Formulation Development and Optimization of Nitrendipine Nanosuspension with Improved Pharmacokinetic Characteristics

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ABSTRACT

Poorly water-soluble drugs such as nitrendipine (~2.0 µg/ml at 37°C in water) offer challenging problems in drug formulation as poor solubility is generally associated to poor dissolution characteristics and thus to poor oral bioavailability. To enhance these characteristics, we prepared and evaluated nitrendipine nanosuspension using the nanoprecipitation technique. The important process parameters, the concentration of nitrendipine in the organic phase, concentration of surfactant in the anti-solvent, and starting speed were evaluated using 33 full factorial designs. It was observed that the concentration of nitrendipine in the organic phase, concentration of surfactant in the anti-solvent, and starting speed significantly affect the particle size as well as dissolution velocity. Differential scanning calorimetry studies confirmed that the crystallinity of the drug was maintained after the nanoprecipitation suggesting that improved dissolution of nitrendipine nanosuspensions could be attributed to reduction in particle size. This formulation may offer a superior pharmacokinetic profile due to nanotechnological design.

KEYWORDS: Nanosuspension; Dissolution Enhancement; Nanoprecipitation; Mean Particle Size.

Introduction

Nitrendipine is a dihydropyridine calcium channel antagonist with a very low solubility for the treatment of hypertension (Santiago et al., 1990). Nitrendipine is classified as a Class II API (poorly soluble and highly permeable) by the Biopharmaceutics Classification System (BCS) (Fude et al. 2010). The absolute oral bioavailability of this drug is reported to range from about 10% to 20%, depending in part on the dosage form (Soons et al., 1991; Sweetmans et al., 2009). The basic challenge faced by the researcher for the formulation of such poorly soluble drugs is the low oral bioavailability and erratic absorption of the drugs from the gastrointestinal tract due to their low saturation solubility and dissolution velocity. The low saturation solubility results in a low concentration gradient between the gut and blood vessel and leads to a limited transport of drug (Merisko-Liversidge et al., 2003). For poorly soluble drugs as seen in BCS Class II, the dissolution of the drugs in the gastrointestinal fluid media is the rate limiting step for the absorption of the drugs (Aguilar-Bryan et al., 1995). Hence, for efficient absorption of drugs from the gastrointestinal tract to improve their therapeutic efficacy, there is an imminent need for studies in designing novel strategies for their dissolution enhancement.

There are a number of formulation approaches such as salt formation, pH adjustment, cosolvency, and complexation used for enhancement of dissolution but none of the approaches has achieved the merits of being universal. Micronization of poorly soluble drugs has been applied for many years to improve dissolution velocity of poorly soluble drugs but reducing the drug to micron size does not increase the saturation solubility of the drug, and at such a low saturation solubility as generally observed in BCS Class II drug, the increment in the dissolution characteristics does not help to a great extent (Muller et al., 1999; Rasenack et al., 2003). Consequently, offlate nanonisation has been employed for treating the BCS Class II drugs. When the drug is being reduced to nanosized level there is an obvious increase in its saturation solubility assisted by improvement in the dissolution characteristics which could be attributed to the effective increase in particle surface area according to the Ostwald Freundlich equation (Patravale et al., 2004). The drug nanoparticles are generally suspended in an aqueous media and are termed as nanosuspensions.

Nanosuspensions can be prepared using various techniques, namely nanoprecipitation, sonication, high speed homogenization, milling, and high pressure homogenization (Gary et al., 1995; Mullar et al., 1998; Masaaki et al., 1995; Hecq et al., 2005; Mittapalli et al.,
In the precipitation technique, the poorly water-soluble drug is dissolved in a suitable solvent and the solution is added to a miscible anti-solvent with stirring and agitation (Xing et al., 2004; Jai et al., 2005; Ji Yao et al., 2006; Sigfridsson et al., 2007). In supercritical crystalization the supercritical fluid expands into a liquid solvent and the dissolved drug precipitates due to decompression of supercritical fluid. The particles’ growth is controlled by cosolvents and polymers (Pathak et al., 2004). Nanosuspension can be prepared by using emulsions as templates and is applicable for those drugs that are soluble in either volatile organic solvent or partially water-miscible solvent (Aguilar-Bryan et al., 1995). In the media milling technique, the drug is milled with milling media in simple glass vials to specific milling chambers for certain hours to some days and nanosuspensions are produced on a principle of high energy and shear forces generated as a result of the impaction of the milling media with the drug (Timothy et al., 1997; Liversidge et al., 1992).

