Bull. Mater. Sci., Vol. 35, No. 3, June 2012, pp. 319–326. © Indian Academy of Sciences.

PLGA 50:50 nanoparticles of paclitaxel: Development, *in vitro* anti-tumor activity in BT-549 cells and *in vivo* evaluation

RANJITH K AVERINENI[†], GOPAL V SHAVI, ARAVIND K GURRAM, PRAFUL B DESHPANDE, KARTHIK ARUMUGAM, NASEER MALIYAKKAL, SREENIVASA R MEKA and UDUPA NAYANABHIRAMA*

Department of Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal 576 104, India
[†]Department of Pharmaceutical Sciences, South Dakota State University, South Dakota, USA

MS received 2 March 2012

Abstract. **Clinical administration of paclitaxel is hindered due to its poor solubility, which necessitates the formulation of novel drug delivery systems to deliver such extreme hydrophobic drug. To formulate nanoparticles which makes suitable to deliver hydrophobic drugs effectively (intravenous) with desired pharmacokinetic profile for breast cancer treatment; in this context *in vitro* cytotoxic activity was evaluated using BT-549 cell line. PLGA nanoparticles were prepared by emulsion solvent evaporation technique and evaluated for physicochemical parameters, *in vitro* anti-tumor activity and *in vivo* pharmacokinetic studies in rats. Particle size obtained in optimized formulation was <200 nm. Encapsulation efficiency was higher at polymer-to-drug ratio of 20:1. *In vitro* drug release exhibited biphasic pattern with initial burst release followed by slow and continuous release (15 days). *In vitro* anti-tumor activity of optimized formulation inhibited cell growth for a period of 168 h against BT-549 cells. $AUC_{(0-\infty)}$ and $t_{1/2}$ were found to be higher for nanoparticles with low clearance rate.**

Keywords. **PLGA 50:50; paclitaxel; BT-549 cells; pharmacokinetics.**

1. Introduction

Solubility of the active ingredient is the major pharmaceutical concern in developing novel drug delivery systems. Paclitaxel (PTX) is one of the best antineoplastic agents derived from nature and has shown significant clinical activity against a wide variety of tumors like ovarian and breast cancer. Due to its hydrophobic character, commercial formulation of PTX is constituted with ethanol and cremphor EL i.e. Taxol®, which has been associated with serious side-effects and leads to hypersensitivity, nephrotoxicity and neurotoxicity in many patients. Investigations revealed that encapsulation of anti cancer drugs into colloidal drug delivery systems minimizes the tissue distribution and improves therapeutic performance. A cremophor free formulation of PTX nanoparticles, Abraxane®, has been approved by FDA for recurrent metastatic breast cancer. In order to achieve desired therapeutic performance of PTX, various formulations were attempted earlier like liposomes (Fetterly and Straubinger 2003), solid lipid nanoparticles (Shenoy *et al* 2009) and polymeric nanoparticles (Danhier *et al* 2009).

Usually solid tumors are covered with leaky vasculature which renders in escape of nanoparticles from the vasculature through the leaky endothelial tissue and then accumulate in certain solid tumors. The said phenomenon i.e. enhanced permeation and retention (EPR) effect is helpful in treating solid tumors via nanoparticulate drug delivery system.

Polymers like PLGA and PCL have the advantage of biodegradability and biocompatibility besides controlling the release of drug which will extend blood circulation time, thereby, enhancing the anti-tumor activity. Also, concurrently it minimizes systemic toxicity by reducing the biodistribution of the drug to healthy tissue compared to pure drug. Several methods were reported previously to formulate nanoparticles like interfacial deposition i.e. nano precipitation method (Fonseca *et al* 2002) and high pressure homogenization-emulsification method (Dong and Feng 2007; Li *et al* 2008).

