Chapter 6

General discussion and conclusions
General discussion:
Nanomaterials possess different novel properties as compared to their bulk counterparts. Nanomaterial synthesis represents a remarkable achievement in nanotechnology research. Nanosynthesis processes, in particular, bottom up approach, offers much more flexibility in terms of creating nanomaterials with desired features (controlled shape and size) by tuning the reaction conditions such as pH, temperature, reaction time, etc. Moreover, strategies to synthesize nanomaterials with desired features with reproducibility are vital for fundamental research and technological applications. Increase in the awareness toward biological process (green chemistry) has led to the development of an eco-friendly process for the synthesis of nanomaterials. Biological systems are environment friendly, effective, flexible and cheaper as compared to other (chemical and physical) methods. Microbial synthesis therefore emerged as an important branch in nanomaterial synthesizing technology. Microbes have their rich diversity and potential for the synthesis of nanomaterials and they could be regarded as potential nanofactories. Microbes are highly organized units in terms of morphology and metabolic pathways and are capable of synthesizing well defined size and structures with reproducibility. Furthermore, the biosynthesized nanomaterials exhibit water dispersible and biocompatible properties, which are crucial for many applications.

The present research work is focused on the biosynthesis of metals (such as gold, silver and platinum) and quantum dot (CdTe) nanoparticles. An attempt has been made to demonstrate bioleaching for the synthesis of silicate nanoparticles from glass cover slips. Size and shape controlled synthesis was also carried out by physical manipulation studies.

Introduction gives a brief idea about nanotechnology including various aspects. Different methods (chemical, physical and biological) and synthesis routes of nanomaterials are discussed with great interest in bio-based approaches. It also presents an account of different properties and applications of nanoparticles. Biosynthesis of gold and silver nanoparticles from *Humicola lanuginosa* was demonstrated. The fungus was isolated and purified in the laboratory. The fungus was maintained on MGYP agar slants and grows at temperature 50°C and pH 9. The fungus was identified by morphological studies and molecular tools in the laboratory and was identified as *Humicola lanuginosa* (*Thermomyces lanuginosus*). The fungus was used for the first time for biosynthesis of metal nanoparticles. When the fungus
was exposed to chemical precursors such as HAuCl₄ and AgNO₃, it reduced the ions and formed gold and silver nanoparticles respectively in the solution. The change in the color of the respective solution indicates the formation of gold and silver nanoparticles. UV spectrum shows specific surface plasmon resonance for the gold and silver nanoparticles. TEM analyses were performed to analyze the size and shape of nanoparticles. The morphology of gold nanoparticles was found to be monodispersed while it was polydispersed in case of silver nanoparticles. XRD analyses showed crystalline nature of the nanoparticles. The produced nanoparticles are thus stabilized by the secreted protein in the reaction mixture. It was also supported by FTIR analyses. The biocompatibility and cytotoxicity of gold and silver nanoparticles was assessed by cell viability assay. These gold nanoparticles were further radiolabelled with Technetium-99m and injected into rat in order to see the biodistribution of gold nanoparticles. It was revealed that gold nanoparticles reached the liver, heart and kidneys and passed out through urine (within 45 minutes). Cardiotoxicity and renal toxicity occur due to doxorubicin. To avoid these complications, doxorubicin was conjugated with protein capped gold nanoparticles with the help of EDC coupling protocol and the conjugate was purified by HPLC.

Targeted therapy has a significant impact in the treatment of some types of cancer and is currently a very active area of research in order to provide a cheaper and effective drug delivery system which can reduce the current therapeutic intake of some of the costly drugs used in cancer therapy. Nanoparticles can be used in targeted drug delivery at the site of disease to improve the uptake of poorly soluble drugs, the targeting of drugs to a specific site and drug bio-availability. Nanomaterials such as polymeric nanoparticles, liposomes, dendrimers and inorganic nanoparticles as reported by several groups hold tremendous potential as carriers for drugs to target cancer cells. Among these, gold and magnetite nanoparticles synthesized by chemical routes are being used for targeted drug delivery. The lack of sufficient stability in water under strong electrolyte conditions and pH changes has impeded the application of these nanoparticles. There are so far no reports of conjugation of biologically synthesized, protein-capped, water-dispersible, inorganic nanoparticles to chemotherapeutic drugs for targeted drug delivery. In this context, we have synthesized a range of inorganic nanomaterials of different chemical compositions using fungi. These biologically synthesized nanoparticles can be used for drug
delivery and targeted drug delivery. Following the biosynthesis, conjugation of these nanoparticles with anti-cancer drug was also achieved. Cytotoxicity of these nanoparticles has been checked on NIH3T3 mouse embryonic fibroblast cell line and MDA-MB-231 human breast carcinoma cell line.

