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Effect of Polymer and Formulation Variables on Properties of Self-Assembled Polymeric Micellar Nanoparticles

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Abstract

Chemotherapy is having various side effects and toxicities, to overcome these problems nanoparticles are formulated. Nanoparticles accumulate in the tumor cells due to enhanced permeation and retention effect. A series of poly (d, l-lactide-co-glycolide) PLGA and bovine serum albumin (Fraction V) BSA formulations were fabricated and used as nanocarriers for delivery of a promising anticancer drug paclitaxel (PTX). The eight formulations of nanoparticles of PTX-PLGA and PTX-BSA were prepared by using 2^3 factorial designs. PLGA (A,) poly vinyl alcohol (PVA) (B) and stirring speed (C) was used as independent variables where particle sizes (Y1), entrapment efficiency (Y2) and % drug release (Y3) were taken as dependant variables. PTX was efficiently encapsulated into the micelles by desolvation technique. The mean diameter of PTX-BSA and PTX-PLGA nanoparticles ranged from 104 to 1150 nm and 110 to 1023 nm respectively. The entrapment efficiency and *in vitro* drug release also depends on the solubility of drug and polymer in solvent. The use of design expert software is systematic tool for optimization technique, and also helps to reduce number of runs. Hence, in the present work, an attempt was made to formulate, evaluate and optimize particle size and entrapment efficiency of PTX-BSA and PTX-PLGA nanoparticles.

Keywords: Paclitaxel; Poly (d, l-lactide-co-glycolide); Bovine serum albumin; Nanoparticles

Introduction

Cancer is a leading cause of death, with more than 10 million people being diagnosed with the disease annually. The difficulty of drug to target in tumor tissue is the main problem of cancer therapy. In cancer cells division is uncontrolled and which have the ability to attack other biological tissues. The invasion is either by direct growth into adjacent tissue or by implantation into distant sites by metastasis or a mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomous and purposeless proliferation of cells [1,2].

Chemotherapy is the chief curative approach for the treatment of cancers. The lack of cite specificity to cancer cells by anticancer drugs, the selective amplified uptake in cancerous tissue would be of massive interest in cancer chemotherapy [3]. Routes of administration, biodistribution and elimination of available chemotherapeutic agents can be optimized by modified drug delivery systems. The focus is on targeted cancer therapy. The novel approaches to cancer treatment not only complement the conventional chemotherapy and radiotherapy but also suppress the damage to normal tissues and avoid drug resistance [1].

The PTX (Taxol®) has been most elective agent against a variety of cancers including ovarian and gastric cancer. The drug concentration and exposure time decide the efficacy PTX. Taxol® has been administered intraperitoneally in the compartment containing the ovaries in order to achieve a maximum concentration. However, Taxol® is formulated with Cremophor® EL (50:50 ethanol: polyoxyethylated castor oil), which has been coupled with considerable toxicity including anaphylactic response [4].

The effective therapy with PTX is thus mainly focused on developing new drug delivery systems to eradicate the toxicity of the Cremophor® EL, improve efficacy and abolish premedication. A successful administration of PTX requires a formulation, which does not utilize noxious adjuvant, releases the PTX over prolonged period of time and alleviates the compound during long term storage and should be practical to produce on a large scale [5].

Since conventional nanoparticles are naturally taken up within reticuloendothelial cells, the delivery of PTX to these macrophages can be achieved by use of biodegradable nanoparticles. This biodistribution can be of benefit for targeting of the chemotherapeutic agents to mononuclear phagocytes system localized tumors (i.e., hepatocarcinoma, hepatic metastasis, bronchopulmonary tumors, myeloma and leukemia). Due to enhanced permeation and retention effect, the nanoparticles accumulate in the tumor cells. Biodegradable polymeric nanoparticles are more useful in controlled and targeted drug delivery to achieve better therapeutic efficacy and fewer side effects [3].

Thus, formulations of biodegradable polymeric nanoparticles, is a comprehensive means to selectively restrain the tumor growth. However, most of the polymers used in the drug conjugation are nondegradable are having high molecular weight, which accumulate in the body because they are macromolecules whose molecular weight is larger than the cutoff value (5000) of glomerular filtration capacity in the kidney. Thus, for the drug conjugation use of biodegradable polymers is desirable. Formulation of microspheres or nanoparticles is possible by conjugation of various drug molecules to PLGA. They demonstrated high drug entrapment and showed a near zero-order

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release. The PLGA nanoparticles can be degraded into glycolic acid and lactic acid that are non-toxic to the human body. It also permits control of the release rate of drug from nanoparticles [6].

Various polymers are available for preparation of nanoparticles. Among these, albumin shows biocompatibility, biodegradability, nonantigenicity, and ease of preparation. Further, large number of drugs can bind to protein carrier in a relatively nonspecific manner. The presence of surface charge causes physical adsorption of drugs onto the protein surface or forms covalent bond to the matrix. Bovine Serum Albumin (BSA) has been extensively used to prepare micro and nanoparticles. Different active or diagnostic molecules have been entrapped into albumin particles to be administered by different routes such as intravenous, intramuscular, nasal, and ophthalmic routes. Albumin can penetrate cancerous tissues by two means, active or passive transport. The incorporation of PTX in albumin nanoparticles was shown to improve drug solubility, with a variety of advantages conferred to the standard PTX therapy [7].

Hence objective of present research was to entrap hydrophobic PTX in to PLGA and BSA by using solvent evaporation and desolvation technique respectively and to study the effect of polymer on properties of formulated nanoparticles by using 2^3 factorial designs.

Materials and Methods

Paclitaxel was kindly gifted by Alchem International Ltd (Haryana, India). Poly (D, L-lactic-co-glycolic) (PLGA, 50:50, Av.MW 10,000) was obtained from Cipla Pvt Ltd (Mumbai, India). Bovine serum albumin (Fraction V) and tween 80 was purchased from Loba Chemie (Mumbai, India). Poly vinyl alcohol (PVA, Av. MW 30,000–70,000) was obtained from West Coast Laboratories, (Mumbai, India). Glutaraldehyde was obtained from Molychem (Mumbai, India). Dichloromethane was supplied by S.D. Fine Chemicals Ltd. (Mumbai, India). Mannitol was purchased from Thomas Baker (Mumbai, India). Methanol was obtained from Merck Ltd, (Mumbai, India). Acetone was supplied by Ranbaxy Fine Chemicals Ltd., (New Delhi, India).All other chemicals were of the best quality commercially available.