The objective of present study was to develop a polymeric drug delivery system for PTX using biodegradable PLGA 50:50 polymer. The formulation is anticipated for safe intravenous administration, which can avoid the adverse events of conventional PTX cremophor EL formulation. The nanoparticles were characterized in terms of size, surface charge and *in vitro* drug release. *In vitro* anti-tumoral activity of PTX-loaded nanoparticles were evaluated using BT-549 cell line. PTX was encapsulated within PLGA 50:50 by single emulsion solvent evaporation technique using poly vinyl alcohol (PVA) as a emulsifier and mannitol as a cryoprotectant, which produced into a sustained release of PTX nanodelivery system which can deliver the drug over a prolonged period of time.

*Author for correspondence (n.udupa@manipal.edu)

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

2. Materials and methods

2.1 *Materials*

Paclitaxel was obtained as a gift sample from Getwell Pharmaceuticals, New Delhi, India. Polymer PLGA 50:50 (Resomer® 503H) 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. BT-549 cell line was obtained from American tissue cell culture (ATCC), USA. All other chemicals and reagents used were of HPLC and analytical grade.

2.2 *Preparation of nanoparticles*

Nanoparticles were prepared by emulsion solvent evaporation technique as described by Xua *et al* (2005), with different ratios of PLGA 50:50 (Resomer® RG503H) to drug (5:1, 10:1, 15:1 and 20:1) were dissolved in sufficient volume of dichloromethane to obtain low viscosity clear solution. The resulting solution (organic phase) was added slowly to the aqueous phase containing poly vinyl alcohol (PVA) solution 1·0% w/v, homogenized using high speed homogenizer (Polytron Mixer, Kinematica) 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. Nanoparticles 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 h at −40°C with a vacuum pressure of <50 mm torr to obtain dry free flowing mass (Ahlin *et al* 2002). The influence of different experimental parameters such as polymer to drug ratio, organic to aqueous phase ratio and sonication time on the encapsulation efficiency of PTX in the nanoparticles was evaluated.

2.3 *Characterization of nanoparticles*

2.3a *Estimation of paclitaxel by HPLC*: A specific and sensitive reverse phase chromatographic method was used for estimation of PTX in nanoparticles using integrated HPLC system, Shimadzu LC 2010 model, with dual wavelength UV spectrophotometer detector (Shimadzu, Kyoto, Japan). The chromatographic data was processed using LC solution 1·24 SP1 software. The mobile phase consists of acetonitrile and 20 mM ammonium acetate buffer of pH 4·5 (60:40) using Grace vydac C_{18} 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, a specific amount (10 mg) of lyophilized nanoparticles was dissolved in 1 ml of dichloromethane (DCM) 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 of drug in the produced nanoparticles with respect to initial amount (mg) used for formulation of nanoparticles (Fonseca *et al* 2002).

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

2.3b *Particle size and zeta potential*: The average particle size, polydispersity index and zeta potential were measured by using Zetasizer (Nano ZS, Malvern Instruments, UK) utilizing dynamic light scattering (DLS) technique, calibrated with standard latex nanoparticles (Zhang and Feng 2006). 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.

2.3c *Thermogram properties*: Phase behaviour of the PTX and drug loaded particles was studied by differential scanning calorimetry, using DSC-60 instrument. The instrument comprised of calorimeter (DSC 60), flow controller (FCL 60), thermal analyzer (TA 60) and operating software TA 60 from Shimadzu corporation, Kyoto, Japan. Approximately 2–10 mg of samples (pure drug and PTX nanoparticles) were placed in aluminium 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 (Musumeci *et al* 2006). Aluminium 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.

2.3d *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 nanoparticles was mounted on metal (aluminium) stubs, using double-sided adhesive carbon tape and fractured with a razor blade (Reddy *et al* 2004). The samples 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.

2.3e *Transmission electron microscopy (TEM)*: Morphology of nanoparticles 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 phosphotungstic acid. After drying, the specimen was viewed under microscope at 10–100 k fold enlargements at an accelerating voltage of 100 kV.

2.3f *In vitro drug release*: *In vitro* release studies were carried out using vial method as reported by Danhier *et al* (2009) and Verger *et al* (1998). Nanoparticles containing 5 mg 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 2 ml 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 PTX 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.

