In-vitro cell line studies

5. In-vitro Cell Line Studies

5.1 Introduction

After the preliminary studies the final objective was to deliver these nanoparticulate carriers by parenteral route to animals. But before the animal studies, the formulations should be evaluated for their safety and efficacy at tissue culture level. The aim of the study was to apply NPs of biodegradable polymers for cancer chemotherapy with a view to explore the possible effects of particle size, conjugation and particle surface coating on the cell uptake of the drug loaded NPs. Hence, the cell uptake and cytotoxicity studies were taken up prior to in-vivo studies. Cell uptake studies were carried out using coumarin-6 (lipophilic fluorescent dye) loaded NPs with the aim of finding whether the NPs are internalized into the cells and to determine the intracellular concentrations of the loaded dye. Cell cytotoxicity assay was carried out to measure the ability of the cells to survive and to continue to proliferate. Here the mitochondial activity or enzyme activity of the cells, which are able to proliferate after long exposure to PTX, was measured. This assay is based on the measurement of the mitochondrial activity of viable cells by the reduction of the tetrazolium salt 3-(4,5-dimethyathiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to a blue product formazan. Formazan is solubilized in isopropanol (IPA).

5.2 Materials

RPMI 1640, phosphate buffer saline (PBS) and 3-(4,5-Dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemicals, India. Fetal calf serum and Penicillin-Streptomycin were purchased from Hyclone, Utah, USA. Cell lysis buffer was procured from Promega, Madison, USA. Isopropanol was purchased from S.D.Fine Chem, India.

5.3 Methods

5.3.1 Cell Culture

C6 rat glioma cell line was purchased from National Centre for Cell Science (NCCS), Pune. The cells come from brain tissue and its morphology is glioma. The rat glioma cell line C6 was cultured as monolayer, growing as adherent monolayer in RPMI-1640 medium

supplemented with 10% fetal calf serum and 1% penicillin/streptomycin at 37°C in the atmosphere of 5% CO_2 and 90% relative humidity. The cells were harvested with trypsin-ethylenediaminetetraacetic acid. Medium was changed every other day.

5.3.2 Fluorescent microscopy and Intracellular uptake of coumarin-6

For fluorescent microscopy, 1 X 10^6 Cells were seeded on a glass slide and allowed to attach overnight. The cells were incubated with 400 µg/ml coumarin-6 loaded nanoparticle suspension for 4 h. The NPs suspension was removed and the cells were washed with PBS and observed under a flourescent microscope (Olympus BX61, Japan) at excitation and emission wavelength of 430nm and 485 nm respectively and images captured using Cytovision 3.1 software.

For the quantitative cell uptake experiment, the cells were seeded at a density of 1 X 10^4 cells/well in the 96-well plate (black) (Nunclone®surface, Nunc, Roskilde, Denmark) and incubated with coumarin-6 loaded nanoparticle suspension (50, 100 and 200 µg/ml in medium). For control coumarin-6 solution was added in the well. Medium in the plate was changed every other day until 80% confluence was reached. The medium was then replaced with 100-µl medium with coumarin-6 loaded nanoparticles of different concentrations. To examine the specificity of Tf receptor-mediated uptake of the Tf-conjugated NPs, cells were incubated with an excess dose of free Tf (50 µg) for 1h prior to incubating with NPs. The plates were incubated for 0.5, 1, 2 and 4hrs. At different time intervals, suspension was removed and the wells were washed three times using PBS to remove uninternalized nanoparticles. After adding 100µl of cell lysis buffer to break the cells, add 100 µl acetonitrile to the wells and shake the plate. The plate was read using a microplate reader (Synergy HT, Biotek, Vermont, USA).The excitation wavelength and emission wavelength was 430 and 485 nm, respectively, for coumarin-6. As coumarin-6 was assumed dispersed evenly in particles, the amount of particles was assumed to be linearly proportional to the microplate readings. The cellular uptake efficiency was given by the ratio between the amount of coumarin-6 that taken up in the cells to that added.

5.3.3 In-vitro cell cytotoxicity assay

C6 rat glial cells were transferred to 96-well plate (Cellstar®, Greiner bio-one, Germany) to ensure 1 X 10^4cells/well and allowed to attach overnight. The medium was changed with 100-µl medium containing PTX loaded NPs and PTX solution of different concentrations (0.1-40ug/ml). After incubation for 24, 48, and 72h suspension was removed and the wells were washed with PBS. 10µl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5mg/ml) and 90 µl of medium were added and incubated for around 3-4h. Media containing MTT was removed, leaving the precipitate. 100µl of isopropanol was added to the wells to dissolve the formazon crystals. Plate was observed at 570nm using microplate reader (Biotek® Powerwave XS, Vermont, USA). Cell viability was determined using the formula in Eq. (1)

$$Cell\ viability = \frac{Absorbance\ of\ test}{Absorbance\ of\ control} * 100 \qquad \text{Eq. (1)}$$

where Absorbance of test and control cells represent the amount of formazan determined for cells treated with the different formulations and for control cells (nontreated), respectively. Statistical evaluations were made using student t-test (α=0.05) and ANOVA and differences greater than $p<0.05$ were considered significant.

