Chapter – 12

CONCLUSION
12.1 INTRODUCTION AND LITERATURE REVIEW

Cancer is the disease state in which normal growth controlling mechanisms are permanently impaired, permitting the progressive growth of cells without reaching growth equilibrium. These cells show unlimited replication potential, abnormal differentiation and improved survival. About 85% of all the cancers are solid tumors. This abnormal behavior arises out of the activation of certain specialized genes called protooncogenes to Oncogenes in the presence of physical, chemical and viral carcinogens. Once group of such cells are activated, these divide unlimitedly, causing the neighbouring normal cells to strive. There are over 200 different known cancers that afflict humans. Morphological changes that take place in a cancer cell and actual pathogenesis were discussed in detail. Cancer is the leading cause of death worldwide, as per WHO worldwide statistics 7.8 million died of cancer in 2008. A total of 1,638,910 new cancer cases and 577,190 deaths from cancer are projected to occur in the United States this year i.e. 2012. Based on the projections, cancer deaths will continue to rise with an estimated 9.0 million people dying from cancer in year 2015 and Deaths from cancer worldwide are projected to continue rising, with an estimated 13.1 million deaths in 2030. The main objective of this research project was to synthesize a nano based system in such a way that enhances the solubility of hydrophobic chemotherapeutic drugs and makes its administration easy. The Chemotherapeutic drug selected was Paclitaxel. The rationale of behind selection of the drug being, the poor bioavailability of Taxanes derivatives (e.g. Paclitaxel and Docetaxel), Hypersensitivity reactions associated the currently marketed formulations of Taxol® and Taxotere®, Patient non-compliance, Highly toxic surfactants of already available formulations (Cremophore EL of taxol is...
hepatotoxic, nephrotoxic etc.). The literature review part actually discussed the various approaches for the treatment of cancer. Recent efforts in cancer research have been geared towards designing highly effective novel drug delivery systems. These are invariably aimed at delivering the potent drug moiety at the tumor site and are currently the major tools in the anticancer armamentarium and are therefore considered as the first vector in any combinatorial approach for cancer therapeutics. These include the produgs, various polymeric drug carriers and specially designed triggered release systems. Emphasis here is laid upon; Various Polymeric drug carriers that included Conjugates, Dendrimers, Micelles, Nanoparticles, Nanogels and Polymerosomes.

RESEARCH OUTCOMES

12.2 PREFORMULATION STUDIES

It included selection of the API. Complete Pharmaceutical profile and Preformulations studies were performed to confirm their identities. The selected drugs were Paclitaxel and Ellagic acid. Reason for selecting Paclitaxel is already discussed. However, Ellagic acid is only taken as a representative or model drug for making a nanosystem which can carry two hydrophobic moieties easily with a size being in nanorange. Although USFDA has clearly categorized it under Fake Cancer Cure, Patients should avoid. Our main intention was only to design a system which is non interacting with Paclitaxel and has the mechanism of action similar to the herbal drugs like Pomegranate Extract, Punica granatum L. Oil; Ext; Ofr which are categorized under Generally Regarded As Safe.

Reasons for selecting Ellagic acid despite of its Fake cancer Cure treatment label, being, its hydrophobicity, Purity and availability. The system is designed with an intention that can carry two hydrophobic moieties. Incorporating a hydrophilic moiety in a formulation is not an issue as it can easily solubilize i.e. go uptil a nanorange and can show its action. Whereas, administration of the hydrophobic drugs has always been a challenge. For this reason, we have not stressed upon taking punicalagins like moieties. Although, as we move ahead in the research work, we have designed systems keeping in mind which have the ability to carry both hydrophilic as well as hydrophobic moieties. Second reason being, the purity and easy availability. For pre formulation studies, physicochemical properties of the drug and various identification techniques have been employed for both Paclitaxel and Ellagic acid. Preformulation studies were then followed by development of analytical methodology.
12.3 DEVELOPMENT OF ANALYTICAL METHODOLOGY

A simple, rapid, precise and accurate isocratic reversed-phase High Performance Liquid Chromatography assay (HPLC) method was developed and validated for the routine analysis of Paclitaxel and Simultaneous determination of Paclitaxel and Ellagic acid in a combinatorial nanoformulation. Separation was achieved by using column with 25 x 4.6 mm, particle size 5 μm C18 reverse phase column (Luna) using a mobile phase consisting of methanol and 0.05% H3PO4 in gradient elution mode of mobile phase with flow rate of 1 mL/min at 230 nm using UV visible detector. A sharp and well defined peak was obtained at the retention time of 13.75 min. and 11.6 min. for Paclitaxel and Ellagic acid respectively. Regression analysis data showed good linear relationship \((r^2=0.996 \pm 0.0011)\) and \((r^2=0.993 \pm 0.0011)\) in a wide range of 5-500μg/ml and 1-500μg/ml for Paclitaxel and ellagic acid, respectively. LOD and LOQ of Paclitaxel were 30ng/ml and 100ng/ml respectively and for Ellagic acid LOD was found to be 300ng/ml and LOQ to be 1μg/ml. The accuracy of the method was determined by recovery studies using standard addition method and was found in the range of 99.61-101.21% and 98.70-102.22% for Paclitaxel and Ellagic acid respectively. The relative standard deviation (RSD) for precision, repeatability and robustness was less than 2%. A case study was performed by taking Punica granatum fruit extract instead of Ellagic acid. The Ellagic acid content in fruits of Punica granatum and combinatorial formulation with Paclitaxel was analyzed and found to be 0.04%w/w and 0.0012% w/w, respectively. The proposed, developed and validated HPLC method for the simultaneous quantification of Ellagic acid and Paclitaxel can be used for the quality control and standardization of several crude drugs and different combinatorial formulations, in which Ellagic acid is present.
RESEARCH OUTCOMES


