Formulation Development of Aceclofenac Nanosuspension as an Alternative Approach for Improving Drug Delivery of Poorly Soluble Drugs

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ABSTRACT

Poor water solubility of active pharmaceutical ingredients is a major problem in drug discovery and formulation development. Nanoparticle engineering process has been seen as a promising approach for the enhancement of drug solubility. In this study, we present an approach of nanosizing a drug/polymeric complex to increase both solubility and dissolution rate of poorly-water soluble drug aceclofenac, which is widely used as anti-inflammatory drug. Polymeric nanosuspensions were prepared by nanoprecipitation method by using biodegradable polymer loaded with aceclofenac, with the aim to improve anti-inflammatory therapy. The prepared formulation was evaluated for drug excipients compatibility study, polydispersity index, particle size analysis, surface morphology, zeta potential, drug release features and stability. These study results indicate the suitability of formulation procedure for preparation of nanosized poorly-water soluble drug formulation with significantly improved in vitro dissolution rate and fast onset of therapeutic drug effect.

KEYWORDS: Aceclofenac; Nanosuspension; Tween 80; Dissolution; Anti-inflammatory drug.

Introduction

Poor water solubility of active pharmaceutical ingredients is an industry wide problem in drug discovery and development. It is well known that the majority of the new chemical entities coming directly from synthesis are poorly soluble (Fichera et al., 2004). It is estimated that 40% of all newly developed drug compounds are poorly soluble or “insoluble” in water, and upto 50% of orally administered drug compounds present formulation problems related to high lipophilicity (Andrej et al., 2009). Poorly water-soluble compounds are difficult to develop as drug products using conventional formulation techniques and are frequently abandoned early in discovery. Consequently, many of these substances have bioavailability problems after oral administration.

Delivering the drug precisely and safely to its target site at the right period of time to have a controlled release and achieve the maximum therapeutic effect remains a yardstick in the design and development of novel drug delivery systems. The concept of drug targeting stems from the very idea of minimizing the risk-to-benefit ratio.

Nanoscience and nanotechnology is an emerging and rapidly developing field that broadly encompasses the fundamental understanding and resulting technological advances arising from the exploitation of materials having at least one dimension at the nanometer length scale (Hao 2004; Rabinow, 2004; Patravale et al., 2004). Nanoengineering of particles surfaces has received considerable scientific and technological interest in recent years (Caruso, 2003). Nanosuspensions and other related colloidal systems have also received increased attention during the last few years. Nanosuspensions by the virtue of their large surface area to volume ratio provide an alternative method to formulate poorly soluble compounds. Nanosuspensions are sub-micron colloidal dispersions of discrete particles that have been stabilized using surfactants, polymers or a mixture of both. Polymer nanosuspensions (NS) of nonsteroidal anti-inflammatory agents (NSAIDs) have often been proposed as controlled drug delivery systems able to solve pharmacokinetic problems and/or the gastric damaging effects typical of most of these drugs (Bakan et al., 1991; Pignatello et al., 2002).

Aceclofenac is rapidly and completely absorbed after oral administration, peak plasma concentrations are reached in 1 to 3 hours after an oral dose. The drug is highly protein bound and highly metabolized in liver. The plasma elimination half-life of the drug is approximately 4 hours (Martindale, 2002). Hence it is an ideal drug candidate for the drug targeting as well as the sustained release formulation.

The aim of this investigation was to formulate aceclofenac nanosuspension by nanoprecipitation technique and to improve anti-inflammatory therapy.
The formulated nanosuspensions were investigated for its characteristics like drug excipients compatibility, particle size distribution, polydispersity index, zeta potential, transmission electron microscopic (TEM) analysis, drug content and in vitro release and kinetic studies.

**Materials and Methods**

**Drugs and Chemicals**

Aceclofenac was obtained as gift samples from Cipla Pharmaceuticals, Mumbai, India; ethanol, hydrochloric acid, Tween 80, benzalkonium chloride, polyvinyl pyrrolidone were obtained from Loba Chemie Pvt., Ltd., Mumbai, India; potassium dihydrogen phosphate, sodium hydroxide and sodium chloride were obtained from SD fine chemicals, India. Dialysis membrane was procured from Sigma Aldrich, USA.

