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# **Influences of antibacterial activity of copper oxide nanoparticles doped in Polyvinyl Alcohol/Polyvinyl Pyrrolidone blend via laser ablation on wound healing applications**

## **Spectroscopic study and antibacterial activity of Polyvinyl Alcohol/Polyvinyl Pyrrolidone blend doped with copper oxide nanoparticles via laser ablation for biomedical applications**

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### **Abstract:**

Polyvinyl Alcohol (PVA)/Polyvinyl Pyrrolidone (PVP) blend has been treated with various ratios of copper oxide nanoparticles (CuO NPs) via laser ablation. The spectroscopic properties of the synthesized nanocomposites were investigated by different routes. XRD confirmed the miscibility between PVA and PVP, and the existence of CuO NPs was confirmed by the presence of diffraction peak at  $2\theta = 38.1^\circ$ . On the one hand, the results of the shifting and broadening of O-H group by the addition of CuO NPs in FTIR-ATR confirmed a strong indication that there was an interaction between PVA/PVP blend and CuO NPs. On the other hand, UV-Vis spectra confirmed the treatment of copper oxide nanoparticles by appearance of additional peak at 336 nm. Furthermore, the responses of the cell line of fibroblasts towards the synthesized nanocomposites revealed the potency of these films requiring it to be recommended for biomedical applications. The values of the inhibition zone of *E. coli* and *S. aureus* microorganisms have been heightened by increasing the ratios of copper oxide nanoparticles inside PVA/PVP to  $18.5 \pm 0.5$  and  $20.4 \pm 6.0$  specifically for blend with laser ablation time (15 min). Thus the high antibacterial activity of the biopolymeric nanocomposites requires further investigation before its clinical application

**Keywords:** PVA/PVP; Nanocomposites; Laser Ablation; Antibacterial Acitivity; Biomedical applications.

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## **1. Introduction:**

Polymer blending is considered the most interesting method employed in a broad variety of applications, such as medical devices, electrochemical cells, energy storage systems, fuel cells and humidity sensors. Due to its unique and sought-after properties, research on polymer blending has brought a fascinating shift in pharmaceutical and industrial practice in recent years (1,2). The presence of polymer in the form of films offers an opportunity to produce another variant of polymer with good mechanical, thermal and inhibitory properties [3].

The importance of Polyvinyl alcohol (PVA) as polymer is highly rated because of its chemical and physical properties and, over the years, research interest in this field has increased. It is existentially found as a powder, film, and fiber. It has a semi-crystalline nature arising from the position of the hydrogen bonds and the OH group. PVA is used widely in medical devices because it possesses, (among other things) adsorption, biocompatibility and high solubility of water characteristics [4, 5]. Also, it is nontoxic and low-cost polymer used in wound dressing, tissue engineering scaffolding and as part of controlled drug delivery systems [6]. Polyvinyl Pyrrolidone (PVP) is a water-soluble polymer that has high polar group, low toxicity, biodegradable and amorphous nature with good film properties. It has two interactive sites N atom and C=O group. It acts as a protecting agent with other surfaces of inorganic compounds [7].

Over the past decade, metal nanoparticles have been extensively studied due to their use in various fields, including chemistry, physics, materials science, biology and medicine. Metal nanoparticles have a very important role in applied science and technology. Numerous methods are used to prepare metal nanoparticles, such as laser ablation technique, method of chemical reduction, method of microorganism arc-discharge, photo-reduction and bio surfactant technique. The attractive and innovative technique of laser ablation is currently and extensively employed to synthesis nano materials. When an intense laser beam hits the surface of the solid target, the nanoparticles are produced. It is a simple and non-contaminated technique [8].

Ionic metal oxides nanoparticles (NPs) such as CuO NPs are very interesting antimicrobial agents, because they have high number of corners and edges, high surface areas and many reactive sites. It shows a variety of possible physical properties, and it can be easily combined with polymers in order to provide special physio-chemical properties to the composites. These nanoparticles which have high surface areas and crystalline structures can be used with certain dose to have antimicrobial effects [9].

