Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 4, 2015

Original Article

FABRICATION OF A NANOCARRIER SYSTEM CONTAINING PLASMA PROTEIN BY OPTIMIZATION USING RESPONSE SURFACE METHODOLOGY

SANJEEV R ACHARYA*, REDDY RV PADMANABHA

Institute of Pharmacy, Nirma University, Ahmedabad 382481
Email: sanjeev.acharya@nirmauni.ac.in

Received: 18 Dec 2014 Revised and Accepted: 15 Jan 2015

ABSTRACT

Objective: the present investigation involves formulation, optimization and *in vitro* characterization of size controlled HSA nanoparticles with reproducible process yield.

Methods: Human serum Albumin (HSA) is 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. HSA nanoparticles, prepared using desolvation technique, have proved to be promising carrier systems for drug delivery to treat brain disorders. The aim of current Owing to the batch-to-batch variability of HSA based carrier systems, the nanoparticles was optimized using response surface methodology supported by statistical tools for designing a robust formulation. Prior to optimization technique, preliminary screening studies were conducted to evaluate the influence of different formulation variables on particle size and percentage process yield of nanoparticles. The critical process variables were further screened using on 3^2 factorial designs for simultaneous optimization of the process parameters by evaluating their impact on quality attributes of nanoparticles.

Results: The nanoparticles were designed and optimized to achieve a mean particle diameter of 144.55±2.2 nm with process yield of 86.13±1.9 % (n=3), respectively. The relationship between the factors and their coefficients was determined mathematically with their respective *p*-values by employing regression analysis and the factors obtaining *p*-values<0.05 were considered as significant.

Conclusion: The present studies suggest that the physicochemical properties of HSA can be better exploited as a drug carrier for numerous therapeutic and medical applications.

Keywords: Human serum albumin (HSA), Particle size, Yield, pH and optimization.

INTRODUCTION

Delivering drugs into the brain to treat neurological diseases and disorders has been a challenge. Nowadays, many research scholars are exploring the potential use of polymeric nanoparticles as drug/ protein carriers for therapeutic applications. Because of their versatility and wide range of applications, biodegradable polymeric nanoparticles are being used as novel drug delivery systems for the delivery of many chemotherapeutic agents to the brain and other target areas [1, 2].

Nanoparticles are sub-micron sized colloidal particles ranging from 10-1000 nm and boast of effective treatment choice for most bodily disorders like cancer, where the physicochemical properties of nanoparticles can be attuned by tailoring their composition, morphology and surface charge [3, 4]. Nanoparticles possess several advantages over conventional drug delivery forms like enhancing the bio availability of drug, increasing the half-life of the drug, improved drug targeting, increasing stability of the drug and improved drug loading etc. [3, 5]. HSA is a most commonly used biodegradable polymer for the controlled delivery of drugs to the brain due to its early use and approval as a compatible biomaterial in humans. Albumin is a hydrophilic biodegradable molecule, well tolerated without exhibiting any serious side effects and non-toxic in nature. Albumin is a single peptide chain with one free sulfhydryl group on residue # 34 and # 17 intrachain disulfide bonds [6, 7]. 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 [8]. The blood brain barrier (BBB), cerebrospinal fluid (CSF) and blood tumor barrier are the principal factors hampering the drug transport to brain. In addition to BBB, blood tumor barrier and CSF, various efflux transporter proteins like P-gp, multidrug resistant protein (MDR), breast cancer resistant protein (BCRP) etc. also potentiate the resistance to drug transport [9, 10] and contribute to the biochemical hurdles that result in the deprived delivery of drug to brain [11].

HSA, owing to its endogenous biocompatible nature, aids in bypassing the efflux mechanism of the P-gp, thereby preventing the exocytosis of the drug from the endothelial cell [12]. The HSA containing nano particles are found to overcome MDR resistance through P-glycoprotein efflux system localized at the endothelium of blood-brain barrier (BBB) [13].

There are several methods available for development of polymeric nanoparticles such as emulsification-diffusion method [14], emulsification-evaporation method [15], desolvation method [16], nanoprecipitation method [17], etc. Among all the techniques available for preparation of HSA nanoparticles, desolvation technique is the most commonly used for poorly soluble drugs. In the present study, an attempt was made to develop and characterize HSA nanoparticles with controlled particle size and process yield using desolvation technique.

