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ORIGINAL ARTICLE

Synthesis, characterization and antibacterial properties of a novel nanocomposite based on polyaniline/polyvinyl alcohol/Ag

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Abstract In this study, a novel nanocomposite based on polyaniline/polyvinyl alcohol/Ag (PANI/PVA/Ag) has been successfully synthesized. The chemical reduction method was used to produce Ag nanoparticle colloidal solution from Ag^+ ions. The polymerization of aniline occurred *in situ* for the preparation of polyaniline (PANI) in the presence of ammonium persulfate. With exposure to Ag nanoparticles on the PANI/PVA composite, a new nanocomposite was obtained. The morphology and particle size of the novel nanocomposite was studied by scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier transform infrared (FT-IR) analyses. According to XRD analysis, the size of nanoparticles was found to be in the range of 10–17 nm. SEM images showed the favored shape of nanoparticles as triangle which is a benign shape for antibacterial analysis. The antibacterial activity of the obtained nanocomposite was also evaluated against Gram positive bacteria *Staphylococcus aureus* (*Staph. aureus*) and Gram negative *Escherichia coli* (*E. coli*) using the paper disk diffusion method. The antibacterial study showed that the PANI/PVA composite did not have a very good antibacterial activity but PANI/PVA/Ag nanocomposites were found to be effective against two bacteria.

1. Introduction

In recent years, the synthesis of nanoparticles has found special attention because of increased surface area to volume ratio, modified structure and increased activity compared to macro molecules (Bardajee et al., 2012; Prashanth et al., 2011; Dror-Ehre et al., 2009). Nanoparticles have many applications in optical, electronic and textile industries, medicine, cosmetic, and drug delivery (Ahmad et al., 2012; Prashanth et al., 2011). The most important nano product in the field of nanotechnology is Ag nanoparticles which are significantly used in textiles and clothing, food packaging, medical and cosmetic ingredients, water, wastewater and air treatment, pesticides and household usage (Honary et al., 2011).

The antibacterial properties of silver have been recognized over 2,000 years ago. In general, Ag nanoparticles are effective

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against many bacteria and can destroy 650 types of bacteria, viruses and fungi due to enhancement of antibacterial, antiviruses, and antifungal activity of Ag at the nano scale even around 100%. These nanoparticles are more sustainable, efficient and simple to process when compared with other antibacterial agents (He et al., 2012; Li et al., 2011a; Pourjavadi and Soleyman, 2011; Akhbari et al., 2010; Jones and Hoek, 2010; Mahltig et al., 2009).

There are various methods to prepare nanoparticles. The most important ones are follows: chemical reduction (Lee et al. (2010)), optical reduction (Khanna et al., 2005), hydrogel method (Thomas et al., 2007), sedimentation method (Noritomi et al., 2010), UV and gamma irradiation (Lee et al., 2010), Micelles (Noritomi et al., 2010), and biosynthesis method (Tripathy et al., 2010). 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 (Ahmad et al., 2011).

Nanocomposites are multiphase materials with one of their components between 1 and 100 nm in size. These materials have physical and mechanical properties including high strength, toughness and heat resistance at a wide range of temperature. By mixing various materials and their features, the novel composites with new applications will be produced (Ahmad et al., 2011). Based on the matrix, types of nanocomposites are: polymer nanocomposites, ceramic and metallic nanocomposites, polymer and ceramic or metallic nanocomposites, ceramic–ceramic nanocomposites, metal matrix nanocomposites, thin film nanocomposites, and nanocomposites based on carbon nano tubes (Prucek et al., 2011; Thostenson et al., 2005; Balazsi et al., 2003; Fischer, 2002).

Polymeric nanocomposites are advanced composites obtained from nanoparticles and polymeric matrix which nanoparticles are coated by polymers and a core–shell structure can be formed. Because of the special shape, chemical nature, and unique structure of polymers, nanoparticles can be distributed in a polymer matrix in the best shapes. By coating of nanoparticles and functionalization of particles, the *Van der Waals* forces between nanoparticles are reduced and adaptability and distribution of nanoparticles in the matrix are increased. Polymers are always the first choice for nanoparticle coating. On the other hand, suitable functional groups in polymers structure can be used as reaction sites to control the one-pot synthesis of nanocomposites (Dallas et al., 2011; Jeon and Baek, 2010; Chandra et al., 2008; Wu and Ke, 2007; Guo et al., 2006; Han and Yu, 2006; Shenhar et al., 2005; Chang et al., 2003). In general, nanocomposites based on organic polymers have many advantages such as long-term stability, good process ability, and outstanding optical, catalytic, electronic and magnetic properties. Therefore, the resultant nanocomposites could potentially provide many applications in various areas such as automotive, aerospace, optoelectronics, etc. (Jeon and Baek, 2010). For the synthesis of polymer nanocopmposites, a polymer such as polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), polyethylene glycol (PEG), peroxyacetic acid (PAA), and polyglycolacid (PGA) is needed (Jovanović et al., 2011; Shin et al., 2008).

