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Original Article

FORMULATION DEVELOPMENT AND OPTIMIZATION OF NATEGLINIDE-LOADED ETHYL CELLULOSE NANOPARTICLES BY BOX-BEHNKEN DESIGN

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ABSTRACT

Objective: Application of nanotechnology in drug delivery system has released leading new areas of research in sustained release of drugs. The objective of the present study is development and optimization of polymeric nanoparticles of Nateglinide (NTG).

Methods: Nateglinide loaded ethyl cellulose (EC) nanoparticles were prepared by the solvent evaporation technique. Response surface methodology (RSM) using the Box-Behnken design was used to optimize the formulation of Nateglinide nanoparticles. The Box-Behnken design consisting of 14 runs, three-factor, three levels and two centre point was used in this study. The particle size, zeta potential and entrapment efficiency of Nateglinide nanoparticles were investigated with respect to three independent variables including stirring speed (X_1), time (X_2) and surfactant concentration (X_3). The optimized nanoparticle is then subjected to characterization studies including morphology, particle size, zeta potential, % Drug Loading (DL) and % Entrapment Efficiency (EE).

Results: Nateglinide nanoparticles under the optimized conditions gave rise to the DL of 14.30±0.27 %, EE of 72.19±0.24 %, mean diameter of 172 nm and zeta potential value of-15.6 mV.

Conclusion: The optimized nanoparticles formulation with improved characteristic properties could be a promising delivery system for Nateglinide.

Keywords: Box-Behnken, Drug delivery, Nanoparticles, Nateglinide, Response surface methodology, Solvent evaporation method.

INTRODUCTION

Nanoparticles represent an effective nano carrier platform for the delivery of hydrophobic and hydrophilic drugs, since the drugs are protected from possible degradation by enzymes. The development of smart nanoparticles can deliver drugs at a sustained rate providing better efficacy and lower toxicity for treatment of various diseases [1]. Recently, nanoparticle engineering processes have been developed and reported for pharmaceutical applications to increase the dissolution rate of low-soluble drugs which in turn may leads to substantial increases in bioavailability and are essential for pharmaceutical industry as an alternative drug delivery system for the treatment of highly prevalent and chronic disease like diabetes mellitus [2].

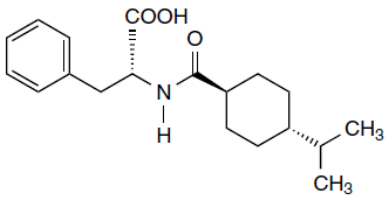


Fig. 1: Chemical structure of nateglinide

Diabetes mellitus is a metabolic disease characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both [3]. Nateglinide (NTG) has been exploited as a new class of oral antidiabetic agent used in the management of Type 2 diabetes mellitus. Nateglinide, (-)-N-[(trans-4-isopropyl-cyclohexane) carbonyl]-D-phenylalanine, is structurally (fig. 1) unrelated to the oral sulfonylurea insulin secretagogues. Nateglinide is a D-phenylalanine derivative recently approved for the management of type II diabetes [4, 5]. In difference to sulfonylureas, Nateglinide increases pancreatic β cell sensitivity to ambient glucose without increasing basal insulin secretion after oral administration. It can be used as mono therapy or in combination with metformin or thiazolidinediones. It has short half-life of 1.5 h, and peak plasma concentration extents at 0.5-1.0 hr. It is metabolized by cytochrome P-450 system to inactive metabolite and eliminated with half-life of 1.4 h [6].

