Introduction

Nanoscience and nanotechnology have emerged as one of the most active area of research all over the globe in the last decades. It is motivated by the desire to formulate materials with novel and superior properties that is likely to impact nearly all areas of science. It is generally accepted that the foundation of the present nano age has occurred with the statement of Richard Feynman that “There is plenty of room at the bottom” during his lecture delivered in 1959 at the meeting of American Physical Society. The nanomaterials have received great attention from the scientific community not only because of their fascinating properties but also due to their many technological applications. Within the nanoscale, the properties of matter are sufficiently different from atoms or molecules and from bulk materials [1]. Nanoparticles exhibit outstanding physical and chemical properties that are not exhibited by their bulk counterparts [2-4]. An increase in surface area with unit mass as a consequence of the reduction in particle dimension alters both physical and chemical properties of a material. The synthesis and characterization of nanomaterials is of primary importance in the advancement of nanotechnology. Since the attractive properties of these materials depend on their size and shape, which in turn depend on the method of synthesis, the controlled synthesis of nanomaterials and manipulating them for various applications is a major challenge in nanotechnology that is to be dealt with in order for this promising field to cope up with the high promise and potential that this field offers.

The noble metal nanoparticles especially silver and gold are being explored for their versatile applications in various fields such as catalysis, optics, sensing, imaging, and biomedical. Several synthetic strategies have been developed for the synthesis of metal nanoparticles including chemical [5], photochemical [6], sonochemical [7], radiochemical [8], electrochemical [9], and biological methods [10]. Among these, chemical reduction of a metal salt in presence of a stabilizing agent to prevent the aggregation of the metal nanoparticles is the most frequently applied method for the generation of nanoparticles as stable colloidal dispersions in water or organic solvents [11]. The major drawback of chemical method is that the various chemical reducing agents such as sodium borohydride, hydrazine, N, N-dimethylformamide, polyols etc. as well as the stabilizers such as synthetic
polymers, surfactants, and dendrimers used in this method causes chemical toxicity and serious environmental problems, thus limiting their utility especially in biomedical applications [12]. Therefore, it is necessary to develop facile and environmentally benign synthetic strategies for the preparation of metal nanoparticles, especially in large scale. Recently, there has been an increased interest on green chemistry approaches with the aim to reduce environmental risks [13]. The selection of a non-toxic reducing agent, a cost-effective and easily renewable stabilizing agent and an environmentally benign solvent system are the three main criteria for a ‘greener’ nanoparticles synthesis [14].

Microwave heating has gained significant attention as a promising new method for the one step synthesis of metallic nanostructures in solutions. The synthesis of metal nanoparticles by microwave irradiation is a novel method for the rapid production of monodispersed nanoparticles with narrow size distribution and is much less exploited especially in biosynthesis of nanoparticles. The microwave-assisted synthesis of nanoparticles is characterized by rapid and homogeneous heating in contrast to a conventional thermal synthesis and thus provides uniform nucleation and growth conditions for nanoparticles [15]. Transfer of energy from the microwave radiation to the reactants is achieved through the interaction of radiation with water or other solvents with high dielectric constant or solvent molecules with large dipole moments [16].

**Objectives of the present work**

The major aim of this work is to synthesize silver and gold nanoparticles by a microwave-assisted method using biopolymers and phytochemicals which can serve as versatile catalysts and antimicrobial agents. Usually, when a strong reducing agent like NaBH₄ is used as reducing agent, the reaction is very fast and has to be cooled to control the rate of reduction in many cases. Moreover, it is highly toxic and synthesis of larger nanoparticles has been found to be difficult [17]. On the other hand, when a mild reducing agent like sodium citrate or ascorbic acid is used, the reduction reaction is slow and has to be carried out at elevated temperatures to enhance the rate of reduction. In addition, they usually yield relatively larger nanoparticles of varying size and shape [18]. This work presents for the first time the
use of hexamethylenetetramine \( ((\text{CH}_2)_6\text{N}_4) \) commonly known as hexamine as reducing agent for the synthesis of silver and gold nanoparticles. Hexamine is non-toxic, low-cost and easy to handle. In this study, silver and gold nanoparticles have been synthesized in aqueous medium by a microwave-assisted method using hexamine as the reducing agent and biopolymers as the stabilizer. Here, the naturally occurring polymers namely agar, starch, pectin, and iota-carrageenan are used to protect and stabilize the nanoparticles. This synthetic strategy is simple, fast, economic and environment friendly. The UV-vis spectroscopic studies are conducted to observe and confirm the formation of nanoparticles. FTIR studies are performed to substantiate the role of biopolymers in stabilizing the nanoparticles. X-ray diffraction study is used to confirm the crystalline nature of nanoparticles. The elemental characterization of the samples is conducted by energy dispersive X-ray (EDX) analysis. The size and morphology of the synthesized nanoparticles are investigated using high resolution transmission electron microscopic (HR-TEM) analysis. The optimization studies are conducted by using varying concentrations of hexamine and metal salt solution.

