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Synthesis and Characterization of Grafted Chitosan Blending with Polyvinyl alcohol / Nanocomposite and Study Biological Activity

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Abstract:

The study of biopolymers and their derivative materials had received a considerable degree of attention from researchers in the preparation of novel material. Biopolymers and their derivatives have a wide range of applications as a result of their bio-compatibility, bio-degradability and non-toxicity. In this paper, chitosan reacted with different aldehydes(2,4 –dichloro- benzaldehyde or 2-methyl benzaldehyde), different ketones (4-bromoacetophenone or 3-aminoacetophenone) to produce chitosan schiff base **(1-4)** . Chitosan schiff base **(1-4)** reacted with glutaric acid or adipic acid in acidic media in distilled water according to the steps of Fischer and Speier to produce compounds **(5-12)** respectively. Grafted chitosan **(5-12)** blended with synthetic polymer PVA to produce compounds **(13-20)**, then these polymers were blended with nano Ag by using a hotplate stirrer for 60 min to produce nanocomposites. The synthesized polymers were identified *via* spectral analysis techniques, including FTIR , ^{1}H-NMR and scanning electron microscope (SEM). Finally, studied anti-bacterial activities of some of the prepared polymers.

Keywords: Grafted chitosan, 2-methyl benzaldehyde, Polyvinyl alcohol, Nanocomposite, Scanning electron microscope (SEM).

Introduction:

Chitosan has a wide-field in biological activities such as antimicrobial, antitumor and used pharmaceutically as an anticoagulant agent [1], also can be useful in several fields such as textiles, environmental protection, water treatment, cosmetics and bio-technology due to its ability to produce bifunctional materials [2-4].

The structure of chitosan is easy to be modified to several derivatives due to -OH and NH_2 groups found [5] which distinct chitosan from cellulose [6]. Amino group can be condensed with the carbonyl group of aldehydes or ketones. The modified chitosan exhibits new properties such as solubility, biological activity [7], biocompatibility and hydrophilicity [8].

Chitosan's anti-bacterial characteristics have been associated with its poly-cationic character [9-11]. The protonated functional groups of the chitosan interacted with the negatively charged micro-organism cell membranes, leading to damage and, ultimately, eradication. On the other hand, those characteristics have been highly dependent upon basic bio-polymer characteristics, like deacetylation degree or molecular weight, in addition to external conditions where material has been utilized [12]. Presently, the interest of scientists' has been increasingly focused on the use of the bio-degradable polymer materials [13]. The chitosan (Ch) and Poly(vinyl alcohol) (PVA) [14] have been considered as best known environmentally friendly polymer types , both of them, and the materials that have been based upon them, found numerous application areas in the pharmaceuticals, medicine, and materials coming in contact with the food, this is particularly a result of their bio-compatibility, bio-degradability, and low or even full lack of the toxicity [13] . The ways by which those polymers have been modified mainly contribute to the enhancement of the materials' characteristics [9]. In cases of the PVA and chitosan, their sufficient miscibility results from bonds of hydrogen that are formed between their functional groups. This is

why, the blending of the Chitosan with the PVA plays a role in receiving homogeneous materials that have anti-microbial characteristics and more sufficient mechanical characteristics compared to the Chitosan [15].The purpose of this work was to prepare new nanocomposites with good biological activity. Resulting from blending grafted chitosan with polyvinyl alcohol and silver ions.

Materials and Methods:

Materials:

The chemicals were supplied from BDH, CDH and SCR.

Instrumentation:

The FT-IR spectra have been registered on Shimadzu FTIR8400-s, ranging between 400cm^{-1} and 4,000cm^{-1}, using the potassium bromide disk.

Preparation of Compounds

Synthesis of Chitosan Schiff base (1-4) [16]

Chitosan (0.5 g.) has been dissolved in glacial acetic acid and ethanol (15mL) with stirring for a period of 30 min at the temperature of the room. After that , variety of aromatic aldehydes (2,4 dichloro benzaldehyde or 2-methyl benzaldehyde) or different ketones(4-bromoacetophenone or 3-aminoacetophenone) were added to the mixture .The mixture has been stirred magnetically then heated at 60˚C for 24h., followed the cooling, the crude product has been washed using ethanol. The product has been dried at room temperature.

