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Development and Characterization of Core Shell Nanoparticle for Enhanced Drug Delivery to Treat Solid Tumor: Preparation and *In-Vitro* Assessment

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ABSTRACT

Mortalities from cancer in the world are projected to continue rising, with an estimated 9 million and 11.4 million people dying from cancer in 2015 and 2030, respectively. Rates are rising as more people live to an old age and as mass lifestyle changes occur in the developing world. With present treating regimen for cancer, dose-limited toxicity is a big reason that reduces the efficacy of cancer treatments. In search for more effective cancer treatments, nanosized drug delivery systems, those are capable of delivering their drug payload selectively to cancer cells such as nanoparticles, solid lipid nanoparticles, liposomes are among the most promising approaches. Core shell nanoparticles are one of the investigated moieties in recent years that are seeking much attention nowadays for biomedical applications including the field of oncology.The present work aims at developing a core shell nanoparticle comprising Poly (D, L –lactide –co –glycolide) (PLGA) core and polyethyleneimine (PEI) shell loaded with anticancer bioactive docetaxel (DTX) for passive targeting of the tumor tissue. It is expected that incorporation of PEI will improve the uptake and subsequent release of the drug in the cytosol due to endosomal escape phenomenon.

Keywords: Solid tumor; nanotechnology; nanoparticle; PLGA

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1. INTRODUCTION

Cancer, a dreadful disease, is one of the chief killers today worldwide. Medical treatment of cancer still has many challenges yet to meet and the medicines used in the treatment of cancer have their own boundaries. The main remedial therapies for cancer, e.g. surgery, radiation and chemotherapy, are generally successful in the early stages of cancer. Once the tissue has progressed to higher stages, these therapies are less successful. Chemotherapy among all the stated therapies is most effective but only for defined period of time. The currently used chemotherapeutic agents are the drugs with the narrowest therapeutic indices in all medicines. Thus, the dose of anticancer agents is restricted by their non-selective toxic effects on healthy cells [1].

Selective and targeted delivery of cytotoxic drugs into malignant tumors may overcome these limitations. Even highly toxic agents could be rendered safer and more effective, if it were possible to direct them only into the tumor because high drug concentrations within the tumor could be obtained while sparing the normal tissues. These harmful side effects can be reduced by developing a drug delivery vehicle that is specific to tumor cells and this may be achieved by employing a strategy which makes advantage of abnormal tumor vasculature. Poorly formed leaky vasculature of tumors contributes for the well-known enhanced permeation and retention (EPR) effect that allows selective accumulation of polymer-drug conjugates/ complexes ranging in size between 10 to 500 nm within tumors when compared to that of free drugs [2, 3].

The polymeric molecules are retained following the accumulation due to their larger size and abnormal lymphatic supply to the vasculature whereas free drug molecules are easily eliminated from the cells [4]. Controlled drug delivery strategies have made a dramatic impact in medicine. Progress in novel targeting strategy is paving way for more safe and effective management of various diseases like cancer, tuberculosis, malaria, arthritis, cardiovascular diseases etc. The ability to transport a large quantity of drug molecules into cytosolic compartments of cancer cells has powerful implications in modern molecular therapeutics because the sites of action of the drugs are often cytosolic organelles. Furthermore, direct cytosolic delivery might offer

a means to evade efflux transporters, such as P glycoproteins. Fast cytoplasmic drug delivery can overcome cancer cells drug resistance and thus have an enhanced therapeutic efficacy. Core-shell architecture represents an effective way to attain multiple functionalities on a nanoscopic length scale. Indeed, a core (template) generally carrying a chemotherapeutic agent can be surroun ded by a shell with different composition and configuration that provides a functional and interacting interface with biological environment. They found application in Drug delivery, both in vitro and in vivo diagnostics, nutraceuticals and production of improved biocompatible materials. The present work aims at developing a core shell nanoparticle comprising Poly (D, L-lactide-co-glycolide) (PLGA) core and Polyethyleneimine (PEI) shell loaded with anticancer bioactive docetaxel (DTX) for passive targeting of the tumor tissue. It is expected that incorporation of PEI will improve the uptake and subsequent release of the drug in the cytosol due to endosomal escape phenomenon.