In this study, structural, optical, and antibacterial activities of PVA/PVP blends treated with various ratios of CuO NPs by changing laser ablation time (3, 5, 10 and 15 min) were studied. Casting technique was employed to form a polymer film with antimicrobial activity against different types of bacteria.

## **2. Materials and Method:**

### **2.1. Materials:**

PVA (M.W.  $\approx$  130,000) and PVP (M.W.  $\approx$  72,000) polymers in powder form purchased from Sigma-Aldrich. Deionized water was used as a solvent for both PVA and PVP. High pure (99.999 %) copper plate was obtained from ACROS.

### **2.2. Preparation of PVA/PVP solution:**

One gram of PVP and one gram of PVA were added to 100 ml of deionized water, and afterwards, the mixture was stirred for 5 h at 50 °C to get a homogenous PVA/PVP solution.

### **2.3. Doping PVA/PVP with CuO NPs by laser ablation:**

CuO NPs have been dispersed in PVA/PVP matrix by laser ablation. Pure copper plate with suitable dimensions has been placed in beaker filled with 20 ml PVA/PVP blend solution. Fundamental Nd:YAG laser beam with parameters of 1064 nm wavelength, 10 Hz repetition rate, and 3.6 W power was streamlined on copper plate by a convex lens to be focused on its surface to support the ablation and formation of CuO NPs in the PVA/PVP matrix. This detailed experiment called one-potential laser ablation process was obtained and employed [1]. Copper oxide nanoparticles have been treated with PVA/PVP blend with various ratios by changing the ablation times (0, 3, 5, 10, and 15 min). The nanocomposite blends were synthesized by casting technique, the resulting PVA/PVP blend solutions were poured into Petri dishes then dried in oven at 35 °C for 36 Hours. The thickness of synthesized nanocomposites was found to be about 0.22 mm.

### **2.4. Characterization Techniques:**

XRD scans of the pure polymers and the PVA/PVP blend films treated with different ratios of CuO NPs have been investigated by PANalytical X-Pert PRO with Copper target with  $K_{\alpha}$  radiation of wavelength ( $\lambda = 1.540 \text{ \AA}$ ) within Bragg angle ( $2\theta$ ) ranging between ( $5-80^{\circ}$ ). FTIR-ATR spectral data obtained in the  $3500-520 \text{ cm}^{-1}$  range using the VERTEX 70/70v (Bruker Company, Germany) spectrometer. UV-Visible electronic spectral data collected in the spectrum range of 190 to 800 nm by a double beam spectrophotometer JASCO Corporation, V-570.

The human osteoblast cell line HFB4 has been grown in Dulbecco's modified Eagle medium (DMEM, Gibco) at  $37^{\circ}\text{C}$  to evaluate the cell viability of PVA/PVP/CuO nanocomposites. Cells seeded at a density of  $5 \times 10^3 \text{ cells/cm}^2$  have been grown on PVA/PVP/CuO nanocomposites in 12-well plates. The medium has been isolated after three days of incubation and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) has been dispersed into each well, then the cell viability has been obtained using an optical analyzer as obtained in the following formula [1]:

$$\text{Viability(\%)} = \frac{\text{Mean optical density of test samples}}{\text{Mean optical density of the control}} \times 100 \quad (\text{Eq. 1})$$

The antibacterial activity of PVA/PVP blend films treated with different ratios of CuO NPs have been tested against *Escherichia coli* (*E. coli*) as Gram-negative bacteria and *Staphylococcus aureus* (*S. aureus*) as Gram-positive bacteria. For disc diffusion antibiotic sensitivity testing, PVA/PVP/CuO NPs were poured into Petri dishes containing Muller-Hinton agar media (sigma Aldrich) and the Petri dishes have been incubated at  $37^{\circ}\text{C}$  for 24 h. The antibacterial activity was calculated by recording the diameter of the inhibition zone after incubation (REF: Al Zoubi, W., Kim, M. J., Kim, Y. G., & Ko, Y. G. (2020). Dual-functional crosslinked polymer-inorganic materials for robust electrochemical performance and antibacterial activity. *Chemical Engineering Journal*, 392, 123654.)

