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Original Article

Polymeric Nanocarrier system Bearing Anticancer Agent for the Treatment of Prostate Cancer: Systematic Development and *in vitro* Characterization

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ABSTRACT

Objectives: Doxorubicin-bearing polymeric (PLGA) nanoparticles (PNPs) were prepared for the treatment of prostate cancer. **Materials and Methods:** These PNPs were prepared using the solvent evaporation method and characterized using UV, NMR, Particle size Analyser, SEM, and TEM for the determination of shape, size, zeta potential, and polydispersity index. Moreover, *in vitro* drug release, SRB assay, apoptosis, and haemolytic study were also performed to prove its potentiality for prostate cancer. **Results:** The mean particle size (MPS), polydispersity index (PDI), and zeta potential (ZP) of PNPs were found to be 101.3 ± 1.23 nm, 0.240 ± 0.28 and -3.11 ± 1.96 mV, respectively. SEM and TEM revealed that the PNPs are spherical in shape and the sizes are approximately 100 nm. The entrapment efficiency, loading efficiency, and percentage drug release of doxorubicin from PNPs were found to be 69.38 ± 1.76%, 4.2 ± 0.64% and 77.56 ± 4.24%, respectively. In addition, cellular apoptosis against PC-3 cell lines was found to be ≥ 5.15 fold and haemolysis toxicity was reduced to ≤ 3.5 fold with PNPs as compared to free drug. **Conclusion:** These findings demonstrated that PNPs have the potential to deliver the anticancer agent to tumor sites and could be an emerging strategy for the treatment of prostate cancer.

Keywords: Polymeric nanoparticles, Targeting, Doxorubicin, PLGA, Prostate cancer.

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INTRODUCTION

Cancer is a fatal and leading source of death all around the world. It has been revealed that current cancer therapy has to be improved because it includes serious side effects such as myelosuppression, nephrotoxicity, and cardiotoxicity as well as the heavy chance to develop drug resistance.[1-3] Prostate cancer is one of the complicated and severe diseases that is spreading rapidly worldwide. The demand for developing new, efficient, and risk-free anti-cancer therapies is heightened by its widespread incidence as well as lethal consequences across the world.[4,5] Hence, the concept of target therapy with the help of various nanocarriers can be envisaged in this context. It only affects the cancer cells and not the healthy cells, and it appears to be a challenging objective to achieve.[6-8]

Doxorubicin (DOX), an anticancer agent that belongs to the anthracycline family, has been used to treat cancer for a very long time. It interferes with macromolecular synthesis by intercalating into the DNA as a method of action. The disadvantages of DOX-based chemotherapy include the fact that it damages healthy cells in addition to cancer cells. Besides, the cancer cells can become resistant to DOX, and that can occasionally result in cell death. Hence, it restricts its clinical use and necessitates the development of new therapeutic formulations such as nanoparticles and other nanocarrier systems.[9,10]

An encouraging tool to realize this goal is nanoparticle. Numerous research teams are concentrating on various nanocarrier systems employing a wide range of materials. A biodegradable, biocompatible, and non-toxic polymeric material like PLGA, or other lactic or glycolic polymers, should be utilized to prepare nanoparticles that have no impact on the healthy cells and tissues.[11,12] It has been investigated that they are in versatile use in drug delivery systems due to their inherent controlled and sustained release properties. Nanoparticles can passively accumulate in tumor tissues based on the EPR effect, which states that angiogenesis is the primary cause of leaky and faulty blood vessels. These include a decreased incidence of adverse effects as well as promising futures for overcoming cancer cell drug resistance, which is a major obstacle in current tumor therapy.[13,14]

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In this work, PLGA was employed as the starting material for the preparation of PNPs and Doxorubicin was taken as the anticancer agent. The PNPs were prepared using solvent evaporation method and systematically optimized using DoE software. They were characterized for various parameters such as particle diameter, polydispersity index, zeta potential, and drug load. Additionally, particular focus was placed on potential variations in drug release behavior, cytotoxicity, and haemolytic toxicity. They were further assessed for cellular apoptosis also.

MATERIALS AND METHODS

Materials

The drug (Doxorubicin) was obtained as a gift sample from Khandelwal Laboratory Pvt. Ltd. Mumbai. PLGA, i.e. poly (D, L-lactide-co-glycolide), 50:50 (mol. Wt. 30,000-60000), poloxamer-407 (PF-127), acetone, methanol, poly (vinyl) alcohol (PVA) were purchased from Sigma Aldrich, India. N, N′-Dicyclohexylcarbodiimide (DCC), and N-Hydroxysuccinimide (NHS) were obtained from Himedia (Mumbai, India). Analytical grade double distilled water, solvents, and chemicals were used in the study.