In the development of nanoparticles, an important issue was to design an optimized pharmaceutical formulation with maximum drug loading (DL), entrapment efficiency (EE) and appropriate mean particle size through minimum trials. For this purpose, a computer aided optimization technique based on a Response Surface Methodology (RSM) was used. Response surface methodology is a collection of mathematical and statistical techniques based on the fit of a polynomial equation to the experimental data, which must describe the behaviour of a data set with the objective of making statistically significant. It can be well applied when a response or a set of responses of interest is influenced by several variables. The objective is to simultaneously optimize the levels of these variables to attain the best system performance. The optimization procedure involved systematic formulation designs to minimize the number of trials, and analyse the response surfaces in order to realize the effects of causal factors and to obtain the appropriate formulations with target goals. Therefore, in order to quickly obtain the optimal formulations with appropriate drug loading (DL), entrapment efficiency (EE) and mean particle size of Nateglinide nanoparticles, RSM was used to evaluate the effects of stirring speed (X_1), time (X_2) and surfactant concentration (X_3). Box-Behnken designs are response surface designs, specially made to require only 3 levels, coded as-1, 0, and+1. Box-Behnken designs are available for 3 to 10 factors. They are formed by combining two-level factorial designs with incomplete block designs. This procedure creates designs with desirable statistical properties but, most importantly, with only a fraction of the experiments required for a three-level factorial. Because there are only three levels, the quadratic model is appropriate [7, 8].

The objective of the study was to fabricate and optimize ethyl cellulose nanoparticles containing Nateglinide to overcome the limitations of the Nateglinide delivery through conventional method.

MATERIALS AND METHODS

Materials

Nateglinide was obtained from Glanmark Pharmaceutics Ltd, Mumbai, India. Ethyl cellulose was received from Himedia Laboratories, Mumbai, India. Polyvinyl alcohol was procured from Fourrts India Laboratories Pvt Ltd, Chennai, India. Methanol (Qualigens Fine Chemicals, Mumbai) was of high performance liquid chromatography (HPLC) grade. All other reagents and solvents were of analytical grade.

Preparation of Nateglinide nanoparticles

The NTG-loaded EC nanoparticles were prepared by the solvent evaporation method. Briefly, weighed NTG and EC were dissolved in mixture of methanol with acetone in 1:2 ratio using a vortex shaker (to mix small vials of liquid) to form the homogeneous organic phase of NTG and EC. This solution was added drop by drop into the 1 % aqueous phase of polyvinyl alcohol using mechanical stirrer at 1000 rpm for 2 h to prepare nano suspension and thoroughly evaporate the organic phase followed by magnetic stirring for 2 hrs under atmospheric pressure at room temperature. Then it was centrifuged at 15,000 rpm for 15 minutes and after centrifugation the supernatant was excreted and the pellets obtained were washed thrice with distilled water and finally freeze-dried to get the powdered nanoparticles [9, 10].

Experimental design

Preliminary experiments indicated that the variables, such as stirring speed, time and surfactant concentration were the main factor that affects the particle size, zeta potential and entrapment efficiency of nanoparticles.

Thus, a response surface methodology-Box Behnken design was used to systemically investigate the influence of these three critical formulation variables on particle size, zeta potential and entrapment efficiency. The details of the design are listed in the table 1. For each factor, the experimental range was selected based on the results of preliminary experiments and the feasibility of preparing the nanoparticles at the extreme values. The range of independent variables and their corresponding levels of actual values are given below.

Table 1: Independent variables and their corresponding levels of NTG-loaded EC nanoparticles preparation for Box-Behnken

Variables	Levels		
	-1	0	+1
Stirring speed (rpm)	500	750	1000
Time (min)	1	2	3
Surfactant concentration (%)	1	1.5	2

Characterisation

Particle size and zeta potential measurement

Particle size of fabricated nanoparticles was measured by particle size analyser (MASTERSIZER 2000, MALVERN Instruments, UK) equipped with MAS OPTION particle sizing software. The measurements were made at a fixed angle of 90 ° for all samples. The samples were suitably diluted with Milli Q water for every measurement. Zeta potential measurements were measured by Malvern zeta sizer (MAL 1054413 Zetasizer Version 6.20 Instruments, UK). For zeta potential determination, samples of all formulations were diluted with 0.1 mM KCl and placed in the electrophoretic cell, where an electric field of about 15 V/cm was applied. The mean hydrodynamic diameter (Dh) and polydispersity index (PI) of the particles were calculated using the cumulative analysis after averaging the three measurements [11].