With increasing emphasis on green chemistry, biological methods of nanoparticle synthesis have aroused much interest as a feasible alternative to conventional physical and chemical methods because of its ease, environment benign nature and cost effectiveness. Plant extracts and several microorganisms such as bacteria, fungi and yeast have been used for nanosynthesis [19-22]. Even though, phytochemical mediated biosynthesis can be carried out at ambient conditions, the time required for nanosynthesis is much longer than the chemical methods. Microwave-assisted synthesis is particularly important in this regard. Microwave-assisted synthesis using phytochemicals as both reducing and capping agents is a feasible way for the rapid and facile green synthesis of silver and gold nanoparticles. In this work, we also report a novel microwave-assisted method for the biosynthesis of silver and gold nanoparticles using the phytochemicals extracted from three medicinally important plants namely, *Alpinia galanga*, *Biophytum sensitivum*, and *Aerva lanata* as both reducing and capping agent. The nanoparticle synthesis is also carried out at room temperature without microwave irradiation to study the effect of microwave heating upon the size and rate of formation of nanoparticles. The
synthesized nanoparticles are characterized using UV-vis., FTIR, XRD, and HR-TEM analysis.

Promising new avenues are emerging in the field of catalysis based on nanotechnology approaches. The efficient, size controlled and cost-effective synthesis of catalysts is thus a goal of great importance. Novel catalytic properties such as greatly improved reactivity and selectivity have been reported for nanocatalysts as compared to their bulk counterparts [23]. Here, an attempt is made to evaluate the catalytic efficiency of the nanoparticles synthesized by the above mentioned methods in the reduction of 4-nitrophenol, methyl orange, methylene blue, and rhodamine B by NaBH₄. The kinetics of these reactions as well as effect of catalyst concentration has been studied using UV-vis. spectroscopic analysis.

The antimicrobial properties of silver have been known for thousands of years. It has been used to treat a wide variety of infections. But it was found that high doses of silver when administered intravenously could cause convulsions and even death [24]. Recently, silver nanoparticles have gained more attention. Due to their small size and hence large surface area, they can undergo more efficient binding with the microorganism. Thus the dose of silver used in medical applications can be reduced which in turn can minimize their toxicity. Considering the possible application of silver nanoparticles in various biomedical fields, the antimicrobial activity of the synthesized Ag nanoparticles have been evaluated by well diffusion method. The clinical isolates of human pathogenic bacterial strains (including both Gram positive and Gram negative) and fungal strains isolated from contaminated food are explored in this study. The synergetic effect of silver nanoparticles with antibiotics has also been studied by combining them with antibiotics against Gram positive and Gram negative bacteria.

Experimental

**Microwave-assisted synthesis of silver nanoparticles using biopolymers as stabilizing agents**

In a typical procedure, 0.1 g of biopolymer was dissolved in 90 mL of double distilled water in a 250 mL beaker. To this, 10 mL of 0.05 M AgNO₃ solution was added drop wise and was stirred for 15 minutes. This was followed by the addition
of a definite amount of hexamine and the mixture was placed in a domestic microwave oven (Sharp R-219T (W)) operating at a power of 800 W and frequency 2450 MHz. No hexamine was added when iota-carrageenan was used as the biopolymer. The solution was subjected to microwave irradiation for a definite time. The formation of AgNPs was monitored using UV-vis. spectrophotometer by analyzing the reaction mixture after different irradiation time. The nanoparticles were separated from the reaction medium by repeated centrifugation. In each case, the supernatant was replaced with double distilled water.

**Microwave-assisted synthesis of gold nanoparticles using biopolymers as stabilizing agents**

To 90 mL aqueous solution containing 0.1 g of biopolymer in a 250 ml beaker, 10 mL 0.01 M hydrogen tetrachloroaurate (III) trihydrate solution was added in a drop wise manner and was stirred for 15 min. To this, a specific quantity hexamine was added and the reaction system was irradiated with microwave radiation by placing in a domestic microwave oven. As in case of AgNP synthesis, no hexamine was used with iota-carrageenan. The reaction mixture was subjected to spectrophotometric analysis after different times of microwave heating in order to authenticate the formation of nanoparticles. The solution was repeatedly centrifuged at in order to separate the nanoparticles.

