OPEN ACCESS JOURNAL

BIOINTERFACE RESEARCH IN APPLIED CHEMISTRY

www.BiointerfaceResearch.com

ISSN 2069-5837

Volume 1, Issue 2, 2011, 057-063

Received: 30.03.2011 / Accepted: 10.04.2011 / Published on-line: 15.04.2011

Bactericidal action of N doped ZnO in sunlight

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ABSTRACT

ZnO based fabrics for antibacterial activity is based on absorbance in the UV region of the electromagnetic spectrum, process requiring activation under UV light. In the present study, ZnO is synthesized by hydrazinated oxalate method. The synthesis introduces nitrogen in the lattice of ZnO which shifts its absorbance in the visible region. This N doped ZnO shows enhanced biocidal activity towards *Escherichia coli.* The textiles based on N doped ZnO can be effectively used as antimicrobial under solar radiations.

Keywords: *zinc oxide, antibacterial properties, Escherichia coli, disk diffusion, minimum inhibitory concentration*

1. Introduction

There is a tremendous increase in the rate and spread of infectious diseases, as well as in the number of antibiotic resistant microorganisms. It has thus become more important to prevent the infections than to cure them. The various inorganic metal oxides such as TiO_2, ZnO, MgO and CaO are of particular interest as they are not only stable under harsh process conditions, but also generally regarded as safe materials to human beings and animals [1-3]. The infectious agents are many times carried by clothes. Hence the need is to design textiles with antimicrobial properties. ZnO is effective against a wide range of microorganisms [4-6] and so the fabrics treated with ZnO prove to be resistant to a large number of microbial strains. ZnO treated cotton fabrics [7] are widely studied for antibacterial activity. ZnO being a wide band gap semiconductor is extensively studied as an antimicrobial agent due to its photocatalytic activity under UV light. ZnO coated fabrics are also used as UV absorbers [8-9]. In the recent years, visible light absorbing photocatalysts with $Ag/AgBr/TiO_2$ have proved to be effective in killing *Staphylococcus aureus* and *Escherichia coli* [10]. Aerogel-derived halogenated (Cl_2 and Br_2) adducts of nanocrystalline metal oxides such as MgO and CaO are reported to be successful against *Escherichia coli*, *Bacillus cereus* and *Bacillus globigii* as well as for decontaminating MS2 bacteriophage in dry powder form or as an aqueous slurry [11]. To increase the photocatalytic activity of ZnO, it is doped with different metals and non-metals [12-15]. Among the non-metals, Nitrogen is regarded as the most promising dopant, exhibiting properties similar to oxygen [16]. In the present study, an attempt is made to synthesize N doped ZnO by hydrazinated oxalate method and to study its antibacterial activity against *E. coli*, a Gram negative bacterial species. The results are also compared with the antibacterial activity of commercially available ZnO.

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2. Experimental section

2.1 Synthesis of Hydrazinates of the oxalates

2.1.1. Solution method. Oxalic acid and hydrazine hydrate in the desired proportion were mixed thoroughly using magnetic stirrer under inert atmosphere. A required amount of the metal chloride solution was run down into the mixture in a three necked reactor.

2.1.2 Equilibration method. Zinc oxalate was synthesized mixing equimolar solutions of $ZnCl_2 \cdot 2H_2O$ and ammonium oxalate and stirring on a magnetic stirrer. A well dried zinc oxalate precursor was spread over a Petri-dish and kept in a desiccator saturated with hydrazine hydrate (Merck, 99%) vapors. The hydrazine uptake was monitored by KIO_3 titration [17].The prepared precursors were decomposed to get the oxides. Commercial ZnO ultrafine powder (> 1μm) of 99% purity was obtained from British Drug House (BDH).

2.2 Characterization. Phase identification of the metal oxide products was carried out by X-ray diffraction (Rigaku Powder X-ray diffractometer). SEM images of the samples were recorded on the instrument JEOL JSM – 5800. Diffuse reflectance spectrum was also recorded on Shimadzu UV-2401-Rev A 6700 to determine the absorption in the range of 200-800 nm. XPS analysis was done at 298K using MgK_α radiation on a VG Microtech Multilab ESCA 3000 spectrometer.

2.3 Antibacterial studies. For the antibacterial studies, *Escherichia coli* was selected as a test organism. The bactericidal activity was tested by disk diffusion method in solid agar medium. The Minimum Inhibitory Concentration (MIC) was assessed using liquid broth dilution method. A protocol applicable to the inorganic metal oxides and composites was followed [18-20]. All disks, media and materials were autoclaved before the experiments. All experimental work was carried out under sterile conditions in a laminar air-flow chamber. *E. coli* culture was grown in nutrient broth for 18 hours at 37 ^oC at 100 rpm on a rotary shaker. The number of bacterial cells was estimated by measuring the absorbance of the suspension at 600 nm. A culture having A_{600} = 0.8-1.0 corresponding to 10^5 cfu/ml was used for all the antibacterial studies. The experiments were done in triplicate and the results were averaged.

