STM JOURNALS

International Journal of
Crystalline Materials

Volume 1, Issue 1, 2024
January—June
DOI (Journal): 10.37591/IJCM

https://journals.stmjournals.com/ijcm

Review IJCM

# Zn Doped CuO Nanoparticles for Increasing the Longevity of The Agricultural Products†

Vatsal Jhaveri [1], Indra Neel Pulidindi [2], Suresh Selvaraj [3], Prakash Vaithyanathan [4,*]

***Abstract***

*Nanotechnology is an enabling technology at the service of mankind. Indeed, enhancing the long-term storage of agricultural products (post-harvest during transportation and storage) in general and apples and tomatoes, in particular, is a challenge. Zn doped CuO is a proven nanomaterial with special ability to kill plant pathogens as well as a variety of microorganisms (bacteria, virus and fungus). Much of the review is focused on the landmark papers published by Professor Aharon Gedanken and his co-workers on the sonochemical synthesis of Zn doped CuO and its application for killing a variety of multi drug resistant (MDR) and extremely drug resistant (EDR) bacteria. Moreover Professor Gedanken's papers contained a wealth of information on the mechanism of killing of the bacteria by the potential antibacterial, antiviral, and antifungal material, namely, Zn doped CuO ($Cu0_{.88}Zn_{0.12}O$). Classical analytical techniques like the X-ray diffraction (XRD), differential scanning calorimetry (DSC), electron paramagnetic resonance (EPR) spectroscopy, high resolution scanning electron microscopy (HRSEM) and high resolution transmission electron microscopy (HRTEM) were judiciously used to drive the point home that sonochemical method is a potential technique to obtain smaller crystallites of $Cu_{0.88}Zn_{0.12}O$ and with high purity, provided, an appropriate mole ratio of the $Cu^{2+}$ and $Zn^{2+}$ precursors (3:1) is chosen. Anything other than this ratio, would lead to the presence of the individual phase of ZnO apart from the desired phase of Zn doped CuO. At this special mole ratio (3:1), as high as 12 %, (we repeat), no more than 12 % of the $Cu^{2+}$ can be replaced by $Zn^{2+}$ in the monoclinic crystal lattice of the CuO. DSC analysis revealed the presence of oxygen vacancies in the crystal lattice of $Cu_{0.88}Zn_{0.12}O$ as one of the factors contributing to the generation of the reactive oxygen species (ROS) with others being the co-existence of the amorphous phase of zinc doped CuO and crystalline phase of Zn doped CuO.*

**Keywords:** Zn doped CuO; nanoparticles; antimicrobial; antibacterial; antiviral; antifungal; longevity; agricultural products; vegetables.

***Author for Correspondence**
Prakash Vaithyanathan
E-mail: pvaithyanathan@gmail.com
[1]Graduate, School of Chemical Engineering, GSFC University, Vadodara, India.
[2]Scientific consultant, Jesus' Scientific Consultancy for Industrial and Academic Research (JSCIAR), Tharamani, India
[3] Associate Professor, Department of Science and Humanities, ACE Engineering College, Ghatkesar, Secunderabad, Telangana, India.
[4] Science Teacher and Innovator 50, L B Road, Adyar, Chennai, India
Received Date: August 12, 2024
Accepted Date: August 21, 2024
Published Date: September 12, 2024.

**Citation:** Vatsal Jhaveri, Indra Neel Pulidindi, Suresh Selvaraj, Prakash Vaithyanathan. Zn doped CuO nanoparticles for increasing the longevity of the agricultural products. International Journal of Crystalline Materials. 2024; 1(1): 14–29p.

## INTRODUCTION

Zn doped CuO is a magic material that generates large amount of reactive oxygen species (ROS) that kill the microorganisms causing corruption and thereby keep the surroundings clean. Owing to their unique antimicrobial, antibacterial, antiviral, and antifungal properties, this material coated on fabrics is recommended for applications in day-to-day life, including hospitals, the under-garments of the soldiers, and many more life-saving applications. However, its use in agricultural sector for improving the shelf-life of the agricultural products like vegetables, and staple food like rice and wheat is under-utilized and this aspect forms the central subject of the current review. In spite of the astounding properties and applications of Zn doped CuO, the material was under-exploited as evident

from web of science search with the keywords, "Zn doped CuO" that gives only 369 results (as on 11th September 2024). A few of the landmark papers related to the title were reviewed [1-18]. The chemists have miles to go in this direction to fully harvest the potential of this wonder material for various applications in day-to-day life. In the words of poet Robert Lee Frost, "The woods are lovely dark and deep; but I have promises to keep; And miles to go before I sleep; And miles to go before I sleep."

## SYNTHESIS AND CHARACTERIZATION OF Zn DOPED CuO

Gedanken et al., reported on the sonochemical synthesis and characterization of Zn doped CuO that exhibited outstanding antimicrobial activity with commercial utility [1-8]. Typical process comprises of the sonochemical irradiation (using a high intensity ultrasonic Ti horn, 20 kHz, 750 W at 40 % efficiency, Sonics and materials VCX600 Sonofier) of copper acetate monohydrate and zinc acetate dihydrate (in a mole ratio of 3:1) in water-ethanol solvent mixture (10:90 v/v ) for 1 h. The pH during the synthesis process of $Cu_{0.88}Zn_{0.12}O$ was maintained at 8 using $NH_4OH$ (~ 30 wt.%). Throughout the experiments an ultrasonic intensity of 45 $Wcm^{-2}$ is maintained. To maintain a reaction temperature of 30 °C, the reaction cell was kept in an ice bath, as sonication causes an increase in the temperature as high as 55 °C. A perceptible colour change of the reaction mixture from pale blue to dark blue is noticed upon the addition of $NH_4OH$ due to the formation of tetra aqua ammonium complex of $Cu^{2+}$, $[Cu(NH_3)_4]^{2+}$. It should be noted that the rate of formation of $[Cu(NH_3)_4]^{2+}$, is much faster than the formation of $[Zn(NH_3)_4]^{2+}$, though $Cu^{2+}$ and $Zn^{2+}$ precursors were simultaneously present in the reaction mixture under sonication. So it is surmised that in the beginning monoclinic CuO crystal lattice is formed into which $Zn^{2+}$ ions are doped for $Cu^{2+}$ resulting in the formation of Zn doped CuO crystal lattice. This is supported by the XRD result showed in Figure. 1, where in the hexagonal Wurtzite phase of ZnO is completely absent in the Zn doped CuO sample. Such phase pure material is much wanted for deducing the mechanism of the killing of microorganisms by the Zn doped CuO nanoparticles [2]. The resulting product (Zn doped CuO) upon sonication for 1 h was washed repeatedly with double distilled water and ethanol to get rid of the impurities like $NH_3$. The material was dried under vacuum overnight to obtain the $Cu_{0.88}Z_{0.12}O$. Similar synthetic strategy was followed for the production of nanoparticles of CuO and ZnO wherein copper acetate monohydrate and Zn acetate dihydrate were used as $Cu^{2+}$ and $Zn^{2+}$ precursors respectively. For comparison, the commercial CuO (bulk, product no. 1317-38-0) and ZnO (bulk, product no. Z-0875) were procured from Aldrich Chem Co, Israel. The structural features of the Zn doped CuO were characterized using XRD. For comparison, the XRD patterns of nanoparticles (NPs) of CuO, and ZnO as well as their bulk counterparts (CuO and ZnO commercial samples) were shown in Figure 1. The beauty of the sonochemical synthesis is that the formation of Zn doped CuO with highest phase purity is achieved. The XRD pattern of Zn doped CuO showed only the single phase of the monoclinic CuO as noticed from the 2θ values of the peaks which were indexed to the crystal planes typical of the monoclinic CuO (JSPDS file: 80-1916). No individual phase of ZnO was noticed in the XRD pattern from the Zn doped CuO sample. Moreover, compared to the XRD peaks of CuO bulk, the peaks of Zn doped CuO were slightly shifted to higher 2θ values and were broadened as well. The shift in the peak position in Zn doped CuO (a = 4.6829; b = 3.4201 and c = 5.1429) compared to CuO NPs (a = 4.6890; b = 3.4200; c = 5.1300) or the CuO bulk is attributed to the change in the lattice constant values (in Å). The observation of the changes in the lattice constant values as well as the reduction in the crystallite size is attributed to the doping of $Zn^{2+}$ (partial replacement of $Cu^{2+}$ by $Zn^{2+}$ in the CuO lattice). Under the sonochemical synthetic conditions, Zn doped CuO was the preferred structure compared to either CuO or ZnO and this is attributed to the choice of the specific mole ratio of $Cu^{2+}$ to $Zn^{2+}$ precursors, namely, 3 [2]. As expected the XRD pattern of CuO NPs and ZnO NPs produced by sonochemical irradiation showed the monoclinic (JSPDS file: 80-1916) and hexagonal crystal lattices (JSPDS file: 89-7102) respectively (Figure. 1).

High resolution transmission electron microscopy (HRTEM) analysis revealed the initial formation of rectangular shaped nanostructures of Zn doped CuO that aggregated into laminar thin film like nano-objects with irregular geometrical forms (Figure 2a). The particle size distribution studies using the image J software in the HRTEM with over 100 Zn doped CuO NPs resulted in a near normal distribution curve with a maximum at around 6 nm. Highly crystalline nature of the Zn doped CuO NPs with an inter-planar spacing (d spacing) value of 0.26 nm is observed (Figure 2b) and the result is in agreement

with the XRD studies (Figure. 1). In addition to the nanostructure, the surface morphology of the Zn doped CuO NPs were also studied using scanning electron microscopy (SEM). Nano aggregates of lamellae of Zn doped CuO with a smooth surface were seen (Figure. 2c). The uniform distribution of Cu (Figure 2d), Zn (Figure 2e) and oxygen (Figure 2f) were seen in the energy dispersive X-ray analysis (EDXA) of the selected region in the SEM image (Figure 2c). SEM with EDXA result is in agreement with the XRD result that revealed the doping of Zn in the CuO lattice (Figure. 1).

