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RESEARCH PAPER

Fabrication of Nanofiber-Based Filters Using Copper Oxide for Antibacterial Purposes

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ABSTRACT

The aim of this study is to produce antibacterial nanofiber filters that were used for the purification process. Copper oxide nanoparticles were synthesized by a chemical reduction method using sodium borohydride as a reducing agent and polyvinylpyrrolidone as a stabilizing agent. Subsequently, the nanofibers were prepared by electrospinning with four different concentrations of copper oxide nanoparticles using polyvinyl alcohol (PVA). The nanoparticles and nanofibers were characterized by UV-Vis spectroscopy, dynamic light scattering (DLS), X-ray diffraction (XRD) and scanning electron microscopy (SEM). The antibacterial activity of the nanofibers was tested against Escherichia coli ATCC 25922 and Bacillus subtilis ATCC6633 using the disk diffusion method. In addition, the effect of different concentrations of nanoparticles in the nanofibers on the antibacterial activity was investigated. According to the results of the present study, the optimal concentration of copper oxide nanoparticles in nanofibers was 5% for maximum antibacterial activity. Finally, the nanofibers showed very good antibacterial activity against gram-negative and gram-positive bacteria.

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INTRODUCTION

A fiber is a natural or artificial material that is significantly longer than it is wide. Fibers are often used to make other materials and are used in various industries. Fibers are generally divided into natural and synthetic fibers. It is very important to study synthetic fibers as the supply of natural fibers is limited. The diameter of fibers is usually in the range of 5 to 500 µm. In recent years, nanofibers have attracted much attention among researchers with the advancement of nanotechnology and nanoscale materials [1,2]. Nanofibers are defined as fibers with a diameter of less than 100 nm. The surface-to-volume ratio of nanofibers is very high, and they also have excellent mechanical properties such as toughness and tensile strength compared to conventional fibers. These valuable properties have made nanofibers a good option for many important applications. Various methods are used to produce such fiber-like structures. Among these methods, electrospinning is the simplest method that can be used to produce very thin and continuous fibers. Electrospinning is a simple and straightforward technique to produce polymer nanofibers with diameters ranging from a few micrometers to

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a few nanometers by forcing polymer liquids (solution or melt) through a spinneret with an electric field [3-5]. One of the promising areas of nanofiber is that mainly polymeric materials are used. During the process, the polymer solution/ melt is extruded, drawn or split into very fine fibers using external chemical or physical methods such as an electric field, drawing force or splitting into smaller pieces [6]. There are many biodegradable polymers that can be used for the production of nanofibers, e.g. natural, synthetic and mixtures of these two. Biodegradable polymers have been used to develop nanofibers with different functions depending on the requirement. Some can be used for a temporary function, such as drug carrier, cell carrier and for a short time. At a certain point in the process of electrospinning with synthetic polymers, it is promising to control their trajectory using an electric field due to the charge of the polymer solution [7]. Nanotechnology is playing an increasingly important role in various technologies of the new millennium. In general, there are various methods for synthesizing copper oxide nanoparticles. Many methods are based on the reduction of Cu^{2+} ions in a solution. In these methods, a reducing agent is (mainly) used for the reduction of Cu^{2+} ions in solution. One could explain the difference between these methods based on the same reducing agent [8-10].

