DESIGN, DEVELOPMENT AND CHARACTERIZATION OF SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM (SNEDDS) OF NATEGLINIDE

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ABSTRACT

Poor water solubility and dissolution rate are issues for the majority of upcoming and existing biologically active compounds. In the present investigation an attempt was made to enhance the solubility and dissolution of poorly soluble drug, Nateglinide, by formulating self-nanoemulsifying drug delivery systems (SNEDDS). Phase solubility of Nateglinide was evaluated in various non-aqueous carriers that included oils, surfactants, and co-surfactants. Pseudo ternary phase diagrams were constructed to identify the optimized self-nanoemulsification region. Preliminary screening was carried out to select proper components combination i.e. oil, surfactant and co-surfactant respectively. Nateglinide SNEDDS was prepared by using Capmul MCM C-8 (oil), Cremophor EL (surfactant), and Transcutol HP (co-surfactant). Two different adsorbents with high specific surface areas were used i.e. NeusilinUS2, NeusilinUFL2 (magnesium aluminometasilicate). The formulations were characterized for self-emulsification assessment, globule size, polydispersity index, zeta potential, % transmittance, drug content, thermodynamic stability and in-vitro dissolution study. The optimized Nateglinide SNEDDS composed of 20.23% Capmul MCM C-8, 55.77% Cremophor EL and 23% Transcutol HP. The rate of dissolution of optimized SNDDES showed better result (91.12%) in 35 min when compared with marketed tablet 72.3% and pure drug (32.54%). The results from this study demonstrate the potential use of SNEDDS in enhancing solubility and dissolution rate of Nateglinide.

Keywords: Nateglinide, SNEDDS, pseudo ternary Phase diagram, dissolution rate, NeusilinUS2 etc.
INTRODUCTION

Most of drugs are frequently administered through oral route but approximately 40 % of new drugs have poor-water solubility and bioavailability, high intra and inter subject variability and not have dose linearity.\textsuperscript{[1, 2]} This problem overcome by varieties of strategies have been developed including the use of surfactants,\textsuperscript{[3]} lipids,\textsuperscript{[4]} permeation enhancer, micronization,\textsuperscript{[5]} saltformation,\textsuperscript{[6]} cyclodextrins complex, nanoparticles and solid dispersions\textsuperscript{[4]} etc. Lipid based formulations indicates that a drug present in liquid form, among these lipid base system the self-nanoemulsifying drug delivery system SNEDDS are promising technology to improve the dissolution rate and rate and extent absorption of poorly water soluble drugs.\textsuperscript{[7,8]} SNEDDS are isotropic mixture of drug lipids and surfactant atusually with one or more hydrophilic co-solvent or co-surfactant that forms fine oil in water nanoemulsion upon mild agitation in an aqueous medium with a droplet size range form 20-200 nm.\textsuperscript{[9,10]} These Nano-sized droplets may offer an improvement in dissolution rate and oral absorption so ultimately improvement in bioavailability which results in more reproducible blood–time profiles. In the gastrointestinal tract, environmental (fluids and motility) this system spontaneously emulsify.\textsuperscript{[11]}

Other important advantages include high stability, 100% drug entrapment efficiency, decreased dose and dosing frequency (due to improved bioavailability), potential to provide protection to drugs against degradation in the hostile environment of the gut and ease of manufacturing and scale-up.\textsuperscript{[12]}

The solid SNEDDS (S-SNEDDS) are highly sought after owing to their myriad benefits like better portability, improved stability and higher drug loading coupled with and economy of their production.\textsuperscript{[13-15]} In recent years low density porous carriers with large surface area composed of magnesium aluminometasilicate i.e. NeusilinUS2 as well as NeusilinUFL2 are used in order to improve dissolution rate and bioavailability of poorly soluble drugs such as carvidilol, indomethacin.\textsuperscript{[16-17]}

