Pharmacokinetic Study of Zaltoprofen Spherical Agglomerated Dense Compacts Canvassed with Commercial Product

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Abstract
Pharmacokinetic parameters including AUC0-24t, AUC0-24d, Cmax, Tmax, T1/2 and Elimination rate constant (K) were determined from plasma concentrations of Zaltoprofen spherical agglomerated dense compacts by 24 factorial design followed by & determination of pharmacokinetic parameters for optimized formulation. Foremost spherical agglomerates of Zaltoprofen prepared with PEG 6000 which is hydrophilic polymer and evaluated for different compressibility parameters and in vitro dissolution studies, studies indicated that the drug release can be modulated by using hydrophilic polymers and the best one compressed as dense compacts and appraised for pharmacokinetic parameters in rabbit models. Sensitive and selective high performance liquid chromatographic method was used to determine drug plasma concentrations with good linearity in the range of 0.5 to 20 μg/mL (r2=0.995, n=7). Study ascertained mitigated values of AUC0-24 (15.789 ± 12.9, 97.334 ± 7.478).

Keywords: Pharmacokinetic study; Zaltoprofen; Spherical agglomerates; Spherical crystals; Rabbit model

Introduction
The conventional tableting method used involves first making granules and then compressing into tablets, but the need in recent years for process validation, GMP and automation of production processes has focused renewal of attention on the direct tableting, which involves few steps [1]. Zaltoprofen, is a novel NSAID, selective COX2 inhibiting drug used in the treatment of rheumatoid arthritis and osteoarthritis and to relieve inflammation and pain after surgery [2]. Zaltoprofen exhibits poor flow and compression characteristics and hence Zaltoprofen selected as suitable candidate for spherical crystallization process to improve flow properties and compressibility. Further, Zaltoprofen shows poor oral bioavailability due to low aqueous solubility [3] hence in such case enhancing bioavailability is a valuable goal to improve therapeutic efficacy. Estimation of Zaltoprofen in plasma by methods like UV-visible spectrophotometric [4], Mass spectrometry in human plasma [5] was reported.

In the present study intended to prepare spherical crystals of Zaltoprofen to improve aqueous solubility, dissolution rate and bioavailability besides improving it micromeritic properties using PEG-6000 which is hydrophilic polymer [6] the best formulation compacted into dense compacts and in this study further intended to determine the pharmacokinetic parameters for optimized formulation by factorial design an followed by in vivo study, rabbit as an animal model and comparative to a marketed formulation.

Materials and Methods

Materials
Zaltoprofen (99.3% purity), as a gift sample was received from M.S Hetero pharmaceutical, Hyderabad. Acetonitrile and water were HPLC grade from RFCL Ltd (Mumbai, India). Bath sonicator was used for degassing (PCI Analytics, Mumbai, India). Elico pH meter was used for adjustment. Ten station rotator tablet punching machine used for compression of tablets with 7 mm flat shaped punch (Elite tablet punching machine, Mumbai) REMI R-2 research centrifuge and REMI-101 cyclo mixer were used for extraction of drug from plasma (REMI Dectrotechnik Ltd., Vasai, India).

Formulation of Zaltoprofen spherical agglomerates
A solution of Zaltoprofen (500 mg) in acetone (3 mL) was added to a solution of Poly ethylene glycol 6000 (1-4% w/v) in 100 mL distilled water. The mixture was stirred continuously using digital mechanical stirrer (IKA motors. Mumbai) at 500 rpm, the bridging liquid (Dichloromethane; 0.5 mL) was added drop wise (Table 1) and stirring was continued for 30 min. The agglomerates were separated by filtration using whatman filter paper (No.1) and dried for 24 hours at room temperature.

Compression of dense compacts
Amid of all spherical agglomerates, the best performed agglomerates in terms of compressibility properties selected as optimized batch. It was thought of interest to study the effect of super disintegrants such as Ac-Di-Sol (CCS), Polyplesdone XL (CP) and Explotab (SSG) on the drug release of Zaltoprofen.

