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Synthesis and Study of the Antimicrobial Activity of Modified Polyvinyl Alcohol Films Incorporated with Silver Nanoparticles

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Abstract:

A new class of biologically active nanocomposites and modified polymers based on poly (vinyl alcohol) (PVA) with some organic compounds [II, IV, V and VI] were synthesized using silver nanoparticles (Ag-NPs). All compounds were synthesized using nucleophilic substitution interactions and characterized by FTIR, DSC and TGA. The biological activity of the modified polymers was evaluated against: gram (+) *(staphylococcus aureus) and gram (-): (Es cherichia coli bacteria)*. Antimicrobial films are developed based on modified poly (vinyl alcohol) MPVA and Ag-NPs nanoparticles. The nanocomposites and modified polymers showed better antibacterial activities against *Escherichia coli (Gram negative) than against Staphylococcus aureus (Gram positive*). This work also studied the effect of using different amounts of nanoparticles on the effectiveness against bacteria and it was found that nanocomposite (P2/Ag 5%) has superior antibacterial properties against *Escherichia coli.*

Keywords: Antibacterial activities, Modified, Nanocomposites, Poly vinyl alcohol, Silver nanoparticles.

Introduction:

The nanocomposites are one of the important industrial polymers, the term Nanocomposite appeared which means material composed of two or more components where one a polymeric matrix as a continuous phase and the others as a reinforcement, which is usually the discontinuous phase and has dimensions in the nanoscale range in order to access unique properties which cannot be provided by any of the components separately[1- 3]. Polyvinyl alcohol (PVA) is one of the highly desirable industrial polymers to synthesis nanocomposite for the importance of PVA applications such as medical where used PVA due to has a lot of advantages such as biodegradability, biocompatibility and it has a water -loving and retain property, which ensures a prolonged moist environment, this led to its use in many medical products contact lenses production, drug systems and artificial organs. Also we use the PVA in the industrial applications due to its ownership to elasticity which can be used to coating of Ag, cellulose , titanium dioxide and others [4,5] the modification of polymers is another methods used to form polymers tailored for specific needs, the hydroxyl groups in PVA which can be used to modification the polymer by common modification reactions are esterification, etherification or acetalization.[6-14] In general the modification of polymers had been have special attention and represented large ratio of the global scientific production dealing with polymers because modified polymers effectively entered into advanced application fields due to their increasing activity compared to individual polymer and capability of being tailored for specific needs. The modification processes were could be include inserting some small or big functional groups onto the main backbone of Polymeric chain in order to improve its chemical, physical and biological properties as well as maintaining on the desirable properties for original polymer. . There are two methods to the surface modification, chemical and physical methods, It is important to focus on the extent of biocompatibility [15] and combination nanoparticles

and modified polymers results in improved many properties[16,17]. In earlier reports, PVA was shown to be able to increase capture or isolate bacteria and fungi from aquatic environments[5,18]so the investigation of the antibacterial activities of the biodegradable polymer attracts many researchers especially after loaded with nanoparticles due to an increase of bacterial resistance.[19,20] The main aim of this research was therefore to study the result of the effect of loading different concentrations 0%, 1%, 3% ,5% and 7% from nanoparticles with modificated PVA on the biological properties of PVA, to be used for medical applications.

Material and method:

Materials

Polyvinyl alcohol (PVA) was purchased from merck-schuchardt, PVA molecular weight=72000 g/mol, All the raw materials were supplied from Merck and SIGMA-ALDRICH CO.

FTIR spectroscopy

The FTIR spectrum of samples were recorded on a Shimadzu type (8400s) via using KBr disc, FTIR spectrophotometer at wave number range 4000–500 cm-1 with a resolution of 4.0 cm-1at 25˚C.

TG-analysis and DSC

Thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC) were carried out on STAPT-1000 LINSEIS, German origin instrument with a heating rate of 10 ˚C/min under nitrogen atmosphere and temperature range 0-600 ˚C.

