PA

Enhanced green luminescence properties of Cu doped ZnO nanoflowers and their improved antibacterial activities

Hind Neelamkodan^{1,2,*}, Unikoth Megha^{1,2}, Manathanath Puzhakkara Binitha¹

¹Department of Physics, Govt. Arts and Science College, (Affiliated to Calicut University), Meenchanda, Calicut-673018, Kerala, India

²Department of Physics, DGMMES Mampad College (Affiliated to Calicut University), Mampad P.O, Malappuram-676542, Kerala, India

Received 3 August 2022; received in revised form 23 November 2022; Received in revised form 20 February 2023; Accepted 1 March 2023

Abstract

The copper doped ZnO ($Cu_x Zn_{1-x}O$, where x = 0, 0.01, 0.02, 0.04, 0.05) nanoflowers were produced by using co-precipitation process. Hexagonal wurtzite nanocrystalline phase of both pure and Cu-doped samples was confirmed by X-ray diffraction analysis. Field emission scanning electron microscopy results confirmed the emergence of fully dispersed nanoflower like morphologies in the Cu-doped ZnO and uniform dispersion of particles. Band gap energies of the prepared samples were calculated using the Tauc's plot. All of the generated samples' room temperature photoluminescent spectra show two emission peaks, one excitonic peak in the UV region and another broad defect level peak in the visible area. The yellow and green luminescence characteristics of both pure and doped samples were analysed via the PL spectra in visible light region, making them appropriate for optoelectronic applications. The antibacterial potential of the produced nanostructures against the bacterial strains of Enterococcus Faecalis (gram positive) and Pseudomonas Aeruginosa (gram negative) were assessed. When compared to the pure ZnO nanostructures, the doped samples exhibit enhanced antibacterial activity. For the samples with 5 at.% Cu, the greatest zone of inhibition against Pseudomonas Aeruginosa was noted to be 25 mm. For the samples doped with 5 at.% Cu, the largest zone of inhibition against Enterococcus Faecalis was 21 mm. The findings indicate that the obtained samples have higher antibacterial potential against gram negative than gram positive bacteria. The prepared doped ZnO nanoflowers were therefore recommended as excellent green nanophosphors for the production of white LEDs and effective nanoantibiotics against gram-negative bacteria.

Keywords: Cu-doped ZnO, nanoflowers, optical properties, nanophosphors

I. Introduction

The vast band gap energy (3.37 eV), electricalthermal stability, high exciton binding energy (60 meV) and high saturation velocity of ZnO make it a highly researched material in the field of nanoscience and technology [1,2]. ZnO nanoparticles' special features are advantageous in a variety of fields, including electronics, optoelectronics, agriculture, communications and healthcare. The potential optical and biomedical characteristics of ZnO nanoparticles can be employed in optoelectronic devices, solar cell appliances, diagnostic tools, antimicrobial agent, bio-imaging, drug delivery as well as in cancer treatment [3].

The remarkable photoluminescence characteristics of the ZnO nanoparticles (NP) make them suitable for photocatalysis and optoelectronic applications. The physical and chemical properties of metal oxide nanoparticles can be improved by doping with several types of dopants. Different physical, chemical and bactericidal efficacies have been observed depending on the types of dopants used, the synthesis methods used, the experimental settings, etc. The photo luminescent and antimicrobial properties of ZnO NP can be enhanced by dopants, such as transition metals Fe, Cu, Mg and Mn, among others. Cu has the ability to significantly alter the

^{*}Corresponding author: tel: +91 9633562847

e-mail: hindneelamkodan@gmail.com

morphological, structural, optical, electrical, magnetic and biological aspects of ZnO NPs among these transition elements [4]. Copper is thought to be the source of green fluorescence in ZnO. It is still unclear what specific mechanism controls the green fluorescence in ZnO. However, research suggests that whilst the structureless green emission comes from oxygen vacancies, the structured green emission is caused by copper impurities or other types of donors [5]. Producers of white LEDs use a variety of green luminous Cu-doped ZnO phosphors. However, employing them to make white LEDs has a number of problems because their synthesis process necessitates intricate chemical processes, uses hazardous precursors and results in polydispersed nanoparticles [6].

Because ZnO nanostructures can generate reactive oxygen species (ROS) such hydroxyl radicals (OH[•]), singlet oxygen, or superoxide anion (O^{2-}) , which can seriously harm bacterial cells' internal components including proteins and DNA, they have the potential to be cytotoxic. ZnO NPs have the ability to emit antimicrobial ions, primarily Zn²⁺ ions, and can come into direct contact with bacteria to kill them [7]. ZnO nanostructures' antibacterial efficacy can be changed by increasing ROS generation within the bacterial cell. A very effective way to increase the ROS generation by the nanostructures is to incorporate different dopants into the material. Doping causes lattice imperfections, which boost ROS production and antimicrobial effects. Previous studies show that pure ZnO has lower antibacterial activity than Cu-doped ZnO nanostructures. Copper has an ionic radius similar to that of zinc and is not hazardous to human cells. Additionally, Cu is a cheap, nontoxic and naturally plentiful element, making it a viable doping agent among transition element dopants. Therefore, the purpose of the current study was to develop pure and Cu-doped ZnO nanophosphors with improved structural, optical and antibacterial properties using a straightforward co-precipitation approach.