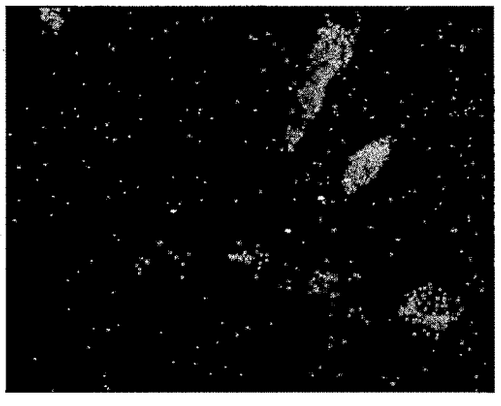
5.4 Results and Discussion

C6 rat glioma cell line was chosen for this study because it is known to express P-glycoprotein which is responsible for the multidrug resistance phenomenon (Lamprecht and Benoit, 2006). PTX is a Pgp substrate hence it gets effluxed out of the cell resulting in a decreased efficacy of the antitumor agent. In order to determine the effect of the nanoparticulate formulations on the Pgp expressing cells as well as the sensitivity of the glioma cells to PTX, the cytotoxic effect of PTX was evaluated by its inhibitory effect on the cell proliferation.

5.4.1 Intracellular uptake efficiency

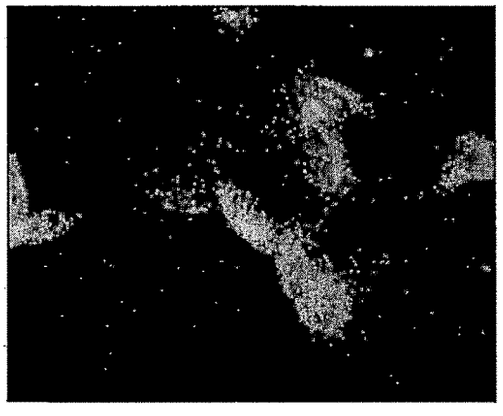
Particles labeled with fluorescent dyes are frequently used to study cellular uptake quantitatively by microplate reader. Particle cellular uptake could be affected by many

factors such as, particle size (Panyam et al., 2003), different cell lines and cell densities (Jung et al., 2000), different compositions of the particles, surface properties (surface hydrophobicity and surface charge) (Jung et al., 2000 and Foster et al., 2001). Coumarin-6 was incorporated as a lipophilic fluorescent marker in NPs to study the intracellular uptake. The advantages of coumarin-6 include the requirement of low dye loading in NPs due to its high fluorescence activity (Panyam et al., 2003). The dye does not leach from the NPs during the experimental time frame and therefore the fluorescence seen in the cells is caused by NPs and not by free dye. The NPs internalize rapidly into the cells.

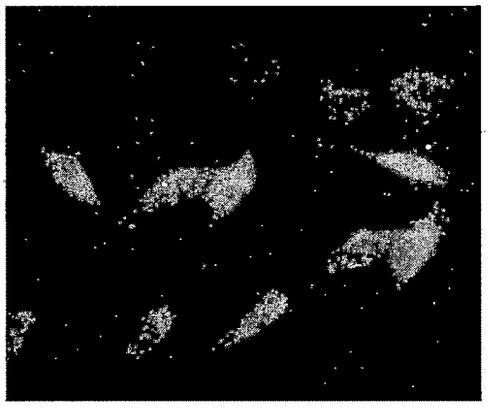


(a) PLGA NPs

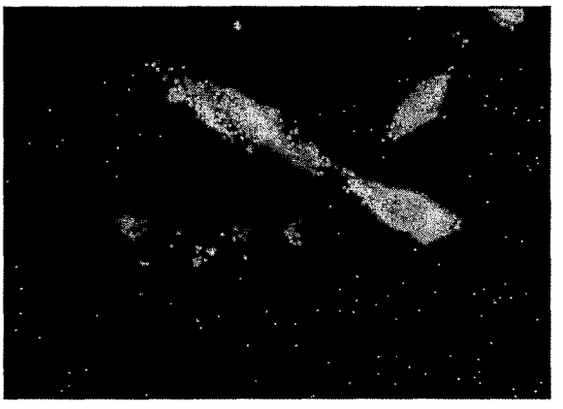
(b) Tf conjugated PLGA NPs



(c) Pluronic®P85 coated PLGA NPs



(d) PBCA NPs



(e) GTS SLN

Figure 5. 1: Fluorescent images of intracellular uptake of coumarin-6 loaded nanoparticle formulations after 4hrs incubation

From the fluorescent images we can observe a green fluorescence in the cell cytoplasm and around the nucleus which reflects the penetration of NPs into the nuclei. Based on this observation, it can be hypothesized that the nanoparticulate carrier may carry PTX like active molecules across the cell membrane by endocytosis as well as transport effectively into cell nuclei. Also the fluorescence intensity was more pronounced in case of Tf-conjugated PLGA NPs (attributed to receptor mediated endocytosis) and Pluronic®P85 coated PLGA NPs in comparison with unconjugated PLGA NPs (Figure 5.1a, b and c). Figure 5.1d and 5.1e depicts the intracellular uptake of PBCA and GTS SLN. In all the cases it is evident that coumarin-6 loaded NPs have internalized into the cell cytoplasm.

