Formulation and Characterization of Nateglinide Nanosuspension by Precipitation Method

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ABSTRACT

Poor water solubility and slow dissolution rate are major issues for the majority of upcoming and existing biologically active pharmaceutical compounds. Nateglinide is Biopharmaceutical Classification System Class-II drug that has low solubility and high permeability. The purpose of the present study was to improve the solubility and dissolution rate of Nateglinide by the preparation of nanosuspension by the nanoprecipitation technique. Nateglinide nanosuspension was evaluated for its particle size, in vitro dissolution study, and characterized by differential scanning calorimetry and scanning electron microscopy. The optimized formulation showed an average particle size of 207 nm and zeta potential of -25.8 mV. The rate of dissolution of the optimized nanosuspension was enhanced by 83% in 50 min relative to micronized suspension of nateglinide (37% in 50 min). This improvement was mainly due to the formulation of nanosized particles of Nateglinide. Stability study revealed that nanosuspension was more stable at room temperature and refrigerator condition with no significant change in particle size distribution. These results indicate that the nateglinide loaded nanosuspension may significantly improve in vitro dissolution rate and thereby possibly enhance the onset of therapeutic effect.

KEYWORDS: Nateglinide; Nanosuspension; Nanoprecipitation; Solubility; Dissolution rate.

Introduction

Amongst the various routes of administration the oral route is the one commonly used and most convenient for the drug delivery. Oral drug delivery system has received more attention in the pharmaceutical field, because of its more flexibility in designing the dosage form than other drug delivery system (Fasinu et al., 2011). More than 40% of the new chemical entities being generated through drug discovery programs are faced the problem for aqueous solubility and become a hurdle for the formulation (Merisko-Liversidge et al., 2003). Nanotechnology can be used to solve the problems associated with these conventional approaches for solubility and bioavailability enhancement (Rajalakshmi et al., 2012). Nanosuspensions are basically suspension where the particle size of the suspended material is within the range of 10-1000 nm (Manju et al., 2006; Krishna et al., 2006). Drugs belong to the Biopharmaceutical Classification System (BCS) Class II and IV is eligible for this approach for increase their solubility and hence partition into gastrointestinal barrier (Dubey, 2006). Nanosuspension platform is an efficient and intelligent drug delivery system for water insoluble drugs as the saturation solubility and the surface area available for dissolution increased (Zheng et al., 2011; Lou et al., 2011). Generally, the biopharmaceutical advantages of water insoluble drugs formulated as nanosuspension including improvement in formulation performance, such as high drug loading, reproducibility of oral absorption, improved dose bioavailability, proportionality, reduced toxicity and side effects and increased patient compliance via reduction of number of oral units to be taken (Shegokar et al., 2010; Das et al., 2011).

Nateglinide is one of the most effective drugs for its treatment in diabetic. It is BCS Class II drug low solubility and high permeability. Nateglinide is non-sulfonylurea drug which blocks K_ATP potassium channel to perform overall glycemic control in type-2 diabetes. It is the selective blocker of pancreatic beta cell (Norman et al., 2011).

Most commonly used stabilizers to stabilize nanosuspension are either polymer like (e.g., polyvinyl pyrrolidone (PVP), crystalline cellulose (Mahmoud et al., 2010), amphiphilic amino acid (Lee et al., 2005), hydroxyl-propyl cellulose (HPC) (Singh et al., 2011), hydroxypropyl methyl cellulose (HPMC) (Van Eerdenbrugh B et al., 2008), and d-α-tocopherol polyethylene glycol 1000 succinate (TPGS 1000) (Ghosh I et al., 2011, Van Eerdenbrugh et al., 2008) whereas surfactant such as ionic are (e.g., sodium dodecyl sulphate (SDS), sodium lauryl sulphate (SLS), poly(ethyleneimine) (PEI) (Dhapte et al., 2011), chitosan (Quan et al., 2012) and non-ionic surfactant [e.g., polysorbate (tween 80), block co-polymer like pluronic] and some food protein are also used as
stabilizers such as soya bean protein isolate, whey protein isolate and β-lactoglobulin.

Nanosuspensions are prepared by two methods first is bottom-up and second is top-down method. In the present work nanosuspension is prepared by bottom up method in which drug is dissolved in a solvent, which is then added to non-solvent that cause precipitation of the fine drug particle and the system is stabilize by polymer and or surfactant to prevent them from aggregation or agglomeration (Mittal et al., 2013). The objective of this work is to formulate Nateglinide nanosuspension by nanoprecipitation method. The response such as particle size, polydispersibility index, zeta potential, drug content and dissolution rate were evaluated in this study.

Materials and Methods

**Materials**

Nateglinide was obtained as a gift sample from Glanmark Ltd., Nashik. Lutrol F68 (Poloxamer 188) obtained gift sample from BASF (Germany). Hydroxypropyl methyl cellulose 3cps (HPMC) was purchased from Samsung fine chemical Co., LTD., Korea.

