

Development of Docetaxel-PLGA-Nanoparticles and *In Vitro* anti-tumor activity in PC3 cells Targeted to Prostate Tumor

Satendra K Dubey, Abhilash Thomas, Malay K Samanta

Abstract

The purpose of this study was to investigate poly (D, L-lactide-co-glycolide) nanoparticles (PLGA-NPs) loaded with Docetaxel (DTXL) as a potent anticancer drug which has shown high antitumor activity for prostate cancer. In this study, we investigated the Docetaxel-PLGA-nanoparticles (DTXL-PLGA-NPs) and their effects on PC3 cancer cell lines were promising. DTXL-PLGA-nanoparticles with particle size within the range of 197.7 ± 19.9 nm were prepared by a nanoprecipitation method. The influence of four different independent variables (amount of polymer, percent of emulsifier, internal phase volume, and external phase volume) on nanoparticle drug-loading was studied. Differential scanning calorimetry was evaluated for physical characterizing. The results of the optimized formulation showed a narrow size distribution, suitable zeta potential and a drug loading. The *in vitro* drug release from DTXL-PLGA-NPs showed a sustained release pattern of up to 168 hours and comparing with DTXL had a significant decrease in initial burst effect. These experimental results indicate that DTXL-PLGA-NPs have a better physicochemical characterization and can be developed as a drug carrier in order to target to prostate cancer cell lines PC3.

Index Terms- Antitumor efficacy, Cytotoxicity, Docetaxel, In-vitro release, Molecular Pharmaceutics, Nano-drug Delivery, Prostate Cancer, Tumor Targeted Drug Delivery System.

1. INTRODUCTION

1.1 Prostate Cancer

Prostate cancer is the second leading cause of cancer death in men and accounts for approximately 30% of all new cancer diagnoses in men. More than 70% of all cancer deaths in 2005 occurred in countries included India. Deaths from cancer in the world are projected to continue rising, with an estimated 9 million people dying from cancer in 2015 and 11.4 million will die in 2030. Prostate-specific membrane antigen (PSMA), also named glutamate carboxypeptidase II (GCPII), is a classic type II membrane glycoprotein and is perhaps the most important enzyme-biomarker and target in prostate cancer research. PSMA can be induced for internalization by bound antibodies or small-molecule inhibitors. Its extracellular domain possess folate hydrolase and N-acetylated-alpha-linked-acidic di-peptidase activities can serve as binding target for antibodies, inhibitors and aptamers. Every single cell that moves to other place may develop into new tumor giving rise to various necrotic regions.

Upon treatment even if a single cell or colony is left out, it can again lead to entire tumor. Present cancer therapy should be based on the philosophy, "even a single cancer cell should not remain untreated in the body and nothing less than complete elimination of tumor from an individual can be accepted" [1]. This forms the objective behind chemotherapy.

The current standard treatment of localized prostate cancer consists of prostatectomy and radiation therapy, sometimes supplemented with hormonal therapies to prevent testosterone production, which include anti-androgens and luteinizing hormone-releasing hormone agonists. In locally advanced and/or widespread prostate cancer, the disease gradually transforms to a metastatic hormone-refractory state. Despite castrate levels of testosterone, the tumor will finally become independent of androgens resulting in death within a few years from diagnosis. In fact, the mortality rate of metastatic prostate cancer is extremely high. Thus, novel therapies are on demand for the treatment of the malignant forms of prostate cancer that recur after initial therapies, including hormone refractory and castration resistant prostate cancer.

1.2 Targeted drug delivery Nanoparticles (NPs)

Targeted therapy is potentially an important mechanism to target at specific site of cancer. Better outcomes for hormone resistant tumors may be achieved by specifically delivering drugs, antigens or toxins to tumors specifically kill tumor cells without affecting surrounding tissue.

