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Biogenic Synthesis of Zinc Oxide (ZnO) Nanoparticles Using a Fungus (*Aspargillus niger*) and Their Characterization

Aisha Shamim[1], Monis Bin Abid[2], Tariq Mahmood[3]

[1]Department of Chemistry, University of Wah, Wah Cantt 47040, Pakistan

[2]Department of Chemistry, University of Prince Mugrin, Medina Al Munawara, Saudi Arabia

[3]Nano Sciences and Technology Department, National Centre for Physics, QAU, Islamabad 45320, Pakistan

Correspondence: Ms. Aisha Shamim, Department of Chemistry, University of Wah, Wah Cantt, Pakistan. E-mail: aishashamim22@gmail.com

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Abstract

Nanoparticles are ultrafine structures with dimensions less than 100 nm. Nanoparticles have diverse applications. There are three important methods of fabrication of nanoparticles namely physical, chemical and biological methods. Physical method is a top down strategy for the fabrication of nanoparticles. It is energy intensive and time consuming. A chemical method is simple, but is expensive and requires expensive chemicals with high purity and also involves hazards of contaminations. Biological synthesis is very simple, cheap and environment friendly, requiring no expensive chemicals, temperature and is time saving. Plants and microorganisms are commonly used in this method. These are available everywhere. In the present work we synthesized Zinc Oxide (ZnO) nanoparticles by biological method using *Aspargillus niger* and zinc chloride ($ZnCl_2$) as precursors. Biogenic synthesis of metallic nanoparticles by fungi is a safe and economical process because of formation of stable and small sized nanoparticles. Fungal biomass secretes proteins which act as reducing and stabilizing agents. The synthesized nanoparticles were characterized by XRD (X-Ray Diffraction), SEM (Scanning Electron Microscopy), UV-Vis (Ultraviolet, Visible) and EDX (Energy Dispersive X-Ray) techniques. Their size was in nm range and morphology of synthesized ZnO NPs was hexagonal. The ZnO nanoparticles are one of the most versatile materials and are used in cosmetics and in Bioenergy production, as a catalyst and as antibacterial material.

Keywords: antibacterial, biogenic synthesis, chemical method, fungi, microorganisms, nanoparticles, zinc oxide

1 Introduction

Inspired by a revolutionary lecture of Richard Feynman 'There's Plenty of Room at the Bottom' the scientific community got a new thought process to miniaturize the prevailing technology and form an advanced branch of science called nanotechnology (Dhand et al., 2015).The term nanotechnology was introduced by Tokyo Science University Professor Norio Taniguchi (Chokriwal et al., 2014). Nanotechnology is an escalating technology that revolutionizes many scientific realms (Fakhari et al., 2019). Nanotechnology deals with the synthesis and applications of nanomaterials that have wide applications in material science, agriculture, food industry, medical, and diagnostics (Siddiqi et al., 2018). Nanoparticles- particles having one or more dimensions of the order of 100 nm or less have attracted much of the interest of researchers due to their fascinating properties and applications advantageous over their bulk counterparts (Li et al., 2011).The characteristic features of nanoparticles are high volume/surface ratio, surface tail or ability and multifunctionality (Chokriwal et al., 2014) and high reactivity. When size of the particles becomes less than 70 nm, Van der Waals force becomes dominant which gives rise to interesting phenomena. The fascinating aspect of nano is change in properties at very small scale (Trybula et al., 2015).

Metallic nanoparticles have unique characteristics like large surface energies, quantum confinement, Plasmon excitation and increased number of kinks, mechanical strengths, optical and magnetic properties (Harish et al., 2018). A variety of physical and chemical methods have been used for the synthesis of metal nanoparticles such as coprecipitation, sol-gel, microemulsion, hydrothermal, electro spray (Din and Rani, 2016), attrition and pyrolysis Thakkar et al., 2010), ultrasonic radiation, laser chemical method, and solid state method (Khandagale and Shinde, 2017), ultraviolet irradiation, aerosol technologies, lithography, laser ablation, and photochemical reduction (Krol et al., 2017). The yield of metallic nanoparticles is quite low in the physical procedures. These methods are expensive and energy intensive