Diabetes mellitus is one of the common problems of this days.\textsuperscript{[18]} 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 KATP potassium channel to perform overall glycemic control in type-2 diabetes. It is the selective blocker of pancreatic beta-cells.\textsuperscript{[19]} The refrain order to effect of drug.
The aim of this study was to investigate self-nanoequilbrating drug delivery system, as a potential drug delivery system of poorly water soluble drug delivery system of Nateglinide. In the present study, we have developed and evaluated a stable SNEDDS formulation of an anti-diabetic drug Nateglinide which is a BCS class II drug with low solubility and high permeability. The SNEDDS consisting of Capmul MCM C-8 /Cremophor EL/Transcutol HP was characterized for the particle size, emulsifying ability as well as solubilisation capacity for Nateglinide. After development of the Nateglinide containing SNEDDS it converts into a solid SNEDDS by adsorbing technique using various types of Carriers, NeusilinUS2, NeusilinUFL2 and Aeroperl 300 pharma. NeusilinUS2 influence of this solid carrier on drug dissolution rate was observed.

MATERIALS AND METHODS

Materials
Nateglinide was obtained as a gift sample from Glenmark Ltd. Nashik. Capmul MCM C-8(Glycerylmonocaprylate), Capmul MCM (Medium Chain Mono and Diglyceride), Capmul PG (Propylene glycol Monocaprylate), Captex-300(Glyceryl Tricaprylate/Tricaprate), Captex- 355(Glyceryl Tricaprylate/Caprate) were obtained as gift samples from ABITEC Corporation (Cleveland,USA). Cremophor-El (MacroglycerolRicinoleate), CremophorRH-40 (MacrogolglycerolHydroxystearate) was gift from BASF (Germany). Transcutol HP (Diethyleneglycol monoethyl ether), were supplied by Gattefosse(France). Tween 80, oleic acid,Brij-35,PEG 400,PG was purchased from S.D fine chemical. NeusilinUS2 and Neusilin FLU2 (magnesium aluminometasilicate) was obtained as a gift sample Fuji Chemical Industry Co.Ltd Japan.

METHODS

Solubility study
The solubility of Nateglinide in various vehicles like oil, surfactant, and co-surfactant was determined by shaking flask method. It was carried out by dissolving an excess amount of drug in 2 ml of the vehicle. Then the mixture was vortexed and kept for 72 hrs.at 25°C in an orbital shaking incubator to facilitate the solubilisation. Than the samples were subjected to centrifugation at 10000 rpm for 10 min, Supernatant layer was filtered through membrane filter using 0.45 μm filter paper. The solution was filtered and diluted with methanol and UV absorbance was measured at 210 nm by UV-spectrophotometer. (UV-1600,Shimadzu,Japan). The drug Concentration in each vehicle was quantified by UV-visible spectrophotometer.
Selection of Surfactant

The solubility of Nateglinide was determined in Tween 80, Cremophor EL, Cremophor RH-40, and Labrasol, Brij 35 by following the same procedure as described for the selection of oil by substituting oils with the surfactants. Thereafter the surfactants were screened based on their emulsification study, water uptake capacity and % transmittance of the selected oil phase. The 200µl of surfactant was added to 200µl of the selected oily phase, mixed thoroughly and then 50µl of this mixture was diluted to 50 ml with distilled water. The ease of formation of emulsions was monitored by the number inversions of volume tric flask required to produce a uniform emulsion. The emulsions were allowed to stand for 2 hrs. and their transmittance was measured at 640.2 nm using UV–Visible spectrophotometer (UV-1600, Shimadzu, Japan) against distilled water as the blank.\textsuperscript{[22]}

Selection of Co-Surfactant

The solubility of Nateglinide was also carried out in different co-surfactant (Propylene Glycol, Transcutol HP PEG-200, PEG -400) by following the above mentioned procedure. Co-surfactant selection was based on their efficacy to improve the nanoemulsification ability of the selected surfactant. For this study 40 µl of surfactant was mixed with 20µl of co-surfactant (S-Cos mix 2:1) select and selected oil 60µl oil phase was added to the this mixture (oil: mix; 1:1) and mixture was heated up to 45 to 50\textdegree C in water bath to allow proper mixing, from this mixture 50 µl was diluted up to 50 ml double distilled water and emulsification monitored by number of flask inversion required to form uniform emulsion. Then nanoemulsion was allowed to stand for 2 hrs. and their transmittance was measured at 640.2 nm by using UV-visible spectrophotometer against distilled water as the blank.\textsuperscript{[12]}

