Manas Journal of Engineering
ISSN 1694-7398 | e-ISSN 1694-7398

Volume 7 (Issue 1) (2019) Pages 13-23

Production of antibacterial polyvinylpyrrolidone nanofibers containing silver nanoparticles via electrospinning method

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ABSTRACT

Since polymeric nanofibers find extensive usage in medical applications, the risks associated with bacterial contamination must be taken into account. In this study, the production of antibacterial polyvinylpyrrolidone (PVP) nanofibers containing silver nanoparticles (AgNPs) was achieved. For this production, AgNPs with the average diameters ranging from 5.69 nm to 14.83 nm were synthesized and characterized firstly. Then, the properties of the nanofibers produced via electrospinning method were investigated by means of scanning electron microscopy (SEM) and X-ray diffraction (XRD) techniques. The effect of AgNPs on the conductivity of electrospinning solutions were investigated. The stability of nanofiber mats were determined to show the crosslinking efficiency. Finally, antimicrobial tests against three types of microorganisms (*E. coli*, *S. aureus* and *A. niger*) were carried out according to zone of inhibition method. Antimicrobial test results proved that nanofibers containing AgNPs show inhibition against *E.coli* and *S. aureus*, while neat nanofibers not. Additionally, any antifungal activity was not observed against *A. niger*.

ARTICLE INFO

Research article
Received: 03.04.2019
Accepted: 25.04.2019

Keywords:
Silver nanoparticles, polyvinylpyrrolidone, electrospinning, nanofibers.

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1. Introduction

Nanotechnology provides an opportunity for production of nanomaterials which can be in the form of metal, ceramic, polymeric or composite with at least one external dimension in the nanoscale or having internal or surface structure in the nanoscale [1]. As the size decreases to nanoscale, surface to volume ratio increases and hence surface atom fraction increases and quantum effect becomes important. Consequently, the properties of nanomaterials such as optical, electrical, mechanical, chemical, magnetic improve and differ from their atomic-molecular or bulk counterparts [1, 2].

Polymeric nanofibers have novel properties due to their large specific surface area, small pore size, high porosity, flexibility and improved mechanical structure [3]. Highly enhanced surface area, continuous and interconnected porous network of nanofiber mats provide efficient fluid absorption, adequate space for cell attachment, transportation of gases, nutrients or drugs, which are important properties in biomedical usage [3, 4]. Nanofibers can be produced by variety of methods such as drawing, template synthesis, phase separation, self-assembly and electrospinning which are based on physical, chemical, thermal and electrostatic techniques [5]. Among these methods, electrospinning is the most attractive because of enabling easy and rapid fabrication of continuous fibers with diameters ranging from tens of nanometers to microns along with the advantages of easy set-up, cost effectiveness, allowing diameter control and variety of material usage [5-7].

Electrospinning technique uses electrostatic forces in order to form nanofibers from liquid polymer solutions or melt [6]. In this technique, the pendant drop which is fed to the nozzle gets charged by applying high voltage and it forms a liquid jet after electrostatic force overcomes surface tension. The jet elongates and dries through solvent evaporation due to undergoing physical instabilities under the electrical field and solid nanofibers are deposited on the collector at the end [6-8]. Morphology and diameter of nanofibers can be controlled by changing the electrospinning parameters which are solution parameters (e.g. concentration, molecular weight, viscosity, surface tension), process parameters (e.g. applied voltage, tip to collector distance, flow rate) and ambient parameters (e.g. humidity and temperature) [9].

Since polymeric nanofibers are notably used in medical areas, the risks associated with bacterial growth or infection must be prevented [3, 10, 11]. Electrospun nanofibers can be converted to outstanding antimicrobial materials via functionalization of the nanofibrous matrix by addition of AgNPs [10, 12]. AgNPs have remarkable physico-chemical properties such as high thermal and electrical conductivity, chemical stability and catalytic activity [13]. Beside of these properties, AgNPs have broad-spectrum antimicrobial effect to many type of bacteria, fungi, and virus despite their low toxicity, they exhibit biocidal effect via multiple mechanism, and release Ag^+ ions slowly, these properties make them strong antimicrobial agent for various biomedical applications [14-17]. Silver nanoparticles can be synthesized with many methods which are mainly classified under three groups as physical, chemical and biological [18]. Chemical reduction method has widely usage which allow to synthesize nanoparticles with various size and shape [18-20]. In this method, reduction of Ag^+ ions to Ag^0 is generally carried out in aqueous or solvent medium containing metal precursor, reducing agent and stabilizer by applying heat and using catalyst, if necessary. [20, 21]. As an alternative to the classical heating that causes an increase in reaction time and thermal gradients in the reaction medium, microwave irradiation provides rapid and uniform heating in order to produce small and monodispersed nanoparticles by accelerating the reaction [22, 23].