(Thakkar et al., 2010). Chemical methods produce high yield of nanoparticles and are of low cost but have drawbacks of the use of toxic solvents, contamination from precursors and hazardous by-products. Green synthesis manipulates a vast array of natural biological resources like viruses, bacteria, fungi, plants and algae that could be employed for the extracellular and the intracellular synthesis of metallic nanoparticles (Thakkar et al., 2010). Biological methods are safe, clean, biocompatible, and eco-friendly and thus deserve merit (Chokriwal et al., 2014). In recent years there has been greater focus on nano semiconductors because of their novelty and wide range of applications in optoelectronics. Among metallic nanoparticles ZnO NPs are proved to be versatile semiconductors because of their significant optical transparency and luminescence in uv-visible regions. The ZnO NPs have been the focus of research due to their excellent thermal and chemical stability (Fakhari et al., 2019), conductivity, catalytic properties, photonics, optoelectronics, antibacterial, and antifungal properties (Beegam et al., 2016).

Zinc oxide is an n-type semiconductor with a large band gap (3.37eV) and high excitation binding energy (60 meV). Zinc Oxide NPs have drawn maximum interest among several types of inorganic metal oxides because they are safe and inexpensive to produce. ZnO NPs have tremendous semiconducting properties like high catalytic activity, UV filtering, optic, and anti-inflammatory. Because of the UV filtering ability it is widely used in cosmetics. ZnO has wide range of applications in electronics, optics, and biomedical systems (Agarwal et al., 2018). Different methods are used for synthesis of ZnO NPs such as spray pyrolysis, hydrothermal synthesis, sol-gel process, physical vapor deposition (PVD), and chemical vapor deposition (CVD) (Farahmandjou and Jurablu, 2014, Fakhari et al., 2019),ultrasonic irradiation, arc plasma method, thermal evaporation (Krol et al., 2017), precipitation, homogeneous precipitation, mechanochemical, Sonochemical, Pechini-polymer complex, and combustion (Lopez and Paez, 2017). Precipitation method is particularly used to fabricate a variety of ZnO NPs with different morphologies. Precipitation method was used for ZnO NPs synthesis using Zn $(NO_3)_2.6H_2O$ as a precursor and Potassium carbonate as a precipitator (Farahmandjou and Jurablu, 2014). S.Sepulveda-Guzman et al synthesized ZnO nanostructures by aqueous precipitation method. J.Wang and LGao synthesized ZnO nanoparticles by a template- free method. Hexagonal ZnO nanoparticles were obtained after annealation at 500C °. The ZnO nanoparticles were synthesized by precipitation method using zinc nitrate and ammonium carbonate (Dawood Raoufi, 2012). Ismail and coworkers reported the formation of spherical ZnO nanoparticles with average diameter of 35 nm by pulsed laser ablation in double distilled water. AmirKhanlou and co scientists reported a very efficient and cost effective high energy ball milling process for the synthesis of ZnO nanoparticles (Haq et al., 2017). Green synthesis is a safe strategy for the production of ZnO nanoparticles because of the least amount of chemicals used. These are energy efficient and cost effective methods. Natural moieties such as plants and microorganisms are used in this method (Haq et al., 2017). They allow large scale production of pure ZnO NPs which show more catalytic activity (Agarwal et al., 2017). The ZnO NPs can be prepared by Biological methods using leaf extract of *Coriandrum sativum*, *Acaphyla indica*, Milky latex of *Calotropis procera* and *Oryza sativa* (Sabir et al., 2014). Leaf extracts of *Azadirachta indica*, *Agathosoma betulina*, *Aloe vera*, *Parthenium hysterophorus* L., *Pongamia pinnata*, and *Costus pictus* D.Don also called insulin plant are also used for biosynthesis of zinc oxide nanoparticles. Plant mediated synthesis is more efficient than that of other organisms. Plant extracts are rich in phytochemicals which act as reducing and stabilization agents(Suresh et al., 2018).Leaves of *Cochlospermum religiosum*, *Plectranthus amboinicus*, *Andrographis paniculata*, the peel of *Nephelium lappacceum*, the root of *Polygala tenuifolia*, the seeds of *Physalis alkekengi* are also reported to synthesize Zinc Oxide NPS (Jiang et al., 2018).Jayaseelan et al explained cost effective and simple biosynthesis of ZnO nanoparticles using bacteria *Aeromonas hydrophilia*.XRD analysis confirmed the presence of spherical