2. MATERIALS

Poly (D, L-lactide-co-glycolide) (PLGA) and DOCETAXEL was obtained as a gift sample from M/s. Sun Pharma Advanced Research Center (SPARC), Vadodra, Gujarat, India. Branched PEI (Polyethyleneimine) (MW: 25 kDa), Pluronic F-68, was purchased from Sigma Aldrich, India. All other reagents and solvents used were of analytical grade. Unless stated otherwise double deionized water was used throughout the study.

3. METHODS

3.1 Preparation of PLGA nanoparticles

PLGA nanoparticles were synthesized by emulsion solvent evaporation method with slight modifications [5]. Figure 1 represents the pictorial presentation of preparation of nanoparticles. In order to obtain stable formulation, different process and formulation parameters were optimized as follow.

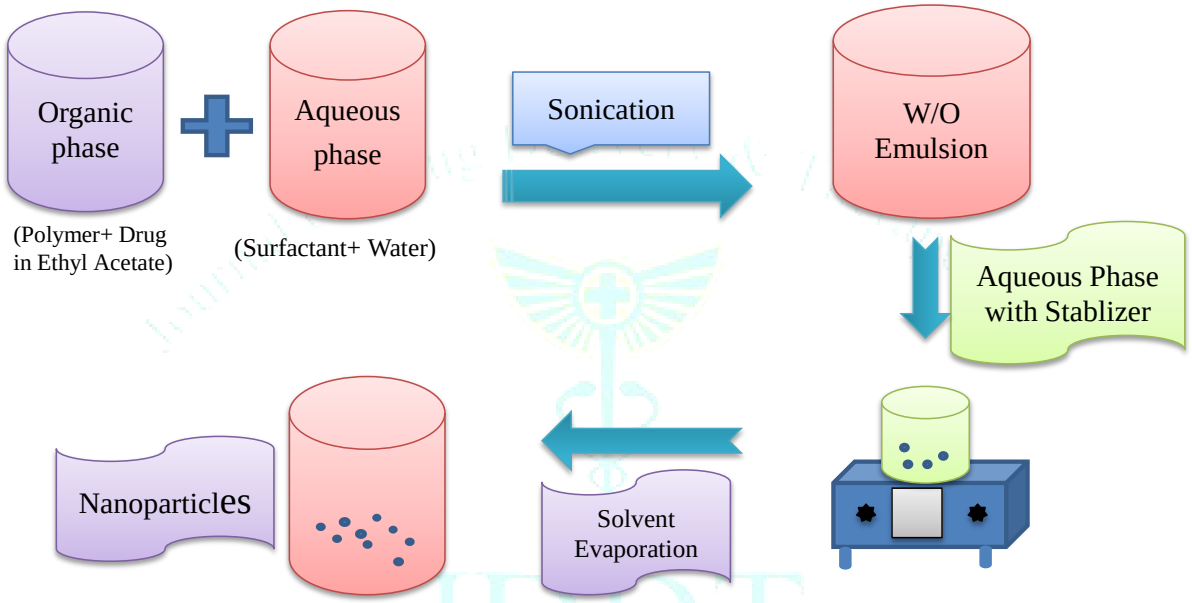

Figure 1 Pictorial presentation of preparation of nanoparticles

3.2 Optimization of Nanoparticle Formulation

It was done by taking into consideration of critical parameters which influence the property and stability of nanoparticles. One parameter was optimized at a time keeping all other parameters constant. These variables are shown below.

3.2.1 Optimization of Formulation Variables

3.2.1.1 Optimisation of polymer concentration

For optimization of Polymer concentration (PLGA), the nanoparticle formulations were prepared with varying concentrations of PLGA keeping other parameters constant. Optimization was done on the basis of average particle size and drug entrapment efficiency of nanoparticle, which were determined using Nanosereis ZS90 zetasizer (Malvern, UK) (Figure 2).

