ORIGINAL ARTICLE

Optimization of size controlled poly (lactide-co-glycolic acid) nanoparticles using quality by design concept

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Quality by design (QbD) is a risk management and science-based approach laid down by the ICH as well as other Regulatory agencies to enhance pharmaceutical development throughout a product's lifecycle. Poly(lactide-co-glycolic acid) (PLGA) is the material of choice for development of depot particulate formulations due to its biodegradable nature and is also considered as the 'green' eco-friendly material due its biocompatibility and non-toxic properties. Further, PLGA based formulations are approved by regulatory agencies and currently in clinical practice. The aim of the current investigation involves formulation, optimization and in vitro characterization of size controlled PLGA based nanoparticles by employing modified nanoprecipitation technique. An initial risk-assessment analysis was conducted with different formulation and process variables along with their impact on critical quality attributes of the formulation which were identified as particle size and percentage process yield. The Ishikawa diagram was employed to determine the potential risk factors and subsequently optimized by statistical experimental design concept. Box–Behnken design was utilized to optimize nanoparticles and further characterizing the optimized nanoparticulate formulation in vitro. From the present study, it can be concluded that PLGA based nanoparticles with controlled particle size and process yield can be obtained by inculcating the concept of QbD in the product development.

Key words: *Box–Behnken design, nanoprecipitation technique, particle size, poly(lactide-co-glycolic acid), quality by design*

INTRODUCTION

Nanoparticulate delivery systems,[1] such as those based on poly(lactic-co-glycolic acid) (PLGA) have been studied extensively for many years. For the past three decades, lot of researchers has explored PLGA to fabricate drug delivery systems for pharmaceutical and biomedical applications due to its biocompatible and biodegradable properties. PLGA, further, has the advantage of being well characterized and commercially used for microparticulate and nanoparticulate drug delivery systems (Allemann and Leroux, 1999). PLGA polymer is one of the most common biodegradable polymers used for the controlled delivery of drugs due to its early use and approval as a compatible biomaterial in humans. Lewis reported that, by varying the molecular weight and lactide/glycolide ratio, the degradation time of the polylactic acid (PLA) and PLGA and the release kinetics of the active agent can be controlled.[2] In aqueous media, degradation of PLGA is triggered by hydrolysis of its ester linkages. Presence of methyl side groups in PLA makes it more hydrophobic than PGA and hence lactide rich PLGA copolymers are less hydrophilic, absorb less water and degrade more slowly and control the release of drug for prolonged duration.[3,4]

Paradigm shift in drug delivery offer unique distribution characteristics or targeting characteristics based on their size. The major advantages of nanoparticles is improved bioavailability by enhancing aqueous solubility, increasing resistance time in the body (increasing half-life for clearance/increasing specificity for its associated receptors and targeting drug to specific location in the body. This is why nanoparticles

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are increasingly used in a variety of applications that includes drug carrier systems and to pass organ barriers such as the blood-brain barrier, cell membrane, etc. The cellular uptake, biodistribution, and circulating half-life are the key factors which are influenced by particle size of nanoparticles. Therefore, particle size becomes primary concern while formulating a nanoparticulate system.[5] Moreover, the particle size thus obtained should be uniform because more uniform the distribution of particles more consistent will be the biodistribution, cellular uptake, and drug release.[6]

There are several methods available for development of polymeric nanoparticles such as emulsification-diffusion method,[7] emulsification-evaporation method,[8] desolvation method,[9] nanoprecipitation method,[10] etc. Among all the techniques available for preparation of nanoparticles, nanoprecipitation technique is the most commonly used for poorly soluble drugs.[11] In the current investigation, PLGA based nanoparticles were formulated with an objective of achieving optimum process yield, as well as minimal particle size with uniform particle size distribution. Therefore, in a view to pursue the aim, PLGA based nanoparticles were prepared by employing nanoprecipitation method. Preliminary screening studies were conducted with respect to the process yield and particle size by screening different parameters such as the polymer concentration and molecular weight, stirring speed, rate of addition of anti-solvent, type of anti-solvent and selection of stabilizer/surfactant. Initially a number of trials were conducted to establish the process variables and studying their influence on the quality attributes, that is, process yield and particle size. These investigations were accomplished by application of statistical optimization techniques to establish the interrelation between independent variables and response variables. Preliminary experiments revealed that polymer concentration, surfactant concentration, and stirring speed are the factors significantly affecting the critical quality attributes (CQAs). Hence, Box–Behnken design was employed in order to optimize the PLGA based nanoparticulate formulation with controlled particle size and process yield.[8,11]

MATERIALS AND METHODS

Poly(lactide-co-glycolic acid) copolymer RG 502-H (lactide:Glycolide ratio of 50:50, molecular weight 7–17 kDa), Resomer RG 504-H (lactide:glycolide ratio of 50:50, molecular weight 38–54 kDa) was obtained as gift sample from Evonik Industries AG, Germany. Poloxamer 188 was supplied as a gift sample from Sandoz Pvt. Ltd., Mumbai. Dichloromethane (DCM) (purity (Not less than) NLT 99% by gas chromatography [GC]), Acetone (purity NLT 99% by GC), methanol (high performance liquid chromatography grade) were procured from Merck and co., Germany. Double distilled water used was filtered through 0.22 μm filter from Millipore (Mumbai, India) All other cited chemicals used were of analytical grade.