Formulation of PTX-PLGA nanoparticles by solvent evaporation technique

Nanoparticles were prepared by using oil in water (O/W) solvent evaporation technique. The method involves preparation of an organic phase containing PTX and PLGA dissolved in dichloromethane (DCM) and aqueous phase containing PVA dissolved in water. The organic phase was added drop by drop into the aqueous phase during homogenization. The emulsion was broken down in to nanodroplets by using high speed homogenizer (Ultra turrex-T25, IKA laboratories, India), which formed O/W emulsion. Solvent was evaporated by heating the suspension on magnetic stirrer (Remi equipments, India). This suspension was then passed through 0.45 um membrane filters (Millipore, Bedford, MA, USA). Filtrate was centrifuged in cooling centrifuge (Remi equipments, India) for 10 min, 15000 rpm. After washing 2-3 times using distilled water, the residue was collected and dispersed in distilled water. The mannitol was added in the suspension and sprays dried by using spray dryer (Labultima LU222, India) Rafati et al. [14]. All formulations in the study were prepared via spray drying on the spray dryer. (Labultima LU222, India). All solutions were filtered through a 0.45 μm filter (Millipore, Bedford, MA, USA) prior to spray drying; to minimize blockage due to any undissolved particles at the spray mesh. The dried powder was collected from the cyclone using a particle scraper and then stored in desiccators at room temperature for further characterization [8,9]. Following conditions were set for spray drying: inlet temperature, 160°; outlet temperature, 130°; aspirator rate, 35 Nm3/h; solution feed rate, 2 ml/min; spraying air flow pressure, 2 bar

Formulation of PTX-BSA nanoparticles by desolvation technique

Albumin nanoparticles were prepared using the desolvation or simple coacervation method. The process involved the intermittent addition of an acetone solution containing PTX to BSA solution containing tween 80. The solution turned milky white. Acetone acts as antisolvent for BSA to reduce its solubility in water and facilitate nanoparticles formation by precipitation. The desolvation factor changes the tertiary structure of protein. On reaching the critical level of desolvation, protein clump formed which on cross linking with gluteraldihyde, results in the formation of nanoparticles. The resulting suspension was homogenized (Ultra turrex-T25, IKA laboratories, India) for 30 min. During homogenization, the nanoparticles formed were cross-linked by drop wise addition of GTA at room temperature. The suspension was kept in an ice bath for 10 min. The suspension was placed on the magnetic stirrer (Labultima LU222, India), and the cross-linking reaction was allowed to continue at room temperature for another three h. The particles were collected by centrifugation at 15,000 rpm for 10 min. using cooling centrifuge (Remi equipments, India). The supernatant was decanted and suspension was washed three times with acetone. The resulting washed suspension was spray dried by using spray dryer [10-12]. All formulations in the study were prepared via spray drying (Labultima LU222, India). Following conditions were set for spray-drying: inlet temperature, 50°; outlet temperature, 35°; aspirator rate, 35 Nm^3/h; solution feed rate, 2 ml/min; spraying air flow pressure, 2 bar.

Experimental Design

The eight formulations of PTX-BSA nanoparticles and PTX-PLGA nanoparticles were prepared by using 2^3 factorial designs (Design expert 8.0.6.1) as mentioned in Table 1a and Table 1b respectively. PLGA (A) and PVA solution (B) as a stabilizing agent, and stirring speed (C) were used as independent variables for PTX-PLGA nanoparticles, where BSA (A), Tween 80 (B) as a surfactant and GTA (C) as a cross-linking agent were used as independent variables for PTX-BSA nanoparticles. Particle size (PS) (Y_1), Entrapment Efficiency (EE) (Y_2) and % Drug Release at the 32^{nd} hour (Y_3) were taken as dependant variables. Each factor was tested at two levels designated as -1 and +1

The values of the factors were transformed to allow easy calculation

Run	BSA	Tween 80	Gluteraldehyde
B1	300	0.5	10
B2	100	3	10
B3	300	3	25
B4	100	3	25
B5	100	0.5	10
B6	100	0.5	25
B7	300	0.5	25
B8	300	3	10

Factor	Name	Units	Low Level	High Level
A	BSA	mg	100	300
B	Tween 80	%	0.5	3
C	Gluteraldehyde	%	10	25

Table 1a: Experimental design of PTX-BSA nanoparticles.

Run	PLGA	PVA	Speed
P1	100	0.5	5000
P2	100	3	5000
P3	300	3	20000
P4	300	0.5	5000
P5	300	0.5	20000
P6	100	3	20000
P7	300	3	5000
P8	100	0.5	20000

Factor	Name	Units	Low Level	High Level
A	PLGA	mg	100	300
B	PVA	%	0.5	3
C	Speed	rpm	5000	20000

Table 1b: Experimental design of PTX-PLGA nanoparticles.

of co-efficient in polynomial equation. To identify the effect of significant variables, the reduced model was generated. Interactive multiple regression analysis and F statistics were utilized in order to evaluate the response. The regression equations for the three responses were calculated using the following equations.

$$Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{23}BC + b_{13}AC + b_{123}ABC \quad (1)$$

The equation was applied on three responses, to describe the principal effects and interaction among the identified variables, A and B. Coded (−1; +1) value were used for each independent variable: the −1 corresponds to the low level of each variable and +1 to the highest level. These limits were selected on the basis of previous studies and the optimization procedure was carried out within these domains. Concerning the equation (1): *Y* is the dependent variable or response, b_1, b_2 and b_3 are the coefficients of the respective independent variable, b_0 is the arithmetic mean response, b_{12}, b_{23}, b_{13} and b_{123} are the interaction term. ANOVA was applied to verify the fitted model. Statistical analysis was considered significant when the p values were less than 0.05 [13].

Evaluation of formulated PTX nanoparticles

Particle size and size distribution: Particles size was determined by dynamic light scattering method using the particle size analyzer (Nanophox NX0088, Sympatec GmbH, Germany). The diameter of about 100 nanoparticles was measured from the photomicrographs of each batch. Finally, average mean diameters of particles were obtained [14].

Drug entrapment efficiency: Loading of PTX in nanoparticles was determined by extracting 10 mg nanoparticles with solvent in which polymer is soluble for 6 h and analyzing the extract by HPLC. PTX was quantified by UV- spectrophotometrically at 227 nm. Entrapment efficiency was expressed as the percentage of drug versus the amount of drug in organic phase [14,15]

Shape and surface morphology: The morphology of the prepared nanoparticles was investigated by scanning electron microscopy (JEOL Model JSM - 6390 LV). The nanoparticles were fixed on adequate supports and coated with gold under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Observations under different magnifications were performed at 15 kV [8].

In vitro drug release study: The *in vitro* release of drug from the nanoparticulate formulations was determined using membrane diffusion technique. spray dried product equivalent to 3 mg of drug from each batch were taken and suspended in 10 ml of phosphate buffer pH 7.4 saline solution. The time at which diffusion was initiated was noted and 10 ml of diffusate was withdrawn with pipette at various time intervals of 0.5, 1, 2, 4, 6, 8, 12, 24th h, and replaced by the same volume of fresh phosphate buffer to maintain a sink condition. These samples were filtered through 0.22 µm membrane filter. The obtained solution was analyzed UV-spectrophotometrically (Jasco V-630, Japan) at 228 nm after suitable dilution if necessary, using appropriate blank [16].

