1. Cisplatin as a model drug for cancer:-

The use of cisplatin is restricted due to its high toxicity i.e. nephrotoxicity, gastrointestinal toxicity, ototoxicity, cardiotoxicity and neurotoxicity when it is given in nonsite specific manner, thus it limits its therapeutic potential. Past researches (Li et al., 2008) confirmed that cisplatin if given in site specific manner proves it a superior antitumor activity producing drug.

So that from our approaches we found its cytotoxic activity against different cell lines. Targeted encapsulated cisplatin produces more cytotoxicity as compared to plain drug solution. Encapsulated cisplatin also found to disrupt the tumor nucleolus vasculature if given in site specific manner.

2. Making of copolymer :-

In the present investigation we made different copolymers (Folate-PEG-PLGA, Galactose-PEG-PLGA and, Heparin-PEG-PLGA). The synthesis of copolymer were successful and the % yield was also acceptable. In our approach we made copolymer by two methods-

I. Graft copolymerization method

II. Bioconjugation method

Both the methods were successfully applied and both methods were used to produce copolymers.

The comparative % yield conforms that graft polymerization produces high % yield of copolymers whereas conjugation method also gives copolymers with acceptable % yield but the % yield was low as compared to graft polymerization method.

First time copolymer of Galactose-PEG-PLGA, Heparin-PEG-PLGA was synthesized and compared.

3. Making of nanoparticles:-

Till date no studies have been reported of Galactose targeted PEGylated NPs and Heparin targeted PEGylated NPs. First we did the conjugation and made the NPs of Galactose targeted and Heparin targeted NPs.
First time in our approach we used conjugated copolymer as a polymer matrix to form NPs. Before that past researchers conforms the attaching of ligands either by coupling reaction or by cross linking method or some researchers are also used preformed conjugates (Folate-PEG-PLGA) to the polymer solution so that the hydrophobic portion will orient towards the core.

In our study we prepared NPs directly from prepared conjugates. This is first innovation for us.

**4. Enhancement of entrapment efficiency:-**

Past research confirms that hydrophilic drug gives low entrapment efficiency within the lipophilic polymer. But from our study it can be conformed that making of hydrophilic coating improves the encapsulation efficiency of drug from $78\pm0.05\%$ to $80\pm1.01\%$. So that the present approach can be used to improve the encapsulation efficiency of hydrophilic drugs in hydrophobic core.

**5. Sustained release:-**

From our study it is clear that the non PEGylated NPs (PLGA NPs) showing initial burst release whereas PEGylated NPs (PEGyNPs, FPNPs, HPNPs and GPNPs) conforms reduction in burst release. The drug release mechanism were more sustained in PEGylated NPs & can be controlled (zero order) due to presence of one additional layer over the polymeric core. The presence of hydrophilic additional layer makes the carrier more sustained as compared to plain NPs (without PEGylated i.e. PLGA NPs).

**6. Site specific delivery of carrier:-**

From present study the formation of ligand anchored PEGylated NPs were conformed and evaluated by SEM and TEM. The capabilities of endocytosis by receptors were also evaluated using different ligands (i.e. folate, galactose and heparin). Result conforms the potency of ligands to recognize their specific receptors in the cell. The internalization process of carrier was also evaluated.

**7. Nuclear disruption and induction of Apoptosis:-**
The acridine orange study photomicrograph conforms that the ligand anchored PEGylated nanoparticles conforms the delivery of nanocarriers into the deeper portion of malenoma cells (near to nuclear portion) which enables them to detain in the cellular portion nearby nucleolus in which the efflux mechanism of cancer cells unable them to efflux out from the cell due to deeper cytosolic presence of carriers. The photomicrograph taken after acridine orange study confirms that the ligand anchored PEGylated NPs disrupts the nucleolus & induces the apoptosis mechanism.

8. Receptor specific cell uptake:
The three different cell line studies confirm that expression of receptors plays important role during the internalization of nanocarriers. In one additional study of receptor blockage by ligand saturation to the cell effects the internalization of nanocarriers and the uptake was found to be low.

Future opportunities:

1. SURFACE MODIFIED CISPLATIN:

As now a day’s some of the drugs are in clinical trial which were directly linked to the surface modifying agent (like – surfactant (poly ethylene glycol, Tween, PVA) polymer (PLGA, PGA, PLA, HPMA, Gelatin, Eudragit, Starch), copolymer (L-glutamic acid bound drug, N-(2-hydroxypropyl)-methacryl-amide (HPMA) copolymer conjugates with drug, Cisplatin-PLGA-PEG, drug-PLGA-PEG-ligand, to give better pharmacological effect or to revert their demarits.