Construction of Pseudo Ternary phase Diagrams

The Pseudo-ternary phase diagram was constructed by titration of homogeneous liquid mixture of oil, surfactant and co-surfactant, with water phase, at 37 \textdegree C.\textsuperscript{[23]} The weight ratio of surfactant to co-surfactant (km) was varied as 1:1.2:1 and 3:1. for each pseudo ternary phase diagram at a specific surfactant/co-surfactant weight ratio, oil and surfactant /co-surfactant mixture were mixed thoroughly in different weight ratios (1:9 to 9:1).\textsuperscript{[24]} The each mixture of titrated with distilled water until they exhibited turbidity. At the same time samples were visually examined for transparency. Only single-phased, transparent, low viscous mixtures were considered as nanoemulsions. After the study of nanoemulsion region in the phase diagrams were done, constructed by using CEMEX school software.
Formulation of liquid SNEDDS and solid SNEDDS

On the basis of solubility study and pseudo ternary phase diagrams plot, Capmul MCM C-8,CremophorEl, and Transcutol HP were selected as oil, surfactant and co-surfactant respectively . The liquid SNEDDS was prepared as reported in literature.[25] The calculated amount of surfactant/co-surfactant (3:1) and oil to S/COS ratio (2:8) was taken to formulate four batches. Nateglinide (60 mg) in accurately weighed amount of lipid (oil) into a screw-capped glass vial and heated in water bath up to 40°C. The surfactant and co-surfactant mixture (S-Cos mix) were added to lipid mixture using pipette and stirred with vortex to obtain homogenous solution. The prepared SNEDDS was stored in room temperature in sealed transparent bottles until used.[26]

S-SNEDDS of Nateglinide were prepared by adsorbing technique by using various novel adsorbent previously reported in literature.[22]

Characterization of Liquid SNEDDS

Visual observation, phase separation of Nanoemulsion

Each formulation of SNEDDS containing Nateglinide was diluted with 250 ml of distilled water at 37°C to check visual appearance, the diluted preparation was vortexed for 5 min, and then the mixtures were stored for a period of 24 hrs and phase separation and precipitation observed visually.[27] Those formulations exhibiting a negligible phase separation were used for further study.

Self-nanoemulsification efficiency test

The stable SNEDDS were further subjected to self-nanoemulsification efficiency test. The self-nanoemulsification efficiency of Nateglinide loaded SNEDDS was assessed using a standard USP XXII dissolution apparatus II.[28] The self-nanoemulsification performance of each SNEDDS was visually assessed using different grading systems like grades A, B, C, D and E as reported previously.[27-29] Formulations those passed self-nanoemulsification efficiency test in grades A and B were selected for further evaluation.

Thermodynamic stability test

In this test formulation solved problem of stability, metastable formulation was found. The optimize formulations were subjected to different thermodynamic stability study tests viz. centrifugation, heating and cooling cycle and freeze thaw cycles.[30]
% transmittance test
The Nateglinide SNEDDS were reconstituted with distilled water (1:100) and the resulting nanoemulsion was observed visually any precipitation. Thereafter, its % transmittance was measured at 640.2 nm using UV-visible spectrophotometer against distilled water as blank.

Globule size and PDI
The globule size and PDI of reconstituted Nateglinide SNEDDS were determined using MalvernZetaseizer(Nano ZS 90, UK).the sample were put in folded capillary cells and results obtained for size, PDI were recorded.

Zeta-potential
The stability of nanoemulsion is directly related to the magnitude of the surface charge. The zeta potential of formulations was determined by laser diffraction analysis using particle size analyzer (Malvern Zetasizer Nano series ZS 90 UK).The samples were diluted with a ratio 1:100 (V/V) with distilled water.

