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Research Article

Surfactant Assisted Synthesis of ZnO Nanoparticles, Characterization and its Antimicrobial Activity against *Staphylococcus aureus and Escherichia coli*

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Abstract

Present work deals with a simplistic surfactant assisted combustion synthesis of ZnO NPs (Zinc Oxide Nanoparticles) where Zinc nitrate solution is used as a precursor and Glycine as fuel with TWEEN 80, a non ionic surfactant. Characterizations carry out to study different properties of the acquired particles the crystalline size and shape analysis by X-ray diffraction, UV–Vis absorption spectroscopy to monitor peak absorbance wavelength and nanoparticles size determined by using the mathematical model of effective mass approximation equation, Thermogravimetric (TG) analysis for weight loss, surface morphology by Transmission Electron Microscope, and particle size by Particle Analyzer. Antimicrobial activity of ZnO Nanoparticles against Staphylococcus aureus and Escherichia coli has been tested by disc diffusion technique. ZnO Nanoparticles exhibited a very strong antibacterial activity against bacterial species.

Keywords: *ZnO Nanoparticles, surfactant assisted, TEM, Staphylococcus aureus, Escherichia coli antibacterial activity.*

1. Introduction

Nanotechnology envelops material science, physics, biology, chemistry, and engineering operating at the nano level along with constructing materials on the atomic level and alterations at molecular level. In many areas of chemistry, physics and material science transition metal oxides with nano structure have attracted substantial interest during the last few years because of their novel optical and electrical properties as well as semiconductors crystals with a large binding energy (60meV) (Jiangtao Hu et al, 1999). In diverse of applications Zinc oxide Nanoparticles are used such as catalyst (Winn-Jung Huang et al, 2005) , antibacterial treatment (Laura Sánchez et al, 1996), and photocatalyst (Ramiah Annapoorani et al, 1997). Different physical methods such as pulse laser deposition (Yoshiki Nakata et al, 2002, Y.Z. Yoo et al, 2002) , vapor phase transparent process (B.J. Chen et al, 2004), vapor transparent deposition and chemical vapor deposition (Y.J Li et al, 2004) have been established for synthesis of nano ZnO. Sol-gel method is one of the conventional procedures for the research of metal oxide nanoparticles (Xin-Li Yang et al, 2004) which is found on the reactive metal precursor hydrolysis. A number of techniques are available for the synthesis of ZnO Nanoparticles for example, chemical and photochemical reactions in reverse micelles, thermal decomposition of Zinc silver compounds, microwave assisted process, reduction in solutions, and of late via green chemistry route. But there are very few who worked with the surfactant assisted method.

In this paper a simplistic surfactant TWEEN 80, zinc nitrate and glycine are used to synthesize ZnO Nanoparticles and the obtained Nanoparticles are characterized by various techniques like X-Ray Diffraction, UV-Visible Spectroscopy, Thermogravimetric Analysis, Particle Analyzer, Transmission Electron Microscope to find the size, morphology and weight loss.

The significance of bactericidal nanomaterials is due to the amplified resistant strains of bacteria against most potent antibiotics and has promoted the research in the antibacterial properties of Zinc Oxide Nanoparticles.

Staphylococcus is a genus of Gram-positive bacteria. Under the microscope, they appear round (cocci), and form in grape-like clusters. *Staphylococcus* can cause a wide variety of diseases in humans and other animals through either toxin production or penetration. Staphylococcal toxins are a common cause of food poisoning, as they can be produced by bacteria growing in improperly-stored food items. The most common sialadenitis is caused by staphylococci, as bacterial infections.

Escherichia is a genus of gram-negative bacteria, rod shaped and non-spore forming bacteria. Most of these are pathogenic. They are the inhabitants of gastrointestinal tracts of warm-blooded animals.

2. Experimentation

2.1 Synthesis of Zinc Oxide Nanoparticles

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Freshly prepared aqueous solutions of zinc nitrate, non-ionic surfactant and glycine were used for the synthesis of nanoparticles. At room temperature the chemicals are added one by one with the 0.1 M solution of zinc nitrate, 0.15 M solution of glycine and 0.025 M solution of non-ionic surfactant respectively. The mixture of chemicals was then heated on a hot plate in separate beakers which led the chemical mixture to self-combustion. After combustion the final precipitate is calcinated for 1 hr at 400 ^{0}C. Thus we successfully obtained a pure ZnO nano powders in this synthesis.

2.2 Disc diffusion method

Materials used for antimicrobial activity of nano Zinc Oxide was Nutrient broth 1.3 g, Agar-agar 1.5g, petriplates, cotton swabs, *Staphylococus aureus*, *Escherichia coli*. Disc diffusion method was used to test the antimicrobial activity of nano Zinc Oxide.

