Research Article

Formulation and Optimization of Nateglinide Loaded polymeric Nanoparticles using Response Surface Methodology

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ABSTRACT

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 to design and optimize the formulation of Nateglinide loaded polymeric Nanoparticles using Response Surface Methodology (RSM). Nateglinide-loaded ethyl cellulose nanoparticles were prepared by solvent evaporation technique. Response surface methodology using the central composite rotatable design (CCRD) model was used to optimize the formulations of Nateglinide nanoparticles. The CCRD consisting of three-factor factorial design with three levels was used in this study. The effect of Polymer concentration (X_1), Percentage of Surfactant (X_2) and Stirring speed (X_3) on the particle size, polydispersity index and surface area of Nateglinide loaded Nanoparticles were investigated. The optimized nanoparticles is then subjected to characterization studies including morphology, particle size, zeta potential, % drug content (DC) and % entrapment efficiency (EE). Nateglinide nanoparticles under the optimized conditions provides the DC of 85.82 ± 0.28 %, EE of 71.16 ± 0.24 %, mean diameter of 172 nm and zeta potential value of -15 mV. The optimized nanoparticles formulation with improved characteristic properties could be a promising delivery system for Nateglinide.

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

INTRODUCTION

Nanoparticles represent an effective nanocarrier 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]

Diabetes mellitus is a metabolic disease characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both.[3] Nateglinide has been exploited as a new class of an oral antidiabetic agent used in the management of Type 2 diabetes mellitus. Nateglinide, (-)-N-[(trans-4-isopropylcyclohexane) carbonyl]-D-phenylalanine, is structurally 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 monotherapy 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 h. It is metabolized by cytochrome P-450 system to inactive metabolite and eliminated with half-life of 1.4 hrs.[6]

In the development of nanoparticles, an important issue was to design an optimized pharmaceutical formulation with maximum drug content, entrapment efficiency, and appropriate mean particle size through minimum trials. For this purpose, a computer aided optimization technique based on a Response Surface Methodology 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 content, entrapment efficiency and mean particle size of Nateglinide nanoparticles, RSM was used to evaluate the effects of polymer concentration (X_1), surfactant concentration (X_2) and stirring speed (X_3).

Central composite rotatable design (CCRD), originally developed by Box and Wilson[7] and improved upon by Box and Hunter,[8] is an ideal tool for process optimization,[9] and its rotatable characteristic enables it to identify

optimum responses around its centre point without changing the predicting variance.

Objective of this study was to use response surface methodology in conjunction with central composite rotatable design to establish the functional relationships between three operating variables of polymer concentration (X_1), surfactant concentration (X_2) and stirring speed (X_3) and three responses of mean particle size, polydispersity index and surface area of nanoparticles, respectively. In order to optimize Nateglinide nanoparticles, mathematical model equations were derived by computer simulation programming Design-Expert® 8.0.1. For a better understanding of the three variables for the optimal Nateglinide nanoparticle performance, the models were presented as three-dimensional (3D) response surface graphs. Furthermore, morphological characteristics, particle size, particle size distribution, zeta potential value and surface morphology of optimized nanoparticles were evaluated.

MATERIALS AND METHODS

Materials

Nateglinide (NTG) was obtained as a gift sample from Glanmark Pharmaceutics Ltd, Mumbai. Ethyl cellulose (EC) was received from Himedia Laboratories, Mumbai. The following materials were procured from the indicated suppliers and used as received: Polyvinyl alcohol (PVA) (Fourrts India Laboratories Pvt Ltd, Chennai), Methanol (Qualigens Fine Chemicals, Mumbai), Acetone, Sodium di-hydrogen phosphate (NaH_2PO_4) and 85% *ortho*-phosphoric acid (H_3PO_4) of analytical-reagent grade were purchased from S.D. Fine Chemicals Ltd. (Mumbai, India). All other materials and reagents used were of analytical grade.