Because of its low cost, non-toxicity, low temperature requirement and high productivity, co-precipitation method was chosen for this study among the numerous types of ZnO nanoparticle synthesis techniques. To study the optical properties of the material, UV-Vis and photoluminescent spectra of the generated samples were measured. Additionally, the antibacterial efficacy of synthesized materials against gram positive and gram negative bacteria using the agar well diffusion method was analysed.

II. Experimental

Nanosized pure and Cu-doped ZnO powders $(Cu_x Zn_{1-x}O)$, where x = 0, 0.01, 0.02, 0.04, 0.05) were synthesized using co-precipitation technique (Fig. 1). Sigma Aldrich 0.5 M zinc acetate dehydrate solution (purity >99%) and aqueous solution containing 1 M NaOH (purity >99%) were combined and stirred continuously to create pure ZnO nanostructures and pH



Figure 1. Illustration of synthesize process of Cu-doped ZnO nanoparticles

was maintained at 11. Doping was carried out by combining various concentrations of zinc acetate dehydrate solution with copper acetate dehydrate solution (Sigma Aldrich, purity >99%). Cu-doped ZnO nanoparticles were precipitated by gradually adding 1 M NaOH to this mixture and pH was kept at 11. The ensuing pale blue suspension was aged for a further reaction to occur and filtered the next day. To remove by-products, the precipitate was washed in distilled water and ethanol and then calcined at 200 °C for 2 h. The calcined samples were ground using an agate mortar, before being submitted to several characterisation experiments.

XRD patterns were collected by X-ray diffractometer (X'Pert3 Powder) with CuK α radiation, (λ = 0.15406 nm). The surface morphology of the Cudoped ZnO nanostructures was investigated by FESEM (MAIA3 XMH) with Schottky emitter resolution of 0.7 nm at 15 kV. The room temperature absorption spectra of the pure and Cu-doped ZnO nanopowders were captured in the region 200–650 nm using Lambda 650, Perkin Elmer UV-Vis spectrometer. Photoluminescence spectra (PL) of the undoped and doped ZnO nanostructures stimulated at 340 nm were obtained using a Horiba Fluorolog 3 Fluorescence Spectrometer with TCSPC.

III. Results and discussion

3.1. X-Ray diffraction analysis

XRD patterns of the pure and Cu-doped ZnO nanoparticles are shown in Fig. 2. The pure ZnO has extremely firm and intense peaks, which show that the sample is well-crystalline. Peak intensities were rapidly decreased as Cu was added. The existence of defects or disorders caused by the copper ions in the hexagonal wurtzite ZnO structure was the cause of the peaks' reduced intensity [8]. The ICDD card number (36-1451) was found to coincide exactly with the (100), (002), (101), (102), (110), (103), (200), (112) and (201) peaks for the pure and doped samples.

A shift in peak positions towards higher angles and broadening of diffraction peaks with an increase in doping concentration was observed (Fig. 2b). This was due



Figure 2. XRD patterns of Cu-doped ZnO nanoparticles (a) and enlarged 2θ region showing peak shifting (b)

Doping concentration	Lattice constants			Cell volume	C
[at.%]	a [Å]	<i>b</i> [Å]	<i>c</i> [Å]	[Å ³]	Space group
0	3.2533	3.2533	5.2073	47.73	P63mc
1	3.2495	3.2495	5.2069	47.62	P63mc
2	3.2495	3.2495	5.2069	47.62	P63mc
4	3.2495	3.2495	5.2069	47.62	P63mc
5	3.2490	3.2490	5.2070	47.60	P63mc

Table 2. Average crystallite size from Scherer equation D_S and W-H plot D_{WH} , microstrain and dislocation density δ of prepared samples

Doping concentration [at.%]	D_S [nm]	D_{WH} [nm]	Microstrain	$\delta [\mathrm{nm}^{-2}]$
0	29.5	60.9	0.00157	3702.72
1	17.7	37.8	0.00289	1429.60
2	16.6	36.1	0.00295	1303.93
4	16.1	32.5	0.00291	1054.30
5	14.3	25.6	0.00272	656.90

to the different ionic radii of Zn and Cu ions. Introduction of Cu into the Zn lattice caused a compressive strain within the structure, which shifted the peak locations of the X-ray diffractograms towards higher diffraction angles. All of these differences resulted from the increased lattice defects and micro strain of the sample with doping [9]. The complete substitution of Cu ions into the Zn lattice was therefore confirmed by the peak shift and peak width broadening.

