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Self Nano Emulsifying Formulation of Nateglinide with Improved Drug Solubility and Dissolution

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ABSTRACT

The objective of the present work was to formulate and evaluate novel self-nano emulsifying drug delivery system (SNEDDS) of poorly soluble drug Nateglinide. Poor water solubility and slow dissolution rate are major issues for most upcoming and existing biologically active pharmaceutical compounds. Nateglinide is Biopharmaceutical Classification System Class-II drug that has low solubility and high permeability. Surfactants and oil was selected based on solubility studies were further screened for their efficiency in formulation. Acrosyl K-135 was used as oil phase and Kolliphor RH 40 and Transcutol P were used as surfactant and co-surfactant respectively for formulation. Formulation F13 was found to be optimized formulation on the basis of in vitro dissolution studies, particle size and zeta potential. The particle size of the optimized SNEDDS formulation was found to be 74.6 nm and Z-Average was found to be 43.1 nm, indicating all the particles were in the nanometer range and the zeta potential of the optimized SNEDDS formulation was found to be -18.4 mV. The optimized formulation was then subjected to stability studies and was found to be stable after 6 months. Thus, the study confirmed that the SNEDDS formulation can be used as a possible alternative to traditional oral formulations of Nateglinide to improve its solubility.

Keywords: Nateglinide, Diabetes mellitus, SNEDDS, Solubility, Particle size & Zeta potential

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INTRODUCTION

More than 40 percent of the drugs coming from high-throughput screening are poorly soluble in water¹. Compounds with poor solubility are increasingly posing challenges in the development of new drugs, since many drugs coming directly from synthesis or from high throughput screening have a very poor solubility². Recent years Self-Nano emulsifying drug delivery system (SNEDDS), self-micro emulsifying drug delivery system and self-emulsifying drug delivery systems is used to improve the aqueous solubility of poorly water-soluble drugs³.

Self-nano emulsifying drug delivery system (SNEDDS) is isotropic mixture of natural or synthetic oil, surfactants and co-surfactants that have a unique ability of forming fine oil-in-water (O/W) nano-emulsions under mild Agitation followed aqueous media⁴.

The SNEDDS is one of the Stable Nano emulsion is important to provide a large interfacial area for partitioning of drug between oil and aqueous phase, having better rate of drug dissolution and increases bioavailability of drug formulation⁵. The Self-Nanoemulsifying drug delivery system is thermodynamically Stable and Transparent or Translucent Non-Ionized Dispersion of (o/w) and (w/o) Nano emulsion was stabilized by addition of Surfactant and Co-surfactant Molecule⁶.

Diabetes mellitus is one of the common problems of this day⁷. 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 glycaemic control in type-2 diabetes. It is the selective blocker of pancreatic beta-cells⁸. The aim of this study was investigating self-nano emulsifying drug delivery system, as a potential drug delivery system of poorly water-soluble drug delivery system of Nateglinide. The SNEDDS consisting of Acrysol K-135, Kolliphor RH 40 and Transcutol P was characterized for the particle size, emulsifying ability, solubilisation capacity and all other characterization.

MATERIALS AND METHOD

Materials:

Nateglinide, Labrafac PG, Labrafil M2125CS Labrafil M 1944 Labrasol was gifted from Aurobindo Pharma limited, Hyderabad. Acrysol K-135 Acrysol K-150 Capryol PGMC Kolliphor ELP Captex 355 procured from Granules India limited, Hyderabad. Propylene glycol PEG 400, Transcutol P and oleic acid was purchased from SDFCL, Mumbai. PEG 600 and Kolliphor RH 40 were gifted from BASF, Mumbai.

Preparation of Standard Stock Solution

Working standard Nateglinide 60mg was weighed accurately and transferred to a 10ml volumetric flask and dissolved in 1 ml of phosphate buffer pH 6.8. The flask was shaken and volume was made up to the mark with phosphate buffer to give a solution of 1000 μ g/ml. It was further diluted with phosphate buffer to get a concentration of 100 μ g/ml. From this solution, a series of aliquots were prepared for further method development.

Absorption Maxima Method:

For the selection of analytical wavelength, 10 μ g/ml solution of Nateglinide was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200nm to 400nm. From the spectrum λ_{max} of Nateglinide, 220nm was selected for the analysis. The calibration curve was prepared in the concentration range of 5-30 μ g/ml at 220nm. The calibration curve for Nateglinide was plotted in the concentration v/s absorbance and regression equation was calculated.

Solubility Studies:

The solubility study was used to find out the suitable oil, surfactant and co-surfactant that possess good solubilizing capacity for Nateglinide⁹. The solubility of Nateglinide in various oils (Acrysol K-135, Acrysol K-150, Oleic acid, Captex 355, Capryol PGMC), surfactants (Kolliphor RH 40, Labrafac PG, Labrafil M2125CS, Kolliphor ELP, Kolliphor PS 80, Labrafil M 1944, Labrasol) and Co-surfactants (PEG 600, Propylene glycol, PEG 400, Transcutol P) were determined by mixing excess amount of Nateglinide (approximately 60 mg) with 2 ml of each of the individual components. The drug was added to a 5-ml capacity stoppered glass vial and mixed for 10 min with each component using a vortex mixer. The mixture vials were then kept at $37 \pm 1.0^{\circ}\text{C}$ in an isothermal shaker for 72 h until homogeneity. The homogenate samples were then centrifuged at 18,000 rpm for 30 min at 4°C . The supernatant was removed by pipetting and the drug concentration was determined by UV Visible spectrophotometrically at 220 nm.

Ternary Diagram

The use of pseudo ternary diagrams is not recent. This technique was mainly used to map the microemulsion areas (composition ranges). Pseudo ternary phase diagram is used to map the optimal composition range for three key excipients according to the resulting droplet size following self-emulsification, stability upon dilution and viscosity¹⁰.

Construction of ternary phase diagrams

Based on the solubility study of drug, oil, surfactants and co-surfactants were used for construction of phase diagram. Oil, surfactant, and co-surfactant are grouped in three different combinations for phase studies. Surfactant and co-surfactant (Smix) in each group were mixed in different weight

ratio (1:1, 2:1 and 3:1). These Smix ratios are chosen in increasing concentration of surfactant with respect to co-surfactant and in increasing concentration of co surfactant with respect to surfactant for detail study of the phase diagram for formulation of micro emulsion. For each phase diagram, oil, and specific Smix ratio are mixed thoroughly in different weight ratio from 1:9 to 9:1 (1:9, 2:8, 3:7, 4:6, 5:5, 6:4,7:3, 8:2, 9:1) in different glass vials. Different combination of oils and Smix were made so those maximum ratios were covered for the study to delineate the boundaries of phase precisely formed in the phase diagrams. Pseudo-ternary phase diagram was developed using aqueous titration method. Slow titration with aqueous phase is done to each weight ratio of oil and Smix and visual observation is carried out for transparent and easily flow able o/w micro emulsion. The physical state of the micro emulsion was marked on a pseudo-three-component phase diagram with one axis representing oil, the other representing surfactant and the third representing co-surfactant at fixed weight ratios ¹¹.

