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Green synthesis of zinc nanoparticles using soybean seed extract

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Abstract
An eco-friendly green mediated synthesis of inorganic nanoparticle is a fast growing research in the limb of nanotechnology. This study reports the synthesis of zinc nanoparticle through ultra-sonication method by using soybean seed extract which act as both reducing and capping agent without the use of toxic chemicals. Where, the reaction mixture of 25 ml of zinc acetate (0.02 M) and 5 ml of soybean seed extract (24%) was ultrasonicated for 30 min. An intense peak was observed in the UV-spectrophotometer at 250 nm. Further, zinc nanoparticle formation was confirmed by Atomic Force Microscope (AFM) and Particle Size Analyzer (PSA). On determination of size of ZnNPs by PSA, it was found to be 14.5 nm. This novel green approach is a rapid, facile and used for large scale production of zinc nanoparticle.

Keywords: Soybean seed extract, Green synthesis, zinc nanoparticles and particle size analyzer

Introduction

Zinc (Zn) is typically the second most abundant transition metal in organisms after iron and the only metal represented in all six enzyme classes (Oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases) (Auld, 2001; Brown *et al.,* 1993, Fageria *et al.,* 2002 and Marschner, 1993) [1, 2, 3, 4].

Zinc plays very important role in plant metabolism by influencing the activities of hydrogenase and carbonic anhydrase, stabilization of ribosomal fractions and synthesis of cytochrome. Plant enzymes activated by Zn are involved in carbohydrate metabolism, protein synthesis, regulation of auxin synthesis and pollen formation. The regulation and maintenance of the gene expression required for the tolerance of environmental stresses in plants are Zn dependent. Its deficiency results in the development of abnormalities in plants which become visible as deficiency symptoms such as stunted growth, chlorosis and smaller leaves, spikelet sterility. Zn deficiency can also adversely affect the quality of harvested products; plant's susceptibility to injury by high light or temperature intensity and infection by fungal diseases can also increase. Zinc seems to affect the capacity for water uptake and transport in plants and also reduce the adverse effects of short periods of heat and salt stress. Zinc is required for the synthesis of tryptophan which is a precursor of IAA; it also has an active role in the production of an essential growth hormone, auxin. Zinc is required for integrity of cellular membranes to preserve the structural orientation of macromolecules and ion transport systems, chlorophyll production, pollen function, fertilization and germination. Its interaction with phospholipids and sulphydryl groups of membrane proteins contribute for the maintenance of membranes. The Zn finger-transcription factors are involved in the development and function of floral tissues such as anthers, tapetum, pollen and pistil secretory tissues in many plant species. It also plays a key role in both flower and fruit development.

Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties, they are gaining the interest of scientist for their novel methods of synthesis.

Green synthesis of nanoparticles is an eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles. This kind of a synthesis includes usage of plant or microbes as a source. Among this, plants are considered as a best suited option as they can be made available easily for large scale biosynthesis of nanoparticles.

Nanoparticles synthesized by plants are more stable and faster. As the nanoparticles are synthesized from plants, these nanoparticles seem to incorporate the plant peptides as capping agents during synthesis, which stabilizes the nanoparticles as well as enhances their antagonistic properties. A variety of techniques including physical and chemical methods have been developed to synthesize zinc nanoparticles. The physical methods are highly expensive and chemical methods are harmful to the environment. Chemicals used for nanoparticles synthesis and stabilization are toxic and lead to non-ecofriendly by products (Garima Singhal *et al*., 2010) [5].

Therefore, there is a growing need to develop eco-friendly nanoparticles that do not use toxic chemicals in the synthesis. Synthesis of zinc nanoparticles from soybean seed and leaves have already been reported. As the nanoparticles are synthesized from plants, these nanoparticles seem to incorporate the plant peptides as capping agents during synthesis, which stabilizes the nanoparticles as well as enhances their antagonistic properties. Zinc nanoparticles (ZnNPs) have become the focus of intensive research owing to their wide range of applications in physical and biological sciences including agricultural and allied fields. Zinc nanoparticles exhibit improved properties depending upon their size, morphology and distribution. Plants are the reservoir of various phytochemicals and their extracts have been used for the synthesis of metal nanoparticles. Keeping this in view a present investigation was taken on green synthesis of zinc nanoparticles using soybean seed extract.

