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Development and Optimization of Nateglinide Solid Dispersions By Design of Experiment

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ABSTRACT

Nateglinide is an anti-diabetic drug that lowers the blood glucose levels by stimulating insulin secretion from pancreas. Because of low solubility and bioavailability, its usage is limited. In the present study solid dispersions of Nateglinide were prepared by solvent evaporation method and evaluated through various steps for biological correlation. Nateglinide solid dispersions were prepared using PEG 6000, Pluronic F 127 and Labrafil M 1944. A 3-factor, 3-level Central composite design employed to study the effect of each independent variable on dependent variables. X-ray diffraction was used to analyze the crystallinity and FTIR was used to analyze the drug and excipient compatibility. Scanning electron microscopy was performed to analyze the surface of solid dispersion samples. The correlation coefficient showed that the release profile followed Higuchi model (R2= 0.95836). From Korsmeyer peppas model, the release exponent, n was found to be 0.80635 ($0.43 < n < 0.85$) and followed anomalous behaviour and hence release mechanism was indicative of diffusion. From in vitro dissolution studies it was proved that a Nateglinide solid dispersion may achieve good formulation capability for pharmaceutical manufacturer by increasing solubility and dissolution rate.

Key words: Nateglinide, Diabetes mellitus, solid dispersions, solubility, Central composite

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INTRODUCTION

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Diabetes mellitus is a metabolic disorder characterized by high blood glucose level as a result of improper or insufficient insulin secretion, insulin action or both[1]. Nateglinide is being used extensively as an oral anti-diabetic agent used for the management of diabetes mellitus. Upon oral administration, Nateglinide increases pancreatic β cell sensitivity to ambient glucose without increasing basal insulin secretion. Chemically Nateglinide is (-)-N-[(trans-4-isopropylcyclohexyl) carbonyl]-D-phenylalanine [2, 3]. Nateglinide has a short half-life of 1.5 hrs, peak plasma concentration at 0.5 to 1 hr and metabolized by cytochrome P 450 system[4].

Nateglinide is practically insoluble in water. Numerous formulation methods have been developed to increase its solubility and prolong the duration of action of this drug. Solid dispersion formulation by solvent evaporation method is easy to scale up and stabilize. Hence this study was carried out to fabricate and optimize solid dispersions of Nateglinide and overcome the limitations of drug delivery via conventional methods.

The solubility and bioavailability of drugs can be enhanced using various polymers. Polyethylene glycol (PEG) is the most commonly used excipient to increase solubility[5]. With a HLB value of 19, PEG exhibits excellent water solubility and varies significantly in molecular weight, ranging from 200 to >300,000. The molecular size of favors the formation of interstitial solid solutions. It is often employed as vehicles due to their low toxicity, low melting point, rapid solidification rate, high aqueous solubility, availability in various molecular weights, economic cost, and physiological tolerance[6]. Pluronic F 127 is a non-ionic bioreagent. It was used to enhance the solubilization of compounds with low aqueous solubility like ketoconazole[7] and itraconazole[8] previously. Labrafil M 1944 is polyglycolyzed glyceride used in order to enhance the stability of solid dispersions and prolonging the half-life of drugs.

Usually to develop a formulation, conventional experiments need more time and materials, so the target was to design a pharmaceutical formulation in shorter time and with minimum trials and maximum penetration rate[9]. Response surface method (RSM) is the most accepted method in drug development which aims at approximate regression model that is closest to the actual regression model. In the present study solid dispersion formulation was developed as an approach to illustrate the interactions caused by three different factors - Concentrations of PEG 6000, Labrafil M 1944 and Pluronic F127 by the aid of Box-Behnken experimental design. The responsibility of each factor towards drug solubility has been defined and the results have been discussed statistically.

MATERIALS AND METHOD

Materials

Nateglinide was a generous gift from Aurobindo Pharma Ltd., Hyderabad. Pluronic F127, Oleic acid, Gelucire 44/14, Labrasol, Labrafac PG and Transcutol P were also obtained from Aurobindo Pharma Ltd. Kolliwax GMS II was procured from Signet Chemical Corp. Pvt. Ltd, Mumbai. Ethanol, HCl, Labrafil M 1944, Propylene glycol, PEG 8000 and PEG 6000 were obtained from SDFCL, Mumbai. Kolliphor RH-40 and Captex were given by BASF, Mumbai. All other excipients used were of analytical grade.

Methods

Preliminary Solubility Studies

Drug solubility studies were performed in triplicate by adding excess amounts of nateglinide water soluble carriers mentioned in **Table 4** with 1:1 ratio and solutions containing flasks were kept on a rotary shaker at constant speed at were kept on a rotary shaker at constant speed at 25 °C ± 2 °C for 24 h. The saturated solutions after equilibration for 24 hr were filtered through a membrane filter having pore size of 0.45 µm. Filtrates were suitably diluted and estimated using UV spectrophotometer at 220 nm. Simultaneously an excess amount of nateglinide was added to 25ml of aqueous solution of water soluble carriers in the various ratios in screw capped bottles. Samples were shaken for the 24 hours at room temperature. Subsequently, the suspensions were filtered through a Whatman filter paper no 1. Filtered solution analyzed for the Nateglinide using UV method[11].

Table 1: Solubility studies of Nateglinide in different polymers

Sample (Physical mixtures)	Drug & Polymer ratios	Solubility (µg/ml)
Pure Drug	-	6.23 ± 0.072
Drug: Pluronic F 127	1:1	55.87±0.21
Drug: Oleic acid	1:1	18.56±0.019
Drug: Captex	1:1	17.61±0.011
Drug: Gelucire 44/14	1:1	27.25±0.089
Drug: Kolliphor RH 40	1:1	14.215±0.023
Drug: Kolliwax GMS 2	1:1	17.067±0.067
Drug: PEG 6000	1:1	42.72 ±0.045
Drug: PEG 8000	1:1	32.31±0.056
Drug: Transcutol P	1:1	21.382±0.078
Drug: Propylene Glycol	1:1	18.536±0.081
Drug: Labrasol	1:1	21.872 ± 0.081
Drug: Labrafac PG	1:1	37.632 ± 0.0132
Drug: Labrafil M 1944	1:1	49.34 ± 0.027

Phase Solubility Studies

The aqueous solubility of nateglinide in water was determined at increasing concentrations of different carriers. The solubility method of Higuchi and Connors was used. An excess amount of nateglinide was added into the screw capped amber vials of aqueous solutions of each carrier in 50 mM citric acid/100 mM sodium phosphate dibasic buffer with a pH of 4.5 containing increasing concentrations of the individual carrier (i.e., 0.1%, 0.25%, 0.5%, 0.75% and 1% w/v). The vials were rotated at 60 rpm while being kept at 37 °C. After equilibrium was reached (24 h), the solutions were filtered through 0.45 µm cellulose acetate filters and analyzed spectrophotometrically (Shimadzu 1601, Japan) at 220 nm[12].

Drug-excipients compatibility study

The Drug-Excipients Compatibility Studies were performed in order to check and interaction between drug and excipients. Physical mixtures were prepared by grinding Nateglinide and individual polymeric carriers in a mortar. The FTIR spectra of drug sample and its physical mixtures were carried out by potassium bromide disc method using Tensor 27 FTIR Spectrophotometer (Bruker Optics, Germany) in the region of 4000 to 600 cm^{-1} [13].

