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Influence of Process Variables on Budesonide Nanoparticles Using Factorial Design

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Authors' contributions

This work was carried out in collaboration among all authors. Author AK performed the statistical analysis and supervision of the study. Author JJ managed the literature searches, designed and performed the study and wrote the first draft of the manuscript. Authors AK and AG managed the review and editing of the manuscript. Author BK managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Nanoparticles are the colloidal carrier systems for delivery of poorly soluble drugs. Budesonide. (BUD) a corticosteroid practically insoluble in water is used in asthma treatment. The aim of the present research work was to develop and evaluate BUD nanoparticles.
Methodology: The prepared formulation was analyzed for % encapsulation efficiency, particle size analysis, zeta potential, polydispersity index (PDI), scanning electron microscopy and transmission electron microscopy. Poloxamer-188 was found in stabilizing BUD nanoparticles.
Results: The observed % encapsulation efficiency of the optimized batch was (82.95) %, particle size was 271.8 nm with PDI 0.456. Solvent injection method was successfully implemented to developed BUD nanoparticles poloxamer-188. Sonication time and amplitude played an important role in governing the particle size.
Conclusion: It can be inferred from the study that nanoparticles are a potential drug delivery method for poorly water-soluble drug delivery which can not only get impacted by formulation variables but also by process variables.

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Keywords: Budesonide; factorial design; nanoparticles; particle size analysis; solvent injection method.

1. INTRODUCTION

Due to high surface area, lung permeation, avoidance of hepatic first pass metabolism and non-invasive route for drug administration, the pulmonary drug delivery system is jointly defined by the chosen drug delivery systems. It is observed to be the most effective treatment route for asthma, chronic pulmonary obstructive disease, and cystic fibrosis [1,2]. The efficacy of nanoparticles in the body depends on the size and charge of the particles on the surface. The clearance from the lungs of inhaled nanoparticles is dependent on the particle size. Compared with larger particles, small particles are cleared slower, with further translocation to interstitial sites and lymph nodes. To prevent macrophages from phagocytosis, particles smaller than ~ 250 nm are often widely recognized. In addition, for an extended period of time, nanosized particles adhere to the lung's mucosal surfaces [1-5].

Nanotechnology is a multidisciplinary scientific practice that includes the assembly and use of nanotechnology for products, equipment or systems. Over the past few decades, there has already been significant research interest in the area of drug delivery, using particle delivery systems as carriers for small and large molecules [5,6]. Nanoparticles offer a promising method of controlled and targeted release for drug delivery. In order to observe and improve the pharmacokinetic and pharmacodynamic properties of assorted types of drug molecules, nanoparticles are used as a physical method. For the delivery of therapeutic drugs, nanoparticles attract great deal of attention. It is now possible to choose the easiest type of preparation with the most active polymer in order to successfully capture the drug, depending on the physicochemical characteristics of the product. There are various methods available for arranging nanoparticles, including evaporation of solvents, nanoprecipitation, emulsification of solvents, dialysis. [7-9].

Budesonide (BUD), a corticosteroid used to treat obstructive coronary pulmonary disease as a first-line treatment [2,10]. For BUD, the optimum dose ranges between 200 µg and 800 µg. It is a potent corticosteroid that is non-halogenated and has the highest effect on glucocorticoid receptors. The first-pass liver metabolism of Budesonide is about 90 percent, the highest reason for its poor oral bioavailability of 6 to 11% with a half-life of 2 to 3 hours [11]. High doses of corticosteroids, with long-term administration, cause severe side effects [12,13]. There is a need for controlled release of BUD nanoparticles. Such a formulation can reduce systemic side effects by achieving elevated local lung concentration and improving patient compliance. The goal of the research is the production of BUD nanoparticles [14]. In addition, the goal was to optimize it to achieve the desired particle size with maximum percent trapping efficiency for independent variables (% EE) [15,16]. Factorial architecture makes it possible to vary all the variables at the same time. Using factorial architecture, several researchers have optimized nanoparticulate formulations.

2. MATERIALS AND METHODS

2.1 Materials

Avik Pharmaceutical, Vapi supplied Budesonide as a gift sample, Poloxamer-188 was obtained as gift sample from BASF, Mumbai. The other reagents were obtained from S. D. Fine Limited Chemicals, India, Mumbai.

2.2 Methods

2.2.1 BUD nanoparticle – design of experiments

Preliminary studies were performed to determine the impact of different polymers and main process parameters in which they are placed in the optimal range. The effect of two process variables (sonication time and amplitude) were established on the particle size and entrapment efficiency. Based on the preliminary runs, a 3-level factorial design was used to study the effect of each independent variable on the dependent variable (particle size). The independent factors and dependent variables are represented in Table 1. [17-19]. Using Stat-Ease Design Expert version 13 software, the design response was analyzed.