The fungus *Fusarium oxysporum* was exploited for the synthesis of various nanomaterials such as gold, silver, gold-silver alloy, CdS, CdSe, silica, titania, zirconia, magnetite, CaCO$_3$, barium titanate, etc. An attempt has been made for the production of platinum nanoparticles using the fungus *Fusarium oxysporum*. The fungus reduces the ions in the solution which are stabilized by the secreted proteins. The morphology of the nanoparticles was found to be spherical bearing in the dimensions of ~15 ± 5 nm.

Quantum dots were synthesized by chemical and physical routes but their increasing demand in bio-based approach due to their eco-friendly nature has witnessed the highly stable, water dispersible nanoparticle synthesis. In an extension to our previous work on the biosynthesis of quantum dot nanoparticles (CdS, CdSe) using the fungus *Fusarium oxysporum*, the fungus was reacted with a mixture of CdCl$_2$ and TeCl$_4$ resulting in the formation of stable and highly fluorescent CdTe nanoparticles. These particles are crystalline in nature which was supported by XRD data. FTIR analysis showed that these particles are capped by protein layer, which makes them water dispersible.

Laboratory methods for the synthesis of silica nanoparticles used chemical precursors (bottom up approach), which then extended to bioleaching approach (top-down approach) using naturally available materials (white sand, zircon sand) and agro-industrial by-products (rice husk). To follow up bioleaching work, the fungus was employed for the bioleaching of glass cover slips. The fungus leached out water dispersible and nearly monodispersed silicate nanoparticles with ~5 ± 0.5 nm size dimensions. These nanoparticles were capped with secreted proteins in the solution. Bioleaching by the fungus showed that glass surface also undergoes morpho-chemical modifications.

Synthesis of nanomaterials of different chemical compositions, sizes and shapes is an important developed area in nanotechnology. Although several synthesis methods are available for the nanomaterials, synthesis with desired features are still a constant challenge in nanotechnology. Percolative microcavity synthesis scheme was
developed for the size and shape controlled synthesis. Physical manipulation was performed for the solution based approach. The percolative cavity method ensures connectivity and formed a confined reaction zone for synthesis. Gold nanoparticles synthesized by biological and chemical methods are used to compare the percolative microcavity synthesis scheme. It represents a simple solution based process and is further exploited for synthesis of nanomaterials of different chemical compositions.

**Future Prospects:**
Microbial systems are found to associate with metals through metal-microbe interactions. Most of the microorganisms reduce the inorganic metals in solution. As compared to the other chemical and physical methods, biological methods attract greater attention and are regarded as the natural nano-factories. Although biosynthesis processes are emerging as a new and interesting field in nanotechnology, some crucial issues of concern have to be addressed. The major concern is that of the pathways leading to metal reduction and formation of nanomaterials. The identification of secreted biomolecules (enzymes) which reduce the metal ions and cap the nanomaterials as well as their nature and function has to be completely characterized. Surface chemistry also plays a pivotal role in different applications and therefore complete understanding about surface chemistry is also an equally important factor. Currently, the accessible ranges of chemical compositions of nanomaterials are confined to metals, sulfides, carbonates and oxides. Extension of these processes to enable reliable synthesis of multifunctional nanoparticles, physically and chemically hard to synthesize nanoparticles, etc. could make this process a commercially viable approach.

Some sort of questions related to the metal ion reduction reaction process in metabolism and the equally important possible role of the formed nanoparticles in cellular activity or these nanoparticles are formed as the by-products of the reduction process. Among the microbes, fungi are not normally exposed to the high concentrations of metal ions such as Cd$^{2+}$, AuCl$^{4-}$ and Ag$^+$. This stress induces cellular functions such as secretion of biomolecules (enzymes) when reacted, having the ability to reduce the metal ion and formation of respective nanoparticles which may help in the understanding of the process. Nanomaterials are being used for a variety of applications, especially in the field of medicine for drug delivery applications. It is very important to take care about the toxicity issues related to the
nanoparticles. Toxicological studies are needed for the nanomaterials (both \textit{in vitro} and \textit{in vivo}) for further applications. It is immature at present stage to talk about the various aspects of biosynthesized nanomaterials and more efforts are required to understand these issues. It is believed that the future research is of great interest and importance, because nanoparticles can be synthesized with the desirable features according to their applications that make an impact on different fields such as chemistry, physics, biology and medicine.

The nanoparticles which we have synthesized using fungi are capped with natural proteins making them water-dispersible and may bind to integrins or VEGFs (vascular endothelial growth factors). Therefore, targeting integrins and VEGFs is a novel anti-angiogenesis strategy for treating a wide range of solid tumors. Since these nanoparticles are capped by natural proteins, they may bind to various receptors such as LHRH, EGFR and EpCAM without targeting agent. Hence, these nanoparticles can be used directly as drugs in the future. They may fulfill the emerging need for cheaper drugs with no side effects.
Publications:


8. Sreekanth D.; Gupta, S. K.; Syed, A.; Khan.B. M.; Ahmad, A. Molecular and morphological studies of a Taxol producing endophytic fungus, Gliocladium sp. from Taxus baccata. *(Under revision)*