- Satendra K. Dubey, Department of Pharmaceutical Biotechnology, JSS University Mysuru, JSS College of Pharmacy, Ooty, 643001 INDIA. E-mail: publication.skdg@gmail.com
- Abhilash Thomas, Department of Pharmaceutical Biotechnology, JSS University Mysuru, JSS College of Pharmacy, Ooty, 643001 INDIA.
- Malay K. Samanta, Prof. & Registrar, University of Kalyani, Kalyani, 741235 INDIA

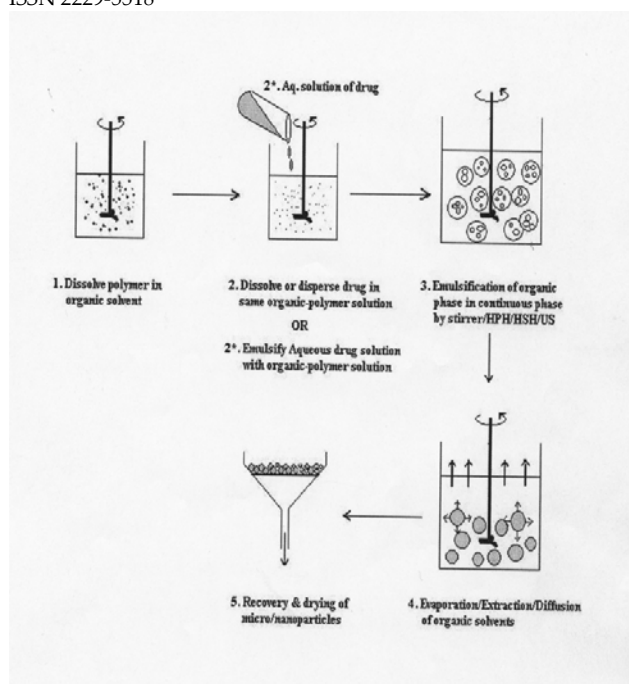


Figure 1: Schematic diagram of solvent evaporation technique for the preparation of DTXL-loaded nanoparticles adopted by Nak 2012 [9].

Targeted therapy is dependent on specific marker for the cancer cells that can be identified with affinity agents such as antibodies for the particular antigens [2]. Targeted drug delivery system designed by three important parameters on which the selection of the most suitable drug delivery system is based on the drug, the disease state, and the latter's location in the body. It enjoys the advantages of small molecular size, solubility, and permeability, which are favorable for passive membrane diffusion. We had chosen this drug delivery system because researchers have succeeded in part in controlling the drug absorption process to sustain adequate, effective plasma drug levels and better cellular uptake or drug targeting to specific tissues for those drugs with poor bioavailability over a prolonged period of time by designing delayed or controlled release nanoparticulate delivery systems intended for either parenteral administration [3].

In this study, we have chosen emulsification solvent evaporation technique, which is simple beaker-stirrer lab scale, economic, robust, and well controlled method which also gives preliminary idea about the controlled factors and parameters required for the production in large scale. The solvent evaporation technique is fully developed at the end of 1970s [4]. This classic technique showed different recent variations are commonly used for encapsulation of various substances from simple pharmaceutical products to protein and DNA. Solvent evaporation technique is based on the evaporation of internal phase of an emulsion by agitation, therefore neither requires elevated temperatures nor phase separation inducing agents [5-8].

DTXL-PLGA-NPs prepared by single solvent evaporation technique showed in Fig. 1 as dispersion of Docetaxel drug in organic-polymers solution and Emulsification of organic phase in second continuous phase involved in this method for the preparation of drug loaded nanoparticles. Targeted NPs therapeutics have shown great potential for cancer therapy, as they provide enhanced efficacy and reduced side effects. NPs drug delivery can be either an active or passive process. Passive delivery refers to NP transport through leaky tumor capillary fenestrations into the tumor interstitium and cells by passive diffusion or convection. Selective accumulation of Nanoparticles with the drug then occurs by the already mentioned characteristics of the tumor micro-environment showed in Figure 2.