The lattice parameters, cell volume and space group of the pure and doped materials were retrieved with the QualX software and displayed in Table 1. The lattice parameters of the doped samples obeying the Vegard's law also somewhat decreased as a result of the difference in the ionic radii of Zn and Cu. For the doped samples, there was also a modest decrease in the cell volume.

The average crystalline size of all pure and doped samples was determined from the XRD patterns using the Scherer formula and Williamson Hall (W-H) plot. In the Scherer formula $D = K \cdot \lambda / \beta \cdot \cos \theta$, *D* is the crystallite size, *K* is the Scherer constant, λ is the X-rays wavelength, β is the full width at half maximum (FWHM), and θ is the Bragg's angle. Table 2 lists the crystallite size results for samples of the pure and Cu-doped ZnO NPs. It has been discovered that when the doping concentration increases, the average crystallite size decreases. Impurities will cause distortion of the host material's crystal lattice, which will slow down the nucleation and growth rates. This feature is explained by the Zener pinning theory, which shows how grain boundaries move across the grain's edges. This idea states that the edges of the crystallite impose an imminent force on the moving boundaries and these moving borders may become stuck if Cu ions replace Zn atoms. Because the obstructing power is greater than the E_g primary driving force for grain growth, crystallite growth is therefore prevented. Due to the doping of Cu in ZnO, there may be some charge imbalance and crystal defects around the dopants. Therefore, the presence of Cu altered the ZnO crystal lattice and caused the crystallite size to decrease as doping concentration increased. This reduction in crystallite size suggests promising applications in gas sensor, spintronic and optoelectronic devices [10].

There is a significant discrepancy between the average crystallite size values determined using the Scherer equation and W-H plot. The crystallite size determined from the W-H plot was found to range from 60.8 to 25.6 nm, whereas according to the Scherer equation it was between 14.3 and 29.5 nm. The discrepancies in how the particle dispersion was averaged led to this variation [11].

The dislocation density has a significant impact on the physical characteristics of materials, such as grain size and hardness. According to the literature review



Figure 3. SEM images of $Cu_x Zn_{1.x}O$ NPs, where x is: a) 0, b) 0.01, c) 0.02, d) 0.04 and e) 0.05, as well as f) enlarged image of $Cu_{0.05}Zn_{0.95}O$ NPs with g) brush like structure on petals

[12], the material's hardness is inversely proportional to its grain size. With increasing the incorporation of Cu into the ZnO lattice, a little drop in the dislocation density has been observed since doping increases the microstrain of the material. Table 2 lists the microstrain and dislocation density of the samples as determined by the W-H plot.

3.2. FESEM analysis

The surface morphology of the Cu_xZn_{1-x}O NPs was investigated by FESEM (Fig. 3) which showed the emergence of fully dispersed nanoflower like morphologies for the samples doped with 0, 1 and 2 at.% Cu with a diameter of 200–500 nm. The produced flower like structures composed of petals having numerous nanobrushes with diameters of 40–50 nm and length from 100–140 nm. The diameter of petals increases to 1–1.5 µm with the increase in doping concentration to 4 and 5 at.% Cu. Nanoflowers can be used for a wide range of biological applications, including catalysis, bioremediation etc. due to their extremely high surface area to volume ratio.

3.3. Optical analysis

UV-visible spectra analysis

The room temperature absorption spectra of the pure and Cu-doped ZnO nanopowders are shown in Fig. 4. The image makes it obvious that all of the samples exhibit noticeable absorption in the range of 255 to 404 nm. Band gap energies of the prepared samples were calculated using the Tauc's relation $(\alpha hv) \sim$



Figure 4. Absorption spectra as well as the Tauc's plots of pure and Cu-doped ZnO nanoparticles