As reported in the literature, antibacterial electrospun nanofibers containing silver nanoparticles were prepared with different types of polymers such as polyacrylonitrile (PAN) [12], poly(butylenes succinate) (PBS) [24], chitosan [25], polyvinyl alcohol/chitosan blend [26], nylon 6 [27], polycaprolactone (PCL) [28], chitosan/poly(ethylene oxide) blend [29], gelatin [30]. Being a hydrophilic, biocompatible, soluble in water and various solvents, non-toxic and a good compounding properties as well as a good dispersant, polyvinylpyrrolidone (PVP) has been used to form nanofibers alone or blending with different polymers in order to improve spinnability and/or introduction of AgNPs to the matrix [24, 31-33]. In this study, PVP nanofibers containing AgNPs were produced via electrospinning method for potential usage in biomedical applications. In this context, AgNPs were synthesized from $AgNO_3$ salt in ethanolic solutions of low molecular weight PVP and the effect of PVP concentration on the AgNPs synthesis process was investigated firstly. Then, the effect of electrospinning solution composition on the neat and AgNPs containing PVP nanofiber structures were determined and compared in detail. Finally, antimicrobial properties of neat and AgNPs containing nanofibers were tested against *E. coli*, *S. aureus* and *A. niger*.

2. Materials and methods

2.1. Materials

For the synthesis of AgNPs silver nitrate ($AgNO_3$) was used as silver precursor and PVP $(C_6H_9NO)_n$ with the average molecular weight of 40000 was used as stabilizing agent. On the other hand PVP with the average molecular weight of 360000 was used for the preparation of electrospinning solution. Avoiding any confusion in the text, PVP with the average molecular weight of 40000 and 360000 were written as L-PVP and H-PVP, respectively. Ethanol (C_2H_5OH) was used as reducing agent for the synthesis of AgNPs and also solvent for PVP. All chemicals mentioned above were analytical grade and used directly without further purification.

2.2. Synthesis of AgNPs

For the synthesis of AgNPs, 1% (w/v) L-PVP solution was prepared first by dissolving 0.15 g L-PVP in 15 mL ethanol and kept on a magnetic stirrer at room temperature for 24 h. Then, $AgNO_3$ was added with a PVP/$AgNO_3$ weight ratio (R) of 10 and 50 and the solution was stirred until ensuring complete dissolution. Resultant solution was placed into a domestic microwave oven operating 2450 MHz frequency and treated at different conditions which were given in Table 1. To prevent intense boiling of the solution microwave irradiation was carried out discontinuously.

Table 1. *Conditions applied for AgNPs synthesis in microwave.*

R	Microwave power (W)	Irradiation time (s)
10	180	30
	180	60
	600	30
	600	60
50	180	30
	180	60
	600	30
	600	60

R = PVP/$AgNO_3$ weight ratio

To investigate the effect of polymer concentration on the properties of synthesized AgNPs, 5%, 7.5% and 10% (w/v) L-PVP/ethanol solutions were also prepared. The addition of $AgNO_3$ salt to these colourless solutions with a constant R value of 50, a change in the colour from yellow to brown was observed which indicates the AgNPs formation in the solution. Because of this observation microwave treatment was not applied to these solutions.

2.3. Preparation of Electrospinning Solution

Electrospinning was firstly applied to the L-PVP solutions containing AgNPs but densely accumulated nanofiber mats cannot be produced because of their low viscosity. In order to solve this problem, viscous H-PVP solutions were prepared by dissolving different amounts of H-PVP in ethanol with continuous stirring for 24 h. Then, electrospinning solutions having different compositions as given in Table 2 were prepared by mixing H-PVP solutions with the same volume of L-PVP solutions and stirring them for 1 h.

Table 2. *Composition of the electrospinning solutions.*

Total PVP conc. % (w/v)	Concentration % (w/v) of the solutions		[a]Amount of $AgNO_3$ (%)
	L-PVP	H-PVP	
5	1	9	1
5	1	9	0
10	1	19	0.5
10	1	19	0
5	5	5	1
5	5	5	0
7.5	7.5	7.5	1
7.5	7.5	7.5	0
10	10	10	1
10	10	10	0

[a] With respect to the total PVP

2.4. *Production of Nanofibers*

Electrospinning device (Nanospinner 24-XP, Inovenso) based on a bottom-up spinning technique was used to produce nanofibers. Electrospinning solutions were transferred to a 5 mL plastic syringe which was connected to syringe pump and flow rate was set as 3.5 mL/h. Constant voltage of 18 kV was applied to form an electrical field at the distance of 16.5 cm between tip of syringe and grounded plate wrapped with aluminium foil. During nanofiber production process relative humidity was kept lower than 60% because of the highly hygroscopic nature of the polymer.

Nanofibrous mats were exposed to thermal treatment at different conditions (150°C for 3 h, 175°C for 2 h and 200°C for 2 h) in order to prevent their solubility in water and other solvents.

