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Surface Modification of Cu/PS Nanocomposite Films: A Comparative Study of DC N_2 Plasma and Gamma Radiation and their Effects on the Films' Antibacterial Activity

Naglaa M. El-Sayed and Nora A. Eid

Physics Department, Faculty of Science, Zagazig University, Zagazig, Egypt

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In this work, a comparative study on the efficiency of surface modifications of Copper/Polystyrene (Cu/PS) nanocomposite films, induced by N_2 DC plasma and gamma radiation to enhance their surfaces' antibacterial activity was conducted. Cu/PS nanocomposite films, with different concentrations of copper 0.4, 0.8 and 1 wt.%, were prepared by the solution casting method at room temperature. The samples were exposed to gamma rays at different radiation doses and N_2 DC plasma for different treatment time intervals. The structural and morphological properties of the samples were investigated before and after treatment by, water contact angle measurements, weight loss and scanning electron microscopy. The surfaces of the films were found to be more sensitive to N_2 DC plasma compared gamma radiation. The antibacterial properties of Cu/PS nanocomposite films against *Staphylococcus aureus 15* have been evaluated by the optical density and colony forming unit's techniques. The results indicated that the N_2 DC plasma treatment enhanced more antibacterial properties than those induced by gamma radiation.

Keywords: *N_2 DC Plasma, Gamma radiation, Polymer surface modification, Cu/PS Nanocomposites films, Antibacterial properties*

Introduction

Synthesis, modification and applications of metal-polymer nanocomposites has gained an extensive interest in the last few decades in the field of materials science research [1-5]. The incorporation of metal nanoparticles in a polymer matrix may combine favorable features of the constituents producing new materials with novel properties including optical, electrical, magnetic, mechanical, plasmon resonance, and antimicrobial characteristics. Such behavior allows their application in many fields such as electronics, optoelectronics, biomedicine, energy, etc. This can be considered an adequate solution to many present and future technological challenges.

Copper nanostructure was reported to have antibacterial activities which increase with increasing Cu content [6,7]. Recardo J.B. Pinto [8] studied the antibacterial activities of Cu filled cellulose and suggested further investigation to prove that the behavior depends on the morphological characteristics of the Cu particles. The antimicrobial activity of the copper/polystyrene nanoparticles was investigated by Isha R. Kamrupi [9]. They confirmed the sensitivity of different bacterial strain against the copper encapsulated polystyrene.

The particle size of copper was found to affect the antibacterial behavior of cu/polymer composites. Due to the fact that nanoscale materials are characterized by the higher surface to volume ratio compared to microscale counterparts, this makes these particles more efficient having the ability to

Corresponding author: nagla68mohammed@gmail.com
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attach to the microbial molecules and bacterial cell membrane causing cell death. The antimicrobial activity of nanoscale materials has been investigated and showed positive results as growth inhibitors [10, 11, 12]. Humberto Palza et al. [13] confirmed the advantage of nano-scale cu-polypropylene composite, over the micro-scale one, against *Staphylococcus aureus and Pseudomonas aeruginosa--bacteria*.

Many polymeric materials have been used as polymeric hosts for the CuNPs to form composites with antimicrobial activities [14-17]. In this study, the authors used polystyrene as the host matrices. Polystyrene can be fabricated easily to form products with good mechanical and thermal properties so that it is one of the most widely used plastic materials in the world including food packaging and medical instruments such as tissue culture trays, test tubes, petri dishes, diagnostic components, and housing for test kits. This makes increasing the hygienic quality an important manufacturing factor.

The antimicrobial activity of any substrate is known to be affected by its chemical composition, surface roughness, surface charge, and surface free energy. These factors could be altered by means of numerous methods such as plasma, electron beam, ion beams, UV radiation, chemical reactions and gamma irradiation [18-23]. In this work, the effect of N_2 glow discharge plasma and gamma radiation on the surface properties of the Cu/PS nanocomposite films were investigated. The plasma treatments were performed for different treatment time intervals at fixed discharge power and gas pressure. Meanwhile the gamma irradiation is performed with different doses. In addition, the antimicrobial properties of the untreated and treated Cu/PS nanocomposite films have been evaluated..