Preparation of nanoparticles

The nanoparticles were developed by employing nanoprecipitation technique as described by Fessi, *et al*.[12] using PLGA copolymer. Briefly an organic solution containing PLGA polymer (5 mg/mL, 40 mL) was added to an aqueous solution containing poloxamer 188 (0.1% w/v) in a dropwise manner under vigorous stirring, followed by magnetic stirring at room temperature. Later the dispersion was kept for magnetic stirring for 6 h at room temperature to evaporate the organic solvent. The precipitated nanoparticles were separated by ultracentrifugation and dried using vacuum drying to obtain free flowing dried nanoparticles.[13]

In the present study, various formulation and process parameters such as type of anti-solvent, type and concentration of stabilizer, concentration and molecular weight of polymer, homogenizer type, rate of addition of anti-solvent, etc., were screened as independent factors, whereas particle size and percentage process yield were chosen as dependent variables and later optimized using Box–Behnken design to evaluate the factors having considerable effect on particle size as well as process yield of PLGA nanoparticles.[14] Each batch of formulation was prepared in triplicate using the aforementioned technique.

Physicochemical characterization

Particle size and size distribution analysis

The average particle size and size distribution of PLGA nanoparticles was measured by dynamic light scattering technique using a particle size analyzer by photon cross-correlation spectrometry (Nanophox, Sympatec GmbH, System-Partikel-Technik, Clausthal-Zellerfeld, Germany). The measurement was done, using laser light scattering which was monitored at a scattering angle of 90° at wavelength of 635 nm. The measurements were repeated three times and average was taken. The nanoparticle sample was diluted in distilled water and sonicated gently for about 3–5 min in a bath-type sonicator and the dispersion thus obtained was analyzed for particle size by loading into 1 cm^2 cuvettes in a thermostated chamber at 25°C. The size distribution obtained is by plotting the relative intensity of light scattered by particles in various size classes and is therefore known as an intensity size distribution. The particle size distribution is exhibited in terms of span value which is obtained using formula:[15]

$$\text{Span value} = \frac{(\text{D90-D10})100}{\text{D50}}$$

Zeta potential

Zeta potential is a scientific term for electro-kinetic potential. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in the dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability,

that is, the solution or dispersion will resist aggregation. When the potential is low, attraction exceeds repulsion, and the dispersion will break and flocculate. Hence, colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate or flocculate.[16,17] Zeta potential was measured using Malvern Instruments Ltd., (Malvern, UK) after dispersion of the sample (10 mg) ion distilled water and sonicated gently for about 3–5 min in a bath-type sonicator.[18,19]

Quality by design

Quality has always been a prime concern for all the pharmaceutical industries and for improving the quality it is needed to be built up in the product. Hence, several elements have to be studied and evaluated such as the quality target product profile (QTPP), scientific knowledge, design space, design of experiments (DOEs), etc., quality by design (QbD) means a series of events leading to a robust formulation by proper design and sound knowledge space.[20,21]

The QTPP is a vital element of QbD principle, and it includes all the formulation attributes that ensure the quality, safety and efficacy of the product. The foremost step in developing a formulation by QbD process was to define the QTPP of the drug product which included details regarding the indication, treatment, usage and dosage, route of administration, contraindications, references, etc. For developing a robust formulation it was necessary to identify a target formulation and the impact of the various formulation variables and process conditions on the quality were studied. To identify key quality attributes among an array of active pharmaceutical ingredient and drug product quality attributes that might later prove to be primary determinants impacting intended safety, efficacy, or performance characteristics of a final product. This was achieved through establishing a list of possible CQAs and critical process parameters (CPPs) affecting the product CQAs which include polymer type and concentration, organic solvent, stabilizer, aqueous solvent, electrolyte concentration, dielectric constant, pH, ionic concentration, stirring speed and time, drying temperature and time, T_g, etc. These CPPs affecting the CQAs were further refined and optimized using DOEs concept by employing Box–Behnken design in order to obtain the limits for construction of design space.[22]

Risk-assessment

After establishing of QTPPs, QbD concept was applied by prior knowledge and past experience with the similar polymer as well as method of preparation with respect to various constraints and an initial risk-assessment was accomplished. For construction of design space (a key element of QbD), it is imperative to identify the CPPs causing variabilities in the CQAs and this is possible through thorough understanding of the process so that the variations can be minimized by controlling the CPPs. This is possible by prior knowledge and risk-assessment of all the CPPs and critical material attributes (CMAs) which have the potential of hampering the quality of the product. Initial risk-assessment included evaluation of every CQA, and the severity of the failure was measured by preliminary hazard analysis tool. All the control strategies developed ensured that the CQAs were within the limits.[23]

Optimization of nanoparticles by Box–Behnken design

In the current investigation, the formulations were optimized using Box–Behnken design to evaluate the influence of three independent factors, that is, polymer concentration (X_1), stirring speed (X_2) and surfactant concentration (X_3) that classified to low, medium, and high values on the response variables, that is, the particle size (Y_1) and percentage process yield (Y_2).