Design of experiments

Initially, preliminary experiments (one factor at a time approach) were performed to determine the main factors and the appropriate ranges in which the optima lie. From the preliminary studies, it was observed that the concentration of polymers affects the formulations of solid dispersion. Based upon this, three factors were selected: Further, the effects of three factors (Concentrations of PEG 6000, Labrafil M 1944 and Pluronic F127) on % Cumulative drug release (%CDR) and solubility were tested. Through preliminary screening the concentrations of PEG 6000, Labrafil M 1944 and Pluronic F127 were identified as the most significant variables within the range of 35-45 mg, 25-35 mg, and 20-30 mg, respectively. On the basis of the preliminary trials a 3-factor, 3-level rotatable central composite design (CCD) was employed to study the effect of each independent variable on dependent variables (% CDR, and solubility). The range of level of each independent variable was set according to the preliminary experiments and is listed in **Table 1.** On the basis of the central composite design model provided by Stat-Ease Design Expert® software V8.0.1, 20 model experiments were randomly arranged. For all the experiments the concentration of drug and the volume of the solvent were kept constant. The experiments were conducted as for the design and the obtained responses for the dependent variables (% CDR, and solubility) were presented in **Table 1.**

Table 1: List of dependent and independent variables in central composite design

Independent variables			**Levels**			
Variable	Name	Units	-1	+1	-α	+α
A	Concentration of PEG 6000	Mg	35	45	31.59	48.41
B	Concentration of Labrafil M 1944	Mg	25	35	21.591	38.41
C	Concentration of Pluronic F127	Mg	20	30	16.59	33.41
Dependent variable			Goal			
Y1	CDR	%	Maximize			
Y2	Solubility	µg/ml	Maximize			

Regression analysis:

The targeted response parameters were statistically analyzed using Stat-Ease Design Expert ® software V8.0.1 by applying one-way ANOVA at 0.05 levels. The individual parameters were evaluated using the F test and quadratic models of the form $Y = \beta + \beta_1X_1 + \beta_2X_2 + \beta 3X_1X_2 + \beta_4X_1^2 + \beta_5X_2^2$ were generated for each response parameter using multiple linear regression analysis (MLRA), where Y is the level of the measured response; β is the intercept β_1 to β_2 are the regression coefficients. X_1 and X_2 stand for the main effects[14]. $X_1X_2$is the interaction between the main effects; X_1^2 and X_2^2 are the quadratic terms of the independent variables that were used to simulate the curvature of the designed sample space. The value of coefficients reflected the effect of independent variables and their interaction on the dependent variables. A positive coefficient indicates a synergistic effect; meanwhile, a negative one reflects an antagonistic effect. The significance of individual coefficients was determined by ANOVA test, and one was considered significant if the p value was $\leq$ 0.05.A backward elimination procedure was adopted to fit the data into different predictor equations[15]. The quadratic models generated by regression analysis were used to construct the 3-dimensional graphs in which response parameter Y was represented by a curvature surface as a function of X.

For the purpose of finding the best compromising formulation for all responses, the multi criteria problem can be treated as a single criterion problem by using the desirability function approach[16]. The desirability function combines all the responses into one variable to predict the optimum levels for the independent variables. A desirability value of 0 represents an unacceptable value for the responses, and a value of 1 represents the most desired value for the responses[17]. The optimized formulation was obtained which have the maximum % CDR and solubility. The optimized batch(s) was further investigated by XRD and SEM.

Preparation of Nateglinide solid dispersions

Solid dispersions containing nateglinide were prepared by solvent evaporation method. The polymer mixture consisting of PEG 6000, Labrafil M 1944 and Pluronic F127 was accurately

weighed and dissolved in absolute ethanol to get a clear solution. Accurately weighed amount of drug was dissolved in the polymer solution and stirred on a magnetic stirrer (model 2 MLH, Remi motors, Mumbai) at room temperature for duration of 4 to 6 h[18]. The solvent was removed under reduced pressure (100 mm Hg) in a rotary flash evaporator (Buchi type, Servewell instruments Inc., Bangalore) at an external maximum temperature of 40 °C. The solid residue obtained was dried in a vacuum oven (Lab model, Servewell instruments Inc., Bangalore) at 30 °C for 24 h. The powder was passed through #44 mesh and stored in closed glass container. Twenty batches of solid dispersions (F1 to F20) were prepared as per experimental design by varying the polymer concentrations and maintaining the other experimental conditions constant[19, 20].

Determination of solubility of solid dispersions

The saturation solubility studies were carried out to determine the solubility of pure drug and solid dispersions. Weighed amount of solid dispersions were added to 250 ml conical flasks containing 25 ml of distilled water. The sealed flasks were shaken for 24 hrs at 37±0.5ºC. Then aliquots were filtered through Whatman filter paper. The concentration of Nateglinide was determined by UV spectrophotometer at 220 nm [21].

Drug content of solid dispersions

Solid dispersions equivalent to 2 mg drug were taken and dissolved in minimum quantity of methanol and volume was made up to 50 ml. From this solution, 5 ml was taken and again diluted with methanol up to 50 ml. The samples were filtered through Whatman filter paper 0.45 µm. The filtrate was analyzed using a UV spectrophotometer at 220 nm against a blank after appropriate dilutions (Model UV-1700, UV Visible spectrophotometer, Shimadzu, Kyoto, Japan). The polymers did not interfere with the drug extraction and determination at the specified wavelength.

In vitro dissolution studies of solid dispersion

In vitro release studies of nateglinide solid dispersions (equivalent to 2 mg nateglinide filled in hard gelatin capsule) were performed using USP XXII tablet dissolution test apparatus at the basket rotation speed of 75 rpm in 900 ml 50 mM citric acid/100 mM sodium phosphate dibasic buffer with a pH of 4.5 at 37 ºC[22, 23]. At the specified time intervals, 5 ml of samples were withdrawn, filtered and assayed for nateglinide using specrophotometry method by measuring the absorbance at 220 nm. An equal volume of dissolution medium kept at the same temperature was added to maintain the sink conditions. Dissolution profiles of the formulations were analyzed by plotting drug release versus time[24].

Drug release kinetics

To elucidate the mode and mechanism of drug release, the data from the in-vitro release study were fitted into various kinetic models, like zero order, first order, Higuchi's and Korsmeyer Peppas model[25].

X-Ray powder diffraction Studies (XRPD)

X-ray powder diffraction patterns of nateglinide and optimized formulations were recorded on X-ray diffractometer (Bruker D8 Advance) at a scan rate of 5 °/ min in the 2θ range from 2.5° - 60°.

Scanning electron microscopy

SEM (Jeol JSM 5600 LV, Jeol, Tokyo, Japan) was used to visualize the external and surface morphology of plain nateglinide and optimized SD formulation. The samples were coated with the platinum sputter coater 208 HR (Cressington Scientific Instruments Ltd., Watford, UK) to assure conductivity.

