FORMULATION AND CHARACTERIZATION OF NATEGLINIDE LOADED SOLID LIPID NANOPARTICULATE CAPSULES FOR THE TREATMENT OF TYPE II DIABETES MELLITUS

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ABSTRACT

Nateglinide is a novel non-sulfonylurea oral hypoglycaemic agent which has outstanding clinical effectiveness in the treatment of Type II diabetes mellitus. The aim of the present study was to prepare and characterize Nateglinide loaded Solid lipid nanoparticulate capsules to improve the therapeutic efficacy and reducing the frequent dosing. Hot homogenization followed by ultra sonication method was employed using glyceryl behenate as a lipid with various drug-lipid ratios for the preparation of solid lipid nanoparticles and optimized formulation filled in hard gelatin capsules. Characterization techniques followed for the formed solid lipid nanoparticles were drug content, entrapment efficiency; in vitro drug release, particle size analysis, morphology and optimized formulation were filled hard gelatin capsules and evaluation of nanoparticulate capsules, kinetic drug release and stability studies. The drug-lipid ratio showed remarkable impact on drug content, entrapment efficiency. The solid lipid nanoparticles were then loaded in capsules followed by in vitro drug release study, SEM micrographs revealed that solid lipid nanoparticles are circular in shape with smooth surface. Which depicted that solid lipid nanoparticles with drug - lipid ratio 1:3 was more proficient to give controlled release at the end of 12 h. the flow property measurements for optimized formulation observed good flow were its filled in capsules and evaluate uniformity of weight, disintegration test, drug content and in vitro drug release for capsules. The uniformity of weight, disintegration test, drug content complies with official specifications and Accelerated stability studies revealed that the formulation is stable as per International Council on Hormonisation guidelines.
KEYWORDS: Solid lipid Nanoparticles, Nateglinide, Hot homogenization method, Glyceryl behenate (Compritol 888 ATO), Poloxomer.

INTRODUCTION
The SLNs are sub-micron colloidal carriers (50-1000 nm), which are composed generally of physiological lipid dispersed in water or in aqueous surfactant solution. (SLNs) as colloidal drug carrier, combine advantages of polymeric nanoparticles, fat emulsions and liposomes and are simultaneously capable of avoiding some of their disadvantages. To overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid was replaced by a solid lipid, which is eventually transformed into solid lipid nanoparticles a clear advantage of SLNs is the fact that the lipid matrix is made from physiological lipids which decrease the danger of acute and chronic toxicity. The use of solid lipid instead of liquid lipid is beneficial as it has been shown to increase control over the release kinetics of encapsulated compounds and to improve the stability of incorporated chemically-sensitive lipophilic ingredients. The mobility of reactive agents in a solid matrix is lower than in a liquid matrix and so the rate of chemical degradation reactions may be retarded. The absorption of poorly absorbed bioactive compounds has been shown to be increased after incorporation into solid lipid nanoparticles. Therefore it is preferable to develop a SLNs to deliver the drug in a controlled manner for prolonged period.[1]

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism. As the disease progresses tissue or vascular damage ensues leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration. Thus, diabetes covers a wide range of heterogeneous diseases. Diabetes mellitus may be categorized into several types but the two major types are type I and type II. On the basis of aetiology, the term type I and type II were widely used to describe IDDM and NIDDM, respectively. On the basis of etiology, type I is present in patients who have little or no endogenous insulin secretory capacity and who therefore require insulin therapy for survival. Type II diabetes is the commonest form of diabetes and is characterized by disorders of insulin secretion and insulin resistance. There is a higher incidence of type II Diabetes mellitus in urban than in rural areas. Type II Diabetes is increasingly becoming a problem among adolescents and even
children. In some countries, childhood diabetes Type II is more common than Type I. The disease is usually controlled through dietary therapy, exercise and hypoglycaemic agents.\textsuperscript{[2]}

