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Antibacterial chitosan-copper nanocomposite coatings for biomedical applications

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Abstract

The purpose of this study was to improve the antibacterial activities of chitosan by adding copper nanoparticles at different concentrations. The physical and chemical properties of the synthesized nanoparticles and nanocomposite coatings were assessed using different characterization techniques, including X-ray diffraction, transmission electron microscopy, Fourier transform infrared spectroscopy, and scanning electron microscopy. The antibacterial properties of the nanocomposite coatings were investigated using several Gram-positive and Gram-negative microorganisms of interest, including methicillin-resistant Staphylococcus aureus and Escherichia coli. The size of the obtained copper nanoparticles was about 11 nm, with chitosan as a stabilizer. The best result of antibacterial activity was related to the sample subjected to 2 M of chitosan solution (0.5 wt. % Cu nanoparticle) for 5 min at 5 volt.

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Keywords: Chitosan; Copper nanoparticle; Nanocomposite; Antibacterial; Electrophoretic deposition.

Nomenclature

XRD	X-Ray Diffraction
TEM	Transmission Electron Microscopy
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Infrared (spectroscopy)

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$CuSO_4.5H_2O$	Copper Sulfate Pentahydrate
$(C_6H_{11}NO_4)n$	Chitosan
CH_3CO_2H	Acetic Acid
NaOH	Sodium Hydroxide
$C_6H_8O_6$	Ascorbic Acid
NH_2NH_2	Hydrazine Hydrate

1. Introduction

Nanotechnology is a field of science with vast potential in medicine. Materials in the range of 100 nm or less are considered to be nanoparticles. They exhibit a wide range of properties, including optical, electrical, catalytic, magnetic and biological activity [1]. Some of the biological properties of nanoparticles have been explored by antimicrobial susceptibility testing of nanoparticles produced from different metals using different synthetic methods. It has been reported that metal nanoparticles (Ag, Cu, Au) exhibit a wide spectrum of antimicrobial activity against different species of microorganisms, including fungi and Gram-positive and Gram-negative bacteria [1].

The availability of copper (Cu) has made it a better choice to work with, because it shares properties similar to those of other expensive noble metals, including silver and gold. However, copper nanoparticles have major limitations, which include rapid oxidation on exposure to air. Therefore, alternative pathways have been developed to synthesize metal nanoparticles in the presence of polymers (eg, polyvinylpyrrolidone, polyethylene glycol, and chitosan) and surfactants (cetyl trimethyl ammonium bromide) as stabilizers, and to form coatings on the surface of nanoparticles [2,3]. Chitosan is a biocompatible, biodegradable, and nontoxic polymer with various applications in the pharmaceutical and biomedical fields. These properties make the chitosan polymer a good candidate for medical applications and research [4,5]. On the other hand, due to releasing ions and infections caused as a results performing implants especially stainless steel, usage of antibacterial coatings have been extensively used. In this regard, nanocomposite coatings due to wide range of effects on bacteria have been attracted. One of the most applicable methods of coating is electrophoretic deposition. Easy process and flexibility for any types of coatings such polymers, metals and ceramics [6,7]. Generally, the aim of this study was to synthesize copper nanoparticle by chemical reduction and fabricate nanocomposite antibacterial coating of chitosan/copper and evaluate physical and chemical properties of coating.

2. Materials and Methods

In this research, copper sulfate pentahydrate ($CuSO_4.5H_2O$) as precursor, chitosan with medium molecular weight ($(C_6H_{11}NO_4)_n$), acetic acid (CH_3CO_2H), sodium hydroxide (NaOH), ascorbic acid ($C_6H_8O_6$) and hydrazine hydrate (NH_2NH_2) which were purchased from Merck, Germany, were used. Distilled water was used to prepare solutions. In order to make a coating by electrophoretic method using power supply (SL20200J IPC) which stainless steel 316 low carbon was used as cathode and anode with dimension of $20\times10\times1$ mm^3, in order to synthesize copper nanoparticles, at the first 10 ml of 0.05 M of $CuSO_4.5H_2O$ added to 40 ml of 0.1 M acetic acid included different concentrations of chitosan (0.5, 1, and 4 g/l) until color of solution switched to blue (fig-1.a). Secondly, 0.5 ml of 0.05 M ascorbic acid incorporated to the solution. Then 2 ml of 0.6 M NaOH solution added to the solution until the solution became green (fig-1.b). Finally, 0.5 ml of hydrazine hydrate added to the solution to obtain dark brown color (fig-1.c). Upon the process, temperature of the solution was kept constant at 70°C and stirred magnetically in high speed [8].