Response surface analysis: Response surface methodology is a collection of mathematical and statistical techniques used for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response. The experimental results were analyzed using Design Expert® software.

***In vivo* tissue distribution studies:** This study was carried out after obtaining the due permission for conduction of experiments from relevant ethics committee (K.L.E.S's College of Pharmacy, Belgaum) which is registered for "Teaching and Research on Animals" by committee for the purpose of control and supervision of experiments on animal, Chennai (Registration number 221/CPCSEA).

Dose of paclitaxel to be administered to rats was calculated according to body surface area ratio of human being. Nine healthy adult *Sprague dawley* rats weighing 200-250 g were selected, a constant day and night cycle was maintained and they were fasted for 12 h. The animals were divided into 3 groups, each containing 3 rats. Group I received nanoparticles equivalent to 300 µg/kg of paclitaxel intravenously in the tail vein after dispersing them in sterile phosphate buffer saline solution, F-4 (optimized) batch were selected for the study. Group-II rats received 300 µg/kg of pure paclitaxel intravenously. Group-III rats were treated as solvent control and were injected intravenously with sterile phosphate buffer saline solution.

After 24 h, the rats were sacrificed and their liver, lungs, spleen, kidney, heart and brain were isolated. The individual organs of each rat were homogenized separately by using a tissue homogenizer and the homogenate was centrifuged at 17609×g for 30 min. The supernatant was collected and filtered through 0.22 µ filters (Minisart, Germany) and analyzed by UV Spectrophotometer at 227 nm [17,18].

Results and Discussion

Particle size and size distribution

The mean particle size of nanoparticles formulation was in the range of nm. Formulation B3 of PTX-BSA nanoparticles and formulation P4 of PTX-PLGA showed relatively large particle size i.e. 1150 nm and 1023 nm respectively, whereas formulation B8 of PTX-BSA nanoparticles and P1of PTX-PLGA nanoparticles showed relatively small size i.e. 104 nm and 110 nm. In case of formulations prepared by BSA, the size of particles can be affected by concentration of polymer, pH, ratio of acetone/BSA and surfactant Tween 80. The particle size increased with increase in the concentration of BSA. Where, the nanoparticles formulated using PLGA, the particle size of different batches was varied. The main variables were polymer concentration (PLGA) and homogenization speed. When PLGA concentration was increased the size of nanoparticles increased.

Drug entrapment efficiency

The drug entrapment efficiency of different batches of nanoparticles was found in the range of 33.57 % to 78.36 % for PTX-BSA nanoparticles. The formulations prepared by PTX-PLGA showed drug entrapment efficiency in the range of 31.25% to 70.81%. It was observed that the entrapment efficiency increases with the increase in concentration

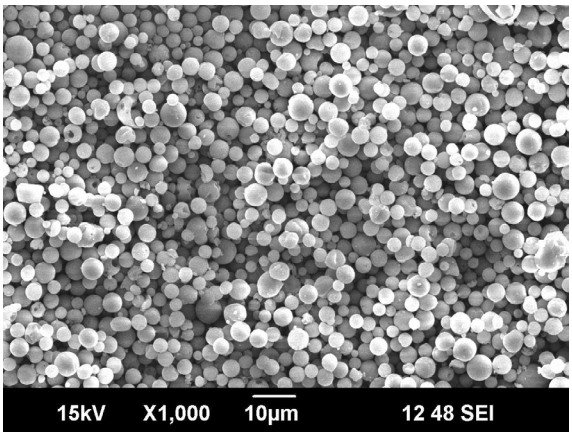

Figure 1a: SEM image of formulation B9 prepared by PTX-BSA.

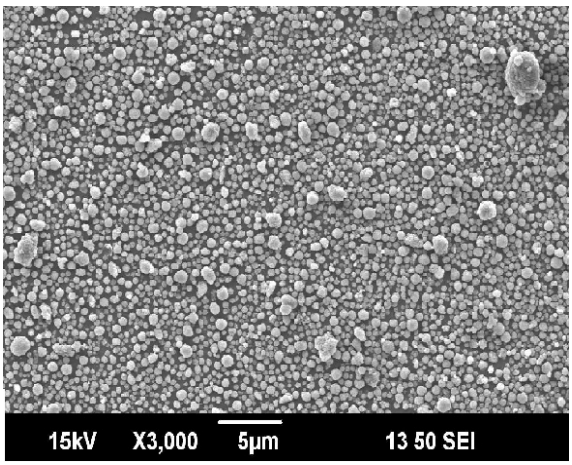

Figure 1b: SEM image of formulation P11 prepared by PTX-PLGA.

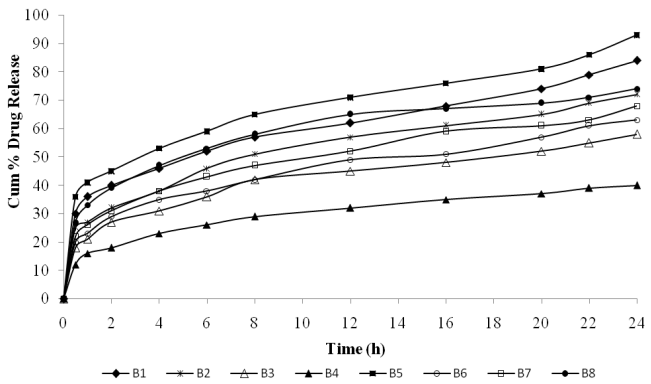

Figure 2a: *In vitro* drug release study of PTX-PSA loaded nanoparticles after 24th h. Formulation B_4 shows the controlled and slow release as compare to other formulation.

of GTA in the formulations and also it decreases with increase in the Tween 80 concentration. It was observed that the entrapment efficiency increases with the increase in homogenization speed in the formulations prepared with PLGA and also it increases with increase in PLGA concentration. Surfactant concentration also plays major role in entrapment of drug. It was also observed that relatively more drug was entrapped in nanoparticles prepared by BSA than PLGA. The effect may be due to inclusion of surfactant as well as cross linking agent in the formulation. In BSA nanoparticles, BSA forms stable nanosuspension with the help of surfactant tween 80. The gluteraldehyde acts as cross linking agent, which closes up the apertures of the particles thereby increasing resistance for drug leakage.

Shape and surface morphology

Morphological analysis of the PTX-BSA nanoparticles and PTX-PLGA nanoparticles showed regular and isolated particles showed in Figure1a and 1b respectively**.** SEM analysis of the samples revealed that all nanoparticles prepared were spherical in shape.