Spherical crystals were made into dense compacts with 4% w/w CCS, CP and SSG separately using Elite tablet punching machine, 10-station rotary punching machine (7 mm punch and die set) at a

\[ \text{Table 1: Formulation of Zaltoprofen Spherical agglomerates.} \]

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zaltoprofen (mg)</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>PEG 6000 (%w/v)</td>
<td>1%</td>
<td>2%</td>
<td>3%</td>
<td>4%</td>
</tr>
<tr>
<td>Acetone (mL)</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>DCM (mL)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Water (mL)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

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plasma was collected and stored at -20°C until HPLC analysis. After each sample was taken and centrifuged immediately, 0.2 mL of heparinized 0.9% NaCl solution was injected into the cannula just 1, 2, 4, 8, 12, 24 hours after the oral administration of the drug. About 20 mL of water was given to each rabbit to help them swallow the minicapsule. In a single-dose, open-label, crossover study, twelve rabbits bisected randomly in two groups, six rabbits in each group. White Rabbits were assigned to one in a cage for one day to allow them to recover from surgery. The rabbits were fasted overnight for at least 6 hours before the experiment. With full access to water, the rabbits were assigned to one of two groups each of 6 rabbits in each group. A mini capsule filled with spherical agglomerates formulation and marketed formulation after the oral administration were maintained at 40°C and auto sampler chamber was sustained at 4°C. The dissolution medium used was 900 mL, mixture of phosphate buffer solution pH 6.8 and water (1:1) used as dissolution medium. The agglomerates containing 80 mg of Zaltoprofen were weighed and then introduced into the dissolution medium. The medium was stirred at 50 rpm using paddle at 37 ± 0.5°C. The samples were collected, filtered through whatman filter paper (0.45 μM) and analyzed spectrophotometrically at 340 nm.

In Vitro dissolution studies

The in vitro dissolution studies [7] were carried out using 8 station USP XXIII dissolution testing apparatus (Electrolab, Mumbai, India). The dissolution medium used was 900 mL, mixture of phosphate buffer solution pH 6.8 and water (1:1) used as dissolution medium. The agglomerates of 80 mg of Zaltoprofen were weighed and then introduced into the dissolution medium. The medium was stirred at 50 rpm using paddle at 37 ± 0.5°C. The samples were collected, filtered through whatman filter paper (0.45 μM) and analyzed spectrophotometrically at 340 nm.

In Vivo Studies

In vivo chromographic conditions of HPLC

The HPLC system included system was automatically processed with and fully automated processor with Elite program. The pre column used in HPLC system was Capcell pak and the main analytical column used in the system was Capcell pak C18. Mobile phase A (a 12.88 v/v mixture of Acetonitrile and 10 mM Phosphate buffer) and Mobile Phase B (A 35.65 v/v mixture of Acetonitrile and 10 mM Phosphate buffer, pH 6.8) was pumped into the HPLC system at a flow rate of 500 μL/min and 90 μL/min. The Zaltoprofen concentrations in effluent of the system were discovered with a UV detector at 340 nm. The HPLC system included system was automatically processed with and fully automated processor with Elite program. The pre column used in HPLC system was Capcell pak and the main analytical column used in the system was Capcell pak C18. Mobile phase A (a 12.88 v/v mixture of Acetonitrile and 10 mM Phosphate buffer) and Mobile Phase B (A 35.65 v/v mixture of Acetonitrile and 10 mM Phosphate buffer, pH 6.8) was pumped into the HPLC system at a flow rate of 500 μL/min and 90 μL/min. The Zaltoprofen concentrations in effluent of the system were discovered with a UV detector at 340 nm and the temperatures in the system of pre and analytical columns were maintained at 40°C and auto sampler chamber was sustained at 4°C.

Extraction procedure

The Zaltoprofen solutions were added to blank plasma samples of rabbits, 5 mL of ethyl acetate and 50 μL of amyl alcohol were added and adjusted the pH to 7.4 with 0.2 mol NaOH for absolute recovery followed by centrifuge at 2500 r/min at 40 min. The ethyl acetate phase was separated and evaporated under the nitrogen stream, reconstituted with 2 mL of mobile phase and stored at -20°C till analyzed by RP-HPLC system.

Study Design and Pharmacokinetic Analysis

Methods

In a single-dose, open-label, crossover study, twelve rabbits bisected randomly in two groups, six rabbits in each group. White Rabbits weighing 1.4-1.6 kg were allowed free access to a normal chow diet and water for at least one week before the experiments. The rabbits were anesthetized with diethyl ether, and the common central ear vein was cannulated with polyethylene tubing for blood sampling. The cannula was flushed and filled with heparinized isotonic saline (100 IU/mL) to prevent the blood from clotting. The rabbits were housed individually in a cage for one day to allow them to recover from surgery.

The rabbits were fasted overnight for at least 6 hours before the experiment, with free access to water. The rabbits were assigned to one of two groups each of 6 rabbits in each group. A mini capsule filled with Spherical agglomerates formulation and marked formulation equivalent to 6.5 mg of Zaltoprofen was administered orally with a mini capsule injector. Immediately after oral dosing, 5 mL of distilled water was given to each rabbit to help them swallow the minicapsule. About 1 mL of blood was collected from the each rabbit at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24 hours after the oral administration of the drug. About 2 mL of heparinized 0.9% NaCl solution was let into the cannula just after each blood sample was taken and centrifuged immediately; the plasma was collected and stored at -20°C until HPLC analysis.