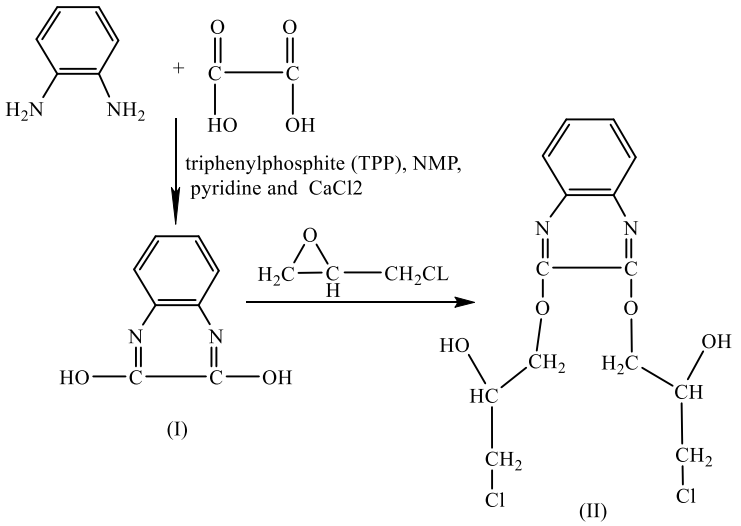
Antibacterial Activity test

The Biological test was examined using S. aureus and E. coil supplied by Microbiology Laboratory (central environmental laboratory) in College of Science/University of Baghdad.

Method

Preparation compounds [II, IV and V]

The compound 3, 3'-(quinoxaline-2,3-diylbis(oxy))bis(1-chloropropan-2-ol) (II) was prepared according to the literature[21]. The reaction sequence leading to the formation of compound II were outlined in Scheme 1.



Scheme 1. Reaction pathway for the synthesis compound II

The compound 2.1. 4-((4-hydroxybenzylidene) amino) benzoyl chloride (IV) was prepared according to the literature [21]. The reaction sequence leading to the formation of compound (IV) were outlined in Scheme 2

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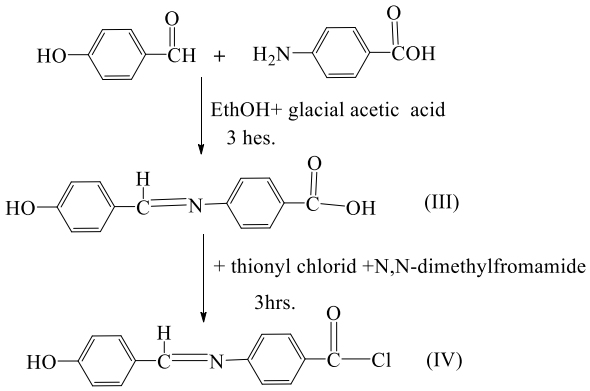
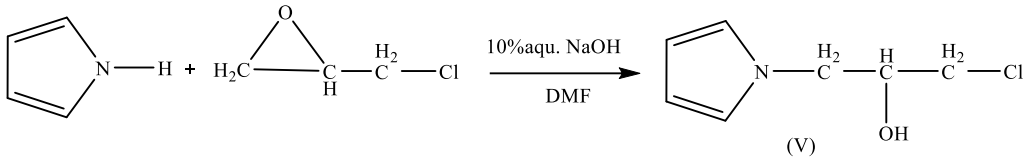


Scheme 2. Reaction pathway for the synthesis compound IV

The compound 1-chloro-3-(1H-pyrrol-1-yl) propan-2-ol (V) was prepared according to the literature [22] and the reaction leading to the formation of compound (V) was outlined in Scheme 3.



Scheme 3. Reaction pathway for the synthesis compound V

While the compound 4-methoxybenzoyl chloride (VI) is shown in Fig1. was supplied from Merck Co.

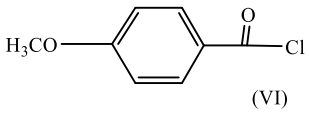


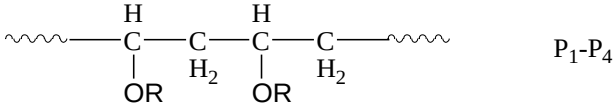
Figure 1. Chemical structure for 4- methoxy benzoyl chloride (VI)

Synthesis of modified Polymers P_2, P_4

Modified Polymers (P_2 and P_4) were synthesized by esterification of polymer PVA. The compound acid chloride IV or VI, 1 mol was added into solution polymer composed of o.1 mol PVA in 3 mL triethylamine in mixture of 5 mL DMF: 10 mL THF with stirring at 0-4 ˚C, after mixing compounds, stirring for 5 hours then the mixture was poured onto 100 mL solution cold from dilute HCl and filtered the precipitate, collected and washing by adding 50 mL of diethyl ether[23].