NTG is a novel non-sulfonylurea oral hypoglycaemic agent which has outstanding clinical effectiveness in the treatment of Type II diabetes mellitus. After oral administration, NTG is rapidly absorbed from the gastrointestinal tract and rapidly eliminated from plasma with a half-life of approximately 1.5 to 2 h. NTG has low bioavailability. NTG belongs to BCS Class II (low solubility and high permeability), thus selected to augment the bioavailability of the drug. NTG is characterized by rapid clearance due to its shorter half life and thus warrants the use of sustained release formulation for prolonged action to improve its patient compliance.\textsuperscript{[3]}

**MATERIALS AND METHODS**

**Materials**

Nateglinide was received as a generous gift from Dr. Reddys laboratories Ltd., A.P. Glyceryl behenate obtained as gift sample from Orchid Pharmaceuticals, Chennai. Poloxomer were purchased from Lab Chemicals, Chennai. The other chemicals used were of analytical grade.

**Methods**

**Preparation of Solid Lipid Nanoparticles**

Nateglinide loaded solid lipid nanoparticles is prepared by hot homogenization followed by ultra sonication method. Drug and lipids of different ratio are weighed accurately for the preparation of solid lipid nanoparticles. In hot homogenization technique the solid lipid is heated above its melting temperature and mixed with surfactant with a low hydrophilic-lipophilic balance (HLB), under magnetic stirring. A pre-emulsion of the drug loaded lipid melt and the aqueous emulsifier phase the mixture homogenization was performed 2500 rpm by using high speed homogenizer for 60 min.\textsuperscript{[4]} The coarse oil in water emulsion was obtained to ultrasonicated using probe sonicator the amplitude in a pulse regime (2s on, 1s off) during 25 min. forming nanoemulsion was centrifuged in high speed cooling centrifugger at 10,000 rpm. The nanoparticles are seperated and subjected to lyophilization (Table 1)

<p>| Table No. 1: Composition of Nateglinide SLNs. |</p>
<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug Lipid Ratio (Nateglinide : Glyceryl Behenate)</th>
<th>Surfactant (Poloxomer % w/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLN1</td>
<td>1:1</td>
<td>2</td>
</tr>
<tr>
<td>SLN2</td>
<td>1:2</td>
<td>2</td>
</tr>
<tr>
<td>SLN3</td>
<td>1:3</td>
<td>2</td>
</tr>
<tr>
<td>SLN4</td>
<td>1:4</td>
<td>2</td>
</tr>
<tr>
<td>SLN5</td>
<td>1:5</td>
<td>2</td>
</tr>
<tr>
<td>SLN6</td>
<td>1:6</td>
<td>2</td>
</tr>
</tbody>
</table>

**Physical compatibility study**

100mg each of powder drug, lipid, and surfactant were weighed. Individual drug, lipid (glyceryl behenate) poloxomer along with admixture of drug and excipients in airtight screw cap vials, kept at room temperature as well as 40°C±2°C / 75% ± 5% RH for 30 days.\(^5\)

**Chemical compatibility study by Fourier Transform Infra-Red Spectroscopy**

FT-IR spectra of Nateglinide drug, Glyceryl behenate and their physical mixtures were recorded by grinding and dispersing the samples with micronized IR grade Potassium bromide powder.\(^6\) The mixture was dissolved in chloroform and casted on a sodium chloride disk and subjected to FT-IR measurement over the range of 4000-400 cm\(^{-1}\).

**Standard curve for Nateglinide in ph 6.8 buffer**

100 mg of NTG was weighed and transferred to 100 ml of volumetric flask. The drug was dissolved in 10 ml of methanol and volume was made up to 100 ml using phosphate buffer pH 6.8 to obtain a stock solution of 1000 µg/ml (stock solution I). 10 ml of this stock solution was again diluted with phosphate buffer pH 6.8 up to 100 ml to obtain a solution of 100µg/ml (Stock solution II). From stock solution II of 2,4,6,8 10 ml were transferred to series of 100 ml volumetric flasks. The volume was made up with phosphate buffer pH 6.8. The absorbance of these solutions was measured at 210 nm against blank.\(^{[7,8]}\)