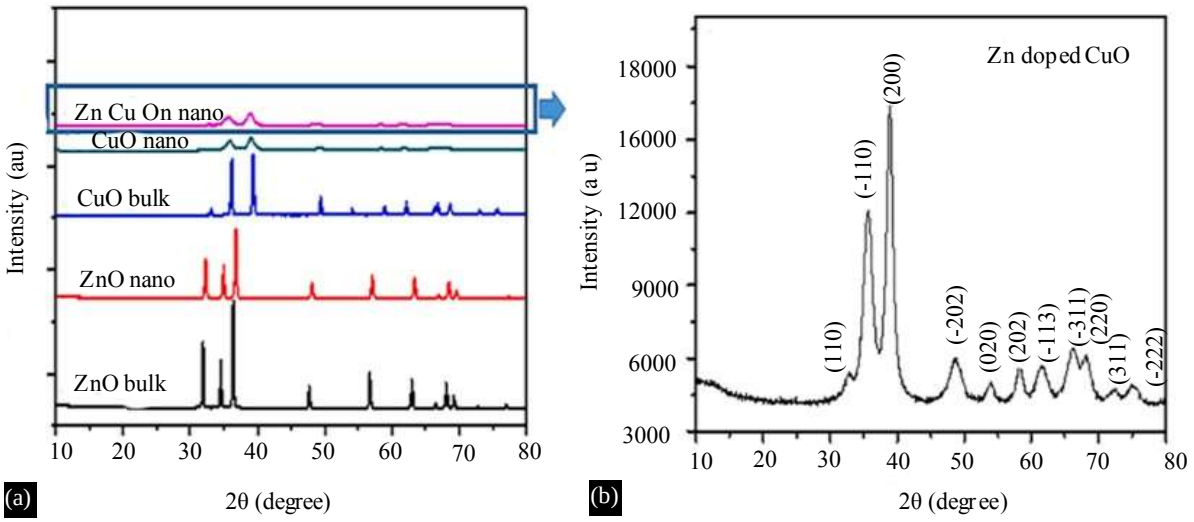
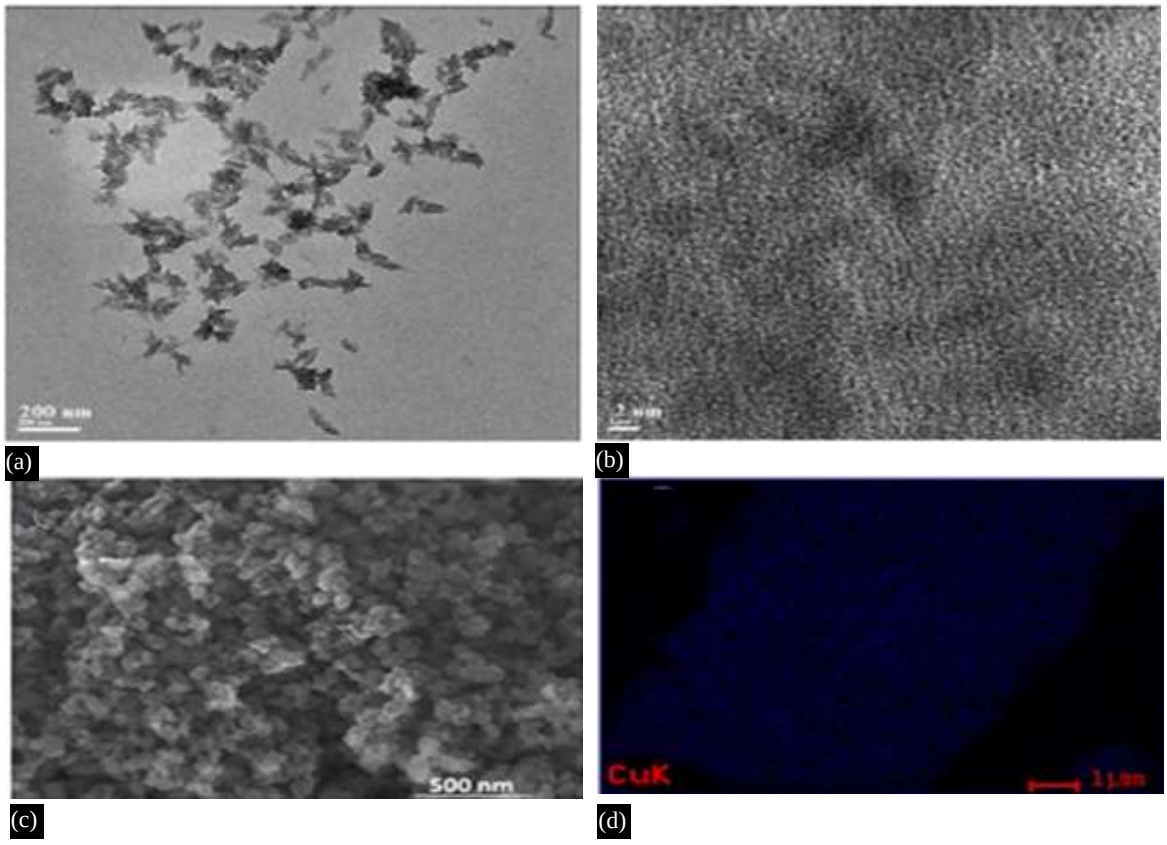
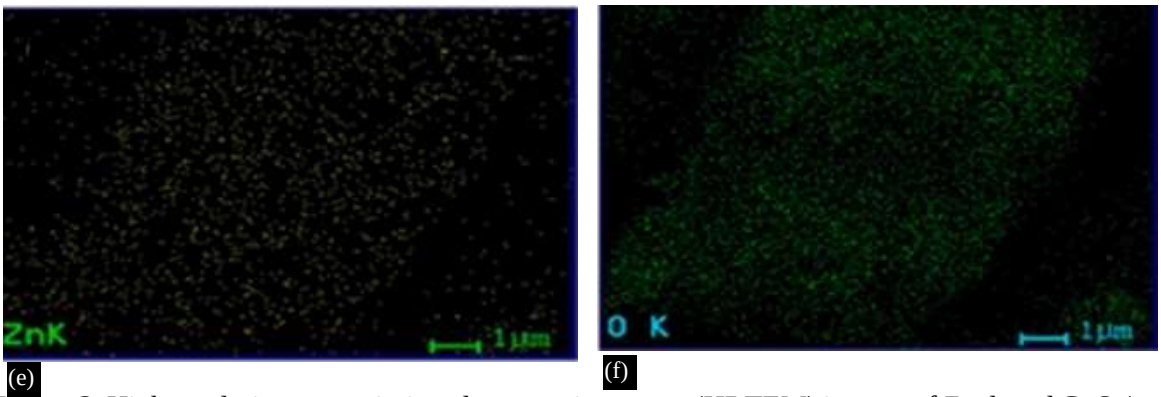



**Figure 1.** XRD pattern of (a) ZnO (bulk), ZnO (nano), CuO (bulk), CuO (nano) and Zn doped CuO nano and (b) enlarged version of the XRD pattern of Zn doped CuO nano [Reproduced from reference 3 with permission from Elsevier].






(e) (f)

**Figure 2.** High resolution transmission electron microscopy (HRTEM) images of Zn doped CuO (a and b); high resolution scanning electron microscopy (HRSEM) image (c) and the corresponding energy dispersive X-ray analysis (EDXA) images of Zn doped CuO (d, e and f) [Reproduced from reference 3 with permission from Elsevier].

Bruno et al., synthesized Zn doped CuO using microwave assisted precipitation technique using $CuCl_2$ $2H_2O$ and $ZnCl_2$ as precursors for $Cu^{2+}$ and $Zn^{2+}$ in alkaline medium (KOH). Structural features (lattice constants and interlayer spacing) were characterized using XRD and were compared with the JCPDS data. Increase in the band gap and reduction in crystallite size were noticed with increasing the amount of dopant [9].

**Application of Zn Doped CuO**

***Antimicrobial activity of Zn doped CuO***

Doping of lattice of CuO NPs with $Zn^{2+}$ enhanced the anti-biofilm formation compared to CuO NPs. Moreover, while CuO NPs as such did not exhibit any antibacterial activity against *Streptococcas mutans*, Zn doped CuO NPs exhibited antibacterial activity, though not complete eradication. The anti-biofilm and anti-bacterial activity of Zn doped CuO NPs against the bacterial strains, namely, *Streptococcas mutans*, was attributed to the increased number of structural defects and dislocations in the Zn doped CuO ($Cu_{0.88}Zn_{0.12}O$) leading to the formation of reactive oxygen species (ROS) that are responsible for either enhanced ability to prevent biofilm formation or to exhibit antibacterial activity against *Streptococcas mutans* compared to CuO NPs. The ROS cause the rupture of the cell membrane and give way to more of the nanoparticles of Zn doped CuO into the cell and kill the bacterial cells (*Streptococcas mutans*). The antibiofilm activity of the CuO NPs as well as the Zn doped CuO NPs was tested by coating them on artificial teeth using sonochemical irradiation [4]. The artificial acryl tooth were obtained from the school of dental medicine at the Tel Aviv university. The teeth uncoated with either CuO or Zn doped CuO nanoparticles supported massive biofilm formation. With CuO NPs and Zn doped CuO coating on the teeth, the biofilm formation was reduced by 70 % and 88 %. The enhanced performance of Zn doped CuO NPs for hindering the biofilm formation is attributed to the generation of higher amount of ROS compared to CuO NPs. The antibiofilm activity of the NP coated teeth was tested using the static biofilm assay wherein the artificial teeth were placed in a 24-well plate (Greiner Bio-one). Each well plate contained a 3 mL bacterial suspension of *Straptococcas mutans*. The teeth were incubated in brain-heart (BH) medium in the bacterial suspension for 24 h at 37 °C. Subsequently the teeth were washed twice with double distilled water to remove the weakly bound bacterial cells. The biofilm mass present on the teeth was stained with 1 % crystal violet for 15 minutes at room temperature. Subsequently, the unbound dye was removed by washing the stained teeth with double distilled water for five times. The crystal violet dye held to the bacterial mass on the teeth was then eluted with absolute ethanol for 15 minutes and the amount of the biofilm deposited on the uncoated, CuO NP coated and Zn doped CuO NP coated teeth was deduced by measuring the absorbance at $OD_{595}$ [4].