Preliminary experiments involved numerous trials conducted to identify and define the possible number of independent variables that can evolve during formulation and studying its effect on the dependent variables at different levels of factors. The response(s) obtained are measured for each experiment and analyzed by either linear or multiple regression models. However, the change in one independent variable and its influence on the responses can be studied by employing response surface methodology (RSM). The RSM and contour plots can be obtained by employing the regression equation and the impact of each variable on the response variables can be studied effectively. Regression analyzes were carried out to derive a polynomial model for the estimation of the average particle size and percentage process yield.[24]

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots\ldots + \beta_i X_{i.} \quad (1)$$

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 \ldots\ldots + \beta_i X_i + \beta_{12} X_1 X_2 + \beta_{23} X_2 X_3 + \beta_{13} X_1 X_3 + \ldots\ldots + \beta_{ij} X_i X_{j.} \quad (2)$$

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 \times_2 + \beta_3 X_3 \ldots\ldots + \beta_i X_i + \beta_{12} X_1 X_2 + \beta_{23} X_2 X_3 + \beta_{13} X_1 X_3 + \ldots\ldots + \beta_{ij} X_i X_j + \ldots\ldots + \beta_{ij.} \quad (3)$$

Where, Y represents the response while X_1, X_2 and X_3 denoting the main effects of factors. Equation (1) above signifies simple linear regression whereas Equation (2) represents interactive and Equation (3) as quadratic regression equation. β_0 is a constant and β_1, β_2, β_3 are the coefficients of the factors. The P values of the regression coefficients as well as ANOVA were determined in order to evaluate the significance of the variables on the CQAs and significance of the model, respectively.

The design space may be obtained from overlay plots after plotting contour plots and response surface plots based on the desired range of values for the CQAs after which the formulation and process was optimized with respect to particle size and percentage process yield. Several trials were conducted to ascertain the correlation between the predicted and the practical values in order to validate the optimized process or formulation.[25]

RESULTS AND DISCUSSION

Physicochemical properties of poly(lactide-co-glycolic acid) nanoparticles

In the current study, PLGA based nanoparticles were prepared using nanoprecipitation technique which encompasses the addition of polymer in a water-miscible organic solvent with the subsequent addition to the excess amount of anti-solvent (aqueous). The nanoparticles are formed by the diffusion of solvent into anti-solvent. The prepared nanoparticles were then characterized by their particle size, process yield, PDI and zeta potential. Initially several factors such as polymer type and concentration, organic solvent, stabilizer, aqueous solvent, electrolyte concentration, dielectric constant, pH, ionic concentration, stirring speed and time, drying temperature and time were screened and their possible impact on the Physicochemical properties of PLGA nanoparticles were evaluated [Table 1].

Poly(lactide-co-glycolic acid) nanoparticles were prepared using different solvents like acetone, methanol and DCM differing in their polarity and dielectric constant. Among these solvents, it was found that nanoparticles prepared using acetone as solvent obtained lower sized nanoparticles which can be attributed to the rapid diffusion of acetone in water.

The stirring speed used in the process of nanoprecipitation was found to significantly affect the particle size as well as yield of the nanoparticles. Stirring speed as low as 1000 rpm produced nanoparticles with larger particle size as compared to nanoparticles obtained at 2500 rpm speed. Even the concentration of PLGA plays a vital role in the physicochemical properties of nanoparticles obtained by nanoprecipitation technique. From the results it was evident that as the concentration of PLGA was increased from 5 mg/mL to 15 mg/mL, the particle size was found to increase from 105 nm to 124 nm. This may be attributed to the number of polymer chains per unit volume of the solvent leading to the formation of aggregated or large sized particles owing to a more polymer-polymer interaction.

The concentration and type of surfactant is an important factor as it stabilizes the system by reducing the surface tension and control the particle size. Various surfactants differing in their HLB values were selected and subsequently evaluated for their possible impact on the particle size and process yield of the PLGA nanoparticles. Poloxamer 188 gave lowest particle size when compared to tween 80 and sodium lauryl sulfate (SLS). Since, SLS is known to have a deleterious effect on the liver; it is therefore omitted from the study.

From the results of zeta potential, it was found that all the formulations exhibited negative charge ranging from −32.5 to −38.9 mV [Table 2]. These negative charges on the particles suggest that the formulations will remain fairly stable due to the repulsive forces between nanoparticles, thus preventing agglomeration.

Risk identification and analysis

The potential risk of formulation variables on the CQAs (i.e., the particle size and percentage process yield) of the final product was established by Ishikawa diagram. From the preliminary trials and knowledge space, seven potential risk factors were identified as shown in Figure 1 and screened further. The screening studies were conducted in order to establish the significant formulation variables affecting the response variables. Among the variables polymer concentration, stirring speed and surfactant concentration were found to have a significant impact on the particle size and percentage process yield which were further evaluated statistically.

Feasibility of nanoparticles formulation was evaluated by employing three different surfactants, that is, SLS, tween 80 and poloxamer 188 at different concentration levels of the aqueous phase. The inherent property of surfactants of preferentially adsorbing at interface beyond critical micelle concentration on addition to dispersion and reducing the surface tension assists in obtaining smaller particle size of nanoparticles. This can also be attributed to increase in viscosity of the solution thereby hydrodynamically stabilizing the system by preventing coalescence of the particles. This was more evident when poloxamer 188 was used as a surfactant.

Table 1: Preliminary screening studies of the significant factors with their levels

Factors	Code	Levels		
		Low	Medium	High
Polymer concentration (mg/ml)	X1	5	10	15
Surfactant type	X2	SLS	Tween 80	Poloxamer 188
Surfactant concentration (% w/v)	X3	0.1	0.2	0.3
Homogenizer type	X4	Magnetic stirrer	Remi mixer	Homogenizer
Type of organic solvent	X5	DCM	Methanol	Acetone
Molecular weight of PLGA polymer (kDa)	X6	7-17	-	38-53
Stirring speed (rpm)	X7	1000	1750	2500

PLGA: Poly (lactide-co-glycolic acid), SLS: Sodium lauryl sulfate, DCM: Dichloromethane

Table 2: Effect of the independent variables on the physicochemical properties of PLGA nanoparticles