Materials and methods

Preparation of cu/ps nanocomposite films

Samples were prepared by solution casting method. PS of molecular weight (135, 000 g.mol^{-1}) in the form of grains and copper nano particles (CuNPs) of particle size 60 nm were purchased from Sigma-Aldrich Company. PS and copper (Cu) were dissolved separately in toluene of 99.99% purity, using a magnetic stirrer. After complete dissolution, the two solutions were added to each other and the stirring continued until the solution was well mixed. The solution mixture then was cast onto a clean glass Petri dish and let to dry in air at room temperature for one week. The samples, with thickness ranges of (0.22-0.25 mm), were cut into slides of (1×2 cm) dimension. PS/Cu nanocomposites films were optically opaque, with black color and the intensity of its color depends on the Cu concentration, while pure PS sample was colorless. The films were prepared with three copper content values of 0.4, 0.8 and 1wt.%, .The samples were denoted as S1, S2 and S3 for simplicity.

Sample treatments

Gamma irradiation was carried out using the gamma cell 220 Excel Co_{60} irradiation. The absorbed irradiation dose rate of the γ–cell was measured using the National Physical Laboratory (NPL) alanine reference dosimeter. The irradiation dose rate was 1.35 KGY/h. The samples were subjected to gamma radiation doses of 10, 30, and 50 KGy.

The schematic diagram of the DC glow discharge setup used for surface treatment of the composite films was described in detail in a previous work [24]. The discharge parameters were kept unchanged through the whole experiment. The pressure of N_2, as the working gas, was 0.4 Torr, and the input power was about 3.5 Watt. The treatment time of the samples ranged from 15 to 60 min. The sample was supported on a glass holder and located at the edge of negative glow region of the discharge in front of the cathode during the treatment and its position is perpendicular to the flow.

Characterization techniques

Surface wettability of the films was examined by water contact angle (WCA) measurements. The contact angle of 5 □l distilled water droplets on the surfaces was measured using a travelling microscope with repeating measurements about 8 times at different locations on the same sample to check the accuracy. Etching effect of both treatment methods was indicated by measuring the weight loss percentage for the treated samples by weighing each sample, with a microbalance, before (W_o) and after (W_1) treatment using equation (1) [25]:

$$Weight\ loss\ (\%) = \frac{W_o - W_1}{W_0} \times 100 \qquad (1)$$

The size and distribution of cu nanoparticles in addition to surface morphologies of the treated films were scanned using scanning electron microscope (SEM) quanta fei 250, made in the Czech Republic, were used.

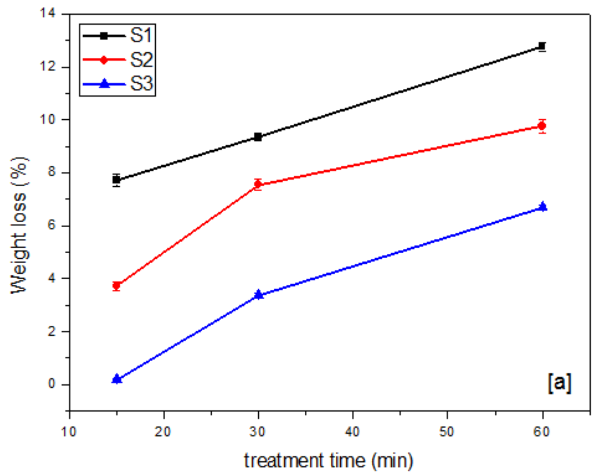
Microbiological test

The tested organism, a multi-drug resistant *Staphylococcus aureus 15 (KT337489)* strain, was selected from a previous study by one of the present authors [26]. The tested organism *S. aureus 15* was grown in a 5ml of sterile nutrient broth which incorporated separately with pure PS film and treated with plasma at different exposure time intervals (0, 15, 30 and 60 min) and at different exposure doses of gamma ray (0, 10, 30 and 50 KGy). All tubes were incubated at 37°C for 24h, then 0.1 ml from each tube was spreading on nutrient agar plates and these plates were incubated at 37°C for 24h. After the incubation period, the number of colony forming unites (CFU/ml) were determined for each treatment. Nutrient agar medium composed of (g/ L): 2.0 yeast extract, 5.0 peptone, 5.0 sodium chloride and 15.0 agar and dissolved in distilled water up to 1000 ml. The pH of the medium was adjusted to be 7.0 [27].