### 3. Results and discussion:

#### 3.1. X-ray Diffraction Analysis (XRD):

XRD analysis offered a lot of valuable information about the compounds' scale, orientation and crystal structure. XRD pattern of PVA, PVP and PVA/PVP blend films treated with CuO NPs at different laser ablation time (0, 3, 5, 10, 15 min) at room temperature are presented in Figure 1. Due to the semicrystalline structure of PVA supported by the intermolecular and intramolecular hydrogen bonds, PVA has a diffraction peak of  $2\theta = 19.6^{\circ}$  and  $40.5^{\circ}$ . There is another diffraction peak at  $2\theta = 22.7^{\circ}$  with low intensity [10- 12]. Hence, PVP has two semicrystalline diffraction peaks at  $2\theta = 10.3^{\circ}$  and  $21.4^{\circ}$  [13, 14].

The PVA/PVP blend film at laser ablation time (0 min) has two diffraction peaks at  $2\theta = 10.4^{\circ}$  and  $19.6^{\circ}$ , this affirmed the interaction and miscibility between PVA and PVP because of the presence of O-H group in PVA and C=O group in PVP and the strong interaction between them [15].

For PVA/PVP blend films treated with CuO NPs at different laser ablation time (3, 5, 10, 15 min), there is an additional diffraction peak at  $2\theta = 38.1^\circ$  that is paralleled with CuO NPs [16]. Also, there is a shift in the diffraction peak at  $2\theta = 10.4^\circ$  towards lower diffraction angle. As seen in Figure (1c-1f), the intensity of diffraction peak at  $2\theta = 38.1^\circ$  decreased with increasing the ablation time. This means that as more bonds were broken, new bonds were built in the polymer matrix between the blend groups and CuO NPs. Also the peak broadening increases with increasing laser ablation time and this established the interaction between PVA/PVP polymer blend and CuO NPs.

### 3.2. Fourier Transform Infrared –Attenuated total Reflection Analysis (FTIR-ATR):

Figure 2 shows FTIR-ATR spectra of PVA, PVP and PVA/PVP blend films treated with CuO NPs at different laser ablation time (0, 3, 5, 10, 15 min) at room temperature in the range 4000-400  $\text{cm}^{-1}$ . For PVA, the O-H stretching vibration was observed at 3305  $\text{cm}^{-1}$ . The symmetric and asymmetric stretching vibrations of C-H group were seen at 2923  $\text{cm}^{-1}$  and 2852  $\text{cm}^{-1}$ , respectively. The vibrational band at 1725  $\text{cm}^{-1}$  was attributed to C=O stretching vibration while O-H bending vibration was observed at 1651  $\text{cm}^{-1}$ . The banding and wagging vibration of C-H group were seen at 1431  $\text{cm}^{-1}$  and 1375  $\text{cm}^{-1}$ , respectively. At 1242  $\text{cm}^{-1}$ , the C-H wagging of acetate residue was observed and C-O stretching vibrations were located at 1087  $\text{cm}^{-1}$  and 1023  $\text{cm}^{-1}$ . At 845  $\text{cm}^{-1}$ , the rocking vibration of  $\text{CH}_2$  was seen [17-19]. For PVP, the stretching vibration of O-H group was observed at 3405  $\text{cm}^{-1}$ . The asymmetric and symmetric stretching vibration of  $\text{CH}_2$  were noted at 2948  $\text{cm}^{-1}$  and 2885  $\text{cm}^{-1}$ , respectively. The C-O stretching vibration was shown at 1644  $\text{cm}^{-1}$ . The C-H bending vibrations have two bands at 1427  $\text{cm}^{-1}$  and 1373  $\text{cm}^{-1}$ . The C-N bending vibration was observed at 1278  $\text{cm}^{-1}$ .  $\text{CH}_2$  twisting and rocking vibrational bands were seen at 1220  $\text{cm}^{-1}$  and 1012  $\text{cm}^{-1}$ . The band at 564  $\text{cm}^{-1}$  is assigned to N-C=O bending vibrations [20-23].