Apart from antibiofilm activity, Zn doped CuO NPs also showed antibacterial activity while CuO NPs showed no antibacterial activity against *Streptococcas mutans*. Zn doped CuO NPs reduced the

viability of *Streptococcas mutans* (from 1.7 x $10^8$ CFU/mL to 6.3 x $10^6$ CFU/mL) though the bacterial is not completely eradicated. Transmission electron microscopy was effectively used to study the mode of action of Zn doped CuO NPs in killing the bacteria and reducing their viability (Figure 3 c and f). In the case of either the control (untreated *Streptococcas mutans*) (Figure 3 a and d) or in the CuO NP treated *Streptococcas mutans* (Figure 3 b and e) no disorganization or injury to the cell membrane is observed. However, in the case of Zn doped CuO NPs treated *Streptococcas mutans* the localization of the NPs either on the cell surface or within the cell membrane is observed. As a result of the release of the ROS, the cleavage, rupture, and injury to the cell wall (membrane) were clearly observed. Moreover, some Zn doped CuO NPs remained bound to the cell membrane even after washing the bacteria prior to fixation for microscopy examination [4].

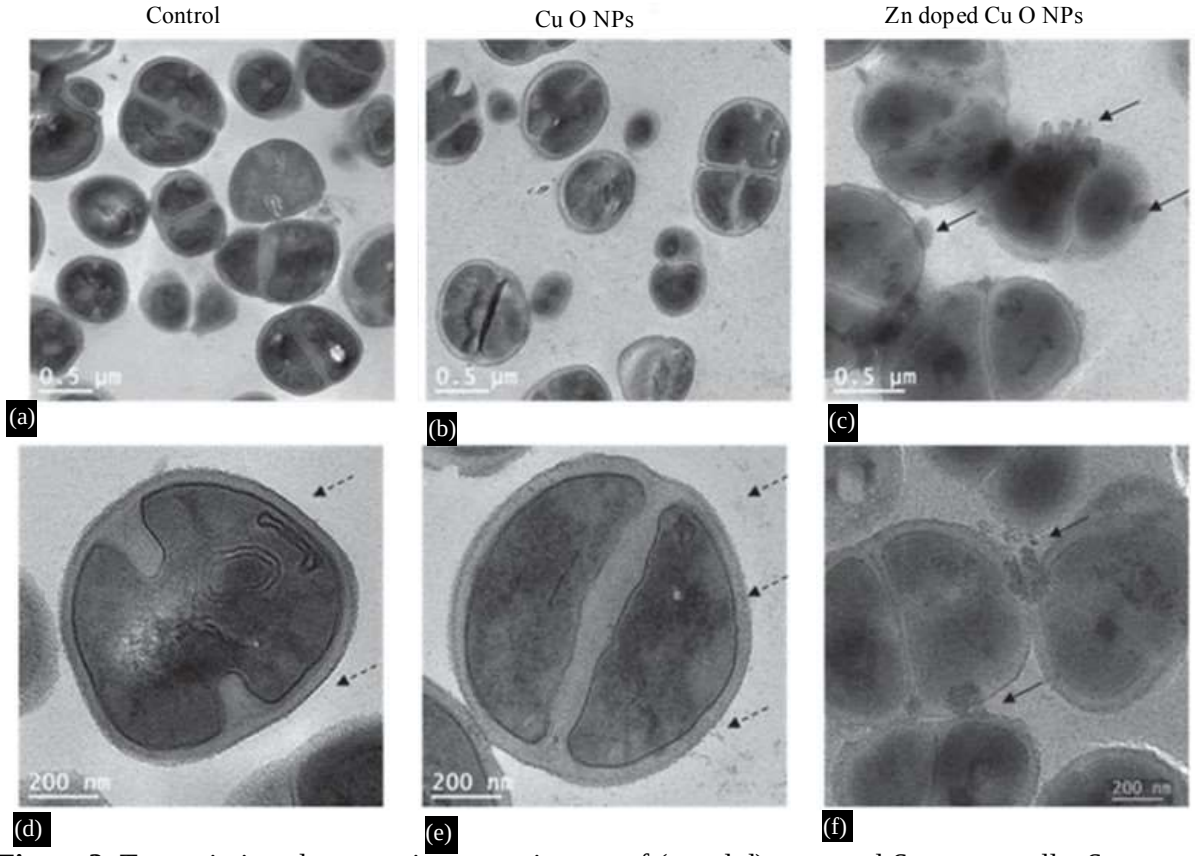



**Figure 3.** Transmission electron microscope images of (a and d) untreated *S. mutans* cells; *S. mutans* cells treated (b and e) with CuO nanoparticles, NPs (1 mg/mL), and (c and f) Zn doped CuO nanoparticles (NPs) (1 mg/mL). The broken arrows indicate normal cell membranes; the unbroken arrows indicate Zn doped CuO nanoparticles [Reproduced from 4 with permission from John Wiley and sons].

Gedanken et al., demonstrated the high antimicrobial potential of Zn doped CuO composited with polydopamine (PDA) even in the micro meter size particles (1-5 µm). In fact, the antimicrobial activity of the PAD-Zn doped CuO composite is much higher than the native Zn doped CuO nanoparticles. The enhanced activity of the PDA composite of Zn doped CuO micro meter sized particles is attributed to two modes of action: via the binding of the PDA polymer to the bacterial cell surface owing to the unique biocompatibility and adhesive nature of the PDA and through the release of the reactive oxygen species (ROS) from the Zn doped CuO encapsulated in the PDA. Upon binding to the surface of the membrane of the bacterial cells, the PDA will restrict the diffusion of the essential nutrients into the cell and also prevents the outflow of the wastes from the cell via the membrane causing cell death. The second mode of killing is by the release of the ROS from the Zn doped CuO contained in the PDA matrix. This double action of killing the bacteria is responsible for the enhanced activity of the PDA-

Zn doped CuO composite relative to the native Zn doped CuO nanoparticles. The antimicrobial activity was tested with four bacterial strains, namely, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300, multi drug resistant *Escherichia coli* (MDR *E. coli*) ATCC BAA-24512 (resistant to Imipenem and Ertapenem), *Escherichia coli* ATCC 25922 and *Staphilococcus aureus* ATCC 29213. The very poor killing ability towards the bacterial strains, namely, *Escherichia coli* ATCC 25922 and *Staphilococcus aureus* ATCC 29213, by the bare PDA without the Zn doped CuO is shown in the SEM images in Figure 4. (a and b). In contrast, when the PDA is composited with the Zn doped CuO resulting in the PDA encapsulated micro meter particles of Zn doped CuO, the killing effect is remarkably enhanced as seen from the rupture of the cell membrane and destruction of the integrity of the bacterial cell structure (Figure 4 c and d).

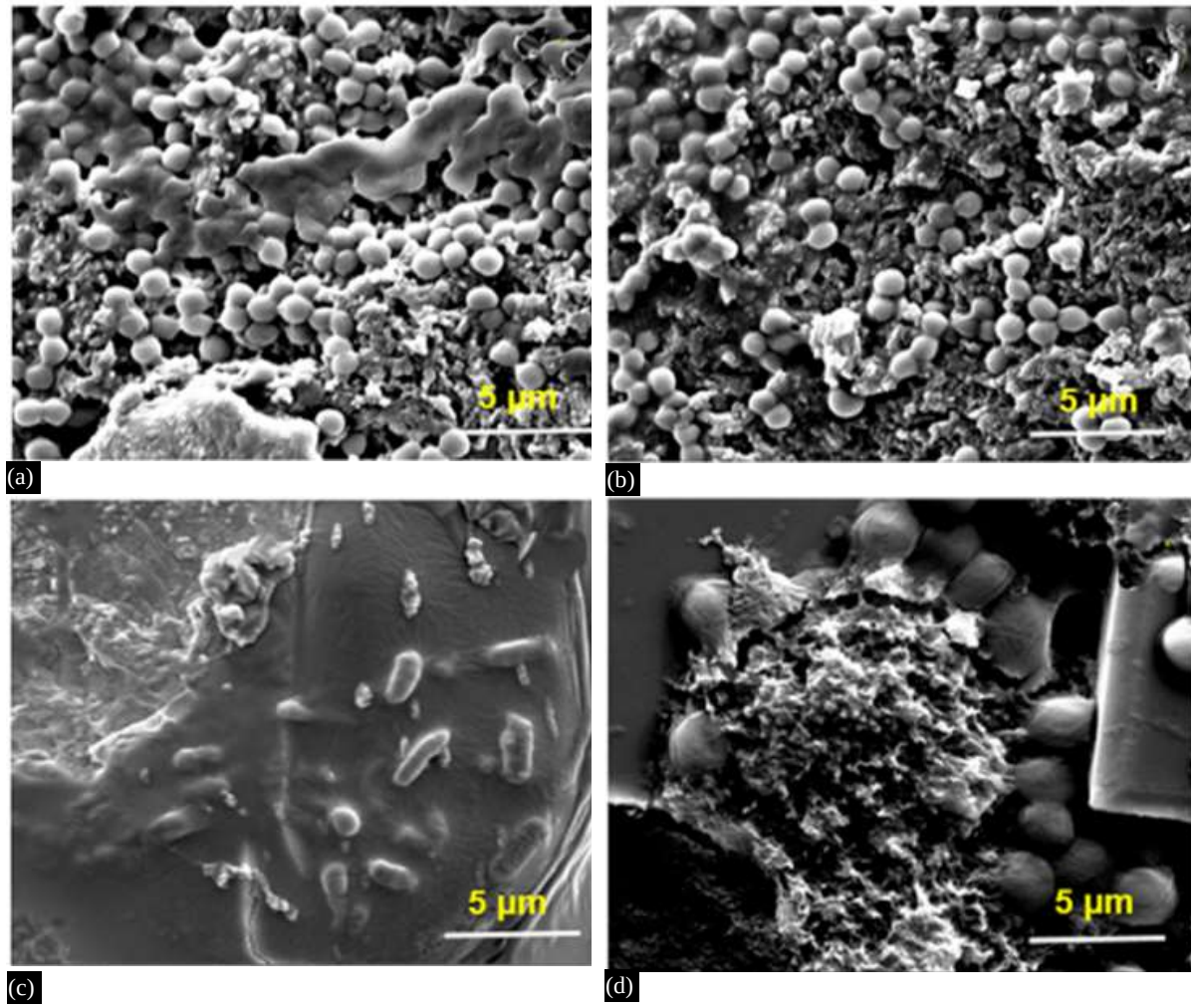



**Figure 4.** Antimicrobial effect of polydopamine (PDA) (a and b) and PDA-Zn doped CuO micro-meter particles (c and d) against the growth of (a) *Escherichia coli* ATCC 25922 and (b) *Staphilococcus aureus* ATCC 29213 and antimicrobial effect of PDA-Zn@CuO against the growth of (c) *Escherichia coli* ATCC 25922 and (d) *Staphilococcus aureus* [Reproduced from 5 with permission from the American Chemical Society].