Nutrient broth (1.3g in 100 ml D/W) was prepared in two conical flasks and sterilized. In one conical flask clinically isolated strain of *Escherichia coli* was inoculated and in the other conical flask clinically isolated strain of *Staphylococcus aureus* was added. The bacterial culture inoculated nutrient broth was kept on rotary shaker for 24 hours at 100 r.p.m.

Nutrient agar was prepared (1.3g nutrient broth, 1.5g Agar Agar in100ml distilled water) and sterilized. The agar suspension was transferred into sterile Petri plates and allowed to solidify. Then the two pathogenic strains *E.coli* and *Staphylococcus aureus* were taken and spread evenly on the entire surface of the plate. Before applying the sample plates were allowed to dry. Filter paper discs were used to impregnate the control and test samples.

Discs were coated with equal volume (20µl) of control and different concentrations of the test sample. Then these discs were placed on the Petri plates. All the test plates were incubated for 19-24 hrs at 37^{0}c. The activity was clearly visible from19-24 hrs on the plates. The zone of inhibition was measured and the sample of the nano Zinc Oxide showing maximum antimicrobial activity was observed and noted.

3. Characterization

The crystal phases of the synthesized powders were determined by X-ray diffraction (XRD, Bruker D& Advance, Germany) using CuKα as radiation source (40 kV, step size 0.02o, scan rate 0.5° min^{-1}, 20° $\leq 2\theta \leq$ 80°). The optical properties of the samples were characterized by UV-VIS Spectroscopy (Systronics). Thermal analyses of the samples were done with TG-DTA (EXSTAR TG/DTA6000 series). The particle size is measured by Nano Particle Size Analyzer (SZ-100 Nanoparticle, Horiba, Germany). The thermal decomposition behaviors of the samples were investigated by thermo gravimetric analysis (TGA/DTA, A6300R). The particle size and morphology of the synthesized powder were examined by scanning electron microscope (SEM, Hitachi VP-SEM S-3400N, Germany). The surface morphology of ZnO nanoparticles were studied using Transmission Electron Microscope (Tecnai 20 T G2 (FEI)).

4. Results and Discussion

4.1 XRD Analysis

The crystallite size is calculated from full width at half maximum (FWHM) of the peaks (1 0 0) (0 0 2) (1 0 1) (1 0 2) (1 1 0) (1 0 3) (1 1 2) and (2 0 1) using the Debye–Scherrer's Eqation (Cullity BD, 1978).

$$D = \frac{0.94\lambda}{\beta \cos\theta}$$

Where D is the average crystallite size perpendicular to the reflecting planes, λ is the -ray wavelength, β is the full width at half maximum FWHM, and θ is the diffraction angle. All the diffraction peaks can be assigned to hexagonal phase with Wurtzite structure with space group (P63mc), JCPDS card No.36-1415 and unit cell parameters a = b = 0.3249 nm and c = 0.5206 nm. The average crystalline size is 12 nm (Figure.1).

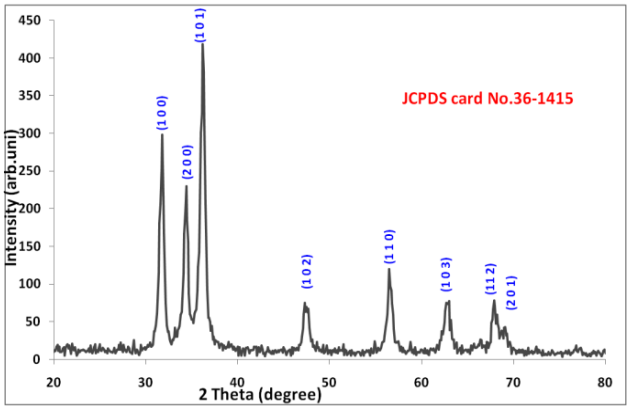

Fig.1 The XRD pattern for the nano ZnO with fuels Gylcine and urea.

4.2 UV-Vis Spectroscopy

ZnO nanoparticles are dispersed in ethanol solution and absorption spectrum is seen in figure 2. At 372 nm the absorption is observed. The average particle size present in the nanoparticles can also be determined by using the mathematical model of effective mass approximation equation (N.S. Pesika et al, 2003, Louis Brus, 1986) where the particle size (r, radius) and peak absorbance wavelength (λp) for monodispersed ZnO nanoparticles.

$$r(nm) = \frac{-0.3049 + \sqrt{-26.23012 + \frac{1024072}{\lambda_p(nm)}}}{-6.3829 + \frac{24832}{\lambda_p(nm)}}$$

During the derivation of equation, m_e = 0.26 m_o, m_h = 0.59m_o, mo is the free electron mass, ε = 8.5, and E_g bulk = 3.3 eV. The prepared ZnO nanoparticles show peak absorbance at 372nm which corresponds to average particle size of 5 nm.