There are many studies for the synthesis of polymeric nanocomposites in the literature. For example, Bryaskova et al. (2011) synthesized Ag/polyvinylpyrrolidone nanocomposites by thermal or chemical reduction of silver ions to silver nanoparticles. The antimicrobial activities of the synthesized nanocomposites were tested against various bacterial and fungal strains. The results showed a strong antimicrobial property against the tested strains. The polyacrylonitrile/montmorillonite/Ag nanocomposites were also prepared using the chemical reduction of Ag^+ (*in situ*) by Hwang and Ma (2012). The antibacterial activities of the silver nanoparticle solution, which was obtained by soaking the polyacrylonitrile/montmorillonite/Ag nanocomposite films in distilled water, were tested using the paper disk diffusion method. The results showed that the silver nanoparticle solution was quite effective against tiny bacteria such as *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia*.

Polyvinyl alcohol (PVA) has several advantages such as high biocompatibility, biodegradability, hydrophilicity, and ability to form fiber. Because of these features, 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 Ag (Shin et al., 2008), cellulose (Gea et al., 2010), titanium dioxide (Hebeish et al., 2012), and copper(I) sulfide nanoparticles (Kumar et al., 2002).

Polyaniline (PANI) is another polymer which is also used for coating of Ag (Barkade et al., 2011; Porramezan and Eisazadeh, 2011; Khanna et al., 2005), zeolite (Shyaa et al., 2012), silica gel (Stejskal et al., 2002), and nano fibers, especially gelatin nano fibers (Fan et al., 2012; Li et al., 2006).

The aim of this study is to synthesize a novel nanocomposite based on Ag nanoparticle coating by PANI and PVA polymers. The Ag nanoparticles are prepared using the chemical reduction method, a fast, simple and low cost method. Finally, the antibacterial property of the obtained nanocomposite is evaluated against two pathogenic bacteria, including *Staph. aureus* (Gram positive) and *E. coli* (Gram negative) by using the agar disk diffusion method. To the best of our knowledge, this is the first work on the synthesis of PANI/PVA/Ag nanocomposite by *in situ* polymerization of aniline in the presence of PVA and Ag nanoparticles.

2. Materials and methods

2.1. Chemicals

All chemicals were purchased from Merck (Darmstadt, Germany). All chemicals were of analytical reagent grade and used without further purification. Silver nitrate ($AgNO_3$) was used as Ag^+ source and aniline in aniline hydrochloride form was used as monomer for synthesis of polyaniline (PANI).

2.2. Microorganisms

The antibacterial activity of prepared nanocoposites was determined using two different bacterial strains including *Staph. aureus* and *E. coli*. All the bacterial strains were obtained from the School of Pharmacy, Medical Science University of Zabol, Zabol, Iran.

2.3. Preparation of Ag nanoparticles

Nano sized Ag particles were synthesized by the chemical reduction of $AgNO_3$ using $NaBH_4$ (1:4) in deionized (DI)

water according to the procedure described by Ahmad et al. (2012). Silver nitrate solution was separately prepared at different concentrations (0.0025, 0.005, 0.007, 0.01 and 0.0125 M) in DI water. The aqueous solution of $NaBH_4$ was maintained at 0–3 °C for 20 min and was slowly titrated by $AgNO_3$ solution (200 ml) under constant stirring. The reduction reaction was continued for 30 min at room temperature. Finally, the yellowish-red colloid of Ag nanoparticle solution was obtained.

2.4. Preparation of aniline hydrochloride

Aniline hydrochloride was synthesized (Bhadra and Sarkar, 2010) as follows: pure aniline and concentrated hydrochloric acid was mixed in the ratio of 1:2. First, 20 ml of aniline was heated and 40 ml of hydrochloric acid was added dropwise and the mixture was stirred consequently by a magnetic stirrer. After the color changed from colorless to violet, the solution was allowed to cool at room temperature. Then, the solution was filtered and washed by concentrated hydrochloric acid. Finally, the obtained sediment was dried at 40–50 °C in an electrical oven.