However, all the published content, whether by the celebrated ACS or the Elsevier should not be considered as the gospel truth and must be taken with a pinch of salt and that is the very purpose of reviews on the published work of famous scientists like Professor Aharon Gedanken. One of the co-authors of this paper, INP, has the unique advantage of refereeing 324 papers, that enriched him with the special ability of scrutinizing the scientific literature. For instance, the SEM image in Figure 4 (a) labelled as *E coli*, in high probability, could have been mistaken for *S aureus*. The reason for doubt is

due to the representation of *E coli* in two morphologies, namely, spherical (in Figure 4 a) and rod shaped (in Figure 4 c). Irrespective of the labelling, the enhancement of antimicrobial activity of PDA-Zn doped CuO over bare PDA is beyond any doubt as evident from the SEM images in Figure 4 [5].

The nature and amount of the reactive oxygen species (ROS, the short-lived free radicals) were examined using the electron paramagnetic resonance (EPR) spectroscopy with the famous spin-trap method. The famous 5, 5-dimethyl pyrroline N oxide (DMPO) was used as the spin trap that forms an adduct with either hydroxyl ($OH^{\cdot}$) or superoxide anion ($O_2^{-\cdot}$) radicals. The intensity of the EPR signal is a measure of the amount of ROS ($OH^{\cdot}$, $O_2^{-\cdot}$) generated by the material under test and is a reflection of the antimicrobial ability. The trapping of either the hydroxyl ($OH^{\cdot}$) or superoxide anion ($O_2^{-\cdot}$) radicals results in the formation of the DMPO-OH as the final adduct and appears as a quartet with the relative intensity ratio of 1:3:3:1. From a comparison of the intensity of the EPR signals of the samples, namely, PDA, PDA-Zn doped CuO and Zn doped CuO, it is evident that the ROS generation in PDA-Zn doped CuO is 3.5 fold and 2 fold higher compared to PDA and Zn doped CuO respectively [Figure 5]. This explains the enhanced antimicrobial activity of PDA-Zn doped CuO [5].

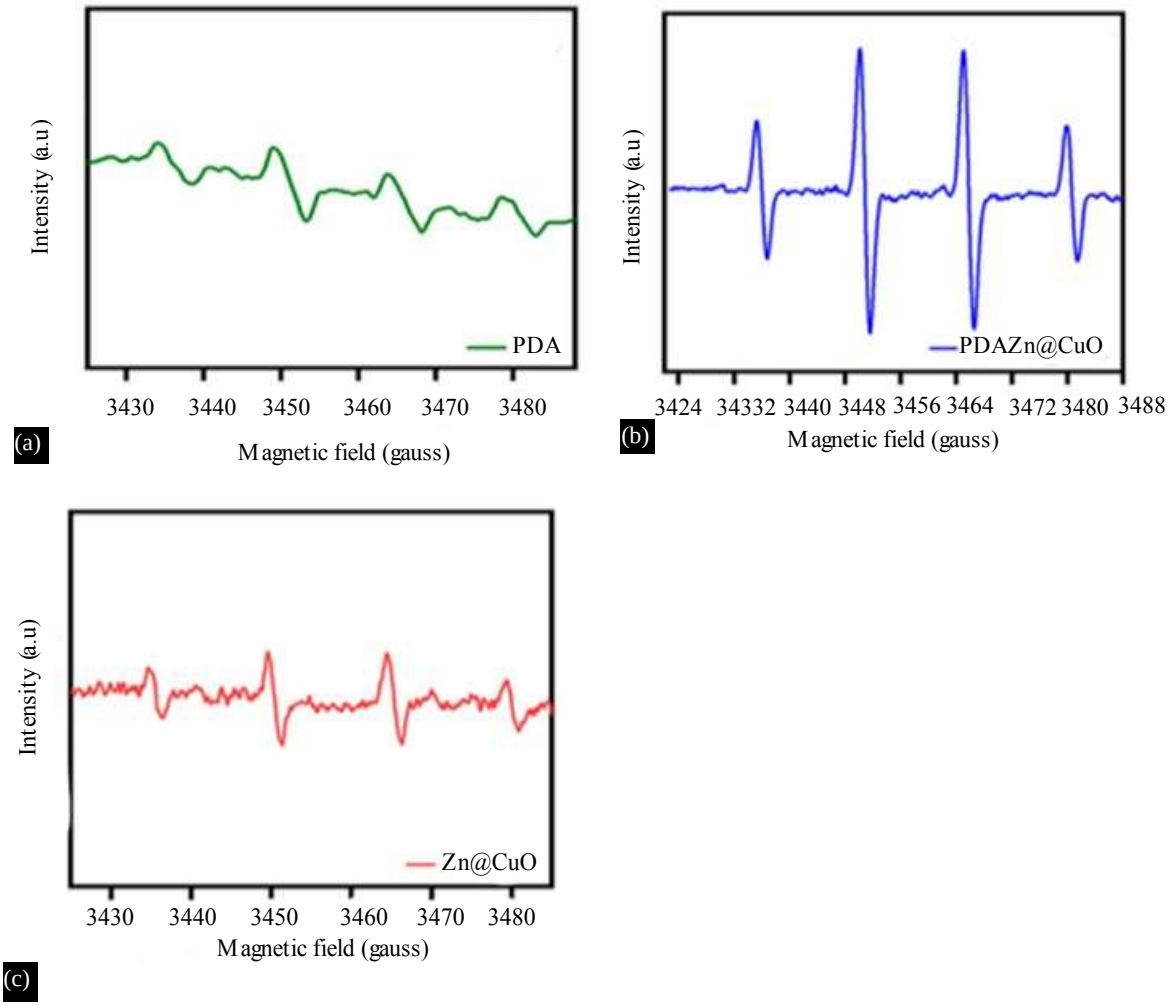



**Figure 5.** Electron paramagnetic resonance (EPR) spectra of (a) PDA, (b) PDA-Zn@CuO, and (c) Zn@CuO [Reproduced from 5 with permission from the American Chemical Society].

Catheter related urinary track infections due to bacterial biofilm growth were suppressed by the use of Zn doped CuO coatings on the catheters. The bacterial species responsible for the biofilm formation on catheters are *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. Suppression of biofilm formation is an evidence

for the antibacterial activity of Zn doped CuO ($Cu_{0.88}Zn_{0.12}O$) against the afore mentioned multiple bacterial strains [6]. Moreover, the catheters coated with Zn doped CuO (using sonochemical irradiation) are not cytotoxic and are biocompatible.

Gedanken et al., developed ointments based on Zn doped CuO for wound healing application that destroyed the biofilm formation. The performance of the ointment was better than the commercial gentamicin [7]. The antibacterial activity was tested with *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228 (*S. aureus* and *S. epidermidis*) and gram-negative *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*).

**Antibacterial Activity of Zn Doped CuO**

In their landmark paper, Professor Aharon Gedanken and co-workers in the year 2013, reporting the four orders of magnitude enhancement of the antibacterial activity of Zn doped CuO NPs compared to CuO NPs, they cited the founding contribution of Prabhakaran and Boothroyd [19, 20] reporting the growth of single crystal of Zn doped CuO by floating zone technique. This is analogous to Professor C S Lewis, an expert in linguistics from the Magdalen college, Oxford university referring to the work of a layman John Bunyan, for his commendable work, namely, the Pilgrim's progress, astonishing that how such a historical masterpiece originated from a layman. In fact, the work of Prabhakaran and coworkers as well as the report from Brozi's group, on the synthesis, characterization of magnetic properties of Zn doped CuO based on high temperature superconductors, opened a new avenue leading to unfolding of the application of the material in agriculture killing plant pestilents [17, 19, 20]. This is one of the classical examples of the significance of interdisciplinary and cross-disciplinary research leading to break-throughs in Science.