In order to optimize the HSA nanoparticulate formulation, several formulation variables were screened initially such as polymer concentration, type and concentration of stabilizer, pH, type and concentration of desolvating agent, stirring rate, amount of glutaraldehyde and time for cross linking etc. these preliminary studies revealed an amount of desolvating agent and pH as critical process parameters which were further optimized by using 3^2 full factorial design for simultaneous evaluation of the independent variables. As discussed earlier, it is extremely important to develop size controlled polymeric nanocarriers as it influences the bio distribution and physical stability of the formulation in dispersion form. The objective of the present study is the optimization of the desolvation procedure for the preparation of HSA-based nanoparticles which show a controllable particle size below 200 nm and exhibit narrow size distribution as well as enhanced process yield of nanoparticles. In a view to achieve the objective, attempts

have been made to identify the critical quality attributes and studying their impact on responses by application of statistical design of experiments (DOE) approach. This method is efficient for evaluating the impact of formulation as well as process parameters on response variables and subsequently optimization of these parameters with respect to the desired specifications.

MATERIALS AND METHODS

Materials

Human Serum Albumin IP (20% total protein) was procured from Reliance Life Sciences Pvt. Ltd. Navi Mumbai. Poloxamer 188, sodium lauryl sulphate (SLS) and tween 80 were supplied as a gift sample from Sandoz Pvt. Ltd, Mumbai. DCM (purityNLT 99% by GC), Acetone (purity NLT 99% by GC), methanol and ethanol (HPLC 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 paclitaxel loaded HSA nanoparticles

HSA nanoparticles were prepared by employing desolvation technique as described by Marty et al., 1978 and Langer K et al., 2003) [18, 19]. 20% w/v of HSA was prepared in 20 mL of double distilled water containing 0.1% of surfactant (poloxamer 188). Around 20 mL of desolvating agent (acetone) was added dropwise to the above aqueous solution containing polymer HSA at room temperature both maintained at pH 8-9. The mixture was stirred using blade homogenizer at 2500±200 rpm for 15 minutes (Remi equipment, Mumbai, India). Later, crosslinking was achieved by addition of 1 mL of glutaraldehyde drop wise and the resultant nanoparticles dispersion was kept under stirring at room temperature for 6 hours for evaporation of organic solvent from the system. Finally, the nanoparticles were obtained by drying in vacuum dryer.

Optimization of HSA nanoparticles

In the present investigation, comprehensive process understanding was achieved through statistical DOE technique by employing 3^2 full factorial design establishment of design space for developing nanoparticles [20]. Initially, several process parameters were evaluated to establish their possible impact on the critical quality attributes (CQAs) i.e. particle size and process yield of nanoparticles. Preliminary screening studies help in identifying the critical process parameters (CPPs) posing significant influence on quality attributes of nanoparticles which was followed by optimization of the independent variables by simultaneous evaluation of two formulation variables and their interaction at three different levels (low, medium and high) [21]. Minitab®16 was used for the statistical evaluation and experimental design. The main effects and interaction effects are calculated for the responses of particle size and percentage process yield [22]. 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 were optimized with respect to particle size and % 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 [23].

Characterization of HSA nanoparticles

Determination of process yield

The percentage process yield of HSA nanoparticles was determined by the weight of final dry powder with respect to the initial total amount of the polymer (HSA) and other solid excipients used for the preparation of nanoparticles. The percentage process yield is calculated by using the formula:

$$\text{Percentage yield} = \left(\frac{\text{Practical yield}}{\text{Teoretical yield}}\right) \times 100$$

Particle size, size distribution analysis and zeta potential

The average particle size and size distribution of HSA nanoparticles were 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 by using laser light scattering which was monitored at a scattering angle of 90^0 at the 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 minutes in a bath-type sonicator and the dispersion thus obtained was analyzed for particle size by loading into 1 cm^2 cuvettes in a thermaostated chamber at 25^0C. 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 by using formula [24]:

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

Where, $D_{90\%}$, $D_{50\%}$ and $D_{10\%}$ are the mean diameter at which 90, 50 and 10 % (cumulative %) of the nanoparticles are counted and calculated. The zeta potential of HSA-nanoparticles was determined by using Zetasizer 3000 (Malvern instruments, UK).

RESULTS AND DISCUSSION

Effect of desolvation process parameters on properties of nanoparticles

In the present investigation, an attempt was made to closely perceive the process of desolvation technique by evaluating the influence of several process parameters such as type and amount of desolvating agent, pH of the aqueous phase, type and amount of stabilizer, rate of stirring, concentration of polymer, amount of crosslinking agent and time for crosslinking etc. on the quality attributes ie. particle size and percentage process yield. Particle size is considered as a crucial parameter while formulating nanoparticles as the particle size influences the biodistribution pattern and interaction with the biological membrane. The particle size of the formulations was obtained in the range of 145.4 nm to 463.7 nm.