Synthesis of modified Polymers P_1, P_3

The modification polymers P_1 and P_3 were synthesized by etherification, 0.1 mol PVA polymer which was dissolved in 10 mL DMF with 10 % aq. NaOH as a catalyst, then 1 mol of compound II or VI was added to the polymer solution and stirred with it under reflux for 4 hours. The mixture was poured into the cold water, filtered and washed with a little sodium bicarbonate[22]. Scheme 4. illustrates general formula for modified polymers. The characteristic FTIR absorption bands of compounds [II, IV and V] and the modified Polymers P_1-P_4 were listed in Table1. Also, the physical properties, structure of these compounds and the modified Polymers were listed in Tables 2.

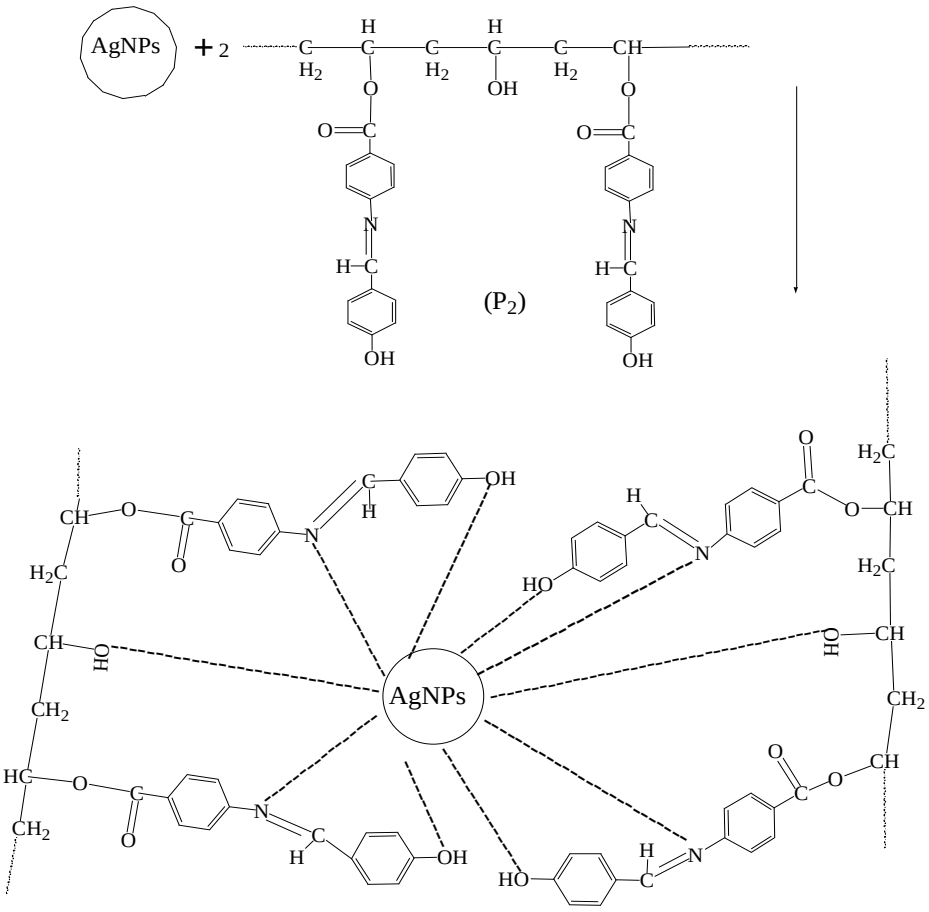


Scheme 4. Structure of modified polyvinyl alcohol with different R

Nanocomposite synthesis

To prepare the nanocomposites by the solution casting method, 1 gm of P_1, P_2, P_3 and P_4 was placed in 20 ml of DMSO with stirring using magnetic stirrer for 24 hours. Then, nanoparticles AgNPs in concentration of 5% was disperse the polymer media, ultrasonic for 4 hours at 25ºC was used to ensure preparation of homogenous mixture of nanoparticles and the modification polymer, and then the mixture was poured into petri dishes[24]. By following the same

method, the nanocomposites from P_2 with different weights 1, 3, 5 and 7% of nanoparticles were prepared. The process of synthesizing nanocomposite is shown in Fig. 2.