Stability Studies

Stability testing is an integral part of formulation development. It provides evidence on how the quality of a drug substance or a drug product varies with time under the influence of a variety of environmental factors. It establishes a re-test period for the drug substance or a shelf-life for the drug product and is used to recommend storage condition. The selected optimized formulations were packed in amber colored bottles, which were tightly plugged with cotton and capped with aluminum. They were then stored at 25ºC / 60% RH, 30ºC / 65% RH, & 40ºC / 75% RH for 6 months and evaluated for their drug content and dissolution study.

Data analysis

Data are expressed as the means ± standard deviation (SD) of the mean and statistical analysis was carried out employing the one-way analysis of variance (ANOVA). A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

FTIR Spectroscopy

The principal peaks were observed at wave numbers- 700.03, 756.92, 1240.97, 1540.85, 1649.80, 1713.44, 2859.92, 2924.52 and 3299.61 confirming the purity of drug as per established standards.

Preliminary solubility studies of Nateglinide

Solubility measurements of Nateglinide were performed according to a published method (Higuchi and Connors 1965). Solubility of nateglinide in water was found to be 0.0623 mg/ml indicating it is practically insoluble in water. Nateglinide showed pH-dependent solubility at physiological pH

range, which gradually increased from acidic to alkaline pH. At 25°C, aqueous solubility of Nateglinide was found to be increasing with physical mixtures of different polymers (1:1 ratio). The solubility values are as shown in Table 4. Among all the carriers PEG 6000, Labrafil M 1944 and Pluronic F 127 have shown maximum increase in solubility.

Selection of Polymers

Based on the solubility parameters, PEG 6000, Labrafil M 1944 and Pluronic F 127 are selected for the formulation of Nateglinide solid dispersions.

Drug-excipients compatibility study

In order to test for possible intermolecular interaction between nateglinide and the excipients of the dispersion matrix, FTIR was carried out. When the FTIR spectrum of pure Nateglinide is taken into account it showed an absorption band at 2924.52 cm^{-1} (aliphatic C-H stretching; asymmetric), 2859.92 cm^{-1} (aliphatic CH stretching; symmetric), 1649.80 & 1713.44 cm^{-1} (C=O stretching for Ketone). Conformation of C-O stretching OH bending of carboxylic acid spectra was given by the band at 1240.97 cm^{-1} owing to hydrogen bonded O-H of COOH. The peak at 3299.61 cm^{-1} is attributed to secondary amide (-NH stretching). The absorption band at 1540.85 cm^{-1} corresponds to alkene C=C stretching bonds. The sharp band at 756.92 cm^{-1} & 700.03 cm^{-1} indicates the mono-substituted benzene. The FTIR spectrum of nateglinide with Labrafil M 1944, poloxamer 188 and PEG6000 showed all the peaks for Nateglinide (**Figure 1, 2**). There is no significant change in the absorption bands; hence no interaction was observed between them. However, some additional peaks were observed with physical mixtures, which could be due to the presence of polymers.

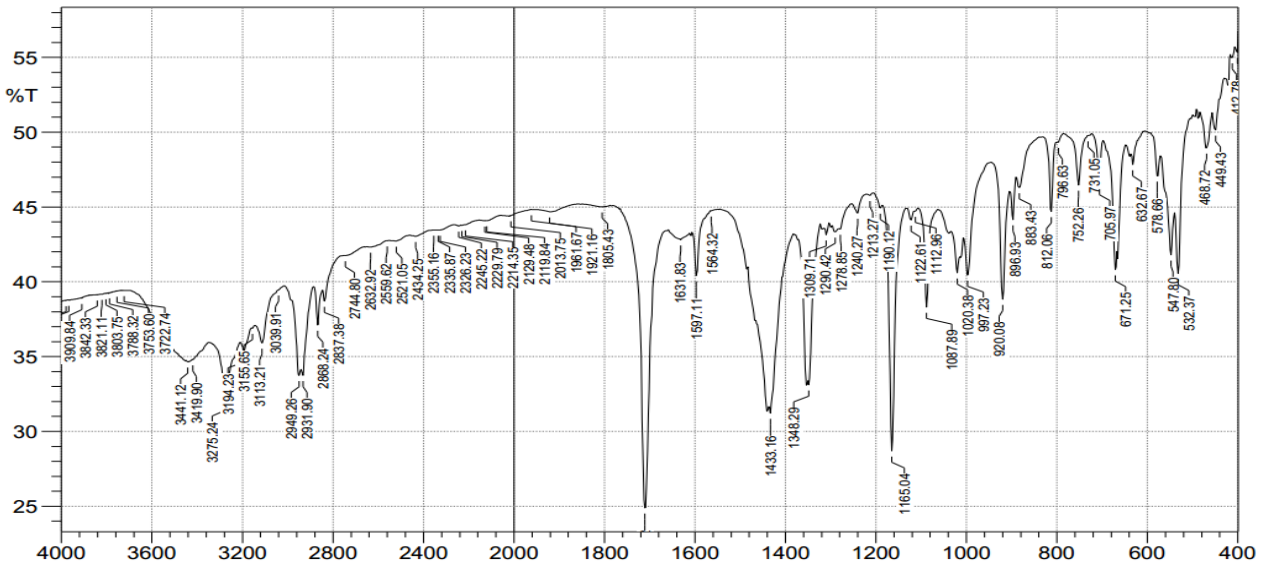


Figure 1: FTIR Spectrum of Nateglinide pure drug

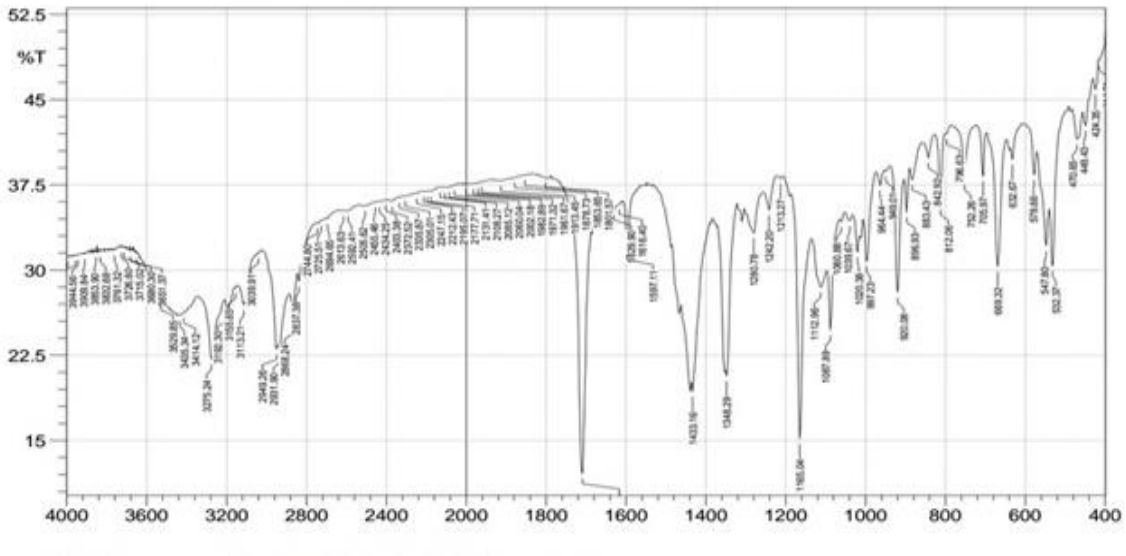


Figure 2: FTIR spectrum or Nateglinide physical mixture with polymers

Phase Solubility Studies

Phase-solubility diagrams (**Figure 3**) show that the concentration of nateglinide in pH 4.5 buffer increases as a function of increase in the concentration of PEG 6000, Labrafil M 1944 and Pluronic F127. All the curves obtained in the present studies are AL type because of linear increase in solubility with the value of R^2 closed to unity. Hydrophilic carries are known to interact with drug molecules mainly by electrostatic forces and occasionally by other type of forces like hydrogen bonds. The solubility enhancement of nateglinide with various carries was found to be in the order of Pluronic F127> Labrafil M 1944> PEG 6000. At 1% w/v concentration of the polymer, Pluronic F127 showed 10-fold enhancements in the solubility of pure drug, attributable to be the micelle solubilization of drug.

