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Zinc Oxide-Supported Copper Clusters with High Biocidal Efficacy for Escherichia coli and Bacillus cereus

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Supporting Information

ABSTRACT: Cu clusters on ZnO have been prepared by a simple low-temperature solidstate reaction from their respective acetate precursors. The formation of metallic Cu along with a small quantity of CuO was influenced by the presence of the zinc acetate precursor. Although there is a lack of formation of any metallic Cu in the absence of zinc acetate, increase in the heating duration helps in the formation of increased metallic Cu. A mechanism for formation of the Cu@ZnO nanocomposite has been suggested. The prepared Cu@ZnO nanocomposite, with metallic Cu, was identified by X-ray diffraction studies followed by confirmation of clusters of the kind Cu₉ and Cu₁₈ by transmission electron microscopy and matrix-assisted laser desorption ionization time-of-flight mass spectrometry. The photoelectron spectroscopy is able to clearly distinguish the Cu from CuO, which is very well complimented by electron spin resonance analysis. The morphological feature of ZnO changes from flakes to rods on increasing the duration of heating, as shown by scanning electron microscopy (SEM) analysis. The observed Cu plasmonic band in UV-vis diffuse reflectance gets blue-shifted to 463 nm from its



normally observed position of 550-580 nm possibly due to cluster formation and interaction with ZnO, the band gap of the latter getting red-shifted to 3.2-3.0 eV. The antibacterial activity of the synthesized Cu cluster-ZnO nanocomposites was investigated against Escherichia coli ATCC-25922 for Gram-negative and Bacillus cereus ATCC-10876 for Gram-positive bacteria. Tests were performed on a nutrient agar medium and liquid broth supplemented with different concentrations of nanoparticles. SEM analysis of the native and treated Gram-positive and Gram-negative bacteria established a high efficacy of biocide activity in 24 h, with 200 μ g/mL of Cu@ZnO nanocomposites.

1. INTRODUCTION

Noble metal nanoparticles (MNPs)^{1,2} have found innumerable applications in the field of medicine, energy, electronics, manufacturing materials, catalysis, etc. Of the three noble metals, namely, gold (Au), silver (Ag), and copper (Cu), Cu is the least expensive and more abundant in nature. The noble MNPs exhibit entirely different physicochemical properties from their bulk metal. Cu NPs find application as a conducting medium in the form of conductive inks,³ lubricant additives,² thermal conductors,⁵ highly selective and efficient catalysts in catalysis,⁶ fillers⁷ when added to plastics, coatings, and textiles, and also as antibacterial agents.⁸ Other practical applications of Cu NPs are biosensing,⁹ optical cloaking,¹⁰ superlensing,¹¹ parasite therapy,¹² etc. using the principles of plasmonics.^{13,14} Chemical reduction, microemulsion, photolithography, etc. are some of the physicochemical methods¹⁵⁻³⁰ employed for the synthesis of MNPs, which have mushroomed to a large number in recent times. Of all of the above mentioned procedures and methodologies, thermal degradation is the simplest and less time consuming without using any expensive chemicals and an environmentally less polluting method. In our current study, we report the preparation of metallic Cu NPs along with ZnO by simple low-temperature solid-state reaction of Cu and zinc acetate salts and study the effect of temperature and duration of

thermal treatment on the physicochemical properties of NPs. It is of interest to note that ZnO-supported metallic Cu, sometimes along with CuO, has been identified as clusters of the type Cu₉ and Cu₁₈ using a variety of characterization methods, including transmission electron microscopy (TEM) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF). The nuances of preparatory methods and characterizations of the prepared Cu@ZnO nanocomposites are presented (Figure 1).

Cu is an essential micronutrient required for normal functioning of the aerobic microorganisms.³¹ However, excess amount of Cu in the human body causes Cu toxicity or copperiedus, which can be of a genetic or nongenetic origin. According to the U.S. Environmental Protection Agency, the maximum contaminant level of Cu in drinking water is 1.3 mg/ L. About 0.9–1.3 mg/day is the recommended intake of Cu in North America. Metal and metal oxide NPs, such as Ag, Au, Cu, TiO₂, CuO, ZnO, etc., are known to possess good antibacterial properties. Many research works on antibacterial studies have shown that doped metal oxide NPs have better antibacterial

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Zn and Cu acetate Before Mixing After Mixing

After thermal treatment @ 300 deg/3 h @ 4 deg/min

Figure 1. Preparation of Cu@ZnO nanocomposite materials.

properties than those of undoped ones.^{32–34} Cu as a biocide has been known since the metal age. Cu and Cu-based NPs such as CuO and Cu₂O have adequately shown antibacterial effects in lesser amounts but toxic effect in large amounts.³⁵ Whereas one of the reports³⁶ of the World Health Organization suggests an acceptable intake of Cu to be approximately 1–1.5 mg/day.

Several studies report the nontoxic nature of ZnO NPs to human cells.^{37–39} ZnO NPs are noxious to microorganisms, have good biocompatibility to human cells, and are used as effective antibacterial agents.⁴⁰ ZnO NPs are also known to have good antibacterial property and have been tested on a number of Gram-positive and Gram-negative bacteria as well by varying different factors, such as the concentration, particle size, and specific surface area.^{41,42} Hajipour et al.⁴³ in their critical review, discussed about the functioning of NPs against bacteria and their role in antibacterial activity. A recent antibacterial study of E. coli, Staphylococcus aureus, and Streptococcus pyogenes by Bhuyan et al.⁴⁴ using pure and Cu-doped ZnO NPs showed that Cu-doped ZnO NPs have superior antibacterial action than that of pure ZnO NPs. In another study by Gupta et al.,⁴⁵ on exposing the E. coli cells to N and Cu co-doped ZnO NPs under visible light irradiation, E. coli becomes inactive. The synergistic antibacterial effect of Cu@T-ZnO nanocomposites for both Gram-negative and Gram-positive bacteria was shown by Yu et al.⁴⁶ Most importantly, the authors have positively proven them to have significant potential as bactericidal agent, exemplifying a biocidal action of these particles against E. coli ATCC-25922 and Bacillus cereus ATCC-10876. The aim of this work was to study the interaction between bacteria and NPs by scanning electron microscopy (SEM) and optical method.