Nanosuspensions can also be prepared by dry-grinding poorly soluble drugs with soluble polymers and copolymers (Wongmekiat et al., 2002; Mura et al., 2002; Pengpeerapat et al., 2003) In high pressure homogenization, the drug powder is first dispersed into an aqueous surfactant solution and passed through a homogenizer to obtain desired size range (Muller et al., 2000; Grau et al., 2000 and Mochwitzer et al., 2004). The dissolution is the rate-limiting factor for absorption of nitrendipine. Hence nitrendipine nanosuspension has been achieved using nanoprecipitation technique to enhance the dissolution velocity.

In this study, we prepared and evaluated nitrendipine nanosuspension using the nanoprecipitation technique. This formulation appears to offer a superior pharmacokinetic profile due to nanotechnological design.

**Material and Methods**

**Chemicals and Drugs**

NT was obtained as a gift sample from Spansules Formulations, India. Hydroxy propyl methyl cellulose (HPMC 6cps) was obtained from Ruitai Pharmaceutical Co, China. Polyvinylpyrrolidone (PVPK-30), Polyvinyl Alcohol (PVA) polysorbate 20 and sodium lauryl sulphate were supplied by Loba Chemie. Pvt. Ltd., Mumbai. All the reagents used were of AR grade and double distilled water was used throughout the study.

**Preparation of Nanosuspensions**

Nanosuspensions were prepared by the solvent evaporation technique. NT was dissolved in a 5ml mixed solvent of PEG 200 and acetone (ratio of 1:1, v/v) at room temperature. This was poured by means of a syringe positioned with the needle directly into 20 ml water containing a different amount of surfactant maintained at a temperature below 5°C and subsequently stirred at ranging agitation speed for 2 hr to allow the volatile solvent to evaporate (Remi, High speed stirrer, India.). Organic solvents were left to evaporate off under a slow magnetic stirring of the nanosuspensions at room temperature for 2 hours.

**Particle Size and Size Distribution**

The mean particle diameter and size distribution of the prepared nanosuspension were measured using a Mastersizer 2000 (Malvern Instruments, UK). The particle size detection range for Malvern SM 2000 is 0.02 to 2000 μm. The average particle size was measured after performing the experiment in triplicate.

**Differential Scanning Calorimetry (DSC)**

The phase transition of NT nanosuspension and NT pure drug was analyzed by differential scanning calorimetry (DSC- Shimadzu 60, Shimadzu Co., Kyoto, Japan). In DSC analysis, the samples were weighed (5 mg), hermetically sealed in flat bottom aluminum pans, and heated over a temperature range of 50 to 300°C at a constant increasing rate of 10°C/min in an atmosphere of nitrogen (50 mL/min).

**In Vitro Dissolution Profile**

**In vitro** dissolution studies were performed using USP dissolution test apparatus-II (paddle assembly). Dissolution was carried out on an equivalent of 10 mg of nitrendipine. Water containing 0.1M hydrochloric acid and 0.1% SDS was selected as dissolution medium. The volume and temperature of the dissolution medium were 900 ml and 37.0±0.2°C, respectively. Samples (5 ml) were withdrawn at regular intervals of 5 min for 60 min and replaced with fresh dissolution medium. Samples were filtered through 0.2μm whatman filter paper and assayed spectrophotometrically on SHIMADZU UV-VISIBLE spectrophotometer at 236 nm wavelength.