Preparation of polymeric nanoparticles (PNPs)

PNPs were prepared using the solvent evaporation method (Figure 1) as reported.[15] In brief, 60 mg of PLGA was taken and dissolved in 3.0 ml of acetone and 3.0 mg of DOX was dissolved in 1 ml of methanol. Then, both solutions were mixed properly to form an organic phase. This organic phase solution was poured into PVA solution (5 ml, 2.5% m/V) with continuous stirring and simultaneously, the addition of a non-ionic copolymer surfactant solution (slowly) i.e. poloxamer-407 (F-127) solution (2%, m/V in distilled water). After stirring overnight, the resultant PNPs were separated by centrifugation at 25,000 rpm for 20 min and redispersed in purified water and this washing process was repeated three times. After the final washing, the nanoparticles were centrifuged, sedimented, and collected. Then, PNPs were lyophilized and stored at 0-4°C.[15,16]

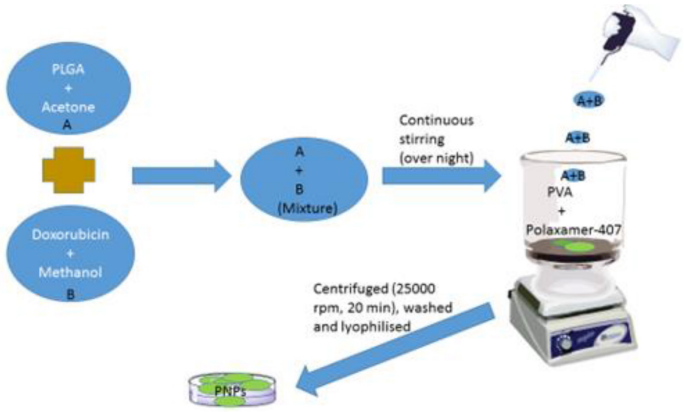

Figure 1: Preparation of PNPs by a solvent evaporation method.

Optimization of nanoparticles

In the current experimental study, optimization was comprised of multiple center points with three independent factors, three dependent factors, and three levels. Further, it included 17 experimental runswere applied to obtain second-order polynomial equations to optimize batches with desirable characteristics (Table 2). The Box Behnken Design (BBD) applied to achieve the quality target product profile (QTPP) in a minimum number of experimental runs. This design was employed to assess the coinciding effect of critical process parameters (CPP) on critical quality attributes (CQA) or responses using Design Expert Software (Version 7.16, Stat-Ease, Minneapolis, MN, USA). The important CPP, which were used in this experiment were molar concentration of PLGA (X1), molar concentration of PVA (X2), and molar concentration of Poloxamer-407 (X3). Three respective levels were selected i.e. −1 (low), 0 (medium) and +1 (high). Similarly, three CQA were also selected i.e. mean particle size (MPS) (nm), Polydispersity index (PDI) and percentage entrapment efficiency (% EE) as shown in Table 1. Analysis of variance (ANOVA) and a p-value < 0.05 were used to forecast that each independent variable would have a sizable impact on the dependent variable given its relative levels. A number of model characteristics, including the multiple correlation coefficient (R^2), adjusted multiple correlation coefficients (adjusted R^2), coefficient of variation (CV), and anticipated residual sum of the squares, were researched and compared in order to evaluate the best model that was generated. Response surface analysis (RSA) wasperformed using the Design-Expert® application by amending 2D contour plots and 3D response surfaces.[17,18] as illustrated in Figure 2. (a), (b), and (c).

CHARACTERIZATION OF PNPs

PNPs were examined for many characteristics, including mean particle size, zeta potential, polydispersity index (PDI), TEM, SEM, NMR, percentage entrapment efficiency (% EE), loading

Table 1: Box-Behnken design (BBD) factors and their levels for PNPs optimization.

Factors (Independent variables)	Levels		
	Low (-1)	Medium (0)	High (+1)
Concentration of PLGA: X1 (mg/ml)	40	50	60
Concentration of PVA: X2 (%)	1.5	2.5	3.5
Concentration of Polaxamer-407 : X3 (%)	1.0	2.0	3.0
Responses (Dependable variables)	Constraints		
Mean Particle Size: Y1 (nm)	Minimum		
Polydispersity Index: Y2	Minimum		
Percentage Entrapment Efficiency (% EE): Y3	Maximum		

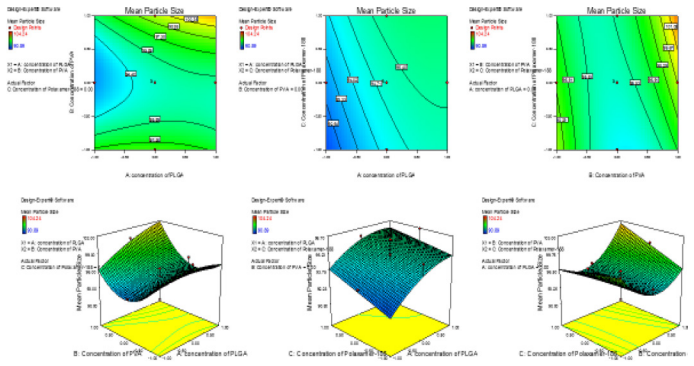

Figure 2 (a): 2D contour plots and 3D response surfaces for MPS.

