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RESEARCH ARTICLE

Preparation and Characterization of Bovine Serum Albumin Nanoparticles of Curcumin using a Chemometric Approach

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Abstract

Bovine Serum Albumin (BSA) has been presupposed to be a versatile protein polymer for targeted drug delivery. BSA nanoparticles can lead to passive targeting of drugs to the inflamed joint via the Enhanced Permeability and Retention (EPR) effect and due to their specific affinity towards the inflamed joint because of the inadequacy of protein in the affected region. Hence, the aim of the study was to develop BSA nanoparticles loading curcumin (BSA_CUR_NPs) by nanoparticle albumin-bound technology and its optimization was conducted by 3^3 Box Behnken Design (BBD) in order to achieve the desired particle size and entrapment efficiency. Further, the optimised nanoparticles were also assessed for polydispersity index, zeta potential, total drug content, and *in-vitro* drug release study. The response surface plots and equations generated by BBD predicted the relationship between variables under study. The optimised formulation C_{12} was found to have a particle size of 207.1 ± 1.36nm, PDI of 0.138 ± 0.03, entrapment efficiency of 75.04 ± 0.06 %, total drug content of 91.40 ± 0.08% and zeta potential of -32.9mV. The optimised nanoparticles exhibited good sustained release for up to 8 days. The use of a chemometric approach led to the development of BSA_CUR_NPs with the desired characteristics with a less experimental procedure. Therefore, it presents an important model for producing the nanoparticles of the desired characteristics using albumin as a polymer for the enhanced and sustained delivery of loaded drugs to the inflamed joint.

Keywords: Bovine Serum Albumin Nanoparticles, Box Behnken Design, Curcumin, EPR Effect

1. Introduction

The nanotechnology field has had an enormous impact in the last two decades in the field of pharmaceutical health sciences, where drug delivery through nano-based carriers has shown favourable developments in the therapeutic effects of drugs for the treatment of various life-threatening diseases[1,2]. Indeed, the therapeutic efficacy of most drugs is compromised of various reasons, viz., poor solubility, degradation at acidic and alkaline pH, poor absorption, delivery at non-specific sites, short-half-life and high toxicity. Researchers are heavily researching nanoparticulate drug delivery systems as a strategy for producing nanosystems loaded with various drugs in order to improve the bio-distribution of the drug to the desired site of action, i.e., specific tissue or cells, and to prevent systemic degradation of the drug[3]. Further, higher therapeutic levels of the drug at lower doses can be achieved, thereby reducing off-target toxicity of the drug. The targeted action potential of the nanosystems depends upon the physicochemical properties of the nanosystem and majorly upon the type of polymer used. There is a wide variety of natural and artificial polymers that can be used in the production of nanosystems. However, polymers that are biodegradable, biocompatible, and non-immunogenic are most preferred. Bovine serum

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albumin is one such wonder protein polymer that is biodegradable, biocompatible, non-immunogenic, guards drug molecules against degradation, increases water solubility and permeability, and can alter the drug distribution kinetics. Albumin nanoparticles of definite size i.e., 20–200 nm can extravasate and accumulate in the interstitial space of the inflamed tissue due to Enhanced Permeability and Retention (EPR) effect, wherein there is increased vascular permeability in the target tissues due to inflammation, further inflamed joint affected with rheumatoid arthritis develops hypoalbuminemia hence have a high rate of albumin consumption. Therefore, albumin becomes an attractive agent for carrying the drug to the joint affected with Rheumatoid Arthritis (RA)[4,5]. Current literature carries strong evidence in support of natural products for the effective treatment and management of rheumatoid arthritis. Curcumin is one such wonder drug because of its anti-inflammatory property, which can be used for the effective management of RA. Unfortunately, the drug suffers from extremely low bioavailability due to poor solubility and rapid metabolism[6,7]. Optimizing the preparation of nanoparticles is complex and tedious as many controlling factors are involved that affect the particle size of nanoparticles. The chemometric approach is utilised by many researchers, as it allows for systematic development of formulations with fewer experiments. It can also correlate the dependent and independent variables in the study and asses the interacting factors. Subsequently, the size of the nanoparticles plays a major role in deciding the fate of nanoparticle accumulation in the inflamed joint. Hence, on account of the above facts, the study was initiated to formulate the bovine serum albumin nanoparticles of curcumin by the nanoparticle albumin-bound technique (Nab) and apply box-behnken design to determine the effect of independent variables on dependent variables.

2. Materials and Methods

2.1 Materials

Curcumin was procured from Konark Herbals and Health care Ltd. Bovine serum albumin, sodium hydroxide, and dialysis membrane were purchased from Hi-Media Ltd., India. All the other chemicals used in the study were of analytical grade.

2.2 Preparation of Bovine Serum Albumin Nanoparticles Loaded with Curcumin (BSA_CUR_NPs) by Nab Technology

Accurately weighed curcumin is dissolved in acetone and BSA in distilled water separately. Both the solutions are gently mixed together and the emulsion is subjected to homogenization using an ultra-turrax homogenizer. The resulting dispersion is rotary evaporated to remove acetone. All the formulations were lyophilized to get a free-flowing powder[8].

2.2.1 Optimization of Bovine Serum Albumin Nanoparticles Loaded with Curcumin (BSA_CUR_NPs) by 3^3 Box-Behnken Design

The formulations were developed as per 3^3 Box-Behnken Design (BBD), allowing the simultaneous assessment of selected independent formulation and process variables. A 3-factor, the 3-level design was implemented to determine the influence of three independent variables: drug: albumin ratio (X_1), the volume of organic solvent (X_2), and homogenization speed (rpm) (X_3) on dependent variables viz., particle size (Y_1) and entrapment efficiency (Y_2). Based on preliminary screening experiments that determined the main variables and their suitable optimum range, the factors were evaluated at three levels: low (-1), middle (0), and high (+1). The coded and actual values of the variables are given in Table 1. A total number of 17 experiments were performed generated by the Box-Behnken design matrix. The results of the experiments were analysed as per Design Expert software version 7.0.0. Step-wise linear regression analysis developed polynomial equations for dependent variables which are represented in the form of equation-1:

$$Y = b_0+b_1X_1+b_2X_2++b_3X_{3+}b_{11}X_1^2+b_{22}X_2^2+b_{33}X_3^2+b_{12}X_1X_2+b_{13}X_1X_3+b_{23}X_2X_3\ldots \quad (1)$$

Here, Y represents the dependent variable, the dependent variable; b_0 is the intercept; b_1, b_2 and b_3 are the linear coefficients of independent variables for factors X_1, X_2 and X_3 while b_{11}, b_{22}, and b_{33} are their squared coefficients; and b_{12}, b_{13} and b_{23} are interaction coefficients of X_1X_2, X_1X_3, and X_2X_3, which indicate the variation in response when X_1, X_2 and X_3 are simultaneously varied.

