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ISSN 0543-5846
METABK 63(2) 245-248 (2024)
UDC – UDK 669.3:669.3.6/669.3.5:621.41:541.451:576.8=111

THE ROLE OF SURFACE WETTABILITY OF COPPER AND ITS ALLOYS CuSn6, CuZn37 IN ANTIMICROBIAL EFFICACY STANDARDIZED TESTS

Received – Primljeno: 2023-09-25
Accepted – Prihvaćeno: 2023-12-05
Original Scientific Paper – Izvorni znanstveni rad

The spread of bacterial infections often occurs through indirect contact with infected individuals. Thus, surfaces with antimicrobial properties have gained prominence in healthcare and public spaces. Testing standards exist for assessing the antibacterial effectiveness of these materials, but they do not consider surface properties, particularly surface wettability during microbiological tests. An experiment was conducted to modify copper and its alloys' surfaces through chemical treatment, altering contact angles. The results revealed that contact angles significantly influence the contact area between droplets and test surfaces, as well as the evaporation time of droplets. These factors can ultimately impact the results of antimicrobial efficacy tests.

Keywords: copper, copper alloy, copper sheet; copper oxide, antimicrobial surface

INTRODUCTION

Disease-causing bacteria – pathogens – can be spread or transmitted among human population by several routes. One of the pathways for spread of pathogens is through indirect contact when an infected person touches a surface such as a handrail, doorknob or light switch leaving microbes on it, that are then transmitted to another person who touches that surface [1 – 3]. In the recent years, there is a growing interest in touch surfaces, which have intrinsic antimicrobial properties. Researchers, hospital personnel, government agencies and hardware manufacturers are focusing on application of such materials in public areas in order to brake the chain of infections, transmitted through indirect contact [4, 5].

The proper selection of material for this kind of application, arises from its antimicrobial performance, which currently is often determined by only one international standard established originally in Japan – JIS Z 2801 [6, 7]. According to the Japanese standard, test samples should be square in shape with dimensions of 50 mm x 50 mm and up to 10 mm thick. After sterilization with alcohol test samples are being contaminated with 400 µl bacterial suspension of Staphylococcus aureus or Escherichia coli. In the next step the microbial inoculum on the test samples is covered with a sterile polyethylene film. The film allows the inoculum to spread on sample surface and prevents it from evaporating. Then the inoculated samples are being incubated at 35 +/- 1 °C and at 90 % relative humidity for 24 +/- 1 hours. After the time, microbial concentration is determined by elution of the microbes from the test sample, followed by dilution and plating on agar media [8]. The above mentioned methodology is often criticized in the literature [9, 10], especially because of relatively high incubation temperature of 35 °C and humid environment, which is very unlikely to be experienced in real life conditions, and also because of usage of film for covering the inoculum, which is not happening during real life microbes contamination event.

Therefore attempts are still being made for developing new standard, which by definition should better reflect the real conditions of contamination event by infected individual. There are few examples to be mentioned, one of which is standard developed by US Environmental Protection Agency [11] or other standard developed by private owned laboratory [12]. In both standards methodology comprises placing the microbial suspension of much smaller volume of 20 µl on the sample surface and letting it to dry at ambient conditions. Also in own research, attempts are being made on developing standard with even smaller volume of the bacterial inoculum of 10, 5 or 2 µl, which aimed on reducing the total count of the bacteria to be spread, in order to reflect conditions of real life contamination event.

The present work is focused on some technical aspects of a microbiological test, in which inoculum drop is left to dry, and which were not reflected in the current microbiological standards. In particular the current research focuses on the fact that the surface wettability of given materials can be altered, by for example chemical oxidation. Changing wettability, which is defined by the

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contact angle between surface and droplet of a given volume, may seriously affect contact area between bacterial inoculum and antimicrobial substrate, which in turn may affect drying time of the inoculum droplet and as a result of above factors the whole antimicrobial test results [13].

EXPERIMENTAL PROCEDURE

Materials selection

In the current experiment commercially available Cu-ETP copper and selected copper alloys (CuSn6, CuZn37) sheets were used. Table 1 lists the materials, together with the chemical composition and their UNS (Unified Numbering System) designation. From 0,5 mm thick sheets, square 25 mm x 25 mm samples were cut out, which were then mechanically polished to remove corrosion inhibitor, with which commercial copper sheets are covered. The materials were then cleaned in an ultrasonic bath filled with acetone and then rinsed in distilled water.