2.5. *Characterization of AgNPs and Nanofibers*

Dynamic light scattering (DLS) technique was used to determine the size of AgNPs via Nano-flex particle size analyzer. The UV-visible spectra of the AgNPs solution was measured with a Hach Lange-DR 5000 spectrophotometer to confirm AgNPs formation. The morphology of the electrospun nanofibrous mats was investigated by scanning electron microscopy (SEM, FEI Nova NanoSEM 450). The average fiber diameter and the standard deviation were calculated using Image-J software program from 50 randomly selected measurements for each SEM images. The crystalline structure of nanofibers containing AgNPs was determined by X-ray diffraction analyzer (XRD, Rigaku).

2.6. *Antimicrobial Activity Test*

Antimicrobial properties of the thermally treated nanofiber mats against three types of microorganism were tested with zone of inhibition method. In this method, *Escherichia coli (E. coli)* as a gram-negative bacteria, *Staphylococcus aureus (S. aureus)* as a gram-positive bacteria and *Aspergillus niger (A. niger)* as a fungus were inoculated to selective agar. Then, samples were placed on the middle of the petri dishes and incubated for 24 h. At the end of this period, the resulted zone of inhibition around the sample was observed.

3. Results and discussion

3.1. *Synthesis and Characterization of AgNPs*

Fig. 1 shows the appearances of 1% L-PVP solutions obtained after AgNPs synthesis in microwave at different conditions as indicated in Table 1. It is clearly seen that L-PVP-EtOH solutions which were colourless initially, turned to a hardly visible yellow colour after dissolution of $AgNO_3$ and gradually darkened with increasing microwave power and irradiation time. AgNPs have different colours depending on the particle size and the shape through absorption and scattering effects resulted from strong interaction between light and nanoparticles [21, 34]. Colour change to yellow-brown is a physical evidence of the formation of AgNPs in solutions [12, 24, 32, 35, 36]. Considering the changes in colours of solutions, it is deduced that 180 W microwave power was not adequate to reduce Ag^+ ions. Therefore, microwave oven should been operated at 600 W for AgNPs synthesis in 1% L-PVP solutions.

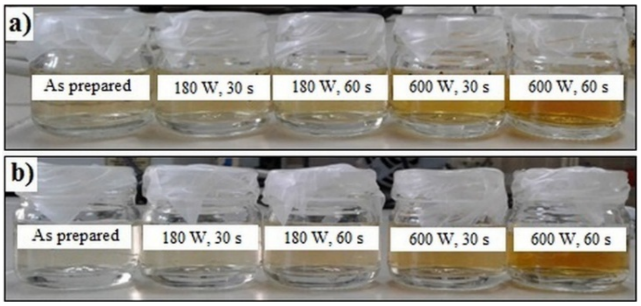


Figure 1. *1% L-PVP solutions containing $AgNO_3$ with the ratio of R = 10 (a) and R = 50 (b).*

Ethanol is a proper solvent for nanoparticle synthesis due to its ability of reduction of strongly electropositive metal ions and having high dielectric losses which enable to heat under microwave irradiation [22, 37]. On the other hand, PVP is one of the most used stabilizing agent in the synthesis of silver nanoparticles [38]. Being an amphiphilic non-ionic polymer, PVP has a structure of consisting of hydrophilic amide groups in its pyrrolidone rings and hydrophobic polyvinyl backbone. Thus, PVP provides steric stabilization by binding nanoparticle surface due to the high affinity of N and O atoms for silver ions and nanoparticles while polymer chain surrounds the nanoparticle surface to prevent agglomeration [20, 39-41]. Moreover, the ability of this polymer to reduce metal ions has also been known [24]. The studies in which the effects of molecular weight of PVP on AgNPs synthesis were investigated have shown that increase in molecular weight resulted to increase in nanoparticle size [42, 43]. This was explained by the effective interaction with silver ions in the case of lower molecular weight PVP which has shorter molecules resulting increased number of polymer chains in the medium [42, 43].

As shown in Fig. 2, the colours of the solutions having L-PVP concentrations of 5%, 7.5% and 10% and R value of 50 were changed significantly right after the dissolution of $AgNO_3$ without any microwave treatment. It was also observed that the solutions turned to a darker colour with increasing the amount of silver ions in the solution. It was reported that the reduction rate of silver ions in PVP-EtOH solutions increases with the increase in PVP and $AgNO_3$ concentration and temperature; moreover, the reaction time decreases significantly with increasing the amount of PVP in the solution [41]. Chen et al. synthesized AgNPs via mixing $AgNO_3$-EtOH solution with the same volume of PVP-EtOH solution and they observed that increasing the concentration of PVP accelerates the reaction and increasing concentration of $AgNO_3$ leads to increase the number of AgNPs formed [40].

Figure 2. *5%, 7.5% and 10% L-PVP solutions containing $AgNO_3$ with the ratio of R = 50.*

In the present study formation of AgNPs was also confirmed by measuring the absorption spectra of 7.5% L-PVP solution having R value of 50. As shown in Fig. 3 an absorption band at 414 nm was observed which is attributed to characteristic surface plasmon resonance (SPR) of spherical AgNPs [24, 35]. Collective oscillation of conduction electrons on nanoparticle surfaces after exposed to light at specific wavelengths leads to exhibition of SPR [21, 34].