ZnO nanoparticles with an average size of 57.72 nm. Lactobacillus sporogens was investigated to produce ZnO NPs of diameter of 5-15 nm. Fungal biomass are incubated with a zinc salt solution and kept in the dark for a particular time in the intracellular synthesis while in the extracellular synthesis fungal filtrate is treated with salt solution. Jain et al demonstrated that *Aspergillus aeneus* isolate NJP12 exhibited highest capacity for extracellular synthesis of ZnO nanoparticles (Haq et al., 2017). Jacob and companions synthesized ZnO nanoparticles by using fungal filtrate of *Aspergillus niger*. These nanoparticles were spherical with an average diameter of 39.4-114.6 nm (Haq et al., 2017). The ZnO nanoparticles were biosynthesized extracellularly by Baskar and coworkers using filtrate of *Aspergillus terreus*. The obtained nanoparticles have a spherical morphology with the diameter in the range of 54.8-82.6 nm (Haq et al., 2017).

Microbes are potent eco-friendly nanofactories and have potential to control the size and shape of biological nanoparticles (Li et al., 2011). Over the last decade there have been great advancements in microorganism- generated nanoparticles and their applications. However, efforts are needed to improve the synthesis efficiency and control of particles size and shape (Li et al., 2011). Synthesis of NPs by microorganisms is a slow process which requires several hours and few days compared to physical and chemical methods. This route can be more attractive by reducing the time of synthesis. Effective control of size and monodispersity must be explored. Nanoparticles synthesized by microorganisms are generally unstable and decompose after a certain time, so this problem needs further study and should be removed. Biological method with a strict control on particle shape would be more advantageous (Li et al., 2011). The rate of reduction of metal ions using biological agents (plants and microorganisms) is much faster at ambient conditions of temperature and pressure (Chokriwal et al., 2014). Nanoparticles synthesized by microbes have tremendous applications in the fields of bioremediation, bio-mineralization, bioleaching, and bio-corrosion (Chokriwal et al., 2014). Biological Synthesis of metallic nanoparticles can be split into two categories, bioreduction and biosorption. In bioreduction metal ions are biologically reduced into more stable forms which is coupled with oxidation of enzymes. Biosorption involves the binding of metal ions from an aqueous solution or soil sample onto the organism, it does not require energy (Pantidos and Horsfall, 2014). Nevertheless only a few microbes are reported to have synthesized ZnO NPs. There is a need to investigate more potential microbes for the synthesis of ZnO NPs (Yusof et al., 2019).

Zinc Oxide is considered to be a magic material because of its versatile applications. Zinc oxide NPs are used in biomedicine like biomedical imaging, drug delivery, gene delivery, and bio sensing ((Zhang et al., 2012). The ZnO is used in ceramics, as filler in rubber and plastics, cosmetics, electrical and optoelectronic devices, and pigments (Sarivastava et al., 2013).

In this study ZnO NPs were synthesized by a biological route using fungus (*Aspargillus niger)* as a biological system and zinc Chloride as a precursor. Fungus mediated synthesis is more advantageous because fungi are hyper accumulators and show economic viability and easily scaled up synthesis (Thakkar et al., 2010).

