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RESEARCH ARTICLE

BIOSYNTHESIS OF ZINC OXIDE NANOPARTICLES USING TURBINARIA CONOIDES—A GREEN APPROACH

Khoushika Raajshree, R. and *Brindha Durairaj

Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India

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ABSTRACT

Green synthesis of metal oxide nanoparticles from seaweed extract is a promising alternative to the traditional method of chemical synthesis. In this paper, we report the synthesis of nanostructured zinc oxide particles by a biological method. Highly stable and spherical zinc oxide nanoparticles (ZnO-NP's) are produced by zinc acetate utilizing the biocomponents of crude extract of *Turbinaria conoides*. Formation of ZnO-NP's has been confirmed by UV-Vis absorption spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), zeta potential study and Scanning Electron Microscope with the Energy Dispersive X-ray studies (EDX). The ZnO-NP's from *Turbinaria conoides* are expected to have applications in pharmaceutics, biomedical, cosmetic industries, biotechnology, sensors, medical, catalysis, optical device, coatings, drug delivery and water remediation. This new eco-friendly approach to synthesis is a novel, cheap and convenient technique suitable for large-scale commercial production.

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INTRODUCTION

Nanotechnology is an important branch in the major fields of biology, chemistry, physics and material sciences (Bhumi and Savithramma, 2014). Nanoparticles are of great interest due to their extremely small size and large surface area to volume ratio, which lead to both chemical and physical differences in their properties (e.g. mechanical properties, biological and sterical properties, catalytic activity, thermal and electrical conductivity, optical absorption and melting point) compared to bulk of the same chemical composition. Therefore, design and production of materials with novel applications can be achieved by controlling shape and size at nanometre scale. These particles also have many applications in different fields such as medical imaging, nanocomposites and drug delivery (Zharov *et al*., 2005). Over the past few decades, inorganic nanoparticles, whose structures exhibit significantly novel and improved physical, chemical, and biological properties and functionality due to their nano-scale size, have elicited much interest (Long *et al.,* 2006). Among the metal oxide nanoparticles, ZnO-NP's have drawn the attention of many researchers for their unique optical and chemical behaviours which can be easily tuned by changing the morphology and have been used in various cutting edge applications like electronics, communication, sensor, cosmetics, environmental protection, biology and medicinal industry.

Corresponding author: **Brindha Durairaj,**
Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India

Moreover, ZnO-NP's have a tremendous potential in biological applications like biological sensing, biological labelling, gene delivery, drug delivery and nanomedicines (Dagdeviren *et al*., 2013). The nanomaterials can be synthesized by different methods including chemical, physical, irradiation and biological methods. The development of new chemical or physical methods has resulted in environmental contaminations since the chemical procedures involved in the synthesis of nanomaterials generate a lot of hazardous byproducts. Thus, there is a need for "green synthesis" that includes a clean, safe, eco-friendly and environmentally nontoxic method of nanoparticle synthesis. Moreover, in this method there is no need to use high pressure, energy, temperature and toxic chemicals (Bhumi and Savithramma, 2014). *Turbinaria conoides* belongs to the family of Sargassaceae (brown algae). It has traditionally been used as antipyretic and antibacterial agents. These macrophytic marine algae produce a great variety of secondary metabolites having a broad spectrum of biological activities. Hence, in the present investigation, a biological approach using *Turbinaria conoides* as a reducing as well as a surface stabilizing agent for the synthesis of ZnO-NP's has been reported. Synthesized product was characterized by the standard characterization techniques.

MATERIALS AND METHODS

Collection and Preparation of Seaweed

The brown seaweed, *Turbinaria conoides* was collected from Mandapam coastal region, Gulf of Mannar, Southeast coast of

India. The algal samples were washed thoroughly with running tap water followed by distilled water to remove adhering salts and associated biota. The washed samples were dried under shade at room temperature for a week. The dried materials were ground to fine powder using mixer grinder and stored in airtight container for further analysis.

Preparation of crude algal extract

The pure algal extract (PAE) was prepared by adding 10 g of algal powder into 100 ml of 50% ethanol and kept in the rotatory shaker for 24 hours. Filtered, collected the solvent and was used for further analysis.