Figure 5.2 to 5.7 represents the quantitative uptake of coumarin-6 solution and NPs in C6 rat glioma cells. The uptake of coumarin-6 was found to be concentration and time dependent. Both had a positive effect on uptake efficiency. The fluorescence intensity and hence the intracellular uptake efficiency increased with incubation time in the cells treated with dye loaded NPs. The uptake efficieny of Tf-conjugated NPs and Pluronic®P85 coated PLGA NPs was found to greater compared to the unconjugated NPs. The specificity of Tf receptor mediated binding of conjugated NPs was evident from the reduced uptake of Tf-conjugated NPs in the presence of free Tf. The increase in fluorescence intensity in the cells treated with coumarin-6 loaded NPs seems to be due to slow intracellular release of the encapsulated dye from the NPs that are localized

inside the cells. (Sahoo et al., 2005), untreated cells did not show any autofluorescence. At high concentrations at four hours there is not much increase in uptake efficiency suggesting that the cells were reaching their saturation capacity. The uptake efficiency of coumarin-6 solution and coumarin-6 loaded NPs are shown in table 5.1.

Table 5. 1: Uptake efficiency (%) of coumarin-6 solution and coumarin-6 loaded NPs (n=3)

Time (hrs)	PLGA NPs			PLGA NPs coated with Pluronic®P85		
	Uptake efficiency (%)			Uptake efficiency (%)		
	50ppm	100ppm	200ppm	50ppm	100ppm	200ppm
0.5	10.30 ± 1.06	14.67 ± 2.04	20.36 ± 1.67	21.31 ± 0.45	32.47 ± 2.09	38.14 ± 1.20
1	16.87 ± 1.47	27.58 ± 1.12	32.47 ± 0.58	38.52 ± 1.43	50.21 ± 1.32	63.01 ± 0.97
2	34.29 ± 0.94	38.31 ± 1.28	43.18 ± 0.93	47.23 ± 2.87	56.94 ± 1.78	67.45 ± 1.04
4	43.56 ± 1.02	56.43 ± 0.93	57.23 ± 0.82	55.82 ± 1.74	61.27 ± 1.08	68.43 ± 0.99
Time (hrs)	Tf-PLGA NPs			Competitive uptake of Tf-PLGA NPs		
	Uptake efficiency (%)			Uptake efficiency (%)		
	50ppm	100ppm	200ppm	50ppm	100ppm	200ppm
0.5	25.46 ± 2.24	36.14 ± 0.87	42.46 ± 0.59	12.43 ± 1.63	17.39 ± 2.65	21.45 ± 2.87
1	43.25 ± 1.87	53.62 ± 1.27	63.67 ± 0.94	17.93 ± 1.89	29.84 ± 1.37	33.87 ± 3.01
2	57.87 ± 1.94	71.39 ± 1.15	72.16 ± 1.46	35.71 ± 3.19	38.69 ± 2.31	45.61 ± 1.24
4	60.35 ±0.96	75.70 ± 1.63	76.84 ± 0.97	42.89 ± 0.94	59.02 ± 1.19	59.94 ± 1.89
Time (hrs)	PBCA NPs			GTS SLN		
	Uptake efficiency (%)			Uptake efficiency (%)		
	50ppm	100ppm	200ppm	50ppm	100ppm	200ppm
0.5	24.78 ± 1.96	35.89 ± 0.68	40.28 ± 1.47	16.84 ± 0.75	18.23 ± 1.42	25.64 ± 1.49
1	45.23 ± 1.18	50.17 ± 1.27	62.45 ± 2.58	20.36 ± 0.97	26.31 ± 1.78	38.12 ± 2.82
2	54.70 ± 2.83	70.25 ± 2.51	71.35 ± 1.96	37.21 ± 1.14	40.92 ± 1.69	45.37 ± 2.15
4	61.28 ± 1.49	74.26 ± 1.74	73.68 ± 1.87	45.68 ± 1.87	60.49 ± 2.63	58.21 ± 1.67