(Nanocomposite (NCs) from Ag nanoparticles and P_2)

Figure 2. Process of synthesizing nanocomposite (NCs) from Ag nanoparticles and P_2

Result and discussion:

FTIR Characterization

The characteristics FTIR spectra of the polymers are listed in Table 1.

Table 1. Summary of identification of infrared bands for organic compounds and modified polymers P_1-P_4

Compound index	cm^{-1}								
	OH	C-H aromatic	C-H aliphatic	C=O	C=N	C=C	C-N	C-O-C	C-Cl
I	3402	3010	—	—	overlap 1627		1350	—	—
II	3419	3025	2960-2800	—	1630	1595	1311	1240	750
P_1	3434	3030	2923-2800	—	1627	1595	1321	1255	—
III	3248-2542	3043	—	1680	1600	1585	1320	1249	
IV	3414	3012	—	1770	Overlap 1600		1319	1255	771
P_2	3427	3053	2977-2807	1710	Overlap 1604		1340	1253	—
V	3406	3050	2981-2800	—	—	1597	1355	1230	735
P_3	3373	3010	2935-2879	—	—	1577	1334	1240	—
P_4	—	3026	2981-2842	1727	—	1590	—	1226	—

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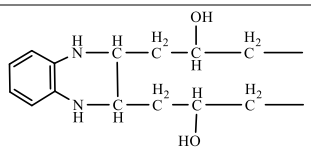
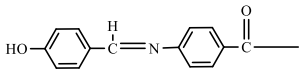
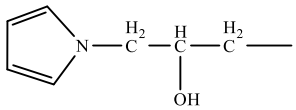
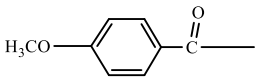
Table 2. Summary of physical properties for compounds, modified polymers and nanocomposite.

Modified polymer N.	R	Color of R	M.P. of R	Color of P	Color of NCs (P+Ag 5%)
P_1		Gary	> 300	brown	Dark brown
P_2		yellow	195	orange	Dark orange
P_3		brown	Liquid	Dark brown	Dark brown
P_4		colorless	Liquid	Pale gray	Gray

Antibacterial Activity test

The inhibition rate of all compounds and Nanocomposits with different loads were investigation against two types of bacteria; *Escherichia coli (G-) Staphylococcus aureus (G+),*were performed according to the agar diffusion method, used DMSO to prepare solutions of compounds and the petri dishes were sterilized for 25 min at 37ºC. All plates were incubated at 37 for 24 hours, after that the plates were then taken out. In this study, A comparison between the inhibition rate of PVA, modified polymers (P_2) and nanocomposites (P_2/Ag 5%) against two types of bacterial species; *(Esherichia Coli (Gram negative)* and *staphylococcus aureus (Gram positive))* showed activity from low, Moderate to high activity, as shown in Table 3. and Fig.3. Nanocomposite (P_2/Ag 5%) showed higher activity against *E.coli* bacteria ,while it showed lower activity against staphylococcus , the experiment was repeated for P_2 with different loading ratios of silver nanoparticles 1, 3, 5, and 7% to observe the effect of different amounts from nanoparticles to develop antimicrobial polymeric films. The results are summarized and presented in Table 4.and Fig. 4. According to Fig. 4, it is observed that the load 1% did not produce sufficient inhibition while for the percentage of 3% and 5% they showed distinct efficacy against *E.coli* better than other[24]. However, the samples had low activity to moderate against *staphylococcus* bacterial.

