GREEN SYNTHESIS OF METAL BASED NANOPARTICLES AND THEIR ANTIMICROBIAL APPLICATIONS – A REVIEW

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Abstract- Recently, green approaches for the synthesis of metal-based nanoparticles are gaining popularity due to their cost-effectiveness and environment friendly nature as compared to physical and chemical methods where different toxic chemicals are used which directly affect the environment. During synthesis of metal-based nanoparticles through green approach, various biological components like leaves, stems, roots, flowers etc. are used as stabilizing as well as reducing agents. According to WHO report, metal-based nanoparticles have been used as an effective agent against different pathogens due to which they are also investigated for their biomedical applications. This review summarizes the overview of synthesis of metal-based nanoparticles (Ag, Au, Fe, Zn, Pd and Ni) and their antimicrobial activities. The novelty of this review is based on the comparative studies of different types of metal-based nanoparticles and their development as more effective antibacterial agents against multi-resistant gram-negative and gram-positive bacteria.

Keywords: Antimicrobial activity, Metal-based nanoparticles, Green synthesis, Gram-negative, Gram-positive

1. INTRODUCTION

The highly adaptable and first living organism on the earth was bacteria. During 20th century, one of the most significant achievement by human being in medical field is the discovery of antibiotics. The discovery of antibiotics started with finding of first non-toxic medicine, Salvarsan, which was used to cure syphilis, an infectious disease followed by Penicillin (discovered by Alexander Fleming in 1928) and reached on peak from 1950-1960 [1]. From last few decades, new compounds with antimicrobial properties against contamination of microbes have been revealed. The pathogenic microorganism elimination for declining nosocomial infection and foodborne diseases and to prevent health has been foremost target in public and private sector of research and medical field. The antibacterial efficiency of a given compound is defined by two terms: bacteriostatic where there is delay in bacterial growth while maintaining the growth in initial stage for a longer period and bactericidal where bacteria die due to inhibition in their growth. Moreover, the compounds exhibited both these effects in in-vitro process depend on many parameters like duration of test, condition where growth takes place, density of bacteria etc [2]. The efficiency of antibacterial compounds also depends on bacterial form for example gram-positive and gram-negative bacteria are different as thickness of their peptidoglycan layer is different [3]. As a result, it has become pressing requirement to develop advanced and efficient antimicrobial agents. Therefore, the scientist and researchers are focusing on nanomaterials, especially metal-based nanoparticles in order to assess their feasibility and antimicrobial properties for elimination of contaminated sources and diseases produced from those sources. The synthesis of nanoparticles are being done on nanoscale in order to enhance their physical, chemical and biological properties [4], [5], [6].

2. SYNTHESIS OF METAL-BASED NANOPARTICLES

Synthesis of metal-based nanoparticles is not a new technique. The natural synthesis of metal-based nanoparticles using microorganism for detoxification of heavy metals has already described over last decades. The versatility of this techniques is widely used for the cosmetics production resulting increasing the attention of scientist and researchers where they started to search their different synthesis method with new composition and applications. Although researchers have been focusing on some particular metals like gold, silver, zinc, nickel, palladium and iron. The most suitable candidates for synthesis of metal-based nanoparticles are transition elements as they have partially filled d-orbitals due to which they can easily undergo reduction (reduced to zero-valent atoms) results in enhancement of aggregation of nanoparticles.

Two main approaches used for nanoparticles synthesis are: top-down and bottom-up approach (Fig. 2.1). In top-down approach, synthesis takes place by reduction of size of particles (fine particles) from their bulk size through disintegration. This process mainly involves physical and chemical methods such as lithographic, sputtering, pulsed laser ablation etc. Although, this approach is very simple, but it also generates nanoparticles with equally dispersed size distribution which is not considered a appropriate for metal-based nanoparticles synthesis where size is determining parameter for their activity. On the other hand, this approach is very lengthy including energy demanding and highly expensive [7]. The major disadvantage is defect in the surface structure of nanoparticles. In bottom-up approach, smaller atoms and molecules assembled and generates nanoparticles in diverse range. For example, chemical precipitation, sol-gel processing, chemical vapor deposition and bio-assisted methods [8]. However, all these methods are non-eco-friendly, require high energy for completion of reactions and the chemicals used during the reaction as well as products formed are hazardous and highly toxic. Most of chemical methods results in exploitation of harmful reducing agents like hydrazine, sodium citrate, ethylene glycol but they show...
poor control over size of particles and their size distribution thus additional stabilizing or capping agents are required for prevention of nanoparticles agglomeration.

