Conclusion

Conventional cancer chemotherapy does not prove effective as drug molecule does not reach the target site at therapeutic concentration. An array of approaches and systems have been suggested to circumvent the goal of tumor targeting such as cellular carriers, vesicular systems, polymeric systems, dendrimers, etc. By virtue of their size, these carriers are cleared by the mononuclear phagocytic cell of the RES following intravenous administration. The passive vectoring as done by these carriers make ideal for tumor targeting. These carriers can be directed to tumor by means of extracorporeal guidance. Receptor based tumor selective delivery of therapeutic agent is a promising strategy for improving both the therapeutic efficacy and therapeutic index of cytotoxic drugs that exhibit dose-limiting toxicity.

NPs are attracting major attention as a promising colloidal drug carrier in the recent years. NPs are particulate dispersions or solid particles within size range of 10-1000nm. Nanoparticle formulation of anticancer drugs offers a number of advantages over the conventional dosage forms. In particular, it provides a sustained release of the drug targeted the desired site. Therefore, it would not only increase the therapeutic efficiency of the drug but also allow a reduction in the amount of drug administered and hence minimize the undesirable side effects. The uptake can also result from a specific recognition in case of ligand anchored NPs.

The most promising application of NPs is to deliver chemotherapeutic agents to the tumor. Enhanced endocytic activity and leaky vasculature of tumor favors accumulation of intravenously administered NPs. The anchoring of target specific ligand system to the NPs surface may facilitate their delivery to specific tumor sites. Selectivity in drug targeting can be achieved by the attachment of receptor specific ligand system (eg. folate, transferrin, ferritin, argenin, galactose, heparin etc.) as a site directing device. Folic acid receptors are overexpressed by various tumor cells including carcinoma of breast, prostate, pancreas, stomach, colon rectum, leukemia and lungs.

PLGA nanoparticles have enormous potential particularly in the field of drug delivery because of its unique properties. In last few years, nanoparticle based drug delivery has been dumped into the engineering science of these carriers to improve the physicochemical and biological behavior of the drug. PLGA nanoparticles are one of
these wonderful nanoconstructs used for drug delivery. But the higher RES uptake restricts their use, which is mainly responsible for low efficiency of these nanoparticles. To resolve these problems surface engineered nanoparticles are being developed. Targeted PEGylated nanoparticles consists PEGylation, which is one of such approach to reduce the RES uptake of these nanoconstructs.

Identification studies showed that the drug supplied by M/s Khandelwal Laboratories, Mumbai, matched with standared as prescribed in I.P. and Florey (1973). Drug was found to be yellow, crystalline powder, which matched with the description as per Florey (1973) (Table 3.1.1). The absorption maxima of Cisplatin in 0.1 N HCl was measured using spectrophotometer (Cintra 10 GBC UV, Japan) and was found to be 301 that matched exactly with that reported in Florey (1973) (Fig.3.1.1).

The solubility of Cisplatin in different solvents was evaluated and observation were analogous to that reported in Florey (1973) (Table 3.1.2). The drug was freely soluble in DMSO, soluble in DMF, insoluble in Chloroform, Acetone, Alcohol and methanol. The solubility of drug in water and PBS (pH 7.4) mainly depends on time as the time increases the solubility also increases (Florey 1973). The partition coefficient of Cisplatin in n-octanol:PBS (pH 7.4) and n-octanol:water showed that the was hydrophilic in nature, which makes it a good candidate for incorporation in PLGA nanoparticles (Table 3.1.3).the FTIR of Cisplatin conforms that its its important peaks was exactly matched with the reference peaks (Florey 1973) (Table, 3.1.4)

The standard curve of Cisplatin was prepared in PBS (pH 7.4) to facilitate quantitative estimation. The curve was found to be linear and straight line was observed in the concentration range of 0.2-20 µg/ml. the identity, purity, strength and quality (IPSQ) driven studies suggest that the drug under investigation is of required IPSQ standard and can be used for the formulation development.

The compatibility of the drug with Folic acid, Galactose, Heparin, mPEG and PLGA was observed spectrophotometrically. It was found that drug is compatible with the all and there was no significant change in absorption maxima was observed in any case (Table 3.1.8).
The nanoparticles were prepared by Solvent evaporation method (Patil et al., 2008; and Song et al., 2008) with slight modification, to obtain nanoparticles of optimal drug loading and particle size (Song et al, 2008). First of all folate conjugated-PEG-PLGA copolymer, galactose conjugated-PEG-PLGA copolymer, Heparin conjugated-PEG-PLGA copolymer and mPEG-PLGA copolymer were prepared using the standard method (described in the concerned section). The synthesis was confirmed by FTIR and, $^1$H NMR analysis (Fig, 3.2.1.9, 3.2.2.6, 3.2.3.7 and 3.3.1.1).