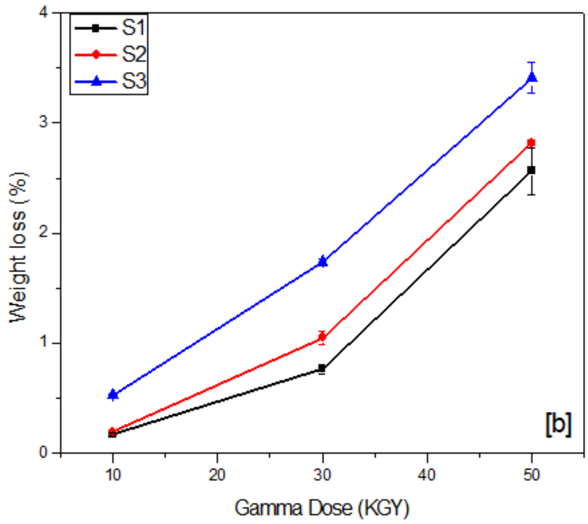
Results and discussion

Weight loss measurements

The weight loss percentages of samples S1, S2, and S3 exposed to both N_2 plasma and γ -irradiation were plotted versus the plasma treatment time in Fig. (1-a) and γ-dose in Fig. (1-b). According to the results, all samples experienced weight loss with increasing both plasma treatment time and γ-dose. However the loss due to plasma irradiation is much larger compared to γ-rays. This is attributed to the fact that plasma has an etching effect, as for most polymers when exposed to plasma, an interaction takes place between plasma species and the polymer surface molecules leading to abstraction of hydrogen atoms and formation of free radicals. These free radicals can weaken the bond strength leading bond breakage or chain scission. As a result, low-molecular-weight volatile fragments can be formed and thus weight loss occurs in the exposed sample [28]. However, the weight loss due to γ-rays may be resulted from either local heating in the samples subsequently, losing their moisture and residual solvent or due to structural decomposition in the matrix of polystyrene [29].



(a)



(b)

Fig. (1): The weight loss for samples S1, S2 and S3 that [a] treated with N_2 plasma and [b] treated with gamma radiation

It was also observed that for plasma treated samples, as the CuNPs content in PS-Cu composites film increases, the weight loss % decreases. This can be explained as; the etching process is predominant on the amorphous regions of the surface than on the crystalline regions [30]. Therefore, increasing CuNPs content in PS-Cu composites film leads to increasing the crystallinity of film's surface, which in turn, decreases weight loss %. However, CuNPs concentration does not play a clear role towards the weight loss % for gamma treated samples.

Surface wettability and water contact angle

The results of WCA are presented in Table (1) from which it can be seen that values of WCA for all the treated samples with both methods are reduced after treatment. However the effect of plasma is higher than that of gamma rays. The main reasons for this reduction are always attributed to the surface roughness as well as the creation of functional groups [26,31,32]. The reduction in WCA is a good indicator for enhancing surface wettability or increasing the hydrophilicity of the treated films.