2.2.2 Preparation of BUD nanoparticles

Using a solvent injection technique with ethanol as the organic solvent, the BUD nanoparticles were prepared. 50 mg of Poloxamer-188 was dissolved in water to form a clear solution. BUD

(2 mg) was dissolved in ethanol. With stirring, the organic phase was introduced dropwise to the aqueous solution containing the polymer by means of a hypodermic needle. The mixture was then sonicated for a variable duration and amplitude to obtain nanoparticles. Using a similar process, the sonication time and amplitude of BUD nanoparticles were prepared [20-23].

2.3 Evaluation of BUD Nanoparticles

2.3.1 Particle size analysis

Particle size analysis was determined using laser diffraction technique (Malvern 2000 SM, Instruments, UK). The particle size measure ments were carried out at a 90° scattering angle. In distilled water, the samples were dispersed. In terms of d(0.9) nm, the average particle size was calculated and expressed [24].

2.3.2 Zeta potential

The zeta potential was measured at a temperature of 25°C using the Doppler laser electrophoretic mobility measurement technique (Zeta Potential Measurement ZS 90, Malvern Instruments, UK) [24].

2.3.3 Entrapment efficiency

The sample of freshly prepared BUD nano particles was centrifuged and examined to quantify supernatants. The UV-Spectrophoto meter at Lambda max 246 nm was used to evaluate the unentrapped drug [25].

EE%=Total amount of drug added−Unloaded Drug / Total amount of drug added × 10 (1)

2.3.4 Differential scanning calorimetry (DSC)

To obtain melting point endotherms from the formed BUN nanoparticles, the DSC thermogram was carried out using the (Hitachi 7020) instrument. At a nitrogen flow rate of 30 ml/min, approximately 3-5 mg of the sample washeated in an aluminum pan at a heating rate of 10°C/min. Analyses of thermal data were then carried out by a DSC thermogram.

2.3.5 Transmission electron microscopy (TEM)

Transmission electron microscopy was used to morphologies BUD nanoparticles (Tecnai G2 Ultra twin FEI, Netherland). A drop of the sample was placed on a coated carbon grid to create a thin layer of liquid. The excess solution was extracted and the sample was tested at an accelerating voltage of 120 KV and photographed.

2.3.6 Scanning electron microscopy (SEM)

Scanning electron microscopy has determined the external morphology (Oxford Instruments, INCA X Sight, UK). On double-faced adhesive film, spray dried samples were mounted and analyzed with a thin gold-palladium layer by a sputter-coated device and surface topography.

3. RESULTS AND DISCUSSION

3.1 Statistic Experimental Data Analysis by Design Expert Software

BUD nanoparticles were prepared using a solvent injection method followed by sonication. Sonication time and amplitude were the two factors that were evaluated for the response's particles size and entrapment efficiency. It was observed that both the factors were showing significant effect on the responses. The results of design were analyzed using Design Expert software showing relevant data of statistical design [17, 26,27].

Based on the results obtained for particle size, the polynomial equation and response surface plots were determined investigate the response [28]. Nonsignificant terms were eliminated from the obtained ANOVA to make the model significant and reduced quadratic model was obtained

$$Y_{\text{Particle Size}} = -2608.25 + 385.03\,X_1 + 18.84\,X_2 - 1.98\,X_1 X_2 - 8.72\,X_1^2;\ R^2 = 0.9164 \quad (2)$$

The ANOVA results showed the effects have p-values less than 0.05, which indicates that factors are significantly impacting the response. As per equation (2) and Fig. 1. the values of coefficient indicate a good fit. The coefficient's negative sign confirms the aggregation of nanoparticles, resulting in an increase in particle size[29]. Although the amplitude shows a positive effect on the response but this value is comparatively very small than the positive effect of sonication time. This explains the significant effect of the sonication time on the particle size. The same is reflected in the response surface plot Fig. 1. the sonication time increases the particles size also increases. This decrease in the particle size may be because of the high

concentration of the polymer present in the formulation at a specific run. But at the same time in the process variables, when the formulation is subjected at high sonication time (20 mins) and amplitude (60 %) aggregates with increased particles size were observed which is also reflected. Equation 2 depicts the negative sign of the interaction effect between the two factors and individual interaction effect of sonication time. Hence, Batch 3 from the optimized batched was selected for further characterization studies [15,30].

The desirability function was calculated using the criteria given in Table 2. and the contour plot of desirability function as given in Fig. 2(a). Maximum desirability that can be achieved is 1. Desirability above 900 in the contour plot is represented by red colored region. The desirability had increased with the maximum duration of sonication time above 15 hrs. as reflected from Fig. 2(b). An exact depiction of the operating condition and corresponding desirability could be obtained from a graphical representation of the numerical optimization results. This could be utilized to identify the optimum operating conditions (sonication time and amplitude) and overall desirability to achieve optimum particle size. Accordingly, the optimum conditions for achieving a range of particle size of 271.8 - 996.1 were 18.14 sonication time (mins) and 33.21 amplitude (%) as shown in the Fig. 3(a). Fig. 2(a) shows overall desirability achieved under optimum conditions was 1. Bar graph as represented in the Fig. 3(b) shows how well each process variables satisfied the criteria of the individual process variable values near one are good [31,32].

