CHAPTER 5

CONCLUSIONS AND SCOPE FOR FUTURE WORK

5.1 Conclusions

Major conclusions drawn out of the research work carried out to meet the objectives of this thesis are as follows:

Well dispersed, uniform SNPs with controllable size and narrow size distribution are prepared by chemical reduction technique. Nanoparticles’ yield, their morphology, particle size, polydispersity and agglomeration are strongly correlated with concentration of reducing capping agent (oleylamine) and other kinetic parameters like nucleation temperature, nucleation time, growth temperature and growth time. Optimized conditions for these parameters have been established for which smallest particle size (< 10 nm), narrowest polydispersity (≈ 0.12) and highest yield (≈ 46%) of SNPs were obtained. Nucleation at 200 °C for 30 min and growth at 150 °C for 4 h are the optimum processing parameters for highest nanoparticle yield, lowest particle size and polydispersity index with no or very little aggregation.

Present study also reveals that oleylamine traditionally known as reducing and capping agent, also functions as grain growth inhibitor. 15 mM is the optimized concentration of reducing agent to prepare well dispersed self-assembled spherical SNPs. Below this concentration, SNPs aggregate strongly due to the lack of monolayer coating of oleylamine. Growth of SNPs follows the classical condensation mechanism. Oleylamine coating on SNPs make them hydrophobic. For medical applications, nanoparticles need to be hydrophilic. Hence, a facile phase transfer protocols have been developed to transfer hydrophobic SNPs into hydrophilic by ligand-exchange reaction using pluronic F-127. No deviation in the physical or surface properties of SNPs has been observed after the phase transfer.

Well dispersed, uniform colloidal CNPs with controllable hydrodynamic size and narrow particle size distribution were obtained by reducing copper (II) chloride with a combination of strong (NaBH₄) and weak (L-ascorbic acid) reducing agents in the presence of a surfactant (PVP). Concentration of strong reducing agent, weak reducing
agent and capping agent has major impact on the crystal phase, size and size distribution of CNPs. Absence of the strong reducing agent or the weak reducing agent or the surfactant either does not form CNPs or the formed nanoparticles are not stable and get oxidized. Present study reveals that in addition to strong reducing agent, a secondary weak reducing agent is essential to simulate dynamic equilibrium around the surface of CNPs, which prevents their oxidation and stabilizes the metallic copper phase. Spherical, ultra-small CNPs with uniform physical size (≈ 2 nm) are synthesized at 80 °C under optimized conditions when concentration of each PVP, NaBH₄ and L-ascorbic acid is 10 mM. The protocols developed here for the synthesis of air stable CNPs do not require any inert environment / protective gases. As-synthesized CNPs form stable aqueous colloidal dispersion, which do not show any sign of aggregation or oxidation even after their storage under ambient conditions for 120 days.

Pristine and cobalt doped TNPs are prepared by sol-gel technique. Titanium (IV) isopropoxide is hydrolysed and condensed into amorphous titanium dioxide gel by water / ethanol under acidic conditions. Amorphous TiO₂ gel is crystallized into anatase crystallographic phase when calcine at 500 °C. The optical band gap of pristine (0% Co doped) TNPs is 3.03 eV (λ = 409 nm), which decreases upto 1.93 eV (λ = 642 nm) when Co concentration in TiO₂ matrix increases from 0 to 2%. Co(+2) substitution at Ti(+4) site generates additional oxygen vacancies in the titania unit cell, which introduces extra energy levels in the forbidden band that reduces the indirect energy band gap of TNPs. Irrespective of the Co concentration, TNPs always crystallize into anatase phase when calcined at 500 °C. No signature of other isomorphous phases i.e. rutile or brookite are detected. With increasing Co doping, the amorphous to crystallization anatase phase transition temperature increases from 404 °C to 454 °C. The average physical size obtained from SEM measurement is ≈ 10 nm. Co doping in TNPs make them sensitive to visible radiation and, hence their photoresponse is expected to be better under sun light than pristine bulk titania, which is active in the UV-region of the electromagnetic spectrum.

Antibacterial activities of as-synthesized SNPs, CNPs and TNPs have been investigated against clinically important pathogenic strains (E. coli, S. aureus and P. vulgaris) and eco-friendly microorganisms (B. subtilis and P. fluorescens). No biocidal activities of pristine and cobalt doped TNPs are observed against microorganisms under
test for TNPs concentration as high as 500 µg/mL both under dark and light conditions. Strong antimicrobial activities are observed in case of SNPs and CNPs against both pathogenic and non-pathogenic strains under investigation. Amongst SNPs and CNPs, CNPs report enhanced biocidal activities against all tested microorganisms (pathogenic and non-pathogenic), whose minimum inhibitory concentration values lie in the range of 20 - 50 µg/mL. For SNPs, the minimum inhibitory concentration values against the same set of microorganisms fall in the range of 25 -150 µg/mL. CNPs are equally effective against all tested microorganisms as no significant variation in their MIC values is observed. SNPs exhibit strongest biocidal activities against both the eco-friendly strains and one pathogenic strain (E. coli). S. aureus is least vulnerable to SNPs (MIC = 150 µg/mL). Results reported here are either better or comparable to those found in literature.