2.5. Preparation of PANI/PVA/Ag nanocomposites

The PANI/PVA/Ag nanocomposites were synthesized by *in situ* chemical oxidation polymerization of aniline monomer in the presence of PVA and Ag nanoparticle colloidal solution. In a typical synthesis process, aniline–hydrochloride was added to the prepared Ag nanoparticles colloidal solution (200 ml). The obtained mixture was stirred for 10 min and then the PVA aqueous solution (which dissolved with 0.1 M HCl) was added and the mixture was stirred for 30 min. By addition of the aqueous solution of ammonium persulfate, $(NH_4)_2S_2O_8$, the mixture was allowed to react for 12 h under constant stirring at −3 °C.

2.6. Characterization

2.6.1. UV–Vis spectroscopy

The absorption spectrum of the reaction mixtures was recorded at room temperature using UV–Vis spectrophotometer (Rayleigh, UV-2100) at a resolution of 1 nm.

2.6.2. FT-IR spectroscopy

The products were analyzed by Fourier transform infrared (FT-IR) spectroscopy (Bruker Optics Ft Tensor 27, Germany) using KBr disks.

2.6.3. X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis of the products was also conducted using a Bruker D8 X-ray diffractometer. The X-ray beam was Ni-filtered Cu Kα radiation from a sealed tube. PANI/PVA/Ag nanocomposites were analyzed in a 2θ range of 1–80°, at the scanning rate of $1.5°min^{-1}$.

2.6.4. Field emission scanning electron microscopy (FESEM)

FESEM was applied to observe the surface morphology of PANI/PVA/Ag nanocomposite materials using a Hitachi S4160 instrument. Thin films of the samples were prepared on graphite adhesives; then, the surface of the samples was coated by gold powder using sputter hummer instrument.

2.7. Antibacterial activity study

The antibacterial activity of the PVA/PANI/Ag nanocomposites was tested against *E. coli* and *Staph. aureus* microorganisms using the paper disk diffusion method according to the procedure described by Hwang and Ma (2012). This method is indeed a means of measuring the efficiency of an antibacterial agent against the mentioned bacterial growth. The suspensions of the bacteria culture were prepared and their concentrations adjusted by comparing them to the concentrations in standard tubes with a McFarland turbidity of 0.4–0.5 (1.5×10^8 CFU). The Muller–Hinton agar (MHA) powder was used as a culture medium for bacterial growth. 19 g of agar was dissolved in 500 ml of distilled water; then the clear brown solvent was obtained by boiling the solution. The MHA medium (15 ml) was sterilized at 120 °C for 60 min in autoclave, cooled to room temperature, and then poured into sterilized Petri dishes (10 mm × 90 mm). The bacteria of interest are swabbed uniformly across a culture plate, while the Petri dishes are cooled over 24 h. Filter-paper disks according to the number of samples, were placed on the surface of the medium and 40 µl of each concentration of PVA/PANI/Ag samples were dropped over disks by sampler to investigate antibacterial activity. If the compounds are effective against bacteria at a certain concentration, then no colonies will grow and the concentration in the agar is greater than or equal to the effective concentration. This region is called the zone of inhibition. 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 tests have been done under laminar flow hood. Finally, all Petri dishes contained bacteria and antibacterial reagents were incubated and maintained at 37 °C for 24 h. After this period, the diameters of the inhibition zones formed around each disk were determined and presented in mm. The results concerning antibacterial activity were ordered as strong activity (> 13 mm), moderate activity (6–12 mm), weak activity (5 mm) or no activity (inhibition zone < 5 mm).

3. Result and discussion

Metal nanoparticles are generally obtained from noble metals like silver, gold, platinum, titanium, cupper and tin. Among the noble metals, silver has the most widely required properties for various applications (He et al., 2012; Honary et al., 2011; Li et al., 2011b; Pourjavadi and Soleyman, 2011; Savithramma et al., 2011; Akhbari et al., 2010; Mahltig et al., 2009; Wu and Ke, 2007).

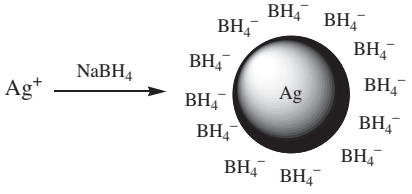

Figure 1 Schematic of the chemical reduction of silver ions with $NaBH_4$ as reducing as well as stabilizing agent.

In this study, silver nanoparticle colloidal solution was synthesized using $NaBH_4$ as a reducing agent. The chemical reduction of Ag^+ ions to Ag nanoparticles by $NaBH_4$ is as follows (Solomon et al., 2007):

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

A schematic of the chemical reduction is shown in Fig. 1. It is observed how Ag nanoparticle was cached by the reducing agent.

