GREEN SYNTHESIS OF ZINC OXIDE NANOPARTICLES - REVIEW PAPER

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ABSTRACT

Plants have been used in the synthesis of metallic nanoparticles because they are more ecofriendly. These plant extracts also allow a controlled synthesis. Organic chemical solvents are toxic and require extreme conditions during nanoparticle synthesis. Plant extracts function as stabilizing, capping or hydrolytic agents. The ZnO nanoparticles are of significant interest as they provide many practical applications worldwide. The most important application of ZnO nanoparticles would be as antibacterial agents. The increases surface area and smaller size of these particles make them an ideal antibacterial agent. In this review, the overview of green synthesis of ZnO nanoparticles along with their antimicrobial activity. The mechanism of this activity was also reviewed. The green synthesis of ZnO nanoparticles from Azadirachta indica, Aloe vera, Murraya koenigii and Anissochilus carnosus were also highlighted.

KEYWORDS: Green synthesis, nanoparticles, zinc oxide nanoparticles, antimicrobial activity.

GREEN SYNTHESIS OF NANOPARTICLES

Lot of attention has been diverted to the green synthesis of metal nanoparticles using biological material as the reducing and stabilizing agents and due to the usage of ecofriendly,
non-toxic and safe reagents during the biosynthesis process, green synthesis has been considered in the field of toxic chemical and physical methods (Moritz et al., 2013; Rajiv et al., 2013; Caruthers et al., 2007; Nath and Banerjee, 2013; Salam et al., 2012). In the biological method, plant extracts are used for controlled and precise synthesis of several metallic nanoparticles (Rajiv et al., 2013). High surface and a large fraction of surface atoms are responsible for the nanoparticles’ atom-like behavior (Dijiken et al., 2000; Singhal et al., 2012). Despite the fact that conventional methods use less time for synthesizing nanoparticles, they contribute to environmental toxicity because they require toxic chemicals as capping agents. Green nanotechnology is an eco-friendly alternative and is cost effective (Chandran et al., 2000; Shankar et al., 2004; Huang et al., 2007) and utilizes proteins as natural capping agents. Synthesis of metal nanoparticles by plants utilize various secondary metabolites, enzymes, proteins and or other reducing agents.

**ZnO NANOPARTICLES**

Zinc oxide (ZnO) are a class of inorganic metal oxides available and exhibit a wide range of nanostructures. Photocatalytic and photo oxidizing ability against chemical and biological species are used to characterize these metal oxides (Szabo, 2003). U.S. Food and Drug Administration have recognized ZnO as safe (Premanathan et al., 2011). Lower cost, UV blocking properties, high catalytic activity, large surface area, white appearance and their remarkable applications in the field of medicine and agriculture are the advantages of ZnO particles (Kairyte et al., 2013; Kumar et al., 2013; Kajbafvala et al., 2012). Recently, ZnO have been used extensively in environmental remediation and antibacterial activity (Kuriakose et al., 2013).

ZnO nanoparticles exhibit strong strong antibacterial activity against high temperature and pressure resistant spores (Nicole et al., 2008; Neal, 2008). It is postulated that the generation of hydrogen peroxide or due to the electrostatic binding of the particles on the microbial surface contribute to the antimicrobial activity of ZnO nanoparticles (Zhang et al., 2007). Antibacterial activity of ZnO nanoparticles is of remarkable applications in designing microbial resistant articles (Sharma et al., 2010) for preserving food and wood products (Singhal et al., 2012), cosmetics, novel nanomedicines (Dijiken et al., 2000) wound dressing (Shalumon et al., 2011) and disinfecting agents (Dijiken et al., 2000). Photocatalytic activity of ZnO nanoparticles offers a promising method for waste water treatment (Reddy et al., 2012). Toxic water pollutants released from textile and dying industries by utilizing natural
source of energy, sunlight are degraded by ZnO and exhibit photochemical reactivity. This could be because of the presence of many active sites and fabrication of hydroxyl radicals on ZnO surface (Baruah et al., 2009; Kajbafvala et al., 2012).

Zinc oxide has vast applications in optical, piezo electric, magnetic, and gas sensing. They exhibit high catalytic efficiency, strong adsorption ability and used in sunscreens manufacture (Seshadri et al., 2004), ceramics and rubber processing, waste water treatment, and fungicide (Theodore, 2006; Wang et al., 2008). ZnO nanoparticles can absorb both UV-A and UV-B radiation and therefore offers better protection and improved opaqueness (Theodore, 2006).

