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Original research paper

Design and development of paclitaxel-loaded bovine serum albumin nanoparticles for brain targeting

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Bovine serum albumin (BSA) nanoparticles loaded with paclitaxel (PTX) were prepared using a desolvation technique. A 3^2 full factorial design (FFD) was employed to formulate nanoparticles. Nanoparticles were characterized for particle size by photon correlation spectroscopy and surface morphology by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Encapsulation efficiency, zeta potential and particle yield were also determined. Response surface linear modelling (RSLM) was used to predict the optimal formulation. Various models were applied to determine the release mechanism from PTX nanoparticles. The effect of drug-polymer ratio on the release profile of formulations was observed and was applied to determine the suitability of the predicted optimal formulation. A preliminary study to determine the feasibility of targeting the prepared nanoparticles to brain was also carried out using mice as *in vivo* models.

Keywords: paclitaxel, bovine serum albumin, nanoparticles, desolvation, brain targeting, factorial design

Paclitaxel (PTX) is an important anticancer drug, isolated from the bark of *Taxus brevifolia (Taxaceae)*, with effective chemotherapeutic and cytotoxic activity against ovarian, breast, lung and brain cancers (1). It acts by binding to the micro tubules in the cells and preventing formation of normal mitotic apparatus in the G_2/M phase of cell cycle (2). However, like all other chemotherapeutic agents, PTX has various disadvantages in terms of normal cell toxicity, severe adverse reactions, drug interactions and unfavourable physicochemical properties for the development of a suitable delivery system (3). PTX belongs to the biopharmaceutical class IV and has poor aqueous solubility and permeability. Poor permeability across the blood brain barrier (BBB) limits its application in the treatment of brain tumours. BBB is characterized by relatively impermeable endothelial cells with tight junctions, enzymatic activity and active efflux transport systems through which only selective and essential molecules can pass (4, 5). However, several novel

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drug delivery systems, such as nanoparticles and liposomes, have been developed and modified to target impermeable drugs across BBB. Nanoparticles coated with polysorbate 80/Tween 80 (P80) can cross BBB and have been used to target many poorly permeable drugs (6). A commonly observed problem with controlled release particulate drug carriers is the »burst effect«, *i.e.*, excessive drug release from the polymer matrix of particles into the dissolution media over a comparatively short period of time. This could produce adverse and toxic effects *in vivo* especially in case of potent and low therapeutic index drugs. Various mechanisms and strategies have been reported by researchers to explain and prevent burst release (7). The presence of a portion of the drug on particle surface and addition of a post production washing step could ideally explain the problem and its solution. Moreover, the biphasic pattern of such a formulation may also lead to false interpretation of the release mechanism; hence, it may not fit any single model expression and requires application of more than one release model. However, suitability of the selected fitting method should be established by comparison of their R^2 or R values. Thus, the present study includes quantification and interpretation of the drug release profile involving burst effect, both by application of one uniform model and two separate models (as in biphasic release) for curve fitting. The objective of the present study was to formulate PTX nanoparticles with different drug-polymer (macromolecule) ratios and to evaluate the effect of formulation variables on the particle size, zeta potential, drug entrapment and drug release. The specific goal was to predict and practically determine the *in vitro* behaviour of the formulations and to identify the one with the optimal and desired controlled release profile. These objectives were achieved by experimental design using a simple 3^2 randomized full factorial design (FFD), response surface methodology (RSM) and optimization techniques. The selected formulation was also subjected to preliminary targeting studies in order to determine the effect of P80 on the brain delivery of prepared nanoparticles.

EXPERIMENTAL

Materials

PTX was obtained as a gift sample from Venus Remedies Limited, India, bovine serum albumin fraction V (BSA) was purchased from the Central Drug House, India. Acetonitrile, methanol and ethanol were of HPLC grade and were purchased from SD Fine Chem Ltd., India. AR grade reagents and chemicals were used. Membrane filters of 0.45 and 0.2 μm pore size were obtained from Advanced Microdevices Pvt. Ltd., India. The buffers used during the entire study were 0.15 mol L^{-1} pH 7.4 phosphate buffer saline (PBS) and pH 7.0 phosphate buffer.

HPLC analysis

The samples were analyzed for PTX concentration by the HPLC method (8). The samples were injected manually using a Rheodyne injector with a 20-μL loop. The separation was achieved using a Waters 10 μm Bondapak C_{18} (3.9 × 300 mm) column (Waters, USA). A Shimadzu SCL10A system (Japan), controller equipped with solvent delivery

pumps and an SPD 10MA UV detector set at 227 nm, was used. All the samples were prepared in triplicate. All the solvents and triple distilled water used for all aqueous solutions and buffers were filtered through 0.45-μm membrane filters. The mobile phase was acetonitrile/water (50:50), the flow rate 1 mL min^{-1} and the run time for each sample was 10 min.