2.3.1. Disk diffusion assay. Nutrient agar medium was used as basal medium in the Petri plates used for the assay. A 100 μl of the bacterial suspension was added in 5 ml of top agar, mixed well and poured evenly over the basal medium. The *E. coli* culture was allowed to grow at 37 ^oC for 30 minutes. 0.025g of the oxides were coated on filter paper disks of 6 mm diameter and placed on the nutrient media in the Petri plates. Filter paper disk without the oxide coatings was used as a control. The Petri plates were incubated for 18-24 hours and then the diameters of inhibition zones were measured.

2.3. 2. Minimum inhibitory concentration (MIC). ZnO powders ranging from 0.01 to 1.0 mMoles were added to five tubes, each containing 5 ml of L. B. (Luria Bertani) broth. 100 μl of *E. coli* culture was added to each tube and the resulting solutions were stirred overnight (18-24 hours) on a rotary shaker at 37 ^oC. Positive and negative controls were also prepared. Aliquots of 100 μl from all the tubes were plated on Mac Conkey agar. The plates were incubated at 37 ^oC for 18-24 hours and checked for growth. MIC was determined based on the culture growth intensity.

3. Results section

The diffraction patterns of oxides obtained from the decomposition of hydrazinated oxalates matched well with the JCPDS card no 5- 664, for Wurtzite ZnO (Figure 1).

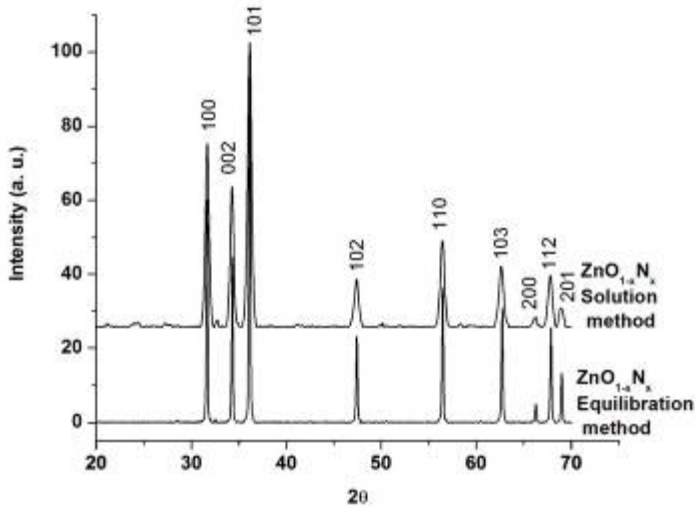

Figure 1. X-ray diffraction patterns of ZnO synthesized by hydrazinated oxalate method.

SEM analysis (Figure 2) showed particle sizes of 69 and 38 nm for ZnO prepared by equilibration and solution methods respectively. Broadening of XRD peaks in case of the sample prepared solution method is due to small particle sizes.

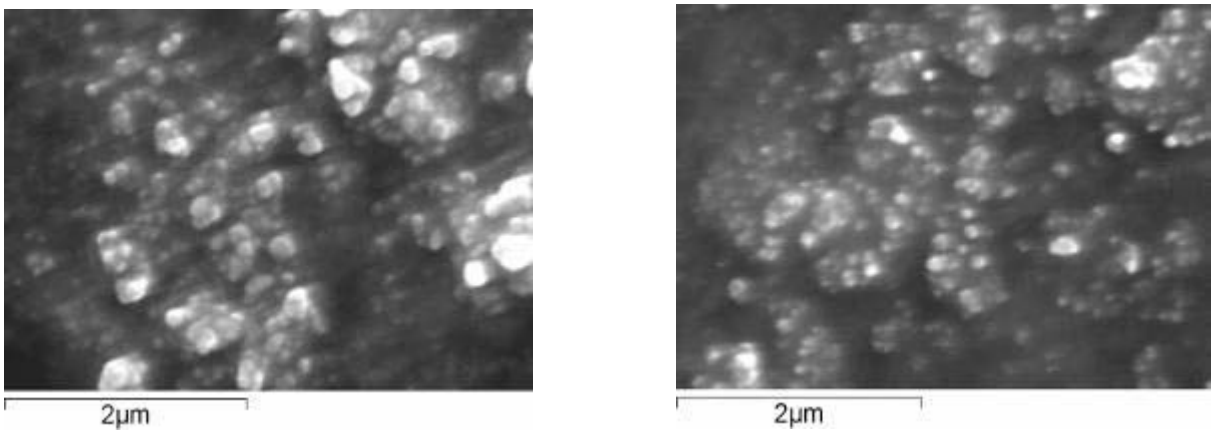

Figure 2. SEM images of $ZnO_{1-x}N_x$ prepared by hydrazinated oxalate
1) Equilibration method 2) Solution method