**Factorial Design**

A statistical model incorporating interactive and polynomial terms was used to evaluate the responses: 

\[ Y=b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_12X_1X_2 + b_13X_1X_3 + b_23X_2X_3 + b_123X_1X_2X_3 + b_11X_1^2 + b_22X_2^2 + b_33X_3^2 \]

Where, Y is the dependent variable, bo is the arithmetic mean response of the 27 runs, and bi is the estimated coefficient for the factor Xi. The main effects (X1, X2 and X3) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X1X2), (X1X3), (X2X3) and (X1X2X3) show how the response changes when two or more factors are simultaneously changed. The polynomial terms (X^2), (X^2) and (X^3) are included to investigate nonlinearity. On the basis of the preliminary trials a 3^1 full factorial design was employed to study the effect of independent variables Concentration of drug (X1), Concentration of Stabilizer (X2), and Stirring speed (X3) on dependent variables Mean Particle Size and Drug release in 5 min.
Result and Discussion

The influence of different stabilizers was investigated in nanoprecipitation technique with a fixed concentration of the drug. The type of compound and their amount employed for stabilization has a prominent effect on particle size. Small particles, which spontaneously aggregate to decrease the surface energy, were stabilized by a layer of surfactant or/and protective polymer.

Four stabilizers (SLS, HPMC 6cps, PVA and PVPK-30) were tested for their stabilization potential. An important function of the stabilizers is that they can form a substantial mechanical and thermodynamic barrier at the interface that retards the approach and coalescence of individual nanoparticles. As the data shows in Table 1, it may be concluded that mean particle size varies with stabilizer and with HPMC 6cps it shows lowest size.

<p>| Table 1 |
| Effect of various stabilizers particle size and size distribution. |</p>
<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Stabilizer</th>
<th>Concentration of Drug (mg/ml)</th>
<th>Drug to Stabilizer ratio</th>
<th>Mean Particle Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT 1</td>
<td>SLS</td>
<td>20</td>
<td>1:0.2</td>
<td>328</td>
</tr>
<tr>
<td>NT 2</td>
<td>HPMC 6cps</td>
<td>20</td>
<td>1:0.2</td>
<td>300</td>
</tr>
<tr>
<td>NT 3</td>
<td>PVA</td>
<td>20</td>
<td>1:0.2</td>
<td>337</td>
</tr>
<tr>
<td>NT 4</td>
<td>PVPK-30</td>
<td>20</td>
<td>1:0.2</td>
<td>324</td>
</tr>
</tbody>
</table>

As shown in Table 2, an appropriate amount of stabilizer is required to achieve smaller particle size. The crystal growth was protected by the adsorbed stabilizers, and the quantity of stabilizer should be enough to cover the crystal surface to provide enough steric repulsion between the crystals. Inadequate surface coverage of stabilizer could result in rapid crystal growth and agglomeration, while high concentration of stabilizer could result in enhanced viscosity of the solution which would obstruct the diffusion between the solvent and anti-solvent during precipitation (Singh et al., 2011). At low drug concentration, the particle size was smaller with a narrow size distribution. However, at higher drug concentration, due to greater supersaturation, a higher diffusion controlled growth and agglomeration rate were achieved, resulting in larger crystals. Stirring speed is obviously affecting the particle size, as increasing the stirring speed, decreases the mean particle size because of high shear force (Fude, et al. 2010).

<p>| Table 2 |
| The 3^3 full factorial design lay out. |</p>
<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Variable level in coded form</th>
<th>Mean Particle Size</th>
<th>Drug release in 5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT 5</td>
<td>-1 -1 -1</td>
<td>455</td>
<td>82.06</td>
</tr>
<tr>
<td>NT 6</td>
<td>-1 -1 0</td>
<td>363</td>
<td>88.25</td>
</tr>
<tr>
<td>NT 7</td>
<td>-1 -1 -1</td>
<td>257</td>
<td>93.02</td>
</tr>
<tr>
<td>NT 8</td>
<td>-1 0 -1</td>
<td>468</td>
<td>80.87</td>
</tr>
<tr>
<td>NT 9</td>
<td>-1 0 0</td>
<td>361</td>
<td>88.14</td>
</tr>
<tr>
<td>NT 10</td>
<td>-1 0 1</td>
<td>364</td>
<td>88.02</td>
</tr>
<tr>
<td>NT 11</td>
<td>-1 1 -1</td>
<td>471</td>
<td>80.12</td>
</tr>
</tbody>
</table>