**Percentage Yield**

Solid lipid Nanoparticles are weighed and the percentage yield of each formulation was calculated by taking into consideration the total weight of the drug and lipid used for the preparation of solid lipid nanoparticles.\(^9\)

\[
\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100
\]

**Determination of drug content**
The total drug content of SLN dispersion was determined by spectrophotometric analysis. 180 mg equivalent of Nateglinide loaded SLNs is dissolved and made up to 100 ml with phosphate buffer pH 6.8. The solution was kept for 24 h and filtered to separate the fragments. Drug content was analyzed after suitable dilution by UV spectrophotometer at a wavelength of 210 nm against phosphate buffer pH 6.8 as blank. From the absorbance the drug content of each formulation was calculated using the following equation. \[ \text{Actual Drug content} \times 100 \]

\[ \frac{\text{Percentage Drug Content}}{\text{Theoretical drug content}} \]

**Determination of drug entrapment efficiency**

Nateglinide loaded SLNs dispersion was centrifuged at 20000 rpm for 30 mins using high speed cooling centrifuge at 4°C. The clear supernatant was removed from the residue and after suitable dilution the absorbance was recorded at 210 nm using UV spectrophotometer.\[11\]

The percentage entrapment efficiency (%EE) is calculated by following formula:

\[ \%\ EE = \frac{\text{Total amount of drug taken} - \text{Amount of drug in supernatant}}{\text{Total amount of drug taken}} \times 100 \]

**In vitro drug release studies**

*In vitro* release rate studies of Nateglinide loaded SLNs were carried out by using dissolution test apparatus Type-I (basket). Nateglinide loaded SLNs filled equivalent amount in capsule and placed in a dissolution jar. Phosphate buffer pH 6.8 was used as dissolution medium and rotated at 50 rpm. 10 ml of samples were withdrawn predetermined intervals of 1 h and replaced with equal amount of phosphate buffer pH 6.8 for further dissolution testing the absorbance determined by spectrophotometrically at 210 nm.\[11,12\]

**Morphology of Solid Lipid Nanoparticles by scanning electron microscopy**

Scanning electron microscopy (SEM) is an excellent tool for physical observation of morphological features of particle both initially and degradation process. It is helpful to examine particle shape and surface characteristics such as surface area and bulk density. The sample was mounted directly onto the SEM sample holder using double sided sticking tape and images were recorded at different magnifications at acceleration voltage of 10 kV using scanning electron microscope.\[13\]
Particle size
Particle size (Z-average diameter), Polydispersity index (as a measure of the width of the particle size distribution) of Nateglinide loaded SLNs dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000 Nano S (Malvern instruments, UK) at 25°C. Prior to measurements all samples were diluted using ultra-purified water to yield a suitable scattering intensity. The diluted nanoparticle dispersion was poured into the disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analyzed. Air bubbles were removed from the capillary before measurement.

Zeta potential
Zeta Potential is a crucial factor to evaluate the stability of colloidal dispersion surface charge on the Nateglinide loaded SLNs were determined using Malvern Zetasizer. 1 ml of sample of Nateglinide suspension was filled in clear disposable zeta cell, ensured there was no air bubble within the sample and the system was set at 25°C temperature and the test can be carried.

PREFORMULATION STUDY OF OPTIMIZED SLNs
Flow property measurements
The flow properties are critical for an efficient tabletting and capsule filling operation. A good flow of the powder or granules is necessary to assure efficient mixing and acceptable weight uniformity for the compressed tablets and capsules. The flow property measurements include bulk density, tapped density, angle of repose, compressibility index and Hausner’s ratio. The flow property measurements of Nateglinide SLNs are determined.

Optimized SLNs filled in hard gelatin capsules
The optimized Nateglinide solid lipid nanoparticles unstable in suspension form so it is lyophilized and converted into powder form. The dried powder is filled into “00” size hard gelatin capsules and each capsules containing 180 mg equivalent of Nateglinide.