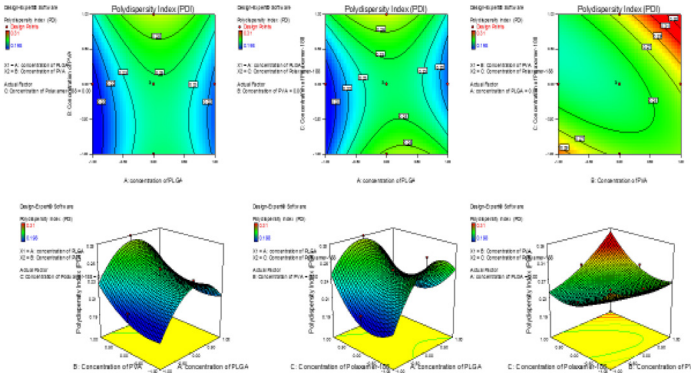

Figure 2 (b): 2D contour plots and 3D response surfaces for PDI.

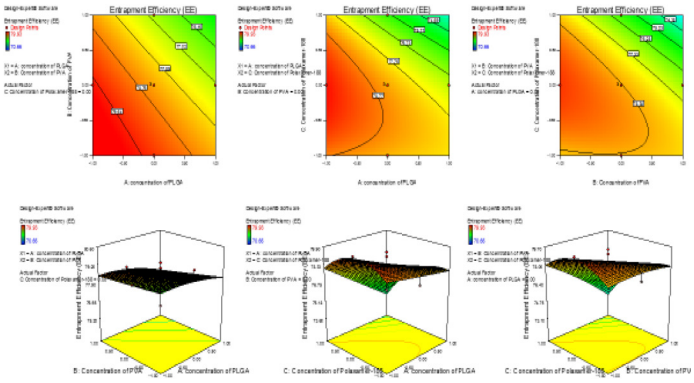

Figure 2 (c): 2D contour plots and 3D response surfaces for % EE.

efficiency, *in vitro* percentage drug release, SRB assay, apoptosis, and haemolytic investigations.

NMR spectrophotometry

Using DMSO as the dissolving solvent and TMS as the internal standard, the optimized DOX carrying PNPs were scanned on a ^{1}H NMR spectrophotometer (JEOL 500 MHz, NMR Spectrophotometer, JAPAN) and the scan is reported in Figure 3 (c).

Table 2: Design matrix for the formulation of PNPs.

Runs	Factors			Responses		
	Concentration of PLGA (mg/ml)	Concentration Of PVA (%)	Concentration Of Polaxamer-407 (PF-127) (%)	MPS (nm)	PDI	%EE (%)
1	1.00	1.00	1.00	104.24	0.292	70.66
2	0.00	1.00	0.00	100.13	0.282	76.90
3	0.00	0.00	0.00	90.89	0.249	76.19
4	1.00	-1.00	1.00	95.34	0.201	75.64
5	1.00	-1.00	-1.00	98.32	0.281	78.34
6	-1.00	1.00	-1.00	93.12	0.201	79.14
7	0.00	0.00	0.00	95.12	0.249	79.43
8	-1.00	0.00	0.00	93.14	0.206	79.89
9	0.00	0.00	1.00	95.98	0.282	76.89
10	1.00	0.00	0.00	96.65	0.215	77.24
11	0.00	0.00	0.00	96.43	0.198	79.04
12	1.00	1.00	-1.00	98.99	0.246	77.23
13	0.00	-1.00	0.00	98.96	0.249	79.67
14	-1.00	-1.00	1.00	96.59	0.203	79.93
15	0.00	0.00	-1.00	95.23	0.278	77.58
16	-1.00	-1.00	-1.00	98.34	0.226	78.34
17	-1.00	1.00	1.00	99.13	0.310	75.10

Table 3: Statistics of all responses measured as per Box-Behnken design (BBD).

Coefficient Code	Polynomial coefficients for response variable		
	MPS	PDI	%EE
β1	+94.99014	+0.24259	+78.40873
β2	+1.32200	+8.90000E-003	-1.33700
β3	+0.80600	+0.017100	-1.28100
β4	+0.72800	+5.60000E-003	-1.23300
β5	+1.53125	-3.25000E-003	-0.26750
β6	-0.24875	-0.015000	-0.86250
β7	+1.99875	+0.032250	-1.17750
β8	-0.72775	-0.040035	+0.014718
β9	+3.92225	+0.014965	-0.26528
β10	-0.017746	+0.029465	-1.31528

Particle Size, zeta potential, and size distribution

After dilution of the samples of nanoparticles to 1:9 (v/v) with deionized water, the mean particle size and size distribution of the produced PNPs were determined by photon correlation

Table 4: Constraint for numeric optimization and predicted solution.