Preparation of Nateglinide nanoparticles

The Nateglinide-loaded ethyl cellulose nanoparticles were prepared by the solvent evaporation method. Briefly, weighed NTG and EC were dissolved in suitable organic solvent mixture of methanol with acetone in 1:2 ratio using a vortex shaker (to mix small vials of liquid) to form 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 3 hrs to prepare nanosuspension and thoroughly evaporate the organic phase followed by magnetic stirring for 2 hrs under atmospheric pressure at room temperature. The solution was centrifuged at 15,000 rpm for 15 Min.

After centrifugation the supernatant was excreted and the pallets obtained were washed by using the same volume of distilled water as of the supernatant and again centrifuged at 15,000 rpm for 5 Min.

The precipitate was washed thrice with distilled water and finally freeze-dried to get the powdered nanoparticles.[10,11]

Experimental Design

Preliminary experiments indicated that the variables, such as polymer concentration, surfactant concentration and stirring speed were the main factor that affects the particle size, polydispersity index and surface area of nanoparticles. Thus, a central composite rotatable design-response surface methodology (CCRD-RSM) was used to systemically investigate the influence of these three critical formulation variables on particle size, polydispersity index and surface area of the nanoparticles. 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.

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.[12]

Scanning electron microscopy

Scanning electron microscopy (SEM) of the nanoparticle formulation was performed to evaluate the surface morphology of nanoparticles. Images were taken using JEOL JSM-5610LV (Tokyo, Japan) at 25 kV with 2,000 and 5,000 magnification, and 1µm & 200 nm scale bar was used.[13]

Chromatographic Conditions

Nateglinide estimation was carried out by reverse phase high pressure liquid chromatographic (RP-HPLC) based on the reported method by Madhavi.[14] An isocratic 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 ACN: 10 mM Sodium di-hydrogen phosphate (NaH_2PO_4) buffer solution [phosphate-buffered saline (PBS); adjusted to pH 3.0 with H_3PO_4] (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 content and entrapment efficiency

A 10 mg sample of the formulated nanoparticles was dissolved in 10 mL acetonitrile (as common solvent for both the drug and polymer) and from the above solution 20 µl was taken. The amount of drug in the solution was calculated using standard graph of Nateglinide in pH 7.4 PBS buffer 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.[14] Drug content (% w⁄w) and drug entrapment (%) were represented by equations 1 and 2, respectively.[15]

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

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

RESULTS AND DISCUSSION

Optimization of NTG-loaded EC nanoparticles by Response surface methodology

Experimental design, data analysis and desirability function calculations were performed by using Design-Expert® version8.0.1 (Stat-Ease Inc., Minneapolis). Before starting an optimization procedure, it is important to investigate the curvature term using central composite rotatable design response surface methodology. 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. CCRD-RSM 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 Polymer concentration (X_1), Surfactant concentration (X_2) and Stirring speed (X_3). The Particle size, Polydispersity index and Surface area were selected as responses.

All experiments were conducted in randomized order to minimize the effects of uncontrolled variables that may introduce a bias on the measurements. Replicates (n=6) 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).

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

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^2 always decreases when a regressor variable is eliminated from a regression model, in statistical modelling the adjusted R^2 which takes the number of regressor variables into account, is usually selected.[16,17]

In the present study, the adjusted R^2 were well within the acceptable limits of $R^2 \geq 0.8044$ 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. 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 was found to be more than 10%.

In Figure 1 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. Figure 1a shows that stirring speed alone affects the particle size. In Figure 1b, polydispersity index is highly affected by polymer concentration followed by stirring speed and surfactant concentration. Figure 1c shows that surface area is mainly affected by stirring speed followed by polymer concentration and surfactant concentration.

Response surfaces plots for particle size, polydispersity index and surface area are illustrated in Figure 2 (Polymer concentration, surfactant concentration and stirring speed were plotted against particle size, polydispersity index and surface area held at constant at the centre value). Analysis of the perturbation plots and response plots of optimization models revealed that polymer concentration and stirring speed had the significant effect on the polydispersity index and particle size.

