GREEN SYNTHESIS OF ANTIBACTERIAL: A NOVEL NANOCOMPOSITE FROM POLYVINYL ALCOHOL/POLYANILINE/AG

Neama Ahmed Sobhy Ahmed[1] and Marwa Mahmoud El-Tayeb[1]

ABSTRACT

A new nancomposite based on polyvinyl alcohol/polyaniline/Ag (PVA/PA/Ag) has been synthesized. An eco-friendly chemical reduction method was used to produce Ag nanoparticle solution from Ag^{+} ions. The polymerization of aniline occurred for the preparation of poly aniline (PA) in the presence of ammonium persulfate. With exposure to Ag nanoparticles on the PVA/PA composite, a new nanocomposite was obtained. The morphology of the new nanocomposite was studied by scanning electron microscopy (SEM), fourier transorm infrared (FT-IR) analysis, and x-ray diffraction (XRD). According to XRD analysis, the size of nanoparticles was found to be in the range of 15-20 nm. SEM images showed the shape of nanoparticles as triangle which is a benign shape for antibacterial analysis. The bacterial activity of the obtained nanocomposite was also evaluated against Gram negative Escerichia coli (E.coli) and Gram positive bacteria staphylococcus aureus (staph. Aureus) by the disc diffusion method. The antibacterial study showed that the PVA/PA/Ag composite had good antibacterial activity and PVA/PA/Ag nanocomposites were found to be effective against two bacteria.

Keywords: *Nanocomposite; Polyvinyl Alcohol; Poly aniline; Ag nanoparticles; Antibacterial activity.*

INTRODUCTION

In recent years, noble metal nanoparticles have been the subjects of focused researchers due to their unique electronic, optical, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials [1]. The preparation of colloidal AgNPs is of great interest for their unusual chemical and physical properties and their applications [2].

[1]Sanitary & Environmental Engineering Institute, Housing & Building National Research Center

Silver nanoparticles (AgNPs) are well known for strong antibacterial properties and no harm to human cells [3]. The main advantage of AgNPs is that even molar concentrations are effective than micro molar concentration of silver ions [4]. In addition, AgNPs were found to be relatively non toxic to human cells [5]. There are various methods to prepare nanoparticles. The most important ones are follows: chemical reduction [6]. Optical reduction [7], hydrogel method [8], Sedimentation method [9], UV and gama irradiation [6] and biosynthesis method [10]. Among these methods, the chemical reduction process is the most common industrial method for Ag nanoparticle synthesis. This method has the highest production efficiency and ability for use in a wide range of nanoparticle and nanocomposite production methods [11].

Polymer blending technology is an effective way to obtain new polymeric materials with optimized properties. The advantages of this technology include versatility, simplicity, and inexpensiveness [12,13], numerous polymers have been employed to prepare polymer-silver nanocomposites and have been successfully used for the synthesis of metal nanoparticles [14,15]. Polymer stabilizes the metal nanoparticle-polymer composite, and hence, the nanoparticles attached to the polymer chains will disperse in the solution when the composite dissolve [16]. Therefore, the synthesis protocol often involve a stabilization process by reducing the silver ions in the presence of surfactant, polymers and hydrogels [14]. There are many studies for the synthesis of polymeric nanocomposites in the literature. For example, The polyacrilonitrile/montmorillonite/Ag nanicomposites were prepared using the chemical reduction of Ag^+ (in situ) [17]. The antibacterial activities of the silver nanoparticle solution, which was obtained by soaking the polyacrilonitrile/montmorillonite/Ag nanocomposite films in distilled water, were tested using the paper disc diffusion method. The results showed that the silver nanoparticle solution was quite effective against tiny bacteria such as Staphylococcus aureus, Escerichia coli and klebsiella pneumonia.

Polyvinyl alcohol (PVA) have several advantages, such as high biodegradability, biocompatibility, hydrophilicity and ability to form fiber. So that, PVA has a lot of medical applications and is used by virtue of elasticity and tensile strength in some polymers like chitosan. It can be used for coating of cellulose [18], Titanium dioxide [19]. polyaniline (PA) is another polymer which also used for coating of Ag [20,21], zeolite [22], silica ge [23], and nanofibers, especially gelatin nano fibers [24,25].