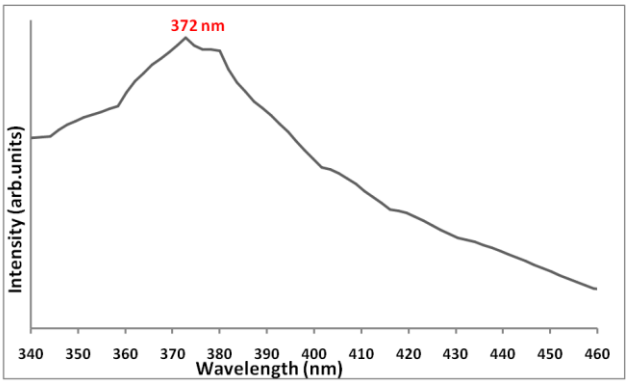

Fig.2: Shows the absorption spectrum of ZnO nanoparticles

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4.3 Thermal Properties

Thermogravimetric (TG) analysis is performed between 30^{o}C to 800^{o}C at the rate of 20^{o}C / min for the sample and the weight loss is observed (Figure 3). Between the temperatures 30^{o}C to 150^{o}C was about 1.09% due to removal of water molecules on the surface, between 150^{o}C to 550^{o}C was about 2.05% due to decomposition of carbon and nitric compounds and between 550^{o}C to 800^{o}C the weigh loss was due to loss of oxygen.

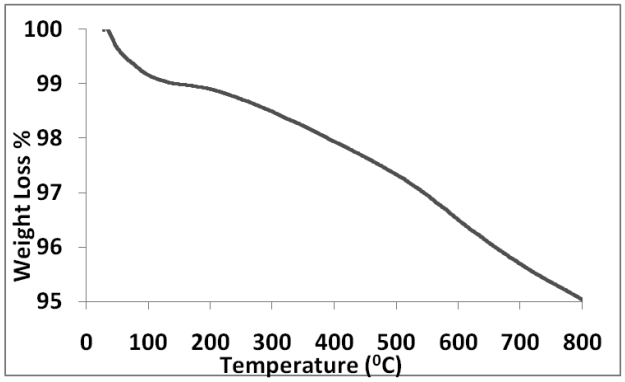

Fig.3: TGA curve for ZnO powder.

4.4 Nano Particle Analyzer and TEM

The size of the nanopowders is measured using Nano Particle Analyzer (SZ100). The average particle size for sample is shown with histogram in figure.4.

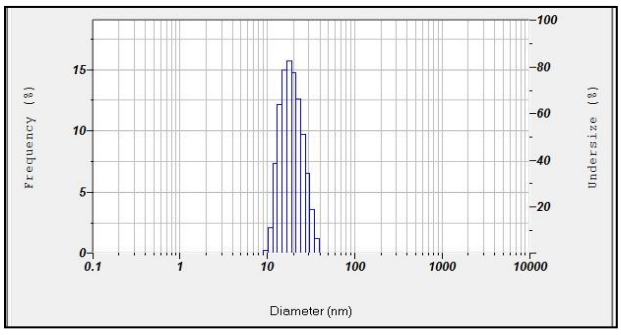

Fig.4: Particle analyzer histograms of ZnO powder.

The result from particle analyzer is in good agreement with the XRD result of crystallite size.

Size and morphology of ZnO particles are analyzed from the TEM (Figure 5). The TEM image discloses that the average size of 20-30 nm of the sample is in good agreement with Scherer formula based XRD results.

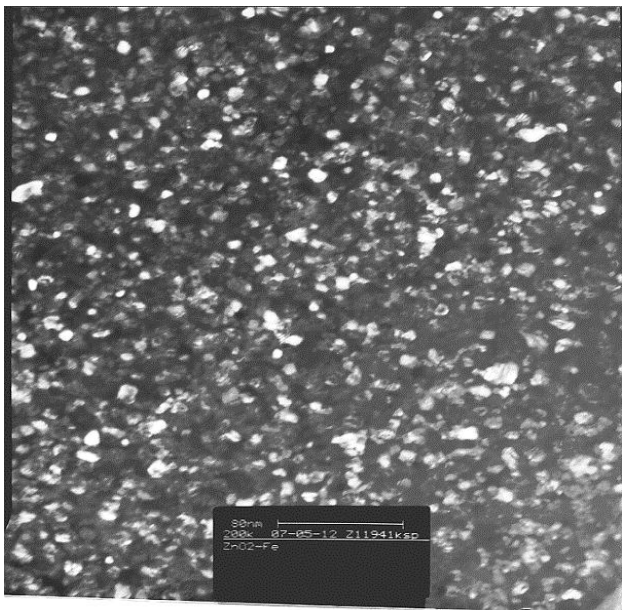

Fig.5: The dark field TEM image of nanocrystalline ZnO.