% Transmittance

The Nateglinide SNEDDS were reconstituted with distilled water and the resulting microemulsion was observed visually for any turbidity. Thereafter, its % transmittance was measured at 220 nm using UV spectrophotometer against distilled water as the blank ¹².

Emulsification Time

A visual test to assess the self-emulsification properties was modified and used in the present study. With the use of this method, a predetermined volume of mixture (0.2 ml) was added to 300 ml of water in a glass beaker under stirring and temperature was maintained at 37⁰C using a magnetic stirrer. The tendency of formation of emulsion was observed. If the droplet spreads easily in water was judged as 'good' and judged as 'bad' when there was milky or no emulsion or presence of oil droplets. The assessment for the efficiency of the emulsion system is also made according to the following grading system ¹³.

Development of SNEDDS Formulations

The following should be considered in the formulation of a SNEDDS:

1. The solubility of the drug in different oil, surfactants and co-surfactants.
2. The selection of oil, surfactant and co-solvent based on the solubility of the drug and the preparation of the phase diagram ¹⁴.
3. The phase diagrams were constructed at different Km values and the Km value at which high microemulsion region obtained was selected for formulation of Liquid SNEDDS.

Here, Acrosyl K-135 was used as oil phase and Kolliphor RH 40 and Transcutol P were used as surfactant and co-surfactant respectively. The compositions were given in the **Table 1**. In brief,

Nateglinide (60mg) was added in accurately weighed amount of oil into screw-capped glass vial and heated in a water bath at 40°C. The surfactant and co-surfactant were added to the oily mixture using positive displacement pipette and stirred with magnetic bar. The formulation was further sonicated for 15mins and stored at room temperature until its use in subsequent studies.

Table 1: Formulation Trials of Liquid SNEDDS:

Smix(Surfactant: Co-surfactant)	Oil:Smix	Formulation code	Drug (Nateglinide) (mg)	Oil(Acrysol K-135) (ml)	Surfactant (Kolliphor RH40)(ml)	Cosurfactant (Transcutol P)(ml)
1:1	2:8	F1	60	0.3	0.6	0.6
	3:7	F2	60	0.45	0.525	0.525
	4:6	F3	60	0.6	0.45	0.45
	5:5	F4	60	0.75	0.375	0.375
	6:4	F5	60	0.9	0.3	0.3
2:1	1:9	F6	60	0.15	0.9	0.45
	2:8	F7	60	0.3	0.8	0.4
	3:7	F8	60	0.45	0.7	0.35
	4:6	F9	60	0.6	0.6	0.3
	5:5	F10	60	0.75	0.5	0.25
3:1	5:5	F11	60	0.75	0.562	0.187
	6:4	F12	60	0.9	0.45	0.15
	7:3	F13	60	1.05	0.337	0.112
	8:2	F14	60	1.2	0.225	0.075
	9:1	F15	60	1.35	0.112	0.037

Thermodynamic Stability Studies

The objective of thermodynamic stability is to evaluate the phase separation and effect of temperature variations on SNEDDS formulations.

Freeze Thawing

Formulations were subjected to freeze cycle (-20°C for 2days followed by 40°C for 2days). Only stable formulations were selected for further studies ¹⁵.

Centrifugation

Centrifugation was performed at 3000 rpm for 5 minutes. The formulations were then observed for phase separation. Only formulations that were stable to phase separation were selected for further studies ¹⁶.

Determination of Drug Content

Accurately measured dose of Nateglinide formulation (0.2ml) equivalent to 60mg was taken in volumetric flask and the volume is made to 100ml with phosphate buffer pH 6.8. From this 1ml of solution is taken in a 10ml volumetric flask and is made up to 10ml with buffer. This solution is

diluted to 10 μ g/ml and absorbance was measured at λ_{\max} 220 nm against blank. The amount of drug present in 0.2ml of formulation was determined by using UV spectrophotometric method and drug concentration was determined from standard graph.

$$\% \text{ Drug content} = \frac{\text{Actual amount of drug in SMEDDS}}{\text{Theoretical amount of drug in SMEDDS}} \times 100$$

In Vitro Dissolution Test of SNEDDS:

The dissolution test was undertaken with paddle method in 900 ml of pH 6.8 phosphate buffer containing various concentrations of Nateglinide at 37 $^{\circ}$ C with a paddle speed of 50 rpm. The liquid SNEDDS equivalent to 60 mg of Nateglinide were filled into hard gelatin capsules (capsule No. 00) samples for analysis were collected at appropriate time intervals at 2,5,10,15,20,25,30,45,60min through filters and the concentration of Nateglinide was determined in at 220nm by UV.

Characterization of SNEDDS

Drug-Excipient Compatibility Studies

The Drug Excipient Compatibility Studies were carried out by Fourier Transform infrared spectroscopy (FTIR) method.

Fourier transform infrared spectroscopy (FTIR)

An FTIR-8400S Spectrophotometer (Shimadzu, Japan) equipped with attenuated total reflectance (ATR) accessory was used to obtain the infrared spectra of drug in the isotropic mixtures of excipients. Analysis of pure drug i.e., Nateglinide and physical mixtures of the drug with the excipients were carried out using diffuse reflectance spectroscopy (DRS)-FTIR with KBr disc. All the samples were dried under vacuum prior to obtaining any spectra to remove the influence of residual moisture. For each the spectrum, 8 scans were obtained at a resolution of 4 cm^{-1} from a frequency range of 400–4000 cm^{-1} .

Determination of Droplet Size

The average droplet size of Nateglinide SNEDDS formulations were determined by Photon correlation spectroscopy (Malvern Instrument UK) able to measure sizes between 10 and 5000 nm. The selected formulations were diluted with deionized water and placed in an electrophoretic cell for measurement ¹⁷.

Determination of Zeta Potential

The emulsion stability is directly related to the magnitude of the surface charge. In conventional SNEDDS, the charge on an oil droplet is negative because of the presence of free fatty acids. The zeta potential of the diluted SNEDDS formulation was measured using a zeta meter system. The

SNEDDS were diluted with a ratio 1:2500 (v/v) with distilled water and mixed with magnetic stirrer. Zeta-potential of the resulting micro emulsion was determined using a Zetasizer¹⁸.

SEM

Shape and surface morphology of microspheres was studied using scanning electron microscopy (SEM). The SNEDDS after converting to emulsion were mounted on metal stubs and the stub was then coated with conductive gold with sputter coater attached to the instrument. (HITACHI, S-3700N)¹⁹.

