CHAPTER: 5

SUMMARY

AND

CONCLUSION
This chapter summarizes all the results obtained during the course of the investigation. The significance, scope and future prospects of the work are also discussed. Some of the possible approaches to improve the current strategy are also discussed.

i. **Enzyme mediated synthesis and control of geometry of fluorescent nanoparticles:**

The work presented here reports the syntheses of fluorescent nanoparticles such as CdSe, Co$_3$S$_4$, PbS, and Ni$_7$S$_6$ utilizing enzymes purified from the extra-cellular broth of fungus, *Fusarium oxysporum*. Nitrate reductase was used for the synthesis of CdSe while metal sulphide nanoparticles were synthesized using Sulphite reductase. Both the enzymes require NADPH as the co-factor for their activity. All the nanoparticles required one of the synthetic peptides of general structure \((\gamma\text{-Glu-Cys})_n\text{-Gly}\), where \(n = 2, 3, 4\) or \(5\) for their synthesis which in turn bind to the nanoparticles thereby preventing the nanoparticles from aggregation. The mechanism of synthesis of fluorescent nanoparticles has been hypothesized. The syntheses of the nanoparticles involve the reduction of precursor cations that subsequently gets bound by capping peptides which in turn restricts the as-synthesized nanoparticles into specific sizes and shapes. The size and shape of the as-synthesized nanoparticles were dependent on the length and structure of the synthetic peptides.

a. **CdSe nanoparticles:**

- CdSe nanoparticles were synthesized using enzyme NADPH-dependent nitrate reductase that utilizes sodium nitrate as the substrate. CdCl$_2$ and SeCl$_4$ served as the precursors.
- The synthesis requires synthetic peptides of general structure \((\gamma\text{-Glu-Cys})_n\text{-Gly}\), where \(n = 2, 3, 4\) or \(5\).
- Synthetic peptides of structure, \((\gamma\text{-Glu-Cys})_2\text{-Gly}\) were able to template the synthesis of quasi-spherical CdSe nanoparticles in the size range of 3.9 – 9.0 nm with an average size of 5.5nm.
- The as-synthesized CdSe nanoparticles were crystalline and were in wurtzite form of CdSe as deduced from X-Ray diffraction studies.
- The CdSe nanoparticles showed absorbance and emission maxima of 366nm and 454nm respectively.
The chemical properties of as-synthesized CdSe nanoparticles matched with the standards

The CdSe nanoparticles have been found to contain reactive amino group

**b. Metal sulphide nanoparticles:**

- Metal sulphide nanoparticles such as Co$_3$S$_4$, PbS and Ni$_7$S$_6$ nanoparticles have been synthesized in vitro using enzyme sulphite reductase that utilized sodium sulphite as the substrate
- CoCl$_2$, PbCl$_2$ and Ni(NO$_3$)$_3$ served as the precursor salts for Co$_3$S$_4$, PbS and Ni$_7$S$_6$ nanoparticles respectively
- The particle sizes of CdS, Co$_3$S$_4$, PbS and Ni$_7$S$_6$ nanoparticles were controlled by utilizing the synthetic peptides of varying sizes
- PbS nanoparticles were size controlled to quasi-spherical particles in the range of 3-7nm using ($\gamma$-Glu-Cys)$_4$-Gly
- Co$_3$S$_4$ nanoparticles were size controlled synthesized to spherical particles in the presence of ($\gamma$-Glu-Cys)$_4$-Gly
- CdS nanoparticles were controlled to particles in the size range of 5-8nm in the presence of ($\gamma$-Glu-Cys)$_5$-Gly
- Synthesis of Ni$_7$S$_6$ nanoparticles in the presence of ($\gamma$-Glu-Cys)$_3$-Gly yielded ‘tear-drop’ shaped nanoparticles
- All the as-synthesized metal sulphide nanoparticles were characterized for their structural properties (CdS - orthorhombic, Co$_3$S$_4$ – linnaeite (cubic), PbS – tetragonal, Ni$_7$S$_6$ - orthorhombic)
- Absorbance properties of all the nanoparticles showed blue shifts characteristics of nanoparticles of corresponding bulk materials (CdS – 339nm, Co$_3$S$_4$ – 333nm, PbS – 342nm, Ni$_7$S$_6$ – 407nm)
- Emission spectra of all the nanoparticles showed maxima characteristics of large band gap semiconductors (CdS – 418nm, Co$_3$S$_4$ – 380nm, PbS – 393nm, Ni$_7$S$_6$ – 473nm)
- X-ray photoelectron spectroscopic analyses confirmed the presence of corresponding elements in the as-synthesized nanoparticles
c. Conjugation of nanoparticles with glycopeptides:

- CdS and CdSe nanoparticles synthesized using enzymes were conjugated with bi- /tri-antennary glycopeptides using a modified EDC mediated coupling method
- The nanoparticles-glycopeptide conjugates were purified using size exclusion chromatography
- The emission maxima of CdSe-glycopeptides conjugates (CdSe-bi-antennary glycopeptide – 443 nm and CdSe-tri-antennary glycopeptides – 437 nm) showed blue-shifts with respect to the bare CdSe nanoparticles (454nm). But little or no shift (1-2nm) is observed in the case of CdS-glycopeptides conjugates

ii. Significance of the current investigation

Nanomaterials have attracted lots of attention because of the novel properties associated with the decrease in size of the material. In order to realise the full potential of nanomaterials with tailor-made properties needs to be produced. Controlled production of nanomaterials involves a wide range of techniques that can be classified into three basic approaches viz. physical, chemical and biological methods. Physical and chemical methods are characterized by high efficiency and specificity. On the other hand, both of these approaches require sophisticated instrumentations, involve harsh conditions such as high temperature and pressure, utilise toxic chemicals and produce toxic wastes. Hence, the need for alternative synthetic approaches that overcomes these draw-backs without compromising the end-products is imperative. The use of biological entities such as microbes that function under ambient conditions has been considered a good alternative for the current methods of controlled synthesis of nanomaterials. But their inherent complexity of the synthetic machinery and/or the factors responsible over-shadows their ability to replace ‘harsh’ synthetic methods. Hence, enzyme mediated synthesis of nanomaterials gains importance. Enzyme mediated synthesis of nanomaterials works on similar principles of the physical and chemical methods but function under ambient conditions. In general, reductase enzymes involved in the synthesis of nanomaterials reduces the precursor(s) resulting in the synthesis of nanomaterials. The careful selection of template molecules produces the end-products of defined shapes and sizes.
Enzyme mediated controlled synthesis of nanomaterials possess the following advantages:

- Require ambient conditions – majority of the enzymes require ambient temperature and pressure
- Show high specificity – enzymes are substrates specific
- Economical and scalable – enzymes can be produced in large scale
- Versatile – the capping molecules can be replaced with any potential molecule

iii. Scope and future prospects

Enzyme mediated synthesis of nanomaterials has developed as a potential alternative strategy for hazardous synthetic methods. Currently, this method experiences shortcomings that need to be rectified before utilising it for large scale applications. Molecular biological approaches to enhance and/or alter the efficiency and specificity of enzymes might have a positive impact on the utility of this method. Elucidation of mechanism of synthesis of nanomaterials using enzymes and the role of synthetic molecules including peptides would open up lots of opportunities for this method in the production of nanomaterials of complex shapes and sizes and with precise properties suited for various applications. An interdisciplinary approach spanning in-vitro, in-vivo and in-silico approaches would provide us with great deal of information to tap the full potential of this novel method. The study of surface properties of the biogenic nanomaterials (synthesized using enzymes and bound by capping peptide) would expand the utility of bio-synthesized nanomaterials. The utility of this method is currently confined to metals, metal sulphides and some oxides. The use of this method for the production of other oxides, nitrides and carbides would be exciting. The use of fluorescent nanoparticles attached to individual biomolecules as tracers would help in the better understanding of the molecular processes underlying various biological processes. This might expand the opportunities available in the field of diagnostics, treatment and follow up of various medical complications.