

Green synthesis of nanomaterials with special reference to environmental and biomedical applications

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Environmental benign bioinspired and/or biomimetic synthetic process of metal nanoparticles have been developed for water purification and biomedical applications. In the synthetic process, the biomolecules acted as shape directing agent determining the crystal structure of the nanoparticles and also control the surface properties of the nanomaterials.

Keywords: Biomedical applications, environmental management, green synthesis, metal nanoparticles.

Research highlights

IN recent years, considerable efforts have been made in the design and synthesis of nanostructured materials. In particular, metal nanoparticles (MNPs) with at least one dimension having size of 1–100 nm possess wide application potential in optical, electronic, catalysis, environmental and biomedical fields. Precise control over the synthesis and surface functionalization of MNPs is crucial to govern their stability, physico-chemical properties and biological behaviour. Although significant success has been achieved in the size and shape-controlled synthesis of MNPs, use of toxic chemicals and highly flammable organic solvents is a major concern for environmental and safety issues.

In an effort towards minimizing the use of toxic chemicals and reduce the generation of hazardous waste while obtaining metal nanoparticles, CSIR–Central Leather Research Institute (CLRI), Chennai has found suitable biosynthetic and/or biomimetic processes of metal nanomaterial synthesis for environmental and biomedical applications.

Sustainable metal nanoparticles for environmental management

The water resources of the Earth are being increasingly contaminated due to rapid industrialization leading to

reckless pollution of the water bodies. The industrial effluents of tanneries, textile and paper industries contain high amount toxic and carcinogenic dyes used for colouring, posing severe threat to aquatic and human life. As life on the Earth depends on water, the accessibility of pure surface and groundwater is becoming difficult due to contamination with effluents. Thus, efficient water purification technology is an urgent need for pollution control management. The present technologies frequently used for the treatment of wastewater can be classified into physical, chemical and biological methods. However, these technologies, which include ion-exchange, reverse osmosis, ultra-filtration, ozonation, oxidation, adsorption, etc. suffer from certain limitations such as high operation cost, low removal efficiency, generation of toxic sludge and requirement of high energy. Moreover, removal of pathogens from treated water requires additional processes like chlorination, ozonation, etc. which increase the cost of treatment and raise health issues. Recent studies by the Bhabha Atomic Research Centre, Mumbai demonstrated that decontamination of water by chlorination and ozonation processes generates harmful by-products. Moreover, microbial growth in the treatment plants leads to biofilm formation resulting in higher energy consumption, operational expenditure, accelerated corrosion and generation of antibiotic-resistant strains. These create significant health and economic losses. Recent developments in nanoscience and nanotechnology paved the way to solve the technological constraint of water purification. Being small in size, the nanomaterials possess a series of unique physical and chemical properties making them an ideal candidate for environmental applications.

A research team from CSIR–CLRI has achieved a breakthrough in the development of nanotechnology-based cost-effective and eco-friendly water purification for the treatment of dye bearing contaminated water. A facile, environmentally benign and energy-efficient biosynthetic process has been developed to prepare silver–silica nanocomposite for treatment of wastewater¹. Silver nanoparticles were synthesized on nano-silica surface upon reduction of silver ions bound on nano-silica utilizing intracellular proteins of *Rhizopus oryzae*. The synthesis process is closely related to the natural biomineralization

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process. It does not require any chemical, high temperature or pressure and thereby protects the environment from toxic chemicals, often required for nanomaterial synthesis. The protein coating on the nanoparticle surface also prevents aggregation of nanoparticles and leaching of metal ions and hence, provides high stability to the nanocomposite. The as-prepared silver–silica nanocomposite was finally used to remove dyes and pathogenic microorganisms from contaminated water. Results show excellent dye-adsorption capacity of the nanocomposite both in single and multi-component systems and it removed 99% of dyes even from higher concentration of dye solution (Figure 1). The dye adsorption capacity of the nanocomposite was estimated to be six times higher than the conventional activated carbon. Apart from dye removal ability, the said nanocomposite showed excellent antimicrobial efficacy and killed *Escherichia coli* and *Pseudomonas aeruginosa*, which are common pathogens in wastewater. In addition to antibacterial activity, the nanocomposite also prevented bacterial attachment on the surface and ultimately inhibited biofilm formation. Scanning electron and fluorescence microscopic studies revealed that cell death occurred due to irreversible damage of the cell membrane upon electrostatic interaction of positively charged nanocomposite with the negatively charged bacterial cell membrane. The tests performed in our laboratory showed no leaching of silver ions from the hybrid, which makes the material safe for use in water treatment. The purified water is devoid of silver and other contaminants, making it suitable for domestic purposes and human consumption. The nanocomposite does not require high-end instruments, power or other chemicals to purify water, and thereby reduces the cost of water treatment. Moreover, dyes and microbial contaminants can be removed simultaneously, making the water treatment

process cost-effective. The high dye-adsorption capacity, reusability, antibacterial and antifouling properties of the nanocomposite may therefore provide an opportunity to develop cost-effective and high-efficiency water-purification processes.