The present study reported a facile synthesis of nanocomposite based on Ag nanoparticles coated by Poly Vinyl Alcohol and Poly Aniline polymers. Ag nanoparticles are prepared using chemical reduction method, a simple, low cost and facile method. The developed nanocomposite was evaluated for their antibacterial applications against two pathogenic bacteria, including saph. Aureus (Gram positive) and E.coli (Gram negative) by using disk diffusion method.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals were of analytical reagent grade and used without further purification. All chemicals were purchased from Merck (Mumbai, India). Silver nitrate (AgNO3) was used as Ag+ source and aniline in aniline hydrochloride form was used as monomer for polyaniline (PA) synthesis.

2.2. Ag nanoparticles preparation

Ag nanoparticles were synthesized by the chemical reduction of $AgNO_3$ using $NaBH_4$ (1:3) in deionized water according to procedure described by [26]. Silver nitrate solution was prepared with different concentrations (0.005, 0.008, 0.01 and 0.012) in DI water. The aqueous solution of $NaBH_4$ was maintained at 5°C for 20 min. and was titrated using $AgNO_3$ solution (200 ml) under constant stirring. The reduction reaction was continued for 40 min. at room temperature. Then, the yellowish-red colloid of Ag nanoparticle solution was obtained.

2.3. Aniline hydrochloride preparation

Aniline hydrochloride was synthesized [27] as follows: aniline and hydrochloric acid was mixed in the ratio 1:2. A 20 ml of aniline was heated and 40 ml of hydrochloric acid was added drop wise, the mixture was stirred by magnetic stirrer, the color changed from colorless to violet. Then, the solution was filtered and washed using concentrated hydrochloric acid. Then, the sediment was dried at 60°C in an oven.

2.4. PVA/PA/Ag nanocomposites

The PVA/PA/Ag nanocomposites were synthesized by chemical oxidation polymerization of aniline in presence of PVA and Ag nanoparticle solution. Aniline-hydrochloride was added to the prepared Ag nanoparticles colloidal solution (250 ml). The mixture was stirred for 15 min. and the PVA aqueous solution (dissolves with 0.1 MHCl) was added and the mixture was stirred for 30 min. Then adding aqueous solution of ammonium persulfate, $(NH4)_2S_2O_8$, the mixture was allowed to react for 10 h under constant stirring at -3°C.

2.5. Micro organisms

The antibacterial activity of prepared nanocomposites determined using two bacterial strains, E.coli and staph. Aureus.

2.6. Characterization

2.6.1 FT-IR spectroscopy

The nanocomposite formed were analyzed by fourier transform infrared (FT-IR) spectroscopy (Jasco FTIR-4100, Jasco, Japan) using KBr disks.

2.6.2 UV-vis spectroscopy

The absorption spectrum of mixtures was recorded at room temperature using UV-vis spectrophotometer (Shimadzu 3600, NIR UV, Kyoto, Japan) at a resolution of 1 nm.

2.6.3 X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis of the products was measured using (X Pert Pro, Panalytical, Holland) at room temperature, using Nickel filtered Cu kα radiation generated at 45 kV, and 50 mA. The diffraction patterns were determined over a diffraction angle range of 2 θ = 5°-80°.

2.6.4 Scanning electron microscopy (SEM)

SEM was applied to observe the surface morphology of PVA/PA/Ag nanocomposite materials using (Inspect S, FEI Ltd., Holland) after gold coating.

2.7. Antibacterial activity study

The antibacterial PVA/PA/Ag nanocomposites was tested against E.coli and Staph. Aureus microorganisms by paper disk diffusion method according to procedure described by [17].