3.2 Evaluation of BUD Nanoparticles

3.2.1 Particle size analysis

As shown in Fig. 4, the observed particle size ranged from 271.8 to 996.1 nm. Ultrasound sonic waves lead to the creation of cavitation forces, which disrupt the particle structure. Until the size of the nanoparticles decreases, optimum pressure and ultrasonic time are retained and sustained cavitation forces and exposure of the particles to these conditions for a longer period of time leads to particle aggregation. For a prolonged period of time, excessive cavitation forces and exposure of particles to these conditions contribute to particle aggregation and optimum pressure and ultrasound exposure until the size of nanoparticles decreases [33]. The increase in ultrasound time and amplitude at a given point indicates a decrease in the average particle size, but an increase in ultrasound time contributes to a decrease in the average particle size. With a PDI of 0.456, Batch F3 gave a minimum average particle size of 271.8 nm, with a PSD of less than 0.6 suggesting particle monodispersity [34].

3.2.2 Zeta potential

Observed zeta potential values ranged from -2.17 to -11.3 mV. Compared to other samples, the formulation containing a zeta potential of -11.3 mV is considered a stable lot, as shown in Fig. 5. [33].

3.2.3 Entrapment Efficiency

The drug entrapment efficiency percentage was between 82.95 and 99.80%. Based on its variations in the solubility of the PEO and PPO groups, Poloxamer forms a thermodynamically stable self-assembly in an aqueous solution. Poloxamer in aqueous solution to adhere to the PEO-PPO-PEO unimer solution, which helps to form self-assembly due to hydrogen bonds between bending and breaking water molecules. The self-assembly property of the aqueous solution of poloxamer is responsible for greater trapping efficiency [34,33].

3.2.4 Differential Scanning Calorimetry (DSC)

Due to the drug's melting point, the DSC thermogram (Fig. 6) of budesonide displayed a high endothermic peak at 260°C. At 165°C, BUD nanoparticles show an endothermic peak. The lack of an endothermic peak of budesonide across the formulation continuum meant that the drug content was completely stuck in the formulation [35,36].

3.2.5 Transmission electron microscopy (TEM)

It was evident from Fig. 7. that a round and homogeneous form was revealed by the particles. In the figure, the form of the nanoparticles trapped with the model drug is shown.

3.2.6 Scanning electron microscopy (SEM)

As shown in Fig. 8, the surface morphology and the nature of the formulated nanoparticles have been confirmed by the SEM technique. In surface and spherical form, the optimized BUD nanoparticles observed were smooth [37,38].

Table 1. 3^2 Factorial design for BUN nanoparticles

Runs	X_1: Sonication time	X_2: Amplitude	Y: Particle Size
	mins	%	nm
1	15	60	621.8
2	10	30	299.9
3	10	60	271.8
4	10	45	399.0
5	15	45	556.2
6	20	30	996.1
7	20	45	620.5
8	20	60	372.0
9	15	30	955.9

Table 2. Desirability analysis of particle size: Goal settings of each variable and response

Factors	Goal	Goal Setting
Sonication time (mins)	In range	10 – 20
Amplitude (%)	In range	30 – 60
Response		
Particle Size (nm)	In range	271.8 - 996.1