Evaluation of optimized capsules
Uniformity of weight
Intact capsule were weighed. The capsules were opened without losing any part of the shell and contents were removed as completely as possible. The shell was washed with ether and the shell allowed to stand until the odour of the solvent was no longer detectable. The empty shell was weighed. The procedure was repeated with a further 19 capsules. The average weight was determined. Not more than two of the individual weights deviate from the average weight by more than the percentage deviation and deviates by more than twice that percentage.\textsuperscript{[18]}

**Disintegration test**

One capsules introduced in to each tube of the disintegration test apparatus. a disc may be added if necessary. The basket rack assembly is suspended in the beaker containing the liquid medium. The apparatus is operated and the time for disintegration is noted.

**Drug content**

Five capsules were selected randomly and the average weight was calculated. The powder is removed completely. An amount of powder was equivalent to 180 mg made upto 100 ml with phosphate buffer pH 6.8. It was kept for overnight. 1 ml of solution was diluted to 100 ml using phosphate buffer pH 6.8 in separate standard flask. The absorbance of solution was recorded at 210 nm.

**In vitro drug release studies**

In vitro release rate studies of Nateglinide loaded SLNs were carried out by using dissolution test apparatus Type-I (basket). Nateglinide loaded SLNs filled equivalent amount in capsule and placed in a dissolution jar. Phosphate buffer pH 6.8 was used as dissolution medium and rotated at 50 rpm.10 ml of samples were withdrawn predetermined intervals of 1 h and replaced with equal amount of phosphate buffer pH 6.8 for further dissolution testing the absorbance determined by spectrophotometrically at 210 nm.

**Stability studies**

The optimized Nateglinide solid lipid nanoparticles filled in hard gelatin capsules and kept under accelerated conditions (temperature 40°C±2°C and RH 75±5%) according to ICH guidelines using stability chamber for the period of one month. the samples were withdrawn at 15 days predetermined intervals and evaluated for their physical appearance, drug content entrapment efficiency and disintegration test of capsules.\textsuperscript{[19,20]}
RESULTS AND DISCUSSION

Physical compatibility study showed that the drug and excipients were physically compatible with each other. The chemical compatibility study of Nateglinide with excipients were analyzed by using FTIR Spectrometer. The results of the FTIR study proved that there is no interaction between the drug and lipids.

FT-IR spectroscopic studies

FT-IR spectroscopy gives the possible information about the interaction between the drug and excipients.

**Figure 1:** FT-IR spectrum of Nateglinide.

**Figure 2:** FT-IR spectrum of Nateglinide and Glyceryl behenate admixture.
Standard graph was drawn for Nateglinide and it was found that the solutions show linearity (0.999) and obeyed Beer’s and Lambert’s law.
The formulations SLN1, SLN2, SLN3, SLN4, SLN5 and SLN6 were prepared using Glyceryl behenate as lipid in the ratio of (Drug: Lipid- 1:1, 1:2, 1:3, 1:4, 1:5 and 1:6) and the poloxomer as a surfactant at 2% w/v concentration respectively.

All 6 formulations characterized for percentage yield were found to be within the range of 61.8 % to 69.16 and the drug content of the formulations was observed between 98.04 % to 99.34 %.

The entrapment efficiency of all the formulations was observed to be between 66.5% and 75.83 %. The results showed that the increase in lipid concentration increased the drug entrapment efficiency. The entrapment efficiency was found to be higher in SLN3-75.83% comparatively with other formulations.

