

Antibacterial Poly(vinyl Alcohol) Film Containing Silver Nanoparticles: Preparation and Characterization

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Received 19 February 2008; accepted 30 June 2008

DOI 10.1002/app.28908

Published online 8 September 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The importance of antibacterial materials for biomedical applications is growing nowadays. The presented article deals with the characterization of structural, mechanical and thermal properties and of antibacterial polymeric films based on polyvinyl alcohol (PVA) and silver nitrate, which can find their applicability in wound dressing components and protective coating. The methods of transmission electron microscopy, UV-vis and XRD spectroscopy, optical microscopy, differential scanning calorimetry, stress-strain analysis, and agar diffusion test were used to characterize the polymer films prepared. The results showed strong antibacterial activity against *Escherichia coli*

and *Staphylococcus aureus* already at the lowest addition level of silver nitrate. An improvement of mechanical properties (Young's modulus) was also noticed due to a modification of PVA with silver nitrate up to 1 wt. % of silver content. Furthermore, the results show a strong effect of the thermal history of the sample preparation on the degree of silver-ion reduction and formation of nanoparticles. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 110: 3178–3185, 2008

Key words: polyvinyl alcohol; silver nitrate; antibacterial; polymer film; TEM; DSC; UV-vis; mechanical properties; nanoparticles

INTRODUCTION

There has recently been a growing interest in developing antibacterial medical polymer materials. The reason for this attempt is the effort to reduce health complications caused by bacteria commonly found on various types of medical equipment.¹ As the most types of the commonly applied polymers have no antibacterial action, they have to be modified to obtain polymer materials with the desired properties.²

The modification of virgin polymer with a bioactive agent is a possible method. In this case, the polymer is a carrier, providing transport and controlled release of bioactive substances into the environment where they are needed.^{3,4} Numerous methods have been used to develop antibacterial

polymer materials.⁵ Besides various agents such as chemicals with antibacterial effects and antibiotics, silver and its compounds can be applied to medical polymer devices. Silver compounds have been known as disinfectants for many years and have a broad spectrum of antibacterial activity.⁶ Their antibacterial activity can already be noticed at low concentrations. The mechanism of antibacterial action of silver are not clearly understood yet. However, some papers deal with the mechanisms of inhibitory action of silver on silver ions (Ag^+). They can interact with bacterial DNA, which loses its replication ability.⁷ It has also been reported that Ag^+ affects the function of membrane-bound enzymes, such as those in the respiratory chain.^{8–10}

Silver nitrate (AgNO_3) is one of the sources of silver ions. It is soluble in water and because of its light sensitivity, it is used in manufacturing of photographic films, mirror silvering, hair dyeing, silver plating as well as in medicine as cauterizing and antiseptic. As all water soluble silver salts, it tends to be very toxic to bacteria and other lower life forms.¹¹ C.A. Moyer revived interest in silver nitrate solution in 1965 already. On the basis of *in vitro* and *in vivo* studies, he concluded that a 0.5% solution represented the lowest concentration at which

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Contract grant sponsor: Ministry of Industry and Trade of the Czech Republic; contract grant number: 2A-ITP1/126.

Contract grant sponsor: Ministry of Education, Youth, and Sports of the Czech Republic; contract grant number: MSM6198959223.

antibacterial activity (against *Staphylococcus aureus*, *Hemolitic streptococci* and, in general, against *Pseudomonas aeruginosa* and *Escherichia coli*) was displayed. He and his coworkers used silver nitrate solutions for all kinds of burn therapies as a wound dressing.¹²

Poly(vinyl alcohol) (PVA) belongs to the group of polymers which can be used in combination with silver nitrate. PVA is one of the synthetic, biodegradable, biocompatible, water-soluble polymers utilized in medical applications such as wound dressings, artificial skin, coatings, transdermal patches, cardiovascular devices, and drug delivery systems. Moreover, it has good barrier properties against scents, oils, and fats. The physical characteristics of PVA are dependent on its method of preparation by hydrolysis or partial hydrolysis of poly(vinyl acetate).^{13–18}

The combination of PVA/AgNO₃ has been reported to be a suitable material for preparation of antibacterial nanofibers. The authors prove the nanofibers prepared to have strong antibacterial activity against *S. aureus* and *Klebsiella pneumoniae*.¹⁹ Other references mostly mention the electrical characteristics of this material.¹¹ That encouraged us to develop a new antibacterial polymer film based on PVA and AgNO₃ which can find applicability in the area of wound dressings and protective packaging as well as in coatings for medical items.

This article is focused on preparation of PVA/AgNO₃ films and on characterization of their structure, mechanical and thermal properties, and, last but not least, on their antibacterial activity against selected pathogenic bacterial species.