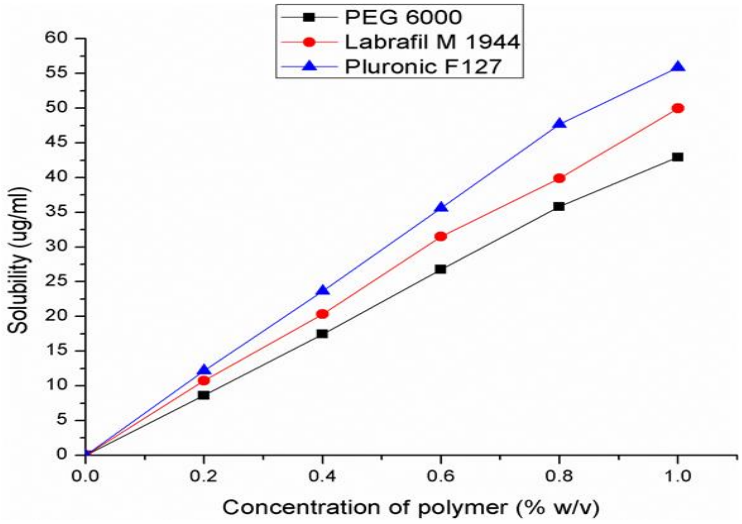


Figure 3: Phase Solubility Diagrams of Nateglinide

Optimization of formulation variables

Through preliminary experiments the Concentration of PEG 6000 (A), Concentration of Labrafil M 1944 (B) and Concentration of Pluronic F127 (C) were identified as the most significant variables influence the % Cumulative drug release and solubility. The formulations were further optimized by considering the parameters like maximum CDR, and maximum solubility (**Table 3**).

Twenty experiments were required for the response surface methodology based on the Central composite design. Based on the experimental design, the factor combinations yielded different responses as presented in **Table 2**. These results clearly specify that the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among all the 20 batches. Data were analyzed using Stat-Ease Design Expert ® software V8.0.1 to obtain analysis of variance (ANOVA), regression coefficients and regression equation. Mathematical relationships were generated using multiple linear regression analysis for the mentioned variables. These equations represent the quantitative effect of Concentration of PEG 6000 (A), Concentration of Labrafil M 1944 (B) and Concentration of Pluronic F127 (C) and their interaction on % CDR (Y1) and solubility (Y2). The values of the coefficients of A, B and C are related to the effect of these variables on the responses Y1 and Y2. Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationship respectively. A positive sign represents synergistic effect, while a negative sign indicates antagonistic effect. A backward elimination procedure was adopted to fit the data to the quadratic model. Both the polynomial equations were found to be statistically significant ($P \leq 0.01$), as determined using ANOVA, as per the provisions of Design Expert software.

Table 2: Factor combinations and response parameters of solid dispersions of nateglinide prepared as per central composite design

Run	Concentration of PEG 6000	Concentration of Labrafil M 1944	Concentration of Pluronic F127	% CDR	Solubility (µg/ml)
1	45	25	20	89.74	48.12
2	40	30	25	91.8	53.73
3	35	35	20	87.12	47.72
4	40	30	25	91.42	54.78
5	45	35	20	77.12	31.46
6	45	25	30	83.46	38.66
7	40	30	25	91.76	53.84
8	40	30	25	91.67	53.65
9	40	30	25	91.12	53.22
10	40	21.59104	25	97.56	54.78
11	31.59104	30	25	98.72	56.23
12	35	25	20	93.69	48.36

13	45	35	30	76.37	35.12
14	35	35	30	85.32	39.72
15	40	30	25	91.92	52.78
16	40	30	16.59104	81.56	40.35
17	40	38.40896	25	83.52	51.43
18	40	30	33.40896	71.12	29.18
19	35	25	30	86.46	38.37
20	48.40896	30	25	79.36	46.72

Table 3: Optimization of Nateglinide solid dispersion.

Constraints			
Name	**Goal**	**Lower limit**	**Upper Limit**
Concentration of PEG 6000	In range	31.59	48.41
Concentration of Labrafil M 1944	In range	21.591	38.41
Concentration of Pluronic F127	In range	16.59	33.41
% CDR	Maximize		
Solubility	Maximize		
SOLUTION (Desirability value 0.935)			
Concentration of PEG 8000	**Concentration of Labrafil M 1944**	**Concentration of Pluronic F127**	**% CDR**	**Solubility (µg/ml)**
37.28	25.00	23.67	96.9321	54.4569

The polynomial equation, describing the % CDR as a simultaneous function of Concentration of PEG 6000 (A), Concentration of Labrafil M 1944 (B) and Concentration of Pluronic F127 (C), is as shown in equation 1.

$$\%CDR\ (Y1) = 91.30 - 4.28A - 3.74B - 2.46C - 1.50AB + 1.37NC - 0.88A^2 - 5.37C^2 \quad (1)$$

The equation represents the quantitative effect of process variables (A, B and C) and their interaction on the response Y1. The values of the coefficient A, B and C are related to the effect of these variables on the response Y1. Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationship respectively. The quadratic model generated revealed that A, B and C have significant negative effect on % CDR. An antagonistic interactive effect was observed between A and B. A Synergistic positive interactive effect was observed between B and C. Higher concentrations of A and C had a significant antagonistic effect on % CDR. The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). The ANOVA indicated a significant ($P < 0.05$) effect of factors on response. The Model F-value of 56.08 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, BC and $C^2$are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

Figure 4 a shows the main effects, interaction effects and quadratic effects of Concentration of PEG 6000 (A), Concentration of Labrafil M 1944 (B) and Concentration of Pluronic F127 (C) on percent cumulative drug release (Y1). The **Figure 4 a** clearly shows that Concentration of PEG 6000 (A), and Concentration of Labrafil M 1944 (B) have the main and the major effect on Y1. The Concentration of Pluronic F127 (C) has a moderate effect on Y1. A careful looking at the **Figure 4 a** we can see that the concentration of PEG 6000 and Concentration of Labrafil M 1944 (B) have the negative effect on % CDR. The % CDR was found to increase from 79.36 TO 98.72 as the concentration of PEG 6000 decreases from 48.408 mg to 31.591 mg. Similarly, the % CDR was found to increase from 83.52 to 97.56 as the concentration of Labrafil M 1944 decreases from 38.408 mg to 21.591 mg. On the other hand, the concentration of Pluronic F127 has the initial positive effect on % CDR at lower concentrations. The % CDR was found to increase from 81.56 to 98.72 as the concentration of Pluronic F127 increases from 16.591mg to 25 mg. But at higher concentrations Pluronic F127 was found to have negative effect on % CDR. The Concentration of PEG 6000 (A) and Concentration of Labrafil M 1944 (B) were found to have significant negative interactive effect on % CDR. Whereas the Concentration of Labrafil M 1944 (B) and the concentration of Pluronic F127 (C) were found to have significant positive interactive effect on %CDR. Three-dimensional response surface plots and corresponding contour plots to study the effect of A and B and their interaction on % CDR (Y1) at a middle level of C were presented in Figure 4 (a and b).