Synthesis of polymers (5-12) [17]

Polymers **(5-12)** were synthesized from (0.5 g.) of compounds **(1-4)** was hung in 25 mL of H_2SO_4 (2M), then added (glutaric acid or adipic acid) into the solution, after that has been refluxed for a period of 6h. followed by cooling to r.t. pH value has been settled to 7 through the neutralization with the sodium bi-carbonate, and after that it has been precipitated in the acetone, followed by filtering, cleaning by acetone, then drying at a temperature of 60^{o}C in the oven. As shown in schemes (1),(2).

Synthesis of Polymers Blend (13-20)[18]

Polymers blend were produced by using the solvent casting method. The solutions of the grafted chitosan **(5-12)** were produced by dissolving **(5-12)** in a 2% solution of aqueous acetic acid with stirring at the temperature of the room. Polyvinyl alcohol (PVA), figure 1 was dissolved in the hot water for the purpose of producing 5 wt% solutions of polymer. Both solutions of the polymers were mixed and a homogenous solution has been made with the use of a hot-plate stirrer for a duration of 60min. the Grafted Cs/PVA blends have been done through the mixing of (one ratio) Grafted Cs: PVA (5:5).

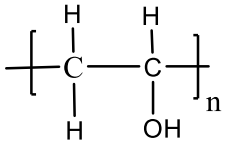

Figure 1. Structure of Polyvinyl alcohol(PVA)

Preparation of Grafted Cs/ PVA Silver Nanocomposites [19]

100mg of the dried Grafted Cs/PVA blend has been put in 50mL of the silver solution of a 250mg/L concentration as well as 1.5h sonication to bond the silver nanometal in blend matrix by the electro-static force.

