Antifungal Studies of ZnO Nanopowder Prepared by Solution Combustion Method

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***Abstract*: ZnO nanopowder was prepared by the simple and cost effective solution combustion method. It was characterized by FTIR, XRD, SEM and HRTEM. The PXRD pattern showed the presence of wurtzite hexagonal structure. Diffraction pattern agreed with the standard JCPDS pattern of Zincite [36-1451]. Transmittance band at 468 cm^{-1} in FTIR spectrum confirmed the presence of Zn-O bonding. Surface morphology of the sample was studied by SEM and HRTEM. The sample was tested against a fungal human pathogen *Candida albicans* and a fungal plant pathogen *Fusarium oxysporum*. The results indicate that the inhibitory efficacy of ZnO nanopowder is very much dependent on its chosen concentration and suggest the importance of nanomaterials in antifungal products.**

Keywords: ZnO nanopowder, Solution combustion synthesis, HRTEM, Antifungal activity, Candida albicans, Fusarium oxysporum.

1. INTRODUCTION

Nanomaterials are defined as materials with at least one external dimension in the size range of approximately 1-100 nanometers. Nanoparticles are objects with all three external dimensions at the nanoscale, which can be exploited for a wide range of applications in biology, food industries and pharmaceuticals possessing anti-inflammatory, anti-hypersensitive and anti-microbial functions [1]-[4]. The antimicrobial activity of nanoparticles has been studied with different pathogenic and nonpathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli* [5], [6] and fungi like *Aspergillus niger*, *Candida albicans* [7], [8].

In recent days controllable synthesis of nanocrystalline materials is one of the most interesting areas of research due to their applications in many fields. Many methods have been used to synthesize ZnO nanoparticles such as precipitation [9], hydrothermal [10], combustion [11], sol-gel [12], sono chemical method [13] etc. Solution combustion synthesis (SCS) is a wet chemical method which has been proved to be an excellent technique for preparing several nanomaterials. It has many advantages like short processing time, optimum processing temperature, low cost and good ability to achieve high purity in making nano metal oxide powders at the as-prepared state [14].

Among the inorganic materials, metal oxides such as MgO, CuO, TiO_2, ZnO etc., are of particular interest because of their unique properties. ZnO is a wide band gap (3.37 eV) semiconductor which has received attention over the past decade because of its wide range applications in photovoltaic [15], humidity sensing [16], nano generators [17], UV shielding materials [18], antibacterial agents [19], biocompatibility [20], etc. Inorganic metal oxides are being increasingly used for antimicrobial applications due to their stability and long shelf life. Thus, ZnO is an inorganic antimicrobial agent that is safer and more stable when compared to other antimicrobial agents, and its antimicrobial properties are explored for novel applications (other than pharmaceutical or cosmetic industries) such as, in replacement of toxic chemicals in leather retanning process and more generally for textile modification, as well [21], [22].

Candida albicans is a diploid fungus that grows both as yeast and filamentous cells, and a causal agent of opportunistic oral and genital infections in humans. *Candida albicans* is normally present on the skin and in mucous membranes such as the vagina, mouth, or rectum. The fungus also can travel through the blood stream and affect the throat, intestines, and heart valves. Pathogenic strains of *Fusarium oxysporum* have been studied for more than 100 years now. The host range of these fungi is extremely broad and consists of animals, ranging from arthropods to humans as well as plants, including a range of both gymnosperms and angiosperms. While collectively, plant pathogenic *Fusarium oxysporum* strains have a broad host range, individual isolates usually cause disease on a narrow range of plant

species. *Fusarium oxysporum* is a universal fungus which includes pathogenic and saprophytic members. The pathogenic members are well known for causing *Fusarium* wilt diseases of many important crops [23].