The most possible approach to stabilize nanoparticles, especially Ag nanoparticles, and preparation of nanocomposites, is using polymers (Travan et al., 2011; Zahir et al., 2009). Polymers are realistically not impeccable and have defects somehow. These deficiencies could be improved or completely resolved by modulating polymers and nanoparticles. An alternative method to improve polymer properties is to combine the desired polymer with other polymers which have better properties. The combination of two or more polymers is the most efficient method to build novel compounds with benign properties and many potential applications (Ahmad et al., 2011). In this method, the polymer with better properties can upgrade the properties of a weak polymer.

PANI has many advantages but has low solubility in several common solvents. There are some suggestions to overcome this problem. Dispersing PANI in the soluble polymeric matrix like polystyrene sulfonic acid, polyvinyl alcohol (PVA), gelatin, hydroxy methyl cellulose and polyethylene oxide is the most ordered due to simplicity and low-cost. There are various studies reported in the literature which have used PVA as advanced matrix (Bhadra and Sarkar, 2010; Ramirez et al., 2009). In fact, PVA improved the solubility of PANI and this novel composite can be applied for Ag nanoparticle coating.

The PVA/PANI/Ag nanocomposite was synthesized by *in situ* polymerization of aniline hydrochloride in the prepared Ag nanoparticle colloid. The process of synthesizing PVA/PANI/Ag nanocomposite is shown in Fig. 2.

3.1. UV–Vis analysis

The successful synthesis of Ag nanoparticle colloidal solution was explored by UV–Vis analysis. All samples have shown an intense peak in the wavelength range of 400–450 nm,

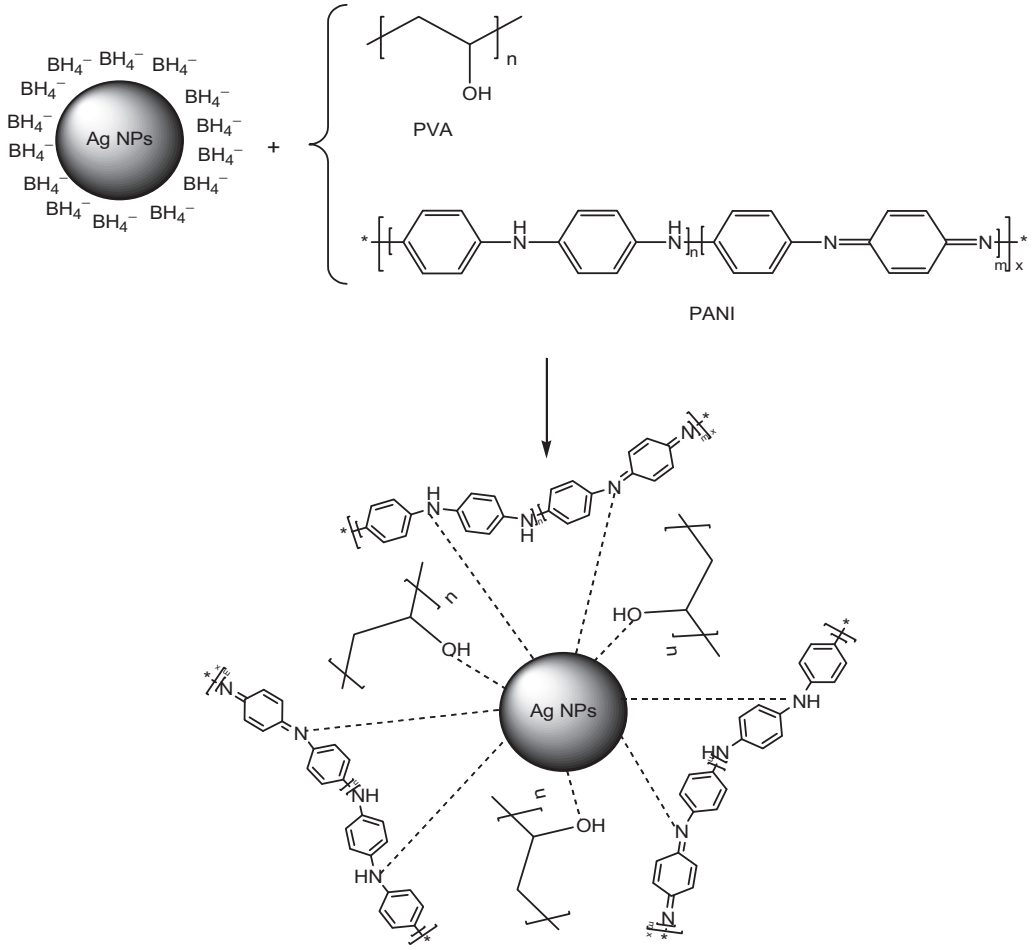

Figure 2 Process of synthesizing PVA/PANI/Ag nanocomposite from Ag nanoparticles, PVA and PANI.

