Evaluation of Reactive Oxygen Species (ROS) Generated on the Surface of Copper Using Chemiluminescence

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Abstract

The antibacterial activity of copper is well-known from an ancient civilization, however, its biocidal mechanism has not been necessarily elucidated. Notwithstanding up to now, mainly 4 processes have been proposed. Among them, it is cleared that 4 kinds of reactive oxygen species (ROS): hydroxyl radical ·OH, hydrogen peroxide H₂O₂, superoxide anion O₂⁻ and singlet oxygen O₁, play an important role for contact-killing of bacteria, viruses and fungi. In this paper, generation of ROS on the surfaces of copper plates heated from room temperature to 673 K for 4.2 × 10² s in air, was investigated using the chemiluminescence. ROS have been evaluated by selecting the most suitable scavengers, such as 2-propanol for ·OH, sodium pyruvate for H₂O₂, nitro blue tetrazolium for O₂⁻ and sodium azide NaN₃ for O₁. At the same time the outermost surface of copper, on which thin film of cuprous oxide Cu₂O was first formed and then cupric oxide CuO was laminated on Cu₂O, was examined by thin-film XRD and TEM analysis to estimate the amounts and kinds of copper oxides. It was found that the most amounts of ROS were obtained for the 573 K-heated Cu plate and they were composed of ·OH, H₂O₂, and O₂⁻.

Keywords

Copper, Microbial Activity, Reactive Oxygen Species, Chemiluminescence, Scavengers
1. Introduction

Copper and its alloys have been widely recognized from ancient to modern civilized societies as natural antibacterial materials [1], and they had been used as such as a water vessel, the building parts of shrine or temples, or a balustrade of bridge in Japan. Ancient civilizations utilized these antimicrobial properties long before the discovery of bacteria in modern civilization. Recently, the mechanism of antibacterial activity of copper and the related alloy has been much attracted again due to the worldwide disaster of Covid-19 [2]. Mainly 4 types of copper’s antibacterial activities have been proposed [3] [4]; 1) bacteriolysis, i.e., membrane breakdown; 2) membrane breakdown under stress; 3) DNA damages caused by reactive oxygen species (ROS) generated on the copper surface; 4) devolution and disaggregation of genome and or plasmid. These killing processes are multifaced with the main mechanism of bactericidal activity generated by ROS, which irreversibly brings damage of membranes and DNA chain in cell.

As far as oxygen concerned, there are 4 kinds of ROS [5], such as hydroxyl radical ·OH, hydrogen peroxide H₂O₂, superoxide anion ·O₂⁻ and singlet oxygen ¹O₂. Especially, ·OH reveals strong antibacterial activity due to its high reactivity which originates from high standard redox potential $E_o = 2.38 \text{ V}$, the second highest followed by active fluorine $E_o = 2.85 \text{ V}$ [6]. Reactive oxygen species are generated on the surface of metal copper and metal oxides such as zinc and titanium oxides too. The present authors have been publishing some papers of the antibacterial activity of ZnO [7] [8] [9] anatase TiO₂ [10], which activity can be sustained even in a dark condition (i.e., no sunlight). And further, a paper of anatase TiO₂ added Cu powders with improved antibiotic properties has been published recently [11]. Microscopically, the surface of metal copper is covered with very thin film of cuprous oxide Cu₂O and cupric oxide Cu₂O [12]. Therefore, ROS could be stated that its generation originates at the metal oxide surfaces [13].

