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ANTIBACTERIAL EFFECT AND STRUCTURAL PROPERTIES OF PVA-PVP-AG NANOCOMPOSITES

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ABSTRACT
Antibacterial activity of Polyvinyl alcohol- Polyvinyl pyrrolidone-Ag (PVA-PVP-Ag) nanocomposite was investigated against Pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* using disk diffusion method and co-culture method to calculated percent reduction of bacteria. PVA-PVP-Ag nanocomposite showed inhibition activity against both gram positive and gram negative bacteria with inhibition zone (8- 11)mm, the reduction of *E.coli* and *S.aureus* growth reached to 100% . Polymer-nanocomposite were prepared by solution cast method using co-polymer (PVA and PVP) as matrix and silver nanoparticle as filler. The X-ray diffraction (XRD) studies showed that the structure of (PVA-PVP-Ag) nanocomposite is polycrystalline, and structure form is face centered cubic (fcc), with a prevailing level in the (111) direction.

KEY WORDS: Polyvinyl Alcohol , Polyvinyl Pyrrolidone , Nanocomposite , Antibacterial Effect.

INTRODUCTION
Polyvinyl alcohol (PVA) is a thermoplastic and biocompatible polymer prepared from the polymerization of vinyl acetate followed by alcoholysis. It has been widely used as films, fibers, paper coating and adhesives due to their attractive properties.[1, 2] PVA is a thermoplastic and biocompatible polymer, it is strongly hydrophilic and soluble in water, which also helps to promote its degradation through hydrolysis.[1,2, 3,4]

Polyvinylpyrrolidone (PVP) is a class of hydrophilic water-soluble polymer which attributing for electronegative groups of the carbonyl in the pyrrolidone structure that are able to form hydrogen bond with water[5], PVP is regarded as bulky, non-toxic, colorless, non-ionic, temperature-resistant, pH-stable, biocompatibility polymer , regard as interesting polymer for its capacity to interact with enormous variety of organic and inorganic compounds.[6,7,8]

Silver and silver ion based materials are widely known for their bactericidal and fungicidal activity. Their antimicrobial effect is due to blockage of respiratory enzyme pathways, alteration of microbial DNA and the cell wall.[9] Silver nanoparticles based antimicrobials have many advantages due to their thermal stability, health and environmental safety.[10] Therefore, silver and silver ion containing materials are used as prostheses, catheters, vascular grafts and as wound dressings.[11,12] Silver nanoparticles are among the most commercialized inorganic nanoparticles due to their antimicrobial potential. They have also been used for a number of applications such as non linear optics, spectrally selective coating for solar energy absorption, biolabelling and antibacterial activities.[13]

Polymer composites using biobased and biodegradable polymers and natural fibers have been widely studied and developed due to the increasing environmental and energy concerns.[14, 15] Polymer nanocomposites are polymers or copolymers having dispersed in its nanomaterials. These nanomaterials may be of different shape (e.g., platelets, fibers, spheroids), but at least one dimension must be in the range of nanoscale (normally less than 100 nm).[4, 16] The combination of silver nanoparticles with water soluble biopolymers will produce new antimicrobials. Based on this, various natural polymers such as gum acacia, starch, gelatin, sodium alginate, carboxy methyl cellulose etc., have been employed to prepare biocompatible polymeric silver nanocomposites.[17]

This study was aimed to evaluate antibacterial activity and structural properties of PVA-PVP-Ag nanocomposites.

MATERIALS AND METHODS
-Polymers: Two polymers were used in this study included Polyvinyl alcohol(PVA) supplied from Shanghai Kaidu Industrial Development Co., Ltd China,

and Polyvinyl pyrrolidone (PVP) supplied from Anhui LeafchemCo., Ltd, China.

-Silver nanoparticles: Silver nanoparticles powder (20nm) with a purity(99.99%) used in this study supplied from (hongwu nanometer).

-Pathogenic bacteria: Pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* obtained from Department of biology/College of Science/Al-Mustansiriyah University/Baghdad / Iraq.