The Muller Hinton agar (MHA) powder was used as a culture medium for bacterial growth. 20 g. of agar was dissolved in 500 ml of distilled water, the clear brown solvent was obtained by boiling the solution. The MHA medium (15 ml) as sterilized at 120^{o}C for 60 min. in autoclave, cooled to room temperature, and poured into sterilized petrie dishes (10mm X 90mm), the petrie dishes are cooled over 24 hours. Filter paper disks according to the number of samples were placed on the surface of the medium and 40 ml of each concentration of PVA/PA/Ag samples were dropped over disks by sampler to investigate antibacterial activity. The size of the zone of inhibition measures the effectiveness of the compounds, a more effective compound produces a larger clear area around the filter disk. All petrie dishes contained bacteria and antibacterial regents were incubated and maintained at 37°C for 24 hours. After this period, the diameters of the inhibition zones formed around each disk were determined and presented in mm. The results concerning antibacterial activity (inhibition zone < 5 mm), weak activity (5 mm), moderate activity (6-12 mm) and strong activity (>13 mm).

3. RESULTS AND DISCUSSION

In this study, nanoparticle colloidal solution was synthesized using reducing agent and ions to Ag nanoparticles by $NaBH_4$ is as follows [28].

$$NaBH_4 + AgNO_3 \longrightarrow Ag + 1/2\ H_2 + NaNO_3 + 1/2\ B_2H_6$$

To improve polymer properties is to combine the desired polymer with other polymers that have better properties. The combination of two or more polymers is an efficient method to synthesize new compounds with benign properties and many applications [11]. The polymer which have better properties can enhance the properties of a weak polymer. However, PA have advantages but has low solubility in common solvents. To overcome this problem, dispersing PA in soluble polymeric matrix like polyvinyl alcohol (PVA), poly ethylene oxide, poly styrene sulphonic acid and hydroxyl methyl cellulose. PVA improved the solubility of PA and the composite can be applied for Ag nanoparticle formation.

The PA/PVA/Ag nano composite was synthesized by polymerization of aniline hydrochloride in Ag nanoparticle colloid.

3.1. FT-IR Spectroscopy

Fig. 1 shows FT-IR spectra of synthesized nanocomposites. The free OH functional group has a broad peak at 3600-3650 cm^{-1}, and the peak goes to 3200 – 3400 cm^{-1}, the presence of C-H and CH_2 bonds in alkanes which are in the PVA structure was confirmed with the intense bending peaks around 2900 – 3000 cm^{-1} and 1465 cm^{-1} and an intense peak in 1000 – 3000 cm^{-1} shows the existence of the C-N bond in polyaniline chains.

The presence of benzene in the polyaniline structure was confirmed with an intense tensile peak around 1500 – 1600 cm^{-1}.

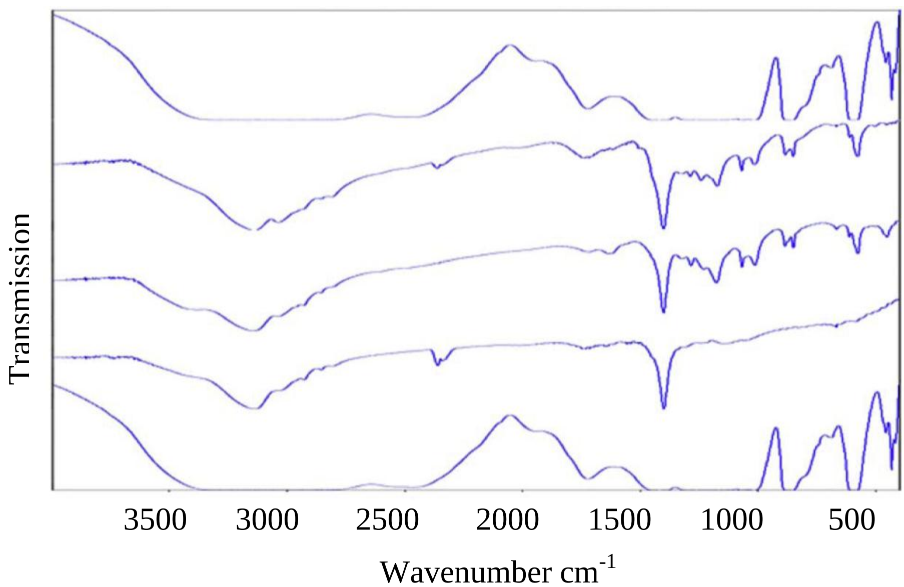


Figure (1) FT-IR spectra of prepared nanocomposites at different concentrations of silver nanoparticles; 10% (A); 15% (B); 20% (C); 25% (D); 30% (E).

