

Synthesis and Characterization of Polyvinyl Alcohol/ Gelatin/ CuO Nanocomposite Film Via γ -Irradiation for Antimicrobial Application

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ABSTRACT

Several polyvinyl alcohol (PVA)/ gelatin nano-copper oxide (nano-CuO) films were prepared by a solution casting method. Changes in the structural properties, with two different irradiation doses were subsequently investigated using TEM, SEM and FTIR. The FTIR spectrum and SEM shows a strong chemical interaction between PVA/ gelatin and nano-copper oxide (nano-CuO) with the formation of new peaks. Moreover, the results indicated that an intermolecular hydrogen bond and a chemical bond Cu- O-Cu were formed in the nano-CuO and PVA/ gelatin. Results showed that all the tested synthesized films have a significant growth inhibition of both Gram positive and negative bacteria and fungi,

Keywords: (PVA/Gelatin/Cuo Nanoparticles), Gamma irradiation, Films, Characterization

1. Introduction

Poly (vinyl alcohol) (PVA) is a synthetic polymer, water soluble, non-toxic, which has been used in various materials in the medical and biomedical areas [1]. It also has excellent film forming properties [2]. PVA is a versatile polymer with any industrial applications for a sizing, adhesives, and an emulsification, and it may be the only synthesized polymer, whose backbone is mainly composed of C–C bond, that is absolutely biodegradable [3].

Gelatin is a heterogeneous system constituted of 20 different amino acids. [4] showed that 50% of the amino acids of a pigskin gelatin were polar, being ionic (e.g. lysine, histidine, aspartic acid and glutamic acid) and non-ionic (e.g. serine, threonine, tyrosine, glutamine)

Gelatin is widely used in the photographic, pharmaceutical and food industries, since it is a protein that can be produced in abundance at relatively low cost and showing interesting functional properties [5]. In addition to its more conventional functional properties, gelatin presents outstanding film forming properties, and as a result has been widely used in studies involving edible films [4].

In theory, metal nanoparticles could be combined with polymers or coated onto surfaces, which may then have a variety of potential antimicrobial applications. The antimicrobial properties of both silver and copper nanoparticles [7] have been previously reported, and both of these have been coated onto or incorporated into various materials [8].

CuO is cheaper than silver, easily mixed with polymers and relatively stable in terms of both chemical and physical properties. Highly ionic nanoparticulate metal oxides, such as CuO, may be particularly valuable antimicrobial agents as they can be prepared with extremely high surface areas and unusual crystal morphologies [9]. The aims of this study were to characterize physically and chemically nano CuO and to investigate this compound with respect to its potential antimicrobial applications.

2.EXPERIMENTAL

2.1. Materials

PVA used in this work was obtained in a powder form C.R.C Fine Chemicals, Ltd.(Italy), with a molecular weight of 450,000 g/mol and degree of polymerization of 1750-50. The gelatin used in this work is type B derived from bovine skin, with Bloom number 50-300 and presents an iso-electric point between 4.7 and 6, was purchased from Fisher Scientific Fine Chemicals, Ltd (Germany) and used without further purification.

Copper Oxide (CuO) is black powder nanoparticle size 40 nm, with molecular weight of 79.546 g/mol , assay > 99%, melting point 1326°C and density 6.3-6.49g/mol at 20°C obtained from MK Nano (Diven. of M K impex Corp.) Chemical Nano Company, Canada. Citric acid used in this work is white powder(C₆H₈O₇.H₂O), with molecular weight of 210.14 g/mol, assay 99.5-100.5%, was obtained from PRS Panreac Company, (Spain). HCl used, which is of analytical grade, purchased from Aldrich. The water used was distilled and de-ionized water. All reagents, which were all of analytical grade, were used as received.

2.2. Antibacterial activity materials

2.2.1. Microbial sources and culture conditions

The antimicrobial activities of the developed polymer blend of Poly (vinyl alcohol)/Gelatin films and nanocomposite film of Poly (vinyl alcohol)/Gelatin/CuO) before

and after irradiated are tested by disc diffusion, spread plate method against different pathogenic microorganisms. Gram positive and negative reference strains of bacteria from the American Type Culture Collections (ATCC) were used in this study as follows: *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 19430, *Klebsiella pneumonia* ATCC 13888, *Shigella flexneri* ATCC 12022, *Pseudomonas aeruginosa* ATCC 278223, and Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300. These bacterial strains were kindly donated from LNH (Luxor National Hospital), maintained on suitable medium at 4 °C and subcultured on MacConkey agar and Mueller Hinton Broth at 37 °C for 18 hrs before testing.

The fungal isolate used in this study was identified as *Aspergillus niger*. The fungus was isolated from infected plants, subcultured on plates of glucose-Czapek's agar medium [10] which contained per liter: glucose, 10 g; NaNO₃, 2 g; KH₂PO₄, 1 g; MgSO₄.7 H₂O, 0.5 g; agar agar, 20 g and 1000 ml distilled water. The medium was supplemented with chloromphenicol (250 mg per liter) [11]. Pure cultures of the tested fungi were transferred to glucose- Czapek's agar slants and used for antifungal activity.