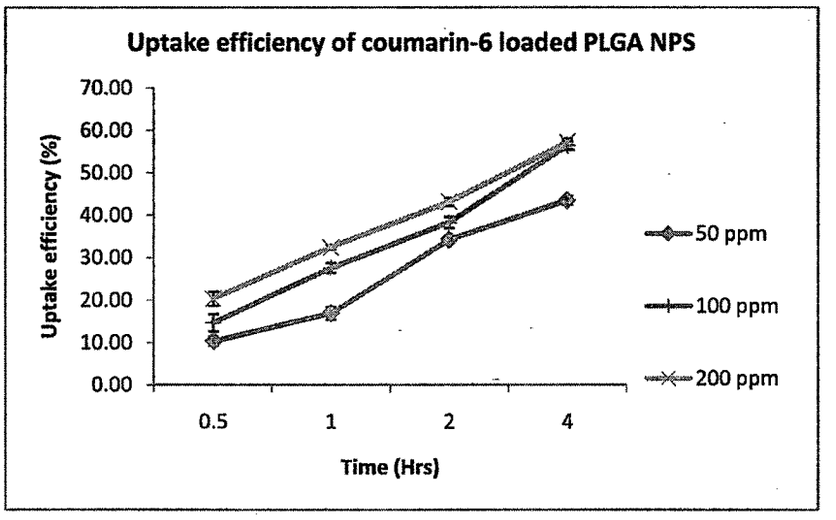


Figure 5. 2: Uptake efficiency of coumarin-6 loaded PLGA NPs

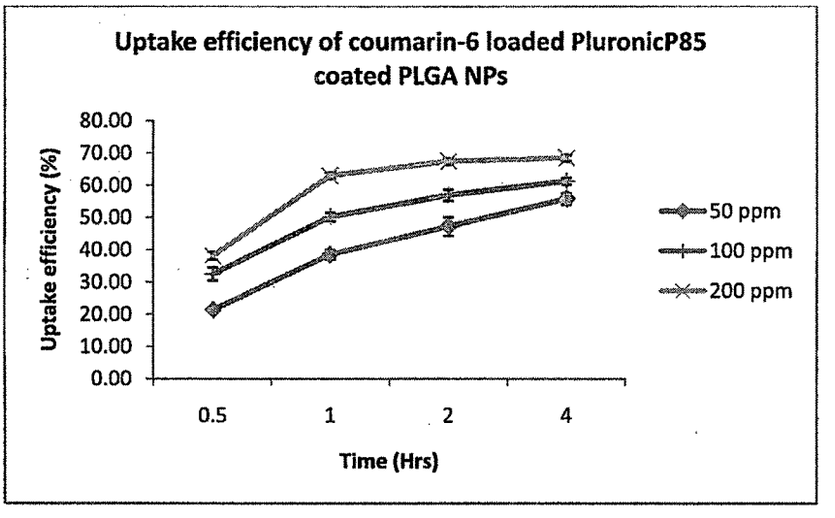


Figure 5. 3: Uptake efficiency of coumarin-6 loaded Pluronic®P85 coated PLGA NPs

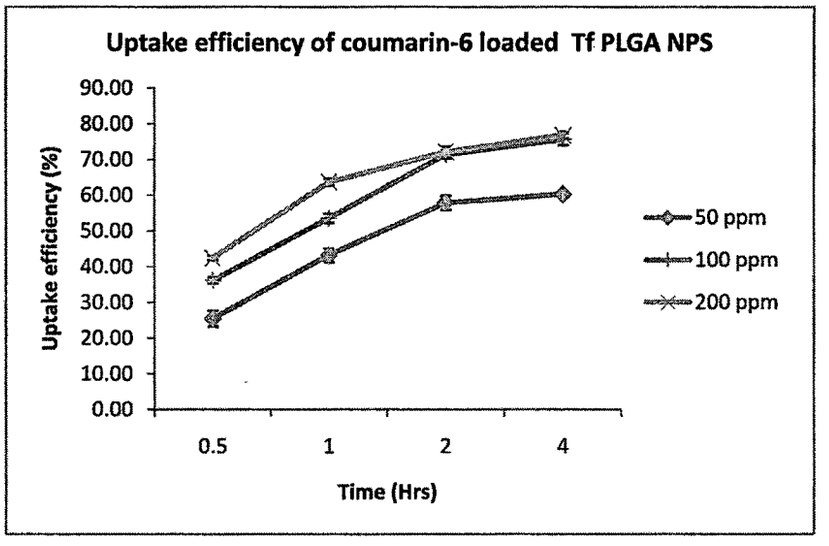


Figure 5. 4: Uptake efficiency of coumarin-6 loaded Tf PLGA NPs

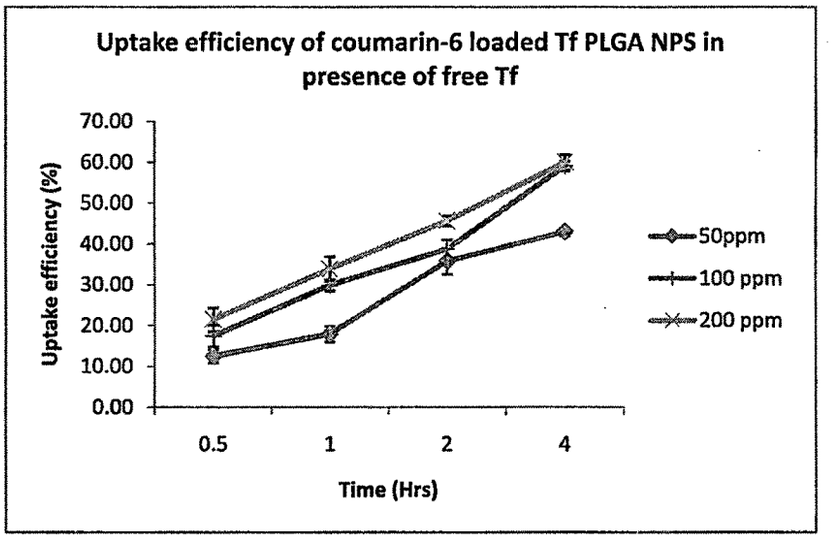


Figure 5. 5: Uptake efficiency of coumarin-6 loaded Tf PLGA NPs in presence of free Tf