From the preliminary studies (table 2), it was revealed that the size of nanoparticles were inversely related to pH of the medium. This was studied by altering the pH from 7.5 to 9.5 and the effect of pH on size nanoparticles was evaluated. It was found that the particle size decreased from 281.8 nm to 145.4 nm when the pH of the medium was increased from 7.5 to 9.5 respectively. The pH value of HSA solution prior to addition of desolvating agent was found to strongly influence the particle size of nanoparticles which can be assumed due to increased ionization of HSA whose isoeletric point (p*I*) is 4.7 [25]. Hence, at higher pH, HSA remains in anionic state unlike in lower pH where it exists in neutral state. Ionized HSA develops repulsive forces which resist particle aggregation leading to smaller sized particles, whereas the neutral state at lower pH invites protein-protein interactions thereby resulting in aggregation of particles [26].

Among different desolvating agents, Acetone yielded nanoparticles with least particle size (178.2 nm) and hence it was selected as desolvating agent of choice. The rate of addition of acetone didn't show significant influence neither on the particle size nor the process yield of nanoparticles, however, the amount of desolvating agent matters much as it considerably affects the particle size and yield of nanoparticles. Besides, the rate of addition of acetone influenced the width of particle size distribution to a lesser extent. Determination of cloud point formation gives an estimate for the yield of nanoparticles. Particle preparations with acetone exhibited intense turbidity, thus indicating greater yield of particles and the turbidity increased with increase in concentration of acetone. This can also be attributed to the fact that acetone acts as a better non-solvent for HSA than ethanol and methanol, thereby, favoring the particle nucleation and growth, thus developing smaller sized nanoparticles by increasing solvent diffusion into anti-solvent phase [26, 27]. Hence, from the results obtained, it was clear that volume

of acetone added is vital with respect to the yield of size controlled nanoparticles. However, there was no considerable effect of ionic strength, type of addition (intermittent or continuous) on the particle size of HSA nanoparticles.

Concentration of HSA (concentration range between 5 mg/ml and 20 mg/ml) unveiled only a slight impact on the particle diameter with a slight reduction in polydispersity of the samples. The zeta potential values of nanoparticles altered with increase in polymer concentration ie. from-32.2 mV to-21.4 mV which is supposed to be the reason for increase in particle size. The change in surface charge of the nanoparticles hindered the repulsive forces thereby leading to growth in particle size by favoring particle aggregation. Surfactants in the formulation are necessary for the stabilization of nanoparticles obtained. Surfactants like sodium lauryl sulphate (SLS), poloxamer 188 and tween 80 were screened (table 2) for their possible role in influencing the particle size and yield of nanoparticles and it was found that poloxamer 188 produced lower particle size (304.1 nm)than SLS (463.7 nm) and tween 80 (333.9 nm). Increase in the concentration of stabilizer, particle size was found to decrease slightly whereas the yield of nanoparticle found to improve to a minimal extent. The poloxamer molecules overlay on the surface of droplets and assist in preventing the coalescence of droplets by reducing the shear stress during the homogenization process. As a result, the stabilizers which preferentially adsorb at the interface of droplets tend to decrease the particle size of nanoparticles by merely reducing the tension at interface [23]. Since, SLS is known to have the deleterious effect on liver; it is therefore omitted from the study. Similarly, the rate of stirring failed in illustrating its significant role in affecting the CQAs. As depicted in fig. 1, the increase in stirring speed from 1000 rpm to 2500 rpm led to decrease in particle size from 225.32 nm to 216.89 nm which was considered insignificant, though the speed of 2500 rpm was selected for the study as it helped in decreasing the width of particle size distribution.

Table 1: Factors and their levels used for preliminary studies of HSA nanoparticles

Factors		Levels		
	Code	Low	Medium	High
Polymer concentration	X1	10 mg/ml	15 mg/ml	20 mg/ml
Surfactant type	X2	SLS	Tween 80	Poloxamer 188
Surfactant concentration	X3	0.1 % w/v	0.2 % w/v	0.3 % w/v
Homogenizer type	X4	Magnetic stirrer	Blade stirrer	Homogenizer
Type of organic solvent	X5	Ethanol	Methanol	Acetone
Concentration of desolvating agent	X6	20 ml	22.5 ml	25 ml
Stirring speed	X7	1000 rpm	1750	2500 rpm
pH	X8	7.5	8.5	9.5
Glutaraldehyde concentration	X9	150 µl	250 µl	450 µl

Glutaraldehyde crosslinking plays a major role in the stability and drug release properties of albumin nanoparticles. The amount of crosslinking agent and time of crosslinking process was evaluated for optimized crosslinking of amino groups of lysine in the HSA molecules of the particle matrix. No impact of crosslinking conditions on the particle size as well as process yield of nanoparticles was observed (fig. 1).