Stability studies

The SNEDDS formulations were put into empty hard gelatin capsules and subjected to stability studies at 25°C/60% relative humidity (RH) and 40°C/75% RH using stability chambers (Thermo lab, Mumbai, India) with humidity and temperature control. They were withdrawn at specified Accelerated conditions for 6 months for long-term conditions. Drug content of the capsules was analyzed using a previously developed and validated stability-indicating UV method.

RESULTS AND DISCUSSION

Solubility Studies

The Nateglinide drug solubility of pure drug was found to be 0.00848 mg/al. The solubility of the Nateglinide drug was tested in different oils phases (Acrysol K-135, Acrysol K-150, Oleic acid, Captex 355, Capryol PGMC) and maximum solubility was determined in Acrysol K-135 0.2968 mg/ml shown in **Table 2** and graphical representation is shown in **Figure 1**. And was selected as oily phase for SNEDDS formulation

Table 2: Solubility of Nateglinide in Different Oils

S.NO	OILS	SOLUBILITY(mg/ml)
1	Oleic acid	0.2121
2	Acrysol K-150	0.1356
3	Capryol PGMC	0.1272
4	Acrysol K-135	0.2968
5	Captex 355	0.2374

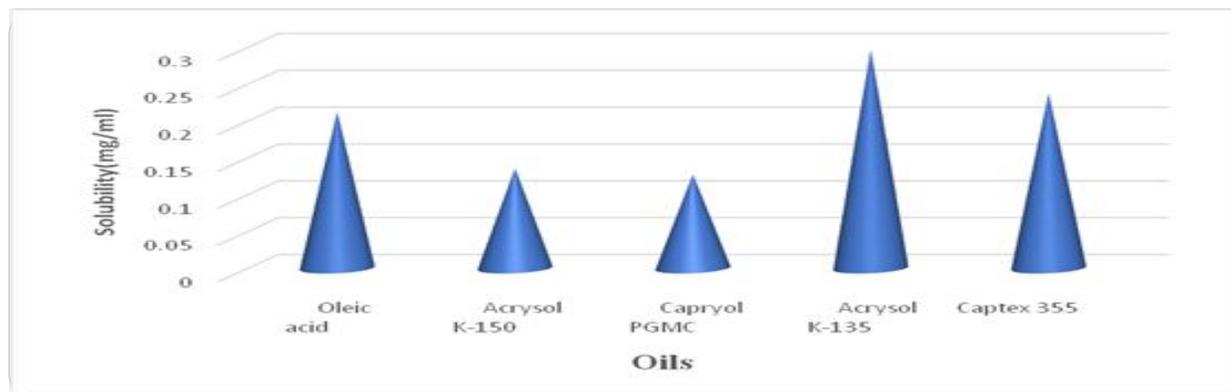


Figure 1: Solubility studies of Nateglinide in oils

The solubility of the drug was tested in different surfactants (Kolliphor RH 40, Labrafac PG, Labrafil M2125CS, Kolliphor ELP, Kolliphor PS 80, Labrafil M 1944, Labrasol) and co-surfactants (PEG 600, Propylene glycol, PEG 400, Transcutol P) and maximum solubility determined 0.2713mg/ml of Kolliphor RH 40 as a surfactant phase (shown in **Table 3 & Figure 2**) and 0.3052mg/ml (shown in **Table 4 & Figure 3**) of Transcutol Pas a co-surfactant phase. It was selected as surfactant and co-surfactant for SNEDDS formulation.

Table 3: Solubility of Nateglinide in Different Surfactants

S.NO	Surfactants	Solubility(mg/ml)
1	Labrafac PG	0.1441
2	Kolliphor RH 40	0.2713
3	Labrafil M2125CS	0.1187
4	Kolliphor ELP	0.241
5	Kolliphor PS 80	0.212
6	Labrafil M 1944	0.1780
7	Labrasol	0.1017

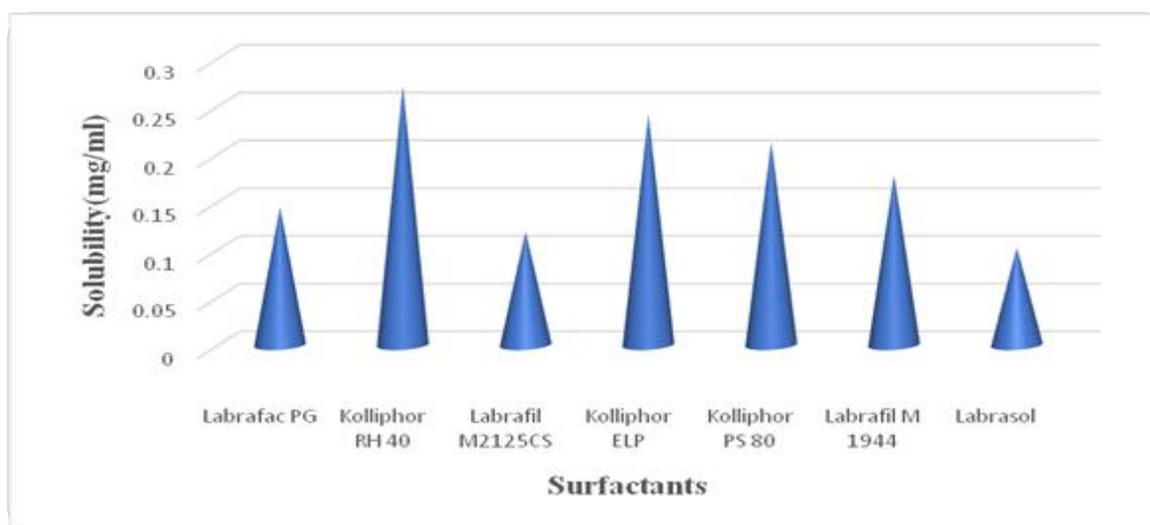
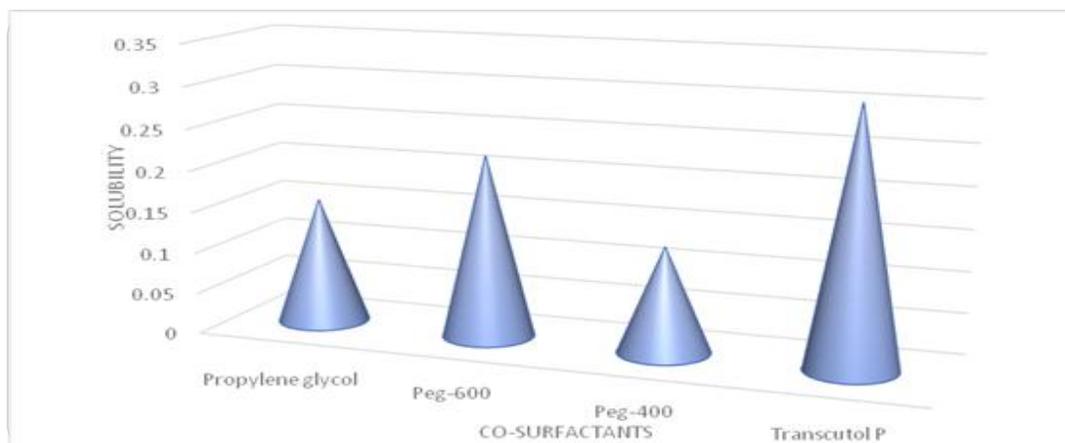