Electrolyte used for coating was prepared by incorporation of 3 g/l acetic acid solution at different concentration of chitosan to previous solution. In this stage, copper to chitosan ratios (W/W) were considered 0.15, 0.3, 0.5 and 1 %, importantly, before performing coating process, the solution was subjected to ultrasonic. Substrates was polished using a polish sheet of number 600 to obtain a substrate with suitable roughness and then subjected to ultrasound waves in acetone, alcohol, and distilled water for 15 min to remove any kind of pollution from the surface. Electrophoretic deposition was carried out with a distance between cathode and anode of 10 mm at several times (1, 5, 10 and 15 min) and voltages 1, 5, 10 and 15 V. Fig-2 shows schematic of electrophoretic deposition set up.

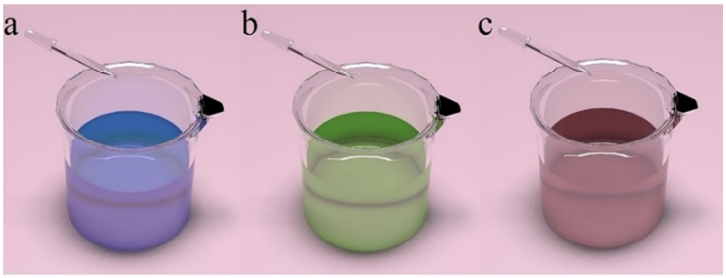

Figure 1: Steps of synthesize of copper nanoparticles.

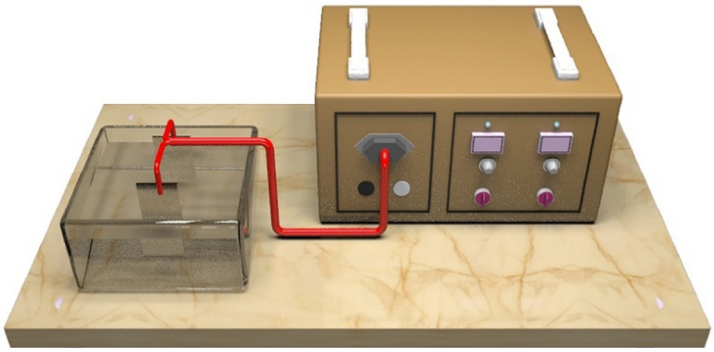

Figure 2: Schematic of electrophoretic deposition set up.

3. Characterization

Characterization of synthesized powder and chitosan was performed using Philips X'pert-MPD System with Cu Kα radiation. In order to determine functional groups existed at synthesized copper powder and compare to coating, Fourier transform infrared (FTIR) spectroscopy using Bruker Tensor-27 In the range of 600-4000 cm^{-1} was performed. Furthermore, to valuate distribution of copper nanoparticles into the coating, scanning electron microscope (SEM, Philips XL30) at 10-15 V was applied. Moreover, to evaluate morphology and particle size of powder obtained from chemical synthesis method, transmission electron microscope (TEM, Philips EM208S 100kV Netherland) at voltage of 10-15 V. the particle size of powders was measured using ImageJ software. Additionally, antibacterial activity of coating prepared against both gram-positive and gram-negative bacteria such as *S.aureus* and *E.coli* was investigated by disc diffusion method.