2.2.2. Composition of bacterial media

Mueller Hinton agar (MHA) (g/L) [12] Beef extract, 3.0 g; Casein hydrolysate, 17.5 g; Starch, 1.5 g; Agar, 17 g, pH = 7.3 ± 0.1 at 25 °C. Muller Hinton Broth (MHB) (Beef extract, dehydrated infusion form, 3 g, Casein hydrolysate, 17.5 g, Starch, 1.5 g, pH = 7.3 ± 0.1 at 25°C. **MacConkey agar (g/L) (Macconkey,1905) [13]** Peptone, 20 g; Lactose, 10 g; Bile salts, 1.5 g; Sodium chloride, 5 g; Neutral red, 0.05 g; Crystal violet, 0.0001 g; Agar, 15 g, pH = 7.2 ± 0.2 at 25°C.

2.2.3. Disc diffusion test

Antibacterial activity was determined against the above bacterial strains and the fungus isolate as model bacteria and fungi using disc diffusion method [14]. The films are cut into disc shape of 6-mm diameter, sterilized by autoclaving for 20 min. at 121 °C. Agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. For bacterial strains, the concentration was approximately 1.2 X 10⁸ CFU/ml. The discs were placed on the Muller Hinton medium suitably spaced apart and the plates were incubated at 37 °C for 24 h. Commercial antibiotic discs (Chloromophenicol, 10 mg disc and tetracycline, 30 mg disc) were used as a positive control. For *A. niger*, 1 cm disc from 14 days fungus culture was placed on glucose-Czapek's agar medium. The film discs were placed in contact within the same plate. Diameter of the inhibition action of films for the microbial growth is then measured by a ruler and expressed in millimeter. All the assays were carried out in triplicate.

2.3. Methods

2.3.1. Preparation of Gelatin / PVA blend films:

Aqueous 10 wt.% Gelatin/PVA solutions were prepared by dissolving 6 g of ST and 4 g PVA in 125 ml water and refluxing these at 90 °C for 30 min. Then (0.5ml of Citric acid 0.1N) was mixed, and the mixture was stirred for 90 min before casting into a plexi glass plate placed on a leveled flat surface. After the blends were allowed to dry at 50°C in an oven for 12 h, the fully dried membranes were peeled away from the glass plate, then they were heated in a thermosetting oven at 95°C for 1 h to induce crosslinking reaction [15].

2.3.2. Preparation of Gelatin / PVA nano-CuO composites films:

The Gelatin/PVA/nano-CuO composites were prepared by the addition of a nano-CuO mixture to the 10 wt.% Gelatin/PVA solutions. The nano-CuO mixture was prepared by mixing nano-CuO and (0.1N) HCl which was stirred at room temperature for 2 h. The nano-CuO mixture was added at a different weight to the polymer weight. Then the Gelatin/PVA/ CuO solutions were mixed together along with 0.5ml of Citric acid 0.1N for each composition, and the mixture was stirred at 90 °C for 2 h. The solutions were then poured onto a plexi glass plate. The cast solutions were allowed to dry at 50 °C for 12 h. After which, the fully dried membranes were peeled away from the glass plate, and then were heated in a thermosetting oven at 95 °C for 1 h to induce the crosslinking reaction.

2.4. Irradiation process:

Irradiation of the prepared samples to the required doses (5 and 20kGy) was carried out in a 60Co gamma cell (made in India) at the National Center for Radiation research and Technology, Cairo, Egypt. Irradiation was carried out under atmosphere at a dose rate 6.92 kGy/h.

2.5. Characterization:

A transmission electron microscope, TEM, of the type JEOL-JEM-1011, Japan was used to determine the particle size distribution of Copper oxide nanoparticles. The scanning electron microscope (SEM) was employed with the scanning electron microscope of JEOL-JSM-5500 LV (Japan). The IR spectra were recorded over the range (400 – 4000 cm⁻¹) at resolution 4 cm⁻¹ by using Fourier transform infrared spectrometry (FT-IR-6300)- Japan, Ultraviolet/visible spectroscopy, (UV/VIS) spectrometer (Jasco/v-560) made in Japan, was used for scanning the absorption spectra in the range 200 nm to 900 nm wavelengths.

3. Results:

3.1. TEM analysis:

The particle size of CuO oxide nanoparticles were determined to be in the range 18-20 nm. Almost all the particles had only one unique grain (See Figure 1.) accordance to study [16].

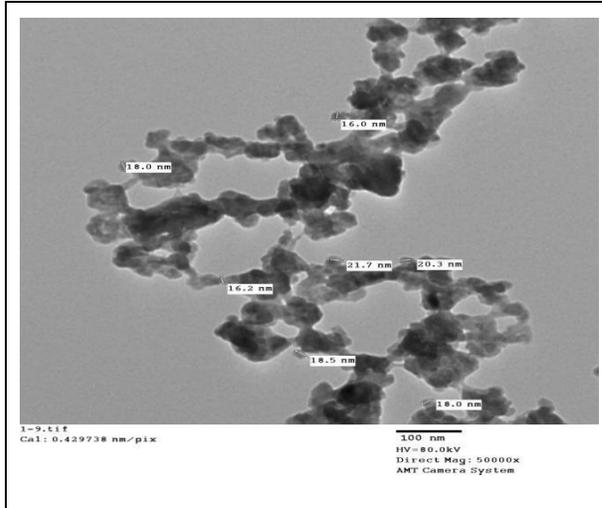
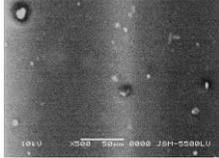
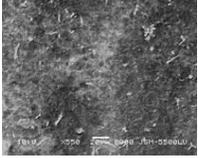
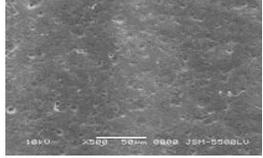