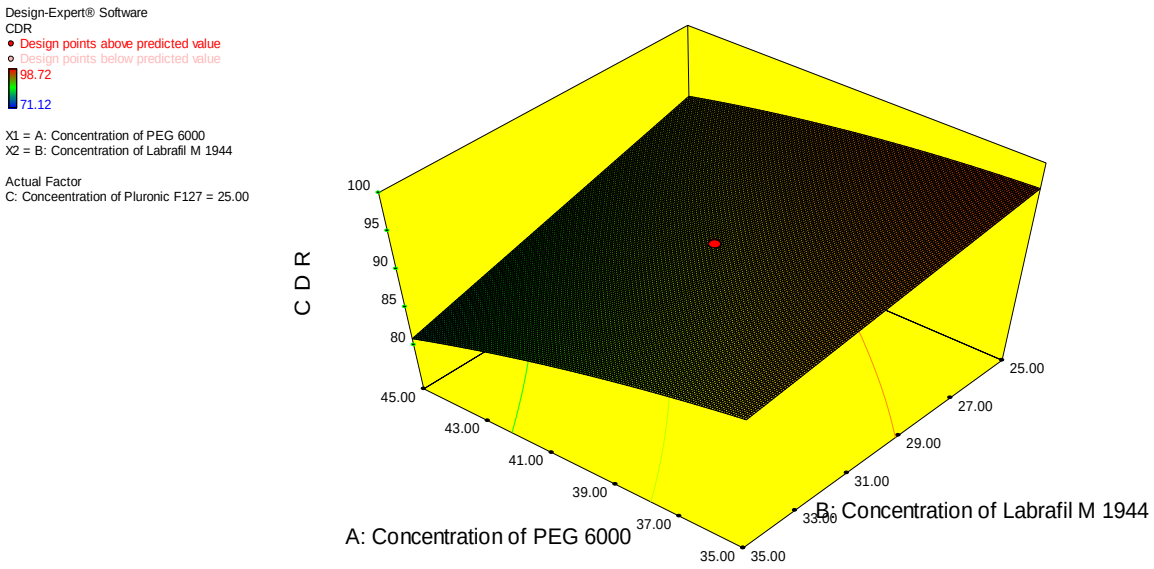


Figure 4 a 3D-Response surface plot showing the influence of Concentration of PEG 6000 (A), Concentration of Labrafil M 1944 (B) on % CDR at constant level of C

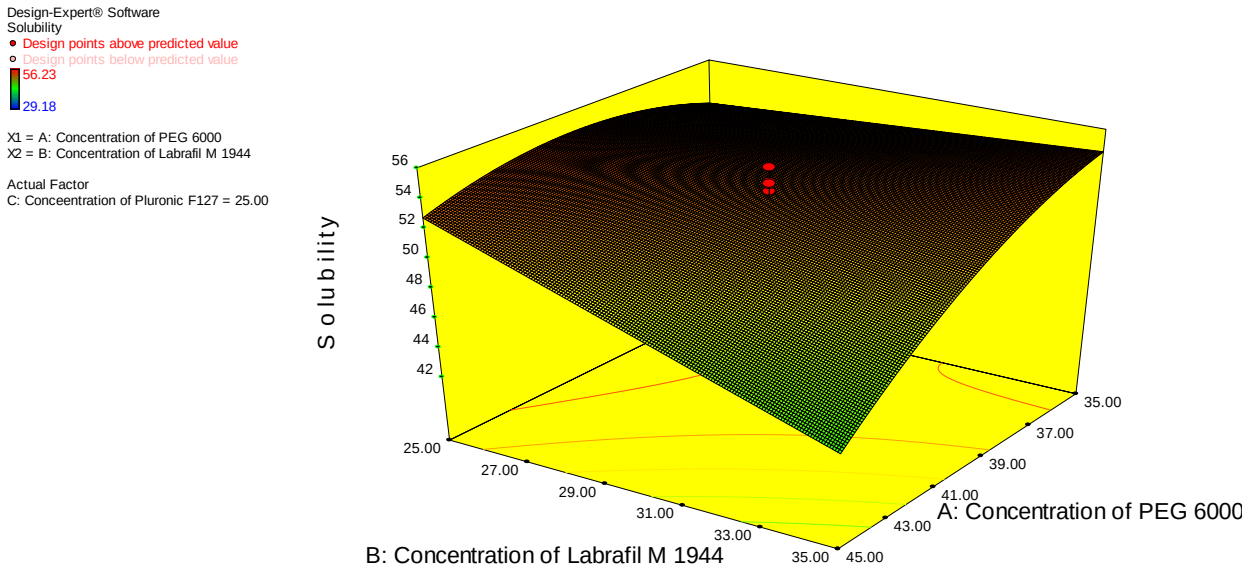


Figure 4 b 3D-Response surface plot showing the influence of Concentration of Labrafil M 1944 (B) and concentration of Pluronic F127 (C) on % CDR at constant level of A

The polynomial equation, describing the solubility as a simultaneous function of Concentration of PEG 6000 (A), Concentration of Labrafil M 1944 (B) and Concentration of Pluronic F127 (C), is as shown in equation 2.

$$\text{Solubility (Y2)} = 52.79 - 2.69A - 1.84B - 3.12C - 2.61AB - 1.72A^2 - 7.63C^2 \quad (2)$$

The equation represents the quantitative effect of process variables (A, B and C) and their interaction on the response Y2. The values of the coefficient A, B and C are related to the effect of these variables on the response Y2. Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationship respectively. The quadratic model generated revealed that A, B and C have significant negative effect on solubility. An antagonistic interactive effect was observed between A and B. Higher concentrations of A and C had a significant antagonistic effect on % solubility. The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). The ANOVA indicated a significant ($P < 0.05$) effect of factors on response. The Model F-value of 14.58 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, $C^2$are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. shows the main effects, interaction effects and quadratic effects of Concentration of PEG 6000 (A), Concentration of Labrafil M 1944 (B) and Concentration of Pluronic F127 (C) on solubility (Y2). It is clearly shows that Concentration of

PEG 6000 (A), and Concentration of Pluronic F127 (C) have the main and the major effect on Y2. The Concentration of Labrafil M 1944 (B) has a moderate effect on Y2. From this we can see that the concentration of PEG 6000 has the negative effect on solubility. The solubility was found to increase from 46.72 µg/ml to 56.23 µg/ml as the concentration of PEG 6000 decreases from 48.408 mg to 31.591 mg. Similarly, Concentration of Pluronic F127 (C) had a moderate negative effect on solubility. The solubility was found to increase from 29.18 µg/ml to 40.35 µg/ml as the concentration of Pluronic F127 (C) decreases from 33.408 mg to 16.591 mg. But at higher concentrations Pluronic F127 was found to have more negative effect on solubility. Concentration of Labrafil M 1944 (B) had a little negative effect on solubility. The solubility was found to increase from 51.43 µg/ml to 54.78 µg/ml as the concentration of Labrafil M 1944 decreases from 38.408 mg to 21.591 mg. The relationship between solubility and independent variables was further elucidated using 3d response surface plots and corresponding contour plots. **Figures 5 (a, b)** shows the interaction between the Concentration of PEG 6000 (A) and Concentration of Labrafil M 1944 (B) and at a fixed level of Concentration of Pluronic F127 (C).