**Methodology**

**Formulation of Nanosuspension**

Nanosuspension was prepared by precipitation technique. Aceclofenac and polyvinyl pyrrolidone were dissolved in ethanol. Drug solution was dispersed in the polymer solution at room temperature and sonicated for 5 minutes. The solution was slowly injected with a help of syringe into an aqueous phase (50 ml) containing various ratio of Tween 80 and 0.02 to 0.04% v/v of benzalkonium chloride which is kept at room temperature. During addition of the drug solution the mixture was mixed by using mechanical stirrer at 4000 rpm for one hour and then the dispersion was ultra sonicated for 10 minutes. Ethanol residues were left to evaporate off under slow mechanical stirring of the nanosuspension at room temperature for about 8 hours. Formulation composition is shown in Table 1.

**Drug-Excipients Compatibility Studies**

Excipients are integral components of almost all pharmaceutical dosage form. Compatibility studies are very important for the successful formulation of any dosage form. Commonly DSC, FT-IR, TLC and UV techniques are used for the determination of drug compatibility. Fourier Transform Infrared Spectroscopy (FTIR) and UV studies were used for the evaluation of physicochemical compatibility and interactions, which helps in the prediction of interaction of the drug with excipients used in the formulation. The earlier reports on drug-excipient interactions recommended that 1:1 ratio of drug and excipient maximizes the possibility of interaction and helps in easier detection of incompatibilities (Loganathan et al., 2003). Therefore, in the present study 1:1 ratio was used for the preparation of physical mixtures and analyzed for compatibility studies.

**Particle size distribution & Polydispersity Index**

Particle size was determined by Photon Correlation Spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, UK). This analysis yields the mean diameter (z-average, measuring range between 20 and 1000 nm) at 25º C, and at an angle of 90 degree (n=10). The PCS analysis yields a mean diameter (z-average) as a light intensity-weighted size of bulk population and the polydispersity index as a measurement for the width of a particle size distribution. Polydispersity index (dimensionless measure for the broadness of a particle size distribution) of the prepared formulation was determined by instrument software.

**Zeta Potential**

The electrophoretic mobility was obtained by a Laser Doppler Anemometer connected with the Malvern zetasizer instrument. A suitable amount of sample (50-100 μL) was diluted with 5 ml of water (0.45 μm) and injected in the electrophoretic cell of the instrument where a potential of ± 150 mV was set. The zeta potential values were calculated by the instrument software using Smoluchosky equation.

**Transmission Electron Microscopic (TEM) Analysis**

TEM helps to visualize the inherent matrix of individual particles and its shape. A drop of the suitably diluted sample was placed on to a holey carbon coated copper grid and left for 10 minutes. Then grid was kept inverted and a drop of phosphotungstic acid (PTA) was applied to the grid for 10 s. Excess of PTA was removed by absorbing on a filter paper and the grid was analyzed using the TECNAI-10 (PHILIPS) operated at 70-80kV at 17500 x magnification.

### Table 1

Composition of various Nanosuspension formulations.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (mg)</th>
<th>Polymer (PVP K-30) (mg)</th>
<th>Surfactant (Tween 80) (% w/w)</th>
<th>Preservative (Benzalkonium chloride) (% w/w)</th>
<th>Solvent (ml) Ethanol</th>
<th>Solvent (ml) Water</th>
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<tbody>
<tr>
<td>F1</td>
<td>200</td>
<td>200</td>
<td>2</td>
<td>0.04</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>F2</td>
<td>200</td>
<td>300</td>
<td>2</td>
<td>0.04</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
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<td>0.04</td>
<td>2</td>
<td>20</td>
</tr>
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<td>2</td>
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<tr>
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<td>1000</td>
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<td>0.04</td>
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</table>
Drug Content

The amount of drug present in the Aceclofenac nanosuspension was determined using phosphate buffer solution at pH 7.4. Measurements of the absorbance were carried at 273 nm in UV-Visible spectrophotometer (Shimadzu, Japan, Model: 1700), finally the percentage of drug content was calculated.