Factors (Independent variables)	Goal	Levels		Importance
		Low (-1)	High (+1)	
X1: Concentration of PLGA (mg/ml)	In range	40	60	***
X2: Concentration of PVA (%)	In range	2	4	***
X3: Concentration of Polaxamer-407 (PF-127) (%)	In range	1	3	***
Response (Dependent variables)				
R1: Mean Particle Size (MPS) (nm)	Minimum	90.98(nm)	104.24(nm)	
R2: Polydispersity Index (PDI)	Minimum	0.20	0.31	
R3: % EE	Maximum	70.66 (%)	79.93 (%)	

***Most significant variables.

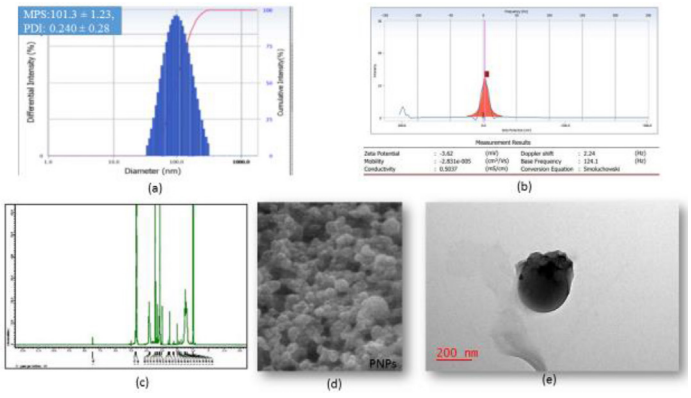

Figure 3: (a) and (b) Mean Particle Size (MPS), PDI and ZP images (c), (d), and (e) NMR Spectroscopy, Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) images of PNPs.

spectroscopy using a particle size analyzer (Nanoplus, Particulate Systems, Norcross, GA, U.K.). After diluting the PNPs with double-distilled water and setting the conductivity to 50 S/cm with a solution of 0.9% NaCl, the zeta potential of the PNPs were measured using the same equipment.[17]

Scanning Electron Microscopy (SEM)

The particle morphology of the PNPs was examined using a scanning electron microscope (NOVA NANOSEM). The PNPs were lightly sprayed onto an adhesive carbon tape, which was positioned on an aluminium stub, to prepare the samples for SEM. The necessary thickness of gold was then applied to the stubs. Additionally, photomicrographs of the samples were collected at various magnifications and evaluated under a SEM at a voltage of 20 kV are shown in Figure 3 (d).

Transmission Electron Microscopy (TEM)

The particle size of the PNPs was studied using TEM (Czech Republic instrument, FEI TACNAI). On a copper grid with a carbon coating, a drop of sample dispersion was applied and the sample was examined at a low vacuum with the copper grid secured into the sample holder, and a photomicrograph of the PNPs was taken at an appropriate magnification, as shown in Figure 3 (e).

Percentage entrapment efficiency (% EE)

The HPLC method was used to estimate the untapped drug that was present in the supernatant, and the entrapment efficiency of the drug (DOX) was calculated. After centrifuging the PNPs suspension at 18,000 rpm for 20 min at 4°C, the supernatant was recovered. Using acetonitrile-water (70:30, 0.1 percent TFA) as the mobile phase on a Phenomenex Luna C-18 (2) column in isocratic mode (flow rate of 1.2 ml/min) with UV detection at 480 nm, the amount of DOX in the supernatant liquid was determined. The percentage of PNPs with drug entrapment and loading was calculated using the algorithm given below:

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Weight of initial drug (DOX)} - \text{Weight of free drug (DOX)}}{\text{Weight of total drug (DOX)}} \times 100$$

$$\% \text{ Loading efficiency} = \frac{\text{Amount of entrapped (DOX) in th PNPs}}{\text{Total weight of PNPs}} \times 100$$

In vitro drug release

To extract the unentrapped drug from PNPs, the prepared PNPs were first centrifuged (25,000 rpm, 20 min) and rinsed with double distilled water. The washed PNPs were then mixed with distilled water, added to the dialysis tube (5 ml), which had a molecular weight of 12 kDa, and submerged in a beaker of PBS (100 mL, at pH 7.4). The beaker's contents were agitated on a magnetic stirrer (REMI, Mumbai, India) at 500 revolutions per minute while the temperature was held at 37 to 100°C. At predefined intervals, the sample (1 ml) was removed and replaced with an equal volume of fresh phosphate buffer solution (pH 7.4). The removed samples were examined for drug content (DOX) using the previously mentioned HPLC technique. The same procedure was performed in PBS (pH 6.5).[19,20]

Cytotoxicity study

The viability of cancer cell lines was measured using sulforhodamine B (SRB) assay technique as reported.[21] Briefly, the human prostate cancer cell lines (PC-3) were seeded (1.0×10^6/well in 96-well plates) and after 24 hr, cells were exposed to different concentrations of samples for 48 hr. The percentages of viable cells were plotted against each sample concentration based on absorbance measured at 540 nm. Inhibition of percent growth was calculated using the following formula:

$$\text{Inhibition of percent growth} = [Zi/X] \times 100\ \%$$

Where (Zi) is the test growth of DOX at different concentrations and (X) is control growth.