Drug Content
The percentage drug content of formulations was determined from the calibration curve of Nateglinide in methanol and assay of the drug was done by UV-visible spectrophotometry.

Viscosity and Refractive index
The SNEDDS is generally administered in soft gelatin or hard gelatin capsules. So, it should be easily pourable into capsules and such systems should not be too thick. The viscosity of optimized formulation was evaluated by Brookfield viscometer. The refractive index of the system is measured by refractometer by putting a drop of solution on slide and comparing it with water (1.333).

Characterization of Nateglinide loaded S-SNEDDS(Solid state characterization of S-SNEDDS)
The solid SNEDDS were characterized for their % transmittance, emulsification time and drug contents described for L-SNEDDS in above section.

Differential scanning calorimetry(DSC)
The thermal properties of pure Nateglinide, physical mixture and S-SNEDDS 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 (Hfusion).

**Scanning electron microscopy (SEM)**
The morphological features of solid Nateglinide SNEDDS are observed by scanning electron microscope at different Magnifications.

**X-ray powder diffraction (XRPD)**
XRD measurements were carried out with an X’Pert PRO diffractometer. The diffractograms of Nateglinide, NeusilinUS2 and S-SNEDDS were obtained for analysis.

**In vitro dissolution studies**
In vitro dissolution/drug release studies were in 900 ml of 0.1 N HCL using USP XXIV method (dissolution apparatus II) at 100 rpm and 37± 0.5°C. 1 ml of each SNEDDS (containing 60mg of Nateglinide) was filled in transparent hard gelatin capsules. 5ml aliquots were removed at predetermined time intervals 0.., 5, 10, 15, 25, 30, 35, 40, 45, 60, 60, 90 and 120 min from dissolution medium an 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.

**Stability study**
Stability of optimized Nateglinide loaded SNEDDS were stored at 40±2 °C and 75±5 %RH for three months where there no obvious change in visual appearance and no change in globule size, emulsification time, % transmittance and drug Content.

**RESULTS AND DISCUSSION**
**Solubility studies**
Results of solubility of Nateglinide in various essential oils are shows Fig. 1. This study allows us to identify the suitable oil to load Nateglinide into formulation. Higher the solubility of drug, higher will be the loading potential. It was observed that the solubility of Nateglinide was significantly higher in novel semi-synthetic medium chain derivatives in comparison to natural medium chain triglycerides. The result suggests that Nateglinide is highly soluble in Capmul MCM C-8 (39.02 mg/ml), Cremophor El (59.12 mg/ml) and Transcutol HP(60.03 mg/ml). Based on solubility study Capmul MCM C-8 selected as oil phase.
Selection of surfactants

Nonionic surfactants are mostly considered safer than the ionic surfactant and they are usually accepted for oral ingestion.[31] They can produce reversible change in intestinal mucosal permeability.[32] So the non-ionic surfactant with above mentioned ranged were selected as emulsifying agent as they have emulsifying ability and solubilize the drug in selected oil phase. Those having HLB 4.3 to 16 were screened on the basics of solubility and emulsifying ability. Hence, different non-ionic surfactants with above mentioned range were selected as emulsifying ability and solubilize the drug in selected oil phase. The higher solubility was observed in high HLB values surfactant.

The selection of final surfactant was based on their emulsifying ability and percent transmittance of resultant nanoemulsion. Hence use of Cremophor RH-40 tween 80 and Cremophor El was found to give better transmittance (<90%) indicating their good emulsification ability Shown in Tab. I. Nateglinide was found to be having better solubility in these surfactants and they were selected for further investigation.