Table 2: Independent variables in different levels selected for the screening studies

Factor	Factor variable	Particle size (nm)	Span value	% Process yield	Zeta potential (mV)
Process	Stirring	350.5±4.4	0.41	71.1±8.5	-38±2.1
	Homogenizer	321.8±4.1	0.36	78.8±6.8	-38±1.4
Type of Desolvating agent	Ethanol	333.9±5.6	0.33	71.5±6.6	-40±1.6
	Acetone	178.2±4.8	0.29	78.5±5.8	-40±1.1
	Methanol	238.9±4.9	0.34	71.75±6.4	-41±1.2
Type of Surfactant	Poloxamer 188	304.1±5.5	0.4	80±6.6	40±1.5
	SLS	463.7±5.8	0.45	90±7.2	39±1.8
	Tween 80	333.9±6.1	0.42	71.5±6.9	39±1.3
Concentration of desolvating agent	20 ml	180.7±3.8	0.22	89.1±6.6	42±1.4
	22.5 ml	230.8±4	0.28	90.1±6.9	41±1.8
	25 ml	279.3±4.4	0.31	84.8±7.4	41±2.1
	27.5 ml	332.7±4.8	0.33	94.7±8.3	42±2.2
Polymer concentration	5 mg/ml	165.3±5.5	0.28	89.1±6.8	32±1.3
	10 mg/ml	220.5±5.7	0.26	90.1±6.9	40±1.6
	15 mg/ml	249.8±5.4	0.29	84.8±7.6	41±1.5
	20 mg/ml	261.2±5.1	0.33	94.7±6.9	24±1.8
pH	7.5	281.8±6.8	0.31	91.63±7.8	-41±1.2
	8.5	188.5±5.6	0.26	87.89±7.2	-42±1.6
	9.5	145.4±4.2	0.22	84.95±6.5	-42±1.4
Surfactant concentration	0.1% w/v	248.9±4.4	0.24	85.7±8.3	40±1.6
	0.2% w/v	232.8±4.6	0.18	86.2±7.7	40±1.8
	0.3% w/v	221.6±4.8	0.23	86.9±6.5	40±2.0
Stirring speed	1000 rpm	225.±4.2	0.22	83.89±7.9	38±2.4
	1750 rpm	220.5±3.8	0.18	84.56±6.5	39±1.9
	2500 rpm	216.89±3.3	0.16	86.1±5.8	39±1.4
Glutaraldehyde concentration	150 µl	185.36±4.4	0.33	84.8±6.6	40±1.5
	250 µl	187.55±4.8	0.36	85.3±6.5	41±1.8
	450 µl	189.1±4.5	0.38	85.8±6.2	41±1.9

Suspensions of nanoparticles have been shown to be stabilized even when the minimum absolute zeta potential of the particles was±21.4 mV. The nanoparticles evaluated for zeta potential measurement exhibited negative charge with values ranging from-21 to-42 mV thus ensuring the stability of the product by preventing agglomeration of particles on storage thereby averting the increased particle size of nanoparticles.