The fundamental work of Professor Aharon Gedanken's group on the use of Zn doped CuO either in aqueous suspensions or in composite form (coated on various materials including cotton fabrics) in killing the bacteria (Gram negative *Escherichia coli*, Gram positive *Staphylococcus aureus*, both regular and multidrug resistant) deals with the mechanism of action of killing the bacteria. Results from differential scanning calorimetry (DSC) as well as from the electron paramagnetic resonance (EPR) spectroscopy were judiciously doctored to hammer upon the head that the antibacterial activity of Zn doped CuO NPs is mainly due to the defective structure (oxygen vacancies, structural defects, dislocations, disorganization and co-existance of the amorphous phase of Zn-doped CuO with the crystalline phase of Zn-doped CuO) to be the origin of the reactive oxygen species (ROS) that cause injury and rapture to the bacterial cell membrane and proliferate into the cell and effectively eradicate the bacteria. This explains the four orders of magnitude enhancement of the antibacterial activity of Zn doped CuO NPs compared to CuO NPs. The DSC plots of Zn doped CuO were recorded both under oxygen deficient ($N_2$ environment) as well as under the oxygen rich (air) atmosphere. The only difference in the DSC traces of Zn doped CuO recorded in $N_2$ environment and in air environment is the appearance of a new sharp and intense exotherm at 420 °C in the air environment. The origin of this new exotherm in DSC trace of the Zn doped CuO recorded in air environment is attributed to the saturation or filling up of the vacancies (defects) present in the lattice of Zn doped CuO with oxygen [2]. Moreover, this sharp intense exothermic peak at 420 °C is irreversible. This means that the peak does not appear in the second heating cycle in the DSC, as all the vacancies (defects) are saturated and filled with oxygen in the first heating cycle. Thus such structural defects (oxygen vacancies) in the Zn-doped CuO are the source (in the latter part it will be disclosed that in fact oxygen vacancies are a minor source of ROS generation with the major fraction of ROS being contributed by the defects and dislocations in the structure of Zn doped CuO, which could not be deducted from DSC analysis alone) for the generation of the ROS as will be proved by the EPR studies discussed in the subsequent paragraphs. Apart from the characteristic sharp intense exothermic peak at 420 °C in the DSC plot of the Zn doped CuO in the air environment, irrespective of the environment (whether oxygen deficient or oxygen rich), three more peaks appeared in the temperature range of 100-250 °C. However, these three peaks centered around 100, 130-165 and 240 °C were broad in contrast to the unique exothermic peak at 420 °C. The broad endothermic peak at 100 °C was attributed to the evaporation of water adsorbed

on the surface of the Zn doped CuO. The two other broad exothermic peaks centered around 130-165 °C and at 240 °C were attributed to the reordering of the structural defects in the Zn-doped CuO due to the thermal induced agitations. Moreover, in particular, the broad exotherm at 240 °C in the DSC trace of Zn doped CuO could also be, in high probability, due to the co-existence of the amorphous phase of Zn-doped CuO and the crystalline Zn-doped CuO phase. It should be noted that all the three peaks in the DSC plot in the temperature range of 100-250 °C are irreversible as they did not reappear in the second heating cycle of the DSC analysis. Thus DSC analysis holds a wealth of information on the unique, atmosphere sensitive, structural features of Zn doped CuO bearing significance on the astounding applications of Zn doped CuO.

The central theme of this review article is the characterization of the reactive oxygen radical species generated by Zn doped CuO NPs that are responsible for the killing and eradication of the rigid bacterial, fungal and viral species that are resistant to pesticides and antibiotics. As these radicals have very short life time much lower than the detection time of the EPR spectroscopy, it is a famous practice to use spin traps. These spin traps, as the name suggest, will capture the radicals generated and will form an adduct and the EPR signal intensity of the adduct is a measure of the amount of ROS generated. Common spin traps well documented in the literature include 5, 5" dimethyl-pyrroline-N oxide (DMPO), 5-diethoxy phosphoryl-5-methyl-1-pyrroline-N-oxide (DEPMPO), 2, 2, 6, 6, tetra methyl piperidine 1-oxyl (TEMPO), 2, 2, 6, 6, tetra methyl piperidine, (TEMP), N-tert-butyl-α-phenyl nitrone or phenyl N-tert-butyl nitrone (PBN), 2-methyl-2-nitroso propane (MNP) depending on the specific radical under test [21-22].

Professor Aharon Gedanken and coworkers made an in depth study on the nature and dynamics of the ROS generated by Zn doped CuO NPs using EPR analysis, including spin traps (DMPO, TEMO, TEMPO) as well as hydroxyl ($OH^{\cdot}$) radical scavenger, namely, dimethyl sulphoxide (DMSO). The EPR signals emerging from the three oxides, namely, Zn doped CuO, CuO and ZnO NPs were shown in Figure 6 (a). As the ROS from the metal oxides is short lived, the intensity of the EPR signals shown in Figure 6 (a) should be taken with a pinch of salt as far as the quantification is concerned, unless and otherwise, proper spin traps are used. However, the relative intensity of the EPR signals from Zn doped CuO, CuO and ZnO NPs is a sure indication of the relative amount of ROS from each of the afore mentioned metal oxides. The order of intensity of the EPR signal is : Zn doped CuO > CuO > ZnO, meaning that the amount of ROS formed from Zn doped CuO is higher. To have a more systematic knowledge on the concentration of the ROS from the metal oxides, namely, Zn doped CuO, CuO and ZnO, the spin trap DMPO was used that forms an adduct selectively with hydroxyl ($OH^{\cdot}$) radicals. The DMPO-OH adduct appears as a quartet with a relative intensity ratio of 1:3:3:1 as shown in Figure 6 (c). Not only the hyperfine splitting pattern but also the parameter, namely, the hyperfine splitting constant (hfsc) which is the peak separation between any two peaks in the quartet is specific to the DMPO-free radical adduct. The hfsc value in the current instance is 14.9 G typical of the DMPO-OH adduct. It was found that hydroxyl radicals ($OH^{\cdot}$) comprises of nearly 40 % of the ROS generated from the Zn doped CuO in which a maximum of 12 % of the $Cu^{2+}$ were replaced with $Zn^{2+}$ ($Cu_{0.88}Zn_{0.12}O$). More than 12 % of $Zn^{2+}$ cannot be accommodated in the CuO lattice and any attempt to dope greater than 12 % of $Zn^{2+}$ leads to appearance of the individual phase of ZnO. Such 12 % dopant concentration yielded optimal antibacterial activity with the generation of maximum ROS [2]. A comparison of the relative intensity of the DMPO-ROS adduct from the three metal oxides namely, Zn doped CuO, CuO and ZnO is shown in Figure 6 (b). The intensity of the EPR signal of DMSO-ROS adduct is the highest from the Zn doped CuO sample that showed 4 orders of magnitude higher antibacterial activity than that of CuO. Now the next important question that need to be answered is that if the ROS comprises of only the hydroxyl ($OH^{\cdot}$) radicals or does the ROS comprise of superoxide anion ($O_2^{-\cdot}$) radicals as well. This question was answered by the addition of DMSO to the DMPO-ROS adduct, as DMSO selectively scavenges the hydroxyl ($OH^{\cdot}$) radicals and treats the superoxide anion ($O_2^{-\cdot}$) radicals as untouchable. As can be seen from the EPR spectra in Figure 6 (c), the addition of DMSO though reduced the signal intensity for the DMPO-ROS adduct, the EPR response is not completely eliminated. This clearly

signals that the DMPO-ROS adduct is not solely due to the DMPO-OH adduct but its due to both DMPO-OH and DMPO-$O_2^{-}\cdot$ adducts. Moreover, it should be in the good books of the readers that the simultaneous presence of the spin trap (DMPO) and the hydroxyl ($OH\cdot$) radical scavenger, namely, DMSO, in the EPR analyte, namely, the Zn doped CuO, results in the formation of DMPO-$CH_3$ that would yield characteristic EPR signals marked by the arrows in Figure. 6 (c).

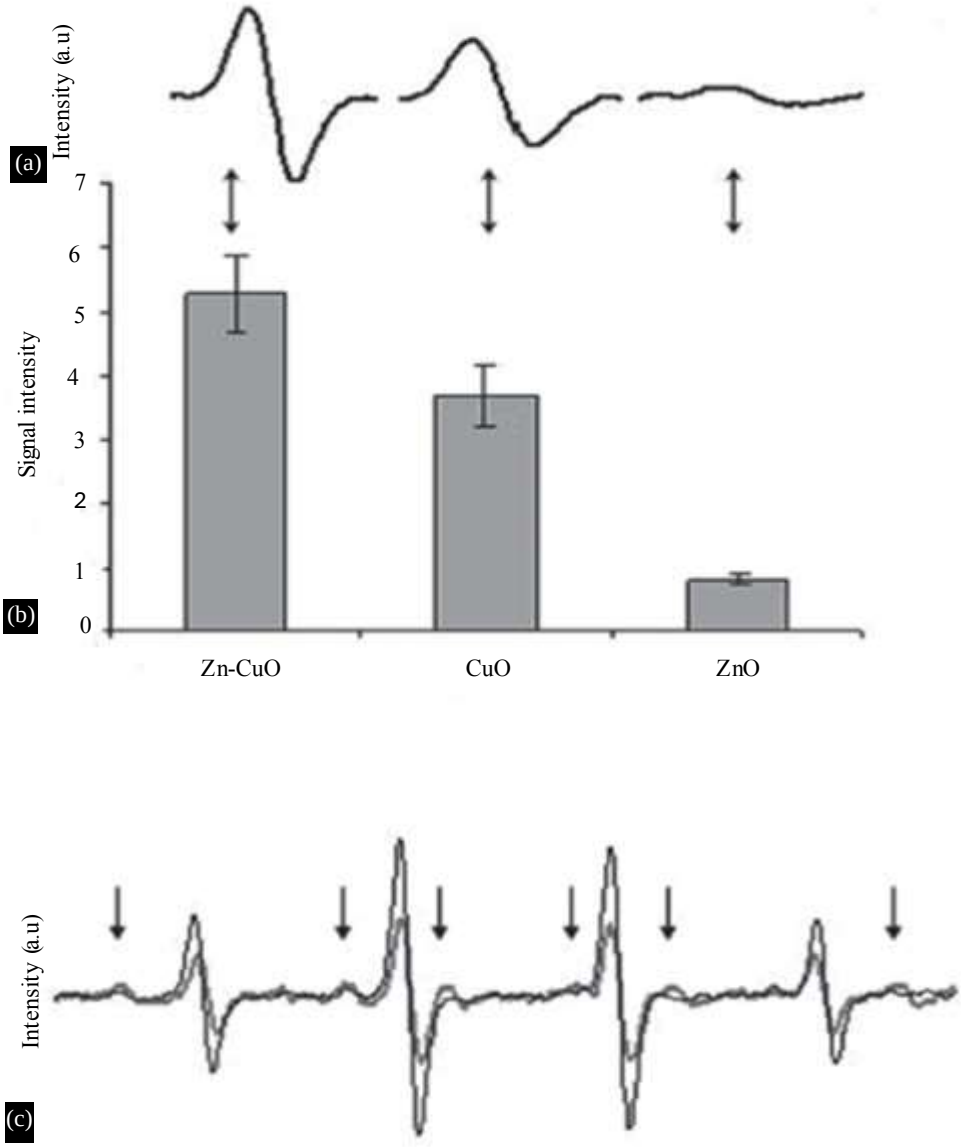



**Figure 6.** (a) ROS generation by Zn dopedCuO, CuO and ZnO NPs; (b) Integrated area of DMPO spin adduct generated from Zn–CuO, CuO and ZnO NPs; (c) ROS formation in a suspension of Zn–CuO nanoparticles (black line) with DMSO (bold gray line) [Reproduced from ref 2 with permission from John Wiley and Sons].