Selection of co-surfactants

This investigation distinguished role of the various co-surfactants, to improve the nanoemulsion ability of selected surfactant. All co-surfactant screened for improving thenanoemulsification ability of selected surfactant were found the spontaneously formation of nanoemulsion. The % transmittance and emulsification time were carried out using various co-surfactants in combination with selected surfactant and Capmul MCM C-8 as oil are given in Tab.I. Among all the co-surfactants screened, Transcutol HP showed highest % transmittance when used with Cremophor El and Cremophor RH40 as a surfactant. The solubility of drug in co-surfactant also was found highest (60.12 mg/ml), the co-surfactant selected on based on its efficacy to improve the nanoemulsification ability of surfactant. Accordingly, Transcutol HP which showed the highest transmittance (99.12%) was selected as a co-surfactant for further investigation.
Fig. 1 Solubility of Nateglinide in (A) Oils, (B) Surfactant, (C) Co-surfactant

Fig. 2 Pseudo ternary phase diagrams of Capmul MCM C-8, Cremophor El, Transcutol HP of various ratios

Fig. 3. Globule Size of optimized formulation (F2) Batch
Fig. 4. Zeta potential of optimized Nateglinide loaded SNEDDS formulation (F2)

Fig. 5. Scanning electron micrographs of (A) Pure Nateglinide (B) Neusilin US2 (C) S-SNEDDS

Fig. 6. DSC of (A) Pure Drug (B) Final formulation Batch (F2)
Fig. 7. XRD of (A) Plain Nateglinide, (B) NeusilinUS2, (C) S-SNEDDS

Fig. 8. In-vitro Drug Dissolution Profile of Nateglinide loaded SNEDDS

Table I. - Emulsification efficiency of Various Sufactants/ Co-surfactants

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Sufactants/ Co-surfactants</th>
<th>Emulsification Time (Sec)</th>
<th>% transmittance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cremophor RH 40</td>
<td>30</td>
<td>85.12</td>
</tr>
<tr>
<td>2</td>
<td>Cremophor El 59</td>
<td>22</td>
<td>98.18</td>
</tr>
<tr>
<td>3</td>
<td>Brij-35 42</td>
<td>59</td>
<td>86.07</td>
</tr>
<tr>
<td>4</td>
<td>PEG-400 19</td>
<td>42</td>
<td>89.23</td>
</tr>
<tr>
<td>5</td>
<td>Transcutol HP 35</td>
<td>19</td>
<td>92.89</td>
</tr>
<tr>
<td>6</td>
<td>PEG-200 40</td>
<td>35</td>
<td>99.12</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td>98.12</td>
</tr>
</tbody>
</table>
Table II - Globule size, polydispersibility index, zeta potential, % transmittance and drug content of developed SNEDDS formulations

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Batch</th>
<th>Globule Size</th>
<th>PDI</th>
<th>Zeta potential</th>
<th>% Transmittance</th>
<th>Drug Content</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>207.01</td>
<td>0.494</td>
<td>-28.12</td>
<td>97.12</td>
<td>91.23</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>137.28</td>
<td>0.310</td>
<td>-35.01</td>
<td>99.12</td>
<td>99.65</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>179.38</td>
<td>0.459</td>
<td>-30.5</td>
<td>96.23</td>
<td>96.12</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>220.89</td>
<td>0.591</td>
<td>25.14</td>
<td>91.23</td>
<td>98.56</td>
</tr>
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</table>

Table III. Stability study of Optimized formulation

<table>
<thead>
<tr>
<th>Time (Month)</th>
<th>Physical appearance</th>
<th>Drug Content (%)</th>
<th>Emulsification time (Sec)</th>
<th>% Transmittance</th>
<th>Particle size</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Clear liquid</td>
<td>99.65</td>
<td>18</td>
<td>97.12</td>
<td>137.12</td>
<td>0.356</td>
</tr>
<tr>
<td>1</td>
<td>Clear liquid</td>
<td>99.03</td>
<td>20</td>
<td>99.12</td>
<td>138.05</td>
<td>0.389</td>
</tr>
<tr>
<td>2</td>
<td>Clear liquid</td>
<td>98.95</td>
<td>19</td>
<td>96.23</td>
<td>137.56</td>
<td>0.390</td>
</tr>
<tr>
<td>3</td>
<td>Clear liquid</td>
<td>98.86</td>
<td>20</td>
<td>91.23</td>
<td>137.96</td>
<td>0.361</td>
</tr>
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</table>