Non targeted nanoparticles (PEGyNPs and PLGA NPs) and targeted PEGylated Nanoparticles (FPNPs, GPNPs & HPNPs) were prepared and formulation were optimized for polymer concentration, drug concentration, sonication time and, stirring time. The variables were optimized on the basis of shape, size and entrapment efficiency. The 1 % w/v concentration of PLGA was found to be optimum because it has appropriate sized range of (171±1.2 nm) NPs with minimum polydispersity index (0.18). The 1 % w/v concentration of mPEG-PLGA was resulted an optimum because it have appropriate sized range of (180±1.4 nm) NPs with minimum polydispersity index (0.29).

Similarly targeted PEGylated nanoparticles showed significant increase in drug loading efficiency in comparison to PLGA nanoparticles and PEGyNPs, might be due to availability of more matrix for encapsulation of drug. Optimized polymer concentration in the case of Galactose-PEG-PLGA copolymer was found to be 1% w/v as it gives optimum particle size of 185±1.83 nm with least polydispersity index (0.21). Whereas for Heparin-PEG-PLGA copolymer and Folate-PEG-PLGA copolymer similar results were obtained. Optimum polymer concentration of Heparin-PEG-PLGA copolymer & Folate-PEG-PLGA copolymer was found to be 1% w/v as it gives optimum particle size of 189±1.39 & 191±1.4 nm with least polydispersity index of 0.22 & 0.20 respectively.

Further, drug concentration was optimized for maximum entrapment efficiency and optimal size of various nanoparticles. For PLGA nanoparticles, at a drug concentration of 200 µl / ml, gives higher entrapment efficiency of 78.8±0.41 % with optimal size of formulation (169.5±1.6) (Table 3.4.3.1, Fig 3.4.3.1). In case of
Conclusion

PEGyNPs the optimum drug concentration was also found to be 200 (µg/ml) as it gives optimum particle size of 184±1.54 with optimum entrapment efficiency of 85.6±1.32 (Table 3.4.3.2, Fig 3.4.3.2).

Similar results were obtained in the case of GPNPs, HPNPs and FPNPs, in each case optimum drug concentration was found to be 200 (µg/ml). GPNPs & HPNPs gives optimum entrapment efficiency of 80.1±1.03 nm & 81±1.02 nm respectively whereas FPNPs gives optimum entrapment efficiency of 79.6±0.51 nm (Table 3.4.3.3 to 3.4.3.5, Fig 3.4.3.3 to 3.4.3.5).

The effect of surfactant concentration was also optimized ranging from 0.5 to 5% w/v (PVA), it resulted that 1% concentration of surfactant was optimum for giving NPs (PLGA NPs, PEGyNPs, GPNPs, HPNPs and FPNPs) having smaller size and having good entrapment efficiency.

The sonication time was optimized from 30 sec. to 150 sec. 120 second sonication time was found to be optimized for PLGA NPs as it gives optimum particle size of 170±6.5 nm and optimum entrapment efficiency of 77.9±2.3 whereas in the case of PEGyNPs optimum particle size was found to be 178±4.01 nm with optimum entrapment efficiency of 79.2±1.63. Similar decrease in particle size and entrapment efficiency was also found in the case of GPNPs, HPNPs and FPNPs, optimum particle size was found to be 179±1.2 nm, 179±2.7 and 181±4.2 nm respectively with optimum entrapment efficiency 78.4±1.6, 78.9±1.8 and 78.9±3.1 respectively.

After that stirring time was also optimized from 4 to 7 Hrs which conforms that the 6 Hrs stirring time results in smaller particles having good entrapment efficiency for all NPs.

The Zeta potential of PLGA NPs was more negative as compared to PEGyNPs, GPNPs, HPNPs and FPNPs that might be due to presence of free –COOH group in PLGA polymer, and the conjugation of free –COOH group from NH₂ group of PEG results in decrease on Zeta potential of all other nanoparticles (Table 3.4.7.1).

The SEM and TEM photomicrograph exhibit that NPs are spherical in shape but the surface of PEGyNPs, GPNPs, HPNPs and FPNPs is less smooth and PEG chains
orientation was clear on that as compared to plain PLGA NPs (Fig 3.4.7.1, 3.4.7.2, 3.4.7.3, 3.4.7.4, 3.4.7.5 for SEM and 3.4.7.6, 3.4.7.7, 3.4.7.8, 3.4.7.9, 3.4.7.10 for TEM).

The drug release profile of optimized nanoparticle formulations (PLGA NPs, PEGyNPs, GPNPs, HPNPs and FPNPs) were checked for in vitro drug release study. Results shown in Table 3.4.7.2 indicates that drug release from optimized formulations were 24.18±1.4 % after 72 hr in case of PLGA NPs, while PEGylated nanoparticles exhibited 23.48±2.26 %, GPNPs exhibited 22.4±2.21 %, HPNPs exhibited 22.43±2.19% and FPNPs exhibited 22.41±1.11 % drug release in 72 hr. Data conforms that the GPNPs, FPNPs and HPNPs gives more sustained release mechanism as compared to PEGyNPs and PLGA NPs.