Characterization of the ROS in a given analyte by EPR is a challenge as the possible ROS that could be formed are many depending on the metal oxide or doped system in question. Now the next question to be asked is, whether any singlet oxygen ($O_2^1$) radicals are formed from Zn doped CuO. This question can be answered by using the unique spin trap, namely, 2, 2, 6, 6-tetra methyl piperidine (TEMP) that selectively reacts with the singlet oxygen radicals ($O_2^1$) and forms the stable free radical, namely, 2, 2, 6, 6-tetra methyl-1-piperidinyl-oxyl (TEMPO) which appears with the finger print EPR signals (three

signals of equal intensity). EPR analysis of Zn doped CuO is carried out in the presence of two spin traps, namely, DMPO and TEMP as shown in Figure 7. As expected, with TEMP the very trace quantities of the singlet oxygen radicals ($O_2^1$) generated from the Zn doped CuO formed TEMPO as shown by the weak signals marked by arrows in Figure 7. The weak triplet marked by arrows (Figure 7) is indicative of the formation of the singlet oxygen radicals ($O_2^1$) species in addition to the major fraction of the hydroxyl ($OH^{\cdot}$) radicals and the superoxide anion ($O_2^{-\cdot}$) radicals.

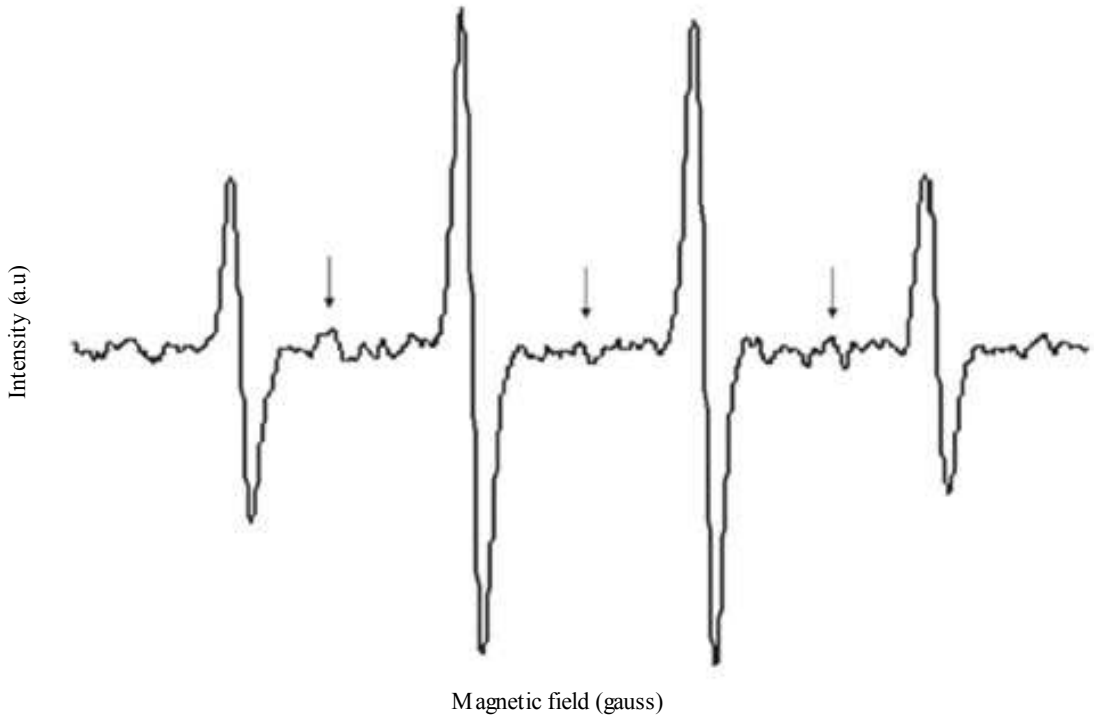



**Figure 7.** Singlet oxygen production in suspensions of Zn–CuO in the presence of 2, 2, 6, 6-tetra methyl piperidine (TEMP) and 5, 5-dimethyl pyrroline N oxide, DMPO (the triplet of, 2, 2, 6, 6-tetra methyl-1-piperidinyl-oxyl, TEMPO signal formed by the reaction of TEMP with singlet oxygen is marked with arrows). [Reproduced from ref 2 with permission from John Wiley and Sons].

The dynamics of the ROS was further probed as a function of environment as well as temperature of heating of Zn doped CuO as shown in Figure 8. The EPR signal from the DMPO-ROS adduct from Zn doped CuO was shown in Figure 8 (a). Upon heat treatment as 300 °C in air, the characteristic DMPO-ROS EPR signal intensity is neither diminished nor eliminated. This imply that the crystal structure with its defects and dislocation is unaltered upon heating the sample in air at 300 °C (Figure 8 b). However, when the sample is heat treated in air at 550 °C, there is a drastic reduction in the EPR signal intensity of the DMPO-ROS adduct from the Zn doped CuO. The reduction in the signal intensity is attributed to the filling of the vacancies with oxygen and that the oxygen vacancies are only a minor factor contributing to the ROS generation. This deduction is further strengthened by the complete absence of the EPR signal from the Zn doped CuO sample heat treated at 550 °C in $N_2$ (Figure 8 d). Under these conditions, the reorganization of the lattice occurs leading to the elimination of crystal defects. Moreover, this material did not show any antibacterial activity indicating that the crystal defects, ROS generation and antibacterial activity of Zn doped CuO are directly related. For comparison the EPR signal arising from the DMPO control is shown in Figure 8 (e).

With its mouldability, Zn doped CuO is indeed a magic material that can kill a variety of bacteria. Gedanken et al., formed the hybrid of Zn doped CuO with nanographene oxide (NGO) and polyethylene glycol (PEG). The NGO modified, pegylated Zn doped CuO (NGO-PEG– ZnCuO) effectively adhered

to the cell membrane of the bacteria *S. aureus* (Figure 9 d) as well as *E. coli* (Figure 9 h) and caused injury to the cell wall and entered into the cell and caused the complete structural disintegration of the bacterial cells in 50 minutes. The change in the morphology of *S. aureus* in the absence of NGO-PEG–ZnCuO (Figure 9 a) and in the presence of NGO-PEG– ZnCuO (Figure 9 d) is distinctly different. Same in the case with *E. coli* (Figure 9 e and h). This example only demonstrates that irrespective of the complexing medium or the adhering surface, the Zn doped CuO NPs will retain their structural defects responsible for the release of the ROS (hydroxyl, super oxide anion and singlet oxygen radicals) and proliferate into the cell via cause rupture of the cell member and kill the bacteria.

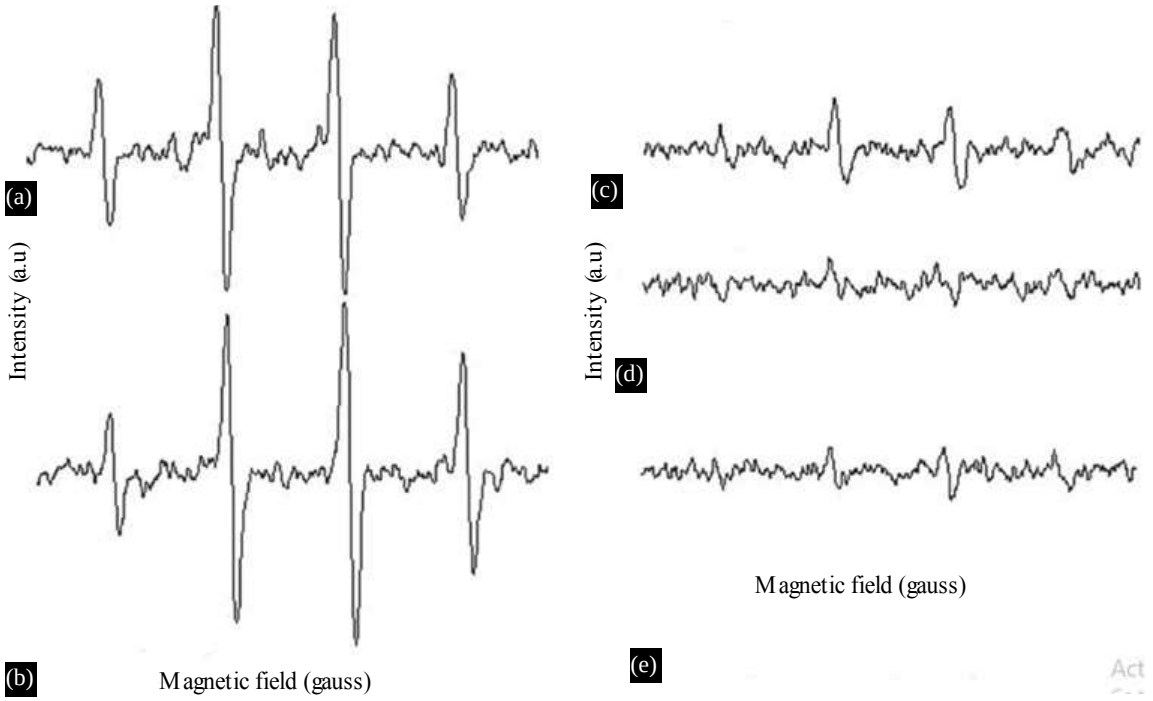



**Figure 8.** (a) Zn–CuO; (b) Zn–CuO after heating at 300 °C under air; (c) Zn–CuO after heating at 550 °C under air; (d) Zn–CuO after heating at 550 °C under $N_2$; (e) DMPO control. [Reproduced from ref 2 with permission from John Wiley and Sons].