In 2020, CuO nanorods were extracted from the fruit extract of Momordica charantia, an environmentally friendly and cost-effective method, and the therapeutic potential of CuO nanoparticles against Corynebacterium xerosis, Streptococcus viridians and the R2B strain of Newcastle disease was reported. Based on the results obtained, it was found that 100 µg/ mL concentrations of CuO nanoparticles were effective against the growth of NDV virus. [11]. Dulta et al (2022) investigated the antibacterial effect of CuO nanoparticles against Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Salmonella typhi. They reported that CuO nanoparticles have excellent application potential for photocatalytic degradation of organic pollutants and for the development of antibacterial materials [12]. The antibacterial activity of ZnO and CuO nanoparticles against Gramme-positive and Gramme-negative strains was investigated by Dadi et al. in 2019. The studies were conducted on Gramme-positive Staphylococcus aureus and Gramme-negative Escherichia coli and Pseudomonas aeruginosa bacteria using disc and well diffusion methods, bioluminescence and optical density analysis. The results showed a strong decrease of the bacterial strains after a short contact with the nanoparticles [13]. The antibacterial activity of the as-synthesised Cu_2O nanoparticles was evaluated by Bezza et al. in 2020 against Gramme-positive B. subtilis CN2 and Gramme-negative P. aeruginosa CB1 strains based on cell viability, zone of inhibition and minimum inhibitory concentration (MIC). They concluded that the lipopeptide-stabilised Cu2O NPs with an ultra-small size of 30 ± 2 nm in diameter exhibited potent antimicrobial activity against both Gramme-positive and Gramme-negative bacteria with a minimum inhibitory concentration of 62.5 µg/mL at pH5 [14]. Various techniques have been developed for the production of polymer fibers, especially in the nanometer range, such as drawing, template synthesis, self-assembly, phase separation, melt-blowing, spun-bonding, fibrillation, carbon dioxide (CO_2) laser supersonic drawing (CLSD) and electrospinning. The technique of electrospinning depends on the complex interaction of surface, shape, rheology and electrical charge. This phenomenon interacts in different ways to create electrified jets from polymer solutions and mixtures. These charges are carried by ions moving through liquids, the ions have the form of positive and negative charges, even if the electric charge between them is equal, the excess arises near the interface between the metallic conductor and the molecules of the solution, the Coulomb repulsions between the charged ions favor the formation of a jet [15, 16]. Nowadays, contact with biological agents in the workplace and living environment increases the likelihood of infectious diseases. This includes hospitals, offices and laboratories. When the filters are coated with nanoparticles such as copper oxide, they have antibacterial properties and a good ability to clean the contaminated environment. The aim of this study is to produce a nanofiber filter [17, 18]. The importance of nanofiber filters is rapidly increasing due to their highly porous structure, their narrow pore size and distribution, their specific surface area and their compatibility with inorganic materials. In this work, a nanofiber filter was fabricated and characterized by UV-Vis spectroscopy, dynamic light scattering (DLS), X-ray diffraction (XRD) and scanning electron microscopy (SEM). In addition,

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the antimicrobial activity of the nanofiber filter against B. subtilis and E. coli was investigated using the disk diffusion method.

MATERIALS AND METHODS

Materials

Copper sulphate pentahydrate ($CuSO_4$-$5H_2O$), sodium borohydride ($NaHB_4$), polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA) and acetone were purchased from Merck Co. Ltd (Germany). Muller Hinton Agar (MHA) powder as growth media was purchased from Sigma-Aldrich (Germany).

Synthesis of copper oxide nanoparticles

Four containers of 100 ml copper sulphate pentahydrate were prepared at concentrations of 0.01, 0.05, 0.1 M and 0.2 M. After addition of PVP and sodium borohydride, the solution was stirred for 30 minutes at 40 °C using a magnetic stirrer. After cooling to room temperature, a brown product was separated by centrifugation. After separation, it was repeatedly washed with deionized water (conductivity 0.05 µS/cm) and pure acetone and finally dried. The copper oxide nanoparticles were analyzed by UV-Vis spectroscopy (Model: Lambda 850, Company: Perkin Elmer,) and DLS analysis (Model: SZ-100z Dynamic Light Scattering & Zeta potential analyzer, Company: Horiba Jobin Jyovin).

The preparation of nanofiber

Electrospinning is a simple and comprehensive process for producing ultrafine fibers from various materials such as polymers, composites and ceramics. The electrospinning system essentially consists of three main components, namely a high-voltage power supply, a syringe with a metal needle and a conductive collector. Polyvinyl alcohol (PVA) is used as a suitable substrate for the adsorption, transportation and release of various materials. This polymer is available in various degrees of polymerization and hydrolysis. PVA can be easily electrospun and produces fibers in micro and nano dimensions that can be used in various fields such as mask manufacturing, filtration and medical clothing. In this work, copper oxide nanoparticles at different concentrations (1 wt%, 2 wt%, 5 wt% and 10 wt%) were added to a PVA solution (9%) and stirred for 3 hours to form a homogeneous solution. A nanofiber electrospinning device (Full Option lab EsII) was used to prepare the nanofiber samples from the solution. The solution was applied to the needle at a constant flow rate of 0.5 ml/h while a potential of 16 kV was applied to the needle.