Construction of Pseudo Ternary phase Diagrams

Pseudo ternary phase diagrams were constructed based on the solubility data to identify the nanoemulsion area for stable and clear formulations in 3:1 S-Cos mix ratio. The proportions of three components in the final SNEDDS preparation i.e. oil, Surfactant and Co-surfactant were selected from pseudo ternary phase diagrams. The different batches of the SNEDDS were prepared by using Phasediagram by varying the concentration. The better nanoemulsion region was observed in Capmul MCM C-8, CremophorEl, TranscutolHP incorporated nanoemulsion. The optimized composition of oil phase (22.12%), Surfactant (55.23%) and co-surfactant (27.23%) had given the globule size <150 nm.

Preparation of solid SNEDDS of Nateglinide

S-SNEDDS of Nateglinide were prepared by Adsorbing technique by using solid adsorbent. Nateglinide loaded SNEDDS pre-concentrate adsorbed on highly porous NeusilinUS2 (magnesium aluminometasilicate). The Nateglinide loaded SNEDDS were added drop wise over the NeusilinUS2 (1:2 by weight). After each addition, the mixture was mixed thoroughly to ensure uniform distribution. The granular mass obtained was passed through sieve with aperture of 40 mesh, to get free flowing and lump free powder. The powder sample was stored in a desiccator for further evaluation.
Characterization of SNEDDS

Self-nanoemulsification efficiency test
The optimized formulation of Nateglinide loaded SNEDDS were further characterized for Self-nanoemulsification efficiency test. In this when SNEDDS were diluted with aqueous medium or GI fluids, possibility of phase separation which could lead to precipitation of drug. In present study, distilled water and 0.1 N HCL were used to check the dispersion and self-emulsification. The formulations that passed this test in grade A and B were selected for further evaluation, as grade A and B formulations will remain as SNEDDS when dispersed in GI fluids. The F1 and F2 batch shows the A and B respectively and other SNEDDS that categorized grade C, D and E were discarded for further characterization.

Thermodynamic stability study
- Heating cooling cycle
The contents of the optimized formulations were subjected to the six cycles between refrigerator temperature -4°C and 45°C with storage at each temperature of not less than 48 hrs. were studied. Those formulations, which were stable at these temperatures, are subjected to centrifugation test.

- Centrifugation study
The optimized formulations were centrifuged at 5000 rpm for 30 min. The resultant formulations were then checked for any instability problem, such as phase separation, creaming or cracking.

- Freeze thaw cycle
Three cycles of formulations subjected to the freezing at-4 0c for 24 hrs and followed by thawing for at40°C for 24 hrs and observed visually for phase separation. The F2 batch passes all tests viz. phase separation, cracking and creaming was not observed during this study. Hence the F2 batch proceeds for further study.

Globule size and polydispersity index (PDI)
The particle size distribution has most important characteristics affecting the in vivo fate of nanoemulsion. The mean droplet size of selected SNEDDS of (F1-F4) ranges was observed from 137.2 to 230 nm. The largest droplet size in F3 which could be due to presence of higher concentration of oil. The droplet size of F2 was found to be lowest (137 nm) shown in Fig.3, which could be due to the lowest concentration of oil, and higher ratio of surfactant/co-surfactant mixture that 3:1. PDI value was to be below 0.5 for all SNEDDS, it indicates
narrow size distribution. The formulation F2 showed lowest PDI (0.310) that indicates good uniformity in droplet size distribution.

**Zeta potential (ZP)**

Zeta potential of Batches F1 to F4 is shown in Table II. ZP governs the degree of repulsion between adjacent or similarly charged and dispersed droplet, it shows the practical application in the stability. ZP-values of SNEDDS F1-F4 were found in the range of 20 to 35mV. F2 Batch shows -35.01mV that is highest, in shown in Fig.4 ZP values of in range of -20 to -35mV in either charge characterizes a stable formulation. The negative charge on all SNEDDS was possible due to the presence of esters and fatty acids in Capmul MCM C-8 which was presence in formulations as oil phase.