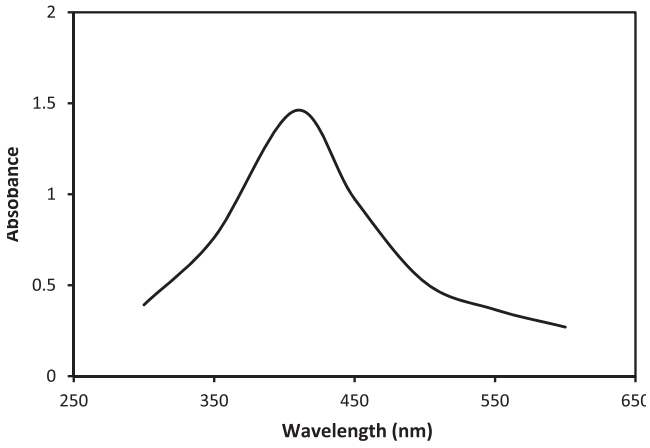

Figure 3 UV–Vis spectrum of Ag nanoparticle colloid in 1:4 ratio of $AgNO_3$ to $NaBH_4$.

especially around 430 nm. Fig. 3 exhibits the UV–Vis spectrum of Ag nanoparticle colloidal solution which is the same for all colloidal samples due to the same ratio of $AgNO_3$:$NaBH_4$ (1:4).

3.2. FT-IR spectroscopy

Fig. 4 shows the FT-IR spectra of prepared nanocomposites. The free OH functional group has a broad peak at 3600–3650 cm^{-1}, and this peak goes to 3200–3500 cm^{-1}, if the OH group is engaged in the formation of hydrogen bond or complex with metal particles. As shown in Fig. 4, the broad peak of hydroxyl groups appeared in this range. 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 2850–3000 cm^{-1} and 1465 cm^{-1}, respectively. A single medium peak in 3100–3500 cm^{-1} and an intense peak in 1000–1350 cm^{-1} showed the existence of the C–N bond in polyaniline chains. The presence of benzene rings in the polyaniline structure was also confirmed with an intense tensile peak around 1475–1600 cm^{-1}.

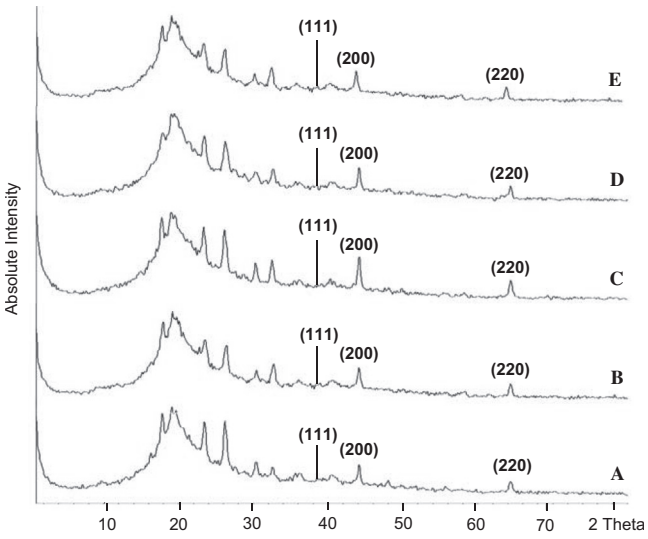

Figure 5 XRD spectra of prepared nanocomposites at different concentrations of silver nanoparticles; 5% (A); 10% (B); 15% (C); 20% (D); 25% (E).

3.3. XRD analysis

XRD patterns of PVA/PANI/Ag nanocomposites are illustrated in Fig. 5. A broad peak appearing at 2θ values in the range of 23–28° is generally confined to the polymeric chains. The sharp and intense peaks around 2θ values of 38, 44 and 64, with 111, 200 and 220 diffraction respectively, are related to benign Ag crystalline structure in the complex which stabilized by polymeric matrix. The XRD patterns clearly exhibit the presence of silver nanoparticles in nanocomposite forms. The average particle size of the PVA/PANI/Ag nanocomposite is