Up to now, many ROS detection methods have been proposed [14]; electron spin resonance (ESR), fluorescence (FL), spectrophotometry, HPLC coupled with UV detection and chemiluminescence (CL). Among them, CL method has attracted much attention because of its unique advantages such as high sensitivity, instantaneity and simplicity of operation [15]. The detecting sensitivity much depends on the luminescence agents. Among emission agents, luminol is the most popular and has high sensitivity, therefore this agent is always used in a crime investigation. However, luminol has disadvantage, i.e., this emission is limited by pH value of aqueous solution which contains antibacterial substance. It is reported that the best pH value is about 11.0 [16]. Furthermore, identification of ROS can be performed by selecting the suitable scavengers for each ROS in CL measurement. Many reports concerning about scavengers used in CL measurement have been published [17] [18]: 1). alcohol [19], mannitol, potassium formate, ascorbic acid, DMSO, and phthalhydrazide [20] [21] for hydroxyl radi-
cal ⋅OH, 2) riboflavin [22] [23], pyruvic acid [17] [24] for hydrogen peroxide \( \text{H}_2\text{O}_2 \), 3) ((2-Methyl-6-(4-methoxyphenyl) imidazo [1,2-a] pyrazin-3(7H)-one, \( \text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2 \), in simple terms as abbreviated, “MPEC” [25] and nitro blue tetrazolium (in simple terms, “nbt”) [17] [18] [26] for superoxide anion \( \text{O}^−_2 \), and 4) 2,5-dimethylfuran, sodium azide “\( \text{NaN}_3 \)” [17], MnTBAP (manganese(III)-tetrakis (4-benzoic acid) for singlet oxygen \( ^1\text{O}_2 \). However, there are some features in each scavenger, for examples, its sensitivity, a combination of emission reagents, and pH dependence of efficiency. For examples, phthalhydrazide and riboflavin were incorrect deemed in this study, furthermore, n-butanol, which was reported to be the suitable scavenger for detecting hydroxyl radical, has been found to not suitable for CL method recently in our investigation. In our previous study, antibacterial activity of \( \text{ZnO} \), anatase \( \text{TiO}_2 \) and Cu powders have been investigated to understand its mechanism and improve the antimicrobial properties, available even in a dark condition for the former two.

Purpose of this study is to inquire what kind of and how amount of ROS are generated on the surface of metal copper, using the combination of light emission reagent and scavengers in CL measurement, and by observation of the outermost surface by TEM and identification of crystalline phases by thin-film XRD.

2. Experimental Procedure

2.1. Preparation of Bulk Cu Plates

Oxygen-free copper (99.99% purity, C1020) plate, a square shape in size \( 25 \times 25 \times 1.0 \text{ mm}^3 \) (Kikukawa Industry Co., Ltd, Tokyo, Japan), were used as starting material. These Cu plates, after wiping their surfaces with ethanol, were heated in air at 373, 473, 523, 573, 623, 673 K for \( 4.2 \times 10^2 \text{ s} \); heat treatment was performed in quick heating and cooling.

2.2. Evaluation

2.2.1. Chemiluminescence (CL) Intensity Measurement and Evaluation of Reactive Oxygen Species (ROS)

Chemiluminescence (CL) of Cu plate in a \( 2.5 \times 10^{-7} \text{ m}^3 \) (0.25 mL) aqueous luminol solution with a concentration of \( 5.0 \times 10^{-1} - 5.0 \times 10^{-6} \text{ mol·m}^{-3} \) (5.0 \( \times 10^{-4} - 5.0 \times 10^{-9} \text{ mol·L}^{-1} \)) mixed with \( 4.0 \times 10^{-6} \text{ m}^3 \) (4.0 mL) carbonic acid buffer solution (NaOH/NaHCO₃, \( \text{pH} = 10.8 - 10.9 \) [27] was observed under dark conditions using a CL detector (CLA-FS3, Tohoku Electronic Industrial Co., Ltd., Sendai, Japan). After dropping the luminol solution in a \( 6.0 \times 10^1 \text{ s} \) warming up of the detector, the intensity of CL was integrated between \( 6.1 \times 10^1 - 6.0 \times 10^2 \text{ s} \). The obtained summation of CL intensity for \( 5.4 \times 10^2 \text{ s} \) (9 min), SCL (9 min), which was calculated as follows: at first estimate summation of the background noise, SCL(BG), from 0 to \( 6.0 \times 10^1 \text{ s} \), and then summarize the CL intensity from \( 6.1 \times 10^1 \) to \( 6.0 \times 10^2 \text{ s} \), SCL (total 9 min), finally, true SCL (9 min) was determined to be as true SCL (9 min) = SCL (total 9 min) – SCL (BG) × 9. This true
SCL (9 min), in simple terms, SCL (9 min) value was utilized to evaluate the amount of ROS generated from the surface of Cu plate.