3.2. XRD Analysis

The XRD patterns of PA/PVA/Ag nanocomposites are shown in Fig. 2. The sharp and intense peaks around 2 θ values of 38, 44 and 64, with 111, 200 and 220 diffraction are related to benign Ag crystalline structure which stabilized by polymeric matrix. A broad peak operating at 2θ values in the range of 23 – 28° is related to the polymeric chains. The XRD patterns inform the presence of silver nanoparticles in nanocomposite forms. The average particle size of PA/PVA/Ag nanocomposite is estimated to be 10 – 17 nm for all samples.

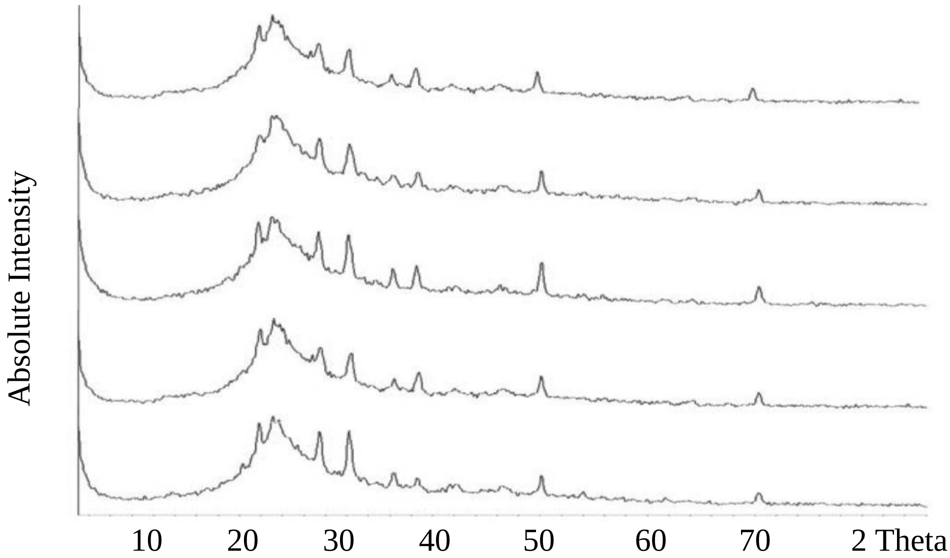


Figure (2) XRD spectra of prepared nanocomposites at different concentrations of silver nanoparticles; 10% (A); 15% (B); 20% (C); 25% (D); 30% (E).

3.3. SEM Analysis

The SEM was used to collect information regarding morphology and cross-sectional structures of the porous nanocomposite. The images shown in Fig. 3 showed that the nanocomposite formed is porous, have crystalline structure. It is clear that some of nanoparticles are triangular which is a good crystalline structure shape for antibacterial tests [29,30,31].

3.4. Antibacterial tests of synthesized PA/PVA/Ag nanocomposites

PA/PVA/Ag nanocomposites were tested for antibacterial activity using Staph. Aureus and E-coli. (Fig. 4) shows the inhibition zones formed by nanocomposite samples. The diameter of the inhibition zones are 8, 9, 14, 10 and 9 mm and 11, 13, 16, 10 and 9 mm against E-coli and Staph. Aureus respectively. The results are presented in Table 1. It is shown that Pa/PVA composite (Sample of F) used as control matrix, that exhibited no antibacterial activity. The plot of the inhibition zone versus various PA/PVA/Ag nanocomposites is shown in Fig. 4. Referring to Fig. 4. The PA/PVA/Ag nanocomposite (C) with 20 % Ag nanoparticle showed better antibacterial activity against Staph. Auresus and E.coli. Silver exhibits antibacterial property that lead to biomedical applications.