4.5 Antibacterial Activity

The formation of Zone of Inhibition is the way to identify the antibacterial activity of the sample. Zone of Inhibition is the area on an agar plate where growth of a control organism is prevented by an antibiotic usually placed on the agar surface. When the testing organism is subjected to the antibiotic then it will not grow where the antibiotic is present. The compound's effectiveness is known by the measurement of the size of the zone of inhibition, larger the clear area around the antibiotic, the more effective the compound. The formation of Zone of inhibition of the sample was observed after 24 hours. The inhibitory activity of ZnO Nanoparticles was confirmed by the presence of zones of inhibition. The zones of inhibition of different bacteria are given in the figure. The clear zones around the samples in the plates show the activity of the sample. Table 1 and Figures 6 - 7 show the Petri dishes with samples of C for control, 1 for 0.04 gm, 2 for 0.06 gm and 3 for 0.08 gm of ZnO nanoparticles with inhibitory zones.

Table 1 Antibacterial activity of ZnO of various concentrations with different bacterial species.

S.No	Bacterial species	Inhibition zone (mm)			
		Control	0.04 gm	0.06 gm	0.08 gm
1	*Staph.aureus*	1.3	1.1	1.4	1.6
2	*E.coli*	1	0.4	0.8	1.2

Fig.6: Inhibitory Zones of Zno Nanoparticles against *Staphylococcus aureus*

Fig.7: Inhibitory Zones of Zno Nanoparticles against *Escherichia coli*.

5. Conclusion

ZnO powder was successfully synthesized by novel surfactant assisted combustion method. The crystallite size calculated from the XRD is 12.82 nm is in good agreement with TEM results. From UV-Vis Spectroscopy the absorption spectrum was 372 nm. Thermal analysis indicated that the sample obtained were of extreme pure in nature, since it was observed that the total weight loss of the sample was only 1.79%. The antibacterial activity of ZnO Nanoparticles was confirmed by Zone of inhibition. As the diameter of the zone of inhibition is high, we can conclude that ZnO is also a very effective antibacterial agent. ZnO Nanoparticles are effective against both the bacteria which gives a conclusion that it is effective against gram +ve and gram –ve bacteria. Therefore we can conclude that ZnO Nanoparticles is a very effective antibacterial agent.

References

Jiangtao Hu (1999), Chemistry and Physics in One Dimension:Synthesis and Properties of Nanowires and Nanotubes. Accounts of Chemical Research, vol 32, pp. 435-445.

Winn-Jung Huang (2005), A nanometer-ZnO catalyst to enhance the ozonation of 2,4,6-trichlorophenol in water, Colloids and Surfaces A: Physicochemical and Engineering Aspects, vol 260, pp 45-51.

Laura Sánchez (1996), Degradation of 2,4-dichlorophenoxyacetic acid by in situphotogenerated fenton reagent. Electrochimica Acta, vol 41, pp 191-198.

Ramiah Annapoorani (1997), An investigation on ZnO photocatalysed oxidation of uracil, Journal of Photochemistry and Photobiology A: Chemistry, vol 111, pp. 215-221.

Yoshiki Nakata (2002), Deposition of ZnO film by pulsed laser deposition at room temperature, Applied Surface Science, vol 197, pp. 368-370.

Y.Z. Yoo, S doping in ZnO film by supplying ZnS species with pulsed-laser-deposition method, Applied Physics Letters, vol 81, pp. 3798.

B.J. Chen, (2004), Growth and characterization of zinc oxide nano/micro-fibers by thermal chemical reactions and vapor transport deposition in air, Physica E, vol 21, pp. 103-107.

Y.J Li, (2004), Synthesis of ZnO nanoparticles on Si substrates using a ZnS source, Journal of Crystal Growth, vol 260, pp. 309-315.

Xin-Li Yang, (2004), Novel efficient and green approach to the synthesis of glutaraldehyde over highly active W-doped SBA-15 catalyst, Journal of Catalysis, vol 229, pp. 259-263.

Cullity BD, Elements of X-ray diffraction, chap-6, Addison-Wesely Publishing Company, Inc., Philippines, 1978.

N.S. Pesika, (2003), Determination of the Particle Size Distribution of Quantum Nanocrystals from Absorbance Spectra, Advanced Materials, vol 15, pp. 1289-1291.