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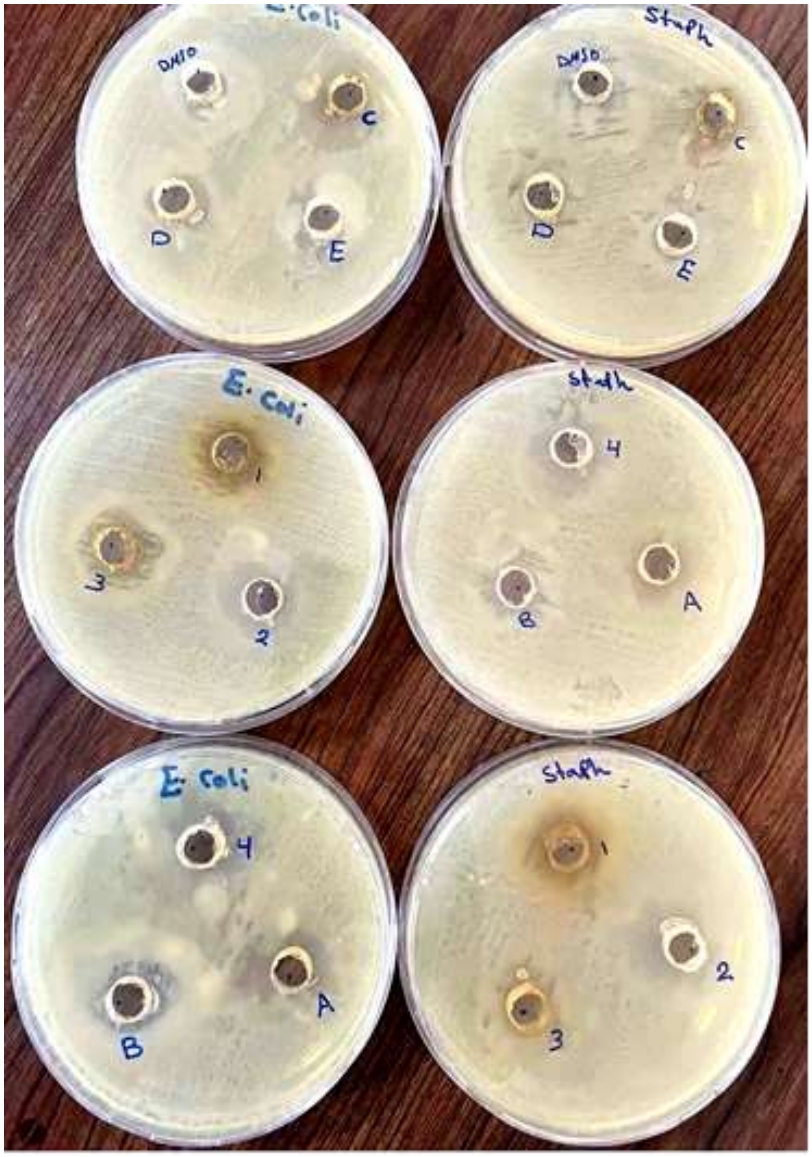

Figure 3. Antibacterial test against *Escherichia coli* and *Staphylococcus aureus*, B= PVA, 1= P_1, 2= P_2, 3= P_3, 4= P_4, A = (P_1/Ag 5%), C = (P_2/Ag 5%), D= (P_3/Ag 5%), E= (P_4/Ag 5%).

Figure 4. Antibacterial test against *Esherichia coli* and *Staphylococcus aureus*, for nanocomposite P_2 with different weight of Ag, a= P_2/Ag 1%, b= P_2/Ag 3%, c= P_2/Ag 5%, d= P_2/Ag 7%.

Table 3. Result of bacterial activity test

Compound	Compound Code	Escherichia Coli	Staphylococcus aureus
PVA	B	12 mm	10 mm
P_1	1	10 mm	10 mm
P_2	2	10 mm	15 mm
P_3	3	10 mm	10 mm
P_4	4	20 mm	10 mm
P_1/Ag 5%	A	18 mm	10 mm
P_2/Ag 5%	C	20 mm	10 mm
P_3/Ag 5%	D	15 mm	10 mm
P_4/Ag 5%	E	10 mm	10 mm

Table 4. Result of bacterial activity test for compound P_2/Ag with different weights of nanoparticles AgNPs

Compound	Ag%	Compound Code	Escherichia Coli	Staphylococcus aureus
P_2/Ag	1%	a	16	12
	3%	b	17	15
	5%	c	20	10
	7%	d	16	12