 $(hv - E_g)^{1/2}$, where hv is the incident photon energy and α is the optical absorption coefficient near the fundamental absorption edge. The measured values of band gap and absorption edges of the samples are reported in Table 3. Doping has been found to widen the bandgap of ZnO nanostructures. According to the Tauc's graphic, the band gap of the pure ZnO is 3.11 eV, whereas the band gap of the sample doped with 1 at.% Cu is 3.26 eV, which are both lower than that of the bulk ZnO. The deviation of band gap in the pure and doped nanostructures from that of the bulk was due to the nanoflower like structures and defects that occurred during synthesis [13,14]. The widening of the bandgap in the doped samples was a result of the quantum confinement effect. The Burstein-Moss effect provides a clear explanation for the band gap widening associated with doping. This effect states that Cu generates free carriers that cause the Fermi level to shift towards the conduction band, resulting in a widening of the band gap. Low energy transitions are therefore not feasible. The decrease in particle size with increased Cu doping is another factor contributing to the bandgap widening [15]. However, it is discovered that the band gaps of the 2, 4 and 5 at.% Cu-doped samples were smaller when compared with the 1 at.% doped sample. The bandgap energy of the 5 at.% Cu-doped sample is determined to be 3.20 eV. The nanoflower morphology and the development of shallow levels inside the band gap caused it to shrink. The overall band narrows as a result of changes in the electron exchange interactions between the band electrons and the *sp-d* exchange interactions of the band electrons to localised d-electrons of Cu ions, which result in a negative correction in the conduction band and a positive correction in the valence band. These shallow levels inside the band gap provided by the doping are one of key components to enhance the photocatalytic activity. Also contributing to the narrowing of the band gap are many-body effects on the conduction and valence bands, i.e. there may be a chance to fuse an impurity band with a conduction band, which results in a narrowing of the band gap [16]. The band gap narrowing originates from the chemical reaction of the host ZnO crystal and it is due to the difference in electronegativity between Zn and Cu.

The pure ZnO exhibits an absorption edge at 403.8 nm. The absorption edges of the Cu-doped materials exhibited a blue shift as a result of an increase in lattice distortion. The absorption edge of the sample doped with 1 at.% Cu is at 387.0 nm. The growing band gap and quantum confinement effect were both confirmed

 Table 3. The band gap and absorption edge of pure and doped samples

Doping concentration [at.%]	Band gap energy [eV]	Absorption edge [nm]
0	3.11	403.8
1	3.26	387.0
2	3.24	392.6
4	3.22	400.0
5	3.20	401.3

by this blue shift in the absorption edges. As the doping concentration increases, it was discovered that the absorption edges of other doped samples (2, 4 and 5 at.%)were moving towards higher wavelengths in comparison to the 1 at.% doped sample. The red shift revealed the presence of surface oxides of Zn and Cu. This red shift indicates the inclusion of Cu ions into the Zn lattice and is a sign of a decrease in bandgap energy. The main causes of the red shift of doped samples include electron-phonon interaction, lattice flaws and the localization of charge carriers caused by point defects [17]. The second order perturbation theory can theoretically be used to explain the red shift in the band gap [18,19]. The red shift of the band gap supports the uniform replacement of Zn ions by Cu ions, according to the perturbation theory. It was also observed that the absorption intensity abruptly decreases while the absorbance increases gradually when the dopant concentration is increased. Additionally, a rise in visible range absorption was seen for the materials that were doped with 2, 4 and 5 at.% Cu. The increase in visible light absorption was caused by the photo-induced transition between the valence, conduction, and impurity states.

Photoluminescence spectral analysis

Photoluminescence (PL) spectra of all samples displayed two emission peaks, a broad peak in the visible range and one excitonic peak in the ultra violet range (340–670 nm). The visible band can be formed by flaws such as impurities, zinc interstitials, oxygen vacancies, etc. but the excitonic band can emerge from the donor acceptor pair transition due to radiative recombination of neutral donor with neutral acceptor. When the pure ZnO was examined, a prominent excitonic peak centred at 386 nm was seen. This peak is associated with near band emission (NBE), which is the recombination of free excitons following an exciton-exciton collision process. The NBE peaks of all doped samples were observed to move towards shorter wavelengths with the peak of 1 at.% Cu-doped sample cantered at 380 nm. The incorporation of Cu atoms into the ZnO lattice is confirmed by the blue shift of the excitonic peaks, which indicates the quantum confinement effect. The incorporation of Cu atoms into the ZnO lattice is confirmed by the blue shift of the excitonic peaks, which indicates the quantum confinement effect and lead to an expansion of the optical band gap. It can be seen (Fig. 5) that the excitonic peaks of the 2, 4 and 5 at.% Cu-doped samples exhibit a minor shift towards a higher wavelengths in comparison to the 1 at.% doped ZnO and are centred at 381 nm as the doping concentration increases. This red shift was the result of a band gap narrowing effect which would lead to increased defect levels between the valance and conduction bands of ZnO [20]. The nanoflower morphology and the incorporation of copper impurity were thought to be the reasons for the band gap narrowing effect. For the previously mentioned UV-Vis absorption spectra, the comparable blue shift and red shift were observed.