Factorial Equation for Mean article Size: The mean particle size varied from 254 nm to 676 nm and showed good a correlation coefficient (0.8873). The particle size of different formulations is shown in Table 2, which clearly indicates the batch NT 19 had less particle size as compare to other formulation.

The batch NT 19 had a Z-average particle size of 254 nm. The particle size distribution pattern of the NT 19 is given in figure 1. Results of the equation indicate that all three independent variables greatly affect the mean particle size. However, drug concentration more significantly affects the mean particle size because at higher drug concentration, due to greater super saturation, higher diffusion controlled growth and agglomeration rate were achieved, resulting in larger crystals. An increase in the stirring speed results in a decrease in particle size because of higher shear force. Mean article size = 385+ 103.9444X1- 42X2 -54.1111X3 -44.4167X1X2-62X3 + 24X1X3 -16.625 X1X2X3+ 75.8333X21+ 26.3333X22+18.3333X23

Factorial Equation for Drug Release in 5 minute: The drug release in 5 min varies 69.14 to 93.21 % with a good correlation coefficient of 0.8757. Results of the equation indicate that all three independent variables greatly affect the drug release. As the size decreases, the effective increase in particle surface area results in an increase in dissolution velocity according to the Nernst Brunner-Noyes Whitney equation:

Drug release in 5 minute = 85.8044 -5.5033X1 - 75.02X2-54.1111X3 - 44.4167X1X2 -62X3 + 24X1X3 -16.625 X1X2X3+ 75.8333X21+ 26.3333X22+18.3333X23
TABLE 3
Summary of results of regression analysis.

<table>
<thead>
<tr>
<th>Coefficient</th>
<th>b0</th>
<th>b1</th>
<th>b2</th>
<th>b3</th>
<th>b12</th>
<th>b23</th>
<th>b13</th>
<th>b123</th>
<th>b11</th>
<th>b22</th>
<th>b33</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPS</td>
<td>385</td>
<td>103.9444</td>
<td>-42</td>
<td>-54.1111</td>
<td>-44.4167</td>
<td>-6</td>
<td>24</td>
<td>-16.625</td>
<td>75.8333</td>
<td>26.3333</td>
<td>18.3333</td>
<td>0.8873</td>
</tr>
<tr>
<td>5 min</td>
<td>85.8044</td>
<td>-5.5033</td>
<td>3.0061</td>
<td>3.75</td>
<td>3.4016</td>
<td>1.1833</td>
<td>-0.735</td>
<td>1.97375</td>
<td>-3.69</td>
<td>-1.175</td>
<td>-1.15</td>
<td>0.8757</td>
</tr>
</tbody>
</table>

Dissolution studies were compared for pure drug and the optimized nanosuspension formulation. The amount of drug released from the optimized nanosuspension formulation was 93.21% within 5 min compared to amount of 18.69% of pure drug after 1 hour in water containing 0.1M hydrochloric acid and 0.1% SDS. The increase in accessible surface area to the dissolution medium and hydrophilic surfactant coating on the particle surfaces may be the reason for increase in dissolution rate. This enhanced dissolution rate can be attributed to the higher surface area of nanocrystals available for dissolution and the decreased diffusion layer thickness.(Hintz et al., 1989)

Conclusions

The nanoprecipitation technique has been successfully utilized as a simple method for drug nanosizing at laboratory scale for preparation of nitrendipine formulation. Particle size is significantly influenced by concentration of drug, concentration of stabilizer, and starring speed. Nanosized nitrendipine dissolved significantly faster than raw drug powder, indicating superior pharmacokinetic profile that was mainly due to nanotechnological design.

References


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