Among 4 kind of ROS, such as ·OH, H$_2$O$_2$, O$_2^−$, and 1O$_2$, scavengers, 2-propanol ("2-pro", for ·OH, Nacalai Tesque Chemicals, Kyoto, Japan) [28] [29] [30], sodium pyruvate [17] ("s-pyr", for H$_2$O$_2$, Fuji-film Wako Pure Chemical Co., Ltd., Osaka, Japan), nitro blue tetrazolium [31] ("nbt", for O$_2^−$, Nacalai Tesque Chemicals) and sodium azide [17] (for short "NaN$_3"", for 1O$_2$, Fuji-film Wako Pure Chemical) were mainly used to determine which ROS is generated. At first these scavengers were solved in the buffer solution into the concentration 5.0 × 10$^{-2}$ - 5.0 × 10$^{-6}$ mol·L$^{-1}$ and then obtained 0.25 mL or 0.50 mL scavenger solution were used. Each solution and a 4.0 mL pure buffer solution were mixed. CL measurement was performed as the same as CL intensity measurement, after a 6.0 × 10$^{1}$ s warming up of the detector, the intensity of CL was integrated between 6.0 × 10$^{1}$ - 6.0 × 10$^{2}$ s. The amount of difference (D) for each ROS was evaluated using the equation of

\[ D = SCL \text{ (9 min, without scavenger)} - SCL \text{ (9 min, with scavenger)} \] (1)

2.2.2. Physicochemical Property

Microstructural observation with a field emission-type scanning electron microscope (FE-SEM; SU8020, Hitachi High-Technologies Co., Ltd., Tokyo, Japan) and a transmission electron microscope (TEM, JEM-2100F, JEOL, Tokyo, Japan) equipped with the energy-dispersive X-ray spectroscopy (EDS, JED-2300T, JEOL) were performed. Then, the Cu samples were cut into a small specimen suitable for TEM observation using a focused ion beam (FIB, FB2200, Hitachi High-Technologies Co., Ltd.). Thin-film X-ray diffraction (XRD; Smartlab, Rigaku, Tokyo, Japan) analysis using CuKα radiation (wavelength of 0.15418 nm) was utilized for identification of the crystalline phases and evaluation of lattice parameter of thin-film copper oxides, Cu$_2$O and CuO. Measuring conditions were as follows: accelerating electric voltage and current were 45 kV and 200 mA, respectively, scanning speed 1°/min, scanning angle 2q: 20° - 80°, angle of incidence 0.5° using a parallel X-ray beam. Under these conditions, the penetration depth of X-ray was estimated to be around 200 nm from the outermost surface [31]. Reference intensity ratio (RIR) analysis [32] was utilized to determine the mass % of Cu$_2$O and CuO.

3. Results and Discussion

Figure 1 shows a brief diagram explaining the flowchart of experimental results.

3.1. CL Intensity of Heated Cu Plates and Their Surface Morphology

As described at 2.2.2 in 2. Experimental procedure, chemiluminescence (CL) of Cu plates was measured using the luminol solution. Figure 2 shows dependence of CL intensity of as-received Cu plate on the concentration of luminol solution.
Based on the previous experimental data [11], their concentrations were varied from $5.0 \times 10^{-4}$ to $5.0 \times 10^{-9}$ mol/L (M). Except for extraordinary high CL intensity curve obtained using luminol’s concentration of $5.0 \times 10^{-4}$ M, the CL intensity was decreased gradually with decreasing luminol’s concentration from $5.0 \times 10^{-5}$ to $5.0 \times 10^{-9}$ M. By considering the suitable intensity and linearity, the aqueous luminol solutions with concentration of $5.0 \times 10^{-5}$ or $5.0 \times 10^{-6}$ M were utilized in the present study. Then the Cu plates were rapidly heated for $4.2 \times 10^{2}$ s and quenched in air to form copper thin-film oxides.