In general, synthesis of nanoparticles takes place using different physical, chemical and biological approaches as antimicrobial properties depends on their synthesis method also. Among all the approaches, biosynthesis, a green synthetic approach, that can be categorized under bottom-up approach is one of the most effective as the nanoparticles can be prepared in large number in short time [9, 10]. This process is just like chemical reduction process where hazardous, toxic and expensive chemical are replaced by plants extracts. Presently, “green method” to synthesize the nanoparticles are receiving much attention due to their low environmental effect. In green synthesis method, different routes by using microorganism and plant can be utilized in healthy, effective and productive manner [11]. From past few years, various metal-based nanoparticles like silver, gold, zinc oxide, nickel oxide etc. have been synthesized which are known for their antimicrobial activities [12, 13].

3. A GREEN APPROACH TO SYNTHESIZE METAL-BASED NANOPARTICLES

The antibiotic-resistant bacteria, resistant to over 100 different forms of antibiotics, have been increased in number due to the development of nanotechnology. This issue has prompted the researchers for developing biosynthetic nanoparticles as antimicrobial agents [14]. Biosynthesis can be considered as green synthetic approach and grouped in bottom-up approach (smaller metal atoms grouped together results in formation of the nanoparticles in diverse range. This process is like chemical reduction process where toxic and harmful chemical are replaced by biological units. The units used to fabricate the nanoparticles are plants extract [15], bacteria, fungi [16], materials from vegetables and fruits [17, 18]. The end product of these methods are known as biogenic while organisms which release proteic materials (reduce metal ions) used during these methods are known as biological nanofactories [19].

Amooaghaie et al performed a comparative study between green synthesis where chemical reduction of silver nanoparticles takes place by using plants extract and conventional wet-chemistry method which is further confirmed by Kumar et al [20], [21]. It was found that silver nanoparticles synthesis using green approach have less cytotoxicity and phytotoxicity as compared to nanoparticles synthesized by wet-chemical method. The comparative studies confirmed that green approach is safer, eco-friendly and used in bio-medical field. Based on these studies, the green approach is gaining more attention in private and public sectors both. Among all biological materials, plant extracts are becoming more interesting for researchers and scientists as they have low cost with simplicity, fast reaction, and ability to reduce the size of metal ions into metal nanoparticles along with their production on large scale. Moreover, the biological units used for nanoparticles preparation control shape, size, structure, morphology and even functions of nanoparticles. Nagajyothiet et al confirmed that gold nanoparticles fabricated using Lonicera Japonica flower extract have different structure like face centered cubic (fcc), quasi spherical with average size 8.02 nm. Recently, Huo et al, confirmed spherical, rodlike and irregular shape of gold nanoparticles synthesized using Chenopodium aristatum L [22].

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**Fig. 2.1 Synthesis of nanoparticles through different approaches**

**Fig. 2.2 Synthesis of metal based nanoparticles by green-approach**
Metal-based nanoparticles mainly provide most promising solution against various microorganisms by targeting over different biomolecules and pose a threat to the growth of resistant strains. Therefore, they are one of the most common inorganic nanoparticles. The major aim of the present review is to explore green approach to synthesize metal-based nanoparticles, their characterization including their morphology and the methods through which their antibacterial properties could be analyzed.