2.3g *In vitro cytotoxic activity*: The effect of pure drug and nanoparticles on the viability of BT-549 (breast cancer cell line) was determined by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay. Cells were cultured in Dubelco's modified essential medium (DMEM) with 10% foetal bovine serum containing pencillin (100 units/ml) and streptomycin (100 μg/ml). In all experiments, cells were maintained at 37°C in a humidified 5% CO_2 incubator. Briefly, 8×10^4 cells per ml were plated in 96 well microtiter plates in triplicates and incubated in 5% CO_2 incubator for 24 h. Then, 100 μl of freshly prepared concentrations (by serial dilution) of pure drug and nanoparticles was added in the growth medium and further incubated for another 48 h under same conditions (Fonseca *et al* 2002). 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 microtiter 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

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

2.3h *In vivo pharmacokinetic studies*: The pharmacokinetic studies were carried out in male wistar rats (150–200 g), obtained from central animal house and study protocol was approved by the Institutional Ethical Committee, Kasturba Medical College (IAEC/KMC/18/2008–2009), Manipal University, Manipal, India. They were housed and maintained at 25°C and 50% RH for 12 h light dark cycle. The animals were divided into two groups ($n = 6$), one group of animals were administered pure drug and other group received nanoparticles, injected intravenously with a dose of 5 mg/kg (Miura *et al* 2004; Straub *et al* 2005). The blood samples were collected at predetermined intervals, into heparinized tubes from the orbital sinus. The blood samples were immediately centrifuged using cold centrifugation (Remi Equipments Ltd., Mumbai, India) at 10,000 rpm for 5 min and the plasma was stored at -70°C until analysis.

2.3i *Bioanalysis of paclitaxel in rat plasma*: The bioanalytical method based on HPLC was used to quantify the drug in rat plasma. Chromatographic conditions are similar as mentioned in previous section. PTX stock solution, 1 mg/ml, was prepared using methanol as a diluent. Working standard solutions were prepared in the concentration range of 0·5–50 μg/ml of PTX and 10 μg/ml of celecoxib as an internal standard. The rat plasma (90 μl) was pipetted into micro-centrifuge tubes and spiked with 10 μl of internal standard (10 μg/ml of celecoxib-IS) and 10 μl of different standard solutions were spiked to get 0·05–5·0 μg/ml of PTX in rat plasma. To this, 200 μl of protein precipitating reagent, acetonitrile: methanol (1:1) mixture was added and vortexed for 5 min and then centrifuged at 10,000 rpm for 5 min at 4°C. To the clear supernatant, 100 μL of mobile phase mixture was added and injected into HPLC system. Standard curves were obtained by using drug/internal standard peak area ratio and theoretical concentration slopes, intercept and correlation coefficients were calculated. Pharmacokinetic parameters i.e. area under curve (AUC), elimination rate constant (k_e), half life ($t_{1/2}$), clearance, volume of distribution (V_d) and mean residence time (MRT) were determined using non-compartmental PK solutions 2 software (Montrose, USA).

2.3j *Statistical analysis*: The data obtained was statistically analysed 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 $p < 0{\cdot}05$.

3. Results and discussion

3.1 *Formulation development*

The choice of a particular method for encapsulation of drug substance in a colloidal carrier is most commonly determined by the solubility characteristics of the drug and

Table 1. Particle size and zeta potential with respect to various polymer: drug ratios.

Polymer: drug ratio	Particle size (nm)	Polydispersity index (PDI)	Zeta potential (mV)
5:1	96·05 ± 10·8	0·425 ± 0·22	−33·5 ± 5·1
10:1	163·7 ± 15·1	0·361 ± 0·12	−47·9 ± 8·6
15:1	178·5 ± 25·4	0·283 ± 0·09	−48·5 ± 9·5
20:1	195·8 ± 20·1	0·163 ± 0·07	−68·9 ± 12·8

polymer. Both, 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 and extreme unaffected particle size with better morphology (in terms of sphericity and discreteness). 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 PTX were successfully prepared. The size and surface morphology of PTX nanoparticles were confirmed by zeta sizer 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 (Mo and Lim 2005a, b). The faster the diffusion rate is, the smaller the particles would result. The reduction of the organic phase viscosity or the interfacial tension can facilitate the solvent diffusion and thus tends to produce the nanoparticles with smaller size. Hence, in the present work, the increased polymer quantity in sufficient solvent resulted in lower viscosity, which thus led to smaller particle size. The formulation was optimized based on physicochemical characterization and further this formulation was used for *in vitro* cytotoxicity against BT 549 cell lines (breast tumor) and pharmacokinetic studies.