Table 1 **Chemical composition of the tested commercial copper and its alloys / wt. %**

Common name	UNS code	Cu	Al	Ni	P	Sn	Zn	As + Bi + Cd + Fe + Pb + Sb + Si
Copper (Cu-ETP)	C110	99,9	0,00	0,00	0,03	0,0	0,0	rest
Yellow Brass (CuZn37)	C274	63,2	0,01	0,06	0,22	0,0	36,7	
Phosphor Bronze (CuSn6)	C519	94,1	0,02	0,01	0,22	5,5	0,1	

Alteration of surface wettability of the test materials

In order to alter the wettability of given materials, all samples were subjected to chemical oxidation in a solution of NaOH sodium hydroxide (2,5 mol/L) and ammonium persulfate $(NH_4)_2S_2O_8$ (0,13 mol/L) at 70 °C for 15 minutes. The samples were then rinsed in ethanol and left to dry. Subsequently, the samples were immersed in 20 mmol/L stearic acid solution in demineralized water in ambient temperature for the of 1 minute, then rinsed in ethanol and left to dry.

Methods of surface properties research

The morphology of the samples surface was observed by scanning electron microscopy. Wettability of materials was determined by placing 5 µl droplet of a demineralized water at 20 °C and relative humidity of 50 % in horizontal microscope with a protractor eyepiece and environmental chamber. Ten measurements of the contact angle were made for each of the samples and the results were averaged.

RESULTS AND DISCUSSION

Copper and copper alloy sheets were subjected to chemical treatment, which aimed at altering wettability of their surfaces. First, samples were subjected to mechanical polishing and in the next step oxidized with solution of $NaOH·(NH_4)_2S_2O_8$ and immersed in stearic acid. Figures 1 – 3 presents Scanning electron microscopy (SEM) micrographs of surfaces of test materials after mechanical polishing (a) and after chemical treatment (b).

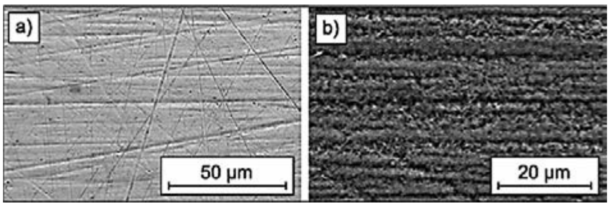

Figure 1 SEM of Cu-ETP sheet after: a) mechanically polished, b) oxidized in $NaOH·(NH_4)_2S_2O_8$ and immersed in $C_{17}H_{35}COOH$

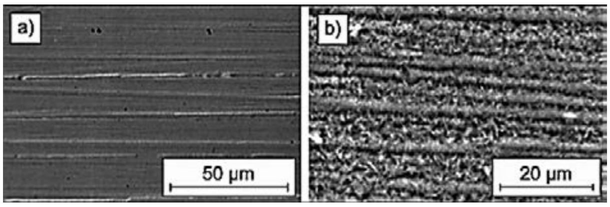

Figure 2 SEM of CuZn37 sheet after: a) mechanically polished, b) oxidized in $NaOH·(NH_4)_2S_2O_8$ and immersed in $C_{17}H_{35}COOH$

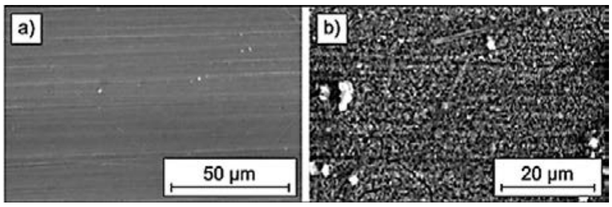

Figure 3 SEM of CuSn6 after: a) mechanically polished, b) oxidized in $NaOH·(NH_4)_2S_2O_8$ and immersed in $C_{17}H_{35}COOH$

It can be seen that the surface of oxidized materials is more complex in comparison to the output ones. From the micrographs it can be also concluded that oxidation solution has had the weakest effect on alloy with the highest content of alloying elements, namely CuZn37 (Figure 2b).

Energy dispersive spectroscopy (EDS) analysis shown in Figures 4 – 6, confirm oxidizing effect of chemical treatment used in current research.

Taking into consideration black color of the oxidized surfaces and the needle-like morphology of the surface it can be assumed, that sheets were covered with copper (II) oxide – CuO as a result of applied chemical treatment.