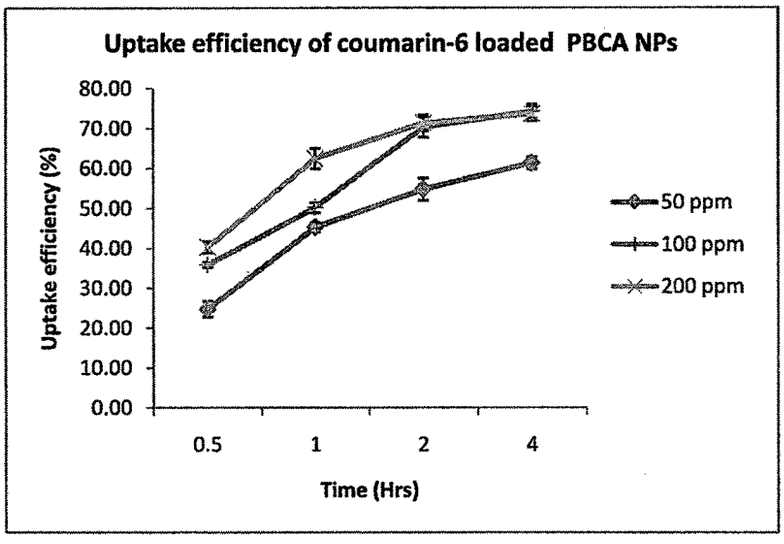


Figure 5. 6: Uptake efficiency of coumarin-6 loaded PBCA NPs

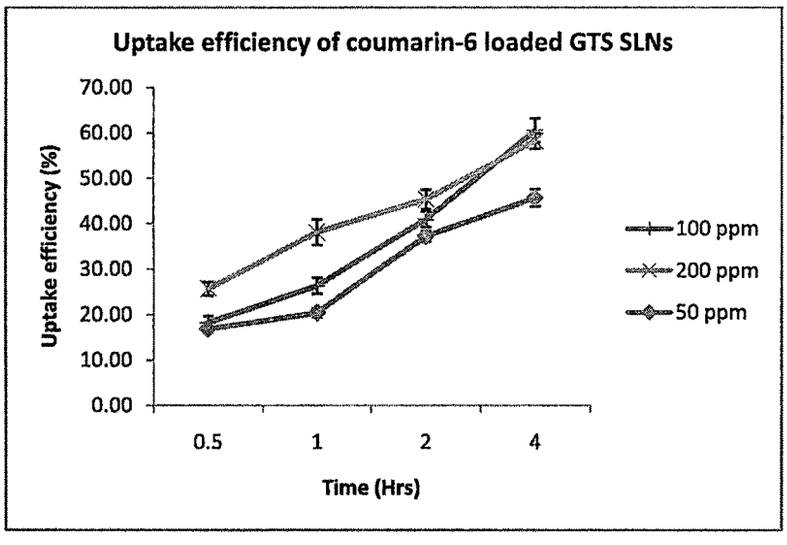


Figure 5. 7: Uptake efficiency of coumarin-6 loaded GTS SLN

5.4.2 In-vitro cell cytotoxicity assay

Widely employed, cell viability test or proliferation test was used to determine the potential carcinogenicity or cytotoxicity of the anticancer drug. The existing well described MTT assays to evaluate the cellular sensitivity to chemotherapeutics drugs was employed because of ease of use, optimal condition, measurement requirement, etc. MTT assay is based on the principle that, a tetrazolium (MTT) salt is added to the

media and upon internalizing into the cells, is reduced to a purple product (formazon) by the mitochondria dehydrogenase into the mitochondria of the live cells. The absorbance is recorded and the extent of the viability was defined as the relative reduction of the absorbance, which is directly correlated with the amount of viable cells in relation to the cell control (= 100 % viability).

The results of the cytotoxicity studies on C6 rat glioma cells confirm that during the nanoparticle engineering process PTX remained stable and cytotoxic. Blank NPs did not exhibit any significant toxicity on C6 cells at the highest concentration of the NPs used in the study which is demonstrated by 100% viability of the cells treated with blank NPs. The IC_{50} values for the free drug and different nanoparticulate formulations were estimated from the cell viability curves at different time points (Table 5.2). The cell viability of PTX and PTX encapsulated into NPs at different concentrations at different time points has been presented in (Table 5.3, 5.4 and 5.5).