3.2 *Encapsulation efficiency (EE)%*

To attain encapsulation of drug in optimum amount, trials were executed in different ratios of polymer to drug. Polymer to drug ratio of 5:1 showed 60·55 ± 3·6% followed by 89·8 ± 4·6%, 90·8 ± 5·8% and 94·5 ± 4·2% for 10:1, 15:1 and 20:1, respectively. 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. PTX-loaded biodegradable drug delivery systems manufactured from single emulsion technique with PLGA 50:50 were reproducible with highest encapsulation efficiency (94·5 ± 4·2) for the polymer to drug ratio of 20:1. The reason being, the high amount of polymer reduced drug loss during fabrication process and showed a drastic drug encapsulation enhancement. This demonstrates that particle size and EE could be significantly affected by the polymer amount when other formulation variables are kept constant. The order for encapsulation was found to be 20:1>15:1>10:1>5:1. The optimized formulation with highest encapsulation efficiency, i.e. 20:1 polymer to drug ratio, was further used for pharmacokinetic studies.

3.3 *Particle size and zeta potential*

The formulated nanoparticles 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 1. For the formulations with polymer to drug ratios, 5:1, 10:1 and 15:1, particle size were found to be 96·05 ± 10·8, 163·7 ± 15·1, and 178·5 ± 25·4 nm with polydispersity index of 0·425 ± 0·22, 0·361 ± 0·12 and 0·283 ± 0·09, whereas for 20:1 polymer to drug ratio having high encapsulation efficiency, particle size was found to be 195·8 ± 20·1 nm (figure 1) with polydispersity index of 0·163 ± 0·07, respectively. The zeta potential values were found to be in the range of −33·5 ± 5·1 to −68·9 ± 12. Size distribution pattern of particles plays an important role in determining the drug release behaviour, their feasibility for intravenous administration as well as their fate after *in vivo* administration (Potineni *et al* 2003). 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

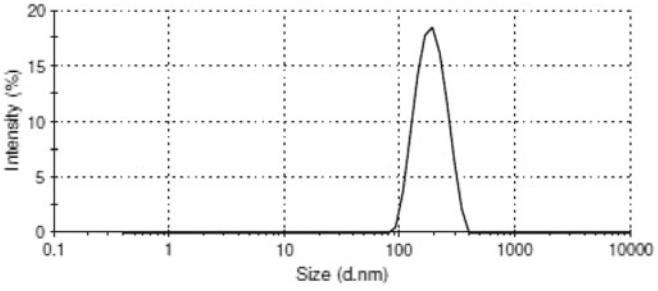

Figure 1. Particle size distribution of nanoparticles (polymer to drug ratio; 20:1).

value of polydispersity index indicates enhanced homogeneity of the nanosuspension which was observed with 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 nanoparticulate formulation through strong electrostatic repulsion of particles with each other. It is also an important factor to determine their interaction *in vivo* with the cell membrane. 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 nanoparticles surface charge could be observed. The value of negative charges on the particles were reduced from $-68{\cdot}9 \pm 12{\cdot}8$ to $-33{\cdot}5 \pm 5{\cdot}1$ mV as the polymer concentration decreased from 20:1 to 5:1 polymer to drug ratio. This may be due to unentrapped 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 (Shah *et al* 2009).

3.4 *Thermogram properties analysis*

The physical status of the native drug in the prepared nanoparticles was performed by DSC analysis. DSC thermogram of the pure drug and formulation are represented in figure 2. Thermal analysis is a useful tool for determining whether solute particles have been dispersed in polymeric matrices (Mu and Feng 2003; Dong and Feng 2007). Pure drug has characteristic endothermic melting peak at ~220°C with high intensity. From the DSC results, it was found that melting temperature of PTX was shifted to lower temperature than that of PTX itself due to amorphous state of PTX in the nanoparticles and also that part of the entrapped drug exists in crystalline form. The melting temperature of hydrophobic drug in the inner core shell type nanoparticles was generally shifted to lower temperature due to amorphous nature of drug in nanoparticles. It also revealed that particles are uniformly dispersed at the molecular level in the nanoparticles.