Figure 5. PL spectra of pure and Cu-doped ZnO nanoparticles

For each sample, a prominent broad defect level peak has been seen in the visible range. The pure ZnO emits light at a wavelength of roughly 573 nm, which corresponds to a yellow luminescence that is typically attributed to deep levels brought on by ionised oxygen vacancies. The visible bands of all doped sample exhibit a blue shift and the centre of the 1 at.% Cu-doped sample is located at 530 nm (2.34 eV), which corresponds to the green luminescence (GL). Cu ion acts as a shallow donor in pure ZnO nanostructures, as shown by the blue shift to the PL peak. According to Raji et al. [20], the GL band was produced by the radiative recombination of Cu⁺ acceptor level holes and donor level electrons that are positioned below the conduction band. As a result, it is possible to conclude that the GL band at 530 nm in the current investigation resulted from the transition between a shallow donor level and a Cu⁺ acceptor state located above the valance band.

The defect level peaks of the higher doped samples (2, 4 and 5 at.%) exhibit green luminescence and are centred at 546 nm (2.27 eV), 553 nm (2.24 eV) and 555 nm (2.23 eV), respectively. The nanoflower like morphology and the increase in copper impurity levels brought on by the increased doping concentration might be what caused the red shift that was noticed. According to Sajjad et al. [21], the oxygen vacancies and inherent defects are what cause the green band to exist near 550 nm. Syed et al. [22], assert that the recombination of photoexcited holes and electrons in singly occupied oxygen vacancy is the cause of the GL band at 546 nm. According to Irimpan et al. [23], anion vacancies caused by copper doping increase ZnO defect levels. They claimed that the transition from the defect level to the copper-induced t_2 level is what causes the amplification of green luminescence in doped materials. According to the earlier findings, the change from deep donor levels to the d^9 state of the Cu²⁺ acceptor was what caused the green emissions at 553 and 555 nm in the current work. Since they were not seen for pure ZnO, the green luminescence bands of all doped sam-



Figure 6. Schematic model of defect levels corresponding to the green emission in Cu-doped ZnO

ples demonstrate that Cu impurity is incorporated into the ZnO lattice. Figure 6 depicts the schematic model of defect levels corresponding to the green emission in Cu-doped ZnO.

Due to the actual decrease in the rate at which photogenerated carriers recombine, it is discovered that all samples up to a doping concentration of 4 at.% lower excitonic and visible band intensities. Copper has the capacity to capture ZnO electrons, improving the separation of electron and hole pairs. Additionally, the addition of Cu results in a reduction in surface flaws like Zn and O vacancies, which lowers the rate of recombination. Because Cu atoms on the surface deflect the excitation light, the visible PL intensity fell as the doping concentration increased. This compound functions better as a photocatalytic material since the presence of Cu impurities also caused a non-radiative recombination process that reduces the PL emission. Since there was too much Cu ions acting as recombination centres, the fluorescence intensity of the sample that with 5 at.% Cu rises. The primary deep donor for the visible luminescence in ZnO is the oxygen vacancy. Higher Cu content results in improved electronic transitions from the Cu shallow-donor level to the O-vacancy deep-acceptor level, which increases the visible photoluminescence intensity [24].

These results suggested that it is possible to create nanoflower-like structures and either reduce or increase the intensity of green luminescence by choosing the right quantity of copper impurity. It has also been demonstrated that the co-precipitation approach can readily adjust the oxygen vacancies in ZnO nanostructures, i.e. the quantity of oxygen vacancies in ZnO can be controlled by this method, and the addition of divalent impurities like copper boosted the luminous capabilities of ZnO. Due to their superb photoluminescence capabilities, the Cu-doped ZnO nanoflowers with green luminescence can be very helpful in the creation of white LEDs. Thus, outstanding green photoluminescence of the Cu-doped ZnO nanophosphors is obtained and the 5 at.% Cu-doped sample synthesised using coprecipitation can be used for the production of white LEDs.

Antibacterial activities

The antibacterial properties of the produced pure and Cu-doped ZnO NPs were assessed using agar well diffusion method. Three different concentrations of the samples (250, 500 and 1000 μ g) were tested for their antibacterial ability against *Enterococcus Faecalis* (gram positive bacteria) and *Pseudomonas Aeruginosa* (gram negative).

The culture media was made by dissolving 33.8 g of Muller Hinton Agar Medium (MHI Agar Media) in 1000 ml of distilled water. The dissolved medium was autoclaved for 15 min at 121 °C and 103 kPa of pressure. This mixture was thoroughly combined before being put (25-30 ml per plate) onto melted 100 mm Petri plates. 13 g of nutrient medium (HI Media) were dissolved in 1000 ml of distilled water to make 11 of nutrient broth, which was then heated to completely dissolve the medium. The medium was distributed and sterilised by autoclaving at 103 kPa of pressure (121 °C) for 15 min. 10 mg/ml of streptomycin was employed as the standard antibacterial agent. The germs Enterococcus Faecalis and Pseudomonas Aeruginosa were planted in Petri plates containing 20 ml Muller Hinton Agar Medium. The growth of the culture was adjusted according to McFarland Standard 0.5%.