In the next part of the research, materials after mechanical polishing and chemical treatment were subjected to contact angle measurements. Test materials were placed in the environmental chamber set up on 20 °C and 50 % of

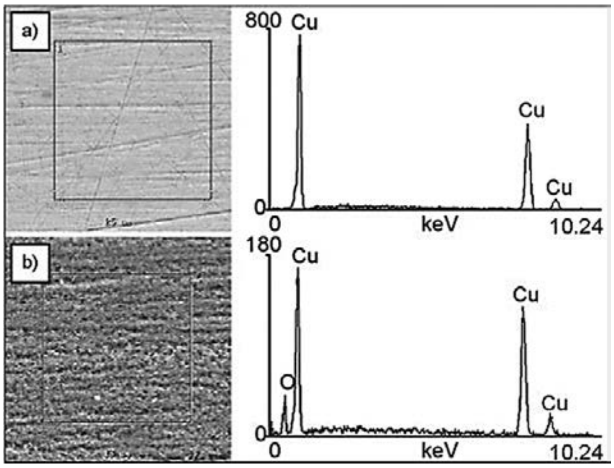

Figure 4 EDS analysis of the chemical composition of the surface layer on the Cu-ETP sheet after: a) mechanically polished, b) oxidized in $NaOH{\cdot}(NH_4)_2S_2O_8$ and immersed in $C_{17}H_{35}COOH$

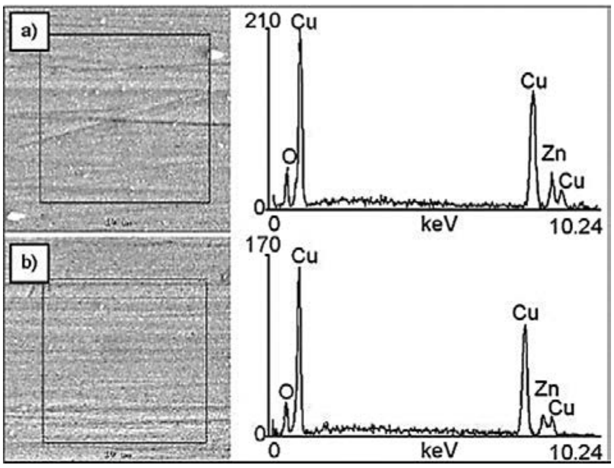

Figure 5 EDS analysis of the chemical composition of the surface layer on the CuZn37 sheet after: a) mechanically polished, b) oxidized in $NaOH{\cdot}(NH_4)_2S_2O_8$ and immersed in $C_{17}H_{35}COOH$

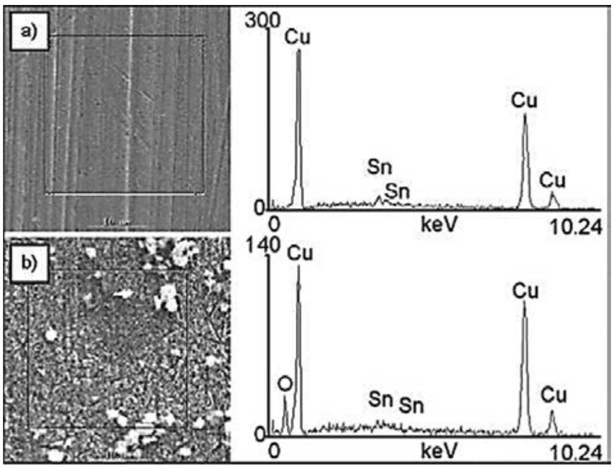

Figure 6 EDS analysis of the chemical composition of the surface layer on the CuSn6 sheet after: a) mechanically polished, (b) oxidized in $NaOH{\cdot}(NH_4)_2S_2O_8$ and immersed in $C_{17}H_{35}COOH$

relative humidity. Precisely controlled environmental condition allowed to conduct measurements of evaporation time of the water droplet of 5 µl volume. Measurement

Table 2 **Results of contact angle measurements between 5 µl demineralized water and test materials after mechanical polishing. Evaporation time of 5 µl droplet at the temperature of 20 °C and relative humidity of 50 %**

Test material	Droplet volume /µl	Contact angle / °C	Tempe-rature / °C	Humidity / %	Evaporation time / s, min
Cu-ETP	5,0	72,5	20	50	2 678, 45
CuZn37		78,2			2 983, 50
CuSn6		73,8			3 091, 52

Table 3 **Results of contact angle measurements between 5 µl demineralized water and test materials after chemical treatment. Evaporation time of 5 µl droplet at the temperature of 20 °C and relative humidity of 50 %**

Test material	Droplet volume /µl	Contact angle / °C	Tempe-rature / °C	Humidity / %	Evaporation time / s, min
Cu-ETP	5,0	133,1	20	50	3 646, 61
CuZn37		107,3			3 408, 57
CuSn6		127,8			3 782, 63