Example of few surface modified moieties which are in clinical trial- Poly(ethylene glycol) (PEG)-bound camptothecin (Conover et al., 1998); poly(L-glutamic acid)-bound taxol (Li et al., 1999); N-(2-hydroxypropyl)-methacryl-amide (HPMA) copolymer conjugates with doxorubicin (Vasey et al., 1994; Thompson et al., 1999); PEG modified adenosine deaminase (Hershfield, 1997) is in use for patients with severe combined immunodeficiency disease; PEG modified L-asparaginase (Holle, 1997) is prescribed for
patients with acute lymphoblastic leukemia, which are hyper-sensitive to the native forms of L-asparaginase).

In our present work we have encapsulated cisplatin in the polymeric core. If we can conjugate the cisplatin with the PEG directly or with the cisplatin-PEG and ligand then this conjugation can also be used for targeting.

2. CAN TREAT MULTIDRUG RESISTANT :-

The ability of cancer cells to become cross-resistant to a variety of structurally and functionally unrelated drugs is termed MDR. This factor is a major hurdle in the fight against cancer as it renders many chemotherapeutic drugs useless. MDR is classified as either intrinsic, if the tumour cell is inherently resistant to chemotherapy, or acquired, if the tumour relapses after treatment (Mansouri et al., 1990). MDR modulators are a group of drugs that can inhibit or reverse the processes that cause cancer cells to become resistant. As there are a multitude of cellular events that can lead to the development of MDR, the pool of MDR modulators is growing steadily. Acquired MDR is commonly caused when the cancer cell i) activates drug metabolizing enzymes, thus prematurely inactivating chemotherapeutics (Bradley et al., 1988; Harris and Hochhauser, 1992; Morrow and Cowan, 1990), ii) activates DNA repair mechanisms, thus undoing the work of many chemotherapeutics (Bradley et al., 1988; Harris and Hochhauser, 1992), iii) blocks the apoptotic signalling cascade, thus inhibiting the cell-death signal (Bradley et al., 1988; Harris and Hochhauser, 1992; Reed 1995; Mueller and Eppenberger 1996), or, most importantly, iv) pumps anticancer drugs out of the cell through efflux pumps from the ATP-binding cassette (ABC)-transporter family, such as P-glycoprotein (P-gp) (Bradley et al., 1988; Harris and Hochhauser, 1992; Gottesman et al., 2002).

**Multi- Drug resistance can be overcome by:-**

1. By achieving selective drug accumulation in tumor cell.
2. By the use of long circulating carriers (PEGylated).
3. By modifying surface property to achieve cytosolic drug delivery.
4. By using specific ligands having deeper penetration on cell.
5. PEG itself work as a Class III glycoprotein pump inhibitor, so it will inhibit the pumps involved in efflux mechanism.

In our present investigation we used some constituents which make them to overcome the difficulties of Multidrug resistance (MDR) i.e. –

**Folic acid and Galactose-**

Provides deeper cellular internalization & nucleus directed release due to caveolin assisted receptor mediated endocytosis (Murthy et al., 2003), (iii) due to strong uptake of folic acid at cellular nucleus (Kim et al., 2007) can manage efflux mechanism of P-glycoprotein (P-gp) causes multidrug resistance (MDR) (Sharon, 1997).

**PEG -**

Coats hydrophilic layer over the surface of carrier thus MDR receptors (hydrophobic vacuum cleaner) will unable to efflux them out (Sharon, 1997).

In our present work the effect of NPs (PLGA NPs, PEGyNPs, FPNPs, GPNPs, HPNPs) on managing MDR is not checked. From the past researches it evident that the targeted PEGylated NPs are potential to manage the MDR cells and thus can be useful to treat the cells with MDR effect.

3. **CAN MAKE MULTIFUNCTIONAL NANOPARTICLE (MNPs)-**

Multifunctional NPs means NPs having ability to do multiple works as compression to single working nanocarriers. In our present designed NPs (TPNPs) if we can also incorporate imaging, contrasting agent on it (i.e.- γ-Fe₂O₃, Fe₃O₄, gold, Gadolinium-157 and, Quantum dots) so that real time in vivo monitoring can be possible.

As we used only one drug (hydrophilic), we can also incorporate another drug on it (hydrophobic) as per previously reported by Song et al., 2008.