In vitro drug release study

The results are shown in Figure 2a for PTX-BSA nanoparticles and Figure 2b for PTX-PLGA nanoparticles**.** Drug release occurs mainly due to diffusion and erosion mechanism. It was observed that the drug release from the PTX-BSA nanoparticles decreases as the GTA and tween 80 concentration increases. All the formulations showed a biphasic release with initial burst effect. The mechanism for the burst release can be attributed to the drug adsorbed on the nanoparticles or weakly bound to the large surface area of the nanoparticles, or due to leakage of the drug from nanoparticles than to the drug incorporated in nanoparticles.

Response surface analysis

Analysis of data was carried out using ANOVA, and the individual parameter was evaluated with the F test. The experimental designs are summarized in Table 1a for PTX-BSA nanoparticles and Table 1b for PTX-PLGA nanoparticles respectively. The predicted values were calculated by using the mathematical model, tabulated in Table 2a and 2b.

The effect of significant factors on response can be measured by use of Pareto chart. The Pareto charts for prepared PTX-BSA nanoparticles show the significance of interaction between factors on particle size and entrapment efficiency as shown in Figure 3a and 3b respectively. The Pareto charts for prepared PTX-PLGA nanoparticles show the significance of interaction between factors on particle size

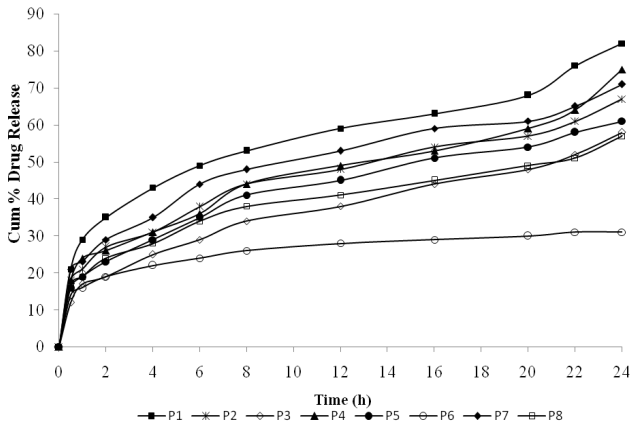

Figure 2b: *In vitro* drug release study of PTX-PLGA loaded nanoparticles after 24th h. Formulation P_6 shows the controlled and slow release as compare to other formulation.

Run	Particle Size		Entrapment Efficiency	
	Observed Value	Predicted Value	Observed Value	Predicted Value
B1	465	466.1	49	48.6
B2	957	955.8	35	35.3
B3	1150	1148.8	47	46.6
B4	370	371.1	33	33.3
B5	163	161.8	71	70.6
B6	871	872.1	57	57.3
B7	659	660.1	78	79.1
B8	104	102.8	67	65.8

*Each value represents mean ± S.E. (n=3)

Table 2a: Observed Response and predicted value for PTX-BSA nanoparticles.

Run	Particle Size		Entrapment Efficiency	
	Observed Value	Predicted Value	Observed Value	Predicted Value
P1	110	108	31	30.75
P2	985	987	44	44.25
P3	140	142	35	34.5
P4	1023	1021	60	60.5
P5	270	262.5	48	48.25
P6	630	637.5	62	61.75
P7	370	377.5	43	43.5
P8	760	752.5	70	69.5

*Each value represents mean ± S.E. (n=3)

Table 2b: Observed Response and predicted value for PTX-PLGA nanoparticles.

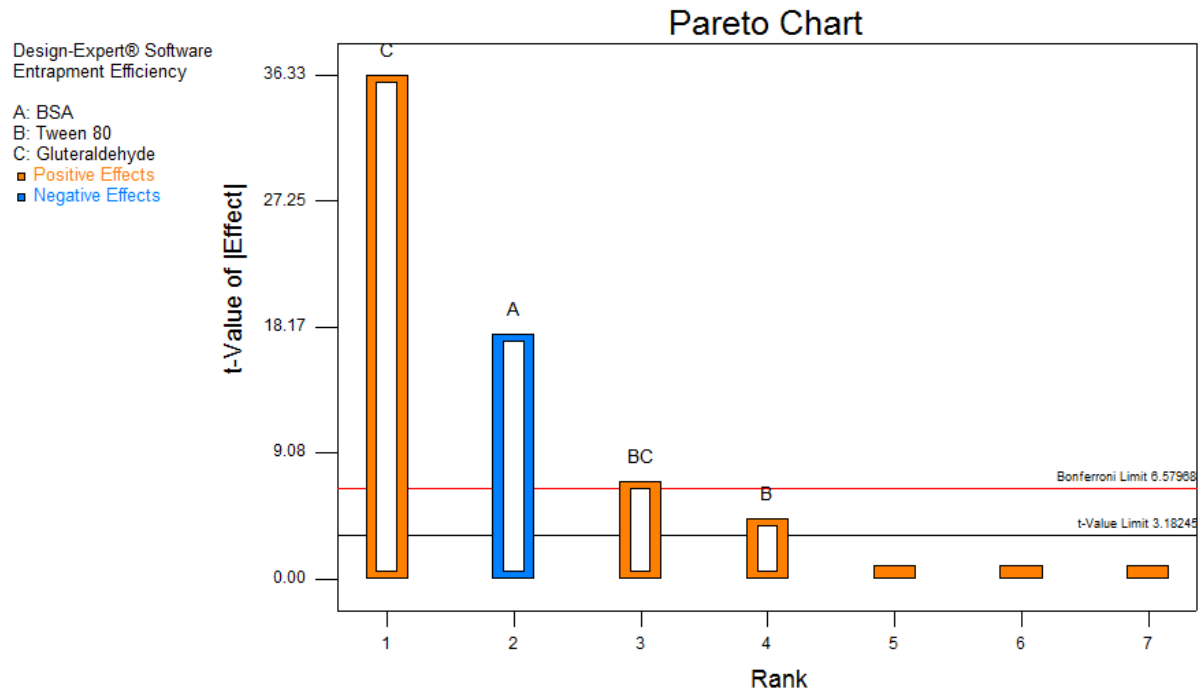

Figure 3a: The Pareto chart for PTX-BSA nanoparticles shows the significance of interaction between factors on particle size. Interaction of factor AB has more impact on the particle size than factor C followed by AC. Where factor B and factor Aalone has a marginal effect on particle size..

and entrapment efficiency as shown in Figure 3c and 3d respectively. Contour plots of most significant factors for particle size analysis, entrapment efficiency of PTX-BSA and PTX-PLGA are shown in Figure 4a-4d respectively.

Diagnostics case statistics of experimental matrix

The values prove that the predicted data, which were obtained from the empirical model for drug loading, are in good agreement with the experimental results due to their low differences.