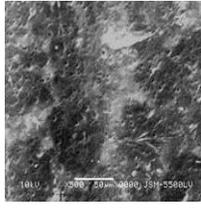
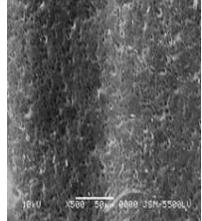
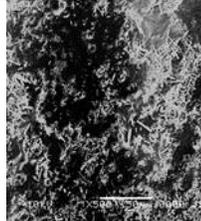
Figure1. Particle size of CuO nanoparticles(18-20nm)

3.2. SEM analysis

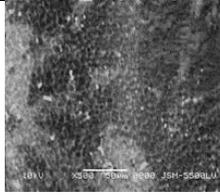
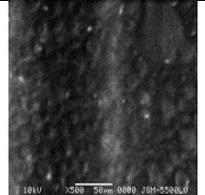
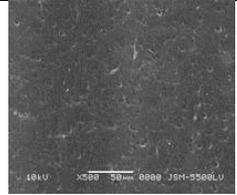
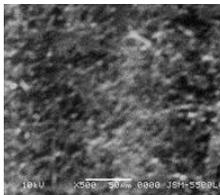
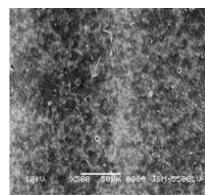
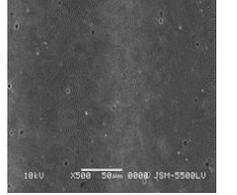
The morphology of unirradiated polymer nanocomposite of (PVA/Gelatin/0.05-0.2gCuO) film inserts was studied through the analysis of images obtained by scanning electron microscope

(SEM) for the unirradiated polymer nanocomposite films ratios (25/75/0.05- 0.2gCuO), (50/50/0.05-0.2gCuO) and (75/25/0.05-0.2gCuO) displayed in Figures (2-7 and 11-13). The photomicrographs of the films obtained by SEM for the upper surface and cross-section showed homogeneity between CuO nanoparticles and (PVA/Gelatin) phases with pores observation. A similar effect observed in previous study [17]. Also, the surface becomes smooth and continuous. The images presented CuO nanoparticles as small white particles dispersed uniformly in polymer bulk.

		
<p>Figure 2. SEM of unirradiated(25/75/0.05CuO).</p>	<p>Figure 3. SEM of unirradiated(50/50/0.05CuO)</p>	<p>Figure 4. SEM of unirradiated(75/25/0.05CuO).</p>

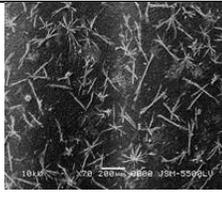
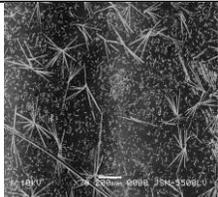
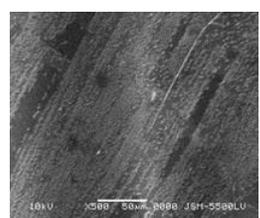
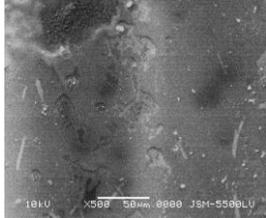
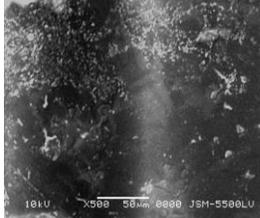
		
<p>Figure 5. SEM of unirradiated (25/75/0.2CuO)</p>	<p>Figure 6. SEM of unirradiated (50/50/0.2CuO)</p>	<p>Figure 7. SEM of unirradiated (75/25/0.2CuO).</p>

Figures (8-13) shows the effect of gamma irradiation doses (5 and 20KGy) on observed polymer nanocomposite ratios of (PVA/Gelatin/0.05gCuO) film, investigated by scanning electron microscope (SEM). The produced images reveal that, the small particles size of CuO nanoparticles in surface were distributed completely on all of the surfaces in regular behavior due to effective of the used doses (5 and 20KGy) of gamma irradiation. Also, homogeneity and smoothing of surface were recorded. No phases separation was observed, indicating good compatibility between insert matrix and CuO nanoparticles as in study [17].