**Methods**

Nanosuspensions were prepared according to nanoprecipitation method. Pure drug nateglinide and HPMC (3cps) was dissolved in (1 ml) acetone at 40 °C to form uniform organic solution. The prepared organic solution was then injected slowly drop wise with the help of a syringe into an aqueous phase (20 ml) containing stabilizers (pluronic F68) under high speed mechanical agitation of 6000 rpm to get desired nanodispersion. Prepared nanosuspensions was then stirred magnetically at 500 rpm at room temperature for 12 hr to evaporate organic solvent. Complete evaporation of acetone was determined by spectrophotometric method. The volume was then adjusted with the addition of triple distilled water to recover loss in keeping other parameters constant (Fessi et al., 1989). The batches were prepared according to the formulation design in Table 1.

**TABLE 1**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug (mg/ml)</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Pluronic F-68 (mg/ml)</td>
<td>3</td>
<td>6</td>
<td>30</td>
<td>3</td>
<td>6</td>
<td>30</td>
<td>3</td>
<td>6</td>
<td>30</td>
</tr>
<tr>
<td>HPMC (3cps) (mg/ml)</td>
<td>6</td>
<td>6</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Acetone (ml)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

**Lyophilisation and redispersibility of nanosuspensions**

Nateglinide nanosuspension were frozen and lyophilized using lyophilizer (Decibel digital, India) for 24 hr (-40 °C). The freeze-dried samples were diluted to original volume with triple distilled water and redispersibility was observed. Freeze-dried samples were further used for solid state characterization.

**Characterization of nanosuspensions**

**(a) Particle size analysis**

Average particle size and polydispersibility index of formulations were determined by Malvern Zetasizer ZS (Nano series ZS 90 UK) using water as dispersion medium. The sample was scanned 100 times for determination of particle size.

**(b) Zeta potential**

Zeta potential of formulation was measured using Malvern Zetasizer ZS (Nano series ZS 90 UK). The samples were diluted 10 times with solvent before analysis. Physically stable nanosuspensions solely stabilized by electrostatic repulsion, a zeta potential of ± 30 mV is required as a minimum. Combined with the steric stabilization, the absolute value of zeta potential about ± 20 mV is sufficient to fully stabilize the nanosuspension system (Wang YC et al., 2010).

**(c) Total drug content**

An aliquot (0.5 ml) was evaporated to dryness. The residue was then dissolved in acetone and filtered with 0.45 µm filter paper. The samples were analyzed using UV spectrophotometer at λmax of 210 nm. Total drug content (TDC) and % TDC were calculated Eq. 1 and 2.

\[
TDC = \frac{\text{Vol. total}}{\text{Vol. Aliquot}} \times \text{Drug amount in aliquot} \times 100
\]

\[
% \text{TDC} = \frac{\text{TDC}}{\text{TAD}} \times 100
\]

Where, Vol. Total/vol. Aliquot are the ratio of total nanosuspension volume to the volume of aliquot taken and the total amount of drug (TAD) taken for the formulation of nanosuspension (Chorny M et al., 2002).

**(d) Scanning electron microscopy**

The morphological features of Nateglinide nanosuspension are observed by scanning electron microscope at different magnifications.

**(e) Fourier transform infrared spectroscopy**

The FT-IR analysis was conducted to check any interaction of chemical bonds between drug and excipients. FT-IR spectrum was performed by using a Shimadzu 8400 spectrophotometer. The samples were scanned in the region between 4000 and 400 cm⁻¹. Solid powder sample were oven dried at around 300 °C, finely crushed, mixed with potassium bromide (1:10 ratio by weight) and pressed at 15000 psig (using a Carver Laboratory Press, Model C, Fred S. Carver Inc., WIS 53051) to make disc and then scanned it (Pavia DL et al., 2001).

**(f) Differential scanning calorimetry**

The thermal properties of pure Nateglinide, physical mixture were characterized by
differential scanning calorimeter (DSC-60, Shimadzu). The samples of about 5 mg were placed in standard aluminum pans, and dry nitrogen was used as effluent gas. All samples were scanned at a temperature ramp speed of 5 min, and the heat flow was set from 0 to 300 °C. Before the experiment, the DSC was calibrated using pure Indium and heat of fusion (H fusion).

(g) X-ray powder diffraction (XRPD)
XRD measurements were carried out with an X'Pert PRO diffractometer. The diffractograms of Nateglinide and lyophilized product were obtained for analysis.