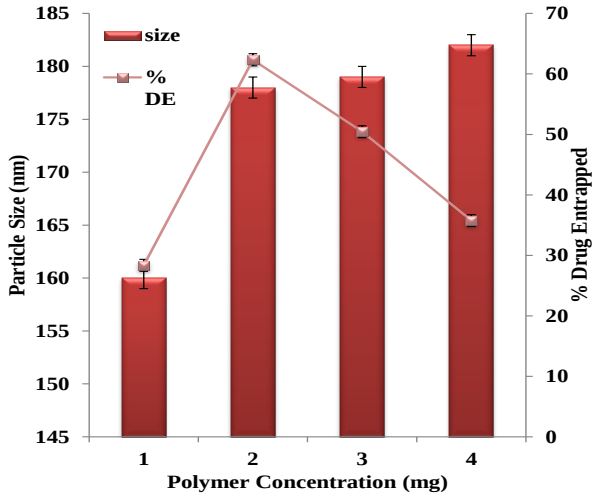

Figure 2 Optimisation of polymer concentration

3.2.1.2 Optimisation of Surfactant Concentration

For optimization of surfactant concentration, different nanoparticles formulations were prepared with different concentrations (0.5%, 1%, 2%, 3%) of surfactant (Pluronic F-68) keeping the other parameters constant. Optimization was done on the basis of average particle size of nanoparticle and % Drug Entrapment (Figure 3).

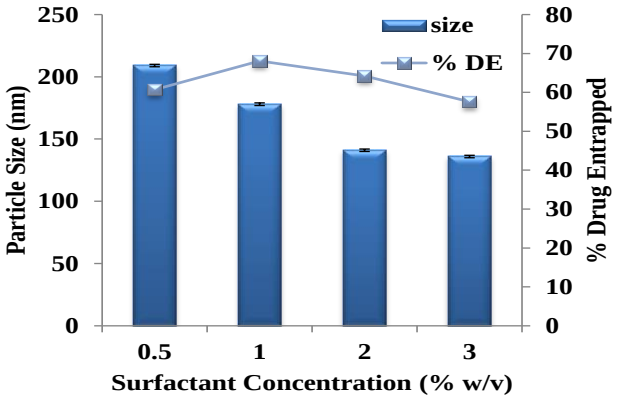

Figure 3 Optimisation of Surfactant Concentration

3.2.1.3 Optimization of Sonication Time

For the optimization of sonication time, the formulation was sonicated (Probe Sonicator) for 60, 120,180, 240 sec using probe sonicator and average particle size and % drug entrapment efficiency were recorded (Figure 4).

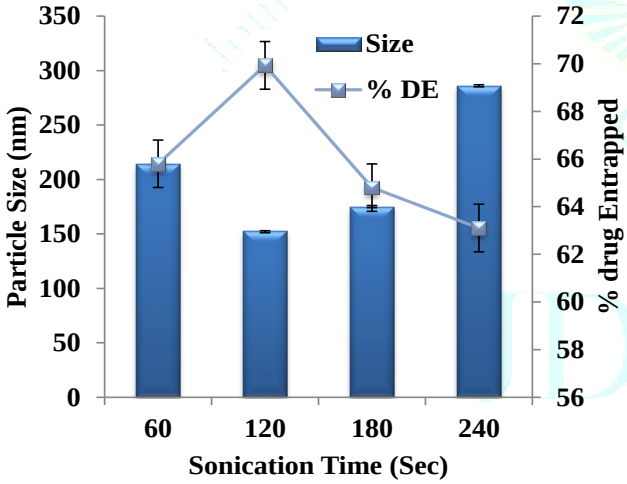

Figure 4 Optimization of Sonication Time

3.2.1.4 Optimization of Aqueous Phase Volume:

Aqueous phase volume also affects the particle size. The volume was varied from 10 to 30 mL and average particle size, % drug entrapment efficiency and PDI were recorded (Figure 5).