The pharmacokinetic parameters of spherical crystals formulation and marketed formulation after the oral administration were determined by non-compartmental analysis using Winnonlin software. The pharmacokinetic parameters estimated in this study are area under the plasma concentration time curve from 0-24 h (AUC0-24), area under the plasma concentration time curve from 0 hour to infinity (AUC0-∞), elimination half-life (t1/2), Volume of distribution (Vd) and clearance (CL), the maximum plasma concentration (Cmax) and the time required to reach Cmax (tmax).

WINNONLIN® (WINNONLIN NONCOMPARTMENTAL ANALYSIS PROGRAM 1.0 Core Version 04 Jun 2007) is the industry standard used for Pharmacokinetic, Pharmacodynamic, and non-compartmental analysis. In addition to its extensive library of built-in PK, PD and PK/PD models.

Results and Discussions

In vitro dissolution studies

The results of in vitro dissolution studies are shown in figures 1 and 2. Compressibility parameters in table 2. Pure Zaltoprofen exhibited very less release at the end of 30 min is 66.6%. The drug release at 30 minutes from the spherical crystals prepared in the presence of 1% to 4% w/v PEG 6000 were 65.2, 72.6, 78.6 and 84.4% respectively, which indicates that the spherical crystals prepared with 4% w/v PEG 6000 exhibited maximum drug release i.e. 84.4%.

This indicates that Zaltoprofen crystals prepared with 4% w/v PEG...
improving the micromeritic properties of the drug, hence selected as
w/v PEG 6000 have shown maximum drug release in addition to
products
Comparison of dissolution of compacts and commercial
Further
the adsorption of PEG 6000 onto the surfaces of crystals. This crystal
may be due to the improved wet ability of the surface of the crystals by
mechanism, although the particle size of the crystals is high. This effect
effect of Zaltoprofen in crystals form may resemble the solid dispersion
6000 exhibit an excellent dissolution and solubility enhancing ability.
The mechanism behind the solubility and dissolution rate enhancing
effect of Zaltoprofen in crystals form may resemble the solid dispersion
mechanism, although the particle size of the crystals is high. This effect
may be due to the improved wet ability of the surface of the crystals by
the adsorption of PEG 6000 onto the surfaces of crystals. This crystal
modification technique can enhance the bioavailability of the drug.
Further in vivo studies are continued to study the improved solubility
and dissolution rate on bioavailability of Zaltoprofen.

Comparison of dissolution of compacts and commercial
products
Out of all spherical crystals, the agglomerates prepared with 4%
w/v PEG 6000 have shown maximum drug release in addition to
improving the micromeritic properties of the drug, hence selected as
optimized batch. The study the effect of super disintegrants such as
Ac-Di-Sol(CCS), Polyplasdone XL (CP) and Explotab (SSG) on the
drug release of Zaltoprofen was done by compressing agglomerates
prepared with 4% w/v PEG 6000 and drug with 4% w/w of above said
super disintegrants.

These spherical agglomerated dense compacts (SADC) along with
market product were subjected to dissolution studies and maintained
same conditions of the spherical agglomerates. The results of drug
release from dense compacts and market product are shown in figures
3-5. The results displayed that the percent release of Zaltoprofen
marketed product were evaluated for
their pharmacokinetics using Winnonlin software. The average
administration of selected formulation i.e. Zaltoprofen spherical
agglomerated dense compacts followed by oral administration of commercial product.

Pharmacokinetic analysis
The concentration of Zaltoprofen in plasma and the respective
average values at different time intervals following the oral
administration of selected formulation i.e. Zaltoprofen spherical
agglomerates prepared in 4% PEG 6000 solution compressed with 4% of Ac-Di-Sol and commercial product were evaluated for
their pharmacokinetics using Winnonlin software. The average
plasma concentration versus time was shown in figures 4-6 and log
concentration versus time curve show in figure 7. Individual and mean
pharmacokinetic parameters (AUC0-24, AUC0-∞, Tmax, Cmax, Kel, t1/2 and
MRT0-24) calculated from the average serum concentrations from the in
vivo experiments are in shown in table 3.