Scanning electron microscopy

Scanning electron microscopy (SEM) analysis of the nanoparticle formulation was performed to evaluate the surface morphology of nanoparticles. Images were taken using JEOL JSM-6701F (Tokyo, Japan) at 3.0 kV with 50,000 magnifications, and 100 nm scale bar was used [12].

Chromatographic conditions

Nateglinide estimation was carried out by RP-HPLC based on the reported method by Madhavi *et al.,* 2008. [13]An isocratic reverse phase high pressure liquid chromatographic (RP-HPLC) with Shimadzu LC-20AD PLC pump and a SPD-M20A photo diode array (PDA) detector were used. Separation was carried out on a Phenomenex C18 column (particle size 5 µm; 150 × 4.6 mm i. d) using Acetonitrile: 10 mM Sodium di-hydrogen phosphate (NaH_2PO_4) buffer solution [phosphate-buffered solution (PBS); adjusted to pH 3.0 with H_3PO_4] in the ratio of 50:50, v/v. The flow rate was 1.0 ml/min at 27 °C and the detection was monitored at a wavelength of 210 nm. The injection volume was 20 µl. Acetonitrile was used as diluent.

Determination of drug loading and entrapment efficiency

A 10 mg samples of the formulated nanoparticles were dissolved in 10 ml acetonitrile (as common solvent for both the drug and polymer) and from the above solution 20 µl was taken for RP-HPLC analysis. The amount of drug in the solution was calculated using standard graph of Nateglinide in pH 7.4 PBS buffers analysed by RP-HPLC method (Phenomenex C18 column 5 µm average particle size; 150 × 4.6 mm i. d). The detection of wavelength was 210 nm [13]. Drug loading (% w/w) and drug entrapment (%) were represented by equations 1 and 2, respectively [14].

$$Drug\ Loading\ \left(\%\frac{w}{w}\right) = \frac{Mass\ of\ drug\ in\ nanoparticles}{Mass\ of\ nanoparticles\ recovered} \times 100 \quad -Eq.(1)$$

$$Entrapment\ Efficiency\ (\%) = \frac{Mass\ of\ drug\ in\ nanoparticles}{Mass\ of\ drug\ added\ in\ formulation} \times 100 - Eq.(2)$$

In-vitro drug release studies

Nateglinide loaded nanoscale solid lipid particles drug release study was carried out by using an incubator shaking method. Accurately weighed quantities of RM-SLNs were suspended in the conical flask containing 50 ml phosphate buffer at pH 7.4 at 37 °C±0.5 °C. The conical flask was sealed tightly and kept in incubator shaker (Lark, India), which was agitated at 50 strokes per minute and maintained at 37 °C±0.5 °C. At schedule time intervals, the 1 ml of the release medium was withdrawn and replaced with the same volume of fresh PBS. The samples were centrifuged and then supernatant was extracted with the syringe and filtered through a 0.45 µm membrane filter (Elix, Mill-Q) and the content of Nateglinde was estimated by HPLC method [15].

RESULTS AND DISCUSSION

Optimization of NTG-loaded EC nanoparticles by Box-Behnken design

Experimental design, data analysis and desirability function calculations were performed by using Design-Expert® version 8.0.1 (Stat-Ease Inc., Minneapolis). Before starting an optimization procedure, it is important to investigate the curvature term using a response surface methodology-Box Behnken design. ANOVA generated shows that curvature is significant for all the responses (X_1, X_2 and X_3) since p-value is less than 0.05. This implies that a quadratic model should be considered. Response surface methodology-Box Behnken design is chosen due to its flexibility and can be applied to optimize by gaining better understanding of

factor's main and interaction effects. The selection of key factors examined for optimization was based on preliminary experiments and prior knowledge from literature. The factors selected for optimization process were stirring speed (X_1), time (X_2) and surfactant concentration (X_3). The particle size, zeta potential and entrapment efficiency was selected as responses.