The rationality of the developed polynomial equations was confirmed by formulating checkpoint formulations[9,10].

2.3 Evaluation of Nanoparticles

2.3.1 Determination of Particle Size and Polydispersity Index (PDI)

The particle size and PDI of all the batches of prepared albumin nanoparticles were measured using a Malvern Nano S-90 Zetasizer (Malvern Instruments Ltd., UK).

2.3.2 Determination of Encapsulation Efficiency and Total Drug Content

The formulated nanoparticles colloidal solution was centrifuged at 8000 RPM for 30 minutes to separate the supernatant phase and the solid pellet of nanoparticles in the bottom of the centrifuging tube. The amount of free drug in the supernatant was determined by the developed method of analysis using a UV-Visible spectrophotometer at 425nm.

The Entrapment Efficiency (% EE) was calculated using the below equation:

$$\textbf{Entrapment Efficiency}(\%) = \frac{Winitialdrug - Wfreedrug}{Winitialdrug} * 100$$

The solid pellet of nanoparticles was then dissolved in a mixture of water and DMSO to dissolve albumin and the loaded drug. The resulting solution was filtered and diluted with methanol before being subjected to analysis by UV spectrophotometry at 425nm[11].

The % TDC was calculated using the below equation:

$$\textbf{Total drug content}\ (\%) = \frac{Wdrug\ in\ residue + Wfreedrug}{Winitialdrug} * 100$$

2.3.3 In-vitro Drug Release Study

In-vitro release studies of the developed nanoparticles were performed using a Franz diffusion cell. The dialysis membrane, molecular weight cut off 12000-14000 Da, (Hi-Media, India) was soaked with buffer and mounted on a diffusion cell. The experiments were performed in the absence of light as curcumin extensively degrades in the presence of light. Phosphate-buffered saline (PBS) (pH 7.4) was used as dissolution media and was stirred on a magnetic stirrer at 37±0.5°C at 50 rpm. Since the stability of curcumin is very low in PBS, therefore ascorbic acid at a concentration of 2% was added to the buffered media to prevent the degradation of curcumin. A nanoparticulate dispersion equivalent to 2 mg of the drug was applied to the donor compartment. Approximately 2ml of the dissolution media from the receptor compartment was withdrawn at specific time periods (0, 4, 8, 12, 24, 48, 72, 96, 120, 144, 168, 192 hours) and was immediately replaced with the same volume of dissolution medium to maintain sink conditions. The withdrawn samples were filtered and analysed for drug release by a developed method of analysis using a UV-Visible spectrophotometer (Shimadzu UV-1700). The release study was performed in multiples of three to maintain accuracy and precision[12,13].

2.3.4 Kinetics Studies of Drug Release Data

In order to determine the drug release mechanism, the *in-vitro* release data was of a selected optimised formulation C_{12} was fitted into a zero-order, first-order, matrix, Higuchi, Hixon-Crowell cube root law and Korsmeyer-Peppas model. Based on the goodness of fit test, the most appropriate model was selected[14].

2.3.5 Zeta Potential

The zeta potential of selected formulation C_{12} was measured using Malvern Zetasizer Nano ZS (Model no: ZEN3600). The sample was subjected to dilution in a 1:10 ratio with distilled water before measurement.

2.3.6 UV- Visible Spectroscopy

UV–visible spectroscopy was used to confirm the encapsulation of CUR within the BSA_NPs. Therefore the spectra of BSA, CUR individually, and BSA_CUR_NPs were taken by scanning the solutions in the 190–800

Table 1. Independent variables and level used for 3^3 BBD

Independent variables	+1(high)	0(medium)	-1(low)
Drug : Albumin ratio	100 mg	50 mg	10 mg
Vol. of Organic solvent	50 %	30 %	10 %
Homogenisation rpm	20,000	15,000	10,000

nm range with a quartz cuvette of 1 cm path length, using a Shimadzu UV–visible spectrophotometer (UV-1700; Shimadzu). From the spectra, the lambda max was measured.

3. Results

3.1 Optimization of Bovine Serum Albumin Nanoparticles Loaded with Curcumin (BSA_CUR_NP's) by 3^3 Box-Behnken Design (BBD)

Box-Behnken design is a quadratic optimization design developed based on a three-level incomplete factorial design, which provides maximum information from a minimum number of experiments. Hence, with a reduced number of experimental trails, an optimum setting for independent variables influencing the process can be identified (Table 2).

3.1.1 Polynomial Equations Obtained by 3^3 BBD

The polynomial equation derived:

Particle size (Y_1) = 257.23+68.16A-20.64B-38.30C+0.35AB-13.37AC+11.53BC-13.23A^2 -22.53B^2 +20.09C^2.

%Entrapment Efficiency (Y_2) = 77.95+11.54A+3.24B+0.97C+0.80AB-0.54AC+0.94BC-2.91A 0.93B^2+2.46C^2

In equation (Y_1) positive sign for coefficient of A and a negative sign for coefficient of B and C indicated that as the concentration of albumin increases, particle size increases, whereas as the volume of organic solvent and homogenization speed increases, particle size decreases. In equation (Y_2), the positive sign for coefficient of A, B and C shows that as the concentration of albumin, volume of organic solvent and homogenization speed increase, %EE increases. The rationality of the above equations was confirmed with the values of the variables obtained by the check point formulation.