Few studies have been reported on the antifungal activity of ZnO. Literature suggests that ZnO powder at the microscale exhibits a weak fungal activity against *Candida albicans,* only at concentrations above 100 mg/ml [24]. Promising results have also been reported on the antibacterial activities of ZnO on oral infections [25]. To the best of our knowledge there are no reports on the antifungal studies of ZnO nanopowders prepared by solution combustion method using sugar as fuel. Hence, here we report the synthesis of ZnO nanopowder and its characterization by different techniques, towards exploring its antifungal activities against C*andida albicans* and *Fusarium oxysporum.*

2. EXPERIMENTAL DETAILS

2.1 Synthesis by Solution Combustion Method using Sugar as Fuel

Calculated amounts of AR grade zinc nitrate [$Zn(NO_3)_2.6H_2O$] and table sugar ($C_{12}H_{22}O_{11}$) were dissolved in 40 ml of double distilled water. Zinc Nitrate and table sugar were used as precursor and the fuel respectively. The petridish containing zinc nitrate and sugar in water was placed in a preheated muffle furnace at 300^0 C $\pm$ 10^0 C. Within a short while the solution boiled to form a gel. Immediately as the reaction was initiated a flame appeared which witnessed the combustion of the fuel. The reaction yielded a white, porous powder. The time span required for completion of the above reaction was about 5 minutes. ZnO powder thus prepared was ground in to a fine powder and characterized by various techniques.

2.2 *Characterization Methods*

The structural properties of as-prepared ZnO nano powders were studied by X-ray diffraction using PANalytica IX'pert diffractometer with Cu Kα radiation (λ=1.5418 A^0) as the source. Metal-oxygen bond formation was measured by FTIR studies which was carried out on IRAffinity-1 (Make: SHIMADZU) spectrophotometer using KBr disc method within the range of 400-3500 cm^{-1}. Surface morphology of the sample was estimated by scanning electron microscopy performed on FEI Quanta FEG 200 - High Resolution Scanning Electron Microscope. Besides using SEM, the surface morphology was investigated by HRTEM also (JEOL 3010 instrument with a UHR pole piece).

2.3 Antifungal Studies

The antifungal activity of ZnO nanopowder was carried out by well diffusion method in potato dextrose agar (PDA) media. Dispersions of nanoparticles with different concentrations from 500 µg/ml to 62.5 µg/ml were prepared. Dispersions were sonicated to break the agglomerations and to make it uniform. Petri plates containing 20ml PDA were seeded with 24hr culture of fungal strains *Candida albicans* and *Fusarium oxysporum* (10^7 cells/ml, OD 660 nm). Wells were cut and 20µl of the dispersions of ZnO (of different concentrations) were loaded. The plates were then incubated at 37°C for 24 hours. The antifungal activity was assayed by measuring the diameter of the zone of inhibition formed around the wells.

3. RESULTS AND DISCUSSIONS

3.1 Crystal Structure

Fig. 1 shows the X-ray diffraction pattern of the as-prepared ZnO nanopowder. Diffraction pattern agrees with the standard JCPDS No. 36-1451 with Zincite pattern. Sharpness of all the peaks implies that the sample is crystalline at room temperature. Absence of other characteristic impurity peaks confirms that the product is in pure phase. The crystallite size was calculated using the Scherrer equation, $D = k \lambda / \beta \cos\theta$, where D is the crystallite size, k is the Scherrer constant (0.9), λ is the X – ray wavelength, θ is the Bragg angle and β is the corrected half-peak width of the sample. The crystallite size of ZnO nanopowder was found to be in the range of 12-18 nm. Diffraction peaks of the XRD can prove that the ZnO sample is of wurtzite structure (p63 mc, a= 3.2455 Å, C= 5.2009 Å) [26]. As can be appreciated from Fig. 1, the crystallite size is a measure of the size of coherently diffracting domains and is not generally the same as particle size due to polycrystalline aggregates.