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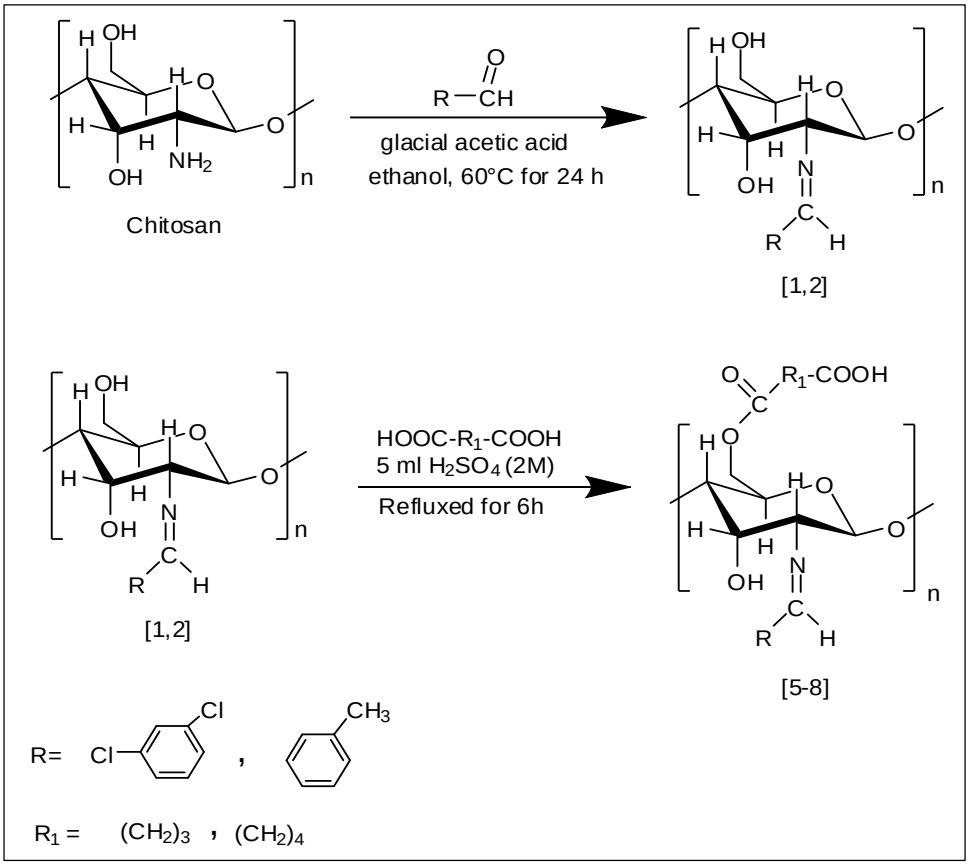
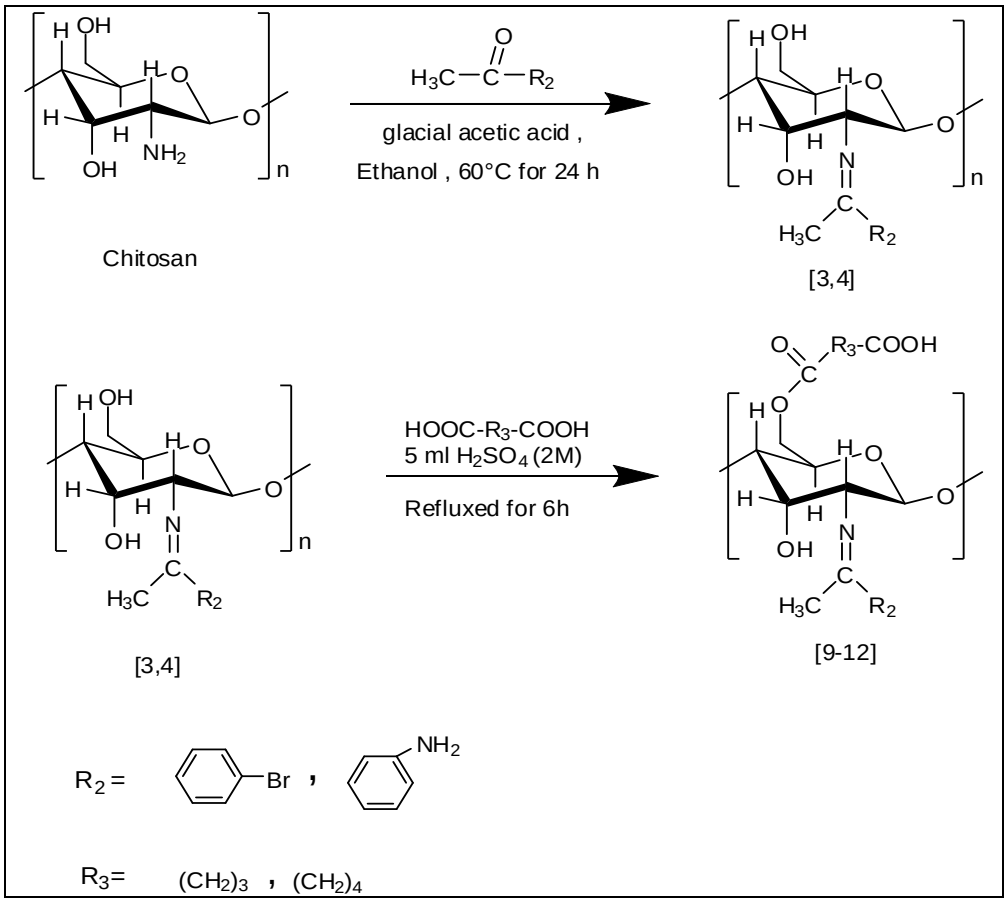


Scheme 1. Synthesis of polymers [1-2], [5-8]



Scheme 2. Synthesis of polymers [3-4], [9-12]

Results and Discussion:

Chitosan Schiff base has been produced from the reaction of the chitosan with the ketone or aldehyde in the ethanol and glacial acetic acid**.** The FTIR spectrum of compound**(1)**, had shown a new band of absorption at 1651cm^{-1} that has been attributed to the (C=N) of the imine group. The band of the absorption at 1595cm^{-1} that has been assigned for C=C of aromatic aldehyde [20], and for the other polymers, the band values are illustrated in table1.