3.1.2 Response Surface Plots

Graphical presentation of the facts can help to expose the relationship between dependent and independent variables. Graphs furnish facts just like the mathematical equations obtained from statistical analysis. The three-dimensional response surface graph is beneficial in getting to know about the principle interaction among the independent variables (factors).

Table 2. Experimental measured values for particle size and PDI and %EE of BSA_CUR_NPs by 3^3 Box Behnken Design

Formulation Code	Drug:Albumin ratio (mg)	Vol. of organic solvent (%)	Homogenization rpm	Particle size (nm)	Entrapment Efficiency (%)
C_1	50	50	15000	211.7	84.81
C_2	50	30	15000	267.6	79.56
C_3	50	10	10000	300.2	72.80
C_4	100	50	15000	242.5	88.38
C_5	50	30	15000	267.6	79.56
C_6	50	30	15000	267.6	79.56
C_7	100	30	10000	383.4	87
C_8	10	30	20000	150.8	65.86
C_9	10	30	10000	208.7	64.94
C_{10}	100	30	20000	272.0	85.77
C_{11}	50	50	10000	257.2	78.91
C_{12}	**50**	**10**	**20000**	**208.6**	**74.96**
C_{13}	100	10	15000	304.4	81.80
C_{14}	10	10	15000	180.4	58.23
C_{15}	50	30	15000	267.6	79.56
C_{16}	10	30	15000	163.9	63.48
C_{17}	10	50	15000	117.1	61.61

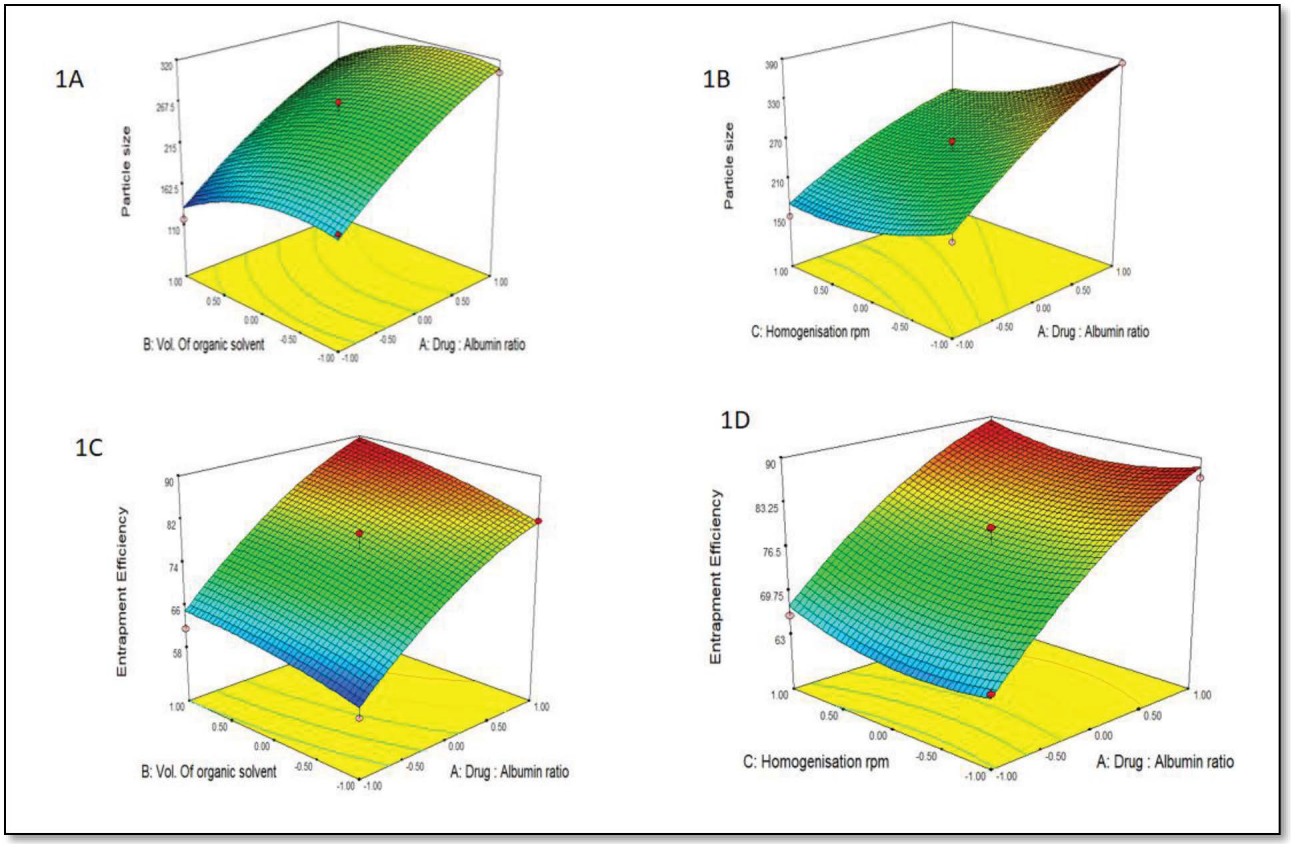

Figure 1. Response surface plot: particle size and entrapment efficiency.

3.1.3 ANOVA for Particle Size and Entrapment Efficiency

Response 1 Particle size

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Block	2015.19	1	2015.19			
Model	57962.25	9	6440.25	5.85	0.0074	significant
A-Drug : Albun	*37169.01*	*1*	*37169.01*	*33.75*	*0.0003*	
B-Vol. Of orga	*3407.25*	*1*	*3407.25*	*3.09*	*0.1125*	
C-Homogenisa	*11735.12*	*1*	*11735.12*	*10.65*	*0.0098*	
AB	*0.49*	*1*	*0.49*	*4.449E-004*	*0.9836*	
AC	*715.56*	*1*	*715.56*	*0.65*	*0.4410*	
BC	*531.30*	*1*	*531.30*	*0.48*	*0.5049*	
A^2	*736.98*	*1*	*736.98*	*0.67*	*0.4345*	
B^2	*2137.27*	*1*	*2137.27*	*1.94*	*0.1971*	
C^2	*1700.25*	*1*	*1700.25*	*1.54*	*0.2455*	
Residual	9912.35	9	1101.37			
Lack of Fit	*1309.40*	*3*	*436.47*	*0.30*	*0.8217*	*not significant*
Pure Error	*8602.95*	*6*	*1433.83*			
Cor Total	69889.79	19				

The Model F-value of 5.85 implies the model is significant. There is only a 0.74% chance that a "Model F-Value" this large could occur due to noise.