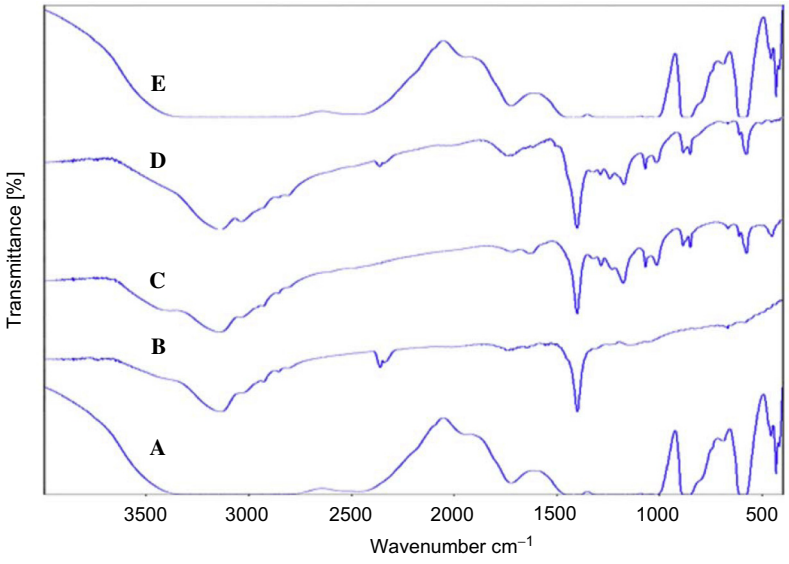

Figure 4 FT-IR spectra of prepared nanocomposites at different concentrations of silver nanoparticles; 5% (A); 10% (B); 15% (C); 20% (D); 25% (E).

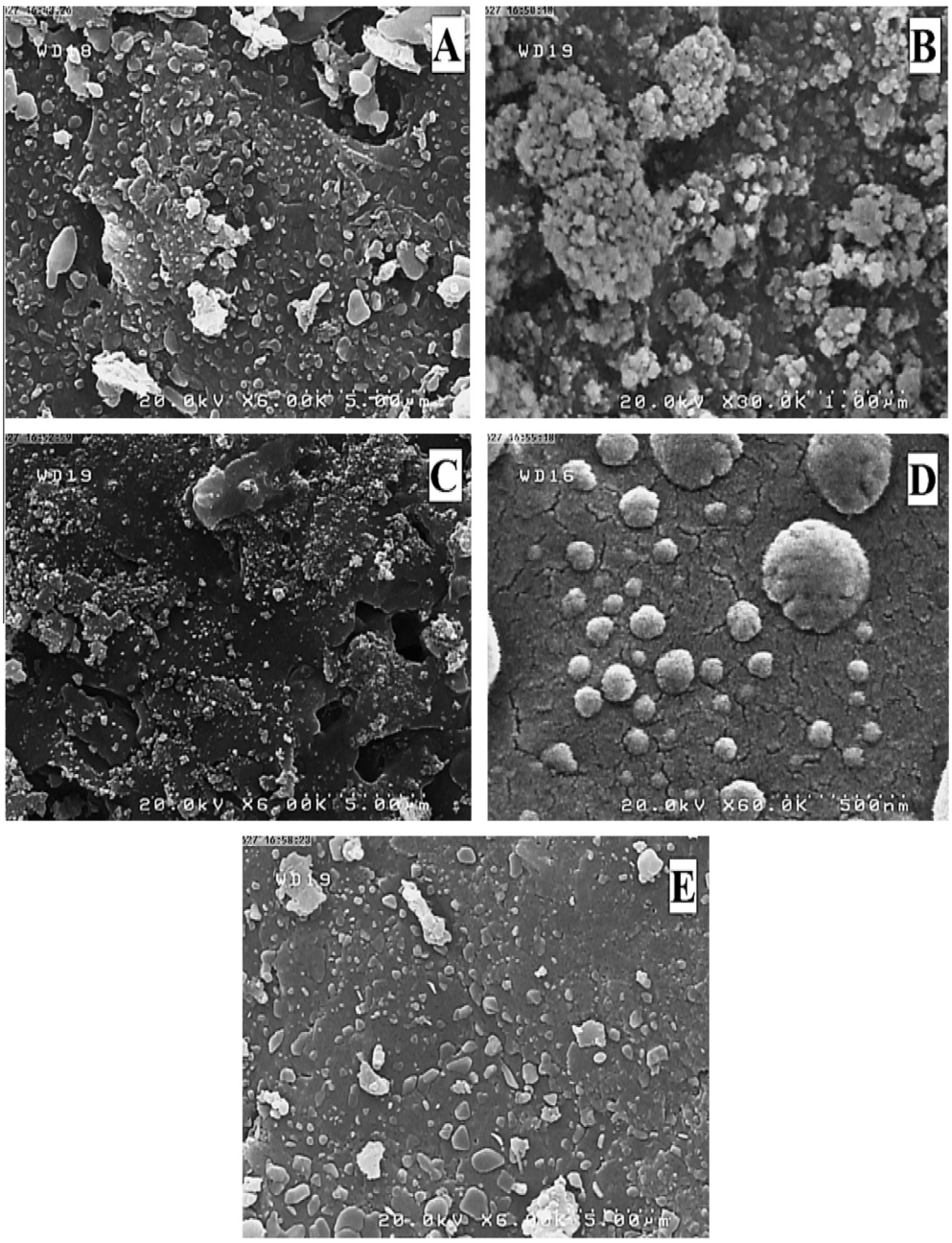

Figure 6 FESEM images of prepared nanocomposites at different concentrations of silver nanoparticles; 5% (A); 10% (B); 15% (C); 20% (D); 25% (E).