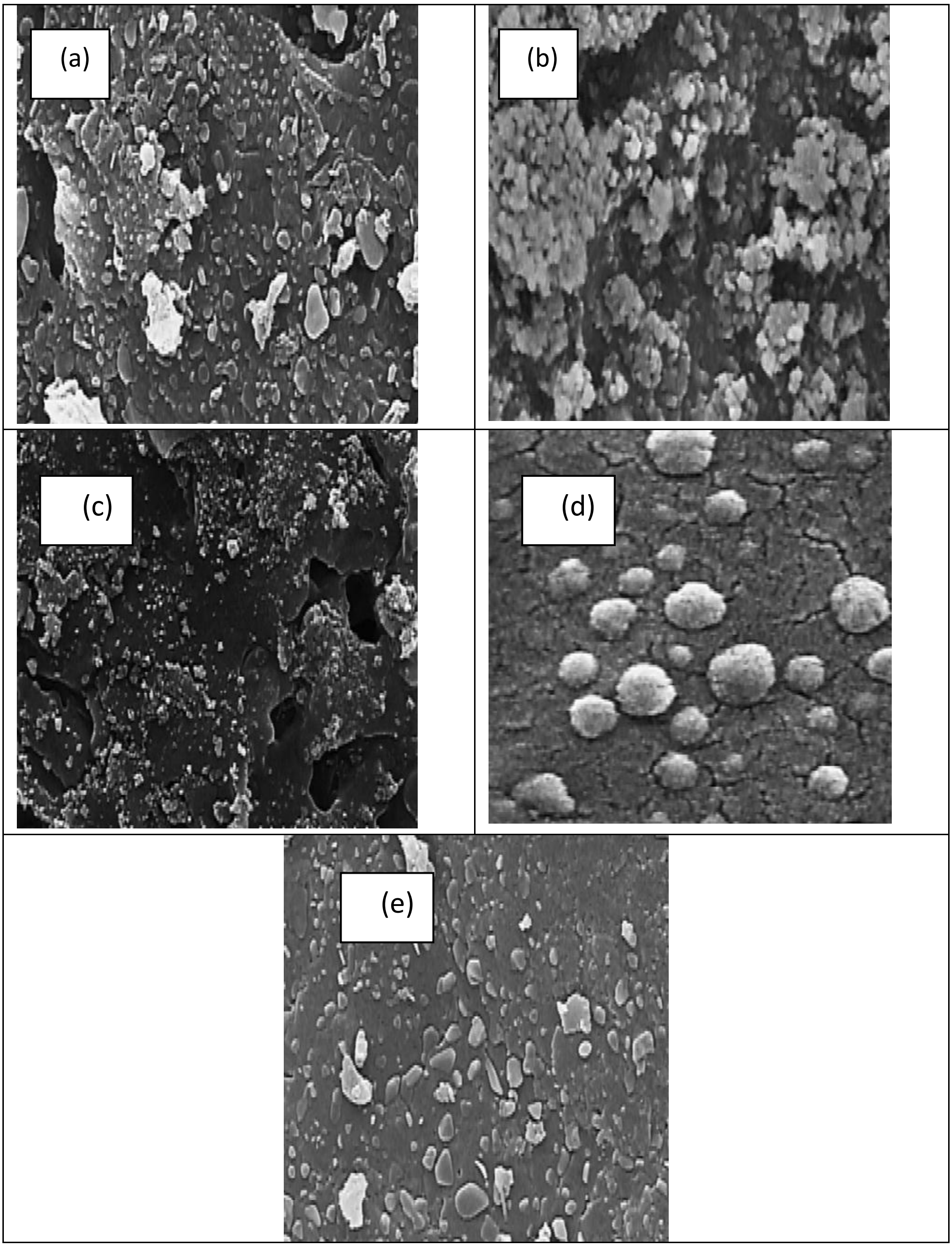
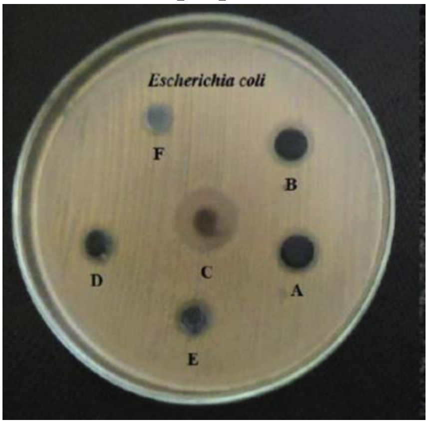


Figure (3) FESEM images of prepared nanocomposites at different concentrations of silver nanoparticles; 10% (A); 15% (B); 20% (C); 25% (D); 30% (E)

At low concentrations of nanoparticles, the interaction of particles with wall of the cell of bacteria decreases and at high concentrations of the particles, the aggregation of particles increases, hence, the effective surface to volume ratio of particles and the interaction between particles and cell wall of bacteria increase (Fig. 4). Shows the releasing Ag nanoparticles at high, medium and low concentrations of nanoparticles. There are various mechanisms on the action of silver nanoparticles on the bacterial cell [32].