Response 2 Entrapment Efficiency

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Block	38.12	1	38.12			
Model	1225.73	9	136.19	5.36	0.0100	significant
A-Drug : Albun	*1065.14*	*1*	*1065.14*	*41.89*	*0.0001*	
B-Vol. Of orga	*83.98*	*1*	*83.98*	*3.30*	*0.1025*	
C-Homogenisa	*7.51*	*1*	*7.51*	*0.30*	*0.6001*	
AB	*2.56*	*1*	*2.56*	*0.10*	*0.7583*	
AC	*1.16*	*1*	*1.16*	*0.045*	*0.8359*	
BC	*3.50*	*1*	*3.50*	*0.14*	*0.7193*	
A^2	*35.61*	*1*	*35.61*	*1.40*	*0.2670*	
B^2	*3.65*	*1*	*3.65*	*0.14*	*0.7137*	
C^2	*25.41*	*1*	*25.41*	*1.00*	*0.3436*	
Residual	228.87	9	25.43			
Lack of Fit	*22.01*	*3*	*7.34*	*0.21*	*0.8840*	*not significant*
Pure Error	*206.85*	*6*	*34.48*			
Cor Total	1492.72	19				

The Model F-value of 5.36 implies the model is significant. There is only a 1.00% chance that a "Model F-Value" this large could occur due to noise.

Figure 2. ANOVA for response - particle size and entrapment efficiency.

3.1.4 Parameters of the Check Point Formulation

The details of the parameters of the check point formulation are given in Figure 3.

3.2 Evaluation of Nanoparticles

Particle size, PDI, % EE and TDC was analyzed for all the batches of formulation and the results are depicted in Table 4.

3.2.1 In-vitro Drug Release Studies

The results of the *in-vitro* release studies for the selected optimised formulations of the prepared nanoparticles in the form of cumulative percentage drug release at the different pre-determined time points are depicted in Figure 5.

Table 3. Parameters of the check point formulation

Formulation C			
Independent Variable	**Dependent variable**	**Predicted value**	**Observed Value**
X_1=74mg X_2=11.7mL X3= 15000 rpm	Particle size (nm)	169.4	170.9
	% Entrapment efficiency	69.35	71.45

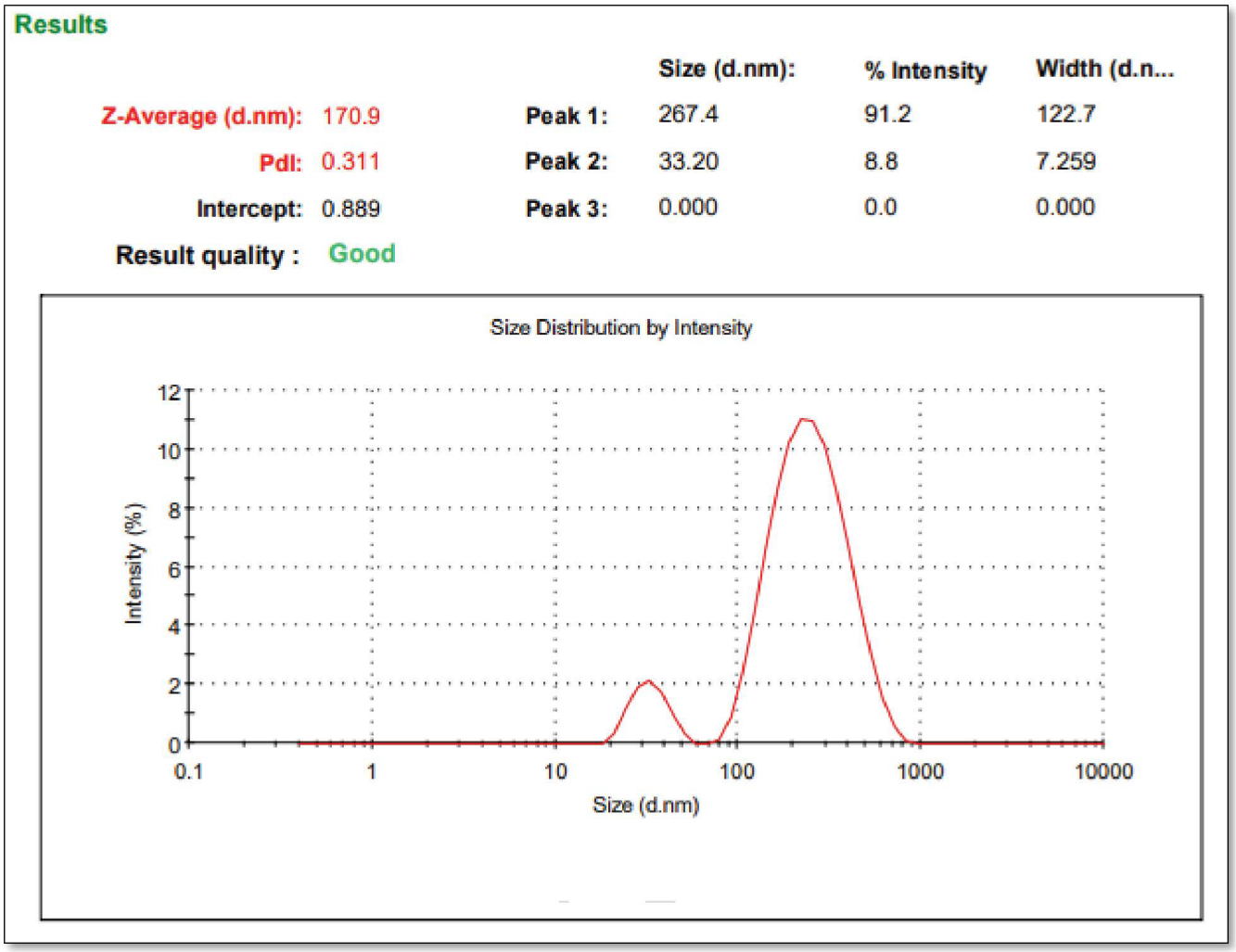

Figure 3. Particle size and PDI of check point formulation.