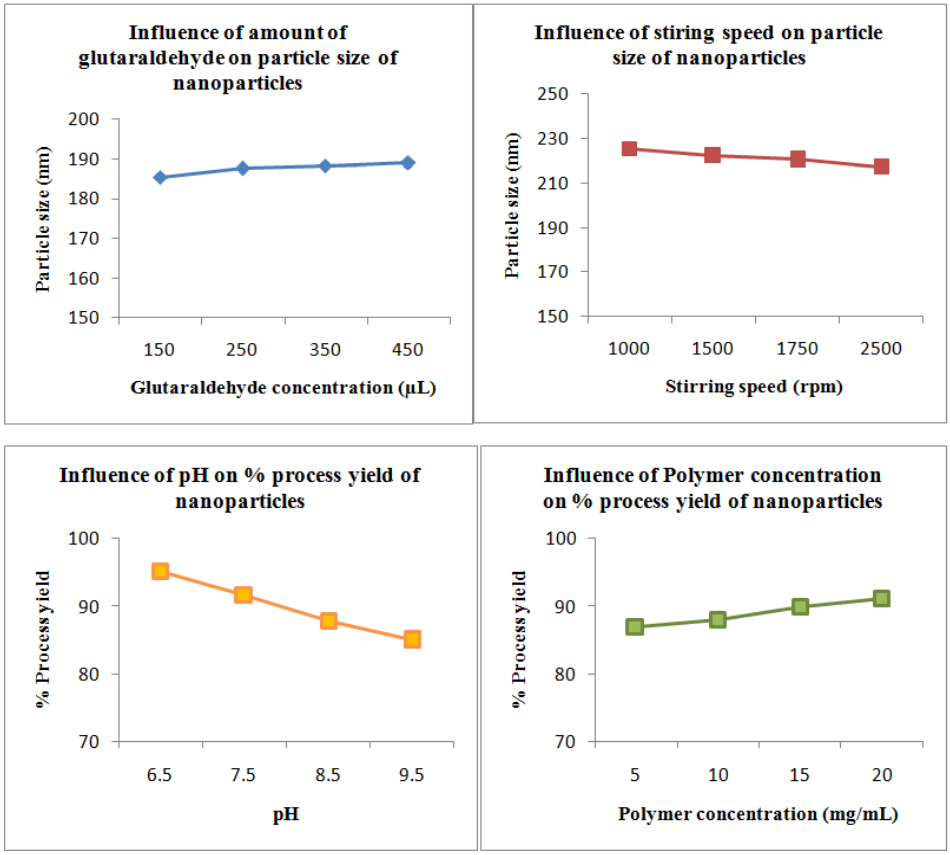


Fig. 1: Influence of process variables on particle size and % process yield of nanoparticles

Optimization of HSA nanoparticles

The preliminary screening of formulation variables encouraged in identifying volume of desolvating agent and pH of the medium as critical process parameters. These parameters were further evaluated by classical design of experiments (DOE) technique using 3^2 full factorial design to develop size controlled reproducible nanoparticles with optimum yield.

The list of factors with their levels used for preliminary studies of HSA nanoparticles is presented in table 1 and 2. The process parameters selected for optimization study are displayed in table 3 along with their coded and actual values. The particle size and percentage process yield followed significant variability ie. 145.4 nm to 463.7 nm and 71.1 % to 94.7 %, respectively. Thisbroad variability in results clearly ensuresthe dependence of response variables on critical process parameters. The design layout along with the coded levels is presented in table 4. From the results of ANOVA it was evident that the independent factors possess great impact on the particle size as the values were well below 0.05. The polynomial equation is used to obtain useful information in evaluation of coefficient while the polynomial model for the estimation of particle size was as below:

$$Y_{particle\ size} = 22.945+38.68\ X_1 - 75.653\ X_2 - 12.035\ X_1X_2 + \text{--------(1)}$$

($R^2 = 0.9193$; $F = 9.104$; $p<0.05$; n=4)

$$Y_{\%\ yield} = 87.078+1.006\ X_1 - 2.74\ X_2 - 1.8\ X_1X_2 + \text{---------(2)}$$

($R^2 = 0.9940$; $F = 138.10$; $p<0.05$; n=4)

From the above equation it was clear that the independent variables amount of acetone (X_1) and pH (X_2) have significant impact on particle size and % process yield of nanoparticles. Equations (1 and 2) are presented in the form of response surface plot and contour plot in fig. 2 for visualizing the effect of the factors on the particle size of nanoparticles. Similarly, the response surface plot and contour plot for envisaging the impact of the factors on the % process yield of nanoparticles is depicted in fig. 3. After the estimation of polynomial equations, the design space was established by setting the target value for particle size (<200 nm) and % EE (>80%) and for this design, contour plots along with the response were established. A total of nine trials were resulted as a result of the optimization design. 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.

Table 3: Independent variables with their levels selected for the study

Independent variable	Actual value	Coded value
Amount of desolvating agent (X1)	20 ml	-1
	22.5 ml	0
	25 ml	1
pH of the system (X2)	7.5	-1
	8.5	0
	9.5	1

From the results, the time of crosslinking of glutaraldehyde was optimized at 6-8 hours with 2500 rpm as rate of stirring and poloxamer 188 concentrations as 0.1 %w/v.

Table 4: Design layout and coded units of 3^2 factorial design

Trial No.	X1	X2	X1	X2	Particle size (Y1)	% yield (Y2)
1	-1	-1	20 ml	7.5	266.56	87.36
2	0	-1	22.5 ml	7.5	315.55	89.51
3	1	1	25 ml	9.5	241.13	83.21
4	0	0	22.5 ml	8.5	204.5	86.89
5	0	1	22.5 ml	9.5	159.63	84.61
6	-1	0	20 ml	8.5	156.68	85.78
7	1	0	25 ml	8.5	201.63	88.58
8	-1	1	20 ml	9.5	141.63	85.19
9	1	-1	25 ml	7.5	384.2	92.58