Biosynthesis of nanoparticles using fungi is a widely used method to produce monodispersed nanoparticles with a wide range of different chemical compositions. Fungi produce large amount of proteins and enzymes and as a result the yield of nanoparticles is high. Extracellular synthesis of nanoparticles by fungi produce protein stabilized nanoparticles (Shah et al., 2015) and allows an efficient way to extract nanoparticles from them. Scalability is another factor responsible for the commercial production of nanoparticles by fungi (Pantidos and Horsfall, 2014). Fungi have a great tolerance to higher metal concentrations and also great binding ability and reduce larger amount of metal ions into metal NPs by secreting a large number of extracellular redox proteins and enzymes (Yusof et al., 2019)

2. Experimental

In the present study we synthesized ZnO NPs by using biological method. This study was carried out at Nanoscience & Technology Department, National Centre for Physics, QAU Islamabad, Pakistan and Department of Chemistry, University of Wah, Wah Cantt Pakistan. These nanoparticles were synthesized by using fungus *Aspargillus niger* and were characterized by using X-ray diffraction (XRD), UV-Visible spectroscopy (UV-Vis), Scanning Electron Microscopy (SEM) and Electron Dispersive X-ray Spectroscopy (EDX). During this work all chemicals were purchased from the local market of Sigma-Aldrich. These were AR-Grade and there was no need of further purification. We used deionized water throughout the experiment.

2.1 Biological Synthesis of Zinc Oxide NPs

During biological synthesis first of all salt solution was prepared by dissolving 3-4 g of zinc chloride ($ZnCl_2$) salt in deionized water and then 2 g crushed powder of fungus *Aspargillus niger* was added into it and the solution was stirred for 30 minutes. After stirring the solution was placed in darkness for 3 days. After 3 days we filtered the solution. Pale white filtrate of ZnO NPs was obtained. The filtrate was characterized by UV-Visible for finding size and concentration of nanoparticles. The filtrate was then dried at 95°Cand calcined at 550°C in muffle furnace for 3 hours. Then calcined material was grinded by mortar and pistil and analyzed by XRD, SEM and EDX.

2.2 Mechanism for the Microbial Synthesis of ZnO NPs by Aspargillus Niger

Aspargillus niger can produce ZnO NPs by extracellular and intracellular routes, however, it has more potential for extracellular synthesis. Extracellular synthesis is enzyme mediated such as a nitrate reductase enzyme which is secreted in the medium and reduces metal ions to metal NPs. Zinc oxide NPs are formed when electrons are transported from NADH by an enzyme NADH reductase to Zn^{+2} which are reduced into Zn^{0} NPs. In intracellular synthesis metal ions are transported within the cell wall where these are reduced by enzymes present there and then nuclei grow to form nanoparticles in the periplasmic space and cytoplasm. Mukherjee et al reported intracellular mechanism for NPs by verticillium sp.consisting of three steps trapping, bioreduction, and capping (Yusof et al., 2019).

2.3 Characterization

The synthesized NPs were characterized by using XRD model D8 ADVANCE BRUKER X-Source Copper/ (anode). UV-Vis was performed on UV-Vis Spectrometer Perkin Elmer; Lambda 25.Both instruments were placed at Nanoscience & Technology Department, National Centre for Physics, QAU Islamabad, Pakistan. All the samples were characterized by XRD and their results were noted in nanometer. The synthesized NPs were also characterized by SEM performed on SEM, TESCAN, VEGA3 placed at Advanced Energy &Material lab NUST Islamabad Pakistan. The SEM study was carried out to find size and morphology of nanoparticles. The EDX was done on EDX Oxford placed at Fracture Mechanics and Fatigue Lab, Mechanical Engineering Department, UET Taxila Pakistan. The EDX was used to find elemental composition and purity of samples.

3. Results and Discussions

3.1 XRD and SEM Analysis

We characterized the prepared sample by XRD. The XRD was used to find the size of particles and crystallinity. The Scherrer formula was used to find the crystallite size of NPs. The XRD analysis of biologically synthesized nanoparticles is described below.