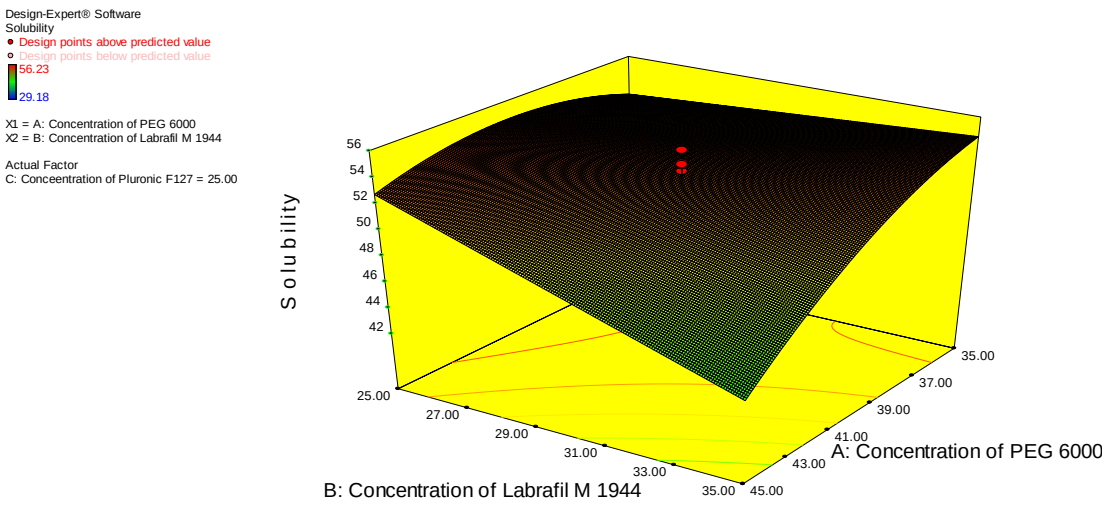


Figure 5 (a): 3D-Response surface plot showing the influence of Concentration of PEG 6000 (A), Concentration of Labrafil M 1944 (B) on solubility at constant level of C

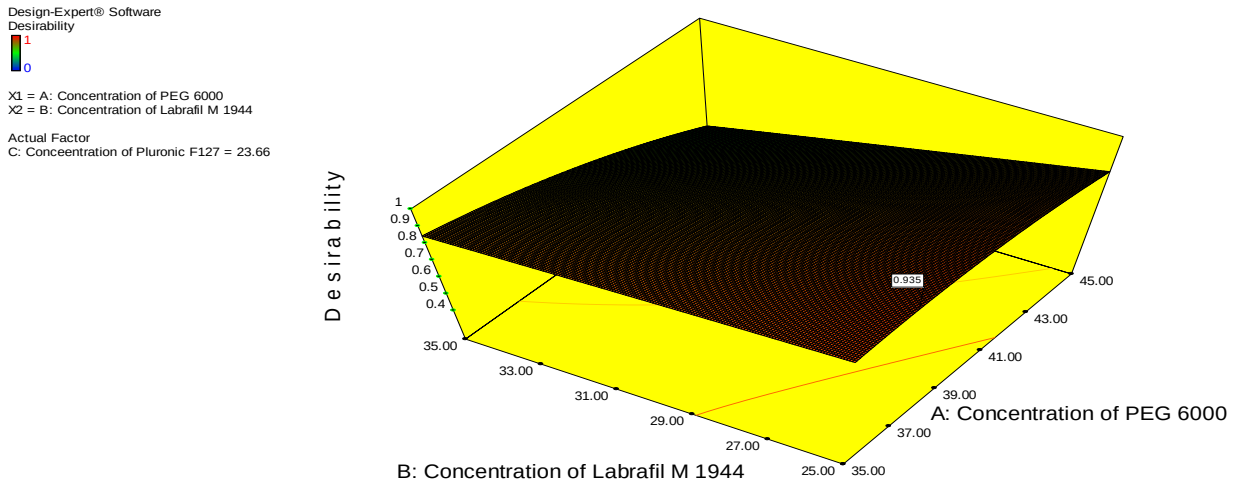


Figure 5 (b): Response surface for overall desirability as a function of Concentration of PEG 6000 and Concentration of Labrafil M 1944

Numerical Optimization

A numerical optimization technique using the desirability approach was employed to develop a new formulation with the desired responses. The perturbation plot and response surface plot for overall desirability as a function of A, B and C. The optimum formulation was selected based on the criteria of attaining maximum % cumulative drug release and optimum solubility (%). depicts the constraints set and the solution provided by the software with desirability value of 0.935. To verify these values, three confirmation batches were prepared according to the predicted levels of A, B and C. Obtained Y1 and Y2 values were in a close agreement with the predicted values, this demonstrated the reliability of the optimization procedure in predicting the concentration of polymers for the formulation of solid dispersions. All the three batches of obtained nateglinide solid dispersions were subjected to further characterization.

Preparation of Nateglinide solid dispersions

Figure 6: Nateglinide solid dispersions Formulation F1

Nateglinide solid dispersions were prepared and shown in **Figure 6**

Drug Content

The drug content ranged between 97.23 ± 0.54% and 98.80 ± 0.438%. The results indicate that the processes employed to prepare solid dispersions in this study were capable of producing formulation with uniform drug content.

Drug release study

Dissolution rates of nateglinide pure drug and solid dispersion formulations were evaluated. All formulations showed an increase in dissolution over pure drug nateglinide, which showed only ≈ 35% release after 60 mins. The Relatively higher dissolution enhancement could be credited to more intimate drug carrier interaction during formulation of solid dispersions. The Invitro drug release pattern of drug from the optimized batches is as shown in **Figure 7 and Table 5**.