Table 2: Characterization of Nateglinide loaded SLNs (Mean ± SD n=3).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Percentage Yield (%)</th>
<th>Drug Content (%)</th>
<th>Entrapment Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLN1</td>
<td>61.8</td>
<td>98.04±0.612</td>
<td>69.77±1.085</td>
</tr>
<tr>
<td>SLN2</td>
<td>63.73</td>
<td>99.00±0.122</td>
<td>71.38±0.470</td>
</tr>
<tr>
<td>SLN3</td>
<td>64.52</td>
<td>98.61±0.246</td>
<td>75.83±1.017</td>
</tr>
<tr>
<td>SLN4</td>
<td>67.64</td>
<td>98.91±0.778</td>
<td>72.77±1.793</td>
</tr>
<tr>
<td>SLN5</td>
<td>68.52</td>
<td>99.34±0.790</td>
<td>69.94±1.481</td>
</tr>
<tr>
<td>SLN6</td>
<td>69.16</td>
<td>96.18±0.696</td>
<td>66.5±1.256</td>
</tr>
</tbody>
</table>

Figure 5: Standard curve of Nateglinide in phosphate buffer pH 6.8.
Table 3: *In vitro* Drug release for all formulations.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>SLN1</th>
<th>SLN2</th>
<th>SLN3</th>
<th>SLN4</th>
<th>SLN5</th>
<th>SLN6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19.50</td>
<td>13.88</td>
<td>12.57</td>
<td>16.24</td>
<td>16.08</td>
<td>11.80</td>
</tr>
<tr>
<td>2</td>
<td>28.36</td>
<td>22.10</td>
<td>21.04</td>
<td>24.66</td>
<td>24.47</td>
<td>19.60</td>
</tr>
<tr>
<td>3</td>
<td>35.13</td>
<td>28.29</td>
<td>26.95</td>
<td>34.88</td>
<td>31.98</td>
<td>27.44</td>
</tr>
<tr>
<td>4</td>
<td>41.59</td>
<td>36.61</td>
<td>35.65</td>
<td>44.57</td>
<td>38.02</td>
<td>36.02</td>
</tr>
<tr>
<td>5</td>
<td>49.41</td>
<td>45.02</td>
<td>44.83</td>
<td>52.94</td>
<td>45.02</td>
<td>45.20</td>
</tr>
<tr>
<td>6</td>
<td>57.05</td>
<td>51.46</td>
<td>51.91</td>
<td>63.73</td>
<td>52.36</td>
<td>53.05</td>
</tr>
<tr>
<td>7</td>
<td>64.64</td>
<td>61.19</td>
<td>59.58</td>
<td>68.54</td>
<td>64.04</td>
<td>60.61</td>
</tr>
<tr>
<td>8</td>
<td>76.44</td>
<td>72.44</td>
<td>67.06</td>
<td>75.61</td>
<td>72.73</td>
<td>64.88</td>
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<tr>
<td>9</td>
<td>85.78</td>
<td>82.65</td>
<td>75.66</td>
<td>78.48</td>
<td>76.22</td>
<td>68.66</td>
</tr>
<tr>
<td>10</td>
<td>97.94</td>
<td>90.51</td>
<td>84.35</td>
<td>83.57</td>
<td>81.03</td>
<td>71.71</td>
</tr>
<tr>
<td>11</td>
<td>-</td>
<td>98.57</td>
<td>91.96</td>
<td>86.25</td>
<td>84.21</td>
<td>75.82</td>
</tr>
<tr>
<td>12</td>
<td>-</td>
<td>-</td>
<td>97.83</td>
<td>90.63</td>
<td>87.02</td>
<td>79.58</td>
</tr>
</tbody>
</table>

The *in vitro* release study was carried out for all 6 formulations. The percentage of drug release in formulation SLN3 was found to be 97.83% at the end of 12 h and the release profile was in controlled manner comparatively with other formulations.

Based on the higher entrapment efficiency and prolonged *in vitro* drug release SLN3 was selected as optimized formulations.

![In vitro drug release of SLN1 to SLN6](https://example.com/in_vitro_release.png)

**Figure 6:** *In vitro* drug release study of Nanoparticles (SLN1 to SLN6).
The optimized formulation SLN3 are characterized for surface morphology, particle size analysis and zeta potential. The shape and surface morphology of optimized formulations were observed in SEM. The image showed that nanoparticles were in spherical and circular shape with smooth surface.