Compatibility

Compatibility of the drug (PTX) with BSA used to produce nanoparticles was established by FTIR (Fourier transform infrared) spectroscopy. FTIR spectral analysis of PTX, unloaded BSA nanoparticles, PTX loaded BSA nanoparticles and a physical mixture of PTX and unloaded BSA nanoparticles (1:1) was carried out by the potassium bromide disc method using finely triturated 1:100, sample KBr physical mixture, on a RX1Perkin Elmer FTIR spectrometer (USA).

Preparation of BSA nanoparticles of PTX by the desolvation method

Design of experiment

Different formulation combinations were designed by applying a randomized 3^2 full factorial interaction design (2FI), which included 2 factors, drug quantity (X_1) and polymer quantity (X_2) in percent (%) as independent variables, evaluated over 3 levels

Table I. Design of the experiment, formulation combinations and response data

Factor	Name	Levels		
		–1	0	+1
X_1	A: PTX (%)	0.10	0.33	0.50
X_2	B: BSA (%)	8.00	10.00	12.00
Batch No.	(X_1, X_2)	Response		
		Y_1 (%) (n = 3)	Y_2 (%) (n = 3)	Y_3 (%) (n = 3)
PLB1	–1, –1	36.7 ± 0.7	60.7 ± 1.4	90.0 ± 2.3
PLB2	0, –1	40.2 ± 1.1	64.8 ± 2.2	91.1 ± 1.6
PLB3	+1, –1	42.7 ± 1.7	69.4 ± 2.3	92.6 ± 2.3
PLB4	–1, 0	35.9 ± 2.4	58.9 ± 1.8	88.7 ± 1.5
PLB5	0, 0	37.5 ± 1.2	62.0 ± 1.4	90.4 ± 3.0
PLB6	+1, 0	39.4 ± 1.4	68.6 ± 0.8	92.6 ± 1.8
PLB7	–1, +1	32.9 ± 2.4	56.6 ± 1.6	84.1 ± 1.1
PLB8	0, +1	35.6 ± 0.9	60.9 ± 1.3	88.0 ± 2.7
PLB9	+1, +1	37.1 ± 1.6	65.2 ± 2.1	92.4 ± 0.9

Y_1 – release after 2 h
Y_2 – release after 12 h
Y_3 – release after 24 h

as shown in Table I. All the evaluations and analyses of response variables (details given under the subheading »*Effect of formulation variables on PTX release*«) were carried out by using the Design Expert® version 7.1 (trial) software of Stat Ease® Inc., Minneapolis, USA.

Nanoparticles were prepared by the desolvation technique, as previously reported by Marty *et al*. (9) and Lin *et al*. (10). Nine batches of nanoparticles were prepared with varying concentrations of BSA and PTX as shown in Table I. Here the masses of drug and polymer changed but the volume of the final solution remained constant for all the formulations. For the preparation of nanoparticles, 5 mL of BSA solution was first filtered through a Millipore membrane filter with pore size of 0.45 μm. The filtrate was collected and its pH was adjusted to 9 with 0.5 mol L^{-1} NaOH solution to get nanoparticles with a reduced particle size. PTX was mixed with BSA solution using a magnetic stirrer, followed by constant, dropwise addition of 20 mL ethanol at the rate of 1 mL min^{-1} from a syringe until turbidity appeared in the solution. Ethanol acts as antisolvent for BSA to reduce its solubility in water and facilitate nanoparticle formation by precipitation. The nanoparticles formed were cross-linked by addition of 587 μL of 8 % (*V*/*V*) glutaraldehyde, followed by continuous stirring at 500 rpm at room temperature for 8 h. After the cross-linking stage, the drug-loaded nanoparticles were filtered though a Millipore filter of 1-μm size. The filtrate was centrifuged using a high speed centrifuge for 20 min at 20,000 *g*. The supernatant was decanted and the suspension was washed three times with distilled water/ethanol (1:1) to remove the adsorbed glutaraldehyde and PTX from the nanoparticles surface. Each redispersion step was performed in an ultrasonication bath. The washed samples were added to glass vials and freeze-dried with sucrose 2 % (*m*/*m*) as cryoprotectant in a lyophilizer. Lyophilized powder was stored at 4 °C and was easily redispersed in aqueous buffer solution by shaking before analysis. P80 1 % (*m*/*V*) was added to a known quantity of PLB7 nanoparticle dispersion and stirred for 30 min to form P80-coated nanoparticles (11). These nanoparticles were found to trick BBB and got the ticket to enter BBB; however, the exact mechanism is not known yet.

Nanoparticle recovery and entrapment efficiency

Nanoparticle recovery was calculated as the mass of dried nanoparticles recovered from each batch compared to the sum of the starting material (12). PTX content of all the batches was determined by adding 5 mg of sample from each batch into 10 mL of pH 7.0 phosphate buffer/ethanol (1:1) solution containing 1 mL of 50 % (*m*/*V*) trichloroacetic acid and incubating it at 4 °C. After 24 h, the samples were centrifuged and the PTX content in supernatant was estimated by HPLC. Drug entrapment efficiency was calculated as the ratio of actual drug content to theoretical drug content (13).