Apoptosis Study

PC-3 cells were used in the apoptosis study, which was carried out using flow cytometry, Annexin V-FITC, and Propidium iodide (PI). In six-well plates, cells were plated at a density of 2×10^5 and incubated for the whole night. The free drug (DOX) and PNPs were then added, and they were incubated for a specified time. Trypsinization, pre-chilled PBS washing, and resuspension of the trypsinized cells in 100 µl of 1 × binding buffer containing 5 µl of Annexin V-FITC and 5 µl of PI for 15 min at room temperature in dark. Following the addition of 400 µl of binding buffer, the cells were filtered through a cell strainer and analyzed using an American BD FACS Aria scanner.[22]

Haemolytic toxicity study

To determine the toxicity of the PNPs induced by the different components of blood cells, a haemolytic toxicity study of the prepared formulations was conducted. The human blood was collected into HiAnticlot collection vials. Centrifugation was used to separate the red blood cells (RBCs) and then resuspended them in a sterile saline solution (10 % haematocrit). Separately, 1 ml of RBC suspension was incubated with distilled water (used as a 100% haemolytic standard) and normal saline (taken as blank for spectrophotometric estimation). Free drug (DOX) and PNPs were dissolved in normal saline to produce a dispersion with a 100 µg/ml drug concentration. With gentle shaking, the sample vials were kept at 37°C for an hour. After centrifuging the tubes for 10 min at 5000 rpm, the supernatant's absorbance was measured at 540 nm, and the percentage of haemolysis was calculated by comparing it to the absorbance of distilled water, a 100% haemolytic standard that was diluted identically.[23]

Statistical Analysis

The experimental design and statistical calculations were done in design expert software. In BBD, 17 runs with 3 center points were implemented to validate the polynomial equation by ANOVA (Analysis of variance). The statistical analysis was completed using the data obtained from studies that were performed in triplicate. The ANOVA test was used to determine whether any results were statistically significant and all results were presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Preparation and optimization of PNPs using DoE

Using the dependent variables i.e. mean particle size (MPS), polydispersity index (PDI), and percentage drug entrapment efficiency (% EE), the Box-Behnken design (BBD) was used to optimize the independent variables such as molar concentration of PLGA (X1), molar concentration of PVA (X2), and poloxamer concentration (X3). Ten polynomial coefficients (β_1- β_{10}) were presumed during specific mathematical modeling with β_0 to β_1 acting as the intercept (Table 3). The polynomial equations were obtained from ANOVA analysis and two-dimensional and three-dimensional response surface plots were generated by the Design Expert that were used to determine the influence of independent factors on responses (Figure 2. a, b and c). The lists of restrictions for numerical optimization and the expected solutions were given in Table 4. The coefficient of regression with a $P \leq 0.005$ indicates the model terms are significant and a value ≥ 0.1 indicates it as insignificant. The quadratic model for the mean particle size was observed to be significant (F value 4.64 and P value 0.027). The predicted R^2 value (0.6713) and adjusted R^2 value (0.6716) which is very close (≤ 0.2) and the model is significant. The quadratic models for PDI were obtained with F value (5.96) and P value (0.0140) and predicted R^2 value (0.7195) and adjusted R^2 value (0.7361) that represents the model to be significant. Similarly, the F value and P value were found to be (6.67 and 0.0102) for quadratic models for % EE. Moreover, it was also noticed that the difference between the predicted R^2 value (0.5875) and adjusted R^2 value (0.7614) was not more than 0.2. All these findings ascribed the selections of the respective models for MPS, PDI, and % EE.[18-24] Additionally, the polynomial equations for MPS (YI), PDI (Y2), and % EE (Y3) were obtained and reported as follows:

Mean Particle Size (MPS) (Figure 2. a, b and c).

$$= +94.99 + 1.32X_1 + 0.81X_2 + 0.73X_3 + 1.53X_1X_2 - \\ -0.25X_1X_2 + 2.00X_2X_3 - 0.73X_1^2 + 3.92X_2^2 - 0.018X_3^2$$

Poly Dispersity Index (PDI)

$$= +0.24 + 8.900E-003X_1 + 0.017X_2 + 5.600E-3.250E-003X_3 - 0.015X_1X_2 \\ +0.032X_1X_2 - 0.040X_2X_3 + 0.039X_1^2 - 0.015X_2^2 + 0.029X_3^2$$

Percentage Entrapment Efficiency (% EE)

$$= +78.41 - 1.34X_1 - 1.28X_2 - 1.23X_3 - 0.27X_1X_2 - 0.86X_1X_3 \\ -1.18X_2X_3 + 0.015X_1^2 - 0.27X_2^2 - 1.32x_3^2$$

NMR of DOX bearing PNPs

The ^{1}H NMR was used to characterize the drug-loaded PNPs. At (δ 7.486 ppm), the distinctive resonances of DOX (CO-NH) protons were seen, indicating the formation of an amide bond between DOX and PLGA. The recorded H^1 NMR spectra are shown in Figure 3 (b).