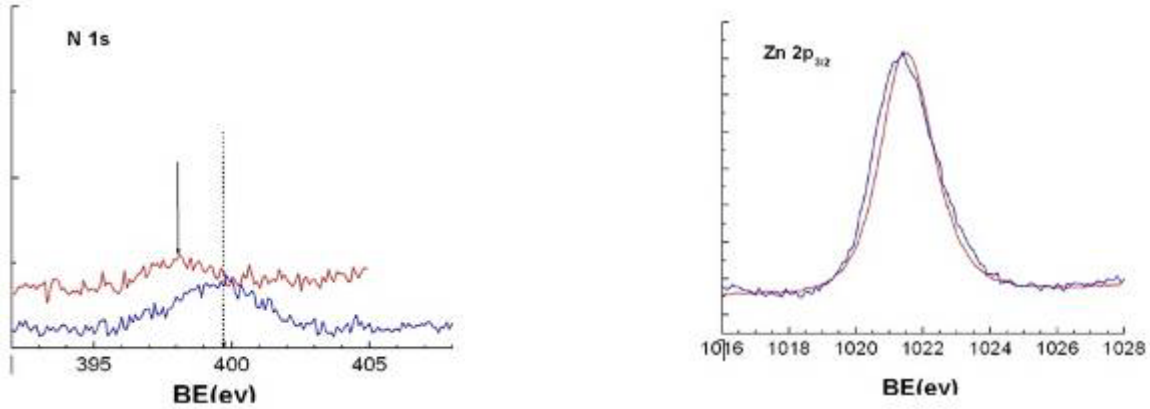

Figure 3. XPS of 1) N 1S electron, 2) Zn $2p_{3/2}$ electron

The ESCA studies (Figure 3) on the ZnO system reveal the presence of nitrogen in the lattice. The nature of nitrogen in the sample prepared by equilibration method is close to nitride type with binding energy (BE) ~ 398 eV, whereas in the sample prepared by solution method, nitrogen seems to resemble that of ammonia (charge density wise) and its binding energy is between 399-400 eV. Zn 2p core level shows BE ~ 1022 eV indicating single type of Zn (II) ions. Based on these, N-doped ZnO is considered to have a stoichiometry, $ZnO_{1-x}N_x$.

In case of synthetic ZnO the N in the lattice modifies the band gap and introduces midgap level which makes it absorb in the visible region [21] as shown in the diffuse reflectance spectra in Figure 4.

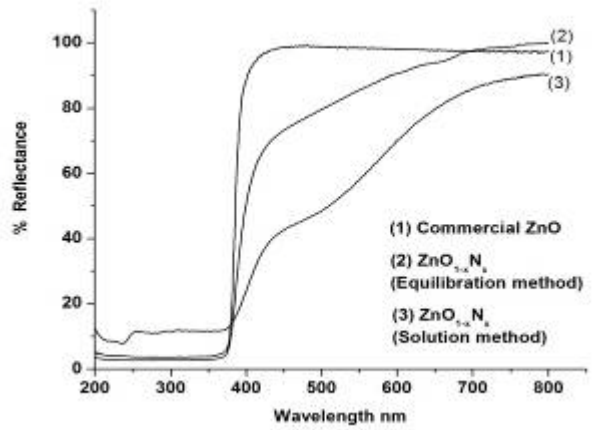

Figure 4. Diffuse reflectance spectrum of commercial and prepared Zinc Oxide.

The results of the disk diffusion studies on ZnO suspensions with different particle sizes are given in Table 1. The presence of an inhibition zone clearly indicates a bactericidal activity. It was found that all ZnO were effective against the tested bacterial strain. The $ZnO_{1-x}N_x$ prepared by equilibration as well as solution method showed a larger inhibition zone (9 mm) as compared to commercial zinc oxide (7 mm) (Figure 5).

Figure 5. Zone of inhibition of $ZnO_{1-x}N_x$ (Solution method)

Table1. Zone of inhibition in the *E. coli* culture for prepared and commercial Zinc oxide samples.