In-Vitro Release Studies

In-vitro release studies were carried out by using dialysis membrane bag method. The dialysis membrane was conditioned by soaking in phosphate buffer 7.4 for 24 hours. Aceclofenac nanosuspension of about 1 ml was taken in the dialysis membrane and immersed in 200 ml of phosphate buffer solution (pH 7.4). A sample of 5 ml was withdrawn from the dissolution setup at regular intervals for 24 hours and an equal volume of phosphate buffer (pH 7.4) was replaced to maintain a sink condition. Samples were analyzed by using UV spectrophotometer at 273 nm and the amount of drug release was calculated and compared with the marketed oral dosage form.

Drug Release Kinetics

The mathematical models are used to evaluate the kinetics and mechanism of drug release from the nanosuspension. The model that best fits the release data was selected based on the correlation coefficient ($r^2$) value in various models. The model that gives high R value was considered as best fit of release data.

Stability Study of ANS

The prepared nanosuspension was tightly sealed in amber colored bottles and kept in a place at room temperature. Then at regular intervals the stability samples was tested for its physicochemical parameters and drug content. The stability study was carried out for a period of 3 months (0, 1, 2 & 3 months).

Results and Discussion

Low oral bioavailability of poorly water-soluble drugs poses a major challenge during drug formulation development. Thus, nanoparticle technology could play a major role in the successful development and marketability of poorly-water soluble drug compounds. Nanosuspensions by the virtue of their large surface area to volume show improved pharmacokinetics and biodistribution of therapeutic agents and thus minimize toxicity by their preferential accumulation at the target site (Alexis et al., 2008).

FT-IR and UV studies were performed to investigate chemical interactions between drug and the excipients. Aceclofenac contains chemical functional groups like carbonyl, carboxyl group and hydroxyl group, the corresponding wave numbers are 1638.4, 1459.2 and 1088.04 cm$^{-1}$ respectively; these characteristic bands were present in the formulation composition. No new bands or shift in characteristic peaks were appeared. IR spectra are shown in Fig. 1a to 1d. In UV technique, the UV spectrum of drug is super impossible with the spectrum obtained with drug excipients mixtures and there is no change in the $\lambda_{max}$ 273 nm between the drug and drug excipients mixtures. FT-IR and UV results revealed that there is no interaction between the drug and the excipients used in the formulation.

Fig. 1a. FTIR spectra of aceclofenac.
Fig. 1b. FTIR spectra of PVP-K30.

Fig. 1c. FTIR Spectrum of Tween 80.
Eight different compositions of formulations were prepared with various ratios of drug and polymer. The best formulation was optimized based on the particle size. Since, reduced particle size helps in the improvement of solubility of poorly soluble drug thereby increase in the dissolution and bioavailability.

Table 2

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Particle size (nm)</th>
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<tr>
<td>F1</td>
<td>255</td>
</tr>
<tr>
<td>F2</td>
<td>263</td>
</tr>
<tr>
<td>F3</td>
<td>102.9</td>
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<td>F5</td>
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<td>F7</td>
<td>271</td>
</tr>
<tr>
<td>F8</td>
<td>346</td>
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</table>

The polydispersity (PDI) of optimized formulation F3 was measured and the value was found to be 0.175; which indicates the broad distribution of particles. An increase or decrease in the particle size of the drug in a formulation can affect its in vitro release and subsequently its bioavailability. For suspension based products, the particle size of droplets of the internal phase have an impact on the stability of suspension itself. The mean particle size was found to be 102.9 nm. The particle size distribution is shown in figure 2.

Table 3

Results of stability study.