Table 4. Experimental measured values for particle size, PDI, %EE and TDC of BSA_CUR_NPs

Formulation Code	*Particle size (nm)	*PDI	*Entrapment Efficiency (%)	*Total Drug Content (%)
C_1	212.1 ± 2.52	0.147 ± 0.04	84.90 ± 0.48	93.63 ± 0.33
C_2	266.2 ± 1.31	0.152 ± 0.04	79.82 ± 0.28	95.11 ± 0.33
C_3	302.7 ± 2.41	0.338 ± 0.08	72.63 ± 0.16	92.63 ± 0.20
C_4	243.1 ± 0.76	0.383 ± 0.11	88.61 ± 0.61	90.40 ± 0.29
C_5	265.8 ± 1.80	0.160 ± 0.04	79.71 ± 0.13	95.24 ± 0.15
C_6	265.8 ± 1.80	0.160 ±0.04	79.67 ± 0.11	95.29 ± 0.13
C_7	383.6 ± 1.31	0.650 ± 0.10	86.92 ± 0.38	91.71 ± 0.40
C_8	151.4 ± 0.93	0.354 ± 0.13	66.13 ± 0.24	96.99 ± 0.35
C_9	207.9 ± 1.65	0.270 ± 0.04	65.17 ± 0.22	98.21 ± 0.33
C_{10}	270.6 ± 1.53	0.505 ± 0.02	85.49 ± 0.30	93.24 ± 0.33
C_{11}	255.4 ± 1.55	0.212 ± 0.03	79.09 ± 0.22	95.99 ± 0.27
C_{12}	**207.1 ± 1.36**	**0.138 ± 0.03**	**75.04 ± 0.06**	**91.40 ± 0.08**
C_{13}	303.4 ± 1.29	0.168 ± 0.01	81.69 ± 0.11	94.37 ± 0.13
C_{14}	181.1 ± 0.81	0.185 ± 0.06	58.53 ± .37	87.00 ± 0.54
C_{15}	266.6 ± 2.09	0.230 ± 0.12	79.89 ± 0.48	95.03 ± 0.57
C_{16}	162.4 ± 1.45	0.264 ± 0.17	63.29 ± 0.25	86.65 ± 0.35
C_{17}	116.1 ± 1.07	0.522 ± 0.07	61.65 ± 0.14	88.96 ± 0.20

***N=3, Average of three determinations**

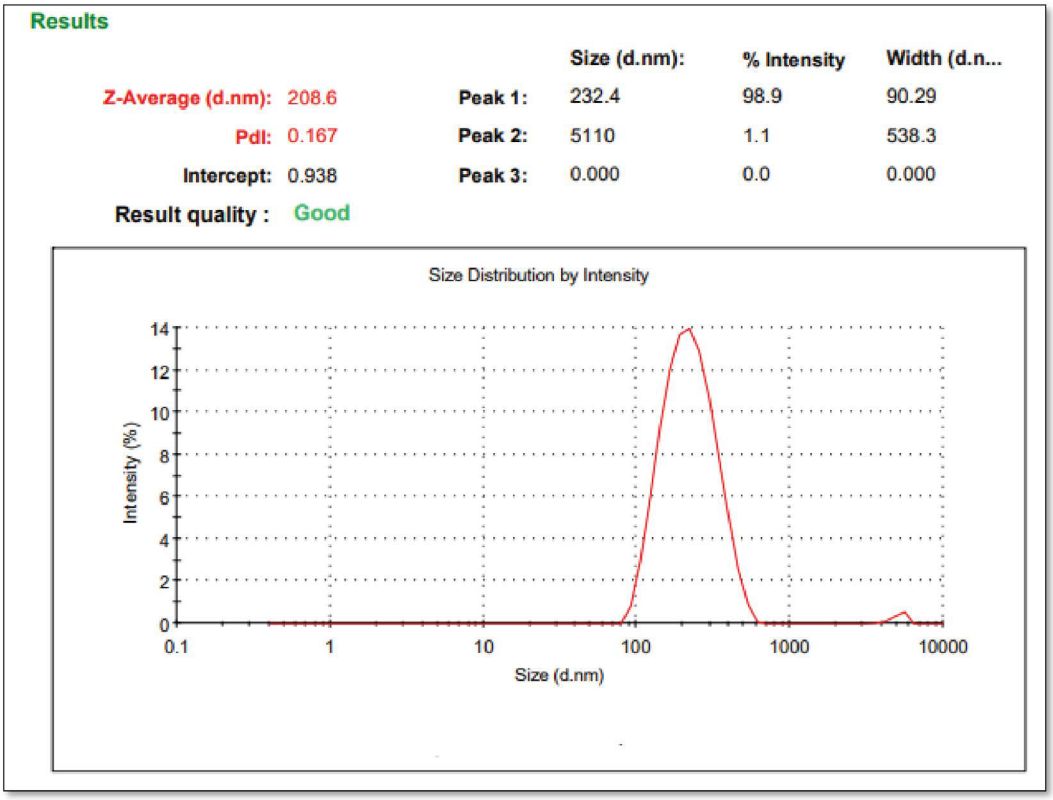

Figure 4. Particle size and PDI of C_{12} formulation.