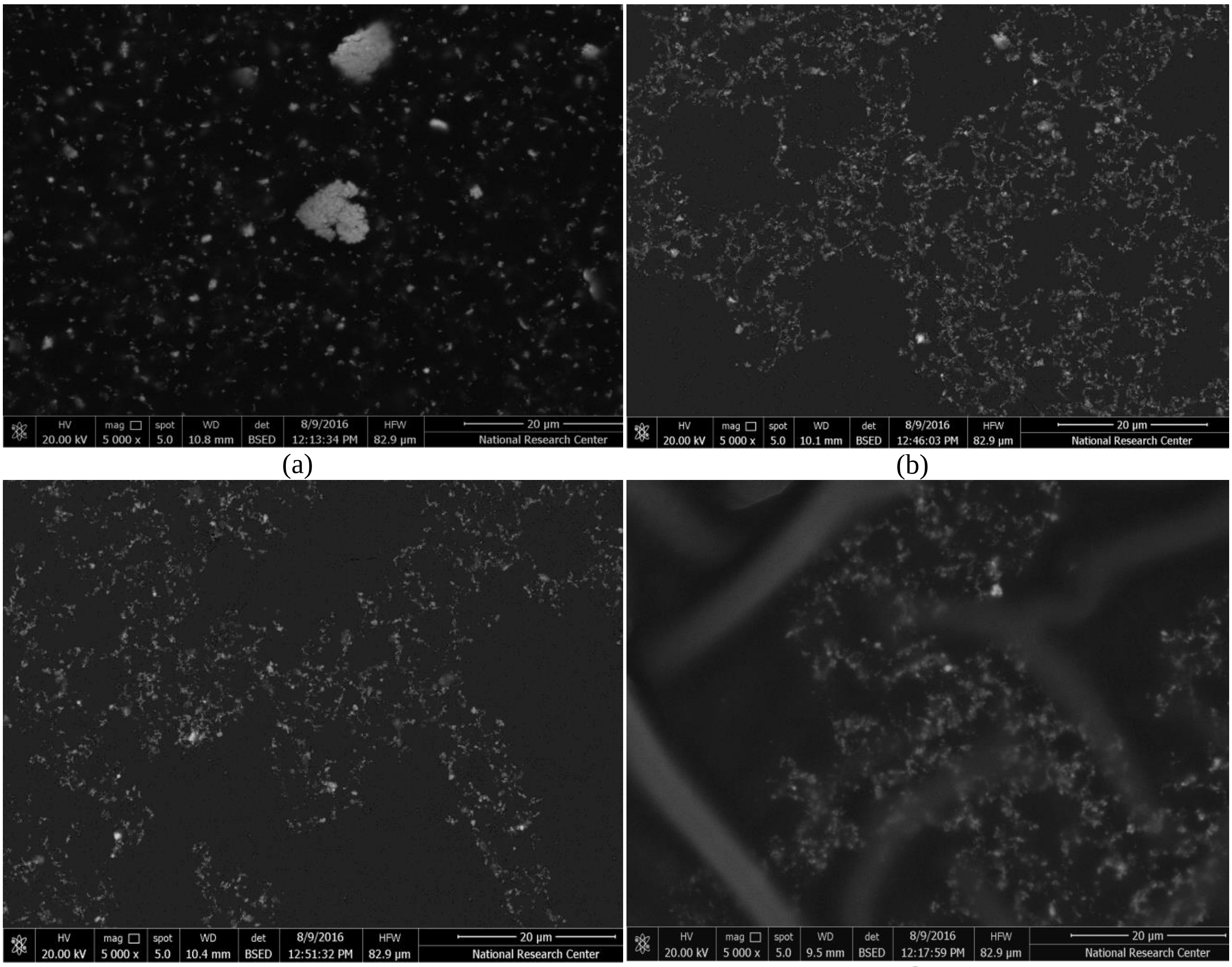
Surface morphology

SEM images indicating surface morphology of the sample S3, irradiated with both plasma and gamma are shown in Figs. (2, 3).

Untreated sample shows smooth surface with relatively homogenous distribution of the CuNPs on the film's surface with average particle size of about 82.5 nm. Generally, it was observed that both plasma and γ- radiation affected the sample the same way. The filler nanoparticles appeared on the films surface showed increasing tendency to agglomerate with increasing the plasma treatment time and γ-dose. For example, the particle size enlarged to about 167.6 nm for sample treated with plasma for 60 min., and 1236 nm for samples treated with 50 KGy. The reason for increasing CuNPs size, due to γ- radiation, is probably attributed to the chain scission of polystyrene matrix [33]. The polymer chain can gain mobility due to chain scission [34] and upon relaxation process stress released and the inter planar spacing may increase giving the CuNPs the chance to accumulate and hence increasing in size. Figure (2-d) shows a creased surface for the film treated with plasma for 60 min. These creases are suggested to increase the surface wettability due to the increased film's contact points and hence surface area. However, samples treated with γ-rad. did not show any creases.