Table 5. 2: IC50 values for PTX loaded NPs and free PTX

Time (hrs)		IC_{50} values (µg/ml)				
	PLGA NPs	P85 coated PLGA NPs	Tf-PLGA NPs	PBCA	GTS SLN	Free Drug
24	40.0	2.23	0.78	0.37	21.0	38.0
48	2.50	0.45	0.085	0.095	0.11	27.0
72	0.07	0.06	0.048	0.085	0.18	40.0

PTX encapsulated into different nanoparticulate carriers showed a very significant increase ($p<0.0001$) in cytotoxicity as compared to the free drug as greater amount of PTX could be delivered intracellularly by endocytosis in the nanoparticulate form. A strong sensitization of the cells with PTX NPs appears to decrease cell resistance. It is hypothesized that the sensitization was related to the ability of the C6 glioma cells to take up NPs with entrapped PTX and thus avoid P-glycoprotein efflux system. Being a Pgp substrate PTX in its free form is recognized by Pgp present on the C6 cells and gets

effluxed out of the cell as soon as it enters into the cells. The ability of the C6 cells to retain PTX loaded into nanoparticles indirectly confirmed that overcoming multidrug resistance by PTX NPs was related to the increased drug flux into the cell by encapsulation of PTX in NPs (Koziara et al., 2004). An important observation was increase in the antiproliferative activity of the drug with incubation time. The cytotoxic effect was found to be dose dependent. The results are in accordance with those reported by Dong and Feng, 2007. In case of free drug the cytotoxicity decreased at 72 hrs in comparison to 48 hrs which is indicative of the cell revival. The cytotoxicity of PTX in solution is less because of the Pgp efflux system. Sustained and increased antiproliferative activity with PTX loaded in NPs could be explained on the basis of the differences in the intracellular drug levels obtained with the plain drugs and drugs encapsulated in nanoparticles. Pharmacological response being directly proportional to the drug concentration, it is reasonable to believe that higher antiproliferative activity is a reflection of higher concentrations of drug available in the cytoplasm. The IC_{50} value for PLGA NPs was greater than free drug at 24 hrs whereas it reduced significantly after 48 and 72 hrs incubation period.

Although there was an increase in cytotoxicity in case of PLGA NPs in comparison to free drug but the effect was significant at 24 hrs ($p<0.05$). In the same case, very significant ($p<0.0001$) differences in cytotoxicities was found at 48 hrs and 72 hrs. Significant differences in cytotoxicities were found between Tf-conjugated PLGA NPs and free drug ($p<0.05$). A significant difference was found in cytotoxicities of unconjugated PTX loaded PLGA NPs and Tf-conjugated PTX loaded PLGA NPs at all time points ($p<0.05$). The greater antiproliferative activity of PTX could be because of greater intracellular uptake of conjugated NPs via Tf receptors than that of unconjugated nanoparticles (Li and Quain, 2002). Alternatively it is possible that Tf conjugated NPs have reduced exocytosis than unconjugated NPs thus resulting in a greater intracellular retention than unconjugated NPs. This greater retention could be a result of difference in uptake pathway between conjugated and unconjugated NPs. Also the toxicity was found to be time dependent. Lopes et al., (1993) have demonstrated that a long exposure to a particular dose is necessary to kill a higher percentage of cells. The longer exposure times allows more cells to enter mitosis phase. This explains the higher efficacy of Tf

conjugated PLGA NPs compared to unconjugated or free drug. Also, conjugated NPs are taken up via Tf receptors compared to the non-specific endocytic pathway of uptake for unconjugated NPs. For example, after binding of Tf to the Tf receptors of on the cell surface, the Tf-receptors complexes are internalized to form endosomes through clatrin-coated vesicles. After internalization, iron loaded Tf releases its iron at low endosomal pH, whereas iron free Tf remains bound to the receptor. These complexes are sorted into exocytic vesicles for delivery back to the cell surface and iron free Tf is released. The entire Tf cycle takes only 4-5 min with a mean transit time of about 10 mins and avoids the lysosomal compartment (Klausner et al., 1983; Bali et al., 1991; Wagner et al., 1994). Since the Tf receptor has an ability to recycle within minutes after endocytosis, multiple intracellular delivery of Tf conjugated NPs through use of a single receptor could occur, thus leading to a greater drug delivery (Sahoo et al., 2004). The IC_{50} value for Tf conjugated PLGA NPs was very low compared to the drug in solution.

Significant differences were found in cytotoxicity of Pluronic®P85 coated PLGA NPs in comparison to PTX solution at 24 and 48 hrs ($p<0.05$) but at 72 hours the difference was found to be very significant ($p<0.0001$). There is a significant difference between Pluronic®P85 coated and uncoated PLGA NPs ($p<0.05$). Although a significant difference was observed between cytotoxicities of Pluronic®P85 coated and Tf-conjugated PLGA NPs at 24 hrs ($p<0.05$) but it is non-significant at 48 and 72 hrs ($p>0.05$). Pluronic block copolymers were shown to be potent biological response modifiers capable of sensitizing multidrug resistant (MDR) cancer cells and enhancing drug transport across cellular barriers (Kabanov et al., 2002a and 2002b). It inhibits drug efflux transporters such as Pgp, multidrug resistance proteins and breast cancer resistance proteins. The hydrophobic PPO chains of Pluronics immerse into the membrane hydrophobic areas, resulting in alterations of the membrane structure, and decrease its microviscosity ("membrane fluidization") (Batrakova et al., 2008). It can be hypothesized that Pluronic®P85 inhibits the Pgp present on the cell membranes of C6 cells and results in an increased intracellular retention of PTX and hence an increased antiproliferative activity.