		
<p>Figure 8. SEM of (25/75/0.05CuO) at 5KGy</p>	<p>Figure 9. SEM of (50/50/0.05CuO) at 5KGy</p>	<p>Figure 10. SEM of (75/25/0.05CuO) at 5KGy.</p>
		
<p>Figure 11. SEM of (25/75/0.05CuO) at 20KGy</p>	<p>Figure 12. SEM of (50/50/0.05CuO) at 20KGy</p>	<p>Figure 13. SEM of (75/25/0.2CuO) at 20KGy.</p>

In other hand a regular straight white line with complete homogeneity, smoothing and absence of pores were observed in irradiated ratio (25/75/0.2CuO) at 20kGy (see Figure

17). Distribution of CuO nanoparticles as a group of white tall thin sticks or rods shape presented in irradiated ratio (50/50/0.2CuO) at 5kGy as shown in Figure 15. The morphology image of irradiated ratio (70/20/0.2CuO) at 5kGy shows shape of flowers distributed completely in the surface (see Figure 16). [18].

		
<i>Figure 14. SEM of (25/75/0.2CuO) at 5KGy.</i>	<i>Figure 15. SEM of (50/50/0.2CuO) at 5KGy.</i>	<i>Figure 16. SEM of (75/25/0.2CuO) at 5KGy</i>
		
<i>Figure 17. SEM of (25/75/0.2CuO) at 20KGy</i>	<i>Figure 18. SEM of (50/50/0.2CuO) at 20KGy.</i>	<i>Figure 19. SEM of (75/25/0.2CuO) at 20KGy.</i>

1.1. FTIR characterization

Figure (20 and 26) shows the FT-IR absorption spectra and their assignment of unirradiated and irradiated polymer nanocomposite ratios of (25/75/0.05-0.2gCuO), (50/50/0.05-0.2gCuO) and (75/25/0.05-0.2gCuO) of (PVA/Gelatin/0.05-0.2gCuO) film. The band at 3345 cm^{-1} is assigned to O—H stretching vibration of hydroxyl groups observed in a strong broad band. Also, this broad band is overlaps the N—H stretching in the same region according to [19].

Band at 2149 cm^{-1} is generally ascribed to the combination frequency of (CH+C≡C) according to [20]. Carbonyl group C=O was present at 1745 cm^{-1} . A weak band is appeared at 936 cm^{-1} assigned for CH₂ rocking as in study [21]. The vibrational absorption peaks of all metal oxides (M—O) bands were observed with low intensity. As well as bands in the low frequency range assigned to the loaded CuO Nanoparticles. The intensity bands at

868 -708 cm^{-1} attributable to Cu—O—Cu in plane vibration according to previous study [21], [22]. The peak at 647 cm^{-1} indicates the vibration of Cu (I)—O in the studied film While, peaks at 556 and 434 cm^{-1} are attributed to Cu(II)—O out of plane vibrations similar to [23-24]. No remarkable changes observed in FT-IR spectra of irradiated (PVA/Gelatin/0.05gCuO) and (PVA/Gelatin/0.2gCuO) as shown in figures (21-22 and 24-25). This may be due to selected low doses (5 and 20kGy) of gamma irradiation used. Moreover, non-changes in films may be due to the stability of investigated polymer nanocomposite according to [25].

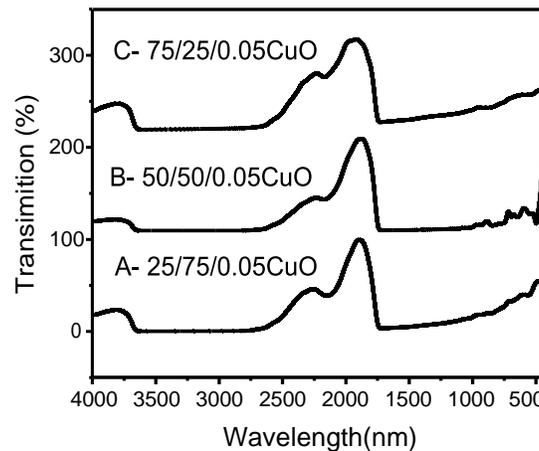


Figure 20. FT-IR Spectra of unirradiated polymer nanocomposite ratios of (PVA/Gelatin/0.05CuO) film.

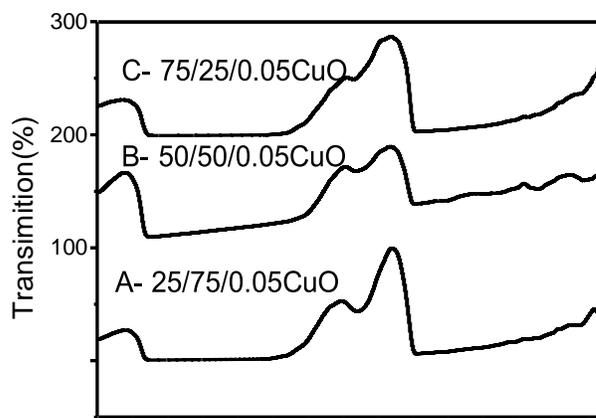


Fig.21.FT-IR Spectra of irradiated polymer nanocomposite ratios of (PVA/Gelatin/0.05CuO) film at 5kGy

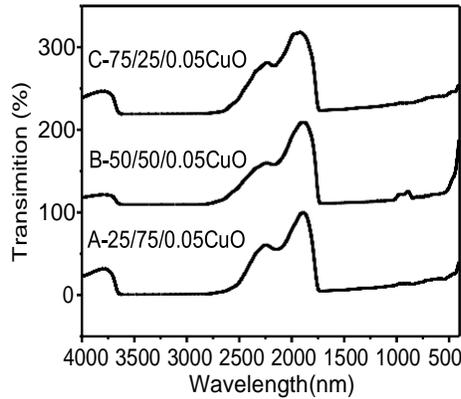


Figure 22. FT-IR Spectra of irradiated polymer nanocomposite ratios of (PVA/Gelatin/0.05CuO) film at 20kGy.

