CHAPTER 7

SUMMARY

& CONCLUSION
7. Summary and Conclusion

Gold nanoparticles are inert, biocompatible and they can be easily synthesized in a variety of shapes including spherical, rod-like, core–shell, and many others with sizes ranging from 1 nm to more than 100 nm. The unique chemical, physical, and photophysical properties of gold nanoparticles can be exploited in innovative ways to control the transport and controlled release of anticancer agents. Thus gold nanoparticles are considered as one of the suitable drug delivery vehicles for efficient cancer therapy. Therefore in Chapter 1 the General introduction has been given with the current interest in this field.

In Chapter 2 Various literatures related to green synthetic methods of gold nanoparticles and its application in cancer drug delivery had been presented.

In Chapter 3 Scope and objectives were presented

In Chapter 4 Green synthesis of gold nanoparticles (GNPs) was achieved by using aqueous leaf extracts of *Pisonia grandis* and *Cardiospermum halicacabum* and fruit extract of *Persea americana* as the reducing and stabilizing agents. Pa-FE effectively reduced the HAuCl₄ and the reaction occurred rapidly at room temperature without the application of any external energy. Zeta potential of Pa-GNPs was found to -35.7 mV indicting that Pa-GNPs were highly stable. Among the synthesized GNPs, Pa-GNPs were found to be having long term stability and suitable for drug delivery applications. FESEM and TEM studies revealed that most of the Pa-GNPs were nearly spherical in shape. Pa-GNPs showed significant biocompatibility on NIH-3T3 fibroblast cell line. In addition, zebrafish studies ensured the *in vivo* safety of the Pa-GNPs. 6-mercaptopurine (MCP) was loaded on the Pa-GNPs and percentage loading was estimated to be about 54.04%. In-
vitro release studies confirmed the sustained release of MCP from the Pa-GNPs. MCP loaded Pa-GNPs exhibited superior anticancer activity than the free MCP in K-562 leukemia cell line. Flow cytometry analysis confirmed the arrest of cells at G2/M phase after treatment with Pa-GNPs-MCP, and western blotting analysis confirmed the occurrence of apoptosis in the K-562 leukemia cell line after Pa-GNPs-MCP treatment. The outcomes of present study indicate that Pa-GNPS could be effectively utilized for anticancer drug delivery applications.

Target drug delivery is the primary focus in cancer treatment to avoid unnecessary side effects, which were observed in conventional cancer treatment. The gold nanoparticles are attractive for targeted cancer therapy as they are appropriate for surface functionalization. Many tumor cells over express folate receptor on their surface that can be targeted using folic acid coupled gold nanoparticles to achieve efficient therapeutic activity.

Chapter 5 described the green synthesis of gold nanoparticles which was achieved by exploiting the antioxidant property of quercetin. The formation of quercetin reduced and dextran sulphate stabilised gold nanoparticles (DS-Q-GNPs) were confirmed by the observation of the surface plasmon resonance band at 520 nm. FE-SEM and TEM were performed to check the morphology of the synthesized GNPs. Both analysis revealed the presence of spherical and well dispersed GNPs with a narrow size distribution. XRD analysis confirmed the crystalline nature of the sample. Zeta potential of the DS-Q-GNPs was -42.45 mV indicating that they are highly stable. In-vitro stability studies indicated that DS-Q-GNPs were extremely stable at pH 7.4 indicating that DS-Q-GNPs were highly suitable for drug delivery applications. MTT assay using NIH 3T3
fibroblasts indicated that the DS-Q-GNP were biocompatible. Similarly in-vivo zebra fish toxicity studies revealed that DS-Q-GNPs were safe in in-vivo and suitable for variety of biomedical applications.

Doxorubicin was successfully loaded on DS-Q-GNPs and decorated with folic acid for targeting efficiency. High drug loading of the drug was achieved (90%) because electrostatic interaction between the anionic DS capped over GNPs and cationic drug DOX. In-vitro drug release studies revealed that the drug was released in controlled manner. It was also found that the DOX release from DS-Q-GNPs-FA was higher at pH 5.7 than pH 7.4 which is highly advantageous in cancer therapy because pH dependent drug release will not only help to achieve better therapeutic activity of drugs in cancer cells but also leads to reduction in the toxic effect of cancer drugs on normal cells.

The therapeutic activity of the doxorubicin loaded gold nanoparticles was evaluated using MCF-7 cell line. Based on IC_{50} values it was found that folate conjugated doxorubicin loaded gold nanoparticles showed better cytotoxic effect than free drug DOX and DOX-DS-Q-GNPs on MCF-7 cells. Moreover apoptosis by AO/EB staining method was carried out using MCF-7 cells and NIH 3T3 fibroblast cell line and this simple study confirmed the folate receptor based cell specific drug delivery of DOX-DS-Q-GNPs-FA. FACS analysis revealed that DOX-DS-Q-GNPs-FA induced the cell cycle arrest significantly at G2/M phase in the MCF-7 cells. Western blotting studies confirmed the increase in the apoptotic protein levels in the DOX-DS-GNPs-FA treated MCF-7 cells. Over all the following studies suggest that doxorubicin-loaded quercetin reduced and dextran sulphate stabilized gold nanoparticles conjugated with folic acid represent a new potential delivery system for targeted breast cancer therapy.
Chapter 6 described the successful synthesis of gold nanoparticles using a carbohydrate polymer, pectin by adopting microwave assisted one pot aqueous synthesis of gold nanoparticles (GNPs). Pectin acted as both as a reducing agent as well as stabilizer. The Pec-GNPs was characterized by PCS, UV-Visible spectroscopy, XRD and TEM. Pectin acted concurrently as both a reducing and stabilizing agent. EDS analysis proved the presence of gold in the sample. XRD analysis confirmed the crystalline nature of the synthesized GNPs. Spherical morphology of Pec-GNPs was demonstrated by TEM analysis. The FTIR spectrum revealed the capping of pectin on the surface of synthesised GNPs. Furthermore, the Pec-GNPs are found to be stable at different pH and electrolytic conditions. In vivo safety of the Pec-GNPs was established through zebra fish toxicity studies. Cationic drug doxorubicin was successfully loaded on the synthesized anionic Pec-GNPs by electrostatic interaction. High amount of DOX loading was achieved. In-vitro release studies confirmed the pH dependent sustained release of the doxorubicin i.e., doxorubicin release was remarkably lower at pH 7.4 than pH 5.7 ensuring that Pec-GNPs will help to reduce toxicity of DOX to the normal cells. DOX loaded Pec-GNPs were decorated with folic acid for achieving targeted delivery. Under in-vitro conditions it was noted that the folic acid functionalized DOX loaded Pec-GNPs showed excellent cytotoxicity on HT-29 colon cancer cells than free DOX. Flow cytometry analysis revealed that FA@Pec-GNPs-DOX caused death of HT-29 cells by G2/M phase arrest. Overall the in-vitro drug release, cytotoxicity, therapeutic efficacy results showed that FA@Pec-GNPs- DOX could be a promising alternative carrier for targeting colon cancer.
The versatile green procedures for the biosynthesis of gold nanoparticles have been shown to be effective in both the production and stabilization of biocompatible gold nanoparticles and it was found that the synthesized GNPs could be a unique drug carrier intended for cancer drug delivery applications.