Antibacterial activity

In the present study, the antibacterial activity against the bacteria E. coli and B. subtilis was investigated using the disk diffusion method. MHA medium plates were prepared, sterilized and solidified. Samples of nanofibers (1 wt%, 2 wt%, 5 wt% and 10 wt% copper oxide nanoparticles) were added to the MHA plate and incubated at 37 °C for 24 hours. At the end, the diameter of each inhibition zone for the prepared samples was measured with a ruler. The experiments were performed three times and the mean values of the zone diameters were determined.

RESULTS AND DISCUSSION

The study of copper oxide nanoparticles by UV-Vis spectroscopy

The adsorption value of the prepared samples was measured in four states in the wavelength range from 200 to 500 nm by UV-Vis spectroscopy. The UV–Vis absorption spectra of the copper oxide nanoparticles showed the absorption peak in the range of 220 to 260 nm. This absorption peak indicates the presence of copper oxide nanoparticles in solution [19,20]. The optical spectrum of these four concentrations is shown in Fig. 1.

Fig. 1 shows that the wavelength of the peaks increased from 230 nm to 260 nm with increasing concentration. This increase in peak wavelength indicates an increase in the size of the copper

Table 1. Chemical standards used: CAS number, supplier and purity.

Component	CAS number	Supplier	Purity
Copper sulphate pentahydrate	7758-99-8	Merck	>98
Sodium borohydride	16940-66-2	Merck	>98
Polyvinylpyrrolidone (PVP)	9003-39-8	Merck	>99
Polyvinyl alcohol (PVA)	9002-89-5	Merck	>99
Acetone	67-64-1	Merck	>97

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oxide nanoparticles. At a concentration of 0.01 M, the nanoparticles with the smallest size and the narrowest size distribution were observed. In addition, at both 0.1 M and 0.2 M concentrations, microparticles also appear to be present. On the other hand, the absorbance at *λmax* decreased with increasing concentration. This indicates a higher production of nanoparticles at lower concentrations.

The study of copper oxide nanoparticles by DLS

The next step is to use DLS analysis to measure the size of copper oxide nanoparticles. Fig. 2 shows the size distribution of the copper oxide nanoparticles obtained. As can be seen in Figs. 2a and 2b, the average size of copper oxide nanoparticles was about 15 nm and 35 nm at concentrations of 0.01 M and 0.05 M, respectively. However, as shown in Figs. 2c and 2d, two absorption peaks were observed at wavelengths of about 9 nm and 190 nm for the 0.1 M concentration and about 15 nm and 405 nm for the 0.2 M concentration, indicating an inappropriate distribution of nanoparticles and the presence of particles larger than 100 nm. DLS analysis showed that the most appropriate concentrations were 0.01 M and 0.05 M. These results were consistent with the results of Uv-Vis spectroscopy.

The study of prepared nanofiber by XRD

Fig. 3 displays the X-ray diffraction patterns of the pure PVA fiber and the copper oxide nanofiber. To confirm the existence of copper oxide nanoparticles in nanofibers, XRD spectra in the scan range 2θ = 10-80° were used for the 5 wt% case. There are obvious diffraction peaks at the diffraction angle of 19.2 º in the both curves Fig. 3a and 3b that indicates the presence PVA. There were more peaks in copper oxide nanofibers which were exhibited at 35.6◦, 39.1◦, 49.2◦, 54.1◦, 59.2◦, 62.5◦, 66.3◦ and 68.4◦. This pattern show that all of the diffraction peaks are in good agreement with the standard diffraction data for CuO (JCPDS 45-0937), and no characteristic peaks were observed for other oxides (such as Cu_2O or Cu_2O_3) [21].