Zn doped CuO ($Zn_xCu_{1-x}O$) exhibited antibacterial activity (*Camphylobacter coli*, *C coli*) with minimum inhibitory concentration (MIC) [10]. Bhosale et al., recommended the use of Zn doped CuO in food industry owing the ability of the material to kill bacteria by the release of reactive oxygen species (ROS) [11]. Guru Prasad et al., reported on the antibacterial activity of Zn doped CuO against the bacterial strains, namely, *Acinetobacter baumanni* and *Klebsiella pneumonia* [12]. Owing to the commercial scale utility of Zn doped CuO nanoparticles for antibacterial applications, intense activity is going on in this direction [13-16].

**Antiviral Activity of Zn Doped Cuo:** Strangely, scientific literature is silent on the antiviral activity of Zn doped CuO deeding a research into the problem.

***Antifungal activity of Zn doped CuO***

Promod Kumar et al., reported on the antifungal activity of the Zn doped CuO against the well-known and notorious plant pathogens, namely, *Alternaria alternate* CGJM3078 and *Alternaria alternate* CGJM3006. The filamentous fungus, namely, *Alternaria alternate*, is known to spoil fruits and vegetables as it is a notorious plant pathogen leading to extensive post harvest loss. Fruits and vegetables like mandarins, grapes, tangerines, strawberries and tomatoes are rotten due to this pathogen and their shelf-life and storability is drastically reduced. Use of Zn doped nanoparticles protect the agricultural produce and prevents the post-harvest losses and ensures food storage, security and sustainability in agriculture sector and in food industry. The antifungal activity of the Zn dopes CuO

was attributed to the defective structure that facilitates the generation of the reactive oxygen species that are detrimental to the plant pathogens that act as predators for the agricultural products. The ROS generated will destroy fungal cell membrane and finally cause the cell death there by protecting the agricultural products. Copper nitrate trihydrate and zinc nitrate pentahydrated were used as precursors for $Cu^{2+}$ and $Zn^{2+}$ and urea is used as a fuel for the combustion synthesis of Zn doped CuO. The resulting material exhibited, in addition to the major phase of monoclinic CuO, the individual and separate Wurtzite phase of ZnO. The crystallite size of Zn doped CuO varied from 32-44 nm depending on the amount of the dopant. With high mole ratios of Cu/Zn precursors, the $Zn^{2+}$ (ionic radii, 0.074 nm) replaced the $Cu^{2+}$ (ionic radii, 0.072 nm) in the lattice of CuO. However, upon increasing the amount of the Zn precursor (with a reduction in the Cu/Zn mole ratio), the $Zn^{2+}$ occupied the interstitial sites resulting in the formation of the secondary phase, namely, the Wurtzite ZnO phase. Thus the mole ratio of Cu and Zn precursors is an important criteria for obtaining the material in high phase purity [17]. Ideally, a 3:1 mole ratio of Cu to Zn is recommended [2].

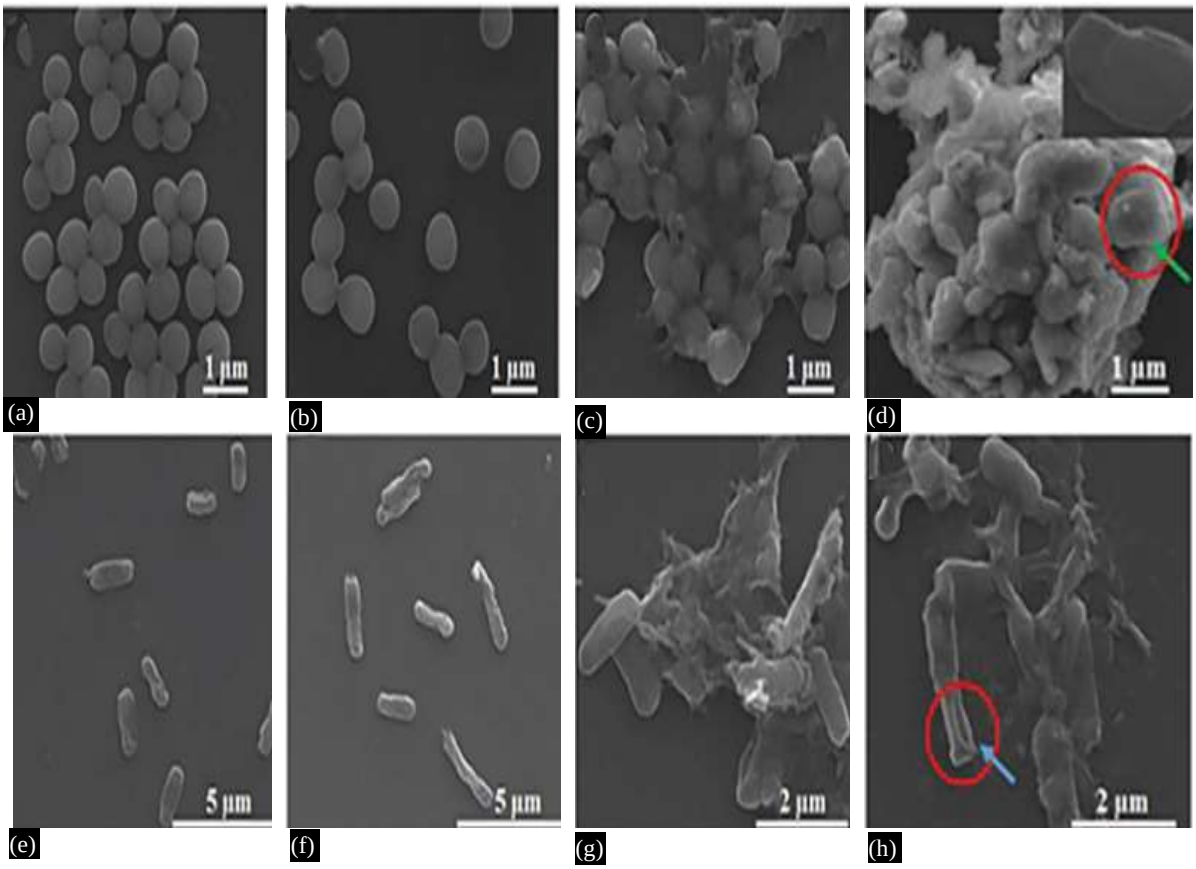



**Figure 9.** Environmental SEM images of bacteria with different solutions at different time points: *S. aureus*: (a) only water (t = 0 min), (b) only water (t = 50 min), (c) NGOPEG–ZnCuO complex (t = 0 min), (d) NGO-PEG–ZnCuO complex (t = 50 min); *E. coli*: (e) only water (t = 0 min), (f) only water (t = 50 min), (g) NGO-PEG– ZnCuO complex (t = 0 min), and (h) NGO-PEG–ZnCuO complex (t = 50 min) [Reproduced from reference 8 with permission from RSC].

## IMPROVING THE LONGEVITY OF AGRICULTURAL PRODUCTS WITH Zn DOPED CuO

Now that the antibacterial, and antifungal activity of the Zn doped CuO along with its mechanism of killing the bacteria is clear, the emphasis should be on using this magic nanomaterial for improving the shelf-life and longevity of the agricultural products. This will lessen the burden on the farmers by preventing easy rottening of the agricultural products, namely fruits and vegetables. The use of this potential ($Cu_{0.88}Zn_{0.12}O$) antifungal, antiviral, and antibacterial material that can protect the post-harvest agricultural products, namely, vegetables and fruits in general and plants in particular should be taken

up on a war-footing. Indeed, it is a great problem that much of the post-harvest agriculture produce, (especially) vegetables and fruits, is rotten due to fungal, viral and bacterial microorganisms. This incurs huge loss not only to the individual farmers but also to the national gross domestic product (GDP) as India is primarily an agricultural economy. Fortunately, nanotechnology based nanomaterials are all-pervading and offers solution to the problem of short-life of the post-harvest agricultural products. The long-life of the agricultural products can be ensured all through the transportation and storage by the use of special and specific nanomaterial offered in this review. The Zn doped copper oxide nanoparticles deposited on high surface area microporous activated carbon material is a ready to use material that can be sprinkled on the agricultural products or coated on fabrics used for packaging the cartons. The Zn doped CuO ($Cu_{0.88}Zn_{0.12}O$) has the potential to kill fungal, viral, and bacterial microorganisms. In the supported form, when they are deposited on high surface area activated carbon materials, the amount of the active component is minimized and these metal oxide nanoparticles could be used more effectively. Moreover their handling in the powdered state will be user friendly, like the conventional fertilizers, namely, urea. These nanoparticle deposited activated carbon powders can be sprinkled onto the tomatoes, for example, that are packed in cartons. As the tomatoes are now in the protective environment of the antifungal, antiviral, and antibacterial Zn doped CuO deposited on activated carbon materials, the microbes that usually cause the rottening of vegetables will not approach the vegetables. As a result, the life-time of the tomatoes will be prolonged during the transportation, and storage. The question still remains. Why not cold storage? These days, cold storage techniques are more dreadful. Most of the dreadful and lathel viruses and bacterial will proliferate under cold conditions. Even refrigeration could not be considered healthy storage method for prolonging the life-time of tomatoes and apples (representative examples of agricultural products). Cold storage including refrigeration has become a home for permafrost. Thus using the solutions offered by nanotechnology and nanomaterials should be the way ahead. Moreover, the nanomaterials, namely, Zn doped CuO have been proven to be antiviral (though not disclosed in open literature), antibacterial and antifungal by the Israeli Scientist Professor Aharon Gedanken. Bringing $Cu_{0.88}Zn_{0.12}O$ in supported form on activated carbon materials by sonochemical coating enhance their ease of handling and also minimizes their utilization and make this material sustainable. This way the technology offered here will serve the Indian farmers to safe-guard their agricultural produce against the killer microbes (fungus, virus and bacteria). Typical synthetic procedure for preparing Zn doped CuO nanoparticles and coating them in situ on high surface area activated carbon material involves taking copper acetate monohydrate and zinc acetate dehydrate salts in 3:1 mole ratio in a water-ethanol (1:9 v/v ratio) and subjecting the contents to ultrasound irradiation (20 kHz) for 1 h by maintaining the pH at 8 and subsequently adding requisite amount of activated carbon and continuing the sonication for 30 more minutes. This would ensure both the formation of Zn doped CuO nanoparticles (a maximum 12 % of $Cu^{2+}$ can be replaced with $Zn^{2+}$ in the CuO lattice) as well as their effective supporting on the carbon material. The contents can then be filtered and the residue is dried and subsequently used as a preservative in powder form for post-harvest agricultural produce in general and apples and tomatoes in particular all through the course of transportation and storage. Implementation of these suggestions and new ideas would prevent the losses incurred by the rottening of vegetables and fruits during transportation and storage.