Particle size and morphology

The size of nanoparticles was determined using photon correlation spectroscopy, which is based on the principle of Brownian motion (14). The analysis was performed at a scattering angle of 90° at 25 °C using appropriately diluted samples. Exactly 3 mg of nanoparticles were dispersed in 10 mL of deionized water, followed by sonication for 7 min and the resulting suspension was introduced into the measurement chamber (12).

Scanning electron microscopy (SEM) (JEOL JSM6100, Japan) and transmission electron microscopy (TEM) (Hitachi H7600, Japan) of prepared nanoparticles were also carried out to determine their morphological characteristics as well as particle size. For scanning electron microscopy, dried nanoparticles were adhered on the stubs covered with silver tape, which were then gold coated in an ion sputter. These stubs were placed in an electron microscope for observation of nanoparticles. Photomicrographs of nanoparticles were taken at different magnifications and randomly selected fields. For TEM, a drop of nanoparticle suspension was placed on copper electron microscopy grids. After 30 s, the excess of sample was removed with a piece of filter paper. The dried sample was then examined under TEM (14).

Zeta potential

Zeta potential *(ζ)* was measured to determine the charge on the surface of nanoparticles. About 3 mg of nanoparticles were dispersed in 10 mL of phosphate buffer (pH 7.0, ionic strength 0.05 mol L^{-1}) by sonication for 7 min. The resulting suspension was placed in the measurement cell of a Nano ZS3600 Zetasizer (Malvern, UK) for zeta potential determination (15).

In vitro *PTX release*

Drug release was determined by using nanoparticles from each formulation equivalent to 1 mg PTX taken in nine separate 15-mL glass culture tubes containing 10 mL of PBS with 0.25 % (*m*/*V*) P80. Drug release studies of all batches were carried out in triplicate to confirm the reproducibility of analysis. The tubes were placed in a shaker-incubator adjusted to 60 horizontal strokes per min at 37 °C. A five-hundred microliter sample was withdrawn from each tube at 0.5, 1, 2, 4, 8, 12, 16 and 24 h and the tubes were replenished with fresh buffer after each withdrawal. All the withdrawn samples were filtered through a 0.45-μm Millipore syringe filter. All the samples were diluted suitably and were analyzed for PTX content by HPLC.

Effect of formulation variables on PTX release. – The effect of independent variables on the dependent (response) variables was evaluated by RSM, linear model with backward elimination regression and ANOVA (analysis of variance) as analytical tools. Response variables Y_1, Y_2 and Y_3 were evaluated for PTX release at 2, 12 and 24 h. The resulting statistical data was diagnosed and interpreted in order to determine the correlation between the predicted and actual drug release data as well as to study the combined behavior of formulations at different, predetermined time points (2, 12, and 24 h). The results were also applied to predict an optimal formulation with maximum probability of offering the desired and controlled PTX release profile.

Mathematical modeling. – The data obtained from *in vitro* PTX release studies was treated by various conventional mathematical models (zero-order, first-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell) to determine the release mechanism from the designed nanoparticle formulations (16–18). Selection of a suitable release model was based on the values of R (correlation coefficient), k (release constant) and n (diffusion exponent) obtained from the curve fitting of release data. Model fitting and statistical

analysis of *in vitro* release studies were carried out using the PCP Disso version 3 software (Poona College of Pharmacy, Bharati Vidyapeeth University Pune, Mahanashtra, India).

In vivo *drug targeting studies*

Male Lacca mice weighing 20 to 25 g were used for the study. The animals were procured from the Central Animal House of the Panjab University. Ethical clearance for the conduct of *in vivo* experiments was obtained from the Institutional Animal Ethics Review Board.

This study was carried out to compare the targeting efficiency of P80-coated PTX-loaded BSA nanoparticles with that of uncoated PTX-loaded nanoparticles and free drug (19, 20). Selected mice were kept on a constant day and night cycle and fasted for 12 h before the study. The animals were divided into 4 groups of 6 mice each and were administered appropriate solution into the tail vein. One batch with the optimal drug release profile (PLB7) was selected for the study. Nanoparticles equivalent to 5 mg PTX were treated with P80. Group I mice were treated as a control and received sterile PBS. Group II mice received 0.1 mL of 20 % of PTX dissolved in PBS containing 0.25 % (*m*/*V*) P80. Group III received nanoparticles equivalent to 20 μg of PTX after redispersing them in sterile PBS. Group IV received P80-coated nanoparticles equivalent to 20 μg of PTX in sterile PBS solution.

After 3 h, the mice were sacrificed and their brains were isolated. The brain of each mouse was homogenized separately with 3 mL of dichloromethane and digested with 1 mL of 50 % (*m*/*V*) tricholoroacetic acid for 12 h to precipitate the proteins. The precipitated homogenate was then cold centrifuged; the supernatant was withdrawn, evaporated to dryness and redissolved in 3 mL of ethanol. The drug content was estimated using HPLC at 227 nm.

The data from *in vivo* study was subjected to statistical analysis using the GraphPad® Prism v5 software of GraphPad® Software Inc., USA. The results of 4 groups were compared on the basis of one way ANOVA.