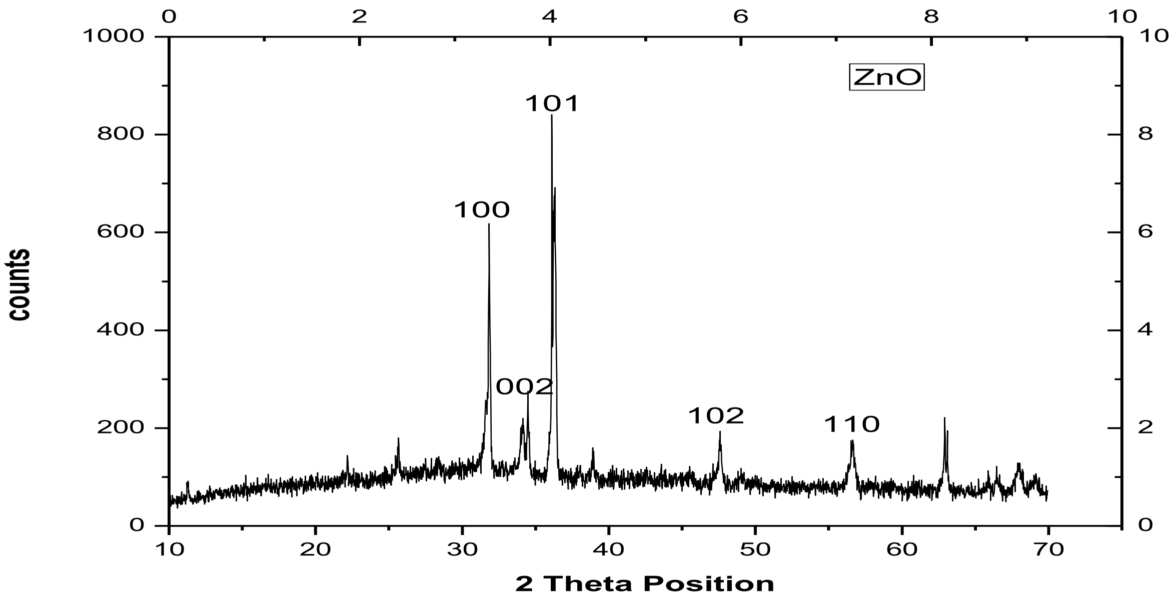

Figure 1. XRD Spectrum of ZnO NPs

The XRD spectrum of ZnO nanoparticles indicates that the peaks are sharp and are located at 2Ɵ values of 31°,34°,36°,38°,47°,56°,62° and 69°.Sharp peaks show good crystallite growth and the position of peaks indicate the formation of pure ZnO nanoparticles. The diffraction lattice planes confirm the hexagonal wurtzite structure of the ZnO NPs. The average particle size was calculated by highest intense peak (101) using the Debye-Scherrer equation. Their size was found to be 40 nm. These results were matched with literature (Farahmandjou and Jurablu, 2014), (Zargar and Arora, 2017), (Fakhari et al., 2019) and also these values were matched with JCPDF # 36-145.

Table 1. XRD Data for ZnO NPs

PEAKS	2Ɵ POSITION	hkl VALUES	d-SPACING
1	31.769	100	2.8143
2	34.421	002	2.6033
3	36.252	101	2.4759
4	47.538	102	1,9111
5	56.602	110	1.6247
6	62.862	103	1.4771
7	66.378	200	1.4072
8	67.961	112	1.3782
9	69.098	201	1.3583

