Development of Acetaminophen Nanosuspension for improved solubility

Introduction:

Acetaminophen (PCM), also known as paracetamol, belongs to the category of COX-2 selective inhibitors. The drug is usually taken for its analgesic and anti-pyretic activity. Fever results from elevated levels of prostaglandin synthesis. The prostaglandins indirectly act on the central nervous system thereby decreasing the body’s overall heat loss with simultaneous increase in heat gain. PCM acts by inhibition of cyclooxygenase and results in reduction of elevated levels of prostaglandins synthesis. Moreover, it produces analgesia by increasing the pain threshold (Eandi et al., 1984). PCM is commonly available as over-the-counter medication in various dosage forms such as solutions, suspensions, tablets and capsules. However, therapeutically PCM has very low aqueous solubility which affects its in-vivo performance, specifically bioavailability (40%). The usual dose administered is 500mg-1g, of which almost 80% is excreted unchanged by kidney. Hence, high doses are required to be administered which can be fatal. Therefore the present delivery system predisposes to liver toxicity (Farre et al., 2008; Biazar et al., 2010).

Various approaches have been tried to improve the solubility profile of PCM such as formation of beta-cyclodextrins (Tasic et al., 1992), size reduction by mechanical activation (Biazar et al., 2009), use of water soluble excipients such as lactose, potassium/ sodium bicarbonate, sodium chloride and tartaric acid (Shaw et al., 2005), solid dispersion technique (Soni et al., 2012; Sheng Qi et al., 2008) and use of poly(ε-caprolactone) layered silicate nanocomposites loaded with PCM (Campbell et al., 2009). It has been reported that the low solubility of a drug is best improved by the use of nanoparticles. The nanosize ranges of the formulations
decrease the particle size and as a consequence the surface-to-volume ratio also increases. This results in better solubility and dissolution profiles (Kaur et al., 2012; Ghosh et al., 2011). Nanosuspensions are recent and novel delivery systems in which pure drug nanoparticles (10-1000nm) are suspended in minimum amount of stabilizers (Zakir et al, 2011; Gao et al., 2012; Kesisoglou and Mitra, 2012). Since nanosuspensions are produced from drug itself, the drug content approaches 100%. Thus they not only improve solubility but also provide better drug loading as well as bioavailability (Sun and Yeo, 2012). Previously nanosuspensions of PCM have been formulated through nanoprecipitation by microfluidic technique (Aghajani et al., 2013). In the present paper, we have formulated PCM nanosuspension by nanoedge method i.e. combination of homogenization and sonication using Tween 80 as stabilizer. The prepared nanosuspension was further evaluated for saturation solubility and dissolution studies.

Objectives:

To increase the solubility of acetaminophen by preparing nanosuspension formulation using Tween 80 as a stabilizer.

Methodology:

Materials:

PCM was obtained as a gift sample from Ministry of Health, Saudi Arabia. Tween 80, dipotassium hydrogen phosphate and potassium dihydrogen phosphate were purchased from S.D. Chemicals Ltd. Ethanol and Ethylacetate were obtained from Sigma Chemicals, USA. All other chemicals were of analytical grade.

Methods:

Preparation of Standard Curve:
Phosphate buffer pH 5.8 was prepared by using 8.5 ml of dipotassium hydrogen phosphate (1M) and 91.5 ml of dihydrogen potassium phosphate (1M). The prepared solution was diluted to 1L using distilled water.

Weigh accurately 100mg of PCM, dissolve in buffer solution using sonicator and make up the volume up to 100ml with the help of pH 5.8 phosphate buffer to make 1000µg/ml solution. Prepare a series of dilute solutions from the above stock solution in 10 volumetric flasks (4, 6, 8, 10, 12 µg/ml). Measure the absorbance of the solutions at 249 nm using UV-Visible spectrophotometer.