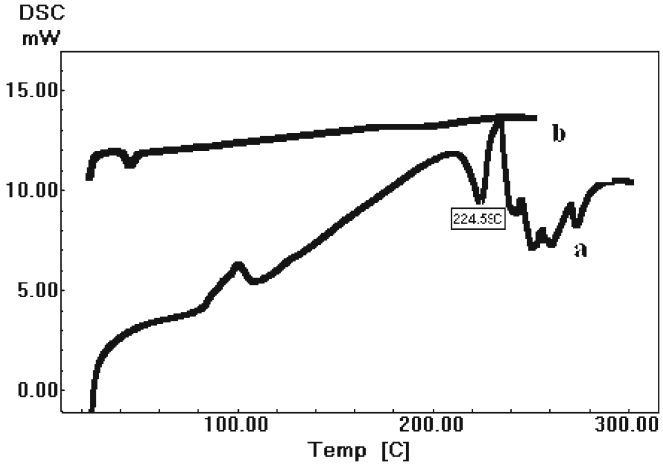

Figure 2. DSC thermogram of **a**. pure drug and **b**. PTX loaded PLGA nanoparticles.

3.5 *Scanning electron micrographs (SEM)*

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 3. The particles have moderate uniformity and all the particles were discrete entities, did not cause aggregation of

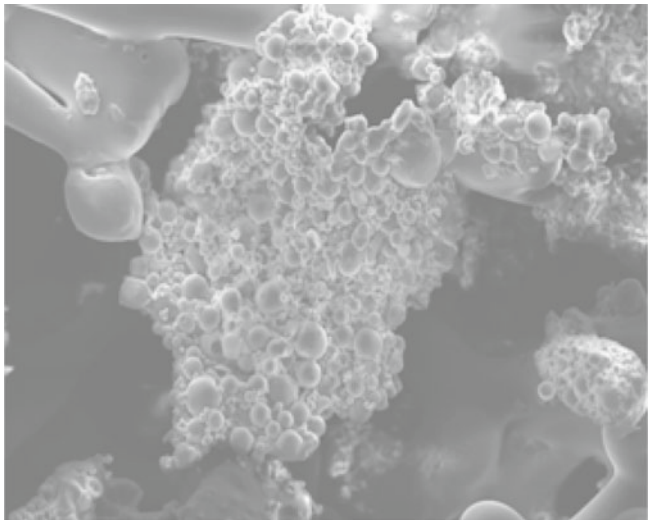

Figure 3. Surface morphology of PTX loaded PLGA nanoparticles.

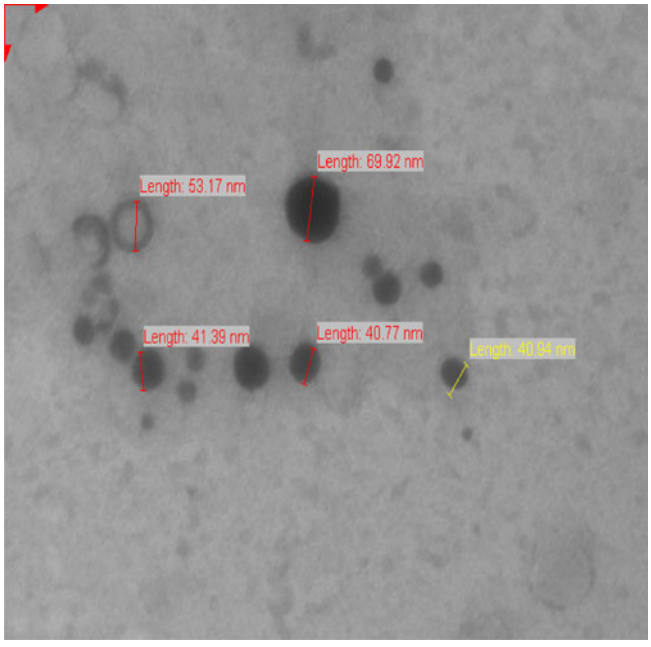

Figure 4. Transmission electron microscopy of PTX loaded PLGA nanoparticles.