The study of prepared nanofiber by SEM

The copper oxide nanofibers were studied

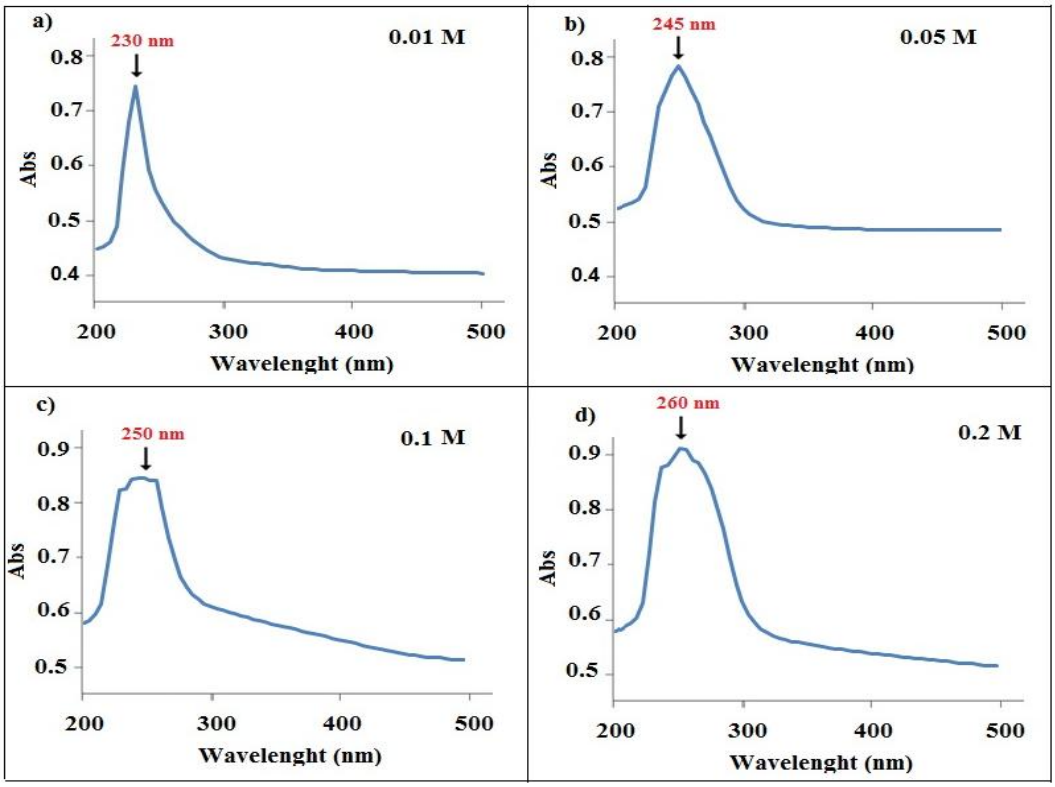


Fig. 1. UV–vis spectra of copper oxide nanoparticles colloids: a) 0.01 M, b) 0.05 M, c) 0.1 M, and d) 0.2 M.

by scanning electron microscopy (SEM) and the images show and confirm copper oxide nanoparticles in the fiber. The SEM images of the nanofiber are shown in Fig. 4. Fig. 4a shows the nanofiber without nanoparticles and Figs. 4b, 4c and 4d show the nanofiber with copper oxide nanoparticles at three concentrations of 1%, 5% and 10%, respectively. As can be seen from the figure, more copper oxide nanoparticles are observed in the nanofiber as the concentration increases.

The study of antibacterial activity of nanofiber by disc-diffusion method

The results were presented in Figs. 5, 6 and 7. As seen in figures, the growth of the bacteria was observed in the control samples (PVA nanofiber), but copper oxide nanofibers caused the inhibition zone of bacteria growth. The copper oxide nanofibers were tested on the bacterial