2. RESULTS AND DISCUSSION

2.1. X-ray Diffraction (XRD) Studies Reveal the Formation of Cubic Cu@ZnO Phase. The formation of metallic Cu and ZnO were confirmed from XRD measurements of the nanocomposite materials. Figure 2 shows the diffraction patterns of the composite material prepared in air atmosphere at different exposure periods at 300 °C. It was observed that the peaks at 2θ values, 43.3 (111), 50.4 (200), and 74.1 (220), correspond to the face-centered cubic (fcc) structure of metallic copper, according to JCPDS no. 851326. The intensity of the Cu peaks increases from 1 to 3 h and decreases from 3 to 6 h on the basis of the duration of exposure. It was also observed that very low intensity peaks at 2θ values, that is, 36.5 (which is merged with the ZnO peak) and 42.6, correspond to CuO, as realized from JCPDS no. 780428, to be an fcc one,47 and the presence of Cu2+ was also confirmed from electron paramagnetic resonance (EPR) and X-ray photoelectron spectros-



Figure 2. (a) XRD patterns of Cu@ZnO nanocomposite materials prepared by simple solid-state thermal degradation at different exposure periods at 300 °C and that of (b) Cu@ZnO nanocomposite S3 sample showing CuO peak at $2\theta = 42.2$ of the monoclinic type.

copy (XPS, vide infra). The increase in the intensity of CuO on increasing the time period from 3 to 6 h suggests that the metallic Cu was being converted to CuO and there is also a phase change of CuO from fcc to monoclinic end center at 6 h of duration. The presence of monoclinic end-centered CuO was confirmed from the XRD peaks at 35.7 and 38.9, according

to JCPDS no. 440706. At the same time, the ZnO peaks (2θ values are 31.7, 34.3, 36.2, 56.5, and 62.7 corresponding to JCPDS no. 891397 of hexagonal wurtzite structure) show continuously increasing formations of ZnO from 1 to 6 h. In the diffraction pattern of 1 h sample, there is no formation of ZnO and they exist as acetates. On increasing the reaction time, Zn acetate oxidizes into ZnO. Once the formation of ZnO is complete, metallic Cu, which is present in the sample, starts getting converted to CuO, as shown in the magnified XRD pattern for S3 in Figure 2b.

2.1.1. Proposed Mechanism for Metallic Cu Formation. It is understood from the works of Afzal et al.⁴⁸ and Lin et al.⁴⁹ that the final products, ZnO from zinc acetate and a mixture of Cu₂O and CuO from copper acetate were obtained when heated separately. But when zinc acetate and copper acetate were heated together at different time durations at 300 °C, we got a mixture of acetates, metallic Cu, Cu(II) oxide, and ZnO. More ZnO and CuO were increasingly formed 2 h onward. However, with no change in temperature but differing the duration of the reaction until 5 h, we witness the formation of ZnO, metallic Cu, and CuO at different ratios; CuO is formed only on heating further to 6 h in air atmosphere, and it is easily inferred that only metallic Cu gets oxidized to CuO, as known from both the reduced ratios of Cu@ZnO and Cu/CuO (see Table 1) as compared to the S5 sample results.

Table 1. Ratio of Metallic Cu Peak to ZnO and Cu₂O^a

sample	Cu peak (deg)	d-spacing (Å)	FWHM (Å)	Cu@ZnO peak intensity ratio	Cu/CuO peak intensity ratio	particle size (nm)
S1	43.4	2.07	0.14	12.75		62.5
S2	43.5	2.08	0.20	11.32		44.5
S3	43.2	2.08	0.09	2.80	10.82	93.2
S4	43.4	2.08	0.10	2.22	8.88	86.1
S5	43.2	2.08	0.09	1.822	9.00	93.3
S6	43.4	2.09	0.13	1.14	4.30	66.9
ac1 c2	62 64	67 10	/	1 11 1	2 2 4 5	1 < 1 (

"S1, S2, S3, S4, S5, and S6 are samples with 1, 2, 3, 4, 5, and 6 h of heating, respectively.

To understand better the reaction mechanism of the formation of metallic Cu and the part played by both zinc and Cu acetates, we kept the amount of Cu acetate (0.5 g) as a constant and changed the quantity of zinc acetate, that is, 0.05, 0.1, 0.25, 0.5, and 0.75 g in separate experiments. After the formation of the products, they were analyzed for XRD studies, and the results are shown in Figure 3.

From Figure 3, it can be found that metallic Cu forms first in the mixture, followed by ZnO and a relatively small quantity of CuO simultaneously. The metallic Cu to cupric oxide ratio (peak positions 43.3-42.6) increases as we increase the amount of zinc acetate. The most possible reason could be that Cu acetate starts decomposing at 115 °C, whereas zinc acetate decomposes at 237 °C. Hence, metallic Cu forms initially on top of zinc acetate, which then decomposes to form ZnOsupported metallic Cu. During the reaction condition, as the zinc acetate gets converted to ZnO, part of the metallic Cu gets exposed to air atmosphere and gets converted to cupric oxide. This is very evident when the amount of zinc acetate increases from 0.05 to 0.75 g. As one can observe from Figure 3, as the amount of zinc acetate increases, the formation of cupric oxide is less and the intensity of metallic Cu is more. This by itself proves that more the amount of zinc acetate, the higher the



Figure 3. XRD patterns of Cu@ZnO nanocomposite prepared by varying the amount of zinc acetate at 300 $^\circ C$ for 3 h.

metallic Cu content. Also, the larger amount of ZnO seems to protect the metallic Cu clusters getting oxidized.

Furthermore, it is necessary to point out that metallic Cu is formed on heating Cu acetate only in the presence of zinc acetate at a higher temperature and longer duration. Heat treatment of pure Cu acetate forms only CuO and Cu₂O, without any trace of metallic Cu at 300 °C. The formation of metallic Cu, as suggested by Afzal et al.⁴⁸ and Lin et al.,⁴⁹ has been taken into consideration in the proposed mechanism, as shown in Scheme 1.

It may, however, be pointed out that a somewhat different method of making Cu in ZnO ended up with the formation of Cu-doped ZnO,⁴⁴ which is distinctly different from metallic Cu in the form of clusters having plasmonic property (vide infra) on the substrate of ZnO, as reported here. In another report, Cu and N are co-doped in ZnO by the solution combustion method.⁴⁵

2.2. XPS Study Confirms the Formation of Metallic Copper (Cu⁰) and CuO (Cu²⁺). To further confirm the presence of metallic Cu, XPS studies performed (survey spectrum shown in Figure S1) show the presence of zinc, Cu, and oxygen in the composite material. The region pertaining to copper and zinc was scanned highly for detection of the oxidation state of the elements. In Figure 4a, the peak position at 932.7 and 933.4 eV corresponds to Cu⁰ and Cu²⁺ states, respectively. From the Cu LMM analysis in Figure 4b, the deconvoluted peak showed the presence of Cu⁰ at 916.4 eV and Cu²⁺ at 915.5 eV. This confirms the presence of metallic Cu and CuO, as obtained from the XRD measurements, as confirmed from the literature resources of Thermo Scientific.⁵⁰

2.3. SEM Analysis Reveals Flakes and Rod-Like Structures of Cu@ZnO. From the SEM analysis of the Cu@ZnO nanocomposite materials, it was observed that the surface morphology of the analyzed samples showed different structures. From Figure S2, the S3 sample showed flake-like structures, whereas the S5 sample showed rod- as well as flake-like structures. The transformation from flakes to rod structure seems to be rather slow and incomplete.

