Photocatalytic antibacterial activity of copper-based nanoparticles under visible light illumination

Zong-Yan Wu¹, Hairus Abdullah¹,², Dong-Hau Kuo¹*

¹Department of Materials Science and Engineering, National Taiwan University of Science and Technology, Taiwan
²Department of Industrial Engineering, Universitas Prima Indonesia, Indonesia

E-mail: *dhkuo@mail.ntust.edu.tw

Abstract. Copper oxide and sulfide nanoparticles after annealing treatment at 400 ºC have been characterized and tested for their bactericidal properties toward Staphylococcus aureus and Escherichia coli under the dark and LED light illuminated conditions. It was found that the nanoparticles with the formation of CuS/Cu₂S/CuO nanostructures exhibited a great capability of killing Staphylococcus aureus and Escherichia coli with or without light illumination. The antibacterial activity of the nanoparticles was demonstrated and simply observed with colony counting method. A mechanism of the antibacterial behaviour had been proposed and elucidated in this work.

1. Introduction
Antibiotics have been mainly used in human life for several decades since 1940s. The utilization of antibiotics has been widely known in many applications such as curing for infectious diseases, combating cancer, compromising with immune system to protect human from cancer, preventing diseases, etc. However, the more the antibiotics used, the more possibility the related bacteria will be resistant to it. The mechanisms to protect bacteria from disruptive effect of antibiotics would be developed by altering the antibiotic target sites or metabolism pathway to reduce the drug accumulation from cell. For the illustration of the antibiotic resistance or inactivation of antibiotic mechanism, the beta-lactamase enzymes as product of bacteria can cleave the beta lactam ring, therefore the beta lactam antibiotics such as penicillin will be neutralized. Salmonella aureus adapted this mechanism or other resistance mechanism to dramatically reduce the antibacterial effects of large classes of traditional antibiotics. To overcome the inactivation of antibiotic mechanism, the works on improving antibiotics need to be constantly carried out. The pathogen resistance against antibiotic medication has appeared as a critical problem. To overcome this medication problem, nanoparticle application to kill bacteria via a mechanism to cause cell membrane rupture could be one of the great methods.

The main mechanism of nanoparticles (NPs) to kill bacteria in the dark and the light illuminated conditions varies with different interactions between nanoparticle and bacteria membrane. Some proposed mechanisms are related to physical contact between nanoparticles and bacteria membrane, release of nanoparticle ions from its surfaces, and the enhanced specific surface area of nanoparticles as particle size decreases. The final result of the nanoparticle interaction causes a cell wall penetration or cell membrane damage. As claimed by many works, chemical interaction, particle shape, particle
size, and zeta potential are the most relevant variables to affect antibacterial activities. The other advanced photochemical interaction between nanoparticles and bacteria cell membrane has been proved with our previous works and many other works.\textsuperscript{1-3, 6, 7} Those works had proposed that the radical species which formed during photo reactions on catalyst surfaces played important roles to disrupt bacteria cell membrane. It has been proposed that active species such OH\textsuperscript{–}, O\textsubscript{2}\textsuperscript{–}, and H\textsubscript{2}O\textsubscript{2}, known as reactive oxygen species (ROS),\textsuperscript{8} would be formed during the photocatalytic bactericide process. The produced ROS would permeate and induce the cell wall rupture as a further effect of cell membrane after an oxidation reaction.

