td>
<td>13.4±3.5</td>
</tr>
<tr>
<td>MRT (hr)</td>
<td>6.9±2.3</td>
<td>5.9±1.1</td>
</tr>
<tr>
<td>$K_e$ (hr$^{-1}$)</td>
<td>0.1678±0.06</td>
<td>0.1457±0.08</td>
</tr>
<tr>
<td>CL (ml/min)</td>
<td>701±18.8</td>
<td>447±15.5</td>
</tr>
</tbody>
</table>

*mean±SD, (n=6)

**CONCLUSION**

As the ketoprofen is having very poor solubility, present study was concentrated on improving the solubility and dissolution characteristics of ketoprofen in acidic medium. Chitosan is a hydrophilic polymer, helps in can be used in enhancing the solubility and dissolution rate of poor soluble drug. In conclusion, the present study demonstrated the method for the enhancement of the solubility and dissolution ketoprofen using chitosan in presence of salting out agents. Future studies may include accelerated stability study for 6 month.

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