Oligodynamic Cu-Zn composite fabricated by powder metallurgy method

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Abstract. The emergence of antimicrobial resistance is becoming a serious threat for the effective prevention from infection caused by bacteria. Materials such as silver, Copper, Zinc, ZnO, CuO, carbon and their composites have recently received much attention due to their oligodynamic properties. This work investigates the properties of Cu/Zn composite fabricated by powder metallurgy method, as oligodynamic materials. By varying the concentration of Zinc in composite, the effect on the samples have been elaborated using X-ray diffractometer (XRD), Optical Microscope and antibacterial activity test. XRD diffractogram shows structure and all unit cell parameter of the samples. The different unit cell parameter is likely due to pressure during samples preparation. The sample exhibit different surface morphology, before and after antibacterial activity test. All samples clearly show different antibacterial activity.

1. Introduction
The emergence of antimicrobial resistance is a serious problem for the effective prevention and treatment of infections caused by bacteria, viruses, fungi and parasites [1,2]. New antimicrobial materials are always searched to control infectious agents, which includes the design of new antimicrobial compounds, materials and drug-delivery vehicles [3, 4]. Oligodynamic effect has been known for decades, and there are several reports on the effective use of this for various applications. Copper is one of excellent material for biocide applications. Copper ions (Cu²⁺) or in copper complexes, have been used for long time to disinfect human tissues, solid or liquid. Nowadays, copper is often used as a water purifier, algaecide, fungicide, antibacterial and antifouling agent [5]. Research showed that by testing a set of metal surfaces, copper is the most effective in inhibiting bacterial viability [6, 7]. Several strains of Staphylococcus aureus were killed in less than 90 min, on the surface of copper. Particle size are important for effective bacterial activity reduction. It is found that copper nanoparticles (CNP) with size of 100 nm have better performance than silver nanoparticles with size of 40 nm against Escherichia coli and Bacillus subtilis [8]. Copper oxide with sizes ranging from 20 to 95 nm also showed activity against several bacterial pathogens, such as Escherichia coli and Staphylococcus aureus [9]. For copper nanoparticle, one of the possible processes is the attachment of metal ions or nanoparticles (NPs) to the outer cell wall of bacterium and then the protein precursor’s layer accumulates on it, which cause disables the proton motive force. Metallic NP could destabilize the outer membrane, following crack production in the membrane of plasma [10], thus reducing the process of metabolism. It destructs the ribosome subunit by t-RNA binding and thus finally induces the total collapse of biological mechanism. Previous researches on the oligodynamic effect of copper...
and silver have confirmed that metal ions are responsible for the inactivation of the bacteria. However, there seems to be no clear-cut explanation of this mechanism taking place within the cells of these microorganisms.

The ultimate goal of our research is to get oligodynamic composite Cu-Zn materials. The selection of Cu and Zn are made due to their ability act as antibacterial. The composition of the composite was varied.

2. Experimental Methods

2.1 Sample preparation

Copper (Merck,-100 mesh, 99.8%) and Zinc powder (Merck,-200 mesh, 99.999%) were placed in a stainless milling vial, along with three 12 g stainless balls, with a ball-to-powder mass ratio of 2. Argon atmosphere were introduced into vial before milling. Then the vial was sealed. The powder was mechanically mixed and alloyed using a SPEX8000 Mixer/Mill (Sytech Corp., Houston, TX) for up to 4 h for each sample. The compositions of the Cu-copper and Zn powders were Cu-10%wtZn, Cu-20%wtZn, Cu-38%wtZn, and Cu-45%wtZn which were chosen based on the Cu-Zn phase diagram. These pelletized powders were annealed at 400°C and non-annealed under Argon atmosphere.

2.2 Characterization

The phase structure was characterized using PAN analytical X-diffractometer (X’Pert Pro, Cu-Kα1, λ = 1.5405 Å) at room temperature. Bacterial activity resistance testing was conducted by Zone inhibition method. Samples were placed in agar as a media for growth of bacteria. Bacterial activity was observed using optical microscope and calculated using inhibition zone method. The clear area in agar indicates the zone without bacteria. The larger clear zone indicates the higher effectiveness of the composite as an oligodynamic material.

3. Results and Discussion

3.1 Phase and Structure

Figure 1 and figure 2 show X-RD pattern of non-annealed and annealed composite. After annealing, a portion of Cu transform into CuZn alloy lead to increase in crystal parameter a and c and then decrease (table 1). Table 1 shows the crystal parameter of Cu in the composite. The lattice constant increases from 3.614 to 3.691 Å. The volume is also increase from 47.201 to 50.290 Å³.