calculated by the Scherrer equation (Eq. (1)) and is estimated to be 10–17 nm for all samples

$$D = \frac{0.94\lambda}{\beta \cos\theta} \tag{1}$$

where D is the average crystallite size, λ is the X-ray wavelength, β is the full width at half maximum (FWHM) and θ is the diffraction angle (Zhu and Zhu, 2006).

3.4. FESEM analysis

The morphology of the prepared PVA/PANI/Ag nanocomposites is shown in Fig. 6. These images clearly showed the porous nanocomposites, crystalline structure of coated nanoparticles and successful particle coating by polymer cavities. It can be seen that some of nanoparticles are triangular which is the best crystalline structure shape for antibacterial tests due to an atomic density higher than other crystalline shapes (Jones and Hoek, 2010; Ramirez et al., 2009; Pal et al., 2007). The synthesis of truncated triangular silver nanoplates has been reported by Chen and Carroll (2002) using cetyltrimethylammonium bromide micelles by solution phase method in the average size of 68 nm. In the other research, the silver triangular nanoplates were synthesized by reduction of silver ions using the end groups of poly(vinyl pyrrolidone) in aqueous media via kinetic control (Washio et al., 2006).

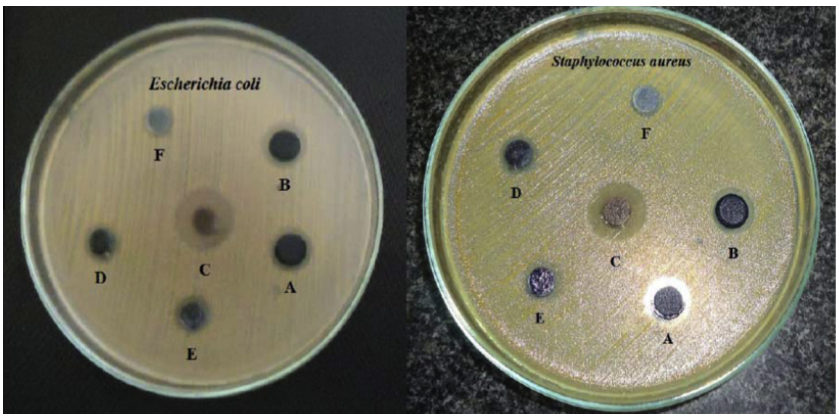

Figure 7 Antibacterial activity of various nanocomposites against two pathogenic strains; *E. coli* and *Staph. aureus* shown by the paper disk diffusion method; 5% (A); 10% (B); 15% (C); 20% (D); 25% (E).

Table 1 Average of inhibition zones obtained from various nanocomposites at different concentrations of silver nanoparticle; 5% (A); 10% (B); 15% (C); 20% (D); 25% (E); 0% (F) against two pathogenic bacteria.

Samples	Average of formed inhibition zones (mm)	
	E. coli	*Staph. aureus*
A	7	10
B	8	12
C	12	15
D	9	9
E	8	8
F	0	0

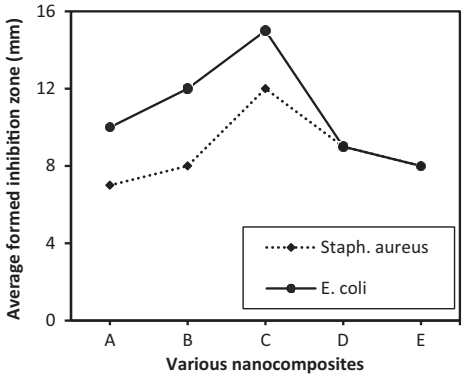

Figure 8 Scatter plot of inhibition zone *versus* various PVA/PANI/Ag nanocomposites at different concentrations of Ag nanoparticles: 5% (A); 10% (B); 15% (C); 20% (D); 25% (E).