The derivative of the *O*-Chitosan Schiff Base derivatives **(5-12)** was synthesized via reaction Chitosan Schiff base with (glutaric acid or adipic acid) in the distilled water in the acidic media. The compound's FT-IR **(5)** had shown a high band at 3402cm^{-1}, referring to O-H and N-H stretch from extra- as well as intra-molecular hydrogen bonding of the molecules of Chitosan, 1651cm^{-1} due to (C=N) and had shown off new bands at 1681cm^{-1}, 1705 cm^{-1} referring to C=O group of the carboxylic acid and C=O group of ester respectively [21] , and for the other polymers, the band values are illustrated in table 2 . ^{1}H-NMR of compound **(5)**shown signal at (1.35ppm) had existed due to existence of the CH_3 of the N-alkylated of the residue of the glucosamine, the quintuple signal (1.63-1.83)ppm due to $CH_2\underline{CH_2}CH_2$, triplet signal at (2.01-2.35)ppm due to CH_2 of $\underline{CH_2}CH_2\underline{CH_2}$, a signal at (2.67) ppm due to (H-2), signals at(3.38-3.50) ppm corresponded to non-anomeric proton (H-1, H-3, H-4, H-5 and H-6) of Chitosan , signals at (4.20) ppm due to (OH) of chitosan, multiple signals at δ(7.04-7.73) for aromatic protons and singlet signals at δ (8.38) for the (H-C=N) group

O-Chitosan Schiff Base derivative (Grafted chitosan) Blended with PVA **(13-20)** were prepared research of characteristics of obtained blends had shown a good level of the miscibility between the PVA and Chitosan that had been shown by FT-IR results of the compound **(13)**, the band broadening in (2400–3600)cm^{-1} region because of a strong inter-molecular bonding of hydrogen that exists between amino groups of Chitosan and PVA's hydroxyl groups, 1635cm^{-1} as a result of (C=N) and 1708 cm^{-1}which meaning C=O ester group [22], and for the other polymers, the band values are illustrated in table 3.

Table 1. FT-IR data of polymers(1-4)

Com. No.	(O-H) and (N-H) cm^{-1}	(C-H) aliph. cm^{-1}	(C=N) .cm^{-1}	(C=C) cm^{-1}
(1)	3406	2943-2912	1651	1595
(2)	3358	2924-2854	1630	1590
(3)	3431`	2943-2904	1624	1566
(4)	3410	2943-2885	1616	1571

Table 2. FT-IR data of polymers(5-12)

Comp. No.	υ O-H) and (N-H)	υ (C-H) aliph.	υ(C=O) ester.	υ (C=N)	υ (C=C)	υ (-CH_2-O-CO)	υ (C-O-C)
(5)	3402	2954-2873	1705	1651	1600	1246	1083
(6)	3406	2943-2912	1708	1651	1577	1249	1091
(7)	3417	2939-2912	1708	1647	1590	1257	1095
(8)	3410	2951-2912	1712	1630	1585	1280	1091
(9)	3346	2937-2881	1718	1643	1589	1285	1080
(10)	3354	2983-2908	1708	1635	1600	1319	1082
(11)	3398	2954-2920	1705	1630	1600	1269	1095
(12)	3414	2912-2800	1707	1639	1585	1269	1076

Table 3. FT-IR data of polymers(13-20)

Comp. No.	υ(O-H) and (N-H)	υ (C-H) aliph.	υC=O) ester.	υ (C=N)	υ (C=C)	υ (-CH_2-O-CO)	υ (C-O-C)
(13)	2400-3600	2985-2885	1708	1635	1600	1284	1064
(14)	3375	2941-2908	1728	1625	1590	1280	1095
(15)	2400-3600	2951-2881	1714	1645	1590	1276	1068
(16)	3419	2949-2918	1720	1647	1602	1274	1091
(17)	3350	2927-2885	1734	1645	1589	1251	1055
(18)	3351	2916-2823	1707	1640	1602	1300	1091
(19)	3418	2999-2914	1716	1635	1583	1273	1083
(20)	3412	2985-2908	1712	1620	1590	1259	1062