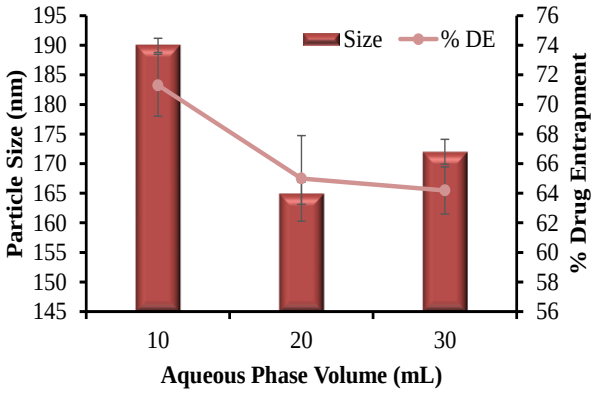

Figure 5: Optimization of Aqueous Phase Volume

3.3 Preparation of PLGA-PEI Nanoparticles

To fabricate PEI-coated PLGA NPs, 5 mg of PEI that was disso lved in 5 mL distilled water (adjusted pH 8) was added to 5 mL of an aqueous solution containing 10 mg of PLGA NPs an d mixed by stirring for 1 hour. The emulsion was washed thr ee times with 10 mL of distilled water by centrifugation (6,50 0×g, 10 minutes) at 4°C, freeze-dried, and stored at 20°C [6].

3.4 Characterization of Formulation

The optimized nanoparticles were characterized on various attributes like particle size, surface charge, PDI, SEM and in-vitro evaluation.

3.4.1 Particle Size Determination and Surface Charge Measurement

The average particle size and size distribution of the PLGA NPs and PEI-PLGA NPs were determined by Nanoseries Zetasizer ZS90 (Malvern) and are represented in figure 6 (A) & (B) and 7 (A) & (B) respectively. The samples of formulation were diluted to 1:9 v/v with deionized water. The particles size and size distribution were represented by average (diameter) of the Gaussion distribution function in the logarithmic axis mode. The zeta potential (ϵ) of the nanoparticles formulation was calculated according to Helmholtz-Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, Zetasizer (NanoPlus) was used.