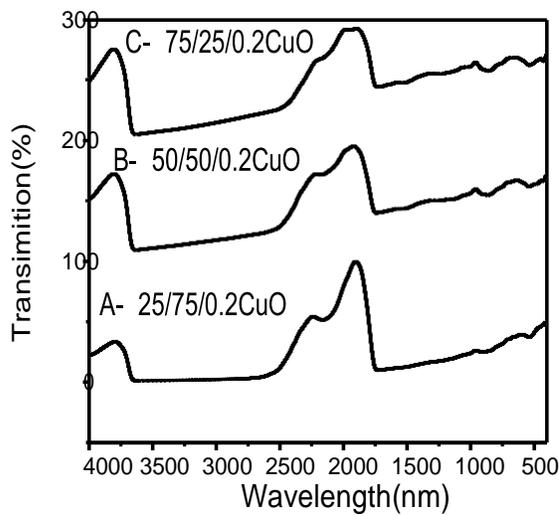


Figure 23. FT-IR Spectra of unirradiated polymer nanocomposite ratios of (PVA/Gelatin/0.2CuO) film.

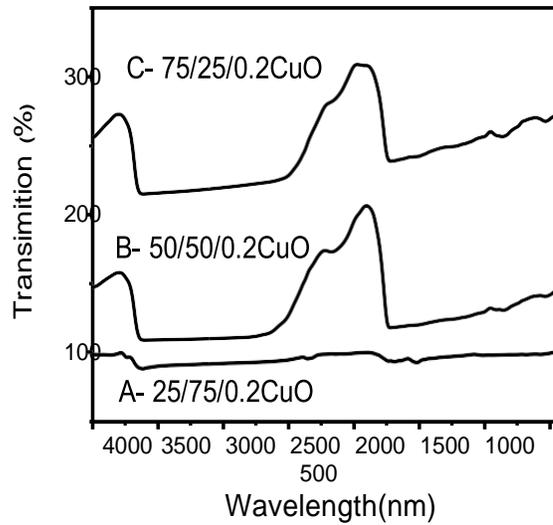


Figure 24. FT-IR Spectra of irradiated polymer nanocomposite ratios of (PVA/Gelatin/0.2CuO) film at 5kGy.

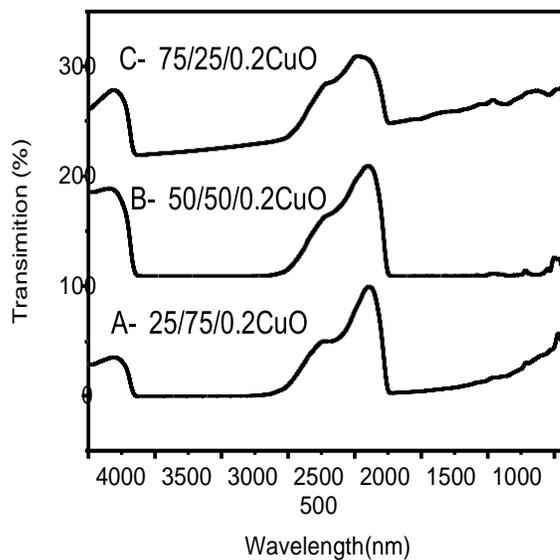


Figure 25. FT-IR Spectra of irradiated polymer nanocomposite ratios of (PVA/Gelatin/0.2CuO) film at 20kGy.

3.3. Biological activity

Antimicrobial activity of the synthesized unirradiated and irradiated nanocomposite

films of (PVA/Gelatin/0.05CuO) and (PVA/Gelatin/0.2CuO) for the selected ratios (25/75), (50/50) and (75/25) with using citric acid plasticizer were investigated.

Antimicrobial activity was applied by using the microorganisms *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 19430, *Klebsiella pneumonia* ATCC 13888, *Shigella flexneri* ATCC 12022, *Pseudomonas aeruginosa* ATCC 278223, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300 and *Aspergillus niger* isolate was examined by the presence or absence of inhibition zone diameters as shown in Figures (32- 44).