Scheme 1. Proposed Reaction Mechanism Depicting the Formation of Metallic Cu in the Presence of ZnO

$$Z_{n}(CH_{1}COO)_{2} : 2H_{2}O + Cu(CH_{3}COO)_{2} : 2H_{2}O \longrightarrow Z_{n}(CH_{3}COO)_{2} + Cu(CH_{3}COO)_{2} + 4H_{2}O + 4H_{2}O$$

Figure 4. XPS spectrum of Cu@ZnO nanocomposite materials (S3) – Cu (a) and Cu_LMM (b) region.

2.4. Confirmation of Cu^{2+} in Cu@ZnO Nanocomposites by Electron Spin Resonance (ESR) Studies. X-band ESR studies of the Cu@ZnO nanocomposite materials show the spectra, as shown in Figure 5a,b. The *g* values, 1.99 and 1.95 m, respectively, correspond to shallow donors, such as zinc interstitials (Zn_i), conduction band electrons (CB_e^-), and oxygen vacancies (V_O).^{51,52} It is clearly evident from Figure 5a,b that S3 sample has less intensity than that of S5 sample at both the *g* values, 1.95 and 1.99. It can be said that at *g* value = 1.95, S3 sample had less concentration of V_O compared to that in the S5 sample. The conduction band electrons, CB_e^- , or native defect, Zn_i, that is, the shallow donors at *g* = 1.99 increase with increase in the reaction time. From the XRD

Figure 5. X-band ESR spectrum of the nanocomposite material, Cu@ ZnO S3 (a) and S5 (b) at 77 K.

Field (mT)

pattern (Figure 2), it can be seen clearly that there is conversion of metallic Cu into CuO in S5 sample. This is the general observation at all heating durations except at 6 h.

Generally, a higher duration of heat treatment leads to the conversion of metallic Cu to CuO; the formation of CuO is such that the EPR of Cu^{2+} is fairly prominent. More V_O is created because of the greater use of oxygen by metallic Cu to form the oxidation product, CuO. The spectral features at fields lower than that of the conduction band are due to CuO.

2.5. MALDI-TOF Study Confirms the Formation of Cu_9 and Cu_{18} Clusters. Metallic Cu cluster formation on the surface of ZnO was confirmed by analyzing the Cu@ZnO nanocomposite materials using MALDI-TOF studies, with dithranol as the matrix. The results are shown in both Figure 6



Figure 6. MALDI-TOF spectra of Cu@ZnO nanocomposite material S3. The mass spectra of Cu clusters Cu₉ and Cu₁₈ are shown in (a) and (b), respectively. The numbers in parenthesis (x, y) refer to the number of 63 Cu and 65 Cu atoms, respectively, for some clusters. The unmarked ones (*) are the protonated forms of the earlier peaks.

and in the Supporting Information, Figure S3 (survey spectrum). There are two series of major peaks observed in the m/z range of 500–1500. They, respectively, are part of the Cu₉ and Cu₁₈ clusters, with each peak corresponding to a combination of isotopic distribution, with ⁶³Cu (62.92 amu, 69.17%) and ⁶⁵Cu (64.92 amu, 30.83%), with the natural abundances given in parenthesis. It is necessary to mention that all S1-S4 samples give identical MALDI spectra. In S5 and S6 samples, there is inclusion of oxygen with the copper clusters, which may have been responsible for the resultant formation of CuO. It is necessary to comment on the analysis of MALDI spectral m/z range of 574–581 as well as 1154–1162. Although we could easily and directly identify the Cu₉ and Cu₁₈ clusters with differing masses of Cu⁶³ and Cu⁶⁵, as marked in Figure 6a,b, respectively, the alternating peaks can be identified by only one of the following rationale: (i) if we can include ZnO, we can assign them to four copper clusters and four ZnO; (ii) the alternating unassigned peaks (*) can be the protonated

form of the earlier peak, justifying its increase by 1 amu due to the capture of a proton from the dithranol matrix. The latter was found to be correct because an exactly similar spectrum was obtained for metallic copper clusters in the absence of ZnO,⁵³ as shown in Figure S4. It is observed from Figure 6a,b that the abundance of the Cu₉ cluster is about 10 times that of the Cu₁₈ cluster.

2.6. TEM Analysis Reveals ZnO-Supported Cu Cluster. An HRTEM analysis of S3 sample is shown in Figure 7, which reveals formation of the Cu cluster on ZnO surface. In Figure 7a, one can find the Cu cluster embedded in the ZnO surface, with cluster size of diameter 3.90 nm, in the form of quantum dots. Figure 7b shows the measured d-spacing values, such as 0.28, 0.24, and 0.26 nm corresponding to the (100), (002), and



Figure 7. Transmission electron micrograph showing Cu cluster (a) and d-spacing values of ZnO (b) in S3.

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(101) phases of the hexagonal Wurtzite ZnO structure, respectively.

2.7. Blue-Shifted Copper Plasmon Coupled with Redshift in the ZnO Band Gap. The Cu@ZnO nanocomposite materials that were prepared at 300° at different time periods were studied for optical property in the reflectance mode. Figure S5 shows the UV-diffuse reflectance spectroscopy (DRS) spectra in absorbance scale for the commercial ZnO, ZnO from zinc acetate, and all of the Cu@ZnO nanocomposite materials. The band gap for the commercial ZnO and ZnO prepared from zinc acetate was calculated to be 3.26 eV, an accepted value in the literature. The band gap of ZnO in the nanocomposite materials gets red-shifted from 3.26 eV to less than 3.0 eV.

The Cu plasmonic band was generally observed at 550-580 nm. The metallic Cu in our experiment shows its surface plasmon resonance band at around 473 nm for all of the Cu@ ZnO nanocomposite materials, as shown in Figure S5. As the reaction time increases, formation of CuO is observed, as there is absorption peak in the spectra as well as in XRD at around 550-580 nm (2.25–2.13 eV). Figure 8 shows the KM function



Figure 8. KM function of the UV–DRS spectra of nanocomposite, Cu@ZnO materials, commercial ZnO, ZnO(C), and the ZnO prepared from zinc acetate, ZnO(Ac).

of the spectra depicted in Figure S5. Almost all of the nanocomposite materials studied here showed the Cu plasmonic band at around 473 nm (2.63 eV), a case of blueshift. Repeated experiments show shifts, as shown in Figure 8. The plasmon band seems to originate from the Cu clusters located on the surface of ZnO, as verified by both TEM and MALDI measurements (vide supra).