Metal nanoparticles as heterogeneous catalyst

Heterogeneous catalysis is mostly prevalent in the chemical industry and more than 90% of all chemical manufacturing is heavily dependent on catalytic processes. It has been estimated that currently catalysis contributes about 35% of the world's gross domestic product. Although the chemistry of noble metal salts as catalyst for organic synthesis was explored during the early 20th century, the catalytic applications of MNPs in chemical reaction have been intensified after the discovery of noble MNPs. Owing to their high surface-to-volume ratio, high surface energy, increased accessibility to surface atoms and lower coordination numbers, MNPs are highly active compared to bulk materials. Since most of the catalytic reactions are carried out in organic solvents, more effort has been devoted recently in the design and synthesis of water-based catalytic system.

Various approaches have been introduced to prepare solid-supported MNPs as heterogeneous catalysts. However, multi-step preparation, aggregation of MNPs, leaching of metal ions and reduced catalytic activity make the processes unsuitable. Eco-friendly *in situ* biosynthesized MNPs on solid support could be the next green answer to aqueous-phase heterogeneous catalysis reaction. In line with biomineralization process, scientists at CSIR–CLRI have developed a simple, cost-effective and facile synthetic protocol for *in situ* production of Pd, Pt and Ag NPs on fungal mycelia as solid support². In making these NPs, the synthesis process was carried out at ambient temperature, pressure and in aqueous phase, without using any external reducing as well as protecting agent. The initial electrostatic binding of metal ions on the cell surface followed by reduction of the bound metal ions by cell surface protein caused formation of MNPs on the fungal mycelia. The cell-surface protein also acted as shape-directing agent governing the size and shape of the biosynthesized MNPs. In case of Pd and Pt, 'flower'-like branched nanoparticles were obtained, while Ag produced spheroidal nanoparticles.

Because of site-specific binding of surface protein on crystal face nanoparticle, the size and shape-controlled formation of MNPs occurred during *in situ* growth process. The competitive and preferential binding of 78, 62 and 55 kDa proteins on the surface of MNPs determines the morphology of MNPs. Since the reduction potential of metal ions and their hydrated species also varies among the metals, the molecular interaction of the surface proteins controls the crystallographic orientation

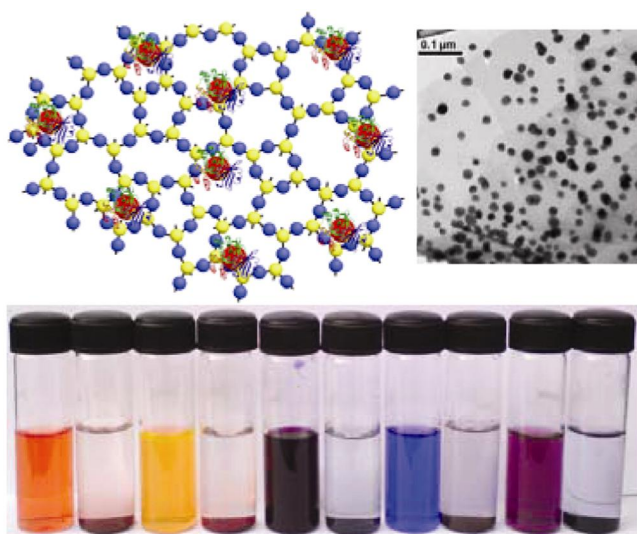


Figure 1. Synthesis of protein-coated nanosilica–silver nanocomposite for wastewater treatment.