Factors	Factor variable	Percentage process yield	Particle size (nm)	Span value	Zeta potential (mV)
Polymer concentration (mg/ml)	5	62.86±4.5	105.2±10.5	0.048	−36.5
	10	76.57±3.6	118.6±8.2	0.059	−36.8
	15	81.28±2.5	124.1±8.5	0.064	−37.1
Stirring speed (rpm)	1000	58.89±1.2	175.4±6.4	1.12	−38.9
	1750	65±2.6	159.7±4.5	0.091	−35.5
	2500	73.41±1.9	106.3±1.9	0.045	−32.5
Type of organic solvent	Methanol	79.8±4.1	210.7±3.3	0.091	−35.2
	Acetone	81.58±2.8	129.7±1.4	0.071	−36.8
	DCM	73.4±3.2	1080±8.5	0.158	−36.1
Surfactant type	SLS	72.8±2.5	210.7±4.4	0.088	−38.9
	Tween 80	77.61±4.8	205.7±8.6	0.031	−33.7
	Poloxamer 188	81.5±3.6	187.1±5.8	0.071	−34.3

PLGA: Poly (lactide-co-glycolic acid), SLS: Sodium lauryl sulfate, DCM: Dichloromethane

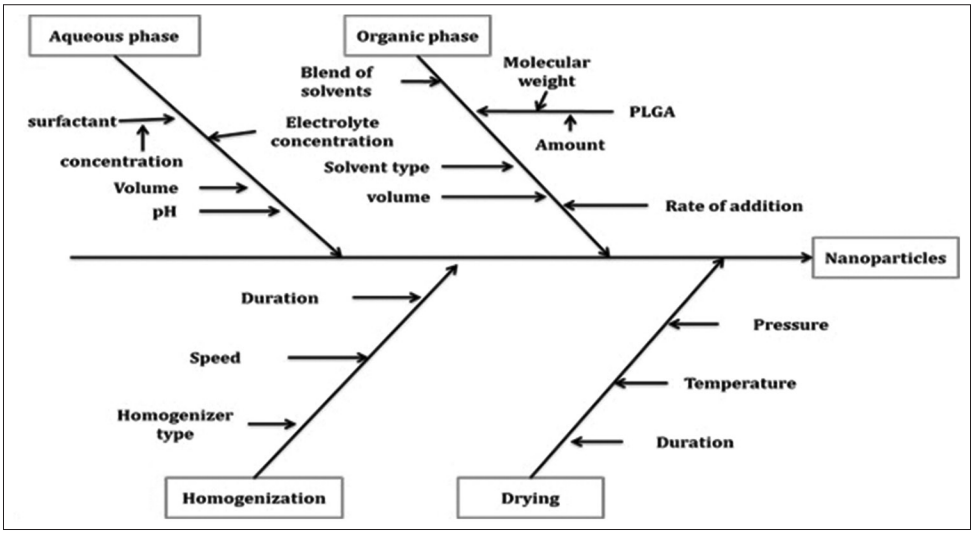

Figure 1: Ishikawa diagram depicting the formulation and process variables having impact on the desired responses of nanoparticles

The polymer concentration was increased from 5 mg/mL, 10 mg/mL and 15 mg/mL and its effect on particle size and process yield of nanoparticles was evaluated. From the results, it was clear that as the concentration of polymer was increased the particle size of the nanoparticles was found to increase. This increase in particle size can be attributed to increased polymer–polymer interaction along with an increase in viscosity of the aqueous phase which prevents effective diffusion of solvent into the aqueous phase thereby increasing the particle size.

The stirring speed of the homogenizer showed a significant difference in the particle size of the nanoparticles. Low-speed homogenizers resulted in larger particle size when compared to high-speed mixers. Moreover, acetone as an organic solvent produced smaller sized nanoparticles when compared to DCM, methanol or combination of the same.

Optimization of poly(lactide-co-glycolic acid) nanoparticles

Design of experiments is an effective tool that can be used in QbD process for developing formulation based on the control strategy which includes CMAs and critical process variables and design space.[25]

In the present investigation, the particle size and percentage process yield were considered as quality attributes, the first step of the QbD process, that is, determination of the CQAs. Subsequently, potential excipients were identified among all the excipients and the process variables that possess the capability to affect the quality of the product. Several such variables were identified such as the concentration and type of surfactant, concentration of polymer, the volumetric ratio of water to anti-solvent, stirring speed. Finally, it was revealed that polymer concentration, stirring speed and surfactant concentration considerably affect the response variables. These variables were tried to link with the CQAs of product

in order to obtain a design space for effective designing of formulation that eventually meets the target profile of the final product.[26]

Box–Behnken design

The primary screening studies revealed polymer concentration, surfactant concentration and stirring speed as significant factors. Therefore, a three factor and three level based Box–Behnken design was applied to understand the impact of polymer concentration (X_1), stirring speed (X_2) and surfactant concentration (X_3) on the particle size (Y_1) and percentage process yield (Y_2) of nanoparticles [Table 3]. The actual and the transformed values of the selected independent variables are presented [Table 4] along with the design layout and the results of the nine experiments [Table 4]. Hence, the broad range of values of responses clearly indicates the dependence of the response variables on the aforementioned independent variables.