**Figure 1.** A brief diagram explaining the flowchart of experimental results.

**Figure 2.** Dependence of CL intensity of as-obtained Cu plate on the concentration of luminol solution.
Figure 3 exhibits the CL curves of these heated Cu plates and the summation of CL intensity SCL (9 min) is displayed; here the luminol’s concentration of $5.0 \times 10^{-5}$ M was adopted. SCL (9 min) increased from $111.6 \times 10^3$ count, i.e., 111.6 k-count, to $736.6 \times 10^3$ k-count with increasing temperature up to 573 K and decreased rapidly to 48.5 k-count at 673 K, suggesting that SCL (9 min) depends much on the amount of copper oxides generated during the heat treatment in air and there might be the most suitable heating temperature. Luminol can emit light in pH value around 11.0 [16], then in order to eliminate pH effect we searched the light emission reagent which can perform in pH around 7. MPEC [25] is a reagent which can perform light emission in neutral pH, however, its intensity is very weak, so we used MPEC with the higher concentration than Luminol. Figure 4 shows SCL (9 min) of Cu plates prepared under various temperatures, which were determined using luminol (the concentration and the amount of instillation, $5.0 \times 10^{-5}$ M and $2.50 \times 10^{-7}$ m$^3$, 0.25 mL) and MPEC ($5.0 \times 10^{-1}$ M, 0.25 mL) under pH = 10.8 and 7.5 buffer solutions (4.0 mL), respectively. Both SCL (9 min) exhibited the highest value around 573 K; the best heating temperature is around 573 K. However, it was found that the top temperature sometimes shifted to the lower temperature, for example, 373 K or even room temperature, depending on the surface conditions of Cu plates as-received. Then, the surfaces of Cu plates heated at various temperatures were observed using an SEM under the magnification around 20,000 and compared. Figure 5 shows their images of various Cu plates; as-received (No. 1), and heated at 373 K (No. 2), 473 K (No. 3), 573 K (No. 4), and 673 K (No. 5) for $4.2 \times 10^2$ s in air. Among the Cu plates from as-received to 473 K, little change has been recognized, however, 573 K-heated Cu plate showed the granular surface. Furthermore, at 673 K a small amount of needle-like granular were observed. As the highest SCL (9 min) value was attained on the Cu plate heated at 573 K, the outermost surfaces of Cu plates heated at 50 K lower and higher than the top temperature (573 K) were observed using the high-resolution TEM. Figure 6 displays the cross-section images of their outermost surfaces, in the top of each photograph upper left gray portion is bulk Cu, along to the right lower direction white thin film and black mass were observed on the surface of bulk Cu. Here, the magnifications of 1) 523 K and 2) 573 K are the same (see the scale bar of 100 nm), however, the right 3) 623 K is a little lower magnification (see the scale bar of 200 nm) because of the thick oxide film of Cu$_2$O, which will be described latter. Here we notice that the heating temperature increased from 523 to 573 K, “island-shaped, nub” film was first formed and grew along the base; “a hetero growth thin film (Cu oxide film on metal Cu)” was observed. This growth might be caused by “Volmer-Weber mode” [33] mechanism, that is, in the case that the surface tension of grown substance (Cu$_2$O) is larger than adhesion force and poor wettablity between Cu$_2$O and Cu.

In the lower images with the higher magnification, the green line indicates the intensity of oxygen content along with a horizontal thin red line. As shown in Table 1, TEM observation gave the thickness of films about 40 to 100 nm for 1)
523 K and 2) 573 K samples, respectively, at 623 K-heated samples “non-uniform” layer. The former thickness values (40 and 100 nm) correspond well to those (40 and 100 nm) of Cu₂O formed on oxygen-free (<5 ppm) Cu heated at 473 K for 25 and 100 min in air, respectively, which is reported by M. Honkanen et al. [12]. Of course in the present study the heating conditions were much different: the temperature (523 K and 573 K) was much higher, and the soaking time (7 min) was very short. M. Honkanen et al. [12] also, described that below 473 K in air Cu₂O was first formed, and then above 473 K, Cu₂O changed into CuO by reacting with O.