Mean particle size and surface charge determination

The mean particle size (MPS) of prepared PNPs was found to be 101.3 ± 1.23 nm. The current data revealed that particle diameter and PDI showed good results due to a monodisperse size distribution due to the solvent displacement method. The prepared PNPs exhibited with a broader size distribution with

PDI (PDI > 0.1) i.e. 0.240 ± 0.28. In addition, the zeta potential value was obtained as -3.11 ± 1.96 Mv. The ZP of the PNPs was negative due to the presence of terminal carboxylic groups of the PLGA polymer and all the PNPs were considered stable due to electrostatic repulsion. Furthermore, PVA and Poloxamer-407 were used during the preparations to sterically stabilize the systems, and hence, unstable and self-aggregating NPs can be excluded.

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

The SEM photomicrographs of PNPs revealed well-formed, well-structured particles with smooth surfaces and no aggregate is depicted in Figure 3 (d). The prepared PNPs were detected using TEM and the images drawn were spherical in shape, shown in Figure 3 (e). No fusion or rupture could be seen in the image of the PNPs, and the shape was preserved. It was anticipated that the DLS mean and median values would be marginally higher than TEM due to the interaction of the dispersant with the hydrodynamic diameter. According to TEM measurements, the size of PNPs was approximately 100 nm.

Entrapment efficiency and Loading efficiency

The drug entrapment efficiency and drug loading efficiency of the prepared PNPs were found to be 69.38 ± 1.76 % and 4.2 ± 0.64 %, respectively.

In vitro drug release

The *in vitro* percentage drug release of free drug (DOX) was 51.34 ± 2.54% at pH 7.4 and 64.56 ± 1.98 % at pH 6.5 after 72 hr using a dialysis tube (molecular cut-off point 3,500) method (at 37 ± 0.5 °C).[20] However, the percentage drug release from PNPs was found to be 65.76 ± 2.84% at pH 7.4, and at pH 6.5, it was 77.56 ± 3.24% after 72 hr. It was observed that the drug release was found to increase at pH 6.5 as compared to pH 7.4 due to the destabilization of PNPs at acidic pH (pH 6.5) which is the pH of the prostate cancer microenvironments. The percentage drug release vs time plotted graphs are shown in Figure 4 (a) and (b).

Cytotoxicity study

Using a SRB experiment on PC-3 cell lines, the *in vitro* cytotoxicity potential of the free drug (DOX) and PNPs were evaluated. It showed a dose-dependent reduction in cell viability. The DOX showed the lowest cytotoxicity at the drug equivalent concentration of 0.018 µMol . The viability of cells treated with PNPs (equivalent to 0.018 µMol drug concentration) was found to 13.6 ± 0.87% while free DOX was showed 58.0 ± 2.8 % at 48 hr. It showed an extensive upsurge ($p < 0.05$) in cytotoxicity as compared to DOX and displayed higher cell uptake by PC-3 cells. The result of the SRB cytotoxicity study of the free DOX and prepared PNPs are shown in Figure 4 (c).

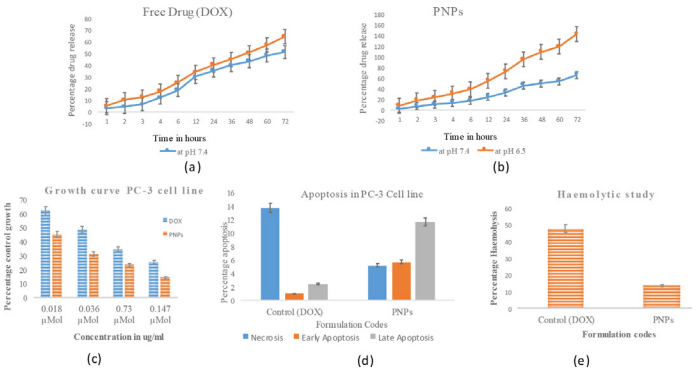

Figure 4: (a) and (b) percentage drug release of DOX and PNPs and (c), (d), and (e) cytotoxicity study, apoptosis, and haemolytic estimation of DOX and PNPs.

Apoptosis Study

The apoptosis studies were also conducted on PC-3 cells and it was observed that the PNPs had significantly higher apoptosis (≥ 5.15 fold) in terms of the apoptotic ratio as compared to the control group treated with a low concentration (2.5 µM) of free DOX. Simultaneously, higher apoptosis was found in PNPs treated groups: 17.38 % (the early apoptotic ratio was 5.71 % and the late apoptotic ratio was 11.67 %) whereas the free drug had 3.37% apoptosis i.e. early and late apoptosis ratio 0.94 % and 2.43 %, respectively which has been depicted in Figure 4 (d). Apart from this, the cellular necrosis of PNPs (5.22 %) was lesser as compared to DOX (13.75 %). It is known that PNPs prevent cells from producing topoisomerase. These findings imply that NPs alter the control of the cell cycle in the S phase, preventing cells from passing through the S-G_2 transition. The observation suggests that there may be some issues with the replication process in the S phase and that excessive DNA damage may possibly be a major factor in cell death. As a result of topoisomerase inhibition and DNA damage, which increases the uptake of DOX and causes apoptosis in PC-3 cells.[25,26]