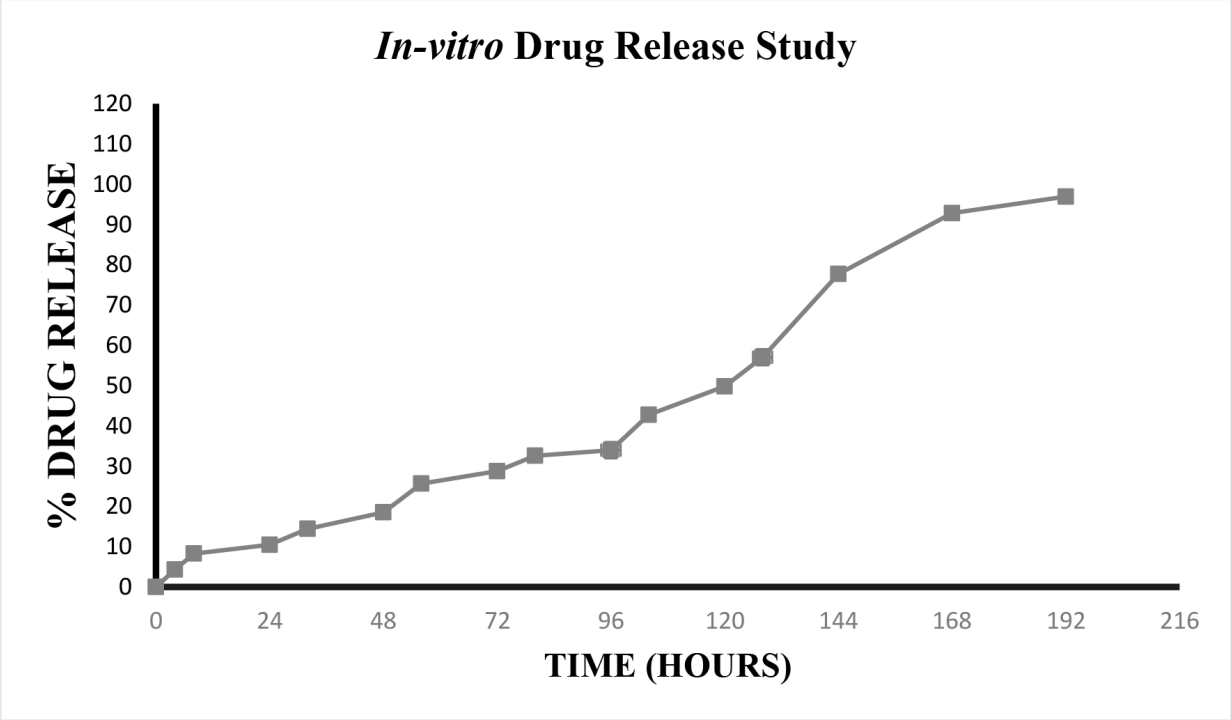

Figure 5. *In-vitro* drug release study of optimized BSA_CUR_NPs (C_{12}).

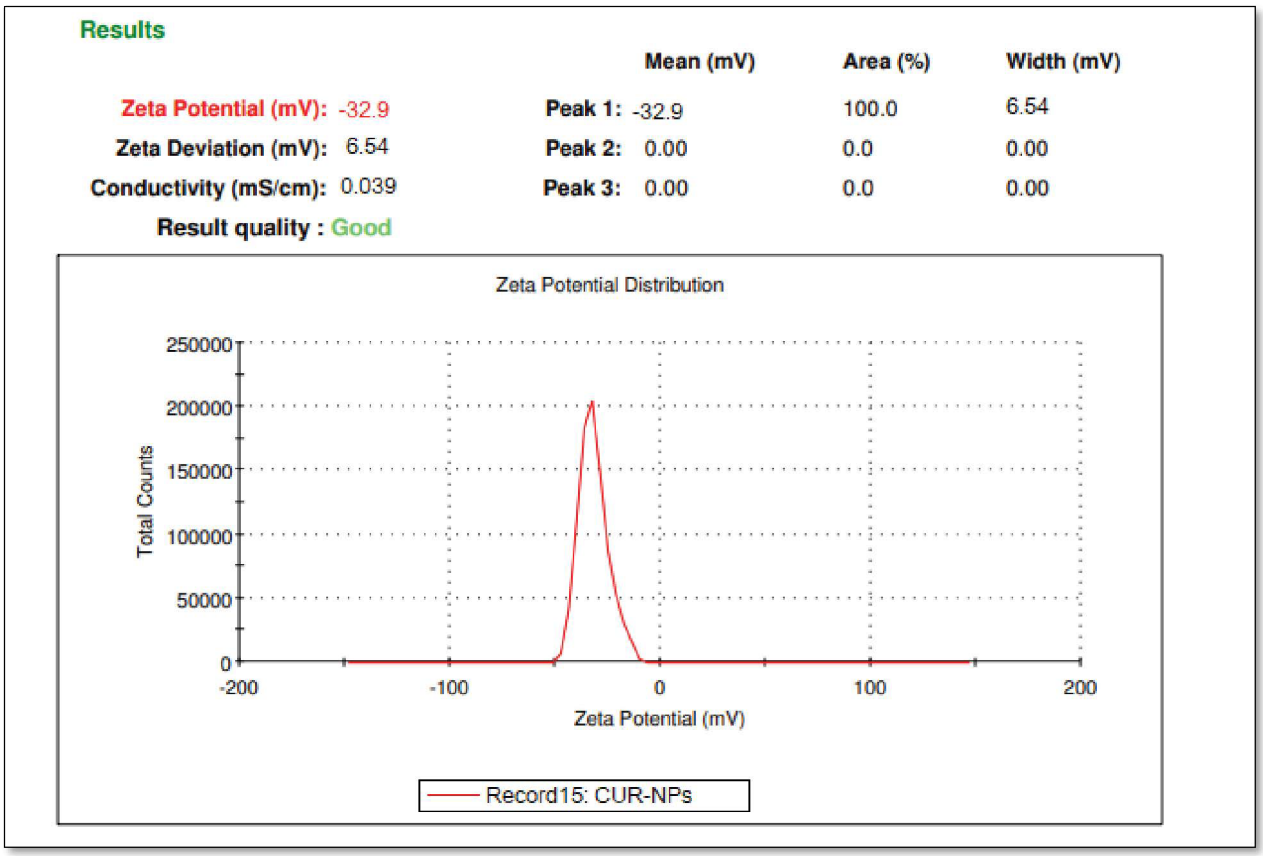

Figure 6. Zeta potential of C_{12} formulation.