The stability data indicates that nanoparticles formulation stored at 4±1°C were more stable than those stored at 27±2°C moreover it was also inferred that targeted PEGylated nanoparticles (TPNPs) were more stable as compared to non PEGylated nanoparticles. This may be attributed to presence of PEG in the surface of TPNPs which results in presence of hydrophilic group in the surface as compared to PLGA nanoparticles and thus aggregation might be increases in PLGA nanoparticles.

Hemolytic toxicity of plain drug solution polymer (PLGA) or copolymer solution (mPEG-PLGA, Galactose-PEG-PLGA, Heparin-PEG-PLGA and, Folate-PEG-PLGA) & drug loaded NPs observed. Result conforms that (Table 3.6.1.1) plain drug solution exhibits highest hemolytic toxicity. Whereas PEGylated nanoparticles (PEGyNPs, GPNPs, HPNPs and, FPNPs) exhibited comparatively lesser hemolysis that could be attributed to reduction in hydrophobicity due to presence of hydrophilic layer around the polymeric core.

MDA-MB-231 cells-

In Fig; 3.6.2.1.1 it shows that drug (Cisplatin) concentration in the range of 12.5 µg/ml was highly cytotoxic than other concentrations. Fig; 3.6.2.1.2 explains that PLGA concentration in the range of 750µg/ml was more cytotoxic to the cells than other polymer concentrations. According to Fig 3.6.2.1.3 it was concluded that PLGA nanoparticles & PEGylated Nanoparticles loaded with drug (optimized drug and polymer
Conclusion

Having less cytotoxic effect as compared to FPNPs, GPNPs, HPNPs loaded with drug. Fig; 3.6.2.1.3 conforms that FPNPs are more cytotoxic then compare to other targeted PEGylated Nanoparticles (GPNPs & HPNPs).

According to Fig. 3.6.2.1.12 highest uptake of rhodamine loaded FPNPs was found to be increased up to 84.45 % in 72 hrs (Fig. 3.6.2.1.12), whereas PLGA nanoparticles and PEGyNPs uptake was found to be 65.26 % & 75.23 % respectively in 72 hrs (Fig. 3.6.2.1.12) which confirms that FPNPs was highly taken up by the cells via receptor mediated endocytosis method and thus uptake was gradually increased as time increases. Other GPNPs and HPNPs also confirmed the receptor mediated endocytosis thus their uptake was increased as compared to PEGyNPs and PLGA NPs, but expression of their receptors are limited as compared to folate receptors thus uptake was found to be low. Result conforms that folate saturated cells (Fig. 3.6.2.1.12) also explained non receptor mediated simple endocytosis mechanism (due to complete saturation of receptors from excess available ligands (folate)) and the results were almost same with slight difference to PLGA NPs & PEGyNPs.

HeLa cells-

In Fig; 3.6.2.2.1 it shows that drug (Cisplatin) concentration in the range of 10.0 µg/ml was highly cytotoxic than other concentrations. Fig; 3.6.2.2.2 explains that PLGA concentration in the range of 750µg/ml was more cytotoxic to the cells than other polymer concentrations. According to Fig 3.6.2.2.3 it was concluded that PLGA nanoparticles & PEGylated Nanoparticles loaded with drug (optimized drug and polymer conc.) having less cytotoxic effect as compared to FPNPs, GPNPs, HPNPs loaded with drug. Fig; 3.6.2.2.3 also confirms the highest cytotoxicity effect of FPNPs as compared to other targeted nanoparticles (GPNPs and, HPNPs).

Almost same cell uptake results were obtained in the case of HeLa cells. Highest uptake was found from Folate targeted PEGylated Nanoparticles might be due to highly expression of their receptors on HeLa cells (Fig. 3.6.2.2.11).

A 549 cells-
In Fig; 3.6.2.3.1 it shows that drug (Cisplatin) concentration in the range of 12.0 µg/ml was highly cytotoxic than other concentrations. Fig; 3.6.2.3.2 explains that PLGA concentration in the range of 750µg/ml was more cytotoxic to the cells than other polymer concentrations. According to Fig 3.6.2.3.3 it was concluded that PLGA nanoparticles & PEGylated Nanoparticles loaded with drug (optimized drug and polymer conc.) having less cytotoxic effect as compared to FPNPs, GPNPs, HPNPs loaded with drug.

In the case of A 549 cells highest cell uptake were obtained from GPNPs as previously reported that galactose targeted carriers are highly accumulated in the lung due to increased expression of Asialoglycoprotein receptors. Other results were almost same as found in other melanoma cells (MDA-MB-231 & HeLa cells) (Fig. 3.6.2.3.11)

From Acridine orange study it is conformed that targeted nanoparticles with excess expression of receptors are localized in the inner portion of cell and thus they produces maximum apoptosis as compared to other Ligand anchored nanoparticles without sufficient expression of their receptors as well plain drug solution.

In this study the FPNPs and GPNPs are found to be efficient to produce apoptosis whereas other NPs and plain drug solution are less effective to produce apoptosis.