Preparation of (PVA-PVP-Ag) Nanocomposites
Weight percentage wt. % of (PVA) and (PVP) were dissolved in (10) ml of distilled water with stirring the solution by magnetic stirrer for 1 hour with temperature 50° C. Adding the weight percentages (1%,2%,3%,4% and 5%) of (Ag) nanoparticles as shown in (Table 1).

Table (1): Composite weight rates

Wight ratio of Ag nanoparticles %	PVA(gm)	PVP(gm)	Ag nanoparticles(gm)
0	0.7	0.3	0
1	0.693	0.297	0.01
2	0.686	0.294	0.02
3	0.679	0.291	0.03
4	0.672	0.288	0.04
5	0.665	0.285	0.05

- Antibacterial activity of PVA-PVP/Ag Nanocomposite
Disk diffusion method: Antibacterial activity of PVA-PVP/Ag nanocomposite was studied against pathogenic bacteria *E. coli* and *S. aureus* using disk diffusion method.[18] Fresh overnight culture of inoculum of each pathogenic bacteria was spread on to Nutrient agar plates. Sterile paper discs of 5mm diameter containing PVA-PVP/Ag nanoparticles composite were placed in each plate. All the plates were incubated at 37°C for (18-24) hours. The zone of inhibition, which appeared as a clear area around the disks, was measured.

Co-Culture Method: Co-Culture technique was used for determination of antibacterial effect of PVA-PVP/Ag nanoparticles composite. The bacterial culture of *E.coli* and *S. aureus* were grown in nutrient broth with PVA-PVP/Ag nanoparticles composite at ratio 1:1 (vol:vol), the control medium contained nutrient broth only. Co-cultures and control were incubated at 37 °C for 24 h. After the incubation 1 ml of each cultures were serially diluted. The 0.1 ml of dilution sample was taken and spreaded on nutrient agar plates, the plates were incubated at 37 ° C for 24 h.[19] The colonies were counted and inhibition effect was assessed and calculated percentage of reduction of bacterial growth using the following equation described as.[20]

R (%)= ((A-B)/A) x 100

where: R :is the reduction of bacterial growth ,A :is the number of bacterial colonies from control ,B :is the number of bacterial colonies from treatments with PVA- PVP/Ag nanoparticles composite.

Structural properties
X-Ray Diffraction(XRD)
For crystalline materials, the periodic arrangement of atoms gives rise to constructive inter of scattered radiation having a wavelength λ comp-arable to the periodicity d when Bragg's law is satisfied.[21]

n λ=2d Sin θ ……. Bragg's law

Where: (n) is an integer and it is the order of reflection, and (λ) is the wavelength of the X-rays, and (d) is the distance between the lattice planes, and (θ) is the Bragg's angle.

Parameters Calculation
1. Full Width at Half Maximum (FWHM) (β)
The FWHM is equal to the width of the line profile (in radian) at the half of the maximum intensity.

2. Average Crystallite Size (D)
The single line method is one of the several line profile analysis methods based on a Voigt function to determine the size–strain parameters (microstrains and crystallite sizes), The average crystallite size (D), which can be estimated using the Scherrer's formula.[22,23]

$$D = \frac{0.9\lambda}{\beta \cos\theta}$$

where: λ: is the X-ray wavelength (Å), β: FWHM (radian), θ: Bragg diffraction angle of the XRD peak (degree)

3. Texture Coefficient (T_C)
To describe the preferential orientation, the texture coefficient, T_C(hkl) is calculated using the expression[24,25]: T_C (hkl) = [(I(hkl) / I_o(hkl)] / [N_r^{-1} ∑ I(hkl) / I_o(hkl)] zhere: I: is the measured intensity , I_o: is the ASTM standard intensity, N_r: the number of diffraction peaks and (hkl) are Miller indices.