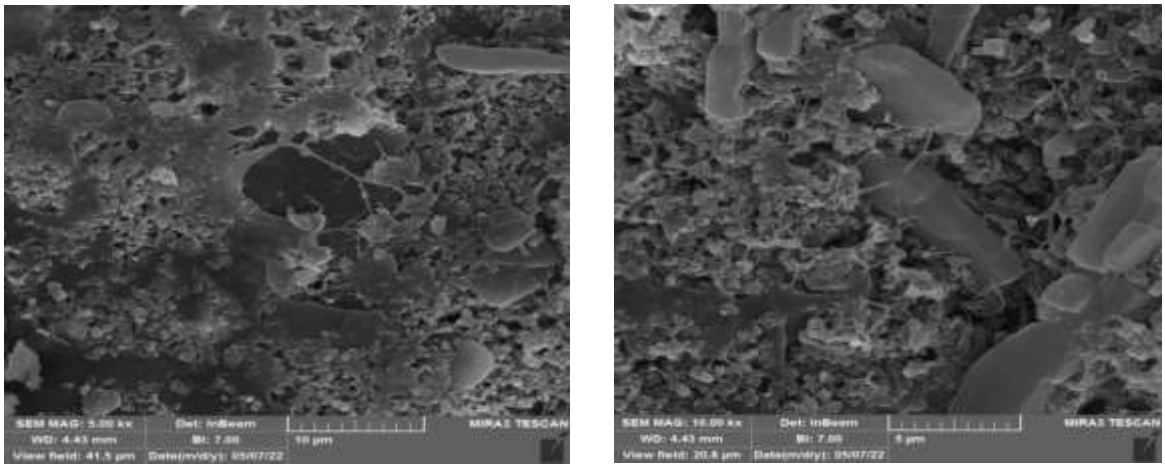
Scanning electron microscope studies (SEM) [23,24]

Surface size, morphology, crystallinity and phase locations of created material could all be researched by examining the SEM. The morphology of the surface varies for Grafted Chitosan figure 2, Grafted Chitosan blend with PVA figure 3, Grafted chitosan /PVA nanocomposite figure 4, that has been loaded with the AgNPs. Adding PVA imparts

roughness to blend membrane (Grafted chitosan /PVA). Adding PVA results in the alteration of the blend membrane surface topography and has a considerable impact on the cell spreading.

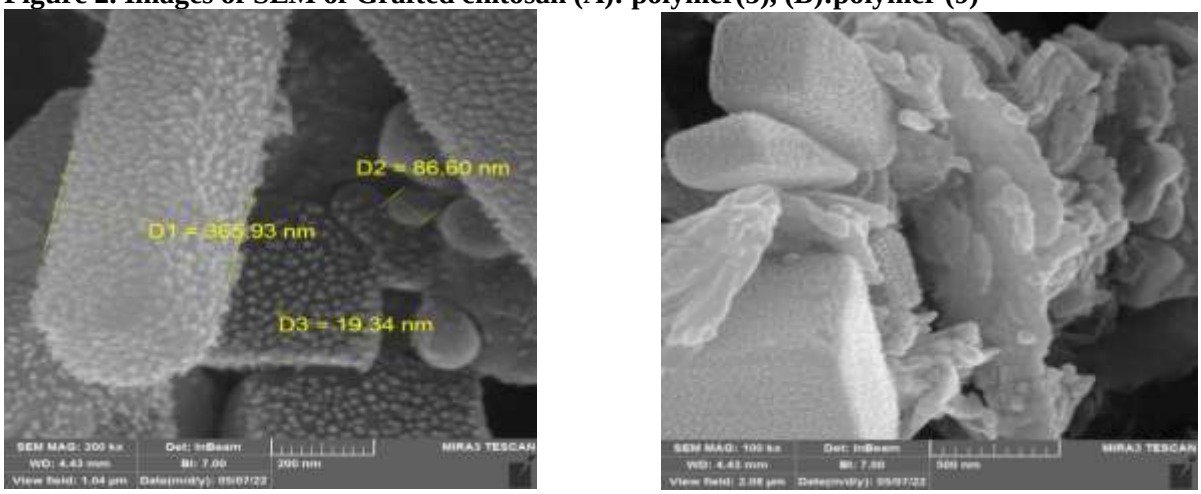
SEM micrograph for presence of AgNPs has been noticed to be with the homogenous distributions on the matrix surface.

The average nano size of the particles is ranged between (21-46) nm for Ag nanoparticles. The SEM images revealed that there were significant changes on the surface of prepared blends after interaction between the polymers. Nanoparticles are in a homogeneous distribution over the matrix's surface. The particles in nanocomposite film were found with almost spherical morphology. However, some of the agglomerations of nanoparticles were also found in the figures and the surface was somewhat rough.