RESULTS AND DISCUSSION

Preformulation studies

FTIR. – IR spectral analyses of pure PTX, unloaded BSA nanoparticles, PTX-loaded BSA nanoparticles and a physical mixture of PTX and BSA nanoparticles were obtained and interpreted. The results are shown in Fig. 1. The spectrum of PTX showed strong absorption bands in the range of 1750–1600, 1300–1180, and 770–630 cm^{-1}. Characteristic peaks of PTX were observed at 1654, 1243 and 710.6 cm^{-1}. The spectra of PTX-loaded BSA nanoparticles showed all the characteristic peaks of PTX with negligible shifts at 1656, 1253 and 708.9 cm^{-1}, which indicated no change in the chemical structure of PTX in the formulation. The physical mixture of PTX and BSA showed peaks at 1658, 1242, 706 cm^{-1}, thus not showing a significant difference from the peaks of the formulation and suggesting the absence of incompatibility between PTX and BSA.

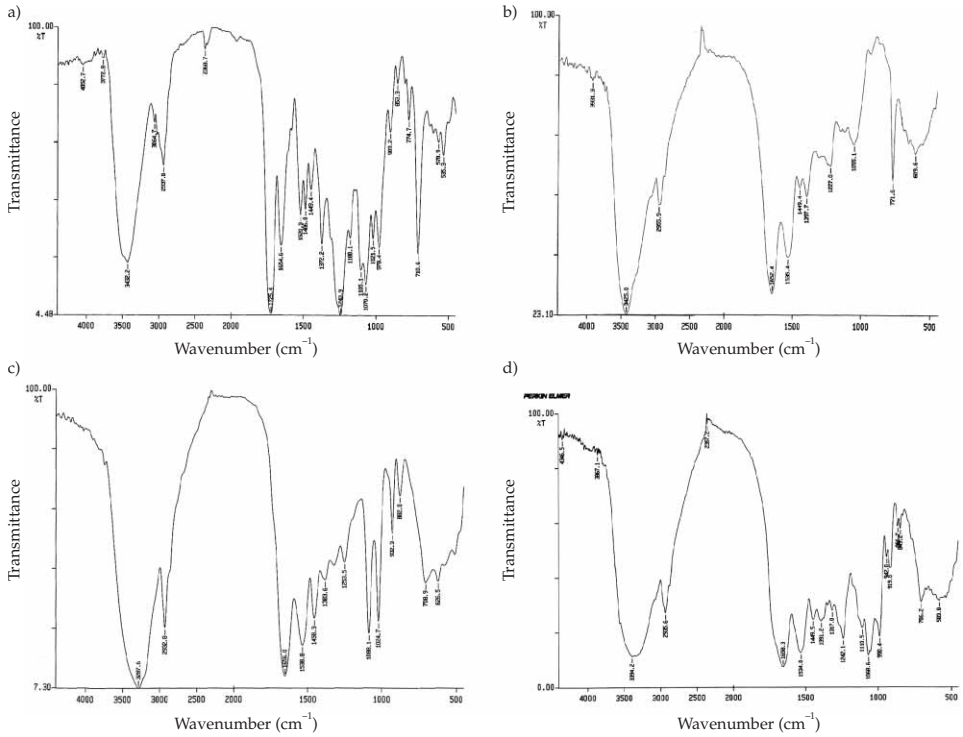

Fig. 1. FTIR spectra of: a) PTX, b) unloaded BSA nanoparticles, c) PTX loaded BSA nanoparticles, d) physical mixture of PTX and unloaded BSA nanoparticles.

Nanoparticulate yield and entrapment efficiency. – Nanoparticulate yield and entrapment efficiency are shown in Table II. Maximum particle yield was found in PLB9 (62.5 %) where the concentration of albumin is highest (12 % BSA + 0.5 % PTX) while the nanoparticle yield is lowest in PLB1 (57.2 %) where the concentration are 8 % BSA + 0,1 % PTX. This reveals entrapment of the poorly soluble drug in the macromolecule matrix instead of its dissolution in aqueous medium. However, encapsulation efficiency decreased with an increase in drug loading with respect to drug-polymer mass ratio. The maximum entrapment efficiency was observed in batch PLB7 (73.4 %) and the lowest in batch PLB3 (61.5 %). This may be due to limited affinity of the drug molecule to the macromolecular material, where further increase in drug concentration would not improve its entrapment in already saturated matrix of the nanoparticles.

