Chapter 5

Summary and Future Perspective
As the wide range of chemical and physical properties achieved within nanomaterials has expanded, so has the number of nanomaterial-containing products. However, synthesis of high quality nanoparticles with respect to chemical purity, crystallinity, phase selectivity and monodispersity in particle size with controlled state of agglomeration in a cost effective procedure is still a challenge. The thesis explores the synthesis of different metal and metal oxide nanoparticles mainly based on environmental benign methods and their interactions with different biological molecules as well as different cancer cell lines. The technological breakthroughs related to innovative nanomaterials in medical and consumer products are accompanied by specific issues regarding the safety and life cycle of nanomaterials in the environment and, more particularly, in the human health. Understanding the interactions of nanomaterials over their entire life cycle is thus a pivotal challenge of nanoscience and a critical issue for societal acceptability.

The thesis describes the synthesis, characterization and their possible application in several fields of science and technology. We described easy synthesis of cuprous oxide nanoparticles and its conjugates with L-tryptophan and their effect on different cancer cell lines. Cuprous oxide (Cu$_2$O) nanoparticles have attracted considerable attention because copper is one of the most important metal in modern technologies and its reasonably large abundance readily available. It has also been reported that excitons can propagate coherently through single crystalline Cu$_2$O. Thus it is possible to convert photons into excitons, which then travel through small apertures or small dimension waveguides with little loss by scattering or diffraction. At the end of the path, the excitons can be converted back into photons. Cu$_2$O is a new type of p-type semiconductor with a direct band gap of 2.0 eV, which makes it a promising material for the conversion of solar energy into electrical or chemical energy. Interesting effect of Cu$_2$O nanoparticles has also been reported in biological filed. There are few reports that that nicely illustated cytotoxic behaviour effect of Cu$_2$O nanoparticles. In our investigation we have demonstrated the effect of Cu$_2$O and its conjugate with amino acid on different cancer cell lines. The spherical shaped Cu$_2$O nanoparticles was made by the hydrolysis of copper sulfate in alkaline medium followed by reduction with ascorbic acid in the presence of sodium dodecyl sulfate as a stabilizing agent and made its surface modification with L-tryptophan. Spectroscopic charaecterization was made.
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by using UV-Vis spectroscopy, FT-IR, TEM, and AFM techniques. We established the interaction of these nanoparticles with different serum proteins using fluorescence and circular dichroism spectroscopy. Finally, we have performed the cytotoxicity test on three different cell lines such as human breast cancer cell line MCF 7, kidney cancer cell line HEK 293, and hepatocellular carcinoma cell line Hep G2. The unmodified Cu$_2$O nanoparticles were found to be toxic to different cultured cancerous cells. Interestingly, the tryptophan conjugated Cu$_2$O nanoparticles attenuate the toxicity as compared to unmodified Cu$_2$O nanoparticles. This result indicated its possible utility in developing a drug candidate for cancer in a controlled fashion. Reduced toxicity also indicated a possible use of the conjugated particle as a drug delivery system.

Metal oxide nanoparticles show ideal pharmacologic activity, especially in tumor therapy. Our study demonstrated that cuprous oxide (Cu$_2$O) nanoparticles (CuNPs) induce apoptosis of tumor cells in vitro. The results showed that CuNPs significantly reduced the growth of cancer cells, but surface modification with tryptophan significantly reduces its toxicity towards those cancer cells. There are few reports available regarding the cytotoxicity study of Cu$_2$O nanoparticle. Particularly, CuNPs can selectively induce apoptosis and inhibit the proliferation of tumor cells and highly sensitive towards melanoma. But, the mechanism of cytotoxicity towards the melanoma cell still unknown. Hence, future work must be done to explore the cytotoxicity mechanism, how this nanoparticle interacts with melanoma cell and also to the other cancer cells. In addition to that, surface modification of Cu$_2$O nanoparticles with specific biocompatible molecules (receptor) to make a composite that may render the particles more specific to the cancer cell targeting causing less distribution and distraction to healthy cells.

This thesis also demonstrates the ‘green’ synthesis of gold nanoparticles and their application in the field of drug carrier and delivery tools. To avoid the toxic reagents, eco-friendly solvents, such as water and biological materials, such as bilirubin have been used as capping/ stabilizing reagents in these synthesis methods. As bilirubin is a normal catabolic breakdown product of heme in vertebrates. We have used this molecule as a reducing agent of Au (III) ion in alkaline medium as well as a stabilizing agent. We have proposed a mechanism for the reduction phenomenon. We have studied thoroughly the interaction of bilirubin with Au (III) ion in aqueous solution using UV-
Vis spectroscopy and fluorescence spectroscopy, and also we have studied the binding interaction of bilirubin on the surface of nanoparticles using FT-IR spectroscopy. The size distribution of the particles was analyzed by TEM image study, the average size of the particles was ~20 mm diameter. Finally, we have performed the cytotoxicity test on three different cancer cell lines, such as Human breast cancer cell line MCF 7, kidney cancer cell line HEK 293 and neuroblastoma cell line Neuro 2a, using MTT assay. The obtained results found that the bilirubin stabilized gold nanoparticles were nontoxic towards those cell lines. It also confirms the nontoxicity of the bilirubin-coated gold nanoparticles and can be used as a carrier for delivering anti-cancer drugs to the target site.

In addition, a unique and illustrative features of silver nanosurfaces (AgNP) coated with billirubin (BR), which is a conjugated π-electronic system. Coated bilirubin or the oxidized form of billirubin, biliverdin hosts and aids to the process of electron transport from silver to specific metal ions such as Fe (III) in aqueous solution. Via electron transfer, AgBR render effective and very specific reduction and detection of Fe$^{3+}$ and Hg$^{2+}$ ions in micromolar concentration levels. In addition, being flat conjugated electron rich π-system, it created an environment to reduce specific ions (Hg$^{2+}$, Fe$^{3+}$) while silver atom was oxidized and the structure of nanoparticles was broken. The oxidation and loss of the nanosurface was evidenced in TEM analysis. Thus, the conjugated π electron system in biliverdin possibly interacts strongly with empty d orbital and effectively transfer electron from silver to empty d orbital of Fe$^{3+}$ followed by the formation of stable complexes of Fe$^{2+}$ with biliverdin. The stability of two complexes has been studied by computational calculation. This observation may have immense implication in making optoelectronics and identification toxic Hg$^{2+}$/Fe$^{3+}$ in water reservoirs.

A thorough examination of gold nanoparticles with different cancer cells and normal cells have been performed. The results show that our prepared nanoparticles are biocompatible, nontoxic and very much stable in the long range of pH and not only that, the particles also quite stable in different types of buffer system. A fundamental issue to address is that the nanoparticles have negatively charged on the surface that may be useful for the loading of anti-cancer drug and will be helpful for the target specific release. If interacting nanoparticles are observed to accumulate at the target
site, then another question to be explored is the impact of surface decoration with different targeting ligands. In the field of antibody therapeutics, the binding-site barrier effect limits the penetration of high-affinity antibodies. However, these questions remain to be answered for nanoparticle formulations and size controlled synthesis. We have shown easy synthesis of several metal and metal oxide nanoparticles and their formulation into surface modification for systemic delivery and sensing of heavy metal ions. Several observations were made that can help direct future research in the field of material science and nanobiology.