4. Results and Discussion

Figure-3 shows X-ray diffraction patterns of copper nanoparticle, chitosan, and coating formed on low-carbon steel 316. In X-ray pattern of copper, characteristic peaks appeared at 2θ:43.298, 50.434, 74.133, 89.934 and 95.143 were related to planes index of (111), (200), (220), (311), and (222) according to standard card (00-001-1241 (ICSD code)), moreover, no peak assigned to secondary phase observed especially copper oxide which is the most likely impurity in synthesis process of copper. In X-ray pattern of copper and according to the pattern of pure chitosan, the peak appeared at 20° attributed to pure chitosan. In the pattern of applied coating on low carbon stainless steel 316, three peaks located at 2θ: 44.354, 64.528 and 81.657 were related to planes index of (110), (200), and (211), in sequences, however, peak appeared at 20° which assigned to chitosan was not detectable in this pattern, that was why severe difference between intensity of peak related to substrate and copper with chitosan [9].

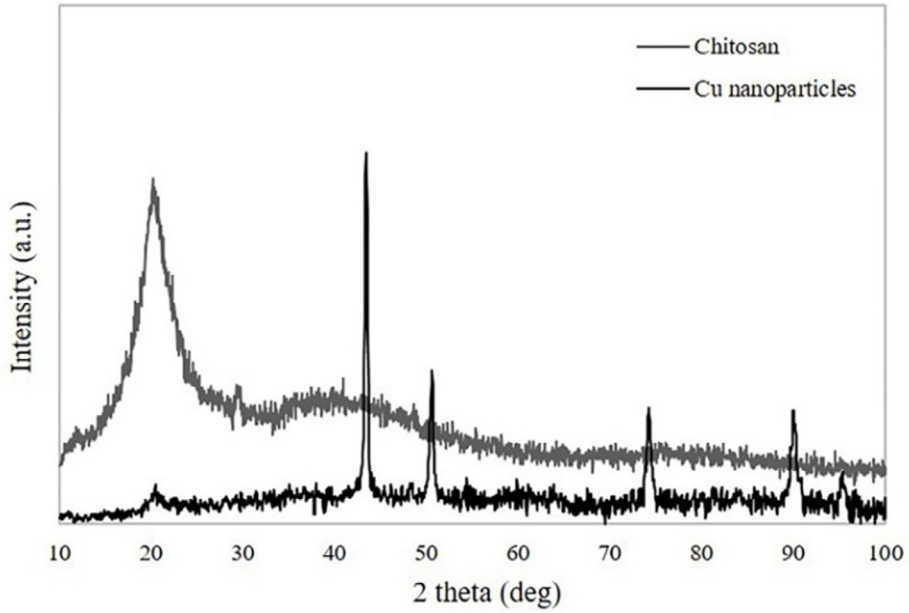

Figure 3: XRD pattern of chitosan, copper nanoparticles and coating.

Chitosan was added to the solution containing copper nanoparticles as stabilizer. Firstly, copper ion is covered by chitosan, then the complex is lessened to copper nanoparticle after incorporation of hydrazine with a core-shell structure which schematically shown in fig-4. This chemical reaction was showed in equation.1. The hydroxyl and amine groups of the chitosan surrounds the copper nanoparticles, which serve as capping, provide stability, prevent Cu_2O formation and likely give rise to a reduction in the size of the particles.

$$2[Cu(Cts)]^{+}_{aq} + N_2H_4 \longrightarrow 2[Cu(Cts)]\downarrow + N_2\uparrow + 2H_2O \quad (1)$$

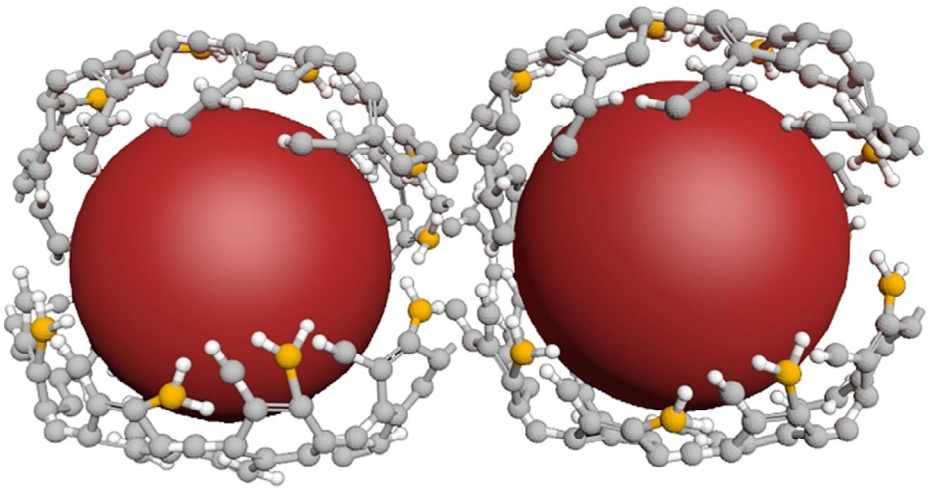

Figure 4: Core-shell structure of copper nanoparticle in chitosan.