The most simple and commonly used method for synthesis of metal-based nanoparticles is through extract of leaves, flowers, barks, fruits, roots of plant. First of all, the plant parts washed in water followed by sterilization using double distilled water (Tween 20 can also be used for sterilization). The parts of plants are then allowed to dry at room temperature and crushed them using pestle mortar. The solution is boiled after adding required concentration of Milli-Q-H$_2$O with continuous stirring. Through Whatman filter paper, filtration was done and the solution obtained after filtration is known as plant extract which is further used for preparation of metal-based nanoparticles. A portion of extract is allowed to mix in metal solution and boiled at appropriate temperature for a certain time to achieve efficient mixing as shown in Fig. 2. During metal-based nanoparticles synthesis, the parameter like pH, temperature, concentration of extract solution and time can be varied for best results. The change in color of the solution during incubation period further confirmed the synthesis of nanoparticles [23].

4. CHARACTERIZATION OF METAL-BASED NANOPARTICLES

Characterization is important to estimate size, structure and morphology of synthesized nanoparticles. The characterization techniques used to investigate size, morphology, surface roughness and texture of NPs are: SEM (Scanning Electron Microscopy), AFM (Atomic Force Microscopy) and TEM (Transmission Electron Microscopy). Characterization tools that are used to recognize and analyze crystalline size of different nanoparticles are: 1) XRD (X-Ray diffractometer) and 2) XPS (X-ray photoelectron spectroscopy). XRD is used for characterization of crystalline material to determine its interatomic distance and angle. As NPs have many applications in the field of optoelectronics therefore it is important to characterize their optical properties. The techniques which are used to investigate optical properties are: (1) UV-Visible Spectroscopy and (2) FTIR (Fourier Transform Infrared Spectroscopy). UV-Visible spectroscopy is employed to find absorbance spectra of a nanomaterial. FT-4787487IR spectroscope is used to detect different functional groups present in the synthesized nanoparticle. To study thermal properties of nanoparticles, common techniques are: 1) DSC (Differential scanning calorimetry) and 2) TGA (Thermal gravimetric analysis). In DSC, measurement of heat capacity (total heat that is required to raise the temperature) of synthesized nanoparticle is done. This is compared with reference as function of temperature. In TGA, change in mass of a nanomaterial with change in temperature with respect to time is measured. Chemical composition analysis i.e. identification of different elements involved in the synthesized nanomaterial is done by EDX (Energy dispersive X-ray)26. Surface charge measurement is also important to explain the properties of synthesized NPs. Examination of colloidal stability and surface charge can be done through zeta potential method [24, 25].

5. ANTIBACTERIAL PROPERTIES OF METAL-BASED NANOPARTICLES

By Romans, Egyptians, Greeks and Persians, the antibacterial properties of metal-based nanoparticles have been studied since ancient times. In present days, the nanoparticles synthesized through green approach are gaining more attention due to their novel functionality specifically, metal-based nanoparticles which provide strong antibacterial properties against wide range of microorganism even at smaller concentration [26]. Table 1 shows some examples of metal-based nanoparticles with their potential antimicrobial properties. The antimicrobial properties are shown against gram-positive (Streptococcus pneumoniae, Staphylococcus aureus, Bacillus subtilis,) and gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae) [27].

| Precursor          | Reducing/Oxidizing Agent | Absorbance Peak, Morphology and Size | Antimicrobial Activity with Zone of inhibition (ZOI) (mm) | References |
|--------------------|--------------------------|-------------------------------------|----------------------------------------------------------|------------|
| Zinc acetate dihydrate | Lippia adenosis          | XRD peak: 360-365 nm Morphology: Uniform spherical particles Size: 19.78 nm | Gram-positive bacteria: S. aureus = 16 E. faecalis =14 Gram-negative bacteria: E. coli = 13 K. pneumonia = 10 ZnO NPs showed strong antibacterial activity against both Gram-positive bacteria | [28]       |
| Zinc nitrate       | Hydro alcoholic clove extract | Morphology: Hexagonal shape of individual and aggregated particles Size: 50 nm | E. coli = 11.12 S. aureus = 12.11 | [29]       |