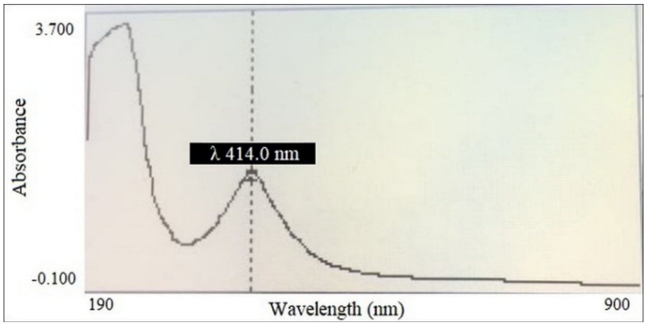


Figure 3. *Absorption spectra of 7.5% L-PVP solution having R = 50 value.*

Mean particle diameters and size distributions of AgNPs were measured with DLS analysis and the results were given in Table 3 and Fig. 4. It was found that the mean particle diameters of AgNPs synthesized in 1% L-PVP solutions ranged from 9.72 nm to 14.83 nm. The mean particle size of AgNPs obtained at the same microwave operation conditions for different R values were found very close to each other (Table 3). Increasing the microwave power from 180 W to 600 W caused a small decrease in particle diameter and an increase in standard deviation. Fig. 4 demonstrates the particle size distributions of AgNPs synthesized from 1% L-PVP solutions at 180 W (Fig. 4a-d) and 600 W (Fig. 4e-h) microwave power. The graphs in Fig. 4a-d confirm that AgNPs have narrow size distributions and standard deviation. By evaluating the DLS measurement results and the colour changes of 1% L-PVP solutions simultaneously, it can be concluded that 180 W microwave power was not adequate for complete reduction of Ag^+ ions in the solution.

Table 3. *DLS results of AgNPs synthesized in 1% L-PVP solutions.*

R	Microwave power (W)	Irradiation time (s)	Mean particle diameter (nm)	Standard deviation (nm)
10	180	30	12.70	2.42
	180	60	14.83	2.69
	600	30	11.90	3.37
	600	60	10.88	3.25
50	180	30	13.71	2.32
	180	60	14.29	2.49
	600	30	9.72	2.56
	600	60	10.25	3.26

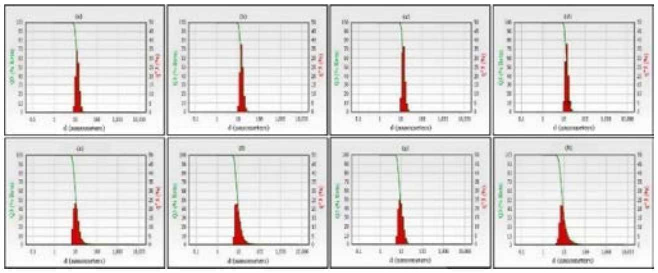


Figure 4. *Size distributions of 1% L-PVP solutions operated at 180 W for (a) R = 10-30 s, (b) R = 10-60 s, (c) R = 50-30 s, (d) R = 50-60 s, 600 W for (e) R = 10-30 s, (f) R = 10-60 s, (g) R = 50-30 s, (h) R = 50-60 s.*

Table 4 and Fig. 5 summarize DLS measurement results obtained for the solutions with 5%, 7.5%, 10% L-PVP concentrations and R value of 50. In respect to these results, AgNPs in the concentrated L-PVP solutions dispersed in two regions as low and high diameter but most of the particles clustered around lower sizes. As given in Table 4; 98.9% of the AgNPs synthesized in 5% L-PVP solution have 5.69 nm mean diameter, 94.5% of the AgNPs synthesized in 7.5% L-PVP solution have 7.4 nm mean diameter and 84% of the AgNPs synthesized in 10% L-PVP solutions have 7.47 nm mean diameter.

Table 4. *DLS results of AgNPs synthesized in 5, 7.5 and 10% L-PVP solutions.*

R	L-PVP conc.% (w/v)	Mean particle diameter (nm)	Percentage of mean particle diameter in solution (%)
50	5	5.69	98.9
		237.7	1.1
	7.5	7.4	94.5
		334	5.5
	10	7.47	84
		416	16