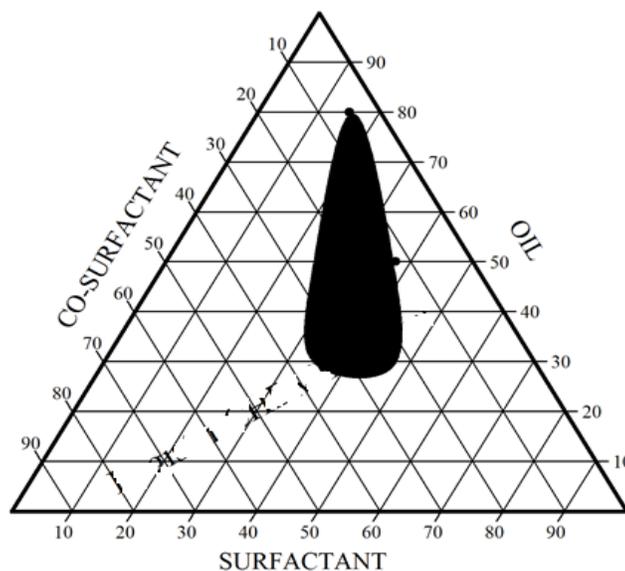
Figure 2: Solubility studies of Nateglinide in Surfactants

Table 4: Solubility of Nateglinide in Different Co-Surfactants

S.NO	CO-Surfactants	Solubility (mg/ml)
1	Propylene glycol	0.1526
2	Peg-600	0.2204
3	Peg-400	0.1272
4	Transcutol P	0.3052

**Figure 3: Solubility studies of Nateglinide in Co-surfactants****Pseudo Ternary Phase Diagram:**

From the solubility studies, Acrysol K 135, Kolliphor RH40 and Transcutol P were selected as oil, surfactant and co-surfactant respectively. From the phase diagram shown in **Figure 4**, it was observed that self-emulsifying region was enhanced with increasing concentrations of surfactant and co-surfactant with oil. Efficiency of self-emulsification was good when the surfactant concentration increased.

**Figure 4: Ternary phase diagram of Acrysol K 135, Kolliphor RH 40 and Transcutol P**

Visual Observation

With the use of visual observation method, the tendency of formation of emulsion was observed. Visual observation test was performed for different ratios by keeping the surfactant and co-surfactant ratio (Smix) as 1:1, 2:1 and 3:1. Grades were given to the ratios based on the tendency of formation of micro-emulsion. Ratios 2:8, 3:7, 4:6, 5:5, 6:4 of Smix 1:1 and 1:9, 2:8, 3:7, 4:6, 5:5 of Smix 2:1 and 5:5, 6:4, 7:3, 8:2, 9:1 of Smix 3:1 showed rapid formation of micro emulsion within a minute having a clear appearance. Therefore, these ratios were selected for the formulation of SNEDDS. The results are tabulated in **Tables 5 & 6** and pictorial representation of the visual observation test was shown in **Figure 5**.

Table 5: Visual observation test for Smix (surfactant: co-surfactant) ratio 1:1

Oil:Smix	Time of self emulsification(Min)	Grade
1:9	<2	III
2:8	<2	III
3:7	<1	I/II
4:6	<1	I
5:5	<1	I
6:4	<1	I
7:3	<1	I
8:2	<2	III
9:1	<2	III

Table 6: Visual observation test for Smix (surfactant: co-surfactant) ratio 2:1

Oil:Smix	Time of self emulsification(Min)	Grade
1:9	<2	III
2:8	<2	III
3:7	<2	III
4:6	<1	I
5:5	<1	I/II
6:4	<1	I
7:3	<1	I
8:2	<1	I
9:1	<1	I/II

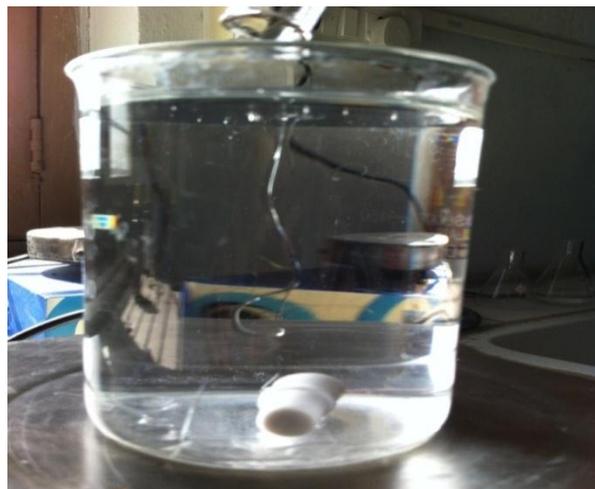


Figure 5: Visual observation test

Preparation of Nateglinide SNEDDS

SNEDDS of Nateglinide were prepared by using Acrysol K 135(oil), Kolliphor RH 40(surfactant) and Transcutol P (co-surfactant). In the present study, fifteen formulations were prepared and their complete composition was shown in **Table 1**. All the formulations prepared were found to be clear and transparent.

Thermodynamic Stability Studies

In thermodynamic stability study, no phase separation and effect of temperature variations on prepared formulations were observed. There was no change in the visual description of samples after centrifugation freeze-thaw cycles. Formulations which are thermodynamically stable only those were selected for further characterization and results are summarized in **Table 7**:

Table 7: Thermodynamic stability studies of the formulations

Formulation code	Centrifugation	Freeze thaw method	
		-20°C for 2 days	+40°C for 2 days
F1	No phase separation	No change	No change
F2	No phase separation	No change	No change
F3	No phase separation	No change	No change
F4	No phase separation	No change	No change
F5	No phase separation	No change	No change
F6	No phase separation	No change	No change
F7	No phase separation	No change	No change
F8	No phase separation	No change	No change
F9	No phase separation	No change	No change
F10	No phase separation	No change	No change
F11	No phase separation	No change	No change
F12	No phase separation	No change	No change
F13	No phase separation	No change	No change
F14	No phase separation	No change	No change
F15	No phase separation	No change	No change

% Transmittance Measurement

The clarity of microemulsion was checked by transparency, measured in terms of transmittance (%T). SNEDDS forms o/w microemulsion since water is external phase Formulation F13 has % transmittance value greater than 99.13%. These results indicate the high clarity of microemulsion. In case of other systems %T values were less than 99.13% suggesting less clarity of microemulsion. This may be due to greater particle size of the formulation. Due to higher particle size, oil globules may reduce the transparency of microemulsion and thereby values of %T. The results of %T are as shown in **Table 8**.