Materials and methods

The experiment was conducted during 2015-16, at the Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad. Green synthesis of zinc nanoparticles, zinc acetate dihydrate ($C_4H_6O_4$ Zn $2H_2O$) was used as a precursor and soybean (DSb-21) seed extract as both reducing and capping agent.

Soybean seed extract method

Genetically pure and fresh seeds of soybean var. DSb-21 were collected from Seed Unit, UAS Dharwad. These seeds were dried for 10 days at room temperature. Dried 60 g seeds were weighed and washed thoroughly with distilled water for 3-4 times. Later washed seeds were placed in clean 500 ml beaker containing 250 ml distilled water. It is boiled for 30 mins on induction coil. After seed leachate was cooled for 10 min and filtered with Whatsman paper no.1. Filtered extract was stored in refrigerator for further use.

Precursor was used in the synthesis of zinc nanoparticles was zinc acetate dihydrate ($C_4H_6O_4Zn\ 2H_2O$) with concentration of 20 mM.

Preperation of zinc acetate dehydrate ($C_4H_6O_4Zn\ 2H_2O$) 20 mM stock solution

The zinc acetate dihydrate ($AgNO_3$) was purchased from Himedia Laboratories, Mumbai, INDIA and it was used as a precursor in the zinc nanoparticle synthesis process. 219.51 g of Zinc acetate dehydrate in 200ml of distilled water gives us 20mM of zinc acetate dihydrate solution.

Preparation of reaction mixture

250 ml of beaker was taken in which 130 ml of Zinc acetate dehydrate is added then 20 ml of soybean seed extract is added.

Method of synthesis

Beaker containing 130ml of zinc acetate dehydrate and 20 ml of soybean seed extract is ultrasonicated for 20 min which preliminarily confirms the synthesis of zinc nanoparticles.

The experiment was conducted during 2015-16, at the Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad. Green synthesis of zinc nanoparticles, zinc acetate dihydrate ($AgNO_3$) was used as a precursor and soybean (DSb-21) seed extract as both reducing and capping agent. Preparation of soybean seed extract by boiling of 60 g of dried seeds in 250 ml of water for 30 min. Extract was filtered by Whatman paper no.1 for removal seed debris and stored at 4 ^oC for further experiment. Green synthesis of zinc nanoparticles was carried out by ultrasonication method. Colour changes was observed from colourless to dark brown it indicate preliminary confirmation of nanoparticles formation as shown in Photo-1. Further, confirmation by UV-Visible spectrophotometer and Atomic Force Microscope (AFM), Particle size analyser (PSA).

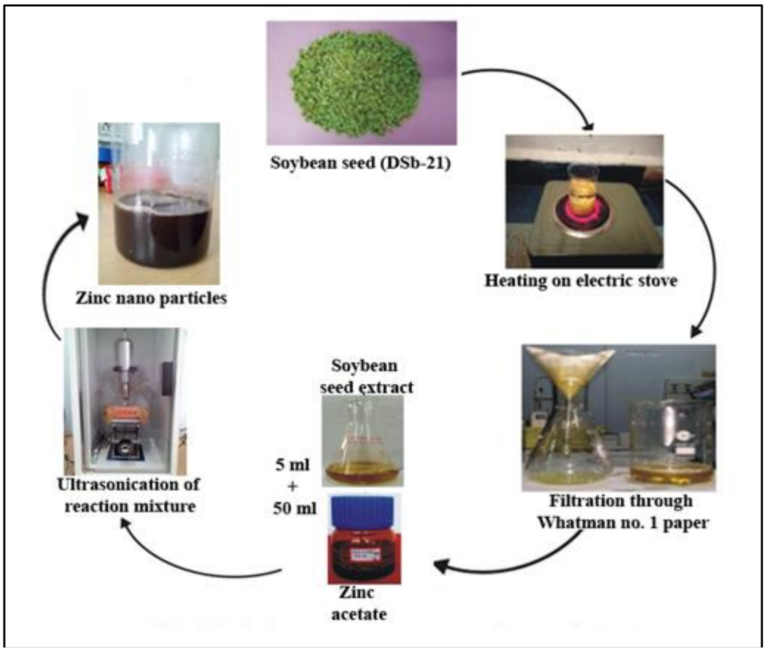

Photo 1: Flow chart of synthesis of zinc nanoparticles using soybean seed extract