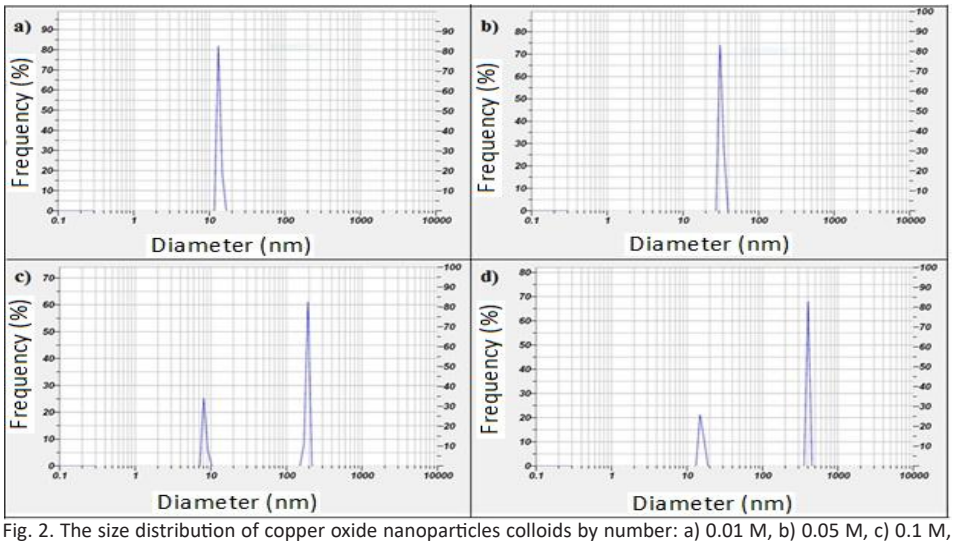


Fig. 2. The size distribution of copper oxide nanoparticles colloids by number: a) 0.01 M, b) 0.05 M, c) 0.1 M, and d) 0.2 M.

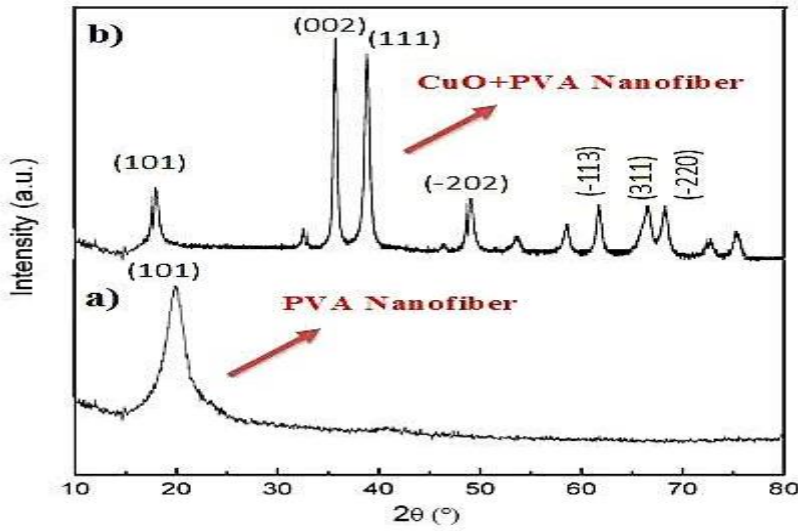


Fig. 3. XRD pattern of the a) pure PVA nanofiber b) Copper oxide nanofiber.

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strains B. subtilis and E. coli. The effectiveness of copper released from copper oxide nanofibers was determined from the zone inhibition of the bacteria. The formation of a clear zone around the hole is a sign of antibacterial activity. The inhibition zone of the diameters was determined. It was 3.4-8.7 mm and 4.1-10.1 mm for B. subtilis and E. coli, respectively. As you can see, the inhibition zone was gradually increased with increasing concentration of copper oxide in nanofibers. Also, the antibacterial activity of the copper oxide nanofibers is stronger on E. coli than on B.