The band structures of metallic Cu and ZnO are shown in Figure 9. From the band structure, one can understand the interaction between metallic Cu and ZnO. It is a well-known fact that the work functions of metallic Cu and ZnO are $\varphi_{Cu} = 4.53 \text{ eV}$ and $\varphi_{ZnO} = 5.2 \text{ eV}$, respectively. When Cu and ZnO come together to make metal-semiconductor contact, electrons from n-type ZnO move toward Cu and a space charge region is created. But in the seminal paper by Bardeen⁵⁴



Zinc Oxide, ZnO



Figure 9. Band structure of copper and zinc oxide before (upper) and after (lower) coming into contact.

and Tung,⁵⁵ it is said that there will be metal-induced gap states or polarization of the bond at the MS interface takes place during the chemical bonding of the metal (Cu) and semiconductor (ZnO). The metal (Cu) donates a large quantity of charge that is absorbed by the highly dense surface states in the semiconductor (ZnO). Henceforth, the semiconductor bands align to a new band level according to the density of the surface states, which pins the Fermi level. This change in the fermi level shows a reduction in the band gap energy of ZnO when in contact with Cu, substantiating the observed redshift. In the same analogy, because Cu had donated charges to ZnO in this case, the free electrons responsible for plasmons become more tightly bound to cause the observed blueshift.

2.8. Highly Effective Biocidal Activity of Cu₉, Cu₁₈@ ZnO, ZnO, Cu, Cu + ZnO. The antibacterial activity of the synthesized Cu@ZnO nanocomposites at different time intervals (S1, S2, S3) were evaluated using the standard well diffusion method. On the basis of the results of our preliminary experiments, sample S3 (as shown in Figure 10), exhibited a

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Figure 10. Antibacterial in vitro effect of Cu@ZnO nanocomposites (S3) at four concentrations on *E. coli* (top) and *B. cereus* (bottom).

larger zone of clearance as compared to that of S1 and S2 (data not shown). It was observed that the zone of inhibition was in the order of S3 > S2 > S1, which can be explained due to the increased content of metallic copper with an increase in time.

On the basis of the promising results of the plate assay, S3 sample was selected to study the dynamics of bacterial growth in a liquid nutrient medium inoculated with *E. coli* ($\sim 10^4 - 10^5$ cells/mL) and supplemented with varying concentrations of these nanocomposites in separate experiments. One control flask was also maintained, which did not have any nanocomposites. The treated bacterial samples were withdrawn at an interval of every 4 h, and the optical density was measured at a wavelength of 600 nm. The above experimental procedure was followed for the Cu@ZnO nanocomposites to find the

antibacterial activity against *B. cereus* also and compared with that in pure ZnO, pristine Cu, and a physical mixture of pure ZnO and pristine Cu.

In the case of *E. coli*, as seen in Figure 11a, the flask which was added with 100 and 125 μ g/mL of nanocomposites



Figure 11. Growth pattern of *E. coli-* (a) and *B. cereus-* (b) treated Cu@ZnO nanocomposites (S3) as compared to control for 24 h duration.

exhibited a nearly similar growth pattern as that of control, which indicated that there was negligible inhibition in growth due to the addition of nanocomposites. However, with an increase in its concentration, there was an increased inhibition in the growth of *E. coli*, which suggests that inhibition takes place at higher concentrations of the Cu@ZnO nanocomposites. Nevertheless, the flask added with 200 μ g/mL of the nanocomposites exhibited complete inhibition in the growth of bacterial cells. For all of the concentrations tested, it was observed that the stationary phase started by the eighth hour of inoculation.

In the case of *B. cereus* (Figure 11b), a similar trend was observed, wherein the flask containing 25 and 50 μ g/mL of the nanocomposites had a meager inhibition in growth. However, when 100 μ g of the nanocomposites were used, an inhibition by almost 50% was observed as compared to that in control followed by 200 μ g/mL of the nanocomposites.

The interesting features observed in the inhibition patterns of both the organisms tested are as follows:



Figure 12. SEM image of control and that treated with 200 μ g/mL of Cu@ZnO nanocomposites (S3) of *E. coli* and *B. cereus* cells just at 8 h of inoculation.

- 1. In the case of *E. coli*, it was seen that when a concentration of 100 μ g/mL of the nanocomposites were used, it showed comparable results to that of control, which indicates that a concentration lower than this may be inadequate to completely inhibit the growth of *E. coli*. Nevertheless, a concentration of 200 μ g/mL of the nanocomposites was highly effective and showed a complete inhibition in their growth, even at lower time scales.
- 2. In the case of *B. cereus*, a different trend was observed, wherein an appreciable amount of inhibition was observed even with a concentration of 75 μ g/mL. However, it was also interesting to note that unlike *E. coli* complete inhibition was not achieved even at a concentration of 200 μ g/mL.
- 3. Pristine ZnO alone seems to be playing a larger role in inhibiting the growth of *B. cereus* compared to that of *E. coli* (cf. Figures 11a and 11b). However, the presence of Cu in Cu@ZnO plays a major role in the destruction of *E.coli* and a minor role in that of *B.cereus*. It is also quite exciting to find that in both Gram-positive as well as Gram-negative organisms ZnO gives a bacteriostatic effect in the initial stage of inoculation, after which the OD value increases. Some reports suggest that this could be due to the surface defects on ZnO.⁵⁶

From these inferences and from the works of Yu et al.,⁴⁶ one can confirm that the presence of both Cu and ZnO in the nanocomposite plays a synergistic effect in inhibiting the growth of bacterial cells, revealing the importance of Cu in the nanocomposite.

To further confirm the results, SEM analysis was used to evaluate the surface morphology of both the native and treated *E.coli* and *B. cereus* in the nutrient medium. The treated bacterial cells were significantly changed and showed total damage in their cell walls that were destroyed and rough (Figure 12). The growth inhibition trend, as observed in the results of optical density measurement in liquid medium for both the organisms, was in good agreement with the SEM images. The above SEM analysis is consistent with the reports of Raffi et al.⁵⁷ More importantly, it must be said that although we have reported the total cell destruction with the use of 200 μ g/mL of nanocomposites, the destruction seems to happen at a much earlier stage, even though the experiment was continued for 24 h.