(h) Saturated solubility
Saturation solubility of pure drug Nateglinide and nanosuspensions formulation was measured in buffer solution having different pH (1.2 to 10) buffers. The solution containing flasks were kept on a rotary shaker (Orbital shaking incubator Remi Lab. India) for 24 hr. After 24 hr, solutions were analyzed using UV spectrophotometer at 210 nm, which was the absorption maxima determined earlier and drug concentrations were calculated (Agrawal et al., 2004; Maheshwari et al., 2012; Soni et al., 2014).

(i) In-vitro drug release
In-vitro dissolution test were performed in USP apparatus Type II (Electro lab Dissolution Tester USP TDT-08L) using paddle method at rotation speed of 100 rpm. Dissolution was carried out in 900 ml phosphate buffer of pH 6.8 as a dissolution medium and maintained temperature 37 ± 0.5 °C. Accurately weighed bulk drug and nanosuspension (all equivalent to 60 mg of Nateglinide) were dispersed in dissolution medium. 5 ml aliquots were removed at predetermined time intervals 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min from dissolution medium and replace with same buffer solution for maintain sink condition and the sample were analyzed for the drug release using UV Spectrophotometer at the wavelength of 210 nm.

(j) Stability study
Stability study of optimized Nateglinide nanosuspension was carried out by placing formulation in glass vials at different temperature condition for 3 months at room temperature (25 °C) and refrigerator (4 °C). After 3 months samples were visually observed for any sedimentation and changes in particle size using zeta sizer and drug content.

Results and Discussion
Nanoprecipitation method has been employed to produce nanosuspension of Nateglinide. Nateglinide is a BCS Class-II drug having low solubility and high permeability. Drug-to-Stabilizer ratio was contributed much towards the change in particle size in nanosuspension formulation. Dissolution rate and saturation solubility of poorly soluble drug of Nateglinide has been enhanced due to reduction of particle size diameter down to the submicron range. Nanosuspension of Nateglinide was prepared as per shown in Table 1. The ratio of solvent/antisolvent was kept constant that is 1:20 and stirring speed 6000 rpm for 8 hr for all batches. The confirmation of formation of colloidal nanodispersion can be visualized by the bluish white transparent in appearance.

**Particle size and Polydispersibility Index (PDI)**
The particle size distribution has most important characteristics affecting the in vivo fate of nanosuspension. The average particle size of F1-F9 batches was observed from the ranges of 207 nm to 556 nm as shown in Table 2. The largest size 556 nm in F1 batch which could be due to the lower concentration of stabilizer, because too little concentration of stabilizer induces agglomeration or aggregation and too much concentration promotes Ostwald ripening. The optimized formulation (F6) showed an average particle size 207 nm as shown in Fig.1.

![Fig. 1. Particle size graph of optimized nateglinide nanosuspension (F6).](image-url)
Polydispersibility index gives degree of particle size distribution. It ranges from 0.257 to 0.567 depending on formulation variables (Table 1). The formulation F1 showed lowest PDI (0.257) that indicates good uniformity in particle size distribution.

### TABLE 2

<table>
<thead>
<tr>
<th>Batch Code</th>
<th>Particle size (nm)</th>
<th>Polydispersity index</th>
<th>Zeta potential (mV)</th>
<th>% Total Drug Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>556</td>
<td>0.373</td>
<td>-8.3</td>
<td>82</td>
</tr>
<tr>
<td>F2</td>
<td>363</td>
<td>0.602</td>
<td>-13.5</td>
<td>87</td>
</tr>
<tr>
<td>F3</td>
<td>237</td>
<td>0.414</td>
<td>-18.2</td>
<td>89</td>
</tr>
<tr>
<td>F4</td>
<td>330</td>
<td>0.549</td>
<td>-16.5</td>
<td>85</td>
</tr>
<tr>
<td>F5</td>
<td>258</td>
<td>0.452</td>
<td>-11.5</td>
<td>88</td>
</tr>
<tr>
<td>F6</td>
<td>207</td>
<td>0.257</td>
<td>-25.8</td>
<td>93</td>
</tr>
<tr>
<td>F7</td>
<td>309</td>
<td>0.566</td>
<td>-12.4</td>
<td>90</td>
</tr>
<tr>
<td>F8</td>
<td>252</td>
<td>0.498</td>
<td>-18.5</td>
<td>75</td>
</tr>
<tr>
<td>F9</td>
<td>230</td>
<td>0.567</td>
<td>-21.5</td>
<td>80</td>
</tr>
</tbody>
</table>

**Zeta potential**

Zeta potential of batches F1 to F9 is shown in Table 2. Zeta potential governs the degree of repulsion between adjacent or similarly charge of dispersed particles. Zeta potential is an important parameter for prediction of stability of nanosuspension. Zeta potential of formulation of F1 to F9 batches was observed between –8 mV to –25.8 mV. Zeta potential of optimized formulation (F6) was found to be –25.8 mV as shown in Fig. 2. Thus, it was concluded that the system had sufficient stability.