Table 8: % Drug Content, % Transmittance for different formulations of Nateglinide SNEDDS

S. No.	Formulation Code	Visual observation	% Transmittance	% Drug content
1	F1	Turbid	67.26	91.22
2	F2	Slightly clear	76.15	92.27
3	F3	Slightly clear	78.97	92.70
4	F4	Slightly clear	75.30	90.99
5	F5	Turbid	66.22	91.74
6	F6	Transparent	91.06	93.66
7	F7	Transparent	90.04	94.98
8	F8	Transparent	91.77	95.45
9	F9	Transparent	92.87	94.21
10	F10	Slightly clear	78.53	93.22
11	F11	Slightly clear	79.01	95.27
12	F12	Slightly clear	76.99	94.12
13	F13	Transparent	99.13	98.23
14	F14	Transparent	92.81	95.57
15	F15	Slightly clear	78.42	93.89

Drug Content of SNEDDS

Actual drug content of all 15 formulations are shown in **Table 8**. The drug content of the prepared SNEDDS was found to be in the range of 90.99 – 98.23 %. Maximum % drug content i.e. 98.23% was found in the formulation F13.

In-Vitro Dissolution Studies of SNEDDS:

The results of in vitro dissolution comparisons of SNEDDS formulations are summarized in **Tables 9, 10 and 11 and Figures 6, 7 and 8**. The faster dissolution from SNEDDS may be attributed to the fact that in this formulation, the drug is a solubilized form and upon exposure to dissolution medium results in small droplet that can dissolve rapidly in the dissolution medium. The release from liquid SNEDDS formulation F13 was faster than other SNEDDS formulations and pure drug substance indicating influence of droplet size on the rate of drug dissolution.

Table 9: In vitro dissolution studies of formulations F1 to F5:

Time (min)	Dissolution media – Phosphate buffer pH 6.8 (% drug release)					
	Pure drug	F1	F2	F3	F4	F5
0	0	0	0	0	0	0
2	4.47±0.115	08.05±0.15	11.21±0.21	13.21±0.27	15.26±0.85	11.66±0.25
5	6.42±0.45	13.27±0.17	19.80±0.19	17.24±0.41	19.27±0.95	16.55±0.17
10	9.18±0.14	23.35±0.12	21.99±0.81	26.24±0.24	28.27±0.91	24.66±0.55
15	13.24±0.51	32.06±0.15	33.24±0.24	36.24±0.13	39.17±0.74	42.74±0.22
20	17.59±0.24	40.25±0.51	43.27±0.19	46.27±0.28	49.33±0.15	51.52±0.66
25	22.46±0.12	53.22±0.26	53.24±0.27	59.27±0.89	60.22±0.71	58.24±0.83
30	27.77±0.34	64.24±0.84	68.67±0.27	71.26±0.56	73.17±0.81	75.55±0.67
45	32.24±0.17	71.35±0.43	74.27±0.61	79.68±0.23	80.55±0.87	82.55±0.30
60	38.99±0.48	80.24±0.25	84.07±0.24	86.65±0.11	89.22±0.74	86.24±0.62

Table 10: Dissolution profiles of formulations F6 to F10

Time (min)	Dissolution media – Phosphate buffer pH 6.8 (% drug release)					
	Pure drug	F6	F7	F8	F9	F10
0	0	0	0	0	0	0
2	4.47±0.115	12.09±0.25	10.54±0.71	14.91±0.17	15.6±0.85	16.66±0.25
5	6.42±0.45	19.22±0.67	21.01±0.79	20.11±0.91	19.27±0.95	21.05±0.17
10	9.18±0.14	24.21±0.42	27.11±0.21	25.22±0.44	28.27±0.91	24.66±0.55
15	13.24±0.51	33.27±0.75	31.01±0.24	37.22±0.13	39.17±0.74	40.09±0.22
20	17.59±0.24	41.55±0.91	43.07±0.19	47.21±0.28	50.27±0.45	52.47±0.66
25	22.46±0.12	55.42±0.16	53.24±0.27	55.21±0.89	61.35±0.41	59.69±0.83
30	27.77±0.34	65.57±0.94	68.67±0.27	72.34±0.55	72.19±0.81	76.77±0.67
45	32.24±0.17	75.15±0.11	74.27±0.61	80.16±0.15	81.66±0.71	83.17±0.30
60	38.99±0.48	81.84±0.25	85.21±0.24	87.71±0.27	89.24±0.41	91.58±0.62

Table 11: Dissolution profiles of formulations F11 to F15:

Time (min)	Dissolution media – Phosphate buffer pH 6.8 (% drug release)					
	Pure drug	F11	F12	F13	F14	F15
0	0	0	0	0	0	0
2	4.47±0.115	10.12±0.11	13.24±0.15	15.69±0.47	15.6±0.85	16.66±0.25
5	6.42±0.45	18.15±0.77	21.55±0.04	24.57±0.20	19.27±0.95	21.05±0.17
10	9.18±0.14	23.66±0.41	25.44±0.55	31.22±0.41	28.27±0.91	25.36±0.55
15	13.24±0.51	34.19±0.75	39.47±0.78	46.24±0.32	39.29±0.74	41.79±0.22
20	17.59±0.24	44.02±0.91	48.13±0.46	51.27±0.20	51.27±0.45	52.47±0.66
25	22.46±0.12	55.42±0.16	59.24±0.83	61.27±0.60	61.35±0.41	59.69±0.83
30	27.77±0.34	65.57±0.94	67.28±0.97	70.55±0.30	73.14±0.81	77.77±0.67
45	32.24±0.17	75.15±0.11	83.26±0.20	89.66±0.71	83.66±0.71	84.57±0.30
60	38.99±0.48	81.84±0.25	93.58±0.61	97.84±0.78	89.24±0.41	92.98±0.62