The obtained results showed that all the tested synthesized films have a significant growth inhibition of both Gram positive and negative bacteria and fungi,

Among Gram negative bacteria, *Shigella flexneri* ATCC 12022 were the most sensitive to all the synthesized irradiated nanocomposite films compared to *chloramphenicol*. The higher antibacterial activity (indicated as zone of inhibition) was recorded for studied irradiated ratios (25/75/0.2CuO), (50/50/0.2CuO) and (75/25/0.2CuO) of nanocomposite film (PVA/Gelatin/0.2CuO) at the applied dose 5K Gy of gamma radiation and also, for the applied irradiated ratios (25/75/0.2CuO), (50/50/0.2CuO) and (75/25/0.2CuO) of nanocomposite film (PVA/Gelatin/0.2CuO) at 20K Gy (32.5, 28.5, 28, 32.5, 33.5, and 30 mm) respectively, while the inhibition zone of *chloramphenicol* was 8.5 mm are shown in Figures (27-40). Hence, the susceptibility of *Shigella flexneri* ATCC 12022 to synthesized irradiated nanocomposite films was more pronounced when compared to the antibiotic *chloramphenicol*.

Next to *Shigella flexneri* ATTC 12022, *Klebsiella pneumonia* ATCC 13888 and *Pseudomonas aeruginosa* ATCC 278223 were very susceptible to the same previous compounds followed by *Escherichia coli* ATCC 25922 and *Salmonella typhi* ATCC 19430as illustrated in Figures (27, 28, 29, 30, 31,32 and 33).

It is worth mention that the same effect for studied irradiated ratios (25/75/0.2CuO), (50/50/0.2CuO) and (75/25/0.2CuO) of nanocomposite film (PVA/Gelatin/0.2CuO) at the

applied dose 5KGy of gamma radiation and also, for the applied irradiated ratios (25/75/0.2CuO), (50/50/0.2CuO) and (75/25/0.2CuO) of nanocomposite film (PVA/Gelatin/0.2CuO) at 20KGy had a significant antimicrobial effect on both Gram positive bacteria and fungi - *Methicillin-resistant Staphylococcus aureus* (MRSA) ATTC 43300 and *Aspergillus niger* isolate.

The most observed antibacterial activity was recorded within the irradiated ratio(75/25/0.2CuO) of nanocomposite film (PVA/Gelatin/0.2CuO) at 5KGy (inhibition zone 36 mm) for *Staphylococcus aureus* (MRSA) ATTC 43300 and irradiated ratio (50/50/0.2CuO) of nanocomposite film (PVA/Gelatin/0.2CuO) at 20KGy (inhibition zone 31.5 mm) as depicted in Figures (34-40).

A few studies have been performed to explain the mechanism of bactericidal action of nanoparticles. For example, [26] suggested that the exposure of Gram-positive bacteria to carboxy fullerene nanoparticles resulted in the puncturing of the bacteria leading to cell death. Another proposed way in which the membrane can be compromised is the alteration of membrane lipid components which is similar to [27]. It is, however, difficult to distinguish between the bactericidal activity of nanoparticles from the ions released by the nanoparticles themselves as in study [28]. The presence of nanoparticles in suspension would ensure continuous release of ions into the nutrient media accordance

to [29].

In addition, study [30] suggested a correlation finding with the size of the nanoparticles and bactericidal effect, also, explained the precise mechanisms of how nanoparticles act as biocidal agent. There are, however, a few mechanisms of nanoparticle toxicity suggested by other works. For example, copper ions released by the nanoparticles may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell death according to [31]. Copper nanoparticles inside the bacterial cells may bind to deoxyribonucleic acid molecules and become involved in cross-linking within and between the nucleic acid strands, resulting in the disorganized helical structure. In addition, copper ion uptake by the bacterial cells has also been found to damage important biochemical processes as seen to study [32].

It is clear from Table (1) and Figures (27 to 40) (maximum zone of inhibition against *Gram positive, gram negative and fungus*) that nanocomposite films with citric acid as plasticizer have shown greater antimicrobial activity against *all of these microorganisms*. The variation in the sensitivity or resistance to both Gram-positive and -negative bacteria populations could be due to the differences in the cell structure, physiology, metabolism, enzymatic activity (in case of the tested fungus) or degree of contact of organisms with nanoparticles. For example, greater sensitivity for Gram-positive bacteria *Methicillin-resistant Staphylococcus aureus* (MRSA) ATTC 43300 to the nanocomposite films with citric acid as plasticizer has been attributed to the greater abundance of amines and carboxyl groups on their cell surface and greater affinity of copper towards these groups according to [33].

Alternatively, Gram-negative bacteria like *Escherichia coli* ATCC 25922, *Salmonella typhi* ATCC 19430, *Klebsiella pneumonia* ATCC 13888, *Shigella flexneri* ATCC 12022, *Pseudomonas aeruginosa* ATCC 278223 have a special cell membrane structure which possesses an important ability to resist antimicrobial agents as in study [34]. Furthermore, other factors such as nanoparticle diffusion rates may also affect

bacterial strain differently. Same results were recorded in previous study [35] using algal extracts against pathogenic gram positive and negative bacteria. Nevertheless, further studies are required to confirm this and it is beyond the scope of this work.

Broadly, interactions between the negative charges of microorganisms and the positive charge of nanoparticles produces an electromagnetic attraction between the microbe and effective levels of active nanoparticles. Such interactions lead to oxidation of surface molecules of microbes resulting in their death. Biodestructive effects such as degradation of deoxyribonucleic acid was observed following exposure of Gram positive

bacteria to silver and copper nanoparticles by other works [30], [36], [37] are in agreement with present findings.