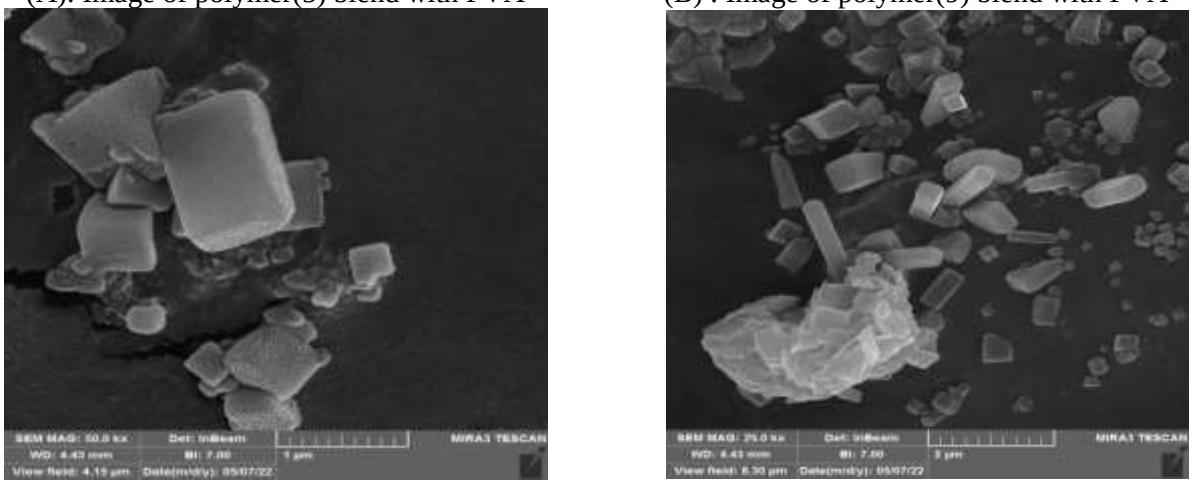


(A): Image of polymer (5) (B): Image of polymer (9)

Figure 2. Images of SEM of Grafted chitosan (A): polymer(5), (B):polymer (9)