For PVA/PVP blend film at laser ablation time (0 min), there was a shift in O-H stretching vibration, also a new band appeared at 1740  $\text{cm}^{-1}$ , 1490  $\text{cm}^{-1}$  and 1250  $\text{cm}^{-1}$  that corresponded to C=O stretching vibration, C-H bending vibration and C-O-C stretching. There was also a shift in the bands at 1644  $\text{cm}^{-1}$  with a decrease in intensity of all bands compared to pure polymers. These results agreed with XRD analysis and confirmed the interaction and miscibility between PVA and PVP [24].

For PVA/PVP blend films treated with CuO NPs at different laser ablation time (3, 5, 10, 15 min), there was a slight shift in O-H stretching vibration that became broader compared to PVA/PVP polymer blend film at laser ablation time (0 min). Also, the intensities of all bands changed irregularly with addition of CuO NPs and ablation time. This indicates that there was an interaction between PVA/PVP and CuO NPs.

### 3.3. Optical properties:

Figure 3 shows UV-Vis spectra of PVA/PVP blend films treated with CuO NPs at different laser ablation time (0, 3, 5, 10, 15 min) at room temperature in the range 190-1000 nm. There was a maximum absorption peak at 225 nm in PVA/PVP blend film at laser ablation time (0 min) spectrum that corresponded to  $\pi$ - $\pi^*$  transition of unsaturated bonds in polymer blend [25].

For PVA/PVP blend films treated with CuO NPs at different laser ablation time (3, 5, 10, 15 min), there was a slight shift toward longer wavelength. This shift correlated with the intermolecular bonding of hydrogen between the adjacent hydrogen groups in the chain of polymer and Cu ions or due to the bonding of hydrogen between C=O of PVP and Cu ions [16]. Also, there was an additional peak at 336 nm and their intensity increased with increasing the laser ablation time that confirmed the interaction between CuO NPs and PVA/PVP polymer.

It is known that Beer-Lambert law demonstrates the transfer of light through the materials according to that relation [26].

$$I = I_0 e^{-\alpha d} \quad (1)$$

where  $I$  and  $I_0$  are the intensities of transmitted and incident light,  $d$  is the thickness of film and  $\alpha$  is the absorption coefficient which must be analyzed in order to examine the existence of any changes in the band structure. This is essential because the absorption of light in any optical medium is determined by its absorption coefficient. So, the above relation can be written as follow [27]:

$$\alpha * d = \ln \frac{I_0}{I} = \ln 10 * \log \frac{I_0}{I} = 2.303 * A \quad \rightarrow \quad \alpha = \frac{2.303}{d} A \quad (2)$$

Where  $A$  is the absorbance.

Figure 4 shows the relation between  $\alpha$  and photon energy ( $h\nu$ ). It is clear that the value of absorption edge of PVA/PVP blend was decreased irregularly by addition of CuO NPs at different laser ablation time (see Table 1). The shift to lower photon energy means that the optical band gap decreased.