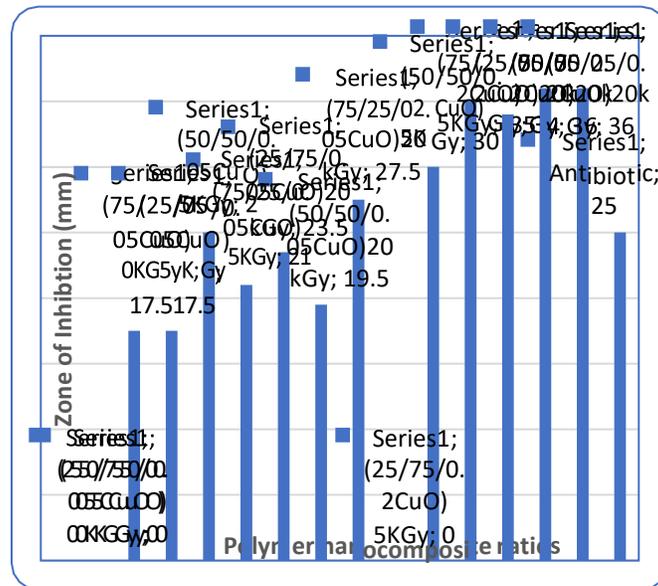


Figure 27. Antibacterial activities of unirradiated and irradiated polymer

Figure 30. Antibacterial activities of unirradiated and irradiated polymer nanocomposite film ratios of (PVA/Gelatin/0.05-0.2g CuO) against *Shigella flexneri* ATTC 12022.

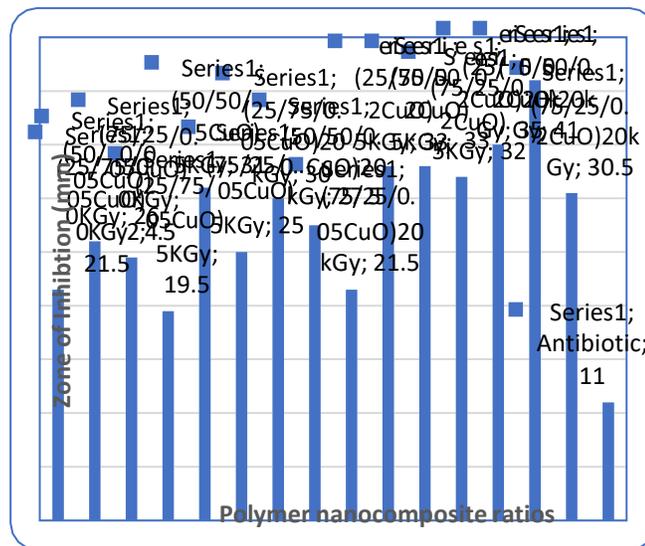


Figure 31. Antibacterial activities of unirradiated and irradiated polymer nanocomposite film ratios of (PVA/Gelatin/0.05-0.2g CuO) against *Pseudomonas aeruginosa* ATCC 278223.

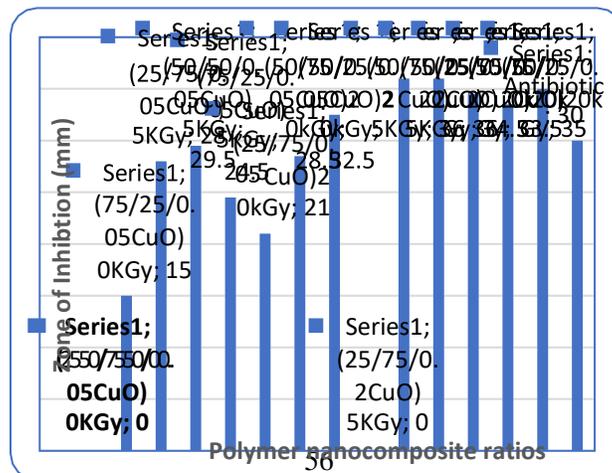


Figure 32. Antibacterial activities of unirradiated and irradiated polymer nanocomposite film ratios of (PVA/Gelatin/0.05-0.2g CuO)) against Methicillin-resistant *Staphylococcus aureus* (MRSA) ATTC 43300.

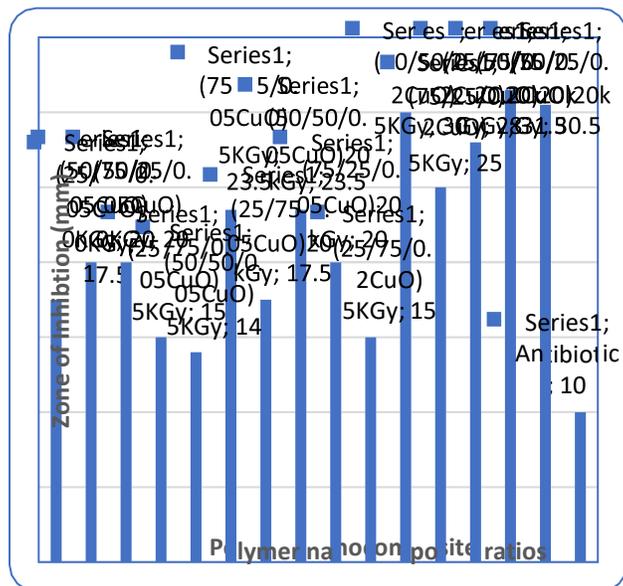


Figure 33. Antifungal activities of unirradiated and irradiated polymer nanocomposite film ratios of (PVA/Gelatin/0.05-0.2g CuO)) against *Aspergillus niger* isolate.