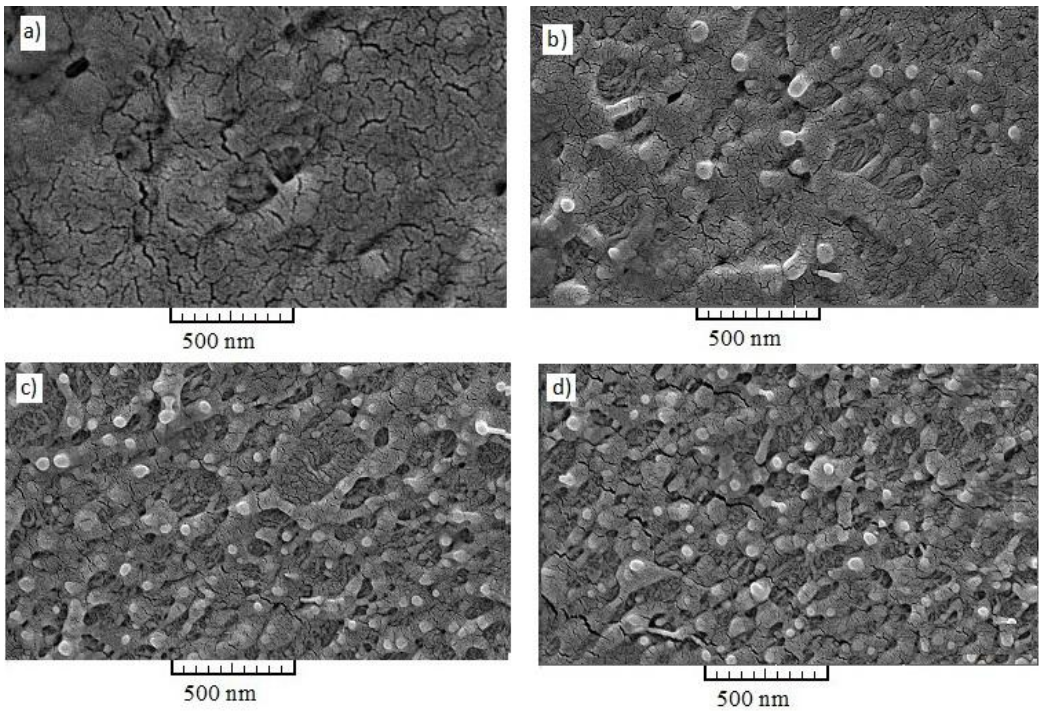


Fig. 4. SEM images of a) Pure PVA nanofiber, b) Nanofiber (1%), c) Nanofiber (5%) and Nanofiber (10%).



Fig. 5. Zone of inhibition for Control samples.

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subtilis. It is clear that the copper oxide nanofibers come into contact with the bacterial wall and show a strong effect against B. subtilis and E. coli. Generally, Copper-containing nanoparticles inhibits bacteria mainly through the release of the copper nanoparticles and ions. Copper-containing

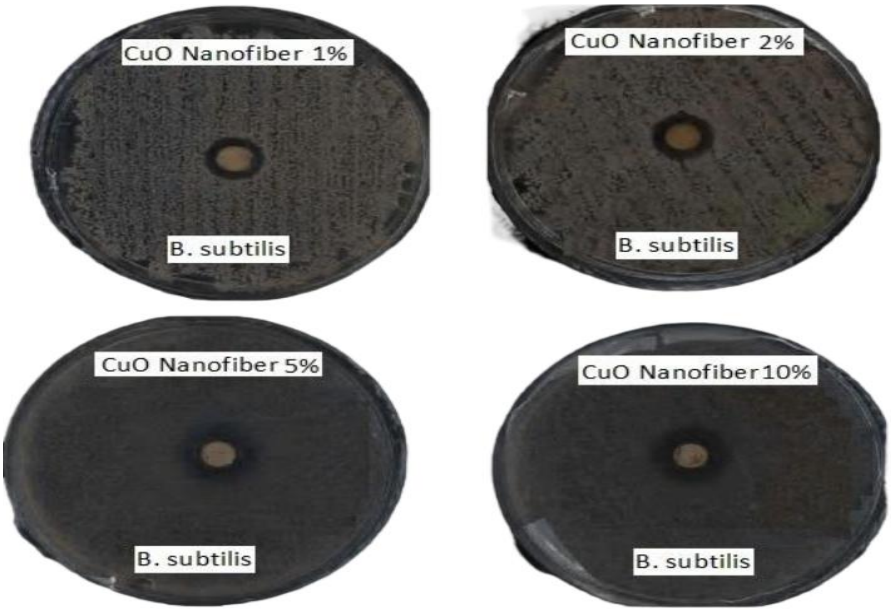


Fig. 6. Zone of inhibition for copper oxide nanofibers against B. subtills.