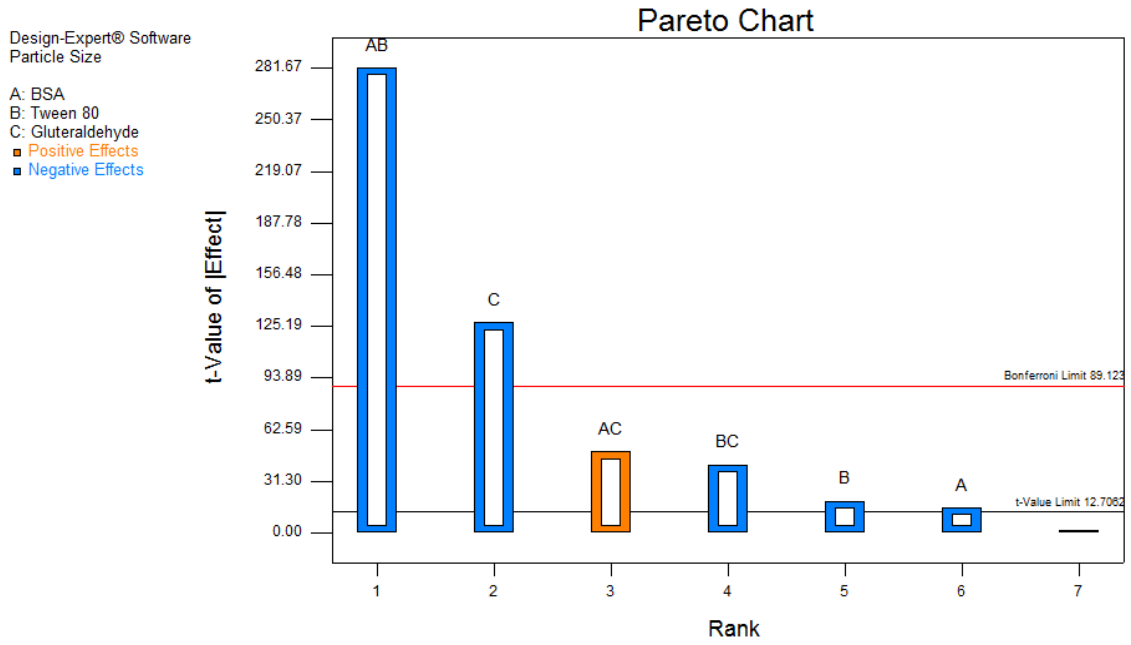

Figure 3b: The Pareto chart for PTX-BSA nanoparticles shows the significance of interaction between factors on entrapment efficiency. Factor C has more impact on on entrapment efficiency followed by factor A and interation of factor BC. Where factor B alone has a marginal effect on entrapment efficiency.

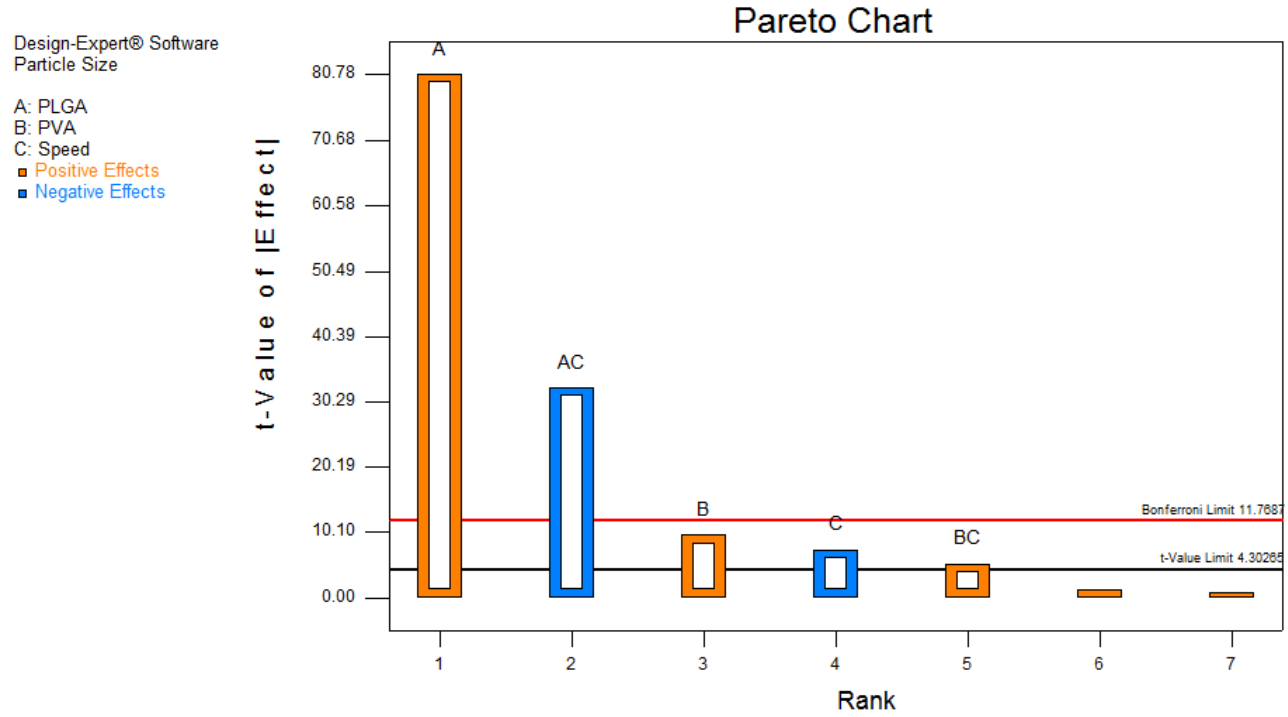

Figure 3c: The Pareto chart for PTX-PLGA nanoparticles shows the significance of interaction between factors on particle size. Factor A has more impact on the particle size than interaction of factor AC. Where factor B and factor Calone and interaction of BC has a marginal effect on particle size.

Desirability approach for optimized solution

It provides flexibility and giving importance for each response individually. According to the final results, the software suggested some formulations and also predicted their responses containing a probability factor named "Desirability" that ranged between 0-1 that the most presumable answer would be the nearest to 1. After