4. Micro Strains (S)
The micro strains are caused during the growth of thin films, and will be raised from stretching or compression in the lattice to make a deviation in the a-lattice constant. So the strain broadening is caused by varying displacements of the atoms with respect to their

reference lattice position.[26,27] This strain can be calculated from the formula[28]

$$\langle S \rangle = \frac{\beta \cos \theta}{4}$$

Where: β: FWHM (radian), θ: Bragg diffraction angle of the XRD peak (degree)

5. Dislocation density (η)

The dislocation density (η) can be calculated using the following relation[29]

$$\eta = \frac{1}{D^2} \quad (\text{lines/nm}^2)$$

6. Number of Crystallites per unit area (N_o)

The Number of Crystalites (N_O) per unit area can be calculated using the following relation.[30]

$N_o = t / (D)^3$ $1/(nm)^2$

RESULTS AND DISCUSSION

-Antibacterial activity of PVA-PVP/Ag Nanocomposite

Disk diffusion method

The results of disk diffusion method showed that PVA-PVP-Ag nanocomposites had inhibition activity against both gram positive and gram negative bacteria and the antimicrobial activity increases with the increase of the weight percentages of silver nanoparticles, reached to 11mm against *E.coli* and *S.aureus* (Table 2).

Table (2): Antibacterial activity of (PVA-PVP-Ag)nanocomposite by Disk diffusion method.

Treatments	Inhibition Zone(mm)	
	Escherichia coli	*Staphylococcus aureus*
0%Ag	8	8
1%Ag	8	8
2%Ag	8	8
3%Ag	11	9
4%Ag	11	11
5%Ag	11	11

Co-Culture Method: The results that were obtained in co-culture method showed high efficiency of Ag nanoparticles against both gram negative bacteria(*E.coli*) and gram positive bacteria(*S.aureus*). The reduction of *E.coli* growth reached to 100% at (1,2,3,4and 5)%Ag compared with control, while the 100% reduction of *S.aureus* growth observed at (3,4and 5)%Ag (Figure 1,2).

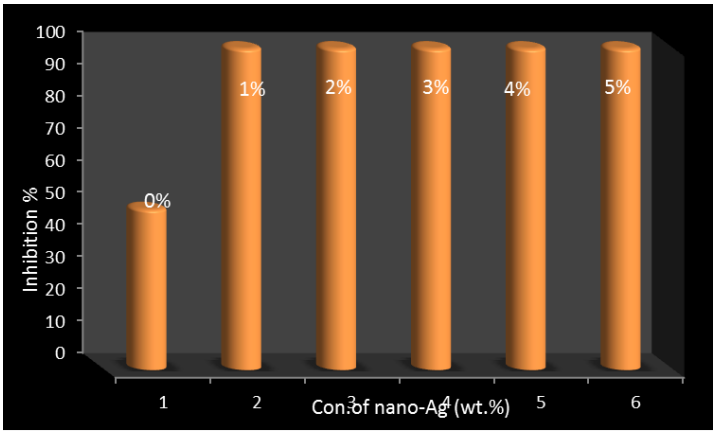

Figure (1): Reduction of *Escherichia coli* growth by PVA- PVP-Ag nanocomposite.

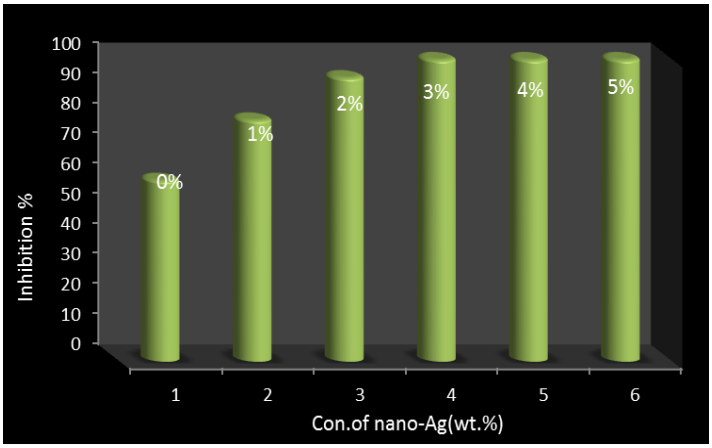

Figure (2): Reduction of *Staphylococcus aureus* growth by PVA- PVP-Ag nanocomposite.