**Total Drug Content (TDC)**

Table 2 shows TDC for the prepared batches. TDC for all batches was satisfactory and was more than 75%, which indicates that loss of drug was lower during preparation process.

**Scanning Electron Microscopy (SEM)**

The morphological features of Nateglinide nanosuspensions observed by scanning electron microscope at different magnifications as shown in Fig. 3.

**Fourier Transform Infrared Spectroscopy (FT-IR)**

IR spectroscopic studies were conducted to determine possible interaction between drug and excipients. IR spectra of pure drug Nateglinide with HPMC and pluronic F68 were obtained. This shows no chemical interaction between Nateglinide and excipients. The results of IR study are shown in Fig. 4.

**Differential Scanning Calorimetry (DSC)**

The DSC thermograms of pure drug Nateglinide and optimized nanosuspension formulation were taken between 20-300 °C at a heating rate of 20 °C/min. The DSC thermogram of pure Nateglinide powder showed a sharp endothermic peak at 110 °C which is due to melting point of the drug. After being precipitated as nanoparticles, 3 peaks are identified. Formulation (F6) was shown endothermic peaks at 52 °C, 109 °C and 220 °C due to melting of pluronic F68, Nateglinide and HPMC respectively. From, thermograms it was concluded that the drug and excipients do not interact with each other. The data was represented in Fig. 5.

**Saturated solubility**

Solubility enhancement ratio of optimized batch (F6) of nanosuspension is 350 in phosphate buffer of pH 6.8. This great increase in saturation solubility of Nateglinide was due to particle size reduction and subsequent increase in surface area. This great increase in saturation solubility of Nateglinide due to particle size reduction can be attributed to enhanced dissolution and justifying the objective of research work. The graph of saturation solubility was shown in Fig. 6.
Fig. 4. FT-IR of (A) HPMC (3cps), (B) Pluronic F68, (C) Nateglinide, and (D) Formulation (F6)

Fig. 5. DSC thermogram of A) Nateglinide and B) Lyophilized nano-suspension of optimized formulation without cryoprotectant.

Fig. 6. Solubility graph of pure drug nateglinide and F6 formulation in different pH buffer.
**In vitro drug release**

The most important feature of nanoparticles is the increase in the dissolution velocity, not only because of increase in surface area but also because of increase in saturation solubility. *In-vitro* drug release data from the nanosuspension were carried out for 60 min and graphically represented as % drug release v/s time profile (Fig. 7). The percentage drug release curve of formulation F6, coarse suspension of Nateglinide and pure drug showed the desired rate in phosphate buffer of pH 6.8 up to 60 min. From that study it was found that formulation of F6 batch gave faster release behavior compared to coarse suspension and pure drug. The drug release of optimized batch (F6) was found to be 83% within 50 min. Thus, from the above results it was found that as the particle size is decrease drug release is increased. So, nanosuspension enhanced rate of dissolution of Nateglinide to a great extent.

![Graph showing drug release](image)

**Stability study**

Physical appearance of the batch of F6 nanosuspension does not change when samples were stored at different temperature condition for 3 months. A loose, thin layer of sediment was observed when nanosuspension was stored at room temperature for 3 months. However, the sediment disappeared with slight hand shaking. Table 3 shows the average particle diameter and drug content of F6 batch after 3 months storage at room temperature (25 °C) and refrigerator (4 °C) respectively. The particle size for the F6 was 207 nm before performing stability study. It can be inferred from the observed data that the prepared nanosuspension F6 was stable after 3 month of storage at different temperature condition.

| TABLE 3 |
|------------------|------------------|------------------|
| **Stability study of optimized formulation (F6) after 3 months.** |
| **Batch F6** | **At 4 °C** | **At 25 °C** |
| **Drug contents** | 93% | 92% |
| **Average particle size** | 207 nm | 209 nm |

**Conclusions**

Nanoprecipitation method was successfully employed to produce stable Nateglinide nanosuspension which can enhance the solubility and dissolution rate. In this process, the particle size of Nateglinide can be obtained in the nano-size ranges, by adjusting the operation parameters, such as surfactant concentration and polymer concentration and agitation speed (6000 rpm) and time (6 hr) was constant. From the above investigation, it is concluded that the drug to stabilizer ratio (1:0.5) and drug to polymer ratio (1:0.3) showed a pronounced effect on particle size reduction. According to optimized batch (F6) the mean particle size and zeta potential was found to be 207 nm and ~25.8 mV respectively and stable at various conditions. The rate of dissolution of the optimized nanosuspension was enhanced by 85% in 50 min, relatively to coarse suspension (37% in 50 min). This improvement of dissolution rate was mainly due to formation of nanosized particles. Thus, Nateglinide nanosuspension formulation prepared by nanoprecipitation method may be therapeutically superior to conventional formulations.

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**References**


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