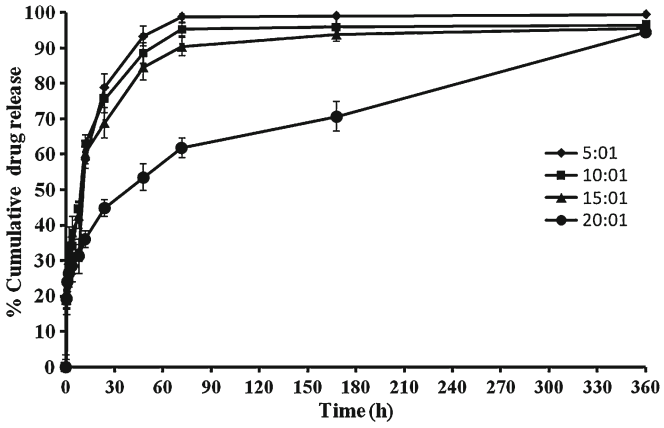

Figure 5. *In vitro* % cumulative drug release (CDR) profile of different formulations.

particles after lyophilization and these particles were readily redispersible.

3.6 *Transmission electron microscopy (TEM)*

The nanoparticles prepared were found to be spherical in shape with nanosize range, supported by SEM analysis as discussed in the above section. TEM image of nanoparticles is shown in figure 4.

3.7 *In vitro release study*

It is evident from the drug release curves that 5:1 polymer to drug ratio showed 93·3 ± 2·4% of drug release within 48 h, followed by 88·6 ± 3·8% drug release for 10:1 and 78 ± 4·3% for 15:1, respectively whereas polymer to drug ratio, 20:1 showed a slower release profile, the release rate was reduced up to 53·5 ± 2·8% 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 PTX from nanoparticles may depend on drug diffusion and PLGA surface and bulk erosion or swelling. The release behaviour of PTX from the developed nanoparticles 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 (Mainardes and Evangelista 2005, Huang *et al* 2007) which is depicted in figure 5. A high initial burst release was observed and can be attributed to the immediate dissolution and release of PTX 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 polymer to drug ratio 20:1 when compared to all other formulations.

3.8 *In vitro cytotoxic study*

In the cytotoxic activity, cells were incubated with concentrations, 0·01, 0·1, 1 and 10 μg/ml of both pure drug and PTX loaded in PLGA nanoparticles and was evaluated by assessing cell viability by the MTT assay using the BT 549 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 6a and b, 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 a marked reduction in cell viability (≈80%) was observed when BT 549 cells were incubated with 10 μg/ml of pure drug at 37°C, no further cytotoxic effect was observed for the longest incubation times (Mo and Lim 2005a, b; Jin *et al* 2009). 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