Polyvinyl-pyrrolidone and polyvinyl alcohol are frequently used as stabilizers, due to its optical clarity, which enables the investigation of the nanoparticle formation. PVP acts as a capping agent and the

antibacterial activities of PVP modified Ag-nanoparticles are significant, because the polymer is most effective in stabilizing the particles against aggregation.[31] The presence of PVA act as a stabilizer of Ag/PVA nanocomposites , Ag nanoparticles (Ag-Nps) have potential antibacterial activity toward both Gram-positive and Gram-negative bacteria. The Ag-Nps with sulphydryl groups in the cellular wall of the microorganism are responsible for the inhibition activity, this activity can be explained in terms of the presence of hydroxyl groups in the PVA chain and it is easy to induce Ag-Nps mobility.[32] For another study, PVA film had antibacterial and antiadhesive activity against pathogenic bacteria *Pseudomonas aeruginosa* and *S.aureus* on plastic and glass plates.[33] The antibacterial effect of AgNPs, was observed against gram negative- *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and gram positive - *Bacillus subtilis and Staphylococcus aureus*.[34] The antibacterial properties are related to the total surface area of the nanoparticles,smaller particles with larger surface to volume ratios have great antibacterial activity.[35,36]

The inhibitory effect of silver ions is higher in case of gram negative bacteria. This might be due to the thickness of the peptidoglycan layer in the cell wall of gram-positive bacteria which may prevent to some extent, the action of the silver ions.[37] The actions of nano-Ag in *E.coli* cells, namely in envelope protein processing, outer membrane permeability, plasma membrane potential and energization.[38] The treatment with nano-Ag destabilizes the outer membrane and disrupts the outer membrane barrier components such as lipopolysaccharide, culminating in the perturbation of the cytoplasmic membrane.[39]

X-RAY Diffraction (XRD)

Figure (3) shows the X-ray diffraction spectra of the (PVA-PVP-Ag) nanocomposites which are made to determine d values, crystallographic structure, lattice parameters, average crystallite size, texture coefficient and others. It can be understand the crystalline growth nature of (PVA-PVP-Ag) composites for samples (pure,1,2,3,4, and 5%) at room temperature. From Figure (4), the pure sample shows that there is a very broadaring and low beak, which can be neglect compared to the other peaks as shown in Figure (3) which indicating that the film is amorphous nature. While samples (1,2,3,4, and 5%) for (PVA-PVP- Ag) nanocomposites are polycrystalline, as shown in Figure (3) produce diffraction pattern with number of peaks with structure form is the face centered cubic (fcc). The data shows diffraction peaks at 2θ = 38.2°, 44.2° are corresponding to the (111), (200) planes of sample. Sharp and highly intense peaks are observed, and the prevailing level is (111) plane for all samples.[40]

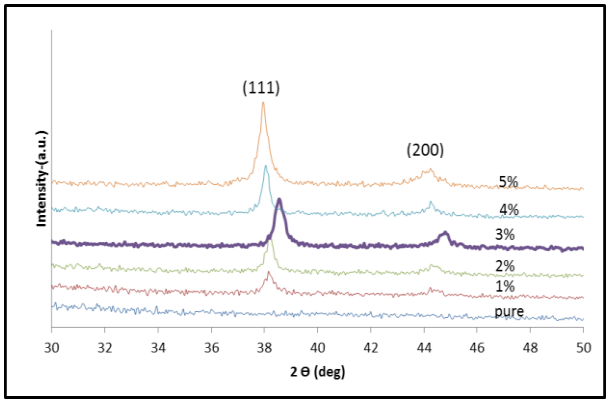

Figure (3): XRD patterns of PVA-PVP-Ag nanoparticles for the samples (pure,1, 2,3, 4, and 5%)