Table (1): Results for WCA for the samples treated with both N_2 plasma and gamma radiation

Sample Cu concentration (wt. %)	WCA for untreated samples (^{O})	Plasma treated		Gamma treated	
		Treatment time (min)	WCA (^{O})	Irradiation Dose(KGy)	WCA (^{O})
0.4	**83.5**	15	64.3	10	75.2
		30	56.4	30	68.5
		60	35	50	62.6
0.8	**83.4**	15	52.8	10	69.6
		30	39	30	66
		60	33.8	50	64.4
1	**83.7**	15	62.6	10	68
		30	55.4	30	65.8
		60	30.1	50	59.4



(a) (b) (c) (d)

Fig. (2): SEM for (a) untreated Cu/PS (1 Wt. %) composite sample and plasma treated samples for different exposure time durations: (b) 15 min, (c) 30 min, (d) 60 min

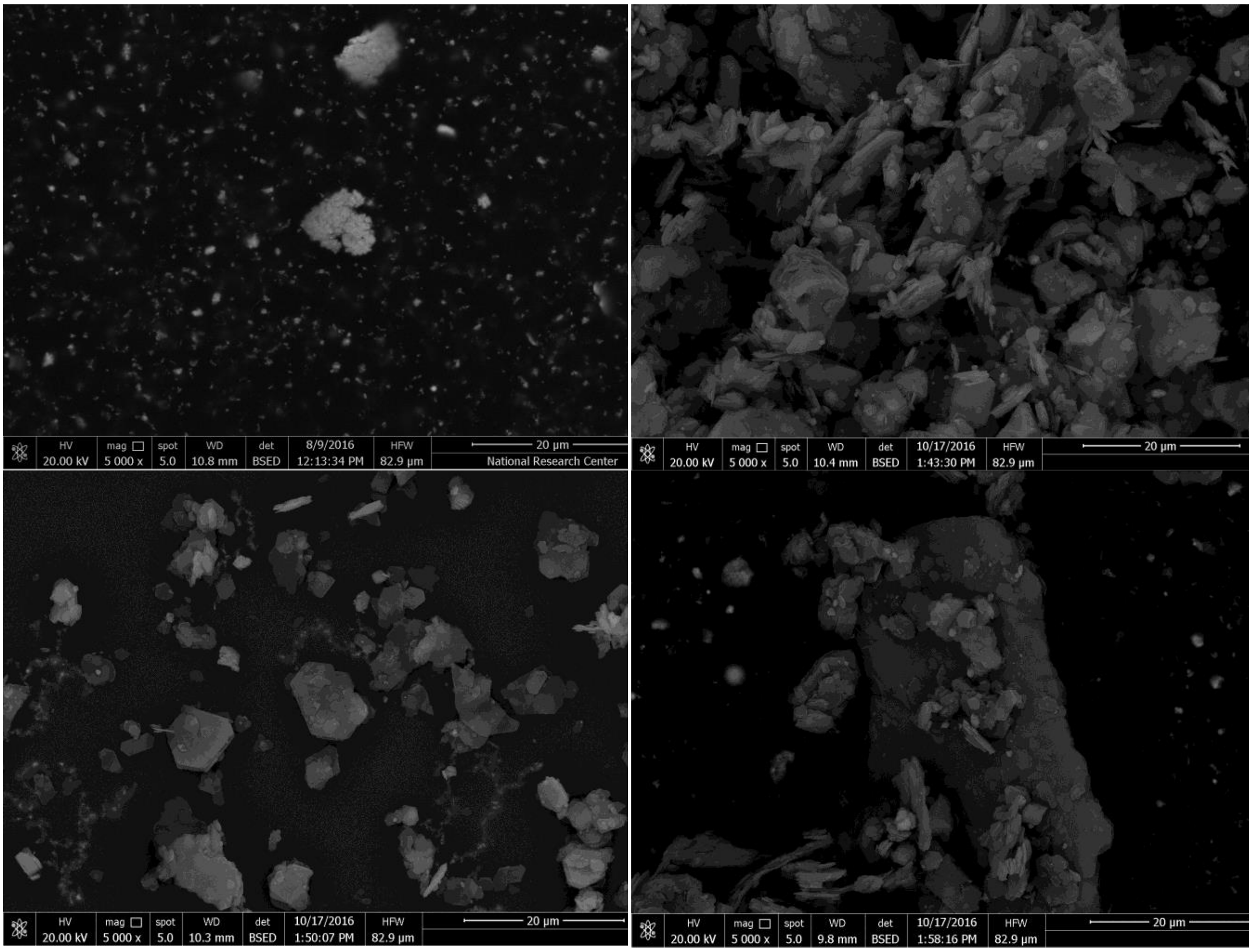


Fig. (3): SEM for (a) untreated Cu/PS (1 Wt %) composite sample and gamma treated samples for different radiation doses: (b)10 KGy, (c) 30 KGy , (d) 50 KGy