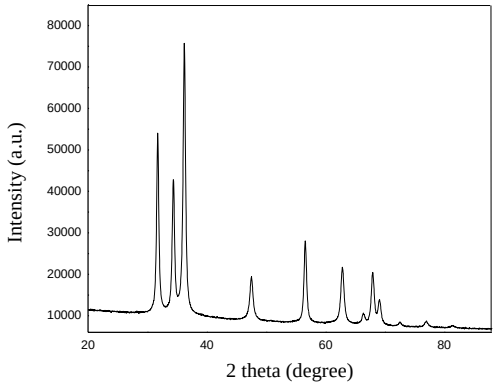

Fig.1. PXRD of ZnO nanopowder

3.2 Morphological Studies

The surface morphology of the as-formed ZnO nanopowder was studied using the Scanning Electron Microscopy. The SEM micrograph as shown in Fig. 2 reveals that the morphology of ZnO seems to be more or less spherical shaped, containing non uniform particles with large agglomeration of very fine particles. Micrograph reveals that besides the spherical crystals the powder also contains several voids, the reason for which can be attributed to the release of hot gases that escape out of the reaction mixture during combustion. It is through pores of various sizes and shapes that the crystallites are interlinked to one another.

Fig. 3 shows the TEM image of the as-formed ZnO nanopowder. It confirms that the particles are spherical. Particle size analysis was conducted by histogram and the mean particle size was found to be around 76 nm.

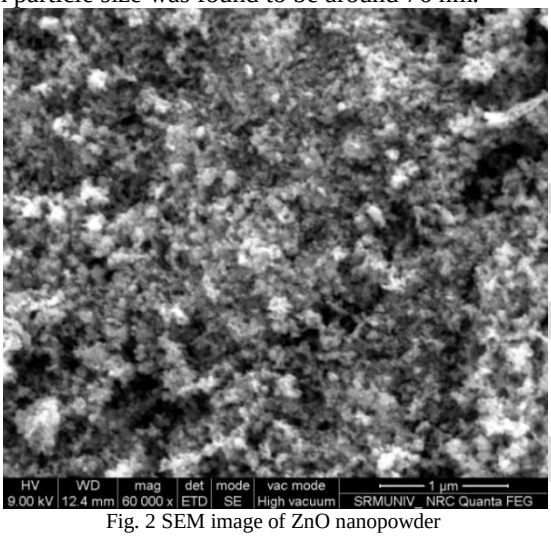

Fig. 2 SEM image of ZnO nanopowder

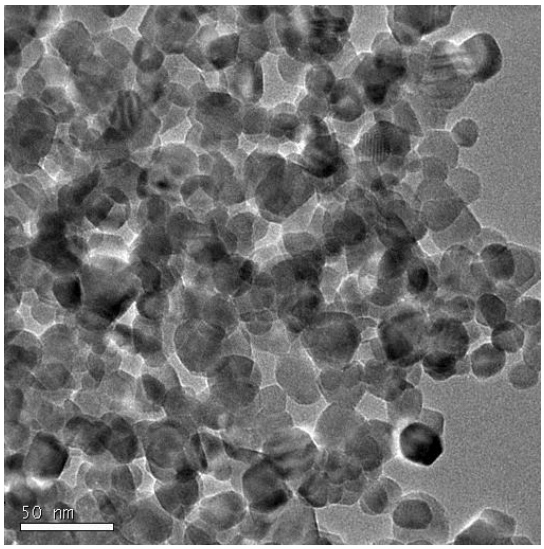

Fig. 3 HRTEM image of ZnO nanopowder

3.3 FTIR Analysis

FTIR spectrum of the sample is depicted in Fig. 4. The absorption bands near 3430 cm^{-1} were due to O-H mode. The peaks in the range 1400-1650 cm^{-1} was attributed to C=O stretching mode. The transmittance band at 468 cm^{-1} corresponds to the Zn-O bonding, and the presence of ZnO particles.