Table 3: Design layout and coded units of Box–Behnken design

Independent variable	Actual value	Coded value
Concentration of polymer (X_1) (mg/mL)	5	−1
	10	0
	15	1
Stirring speed (X_2) (rpm)	1000	−1
	1750	0
	2500	1
Surfactant concentration (X_3) (% w/v)	0.1	−1
	0.2	0
	0.3	1

Table 4: Design layout and coded units of Box–Behnken design

Formulation code	X_1	X_2	X_3	Particle size (nm)	Percentage process yield
T01	−1	−1	0	187.9	67.8
T02	1	−1	0	215.8	82.5
T03	−1	1	0	67.3	59.2
T04	1	1	0	95.7	69.9
T05	−1	0	−1	119.5	64.8
T06	1	0	−1	144.8	79.8
T07	−1	0	1	64.8	63.1
T08	1	0	1	137.6	73.3
T09	0	−1	−1	191.4	73.41
T10	0	1	−1	88.1	68.8
T11	0	−1	1	154.2	72.5
T12	0	1	1	71.5	70.2
T13	0	0	0	105.2	72.2
T14	0	0	0	111.2	71.1
T15	0	0	0	110.8	71.8

The optimization was performed, and the graphs were obtained using Minitab® 16 statistical software (Minitab Inc., USA).

The design space was established with the use of contour plots and an optimum formulation was prepared and evaluated with a desirability function in which constraints were set as the minimum average particle size (<100 nm) and the maximum process yield (>65%). A Box–Behnken design was employed to study the effect of three independent variables concentration of polymer (X_1), stirring speed (X_2) and surfactant concentration (X_3) on the preparation of nanoparticles with optimum particle size and yield.[27,28]

The relationship between the factors and their coefficients were determined mathematically with their respective *P* values by employing regression analysis and the factors obtaining $P < 0.05$ were considered as significant [Table 5].

From the results of ANOVA, it was evident that the independent factors possess a significant impact on the particle size as the values were well below 0.05. The polynomial equation is used to obtain useful information in the evaluation of coefficient while the polynomial model for the estimation of particle size was as below:

$$Y_1 = 109.0667 + 19.3\ X_1 - 53.3375\ X_2 - 14.4625\ X_3 + 0.125\ X_1X_2 + 5.15\ X_2X_3 + 11.875\ X_1X_3 + 11.49 + 21.116 - 3.883. \quad (4)$$

From the above equation it was clear that concentration of polymer (X_1) has positive impact on particle size and process yield of nanoparticles, that is, the particle size and yield was increased with increase in concentration of polymer. Whereas the stirring speed (X_2) and surfactant concentration (X_3) exhibited negative impact on particle size and yield of nanoparticles, that is, on increasing the stirring speed of homogenizer and concentration of surfactant, the particle size and yield of nanoparticles were found to decrease. From the polynomial equation, it was clear that low level of polymer concentration favors the formation of nanoparticles with low particle size. Equation (4) is shown in the form of contour plot [Figure 2] and response surface plot [Figure 3] for visualizing the effect of the factors on the particle size of nanoparticles.[29] The results revealed that the stirring speed and surfactant concentration also contribute significantly in reducing particle size of nanoparticles. No significant quadratic effects were observed for the particle size of nanoparticles with respect to polymer concentration and surfactant concentration as the $P > 0.05$. However, the quadratic effects for stirring speed on particle size was significant as the *P* value obtained was <0.05 (0.014) and possessed high coefficient value. Whereas, no significant quadratic effects for percentage process yield were observed with respect to the independent variables since the *P* value obtained was >0.05 and displayed low coefficient values.

Table 5: Regression coefficients and the respective *P* of the independent variables

Factors	Y1 (average particle size) nm		Y2 (percentage process yield)	
	Co-efficient	*P*	Co-efficient	*P*
β_0 (constant)	109.0666667	<0.001	71.7	<0.005
X_1 (concentration of PLGA)	19.3	0.004513924	6.325	0.001116549
X_2 (stirring speed)	−53.3375	<0.003	−3.51375	0.013640081
X_3 (concentration of poloxamer 188)	−14.4625	0.014530154	−0.96375	0.353779629
X_1X_2	0.125	0.982999644	−1	0.487224589
X_2X_3	5.15	0.398528433	0.5775	0.683114298
X_1X_3	11.875	0.086682551	−1.2	0.409582188
X_1^2	11.49166667	0.104843789	−1.41375	0.35528431
X_2^2	21.11666667	0.014986046	−0.43625	0.766056523
X_3^2	−3.883333333	0.533477147	−0.03625	0.980180967

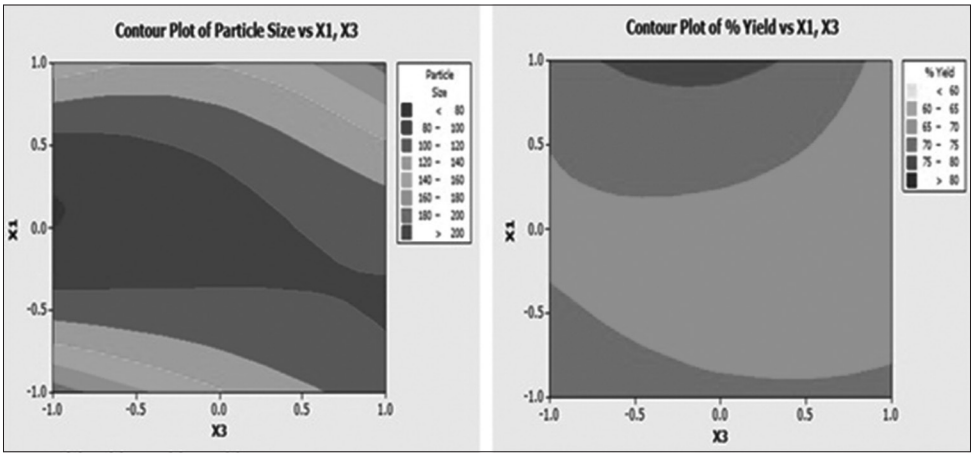

Figure 2: Contour plots showing the impact of polymer concentration (X_1) and surfactant concentration (X_3) on average particle size and percentage process yield of nanoparticles