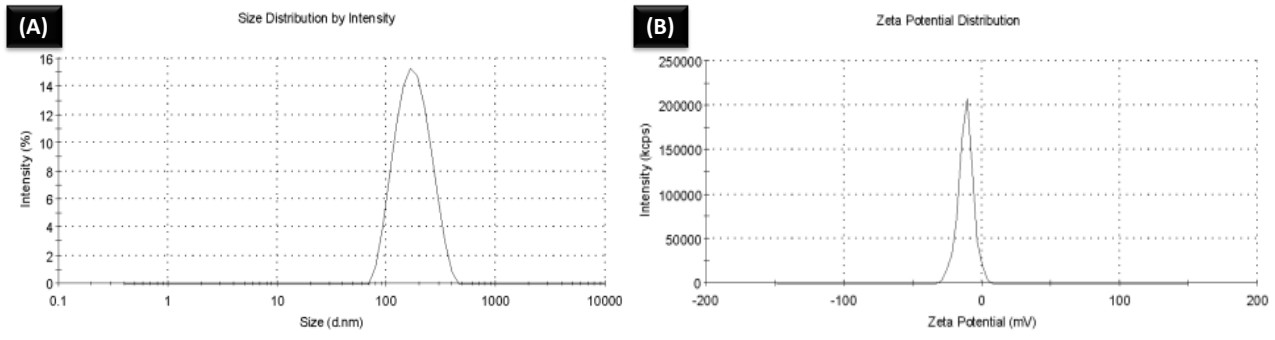

Figure 6(A) Particle size of PLGA-NPs and (B) Zeta Potential of PLGA-NPs

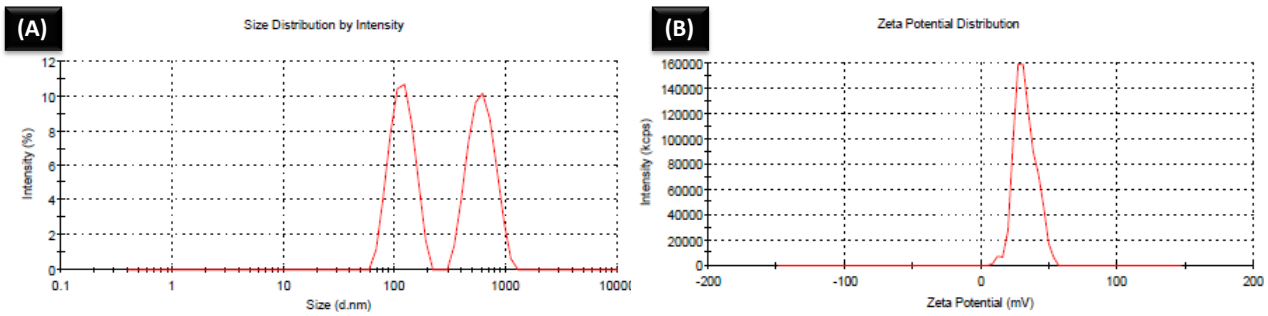

Figure 7(A) Particle size of PLGA-PEI NPs and (B) Zeta Potential of PLGA- PEI NPs

3.4.2 Surface and Shape Morphology

Morphology of drug loaded nanoparticle formulations (D-PLGA NPs and D-PEI-PLGA NPs) was determined by Scanning Electron Microscopy (SEM) at Dr. H.S. Gour Central University, Sagar and at SIL, Dr. H.S. Gour Central University, Sagar (Figure 8 (A) & (B), respectively).

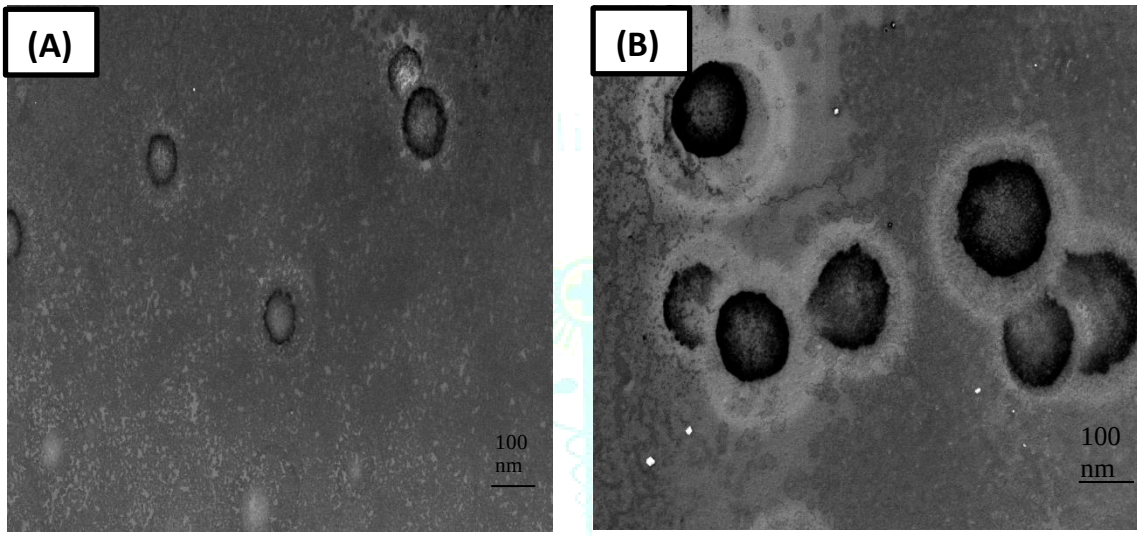

Figure 8 SEM images: (A) PLGA-NPs and (B) PLGA-PEI NPs

3.4.3 Entrapment Efficiency

Entrapment efficiency of the drug loaded nanoparticles was determined by using Sephadex G–50 column [7] (Table 1).

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of total drug - Amount of free drug}}{\text{Amount of total drug}} \times 100$$

3.4.4 Physicochemical characteristics of different drug loaded NPs: A summary

Table 1 represents a brief summary of physicochemical characteristics of different drug loaded NPs.

Table 1: A brief summary of physicochemical characteristics of different drug loaded NPs

Formulation code	Size (nm)	PDI	Zeta potential (mV)	%EE
PLGA	168±3.12	0.180±0.011	-11.7±1.6	69.34±1.53
PLGA-PEI	201±3.81	0.448±0.013	32.3±1.9	72.4±2.26

Values represent mean ± SD (n = 3)

3.5 Cumulative Percent Drug Release

The *in vitro* drug release of entrapped drug from different formulation was determined using dialysis tube method at physiological pH and acidic pH as the atmosphere of tumor tissue is slightly acidic and hence the change in release pattern was studied (Figure 9(A) and (B), respectively) [8].