Green Synthesis of Zinc Oxide Nanoparticles from Crude Algal Extract

20 ml of the crude algal extract was heated at 50˚C for 10 min and 50 ml of 91 mM of zinc acetate solution (1 g of zinc acetate was dissolved in 50 ml of distilled water) was added drop wise. This was then placed on a magnetic stirrer for 2 hrs. Then the precipitate was collected by centrifugation at 16 000 rpm for 10 min at 4˚C. The pale white precipitate was then taken out and washed with distilled water followed by ethanol to get free of the impurities. The ZnO-NP's were obtained after drying at 60˚C in an oven overnight and the sample was stored for further studies.

Characterisation of Zinc Oxide Nanoparticles

The obtained ZnO-NP's were measured for its maximum absorbance using UV-Vis spectrophotometry. The optical property of ZnO-NP's was determined via ultraviolet and visible absorption spectroscopy in the range of 280 – 420 nm. Fourier Transmission-Infrared Spectroscopy analysis was performed to reveal the composition of products by examining the peak variation of amino groups and carboxylic groups. The structure was analyzed by using X-Ray Diffraction (XRD) analysis. The stability of the nanoparticles was checked by zeta potential measurement. External morphology i.e. the shape of the nanoparticles were characterized by Scanning Electron Microscope (SEM). Elemental analysis was obtained from energy dispersive X-ray diffraction (EDX), which was attached with SEM.

RESULTS AND DISCUSSION

Biosynthesis of ZnO-NPs using Brown seaweed

ZnO-NPs were synthesized from the crude extract of *Turbinaria conoides* by green synthesis method, which is more reliable and less toxic when compared with other methods. The formation of pale white colour within 3 hours of preparation indicated the synthesis of ZnO-NPs.

UV-Visible Spectral Analysis

The optical absorption spectra of ZnO-NPs were recorded using UV/VIS 3000+ Double Beam UV-Visible Ratio-Recording Scanning Spectrophotometer from Lab India (SKU: 174-0020) with dimensions of (W × D × H)/Weight = 540 × 440 × 390 mm/36kg. The spectral bandwidth of Spectrophotometer is 0.5, 1, 2, 5 nm and wavelength is in the range of 190 to 1100 nm. Figure 1 shows the UV-Vis absorption spectrum of ZnO-NPs.

The absorption spectrum was recorded for the sample in the range of 280 - 420 nm. The spectrum showed the absorbance peak at 360 nm corresponding to the characteristic band of ZnO-NPs. The UV-Visible spectrum showed the absorbance peak at 340 nm corresponding to the characteristic band of zinc oxide nanoparticles (Imitan *et al.,* 2009).

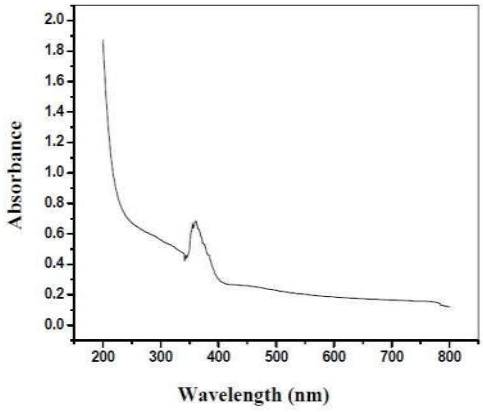

Figure 1. UV-Visible spectrum of synthesized ZnO-NPs

Fourier Transform Infrared Spectroscopy (FT-IR) Analysis

FT-IR is an effective method to reveal the composition of products. Figure 2 is a typical FTIR spectrum of pure ZnO-NP's and Table 1 indicates absorption spectrum with possible assignments. The peak at 533 cm^{-1} is the characteristic absorption of Zn-O bond and the broad absorption peak at 3398 cm^{-1} can be attributed to the characteristic absorption of hydroxyl. The results obtained are in par with the results observed in ZnO nanopowders synthesized using zinc chloride (Srinivasa Rao and Basaveswara Rao, 2015).