Haemolytic study

Biocompatibility with blood and its various components is an early preclinical breakthrough for the development of intravenously delivered nanoparticles. The effects of the prepared NPs on the haemolytic profile of red blood cells were investigated. The free drug (200 µg/ml) was shown to cause 47.60 % of RBC hemolysis whereas PNPs showed hemolysis levels of 13.40 %. Drug integration into PLGA may have reduced the toxicity of the drug, resulting in less hemolysis. The outcomes were in favor of developing a formulation for safer nanocarriers. Overall, the findings of the current study support the formulation's safety as well as its suitability for intravenous delivery for prostate cancer.[27]

CONCLUSION

It is concluded from the studies that DOX is successfully loaded to PNPs for maximum delivery to prostate cancer cells. All the PNPs were systematically optimized, characterized and satisfied the

expected desired results for a promising nanoparticle system. The main advantages of the PNPs include high drug loading as well as good entrapment efficiency. Moreover, PNPs exhibited higher cytotoxicity on PC-3 cell lines, remarkable cellular apoptosis, and showed much less haemolysis on blood components than DOX. Therefore, these PNPs seem to be favorable to reduce the unwanted side effects of the free drug and could be employed as a potential nanocarrier system for the effective delivery of anticancer drug to prostate cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

PCa: Prostate cancer; **PLGA:** Poly (lactic-co-glycolic acid); **DOX:** Doxorubicin; **PNPs:** Polymeric nanoparticles; **BBD:** Box-Behnken design; **MPS:** Mean particle size; **PDI:** Polydispersity index; **ZP:** Zeta Potential; **DMSO:** Dimethyl sulfoxide; **PBS:** Phosphate buffered saline; **SEM:** Scanning Electron Microscope; **TEM:** Transmission Electron Microscope; **NMR:** Nuclear Magnetic Resonance; **SRB:** Sulforhodamine B assay.