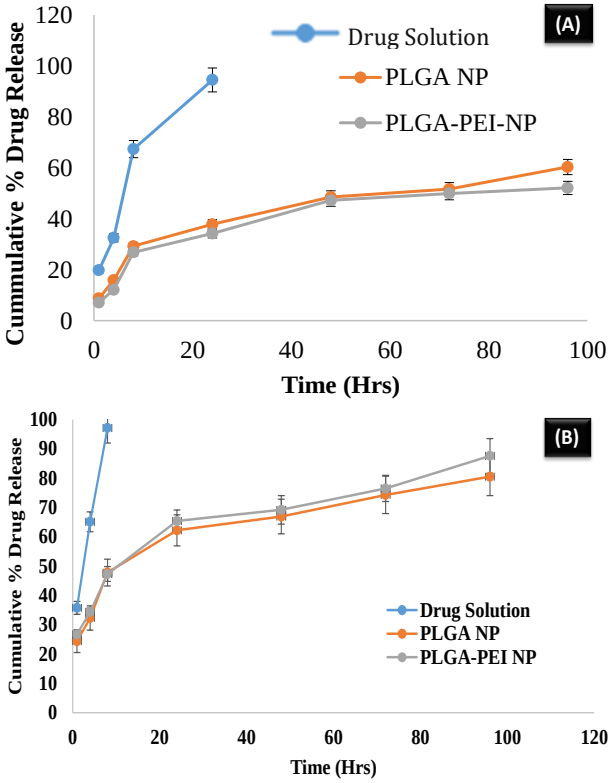

Figure 9 Cumulative percent drug release at (A) pH 7.4 (B) pH 5.0

3.6 MTT cytotoxicity assay

3.6.1 Cell culture

MCF 7cell line was selected for tumor studies. The cells were cultured in RPMI supplemented with 10% Fetal Bovine Serum (FBS), 1% streptomycin/penicillin, 2mM of glutamine, maintained at 37±1°C and 5% CO_2 under humidified conditions [9].

3.6.2 Assessment of cellular toxicity

Inhibition of cell proliferation was studied by tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]- 2-5-diphenyl tetrazolium bromide. Briefly, cells were seeded in a 96 well flat bottom plates and allowed to grow for 24 hr. Then, the cells were incubated with increasing concentration of DTX solution, D-PLGA NPs and D-PLGA-PEI NPs for 24 hr. After 24 hr, the supernatant was removed; MTT and culture medium (100 µL each) were added to each well and incubated for 4 hr at 37◦ C and 5% CO_2 atmosphere. The unreduced MTT and medium were then discarded. Each well was washed with 200 µL of PBS, and 200 µL of DMSO was added to dissolve the MTT formazan crystals. Plates were shaken for 20 min and absorbance was read at 560 nm using a microplate reader (Molecular Devices Corporation, USA). The IC_{50} values (i.e., concentration resulting in 50% growth inhibition) of docetaxel were graphically calculated from concentration-viability curves, considering the optical density of the control well as 100% viable (Figure 10).

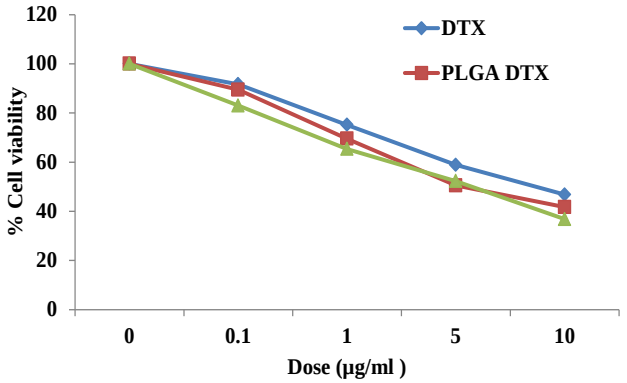

Figure 10 Cell viability (in percent) after treatment of MCF-7 cells with different formulations.