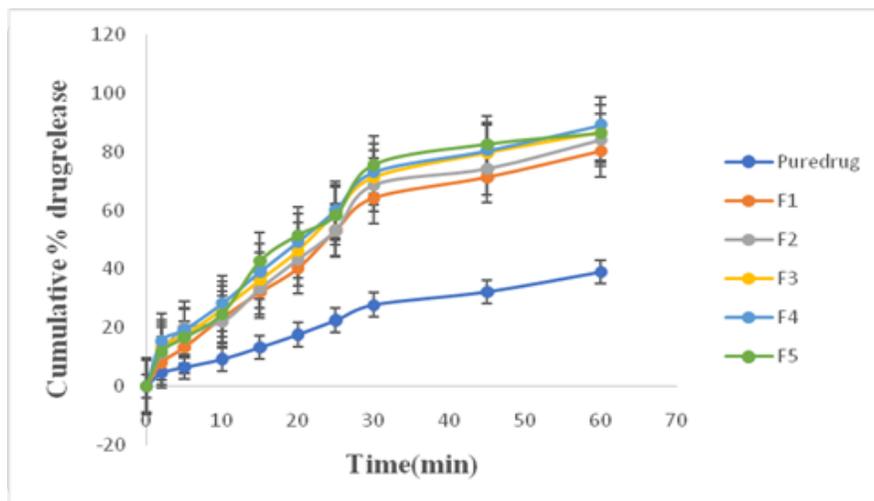


Figure 6: Dissolution profiles of Nateglinide pure drug and formulations (F1 to F5)

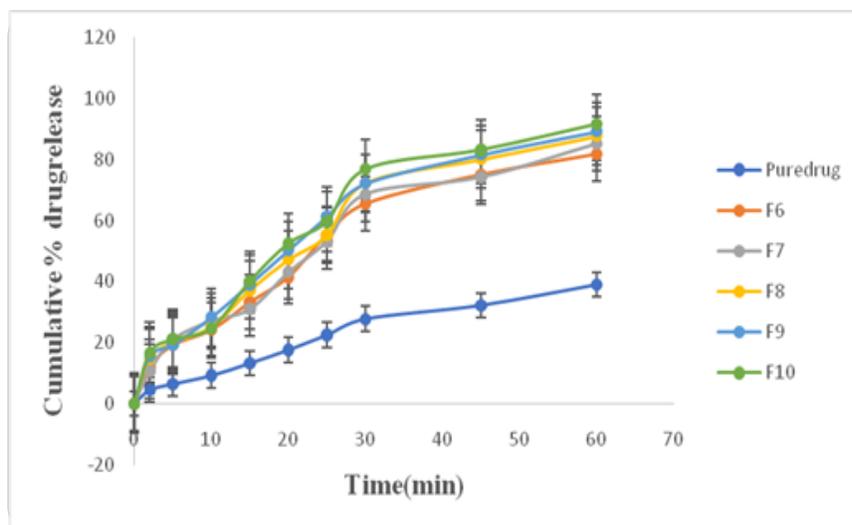


Figure 7: Dissolution profiles of Nateglinide pure drug and formulations (F6 to F10)

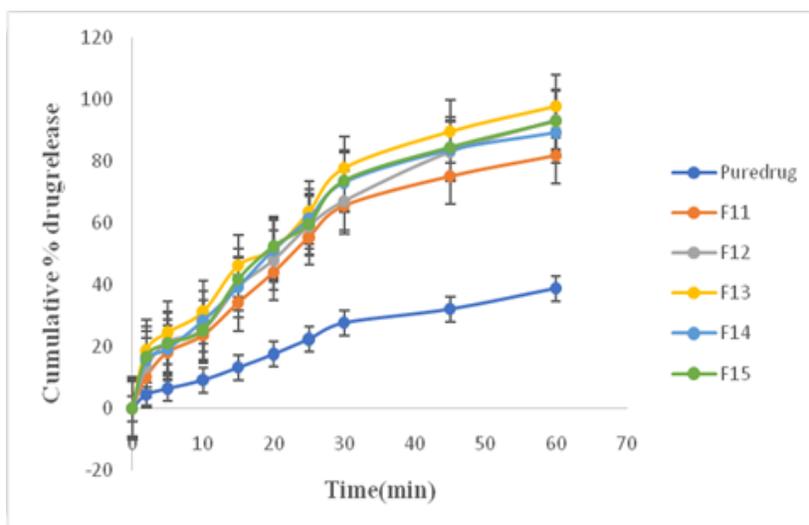


Figure 8: Dissolution profiles of Nateglinide pure drug and formulations (F11 to F15)

Characterization

Drug Excipient compatibility studies by FTIR Spectroscopy:

FT-IR spectrums are mainly used to determine if there is any interaction between the drug and any of the excipient used. The presence of characteristic absorption bands of Nateglinide and the SNEDDS containing Nateglinide suggest that there was no interaction between the drug and excipients used in the formulation shown in the **Figures 9 & 10**.