All experiments were conducted in the randomized order to minimize the effects of uncontrolled variables that may introduce a bias on the measurements. Replicates (n=2) of the central points were performed to estimate the experimental error (table 2), summarizes the conducted experiments and responses. The quadratic mathematical model for three independent factors is given in Eq. (3).

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3 \text{--(3)}$$

Where Y is the response to be modelled, β is the regression coefficient and X_1, X_2 and X_3 represents factors A and B respectively. Statistical parameters obtained from ANOVA for the reduced models are given in table 3. The insignificant terms (P>0.05) were eliminated from the model through backward elimination process to obtain a simple and realistic model. Since R^2always decreases when a regressor variable is eliminated from a regression model, in statistical modelling the adjusted R^2which takes the number of regressor variables into account, is usually selected [16, 17].

Table 2: Experimental responses and Box-Behnken design arrangements

Design points	Factor level			Responses		
	Stirring speed (rpm)	Time (min)	Surfactant concentration (%)	Particle size (nm)	Zeta potential (-mV)	Entrapment efficiency (%)
1	-1	-1	0	236	-0.17	67.17
2	+1	-1	0	182	-14.23	74.43
3	-1	+1	0	208	-11.34	76.12
4	+1	+1	0	180	-15.41	78.27
5	-1	0	-1	226	0.14	76.51
6	+1	0	-1	229	-17.21	79.67
7	-1	0	+1	231	0.583	73.37
8	+1	0	+1	186	-13.23	84.52
9	0	-1	-1	230	-12.21	45.19
10	0	+1	-1	180	-1.33	72.48
11	0	-1	+1	195	0.52	62.51
12	0	+1	+1	191	0.42	68.50
13	0	0	0	174	0.18	47.72
14	0	0	0	175	0.17	47.23
15	0	0	0	175	0.18	46.64
16	0	0	0	175	0.19	46.32
17	0	0	0	172	0.22	43.79

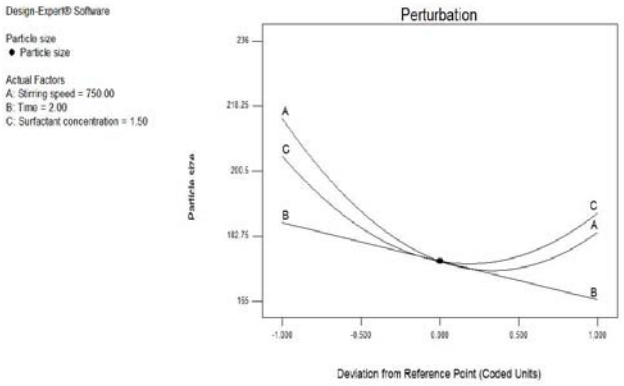
Table 3: Response models and statistical parameters obtained from ANOVA for Box-Behnken design

Responses	Regression model	Adjusted R^2	Model p values	Adequate precision	% CV
Particle size	+175.95-15.50×A-10.50×B-7.75×C-12.00×A×C+11.50×B×C+23.37×A^2+20.87×C^2	0.8961	<0.0001	3.95	12.48
Zeta potential	+0.64-8.45×A-0.21×B-0.33×C-0.29×AB+0.035×AC+0.080×BC-8.43×A^2+0.64×B^2-0.59×C^2	0.9932	<0.0003	3.24	18.16
Entrapment efficiency	+46.04+2.88×A+5.75×B-5.25×B×C+21.98×A^2+5.73×B^2+9.98×C^2	0.9379	<0.0001	3.56	15.66
Acceptance criteria		≥0.80	<0.05	<4%	>10%

In the present study, the adjusted R^2 was well within the acceptable limits of which revealed that the experimental data shows a good fit with the second-order polynomial equations. For all the reduced models, P value of<0.05 is obtained, implying these models, are significant. The adequate precision value is a measure of the signal (response) to noise (deviation) ratio.