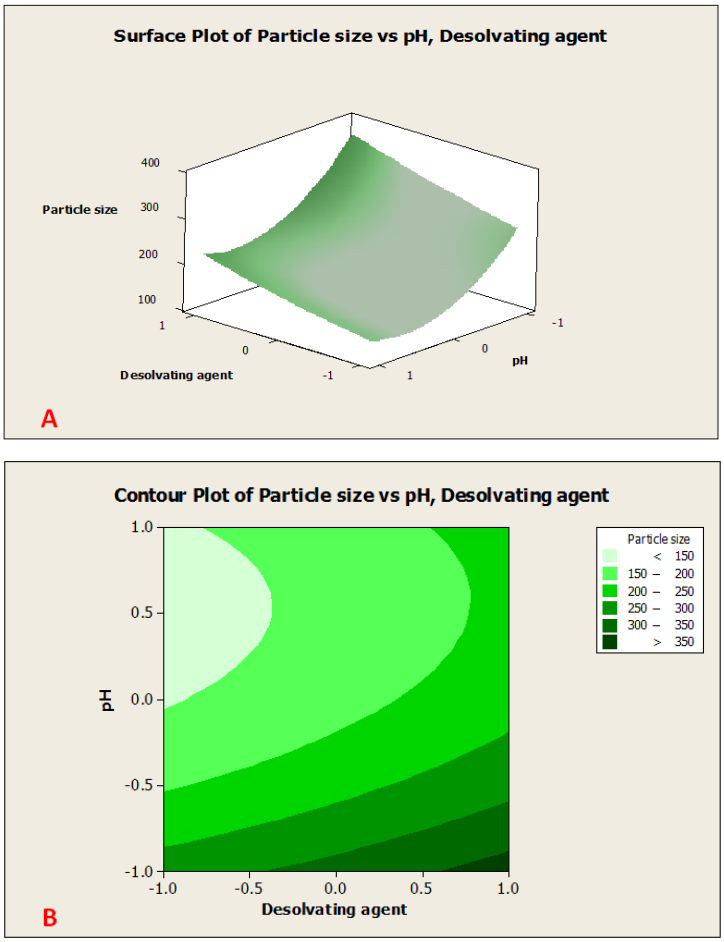
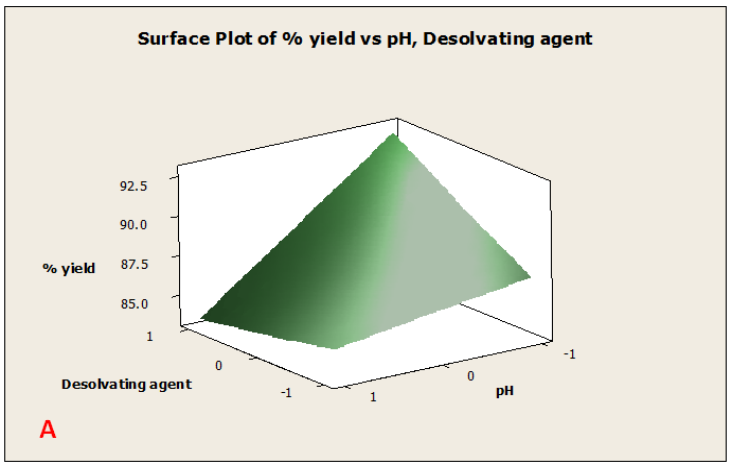


Fig. 2: Response surface plot (A) and contour plot (B) for particle size



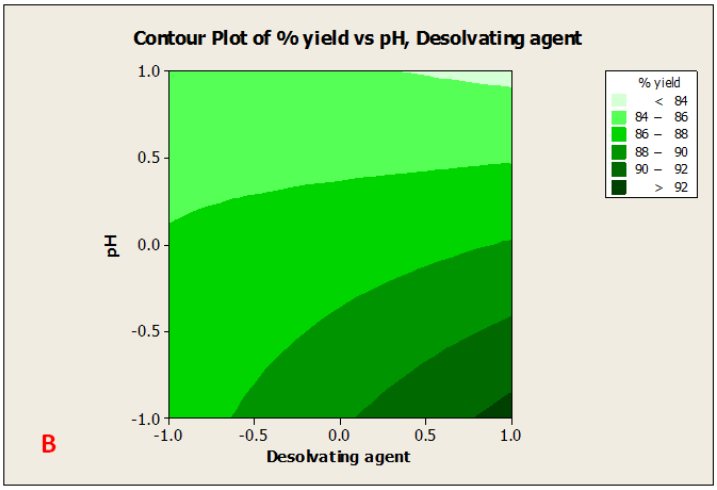


Fig. 3: Response surface plot (A) and contour plot (B) for % Process yield

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. The desirability zone was determined using Minitab® 16 statistical software as shown in fig. 4 depicting the overlay plots for simultaneous optimization of particle size and %process yield shows that the acceptable range of independent variables which meet the given requirements. The optimized formulation obtained exhibited encouraging results showing particle size of 144.55±2.2 (fig. 5) and process yield of 86.13±1.9 (n=3), respectively.