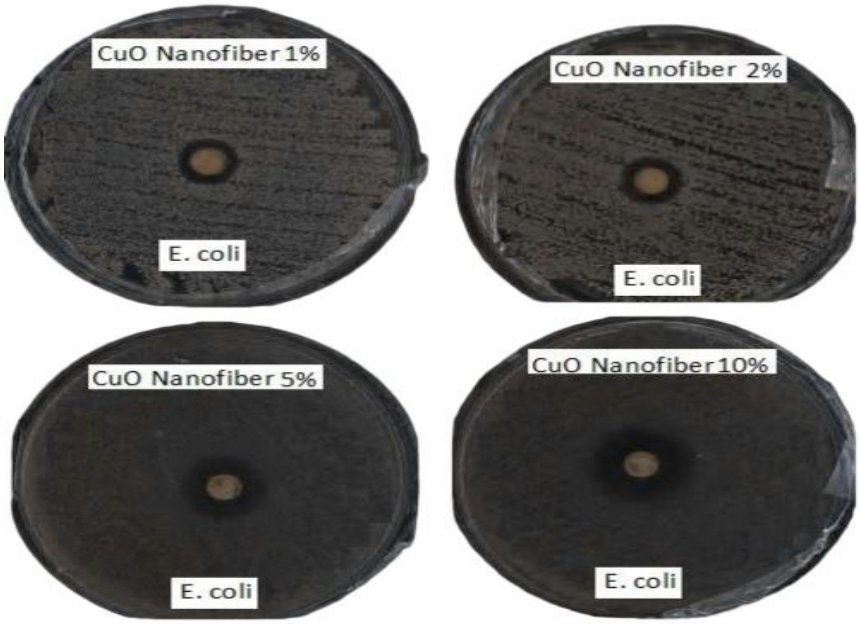


Fig. 7. Zone of inhibition for copper oxide nanofibers against E. coli.

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Table 2. The antibacterial activity of the nanofibers, zone of inhibition (mm).

Organism	Sample control, Nanofiber (0%)	Nanofiber 1%	Nanofiber 2%	Nanofiber 5%	Nanofiber 10%
B. subtilis	0	3.4	5.8	8.5	8.7
E. coli	0	4.1	6.7	9.8	10.1

nanoparticles produce reactive oxygen species (ROS), destroy cell walls and cell membranes, and react with proteins and DNA and kill bacteria that cause antibacterial properties. [22].

In 2017, Taran et al. investigated the antimicrobial properties of CuO nanoparticles on E. coli ATCC 25922 and S. aureus ATCC 43300 using the disk diffusion method. Their results show that two concentration levels of $CuSO_4$ (0.1 and 0.01M) have antibacterial activity on E. coli with a diameter of inhibition zone of 33±0.57 and 6 ±2mm [23]. In another study, the copper oxide nanoparticles showed remarkable antibacterial activity against both Gram-positive (B. subtilis and S. aureus) and Gram-negative (E. coli and P. aeruginosa) bacteria. The zone of inhibition for E. coli and B. subtilis was approximately 14-20 mm and 15-24 mm, respectively [24]. It seems that the antibacterial properties of the copper oxide nanofiber are lower than those of pure nanoparticles due to the distribution of nanoparticles in the fiber. The results of the present work show that the inhibition zone of bacterial growth increased with the higher concentration of copper oxide nanoparticles in the nanofiber (up to 5%), while the inhibition zone remained the same at the concentrations of 5% and 10%. In fact, the optimal concentration for maximum antibacterial activity was 5% for both types of bacteria. So, the copper oxide nanofibers show effective antibacterial activity against gram-negative and gram-positive bacteria. The results are summarized in Table 2.

CONCLUSION

In this study, copper oxide nanoparticles were synthesized by a chemical reduction method using sodium borohydride as a reducing agent and polyvinylpyrrolidone as a stabilizing agent. The copper oxide nanoparticles were characterized by UV-Vis spectroscopy and DLS analysis and the results showed that the most suitable concentrations were 0.01 M and 0.05 M. Subsequently, the nanofibers were prepared by electrospinning with four different concentrations of copper oxide nanoparticles. The antibacterial activity of the nanofibers was tested on the bacterial strains B. subtilis and E. coli using the disk diffusion method. According to the results of the present study, the optimal concentration of copper oxide nanoparticles in the nanofibers was 5% to achieve maximum antibacterial activity. Finally, the nanofibers showed very good antibacterial activity against gram-negative and gram-positive bacteria.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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