A ratio lesser than 4 is desirable. In this study, the ratio was found to be within the range, which indicates an adequate signal and therefore the model is significant [16]. The coefficient of variation (C. V.) is a measure of reproducibility of the model and as a general rule a model can be considered reasonably reproducible if it is greater than 10%. The C. V. for all the models were found to be more than 10%.

In fig. 2 perturbation plots are presented for predicted models in order to gain a better understanding of the investigated procedure. This type of plots show the effect of an independent factor on a specific response, with all other factors held constant at a reference point. A steepest slope or curvature indicates sensitiveness of the response to a specific factor. Fig. 2a shows that the particle size is highly influenced by stirring speed followed by surfactant concentration and then by time. In fig. 2b, the stirring speed contributes the significate influence for zeta potential. Fig. 2c shows that entrapment efficiency is the major contribution by stirring speed followed by surfactant concentration and then by time.



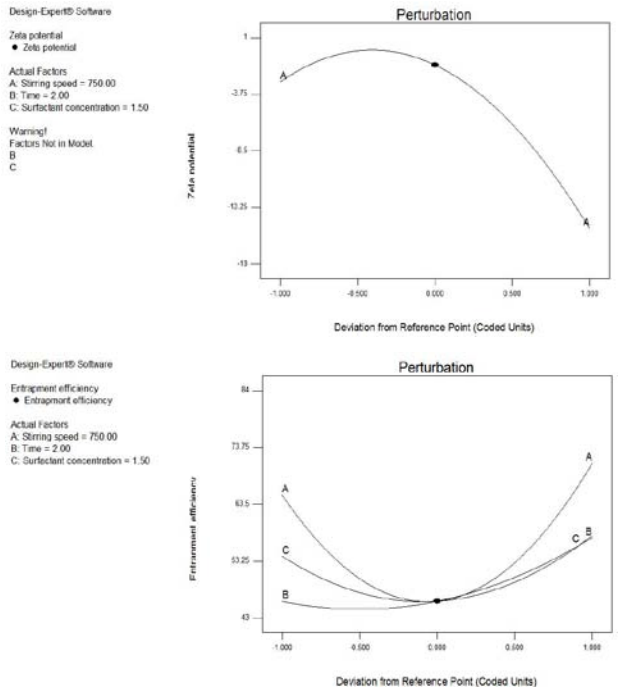
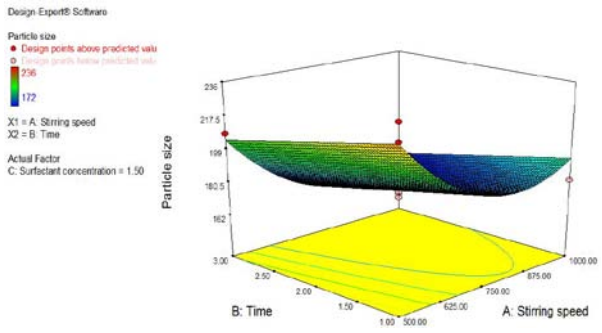
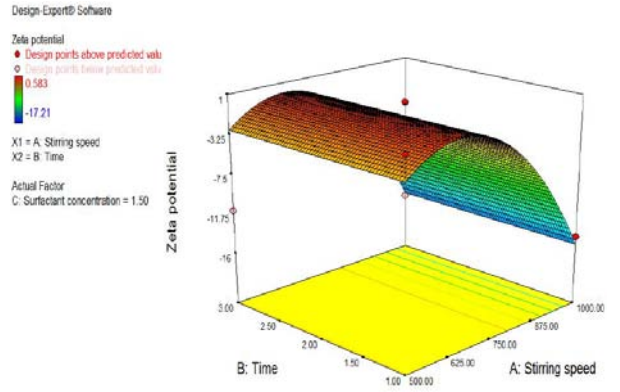


Fig. 2: Perturbation plots showing the effect of each of the independent variable on Particle size, Zeta potential, and Entrapment efficiency

Response surfaces plots for particle size, zeta potential and entrapment efficiency are illustrated in fig. 3 (Stirring speed, time and surfactant concentration were plotted against particle size, zeta potential and entrapment efficiency held at constant at the centre value). Analysis of the perturbation plots and response plots of optimization models revealed that stirring speed and surfactant concentration had the significant effect on the particle size and entrapment efficiency.