Figure 3. CL curves of Cu plates heated at various temperatures for $4.2 \times 10^3$ s in air.

Figure 4. Summation of CL of Cu plates prepared under various heating temperatures, using Luminol ($5.0 \times 10^{-5}$ M, 0.25 mL) and MPEC ($5.0 \times 10^{-1}$ M, 0.25 mL) at pH = 10.8 and 7.5 buffer solutions (4.0 mL), respectively.
Figure 5. SEM photo images of the surfaces of Cu plates heated at various temperatures.

Figure 6. TEM images of the cross-section of surfaces of Cu plates heated at various temperatures for $4.2 \times 10^2$ s in air.

Table 1. Characteristics of oxide films formed on the surface of Cu plate heated at various temperatures.

(a) Characteristics of oxide films

<table>
<thead>
<tr>
<th>Sample</th>
<th>Heating temperature (K) for $4.20 \times 10^2$ s in air</th>
<th>Thickness of films (nm) by TEM observation</th>
<th>Compositional ratio (at%) by EDX</th>
<th>Mass% of oxide films and their crystallite sizes (nm) by XRD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)</td>
<td>523</td>
<td>around 40</td>
<td>Cu 90, O 10</td>
<td>Cu$_2$O 6.0 (19.9), CuO 0.25 (5.1)</td>
</tr>
<tr>
<td>2)</td>
<td>573</td>
<td>around 100</td>
<td>Cu 85, O 15</td>
<td>Cu$_2$O 13.2 (17.4), CuO 0.40 (5.6)</td>
</tr>
<tr>
<td>3)</td>
<td>623</td>
<td>non-uniform</td>
<td>Cu 82, O 18</td>
<td>Cu$_2$O 22.6 (9.2), CuO 6.05 (3.6)</td>
</tr>
</tbody>
</table>
By using the EDX analysis on thin films, the compositional ratios of Cu and O were determined. From the results of thin-film XRD analysis on Cu plates heated, which will be described in next Figure 7, the mass% of Cu2O and CuO and their crystallite sizes were estimated (Table 1(a)). The content of Cu2O was increased from 6.0 to 22.6 mass% gradually with increasing heating temperature up to 623 K, however, that of CuO increased suddenly from 0.40 to 6.05 mass% between 573 and 623 K. On the other hand, the crystallite size of Cu2O reduced in size from 19.9 to 9.2 nm with increasing temperature, which may be reflected by the abrupt growth of CuO between 573 and 623 K. Figure 7 shows the thin-film XRD patterns of Cu plates as-received (300 K) and heated at 473, 573, and 673 K for 4.2 × 10⁴ s in air. XRD peaks of Cu2O and CuO are not recognized on as-received Cu plate, however, heated at 473 K only a small Cu2O peak appeared around 2θ = 36.6°, and at 573 K both Cu2O and CuO peaks are observed. Furthermore, at 673 K many Cu oxides peaks were recognized. Based on these patterns, mass% of Cu2O and CuO and crystallite sizes were estimated [32]. Table 1(b) shows the lattice parameters of cubic Cu2O and monoclinic CuO formed on the Cu plate surfaces, with reported PDF data. At-a-glance, the lattice parameter a (0.42874 to 0.42654 nm) and unit volume V (0.078810 to 0.077605 nm³) of Cu2O phase are larger than those of the reported PDF data (a = 0.42520 nm and V = 0.07884 nm³) and decreased gradually with increasing temperature, however, their changing degrees are very small. On the other hand, those of CuO phase except b values are almost the same with margin for error. Figure 8 displays the Cu2O and CuO contents (mass%) near the outmost surface of the Cu plates heated at various temperatures. Cu2O gradually increased from 373 K and rapidly rose at 573 K, on the other hand, CuO showed little change up to around 573 K, which corresponding to the first Cu2O formation at lower temperature than CuO on the Cu surface during heating in air. From this, temperature of 573 K might be the stage of sudden alternation, suggesting that copper oxides are active for generation of ROS.