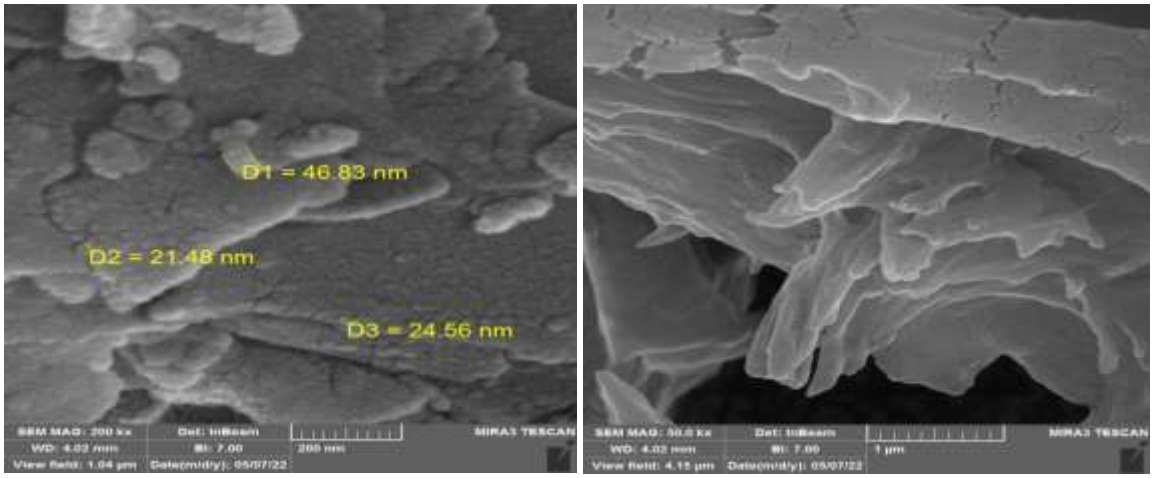


(A): Image of polymer(5) blend with PVA (B) : Image of polymer(9) blend with PVA

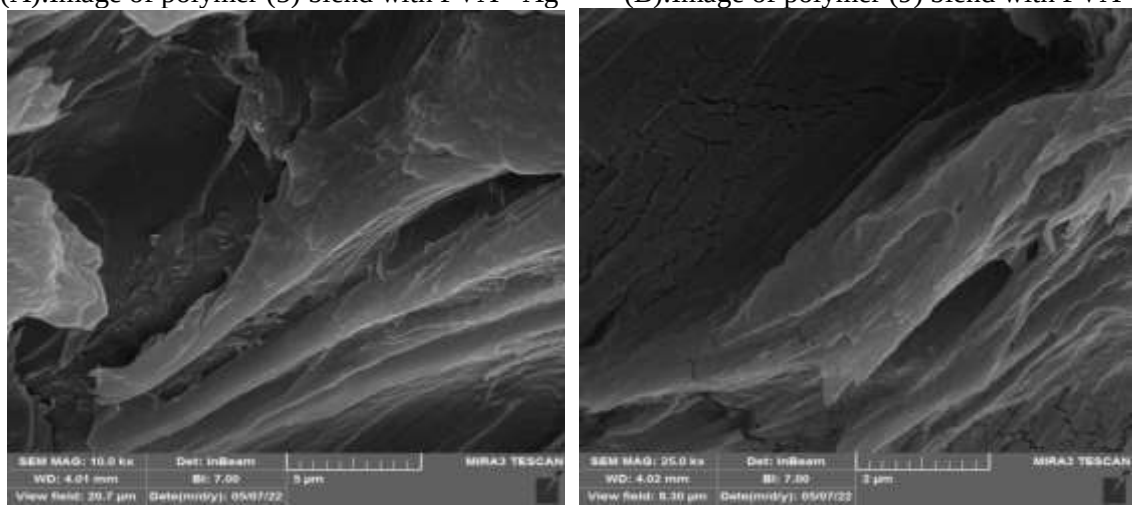


(C, D):Images of polymer(10) blend with PVA

Figure 3. (A, B, C, D)Images of SEM of Grafted chitosan blend with PVA



(A):Image of polymer (5) blend with PVA +Ag (B):Image of polymer (9) blend with PVA +Ag



(C, D):Images of polymer(10) blend with PVA +Ag

Figure 4. (A, B, C, D)Images of SEM of Grafted chitosan blend with PVA nanocomposite

Biological activity [25]

Biological activities of the Grafted Chitosan , Grafted chitosan Blended with PVA , polymer blend of PVA / grafted Chitosan with silver nanocomposite, have been tested against two pathogenic bacteria types (G+) *Staphylococcus aureus* and *E. coli* (G-), utilizing the Diffusion inhibition approach and compare with(Ag) nano. The results of antimicrobial activity are represented in Table 4, Figure 5. When compared to Ag nanocomposites, the ternary mix (PVA / Grafted Chitosan) with Ag nano composite that was exhibited good antimicrobial activities.

For Ag nanocomposite the silver exhibits antibacterial properties which lead to biomedical applications. Silver's antibacterial action is based on Ag^{+}, binding tightly to the electron donor groups in microbial cell walls such as sulfur, nitrogen or oxygen. Ag ions work through the displacement of other important metal ions like Ca^{2+} and Zn^{2+} . Ag nanoparticles have complex impacts on bacterial cells [26, 27]. However, the impact of silver nanoparticles on bacterial cells is mediated in a variety of ways [28] .The following are some of the mechanisms that have been summarized and presented : (i)the capacity of silver nanoparticles to bind to and enter the bacterial cell wall [29], (ii) the production of free radicals by Ag NPs, which may damage and porous the cell membrane [30], (iii) NPs can release Ag ions, which can bind and inactivate thiol groups of several important enzymes [31] and (iv) nanoparticles can alter signal transduction in bacteria, preventing bacteria from growing [32-34].

Table 4. Antibacterial screening data of some synthesized polymers.

Comp.No.	*Escharia .coli*	*Staphylococcus aureus*
Amoxicillin	17	23
Ag nanocomposite	19	19
(1)	21	21
(2)	22	23
(5)	24	24
(6)	22	23
(13)+Ag	25	27
(14)+Ag	27	29