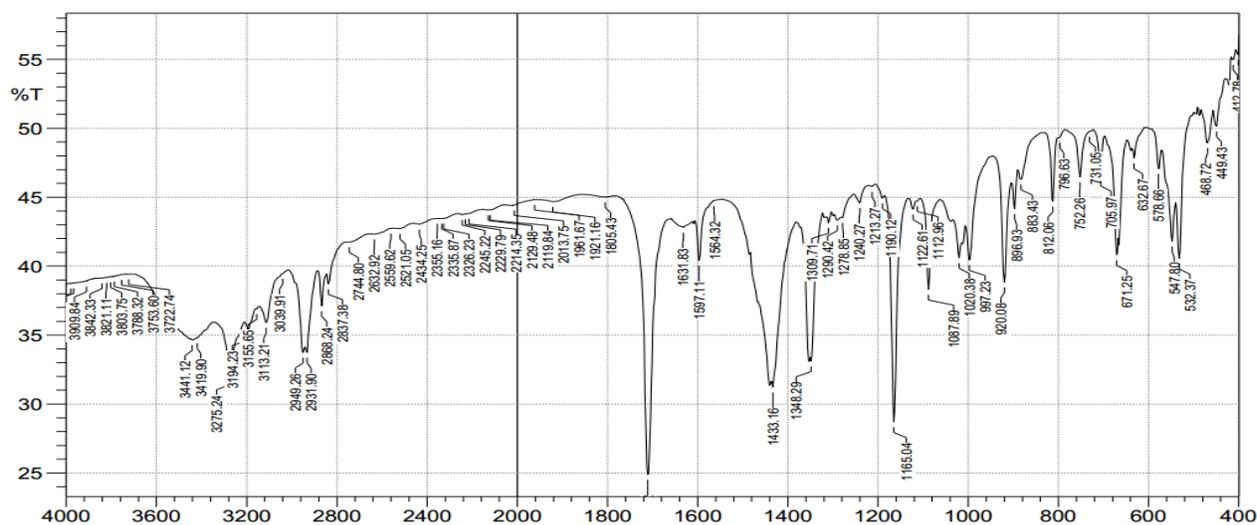


Figure 9: FTIR Spectroscopy of Nateglinide pure drug

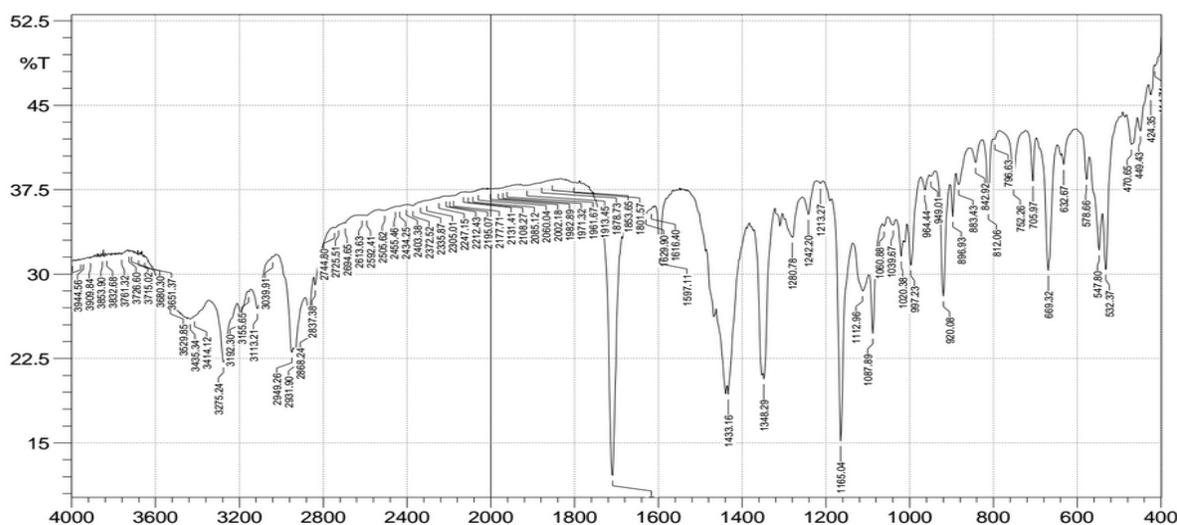


Figure 10: FTIR Spectroscopy of Nateglinide optimized formulation F13

Interpretation of FTIR Data

Particle size analysis of SNEDDS

The droplet size is the crucial factor in the SNEDDS performance because it determines the rate and extent of drug release as well as drug absorption. Moreover, it has been reported that the

smaller the particle size, the larger the interfacial surface area which may lead to more rapid absorption and improve the bioavailability. Systems with a mean droplet size below 200 nm fulfill the criteria of SNEDDS. The particle size of the emulsion is a crucial factor in self-emulsification performance because it determines the rate and extent of drug release as well as absorption. The particle size of the optimized SNEDDS formulation was found to be **74.6 nm** and Z-Average was found to be **43.1 nm**, indicating all the particles were in the nanometer range. **Figure 11** represents the particle size analysis of optimized SNEDDS formulation.

201701192347002.nsz

Measurement Results

Date : 19 January 2017 23:47:34
 Measurement Type : Particle Size
 Sample Name : 2-Netiglinide-SIZE
 Scattering Angle : 90
 Temperature of the holder : 25.0 deg. C
 T% before meas. : 29522
 Viscosity of the dispersion medium : 2.038 mPa.s
 Form Of Distribution : [Standard]
 Representation of result : Scattering Light Intensity
 Count rate : 3363 KCPS

Calculation Results

Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	0.40	8.0 nm	3.7 nm	7.6 nm
2	0.60	116.1 nm	42.1 nm	111.6 nm
3	--	-- nm	-- nm	-- nm
Total	1.00	73.1 nm	62.2 nm	111.6 nm

Histogram Operations

Size (Median) : 74.6 nm
 Mode : 111.6 nm
 % Cumulative (1) : 10.0 (%) - 5.1 (nm)
 % Cumulative (2) : 50.0 (%) - 74.6 (nm)
 % Cumulative (3) : 90.0 (%) - 159.8 (nm)
 % Cumulative (4) : 30.0 (%) - 10.3 (nm)
 % Cumulative (5) : 40.0 (%) - 47.3 (nm)
 % Cumulative (6) : 50.0 (%) - 74.6 (nm)
 % Cumulative (7) : 20.0 (%) - 7.3 (nm)
 % Cumulative (8) : 70.0 (%) - 109.3 (nm)
 % Cumulative (9) : 95.0 (%) - 183.4 (nm)
 % Cumulative (10) : 100.0 (%) - 8510.6 (nm)

Cumulant Operations

Z-Average : 43.1 nm
 PI : 0.550

Figure 11: Particle size analysis of optimized formulation F13

Zeta Potential of SNEDDS

Zeta potential has got practical application in the stability of emulsion since it governs the degree of repulsion between adjacent, similarly charged and dispersed droplets. In general, the zeta potential value of ± 30 mV is sufficient for the stability of a micro emulsion. The zeta potential of the optimized SNEDDS formulation was found to be **-18.4 mV** which comply with the

requirement of the zeta potential for stability. **Figure 12** represents the particle size analysis of optimized SNEDDS formulation.