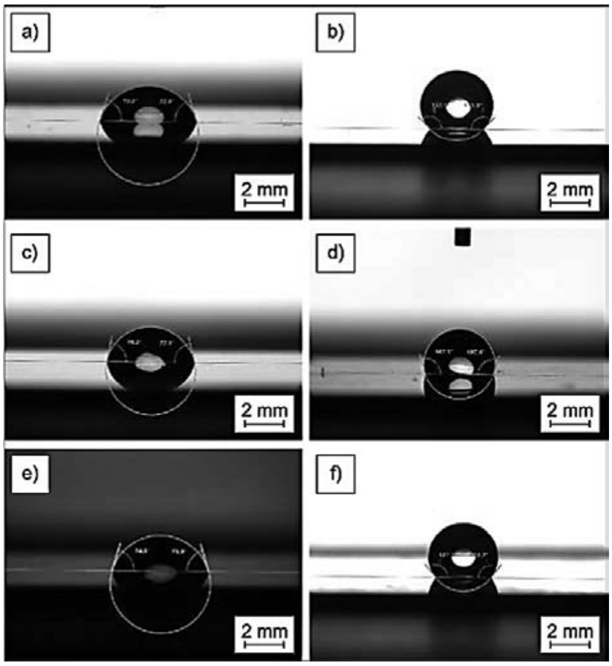

Figure 7 Contact angle measurements of test materials: a) Cu-ETP mechanically polished, b) Cu-ETP oxidized in $NaOH{\cdot}(NH_4)_2S_2O_8$ and immersed in $C_{17}H_{35}COOH$, (c) CuZn37 mechanically polished, (d) CuZn37 oxidized in $NaOH{\cdot}(NH_4)_2S_2O_8$ and immersed in $C_{17}H_{35}COOH$, (e) CuSn6 mechanically polished, (f) CuSn6 oxidized in $NaOH{\cdot}(NH_4)_2S_2O_8$ and immersed in $C_{17}H_{35}COOH$

results are shown in Table 2 for materials after mechanical polishing and in Table 3 for materials after chemical treatment. Photographs of the initial droplets put on the particular materials are shown on the Figure 7.

From above presented results it can be concluded that the initial contact angle on mechanically polished sheets made of copper and its alloys have slightly different contact angles. After chemical treatment, and especially by covering the samples' surface with stearic acid contact angle rises from 72,5 ° to 133,1 ° for ETP copper, from 78,2 ° to 107,3 ° for CuZn37 alloy, and from 73,8 ° up to 127,8 ° for CuSn6 alloy. The smallest increase in the contact angle is for the CuZn37, which can be related to the weakest effect of the oxidation solution on surface morphology of the alloy. Nevertheless oxidation and covering the given materials with stearic acid alters their wettability performance significantly, while chemical composition of materials' bulk remain unchanged.

Worth to mention is also the fact, that the surface chemical treatment by changing wettability of the surfaces of test materials, affected also the time of evaporation of a droplet with given volume of 5 μl. While evaporation of 5 μl water droplet from the ETP copper substrate took on average 45 minutes, at the contact angle of 72,5 °, after chemical treatment it took 61 minutes on average, at the contact angle of 133,1 °. Similar conclusion can be drawn for the other materials by analyzing the data in Table 2 and Table 3.

Based on the assumption that droplet placed on the flat substrate can perceived (in simplification) as a spherical cap, calculations of relation between contact angle and geometry of the droplet were made. From this calculation it can be concluded, that the contact area between droplet and the surface can be couple times larger between extreme values of the contact angle. Also the outer area of the droplet changes significantly with the contact angle and it should be pointed out that it does not change monotonically which was presented in Table 4.

Table 4 **Geometrical calculations of droplet of a given volume, outlined as spherical cap**

Spherical cap (droplet) volume V / μl	Spherical cap (droplet) height h / mm	Sphere radius r / mm	Spherical cap (droplet) total area (base + outer area) Pb + Po / mm^2	Geometric relations between contact angle and sphere radius sin α / -	Contact angle of spherical cap (droplet) α / °
5,00	0,20	39,86	100,04	0,10	6
	0,40	10,08	50,17	0,28	16
	0,60	4,62	33,71	0,49	30
	0,80	2,75	25,67	0,70	45
	1,00	1,92	21,05	0,88	61
	1,20	1,51	18,17	0,98	78
	1,34	1,33	16,81	1,00	90
	1,50	1,21	15,69	0,97	104
	1,70	1,12	14,79	0,85	121
	2,00	1,06	14,19	0,48	151

CONCLUSIONS

The results obtained in the current investigations show that wettability of the surface can have significant implications on methodology of antimicrobial efficacy test, in which inoculum droplet is left to dry, either by the contact area between bacterial inoculum or time of evaporation. The conclusion of the current research should be confirmed in the microbiological procedure on impact of above mentioned factors on antimicrobial performance within the future research.

ACKNOWLEDGEMENTS

This research was supported by The Polish National Center for Research and Development (Grant No. PBS3 /A9/32/2015).

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Note: The translator responsible for English language: Małgorzata Zasadzińska, AGH University of Science and Technology, Kraków, Poland.