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 3 with a D value of 0.830 which is well within the range.

Characterization

Particle size and zeta potential measurement

The mean particle size of nanoparticle was 172 nm. The zeta potential of the nanoparticle was found to be -15m V, and it is sufficiently high to form stable colloidal nanosuspension. The image is shown in Figure 4. The percentage of drug content and entrapment efficiency was found to be 85.82 ± 0.28 % and 71.16 ± 0.24 % respectively.

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 optimal nanoparticle formulation, as shown in Figure 5. The nanoparticles are smooth surface of the particles with round structure.

From the images it was found to be formulated nanoparticles are uniform size and it indicates that the formulation method was efficient.

CONCLUSION

Solvent evaporation method was employed to prepare the nanoparticles. The formulation of NTG-loaded EC nanoparticles were optimized using the central composite rotatable design-response surface methodology 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 DC of 85.82 ± 0.28 %, EE of 71.16 ± 0.24 %, mean diameter of 172 nm and zeta potential value of -15mV.

SEM showed that the nanoparticles are round structure, loading with drug microcrystal uniformly on the smooth surface of and inside the nanoparticles.

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

Variables	Levels				
	-1.682	**-1**	**0**	**+1**	**+1.682**
Polymer concentration (mg)	69.5462	90	120	150	170.454
Surfactant concentration (%)	0.659104	1	1.5	2	2.3409
Stirring speed (rpm)	329.552	500	750	1000	1170.45

Table 2: Experimental responses and central composite rotatable design arrangements

Design points	Factor level			Responses		
	Polymer concentration (mg)	**Surfactant concentration (%)**	**Stirring speed (rpm)**	**Particle size (nm)**	**Polydispersity index**	**Surface area (m^2/g)**
1	**-1**	**-1**	**-1**	202	0.652	51.3
2	**+1**	**-1**	**-1**	263	1.324	49.1
3	**-1**	**+1**	**-1**	249	0.383	42.7
4	**+1**	**+1**	**-1**	237	0.809	37.16
5	**-1**	**-1**	**+1**	141	0.527	51.91
6	**+1**	**-1**	**+1**	102	1.142	52.7
7	**-1**	**+1**	**+1**	147	0.306	54.7
8	**+1**	**+1**	**+1**	161	0.757	53.37
9	**-1.682**	**0**	**0**	183	0.113	54.72
10	**+1.682**	**0**	**0**	214	1.174	53.37
11	**0**	**-1.682**	**0**	148	1.113	55.1
12	**0**	**+1.682**	**0**	177	0.43	50
13	**0**	**0**	**-1.682**	177	0.705	45.1
14	**0**	**0**	**+1.682**	206	0.588	54.6
15	**0**	**0**	**0**	206	0.704	48

Table 3: Response models and statistical parameters obtained from ANOVA for CCD

Responses	Regression model	Adjusted R^2	Model p values	Adequate precision	% CV
Particle size	+192.15- 25.72×C	0.8126	<0.0001	3.28	21.28
Polydispersity index	+0.69 +0.29×A-0.19×B-0.046×C-0.051×A×B+0.037×B^2	0.8563	<0.0001	2.86	10.42
Surface area	+48.76 – 0.77×A-1.88×B+3.54×C+3.00×B×C+1.37×A^2	0.8824	<0.0001	3.12	14.26
Acceptance criteria		≥0.80	<0.05	<4%	>10%

Table 4: Comparison of experimental and predicted values under optimal conditions for final formulation

Polymer concentration (mg)	Surfactant concentration (%)	Stirring speed (rpm)	Particle size (nm)	Polydispersity index	Surface area (m^2/g)
150	1	1000			
Desirability D value			0.830		
Predicted			170	1.203	51.77
Experimental			172	1.212	52.20
Bias (%)			1.17	0.74	0.83
Acceptance criteria = 2%					
Bias was calculated as (Predicted value- Experimental value)/ Predicted value ×100					