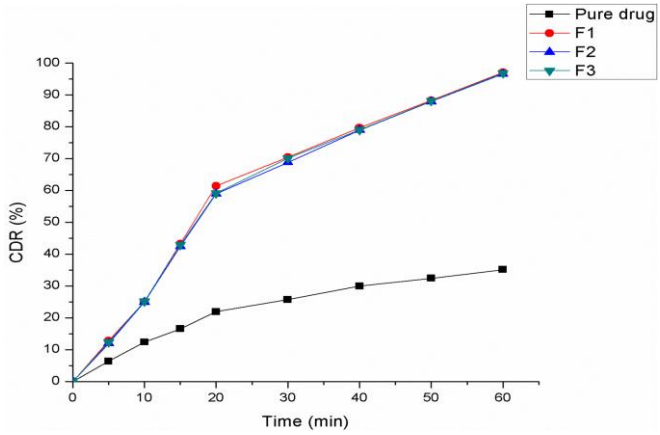


Figure 7: In-vitro release of Nateglinide from solid dispersions

Table 5: Dissolution profile of nateglinide solid dispersions (Optimized batches)

Time (min)	% CDR			
	Pure drug	F1	F2	F3
0	0	0±0	0±0	0±0
5	6.34	12.8±1.28	11.98±2.62	12.31±1.72
10	12.36	25.05±1.52	24.94±1.12	25.17±0.76
15	16.56	43.30±1.25	42.41±1.34	42.92±2.01
20	21.93	61.40±1.19	59.92±0.96	60.12±1.32
30	25.72	70.50±1.27	69.83±1.56	70.12±1.65
40	29.98	79.76±1.28	78.93±2.21	79.02±0.64
50	32.43	88.27±1.58	87.93±1.35	88.12±1.89
60	35.12	97.12±1.52	96.63±0.97	96.91±0.62

Release kinetics

Drug release data for optimized formulation F1 was fitted into various kinetic equations to find out the order and mechanism of drug release. The correlation coefficient showed that the release profile followed the Higuchi model (R^2= 0.95836), Also from Korsmeyer peppas model, the

release exponent, n was found to be 0.80635 (0.43 < n < 0.85) and followed anomalous behaviour and release mechanism was indicative of diffusion

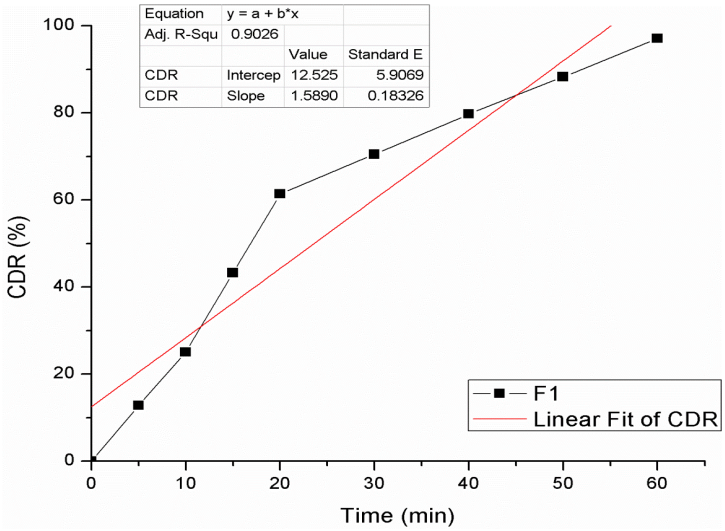


Figure 8: Plot of Zero order release kinetics of the optimized batch F1

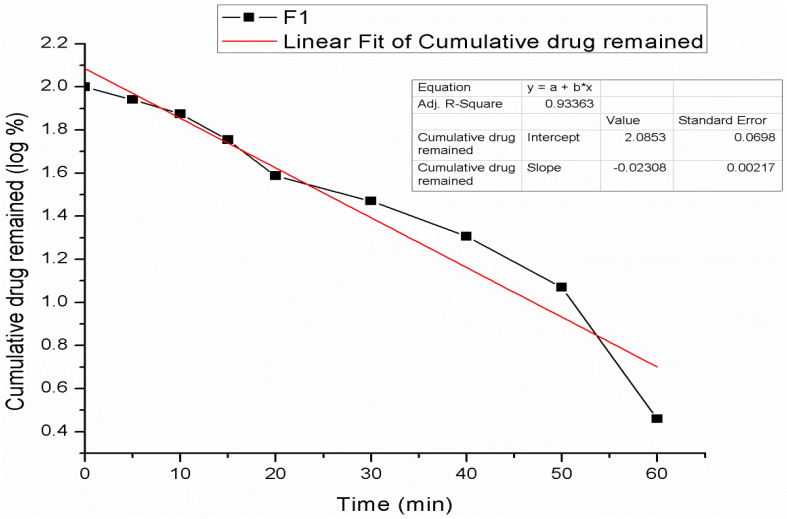


Figure 9: Plot of First order release kinetics of the optimized batch F1

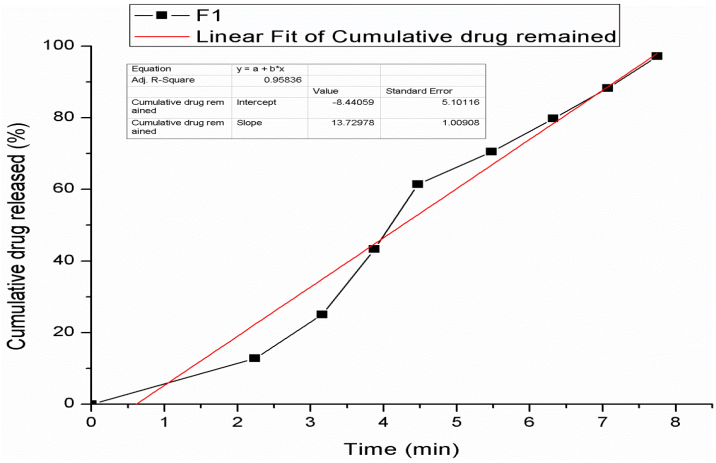


Figure 10: Plot of Higuchi release kinetics of the optimized batch

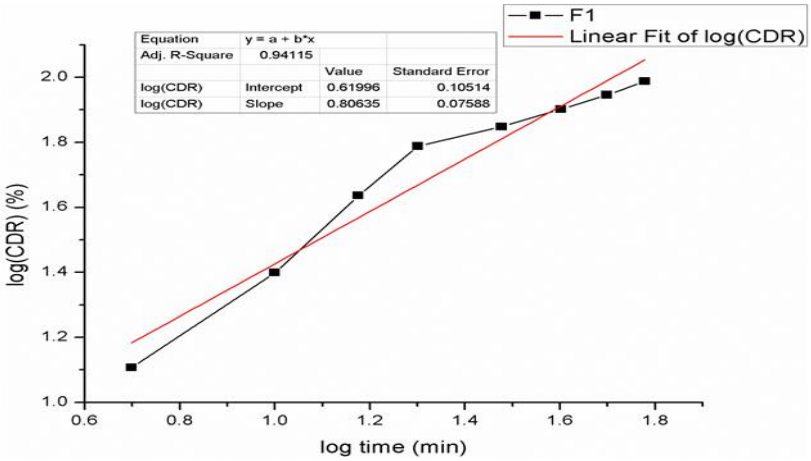


Figure 11: Plot of Korsmeyer- Peppas release kinetics of the optimized batch

From the above results it is apparent that the regression coefficient value closer to unity in case of Higuchi model; indicates that the drug release exponentially to the elapsed time. This data indicates a lesser amount of linearity when plotted by the first order equation. Further, the translation of the data from the dissolution studies suggested possibility of understanding the mechanism of drug release by configuring the data in to various mathematical modeling such as Higuchi and Korsmeyer-Peppas plots. Further the n value obtained from the Korsmeyer-Peppas plots i.e. 0.80635 indicating non Fickian (anomalous) transport thus it projected that delivered its active ingredient by coupled diffusion and erosion (**Figures 8-11 & Table 6**).