DSC and TGA analysis

Thermal analysis of the polymers PVA, P_2 and nanocomposite (P_2/Ag 5%) were examined by DSC and TG. DSC scan for the PVA, P_2 and (P_2/Ag 5%) showed changes in the glass transition temperature (Tg) a round 68, 60 and 90º C respectively, the results of the comparison showed that Tg of the P2 are low compared to the pure polymer PVA, which may be due to an increase in free volume throughout amorphous regions or possibly due to reduce in hydrogen bonding[25]. Figure 5. shows one eminent stages of weight loss in the TGA curve of the PVA, modified polymer and after adding silver nanoparticles with a starting range of 185°C for PVA, 165.°C for P2 and 250°C for (P_2/Ag 5%) with a weight loss of 17% , 38% and 18 % respectively, the presence of Ag-NPs at concentrations of 5 % slightly increases the thermal stability of the nanocomposites compared to that of the unloaded polymer, the reason is that the introduction of nanoparticles AgNs with –OH groups changes the intermolecular and intramolecular interaction between the polymeric chains which in turn changes the composition of the physical structure. similar results have been reported for nanocomposites[26,27]. In general, the reinforcements (silver nanoparticles) improve the thermal stability of the polymeric matrix because they act as a heat barrier, which improves the thermal stability of the system[28].

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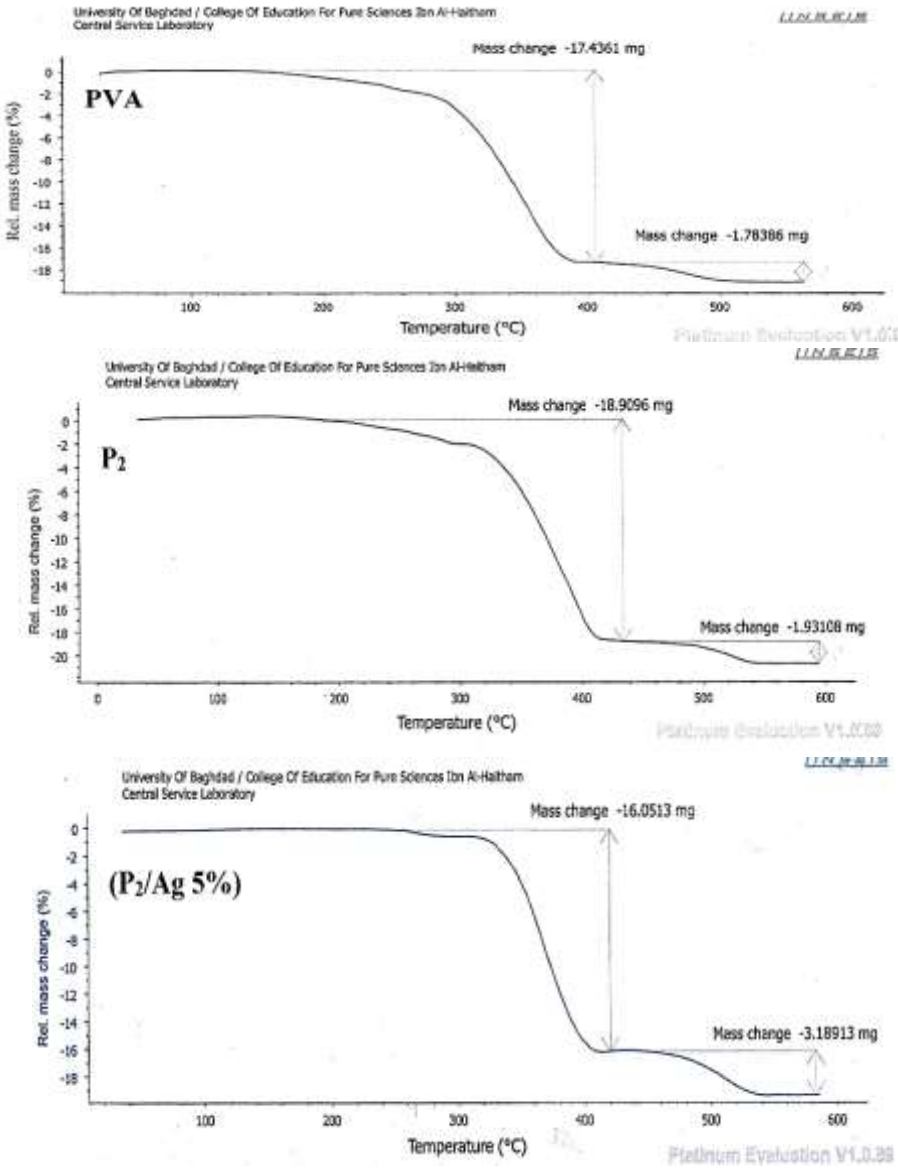