**Scanning electron microscope (SEM) analysis**

![Image 1](Image 1)

**Figure 7: SEM Image of Nateglinide pure drug.**

![Image 2](Image 2)

**Figure 8: SEM Image of optimized formulation SLN3.**

The particle size analysis was done by Malvern particle size analyzer showed that average particle size of formulation SLN3 was 493.1 nm and within nanometric range.
The zeta potential study was done by Malvern zeta sizer. The zeta potential for the optimized formulations SLN3 were found to be (-13.9 mV) respectively and shows that the formulation is stable.

![Size Distribution Number](image)

**Figure 9: Particle size distribution of SLN3 Formulation.**

![Zeta Potential](image)

**Figure 10: Zeta potential of SLN3 Formulation.**

Flow property measurements (Bulk density, Tapped density, Angle of repose, Carr’s index and Hausner’s ratio) were carried out for Nateglinide pure drug and optimized SLNs. It revealed that the flow property of pure drug was very poor, but the Nateglinide SLNs have good flow.
Table 4: Flow property measurements of optimized SLNs (Mean ± SD n=3).

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Bulk density (g/ml)</th>
<th>Tapped density (g/ml)</th>
<th>Carr’s Index (%)</th>
<th>Hausner’s ratio</th>
<th>Angle of repose (θ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure drug</td>
<td>0.273±0.016</td>
<td>0.379±0.013</td>
<td>27.47±1.85</td>
<td>1.37±0.030</td>
<td>40.21±0.665</td>
</tr>
<tr>
<td>SLN3</td>
<td>0.313±0.002</td>
<td>0.358±0.002</td>
<td>12.51±0.56</td>
<td>1.14±0.001</td>
<td>33.28±0.518</td>
</tr>
</tbody>
</table>

The optimized SLNs were filled into “00” size hard gelatin capsules without adding glidant because of its good flow property.

Post formulation parameters (uniformity of weight, disintegration test, drug content, and in vitro drug release) for nanoparticulate capsules were evaluated. The results were found to be comply with official specifications.

Table 5: Evaluation of capsules in SLNs (Mean ± SD n=3).

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Average weight of capsules (g)</th>
<th>Drug content (%)</th>
<th>Disintegration time</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-SLN3</td>
<td>0.905±0.006</td>
<td>99.37±0.826</td>
<td>10 min 02 sec±0.032</td>
</tr>
</tbody>
</table>

The optimized formulations SLN3 were subjected to accelerated stability study (temperature 40°C±2°C and RH 75±5%). No significant change was found in appearance, drug content, and entrapment efficiency and disintegration time at the end of 30 days.

Table No. 6: Stability data for Optimized Nateglinide SLNs in capsules.

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Physical appearance</th>
<th>Drug content (% w/w)</th>
<th>Entrapment Efficiency (%)</th>
<th>Disintegration time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero day 15th day 30th day</td>
<td>Zero day 15th day 30th day</td>
<td>Zero day 15th day 30th day</td>
<td>Zero day 15th day 30th day</td>
</tr>
<tr>
<td>C-SLN3</td>
<td>NC  NC  NC</td>
<td>96.61  96.47  96.18</td>
<td>75.83  75.67  75.22</td>
<td>10 min 17 sec 10 min 10 sec 10 min</td>
</tr>
</tbody>
</table>

*NC-No change

CONCLUSIONS

The present study demonstrated Nateglinide loaded solid lipid nanoparticulate capsules were successfully formulated hot homogenization followed by ultra sonication technique. The formulation was able to enhance the physicochemical characteristic of the drug. The Nateglinide loaded solid lipid nanoparticulate capsules using Glyceryl behenate as lipid (1:3) and releases the drug in a controlled manner for an extended period of time. The current work is reduce the frequency of dosing and to release the drug in a controlled manner to improve the efficacy and patient compliance. The foregoing results attempt to suggest that Nateglinide
solid lipid nanoparticulate capsules can be considered as an alternative to conventional drug delivery for better management of type II diabetes mellitus.

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REFERENCES