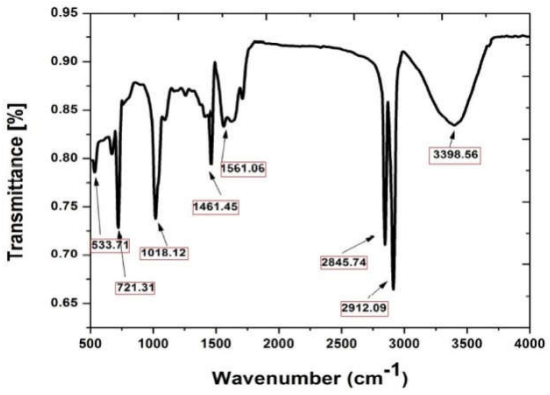

Figure 2. FTIR Spectra of synthesized zinc oxide nanoparticle

Table 1: FTIR spectra with possible assignments

Frequency $(cm)^{-1}$	Possible Assignment
3398.56 cm^{-1}	OH stretching vibrations
2912.09 cm^{-1}	The C-H stretch in alkanes
2845.74 cm^{-1}	O-H stretch in carboxylic acid
1561.06 cm^{-1}	C=C stretch in aromatic ring and C=O stretch in polyphenols
1461.45 cm^{-1}	C-N stretch of amide-I in protein
1018.12 cm^{-1}	C-O stretching in amino acid
533.71 cm^{-1}	hexagonal phase ZnO

X-Ray Diffraction (XRD) Analysis

The X-Ray diffraction (XRD) pattern of synthesized ZnO-NP's is shown in Figure 3.

A normal focus diffractometer source Cu target at 30 kV and 15 mA was used with the scan rate of 3˚/min. The data recorded in the range 5θ -80θ and analyzed using Jade 6.0 software. X-Ray diffraction pattern shows 2θ values at 32.03˚, 34.44˚, 36.52˚, 47.43˚, 56.61˚, 63.29˚ and 68.09˚. All evident peaks could be indexed as the Zinc oxide wurtzite structure (JCPDS Data Card No: 36-1451). Zinc oxide crystallizes in two main forms, hexagonal wurtzite and cubic zinc blende. The wurtzite structure is most stable at ambient conditions and thus most common. It also confirms the synthesized nanopowder was free of impurities as it does not contain any characteristics XRD peaks other than zinc oxide peaks (Bigdeli *et al*., 2010). Similar results were observed in ZnO nanoparticles synthesized from *Catharanthus roseus* (Bhumi and Savithramma, 2014).

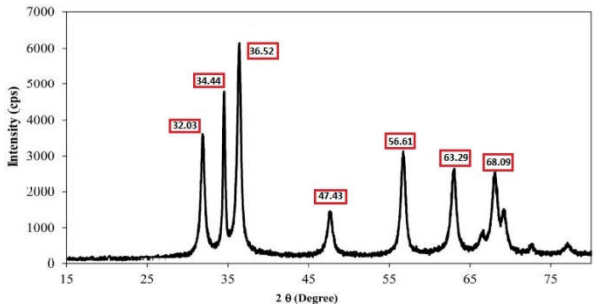

Figure 3. XRD pattern of synthesized zinc oxide nanoparticles

Zeta Potential Analysis

Zeta potential analysis was carried out to detect the surface charges acquired by ZnO-NP's, which can be used to gain further insights into the stability of the obtained colloidal nanoparticles. Zeta potential analyzer has the ability to perform highly accurate zeta potential measurements of sample suspensions in the −500 mV to +500 mV range. The magnitude of zeta potential gives an insinuation of potential stability of colloid. If the particles in a suspension have large negative or positive zeta potential values, particles will repel each other and there will be no aggregation of nanoparticles. On the other hand, if particles have small zeta potential values there is no force to prevent particle coming together and their aggregation. The zeta potential of the synthesized ZnO-NP's was determined in water as a dispersant. The zeta potential was found to be −49.19 mV as shown in Figure 4. It is generally considered that the zeta potential values greater than +30 mV or smaller than −30 mV, result in stable suspensions. The high value confirms the repulsion among the particles and thereby increases the stability of the formulation (Melendrez *et al*., 2010). The observed zeta potential is in good agreement with the work done by Supraja *et al.,* 2016.

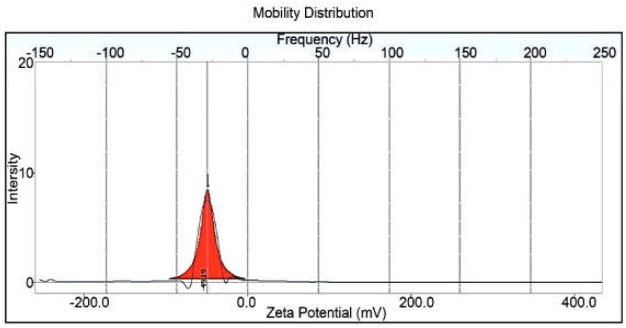

Figure 4. Zeta potential analysis of synthesized zinc oxide nanoparticles

Scanning Electron Microscopy (SEM) Analysis

The morphology of the synthesized nanoparticles was examined using scanning electron microscopy. Figure 5(a) and Figure 5(b) show the surface morphology of the ZnO-NP's under different magnifications. The SEM image showed that most of the nanoparticles are spherical in shape formed within the diameter range of 80 - 130 nm.