EXPERIMENTAL

Materials

Poly(vinyl alcohol) PVA 8-88 ($M_w \sim 67,000$, degree of hydrolysis 86.7–88.7 mol %); the chemicals required for microbiological studies and silver nitrate AgNO₃ (analytical grade) were obtained from Fluka, Sigma Aldrich, and Penta (Czech Republic) respectively. The bacterial species of *S. aureus* (3953) and *E. coli* (3954) were purchased from CCM Brno, Czech Republic.

Sample preparation

Samples of PVA with various concentrations of silver nitrate were prepared by using the solvent cast technique in the following way: Aqueous polymer solution (10 wt % solution of PVA in distilled water) was prepared by dissolving polymer granules in water at the temperature of 80°C for 20 min under continuous stirring. After dissolving the PVA, a rele-

TABLE I
Compositions of the Samples

Sample index	Concentration of AgNO ₃ (wt %)	Calculated content of Ag (wt %)
PVA	0	0
PVA/Ag 0.5	0.78	0.5
PVA/Ag 1	1.57	1
PVA/Ag 3	4.72	3
PVA/Ag 5	7.87	5
PVA/Ag 7	11.02	7
PVA/Ag 9	14.17	9

vant portion of silver nitrate was added (see Table I) to the polymer solution and stirring of this mixture continued for another 30 min. Then samples were poured into an acrylic mold and dried at the temperature of 35°C for 48 h in a temperature controlled incubator. The thickness of the final product was about 200 μm.

Methods

Water content determination

The water content of polymer films of pure PVA and PVA modified with silver nitrate was determined by the gravimetric method. The initial weight (W_i) of the samples was determined before drying (dimensions of 25 × 50 mm before drying). The drying was carried out in a vacuum drying chamber at 60°C and 30 kPa until reaching constant weight (W_d). The water content (W) was then calculated through eq. (1):

$$W(\%) = \frac{W_d - W_i}{W_i} \times 100 \quad (1)$$

Static tensile measurements

The mechanical properties (Young's modulus, tensile strength, and strain at break) of the samples were investigated using a tensile testing machine Instron 8871 at 25°C and at 40% relative humidity. The samples had an initial length of 40 mm, width of 15 mm, and thickness of about 150–200 μm. The speed of moving clamp was 50 mm min⁻¹. The specimens conditioned in a dessicator containing silica gel were studied in each case.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was used to assess the thermal properties of the investigated materials. A small piece of sample (~ 4 mg) was placed in a steel pan and analyzed on a Perkin–Elmer Pyris 1 DSC, calibrated in temperature and heat flow using indium. The experiments were performed according to the following program:

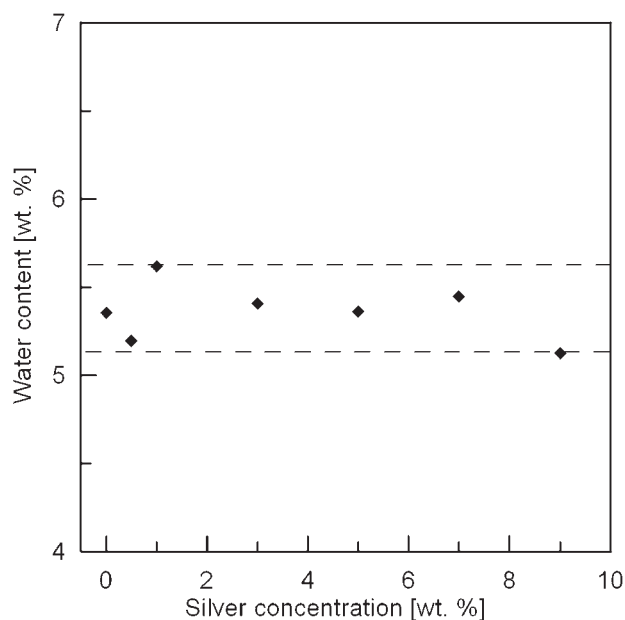


Figure 1 Water content of PVA and PVA/silver nitrate polymer films as a function of recalculated silver content.

- initial temperature of 0°C
- first heating cycle, heating to 150°C (30°C min⁻¹)
- holding at 150°C for 1 min
- cooling to 0°C (30°C min⁻¹)
- holding at 0°C for 1 min
- second heating cycle, heating to 220°C (20°C min⁻¹)

The value of T_g was taken in the second heating scan at the midpoint of a stepwise increase in specific heat associated with the glass transition.