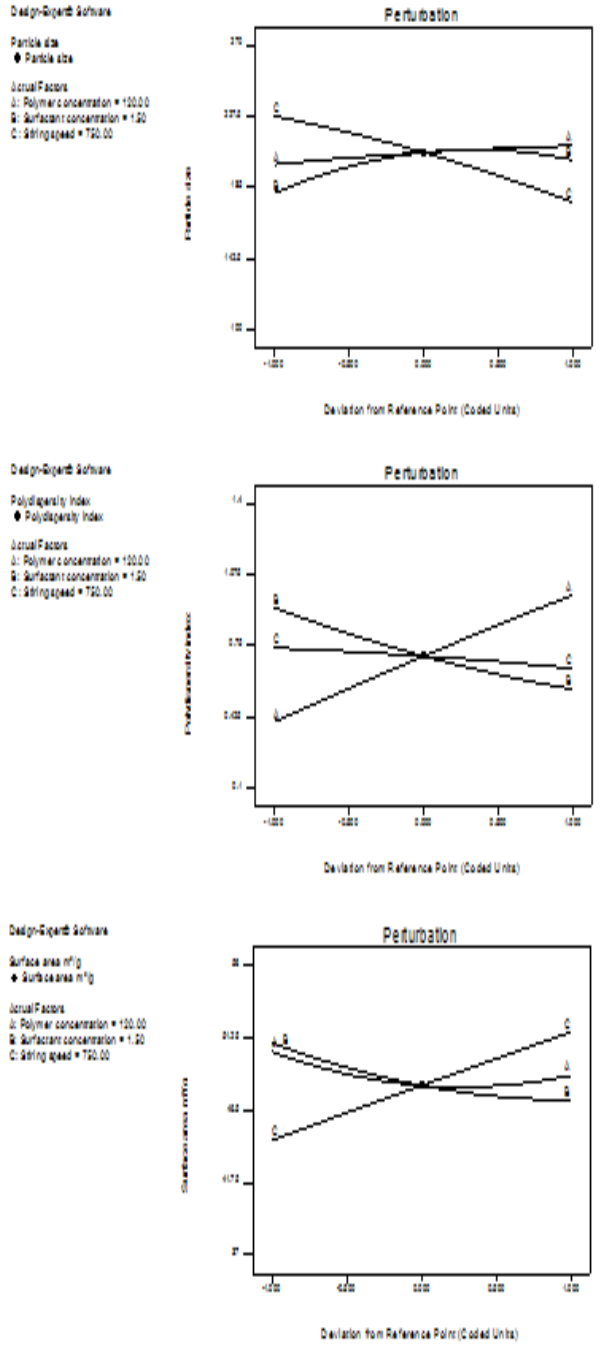

Figure 1: Perturbation plots showing the effect of each of the independent variable on Particle size, Polydispersity index and Surface area.