To find new materials with low cost, durable, cost effective, and efficient antibacterial agents is considerably required. Many works\textsuperscript{1-5, 9-11} revealed nanoparticle semiconductors were great materials to kill bacteria via the photocatalytic mechanism. It is absolutely a different mechanism to inactivate bacteria compared to traditional one. The bactericidal property of nanoparticle semiconductor was regardless of the bacterial resistance mechanism as mentioned above. To the best of our knowledge, once the bacteria can be deactivated with photocatalytic mechanism, no resistance pathway can be developed by bacteria to protect themselves. Therefore, works on developing nanoparticle semiconductors as antimicrobial agents are considerably prospective for future medication. Li et al reported that Ag NPs and Ag\textsuperscript{2+} had strong antimicrobial properties against four bacteria strains, E. coli ATCC 8739, Pseudomonas aeruginosa ATCC 9027, Salmoneella aureus ATCC 6538, and Salmonella epidermidis ATCC 12228.\textsuperscript{12} However, high cost of silver has caused a drawback in large scale industrial application. The other attempts to minimize the utilization of silver without decreasing its property for antibacterial application had been carried out with other metal incorporation.\textsuperscript{2, 13-15} The antibacterial application for NPs could be used in large scale, if the materials involved in their design were not precious metals. Therefore, to progress the utilization of nanoparticles in antibacterial work, materials with the characteristic of low cost, abundance in chemical, high efficiency, low toxicity, and facile powder processing are required. It was found copper sulfides and oxides were promising for further investigation to enhance their antibacterial behavior.\textsuperscript{1, 14}

Based on our previous work\textsuperscript{1} on SiO\textsubscript{2}/CuO for antibacterial application, this work simply focused on improving the antibacterial effect of copper oxide and sulfide NPs with annealing treatments at 400 °C for 3 h. The aim of this work is to indicate copper-based materials with low cost and toxicity are promising to be further improved for antibacterial application. The as-prepared CuO and commercially available CuS materials were tested toward Staphylococcus aureus (S.aureus) and Escherichia coli (E.coli) bacteria for respectively representing gram positive and negative strains with initial bacterial concentration of 10\textsuperscript{8} CFU/mL under the dark and the 20 W LED light illuminated conditions. The bactericidal behaviors of CuO and CuS NPs were demonstrated and elucidated in this work.

2. Experimental procedure

2.1. Materials
All materials in this work were used as received without any further purification treatment.

2.2. Synthesis of copper-based nanoparticles
To synthesize CuO NPs, 4.8 g Cu(NO\textsubscript{3})\textsubscript{2} \cdot 2.5 H\textsubscript{2}O was first dissolved in 300 mL distilled (DI) water by stirring for 30 min, followed by slowly adding 0.8 g NaOH (200 mL DI water) into the solution. The solution was stirred for 2 h at room temperature. The obtained precipitate was washed for three times with alcohol and dried in a rotary evaporator. To form copper oxide NPs, the as-prepared powder was annealed at 400 °C for 3 h in air. The other CuS nanoparticle powder was commercially obtained and annealed with the same condition.

2.3. Characterizations
The morphologies and microstructures of nanoparticles were examined by using field-emission scanning electron microscopy (FE-SEM, JSM 6500F, JEOL, Tokyo, Japan). Powder X-ray diffraction
(XRD) patterns of nanoparticles were recorded by Bruker D2-phaser diffractometer using Cu Kα radiation with a wavelength of 1.5418 Å.

2.4. Luria Bertani (LB) broth and agar preparation
LB broth and LB agar was prepared with 10 g tryptone, 5 g yeast extract, and 10 g NaCl in 1000 mL DI water under vigorous stirring for 1 h. During the preparation, the solution pH was maintained at 7–7.4. To remove all the possible contamination, the as-prepared LB broth solution was heated using autoclave for 20 min with the pressure and temperature were set to 15 psi and 121 °C, respectively. Finally, the LB agar was prepared with the sterilized LB solution by adding 1.5 wt% agar powder. After agar powder was totally dissolved, LB solution will change to solid medium at room temperature. The as-prepared LB broth and agar medium were kept at 4 °C for future use.

2.5. Antibacterial experiment
The bactericidal effects of as-prepared CuO and CuS were carefully examined by a simple colony counting method. S. aureus and E. coli with the concentration of $10^8$ cfu/mL was prepared and used in this work. To examine the bactericide effects, 3 mg of as-prepared nanoparticles was dispersed in 1 mL bacterial solution and illuminated with 20 watt LED lamp at a distance of 30 cm for 3 h or without light illumination under a constant shaking. The intensity of LED lamp is 12 W/m² as measured by photometer. An aliquot of 0.1 mL was taken hourly and spread uniformly on LB agar medium. The LB agar medium was then kept with face-down at 37 °C for 12 h to cultivate the bacteria. The amount of bacterial colonies grown on the agar medium were counted to simply determine the bactericidal abilities of as-prepared nanoparticles.