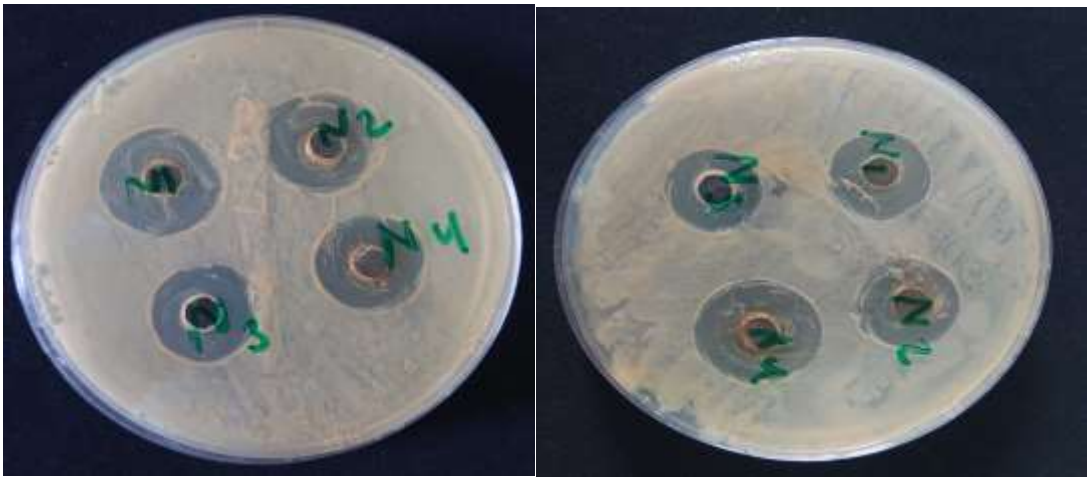


Figure 5. Antibacterial activities of some polymers

Conclusions:

This paper has been aimed at the achievement of Synthesis and Characterization of Grafted Chitosan Blending with Polyvinyl alcohol / Nanocomposite and Study Antibacterial Activity. Results have shown that all of the polymers had higher diameters of the growth inhibition zone. Polymer**(14)** had shown excellent inhibition against *Staphylococcus aureus* and *E. coli.* due to Ag (Nanocomposite). SEM Studies showed the changes in surface morphology of the synthesized polymers due to the new bonds in grafted chitosan and Ag (Nanocomposite)

Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine 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' contributions statement:

R.S. S. has designed the work plan, analyzed the results, wrote the article and reviewed the article. H. G.A. and K. A. O. participated in the practical part and conducted theanalyzes.

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تحضير و تشخيص المتراكبات النانوية لبولي فينايل الكحول مع مزيج الكيتوسان المطعم ودراسة الفعالية البايولوجية

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الخلاصة :

تلقت دراسة البوليمرات الحيوية والمواد المشتقة منها درجة كبيرة من الاهتمام من قبل الباحثين تجاه تحضير مواد جديدة . تحتوي البوليمرات الحيوية ومشتقاتها على مجموعة واسعة من التطبيقات نتيجة لتوافقها الحيوي وقابليتها للتحلل الحيوي وعدم سُميتها. في هذا البحث، تم تفاعل الكيتوسان مع الألديهايدات المختلفة (4،2 ثنائي كلورو بنزالديهايد أو 2-ميثيل بنزالديهايد) ، او كيتونات مختلفة (4-برومو أسيتوفينون أو 3-أمينو أسيتوفينون) لإنتاج البوليمرات (**4-1**) (قاعدة شف الكيتوسان)، ثم تفاعل قاعدة شف الكيتوسان (**4-1**) مع حامض الكلوتاريك أو حامض الأديبيك في وسط حامضي في الماء المقطر وفقًا لخطوات Fischer and Speier لإنتاج البوليمرات (**12-5**) على التوالي. ثم مزج البوليمرات(**12-5**) (الكيتوسان المطعم) بالبوليمر الاصطناعي PVA لإنتاج البوليمرات (**20-13**)، ثم مزج هذه البوليمرات مع جزيئات الفضة النانوي باستخدام hotplate stirrer لمدة 60 دقيقة لإنتاج المتراكبات النانوية Nanocomposites. تم تشخيص البوليمرات المحضرة من خلال تقنيات التحليل الطيفي، بما في ذلك FTIR، ^{1}H-NMR ، مجهر المسح الإلكتروني (SEM). وأخيرا درس النشاط المضاد للبكتيريا لبعض البوليمرات المحضرة.

الكلمات المفتاحية : الكيتوسان المطعم، 2- مثيل بنزلديهايد، بولي فينايل الكحول، المتراكبات النانوية، مجهر المسح الالكتروني .