REFERENCES

1. Saraf S, Tiwari A, Verma A, Panda PK, Raikwar S, Jain A, *et al*. Targeting approaches for the diagnosis and treatment of cancer. Front Anti Cancer Drug Discov. 2019;11:105-38. doi: 10.2174/9789811422133120110007.
2. Abadi AJ, Mirzaei S, Mahabady MK, Hashemi F, Zabolian A, Hashemi F, *et al*. Curcumin and its derivatives in cancer therapy: potentiating antitumor activity of cisplatin and reducing side effects. Phytother Res. 2022;36(1):189-213. doi: 10.1002/ptr.7305, PMID 34697839.
3. Singh R, Kumar S. Cancer targeting and diagnosis: recent trends with carbon nanotubes. Nanomaterials (Basel). 2022;12(13):2283. doi: 10.3390/nano12132283, PMID 35808119.
4. Panda PK, Saraf S, Tiwari A, Verma A, Raikwar S, Jain A, *et al*. Novel strategies for targeting prostate cancer. Curr Drug Deliv. 2019;16(8):712-27. doi: 10.2174/1567201816666190821143805, PMID 31433757.
5. Saraf S, Jain A, Tiwari A, Verma A, Panda PK, Jain SK. Advances in liposomal drug delivery to cancer: an overview. J Drug Deliv Sci Technol. 2020;56:101549. doi: 10.1016/j.jddst.2020.101549.
6. Chen Y, Xu Z, Lu T, Luo J, Xue H. Prostate-specific membrane antigen targeted, glutathione-sensitive nanoparticles loaded with docetaxel and enzalutamide for the delivery to prostate cancer. Drug Deliv. 2022;29(1):2705-12. doi: 10.1080/10717544.2022.2110998, PMID 35980107.
7. Panda PK, Verma A, Saraf S, Tiwari A, Jain SK. Ionically gelled gellan gum in drug delivery. Gels Horizons: from Science to Smart Materials. 2021:55-69. doi: 10.1007/978-981-16-2271-7_3.
8. He Y, Xu W, Xiao YT, Huang H, Gu D, Ren S. Targeting signaling pathways in prostate cancer: mechanisms and clinical trials. Signal Transduct Target Ther. 2022;7(1):198. doi: 10.1038/s41392-022-01042-7, PMID 35750683.
9. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol. 2013;65(2):157-70. doi: 10.1111/j.2042-7158.2012.01567.x, PMID 23278683.
10. Rawat PS, Jaiswal A, Khurana A, Bhatti JS, Navik U. Doxorubicin-induced cardiotoxicity: an update on the molecular mechanism and novel therapeutic strategies for effective management. Biomed Pharmacother. 2021;139:111708. doi: 10.1016/j.biopha.2021.111708, PMID 34243633.
11. Abrigo N, Ruzicka C, Faustino P, Stiber N, NguyenPho A, O'Connor T, *et al*. Development and validation of a headspace GC-MS method to evaluate the interconversion of impurities and the product quality of liquid hand sanitizers. AAPS Open. 2022;8(1):1. doi: 10.1186/s41120-021-00049-8, PMID 35071739.
12. Dhar S, Gu FX, Langer R, Farokhzad OC, Lippard SJ. Targeted delivery of cisplatin to prostate cancer cells by aptamer functionalized Pt(IV) prodrug-PLGA–PEG nanoparticles. Proc Natl Acad Sci U S A. 2008;105(45):17356-61. doi: 10.1073/pnas.0809154105, PMID 18978032.
13. Subhan MA, Yalamarty SSK, Filipczak N, Parveen F, Torchilin VP. Recent advances in tumor targeting via EPR effect for cancer treatment. J Pers Med. 2021;11(6):571. doi: 10.3390/jpm11060571, PMID 34207137.
14. Acharya S, Sahoo SK. PLGA nanoparticles containing various anticancer agents and tumour delivery by EPR effect. Adv Drug Deliv Rev. 2011;63(3):170-83. doi: 10.1016/j.addr.2010.10.008, PMID 20965219.
15. Betancourt T, Brown B, Brannon-Peppas L. Doxorubicin-loaded PLGA nanoparticles by nanoprecipitation: preparation, characterization and *in vitro* evaluation. Nanomedicine (Lond). 2007;2(2):219-32. doi: 10.2217/17435889.2.2.219, PMID 17716122.
16. Kızılbey K. Optimization of rutin-loaded PLGA nanoparticles synthesized by single-emulsion solvent evaporation method. ACS Omega. 2019;4(1):555-62. doi: 10.1021/acsomega.8b02767.
17. Verma A, Sharma G, Jain A, Tiwari A, Saraf S, Panda PK, *et al*. Systematic optimization of cationic surface engineered mucoadhesive vesicles employing Design of Experiment (DoE): a preclinical investigation. Int J Biol Macromol. 2019;133:1142-55. doi: 10.1016/j.ijbiomac.2019.04.118, PMID 31004631.
18. Saraf S, Jain A, Tiwari A, Verma A, Jain SK. Engineered liposomes bearing camptothecin analogue for tumour targeting: *in vitro* and *ex-vivo* studies. J Liposome Res. 2021;31(4):326-41. doi: 10.1080/08982104.2020.1801725, PMID 32718195.
19. Upadhyay KK, Bhatt AN, Castro E, Mishra AK, Chuttani K, Dwarakanath BS, *et al*. *In vitro* and *in vivo* evaluation of docetaxel loaded biodegradable polymersomes. Macromol Biosci. 2010;10(5):503-12. doi: 10.1002/mabi.200900415, PMID 20232310.
20. Park J, Fong PM, Lu J, Russell KS, Booth CJ, Saltzman WM, *et al*. Pegylated PLGA nanoparticles for the improved delivery of doxorubicin. Nanomedicine. 2009;5(4):410-8. doi: 10.1016/j.nano.2009.02.002, PMID 19341815.
21. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc. 2006;1(3):1112-6. doi: 10.1038/nprot.2006.179, PMID 17406391.
22. Ribble D, Goldstein NB, Norris DA, Shellman YG. A simple technique for quantifying apoptosis in 96-well plates. BMC Biotechnol. 2005;5(1):12. doi: 10.1186/1472-6750-5-12, PMID 15885144.
23. Kovshova T, Osipova N, Alekseeva A, Malinovskaya J, Belov A, Budko A, *et al*. Exploring the interplay between drug release and targeting of lipid-like polymer nanoparticles loaded with doxorubicin. Molecules. 2021;26(4):831. doi: 10.3390/molecules26040831, PMID 33562687.
24. Alkholief M. Optimization of lecithin-chitosan nanoparticles for simultaneous encapsulation of doxorubicin and piperine. J Drug Deliv Sci Technol. 2019;52:204-14. doi: 10.1016/j.jddst.2019.04.012.
25. Siddharth S, Nayak A, Nayak D, Bindhani BK, Kundu CN. Chitosan-dextran sulfate coated doxorubicin loaded PLGA-PVA-nanoparticles caused apoptosis in doxorubicin resistance breast cancer cells through induction of DNA damage. Sci Rep. 2017;7(1):2143. doi: 10.1038/s41598-017-02134-z, PMID 28526868.
26. Yallapu MM, Khan S, Maher DM, Ebeling MC, Sundram V, Chauhan N, *et al*. Anti-cancer activity of curcumin loaded nanoparticles in prostate cancer. Biomaterials. 2014;35(30):8635-48. doi: 10.1016/j.biomaterials.2014.06.040, PMID 25028336.
27. Bhadra D, Bhadra S, Jain S, Jain NK. A pegylated dendritic nanoparticulate carrier of fluorouracil. Int J Pharm. 2003;257(1-2):111-24. doi: 10.1016/s0378-5173(03)00132-7, PMID 12711167.

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