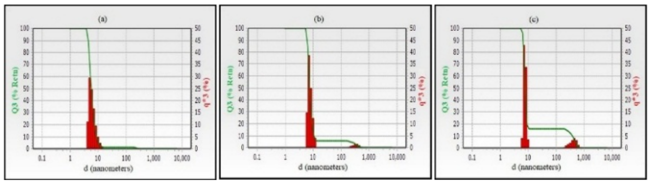


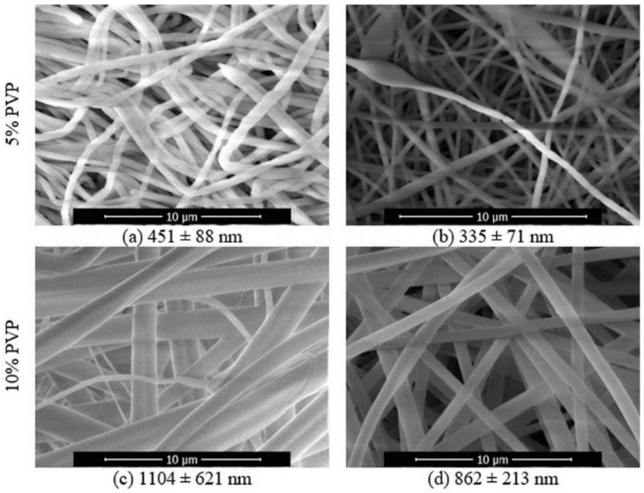
Figure 5. *Size distributions of AgNPs synthesized in (a) 5%, (b) 7.5% and (c) 10% L-PVP solutions.*

In the previous works it was observed that, the mean diameter of AgNPs decreased by increasing concentration of PVP at constant concentration of $AgNO_3$ [40, 41]. This phenomena was explained as the formation of high number of nuclei because of increasing the rate of spontaneous nucleation in the presence of PVP [40]. In this study, while the mean diameters of AgNPs synthesized in 1% L-PVP solutions ranged between 9.72-14.83 nm, this value decreased to 5.69 nm in 5% L-PVP solution. By further increasing L-PVP concentration to 7.5% and 10%, mean diameters became almost constant as 7.4 nm and 7.47 nm, respectively. As a result, increasing concentration of L-PVP gets particle diameter smaller up to a definite size, which is accordance with the study of Chen et al. [40].

3.2. Production of Nanofibers

3.2.1. Effect of the PVP Content of Electrospinning Solution on Nanofiber Structure

In order to investigate the effect of electrospinning solution composition on nanofiber structure various solutions containing different amounts of L-PVP and H-PVP as given in Table 2 were prepared as described in experimental section and used for neat nanofiber production. SEM images of the nanofibers produced from these solutions were given in Fig. 6.



(a) 451 ± 88 nm
(b) 335 ± 71 nm
(c) 1104 ± 621 nm
(d) 862 ± 213 nm

Figure 6. *SEM images of nanofibers produced from 5% and 10% PVP solutions with compositions of (a) 1% L-PVP + 9% H-PVP, (b) 5% L-PVP + 5% H-PVP, (c) 1% L-PVP + 19% H-PVP, (d) 10% L-PVP + 10% H-PVP.*

Fig. 6 shows that all the solutions are suitable to form nanofibers but somewhat different morphologies. The nanofibers produced from 1% L-PVP + 9% H-PVP solution (Fig. 6a) are not smooth and they also tend to accumulate over each other. On the other hand, the nanofibers produced from 5% L-PVP + 5% H-PVP solution (Fig. 6b) are smooth and separated from each other but have a beaded structure. Similar problems as in the nanofibers produced from 1% L-PVP + 9% H-PVP solution can also be seen in the nanofibers produced from 1% L-PVP + 19% H-PVP solution (Fig. 6c). The nanofibers produced from 10% L-PVP + 10% H-PVP solution have long, bead free and uniform structure (Fig. 6d). Since molecular weight of polymer indicates the entanglement of polymer chains, while low molecular weight polymers tend to form beads, smooth fibers can be obtained from the same concentration of higher molecular weight ones [44]. The high content of L-PVP in 5% L-PVP + 5% H-PVP solution compared with 1% L-PVP + 9% H-

PVP solution lead to decrease in solution viscosity and consequently occurrence of bead-on-string structure.

If the mean diameters of the nanofibers given in Fig. 6 are compared, it can be seen that nanofibers produced from 10% PVP solutions have thicker diameters than 5% PVP solutions. Indeed, increase in nanofiber diameter with increasing polymer concentration is an expected result. Furthermore, when the nanofibers produced from 5% and 10% PVP solutions are evaluated within themselves, it is obvious that the solutions having same ratio of L-PVP and H-PVP have thinner fiber diameter. Increasing the content of H-PVP in the solutions leads to increase solution viscosity, which favours the formation of thicker fibers.

By considering all these observation and results, another electrospinning solution with 7.5% PVP content was prepared by mixing same volume of 7.5% L-PVP and 7.5 H-PVP solutions. The SEM image of electrospun nanofiber produced from this solution was shown in Fig. 7. As depicted from Fig. 7 beaded structure was eliminated by increasing the PVP concentration from 5% to 7.5%; furthermore, more uniform and smooth fibers were formed. Mean diameter of the nanofibers produced from 7.5% PVP solution was measured as 605 ± 137 nm which is between the mean diameters of nanofibers produced from 10% PVP solution (856 ± 213 nm) and 5% PVP solution (335 ± 71 nm).