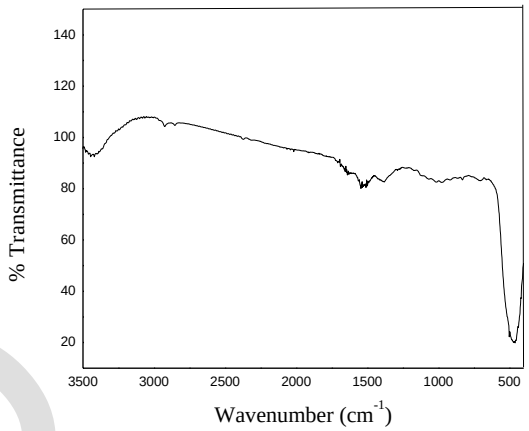

Fig.4 FTIR spectrum of ZnO nanopowder

3.4 Product Formation Mechanism

Stoichiometric amounts of $Zn(NO_3)_2$ (oxidizer) and carbonaceous fuel sugar ($C_{12}H_{22}O_{11}$) were mixed in distilled water. When this mixture was heated to 300^0 C $\pm$ 10^0 C, initially the wet powder underwent thermal dehydration followed by decomposition of zinc nitrate and fuel. Then it ruptured in to a flame and yielded a porous, agglomerated nanopowder. The reaction is self–propagating and the temperature produced was sustained for a length of time ranging from 3-6 seconds. The stoichiometric reaction can be written as

$$24Zn(NO_3)_2 + 5C_{12}H_{22}O_{11} \longrightarrow 24ZnO + 24N_2 + 35H_2O + 60CO_2$$
(aq) (aq) (s) (g) (g)

3.5 Evaluation of Biostudies

The data on the antifungal studies, for the as-formed ZnO nanopowder is indicated in Table 1.

Table 1. Zone of Inhibition (mm)

Species	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	Positive Control (Amphotericin B) 100 µg/ml
Candida albicans	12.00 ± 0.816	7.75 ± 4.573	3.75 ± 4.349	0.00 ± 0.00	36.25 ± 0.957
Fusarium oxysporum	12.00 ± 0.816	9.75 ± 0.957	8.50 ± 0.577	0.00 ± 0.00	36.75 ± 1.258

3.6 Antifungal Mechanism

As can be visualized from Table 1, the zone of inhibition is maximum at 500 µg/ml indicating that at higher concentrations the nanopowders are exhibiting antifungal properties. Few mechanisms have been proposed for the inhibitory action of nanoparticles against fungi. It might be due to the release of extracellular enzymes and metabolites that serve as an agent for their own survival when exposed to stress from toxic materials and temperature variations [27]. Antimycotic activity of silver nanoparticles has been studied against fungi like *Fusarium oxysporum* [28]. It is also reported that the antifungal activity may be due to inhibition of enzymes and toxins used by the fungi for pathogenism [28]. Another report indicates that the inhibitory effect of ZnO nanoparticles may be due to deformation in the structure of fungal hypha [29]. Few studies proposed that ZnO nanoparticles may cause structural changes of microbial cell membrane causing cytoplasm leakage and the death of cells [24]. Literature suggested that the production of mycotoxins by *Fusarium oxysporum* gradually decreased with the increase of ZnO nanoparticles and SEM showed the deformation in the growing mycelia treated with ZnO nanoparticles in *Fusarium oxysporum* [30]. Another report suggested that nanomaterials which can induce reactive oxygen species (ROS) in water suspensions can also be used for fungal eradication [31]. These experiments with ZnO nanopaticles synthesized by our method are especially on the pathogen *Candida albicans.*

CONCLUSION

ZnO nanopowder was synthesized by low cost SCS. Transmittance band at 468 cm^{-1} in FTIR spectrum confirms the presence of Zn-O bonding. The PXRD analysis demonstrates that the nanoparticles have the hexagonal wurtzite structure. Based on the Scherrer equation the crystallite size was found to be around 12-18 nm. The SEM micrograph of the sample reveals that besides the spherical crystals, the powders also contains several voids and pores with large agglomeration of fine particles. The HRTEM image shows that the shapes of the particles are spherical and the mean particle size was found to be around 76 nm. Antifungal studies were carried out against a fungal human pathogen *Candida albicans* and and a fungal plant pathogen *Fusarium oxysporum* in PDA media. The results indicate that, the inhibitory efficacy of nanopowder is very much dependent on its chosen concentration and demonstrates the plausible applications of these nanoparticles in anti-fungal products.

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