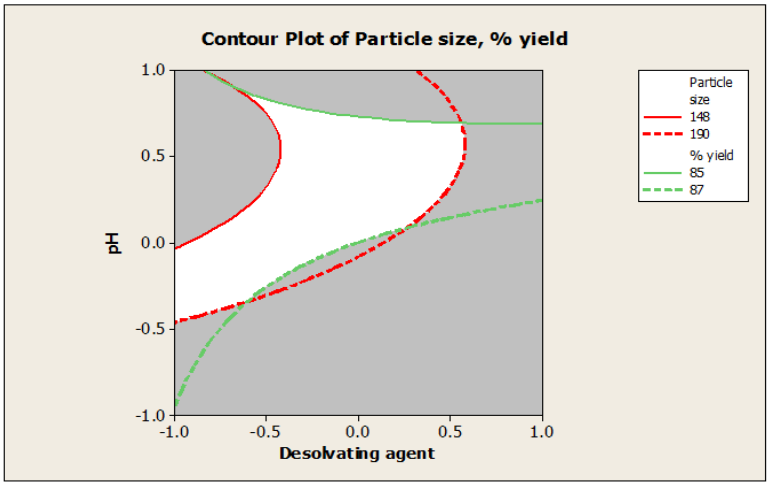


Fig. 4: Overlay plot for simultaneous optimization of Particle size and % Process yield

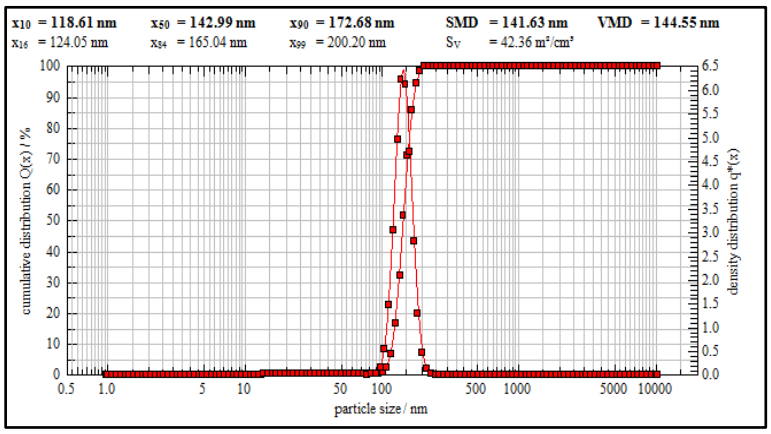


Fig. 5: Particle size report of optimized HSA nanoparticles formulation

Table 5: Checkpoint batches for the validation of regression equation

Independent variable	Particle size (nm)			% Process yield	
	Coded level	Predicted value	Observed value	Predicted value	Observed value
Amount of desolvating agent	-0.88	145.74	147.12	85.18	88.01
pH	0.04	146.74	148.12	86.15	87.89

Table 6: Optimized formulation of HSA based nanoparticle formulation

Responses	Particle size (nm)	% Process yield
Predicted value	143.58	85.01
Experimental value	144.55±2.2	86.13±1.9

Checkpoint batches were prepared to check the predictive productivity of the regression equation as shown in table 5 and the optimized formulation in table 6. A good correlation was observed between the observed and predicted values.

The relationship between the factors and their coefficients was determined mathematically with their respective *p*-values by employing regression analysis and the factors obtaining *p*-values<0.05 were considered as significant [28].

CONCLUSION

The goal of the present study was to develop robust and rugged HSA nanoparticles with controlled particle size and high process yield. The desired particle size with maximum process yield was achieved by simultaneous optimization of the process variables with the aid of statistical design of experiments tool. Initial preliminary experiments were conducted for the better understanding of the desolvation process and identification of key parameters affecting the quality of the final formulation. The critical parameters were further evaluated by 3^2 factorial design and the results obtained were further fortified by statistical models, contour plots and response surface plots. Hence, the objective of obtaining HSA nanoparticles with controlled size and improved process yield is amply achieved through desolvation technique and novel DOE concept. From the present investigation, it was concluded that a robust HSA based nanoparticle formulation can be effectively developed and optimized using 3^2 full factorial design by studying and understanding the formulation and process parameters. Hence our studies suggest that the physicochemical properties of HSA can be better exploited as a drug carrier for numerous therapeutic and medical applications.

ACKNOWLEDGEMENT

The author acknowledge to Institute of Pharmacy, Nirma University for providing the facility for carrying out this research work.

CONFLICT OF INTERESTS

The authors report no declarations of interest.