Compound	Particle size in nm	Diameter of the inhibition zone
ZnO1-xNx (equilibration)	69	9 mm
ZnO1-xNx (solution)	38	9 mm
ZnO (commercial)	> 1μm	7 mm
Control	-----	6 mm

The order of inhibition is $ZnO_{1-x}N_x$ (equilibration) = $ZnO_{1-x}N_x$ (solution) > commercial ZnO.
ZnO is activated by both UV and visible light to create electron-hole pairs (e^-h^+). The holes split H_2O molecules (from the suspension of ZnO) into OH^- and H^+. Dissolved oxygen molecules are converted to superoxide radical anions ($^{\bullet}O_2^-$), which react with H^+ to generate ($HO_2^{\bullet}$) radicals. These hydroxyl radicals upon collision with electrons produce hydrogen peroxide anions (HO_2^-), which react with hydrogen ions to produce molecules of H_2O_2 [4].The generated H_2O_2 can penetrate the cell membrane and kill the bacteria [22]. Since, the hydroxyl radicals and superoxide radical anions are negatively charged particles, they cannot penetrate into the cell membrane and must remain in direct contact with the outer surface of the bacteria, however, H_2O_2 can penetrate into the cell [23].

$$ZnO + h\nu \rightarrow e^- + h^+$$

$$h^+ + H_2O \rightarrow {}^{\bullet}OH + H^+$$

$$e^- + O_2 \rightarrow {}^{\bullet}O_2^-$$

$$^{\bullet}O_2^- + H^+ \rightarrow HO_2^{\bullet}$$

$$HO_2^{\bullet} + H^+ + e^- \rightarrow H_2O_2$$

The photoactivation of ZnO is done with UV/visible light. A normal laboratory environment has fluorescent lighting which emits 4% UV light. So ZnO is 'activated' by this small amount of UV component of the visible light prevalent in the laboratory. In the case of $ZnO_{1-x}N_x$ the absorbance is in the visible light which increases the formation of electron – hole pair and thus facilitates the formation of more hydrogen peroxide and increases the antibacterial action. This explains the increase in the zone of inhibition. It is also argued that size of the particles also plays an important role in inhibiting bacterial growth [1, 18, 24]. The reason for strong antibacterial activity of ZnO with small particle size was explained by Yamamoto as follows: the contact of moisture in the medium per unit ZnO mass increases with the decrease in particle size, because of the increase of specific surface area. This results in the increased H_2O_2 generation from its surface [4]. However some reports indicate no effect of particle size on the toxicity of ZnO particles [25]. All three ZnO samples were tested for bactericidal efficienc, a lower MIC corresponding to higher antibacterial effectiveness. The results are compiled in table 2.

Table 2. Bactericidal action of Zinc oxide

mMoles of ZnO	$ZnO_{1-x}N_x$ (equilibration)	$ZnO_{1-x}N_x$ (solution)	Commercial ZnO
0.01	+++	+++	+++
0.05	+++	+++	+++
0.1	++	+	+++
0.5	+	-	++
1.0	56*	-	++

Legend: * no of colonies, + Matt growth, - No growth

In case of $ZnO_{1-x}N_x$ prepared by equilibration method, few colonies were grown at the concentration of 1 mM. The MIC is above 1 mM showed a more abundant bacterial growth was obtained in the presence of all commercial ZnO concentrations showing its inability to work as an antibacterial agent. $ZnO_{1-x}N_x$ prepared by solution method has an MIC of 0.5 mM.

Figure 5. Bacteriocidal action of 1.0 mM $ZnO_{1-x}N_x$ prepared by
i) solution method and ii) equilibration method in MacConkey broth.

From the above results and reported literature [18], it is known that Zn^{+2} ions act as a nutrient at very low concentration hence ZnO may not be active against *E. coli* in a low concentration range. Zinc traces are an essential cofactor in a variety of cellular processes, but at higher concentrations it is toxic. Hence N doped ZnO is a biocidal agent effective against *E. coli* in normal sunlight as compared to commercial ZnO. N doped ZnO based textiles do not require any photoactivation in UV light and can be used easily and effectively under sunlight for protection against microbes.

4. Conclusions

Synthesis by hydrazinated oxalate method yields ZnO with nitrogen in its lattice. $ZnO_{1-x}N_x$ yellow in colour, absorbs in the visible region of the electromagnetic spectrum. $ZnO_{1-x}N_x$ shows an increase in biocidal activity as compared to commercial ZnO. This enhanced bactericidal activity is attributed to the presence of N in the lattice. Fabrics treated with N doped ZnO can be efficiently used as an antibacterial agent in ordinary sunlight.

5. References

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