### 3.2. CL Intensity of Heated Cu Plates with Scavenger

From the preliminary experiments, as we noticed that there must be the most suitable amount of each scavenger to suppress the generation of ROS, so investigation
Figure 7. Thin-film XRD patterns of Cu plates heated at various temperatures for $4.2 \times 10^2$ s in air.

Figure 8. Contents of Cu$_2$O and CuO formed on the surface of Cu plates heated from room temperature to 673 K for $4.2 \times 10^2$ s in air.

of the relationship between SCL values and the amount of scavengers was performed. Figure 9 displays the variations of SCL as a function of the amount of various scavengers. In this figure, the amount of luminol is constant ($5.0 \times 10^{-5}$ M, 0.25 mL) and, for example, the amount of instillation of nbt solution with the same concentration ($5.0 \times 10^{-5}$ M) as luminol was changed; the bottom point for
SCL is achieved around 0.50 mL, suggesting that the most suitable amount of nbt is 0.50 mL. And as the same manner, the most suitable amount of other scavengers were determined; for 2-propanol 0.25 mL, for s-pyr 0.50 mL and for NaN₃ 0.50 mL tentatively. In the case of the last scavenger NaN₃, the 0.50 mL could reduce SCL value than that without scavenger and 1.0 mL (4 times higher mol ratio) addition might be too much.

Figure 10 summarized CL curves of as-received Cu plates added with the most suitable amounts of scavengers, using a constant amount of luminol (5.0 × 10⁻⁵ M, 0.25 mL) and the buffer solution (pH = 10.9, 5.0 mL); the values of SCL are also shown in this figure. Scavengers such as 2-propanol, nbt, s-pyr revealed the CL reduction, i.e., among ROS which is formed on the Cu plate surface, hydroxyl radial ·OH, superoxide anion ·O₂ and hydrogen peroxide H₂O₂ were contained, however, singlet oxygen ¹O₂ was not recognized. From the difference (D) between SCL values measured without and with scavengers, for example, D·SCL (2-propanol) = SCL (without 111.6) – SCL (with 53.1) = 58.5 k·count might be corresponding to the amount of ·OH. These amounts of each ROS formed on the heated Cu plates will be summarized later. Figures 11-14 are the CL curves measured without and with each scavenger, using Cu plates heated at 373, 473, 573 and 673 K, respectively, for 4.2 × 10² s in air. In Figure 13 and Figure 14, the scavenger’s effect for singlet oxygen ¹O₂ disappeared. Here, we have to mention that each scavenger cannot reduce only one ROS, i.e., one scavenger might eliminate more than one ROS at the same time. Then, we calculate main ROS by normalizing. Figure 15 is the summation of results showing the components of ROS formed on the heated Cu plates. At 573 K the highest amount of ROS was obtained and their main ROS were H₂O₂, ·O₂⁻ and ·OH, however, we should consider the amount of H₂O₂ and ·OH are combined, because the formation of H₂O₂ is strongly related with the amount of ·OH due to the following equation of ·OH + ·OH = H₂O₂. Furthermore, even though some amounts of singlet oxygen ¹O₂ are recognized at both 373 and 473 K, we might ignore its formation.
Figure 10. CL curves of as-obtained Cu plates measured using luminol and various scavengers.

Figure 11. CL curves of 373 K-heated Cu plates measured using luminol and various scavengers.

Figure 12. CL curves of 473 K-heated Cu plates measured using luminol and various scavengers.
Figure 13. CL curves of Cu plate heated at 573 K for $4.2 \times 10^2$ s in air; luminol $5.0 \times 10^{-5}$ M, 0.25 mL, buffer pH = 10.95, 5.0 mL, scavengers, such as, 0.25 mL 2-pro, 0.50 mL nbt and 0.50 mL s-pyr with the same concentration of $5.0 \times 10^{-5}$ M were added.