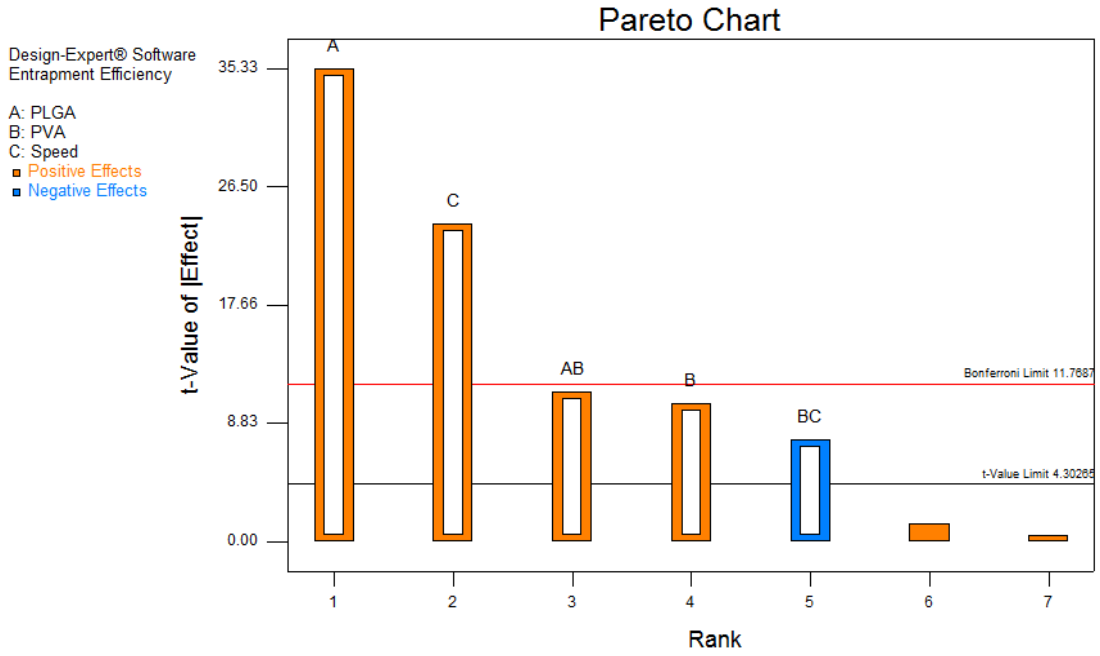

Figure 3d: The Pareto chart for PTX-PLGA nanoparticles shows the significance of interaction between factors on entrapment efficiency. Factor A has more impact on entrapment efficiency than factor C. Where interaction of factor AB, factor B and interaction of factor BChas a marginal effect on entrapment efficiency.

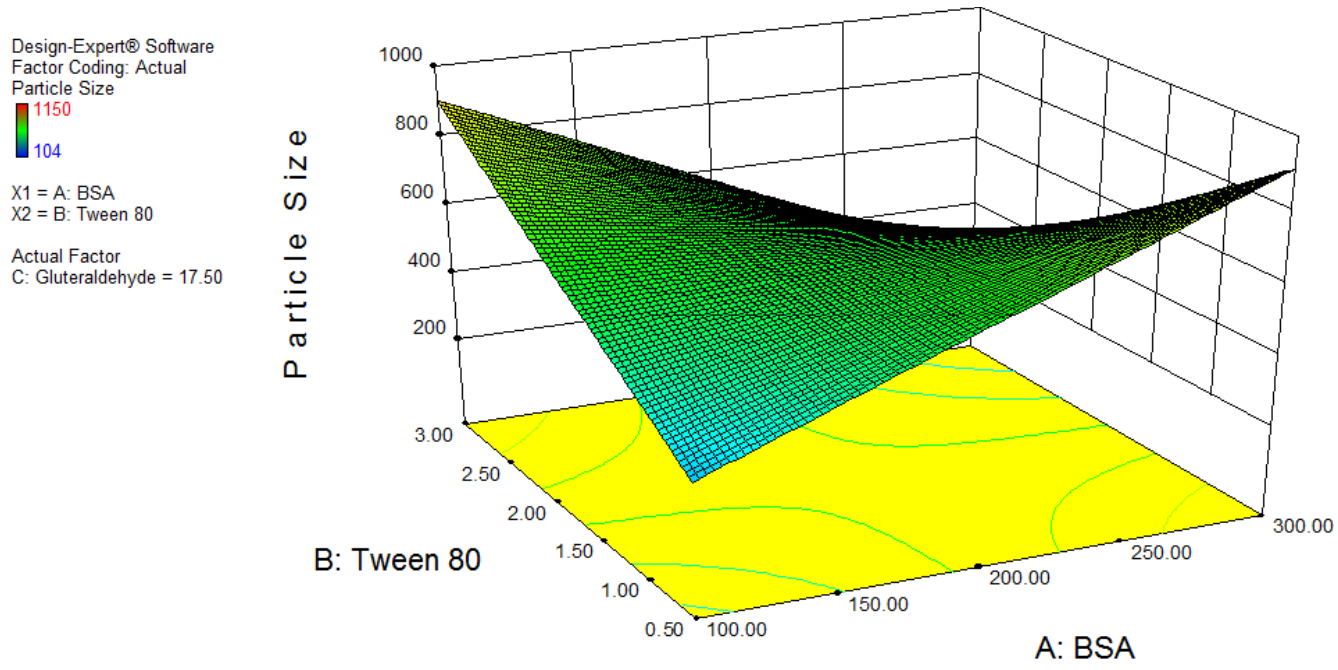

Figure 4a: The contour plot of particle size of nanoparticles prepared by PTX-BSA for most significant factor AB. The plot shows the low level and high level (-1 to +1) of factor A and factor B when C is actual factor kept constant. From the graph it is demonstrated that the particle size depends on the concentration of tween 80 and BSA.

setting the parameters and expected results, the design suggested low and high levels for the optimized batch. Table 3a and 3b include set parameters and some of the suggested formulations of DE 8.0.6.1 and the desirability of each item could be observed. From the suggested solutions three formulations were selected on the basis of feasibility and desirability, and formulated. For the same formulations particle size and entrapment efficiency were calculated, as shown in Tables 4a and 4b.