Characterization of nanoparticles
The synthesised nanoparticles were characterized using UV-Vis Spectroscopy (Spectrum, SP-UV500DB/VDB) over a range of 200-700 nm. Green synthesized zinc nanoparticles particle shape, sizes and distribution characterized by AFM (Nanosurf AG, Swiss, Flex AFM, with 24bit C3000).

UV-Vis Spectroscopy
UV-visible spectroscopy was used to detect and confirm the presence of various ions in a given sample based on their optical absorbance peaks. As the size of the nanoparticles decreases, the band gap increases and thus the optical absorbance increases as compared to that of the bulk particles and therefore their colour changes.

Atomic Force Microscope (AFM)
AFM was carried out to study the size distribution and the shape of the nanoparticles based on the phenomena of transmittance of the electron beam through an ultrathin specimen. The transmitted electrons carried an image of the specimen which was further focused to analyze the shape and the size of the specimen.

Particle Size Analyzer (PSA)
In most applications theoretical calculations predict the relative effects of particle size, particle composition, composition of the surrounding medium and wavelength of light. In order to find out the particles size distribution the ZnNP powder was dispersed in water by horn type ultrasonic processor (Vibronics, VPLP1). The data on particle size distribution were extracted in Zeta sizer Ver. 6.20 (Mal1052893, Malvern Instruments).

Results and discussion
Zinc (Zn) is typically the second most abundant transition metal in organisms after iron and the only metal represented in all six enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases). Zn is now considered the fourth most important yield-limiting nutrient after nitrogen (N), phosphorus (P), and potassium (K). Zinc nanoparticles were synthesized by ultrasonication method by using same soybean seed extract with the slight modification of protocol was followed by Awwad *et al.* (2013) [6].
For this experiment 100 ml of zinc acetate dihydrate (0.02 M) was used as a precursor and soybean seed extract is the reducing agents. For synthesis of ZnNPs, 100 ml of zinc acetate dihydrate was stirred for five min under magnetic stirrer at 500 rpm, later 10 ml of soybean seed extract was added and again stirring was continued another 5 min under magnetic stirrer at 500 rpm. Then, the mixture is ultrasonicated for 20 mins under ultrasonicator, during which sonication the reaction mixture turned to dark colour indicating the synthesis of nanoparticles. The colour occurred due to the surface plasmon resonance phenomenon recorded by UV-vis Spectrometer at 331 nm. The metal nanoparticles have free electrons, which helps in the formation of the surface plasmon resonance absorption band, which is due to the united vibration of the electrons of metal nanoparticles in resonance with light wave (Kanthimathi *et al.*, 2013) [7].
Similar type of green synthesis ZnNPs was reported by Bhumi and Savitramma (2014), where the biologically ZnO-NPs were synthesized by using *C. roseus* revealed that the pale white precipitate was appeared. *C. roseus* leaf has been used for the reducing material as well as surface stabilizing agent for the synthesis of spherical shaped ZnO-NPs.
The ZnO nanoparticles were synthesized by using seed extract of *Abrus precatorius* called as green synthesis. The phytochemicals present in the plants reduced zinc acetate. By UV- visible spectrophotometer we found that the band was observed around 386-450 nm which was identified as "surface plasmon resonance band" and this band is ascribed to excitation of valence electrons of ZnO arranged in the nanoparticles (nanocrystal/ nanosphere) .The size of particles formed were 90 nm and 500 nm studied by the particle size analyzer. (Vishawakarma, 2009) [8].
It is well known that zinc nanoparticles exhibit a yellowish-brown colour in aqueous solution due to excitation of surface plasmon vibrations in zinc nanoparticles. Reduction of zinc ions to zinc nanoparticles could be followed by a colour change and UV-Vis spectroscopy. The technique outlined above has proven to be very useful for the analysis of nanoparticles**)**. Therefore, the progress in conversion reaction of zinc ions to zinc nanoparticles was followed by a colour change and spectroscopic techniques. Figure 1 shows the photographs of sample solutions containing zinc acetate dihydrate and zinc acetate dihydrate in the presence of optimized amounts of soybean seed extract solutions after completion of the reaction. The appearance of a dark brown colour confirms the existence of zinc nanoparticles in the solution.
For further confirmation by UV-Visible spectrophotometer, peak was observed at 250 nm. This is because of a phenomenon called Surface Plasmon Resonance (SPR) exhibited by zinc nanoparticles. Green synthesized zinc nanoparticles reveals particle shape, sizes and distribution by AFM. Zinc nanoparticles sizes ranging from 10-20 nm and spherical in shape.