The Cmax of zaltoprofen from selected formulation and commercial
product were found to be 18.39 ± 2.59 and 15.09 ± 1.87 μg/mL

<table>
<thead>
<tr>
<th>Spherical Crystals</th>
<th>LBD (g/ml)</th>
<th>TBD (g/ml)</th>
<th>Carr’s Index</th>
<th>Hausnar’s ratio (%)</th>
<th>Angle of Repose</th>
<th>True Density (g/ml)</th>
<th>Porosity (%)</th>
<th>Particle Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG 6000 1% W/V</td>
<td>0.42 ± 0.01</td>
<td>0.45 ± 0.01</td>
<td>10.20 ± 1.51</td>
<td>1.18 ± 0.01</td>
<td>29.31 ± 1.23</td>
<td>1.22 ± 0.12</td>
<td>59.55 ± 2.36</td>
<td>201.12 ± 10.18</td>
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<tr>
<td>PEG 6000 2% W/V</td>
<td>0.4 ± 0.02</td>
<td>0.45 ± 0.01</td>
<td>10.10 ± 2.36</td>
<td>1.10 ± 0.02</td>
<td>26.47 ± 2.31</td>
<td>1.25 ± 0.26</td>
<td>65.15 ± 2.16</td>
<td>218.13 ± 12.10</td>
</tr>
<tr>
<td>PEG 6000 3% W/V</td>
<td>0.37 ± 0.01</td>
<td>0.41 ± 0.01</td>
<td>10.00 ± 2.12</td>
<td>1.10 ± 0.01</td>
<td>25.61 ± 1.89</td>
<td>1.28 ± 0.10</td>
<td>71.05 ± 3.01</td>
<td>230.25 ± 11.77</td>
</tr>
<tr>
<td>PEG 6000 4% W/V</td>
<td>0.39 ± 0.01</td>
<td>0.44 ± 0.01</td>
<td>10.10 ± 2.69</td>
<td>1.00 ± 0.01</td>
<td>22.88 ± 1.65</td>
<td>1.25 ± 0.16</td>
<td>74.32 ± 1.36</td>
<td>235.11 ± 12.23</td>
</tr>
<tr>
<td>Pure Zaltoprofen</td>
<td>0.30 ± 0.01</td>
<td>0.52 ± 0.01</td>
<td>42.00 ± 2.36</td>
<td>1.69 ± 0.03</td>
<td>42.61 ± 2.31</td>
<td>1.55 ± 0.46</td>
<td>55.50 ± 2.21</td>
<td>85.55 ± 10.25</td>
</tr>
</tbody>
</table>

Table 2: Micromeritic properties of Zaltoprofen spherical crystals prepared in the presence of different concentrations of PEG-6000 and pure drug.

![Typical HPLC chromatogram of (a) blank plasma (b) plasma spiked with 10 μg/mL of Zaltoprofen (c) Zaltoprofen of a rabbit administered with formulation and (d) Zaltoprofen of a rabbit administered with commercial product.](image1)

![Calibration curve of Zaltoprofen in rabbit plasma by HPLC method.](image2)

![Plasma concentration time curves of Zaltoprofen followed by oral administration of commercial product.](image3)
respectively and the values of the commercial product are below the selected formulations. The $t_{\text{max}}$ values from selected formulation and commercial product were found to be $1.16 \pm 0.51$ and $1.33 \pm 0.50$ hr respectively and the values of the commercial product are below the selected formulations. The $K_e$ values were found to be $-0.807 \pm 0.03$ and $-0.619 \pm 0.02$ hr$^{-1}$ after oral administration of selected formulation and commercial product respectively calculated from the slope of the terminal portion of log plasma concentration of zaltoprofen versus time (Figure 8). The corresponding $t_{1/2}$ values were found to be $0.858 \pm 0.03$ and $1.11 \pm 0.03$ hrs respectively. The $t_{1/2}$ values are almost similar to the reported values.

The values of AUC$_{0-24}$ and MRT$_{0-24}$ of selected formulation and commercial product were $115.789 \pm 3.09$, $97.334 \pm 3.18$ and $5.253 \pm 1.02$, $5.079 \pm 1.16$ respectively.

The AUC values of selected formulation were higher compared to the commercial product indicating the higher bioavailability of the selected formulation. Thus the results of present study indicated the applicability of spherical crystals of zaltoprofen as directly compressible material in direct tableting.

**Conclusion**

This study ascertains that the spherical agglomerated dense compacts were successfully formulated. The use of PEG 6000 in the formulation of spherical agglomerates successfully improved bioavailability of the zaltoprofen compared to commercial product confirmed by *in vivo* studies in rabbits. There lies a great promise that spherical agglomeration technology with use of hydrophilic polymers like PEG 6000 will hasten the drug dissolution and bioavailability of miserable water soluble drugs.

**References**