Holes of about 10 mm were made using a well cutter and various sample concentrations, such as 250, 500 and 1000 μ g, were placed within. After that, the plates were incubated for 24 h at 37 °C. Antibacterial activity of the samples were evaluated (NCCLS, 1993) by measuring the diameter of the inhibition zone that formed around the well. The positive control was streptomycin. Figures 7 and 8 depict the zones of inhibition of pure and Cu-doped ZnO nanostructures. Table 4 provides the measured values for the inhibition zone.

The findings demonstrate that at all of the tested concentrations, Cu-doped ZnO NPs suppress the growth of the tested microorganisms more potently than pure ZnO samples. It has been discovered that as the concentration of copper impurity rises, so does the zone of inhibition. This is brought on by copper's potent antibacterial properties and the fact that crystallite size decreases as copper concentration rises. The nanopowder with 5 at.% Cu exhibits improved antibacterial activity against both Enterococcus Faecalis (gram positive) and Pseudomonas Aeruginosa (gram-negative). This increase in antibacterial activity is brought about by the full development of flower-like structures. Alshraiedeh et al. [25] demonstrated that the shape of nanostructures significantly influences their microbial resistance. Compared to gram positive bacteria, gram negative bacteria are reported to be more susceptible to Cu-doped ZnO nanoflowers. This results from the structural differences between gram positive and gram-negative bacteria [26]. The variance in the wall membranes of these bacterial cells can



Figure 7. Antibacterial analysis of pure and Cu-doped ZnO NPs showing the zone of inhibition of the growth of *Pseudomonas* Aeruginosa (S0 - pure ZnO, S1 - 1 at.% Cu, S2 - 2 at.% Cu, S4 - 4 at.% Cu, S5 - 5 at.% Cu)



Figure 8. Antibacterial analysis of pure and Cu-doped ZnO NPs showing the zone of inhibition of the growth of *Enterococcus Faecalis* (S0 - pure ZnO, S1 - 1 at.% Cu, S2 - 2 at.% Cu, S4 - 4 at.% Cu, S5 - 5 at.% Cu)

Bacteria		Pseudomonas aeruginosa			Enterococcus Faecalis		
Concentration [µg]		250	500	1000	250	500	1000
	Pure ZnO	NIL	12	17	NIL	11	14
Zone of	1 at.% Cu	NIL	15	18	NIL	13	16
inhibition	2 at.% Cu	12	16	20	12	15	19
[mm]	4 at.% Cu	13	18	22	13	17	20
	5 at.% Cu	15	20	25	16	19	21

Table 4. Zone of inhibition of the growth of Pseudomonas Aeruginosa and Enterococcus Faecalis

be used to explain this. When compared to gram negative bacteria, the peptidoglycan cell wall surrounding gram-positive bacteria is many times thicker. Additionally, this cell wall contains several mucopeptides, lipoteichoic acids, murein, etc. As a result, it is extremely difficult to destroy a gram positive bacterial cell by penetrating its cell wall. However, gram negative bacteria have a huge number of protein porins in their peptidoglycan cell walls that assist the nanoflowers diffuse within the bacterial cell and kill the cell. Different processes can be at play in the antibacterial activity of the pure and Cu-doped ZnO nanoflowers. These nanoflowers can generate a variety of reactive oxygen species (ROS) in an aqueous media, including hydroxyl radicals (OH $^{\bullet}$), singlet oxygen, and superoxide anion (O²⁻). These ROS cause oxidative stress and harm to bacterial cells. The Cu-doped ZnO-nanoflowers will produce cytotoxic Zn²⁺, Cu, Cu⁺ and Cu²⁺ ions upon release. Cell death may occur as a result of copper ions' interactions with DNA molecules and disruption of the helical structure [27].

According to the obtained results, antibacterial impact grows as sample concentration rises. This is due to the fact that the nanoflower morphology and a high concentration of Cu ions may negatively affect the functions of bacterial cells, such as glycolysis, transmembrane, proton translocation, acid tolerance, etc. and cause the bacteria to develop more slowly. All pure and doped nanoflowers display better antibacterial activity against gram negative bacteria in the current experiment than they do against gram positive bacteria.

IV. Conclusions

The structural, optical, and antibacterial qualities of pure and Cu-doped ZnO nanoflowers with wurtzite phase synthesized via co-precipitation method were investigated. The nanocrystalline nature of the samples and Cu substitution into the ZnO lattice are both revealed by XRD examination. FESEM research demonstrates the emergence of nanoflower-like structures with increased Cu concentration. The bandgap energies of the synthesized nanoflowers, calculated by the use of the Tauc's plot, raised for the doped samples when Cu was fully incorporated into the zinc lattice and it gradually decreased for samples that were doped with increasing concentrations of Cu compared to the 1 at.% doped sample. High photoluminescent characteristics with green light emission in the doped nanoflowers make them suitable for optoelectronic applications. Due to their superior and highly intense photoluminescent properties, the sample with 5 at.% Cu can be used as green luminescent nanophosphor for the production of white LEDs.