Fig-5 illustrates TEM image of synthesized copper nanoparticle by chemical method and histogram chart of particle size of copper by which it could be figure out that particle size of copper was 11±6 nm.

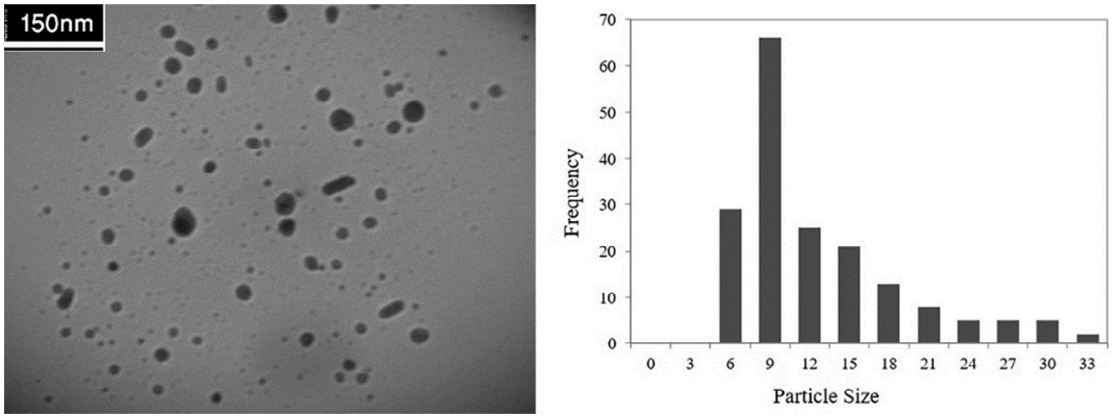

Figure 5: TEM image and histogram chart of particle size of copper nanoparticles.

FTIR test was performed in order to rate interactions of chitosan, copper nanoparticles as well as interactions between both of them. In FTIR pattern of chitosan, wide peak appeared at 3358 cm^{-1} was attributed to overlap O-H bond and N-H stretching bond. Peaks located at 2878, 1658; 1606, 1429; 1358; 1318 and 1028 were related to C-H stretching, N-H bending, C-H bending and C-O stretching bonds. A reduction in intensity and also considerable shift peak appeared at the pattern related to copper/chitosan. Peak attributed to N-H stretching bond shifted from 1606 to 1601 and peak located at 1658 was removed which it could be due to get covered copper nanoparticles by N-H groups of chitosan. Similarly, no peak detected at range of 1318-1429. In addition, new peaks with medium intensity appeared at approximately 629 which assigned to copper nanoparticles which these peaks could possibly indicate interaction between chitosan and copper nanoparticles.