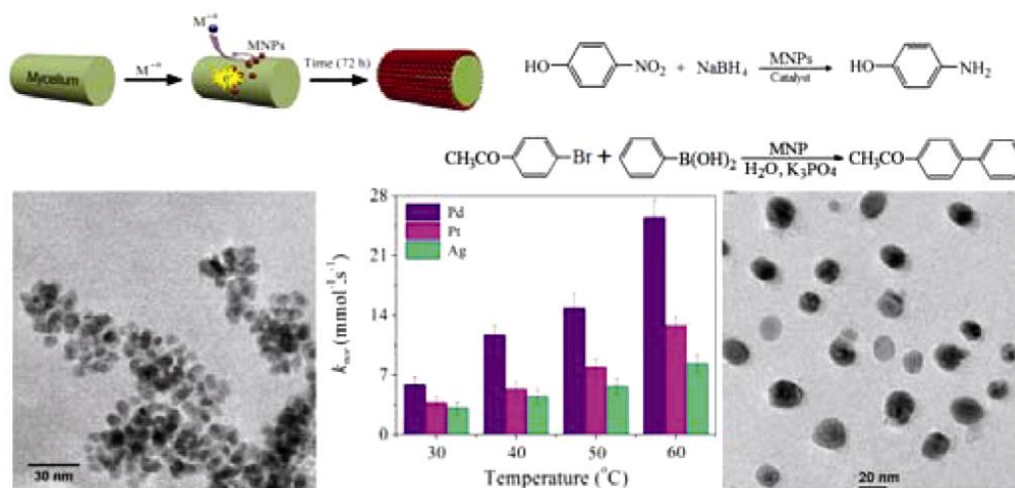


Figure 2. Biosynthesis and catalytic reactions of Pd, Pt and Ag nanoparticles (NPs) at the nano-bio interface.

of the nanoparticles and ultimately governs the shape of the MNPs. In addition, the surface proteins also prevent the leaching of metal ions, providing stability to the MNPs.

At the end, the catalytic applications of MNPs in aqueous phase hydrogenation and coupling reaction were tested (Figure 2). These MNPs exhibited high catalytic activities in hydrogenation and Suzuki coupling reactions in aqueous solution compared to other nanoparticles. It was observed that the catalytic efficiency varied with the type of MNPs, and the high index branched Pd-nanoflower exhibited superior catalytic activity (>99% conversion) in both chemical reactions. Moreover, the nanocatalyst can be easily separated and reused multiple times without significant loss in activity (95% average conversion). High stability of MNPs due to protein coating results in long-term catalytic activity of MNPs. This biosynthetic approach, therefore, provides an alternative for environmentally benign synthesis of shape control-supported nanoparticles as heterogeneous catalysts, which may find potential applications in many industrially important catalytic processes in near future.

Biomedical applications of metal nanoparticles

The global emergence of antibiotic-resistant microorganisms presents one of the greatest challenges in public health care today. Indeed, the third-generation antibiotics such as cephalosporium, are also becoming ineffective in many patients suffering from nosocomial infections. It is therefore necessary to develop new strategies for antibacterial treatment. The emerging advances in nanotechnology provide new opportunities to control pathogenic microorganisms via tailored fabrication of nanomaterials. Various functional nanoparticles (semiconductor quantum dots, metal nanoparticles, magnetic nanoparticles, carbon

nanotubes, etc.) have recently found applications in ultra-sensitive diagnosis, imaging and therapy.

Owing to broad-spectrum antimicrobial behaviour, the application of silver nanoparticles to control the microbial growth and treatment of burns or wounds has been studied. In spite of rapid progress, the biomedical application of Ag NPs in nanomedicine has been restricted due to their potential adverse effects. Upon internalization of Ag NPs, the proteins in the body fluid get bound to the nanoparticle surfaces causing protein corona formation around the Ag NPs. The formation of protein corona induces aggregation of Ag NPs and reduces their efficacy as well. In addition, the bound proteins often undergo conformation changes with subsequent loss of biological activity. In many cases, the structure alteration of the proteins triggers autoimmune diseases. The binding cellular protein with nanoparticles also causes amyloid-like fibrillation, which induces neurodegenerative diseases like Parkinson's disease.

The surface functionalization of Ag NPs may prevent protein corona formation or stability to the adsorbed proteins and this in turn retains the activity of both nanoparticles and proteins. As Ag NPs have excellent affinity towards proteins, great care should be taken in functionalization. We developed an *in situ* synthetic protocol to decorate the Ag NPs with β -hydroxy propylcyclodextrins (β -HPCD-Ag NPs). To understand the impact of surface functionalization of Ag NPs on binding and conformational change, the interaction of protein, viz. haemoglobin (Hb) with β -HPCD-Ag NPs was studied, while haemolytic activity of β -HPCD-Ag NPs was tested on red blood cells (RBC)³.