The difference in cytotoxicities of PBCA NPs and free drug was significant ($p<0.05$) at 24 and 48 hrs. This difference was very significant at 72 hrs ($p<0.0001$). Poly alkyl

The difference in cytotoxicities of PBCA NPs and free drug was significant ($p<0.05$) at 24 and 48 hrs. This difference was very significant at 72 hrs ($p<0.0001$). Poly alkyl cyanoacrylate (PACA) nanoparticles have been proven to be effective in inhibiting Pgp mediated multidrug resistance (Couvreur and Vauthier et al., 1991; Vauthier et al., 2003). Hence we can hypothesize that PTX incorporated into PACA NPs would bypass the Pgp mediated drug efflux hence a greater antiprofliferative activity is observed in comparison with free drug solution.

Although there was an increase in cytotoxicity in case of GTS SLN in comparison to free drug but the effect was non-significant at 24 hrs ($p>0.05$). In the same case, significant differences in cytotoxicities was found at 48 hrs and 72 hrs ($p<0.05$). The enhanced cytotoxicity of PTX incorporated into SLN may be related to fast internalization of PTX loaded SLN followed by the drug's release from the SLN inside the cells, enhancing its action (Serpe et al., 2004). Another possibility that SLN could mask PTX characteristics and thus limit its binding to Pgp, which consequently would lead to cell uptake of the otherwise effluxed drug (Koziara et al., 2004).

The in-vitro drug release data for PLGA NPs (unconjugated and conjugated) suggests that about 60% of the drug is released with the first three days hence the cytotoxicity of PTX PLGA NPs is greater than that of solution. In case of PBCA NPs about 60% of drug is released in just 12 hours hence it is more cytotoxic and in SLN about 50% of the drug is released within 12 hours and sustained upto 48 hrs, it can be concluded that SLN are more toxic than free drug. In all the case at all time points the NPs are more toxic than free drug which can be attributed to sustained drug release from NPs.

Table 5. 3: Cell viability of PTX and PTX NPs at 24 hrs

Concentration (µg/ml)	Cell Viability (%) ± SD (n=3)					
	PLGA NPs	Pluronic® P85 coated PLGA NPs	Tf-PLGA NPs	PBCA	GTS SLN	Free Drug
0.1	80.17 ± 0.14	77.21 ± 0.32	73.43 ± 0.18	81.97 ± 0.29	78.21 ± 0.15	83.86 ± 0.19
1	64.76 ± 0.10	52.34 ± 0.45	47.69 ± 0.21	26.09 ± 0.18	68.32 ± 0.48	76.48 ± 0.15
10	53.84 ± 0.07	45.86 ± 0.98	18.01 ± 0.29	21.22 ± 0.36	54.12 ± 0.23	69.76 ± 0.18
20	56.34 ± 0.07	34.73 ± 1.20	20.34 ± 0.90	23.98 ± 0.08	50.13 ± 0.16	74.70 ± 0.15
40	50.22± 0.07	32.12 ± 0.97	18.17 ± 0.08	23.69 ± 0.12	46.87 ± 0.05	47.34 ± 0.10

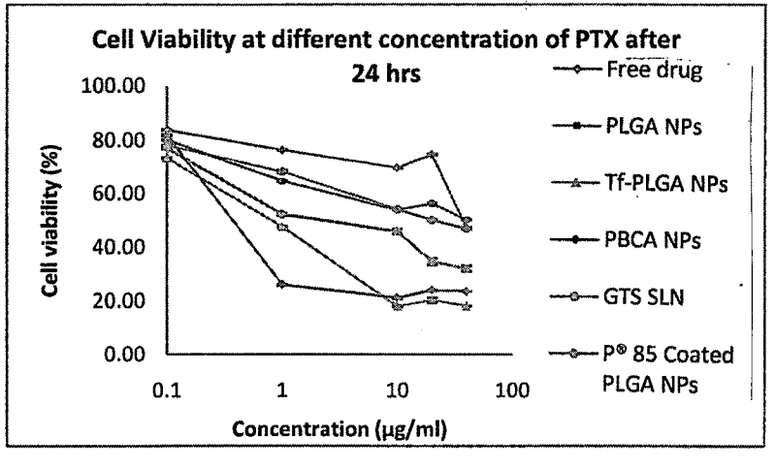


Figure 5. 8: Effect of PTX and PTX loaded into different nanoparticulate carriers on cell viability at 24 hrs

Table 5. 4: Cell viability of PTX and PTX NPs at 48 hrs

Concentration (µg/ml)	Cell Viability (%) ± SD (n=3)					
	PLGA NPs	Pluronic® P85 coated PLGA NPs	Tf-PLGA NPs	PBCA	GTS SLN	Free Drug
0.1	57.88 ± 0.09	53.76 ± 0.89	46.26 ± 0.12	50.30 ± 0.23	49.73 ± 0.06	72.97 ± 0.35
1	51.21 ± 0.08	48.32 ± 0.18	33.85 ± 0.07	35.12 ± 0.17	40.13 ± 0.13	67.03 ± 0.22
10	48.77 ± 0.09	35.83 ± 1.08	29.62 ± 0.18	28.23 ± 0.08	36.24 ± 0.28	64.99 ± 0.19
20	43.13 ± 0.07	30.15 ± 0.67	28.34 ± 0.21	15.28 ± 0.24	30.48 ± 0.09	61.58 ± 0.24
40	31.79 ± 0.05	20.16 ± 1.13	23.97 ± 0.96	17.26 ± 0.47	27.83 ± 0.15	39.60 ± 0.17