2.8.1. Assessing the Inhibiting Effect of Cu@ZnO. To assess which component in the nanocomposite (Cu or ZnO) plays a significant role in inhibition, we further conducted the experiment in a liquid medium with pristine ZnO, pristine Cu, physical mixing of pristine ZnO and Cu, and Cu@ZnO nanocomposites. The antibacterial results are given in Figure S6a,b.

	ref 44	ref 45	ref 46	ref 57	present article
preparation methods	acetate precursor (Cu/Zn = 10%) - thermal decomposi- tion - 500°/4 h	two-step process – solution combustion using glycine + hydrothermal using trietha- nolamine	two-step process – T-ZnO prep- aration + suspension in Cu-tartarate solution and an- nealing in H ₂ atmosphere	pure copper wire using inert gas condensation (IGC) method	acetate precursor (1:1) – solid-state reaction – 300°/3 h
nanoparticles	Cu-doped ZnO	copper- and nitrogen-doped ZnO (CuO, Cu ₂ O, and ZnO)	n-Cu@T-ZnO	pure copper nanoparticles	metallic Cu clusters on the surface of ZnO
cell death	100 μ g/mL – achieved 61% <i>E. coli</i> growth inactivity @ 24 h	250 µg/mL – 8 log reduction of growth of <i>E. coli</i> con- centration	600–800 ppm of the nanoparticle effective for bacterial cells	100 μ g/mL in 10–50 h – Inactivity of <i>E. coli</i> growth	$200 \ \mu g/mL$ — instantaneous inactivity after the intro- duction of nanocomposites through 24 h
SEM analysis			only plate assay studied	SEM image shows pits and cavities in bacterial cell walls	clear SEM image depicting cell death by damage to the cell walls

Table 2. Comparison of Preparatory Methods, Nature, and Antibacterial Activity of Cu(0) Containing NP's and Nanocomposites

From Figure S6a, it is seen that ZnO does play a role as a bacteriostatic agent till 12 h of the experimental period, after which the bacterial cells start growing, as inferred from the increase in the OD values. When pristine Cu is used, the inhibition effects are much less probably due to the aggregation of nanocomposites, as a consequence lesser surface area is available for active inhibition or/and this could be due to the consumption of pristine Cu by the bacterial cell at concentrations of 100 and 200 μ g/mL. However, with increase in time, the latter is seen to inhibit growth of the bacterial cells. A comparison of pristine Cu with pristine ZnO reveals that the former has a lower level of activity than that of the latter. Simple physical mixing of equal amounts (100 μ g/mL) of pristine ZnO and pristine Cu, gives a bactericidal effect, and there is complete mineralization of the bacterial cell medium. After 48 h the entire medium transforms to a green transparent solution, which is due to the presence of Cu^{2+} ions in the solution. The other physical composition (Cu 165 μ g/mL + ZnO 35 μ g/mL) shows no effect till 8 h of experiment, but later there is a sudden decline in the OD values suggesting that bacterial cells are killed partially.

In Figure S6b, the antibacterial effect against Gram-positive *B. cereus* is presented. For ZnO at higher concentration (200 μ g/mL), physical mixing of pristine Cu and pristine ZnO showed very good antibacterial effect. Pristine Cu at higher concentration shows good results only in the later stage of the experiment. But the physical mixture of pristine Cu (165 μ g/mL) and pristine ZnO (35 μ g/mL) completely mineralizes the liquid broth medium after 48 h. Even 100 μ g/mL each of pristine Cu and pristine ZnO yield good antibacterial results but not as good as the former combination.

The mechanism of antibacterial activity of NPs is still not clearly understood, although cell-wall damage due to Cu ions⁵⁸ and hydrogen peroxide production from Cu⁵⁹ and ZnO⁴⁰ are likely possibilities.

A comparison showing superiority of the current studies over the earlier reports is given in Table 2. Similar observations, although not as efficient as ours, have been reported by Raffi et al.,⁵⁷ in studying the antibacterial behavior of Cu NPs against *E. coli*. The sensitivity of bacteria toward Cu NPs was found to vary depending on the type of species. Recently, different investigators have reported the antimicrobial effect of Cu NPs against different bacterial strains.^{12,57,60,61} The cell death reported in this study is substantially higher than that reported by the use of other Cu-doped ZnO.^{44–46} Absolute cell death occurs probably by reactive oxygen species mechanism due to the synergetic effect of both metallic Cu clusters and ZnO in the case of *E. coli*, as already confirmed by Yu et al.⁴⁶ We attribute this greater inhibitive effect for *E. coli* than that for *B.cereus* to the difference in cell-membrane structures, as supported by Yu et al.⁴⁶ An additional advantage of $Cu_{9,18}(a)$ ZnO reported here is that the synthetic procedure is very simple and consumes less time and energy. The antibacterial activity could further be activated with the application of visible light, as reported by Gupta et al.,⁴⁵ wherein the Cu plasmon is expected to play a major role along with ZnO, which will be investigated in the near future.

3. CONCLUSIONS

Cu@ZnO nanocomposites were produced by low-temperature solid-state reactions of Cu and zinc acetate combinations, as a function of heating duration. Interesting Cu clusters in the form of Cu₉ and Cu₁₈ clusters were found on the surface of ZnO that resulted in a blue-shifted copper plasmonic band and redshifted ZnO band gap, showing a strong interaction between the two species in the nanocomposites. Antimicrobial studies revealed that the synthesized Cu@ZnO nanocomposites were capable of destroying the bacterial cell wall, eventually degrading the cytoplasm leading to cell death. The antibacterial activity may be attributed mainly due to the strong adsorption of positively charged Cu ions to bacterial cells, which imparts antibacterial efficacy in a concentration-dependent manner. Furthermore, it is the synergistic effect of both Cu and ZnO, present in the nanocomposites, that leads to the bactericidal effect and not bacteriostatic effect. The authors have contemplated to study bactericidal effects of the Cu@ZnO nanocomposites using light therapy to check the possible effects of Cu plasmon.

4. MATERIALS AND METHODS

4.1. Experimental Methods. *4.1.1. Preparation of Cu@ ZnO Nanocomposite Material.* Metallic salt precursors, such as Zinc acetate dihydrate (0.5 g) and Cu acetate monohydrate (0.5 g), are taken in a silica crucible. They are mixed thoroughly using a pestle and mortar. The silica crucible along with its contents was placed in a muffle furnace under air atmosphere without closing the crucible. The thermal degradation (decomposition) process was initiated by setting the temperature to 300 °C, increasing at the rate of 4 °C per min. After reaching 300 °C, the reaction was allowed for 1, 2, 3, 4, 5, and 6 h, respectively. It was observed that after the respective reaction time periods, the color of the mixture turns from bluish-green to brown. After the sintering process, the synthesized materials were collected and grounded using pestle and mortar. The samples were labeled S1, S2, S3, S4, S5, and S6 for 1, 2, 3, 4, 5, and 6 h reaction time periods, respectively. The preparation of Cu@ZnO nanocomposites is shown in Figure 1.