Table 5. Drug release kinetics of formulation C_{12}

Formulation Code	Zero Order Model	First Order Model	Higuchi Model	Hixon Crowell Model	Korsmeyer Peppas Model		Result
	R square					n	
C_{12}	0.9574	0.9243	0.8307	0.8408	0.955	0.8601	Korsmeyer peppas model

3.2.2 Kinetics Studies of Drug Release Data

The drug release kinetics of the selected optimized formulation $\mathbf{C_{12}}$ was determined and the results are furnished in Table 5.

3.2.3 Zeta Potential

The zeta potential of selected optimised curcumin nanoparticles was found to be -32.9mV indicating good stability.

3.2.4 UV-Visible Spectroscopy

Estimation of spectra of pure BSA, CUR and BSA_CUR_NPs by UV spectroscopy provided confirmation of encapsulation of CUR within the formed nanoparticles of bovine serum albumin.

4. Discussion

4.1 Optimization of Bovine Serum Albumin Nanoparticles Loaded with Curcumin (BSA_CUR_NP's) by 3^3 Box-Behnken Design (BBD)

The goal of the study was to formulate nanoparticles of size less than 200nm. Hence it is important to control the process and formulation parameters affecting the particle size. The applied Response Surface Methodology (RSM) helps in understanding of complex system behavior and region of optimum conditions, where drug: albumin ratio, volume of organic solvent and homogenization rpm were considered as independent variables which were found to influence the dependent variables like particle size and entrapment efficiency. Further, the application of Box Behnken Design gave a statistically systematic approach for the development of nanoparticles with desired characteristics. The design generates results in the form of polynomial equations, ANOVA responses, squared values of dependent variables and parameters about the check point formulation.

4.1.1 Effect of Albumin Concentration on Particle Size and Entrapment Efficiency

BSA is an attractive drug carrier, especially for loading water-insoluble drugs. In our study, we prepared BSA-based nanoparticles by a high pressure homogenization technique, which results in the formation of a new disulfide bond in the free sulfhydryl group of albumin, which results in cross-linking into nanoparticles, thereby forming a tight complex with drugs loaded. The particle size was found to increase with increasing albumin concentration (Figure 1A). This can be due to increased solution viscosity at a higher concentration of albumin, which hinders the process of homogenization during emulsification, leading to a larger particle size of albumin nanoparticles prepared[15,16].

The entrapment efficiency was found to increase with the rise in content of BSA (Figure 1C), as BSA has affinities towards drugs and, due to the presence of charged groups, can bind to drugs through electrostatic interactions. Hence, higher concentration of albumin will result in higher entrapment efficiency.

4.1.2 Effect of Volume of Organic Solvent on Particle Size and Entrapment Efficiency

From Figure 1B, it is evident that the volume of organic solvent has a strong influence upon the particle size of the nanoparticles. Increasing the volume from 10 to 50 % decreased the particle size of the nanoparticles. This phenomenon is likely due to the increased volume of organic solvent providing a larger surface area for the emulsion during the homogenization process, thereby producing smaller globules due to high shear force. As a result, smaller nanoparticles are produced[17].

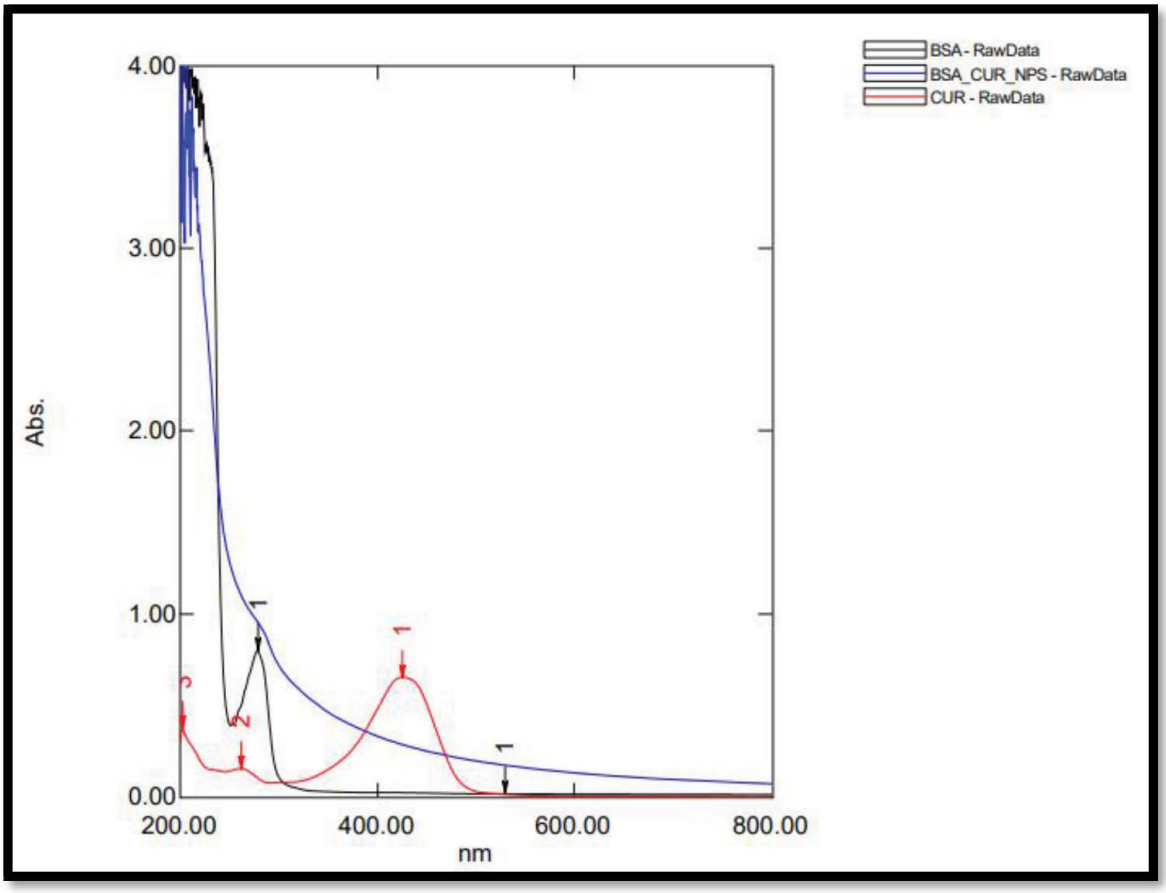

Figure 7. UV absorption spectra of BSA, CUR and BSA_CUR_NP's.