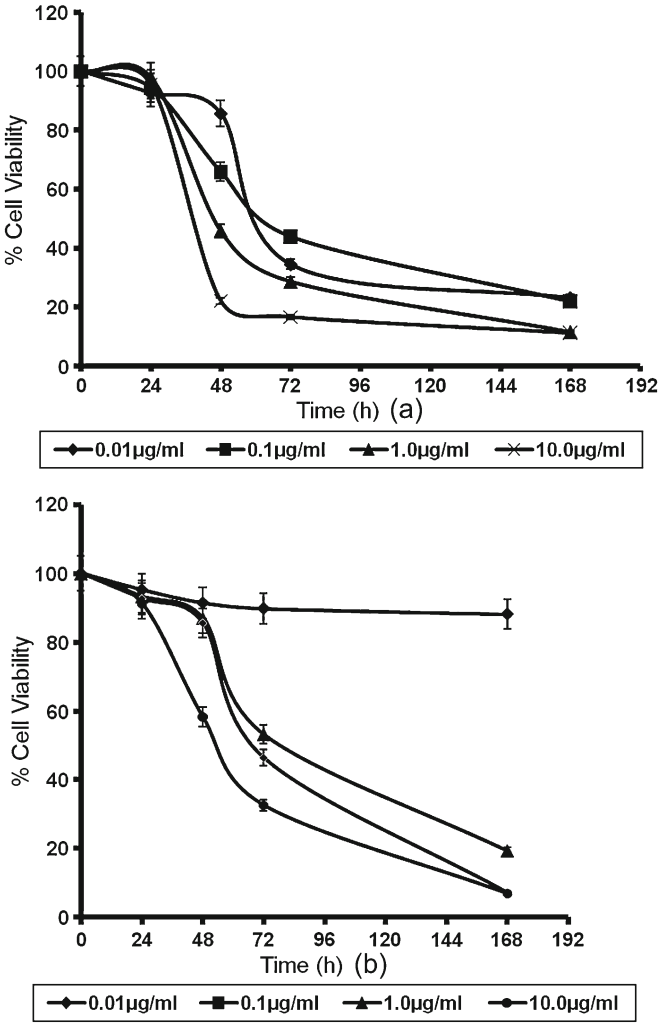

Figure 6. **a.** Percentage cell viability for pure drug with different concentrations and **b.** percentage cell viability for PTX loaded PLGA nanoparticles with different concentrations.

Table 2. Pharmacokinetic parameters of paclitaxel loaded PLGA nanoparticles compared to pure drug.

PK parameters	Units	Pure drug (PTX)	Nanoparticles
$AUC_{(0-\infty)}$	μg.h/ml	6·108 ± 2·81	19·032 ± 5·34**
Elimination rate (Kel)	l/h	0·163 ± 0·09	0·0208 ± 0·002**
Clearance (Cl)	ml h^{-1} kg^{-1}	0·748 ± 1·82	1·273 ± 0·91**
Volume of distribution (Vd)	ml/kg	3·5 ± 2·89	4·56 ± 0·96**
Elimination half life ($t_{1/2}$)	h	5·003 ± 2·86	33·19 ± 5·79**
Mean residence time (MRT)	h	4·6 ± 1·8	26·1 ± 6·2**

** $p < 0{\cdot}05$, at 95% CI

nanoparticles. Cell growth was constantly inhibited for PTX nanoparticles up to 168 h of incubation when compared to pure drug indicating slow release of paclitaxel. 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 μg/ml, whereas for 1 μg/ml and 10 μg/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 PTX loaded nanoparticles were observed.

3.9 *In vivo pharmacokinetic studies*

The effect of optimized formulation over pure drug was subjected to pharmacokinetic analysis. The pharmacokinetic parameters have been summarized in table 2. The values of area under the concentration-versus-time curve ($AUC_{0-\infty}$), mean residence time (MRT) and $t_{1/2}$ of PTX nanoparticles were found to be much higher (3–4 times) for PTX vectored by PLGA (50:50) nanoparticles than for pure drug. The plasma concentration time profile of pure drug and nanoparticles are depicted in figure 7. The plasma drug concentration for nanoparticles was detectable up to 48 h which may be due to the slow clearance rate leading to greater enhancement in elimination half life and correlates well with *in vitro* release data. The results showed nanoparticles had significantly improved the exposure, reduced the clearance, and raised the volume of distribution and mean residence time. This may be attributed to the sustained release of PTX from nanoparticles. The *in vitro* release profile had good correlation to the release results *in vivo* by measuring plasma drug concentration profile in pharmacokinetics experiment by intravenous injection. The reason for prolonged release was considered to be nanoparticles were relatively long circulating ($t_{1/2}$ 33·19 h) with low clearance rate when compared to pure drug. Nanoparticles showed significant changes in pharmacokinetic profile compared to pure drug. It can be observed that there was a statistically significant difference ($p < 0{\cdot}05$) in pharmacokinetic parameters when PTX was formulated in the form of nanoparticles at 95% confidence interval (CI).