ANTIMICROBIAL ACTIVITY OF ZnO NANOPARTICLES

Understanding the mechanism of antibacterial effect of ZnO nanoparticles is necessary to make better use of these nanoparticles in food products and to develop nontoxic, antimicrobial derivatives but the mechanism is not very clear till date. Some studies have showed that morphology and oxidative stress are responsible for the antibacterial activity of zinc nanoparticles activity (Sourabh et al., 2014; Krishna et al., 2011). However, a few studies have suggested that the antibacterial activity might be because of the disruption of cell membrane activity (Brayner et al., 2006).

Another mechanism might be because of the induction of intercellular reactive oxygen species, including hydrogen peroxide (H$_2$O$_2$), which is harmful to bacterial cells (Jones et al., 2008, Sawai, 2003). ZnO have also been reported to be activated by UV and visible light in order to generate highly reactive oxygen species such as OH$^-$, H$_2$O$_2$, and O$_2$$^{2-}$. These radicals and superoxides cannot penetrate into the cell membrane and are likely to remain on the cell surface, but H$_2$O$_2$ penetrate into bacterial cells (Padmavathy and Vijayaraghavan, 2008).

Yamamoto et al., 2000 stated that the presence of reactive oxygen species (ROS) generated by ZnO nanoparticles was responsible for their bactericidal activity. Zhang et al., 2010 further stated that chemical interactions between hydrogen peroxide and membrane proteins, or between other chemical species produced in the presence of ZnO nanoparticles and the outer lipid bilayer of bacteria could be responsible for the antibacterial behaviour of ZnO nanoparticles. The hydrogen peroxide which is produced enters the cell membrane of bacteria and kills them. The study also showed that bacterial growth is inhibited by nano-sized ZnO particles. Further, Padmavathy and Vijayaraghavan, 2008 also proposed that the bactericidal
activity of ZnO nanoparticles was because of hydrogen peroxide generated by ZnO nanoparticles and the nanoparticles remain in contact with the dead bacteria thereby preventing further bacterial action and continue to generate and discharge hydrogen peroxide to the medium.

Phototoxic effect is induced in the aqueous solution of ZnO nanoparticles under UV radiation and produce Reactive Oxygen Species such as hydrogen peroxide (H$_2$O$_2$) and superoxide ions (O$_2^-$) (Zhang et al., 2011). The active species penetrate into the cells and inhibit or kill microorganisms. This is used in bionanotechnology and in bionanomedicine for many antibacterial applications. Therefore, as ZnO absorbs UV light, enhancement of ZnO bioactivity is thought to be as a result of the produced free radicals (Seil et al., 2009).

**ZnO NANOPARTICLES SYNTHESIS**

ZnO nanoparticles have been reported to be synthesized from many plant extracts. In Azadirachta indica, stabilizing agents for the nanoparticle synthesis are flavanones, terpenoids and reducing sugars, the constituents of the Neem leaf broth (Nath and Banerjee, 2013). It is suggested that the aldehyde groups are responsible for reduction of zinc oxide to zinc oxide nanoparticles and also stabilize the nanoparticles (Nath and Banerjee, 2013). Noorjahan et al., 2015 proposed a method to synthesize zinc oxide nanoparticles from the leaf extract of Azadirachta indica and its characterization by FTIR and SEM analysis. It was seen that from FTIR analysis, alcohols, terpenoids ketones, aldehydes and carboxylic acid were surrounded by synthesized nanoparticles. SEM analysis showed stable Zinc oxide nanoflakes and spindle shaped nanoparticles. The size of the ZnO nanoparticles synthesized were found to be 50 μm.

Elumalai and Velmurugan, 2015 reported the MIC, MBC and MFC values of prepared ZnO NPs against bacteria and fungi. Significant inhibition by the ZnO NPs was seen against S. aureus, B. subtilis, P. aeruginosa, P. mirabilis and E. coli and fungi strains such as C. albicans and C. tropicalis with distinct differences in the susceptibility to ZnO NPs in a dose-dependent manner. Among them, S. aureus was found to be more susceptible to ZnO NPs. The mean zones of inhibition ranged from 9.8 ± 0.76 to 23 ± 0.50 (mm). The highest mean zones of inhibition ranged from 14.4 ± 0.76 to 23 ± 0.50 (mm) against S. aureus. The MIC values ranged between to 6.25 to 50 (µg/mL) and MBC and MFC from 12. 5 to 50 (µg/mL). Antimicrobial activities of ZnO NPs increased with increase of concentrations (50, 100 and
200 µg/ml) and was considered to be due to the increase of H$_2$O$_2$ concentration on the surface of ZnO.