From protein-binding assay, it was found that less amount of protein was bound on β -HPCD-Ag NPs compared to the non-functionalized bare Ag NPs (borohydride-mediated synthesis of Ag NPs). The β -HPCD functionalized Ag NPs not only prevented the binding of

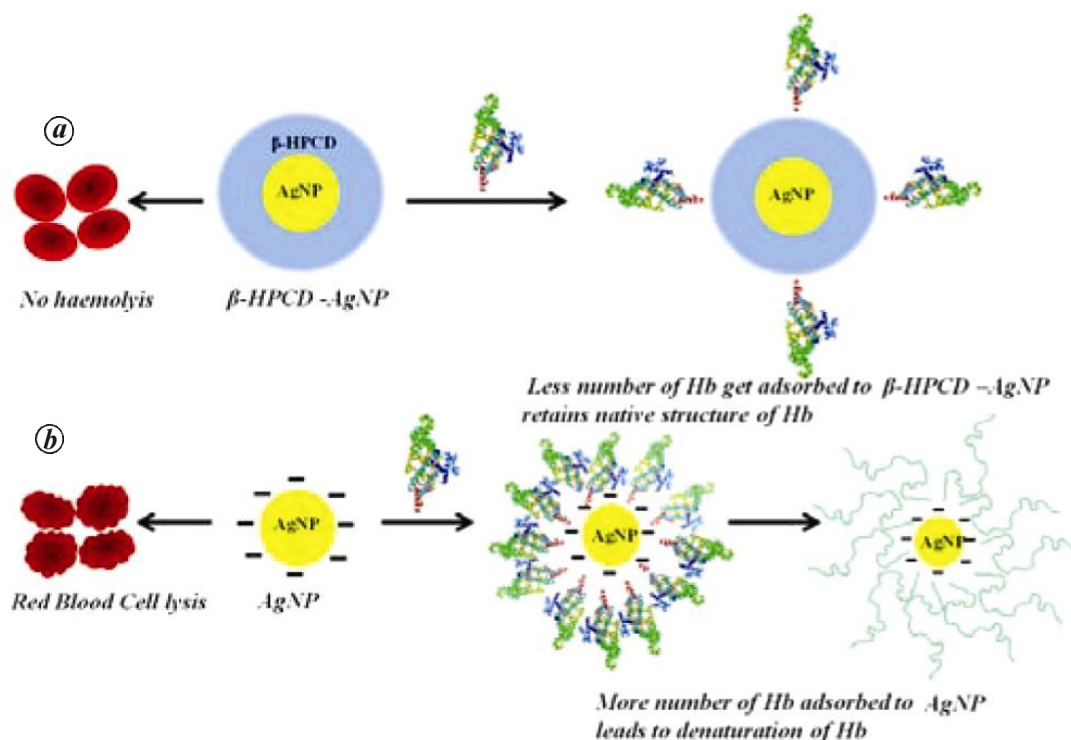


Figure 3. Effect of surface functionalization of Ag NPs on protein corona formation, stability of the secondary structure to the adsorbed protein and haemolysis of RBC: *a*, β -HPCD-Ag NPs; *b*, bare Ag NPs.

Hb on the surface of nanoparticles (Figure 3), but also provided thermal stability to the protein. The higher Soret band intensity of Hb, following binding on β -HPCD-Ag NPs, revealed the native state of Hb; however, binding of Hb on bare Ag NPs caused unfolding of the protein.

The size determination of protein-adsorbed nanoparticles showed that thick layers of protein corona were formed on bare AgNPs, but such a formation was not observed on β -HPCD-Ag NPs. The dichroic study revealed that bare Ag NPs induced considerable change in the secondary structure of Hb, while native state of Hb was retained following interaction with β -HPCD-Ag NPs. Thus, surface functionalization of Ag NPs played a crucial role in the binding and conformation of protein structure dictating overall activity of the protein as well as Ag NPs. The Ag NPs have very high affinity towards the protein, promoting binding of Hb on the nanoparticle surface. On the contrary, β -HPCD functionalization causes masking of the surface of Ag NPs, while retaining their functional property. β -HPCD also has less affinity for the protein. Therefore, direct contact of protein with Ag NPs is prevented in β -HPCD-Ag NPs, resulting in inhibition of corona formation.

Finally, the biocompatibility of functionalized and bare Ag NPs was tested against human blood samples, which revealed that bare Ag NPs cause haemolysis of RBCs, whereas coating of β -HPCD over Ag NPs protects RBCs from NP-mediated haemolysis, suggesting that biocompatibility of β -HPCD-Ag NPs for *in vivo* application without any toxic consequences. The results obtained will therefore help design surface-functionalized, biocompatible nanoparticles for biomedical applications.

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