The pattern of the non-annealed composite shows no new phase appear as Zn content increase. But for annealed composite, it is quite clear that new phase appears such as cuprite (Cu₂O), CuZn, Cu₂₈Zn₁₂. The new phase was confirmed after refining the XRD pattern of Cu-10Zn and Cu45Zn composite (figure 3). The presence of new phase could change the oligodynamic effect of Cu, Zn and their composite.

Table 1. Crystal Parameter of the major phase of the composite

| Parameter | Cu     | Zn     | Cu10Zn | Cu20Zn | Cu38Zn | Cu45Zn |
|-----------|--------|--------|--------|--------|--------|--------|
| a=b (Å)  | 3.614  | 3.6228 | 3.6228 | 3.6436 | 3.6912 | 2.949  |
| c (Å)    | 3.614  | 4.9468 | 3.6228 | 3.6436 | 3.6912 | 2.949  |
| V (Å³)   | 47.2015| 30.3766| 47.5487| 48.37085| 50.2909| 25.6467|
| Space Group | F m -3 m | P 63/m m | F m -3 m | F m -3 m | F m -3m | F m -3 m |
| Rwp (%)  | 8.34   | 5.67   | 6.5    | 6.3    | 7.4    | 8.2    |
| GOF      | 2.3    | 3.2    | 2.5    | 3.2    | 2.5    | 3.2    |
3.2. Optical Microscope and Bacterial activity

Figure 4 shows the surface morphology of Cu-20Zn and Cu-45Zn with and without annealing under argon atmosphere. The Colour of the surface comes from the colour mixture of copper, cuprit, zinc oxide and probably brass (CuZn was confirmed from Rietveld refinement of the samples). Figure 5 shows antibacterial test using inhibition Zone method. By calculating the surface area of the inactive zone, the effectiveness of the antimicrobacterial could be predicted.

The results (figure 5, 6 dan table 2) show that the increase of Zn content lead to increase in inhibition area for non-annealed composite. But for annealed composite, the inhibition seems less effective than the non-annealed one. It can be concluded from XRD analysis that that cuprite and zinc oxide are the effective phase for killing the bacteria. Pure copper could be oxidized at room temperature into Cu₂O and CuO for further oxidation. Cu₂O could transform into CuO if oxygen present around the cuprite. It is possible that the oxygen comes from the environment of the cell and could lead to Oxygen deficiency, making the bacteria inactive or died. These phenomena called stress oxidative. The Zinc Oxide could also become oligodynamic material. If ZnO absorb UV, the electron from the valence band could be excited into the conduction band. The hole in the valence band could make a binding with OH⁻, producing radical which could harm the bacteria.
Figure 3. Rietveld refinement of Cu-20Zn (a) and Cu45Zn (b) composite after annealing at 400°C

Figure 4. Surface morphology of (a) Cu-10Zn and (b) Cu-45Zn composite, after oligodynamic
Figure 5. Inhibition Zone of (a) Cu, (b) Zn, (c) Cu-10Zn and (d) Cu-45Zn composite before annealing

Figure 6. Inhibition zone of (a) Cu, (b) Zn, (c) Cu-10Zn and (d) Cu-45Zn after annealing at 400°C

Table 2. The inhibition zone of the composite

| Composite       | Inhibition Zone (cm²) | Non-annealing | Annealed at 400°C |
|-----------------|-----------------------|---------------|-------------------|
| Cu              | 2.34                  | 2.51          |                   |
| Zn              | 6.36                  | 7.87          |                   |
| (Cu-10%wtZn)    | 7.41                  | 3.59          |                   |
| (Cu-20%wtZn)    | 7.81                  | 4.41          |                   |
| (Cu-38%wtZn)    | 9.15                  | 3.66          |                   |
| (Cu-45%wtZn)    | 9.60                  | 3.44          |                   |

4. Conclusions
Among the studied composites, the non-annealed Co-45Zn possesses the highest antibacterial effect. The resulting inhibition zones are mainly due to the contribution of cupric oxide phase. The oxidized cupric oxide produces CuO which makes bacteria inactive (stress oxidative). Annealed composite does not show good oligodynamic effect due to the existence of ZnO and CuO which cover the surface of the composite.

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