SUMMARY
This summary reflects the salient features of the work presented in the thesis and emphasizes on potential avenues for future research work in this field.
Summary

Study of functionalizing nanoparticles using biomolecules in a facile pot, towards potential applications like anti-bacterial and anti-oxidant activity and analysis of the mechanism of formation and function using biological, physical and chemical methods is presented in this thesis. This is a promising work for enormous future scope of research study.

The work begins with the ‘road not taken’ wherein antibiotics are used for the first time to synthesize stable gold nanoparticles. The use of antibiotics as the capping agent calls for attention due to the major global threat faced by the human population from the highly resistant pathogenic bacteria that is even now evolving, as we develop new means of combating them. Hence this work proves as a new mode of delivering the antibiotic into the environment/system where the bacteria are yet to develop resistance. The capped molecule is unlike the same antibiotic used for the synthesis in two ways:

1. Its redox potential is changed since it participated in the reduction of chloroauric acid into gold nanoparticles
2. It is bound onto the surface of the metal nanoparticle and hence not easily accessible for the lactamases to act upon it, thereby increasing the effective activity against the bacteria.

Further, the system also provides as a simple means of conjugation chemistry which can be used in biological research. Antibiotics being simple biomolecules that are extensively used in the infectious world are easily participating in the reduction reaction in a concentration dependent manner. In addition, antibiotics like cefaclor and ampicillin are also shown for their potential to form stable and active gold nanoparticles.

The synthesis, characterization and preliminary anti-bacterial activity are shown. This new delivery of antibiotics synthesized nanoparticles may not allow the bacteria to quickly gear up their resistance; instead they may get prone to the same.

This synthesis route is also a perfect model system to study the growth of the gold nanoparticles, which is elaborated in following chapter. The simple system is studied
in a temperature dependent manner using light absorption and scattering methods and the electron microscopy. Unique bimodal distribution of gold nanoparticles is reported for the first time in our knowledge using the DLS data. The transmission electron micrograph supported the presence of a large number of smaller 1-3 nm particles as well as larger particles around 25 nm. However, the fact that change in temperature, by an increase of 10 degree order, has nonetheless resulted in bimodal distribution of comparable sizes is highly intriguing. Change in temperature alters every aspect of the reaction. The stability and diffusion rate of the complexes formed in the reaction as well as the binding strength of the surfactant molecules to the growing nanocrystals are all strongly temperature-dependent. Moreover, increasing the temperature greatly decreases the stability of the intermediate complexes formed in solution and the binding of the surfactants to the nanocrystals surface, while increasing the diffusion rates of the complexes. This favors the nucleation and growth. However, too high a temperature may lead to uncontrolled growth so that it is impossible to exploit subtle kinetic or energetic effects to achieve precise control over the size and size distribution of the nanocrystals. Thus choosing an appropriate temperature range is one of the key steps in obtaining control over nanocrystal growth. In our study we see the expected change in the morphology of the particles formed at different temperatures. Hence, the ongoing process of this reaction system is complicated with various parameters affecting the size and shape. This offers as a very good model system that can be exploited to study the energetics involved. According to Howard Reiss’s prediction small crystals will grow more rapidly than larger ones if monomer concentrations are sufficiently high. This prediction on which the ‘size-distribution focusing’ approach is based can be used to explain our observation of the particle morphology at low temperature. The slow growth conditions at low temperature does not allow for change in the size-distribution of the particles, however, the critical size, which depends on monomer concentration, shifts to a smaller value. This work presents a novel and detailed study of different systems of functionalisation of nanoparticles. The different facets of the study make it interesting. Better understanding of these nanosystems holds the key to exploring the exciting opportunities of this field to the full potential of colloidal nanoparticles.

As an effort to functionalize enzymes onto nanoparticles so as to have enhanced efficiency and stability, and improve ease of separation for industrial use, the
A commercially important enzyme, Penicillin acylase was used to functionalize on chemically synthesized nanoparticles in a separate study. The system is characterized and the bioactivity of the enzyme capped on the magnetic nanoparticles is measured. True to the expected data, and industrially accepted limits, the immobilized enzymes had good activity when the enzyme activity was estimated. This is the first report of such a study using the Penicillin acylase enzyme, where numerable studies using polymers and different substrates are used for good immobilisation and activity. The advantage of using magnetic nanoparticles is in the ease of separation for washing and reusing after the biocatalyst has been used. Magnetic nanoparticles are already in the market for such purposes and they prove to be useful in such a utility. Further studies are underway to estimate the leaching percentage, pH stability, re-useability and magnetic sensitivity, to make these nanoparticles useful.

Further, curcumin is used as the functionalizing molecule. Curcumin is a molecule that is making a revolution in the western world, both in the market and in the research field. Name a disease or disorder, curcumin seems to have an effect on it, for the better of it. Curcumin has been used to cap gold nanoparticles. However, like the previous report of synthesis using antibiotics, we observe that curcumin also reduces gold ions to form gold nanoparticles. However, the solubility of curcumin in water has been an issue for biomedical advantages, owing to the high hydrophobic nature of the molecule. Hence the first part of the chapter deals with the study of the physical and spectroscopic behavior of curcumin in water when heated at high temperatures. Such a sample is then used to produce stable gold nanoparticles in aqueous phase. A detailed study of the solubility of curcumin in water at high temperature which is not reported previously is presented in the last chapter. A remarkable change in the temperature dependent electronic transition behavior of the curcumin molecule is observed; however, the absorption spectra after cooling and heating cycles (between 25 °C and 90 °C) remain unchanged. The study indicates that it is perhaps the breaking of the intra-molecular hydrogen bonding which leads to the exposure of the polar groups and hence responsible for the dissolution of curcumin at higher temperature. We believe that formation of inter-molecular aggregates might be responsible behind a better room temperature stability of the molecules after cooling its aqueous suspension from 90 °C to 25 °C. This report warrants further studies to understand the mechanism of the process at depth.
Nonetheless, this curcumin in water is used to synthesize gold nanoparticles which exhibit good anti-oxidant activity compared to curcumin. Their potential use in fight against diseases where anti-oxidants are most helpful can be tested. In vitro studies, as a first step, would throw some light on the effective use of this system as a drug.

These systems need further exploration, to understand them, to verify their usability and to address the toxicity effects if any. Since the first line of exposure comes from the molecules that are functionalized on the nanoparticles, their behavior, properties and acceptance into the biological world depends on the functionalized molecule.