To understand the effect of adsorption of commercial antibiotics (tetracycline and kanamycin) on SNPs and CNPs, antibiotic adsorbed SNPs and antibiotic adsorbed CNPs formulations have also been prepared. The loading efficiency of tetracycline in SNPs is 15.3% while for CNPs, it is 8.6%. The loading efficiency of kanamycin is 6.44% for SNPs and 10.63% for CNPs. The antibacterial activities of antibiotics adsorbed nanoparticles formulations have been evaluated by disk diffusion test, in which zone of inhibition is measured both for pristine (SNPs / CNPs) and antibiotic adsorbed nanoparticles (tetracycline adsorbed SNPs / kanamycin adsorbed SNPs / tetracycline adsorbed CNPs / kanamycin adsorbed CNPs). The percentage enhancement of antibiotic efficiency of SNPs when antibiotic (tetracycline / kanamycin) get adsorbed on their surface is in the range of 66 - 346% for tetracycline and 70 - 289% for kanamycin. Such strong enhancement in the biocidal activities of CNPs is not observed when antibiotics get adsorbed on the surface of CNPs. The enhancement in the biocidal activities of CNPs lies in the range of 0 - 49% for tetracycline and 0 - 20% for kanamycin. The vast difference between the enhancement in the biocidal activities of SNPs and CNPs when antibiotics are adsorbed on their surface might be because of the difference in the site of adsorption of antibiotics on nanoparticles’ surface, which is making them active or inactive. Both tetracycline and kanamycin adsorption on SNPs are equally effective while tetracycline is more effective compare to kanamycin when adsorbed on CNPs. Results of antibacterial tests of nanoparticles and their formulations with antibiotics are summarized in Table 5.1.
Table 5.1 Summary of antibacterial activities of nanoparticles and their formulations with antibiotics tetracycline and kanamycin.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Class</th>
<th>Nature</th>
<th>MIC (µg/mL)</th>
<th>Zone of Inhibition (ZIH) (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SNPs</td>
<td>CNPs</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>Pathogenic</td>
<td>Gram-ve</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>Gram+ve</td>
<td>150</td>
<td>20</td>
</tr>
<tr>
<td><em>P. vulgaris</em></td>
<td>Pathogenic</td>
<td>Gram-ve</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>Non-pathogenic</td>
<td>Gram+ve</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>Non-pathogenic</td>
<td>Gram-ve</td>
<td>25</td>
<td>20</td>
</tr>
</tbody>
</table>
Overall conclusion of the entire study is that out of the three nanoparticle systems designed in the present study, CNPs are most lethal on tested microorganisms. This is in contradiction with the existing literature where highest biocidal activities are reported for SNPs. This could be because of their ultra-small particle size (2 nm) and very high resistance towards oxidation and aggregation. Both nanoparticle systems (SNPs and CNPs) and their formulations with antibiotics are more toxic to eco-friendly strains than to pathogenic ones. Antimicrobial activities of antibiotic adsorbed nanoparticles are higher than the discrete sum of biocidal activities of individual components (nanoparticles and antibiotics). Thus nanoparticle-antibiotic formulations can be used to further enhance the biocidal activities of commercial antibiotics.

5.2 Scope for future work

This thesis reports synthesis of three nanoparticulate systems (SNPs, CNPs and TNPs) and evaluation of antimicrobial activities of these nanoparticles and their formulations with commercial antibiotics tetracycline and kanamycin. Enhanced antimicrobial activities of SNPs and CNPs along with their formulations with commercial antibiotics have been observed. This piece of work could find potential applications in pharmaceutical industries. However, for industrial utilization of these nanostructures and their formulations with commercial antibiotics, synthesis protocols and their subsequent processing with antibiotics need to be modified suitably. In particular, the process needs to be scaled up to make it economically viable for their use as non-traditional antibiotics in health-care industry.

Before commercial utilization of any drug or health-care product, they need to meet certain standards set by food and drug administration. These standards basically define exposure limits of drugs or health-care products. For nanomaterials or nanomaterials based products, such standards are not available. Hence, toxicity of these nanoparticles and their formulations with antibiotics needs to be investigated as per the standard protocols for any potential nanomaterial based new drug. Based on the outcome of this study, exposure limits for these nano-antibiotics needs to be defined.

Antimicrobial activities of metals and their oxides is a vibrant field of research in today’s nanobiotechnology. Most of the investigated nanostructures for their biocidal activities are toxic to human cells and because of this, they cannot be used for in-vivo
purposes. Amongst the biocompatible class of nanomaterials, those showing biocidal activities are of great industrial relevance. Amongst them, SNPs and CNPs are the chief candidates for potential applications in preventive and curative healthcare. There are multiple reports of their biocidal activities along with the present one in this thesis. Despite of wide spread study of this aspect of SNPs and CNPs, the exact mechanism responsible for their biocidal activities are largely unclear. A detailed study needs to be conducted to understand the mechanism responsible for biocidal activities of SNPs and CNPs. The current literature predicts that pathogenic microorganisms, which are developing new strains by mutation are resistant to one or more first line antibiotics, are unlikely to develop such resistance against SNPs and CNPs. However, detailed study of this aspect is completely missing and needs to be evaluated urgently as many health-care products are now using nanostructures at commercial level. Any possible mutation of pathogenic strains that is resistant to these nanostructures would be devastating and very difficult to control.

Nanomaterials are now commercially explored in large number of technical, engineering and medical products. Nanoparticles will eventually leach out from these materials and will accumulate in water and land fields. These accumulated nanostructures may cause severe damage or alteration to terrestrial ecosystems of the earth by interacting with microbes, which are playing critical role in various natural cycles. Such interactions between nanoparticles leaching out from the toxic nanomaterials’ waste and eco-friendly microorganisms present in soil, water, air and plants have potential to extinct these microorganisms. Hence, environmental concerns of nanomaterials need to be addressed urgently and a systematic study is essential that will include impact of commercial nanoparticles on microbes of terrestrial and marine origin. The outcome of such study will eventually define permissible levels of nanomaterials in air, land and water bodies. A separate study can also be conducted on remediation and recycling of nanostructured based pollutants.