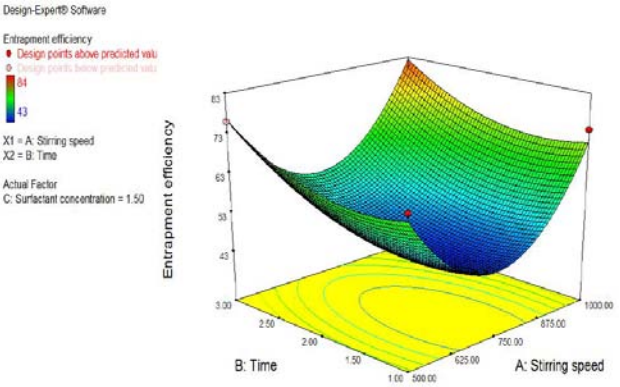


Fig. 3: Three dimensional (3D) response surface plots showing the effect of the variable on the response

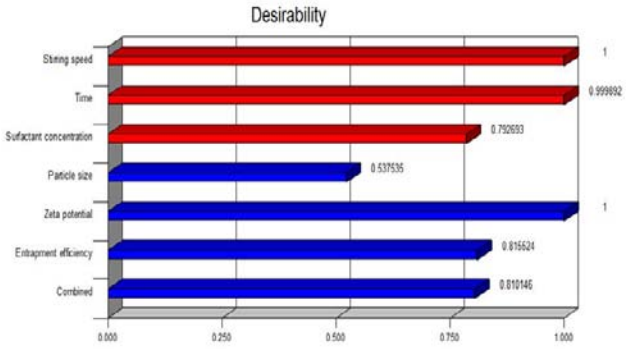


Fig. 4: Graphical representation of overall desirability function

Table 4: Comparison of experimental and predicted values under optimal conditions for NTG-loaded EC nanoparticles formulation

Stirring speed (rpm)	Time (hr)	Surfactant concentration (%)	Particle size (nm)	Zeta potential (-mV)	Entrapment efficiency (%)
1000	2	1			
Desirability D value		0.810			
Predicted			170	-14.5	73.17
Experimental			172	-15.6	72.19
Bias (%)			1.17	-1.3	1.34
Acceptance criteria = 2 %					
Bias was calculated as (Predicted value-Experimental value)/Predicted value × 100					

Table 4. Showed that the experimental values of the nanoparticles prepared within the optimum range were very close to the predicted values, with low percentage bias, suggesting that the optimized formulation was reliable and reasonable and the desirability is graphically represented in figure. 4 with a D value of 0.8302 which is well within the range.

Characterization

Particle size and zeta potential measurement

The mean particle size of optimized NTG-loaded EC nanoparticles formulation was 172 nm. The zeta potential of the nanoparticle was

found to be-15.6 mV, and it indicates that it is sufficiently high to form stable colloidal nano suspension [11]. The image is shown in fig. 5. The percentage of drug loading and entrapment efficiency was found to be 14.30±0.27 % and 72.19±0.24 % respectively.