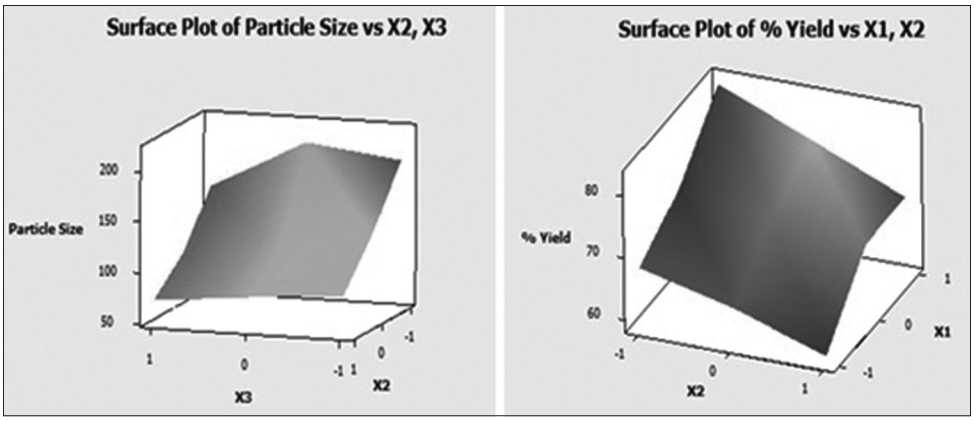

Figure 3: Response surface plots showing the effect of polymer concentration (X_1), stirring speed (X_2) and surfactant concentration (X_3) on average particle size and percentage process yield of nanoparticles

Similarly, a second regression equation was generated for the estimation of percentage process yield of nanoparticles shown as below:

$$Y_2 = 71.1 + 6.325\ X_1 - 3.513\ X_2 - 0.963\ X_3 - 1\ X_1X_2 + 0.5775\ X_2X_3 - 1.2\ X_1X_3 - 1.413 - 0.4362 - 0.03625. \quad (5)$$

After the estimation of polynomial equations, the design space was established by setting the target value for particle size (<100 nm) and process yield (>65%) and for this design, contour plots [Figure 4] along with the response were established.

The two-dimensional plot obtained by contour plots is superimposed for simultaneous optimization of the

independent variables. The desired values for the particle size (<100 nm) and process yield (>65%) were set to obtain the predicted values from the set coded values. From the predicted values obtained by overlay of contour plots of both the responses, the actual values were calculated, and experimental trials were performed for ensuring the proper validation of the process.

The observed and predicted values of optimized nanoparticles formulation were found to be similar from the checkpoint batches, thus ensuring the reliability of the process in obtaining size controlled [Figure 5] robust nanoparticles [Tables 6 and 7].

CONCLUSION

With an aim of achieving controlled particle size and optimum process yield, nanoparticulate system developed by nanoprecipitation technique was optimized by classical DOE technique. In conclusion, QbD ensures quality target profiles, risk analyzes, screening and optimization studies, scale-up studies, and controlled strategies. Risk-assessment approaches, Process Analytical Technology tools, mathematical, statistical and continuous improvement tools are important elements of QbD which mainly focuses on the identification of critical parameters and defining a design space statistically. From the present investigation, it was concluded that a robust PLGA based nanoparticle formulation can be effectively developed and optimized using QbD principles by studying and understanding the formulation and process parameters.

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Table 6: Checkpoint batches for the validation of the regression equation

Independent variable	Coded level	Transformed value	Particle size (nm)		Percentage process yield	
			Predicted value	Observed value	Predicted value	Observed value
PLGA concentration	0.2	11.2 mg/mL	76	71	78	77
Stirring speed	+1	2500 rpm	78	80	82	81
Poloxamer 188 concentration	0.4	0.24%	73	79	75	79

PLGA: Poly (lactide-co-glycolic acid)