Figure 5. The TGA of PVA, P_2 and nanocomposite (P_2/Ag 5%)

XRD analysis

The crystal structures for pure AgNPs, P_2 and nanocomposite (P_2/Ag 5%) are illustrated in Fig.6.that pure AgNPs alone with nanoscale dimensions of 20 nm have four peaks at 2θ =38.08°, 44.27°, 64.43° and 77.37°, which corresponded to are correspond to reflections of crystalline standards 111, 200, 220 and 311 in the Face-centered cubic (FCC) structure for silver metal according to data base on JCPDS with file no.04-0783[29]. XRD for P_2 was recorded two peaks, one sharp at 2θ = 20°and another broad one almost at 2θ = 40° [5], while XRD of the nanocomposite (P_2 /Ag 5%) showed six peaks, four corresponding to the four AgNPs peaks and two corresponding to the P2 peaks, one of two peaks was overlapped with one of the AgNps peaks at 44, with a decrease in the intensity of the peaks compared to the AgNps peaks due to the increase of amorphous regions in the nanocomposite with presence of side compensators.

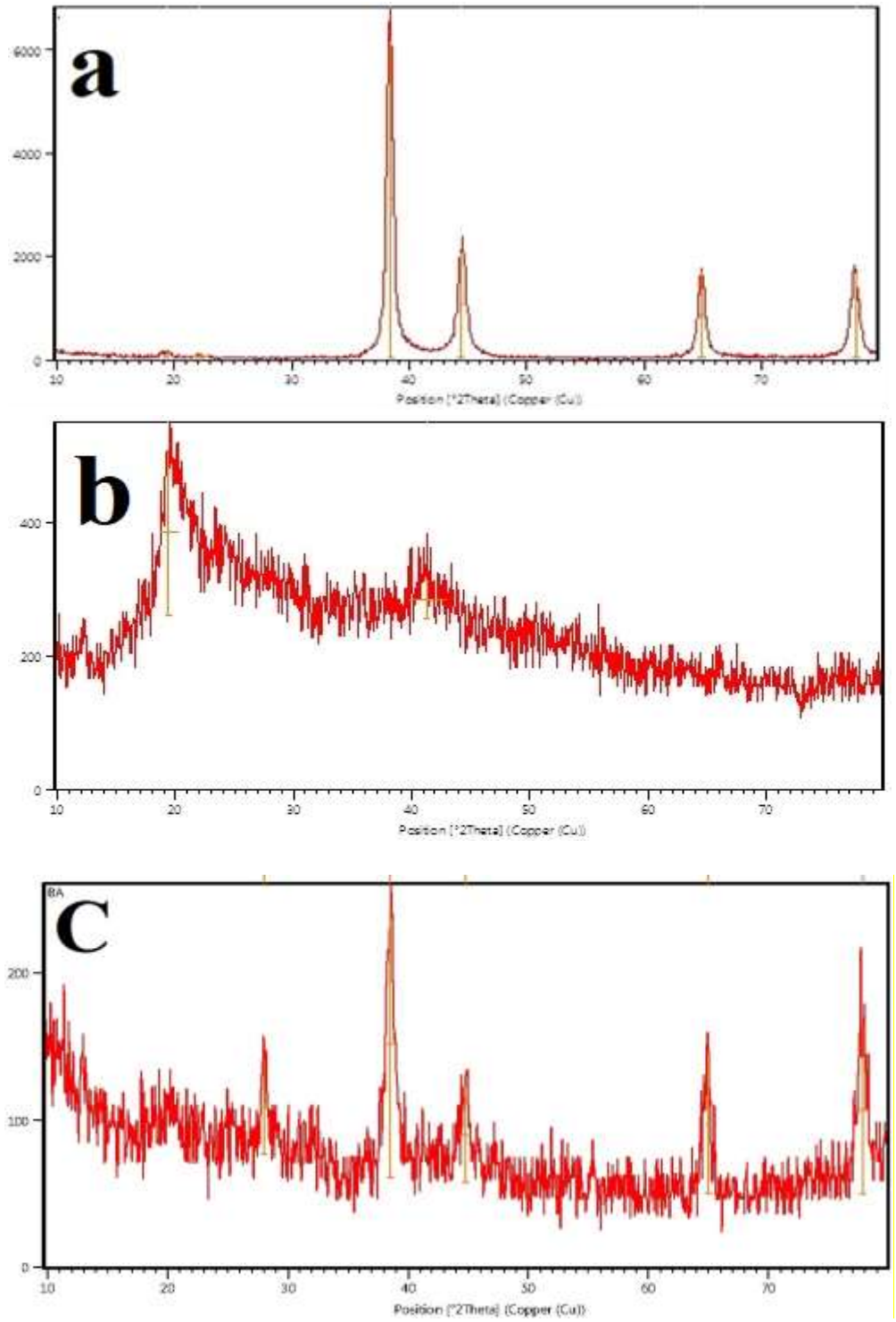

Figure 6. XRD of PVA=a, P_2 =b and nanocomposite (P_2/Ag 5%) = c

Conclusions:

The modification of polyvinyl alcohol was prepared and structurally characterized using FTIR techniques and the nanocomposites of these compounds were prepared using silver nanoparticles. Its efficiency was evaluated in vitro against two bacteria, *gram (+) (staphylococcus aureus)* and *gram (-): (Es cherichia coli bacteria)*, using agar diffusion technique. Some of these nanocomposites showed high antibacterial activity and others did not.

Author's declaration:

-conflicts of Interest: None.

-We hereby confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and images, which are not ours, have been given the

permission for re-publication attached with the manuscript.