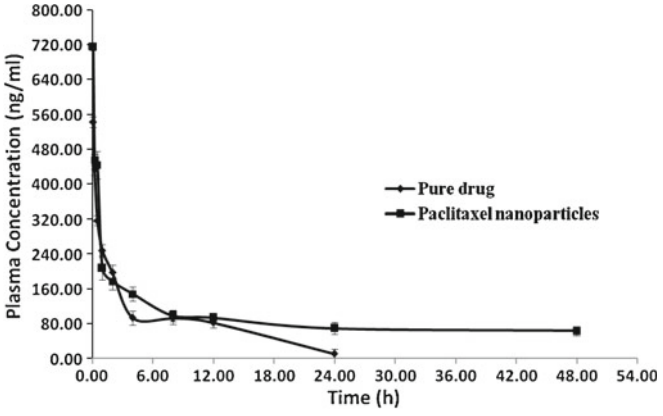

Figure 7. Pharmacokinetic profile of pure drug and paclitaxel loaded PLGA nanoparticles.

4. Conclusion

The objective of the study was accomplished by formulating nanoparticulate drug delivery system for PTX using PLGA with controlled release and anti-tumor activity was established for the first time against BT-549 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 breast cancer.

Acknowledgements

The authors would like to acknowledge Getwell Pharmaceuticals for gift sample of paclitaxel and the Department of Science and Technology (DST), Govt. of India, for providing financial support to conduct research on nano drug delivery systems (Project S.No. SR/NM/NS-47/2008).

References

Ahlin P, Kristl J, Kristl A and Vrecer F 2002 *Int. J. Pharm.* **239** 113

Danhier F, Lecouturier N, Vroman B, Jérôme C, Marchand-Brynaert J, Feron O and Preat V 2009 *J. Control Release* **133** 11

Dong Y and Feng S 2007 *Int. J. Pharm.* **342** 208

Fetterly G J and Straubinger R M 2003 *AAPS PharmSci.* **5** E32

Fonseca C, Sergio Simoes S and Gaspar R 2002 *J. Control Release* **83** 273

Huang C, Chen C and Lee Y 2007 *Int. J. Pharm.* **338** 267

Jin C, Bai L, Wu H, Song W, Guo G and Dou K 2009 *Pharm. Res.* **26** 1776

Li X, Xu Y, Chen G, Wei P and Ping Q 2008 *Drug Develop. Ind. Pharm.* **34** 107

Mainardes M R and Evangelista C R 2005 *Int. J. Pharm.* **290** 137

Miura H, Onishi H, Sasatsu M and Machida Y 2004 *J. Control Release* **97** 101

Mo Y and Lim L Y 2005a *J. Control Release* **107** 30

Mo Y and Lim L Y 2005b *J. Control Release* **108** 244

Mu L and Feng S S 2003 *J. Control Release* **86** 33

Musumeci T, Ventura C A, Giannone I, Ruozi B, Montenegro L, Pignatello R and Puglisi G 2006 *Int. J. Pharm.* **325** 172

Potineni A, Lynn M D, Langer R and Amiji M M 2003 *J. Control Release* **86** 223

Reddy H L, Sharma K R, Chuttani K, Anil Kumar Mishra K A and Murthy R R 2004 *The AAPS Journal* **6** Article 23

Shah N, Chaudhari K, Dantuluri P, Murthy R S and Das S 2009 *J. Drug Target* **17** 533

Shenoy S V, Rajyaguru T H, Gude P R and Murthy R S R 2009 *J. Microencapsul.* **26** 471

Straub A J, Chickering E D, Lovely C J, Zhang H, Shah B, Waud R W and Bernstein H 2005 *Pharma. Res.* **22** 347

Verger M L L, Fluckiger L, Kim Y, Hoffman M and Maincent P 1998 *Eur. J. Pharm. Biopharm.* **46** 137

Xua Z, Gu W, Huang J, Sui H, Zhou Z, Yang Y, Yan Z and Li Y 2005 *Int. J. Pharm.* **288** 361

Zhang Z and Feng S 2006 *Biomaterials* **27** 4025