**Microwave-assisted phytochemical mediated synthesis of silver and gold nanoparticles**

In a typical microwave synthesis, 90 mL of 1 mM silver nitrate solution was taken in a 250 mL beaker. To this, 10 mL plant extract was added and stirred well. It was then placed in a domestic microwave oven and was subjected to microwave irradiation. The formation of AgNPs was monitored using UV-vis. spectrophotometer by analyzing the reaction mixture after different intervals of microwave action. The silver nanoparticle solution was then centrifuged in order to separate nanoparticles from the solution.

The microwave-assisted biosynthesis of gold nanoparticles (AuNPs) was carried out as follows. 10 mL of *Aerva lanata* leaf extract was added to 10 mL of HAuCl₄.3H₂O solution (0.01 M) taken in a 250 mL beaker. The reaction mixture
was diluted to 100 mL with distilled water in order to make the final concentration of gold salt in the reaction mixture as 0.001M. It was stirred well and indulged with microwave radiation for 1 min. The AuNPs were separated from the reaction mixture by centrifugation.

**Catalytic activity of silver and gold nanoparticles**

In order to follow the catalytic activity of nanoparticles, 0.5 mL freshly prepared NaBH$_4$ solution (0.06 M) was added to 2 mL of reactant solution of a definite concentration taken in a quartz cuvette. To this, 0.5 mL of the nanoparticle solution of a specific concentration was added to start the reaction. The reaction rate was investigated by monitoring the change in intensity of the $\lambda_{\text{max}}$ with time using UV-vis. spectrophotometer.

**Antimicrobial activity of silver nanoparticles**

The antimicrobial activity of the AgNPs was evaluated by well diffusion method. Clinical isolates of human pathogenic bacteria strains of *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholera*, *Salmonella typhi*, *Salmonella paratyphi* and fungal strains of *Aspergillus niger* and *Rhizopus stolonifer* isolated from contaminated food were used in this study. The bacterial and fungal strains were sub cultured in liquid nutrient agar and potato dextrose agar (PDA) respectively prior to the experiment. Mueller Hinton agar (MHA) medium and potato dextrose agar (PDA) medium prepared following manufacturer’s instruction were sterilized and transferred into autoclaved Petri dishes placed in a laminar air flow. When the medium was solidified, the culture of each test bacteria was uniformly seeded over the surface of different MHA medium and that of fungus on the PDA medium using sterile cotton swabs. Using a sterile gel puncture, wells of 8 mm diameter were made on the MHA and PDA plates. To these wells, solutions of control, silver nitrate, and different volumes of AgNP were poured using a micropipette. All the MHA plates were incubated at 37°C for 24 hours and the fungal plates were kept at room temperature for 48 hours. Finally, the zone of inhibition around the wells was measured in mm.
Synergistic interaction between antibiotics and silver nanoparticles

The disc diffusion method was used to assay the synergistic effect of antibiotics with silver nanoparticles. In this study, the synergism was evaluated using standard gentamicin (10 µg) discs as a model antibiotic, *Staphylococcus aureus* and *Vibrio cholerae* as representative bacterial strains. Mueller Hinton agar (MHA) medium was prepared by mixing 2.1 g of MHA and 2 g of bacteriological agar with 100 mL of sterile water followed by heating to get a uniform viscous liquid. 20 mL of sterilized MHA medium was then poured into individual Petri dishes placed in a laminar air flow. Hereafter, the Petri plates were uniformly inoculated with overnight cultures of the two bacteria. In order to see the combined activity, gentamicin discs fed with and without 20 µL AgNP solution along with a sterile paper disc impregnated with 20 µL of AgNP solution were placed over the seeded agar medium in different Petri plates. These plates were incubated at 37°C for 24 h and the zones of inhibition around different discs were measured.