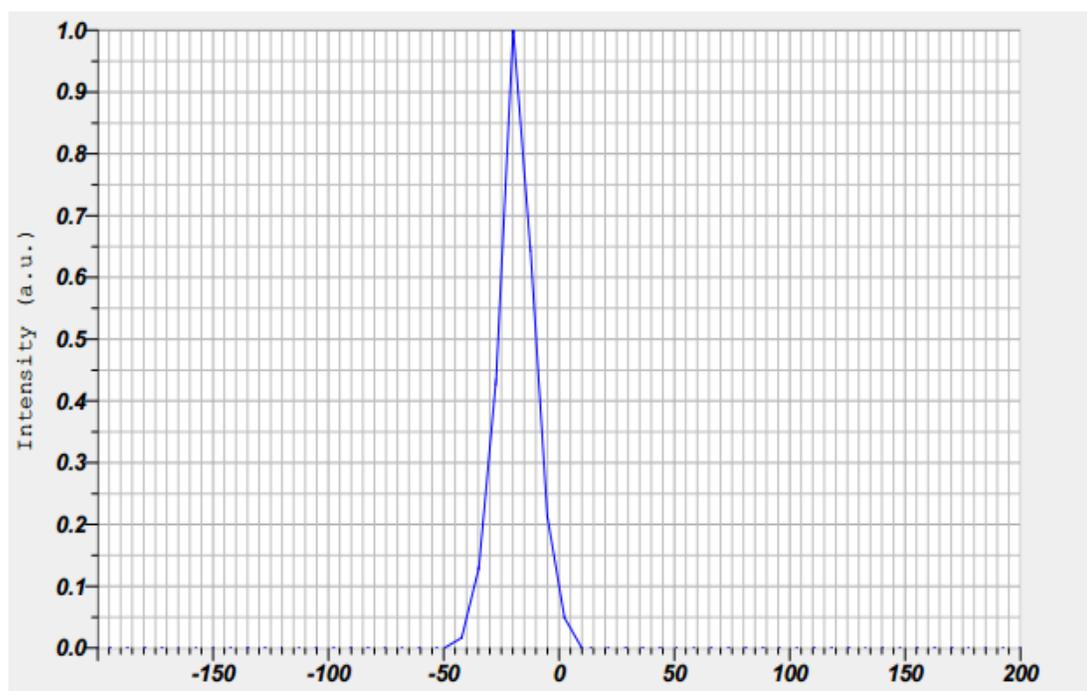
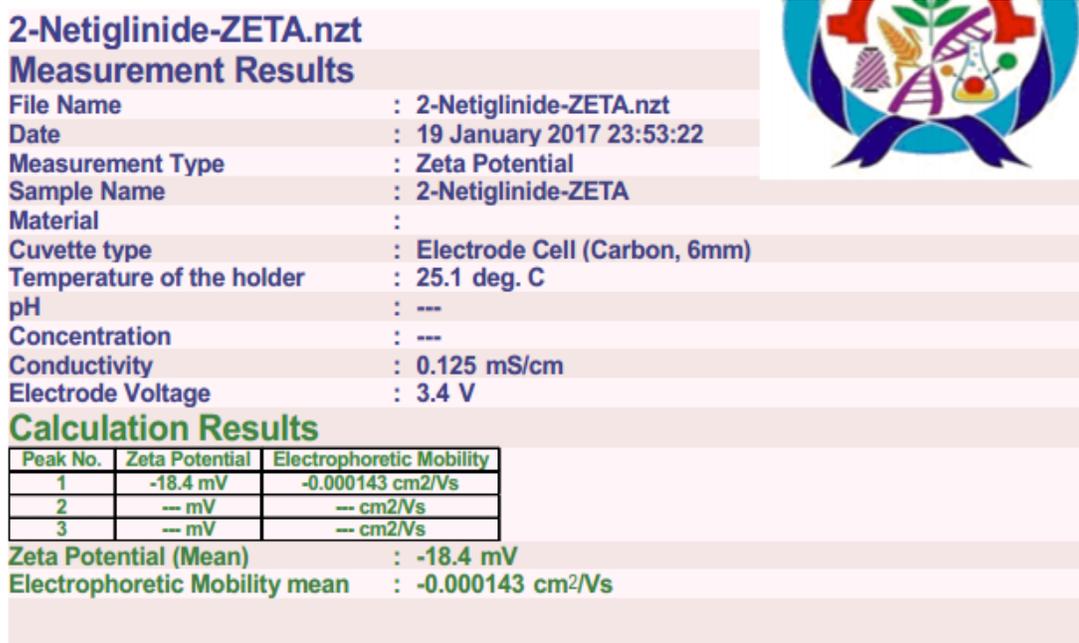


Figure 12: Zeta potential of the optimized formulation F13

Scanning Electron Microscopy (SEM) For Nateglinide SNEDDS

Scanning electron microscope studies of optimized formulation (F13) revealed that oval shaped globule. The size is within nanometers (**Figure 13**). There are no pores observed which shows a clear liquid droplet.

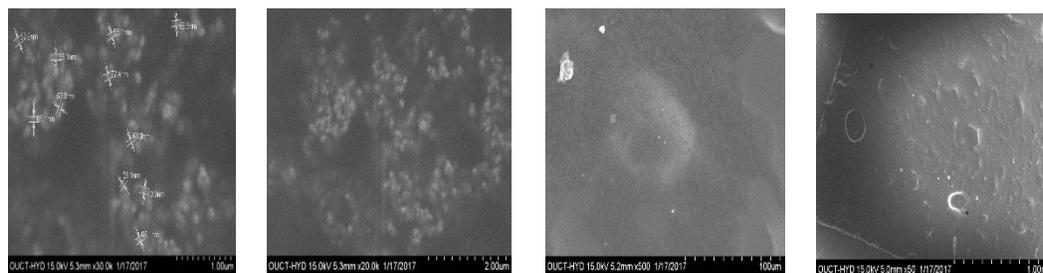


Figure 13: Scanning Electron Microscopy images of Nateglinide optimized formulations (F13)

Stability studies

The Nateglinide SNEDDS were put into hard gelatin capsules as the final dosage form. The formulation (F13) was subjected to stability studies for 6 months. There was no significant change in the drug content, drug release. It was also seen that the formulation was compatible with the hard gelatin capsule shells, as there was no sign of capsule shell deformation. There was no significant change in the appearance, or micro emulsifying property. Thus, these studies confirmed that the formulation was stable and its compatibility with hard gelatin capsules.

CONCLUSION

In this work a SNEDDS formulation of a poorly water-soluble drug, Nateglinide was developed by using different polymers. The solubility study was conducted to find out the suitable oil, surfactant and co-surfactant for Nateglinide and was shown good solubility in Acrysol K 135(oil), Kolliphor RH 40 (surfactant) and Transcutol P (co-surfactant). From Pseudo ternary phase diagram with Acrysol K 135 as oil, Kolliphor RH 40, Transcutol P as a surfactant and co-surfactant, it was observed that self-emulsifying region was enhanced with increasing concentrations of surfactant and co-surfactant with oil. The drug content of all the formulations was performed. Maximum drug content was found in the formulation F13. The *in vitro* dissolution studies were performed for all the fifteen SNEDDS formulations. The release from liquid SNEDDS formulation F13 was faster than other formulations indicating the influence of droplet size on the rate of drug dissolution. Based on visual observation test and faster dissolution rate, formulation F13 was finalized as optimized formulation. The particle size of the optimized SNEDDS formulation was found to be 74.6 nm and Z-Average was found to be 43.1 nm, indicating all the particles were in the nanometer range and the zeta potential of the optimized SNEDDS formulation was found to be -18.4 mV. To get evidence on the possible interactions of drug with the carrier, FTIR analysis was used. The optimized formulation displayed the characteristic peaks at wave numbers nearer to that of pure Drug. There was no alteration in the characteristic peaks of Nateglinide suggesting that there was

no interaction between the drug and polymers. Scanning Electron Microscopy (SEM) studies revealed that the morphology of formed SNEDDS particles appeared as spherical particles having an even and a smooth surface. The present exploratory work successfully illustrates the potential utility of SNEDDS formulation for the delivery of poor water-soluble compounds such as Nateglinide. The results from this study demonstrate the utility of SNEDDS to enhance solubility and dissolution of poorly soluble compounds like Nateglinide which may result in improved therapeutic performance.

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