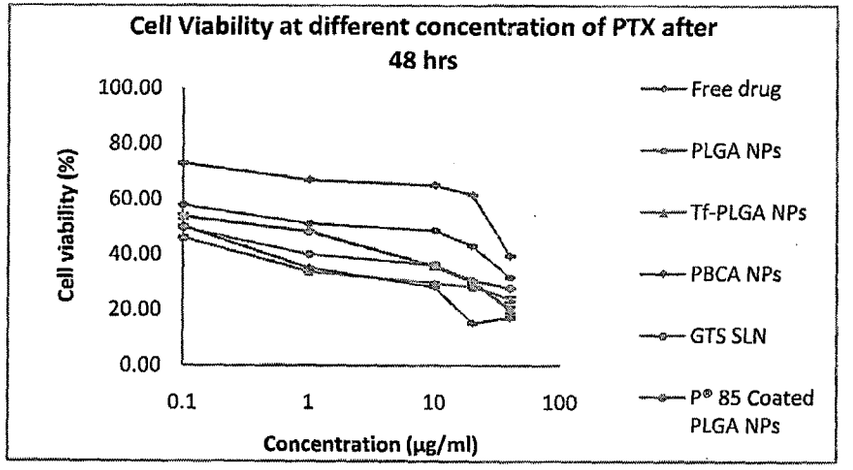


Figure 5. 9: Effect of PTX and PTX loaded into different nanoparticulate carriers on cell viability at 48 hrs

Table 5. 5: Cell viability of PTX and PTX NPs at 72 hrs

Concentration (µg/ml)	Cell Viability (%) ± SD (n=3)					
	PLGA NPs	Pluronic® P85 coated PLGA NPs	Tf-PLGA NPs	PBCA	GTS SLN	Free Drug
0.1	41.32 ± 0.08	34.89 ± 0.09	26.32 ± 0.24	45.76 ± 0.06	56.38 ± 0.09	75.41 ± 0.38
1	39.21 ± 0.11	29.23 ± 0.21	25.27 ± 0.09	30.98 ± 0.09	32.58 ± 0.52	70.16 ± 0.19
10	32.19 ± 0.10	24.87 ± 0.89	26.56 ± 0.12	25.75 ± 0.27	28.76 ± 0.06	67.39 ± 0.17
20	27.48 ± 0.10	20.89 ± 0.32	20.36 ± 0.26	18.92 ± 0.07	22.19 ± 0.27	65.29 ± 0.19
40	24.95 ± 0.10	17.35 ± 0.96	18.37 ± 0.08	14.29 ± 0.13	17.84 ± 0.12	50.47 ± 0.16

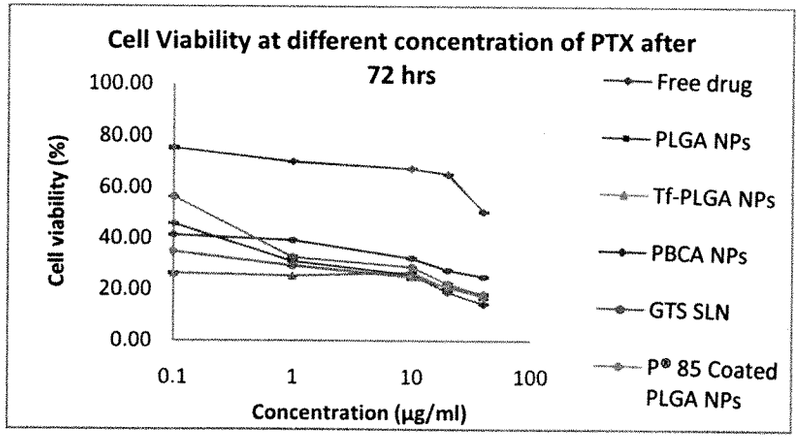


Figure 5. 10: Effect of PTX and PTX loaded into different nanoparticulate carriers on cell viability at 72 hrs

5.5 CONCLUSIONS

Blank NPs exhibited negligible cytotoxicity on the cells suggesting that NPs are suitable carriers for drug without any significant cytotoxic effects. A higher cytotoxicity was found for PTX loaded In NPs in comparison with free drug which could be ascribed to the internalization of the NPs by the cells as shown in the fluorescent images as well as the sustainable drug release feature of the NPs formulation. Also it can be concluded that PTX can be effectively delivered to resistant cells in the form of NPs without the drug getting effluxed out as in the case with free drug as C6 glioma cells express Pgp. In case of Tf conjugated PLGA NPs, receptor mediated endocytosis occurs which results in sustained drug delivery intracellularly. Since the action target of PTX is microtubules in the cytoplasm, the NPs are suitable carriers to deliver PTX into the cells.

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