Results and discussion

Synthesis and characterization of biopolymer stabilized silver and gold nanoparticles

With the intention to make nanoparticles (NPs) through green chemistry practices, silver nanoparticles were synthesized using non-toxic hexamine as reducing agent, easily renewable biopolymers namely agar, starch, and pectin as stabilizing agents, and environmentally benign water as solvent. Gold nanoparticles (AuNPs) were synthesized using the same reducing agent and the biopolymers agar and starch. AgNPs and AuNPs were also produced using the biopolymer iota-carrageenan as both reducing and stabilizing agent and water as solvent. The nanoparticles (NPs) synthesized using all the biopolymers were found to be stable for several months. The UV-vis.spectroscopic studies clearly demonstrated the formation of nanoparticles. All the synthesis processes were very fast. The formation of AgNPs was accomplished within 5 min and that of AuNPs within 4 min. The FTIR spectra of NPs showed some shifts in the position of various absorption frequencies when compared to pristine biopolymers. This shows the involvement of various groups of the biopolymers in synthesize and stabilization of the
nanoparticles. The XRD patterns of the NPs showed diffraction peaks in the 2θ value range of 35-80° which are in good agreement with the standard diffraction pattern of JCPDS file no.04-0783 for silver and 04-0784 for gold, thus indicating their crystalline nature. The characterization of AgNPs was done by EDX analysis which provided evidence for the presence of silver nanoparticles within the biopolymer matrix. The HR-TEM analysis showed that the nanoparticles synthesized using all four biopolymers were uniformly distributed and were more or less spherical in shape. Further evidence for the crystalline nature of NPs was obtained from the high resolution images and SAED patterns. The size of the AgNPs followed the order: AgNP-agar < AgNP-starch ~ AgNP-pectin ~ AgNP-carrageenan while that of AuNPs followed the order: AuNP-agar < AuNP-carrageenan < AuNP-starch. Thus smaller nanoparticles were formed in presence of agar as stabilizing agent in both cases. The optimization study was conducted by varying the concentration of AgNO₃ and hexamine, taking synthesis of AgNP-agar as a representative case. The rate of formation as well as the size of nanoparticles was found to increase with increase in concentration of AgNO₃. Furthermore, the size of the nanoparticles and the reaction time decreased with increase in concentration of hexamine.

**Phytochemical mediated synthesis of silver and gold nanoparticles, their characterization studies**

As compared with chemical methods, biological methods for nanoparticle synthesis are observed to be time consuming. This shortcoming of the biomediated methods need to be rectified if they have to compete with chemical methods. With this intention, we conducted microwave-assisted biosynthesis of silver and gold nanoparticles. Silver nanoparticles were synthesized using the rhizome extract of *Alpinia galanga*, the leaf extracts of *Biophytum sensitivum* and *Aerva lanata*, and gold nanoparticles were prepared using the leaf extract of *Aerva lanata*. The biosynthesis was also performed under ambient condition without microwave irradiation in order to compare the rate of the two methods. All the microwave-assisted synthesis was found to be finished within 3 min. On the other hand, the biosynthesis carried out without microwave assistance took several hours to achieve nanosynthesis. The microwave-assisted biosynthesis is observed to be superior to
room temperature biosynthesis as far as size and rate of formation of nanoparticles is considered. The synthesized nanoparticles were characterized using UV-vis., FTIR, XRD, and HR-TEM studies. The UV-vis. spectra showed strong absorption bands due to surface plasmon resonance (SPR) of metal nanoparticles in the range of 400-450 nm thereby confirming their formation. The nanoparticles were stable in aqueous solution for several weeks. Thus biopolymer stabilized nanoparticles are more stable in aqueous solution than phytochemical stabilized nanoparticles. Evidence for the involvement of phytochemicals in the synthesis and stabilization of nanoparticles was obtained from FTIR measurements. The XRD measurements showed that the synthesized nanoparticles were highly crystalline in nature. From TEM analysis, the AgNPs were found to be more or less spherical in shape. On the other hand, AuNPs were found to be polydispersed in which majority of them were of spherical geometry. The size of the NPs followed the order: AuNP-aerva < AgNP-aerva < AgNP-biophytum < AgNP-alpinia. The HR-TEM and SAED measurements further confirmed the crystalline nature of both AgNPs and AuNPs. Compared to biopolymer stabilized nanoparticles, biosynthesized nanoparticles were found to possess wider size distribution.

**Catalytic activity of silver and gold nanoparticles**

Nanocatalysis is an emerging area of research in nanoscience and nanotechnology. Here, metal nanoparticles are used as catalysts for a wide range of chemical reactions which are otherwise very slow or not feasible. Due to their nanodimensions, special reactivity can be anticipated that can afford specific properties which cannot be achieved with regular non-nano materials. The catalytic activity of the synthesized nanoparticles was evaluated by taking some reactions which are important from the perspective of pollution abatement. The NaBH₄ reduction reactions of 4-nitrophenol, methyl orange, methylene blue, and rhodamine B were studied for this purpose. The silver and gold nanoparticles with redox potential intermediate between those of donor and acceptor were observed to effectively catalyze the reactions by acting as an electron relay system. All these reactions followed pseudo-first order kinetics. The rate of the reactions increased linearly with the increase in concentration of the catalyst. Moreover, an induction time was observed for all the reactions studied when carried out under ambient
condition. The induction time noticed in the case of methylene blue degradation was higher than that in other cases due to the hydrophobicity of methylene blue offered by the four methyl groups which hinders the physical contact of it with NPs to a certain extent. The induction time was found to decrease with the increase in temperature and concentration of the catalyst. The size of the nanocatalysts and its surface characteristics, especially the capping agent played a key role in deciding their catalytic efficiency. In general, the smaller sized nanoparticles were observed to be more effective than larger ones due to their high surface area to volume ratio.