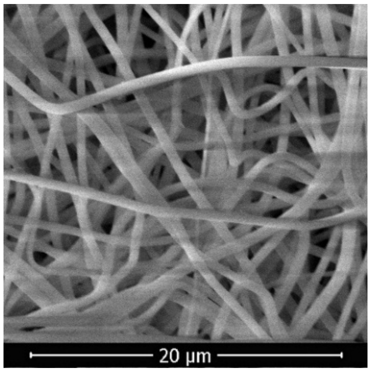
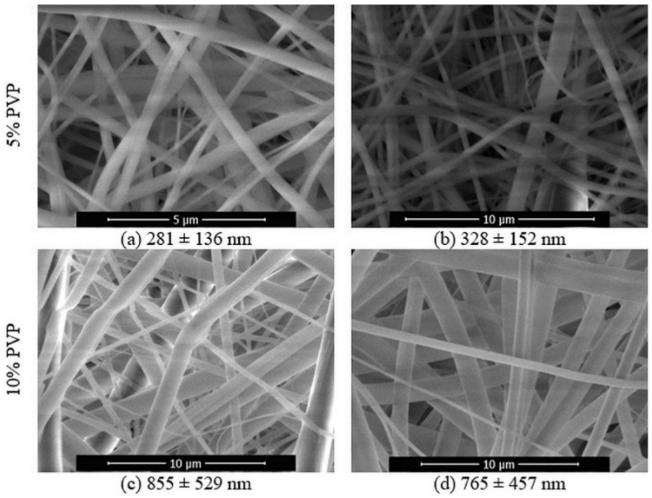


Figure 7. *SEM images of nanofibers produced from 7.5% PVP solution.*

3.2.2. Effect of the AgNPs Content of Electrospinning Solution on Nanofiber Structure

SEM images of the AgNPs containing nanofibers produced from the electrospinning solutions having the composition as indicated in Table 2 were given in Fig. 8.



(a) 281 ± 136 nm
(b) 328 ± 152 nm
(c) 855 ± 529 nm
(d) 765 ± 457 nm

Figure 8. *SEM images of AgNPs containing nanofibers produced from electrospinning solutions with compositions of (a) 1% L-PVP + 9% H-PVP + 1% $AgNO_3$, (b) 5% L-PVP + 5% H-PVP + 1% $AgNO_3$, (c) 1% L-PVP + 19% H-PVP + 0.5% $AgNO_3$, (d) 10% L-PVP + 10% H-PVP + 1% $AgNO_3$.*

Nanofibers produced from 1% L-PVP + 9% H-PVP + 1% $AgNO_3$ solution have more smooth, long and isolated form (Fig. 8a) as compared with the nanofibers produced from 1% L-PVP + 9% H-PVP solution (Fig. 6a), but size distribution became larger while mean diameter decreasing. On the other hand, bead-on string structure of nanofibers produced from 5% L-PVP + 5% H-PVP + 1% $AgNO_3$ solution retained this structure but showing heterogeneity despite having similar mean diameter as seen in Fig. 8b. Similarly, existence of AgNPs reduced the diameter of nanofibers produced from 10% PVP solutions and increased their heterogeneity. Additionally, SEM images of AgNPs containing nanofibers produced from 7.5% PVP solution were given in Fig. 9. As expected, the mean diameter decreased from 605 ± 137 nm to 550 ± 212 nm with widened size distribution by the addition of AgNPs.

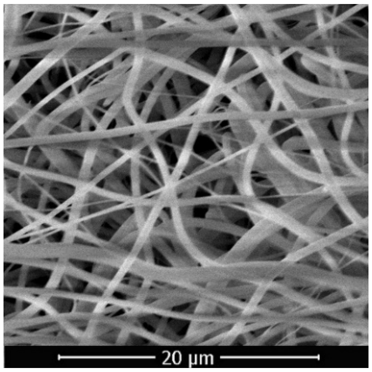


Figure 9. *SEM images of AgNPs containing nanofibers produced from 7.5% PVP solution.*

The changes observed in the diameter and size distribution of nanofibers containing AgNPs can be attributed to increase in the conductivity of the electrospinning solutions by AgNPs addition. Conductivity values of different electrospinning solutions were measured with Multiparameter (Mettler Toledo S470 SevenExcellence) and the results were given in Table 5. As can be seen from Table 5, the conductivity values are found higher for electrospinning containing AgNPs. Hwang and Jeong was reported that AgNPs are more strongly affected by the electrical field produced during the electrospinning process than the polymer and hence the polymer is drawn and stretched by AgNPs resulting in formation of thinner nanofibers [33].

Table 5. *Conductivity values of different electrospinning solutions.*

Total PVP conc. % (w/v)	Concentration % (w/v) of the solutions		[a]Amount of $AgNO_3$ (%)	Conductivity (µS/cm)
	L-PVP	H-PVP		
5	5	5	1	53.2
	5	5	0	10.6
7.5	7.5	7.5	1	58.1
	7.5	7.5	0	12.2
10	10	10	1	61.3
	10	10	0	13.9

[a] With respect to the total PVP

3.2.3. Effect of Thermal Treatment

PVP nanofibers can be crosslinked by photo-crosslinking process via UV-irradiation [33, 45] or thermal treatment between 150 and 200 °C [46]. In the present study, nanofibers were heated at 150, 175 and 200 °C to determine the optimum thermal treatment temperature. Then, the thermal treatment efficiency was determined by observing the solubility of nanofibers, which were cut into squares of 1 cm^2, in deionize water for 24 h.