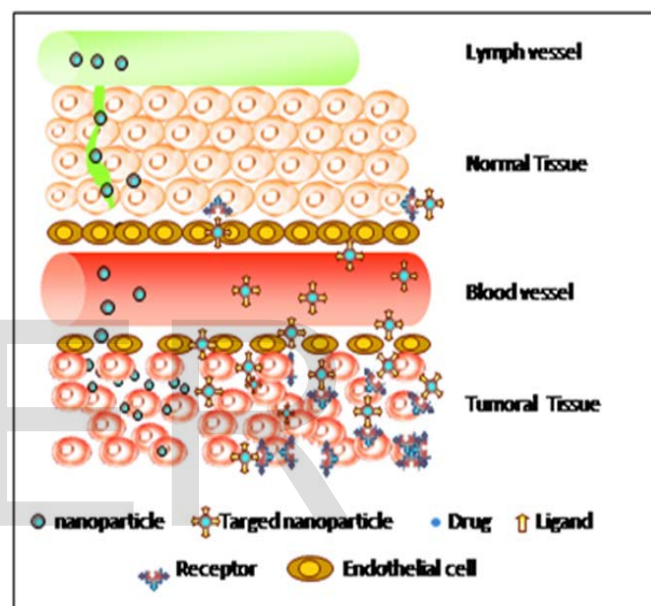


Figure 2: Characteristics of the tumor microenvironment

1.3 Targeting approach of Nanoparticles

Active targeting involves drug delivery to a specific site based on molecular recognition. One such approach is to couple a ligand, such monoclonal antibodies and peptides, to a NP so that the ligand can interact with its receptor at the target cell site showed in Figure 2. Nanoparticles are more able to reach tumor cells through passive targeting due to the characteristics of tumor tissues like vascular disorganization, fenestrations, discontinuous basement membrane, etc.

In normal tissues the lower amount of nanoparticles that able to reach it are removed by lymph vessels while in tumor tissues, lymphatic network is too damaged to perform its function promoting the accumulation of targeted polymer nanoparticles (PLGA-NPs) in the tissue. The functionalized nanoparticles are internalized not only by passive targeting but also by active targeting. This active targeting is more effective in the tumor tissue due to tumor cells overexpress some receptors that allow them a better uptake of functionalized nanoparticles.

2. EXPERIMENTAL METHODS

2.1 Materials

Docetaxel was kindly supplied as a gift sample from Emcure Pharmaceuticals, Pune, India. Polymer PLGA 50:50 was purchased from Boehringer Ingelheim Pharma GmbH & Co., Germany. Poly vinyl alcohol (cold water soluble) with molecular weight, 30,000-70,000 and MTT reagent were procured from Sigma Aldrich, St. Louis, MO, USA. PC3 cell line was obtained from National Centre For Cell Science, Pune. Chloroform and solvents (acetonitrile and water) for high performance liquid chromatography (HPLC) were purchased from Fisher Scientific. All other chemicals and reagents used were of HPLC and analytical grade.

2.2 Formulation of DTXL-PLGA-NPs

Nanoparticles were prepared by single Nano-precipitation cum solvent evaporation method as described by Xua et al [10], with different ratios of PLGA 50:50 to drug formulation (F1, F2, F3 and F4) were dissolved in sufficient volume of dichloromethane to obtain low viscosity clear solution. The resulting organic phase solution was added slowly to the aqueous phase containing poly vinyl alcohol solution 1.0% w/v, homogenized using high speed homogenizer with a speed of 15,000 rpm followed by sonication in ice water bath for 5 min at 80 W amplitude using probe sonicator to form nanoparticles.

The resulting emulsion was kept under stirring for up to 12 h for complete evaporation of organic solvent. PLGA-NPs were separated by centrifuging the suspension at 15,000 rpm for 30 min and the obtained pellet further dispersed in mannitol solution (5 mg/ml) as cryoprotectant and lyophilized for 48 hours at -40°C with a vacuum pressure of <50 mm torr to obtain dry free flowing mass [11].

2.3 CHARACTERIZATION OF DTXL-PLGA-NPs

2.3.1 Estimation of Docetaxel by HPLC

A specific and sensitive reverse phase chromatographic method was used for estimation of DTXL in nanoparticles using Shimadzu LC20AD HPLC system and the LC solutions software. The mobile phase consists of acetonitrile and 20 mM ammonium acetate buffer of pH 4.5 (60:40) using Grace Vydac C18 column (250 × 4.6 mm, 5μ) as stationary phase, maintained at 25°C with a flow rate of 1 ml/min at a detection wavelength of 230 nm. Briefly, 10 mg of lyophilized DTXL-PLGA-NPs was dissolved in 1 ml of dichloromethane and was evaporated under a stream of nitrogen. To the residue, 2 ml of mobile phase mixture was added, centrifuged and the supernatant was used for analysis. Drug encapsulation efficiency (%) analysis was carried out in triplicate and expressed as the Percentage. Percentage of drug in the produced nanoparticles with respect to initial amount (mg) used for formulation of nanoparticles [12].

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Amount of drug in nanoparticles (mg)}}{\text{Initial amount of drug (mg)}} \times 100$$

2.3.2 Particle size and zeta potential

The average particle size, polydispersity index (PDI) and zeta potential of DTXL-PLGA-NPs were measured by using Zetasizer (Nano ZS, Malvern Instruments, UK) utilizing the dynamic light scattering (DLS) technique, calibrated with standard latex nanoparticles [13]. The zeta potential of a particle is the overall charge that the particle acquires in a particular medium.