**Antimicrobial activity of silver nanoparticles (AgNPs)**

Silver and silver compounds have been known to possess strong inhibitory and bactericidal effects as well as a broad spectrum of antimicrobial activities since ancient times. Silver is a safe and effective antimicrobial agent because it is nontoxic to human cells and highly toxic to microorganisms. Recent studies show that antimicrobial formulations of nanosized silver could be used as effective antimicrobial agents because of their broad spectrum activity, high rate of effectiveness and low cost. The total surface area of the nanoparticles is very high compared with bulk metal due to their small size and this gives them high activity to weight ratio. The research activities on the synthesis and characterization of nanosized silver particles open the possibility of formulation of new generation bactericidal materials.

The antimicrobial activity of the synthesized silver nanoparticles were studied using the renowned agar well diffusion method. The antibacterial efficacy was tested against the human pathogenic bacteria namely, *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi*, and *Salmonella paratyphi*. The results of antibacterial study suggest that the AgNPs synthesized using biopolymers as well as phytochemicals are very effective antibacterial agents. They exhibited very good activity against all tested bacterial strains. The efficiency of AgNPs was found to be superior to AgNO₃. Furthermore, the different bacterial strains were inhibited in a size and dose-dependent manner as indicated by the increase in zone of inhibition with decrease in particle size and the increase in AgNP dosage. The biopolymer stabilized AgNPs showed more or less similar activity against both
Gram positive and Gram negative bacteria. On the other hand, the biosynthesized AgNPs inhibited Gram negative bacteria to a greater extent than Gram positive. The AgNPs were also evaluated for their antifungal activity by using two fungal strains isolated from contaminated food namely Aspergillus niger and Rhizopus stolonifer. They showed potent activity against both the fungal species in a size dependent manner. Moreover, they were found to be exceedingly toxic against fungal strains when compared to the bacterial strains due to the weak ability of fungus to resist the attack of AgNPs. The AgNPs were found to be slightly more effective against Rhizopus stolonifer than Aspergillus niger.

**Synergistic interaction between AgNPs and antibiotics**

The emergence of bacterial resistance to antibiotics is a serious and growing phenomenon in current medicine and has appeared as one of the top public health concerns of the 21st century. Silver nanoparticles have been known to possess inhibitory and bactericidal effects against a wide spectrum of Gram positive and Gram negative bacteria especially multidrug resistant bacteria. Thus the association of silver nanoparticles with antibiotics is a very promising strategy to control antibiotic resistant bacteria. The synergistic interaction of antibiotics with AgNPs was investigated by combining silver nanoparticles with gentamicin against Staphylococcus aureus and Vibrio cholerae. The antibacterial activity of gentamicin was increased in combination with all the AgNPs synthesized in the study against both the bacterial strains. In the case of biopolymer stabilized AgNPs, the augmentation in antibacterial activity (i.e. fold increase) was calculated to be high against V. cholerae while for biosynthesized AgNPs, higher fold increase was noticed in the case of S. aureus. In this study as well, fold increase of gentamicin was found to depend on the size of the silver nanoparticles.

**Conclusion**

In short, new methods for the synthesis of silver and gold nanoparticles that fulfill the objectives of green chemistry are presented here. All the methods presented in this work are simple, fast, inexpensive, and environment friendly. The use of microwave as an alternative energy source for the facile synthesis of uniformly small sized silver and gold nanoparticles is found to be successful. These
newer techniques using biorenewable materials appear to be promising as they are deployed of any toxic materials. The potential catalytic and antimicrobial activities exhibited by different nanoparticles make them hopeful candidates for pollution abatement and as new generation antimicrobial agents. The application of these nanomaterials as sensors and in biomedical fields is under consideration. Even though, the use of phytochemicals has shown great potential for the green synthesis of nanoparticles, the exact mechanisms by which these biomaterials are involved in nanosynthesis are still lacking. A progress in this area will open new paths for the size and shape controlled synthesis of metal nanoparticles.