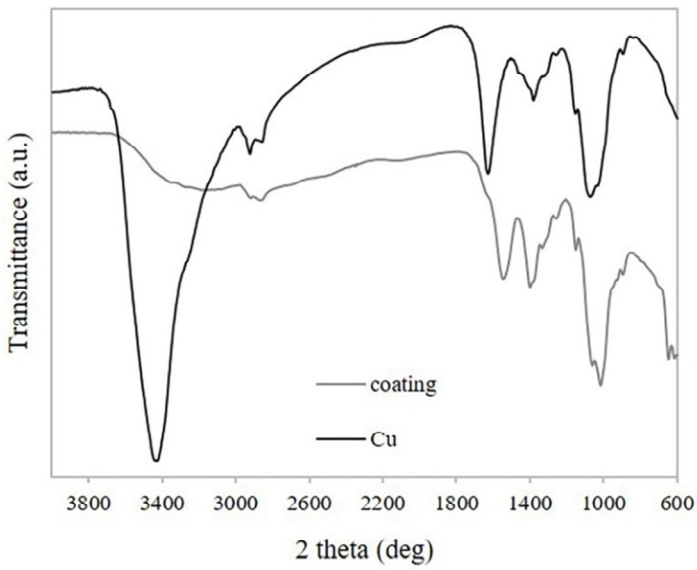

Figure 6: FTIR pattern of copper nanoparticle and coating

Scanning electron microscope (SEM) was carried out to investigate the surfaces of coating and recognize heavy nanoparticles of copper inside light field of chitosan. Fig-7 demonstrates image of back-scattered electron from the surface of nanocomposite coating. It was clear that there was a difference in contrast as a result of difference between atomic mass of chitosan and copper. Light points with higher contrast were related to copper nanoparticles

into coating. Distribution of particles uniformly was detectable in the image. In the other word, the difference in contrast could be due to locate particles in depth of coating.

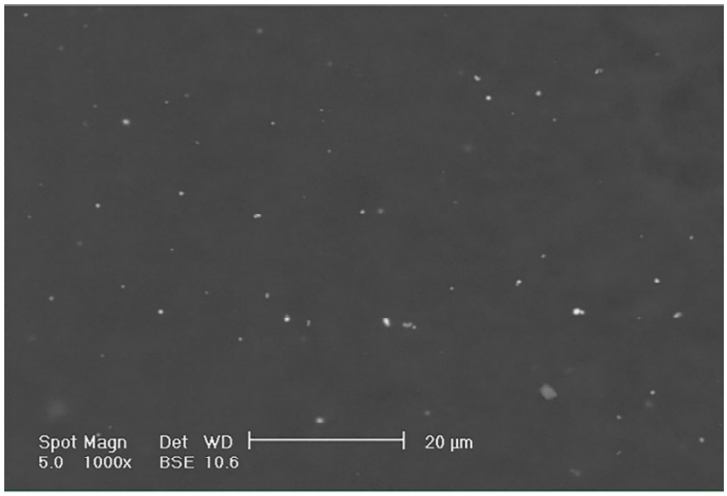

Figure 7: Back scatter image of nanocomposite.

According to fig-8, samples 1-5 were corresponded to steel, chitosan, 0.15 % Cu, 0.3 % Cu and 0.5 % Cu, in sequences. The best results against both gram–negative and gram-positive bacteria related to the highest concentration of Cu (0.5 wt. %).

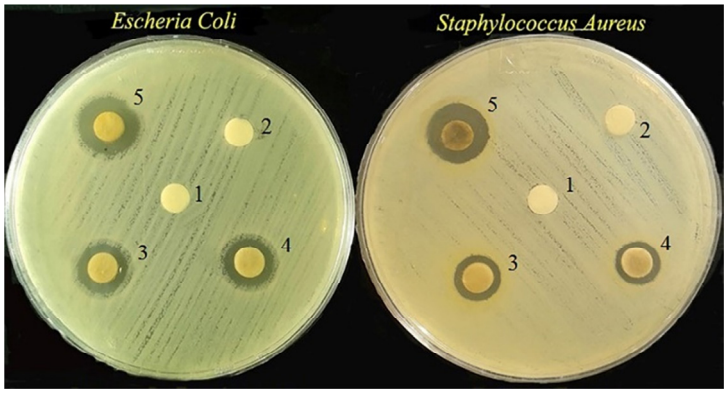

Figure 8. Disk diffusion test of coating.

5. Conclusions

In this study, copper nanoparticles with particle size of 11 nm were synthesized by copper sulfate pentahydrate. Chitosan molecules played a stabilizer role for copper nanoparticles by formation of a weak covalent bond between copper nanoparticles and functional groups existed on its surface, forming a protection layer. In situ synthesis of copper nanoparticles, formation of chitosan/copper composite and deposition were carried out successfully. Deposition was performed by electrophoretic method which the best surface quality and adhesion was related to time of 5 min and voltage of 5 V. The most applicable results of antibacterial test of coating against gram-negative and gram-positive bacteria was attributed to the sample with 0.5 wt. % of Cu.

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