3.5. Antibacterial properties of prepared PANI/PVA/Ag nanocomposites

In this study, PVA/PANI/Ag nanocomposites were tested for antibacterial activity using *E. coli* and *Staph. aureus*. Fig. 7 shows the inhibition zones that were formed by nanocomposite samples. The diameter of the inhibition zones are 7, 8, 12, 9, and 8 mm and 10, 12, 15, 9, and 8 mm against *E. coli* and *Staph. aureus* respectively. The results are summarized and presented in Table 1. It is observed that PVA/PANI composite (sample of F) which was used as a control matrix, exhibited no antibacterial activity when compared with PVA/PANI/Ag nanocomposites. The scatter plot of the inhibition zone *versus* various PVA/PANI/Ag nanocomposites is presented in Fig. 8. According to Fig. 8, the PVA/PANI/Ag nanocomposite (C) with 15% Ag nanoparticle showed better antibacterial activity against *E. coli* and *Staph. aureus*. Silver exhibits outstanding antibacterial property that would lead to biomedical applications. The antibacterial activity of silver is dependent on Ag^+ that binds strongly to electron donor groups on biological molecules like sulfur, oxygen or nitrogen. The silver ions act by displacing other essential metal ions such as Ca^{2+} or Zn^{2+} (Boomi et al., 2013). At low concentrations of nanoparticles, the interaction of particles with the cell wall of bacteria decreases and at the high concentrations of the particles, the aggregation probability of particles increases, as a result, the effective surface to volume ratio of particles and so the resulting interaction between particles and the cell wall of bacteria decrease. Fig. 9 shows the process of releasing Ag nanoparticles at low, medium and high concentrations of nanoparticles. The effects of silver nanoparticles on the bacterial cell are complicated (Kim et al., 2011). However, there are various mechanisms on the action of silver nanoparticles on the bacterial cell (Prabhu and Poulose, 2012). Some of these mechanisms were summarized and presented as follows: (i) the ability of silver nanoparticles to anchor to the bacterial cell wall and then penetrate it (Sondi and Salopek-Sondi, 2004), (ii) the formation of free radicals by the silver nanoparticles which can damage the cell membrane and make it porous (Danilcauk et al., 2006; Kim et al., 2007), (iii) releasing the silver ions by the nanoparticles which can interact with the thiol groups of many vital enzymes and inactivate them (Feng et al., 2008; Matsumura et al., 2003), and (iv) the nanoparticles can modulate the signal transduction in bacteria which stops the growth of bacteria (Shrivastava et al., 2007).

4. Conclusions

In this study, PVA/PANI matrix was first synthesized and used successfully for Ag nanoparticle coating. The results show clearly the good efficiency of synthesized polymers in nanoparticle coating because the particle size was obtained in the

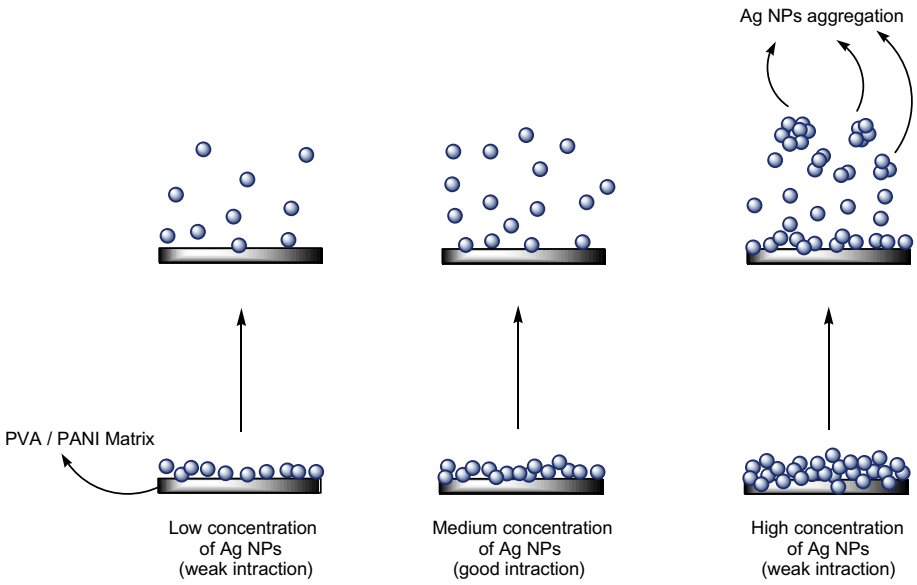

Figure 9 Process of releasing Ag nanoparticles (Ag NPs) at low, medium and high concentrations of nanoparticles.

acceptable range of 10–17 nm. The applied method is simple and of low cost and does not use many chemicals unlike other methods. Both polymers are biodegradable and highly biocompatible; but PANI in generally is not water soluble. Herein, the PVA was used to improve solubility of PANI. The novel nanocomposite has various applications and among its properties, the antibacterial ability is the most important feature. Since the polymers used eco-friendly materials with low bio-contaminations, they are suitable for antibacterial applications. This novel nanocomposite can be added to various drugs as the main or booster component.

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