Figure 34. Inhibition halo zone of irradiated nanocomposite film ratios (25/75/0.2CuO), (50/50/0.2CuO), (75/25/0.2CuO) and (25/75/0.2CuO) at 5KGy and 20kGy respectively against *Pseudomonas aeruginosa* ATCC 27822.

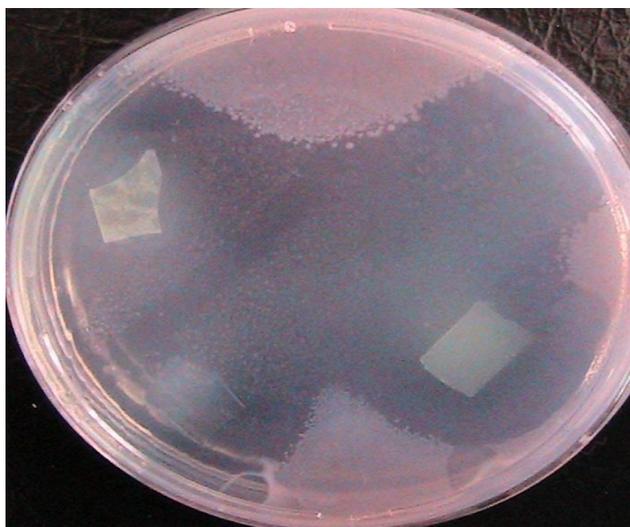


Figure 35. Inhibition halo zone of irradiated nanocomposite film ratios (50/50/0.2CuO), (75/25/0.2CuO), (25/75/0.2CuO) and (50/50/0.2CuO) at 5KGy and 20kGy against *Shigella flexneri* ATCC 12022.

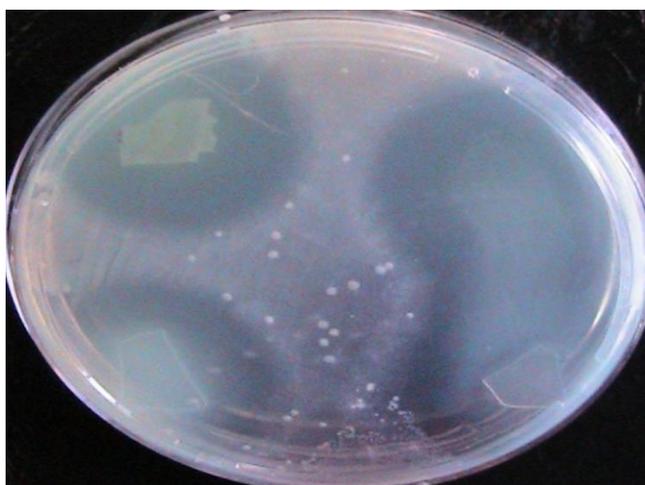


Figure 36. Inhibition halo zone of irradiated nanocomposite film ratios (50/50/0.2CuO), (75/25/0.2CuO) and (25/75/0.2CuO) and (50/50/0.2CuO) at 5KGy and 20kGy against *Salmonella typhi* ATCC 19430.

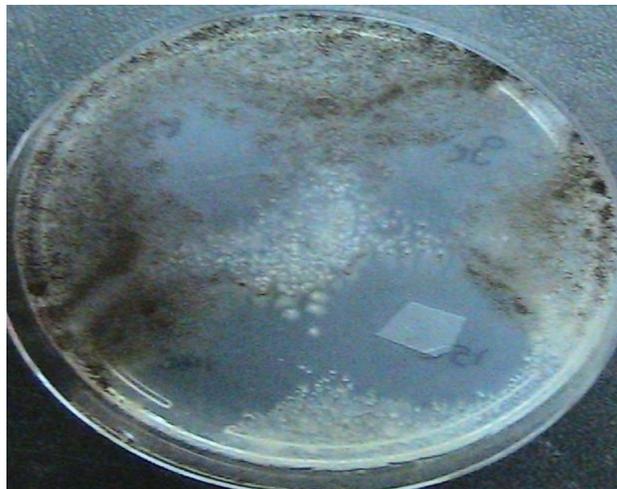


Figure 39. Inhibition halo zone of irradiated nanocomposite film ratios (75/25/0.05CuO) (25/75/0.05CuO), (50/50/0.05CuO) and (75/25/0.05CuO) at 5KGy and 20kGy against A. niger.



Figure 40. Inhibition halo zone of irradiated nanocomposite film ratios (25/75/0.2CuO), (50/50/0.2CuO) and (75/25/0.2CuO) at 20KGy and irradiated blend ratio (25/75/) of (PVA/Gelatin) at 10KGy and 20kGy against A. niger.

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