3. Results and discussion

3.1. X-ray diffraction (XRD) pattern analysis
All the as-prepared and commercially received nanoparticle powders were examined with XRD measurements to determine their phases before and after annealing process. Figure 1 shows the XRD patterns of CuO and CuS before and after annealing at 400 °C.
As shown in Figure 1, Cu(OH)₂ phase (PDF#420746) instead of CuO was formed during the chemical synthesis, however CuO phase (PDF#65-2309) was shown up after annealing process. The XRD peaks of commercial CuS nanoparticles after annealing process showed many major phases of CuS, Cu₂S, and CuO which possibly formed nanoheterostructure particles. The peaks of sulfur in nanoparticle were also appeared due to the effect of 400 °C annealing that induced the liberation of sulfur from CuS NPs. Field emission scanning electron microscopy (FE-SEM) analysis was carried out to examine the morphology of the nanoparticles before and after annealing. Figure 2 shows the nanoparticles of CuO and CuS with the average particle sizes of 100–300 nm and 200–1000 nm, respectively.

Figure 1. XRD patterns of (a) Cu(OH)₂ and (b) CuS before and after annealing at 400 °C and (c) phase identification of CuS after annealed at 400 °C.

Figure 2. FE-SEM images of (a,b) CuO and (c,d) CuS nanoparticles (a,c) before and (b,d) after annealing process.
All the copper-based nanoparticles were tested their antibactericidal properties toward *S. aureus* and *E. coli* with or without light illumination for 3 h, as shown in Figure 3 and 4. The amounts of bacteria colonies grown on agar medium were calculated and summarized in Table 1 and 2. The more amount of bacteria grown on agar medium indicated the worse antibacterial property of the tested nanoparticles. The antibacterial testing results showed the best nanoparticle for simultaneously killing *S. aureus* and *E. coli* was CuS after annealed at 400 °C under LED light illumination. The great bactericide property was related to the nanoheterostuctures formation between Cu$_2$S, CuO, and CuS which formed during annealing process and induced the efficient photo generated electron and hole separation to proceed the formation of ROS which was considerably toxic to bacteria. The observable ability to kill bacteria in dark condition was mainly related to the dissolution of copper ions into solution or to the existing photo carriers which could proceed the photo reaction to oxidize cell membrane of bacteria$^1$.

**Table 1.** Amounts of *E. coli* bacteria cultivated on agar media for each hour after antibacterial experiments as shown in Figure 3.

| Nanoparticles in different condition | *E. coli* amount (cfu) |
|-------------------------------------|------------------------|
|                                     | 1 h     | 2 h     | 3 h     |
| Cu(OH)$_2$ - Dark                   | 3,200   | 0       | 0       |
| Cu(OH)$_2$ – LED Light             | 1320    | 0       | 0       |
| CuS - Dark                          | > 10$^7$| > 10$^7$| > 10$^7$|
| CuS - LED Light                    | > 10$^7$| > 10$^7$| > 10$^7$|
| CuO (annealing at 400 °C) - Dark   | 23540   | 20910   | 19920   |
| CuO (annealing at 400 °C) - LED Light| 21,980  | 20,050  | 8,580   |
| CuS (annealing at 400 °C) - Dark   | 3,280   | 70      | 0       |
| CuS (annealing at 400 °C) - LED Light| 20      | 0       | 0       |
Figure 3. Agar media for E. coli cultivation after exposure to Cu(OH)$_2$, CuS, 400 °C-annealed CuO, and 400 °C-annealed CuS NPs for 1 to 3 h under the dark and LED illuminated conditions.