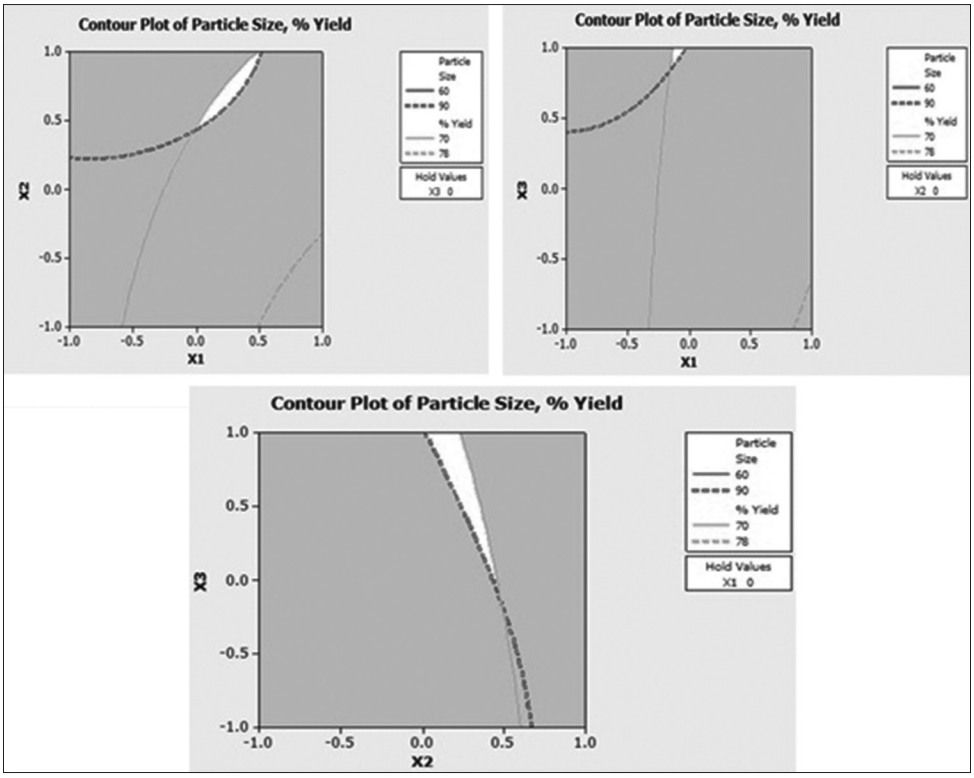

Figure 4: Overlay plots displaying the design space for the formulation of nanoparticles where the white are represents the feasible region obtained after setting the desired values for the particle size and process yield

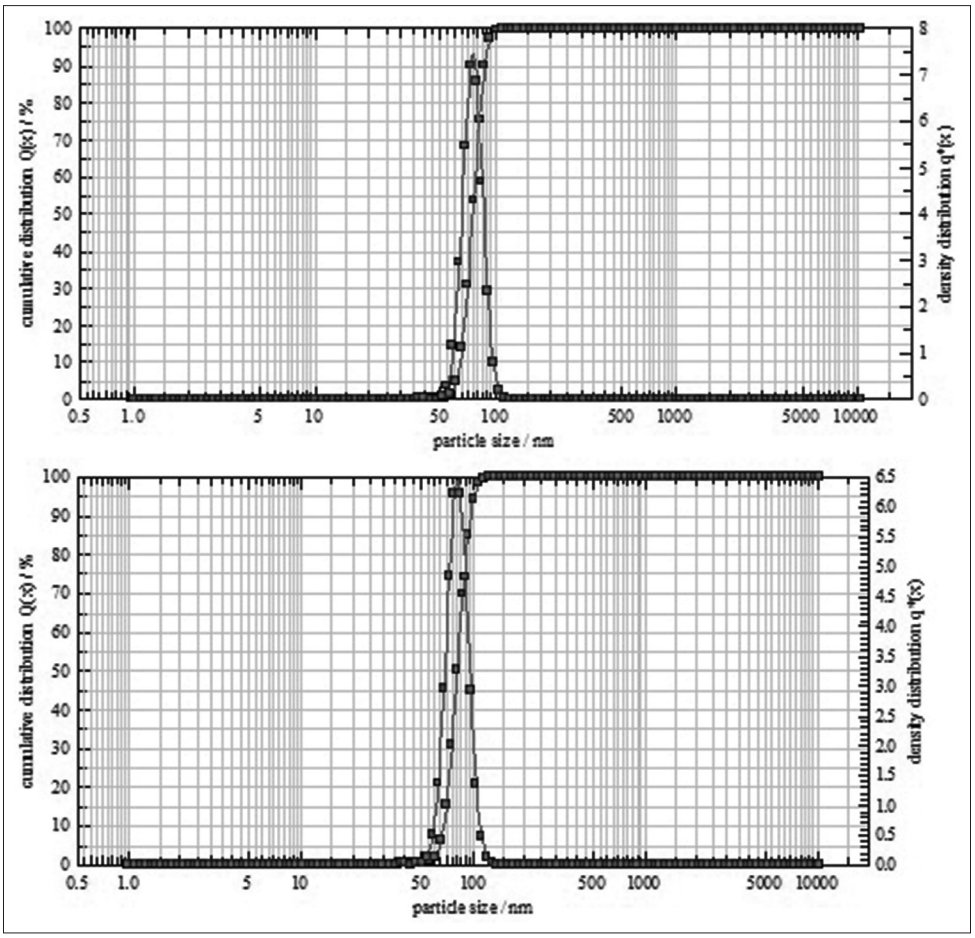

Figure 5: Particle size analysis of the optimized nanoparticle formulation

Table 7: Optimized formulation for PLGA based nanoparticle formulation

Responses	Particle size (nm)	Percentage process yield
Predicted value	75.2	76.8
	77.8	81.8
Experimental value	73.49	75.5
	79.65	77.9

PLGA: Poly (lactide-co-glycolic acid)