UV-Vis spectroscopy
The zinc nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of zinc nanoparticles. The absorption spectrum of the yellowish-brown zinc nanoparticle solution prepared with the proposed method showed a surface Plasmon absorption band with a maximum of 331 nm.

Particle Size Analyzer by diffuse light scattering method
Particle size refers to the details of particles size (d.nm) and zeta potential range based on the count rates. The particle size analysis showed a average size of nanoparticles 14.5 nm as shown in Figure-1.

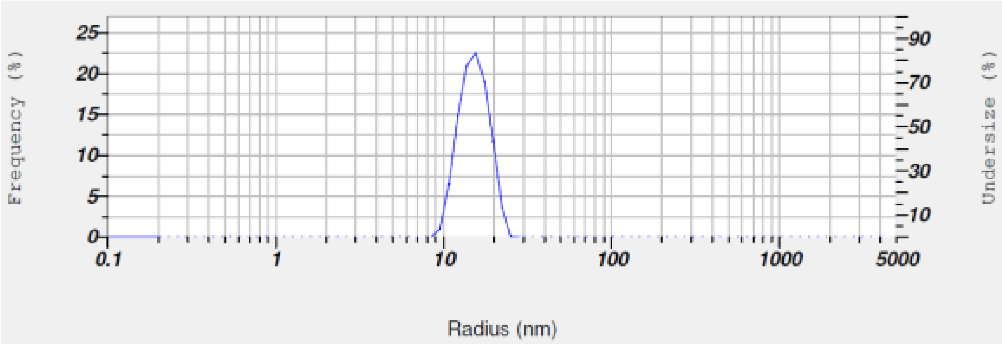

Fig 1: Image of particle size analyzer

Atomic Force Microscope (AFM)

The synthesized zinc were characterized by atomic force microscope with relation to size, shape the data showed 2D and 3D images of nanoparticles. The AFM results of soybean based ZnNPs recorded particle size ranging between 10-20 nm and they were spherical in shape as shown in photograph-2.

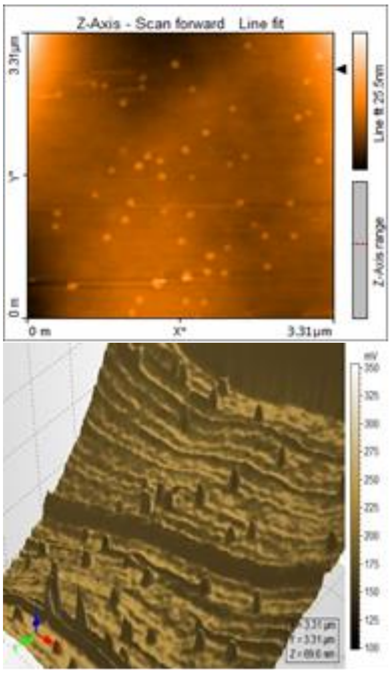

Photo 2: AFM image of ZnNPs

Conclusion

Green synthesis of zinc nanoparticles from soybean seed extract was rapid, eco-friendly and cost effective due to easily available seed source and also act as both reducing and capping agent. Green synthesized zinc nanoparticles was characterized by UV-Vis (250 nm), AFM (10-20 nm) and particle size analyzer (14.5 nm).

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