Gedanken et al., conducted the toxicological studies on the Zn doped CuO material using Frog Embyo Teratogenesis Assay-Xenopus test and found that the nanomaterial is non-toxic [18]. As a result, the use of the nanomaterial ($Cu_{0.88}Zn_{0.12}O$) is recommended as a tool to be used by the farmers in eradicating the pathogens that spoil their agricultural products post-harvest. The ideal way would be using activated carbon powers coated with nanoparticles of Zn doped CuO. However, other ways can also be thought of depending on the application.

## CONCLUSION

Zn doped CuO nanoparticles (NPs), prepared by sonochemical irradiation of $Cu^{2+}$ and $Zn^{2+}$ precursors in 3:1 mole ratio, exhibited four orders of magnitude higher antibacterial activity than CuO NPs prepared in an analogous manner. As high as 12 % of $Cu^{2+}$ in the CuO lattice can be replaced with $Zn^{2+}$

resulting in the formation of Zn doped CuO ($Cu_{0.88}Zn_{0.12}O$). The reason behind the exceptionally high and potential microbial, viral and fungal killing ability of this antimicrobial material is oxygen vacancies as well as structural defects and dislocations. Apart from structural defects, the co-existence of the amorphous phase of $Cu_{0.88}Zn_{0.12}O$ with the crystalline phase of $Cu_{0.88}Zn_{0.12}O$ is surmised. The oxygen vacancies, structural defects and dislocations as well as the amorphous phase (minor amount) have contributed to the formation of the reactive oxygen species (ROS) that are responsible for the killing of even the multi drug resistant (MRD) and extremely drug resistant (MDR) bacteria. Famous plant pathogens, namely, *Alternaria alternate* CGJM3078 and *Alternaria alternate* CGJM3006, were killed effectively by the Zn doped CuO. Whatelse, the magic material, $Cu_{0.88}Zn_{0.12}O$, is under-utilized in agriculture for enhancing the longevity of the agricultural products and also as a pestilent. Efforts should be devoted in this direction for ensuring profit to the framers and increasing their income and standard of living as the great nation India has reently celebrated the 78$^{th}$ year of independence on 15$^{th}$ August 2024.

**Acknowledgement**

Grateful thanks are due to Professor Aharon Gedanken for introducing PIN the new and magic material Zn doped CuO with life-saving applications. Financial support from the Israel Science Foundation (ISF, Grant No. 598/12), from the Ministry of Science, Technology, and Space of Israel (Grant No. 3-9802 and 3-99763), and from the Israel Ministry of National Infrastructures, Energy and Water Resources (Grant No. 3-13442) is acknowledged. Thanks are due to the staff of alumni association IIT Madras for the access to the resources as an alumni. Gratefulness is due to Professor Mahendra N Jadhav, librarian, central library, IIT Madras for providing uncensored access to the commercial resources of the central library. Thankfulness is due to Dr Archana Ramchandra Deokar, GSFCU Vadodara, for the brain-storming discussions on Zn doped CuO material, in general and on antibacterial materials, in particular. Gratefulness is due to Dr Trupti Gajaria, GSFCU, for her original ideas on condensed matter and the defects there in that served as motivation to attempt this review.

†Dedicated to the legendary agricultural scientist, Bharat Ratna, late Professor Mankombu Sambasivan Swaminathan

**REFERENCES**

1 Gedanken A, Banin E, Perelshtein I, et al. Doped metal oxide nanoparticles of and uses thereof, AU 2014264224 B2, 5th July 2018.
2 Malka E, Perelshtein I, Lipovsky A et al. Eradication of Multi-Drug Resistant Bacteria by a Novel Zn-doped CuO Nanocomposite. *Small*. 2013; 9 (23):4069-76p.
3 Mishra RK, Kumar VB, Victor A, et al. Selective Production of furfural from the dehydration of xylose using Zn doped CuO Catalyst. *Ultrasonics – Sonochemistry*. 2019; 56:55-62p.
4 Eshed M, Lellouche J, Gedanken A et al. A Zn-Doped CuO Nanocomposite Shows Enhanced Antibiofi lm and Antibacterial Activities Against Streptococcus Mutans Compared to Nanosized CuO, *Adv Funct Mater*. 2014; 24:1382-90p.
5 Maruthapandi M, Saravanan A, Das P, et al. Antimicrobial Activities of Zn-Doped CuO Microparticles decorated on Polydopamine against Sensitive and Antibiotic-Resistant Bacteria. *ACS Appl. Polym. Mater*. 2020; 2: 5878-88p.
6 Shalom Y, Perelshtein I, Perkas N, et al. Catheters coated with Zn-doped CuO nanoparticles delay the onset of catheter-associated urinary tract infections. *Nano Research.* 2017; 10(2): 520-33p.
7 Deokar AR, Shalom Y, Perelshtein I, et al. A topical antibacterial ointment made of Zn-doped copper oxide nanocomposite, *J Nanopart Res.* 2016; 18:218.
8 Mishra RK, Shalom Y, Kumar VB, et al. Surfactant-free synthesis of a water-soluble PEGylated nanographeneoxide/metal-oxide nanocomposite as engineered antimicrobial weaponry. *J. Mater. Chem. B.* 2016; 4:6706-15p.
9 Bruno E, Haris M, Mohan A, et al. Formation of self-assembled hierarchical structure on Zn doped in CuO nanoparticle using a microwave assisted chemical precipitation approach. *J Mater Sci: Mater Electron.* 2021; 32:19339-351p.

10 Saranya R, Ali MM, Jeyalakshmi K. Evaluation of pharmacological and catalytic activity of CuO and Zn doped CuO nanoparticles, *Indian journal of engineering and materials sciences*. 2022; 29 (1): 124-29p.
11 Bhosale SR, Bhosale RR, Moyo AA, et al. Effect of Zn Doping on Antibacterial Efficacy of CuO *Nanostructures*. 2023; 8 (34):e202301997.
12 Prasad, LG, Janeba T, Raman RG. Investigation on the physicochemical properties and antibacterial activity of pure and Zn doped CuO nanoparticles. *Journal of ovonic research*. 2021; 17 (2):209-16p.
13 Iqbal J, Jan T, Ul-Hassan S, et al. Facile synthesis of Zn doped CuO hierarchical nanostructures: Structural, optical and antibacterial properties. *AIP Advances*. 2015; 5 (127112):277.
14 Wu RR, Zhang HB, Pan JM, et al. Spatio-Design of Multidimensional Prickly Zn-Doped CuO Nanoparticle for Efficient Bacterial Killing. *Advanced materials interfaces*. 2016; 3 (18): 280.
15 Khalid A, Ahmad P, Alharthi AI, et al. Structural, Optical, and Antibacterial Efficacy of Pure and Zinc-Doped Copper Oxide Against Pathogenic Bacteria. *Nanomaterials*. 2021; 11: 285.
16 Tuby R, Gutfreund S, Perelshtein I, et al. Fabrication of a Stable and Efficient Antibacterial Nanocoating of Zn-CuO on Contact Lenses. *Chem Nano Mat*. 2016; 2 (6): 547-51p.
17 Kumar P, Inwati GK, Mathpal MC, et al. Defects induced enhancement of antifungal activities of Zn doped CuO nanostructures, *Applied Surface Science*. 2021; 560: 150026.
18 Mantecca P, Moschini E, Bonfanti P, et al. Toxicity Evaluation of a New Zn-Doped CuO Nanocomposite With Highly Effective Antibacterial Properties. *Toxicological sciences*. 2015; 146(1):16-30.
19 Prabhakaran D, Boothroyd AT. Single crystal growth of Zn-doped CuO by the floating-zone method. *J Cryst Growth*. 2003; 250:77-82p.
20 Borzi RA, Stewart SJ, Punte G, et al. History-dependent magnetic properties in pure and Zn-doped cupric oxide. *Solid State Commun*. 2001; 117:311-14p.
21 Daniel R. Cooper, Nada M. Dimitrijevic and Jay L. Nadeau, Photosensitization of CdSe/ZnS QDs and reliability of assays for reactive oxygen species production, *Nanoscale*, 2010, 2, 114-21p.
22 Steen CJ, Niklas J, Poluektov OG, et al. EPR Spin-Trapping for Monitoring Temporal Dynamics of Singlet Oxygen during Photoprotection in Photosynthesis, *Biochemistry* 2024, 63, 1214-24p.