Nanoparticle size, morphology and zeta potential

The results of nanoparticle size and zeta potential are shown in Table II. PTX nanoparticle size distribution is shown in Fig. 2. Particle size analysis carried out by photon correlation spectroscopy revealed that as the amount of BSA increased, the particle size decreased and as the drug loading increased, the size of nanoparticles increased. The

Table II. Yield, entrapment efficiency, particle size and zeta potential

Batch No.	Yield (%)	Entrapment (%)[a]	Particle size (nm)[a]	Zeta potential (mV)
PLB-1	57.2	70.2 ± 1.2	174 ± 2	–25.0
PLB-2	58.4	65.6 ± 0.9	179 ± 2	–24.6
PLB-3	57.9	61.5 ± 2.1	188 ± 3	–24.2
PLB-4	59.7	71.5 ± 0.9	141 ± 3	–23.8
PLB-5	60.1	66.7 ± 1.3	152 ± 2	–22.4
PLB-6	59.3	66.5 ± 1.0	157 ± 3	–20.0
PLB-7	59.9	73.4 ± 1.1	98 ± 2	–21.3
PLB-8	61.1	69.5 ± 1.1	119 ± 2	–21.5
PLB-9	62.5	68.6 ± 1.2	138 ± 1	–19.6

[a] Mean ± SD, $n = 3$.

particle size of all batches was found to be in the size range of 100–200 nm, which is ideal for brain targeting as the extent of opsonization by organs with reticuloendothelial system (RES) is reported to be low in the size range of 100–200 nm (21). The pH value of the BSA solution prior to addition of ethanol was adjusted to pH 9 in order to get the particle size in the range of 100–200 nm. This observation is in agreement with the work of Lin *et al.* (22). The morphology of PTX nanoparticles was examined with SEM and TEM. Representative SEM and TEM photomicrographs of PTX nanoparticles are shown in Figs. 3a and 3b. It was observed from these photomicrographs that all samples of freeze dried particles were smooth, almost spherical in shape and aggregated to form small clusters.

The values of zeta potential are shown in Table II, indicating that all particles were negatively charged and thus far apart from each other.

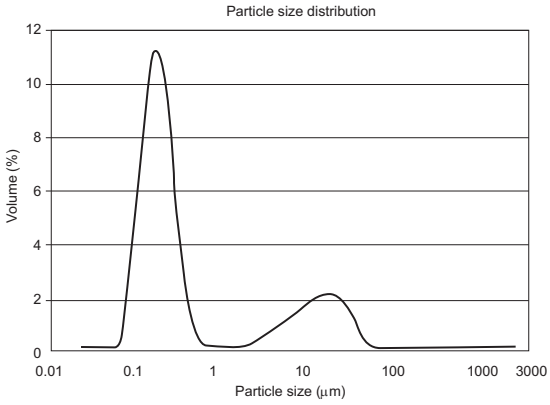

Fig. 2. Particle size distribution of PTX nanoparticles.

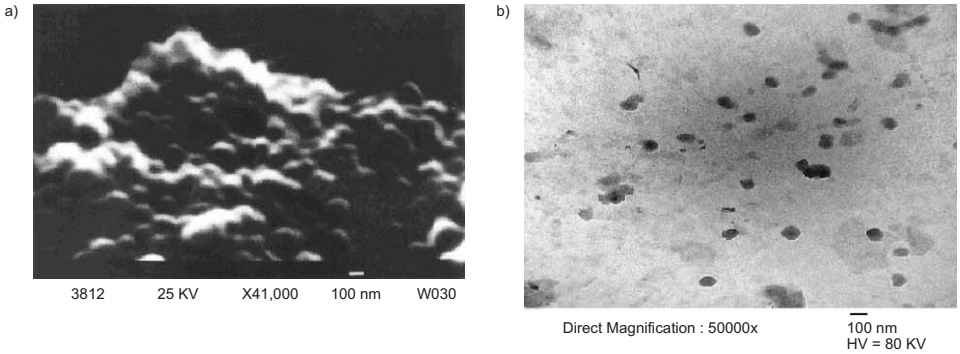

Fig. 3. a) Representative scanning electron micrograph of PTX nanoparticles at magnification of 41000X, b) Representative transmission electron micrograph of PTX nanoparticles at magnification of 50000X.

In vitro *release profile*

The comparative plot of the percent release profile of PTX-loaded BSA nanoparticles is shown in Fig. 4. Effect of formulation variables on PTX release shown in Table I depicts the comparative changes in PTX release at different time points (Y_1, Y_2 and Y_3), which were observed due to quantitative variations in the drug-polymer ratio, represented as 9, different combinations (PLB1 – PLB9) in the 2FI design (Table I). The key statistical results obtained by evaluation of the percent release values are summarized in Table III. As observed in Table I and Fig 4, the overall highest PTX release was observed in the formulation PLB3, which contained 0.5 % PTX and 6 % BSA (92.6 ± 2.28 % after 24 h). This batch also showed maximum burst effect after 2 h (42.7 ± 1.72 %). It can be interpreted from this result that the formulation with the highest drug loading and lowest

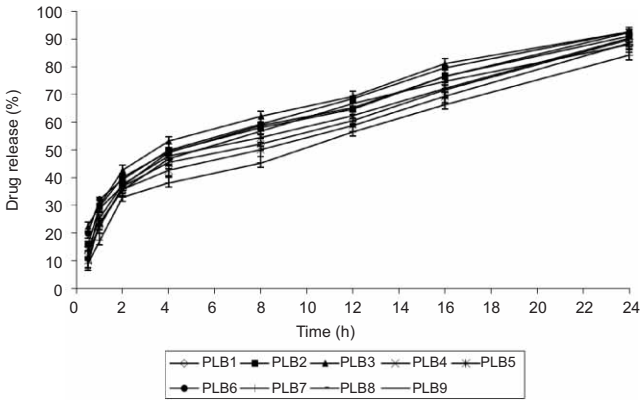

Fig. 4. Cumulative release *vs.* time for various batches of PTX-loaded BSA nanoparticles (mean ± SD, n = 3).