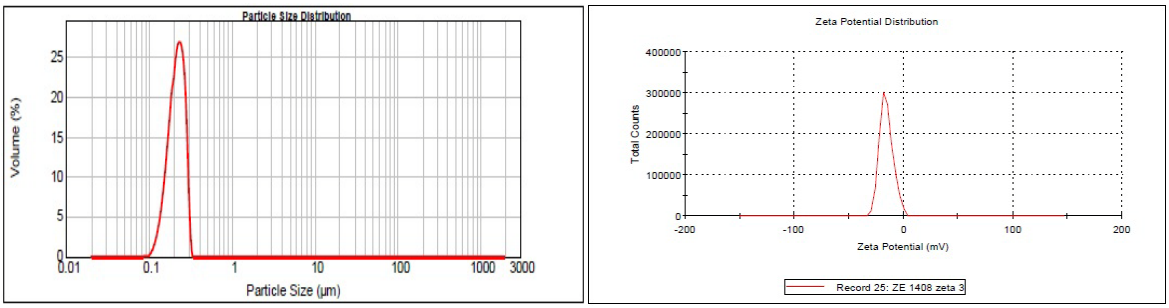


Fig. 5: Particle size distribution and zeta potential of optimized NTG-loaded EC nanoparticles formulation

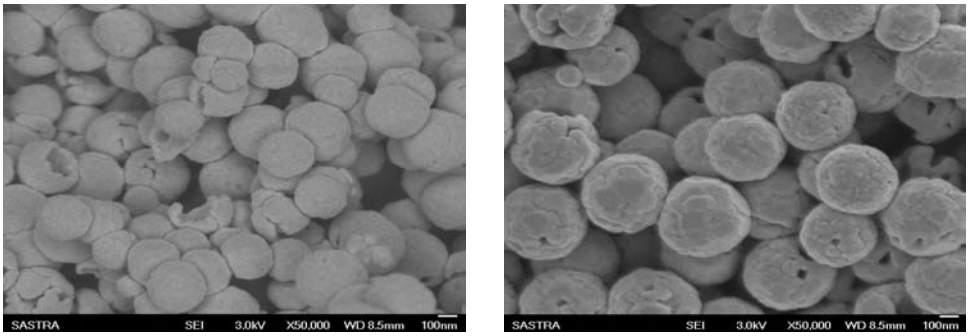


Fig. 6: SEM images of optimized NTG-loaded EC nanoparticles formulation taken at 3.0 kV 50,000 magnifications

Scanning electron microscopy

In order to provide information on the morphology and size of the optimal nanoparticle, SEM was used to take photos of the optimized NTG-loaded EC nanoparticles formulation, as shown in fig. 6. The nanoparticles are smooth surface of the particles with round structure and not aggregated [12]. From the images it was found to be formulated nanoparticles are uniform size and it indicates that the formulation method was efficient.

In vitro release

The percentage of drug release from NTG-loaded EC nanoparticles was studied as a function of time in *in vitro* condition. The drug release study was performed by modified dissolution method. The percentage amount of drug released from NTG-loaded EC nanoparticles formulations was depicted in fig. 7. The formulation shows a significant and sustained release (up to 86.21 % in 12 h) of Nateglinide in nanoparticles [15].

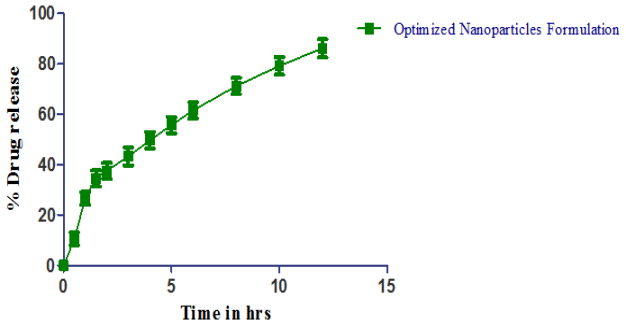


Fig. 7: *In vitro* release profile Nateglinide from Ethyl cellulose nanoparticles

CONCLUSION

Solvent evaporation method was employed to prepare the Nateglinide nanoparticles. The formulation of NTG-loaded EC nanoparticles was optimized using the Response surface methodology-Box Behnken by fitting a second order model to the response data. The experimental results of the nanoparticles prepared under the optimum conditions were well correlated to the predicted values. Nateglinide nanoparticles under the optimized conditions gave rise to the DL of 14.30±0.27 %, EE of 72.19±0.24 %, mean diameter of 172 nm and zeta potential value of-15.6 mV. SEM showed that the nanoparticles are round structure, with smooth surface. The optimized nanoparticles formulation with improved characteristic properties could be a promising delivery system for Nateglinide.