3.2 SEM Analysis

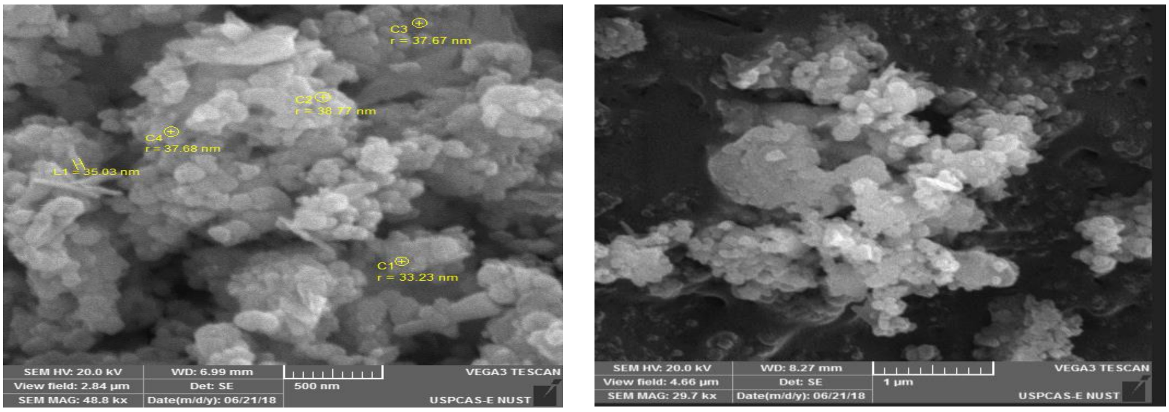

Figure 2. SEM Images of ZnO NPs

The SEM micrographs of ZnO NPs clearly show beautiful white evenly sized crystals with hexagonal morphology. The average size of crystals was 66 nm. Intense agglomeration in case of ZnO NPs is due to the large surface energy of NPs. The particles are legitimately agglomerated due to a typical phenomenon of interaction of moisture and ZnO and inter-particle interactions (Van der Waals, electrostatic and magnetic forces). ZnO NPs exhibit tendency to agglomerate in aqueous solution and develop soft agglomerates but this agglomeration does not create complexity as the application purposes of ZnO depend on particle size and not on agglomerate size (Al- Dhabi and Arasu, 2018).

3.3 UV-Vis Analysis

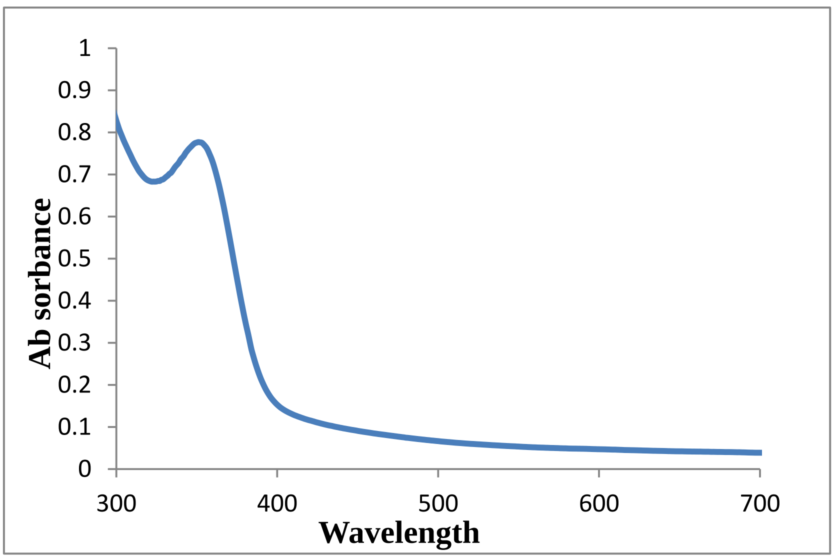

Figure 3. UV-Vis Spectrum of ZnO NPs

The UV-Visible analysis was recorded in the range of 200-800 nm. The UV-Visible analysis indicates that ZnO NPs show maximum absorbance at 357 nm due to the SPR, which occurs due to resonance of collective conduction electrons with incident electromagnetic radiations. This peak is distinct for ZnO NPs due to their large excitation binding energy at room temperature. Bulk Zinc shows absorption at 385 nm, the high blue shift absorption for zinc Oxide NPs is due to high decrease in particle size. The band gap increases with decreasing particle size. The result was matched with (Fakhari et al., 2019). According to Jamdagni et al., 2018 the range of UV spectrum for ZnO NPs is 320-390 nm. Kumar et al., 2017 described the UV spectrum range for ZnO NPs at 380 nm (Kalpana et al., 2018). The result was matched with Sharmila et al., 2018 where SPR peak was observed at 375 nm.