Firstly, AgNPs containing and neat nanofibers produced from 1% L-PVP + 19% H-PVP solutions were heated at 150 °C for 3 h according to the procedure described of Wang et al. [32]. After immersing of these nanofiber mats on deionized water for 24 h, it was observed that while neat nanofiber mat dissolved, AgNPs containing nanofiber mat generally maintained its structure but tended to disintegration. Since the thermal treatment applied at 150 °C for 3 h was not adequate to crosslink of the nanofiber mats, thermal treatment procedure reported by Jin et al. [47] was applied for both 175 °C and 200 °C temperatures. Weight losses determined for the thermally treated nanofiber mats after contact with deionized water for 24 h were given in Table 6. According to these results, when temperature increased from 175 °C to 200 °C, weight losses of AgNPs containing nanofiber mats reduced proportionally with the increasing PVP concentration. If we compare the weight loss values of AgNPs containing and neat nanofibers produced from 10% PVP solutions, it was found that AgNPs containing nanofibers are more stable than neat nanofibers. Thus, it can be concluded that the addition of AgNPs in nanofiber structure makes crosslinking process easy.

Table 6. *Weight losses of thermally treated nanofiber mats after contact with deionized water for 24 h.*

Total PVP conc. % (w/v)	Concentration % (w/v) of the solutions		[a]Amount of $AgNO_3$ (%)	Weight loss (%)	
	L-PVP	H-PVP		175 °C	200 °C
5	1	9	1	55.37	7.33
5	5	5	1	29.55	17.14
10	10	10	1	9.79	9.65
10	10	10	0	41.54	15.87

[a] With respect to the total PVP

Based on these results, AgNPs containing and neat nanofibers produced from 7.5% PVP solutions were thermally treated at 200 °C and SEM images of these nanofibers were shown in Fig. 10. Mean diameter of neat nanofibers was measured as 605 ± 137 nm and 618 ± 109 nm before and after thermal treatment, respectively. On the other hand mean diameter of AgNPs containing nanofibers rose from 550 ± 212 nm to 606 ± 231 nm after thermal treatment. These slight changes occurred in fiber diameter are in the range of standard deviations of the as-spun nanofibers. It shows that thermal treatment has negligible effect on morphology as well as fiber diameter. Meanwhile, weight losses of these neat and AgNPs containing nanofibers were found as 22% and 12%, respectively.

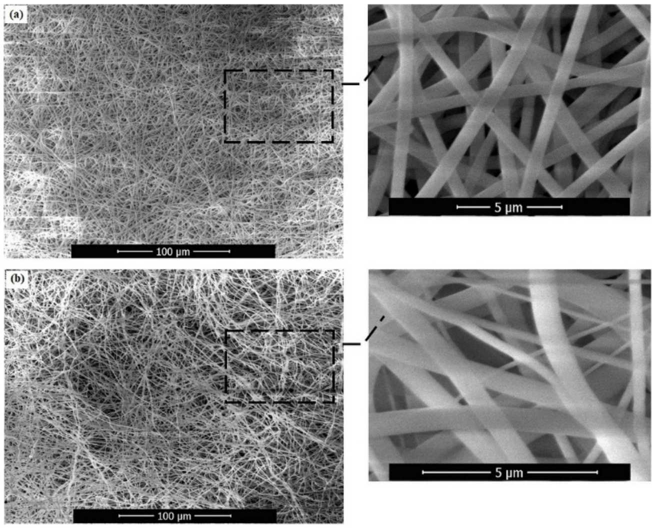


Figure 10. *(a) neat, (b) AgNPs containing nanofibers produced from 7.5% PVP solution after thermal treatment at 200 °C for 2 h.*

3.2.4. XRD Analysis

XRD diffraction analysis of the AgNPs containing nanofibers produced from 7.5% PVP solutions before and after thermal treatment at 200 °C for 2 h was given in Fig. 11. For both fiber mats peaks were observed at 2θ values of around 44.5°, 64.5° and 85° which were associated with (200), (220) and (222) planes of face centered cubic form of Ag [32]. Similarly, Fransis et al. synthesized silver nanoparticles in the size of about 5 nm which is obtained from a peak at (200) plane in the XRD pattern and it was further confirmed with TEM analysis [48].