Table-5.1 Examples of metal-based nanoparticles synthesis through green approach with potential antibacterial activity
| Zinc acetate, Sodium hydroxide, | Rosa indica | UV-Vis peak: 345nm. Morphology: spherical shape Size: 35 nm. | S. aureus = 15 E.coli = 14.5 at 7.5 μg/μl concentration |
|--------------------------------|-------------|---------------------------------------------------------------|----------------------------------------------------------|
| Ferric chloride hexahydrate (FeCl₃·6H₂O). | Carica papaya | Size: 21.59 nm | Klebsiella spp. = 9 ± 1 mm E.Coli = 9 ± 1 mm Pseudomonas spp. = 10 ± 0.5 mm S. aureus = 12.5 ± 0.5 mm at 30mg/ ml dose of synthesized NPs |
| Iron sulphate (FeSO₄). | Lawsonia inermis and Gardenia jasminoides | Size: NPs using Lawsonia inermis = 21 nm NPs using Gardenia jasminoides = 32 nm. | NPs with Gardenia jasminoides were found to be more effective against E. Coli = 15 S. enterica = 12 P. mirabilis = 13 S. aureus = 16 at 30 µg/µl |
| Ferrous sulphate (FeSO₄). | M. ornata | UV-vis peak: in range of 250-350 nm. Size: 43.69 nm S. aureus = 32 S. agalactiae = 28 at 20 mg/ml |
| Nickel nitrate | Moringa oleifera plant | Morphology: Spherical and are slightly agglomerated | E. Hermannii = 5 E. Coli=10 S. Pneumoniae =12 S. Aureus=15 NPs showed greater resistance of Gram negative pathogens over Gram positive bacterial strains |
| Nickel nitrate | Rhamnus virgate. | UV-vis peak: 320 nm Morphology: Spherical Size: 24 nm average size | Synthesized NiO NPs were more effective against gram-negative strains E. coli, P. aeruginosa and K. pneumoniae with MIC: 125 μg/mL |
| Nickel Nitrate hexahydrate | E. heterophylla. | UV-vis peak: 321 nm. Size: Average particles size around 12-15 nm. | E. Coli = 9.23±0.15 S. aureus = 8.23±0.15 P. desmolyticum = 7.33±0.09 K. aerogenes = 7.10±0.21 at 600 µg/mL |
| Silver Nitrate | Glycyrrhiza glabra and Amphipterygium adstringens | UV-Vis peak: 420 nm Morphology: Spherical Size: 9 nm from Glycyrrhiza glabra and 3 nm from Amphipterygium adstringens | NPs synthesized with G. glabra extract exhibited a good antimicrobial activity against E. faecalis |
| Silver nitrate (AgNO₃) and iron III chloride (FeCl₃) | Rhizome extract of Acorus calamus (ACRE) | UV-Vis peak: 421 nm Morphology: Spherical Size: 31.83 nm | B. cereus = 15 B. subtilis = 17 S. aureus = 16 cm respectively |
| Silver Nitrate | Alternanthera dentata | UV-Vis peak: 430 nm Morphology: Individual and aggregated NPs are spherical shape Size: 50-100 nm average particle size | AgNPs were more noticeable toward gram-negative bacteria |
| Compound                          | Plant Name          | UV-Vis peak: | Morphology: | Size:          | Activity and Result                                                                                   |
|----------------------------------|---------------------|--------------|-------------|----------------|------------------------------------------------------------------------------------------------------|
| Gold(III) chloride hydrate (HAuCl₄) | Dracocephalum kotschyi | 536 nm       | spherically shaped | in range of 7.9 to 22.63 nm | No effect of AuNPs on bacterial activity even after on increasing the concentration of nanoparticles. |
| Hydrogen tetrachloroaurate (III) trihydrate (HAuCl₄·3H₂O) | Salix alba L. | 540 nm       | spherically shaped | 63 nm average particle size | Synthesized AuNPs were inactive toward Klebsiella sp. And Bacillus sp. Whereas for S. aureus ZOI was 10±0.58 Excellent antifungal activity against A. solani = 40 ± 0.45 A. niger = 50 ± 0.53 A. flavus = 10 ± 0.56 |
| Gold chloride (HAuCl₄)           | Nigella arvensis    | 546 nm       | spherical shape | 3-37 nm         | Maximum inhibition zone was reported for S. epidermidis (full ZOI) whereas moderate antibacterial activity was shown against E. coli = 17 B. subtilis = 7 S. aureus = 13 |
| Palladium chloride (PdCl₂)       | Filicium decipiens | 650-700 nm   | spherical size | 2-20 nm average particle size | E. coli = 12±0.25 S. aureus = 12±0.4 P. aeruginosa = 24±0.6 B. subtilis = 27±1.2 |
| Palladium(II) chloride, (PdCl₂)  | Melia azedarach    | 280 nm       | spherical size | 10-20 nm        | Maximum ZOI was for B. subtilis = 8.33±0.33 followed by S. aureus, E. coli, P. vulgaris = 7.33 ± 0.33 S. pneumoniae and P. aeruginosa. = 5.33 ± 0.33 |
| Palladium acetate               | Moringa oleifera   | 460 nm       | spherical size | 2-18 nm         | Significant antibacterial activities against E. faecalis with inhibition zone 26±0.6. The cytotoxic effects was more prominent in case of A549 cells as compared to PLs indicating anti-proliferative activities in A549 cells |