From the obtained data of Tables 5a and 5b formulation B9 and

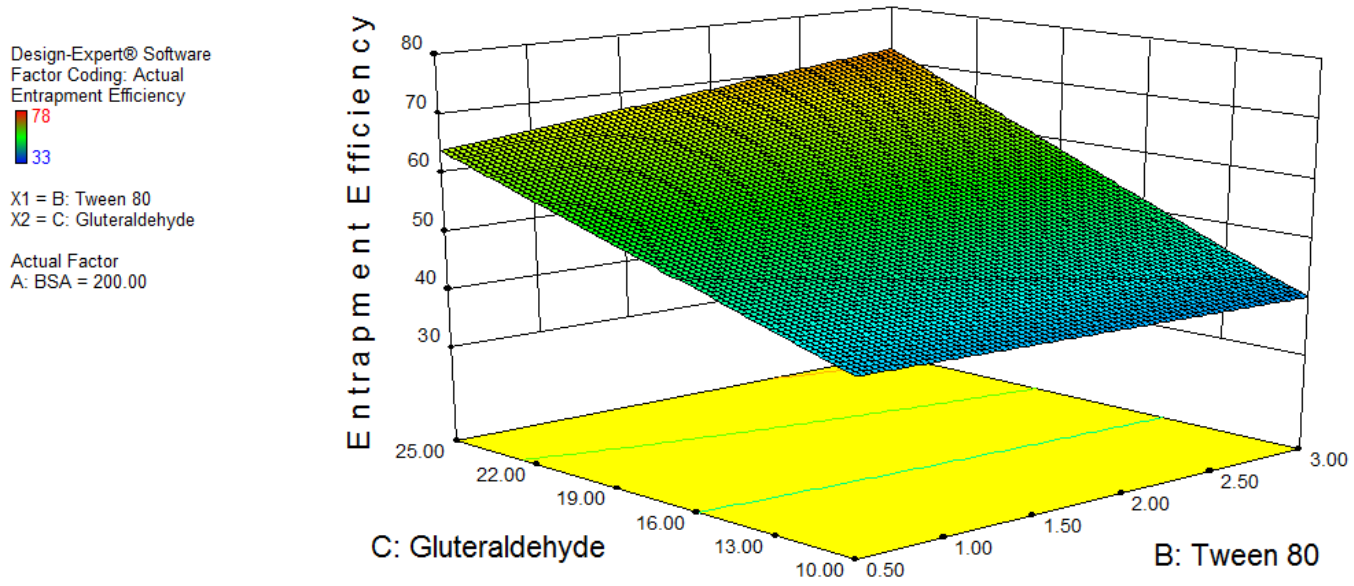

Figure 4b: The contour plot of entrapment efficiency of nanoparticles prepared by PTX-BSA for most significant factor BC. The plot shows the low level and high level (-1 to +1) of factor B and factor C when A is actual factor kept constant. From the graph it is demonstrated that the entrapment efficiency depends on the concentration of tween 80 and BSA.

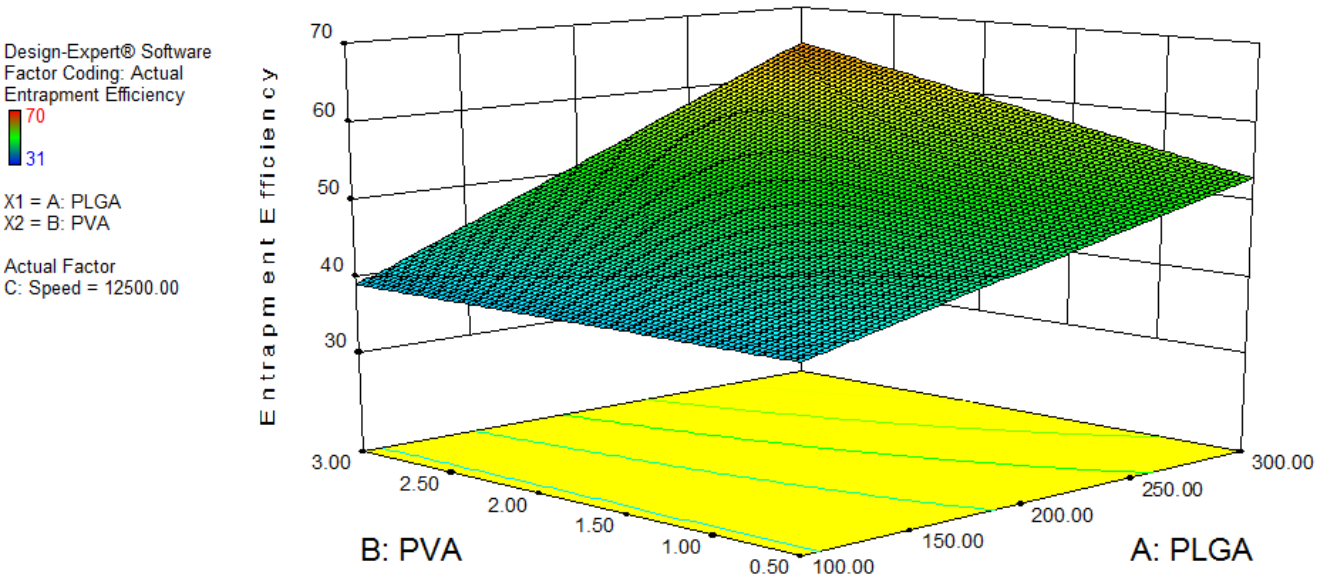

Figure 4c: The contour plot of particle size of nanoparticles prepared by PTX-PLGA for most significant factor AB. The plot shows the low level and high level (-1 to +1) of factor A and factor B when C is actual factor kept constant. From the graph it is demonstrated that the particle size depends on the concentration of PLGA and PVA.

P11 is selected as optimized formula and formulated in triplicate to confirm the optimized formulation. The results obtained are tabulated in Table 6.

Conclusion

In summary, PTX-BSA and PTX-PLGA nanoparticles were prepared by the solvent evaporation technique and Desolvation Technique. The application of factorial design gave a statistically systematic approach for the formulation of nanoparticles with desired particle size, high entrapment efficiency and drug release. Concentration of tween 80 and gluteraldehyde found to influence particle size and drug release in PTX-BSA nanoparticles, where stabilizer (PVA) and speed ratio was found to influence the particle size and drug release of PTX loaded PLGA nanoparticles. The entrapment efficiency was primarily depending