Preparation and Optimization of Nanosuspension:

The nanosuspensions were prepared by nanoedge method (Kaur et al., 2010). Briefly, 500mg of PCM solutions were prepared in 4ml of ethanol and ethyl acetate (in 1:1 ratio), and poured into 98 ml of various concentrations of Tween 80 solutions and stirred with homogenizer (HG-15D, Wisd Labs) at 500 rpm for 5min. The nanosuspensions were then sonicated (WUC-D10H, Wisd Labs) and optimized based on particle size, PDI and zeta potential studies. Ethanol in the formulation was evaporated by keeping under vacuum (−25 in.Hg) at room temperature for 2–3 h.

Physical Characterization:

Size, polydispersity index and zeta potential of nanocrystals were determined by dynamic light scattering method (Zetasizer nanoZS, Malvern Instruments Ltd, Worcestershire, UK) (Tian et al., 2013). The nanosuspension was also viewed under phase contrast microscope (M317E, Nikon).

Drug Solubility Studies:
Saturation solubility of raw PCM and PCM nanosuspension was determined as reported by Talekar et al., 2012. In brief, excess amount of PCM nanosuspension was added into 5 ml Phosphate buffer pH 5.8 and stirred for 48 h at 37±0.5°C. The suspension was then filtered using 0.1 µm membrane filter and analyzed by UV-Visible spectrophotometer (UV1700, Schimadzu Japan) at 249nm.

In-vitro dissolution

The in-vitro drug release profile of PCM nanosuspension was studied for 15 min using USP dissolution apparatus type 2 (Kakran et al., 2012). Samples equivalent to 3 mg PCM were dispersed in the dissolution medium (900ml of phosphate buffer pH 5.8). The system was stirred at 100 rpm and maintained at a temperature of 37±0.5°C. Samples were withdrawn at predetermined time intervals and replaced with the same volume of fresh dialyzing medium. The withdrawn samples were filtered by 0.1µm membrane filter and analyzed by UV-Visible spectrophotometer at 249nm.

Stability studies

The effects of temperature on the stability of nanosuspensions were assessed for a period of 1 week. The nanosuspensions were divided into different batches and stored at different temperatures (4°C, 25°C, 40°C) and examined for changes in size, PDI at regular time intervals (Sahu and Das, 2012).

Results and Discussion:

Calibration curve of PCM:

The calibration curve of PCM was plotted and found to be linear (Fig 1) between 4 to 12 µg/ml thus following Beer-Lambert’s Law. The regression value was found to be 0.999.
Particle size, PDI, zeta potential:
The PCM nanosuspensions were prepared using different concentrations of Tween 80 (0.1%, 0.2%, 0.25%, and 0.3% w/w) and analyzed for physical parameters. As shown in Table 1, the minimum concentration of Tween 80 which gave best results was found to be 0.25%w/w; particle size 321±23.16 nm and PDI -31±0.99. The next step involved optimization of sonication time on the basis of size and PDI data. It was observed that the particle size of the formulation decreased significantly as the sonication time was increased but only up to 6 min (Table 2). The final size and PDI of the optimized formulation was found to be 193±12.54 and 0.249. The zeta potential value of the optimized formulation was found to be -13±0.64, indicating good stability of the formulation. It have been suggested that once nanoparticles begin to form their free energy state increases and they become thermodynamically unstable. As a result the particles try to decrease the Gibbs free energy by agglomeration. This phenomenon is prevented by adding suitable stabilizers such as Tween 80 that improve activation energy (Tian et al, 2013).
Table 1: Optimization of concentration of Tween 80 based on physical parameters.

<table>
<thead>
<tr>
<th>Concentration of stabilizer (% w/w)</th>
<th>Particle size (nm)</th>
<th>Zeta potential (mV)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1%</td>
<td>990±88.91</td>
<td>-35±2.3</td>
<td>0.787</td>
</tr>
<tr>
<td>0.2%</td>
<td>560±46.51</td>
<td>-34±1.4</td>
<td>0.444</td>
</tr>
<tr>
<td>0.3%</td>
<td>788±51.42</td>
<td>-48±0.79</td>
<td>0.686</td>
</tr>
<tr>
<td>0.25%</td>
<td><strong>321±23.16</strong></td>
<td><strong>-31±0.99</strong></td>
<td><strong>0.343</strong></td>
</tr>
</tbody>
</table>

Table 2: Optimization of sonication time based on physical parameters.