Figure 14. CL curves of Cu plate heated at 673 K for $4.2 \times 10^2$ s in air; luminol $5.0 \times 10^{-5}$ M, 0.25 mL, buffer pH = 10.95, 5.0 mL, scavengers, such as, 0.25 mL 2-pro, 0.50 mL nbt and 0.50 mL s-pyr with the same concentration of $5.0 \times 10^{-5}$ M were added.

Finally, the mechanism for generation of hydroxyl radical $\cdot$OH, hydrogen peroxide $\text{H}_2\text{O}_2$, and superoxide anion $\cdot\text{O}_2^-$ on the copper surface are proposed in Figure 16. As already described, a very fine and small amount of thin copper oxide films, Cu$_2$O and CuO are formed on the outermost surface in air. Cu bulk plate is drawn as a rectangular shape and placed in water or air. In the case of former, water contacts with air and the latter, air contains water vapor. Based on some chemical knowledge, i.e., standard electrode potential $E_0(\text{Cu}) = 0.34$ V, SHE [standard hydrogen electrode]) [34], copper’ stability in oxygen dissolved acidity or neutral aqueous solution, and a potential-pH diagram of Cu-$\text{H}_2\text{O}$ at room temperature in air [35], Cu can be oxidized in the alkaline aqueous solution.
Figure 15. Summation of CL, SCL and components of ROS for Cu plates heated at various temperatures for $4.2 \times 10^2$ s in air.

Figure 16. Schematic diagram for "Fenton reaction" to produce "ROS, $\cdot$OH, $\cdot$O$_2^-$" on the surface of Cu plate with thin copper oxides.

with pH = 7 - 14 by dissolved oxygen O$_2$ that is Cu --> Cu$_2$O or CuO. However, these products are supposed to be minute quantity due to a short reaction time for CL measurement of $6.0 \times 10^2$ s at 293 K. Therefore, in Figure 16 (i) Cu in the bulk copper coelute into water at the surface; Cu --> Cu$^+$ + e$^-$ (or Cu$^{2+}$ + 2e$^-$), (ii) O$_2$ in air dissolves into H$_2$O, (iii) and through the reaction $(1/4\cdot$O$_2 + 1/2\cdot$H$_2$O), it (OH) reacts with e$^-$ (or 2e$^-$) at metal surface, (iv) then -OH is formed. Furthermore, (v) two -OH changes into H$_2$O$_2$ + 2e$. On the other hand, [* Cu$^+$ and Cu$^{2+}$ in (i)] reacts with [H$_2$O$_2$ in (v)] into (vi) hydroxyl radical -OH or (vii) superoxide
anion $\cdot O_2^-$. These (vi) and (vii) equations are called “Fenton Reaction” [13]. Here, at the first thin-film layer Cu$_2$O and the second layer CuO formed on the outermost surface of bulk Cu, as presented in upper side of Figure 16, (2Cu$^+$ + 1/2·O$_2$ + 2e$^-$) and (Cu$^{2+}$ +1/2·O$_2$ + 2e$^-$) are produced, respectively. These Cu$^+$ and Cu$^{2+}$ react with H$_2$O$_2$, as presented by the equation (vi) and (vii); “Fenton Reaction” [13] takes place. It should be noted that these reactions tend to take place at “active step” of the edge place near islands of Cu$_2$O/CuO, not continuous thin-film.

4. Conclusion

By focusing on generation of reactive oxygen species (ROS) on the copper surface, the antibacterial activity of copper has been tried to explain. Especially, the change in ROS formed on the heated Cu plate was investigated: hydroxyl radical $\cdot$OH, hydrogen per oxide H$_2$O$_2$, and superoxide anion $\cdot O_2^-$. are the most of ROS. These data concerning about the components of ROS formed on the surface of heated Cu is reported for the first time using Chemiluminescence with the combination of luminol and scavengers. In addition, the generation mechanism of these three ROS on the outermost surface consisting of thin film Cu$_2$O, CuO layer and bulk Cu also is proposed.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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