6. GENERAL MECHANISM OF ANTIMICROBIAL PROPERTIES OF METAL-BASED NANOUPARTICLES

There are some general features of bacteria which play an important role to define their behavior when encounters metal-based nanoparticles [46]. The toxicological effects of antibacterial compounds are due to their direct interaction to cell wall therefore, it is an urgent need to differentiate between cell wall of Gram-Positive and Gram-Negative bacteria as surface charge is same (negatively charged) for both the bacteria [47]. The main difference is between peptidoglycan layer which is formed by cross-linking of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) through 3-5 amino acids sequence and formed a cohesive mesh. Gram-positive bacteria have a dense peptidoglycan layer, while Gram-negative bacteria have a thin layer with a more complicated structure. Therefore, electrostatic interaction between cell wall (negatively charged) and metal-based nanoparticles (positively charged) results in formation of strong bond due to which cell wall disrupt completely results in increase in permeability. Furthermore, metal ion released from their extracellular space, enters into cell wall and inhibits biological processes of bacteria. Reactive oxygen species (ROS) produced in cell either by metal ions or nanoparticles leads to glutathione oxidation and suppressed their antioxidant defense activity against ROS.
Reactive oxygen species are the molecules whose oxidative potential is very large indicating their toxicity against microorganism even though their short lifetime (between $10^{-9}$ to $10^{-3}$ s). The most well-known ROS are Superoxide radicals ($O_2^-$), hydroxyl radicals (•OH), hydrogen peroxide (H₂O₂), and singlet oxygen (O₂) [48, 49]. The general mechanism of interaction of metal-based nanoparticles is shown in Fig. 6.1.

**CONCLUSION**

The market of metal-based nanoparticles has been increasing over the last few years due to their applications in biomedical fields. In present review, we have revised some widely used biosynthesized metal nanoparticles (Zinc, Iron, Nickle, Gold, Silver and Palladium) which are commonly exploited for pharmaceutical and medical fields (used as antibacterial, antiviral, anti-inflammatory, anti-cancer, anti-fungal). These nanoparticles can be used as an alternate over traditional antibiotics due to their well-behaved antimicrobial activities to overcome bacteria resistance. Due to their mechanism of action towards gram-positive and gram-negative bacteria, they differ from classical methods. They have developed antibiotic resistance by targeting various biomolecules which comprises resistant strain development. The main mechanism used by metal-based nanoparticles for elimination of microorganism was oxidative stress. It can be concluded that these green synthesized metal nanoparticles are better alternatives for treatment of bacterial infections in near future.

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**Fig. 6.1 Mechanism of interaction of meta-based NPs with bacterial cell wall**
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