In this technique, a voltage was applied across a pair of electrodes at either end of a cell containing the particle dispersion. Charged particles were attracted to the oppositely charged electrode and their velocity was measured and expressed in unit field strength as their electrophoretic mobility. Samples in triplicate from the prepared suspensions were diluted in Milli-Q water and placed in measurement cell for analysis.

2.3.3 Thermogram properties

Phase behavior of the DTXL and drug loaded particles was studied by differential scanning calorimetry, using DSC-60 instrument. Approximately 2–10 mg of samples (pure drug of DTXL and DTXL-PLGA-NPs) were placed in aluminum pans and were crimped, followed by heating under nitrogen flow (30 ml/min) at a scanning rate of 5°C/min from 25°C to 250°C [14]. Aluminum pan containing same quantity of indium was used as reference. The heat flow as a function of temperature was measured for both pure drug and nanoparticles.

2.3.4 Scanning electron microscopy (SEM)

The shape and surface morphology of the nanoparticles was examined using scanning electron microscopy (JSM-T20, Kyoto, Japan). An appropriate sample of DTXL-PLGA-NPs was mounted on metal (aluminum) stubs, using double-sided adhesive carbon tape and fractured with a razor blade [15]. The samples of nanoparticles were sputter coated with gold/palladium for 120 s at 14 mA under argon atmosphere for secondary electron emissive SEM and observed for morphology at an acceleration voltage of 15 kV.

2.3.5 Transmission electron microscopy (TEM)

Morphology of DTXL-PLGA-NPs was examined using transmission electron microscopic technique. Olympus TEM (Electron Microscope, Eindhoven, The Netherlands) was used as a visualizing aid for nanoparticles. Negative staining TEM method was used to analyse nanoparticles preparations. A 50 μl sample of nanoparticles formulation was taken and placed in parafilm. Samples were dried on carbon-coated grid and negatively stained with aqueous

solution of phospho-tungstic acid. After drying, the specimen was viewed under microscope at 10-100 k fold enlargements at an accelerating voltage of 100 kV.

2.3.6 In vitro drug release

In vitro release studies were carried out using vial method as reported by Danhier et al [16] and Verger et al [17]. DTXL-PLGA-NPs containing 5mg equivalent drug was suspended in vial containing 10 ml of pH 7.4 phosphate buffer with 0.3% tween 80 to improve solubility of drug. The vial was shaken horizontally using water bath shaker at 37°C. In vitro drug release was assessed by intermittently sampling the vial (2 ml) at predetermined time intervals (0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 168 and 360 h) and was replaced with 2ml of fresh pH 7.4 phosphate buffer.

The withdrawn sample was centrifuged at 5000 rpm for 2 min, supernatant was filtered through 0.45 µm membrane filter and injected to HPLC system by using HPLC equipped with UV detector at 230 nm. The amount of DTXL released in each sample was determined using a calibration curve; the reported values are averages of three replicates (n = 3). Results of in vitro drug release studies obtained were tabulated and shown graphically as cumulative % drug release vs. time.

2.3.7 In vitro cytotoxic activity

The effect of pure drug and nanoparticles on the viability of PC3 (prostate cancer cell line) was determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Cells were cultured in Dulbecco's modified essential medium (DMEM) with 10% foetal bovine serum containing penicillin (100 units/ml) and streptomycin (100 µg/ml). In all experiments, cells were maintained at 37°C in a humidified 5% CO₂ incubator. Briefly, 8×10⁴ cells per ml were plated in 96 well micro titer plates in triplicates and incubated in 5% CO₂ incubator for 24 h. Then, 100 µl of freshly prepared concentrations (by serial dilution) of pure drug (DTXL) and equivalent to DTXL-PLGA-NPs was added in the growth medium and further incubated for another 48 h followed by 168 h under same conditions [12].

To evaluate cell survival, 20 µl of MTT solution (5 mg/ml in PBS) was added to each well and incubated for 4 h. At the end of incubation period, medium containing MTT was gently replaced by 200 µl dimethyl sulphoxide (DMSO) to dissolve formazan crystals and the absorbance were measured by a micro titer plate reader (Biotek ELx800-MS) at 540 nm with a reference wavelength of 630 nm. The absorbance of test (treated cells) and the control (untreated cells) were used for the determination of the percentage cell viability. Cell survival in control cells was assumed to be 100%. Cells were maintained in an exponential growth phase by periodic dilutions.