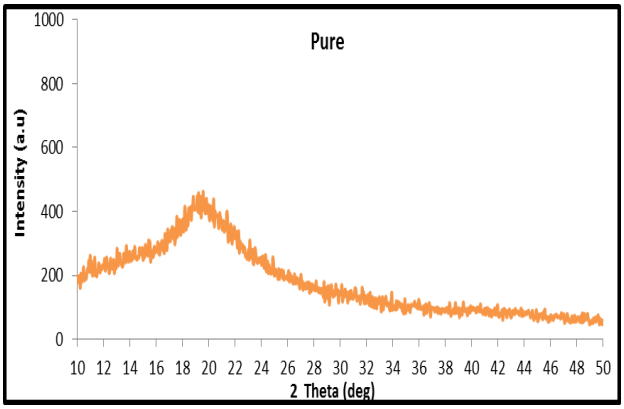

Figure (4): XRD pattern of pure PVA-PVP

Table (3) shows the micro strain and dislocation density of PVA-PVP-Ag nanoparticles films. The micro strain of the films varies from (22.3 to 14.1)*10^{-4} (lines^{-2}.m^{-4}).

The dislocation density of the same films varies from (7.09 to5.17)*10^{15}(lines.m^{-2}). The results reveals that, the maximum value of the micro strain and dislocation density in PVA-PVP-Ag is (1%).

1. Full Width at Half Maximum (FWHM) (β)

The values of full width at half maximum (FWHM) of the preferred orientation (111) of (PVA-PVP-Ag) nanocomposites are obtained from (XRD) pattern. The (FWHM) decrease with increasing PVA-PVP-Ag. This indicates that crystallization of the (PVA-PVP-Ag) nanocomposites progresses gradually as the Ag nanoparticles increases as shown in (Table 3).

2. Average crystallite Size (D): The crystallite size is estimated by using Scherrer's formula, it is observed that the crystallite size increases initially with increasing Ag nanoparticles in the (PVA-PVP-Ag) nanocomposites and reaches the maximum (17.81) nm at (4%) (PVA-PVP-Ag) nanocomposites. The values of crystallite size for different (PVA-PVP-Ag) nanocomposites content are shown in (Table 3).

3. Texture Coefficient (T_c): Texture coefficient (Tc) of(PVA-PVP-Ag) nanocomposites is shown in the (Table 3), the values of (Tc) for samples (1,2,3, 4, and 5%) more than one for level (111) this means that the prevailing level is (111).

4. Micro strains (S): The micro strain decreases with increasing Ag nanoparticles in the (PVA-PVP-Ag) composites. The micro strain depends directly on the lattice constant(a). So the strain broadening is caused by varying displacement of the atoms with respect to their reference lattice position, recorded on the micro strain (Table (3).

5. Dislocation density (η): The calculated values of dislocation density (η) are decreases with increase in Ag content in the (PVA-PVP-Ag) composites (Table 3).

6. Number of Crystallites (N_o): The number of crystallites are decreases with increase of Ag nanoparticles content in the (PVA-PVP-Ag)nano-composites (Table 3).

Table (3): Structural properties of (PVA-PVP-Ag) nanocomposites for samples (1,2,3, 4, and 5%) at room temperature.

Ag wt.%	deg)20)	d (nm)	hkl plane	a (nm)	FWHM (β) (deg)	Average crystallites size (D) (nm)	Dislocation density(η) $*10^{15}$ (lines.m^{-2})	micro strain(S) $*10^{-4}$ (lines^{-2}.m^{-4})	No. of Crystallites (N_o)$*10^{18}$ (m^{-2})	(T_c)
1	38.19 44.24	2.35 2.03	111 200	4.07	0.43	11.69	7.31	21.2	31.29	1.36
2	38.2 44.17	2.35 2.03	111 200	4.07	0.34	16.76	4.56	19.1	11.68	1.2
3	38.5 44.7	2.33 2.02	111 200	4.03	0.44	12.96	5.95	17.7	25.26	1.3
4	38.09 44.25	2.36 2.04	111 200	4.08	0.32	17.81	3.15	21.1	9.73	1.18
5	37.97 44.12	2.36 2.05	111 200	4.08	0.41	13.90	5.17	14.1	20.47	1.42

CONCLUSIONS

PVA-PVP-Ag nanocomposites had antibacterial activity against gram positive and gram negative pathogenic bacteria. The best method for detection of antibacterial effect of nanocomposites is co-culture technique.

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