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CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Vyas SP, Khar RK. Targeted and controlled drug delivery: Novel carrier system. 1st ed. CBS Publishers and distributors; 2007. p. 363.
2. Amulyaratna B, Sunit Kumar S. Development and *in-vitro*, *in-vivo* evaluation of controlled release, biocompatible nanoparticles. Digest J Nanomater Biostruct 2012;7:217-25.
3. Daisy P, Jeeva Kani FG. Evaluation of antidiabetic activity of various extracts of cassia *Auriculata Linn.* bark on streptozotocin-induced diabetic wistar rats. Int J Pharm Pharm Sci 2012;(4, Suppl 4):312-8.

4. Dhana lekshmi UM, Poovi G, Neelakanta Reddy P. *In-vitro* observation of repaglinide engineered polymeric nanoparticles. Digest J Nanomater Biostruct 2012;7:1-18.
5. Jassim ZE, Hussein AA. Formulation and evaluation of clopidogrel tablet incorporating drug nanoparticles. Int J Pharm Pharm Sci 2014;6:838-51.
6. Kaleemuddin M, Srinivas P. Lyophilized oral sustained release polymeric nanoparticles of nateglinide. AAPS Pharm Sci Tech 2013;14:78-85.
7. Box GEP, Wilson KB. On the experimental attainment of optimum conditions. J Royal Statistical Soc: Series B 1951;13:1-45.
8. Box GEP, Hunter JS. Multi-factor experimental design for exploring response surfaces. Ann Math Stat 1957;28:195-241.
9. Jain S, Saraf S. Influence of processing variables and *in-vitro* characterization of glipizide loaded biodegradable nanoparticles. Diabetes Metabolic Syndrome: Clinical Research Reviews 2009;3:113-7.
10. Dhana lekshmi UM, Poovi G, Kishore N, Neelakanta Reddy P. *In-vitro* characterization and *in-vivo* toxicity study of repaglinide loaded poly (methyl methacrylate) nanoparticles. Int J Pharm 2010;396:194-203.
11. Kashanian S, Azandaryani AH, Derakhshandeh K. New surface-modified solid lipid nanoparticles using N‐glutaryl phosphatidylethanolamine as the outer shell. Int J Nanomed 2011;6:2393‐401.
12. Hoa LTM, Chi NT, Triet NM, Nhan LNT, Chien DM. Preparation of drug nanoparticles by emulsion evaporation method. J Phys: Conf Ser 2009;187:1-4.
13. Madhavi A, Reddy GS, Suryanarayana MV, Naidu A. Development of a new analytical method for determination of related components in nateglinide. Chromatogra 2008;67:639-45.
14. Yuan XB, Yuan YB, Jiang W, Liu J, Tian EJ, Shun HM, *et al*. Preparation of rapamycin-loaded chitosan/PLA nanoparticles for immunosuppression in corneal transplantation. Int J Pharm 2008;349:241-8.
15. Marcos F, Soffa N, Karla S, Ana Fernández C, Emilia B. An effective novel delivery strategy of rasagiline for Parkinson's disease. Int J Pharm 2011;419:271–80.
16. Ahn JH, Kim YP, Lee YM, Seo EM, Lee KW, Kim HS. Optimization of microencapsulation of seed oil by response surface methodology. Food Chem 2008;107:98-105.
17. Myers RH, Montgomery DC. Response Surface Methodology. New York: John Wiley and Sons Inc; 1995. p. 700.