4.2. Characterization. XRD patterns were recorded for the synthesized composite materials using Rigaku smartlab in the 2θ range of $20-80^{\circ}$. The surface morphology of the materials was analyzed using FEI quanta FEG 200 SEM. XPS measurements were done using an Omicron ECA probe spectrometer, with polychromatic Mg K α X-rays ($h\nu$ = 1253.6 eV). ESR analyses of the Cu@ZnO nanocomposites were analyzed for oxygen vacancies and conduction band electrons using JEOL model JES FA200. MALDI-TOF analysis was done using a Bruker ultraflextreme equipped with a pulsed IR laser, with 337 nm wavelength; the mass spectra of the Cu@ZnO nanocomposite materials were recorded in the positive reflection mode. A high resolution TEM (HRTEM, JEOL model 3010) was used for the identification of cluster formation and to measure the lattice distance. The band gap of the composite materials was measured using Jasco 650 along with the spherical accessory using the principles of DRS.

4.3. Bactericidal Test. 4.3.1. Well Diffusion Method. To study the bactericidal effect of synthesized Cu@ZnO nanocomposites (S1, S2, and S3) against Gram-negative *E. coli* ATCC-25922 and Gram-positive *B. cereus* ATCC-10876, a loopful of these were inoculated in liquid medium and incubated at 35 ± 2 °C for 16-18 h. Around 200 μ L of these young cultures was spread on nutrient agar plates. Wells of 4 mm were bored with a cork borer under sterile conditions. The Cu@ZnO nanocomposites were dispersed in presterilized deionized water by ultrasonication. Around 25 μ L of these samples was added to the wells, which contained different concentrations of 12.5, 25, 37.5, and 50 mg/mL of nanocomposites and were incubated at 35 ± 2 °C. The plates were observed for formation of the zone of clearance.

4.3.2. Liquid Broth Method. To examine the bacterial growth rate and to determine the growth curve in the presence of Cu@ZnO nanocomposites, a freshly grown axenic culture of *E. coli* (10^4-10^5 cells/mL) was inoculated into flasks containing the liquid nutrient medium supplemented with different concentrations of nanocomposites ranging from 100 to 200 μ g/mL. These flasks were incubated at 35 ± 2 °C at 150 rpm in an orbital shaking incubator. The total volume of the liquid medium in each flask was kept at 50 mL. All experiments were performed in triplicate under sterile conditions. Samples were taken periodically (at regular intervals of 4 h) from the flasks to measure optical density at a wavelength of 600 nm, using UV-vis spectrophotometer. A similar experiment was carried out with *B. cereus* except that the concentration of the nanocomposites ranged from 25 to 100 and up to 200 μ g/mL.

4.3.3. SEM Analysis. Bacterial cells treated with and without Cu@ZnO nanocomposites were collected by centrifugation at 10 000 rpm for 10 min. The bacterial cell biomass was fixed with 0.25% glutaraldehyde and subsequently treated with increasing gradients of ethanol for fixing on aluminum stubs, which were air dried in desiccators before coating with a thin layer of gold. Samples were analyzed by SEM (FEI Quanta FEG 200).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b00214.

XPS survey spectrum, SEM images, MALDI-TOF spectra, UV–DRS spectra of Cu@ZnO nanocomposites, and inhibition effect of bacterial agents (PDF)

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P.T.M. planned and supervised the overall project. P.K. and Y.R. contributed by preparing and characterizing the materials. Y.K. conducted biocidal activity of the materials and wrote that part of the manuscript. P.T.M. and P.K. analyzed the data and wrote the manuscript. All authors discussed, commented, and have given approval to the final version of the article.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Sreeprasad, T. S.; Pradeep, T. In *Handbook of Nanomaterials*; Vajtai, R., Ed.; Springer: Berlin, 2013; Chapter 9, pp 303–388.

(2) Rao, C. N. R.; Müller, A.; Cheetham, A. K. Nanomaterials – An Introduction. In *The Chemistry of Nanomaterials: Synthesis, Properties* and Applications; Rao, C. N. R., Müller, A., Cheetham, A. K., Eds.; Wiley-VCH: Weinheim, Chichester, 2004; Vol. 1, Chapter 1, pp 1–11. (3) Hokita, Y.; Kanzaki, M.; Sugiyama, T.; Arakawa, R.; Kawasaki, H. High-Concentration Synthesis of Sub-10-nm Copper Nanoparticles for Application to Conductive Nanoinks. ACS Appl. Mater. Interfaces 2015, 7, 19382–19389.

(4) Zin, V.; Agresti, F.; Barison, S.; Colla, L.; Gondolini, A.; Fabrizio, M. The Synthesis and Effect of Copper Nanoparticles on the Tribological Properties of Lubricant Oils. *IEEE Trans. Nanotechnol.* **2013**, *12*, 751–759.

(5) Garg, J.; Poudel, B.; Chiesa, M.; Gordon, J. B.; Ma, J. J.; Wang, J. B.; Ren, Z. F.; Kang, Y. T.; Ohtani, H.; Nanda, J.; McKinley, G. H.; Chen, G. Enhanced thermal conductivity and viscosity of copper nanoparticles in ethylene glycol nanofluid. *J. Appl. Phys.* **2008**, *103*, No. 074301.

(6) Isomura, Y.; Narushima, T.; Kawasaki, H.; Yonezawa, T.; Obora, Y. Surfactant-free single-nano-sized colloidal Cu nanoparticles for use as an active catalyst in Ullmann-coupling reaction. *Chem. Commun.* **2012**, *48*, 3784–3786.

(7) Betancourt-Galindo, R.; Reyes-Rodriguez, P. Y.; Puente-Urbina, B. A.; Avila-Orta, C. A.; Rodríguez-Fernández, O. S.; Cadenas-Pliego, G.; Lira-Saldivar, R. H.; García-Cerda, L. A. Synthesis of Copper Nanoparticles by Thermal Decomposition and Their Antimicrobial Properties. J. Nanomater. 2014, 2014, No. 980545.

(8) Kobayashi, Y.; Shirochi, T.; Yasuda, Y.; Morita, T. Preparation of Metallic Copper Nanoparticles by Reduction of Copper Ions in Aqueous Solution and Their Metal-Metal Bonding Properties. *Int. J. Chem., Mol., Nucl., Mater., Metall. Eng.* **2013**, *7*, 769–772.

(9) Anker, J. N.; Hall, W. P.; Lyandres, O.; Shah, N. C.; Zhao, J.; Van Duyne, R. P. Biosensing with plasmonic nanosensors. *Nat. Mater.* **2008**, *7*, 442–453.

(10) Schurig, D.; Mock, J. J.; Justice, B. J.; Cummer, S. A.; Pendry, J. B.; Starr, A. F.; Smith, D. R. Metamaterial Electromagnetic Cloak at Microwave Frequencies. *Science* **2006**, *314*, 977–980.