Table 6: Release kinetics of optimized formulation of nateglinide solid dispersions (F1)

Formulation Code	Zero Order		First Order		Higuchi		Korsmeyer-Peppas	
	R^2	N	R^2	N	R^2	n	R^2	N
F1	0.9026	1.5890	0.93363	0.023	0.95836	13.729	0.94115	0.80635

Powder X-ray diffraction pattern

The X-ray diffraction patterns of the pure drug, physical mixture and final optimized formulation are shown in **Figure 12.** The X-ray pattern of pure nateglinide revealed a drug fingerprint with intense and sharp peaks, indicating its crystalline nature as demonstrated by sharp peaks observed at The X-ray diffractogram of nateglinide exhibited sharp and intense peak at 2θº equivalent to 10º, 13º, 14.25º, 19º, 20º, 23º indicated crystalline in nature. There is a significant difference between the 2θ values of the pure drug and final optimized PSD formulation. A reduction in crystallinity was observed in the optimized formulation.

The diffraction spectrum of the solid dispersion vis-à-vis pure drug indicates the changes produced in the drug crystal structure. The sharp diffraction peaks associated with pure nateglinide are

characteristic of its crystalline form. The absence of diffraction peaks in solid dispersion confirms that the nateglinide is present in amorphous form. No new peaks were observed, suggesting the absence of interaction between the drug and the polymer in the formulation. These data are indicative of the transformation of nateglinide from the crystalline to the amorphous state by formation of solid dispersion. The formation of an amorphous state proves that the drug was dispersed in a molecular state with the polymers.

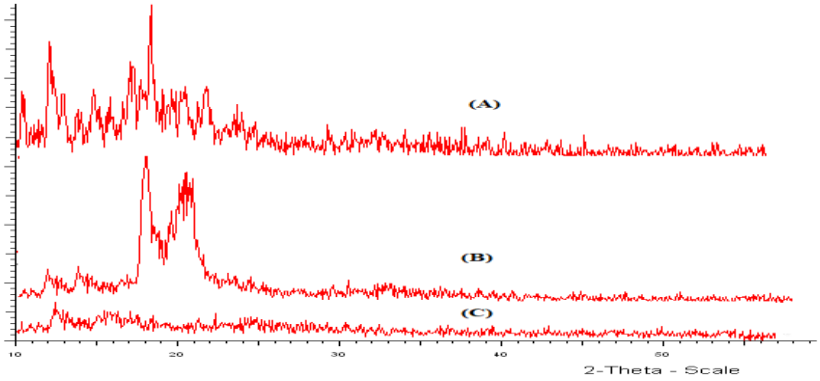
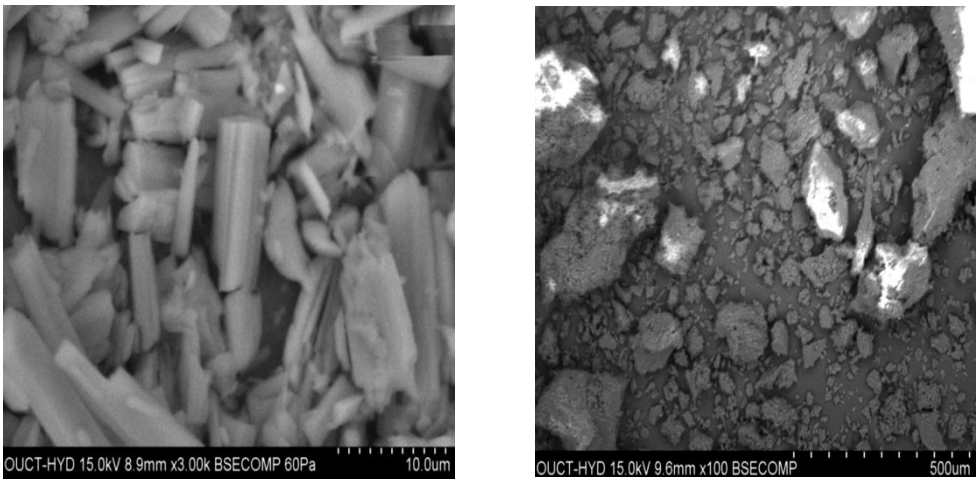


Figure 12: X-Ray powder diffractograms of Nateglinide pure drug (A), Physical mixture (B) and optimized formulation F1 (C)

Scanning electron microscopy

SEM studies were carried out on final optimized formulation to determine the surface morphology and intactness of the drug was observed. The solid dispersions were uniform size and spherical in shape with small porous and little rough surface was observed in optimized formulation. The rough surface is caused due to the rapid loss of moisture from the wet mass with the high liquid content that results in a porous surface structure. The wrinkled surface of the SDs indicated increase in surface area that may play a major role to increase in solubility (**Figure 13**).



a **b**

Figure 13: SEM image of a) Nateglinide pure drug and b) formulation F1 Stability study

There were no physical changes in appearance and flexibility. When subjected to Accelerated Stability Studies, results for optimized formulation F1 showed that there were no major changes in Drug Content, *In Vitro* Drug Release and solubility studies. Hence the formulation was found to be stable (**Table 7**).

Table 7: Parameters after Accelerated Stability Study of Formulation F1

Parameters	**Temperature Maintained at 40 ±2^{0}C Relative Humidity (RH) Maintained at 75%±5%RH**			
	Initial	**After 1 month**	**After 2 months**	**After 3 months**
Drug Content (%)	99.528±0.14	99.46±0.68	99.23±0.37	99.08±0.22
In Vitro Drug Release (%)	97.12±1.52	96.29±1.53	96.05±1.42	96.02±1.35
Solubility studies	54.45±0.013	54.23±0.033	54.18±0.045	54.02±0.025

CONCLUSION

From the above work it was concluded that Nateglinide solid dispersions were prepared by solvent evaporation technique was found to be more feasible than the conventional one, the water-soluble ingredients PEG 6000, Pluronic F 127 and Labrafil M 1944 used in the formulation were increases the solubility of the drug by converting from crystalline to amorphous form. The Central composite design consisting of a 3-factor, 3-level rotatable central composite design (CCD) was employed to study the effect of each independent variable on dependent variables which are percent CDR and solubility. Formulation F1 was found to be optimized on the basis of evaluation parameters. X-ray diffraction was used to analyze the crystallinity of optimized formulation and FTIR was used to analyze the drug and excipient compatibility and found to be compatible. Scanning electron microscopy was performed to analyze the surface of solid dispersion samples. The correlation coefficient showed that the release profile followed Higuchi model (R2= 0.95836). From Korsmeyer peppas model, the release exponent, n was found to be 0.80635 ($0.43 < n < 0.85$) and followed anomalous behaviour and hence release mechanism was indicative of diffusion. From dissolution studies all formulations showed an increase in dissolution (i.e 96.63-97.12%) over pure drug nateglinide, which showed only ≈ 35% release after 60 mins. Thus, we can say that a Nateglinide solid dispersion may achieve good formulation capability for pharmaceutical manufacturer by increasing solubility and dissolution rate.

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