Table III. Statistical summary of response analysis

Summary	Y_1	Y_2	Y_3
Model	RSLM	RSLM	RSLM
SD	0.6	0.75	1.23
Mean	37.57	63.49	89.47
RSD (%)	1.59	1.18	1.38
PRESS	5.78	7.73	24.91
R-squared	0.9678	0.9768	0.9052
Adjusted *R*-squared	0.9571	0.9691	0.8736
Predicted *R*-squared	0.9126	0.9466	0.7407
Adequate precision	26.858	29.171	12.203
F-value	90.18	126.33	28.65
p-value (< 0.05)	< 0.0001	< 0.0001	0.0009

RSLM – Response surface linear model
PRESS – Predicted residual error sum of squares

polymer content showed the fastest release. In contrast, PLB7, which contained 12 % BSA and 0.1 % PTX, showed minimum release (84.1 ± 1.1 % after 24 h) and also minimum burst effect (32.9 ± 2.4 %). Thus, it can be interpreted that the formulation with low drug loading and high polymer content showed the slowest release and minimum burst effect. The statistical model including the highest order polynomial terms was selected for response analysis, as shown in equation 1, which was determined by ANOVA (23):

$$Y = b_0 + b_1X_1 + b_2X_2 \quad (1)$$

where, Y is the response variable, b_0 is the intercept/constant or arithmetic mean response of 9 runs and b_1, b_2 are significant regression coefficients calculated from the response of formulations in the design at a 95 % confidence interval.

The RSLM (response surface linear model) was found to be significant with respect to responses Y_1, Y_2, Y_3. The p-values < 0.05 in all three responses (Y_1, Y_2 and Y_3) for model terms X_1 and X_2, indicate their significance (as shown in Table III). Similarly, high R^2 values, proportionate adjusted and predicted R^2 values, low PRESS values and adequate precision values greater than 4 suggest the suitability of RSLM for Y_1, Y_2 and Y_3. Thus, the final equations 2, 3 and 4 obtained for Y_1, Y_2 and Y_3 in terms of coded factors were:

$$\text{PTX 2 h} = 37.57 + 2.28X_1 - 2.34X_2 \quad (2)$$

$$\text{PTX 12 h} = 63.49 + 4.51X_1 - 1.79X_2 \quad (3)$$

$$\text{PTX 24 h} = 89.47 + 3.76X_1 - 0.58X_2 \quad (4)$$

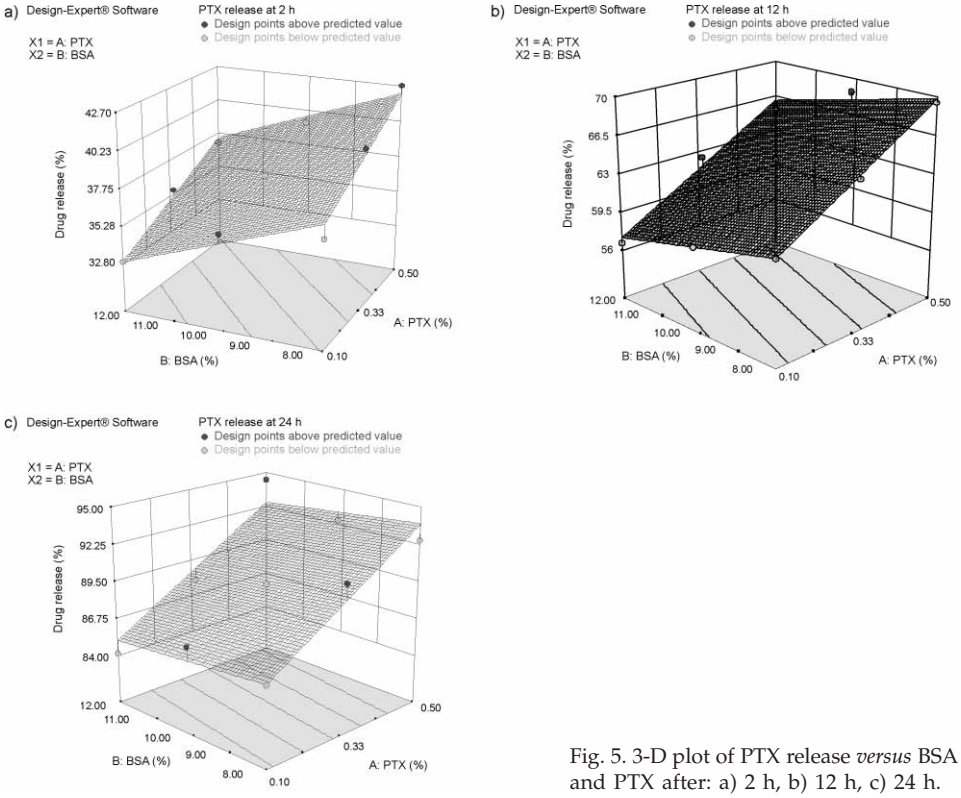

Fig. 5. 3-D plot of PTX release *versus* BSA and PTX after: a) 2 h, b) 12 h, c) 24 h.