REFERENCES

1. Jong DWH, Borm PJ. Drug delivery and nanoparticles: Applications and hazards. Int J Nanomed Jun 2008;3(2):133-49.
2. Cho K, Wang X, Nie S, Chen Z, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. Clin Cancer Res 2008;14(5):1310-16.
3. Mudshingea SR, Deoreb AB, Patil S, Bhalgat CM. Nanoparticles: Emerging carriers for drug delivery. Saudi Pharm J 2011;19(3):129-41.
4. Xia Y, Sun T, Zhang YS, Pang B, Hyun DC, Yang M. Engineered nanoparticles for drug delivery in cancer therapy. Angew ChemInt 2014;53:12320-64.
5. Gelperina S, Kisich K, Iseman MD, Heifets L. The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. Amer J Resp Crit Care Med 2005;172:1487-90.
6. Wang W, Huang Y, Zhao S, Shao T, Cheng Y. Human serum albumin (HSA) nanoparticles stabilized with intermolecular disulfide bonds. Royal Soc Chem 2013:S1-S16.
7. Wang W, Huang Y, Zhao S, Shao T, Cheng Y. Human serum albumin (HSA) nanoparticles stabilized with intermolecular disulfide bonds. Chem Commun 2013;49:2234-6.
8. Sebak S, Mirzaei M, Malhotra M, Kulamarva A, Satyaprakash. Human serum albumin nanoparticles as an efficient noscapine drug delivery system for potential use in breast cancer: preparation and *in vitro*analysis. Int J Nanomed 2010;5:525-32.
9. Muro, Das KS, Raizer JJ. Convection-enhanced and local delivery of targeted cytotoxins in the treatment of malignant gliomas. Techn Cancer Res Treat 2006;5(3):201-13.
10. Smith QR. Carrier-mediated transport to enhance drug delivery to brain. Int Cong Ser 2005;1277:63-74.
11. Ronaldson PT, Covarrubias LS, Slosky LM, Thompson BJ, Davis TP. Transporters at CNS barrier sites: obstacles or opportunities for drug delivery? Curr Pharm Des 2014;20(10):1422-49.
12. Amin ML. P-glycoprotein inhibition for optimal drug delivery. Drug Target Insights 2013;7:27-34.
13. Liang XJ, Chen C, Zhao Y, Wang PC. Circumventing tumor resistance to chemotherapy by nanotechnology. Methods Mol Biol 2010;596:467-88.
14. 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.
15. Arzaluza NMG, Segundob PE, Ronderoa GA, Ballesterosc LD. Single emulsion-solvent evaporation technique and modifications for the preparation of pharmaceutical polymeric nanoparticles. Recent Pat Drug Delivery Formulation 2012;6:209-23.
16. Azarmia S, Huanga Y, Chend H, McQuarriea S, Abramse D, Roa W. 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(1):124-32.
17. 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(4):471-6.
18. Marty JJ, Oppenheimer RC, Speiser P. Nanoparticles-a new colloidal drug delivery system. Pharm Acta Helv 1978;53:17-23.
19. Langer K, Balthasar S, Vogel V, Dinauer N, Briesen HV, Schubert D. Optimization of the preparation process for human serum albumin (HSA) nanoparticles. Int J Pharm 2003;257:169-80.
20. Santos KC, Silva DMF, Filho ER, Fernandes JB, Polikarpov I. Polymeric nanoparticles loaded with the 3,5,3′-triiodothyroacetic acid (Triac), a thyroid hormone: factorial design, characterization, and release kinetics. Nanotech Sci App 2012;5:37-48.
21. Gambhire MS, Bhalekar MR, Gambhire VM. Statistical optimization of dithranol-loaded solid lipid nanoparticles using factorial design. Braz J Pharm Sci 2011;47(3):503-11.
22. Jain A, Jain SK. Formulation and optimization of temozolomide nanoparticles by 3 factor 2 level factorial design. Biomatter 2013;3(2):e25102-13.
23. Zu Y, Zhao Q, Zhao X, Zu S, Meng L. Process optimization for the preparation of oligomycin-loaded folate-conjugated chitosan nanoparticles as a tumor-targeted drug delivery system using a two-level factorial design method. Int J Nanomed 2011;6:3429-41.
24. Murtaza G, Ahmad M, Khan SA. Evaluation of cefixime-loaded chitosan microspheres: Analysis of dissolution data using DDSolver. DissInd 2012:13-9.

25. Lin W, Coombes GA, Davies MC, Davis SS, Illum L. Preparation of sub-100 nm human serum albumin nanoparticles using a pH-coacervation method. J Drug Target 1993;1:237-43.
26. Wang G, Siggers K, Zhang S, Jiang H, Xu Z, Zernicke RF. Preparation of BMP-2 containing bovine serum albumin (BSA) nanoparticles stabilized by polymer coating. Pharm Res 2008;25(12):2896-909.
27. Namasivayam SK, Robin G. Preparation, optimization and characterization of biocompatible nanoalbumin-ofloxacin (bsanp-of) conjugate and evaluation of control release, anti-bacterial activity against clinical isolate of pseudomonas aeruginosa. Asian J Pharm Clin Res 2013;6(3):235-9.
28. Shivhare M, McCreath G. Practical Considerations for DoE Implementation in Quality by Design. Bio Process Int 2010:22-30.