3.4 EDX Analysis

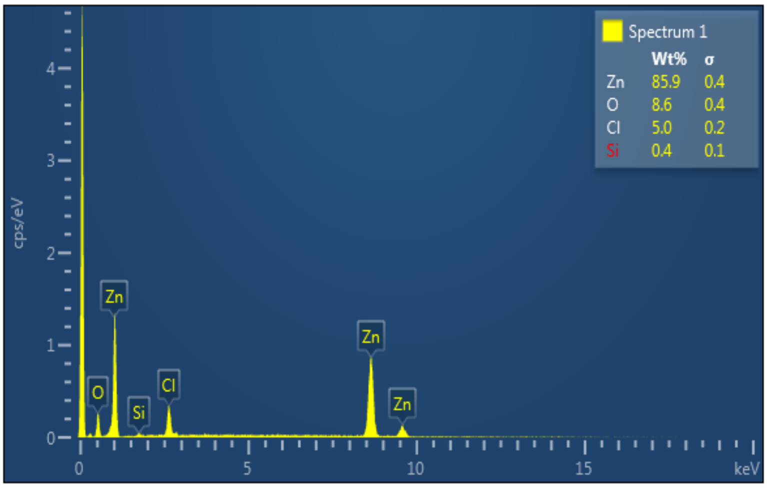

Figure 4. EDX Spectrum of ZnO

The EDX study showed the elements Zn and O. The Zn content was 85.9% while O content was 8.6%.The EDX results indicated that ZnO NPs were pure with only traces of impurities. EDX analysis was used to examine purity, elemental composition and stoichiometry of the synthesized ZnO NPs. The single peak of Zn and O is present between 0 and 2, and the two peaks of zinc are present between 8 and 10. These results match with already reported results with the same peak position in Biosynthesis of zinc oxide using leaf extract of Calotropis gigantea (Chaudhuri and Malodia, 2017) and results also match (Al-Dhabi and Arasu, 2018), however our result is better as they show result with 80 and 64.12 per zinc present on the surface area.

4. Conclusion

The present study shows that Zinc oxide NPs were successfully synthesized by the biological method. Green synthesis is much safer and ecofriendly than the physical and chemical methods. In green approach natural sources act as stabilizing and reducing agent for the synthesis of nanoparticles. This approach leads to the formation of particles of controlled size and shape. ZnO NPs can easily be synthesized using fungi as the biological system. *Aspargillus niger* can be manipulated under controlled conditions and has great potential for extracellular and intracellular synthesis of metallic nanoparticles. The synthesized nanoparticles were stabilized by the proteins released by fungus in extracellular synthesis of ZnO NPs. This method is simple, cheap and without danger of any contaminants and pollution and produced large amount of stable nanoparticles. This method was beneficial for the extracellular synthesis of zinc oxide but this is a slow process and specific conditions are required for the successful synthesis of nanoparticles. The synthesized NPs decompose after a certain period of time, this problem is a challenge for scientists and solution of this should be explored. The synthesized nanoparticles were characterized by analytical techniques like XRD, SEM, and EDX. The XRD study shows that the size of zinc Oxide NPs is 40 nm. The SEM study elaborates that ZnO NPs are hexagonal in shape with average size of 66 nm which deviates from XRD results on account of agglomeration. This agglomeration is due to polarity and electrostatic interactions between zinc oxide NPs. The EDX confirms that synthesized nanoparticles are pure and there only trace of impurities present in the sample. The ZnO is used in ceramics, glass, cement, ointments, paints and lubricants, adhesives, plastics, pigments, batteries and fire retarders. Zinc Oxide shows promising signs in the field of nanotechnology, UV detectors, nanoscale detectors, and actuators. It would replace silicon in chip fabrication. The ZnO has a bright future because of its dual semiconductor and piezoelectric properties. The field of biogenic synthesis of metallic NPs by microbes is new and less explored, there are many aspects of biological methods yet to be discovered and manipulated

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