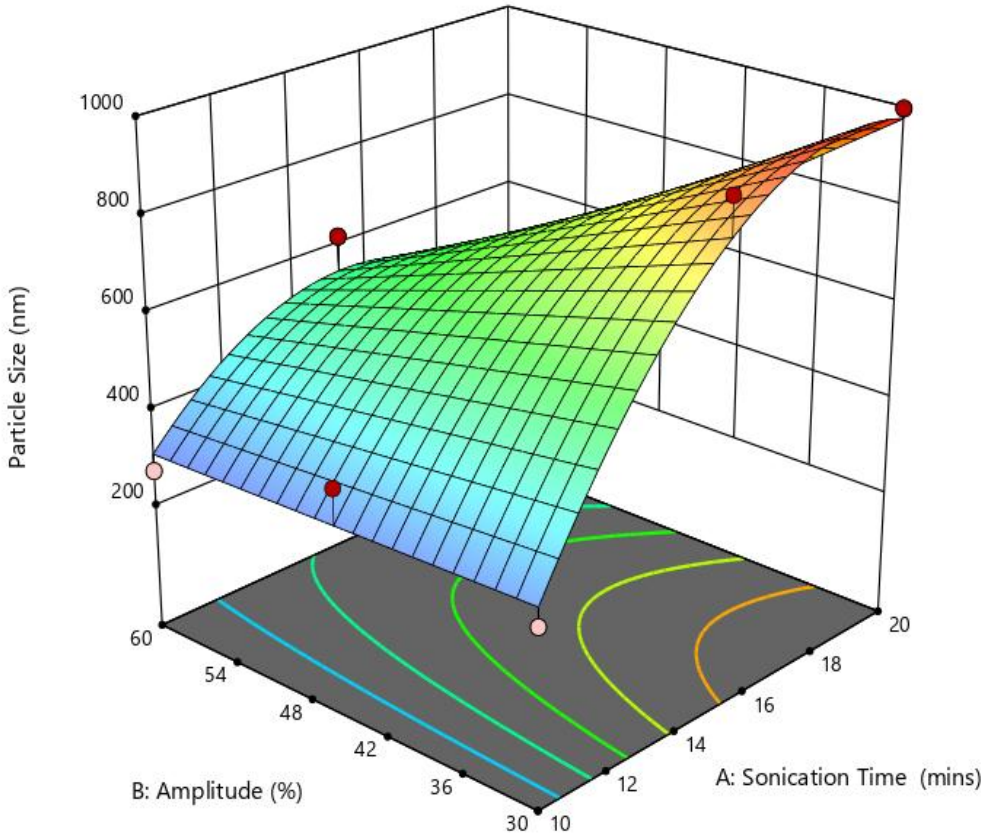

Fig. 1. Response surface graphs showing effect of sonication time (mins) and amplitude (%) on particle size (nm)

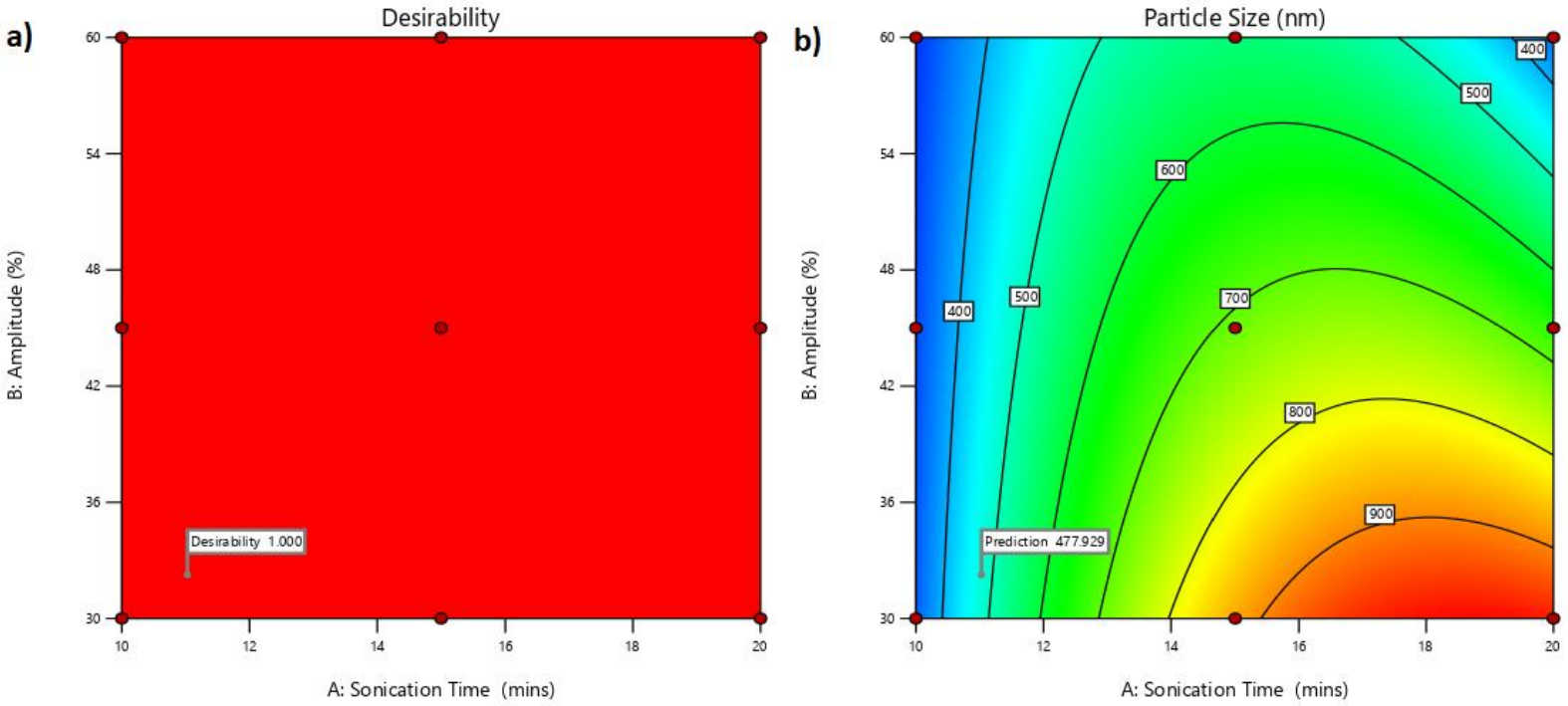

Fig. 2. (a) Desirability function and (b) contour plot of effect of sonication time (mins) and amplitude (%) on particle size (nm)