Figs. 5a, 5b and 5c show the 3-D plots for PTX release after 2, 12 and 24 h with respect to the variation in the amount of PTX and BSA used to formulate different batches of nanoparticles. The wire mesh shows the predicted values and the upper and lower dots represent the design points above and below the predicted values. Table IV shows the predicted points after treating the predicted and actual release data for optimization analysis. The PTX release after 2 h was constrained to the values between 42.7 and 32.9 % with the goal of point prediction set to be minimized whereas the release at 12 and 24 h was not constrained to any specific point. An optimal point of prediction expected to offer the best controlled release profile while maintaining the burst effect to minimum was found to be PLB7 with 12 % BSA + 0.1 % in each formulation.

Mathematical modeling

Correct determination of the release mechanism depends greatly on the selection and application of a suitable model to the release data. Different researchers treat the data differently by either applying one model at a time or considering separate models for the initial burst release and subsequent prolonged release. Both methods lead to different interpretation of results. The present study compares both types of model fitting,

Table IV. Predicted optimal formulation with response statistics

Predicted optimal point: (X_1, X_2) = (–1,+1)					
Response	Prediction	SE mean	95 % CI (low)	95 % CI (high)	SE (pred)
Y_1	32.95	0.40	31.98	33.92	0.72
Y_2	57.19	0.61	50.50	65.98	0.90
Y_3	85.13	0.82	83.12	87.14	1.48

SE – Standard error
CI – Confidence interval

Table V. Comparative model fitting of release

Batch No.	Model fitting PTX release (0–24 h)				Model fitting PTX release, constrained (0–2 h)				Model fitting PTX release, constrained (2–24 h)			
	Model	*R*	*k*	*n*	Model	*R*	*k*	*n*	Model	*R*	*k*	*n*
PLB1	Matrix	0.9792	18.206	0.44	Peppas	0.9999	22.340	0.79	Zero-order	0.9966	2.153	0.32
PLB2	Matrix	0.9800	19.094	0.42	Peppas	0.9999	23.760	1.36	Zero-order	0.9952	2.309	0.33
PLB3	Peppas	0.9736	26.682	0.41	Peppas	0.9998	29.540	0.90	Zero-order	0.9976	14.900	0.30
PLB4	Matrix	0.9813	17.881	0.45	Peppas	0.9999	23.720	1.23	Zero-order	0.9985	2.249	0.34
PLB5	Matrix	0.9785	19.235	0.43	Peppas	0.9997	25.430	0.85	Zero-order	0.9890	2.296	0.33
PLB6	Matrix	0.9778	20.170	0.45	Peppas	0.9999	28.910	1.38	Matrix	0.9983	15.087	0.34
PLB7	Matrix	0.9890	16.896	0.42	Peppas	0.9999	17.234	0.94	Zero-order	0.9990	2.319	0.44
PLB8	Peppas	0.9872	28.094	0.34	Peppas	0.9998	32.180	0.70	Matrix	0.9916	14.255	0.34
PLB9	Peppas	0.9934	29.479	0.35	Peppas	0.9999	30.240	0.44	Matrix	0.9837	15.164	0.34

R – correlation coefficient
k – rate constant: matrix ($h^{½}$), zer-order (% h^{-1}), first-order (h^{-1}), Peppas (h^{-n})
n – diffusion exponent

as shown in Table V. Model fitting from 0–24 h reveals that all the batches follow the matrix or Higuchi and Korsmeyer-Peppas model. The *R* values in the case of batches PLB3, PLB8 and PLB9 were higher for the Korsmeyer-Peppas model. The values of *n* less than 0.45 suggest that all formulations followed the Fickian diffusion controlled PTX release from the nanoparticles. The model fitting data for 0–2 h shows that this release followed the Korsmeyer-Peppas model as well as the value of *R* was found to be 0.9999, indicating goodness of fit for this model. Further, based on the value of *n*, batch PLB9 followed Fickian diffusion, PLB1 and PLB8 anomalous transport, PLB5 case II transport and PLB2, PLB3, PLB4, PLB6 and PLB7 followed super-case II transport. The prepared nanoparticles were spherical in shape and spherical polymeric particles which follow case II transport depend upon zero-order release kinetics, specifically where the relaxation process of the macromolecular chains occurring upon water uptake is the rate controlling step (16, 17). The *R* values of model fitting data for 2–24 h show that PTX release during this

phase followed either the zero-order or matrix/Higuchi model. All *n* values were below 0.45, thus indicating Fickian diffusion controlled release. Overall combined release for 0–2 h and 2–24 h reveals that except PLB1, PLB8 and PLB9, all other formulations displayed no burst effect. Also, details obtained by using separate release models instead of a single uniform model are more precise, informative and offer better correlation with the prediction and analysis of the experimental design responses. However, this may be applicable only in the present case and does not, in any way, lead to any form of generalization.