**% Transmittance**

The optimized formulations F1-F2 of SNEDDS were diluted with distilled water (1:100) and thereafter, its % transmittance was measured at 640.3 nm using UV–visible spectrophotometer against distilled water as the blank. The highest transmittance was observed in F2 batch that is 99.14%. Shown in Tab.II.

**Drug Content**

Drug content for optimized formulations were done by UV-visible spectrophotometric analysis by using standard calibration curve. The F2 batch showed highest percent drug constant (98.05) shown in Tab.II, as compare to other formulations.

**Refractive index and Viscosity**

The refractive indexes of (RIs) of SNEDDS (F1-F4) were carried out at room temperature. The RI of F2 batch was found to be 1.22 and 1.33 respectively. Viscosity of the L-SNEDDS formulations (F1-F4) were found in the range of in range of 200 cps to 221 cps, F2 batch of formulation showed lowest (201.78 cps) as compare to others. The viscosity correlated with either shape or geometry of formulation.

**Differential scanning calorimetry (DSC)**

DSC allows determination of thermotropic phase transition behavior in a quantitative manner. Thermograms recorded during analysis display melting peaks. The narrow peak at 142.02°C showed in fig.6 for pure drug of Nateglinide infers presence of crystalline form of Nateglinide. No representative peaks for drug were observed for solid formulation, indicating the transformation of crystalline structure of Nateglinide as it may be present in molecularly dissolved state in self-emulsifying powder.
Scanning electron microscopy (SEM)
Scanning electron micrograph of Nateglinide, Neusilin US2 and S-SNEDDS are shown in Fig.6. It shows that few particles resembling Nateglinide were visible in case of SNEDDS formulation at 3500 magnification indicating the incorporation in SNEDDS.

X-ray diffraction (XRD)
The physical state of Nateglinide S-SNEDDS was investigated by x-ray powder diffraction measurements and the X-ray diffractograms are depicted in Fig 7. It can be observed that Nateglinide exhibited peaks at 9.143, 17.483 and 20.980 while NeusilinUS2 showed peaks at 18.859 and 23.485. The XRD of Nateglinide, S-SNEDDS showed presence of all the major peaks indicating that the drug retained its crystalline nature even after incorporation in the SNEDDS. However, lowering of intensity of the peaks, point towards reduction in its crystallinity.

In vitro dissolution studies
In vitro dissolution study revealed that Liquidand S- SNEDDS of optimized batch (F2) released more than 85% drug within 15 min and almost 100% up to 30 min irrespective of pH 1.2 of dissolution media, instead of commercial formulation showed 73 % drug release in 45 min and plain drug showed very less release 32.01 % in 0.1 N HCL shown in Fig 8, respectively revealing that the SNEDDS formulation.

Stability study
The developed formulation was subjected into three months stability studies at 40±2°C/75±5 %RH shown in Tab. III. The formulation was found to be stable for three months and there was no significant change in the drug content, emulsification time and % transmittance.

CONCLUSION
Nateglinide loaded Self-nanoemulsifying system consisting of Capmul MCM C-8,CremophorEl, Transcutol HP was formulated, using surfactant/co-surfactant (km)ratio 3:1, as well as surfactant-co surfactant/oil ratio 8:2. The established SNEDDS showed influence on emulsification behavior of investigated system upon water dilution. S-SNEDDS were prepared with three different Adsorbents, NeusilinUS2, Neusilin UFL2, Aeroperl 300 pharma. Formulations were evaluated for in-vitro drug release studies, SEM, DSC and XRD studies confirmed incorporation of drug in SNEDDS. It showed improvement in drug release
rate compared to Marketed formulation and pure drug. The optimized formulations are found to be physically and chemically stable for 3 months.

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