12.4 DEVELOPMENT OF TEMPERATURE SENSITIVE POLYMERIC MICELLES

Temperature-sensitive amphiphilic polymer poly(N-isopropylacrylamide-co-PEG acrylate) has been synthesized and used to encapsulate paclitaxel, a highly hydrophobic anticancer drug, in core-shell nanoparticles formed by a membrane dialysis method. The lower critical solution temperature (LCST) of the nanoparticles is an important factor to control the release of the drug selectively inside the tumor. The polymer so formed has LCST well above the normal body temperature (37°C) under physiological conditions. The critical association concentration of the polymer is determined to be 10 mg/L. Paclitaxel can be easily encapsulated into the nanoparticles. The nanoparticles are spherical in shape, and their size was found to be below 200 nm. Its encapsulation efficiency is affected by fabrication temperature, initial drug loading and polymer concentration. In vitro release of paclitaxel from the nanoparticles is responsive to
temperature changes. Cytotoxicity of paclitaxel-loaded nanoparticles against MCF-7 cells is higher as compared to free paclitaxel. The temperature-sensitive nanoparticles would make a promising carrier for intracellular delivery of anticancer drugs.

12.5 DEVELOPMENT OF TEMPERATURE SENSITIVE POLYMERIC MICELLES FOR COMBINATION OF PACLITAXEL AND ELLAGIC ACID

We have a carrier system in our hand which is thermosensitive, nanosized and has the ability to release the drug at a controlled pace. Moreover its effectiveness on the cell lines also been confirmed. Although it can carry a single therapeutic agent, our next challenge is to use it for a combination therapy. A number of polyphenols have been studied to sensitize the tumor cells towards chemotherapy. In this study, paclitaxel has been combined with a model drug, ellagic acid and the same combination was then formulated with a nanosized delivery system and characterized.

Physical interaction between both the drug molecules was studied using DSC and simple visualization of the mixture at defined intervals. Assay for the simultaneous analysis of both the compounds was developed. NIPAAm based polymeric micelles were formed with outer shell containing ellagic acid and hydrophobic core containing paclitaxel in presence of a suitable initiator and activator, with Nitrogen atmosphere. The same was lyophilized and then reconstituted for further characterization studies. FTIR and NMR were used to study the polymerization reaction and UV analysis was used to study the LCST. Encapsulation efficiency was determined to check the concentration of both the surface coated and encapsulated drug. The formulation was also characterized for particle
size, zeta potential and surface morphology. In vitro release study was performed using
dialysis membrane method to know the simultaneous release of both the drugs.

12.6 DEVELOPMENT OF SOLID LIPID NANOPARTICLES FOR PACLITAXEL

One most important aspect that can’t be overlooked is that we have intentions of making
such system which can carry chemotherapeutic drug in combination with a herbal
 constituent. Herbal constituents being generally impure cannot be administered by
parental route. The study describes the development of solid lipid nanoparticles (SLNs)
as colloidal carriers for paclitaxel, a drug with very low solubility as described before.
SLNs are constituted mainly of bioacceptable and biodegradable lipids, such as that used
in this study is based upon its solubility along with drug; stearic acid with encapsulation
efficiency of 72.33%. SLNs are in the nanometer size range and can be sterilized and
freeze-dried. Release of paclitaxel from SLNs is burst release followed by controlled
release pattern. SLNs are stable over time without precipitation of paclitaxel and can be
proposed for its parenteral administration.

12.7 DEVELOPMENT OF SOLID LIPID NANOPARTICLES FOR ELLAGIC
ACID WITH PACLITAXEL

The study describes the development of solid lipid nanoparticles (SLNs) as colloidal
carriers for paclitaxel and ellagic acid combination. SLNs are constituted mainly of
bioacceptable and biodegradable lipids, such as the one used in this study is based upon
its solubility along with drug; stearic acid was used for encapsulation of paclitaxel as well
as Ellagic acid. SLNs are in the nanometer size range and can be sterilized and freeze-
dried. Release of paclitaxel and ellagic acid from SLNs is very low. SLNs are stable over
time without precipitation of paclitaxel and combination can be given orally

**12.8 BIOLOGICAL STUDIES**

After having two nano sized systems in our hand, that are active upon cancer cell lines
with release kinetics as shown in the previous chapters. We intended to check the
functionality of these two formulations on cancer models. We have designed only a small
study to ensure the effectiveness of the two systems, detailed pharmacodynamics and
pharmacokinetics is beyond the scope of this research project. It should be noted that due
to regulatory reasons we have only checked the efficacy of NIPAAm PEG acrylate
carrier with/without paclitaxel and solid lipid nanoparticles with/without paclitaxel only.
As we have discussed in the previous chapters Ellagic acid does not have GRAS
certification therefore we have not included it for the in vivo studies. Safety analysis
studies have been carried out for the two carriers carrying the drug which included
mainly calculations of maximum tolerated doses of the two formulations. This was
followed by tumor regression studies on the suitable developed models.

**12.9 CONCLUSION**

As a gist of this research work we can say that we have developed a temperature sensitive
micellar formulation that has the capability of carrying two hydrophobic anticancer
moieties together. Temperature sensitiveness would allow the the drug release only at the
tumor site. Moreover, in order to improve upon the quality of life if these formulations
are intended to be given with herbal supplements, which are also helping in overcoming
multidrug resistance, suitable solid lipid nanoparticles were also developed. The
effectiveness of these formulations were studied in cancer cell lines and if we see In-vivo, a marked dose reduction has also been observed.