In vivo *drug targeting*

To determine the extraction factor, PTX was dissolved in methylene chloride to obtain solutions of five different drug concentration, *viz.*, 66.67, 133.33, 200.00, 266.67 and 333.33 μg mL^{-1}. The brains from 5 different untreated mice were removed and homogenized with 3 mL each of five PTX solutions containing known concentrations of the drug. The homogenate was digested with 1 mL of 50 % (*m*/*V*) tricholoroacetic acid for 12 h to precipitate the proteins. The precipitated homogenate was then cold centrifuged; the supernatant was withdrawn, evaporated to dryness and redissolved in 3 mL of ethanol. The drug content was estimated using HPLC at 227 nm. The mean extraction factor of 0.96 (rang 0.93–0.99) was used to determine the actual amount of the drug targeted to the brain after intravenous administration of PTX nanoparticles. It was found that group II mice received 8.2 % of drug after 20 μg of pure PTX was intravenously administered while groups III and IV received 19.4 and 25.2 % of the drug, respectively Fig. 6. The percentage of drug that reached mouse brain revealed that drug targeting to the brain was greater in uncoated and coated nanoparticles compared to the pure drug. It was also observed that the brain targeting of coated nanoparticles was greater than that of uncoated nanoparticles. By applying one-way ANOVA, the amount of PTX present in the group IV mouse brain was found to be significantly higher in comparison with both pure drug and uncoated nanoparticles ($p < 0.01$).

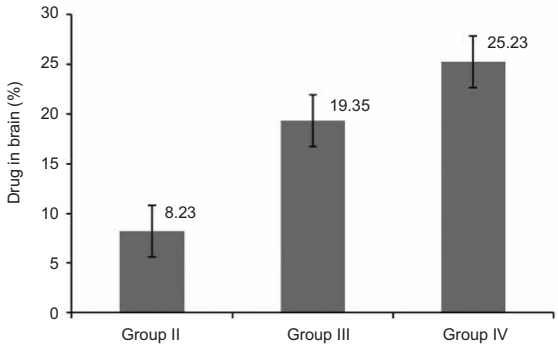

Fig. 6. Drug target to brain (mean ± SD, *n* = 6.)

CONCLUSIONS

It can be concluded from the above studies that it was possible to prepare PTX nanoparticles using bovine serum albumin as a macromolecular material with controlled release up to 24 h. The experiment was designed using 3^2 full factorial design and the effects of variations in the drug-polymer ratio were evaluated through changes in the drug release behaviour of respective formulations. RSLM was selected for the analysis of release profiles. PLB7 was predicted as the optimal formulation with minimum burst effect. The mathematical model fitting of the release data showed that the formulations followed either Fickian diffusion, anomalous or case II transport mechanisms. The *in vivo* studies concluded that PTX nanoparticles coated with P80 were able to reach the mouse brain in an amount higher than that of uncoated PTX nanoparticles or pure drug itself, suggesting the nanoparticle potential to cross BBB. These studies were only preliminary and were carried out to support the need to further elaborate *in vivo* targeting studies in animal models which would also include pharmacokinetic analysis. Detailed *in vivo* studies including exploration of other analytical methods to determine targeting efficiency of the formulations, validation of selected methods and pharmacokinetic studies will be taken up in due course of time.

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S A Ž E T A K

Dizajniranje i razvoj albuminskih nanočestica s paklitakselom za ciljanu isporuku u mozgu

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Opisana je priprava albuminskih nanočestica s paklitakselom (PTX) metodom desolvatacije. Za pripravu je korišten goveđi serumski albumin (BSA) i 3^2 potpuni faktorijalni dizajn (FFD). Pomoću fotonske korelacijske spektroskopije određena je veličina čestica, dok je površina čestica proučavana pretražnom elektronskom mikroskopijom (SEM) i transmisijskom elektronskom mikroskopijom (TEM). Nadalje, određena je učinkovitost kapsuliranja, zeta potencijal i iskorištenje. Linearno modeliranje odzivnih površina (RSLM)

upotrebljeno je za predviđanje optimalne formulacije. Različiti modeli primijenjeni su za određivanje mehanizma oslobađanja iz PTX nanočestica. Proučavan je utjecaj omjera lijeka i polimera na profil oslobađanja i njegova upotrebljivost za predviđanje optimalne formulacije. Mogućnost ciljane isporuke u mozak preliminarno je ispitana *in vivo* na miševima.

Ključne riječi: paklitaksel, goveđi serumski albumin, nanočestice, desolvatacija, ciljano djelovanje na mozak, faktorijalno dizajniranje

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