Aloe vera has been stated to have immune-modulatory, anti-inflammatory, UV protective, antiprotozoal, and wound- and burn-healing promoting properties. Single crystalline triangular gold nanoparticle (~50-350 nm in size) and spherical silver nanoparticles (~15 nm in size) in high yield have been successfully synthesized. This synthesis is by the reaction of aqueous metal source ions (chloroaurate ions for Au and silver ions for Ag) with the extract of the Aloe vera plant. Aloe vera extract was used to synthesize spherical zinc oxide nanoparticles and their optical properties were studied (Sangeethaa et al., 2011).

Lakshmi et al., 2012 have reported the antibacterial study of zinc oxide nanoparticles synthesized from Aloe vera hot extract (ZnO-AH), cold extract (ZnO-AC) and chemical method (ZnO-C) on six clinically isolated strains namely, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi and Staphylococcus aureus. Significant activity was seen in the zinc oxide particles synthesized by chemical method and particles obtained using Aloe vera cold extract. ZnO-AH showed lesser activity. There was a significant difference in the antibacterial activities of ZnO-AH and ZnOAC though both synthesized in a similar manner. This variation was because of the size as the size of ZnO-AH is much more than that of ZnO-AC. The smaller the size of nanoparticles better is their activity (Yamamoto 2001a, Makhluf et al., 2005).

Mariam et al., 2014 reported a novel synthesis for In$_2$O$_3$ and ZnO Nanoparticles with particle sizes in the range of 10 to 30 nm using indium nitrate and zinc nitrate solutions. They utilized A. vera extract as a solvent instead of organic solvents. The antibacterial and antifungal activities of the particles were studied using S. aureus, S. pyogenes, P. aeruginosa, E. coli, and S. typhi and the fungal strains were A. niger, A. flavus, A. fumigatus, Rhizopus indicus and Mucor indicus. Highest inhibitory activity against the tested bacteria were displayed by the extracts with ZnO + ln2O3+ A. vera. A. niger growth was also inhibited by the extract. It was concluded that ZnO nanoparticles mixed with A. vera were effective in inhibiting bacterial growth.

Murraya koenigii has been reported to have hypoglycemic (Khan et al., 1995) and anti-fungal effects (Das et al., 1965) and also against colon carcinogenesis (Khan et al., 1996). The plant has active agents like polyphenols and flavonoids which have strong roles in the synthesis
and stabilization of metal NPs (Roy et al., 2012; Roy et al., 2010). Alam et al., 2014) reported that the contents of polyphenol and flavonoids present in the leaf of M. koenigii are 81.9mg Gallic acid equivalent g⁻¹ and 39.98 mg of quercetin g⁻¹, respectively. These compounds act as reducing agents and as the stabilizing agents by adhering on the surface of the NPs formed, and thereby prevent their aggregation and control the particle size.

Elumalai et al., 2015 reported that to study the antimicrobial activity of the leaf extract of Murraya koenigii the bioassay was carried out using five bacterial strains such as S. aureus, B. subtilis, P. aeruginosa E. coli, P. mirabilis and two fungal strains such as C. albicans and C. tropicalis as per the disc diffusion and dilution technique. It was concluded that the zone of inhibition increased with increase in zinc oxide nanoparticle concentration and decrease in particle size. The ZnO-NPs were found to be effective for both S. aureus and E. coli and P. aeruginosa.

Anbuvannan et al., 2015 reported the ZnO nanoparticle synthesis and antibacterial activity of Anisochilus carnosus. Antibacterial activity was studied against the Gram-negative and the Gram-positive bacteria S. paratyphi, V. cholerae, S. aureus, and E. coli. Inhibition zones of 6mm, 10 mm, 7 mm and 9 mm were observed from the synthesized ZnO nanoparticles against S. paratyphi, V. cholerae, S. aureus, and E. coli, respectively. In the present study, green synthesized ZnO NPs exhibited a greater significant zone of inhibition compared to leaf extract and solvent.

CONCLUSION
The green synthesis of metal nanoparticles is an interesting subject of nanoscience. Also, of latest concern is the biosynthesis of metal nanoparticles using plants for the large-scale biosynthesis. Nanoparticles produced by plants are more stable and more varied in shape and size in comparison with those produced by other organisms. In this review, the synthesis of ZnO nanoparticles were reported. The ZnO nanoparticles have varied applications in all fields. Of special mention is the antimicrobial activity of ZnO nanoparticles. The enhanced bioactivity of ZnO nanoparticles is attributed to the higher surface area to volume ratio. The antimicrobial activity of ZnO nanoparticles were reported with respect to Azadirachta indica, Aloe vera, Murraya koenigii and Anisochilus carnosus. Therefore, based on the reported antibacterial and antifungal activity, it can be concluded that the ZnO nanoparticles constitute an effective antimicrobial agent against pathogenic microorganisms.
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