In an amorphous and disordered materials, the Urbach tail has been observed and is one of the tools that is used for describing the characteristics of electronic transition of these materials. These tails decay exponentially into the band gap. Urbach tail width is a measure of the defect levels between the conduction and valence bands in the forbidden band gap. Previous research showed that the absorption coefficient was represented by Urbach relationship in the exponential-edge region [28, 29].

$$\alpha = \alpha_0 \exp\left(\frac{h\nu}{E_{ur}}\right) \quad (3)$$

where  $\alpha_0$  is a constant and  $E_{ur}$  is the width of the band tails and can be determined from the inverse of the slope of the straight lines of Figure 5. The relationship between  $\ln \alpha$  and  $h\nu$  in Figure 5 was depicted by straight line. This means that the absorption obeys the quadratic interband transition

relationship. Values of  $E_{ur}$  were listed in Table 1, as seen with addition of CuO NPs and increasing laser ablation time,  $E_{ur}$  increased. The increase in  $E_{ur}$  indicates an increase in the amorphous regions in the materials. There are no sharp cutoffs for both conduction and valence bands, in amorphous materials, but in the lower energy region, they have tails of localized states. The increase in tail width can be due to the fact that increasing the CuO NPs content and ablation time in PVA/PVP polymer blend can contribute to the production of ionic complexes, disorders and imperfections. This leads to a rise in localized states within the forbidden band gap of energy [30].

Both insulator and semiconductor materials divided according to their band gap energy into

Sample	Laser ablation time (min)	Absorption edge (eV)	Band Tail $E_{ur}$ (eV)	Energy Gap (eV)
PVA/PVP (0 min)	0	4.74	0.17	4.40
PVA/PVP/CuO NPs (3 min)	3	4.30	0.21	3.64
PVA/PVP/CuO NPs (5 min)	5	4.49	0.24	3.36
PVA/PVP/CuO NPs (10 min)	10	4.49	0.28	2.99
PVA/PVP/CuO NPs (15 min)	15	4.55	0.36	4.29

direct and indirect band gap materials. The top of valence band and the bottom of the conduction band in direct band gap materials are placed in the same zero crystal momentum. In indirect band gap materials, however, the bottom of the conduction band does not relate to zero crystal momentum. So, the movement from valence to conduction band should be always associated with a phonon of the proper magnitude of the crystal momentum. Davis and Shalliday (Year) showed that both direct and indirect transitions can occur near the edge of the basic band, and by plotting  $(\alpha h\nu)^{1/2}$  versus  $h\nu$  can be used for the determination of the optical band gap of the material, the linear portion of the curve corresponding to the electronic transition from the valence band to the conduction band by using this relation [31, 32].

$$(\alpha h\nu)^{1/2} = B (h\nu - E_g) \quad (4)$$

Figure 6 shows the plot of  $(\alpha h\nu)^{1/2}$  versus  $h\nu$ . The extrapolation of the linear portion of the curve with x-axis gives the value of indirect band gap energy. As seen in Table 1 that values of indirect band gap energy decreased compared to PVA/PVP polymer blend at laser ablation time (0 min)., This means that as the amorphous region in PVA/PVP blend increases, by addition of CuO NPs, the disordering increases.

**Table (1): Optical parameters values of PVA/PVP blend embedded by various ratios of CuO NPs at different laser ablation time:**

### 3.4. Cell viability:



The responses of the cell line of fibroblasts towards PVA/PVP/CuO nanocomposite predicts the propensity of these films to be recommended for biomedical applications. The cell viability of these nanocomposite has been investigated as shown in figure 7. It is evident that cell viability of pure PVA/PVP matrix was  $78.1 \pm 3.4$  % and has been increased to  $81.2 \pm 4.2$  % after PVA/PVP matrix embedded by CuO NPs via laser ablation time (3 min). The values of cell viability have been increased by increasing the ratios of copper oxide nanoparticles inside PVA/PVP blend to  $96.7 \pm 3.5$  % for PVA/PVP blend film at laser ablation time (15 min). This is a strong indication that there is a presence of high biocompatibility of CuO NPs in the PVA/PVP blend. The low ratios of copper oxide nanoparticles inside PVA/PVP blend potentiates its degradation without causing fatal adverse effect. This behavior of cell viability could affect the antibacterial activity that could decrease the possible invasion and enables the development and distribution of normal cells instead of death.