REFERENCES

1. Muthu MS. Nanoparticles based on PLGA and its co-polymer: An overview. Asian J Pharm 2009;3:266-73.
2. Lewis DH. Controlled release of bioactive agents from lactide/gylcolide polymers. In: Chasin M, Langer R, editors. Biodegradable Polymers as Drug Delivery Systems. Vol. 4. New York: Marcel Dekker; 1990. p. 1-41.
3. Abhilash M. Potential applications of Nanoparticles. Int J Pharma Bio Sci 2010;1:1-12.
4. Sadat Tabatabaei Mirakabad F, Nejati-Koshki K, Akbarzadeh A, Yamchi MR, Milani M, Zarghami N, *et al.* PLGA-based nanoparticles as cancer drug delivery systems. Asian Pac J Cancer Prev 2014;15:517-35.
5. Decuzzi P, Pasqualini R, Arap W, Ferrari M. Intravascular delivery of particulate systems: Does geometry really matter? Pharm Res 2009;26:235-43.
6. Gu F, Zhang L, Teply BA, Mann N, Wang A, Radovic-Moreno AF, *et al.* Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. Proc Natl Acad Sci U S A 2008;105:2586-91.
7. Murakami H, Kobayashi M, Takeuchi H, Kawashima Y. Preparation of poly(DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsification solvent diffusion method. Int J Pharm 1999;187:143-52.
8. Nava-Arzaluz MG, Piñón-Segundo E, Ganem-Rondero A, Lechuga-Ballesteros D. Single emulsion-solvent evaporation technique and modifications for the preparation of pharmaceutical polymeric nanoparticles. Recent Pat Drug Deliv Formul 2012;6:209-23.
9. Azarmi S, Huang Y, Chen H, McQuarrie S, Abrams D, Roa W, *et al.* Optimization of a two-step desolvation method for preparing gelatin nanoparticles and cell uptake studies in 143B osteosarcoma cancer cells. J Pharm Pharm Sci 2006;9:124-32.
10. Barichello JM, Morishita M, Takayama K, Nagai T. Encapsulation of hydrophilic and lipophilic drugs in PLGA nanoparticles by the nanoprecipitation method. Drug Dev Ind Pharm 1999;25:471-6.
11. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Colloids Surf B Biointerfaces 2010;75:1-18.
12. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 1989;55:R1-4.
13. Govender T, Stolnik S, Garnett MC, Illum L, Davis SS. PLGA nanoparticles prepared by nanoprecipitation: Drug loading and release studies of a water soluble drug. J Control Release 1999;57:171-85.
14. Dos Santos KC, da Silva MF, Pereira-Filho ER, Fernandes JB, Polikarpov I, Forim MR. Polymeric nanoparticles loaded with the 3,5,3'-triiodothyroacetic acid (Triac), a thyroid hormone: Factorial design, characterization, and release kinetics. Nanotechnol Sci Appl 2012;5:37-48.

15. Pinto Reis C, Neufeld RJ, Ribeiro AJ, Veiga F. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine 2006;2:8-21.
16. Storp Bv, Engel A, Boeker A, Ploeger M, Langer K. Albumin nanoparticles with predictable size by desolvation procedure. J Microencapsul 2012;29:138-46.
17. McNaught AD, Wilkinson A. Compendium of chemical terminology. In: IUPAC Recommendations. 2nd ed. Oxford: International Union of Pure and Applied Chemistry, Blackwell Science; 1997.
18. Kashi TS, Eskandarion S, Esfandyari-Manesh M, Marashi SM, Samadi N, Fatemi SM, *et al.* Improved drug loading and antibacterial activity of minocycline-loaded PLGA nanoparticles prepared by solid/oil/water ion pairing method. Int J Nanomedicine 2012;7:221-34.
19. Mahdavi H, Mirzadeh H, Hamishehkar H. The effect of process parameters on the size and morphology of poly (D,L-lactide-co-glycolide) micro/nanoparticles prepared by an oil in oil emulsion/solvent evaporation technique. J Appl Polym Sci 2010;116:528-34.
20. Gómez-Gaete C, Bustos GL, Godoy RR, Saez CK, Novoa GP, Fernández EM, *et al.* Successful factorial design for the optimization of methylprednisolone encapsulation in biodegradable nanoparticles. Drug Dev Ind Pharm 2013;39:310-20.
21. Fangueiro JF, Andreani T, Egea MA, Garcia ML, Souto SB, Souto EB. Experimental factorial design applied to mucoadhesive lipid nanoparticles via multiple emulsion process. Colloids Surf B Biointerfaces 2012;100:84-9.
22. Peter Christoper GV, Vijaya Raghavan C, Siddharth K, Siva Selva Kumar M, Hari Prasad R. Formulation and optimization of coated PLGA - Zidovudine nanoparticles using factorial design and *in vitro in vivo* evaluations to determine brain targeting efficiency. Saudi Pharm J 2014;22:133-40.
23. Abdel-Mottaleb MM, Lamprecht A. Standardized *in vitro* drug release test for colloidal drug carriers using modified USP dissolution apparatus I. Drug Dev Ind Pharm 2011;37:178-84.
24. Lionberger RA, Lee SL, Lee L, Raw A, Yu L X. Quality by design: Concepts for ANDAs. AAPS J 2008;10:268-76.
25. Quality by Design for ANDAs: An example for Modified release dosage forms. The Food and Drug Administration (FDA). Pharm Dev 2011;3.2.P.2:1-159.
26. Jameel F, Khan M. Quality-by-design as applied to the development and manufacturing of a lyophilized protein product. Amer Pharm Rev 2009. Available from: http://www.americanpharmaceuticalreview.com/Featured-Articles/39305-Use-of-Design-of-Experiment-and-Microscale-Down-Strategies-in-Formulation-and-Cycle-Development-for-Lyophilization/. [Last accessed on 2015 Apr 09].
27. Shivhare M, McCreath G. Practical considerations for DoE implementation in quality by design. Bioprocess Int 2010;8:22-30.
28. Mobarak DH, Salah S, Elkheshen SA. Formulation of ciprofloxacin hydrochloride loaded biodegradable nanoparticles: Optimization of technique and process variables. Pharm Dev Technol 2014;19:891-900.
29. Yerlikaya F, Ozgen A, Vural I, Guven O, Karaagaoglu E, khan MA. Development and evaluation of paclitaxel nanoparticles using a quality-by-design approach. Pharm Drug Deliv Pharm Technol 2013;102:3748-61.

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