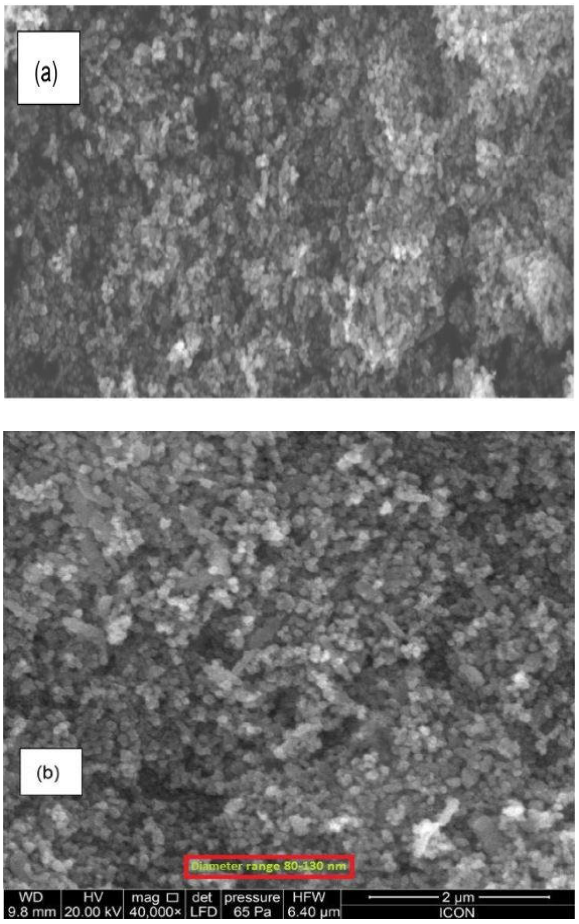

Figure 5[a] & [b]. SEM image of the synthesized zinc oxide nanoparticles

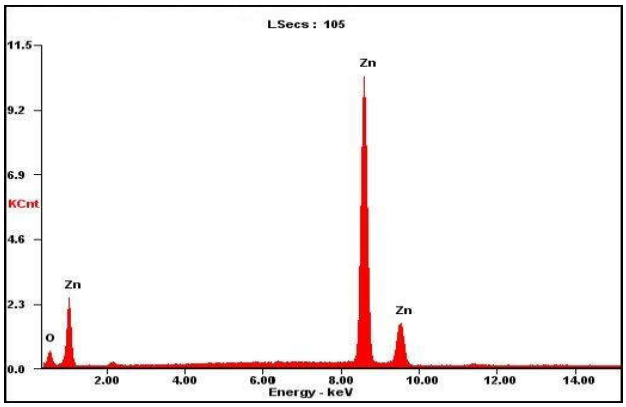

Figure 6. EDX spectrum of synthesized zinc oxide nanoparticles

Energy Dispersive X-Ray Diffractive (EDX) Analysis

The Energy Dispersive X-ray Diffractive (EDX) study was carried out for the synthesized ZnO-NPs to elucidate the elemental composition. EDX confirms the presence of zinc and oxygen signals of ZnO-NPs as depicted in Figure 6.

The results revealed the peaks that correspond to the optical absorption of the produced nanoparticle. The elemental analysis of the nanoparticle yielded 77.32% of zinc and 22.68% of oxygen which proves that the produced nanoparticle is in its highest purified form. The observed results are in good rapport with the SEM-EDX analysis of ZnO nanoparticles synthesized using *Spathodea campanulata* (Ochieng *et al.,* 2015).

Conclusion

The rapid biological synthesis of ZnO-NP's using *Turbinaria conoides* provides an eco-friendly, simple and efficient route for the synthesis of nanoparticles. The synthesized nanoparticle by this method was of high purity with a wide range of applications in nanomedicine mainly for the pharmaceutical industry to develop new drug formulations.

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