Dilutions

$$\% \text{ Cell viability} = \frac{\text{O.D. of test} \times 100}{\text{O.D. of control}}$$

2.3.8 Statistical analysis

The data obtained was statistically analyzed by using independent sample t-test with SPSS software 11.5 version, to stumble out significant differences between two groups. Statistical significance was established at p < 0.05.

3. RESULTS AND DISCUSSION

3.1 Formulation development:

The choice of a particular method for encapsulation of Docetaxel drug substance in a colloidal carrier is most commonly determined by the solubility characteristics of the drug and polymer. Both, the drug and polymer were readily soluble in dichloromethane. The selection of an optimal formulation in the study was based on that which provided highest drug encapsulation efficiency and extreme unaffected particle size with better morphology.

The particles prepared with 5 min of probe sonication showed a monomodal distribution profile. Components of the system described herein include emulsifying and stabilizing agent (polyvinyl alcohol, 1% w/v) used as the aqueous phase with polymer and drug in organic phase. Using oil-in-water single emulsion templates, nanoparticle formulations containing up to 5 mg equivalent of DTXL were successfully prepared. The size and surface morphology of DTXL-PLGA-NPs were confirmed by zetasizer and SEM analysis. It is well accepted that the nanoparticle size is directly dependent on the rate of diffusion of the organic solvent to the outer aqueous environment [18-19].

Table 1: Particle size and zeta potential with respect to various formulations (Polymer: Drug ratio)

Formulation Batch	Polymer: drug ratio	Particle size (nm)	Polydis. Index (PDI)	Zeta pot. (mV)	Encap. Effic. (EE) %
F1	5:1	97.04 ± 10.7	0.417 ± 0.24	-32.8 ± 6.2	59.47 ± 2.4
F2	10:1	165.4 ± 14.2	0.359 ± 0.10	-48.2 ± 8.4	90.1 ± 5.7
F3	15:1	177.1 ± 24.8	0.282 ± 0.08	-48.5 ± 9.7	91.2 ± 3.9
F4	20:1	197.7 ± 19.9	0.162 ± 0.07	-69.1 ± 11.9	95.1 ± 4.1

3.2 Encapsulation efficiency (EE) %

To attain encapsulation of drug in optimum amount, trials were executed in different ratios of polymer to drug. Formulation F1 showed 59.47 ± 2.4% followed by 90.1 ± 5.7%, 91.2 ± 3.9% and 95.1 ± 4.1% for Formulation F2, F3 and F4 respectively showed in table 1. On further increase in the polymer concentration, there was no significant difference in encapsulation efficiency, the drug weight was kept constant (5 mg). The greater amount of drug resulted

in a more viscous dispersed phase, making it difficult for the mutual dispersion of the phases and originating larger particles which lead to precipitation of drug in continuous phase.

DTXL-PLGA-NPs manufactured from single emulsion technique with PLGA 50:50 were reproducible with highest encapsulation efficiency (EE) ($95.1 \pm 4.1\%$) for Formulation F4 containing the polymer to drug ratio of 20:1. Here the reason being, for the high amount of polymer reduced drug loss during fabrication process and showed a drastic change for the drug encapsulation enhancement. This demonstrates that particle size and Entrapment efficiency could be significantly affected by the polymer amount when other formulation variables are kept constant. The order for encapsulation was found to be batch $F4 > F3 > F2 > F1$. The optimized formulation with highest encapsulation efficiency, i.e. 20:1 polymer to drug ratio, was further used for in vitro studies.

3.3 Particle size and zeta potential

The formulated DTXL-PLGA-NPs were evaluated for particle size and zeta potential and results of the same are shown in table 1 and size distribution is depicted in figure 3. For the formulations F1, F2 and F3, particle size were found to be 97.04 ± 10.7 , 165.4 ± 14.2 and 177.1 ± 24.8 nm with polydispersity index of 0.417 ± 0.24 , 0.359 ± 0.10 and 0.282 ± 0.08 , whereas for Formulation F4 having high encapsulation efficiency, particle size was found to be 197.7 ± 19.9 nm with polydispersity index of 0.162 ± 0.07 , respectively. The zeta potential values were found to be in the range of -32.8 ± 6.2 to -69.1 ± 11.9 . Size distribution pattern of particles plays an important role in determining the drug release behavior, their feasibility for intravenous administration as well as their fate after in vivo administration [20].