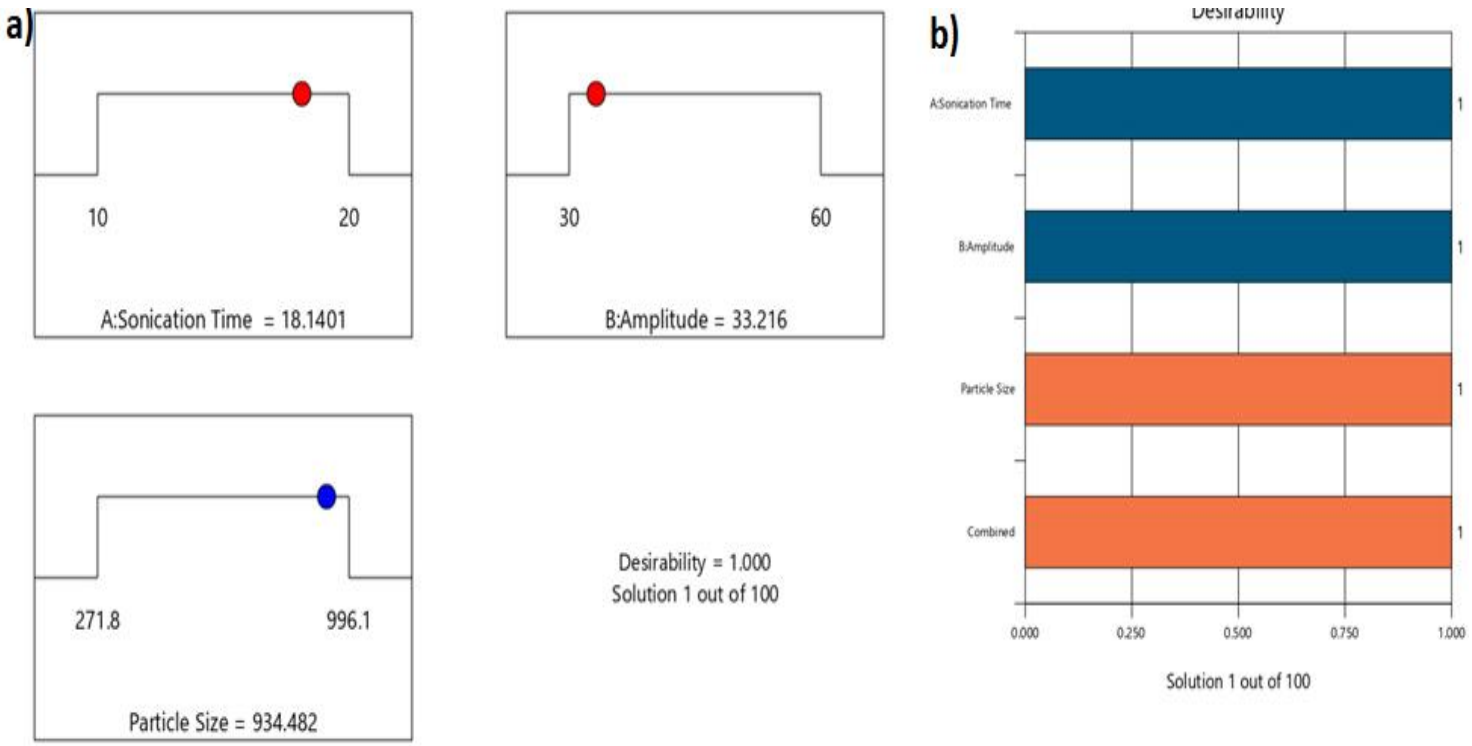

Fig. 3. Desirability functions (a) Graphical representation of desirability ramps (numerical optimization results) for achieving optimum particle size and (b) bar graph showing solution 1 out of 100