<table>
<thead>
<tr>
<th>Sonication time (min)</th>
<th>Particle size (nm)</th>
<th>Zeta potential (mV)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>742±45.35</td>
<td>-33±1.2</td>
<td>0.572</td>
</tr>
<tr>
<td>4</td>
<td>355±22.09</td>
<td>-29±1.1</td>
<td>0.333</td>
</tr>
<tr>
<td>6</td>
<td><strong>193±12.54</strong></td>
<td><strong>-13±0.64</strong></td>
<td><strong>0.249</strong></td>
</tr>
<tr>
<td>8</td>
<td>444±32.11</td>
<td>-18±0.85</td>
<td>0.290</td>
</tr>
</tbody>
</table>
Drug Solubility:
The solubility of PCM nanosuspension was compared with raw PCM in phosphate buffer pH 5.8. Since Tween 80 is a surfactant which also has a solubilizing effect on PCM (Shaw et al., 2005), a physical mixture of PCM and Tween 80 was also prepared and evaluated. The solubility of PCM in phosphate buffer pH 5.8 with and without Tween 80 was found to be 8.4 mg/mL and 129 mg/mL respectively,
Table 3. It was found that the solubility of PCM in nanosuspension increased by up to 40 times. The significant increase in particle size is attributed to particle size reduction and improvement in surface area (Hao et al, 2012).

**Table 3: Solubility studies of different formulations.**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw PCM in phosphate buffer pH 5.8</td>
<td>8.4 mg/mL</td>
</tr>
<tr>
<td>PCM nanosuspension</td>
<td>353 mg/mL</td>
</tr>
<tr>
<td>PCM and Tween 80 in phosphate buffer pH 5.8</td>
<td>129 mg/mL</td>
</tr>
</tbody>
</table>

*In-vitro* dissolution study:

The dissolution profiles of raw PCM, PCM and Tween 80 mixture and PCM nanosuspensions is shown in Fig2. It is clear that the dissolution of PCM from nanosuspension was better than raw drug as almost 87% PCM was dissolved within 10 min as compared to 21%. Similarly the dissolution profile of PCM nanosuspension was statistically significant from PCM and Tween 80 solution by a factor of 2; 62%. The improved dissolution behavior is because of enhanced solubility and reduced particle sizes (Deng-Guang et al., 2010).
Drug Stability studies:

The stability studies were conducted to determine the shelf-life of PCM nanosuspension. Since no significant differences in particle size and PDI were observed at 4°C and 25°C (Fig 3), it can be inferred that the formulation remained stable for a period of 1 week at refrigerator and ambient temperatures. However, the particle size of the nanosuspension increased considerably when stored at 40°C, as the size increased from 193nm to 253nm. Therefore the formulation cannot be stored at higher temperatures.

Fig 2: In-vitro dissolution studies of different PCM formulations.
References:


Fig 3: Stability studies of PCM nanosuspension with 0.25% Tween 80 at various temperatures.


Abstract: Paracetamol (Acetaminophen) is a poor water soluble drug with limited bioavailability. Size reduction is one of the easiest methods to enhance solubility as well as dissolution of any drug. In the present paper, we have formulated acetaminophen nanosuspension using Tween 80 as stabilizer by nanoedge method. The concentration of Tween 80 and sonication time were optimized on the basis of particle size, PDI and zeta potential. It was found that nanosuspension with particle size of 193nm, PDI; 0.249 and zeta potential; -13 mV were formed. The
nanosuspension formulation increased the solubility of acetaminophen to 353 mg/mL. Further, almost 99% of drug was dissolved within 60 min as compared to only 21% for raw acetaminophen. Finally the formulation was evaluated for stability studies at various temperatures and found to be stable at refrigerated and ambient temperature conditions.

Keywords: Acetaminophen, nanosuspension, solubility