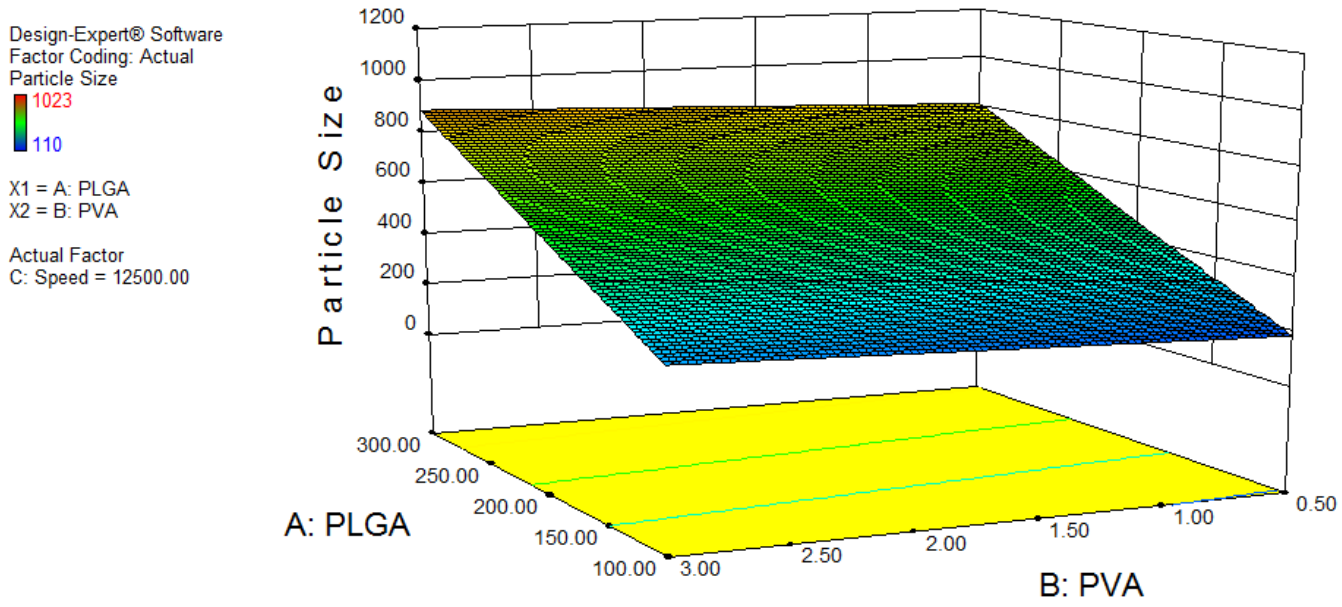

Figure 4d: The contour plot of entrapment efficiency of nanoparticles prepared by PTX-PLGA for most significant factor AB. The plot shows the low level and high level (-1 to +1) of factor A and factor B when C is actual factor kept constant. From the graph it is demonstrated that the entrapment efficiency depends on the concentration of PLGA and PVA.

Source	Particle Size		Source	Entrapment Efficiency	
	F - Value	p-value Prob> F		F -Value	P -value Prob> F
Model	16699.68	0.0059	Model	425	0.0002
A-BSA	225	0.0424	A-BSA	312.1111	0.0004
B-Tween 80	369.4938	0.0331	B-Tween 80	18.77778	0.0227
C-Gluteraldehyde	16185.49	0.0050	C-Gluteraldehyde	1320.111	< 0.0001
AB	79336.11	0.0023	BC	49	0.0060
AC	2401	0.0130	Residual		
BC	1681	0.0155	Cor Total		

*Each value represents mean ± S.E. (n=3)

Table 3a: The ANOVA test to determine the significance of the variables on particle size and entrapment efficiency prepared PTX-BSA nanoparticles.

Source	Particle Size		Source	Entrapment Efficiency	
	F -Value	P -value Prob> F		F -Value	P -value Prob> F
Model	1550.448	0.0006	Model	419.72	0.0024
A-PLGA	6524.963	0.0002	A-PLGA	1248.2	0.0008
B-PVA	92.12033	0.0107	B-PVA	105.8	0.0093
C-Speed	53.92531	0.0180	C-Speed	561.8	0.0018
AC	1054.008	0.0009	AB	125	0.0079
BC	27.22407	0.0348	BC	57.8	0.0169

*Each value represents mean ± S.E. (n=3)

Table 3b: The ANOVA test to determine the significance of the variables on particle size and entrapment efficiency prepared PTX-PLGA nanoparticles.

on solubility of polymer. Comparatively more drug was entrapped in nanoparticles prepared by BSA than PLGA. The effect may be due to addition of surfactant as well as cross linking agent in the formulation. In BSA nanoparticles, BSA forms stable nanosuspension with the help of surfactant tween 80. The gluteraldehyde acts as cross linking agent, which closes up the apertures of the particles thereby increasing resistance for drug leakage. Hence, BSA serves to be good candidate for formulation of PTX nanoparticles.

Name	Goal	Lower Limit	Upper Limit	Importance
A:BSA	Is in Traget	100 mg	300 mg	3
B:Tween 80	Is in range	0.5 %	3%	3
C:Gluteraldehyde	Is in range	10 %	25 %	3
Particle Size	In range	104 nm	1150 nm	3
Entrapment Efficiency	Maximise	33 %	78 %	3

Solution No.	BSA	Tween 80	Gluteraldehyde	Particle Size	Entrapment Efficiency	Desirability
1	100.00	3.00	25.00	660.12	79.12	1
2	102.01	2.99	24.64	664.22	78.19	1
3	103.34	2.81	24.95	616.43	78.15	1
4	120.69	2.56	19.69	540.96	74.69	1
5	115.14	2.98	24.98	614.79	78.01	1
6	104.38	3.00	24.74	656.05	78.25	1
7	157.69	2.76	21.39	430.69	71.69	1
8	103.99	2.98	24.65	657.11	78.05	1
9	100.50	2.82	24.93	624.74	78.31	1
10	112.32	2.99	24.89	628.29	78.05	1

Table 4a: Set parameters and some of the suggested solutions of PTX-BSA nanoparticles.

Name	Goal	Lower Limit	Upper Limit	Importance
A:PLGA	Is in Traget	100 mg	300 mg	3
B:PVA	Is in range	0.5 %	3%	3
C:Speed	Is in range	5000 rpm	20000 rpm	3
Particle Size	In range	110 nm	750 nm	3
Entrapment Efficiency	Maximise	31 %	70 %	3

Solution No.	PLGA	PVA	Speed	Particle Size	Entrapment Efficiency	Desirability
1	299.99	2.95	19999.98	750	69.33	0.98
2	260	2.75	18000	640	71.69	0.98
3	296.18	3.00	19729.60	750	68.84	0.97
4	300.00	2.90	19999.95	747.74	69.17	0.97
5	297.30	3.00	19999.98	747.43	69.14	0.97
6	300.00	2.84	19999.92	744.93	68.99	0.97
7	300.00	2.86	19782.98	749.99	68.92	0.97
8	295.21	3.00	19994.14	743.62	68.87	0.97
9	300.00	2.84	19738.96	749.99	68.84	0.97
10	245	1.26	14999.69	426	75.69	0.98

Table 4b: Set parameters and some of the suggested solutions of PTX-PLGA nanoparticles.

Solution No./ Run	BSA	Tween 80	Gluteraldehyde	Particle Size		Entrapment Efficiency	
				Predicted response (%)	Obtained response (%)	Predicted response (%)	Obtained response (%)
1/B9	100.00	3.00	25.00	660.12	657.69	79.12	78.69
4/B10	120.69	2.56	19.69	540.96	549.69	74.69	75.65
7/B11	157.69	2.76	21.39	430.69	438.65	71.69	70.65

Table 5a: Predicted response and related obtained response of three of selected formulation of PTX-BSA nanoparticles.

Solution No./ Run	PLGA	PVA	Speed	Particle Size		Entrapment Efficiency	
				Predicted response (%)	Obtained response (%)	Predicted response (%)	Obtained response (%)
1/P9	299.99	2.95	19999.98	750	741	69.33	70.05
2/P10	260	2.75	18000	640	649	71.69	72.31
10/P11	245	1.26	14999.69	426	421	75.69	74.36

Table 5b: Predicted response and related obtained response of three of selected formulation of PTX-PLGA nanoparticles.

Solution/Run	Entrapment efficiency (%) (±S.D.)	Particle size (nm)
1/B9	78.69 ± 0.64	657.69 ± 0.76
11/P11	74.36 ± 0.86	421 ± 0.34

*Each value represents mean ± S.E. (n=3)

Table 6: Results of optimized batch formulated with PTX-BSA and PTX-PLGA.

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