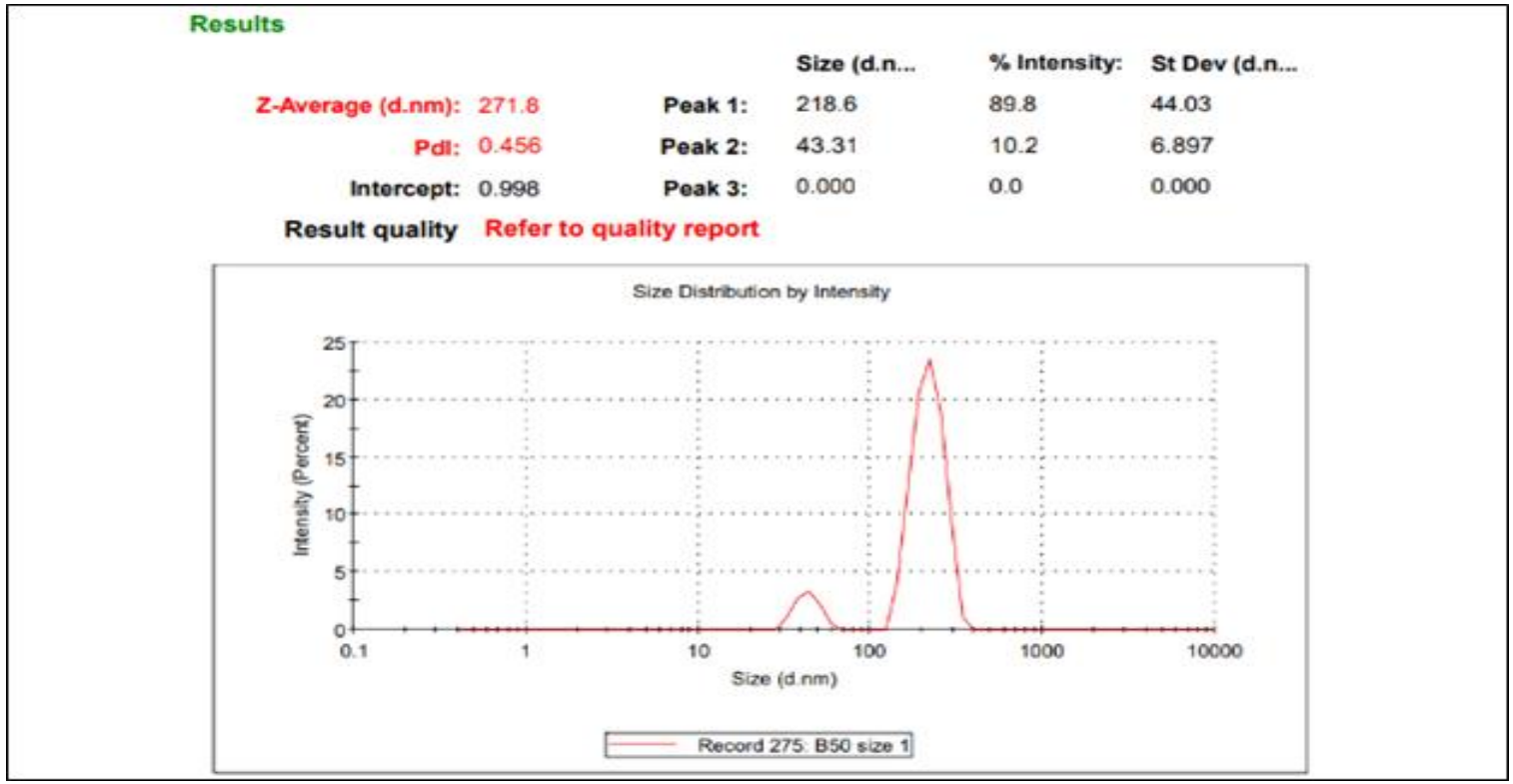

Fig. 4. Particle size distribution of the optimized BUD nanoparticles

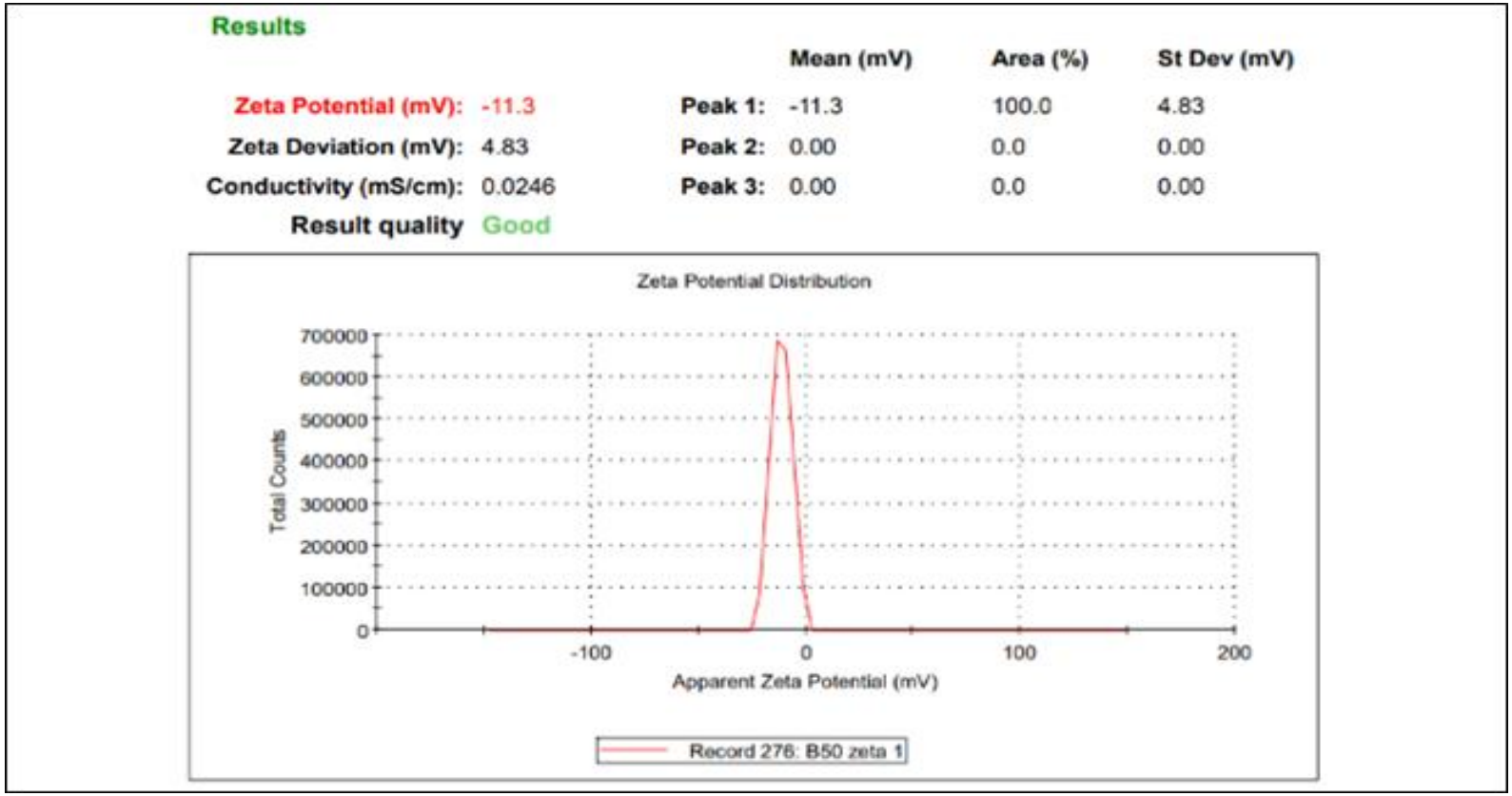

Fig. 5. Zeta potential of the optimized BUD nanoparticles

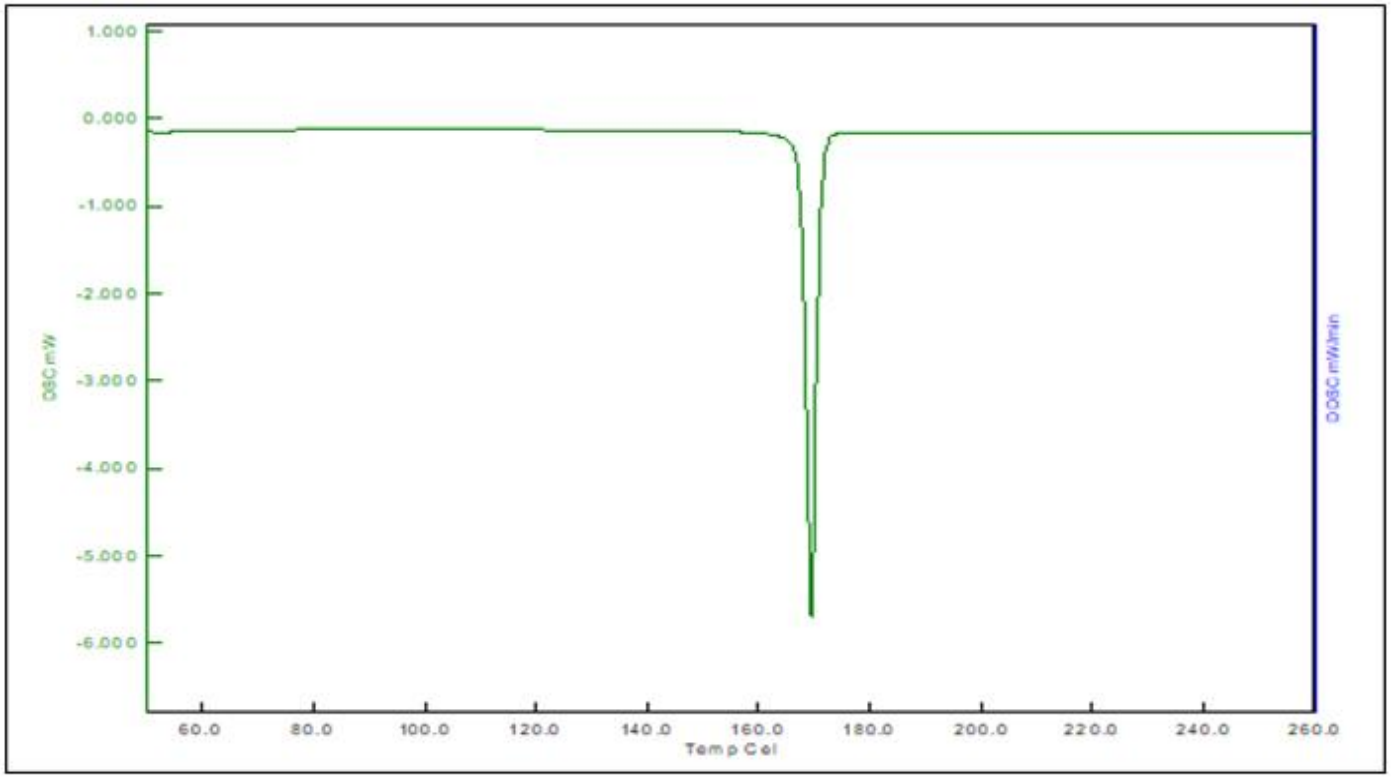

Fig. 6. DSC thermogram of the optimized BUD nanoparticles