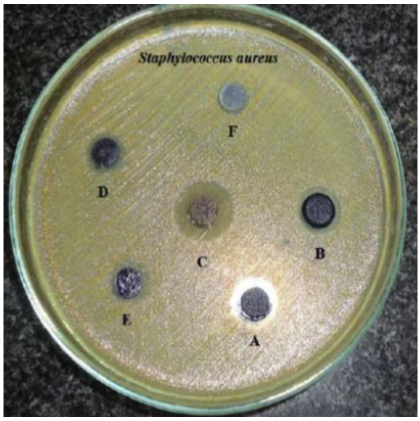


Figure (4) Antibacterial activity of various nanocomposites against two pathogenic strains; E. coli and Staph. aureus by the disk diffusion method; 10% (A); 15% (B); 20% (C); 25% (D); 30% (E).

Some of those mechanisms are presented as follows : (i) The formation of free radicals by silver nanoparticles which can be damage the cell membrane and make it porous [33,34]. (ii) The nanoparticles can modulate the signal transduction bacteria that stops the growth of bacteria [35]. (iii) Releasing silver ions by nanoparticles can interact with the thiol groups of many vital enzymes and inactivate them [36,37].

Table 1 Inhibition zones obtained from various nanocomposites at different concentrations of silver nanoparticle; 10% (A); 15% (B); 20% (C); 25% (D); 30% (E); 0% (F) against two pathogenic bacteria.

Samples	**Average of inhibition zones (mm)**	
	E.coli	**Staph. Aureus**
A	8	11
B	9	13
C	13	16
D	10	10
E	9	9
F	0	0

4. CONCLUSION

The above study presents the synthesis of PA/PVA/Ag matrix and used for Ag nanoparticle coating. The synthesized polymers have good efficiency in nanoparticle coating as the particle size was obtained in the range 12 – 18 nm. This method of low cost, simple. The polymers used Poly Vinyle Alcohol and Poly Aniline are highly biocompatible and biodegradable. The synthesized nanocomposite can be used in different applications, the antibacterial efficiency of the developed nanocomposite was studied since the polymers used eco-friendly materials. The results showed that the synthesized nanocomposite formed have good antibacterial activity.

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الملخص العربي

التصنيع الأخضر لمادة نانونية ذات نشاط مضاد للبكتريا مصنعة من البولي فينيل الكحول والبولي إيثيلين والفضة

نعمة أحمد صبحي أحمد رياض* و مروة محمود الطيب*

في هذه الدراسة، تم تصنيع مادة نانونية من البولي فينيل الكحول والبولي ايثيلين والفضة. وهي مادة صديقة للبيئة باستخدام طريقة الإختزال الكيميائي للفضة للحصول على الفضة في حجم النانو باستخدام أيونات الفضة. يتم تحضير البولي إيثيلين من عملية بلمرة الانيلين في وجود الأمونيوم بير سلفات ويتم تكوين مادة جديدة من الفضة النانونية والبولي فينيل الكحول والبولي أنيلين. وتم توصيف المادة المتكونة باستخدام الميكروسكوب الالكتروني لمعرفة شكل وحجم الفضة النانونية ووجد أنها في المدى : 15 – ٢٠ نانومتر ودراسة الـ FTIR ودراسة الـ X-Ray وتم دراسة النشاط المضاد للبكتريا للمادة المتكونة باستخدام Gram Positive bacteria staphylo coccus aureus وباستخدام Gram negative bacteria Echerichia coli (E.coli) ووجد أن المادة المتكونة لها نشاط مضاد للبكتريا وتم اختبار ذلك بواسطة Disc diffusion method.

***معهد الهندسة الصحية والبيئية - المركز القومي لبحوث الإسكان والبناء.**