(11) Pendry, J. B. Negative Refraction Makes a Perfect Lens. *Phys. Rev. Lett.* **2000**, 85, 3966–3969.

(12) Pissuwan, D.; Valenzuela, S. M.; Miller, C. M.; Cortie, M. B. A Golden Bullet? Selective Targeting of *Toxoplasma gondii* Tachyzoites Using Antibody-Functionalized Gold Nanorods. *Nano Lett.* **2007**, *7*, 3808–3812.

(13) Atwater, H. A.; Polman, A. Plasmonics for improved photovoltaic devices. *Nat. Mater.* **2010**, *9*, 205–213.

(14) Bochenkov, V.; Baumberg, J.; Noginov, M.; Benz, F.; Aldewachi, H.; Schmid, S.; Podolskiy, V.; Aizpurua, J.; Lin, K.; Ebbesen, T.; Kornyshev, A. A.; Hutchison, J.; Matczyszyn, K.; Kumar, S.; de Nijs, B.; Rodriguez Fortuno, F.; Hugall, J. T.; de Roque, P.; van Hulst, N.; Kotni, S.; Martin, O.; Garcia de Abajo, F. J.; Flatte, M.; Mount, A.; Moskovits, M.; Ginzburg, P.; Zueco, D.; Zayats, A.; Oh, S.-H.; Chen, Y.; Richards, D.; Belardini, A.; Narang, P. Applications of plasmonics: general discussion. *Faraday Discuss.* **2015**, *178*, 435–466.

(15) Feldheim, D. L.; Foss, C. A., Jr. Overview. In *Metal Nanoparticles* - *Synthesis, Characterization and Applications*; Feldheim, D. L., Foss, C. A., Jr., Eds.; Dekker: Basel, Switzerland, 2002; Chapter 1, pp 1–16.

(16) Bigall, N. C.; Eychmüller, A. Synthesis of noble metal nanoparticles and their non-ordered superstructures. *Philos. Trans. R. Soc., A* **2010**, *368*, 1385–1404.

(17) Wen, J.; Li, J.; Liu, S.; Chen, Q.-y. Preparation of copper nanoparticles in a water/oleic acid mixed solvent via two-step reduction method. *Colloids Surf.*, A 2011, 373, 29–35.

(18) Liu, Q.-m.; Zhou, D.-b.; Yamamoto, Y.; Ichino, R.; Okido, M. Preparation of Cu nanoparticles with NaBH4 by aqueous reduction method. *Trans. Nonferrous Met. Soc. China* **2012**, *22*, 117–123.

(19) Mott, D.; Galkowski, J.; Wang, L.; Luo, J.; Zhong, C.-J. Synthesis of Size-Controlled and Shaped Copper Nanoparticles. *Langmuir* **2007**, *23*, 5740–5745.

(20) Salavati-Niasari, M.; Davar, F.; Mir, N. Synthesis and characterization of metallic copper nanoparticles via thermal decomposition. *Polyhedron* **2008**, *27*, 3514–3518.

(21) Nishida, N.; Miyashita, A.; Tsukuda, T.; Tanaka, H. Production of Oxidation-resistant Copper Nanoparticles on Carbon Nanotubes by Photoreduction. *Chem. Lett.* **2013**, *42*, 168–170.

(22) Kazuma, E.; Yamaguchi, T.; Sakai, N.; Tatsuma, T. Growth behaviour and plasmon resonance properties of photocatalytically deposited Cu nanoparticles. *Nanoscale* **2011**, *3*, 3641–3645.

(23) Dhas, N. A.; Raj, C. P.; Gedanken, A. Synthesis, Characterization, and Properties of Metallic Copper Nanoparticles. *Chem. Mater.* **1998**, *10*, 1446–1452.

(24) Yeh, M.-S.; Yang, Y.-S.; Lee, Y.-P.; Lee, H.-F.; Yeh, Y.-H.; Yeh, C.-S. Formation and Characteristics of Cu Colloids from CuO Powder by Laser Irradiation in 2-Propanol. *J. Phys. Chem. B* **1999**, *103*, 6851–6857.

(25) Shah, M.; Fawcett, D.; Sharma, S.; Tripathy, K. S.; Poinern, E. G. Green Synthesis of Metallic Nanoparticles via Biological Entities. *Materials* **2015**, *8*, 7278–7308.

(26) Qi, L.; Ma, J.; Shen, J. Synthesis of Copper Nanoparticles in Nonionic Water-in-Oil Microemulsions. J. Colloid Interface Sci. 1997, 186, 498–500.

(27) Park, B. K.; Jeong, S.; Kim, D.; Moon, J.; Lim, S.; Kim, J. S. Synthesis and size control of monodisperse copper nanoparticles by polyol method. *J. Colloid Interface Sci.* **2007**, *311*, 417–424.

(28) Jamali Sheini, F.; Singh, J.; Srivasatva, O. N.; Joag, D. S.; More, M. A. Electrochemical synthesis of Cu/ZnO nanocomposite films and their efficient field emission behaviour. *Appl. Surf. Sci.* **2010**, *256*, 2110–2114.

(29) Chan, G. H.; Zhao, J.; Hicks, E. M.; Schatz, G. C.; Van Duyne, R. P. Plasmonic Properties of Copper Nanoparticles Fabricated by Nanosphere Lithography. *Nano Lett.* **2007**, *7*, 1947–1952. (30) Ryasnyansky, A. I.; Palpant, B.; Debrus, S.; Khaibullin, R. I.; Stepanov, A. L. Nonlinear optical properties of copper nanoparticles synthesized in indium tin oxide matrix by ion implantation. *J. Opt. Soc. Am. B* **2006**, *23*, 1348–1353.

(31) Scheiber, I.; Dringen, R.; Mercer, J. F. B. Copper: Effects of Deficiency and Overload. In *Interrelations between Essential Metal Ions and Human Diseases*; Sigel, A.; Sigel, H.; Sigel, R. K. O., Eds.; Springer: Netherlands, Dordrecht, 2013; pp 359–387.

(32) Nordberg, G. F.; Fowler, B. A.; Nordberg, M.; Friberg, L. T. Introduction—General Considerations and International Perspectives. In *Handbook on the Toxicology of Metals*, 3rd ed.; Nordberg, G. F., Fowler, B. A., Nordberg, M., Eds.; Academic Press: Burlington, 2007; Chapter 1, pp 1–9.

(33) Hsieh, L.; Amin, A. Antimicrobial Stewardship: Hospital Strategies to Curb Antibiotic Resistance A2 - Kon, Kateryna. In *Antibiotic Resistance*; Rai, M., Ed.; Academic Press, 2016; Chapter 1, pp 1–18.