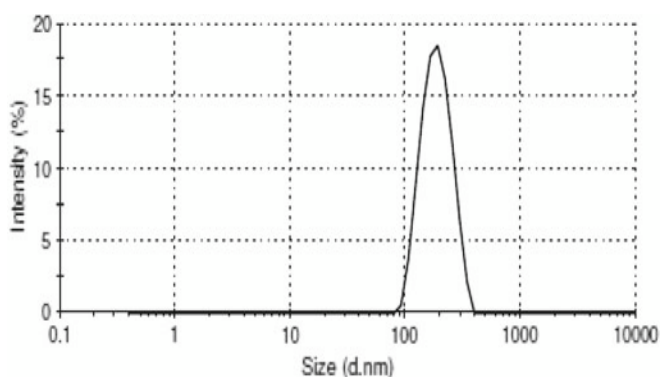


Figure 3: Particle size distribution of DTXL-PLGA-NPs Formulation F4 (polymer to drug ratio; 20:1)

Due to smaller particles (<200 nm), they tend to accumulate in the tumor sites due to the facilitated extravasation, which can prevent spleen filtering. In addition, their sterilization may be simply done by filtration. Polydispersity (PDI) of the formulation increased as polymer to drug ratio decreased. Less value of polydispersity index indicates enhanced homogeneity of the nanosuspension which was observed with formulation F4 (20:1 polymer to drug ratio).

Knowledge of the zeta potential for nanoparticles preparation can help to predict the fate of the nanoparticles in vivo and to assess the stability of colloidal systems. Zeta potential reveals the physical stability of the formulation i.e. surface charge on the particles could control the particles stability of the nanoparticles formulation through strong electrostatic repulsion of particles with each other. In addition, from the zeta potential measurement, we can roughly know the dominated component on the particles surface. A great increase in the absolute value of the PLGA-DTXL-NPs surface charge could be observed. The value of negative charges on the particles were reduced from -69.1 ± 11.9 to -32.8 ± 6.2 as the polymer concentration decreased from 20:1 to 5:1 in F4 to F1 formulations polymer to drug ratio. This may be due to un-entrapped drug of nanoparticles, reduced the negative charges which might be explained by the shielding effect of carboxylic groups by drugs molecules on the particle surface [21].

3.4 Thermogram properties analysis

The physical status of the native drug in the prepared DTXL-PLGA-NPs was performed by DSC analysis. DSC thermogram of the pure drug and formulation are represented in figure 4.

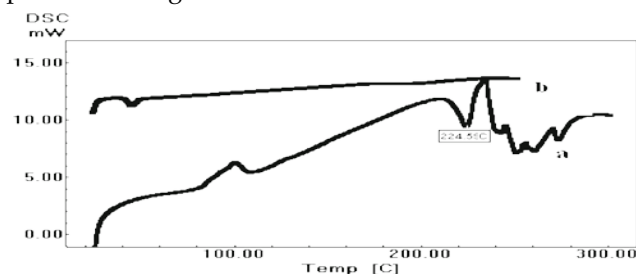


Figure 4: DSC thermogram of (a) Pure drug DTXL and (b) DTXL Loaded PLGA Nanoparticles.

Thermal analysis is a useful tool for determining whether solute particles have been dispersed in polymeric matrices [22-23]. Pure drug has characteristic endothermic melting peak at 220°C with high intensity. From the DSC results, it was found that melting temperature of drug DTXL was shifted to lower temperature than that of DTXL itself due to amorphous state of DTXL in the nanoparticles and also that part of the entrapped drug exists in crystalline form.

3.5 Scanning electron micrographs (SEM) and Transmission electron microscopy (TEM)

It was observed that the nature of particles appears to be homogeneous, smooth and spherical in shape which is confirmed by surface morphology studies as shown in figure 5. The particles have moderate uniformity and all the particles were discrete entities, did not cause aggregation of particles after lyophilization and these particles were readily redispersible. The nanoparticles prepared were found to be spherical in shape with nanosize range, supported by SEM analysis as discussed in the above section.