Table 2. Amounts of S. Aureus bacteria cultivated on agar media for each hour after antibacterial experiments as shown in Figure 4.

| Nanoparticles in different condition | S. aureus amount (cfu) |
|-------------------------------------|------------------------|
|                                     | 1 h | 2 h | 3 h |
| Cu(OH)$_2$ - Dark                   | 13,890 | 370 | 0 |
| Cu(OH)$_2$ – LED Light              | 70 | 40 | 0 |
| CuS - Dark                          | > 10 7 | > 10 7 | > 10 7 |
| CuS - LED Light                    | > 10 7 | > 10 7 | > 10 7 |
| CuO (annealing at 400 °C) - Dark   | > 107 | > 107 | > 107 |
| CuO (annealing at 400 °C) - LED Light | > 107 | > 107 | > 107 |
| CuS (annealing at 400 °C) - Dark   | 23,560 | 70 | 0 |
| CuS (annealing at 400 °C) - LED Light | 20 | 0 | 0 |
Figure 4. Agar media for *S. aureus* cultivation after exposure to Cu(OH)$_2$, CuS, 400 °C-annealed CuO, and 400 °C-annealed CuS NPs for 1 to 3 h under the dark and LED illuminated conditions.

4. Conclusions
The great photocatalytic bactericidal properties of copper-based NPs were found for the CuS/Cu$_2$S/CuO nanoheterostructure which induced efficient photo carrier separation to further provide a photo reaction to form ROS leading to rupture cell membrane of bacteria. Copper-based NPs with the characteristics of low cost, abundance in chemical, high efficiency, low toxicity, and facile powder processing were found as prospective materials to combat bacterial infected diseases.

References
[1] Hou YX, Abdullah H, Kuo D H, Leu S J, Gultom N S and Su C H 2018 Compos. B Eng. 133 166.
[2] Chuang K T, Abdullah H, Leu S J, Cheng K B, Kuo D H, Chen H C, Chien J H and Hu W T 2017 J Photochem. Photobiol. A: Chem. 337 151.
[3] Seil J T and Webster T J 2012 Int. J. Nanomedicine 7 2767.
[4] Shahidi S, Rashidian M and Dorranian D 2017 Opt. Laser Technol. 99 145.
[5] Ioniță M, Vlăsceanu G M, Watzhawek A A, Voicu S I, Burns J S and Iovu H 2017 Compos. B Eng. 121, 34.
[6] Malato S, Maldonado M I, Fernández-Ibáñez P, Oller I, Polo I and Sánchez-Moreno R 2016 Mat. Sci. Semicond. Process. 42, 15.
[7] Scuderi V, Buccheri M A, Impellizzeri G, Di Mauro A, Rappazzo G, Bergum K, Svensson B G and Privitera V 2016 Mat. Sci. Semicond. Process. 42 32.
[8] Abdullah H, Gultom N S and Kuo D H 2017 New J. Chem. 41 12397.
[9] Goryunova N A, Anshon A V, Karpovich I A, Leonov E I and Orlov V M 1971 Phys. Status Solidi A2, K117.
[10] Mazhar ME, Faglia G, Baratto C, Comini E, Zappa D, Kumar R and Sberveglieri G 2014 Procedia Eng. 87 16.
[11] Wu C Y, Pan Z Q, Liu Z, Wang Y Y, Liang F X, Yu Y Q, Wang L and Luo L B 2017 J. Nanopart. Res. 19 35.
[12] Li W R, Sun T L, Zhou S L, Ma, Shi Q S, Xie X B and Huang X M 2017 Int. Biodeterior. Biodegradation 138 304.
[13] Guldiren D and Aydin S 2017 Mat. Sci. Eng. C 78 286.
[14] Yamamoto L M, Nunes J H B, Ribeiro M A, Ferreira A M d c, Lustri W R and Corbi P P 2017 Polyhedron 138 168.
[15] Fakhri A, Pourmand M, Khakpour R and Behrouz S 2015 J. Photochem. Photobiol. B: Biol. 149 78.

Acknowledgement
This work was supported by the Ministry of Science and Technology of Taiwan under Grant numbers MOST 105-2218-E-011-013.