### 3.5. Antibacterial assessment:

The potential to facilitate wound healing and other biomedical applications relies heavily on bacterial invasion inhibition, as the latter can deteriorate the cohesion of the wound and thus delay healing. The antibacterial activity of PVA/PVP blend treated with various ratios of CuO NPs by laser ablation has been investigated against *E. coli* and *S. aureus*. As shown in figure 8, pure PVA/PVP blend (at laser time ablation (0 min)) represents the low antibacterial inhibition zone;  $8.2 \pm 0.5$  and  $9.5 \pm 0.4$  against *E. coli* and *S. aureus*, respectively. Moreover, the inhibition zone of PVA/PVP blend embedded in CuO NPs via laser ablation time (3 min) was  $13.2 \pm 0.4$  and  $15.4 \pm 0.6$  against *E. coli* and *S. aureus*, respectively. The values inhibition zones have been increased by increasing the ratios of copper oxide nanoparticles inside PVA/PVP blend to  $18.5 \pm 0.5$  and  $20.4 \pm 6.0$  against *E. coli* and *S. aureus*, respectively for PVA/PVP blend at laser ablation time (15 min). The antibacterial activity has been attributed to increase in the copper oxide nanoparticles inside PVA/PVP blend and it depends on the capability of the released ionic CuO to degenerate bacterial cells [1]. The high antibacterial activity of the polymeric nanocomposite requires further investigation before it can be used in clinical applications.

**Table 2: Antibacterial activity of PVA/PVP blend embedded by various ratios of CuO NPs against *E. coli* and *S. aureus***

Sample code	Inhibition zone diameter (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
PVA/PVP (0 min)	$8.2 \pm 0.5$	$9.5 \pm 0.4$

PVA/PVP/CuO NPs (3 min)	13.2±0.4	15.4±0.6
PVA/PVP/CuO NPs (5 min)	15.1±0.6	17.3±0.4
PVA/PVP/CuO NPs (10 min)	16.6±0.6	18.0±0.5
PVA/PVP/CuO NPs (15 min)	18.5±0.5	20.4±6.0

### Conclusion:

The characteristic properties of PVA/PVP matrix embedded in CuO NPs via laser ablation were investigated. The peak at  $2\theta = 10.4^\circ$  broadening increase was observed by increasing the ratio of copper oxide nanoparticles and thus confirmed the complex relationship between PVA/PVP polymer blend and CuO NPs. According FTIR-ATR, the intensities of all bands changed irregularly with addition of CuO NPs and ablation time when compared with PVA/PVP polymer blend. This revealed and established an interaction between PVA/PVP and CuO NPs. The optical properties also showed an additional peak at 336 nm and its intensity increased by increasing the ratio of copper oxide nanoparticles. This also indicated that there was an interaction between CuO NPs and PVA/PVP polymer. The values of cell viability have been increased with increasing the ratio of copper oxide nanoparticles inside PVA/PVP blend till reach  $96.7 \pm 3.5\%$  for PVA/PVP blend film at laser ablation time (15 min) that indicated the high biocompatibility of PVA/PVP/CuO nanocomposites. The antibacterial activity test indicated that the highest inhibition zones have been achieved for the highest ratio of CuO NPs inside PVA/PVP blend with  $18.5 \pm 0.5$  and  $20.4 \pm 6.0$  against *E. coli* and *S. aureus*, respectively for PVA/PVP blend film at laser ablation time (15 min). However, while evidence abounds that polymeric nanocomposite possesses high antibacterial activity, further empirical investigations are still required to be performed before engaging its application in clinical world.

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### Conflicts of interest:

The authors declare that they have no conflict of interest.

### References:

Abd Elkader, M. F., Almogbel, M. S., & Elabbasy, M. T. (2020). Survival and reduction in foodborne bacteria using methyl cellulose film doped with europium oxide nanoparticles. *Food Science & Nutrition*, 8(1), 291-298.)