Both gram negative (*Pseudomonas Aeruginosa*) and gram positive bacteria (*Enterococcus Faecalis*) were effectively combated by the pure and Cu-doped samples. The nanoflowers with 5 at.% Cu dopant have 25 mm maximal zone of inhibition against *Pseudomonas Aeruginosa* at a sample concentration of $1000 \,\mu\text{g}$. The largest zone of inhibition against *Enterococcus Faecalis* (21 mm) displayed the samples with 5 at.% Cu. According to antibacterial research, the doped nanoflowers have stronger antibacterial ability against gram negative bacteria than gram positive bacteria. The overall findings of this study point to the possibility of using Cu-doped ZnO nanoflowers as potential antibacterial agents against gram negative bacteria and outstanding nanophosphors with green fluorescence.

Acknowledgements: The authors gratefully acknowledge the SAIF and IUIC, M.G University, Kerala, India, for PL and U-V spectral measurements.

References

- M.J. Haque, M.M. Bellah, M.R. Hassan, S. Rahman, "Synthesis of ZnO nanoparticles by two different methods & comparison of their structural, antibacterial, photocatalytic and optical properties," *Nano Express*, 1 [1] (2020) 10007.
- S. Alamdari, M.S. Ghamsari, C. Lee, W. Han, H.H. Park, M.J. Tafreshi, H. Afarideh, M.H.M. Ara, "Preparation and characterization of zinc oxide nanoparticles using leaf extract of sambucus ebulus", *Appl. Sci.* **10** [10] (2020) 3620.
- N.B. Raj, N.T. PavithraGowda, O.S. Pooja, B. Purushotham, M.R.A. Kumar, S.K. Sukrutha, C.R. Ravikumar, H.P. Nagaswarupa, H.C.A. Murthy, S.B. Boppana, "Harnessing ZnO nanoparticles for antimicrobial and photocatalytic activities", *J. Photochem. Photobiol.*, 6 (2021) 100021.
- A. Khalid, P. Ahmad, A.I. Alharthi, S. Muhammad, M.U. Khandaker, M.R.I. Faruque, I.U. Din, M.A. Alotaibi, A. Khan, "Synergistic effects of Cu-doped ZnO nanoantibiotic against Gram-positive bacterial strains", *PLoS One*, 16 (2021) e0251082.
- V.J. Shukla, A. Patel, "Synthesis, optical, and photoluminescence properties of undoped and Cu-doped ZnO thin films by colloidal solution route", *Mol. Cryst. Liq. Cryst.*, 712 [1] (2020) 62–75.
- A. Vanaja, M. Suresh, J. Jeevanandam, Venkatesh, Sk. Gousia, D. Pavan, D. Balaji, N. Bhanu Murthy, "Copperdoped zinc oxide nanoparticles for the fabrication of white

LEDs", Prot. Met. Phys. Chem. Surfaces, 55 [3] (2019) 481–486.