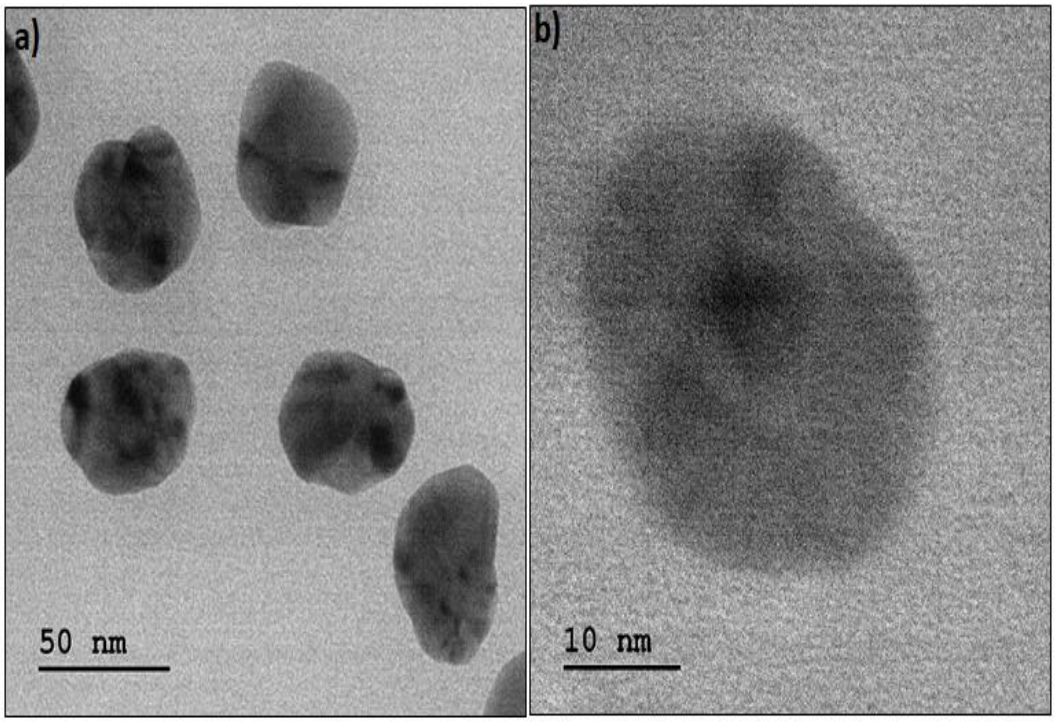

Fig. 7. TEM images of the optimized BUD nanoparticles

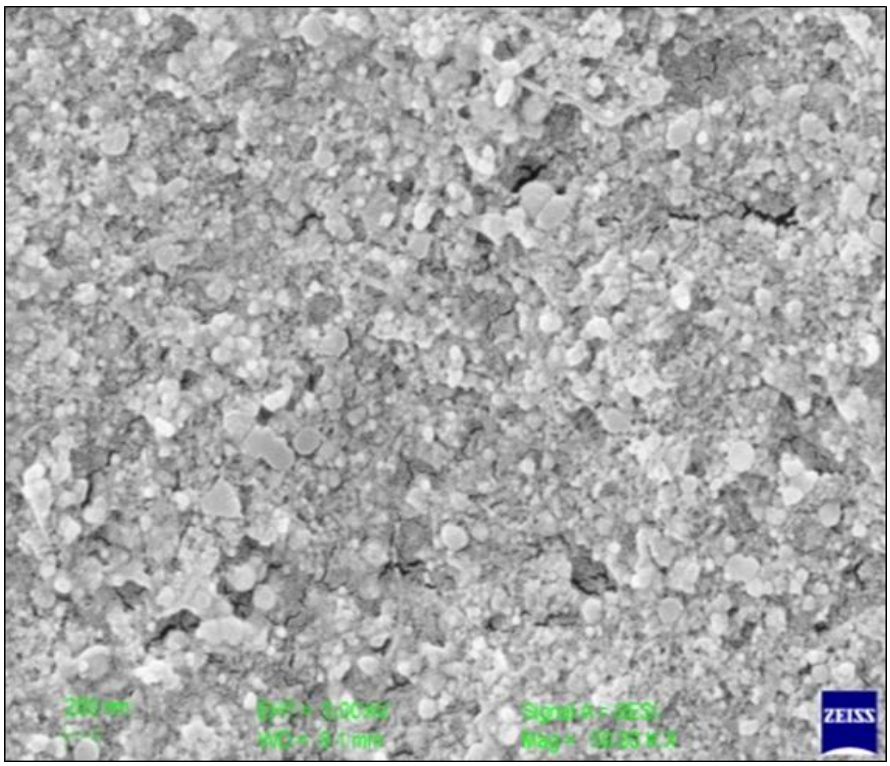

Fig. 8. SEM images of the optimized BUD nanoparticles

4. CONCLUSION

BUN nanoparticles have been successfully developed to provide optimized formulations of nano particle size and percentage of entrapping efficiency. The use of 3^2 factorial design allowed an acceptable formulation to be developed, using the required number of trials with a minimum time limit. Influence of the process variables was observed on particles size.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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