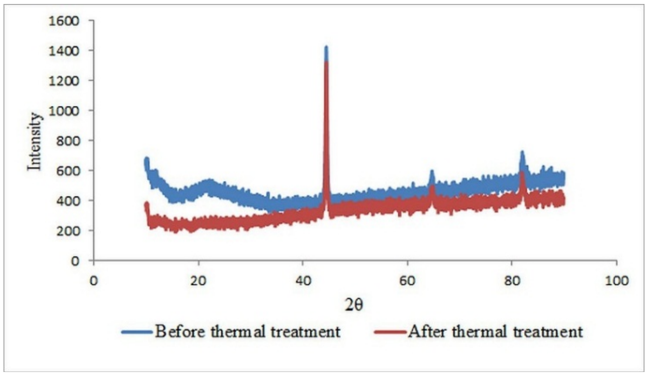


Figure 11. *XRD patterns of AgNPs containing nanofibers produced from 7.5% PVP solutions before and after thermal treatment.*

3.3. *Antimicrobial Activity*

Although silver and silver compounds have been used for antimicrobial purposes since ancient times, the mechanism of the action of silver has not been clarified exactly [49-51]. However, antimicrobial effects of silver ions are based on the interaction between metal ions and electron donor functional groups (such as thiol, carboxylate, phosphate, hydroxyl, amines etc.), which results in binding of silver ions with proteins and nucleic acids [16, 52]. Most commonly emphasized mechanisms of silver nanoparticles to bacteria are comprise of (1) release of free silver ions gradually cause to damage in ATP production and DNA replication, (2) reactive oxygen species generation from both silver ions and nanoparticles and (3) direct cell membrane damage by silver nanoparticles [16, 17].

Antimicrobial properties of neat and AgNPs containing thermal treated nanofibers produced from 7.5% PVP solutions were investigated against *E. coli*, *S. aureus* and *A. niger* and the results were demonstrated in Fig. 12. As can be seen from Fig. 12, while neat nanofiber mats allowed microbial growth on their surfaces for all types (Fig. 12a-c); AgNPs containing nanofiber mats inhibited the *E. coli* and *S. aureus* growth by forming small zones of inhibition around their surroundings (Fig. 12d-e). Nevertheless, no antifungal activity was observed against *A. niger*.

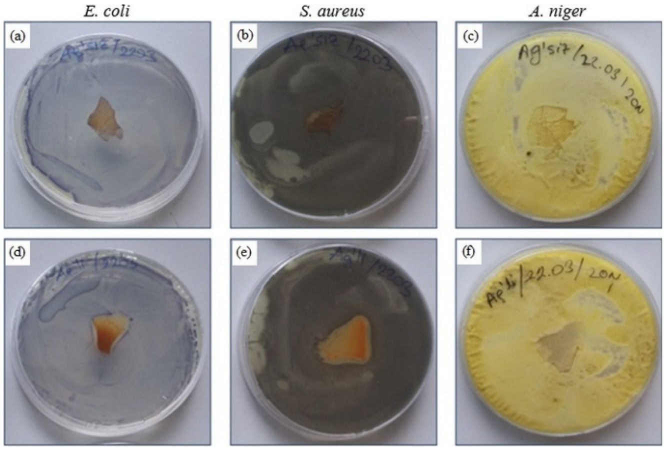


Figure 12. *Zone of inhibition test against E. coli, S. aureus and A. niger. (a-c) neat nanofibers, (d-f) AgNPs containing nanofibers.*

4. Conclusion

In the line of the aim that preparation of antibacterial polymeric nanofibers for biomedical applications, PVP nanofibers containing AgNPs were successfully produced by electrospinning method. To reduction of Ag^+ ions to Ag^0 atoms, microwave treatment was applied for 1% L-PVP solutions; any microwave treatment was not necessary to produce AgNPs for 5%, 7.5% and 10% L-PVP solutions because of increased reaction rate by increasing L-PVP amount in the solution. Microwave treatment at 180 W was not sufficient to reduce the Ag^+ ions in 1% L-PVP solutions. DLS results showed that all the AgNPs solutions have an average particles size ranging from 5.69 nm to 14.83 nm together with a trend as decreasing size by increasing the L-PVP concentration to a certain point. The UV spectrum of the AgNP solution in 7.5% L-PVP solution confirmed the AgNPs formation clearly.

H-PVP was used to obtain required solution viscosity for the electrospinning process to form a densely accumulated nanofiber mats. SEM images showed that increase in polymer concentration and H-PVP ratio in the electrospinning solution lead to increase in nanofiber diameters. Based on these results, optimum amount of total PVP was determined as 7.5% PVP solution prepared by mixing L-PVP and H-PVP solutions with the same concentration. Existence of AgNPs in the electrospinning solution was resulted a decrease in the nanofiber diameter due to increasing conductivity of electrospinning solution. PVP nanofibers containing AgNPs were effectively crosslinked by thermal treatment at 200 °C. Furthermore, XRD patterns of the nanofibers gave (200), (220) and (222) crystal faces of AgNPs. Disk diffusion test results showed that while neat PVP nanofibers allowed microbial growing on their surface; AgNPs containing nanofibers formed a small zone of inhibition against *E.coli* and *S. aureus*.

However, both neat and AgNPs containing nanofibers showed no antifungal effect.

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