(34) Stoimenov, P. K.; Klinger, R. L.; Marchin, G. L.; Klabunde, K. J. Metal Oxide Nanoparticles as Bactericidal Agents. *Langmuir* **2002**, *18*, 6679–6686.

(35) Ramyadevi, J.; Jeyasubramanian, K.; Marikani, A.; Rajakumar, G.; Rahuman, A. A. Synthesis and antimicrobial activity of copper nanoparticles. *Mater. Lett.* **2012**, *71*, 114–116.

(36) WHO/FAO/IAEA. Trace Elements in Human Nutrition and Health; World Health Organization: Geneva, 1996.

(37) Colon, G.; Ward, B. C.; Webster, T. J. Increased osteoblast and decreased Staphylococcus epidermidis functions on nanophase ZnO and TiO2. *J. Biomed. Mater. Res., Part A* **2006**, *78*, 595–604.

(38) Yang, H.; Liu, C.; Yang, D.; Zhang, H.; Xi, Z. Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: the role of particle size, shape and composition. *J. Appl. Toxicol.* **2009**, *29*, 69–78.

(39) Xia, T.; Kovochich, M.; Liong, M.; Mädler, L.; Gilbert, B.; Shi, H.; Yeh, J. I.; Zink, J. I.; Nel, A. E. Comparison of the Mechanism of Toxicity of Zinc Oxide and Cerium Oxide Nanoparticles Based on Dissolution and Oxidative Stress Properties. *ACS Nano* 2008, *2*, 2121–2134.

(40) Nagarajan, P.; Rajagopalan, V. Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. *Sci. Technol. Adv. Mater.* **2008**, *9*, No. 035004.

(41) Zhang, L.; Ding, Y.; Povey, M.; York, D. ZnO nanofluids – A potential antibacterial agent. *Prog. Nat. Sci.* **2008**, *18*, 939–944.

(42) Li, M.; Zhu, L.; Lin, D. Toxicity of ZnO Nanoparticles to *Escherichia coli*: Mechanism and the Influence of Medium Components. *Environ. Sci. Technol.* **2011**, *45*, 1977–1983.

(43) Hajipour, M. J.; Fromm, K. M.; Akbar Ashkarran, A.; Jimenez de Aberasturi, D.; Larramendi, I. R. d.; Rojo, T.; Serpooshan, V.; Parak, W. J.; Mahmoudi, M. Antibacterial properties of nanoparticles. *Trends Biotechnol.* **2012**, *30*, 499–511.

(44) Bhuyan, T.; Khanuja, M.; Sharma, R.; Patel, S.; Reddy, M. R.; Anand, S.; Varma, A. A comparative study of pure and copper (Cu)doped ZnO nanorods for antibacterial and photocatalytic applications with their mechanism of action. *J. Nanopart. Res.* **2015**, *17*, No. 288.

(45) Gupta, R.; KrishnaRao Eswar, N.; Modak, J. M.; Madras, G.
Visible light driven efficient N and Cu co-doped ZnO for photoinactivation of *Escherichia coli. RSC Adv.* 2016, *6*, 85675–85687.
(46) Yu, Q.; Xu, X.; Wang, C.; Ma, Y.; Hui, D.; Zhou, Z. Remarkably improvement in antibacterial activity by synergistic effect in n-Cu@T-

ZnO nanocomposites. Composites, Part B 2017, 110, 32–38.

(47) Schmahl, N. G.; Eikerling, G. F. Über Kryptomodifikationen des Cu(II)-Oxids. *Z. Phys. Chem.* **1968**, *62*, 268.

(48) Afzal, M.; Butt, P. K.; Ahmad, H. Kinetics of thermal decomposition of metal acetates. *J. Therm. Anal.* **1991**, *37*, 1015–1023. (49) Lin, Z.; Han, D.; Li, S. Study on thermal decomposition of copper(II) acetate monohydrate in air. *J. Therm. Anal. Calorim.* **2012**, *107*, 471–475.

(50) http://xpssimplified.com/elements/copper.php.

(51) Drouilly, C.; Krafft, J.-M.; Averseng, F.; Casale, S.; Bazer-Bachi, D.; Chizallet, C.; Lecocq, V.; Vezin, H.; Lauron-Pernot, H.; Costentin,

2534

G. ZnO Oxygen Vacancies Formation and Filling Followed by in Situ Photoluminescence and in Situ EPR. *J. Phys. Chem. C* 2012, *116*, 21297–21307.

(52) McCluskey, M. D.; Jokela, S. J. Defects in ZnO. J. Appl. Phys. 2009, 106, No. 071101.

(53) Raju, Y.; Krishnamurthi, P.; Manoharan, P. T., unpublished work.

(54) Bardeen, J. Surface States and Rectification at a Metal Semi-Conductor Contact. *Phys. Rev.* **1947**, *71*, 717–727.

(55) Tung, R. T. Formation of an electric dipole at metalsemiconductor interfaces. *Phys. Rev. B* 2001, *64*, No. 205310.

(56) Sirelkhatim, A.; Mahmud, S.; Seeni, A.; Kaus, N. H. M.; Ann, L. C.; Bakhori, S. K. M.; Hasan, H.; Mohamad, D. Review on Zinc Oxide Nanoparticles: Antibacterial Activity and Toxicity Mechanism. *Nano-Micro Lett.* **2015**, *7*, 219–242.

(57) Raffi, M.; Mehrwan, S.; Bhatti, T. M.; Akhter, J. I.; Hameed, A.; Yawar, W.; ul Hasan, M. M. Investigations into the antibacterial behavior of copper nanoparticles against *Escherichia coli. Ann. Microbiol.* **2010**, *60*, 75–80.

(58) Karlsson, H. L.; Cronholm, P.; Hedberg, Y.; Tornberg, M.; De Battice, L.; Svedhem, S.; Wallinder, I. O. Cell membrane damage and protein interaction induced by copper containing nanoparticles— Importance of the metal release process. *Toxicology* **2013**, *313*, 59–69.

(59) VanWinkle, B. A.; De Mesy Bentley, K. L.; Malecki, J. M.; Gunter, K. K.; Evans, I. M.; Elder, A.; Finkelstein, J. N.; Oberdörster, G.; Gunter, T. E. Nanoparticle (NP) uptake by type I alveolar epithelial cells and their oxidant stress response. *Nanotoxicology* **2009**, *3*, 307–318.

(60) Ramyadevi, J.; Jeyasubramanian, K.; Marikani, A.; Rajakumar, G.; Rahuman, A. A. Synthesis and antimicrobial activity of copper nanoparticles. *Mater. Lett.* **2012**, *71*, 114–116.

(61) Ruparelia, J. P.; Chatterjee, A. K.; Duttagupta, S. P.; Mukherji, S. Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta Biomater.* **2008**, *4*, 707–716.