<table>
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<th>Parameters</th>
<th>Formulation F3 stability</th>
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</thead>
<tbody>
<tr>
<td>Drug content</td>
<td>99.6 99.1 98.8 98.5</td>
</tr>
<tr>
<td>Sedimentation</td>
<td>No No No No</td>
</tr>
<tr>
<td>Microbial degradation</td>
<td>No No No No</td>
</tr>
<tr>
<td>Physical change</td>
<td>No No No No</td>
</tr>
</tbody>
</table>

TEM for the prepared formulations was examined using TECNAI-10 (PHILIPS) operated at 70-80 kV at 17500 x magnification. The TEM results showed that the particles were of nanometer in size range with uniform, spherical with smooth surface. The TEM image is shown in figure 3.

The zetapotential was determined by using Malvern zetasizer, it was found to be −7.99 mV; the zeta potential greater than +30 mV and smaller than −30 mV is normally considered stable. The formulation shows an accepted value for good stability, the zeta potential distribution is shown in figure 4.
Fig. 2. Polydispersity index and particle size distribution.

Fig. 3. TEM image of aceclofenac nanosuspension.

Fig. 4. Zeta potential distribution.
In vitro release studies are generally employed as a quality control tool for product release and in predicting toxicity of the formulations. In order to assess the ability of a formulation to deliver a drug, it is important to determine the drug’s release rate from its vehicle. In vitro drug release study of aceclofenac nanosuspension was performed in open tube method by using dialysis membrane. The cumulative percent release was found to be 99.56 % for 24 hrs. A comparative study was performed with the marketed tablet to know the pattern of drug release.

Comparative studies done by drug release profile the Nano suspension formulation were shown 99.56% at 24 hours and the marketed tablet were shown 99.45 % release at 5 hours. The release results revealed that Aceclofenac nanosuspension showed faster onset of action and sustained drug release pattern up to 24 hrs. This sustained release helps in the reduction of aceclofenac daily dose. This also proves the advantage of a polymeric nanosuspension in delivering Aceclofenac for better anti-inflammatory therapy. The comparative in vitro release results are shown in figure 5.

![Graph showing in vitro release of aceclofenac nanosuspension and market product.](image)

![Graph showing zero order kinetics plot for aceclofenac.](image)
Fig. 6b. First order kinetics plot for aceclofenac.

Fig. 6c. Higuchi kinetics plot for aceclofenac.

Fig. 6d. Korsmeyer kinetics plot for aceclofenac.
The regression coefficient ($R^2$) for the drug release kinetic studies were found to be 0.8896, 0.2796, 0.7474, 0.5630 and 0.8477 for Hixon crowell model, Korsmeyer peppas, Higuchi plot, Zero order and first order model respectively. The study shows that the Aceclofenac nanosuspension follows Hixon crowell model, Higuchi model and first order model. The Korsmeyer peppas model shows non-fickian release. Hence it was concluded that ANS exhibits first order drug release by dissolution mechanism in a sustained manner.

Stability of the suspensions is dependent on the particle size. As the particle size reduces to the Nano size, the surface energy of the particles will be increased and they tend to agglomerate. So stabilizers are used which will decrease the chances of Ostwald ripening and improving the stability of the suspension by providing a steric or ionic barrier. The physicochemical parameters like appearance, particle size and in vitro release were found to be satisfactory, and there is no change with respected to the initial analysis results.

Conclusions

Dissolution rate and solubility are two of several factors that affect oral bioavailability of poorly water-soluble compounds. Nanosizing techniques have been used to increase dissolution rate, which improves the low oral bioavailability of these compounds. In this study we demonstrated an approach to prepare Aceclofenac sodium nanosuspension with PVP K30 by nano-precipitation technique to increase the dissolution rate and bioavailability of a poorly water-soluble compound. These nanosized particles with a very large interfacial area can influence the transport and delivery properties of the incorporated drugs and provide for site-specific targeting. The results clearly indicated the suitability of formulation procedure for preparation of nano-sized poorly water soluble drug with significant improvement of the in vitro dissolution rate, and thus possibly improve their oral bioavailability.

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References


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