Increased volumes of organic solvent values had a positive effect on the %EE (Figure 1C). It is a well-known fact that water miscible organic solvent allows the fast mass-transfer between the dispersed phase and the continuous phase, leading to faster solidification or precipitation of protein, thereby increasing the entrapment efficiency. The organic solvent (i.e., acetone) used for the preparation of BSA_CUR_NPs is miscible with water.

4.1.3 Effect of Homogenization Pressure (rpm) on Particle Size and % Entrapment Efficiency

The most important step in the nanoparticle preparation is the emulsion formation, as the size of the globule formed is directly proportional to the final nanoparticle size. The emulsion is created by combining an organic phase containing the drug with an aqueous phase containing the polymer and homogenising it to break it down into fine droplets. Hence, homogenization rpm plays an important role in producing smaller sized nanoparticles (Figure 1B). It was found that homogenization rpm had a positive effect on the size of the nanoparticles. With an increase in homogenization rpm, the particle size is reduced as the shear pressure breaks down the larger globules into smaller ones with the mechanical collision against the wall due to excessive fluid acceleration and the shear stress within the gap between the rotor and the stator owing to the rapid rotation of the stator. Hence, the resulting smaller-sized nanoparticles[18–20]. Further, the entrapment efficiency was increased with an increase in homogenization rpm.

4.1.4 ANOVA for Response Surface Quadratic Model of Particle Size and Entrapment Efficiency

The model was validated by analysis of variance (ANOVA) employing design expert software, which was used to develop the experimental plan for RSM. The ANOVA data (Figure 2) show that a model is significant ($p < S0.05$) and that no-significance of lack of fit is required for using a specific model.. For particle

size and %EE, the model F value was found to be 5.85 and 5.36, respectively, which suggests that the model is significant. The R squared value for the model was 0.8540 and 0.8427 respectively; the adjusted r^2 value was 0.7079 and 0.6853 respectively. Predicted r^2 values were 0.4933and 0.5357 respectively, and adequate precision was found to be 10.223 and 7.903 respectively. The predicted r^2 value was in good agreement with the adjusted r^2 value. Adequate precision signifies the signal to noise ratio. A ratio greater than 4 is desirable. This model for particle size and %EE provided a ratio of 10.223 and 7.903 respectively, which indicates an adequate signal and thus the model was used to navigate design space.

4.2 Evaluation of Nanoparticles

4.2.1 Particle Size, PDI, % Entrapment Efficiency and Total Drug Content

Particle size and PDI of all the batches of formulated nanoparticles were carried out using a Malvern zetasizer. The investigation led to producing the smallest particle size ever, as small as 117.1nm. The Polydispersity index (PDI) for all the batches was found to be between 0.1 to 0.5. Generally, PDI below 0.3 designates homogeneity in the size distribution. A PDI of less than 0.3 is considered good, and 0.3 to 0.7 is acceptable. Entrapment efficiency was found to be between 58.53 to 88.61 % and total drug content was found to be between 88.96 to 98.21% (Table 4). Based on the results of particle size, PDI and entrapment efficiency, formulation C_{12} was found to be most satisfactory and was subjected to further analysis.

4.2.2 In-Vitro Drug Release Studies

Drug release from nanoparticles and subsequent biodegradation are vital characteristics to be considered for developing a successful formulation. The release rates of nanoparticles depends upon the diffusion of the drug through the polymer wall or nanoparticle matrix, polymer erosion, desorption of the drug and a combined diffusion/erosion process. Hence, diffusion and biodegradation direct the drug release from nanoparticles. The release profile of the C_{12} formulation was found to sustain the release of the drug for a longer period of time i.e. 192 hours.

4.2.3 Kinetics Studies of Drug Release Data

In order to establish the pattern and mechanism of drug release, the experimental *in-vitro* drug release data of the C_{12} formulation was fitted to various release models. Based on the correlation coefficient (r) values, the suitable model describing drug release from the BSA_CUR_NP's was selected. The selected optimised nanoparticles follow the Korsmeyer Peppas model, which indicates that the drug release from the nanoparticles follows a zero-order release pattern.

4.2.4 Zeta Potential

The zeta potential of selected optimised curcumin nanoparticles C_{12} was found to be -32.9mV (Figure 6), indicating good colloidal stability as negatively charged particles lead to higher stability.

4.2.5 UV Spectroscopy

The UV-Vis spectra of BSA shows the peak at around 278nm and CUR exhibits a peak at 425.5nm (Figure 7). The formulation, i.e., bovine serum albumin nanoparticles loaded with curcumin exhibited only the characteristic peak of BSA and there was no peak of CUR, indicating the encapsulation of CUR within the nanoparticles.

5. Conclusion

Bovine serum albumin nanoparticles loaded with curcumin were successfully developed by Nab technology. The best conditions for the preparation of BSA_CUR_NP's with a size range 100-300 nm and maximum entrapment efficiency were determined by a chemometric approach involving a minimum number of experiments. Furthermore, the experiments performed as per the suggested experimental design allowed the identification of the independent variables affecting the responses. In addition, the *in-vitro* drug delivery studies indicated a sustained release profile for up to 8 days. Hence, it can be concluded that the prepared BSA_CUR_NPs, owing to its composition, can deliver curcumin to inflamed joints at a concentration that will lead to optimum therapeutic efficacy while reducing its degradation and improving stability.

6. Declaration of Interest

The authors report no conflicts of interest.

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