-Ethical clearance: The project was approved by the local ethical committee in University of Baghdad.

Authors Contribution:

Basma J. Ahmed, Maida H. Saleem and Fadhel S. Matty

Basma J. Ahmed, Maida H. Saleem and Fadhel S. Matty certify that we have participated title of MS (Synthesis and Study of The Antimicrobial Activity of Modified Polyvinyl Alcohol Films Incorporated with Silver Nanoparticles) in different roles as follows: Conception, design, acquisition of data, analysis, interpretation, drafting the MS, revision and proofreading

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تحضير ودراسة النشاط المضاد للميكروبات لأغشية البولي فينيل الكحول المحورة المدمجه مع جسيمات الفضه النانويه

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قسم الكيمياء، كلية التربيه للعلوم الصرفه ابن الهيثم، جامعة بغداد، بغداد، العراق.

الخلاصة:
صنعت سلسلة من المتراكبات النانوية والبوليمرات االمحوره على أساس بولي (فينيل الكحول) مع بعض المركبات العضويه [II, IV, V and VI] وباستخدام جزيئات الفضة النانوية (Ag-NPs) .صنعت جميع المركبات باستخدام تفاعلات الاستبدال النكليوفيل وشخصت بتقنيات بـ FTIRو DSC و TGA وكما تم تقييم النشاط المضاد للبكتيريا للبوليمرات االمحوره ضد بكتيريا العُنْقودِيَّة الذَّهَبِيَّة (*Staphylococcus aureus*) الجرام (+) وبكتريا الإشريكية القولونية *Escherichia coli (E. coli)* الجرام(-) . طورت الأفلام المضادة للميكروبات على أساس جزيئات البولي فينيل الكحول المحوره MPVA و Ag-NPS النانوية. أظهرت المتراكبات النانوية والبوليمرات المعدلة نشاطًا مضادًا للبكتيريا أفضل ضد *الإشريكية القولونية* (سالبية الغرام) مقارنة ضد *المكورات العنقودية الذهبية* (موجبة الجرام). كما درس تأثير استخدام كميات مختلفة من الجسيمات النانوية على الفعالية ضد البكتيريا ووجد أن المتراكبه النانويه5 Ag / P_2) ٪ (له خصائص مضادة للبكتريا فائقة ضد الإشريكية القولونيه.

الكلمات المفتاحية: نشاط مضاد للبكتريا، محورة، متراكبه نانويه، بولي فينيل الكحول ،جسيمات الفضه النانويه