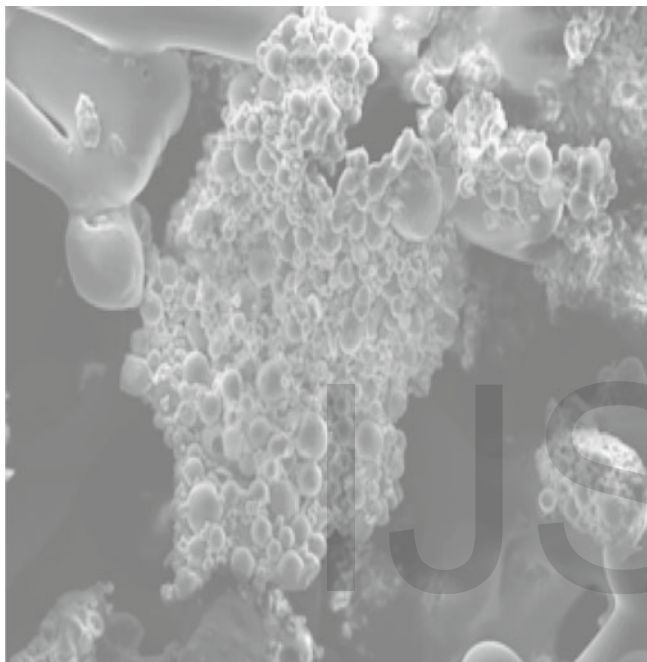


Figure 5: Surface morphology of DTXL-loaded PLGA nanoparticles.

3.6 In vitro Drug release

It is evident from the drug release curves that Formulation F1 (5:1 polymer to drug ratio) showed $92.9 \pm 1.9\%$ of drug release within 48 h, followed by $89.1 \pm 3.7\%$ drug release for F2 (10:1 ratio) and $77.0 \pm 3.9\%$ for F3 (15:1 ratio), respectively whereas in F4 (20:1 ratio) showed a slower release profile, the release rate was reduced up to $54.7 \pm 3.0\%$ in 48 h due to high encapsulation of drug with low swelling of polymer in the release media leading to slow diffusion of drug particles from the polymeric matrices. The degradation of PLGA 50:50 is slow. Therefore, the release of DTXL from nanoparticles may depend on drug diffusion and PLGA surface and bulk erosion or swelling. The release behavior of DTXL from the developed DTXL-PLGA-NPs exhibited a biphasic pattern characterized by an initial rapid release during the first 24 h, followed by a slower and continuous release at extremely slow rates for a period of 15 days [24-25] which is depicted in figure 6.

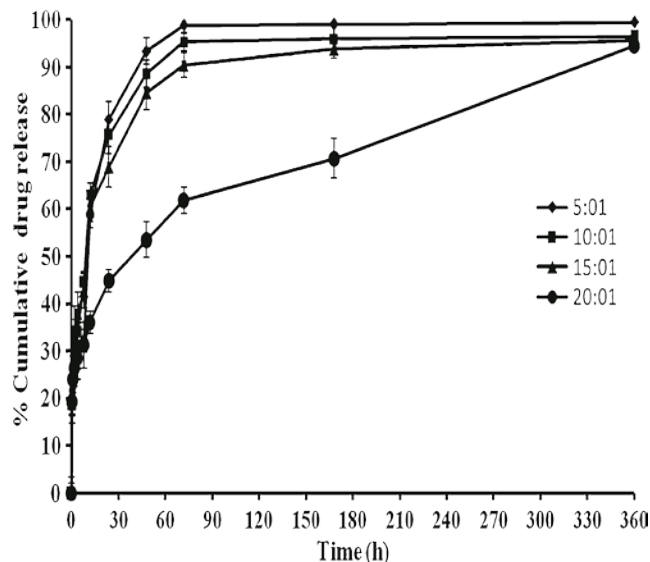


Figure 6: In-vitro % cumulative drug release (CDR) profile of different formulations.

A high initial burst release was observed and can be attributed to the immediate dissolution and release of DTXL adhered on the surface and located near the surface of the nanoparticles. An important phenomenon observed here is that as larger the amount of drug present on the surface of nanoparticles then more quickly the release occurred, and the particles with highest encapsulation exhibited a release in a more sustained fashion. A significant difference was observed in release pattern for formulation F4 (polymer to drug ratio ratio 20:1) when compared to all other formulations [26].

3.7 In vitro cytotoxic activity

In the cytotoxic activity, cells were incubated with concentrations, 0.01, 0.1, 1.0 and 10.0 $\mu\text{g/ml}$ of both pure drug DTXL and DTXL-PLGA-NPs and were evaluated by assessing cell viability by the MTT assay using the prostate cancer PC3 cell lines. These range of concentrations were selected because it corresponds to plasma levels of the drug, was achievable in preclinical studies.