4. RESULTS AND DISCUSSION

PLGA nanoparticles were fabricated by a modified emulsion/ solvent evaporation method (Figure 1) using after optimizing the different formulation variables using one factor at a time approach wherein the factors were optimized varying one parameter at a time keeping other constant as per the literature available. Polymer concentration, surfactant concentration, sonication time and aqueous volume ratio were the factor optimized (Figure 2, 3, 4 and 5, respectively). Particle size and % drug entrapped were the parameters observed for optimizing the plain and drug loaded formulation. The optimized methodology used for nanoparticle fabrication includes the use of 2% *w/v* PLGA in ethyl acetate as organic Phase and 1 % *w/v* Pluronic F-68 in water as stabilizer. Drug loading was done by dissolving 2% w/w drug in organic phase. Particle size, size distribution and surface charge of nanoparticles were determined using Zetasizer (Malvern ZS 90, UK). The average size of the PLGA nanoparticles (PLGA-NPs) and PLGA-PEI nanoparticles (PLGA-PEI-NPs) was observed to be 168±2.7 nm and 201±3.81 nm respectively [Figure 6 (A) & 7(A)]. Zeta potential is one of the most important indices to evaluate nanoparticulate suspension stability. The value of the zeta potential was found to be -11.7 mV for PLGA-NPs due to the presence of terminal carboxylic groups in the polymer. For PLGA-PEI-NPs it was found to be 32.3±1.9 mV [Figure 6(B) & 7(B)] which is indicative of the presence of PEI imine group on the surface. SEM image of nanoparticles showed that particles were spherical in shape and do not show considerable variation in shape [Figure 8 (A) & (B)]. The results are in accordance with previous report [10-12]. Drug loading was optimized on the basis of particle size and percentage entrapment efficiency so as to load the maximum possible amount of the drug in the system. Percent drug entrapment was calculated using Sephadex G-50 column. Formulation with highest percentage of loaded drug was selected for *in vitro* release behavior in media of various ionic strengths (pH7.4 and pH 5.0) [Figure 9(A) & 9(B)]). At acidic pH, the system showed comparatively faster release due to the protonation of the various groups present on the surface of carrier system. The formulations were also studied for MTT assay. In order to evaluate the cytotoxic potential of developed formulations for MCF-7 cell at various time periods are presented in figure 10. The antiproliferative activity of different DTX-NPs formulation on MCF-7 cell line was observed at 24 hr in concentration range of 0.1µg/ml to10 µg/ml. The results showed that the free DTX have slightly higher IC_{50} value than DTX PLGA nanoparticles after 24 hr with values of 6.1±0.12, 4.2±0.22 and 2.1±0.24 µg/ml after 24 hr for DTX, D-PLGA-NPs and D-PLGA-PEI NPs

respectively. PEI coated nanoparticles have lower IC_{50} value and there was significant difference in the IC_{50} values of PEI coated nanoparticles as compared to other formulations, suggesting that PEI coated nanoparticles showed highest cytotoxicity against these cells at all concentrations as compared to PLGA NPs and free drug.

CONCLUSION

In our current study, we used modified core shell nanoparticle comprising the PLGA core and polyethylenimine (PEI) in order to improve the therapeutic index of docetaxel against MCF-7 cancer cells. The nanoparticles were prepared by emulsion/solvent evaporation technique followed by their characterization based upon size and surface charge. A rise in the surface zeta potential of the nanoparticles confirms the electrostatic binding of PEI with the surface of PLGA nanoparticles. Scanning electron microscopy (SEM) was employed to observe the shape, dispersion, and morphology of the nanoparticles. We found that PEI coated PLGA NPs was nanometric in size, uniform in shape and showed good entrapment efficiency of 72.4%. The developed formulation showed sustained drug release. PEI- coated PLGA nanoparticles demonstrated a more potent cytotoxic effect on MCF-7 cancer cells over longer time duration. The results shown in this study are promising and set a platform for further examining the suitability of this PEI-enhanced delivery system for cancer therapy.

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Conflict of Interest

There is no conflict of interest.

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