- A. Sirelkhatim, S. Mahmud, A. Seeni, N.H. M. Kaus, L.C. Ann, S.K.M. Bakhori, H. Hasan, D. Mohamad, "Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism", *Nano-Micro Lett.*, 7 [3] (2015) 219– 242.
- 8. C. Rojas-Michea, M. Morel, F. Gracia, G. Morell, E. Mosquera, "Influence of copper doping on structural, morphological, optical, and vibrational properties of ZnO nanoparticles synthesized by sol gel method", *Surfaces Interfaces*, **21** (2020) 100700.
- M.F. Manzoor, E. Ahmad, M. Ullah, A.M. Rana, A.S. Malik, M. Farooq, I. Ahmad, M. Hasnain, Z.A. Shah, W.Q. Khanf, U. Mehtab, "Impact of copper doping on the structural, electrical and optical properties of auto-combustion synthesized ZnO nanocomposites", *Acta Phys. Pol. A*, 135 [3] (2019) 458–466.
- S.J. Charde, S.S. Sonawane, A.P. Rathod, S.H. Sonawane, N.G. Shimpi, V.R. Parate, "Copper-doped zinc oxide nanoparticles: Influence on thermal, thermo mechanical, and tribological properties of polycarbonate", *Polym. Compos.*, **39** (2018) E1398–E1406.
- S. Mustapha, M.M. Ndamitso, A.S. Abdulkareem, J.O. Tijani, D.T. Shuaib, A.K. Mohammed, A. Sumaila, "Comparative study of crystallite size using Williamson-Hall and Debye-Scherrer plots for ZnO nanoparticles", *Adv. Nat. Sci. Nanosci. Nanotechnol.*, **10** [4] (2019) 045013.
- U. Megha, G. Varghese, K. Shijina, "Effect of Bi and Sr doping on morphological and magnetic properties of LaCo_{0.6}Fe_{0.4}O₃ nanosized perovskites", *Bull. Mater. Sci.*, **39** [1] (2016) 125–131.
- K. Davis, R. Yarbrough, M. Froeschle, J. White, H. Rathnayake, "Band gap engineered zinc oxide nanostructures: Via a sol-gel synthesis of solvent driven shape-controlled crystal growth", *RSC Adv.*, 9 [26] (2019) 14638–14648.
- M. Nafees, W. Liaqut, S. Ali, M.A. Shafique, "Synthesis of ZnO/Al:ZnO nanomaterial: structural and band gap variation in ZnO nanomaterial by Al doping", *Appl. Nanosci.*, 3 [1] (2013) 49–55.
- A.N. Kadam, T.G. Kim, D.S. Shin, K.M. Garadkar, J. Park, "Morphological evolution of Cu doped ZnO for enhancement of photocatalytic activity", *J. Alloys Compd.*, **710** (2017) 102–113.
- L. Ben Saad, L. Soltane, F. Sediri, "Pure and Cu-doped ZnO nanoparticles: Hydrothermal synthesis, structural, and optical properties", *Russ. J. Phys. Chem. A*, **93** [13] (2019) 2782–2788.

- K. Shijina, G. Varghese, U. Megha, "Surface passivation effect on structure, UV and visible emission of ZnNiPdO nanorods", *Mater. Sci. Semicond. Process.*, 34 (2015) 21– 26.
- S. Muthukumaran, R. Gopalakrishnan, "Structural, FTIR and photoluminescence studies of Cu doped ZnO nanopowders by co-precipitation method", *Opt. Mater.*, 34 [11] (2012) 1946–1953.
- R.B. Bylsma, W.M. Becker, J. Kossut, U. Debska, D. Yoder-Short, "Dependence of energy gap on x and T in Zn_{1-x}Mn_xSe: The role of exchange interaction", *Phys. Rev. B*, **33** [12] (1986) 8207–8215.
- R. Raji, K.G. Gopchandran, "ZnO:Cu nanorods with visible luminescence: Copper induced defect levels and its luminescence dynamics", *Mater. Res. Express*, 4 [2] (2017) 025002.
- M. Sajjad, I. Ullah, M.I. Khan, J. Khan, M.Y. Khan, M.T. Qureshi, "Structural and optical properties of pure and copper doped zinc oxide nanoparticles", *Results Phys.*, 9 (2018) 1301–1309.
- S. Syed Zahirullah, J. Joseph Prince, P. Fermi Hilbert Inbaraj, "Structural and optical properties of Cu-doped ZnO nanorods by silar method", *Mater. Technol.*, **32** [12] (2017) 755–763.
- L. Irimpan, V.P.N. Nampoori, P. Radhakrishnan, "Enhanced luminescence and nonlinear optical properties of nanocomposites of ZnO-Cu", *J. Mater. Res.*, 23 [11] (2008) 2836–2845.
- M. Fu, Y. Li, S. Wu, P. Lu, J. Liu, F. Dong, "Sol-gel preparation and enhanced photocatalytic performance of Cudoped ZnO nanoparticles", *Appl. Surf. Sci.*, 258 [4] (2011) 1587–1591.
- N.H. Alshraiedeh, O.F. Ammar, M.M. Masadeh, K.H. Alzoubi, M.G. Al-Fandi, R.J. Oweis, R.H. Alsharedeh, R.A. Alabed, R.H. Hayajneh, "Comparative study of antibacterial activity of different ZnO nanoparticles, nanoflowers, and nanoflakes", *Curr. Nanosci.*, **18** [6] (2022) 758–765.
- 26. G. Sharmila, C. Muthukumaran, K. Sandiya, S. Santhiya, R.S. Pradeep, N.M. Kumar, N. Suriyanarayanan, M. Thirumarimurugan, "Biosynthesis, characterization, and antibacterial activity of zinc oxide nanoparticles derived from Bauhinia tomentosa leaf extract," *J. Nanostructure Chem.*, **8** [3] (2018) 293–299.
- M. Carofiglio, M. Laurenti, V. Vighetto, L. Racca, S. Barui, N. Garino, R. Gerbaldo, F. Laviano, V. Cauda, "Iron-doped zno nanoparticles as multifunctional nanoplatforms for theranostics", *Nanomaterials*, **11** [10] (2021) 2628.