The results of cell viability are shown in figures 7, after 24 h of incubation time, no cytotoxic effect was observed for all concentrations tested both for pure drug and formulation. But after 48 h found a marked reduction in cell viability ($\approx 80\%$) was observed when PC3 cells were incubated with 10 $\mu\text{g/ml}$ of pure drug at 37 $^{\circ}\text{C}$, no further cytotoxic effect was observed for the longest incubation times [18-19,27]. At this concentration cell growth was almost inhibited for pure drug. However, for each of the concentrations there was enhancement in cytotoxicity with increasing time of incubation for nanoparticles. Cell growth was constantly inhibited for DTXL-PLGA-NPs up to 168 h of incubation when compared to pure drug indicating slow release of Docetaxel.

In this case, the incubation time was found to be the most critical parameter for cytotoxic activity. For longer incubation times no significant differences in cytotoxicity was observed among the concentrations, 0.01 and 0.1 $\mu\text{g/ml}$, whereas for 1.0 $\mu\text{g/ml}$ and 10.0 $\mu\text{g/ml}$ cell after 168 h of incubation, a reduction of 90-95% in cell viability was detected. It was observed that cell growth was never completely inhibited for any concentrations of pure drug (20-30%). From the results it can be concluded that for the higher concentrations tested, significant differences in the cytotoxicity effect of DTXL-PLGA-NPs were observed.

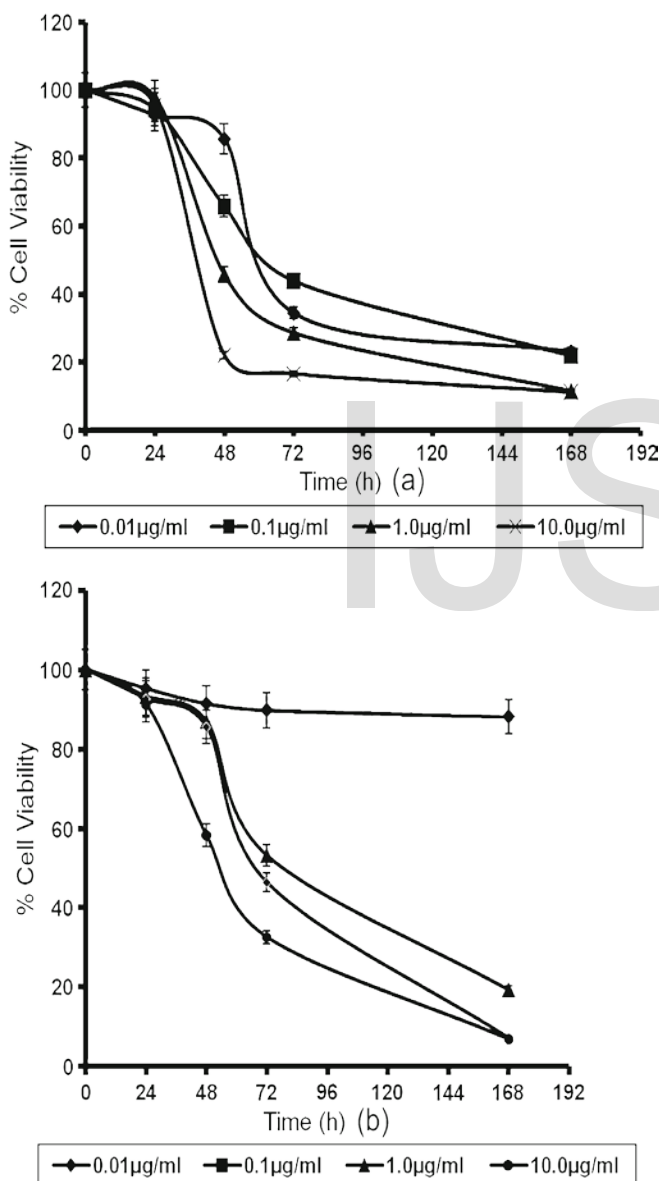


Figure 7: (a) Percentage cell viability for pure drug with different concentrations and (b) percentage cell viability for DTXL-PLGA-NPs with different concentrations.

4. CONCLUSION

The objective of the study was accomplished by formulating nanoparticulate drug delivery system for DTXL using PLGA with controlled release and anti-tumor activity was established for the first time against PC3 cell lines. The methodology selected in this work allowed instantaneous and reproducible fabrication of nanoparticles with homogenous and spherical morphology. Hence it can be concluded that the formulation developed in this study may be considered promising and effective anticancer drug delivery system for long term treatment of prostate cancer.

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