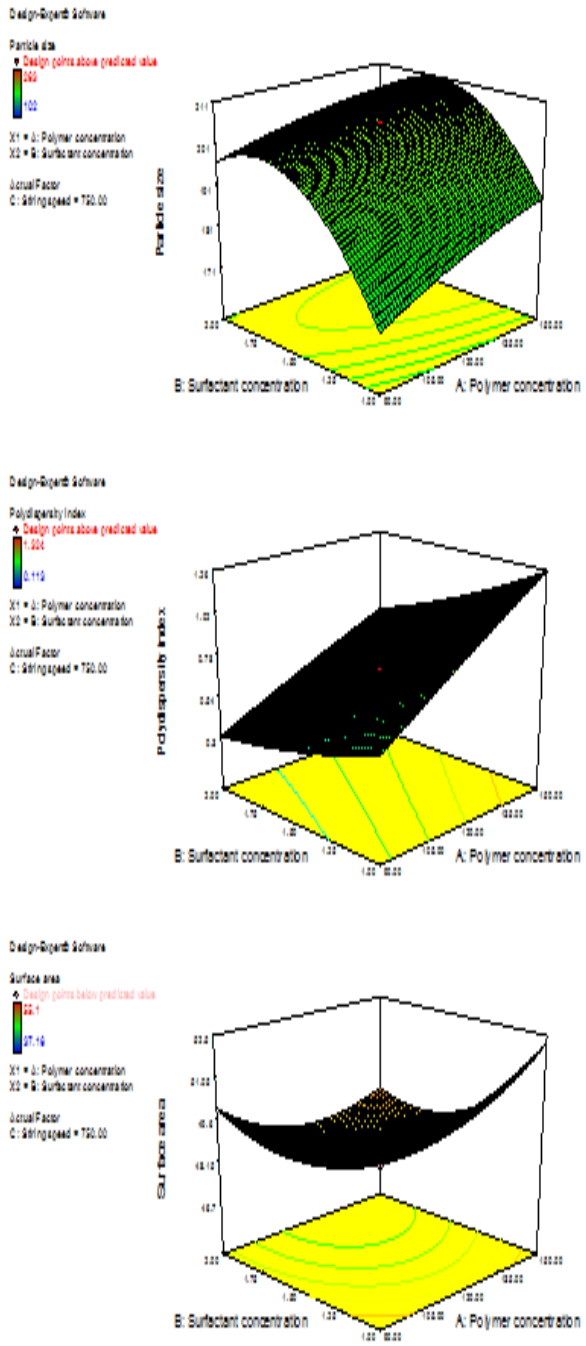

Figure 2: Three dimensional (3D) response surface plots showing the effect of the variable on the response

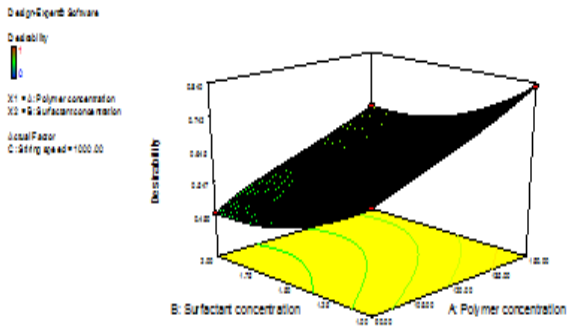

Figure 3: Graphical representation of overall desirability function

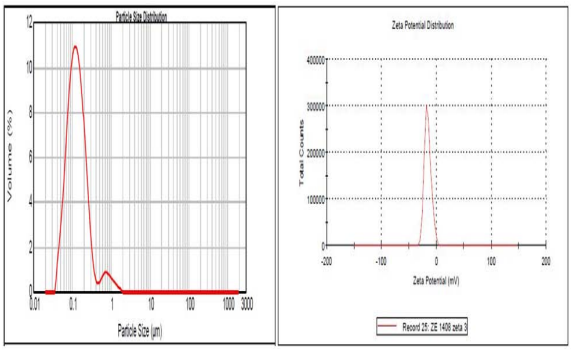

Figure 4: Particle size distribution and zeta potential of the nanoparticles

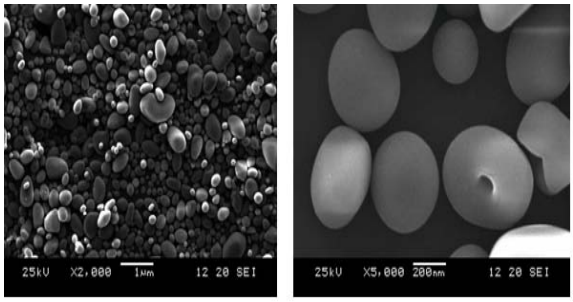

Figure 5: SEM of nanoparticles with magnification ×2000 and magnification ×5000

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