Antibacterial properties

The antibacterial activity for PS-Cu composite film (1wt. %) was carried out for the untreated, N_2-plasma and gamma treated samples. The experiment was performed against *Staphylococcus aureus 15* bacteria and analyzed by the optical density (OD) and colony forming units (CFU) techniques.

The results shown in Table (2) revealed thatin case of plasma treatment, the CFU/ml of tested *S. aureus* 15 was decreased by increasing the exposure time compared with untreated composite film. The CFU/ml of tested strain showed a relatively large inhibition for the sample treated for 30 min however, with increasing the time duration to 60 min, the bacterial accumulation increases but still lower than untreated sample. This can be interpreted in the light that exposing the samples to plasma causes physiochemical changes affecting the bacteria and its adhesion on the film's surface. Due to plasma etching effect, some of superficial polymer molecules were removed leaving the film's surface with a large number of CuNPs. Copper nanoparticles known to be active against bacteria and fungi [35-37]. The antimicrobial activity of CuNPs can be attributed to their morphology, mainly their small size and high surface area to volume ratio, which allows them to interact directly with the bacterial outer membrane, causing it to rupture and hence killing the bacteria. The observed relatively high CFU with increasing the plasma treatment time period to 60 min may be attributed to increasing hydrophilicity and roughness over the action of CuNPs concentration, consequently, resulting in more bacterial attachments [26], in addition to increasing the particle size due to particle agglomeration.

Table (2): Effect of plasma treatment time and gamma radiation dose on the growth of S. aureus 15 strain

Plasma treated			Gamma treated		
Treatment time (min)	**O.D (at 600nm)**	**No. of CFU/ml (N)**	**Irradiation Dose(KGy)**	**O.D (at 600nm)**	**No. of CFU/ml (N)**
0	0.412	$45x10^5$	0	0.412	$45x10^5$
15	0.361	$87x10^4$	10	0.320	$25x10^5$
30	0.173	$2.5x10^2$	30	0.369	$36 x10^5$
60	0.289	$2x10^5$	50	0.385	$42x10^5$

In case of gamma irradiation, it was found that the CFU/ml of tested strain was slightly decreased on the sample exposed to a dose of 10 KGy, with increasing γ-dose from 10 to 50 KGy, an increase in CFU was observed. According to the current results, it is obvious that γ-treatment has no touchable effect on the antibacterial activity of the films under investigation. This result may be attributed to changing the Cu particle size from the nano-scale to micro-scale with increasing γ-dose, as it was reported that micrometric metal copper did not cause cell damage as compared with highly biocidal copper nanoparticles at the same mass [38].

Conclusion

Cu/PS nanocomposite films with different concentrations of Cu NPs were prepared by solution casting method. The law pressure DC N_2 plasma for different treatment time periods and Co_{60} γ-radiation at different doses were used to enhance the surface properties, as well as, their antibacterial activity. The comparative study demonstrated that both treatment methods exhibit a significant capability in changing the surface properties as a function of plasma treatment time and γ dose. However, plasma was found to be more efficient than gamma radiation. This result was confirmed by analyzing the treated surfaces by SEM, weight loss and WCA. The results of WCA and weight loss demonstrated that plasma has a higher etching effect than gamma radiation. The size of CuNPs, recorded by the SEM, showed higher values in case of gamma rays than the N_2 plasma. These changes in the surface characteristics are reflected on the antibacterial activity against *Staphylococcus aureus* 15 bacteria as the CFU/ml and OD for the tested strains showed a relatively large inhibition for the sample treated with N_2 plasma for 30 min however there was no touchable effect for samples treated with gamma radiation at different Doses.

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