Antibacterial properties

The antibacterial properties of the poly(vinyl alcohol) /silver nitrate-based films were assessed by using the agar diffusion test.²⁰ A piece of sample (8 mm in diameter) was placed on the surface of an individual nutrient agar plate, where bacterial solutions (4×10^8 CFU mL⁻¹) of the microorganisms chosen (*E. coli* 3954 and *S. aureus* 3953) had been swabbed uniformly. After 24 h' incubation at 37°C, the dimensions of the inhibition zones around the samples were measured in five directions, and the average values were used to calculate the circle zone area.

Optical microscopy

The heterogeneous nature of PVA and its composites with CL was investigated by using the optical microscope OPTECH Mod. B4. Polymer-modifier interactions had been made visible by staining the samples with methyl orange before taking photos of the sam-

ples with a digital camera NIKON Coolpix E8400. The images have been converted into black and white using the Microsoft Photo Editor 3.0.2.3.

Spectroscopic measurements

To characterize the structure of the samples, UV-visible absorption and X-ray diffraction (XRD) spectroscopy were used. UV-vis spectra were taken by using a fiber optic UV-vis spectrometer, namely the AvaSpec 2048 (Avantes), in the transmission mode. The absorbance of the PVA/Ag 7 and 9 films was too large, and therefore, the UV-vis absorption spectra were not taken in these materials. XRD patterns of the films were obtained using an HZG 4 diffractometer (Freiberg Präzisionsmechanik) with a back monochromator and a Cu anticathode (step 0.1°).

TEM analysis

A small piece of material was embedded in epoxy resin Durcupan (ACM) (Fluka) and polymerized at 65°C. Ultra-thin sections of about 80 nm were made on an ultramicrotome Ultracut (Leitz) with a diamond knife. Ultra-thin sections were fixed to EM grids without a support film to eliminate contaminations. Samples were examined under transmission electron microscope JEM 2010 (JEOL, Japan) at acceleration voltage of 160 kV and with a digital camera Keen View.

RESULTS AND DISCUSSION

PVA is known to have a very strong affinity for water. Therefore, this property cannot be omitted from this study because, for example, the storage conditions for the samples can have a crucial effect on the mechanical properties observed. For this reason, the samples were stored inside dessicator containing silica gel and their water content was determined before further investigation. The water content of the samples against modifier concentration is shown in Figure 1. As can be seen, it varies within 1%. This fact proves that the factor of water sensitivity of materials has equal effects on the data presented below.

The influence of silver nitrate (AgNO₃) on mechanical properties of the polymeric films prepared was assessed from the viewpoint of Young's modulus, E (Fig. 2), tensile strength (Fig. 3), and tensile strain (Fig. 4) changes which had been observed due to an increasing content of AgNO₃ in the systems. As shown in Figure 2, the value of E modulus increases up to 1 wt % of Ag content in the sample (PVA/Ag 1). At this concentration of modifier, the incremental value amounts to more than 10% in comparison with that of virgin PVA film. Further

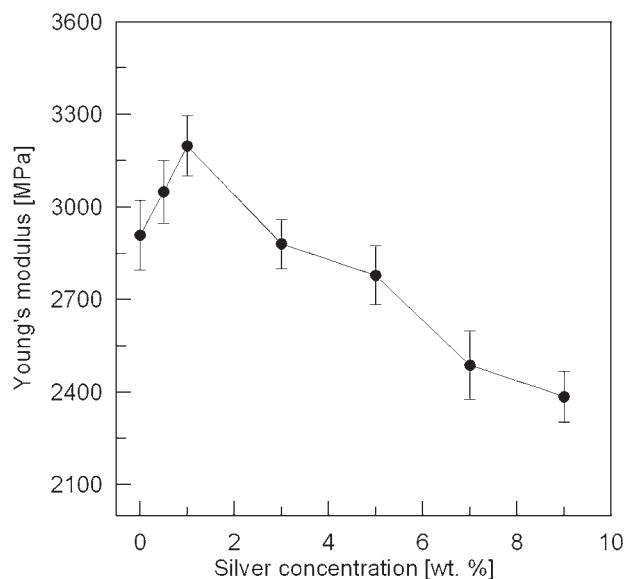


Figure 2 Young's modulus versus recalculated silver content.

additions of AgNO_3 cause a slight decrease in value, and that from 3200 MPa (PVA/Ag 3) to 2400 MPa (PVA/Ag 9). A similar trend was observed in case of the tensile strength course (Fig. 3). However, its increase was solely detected in the sample containing the lowest investigated modifier concentration (PVA/Ag 0.5). A higher level of modification results in a gradual decrease; the same occurs in the previous case. The tensile strength of PVA/Ag 9 is lower than 60 MPa, which corresponds to a drop of approximately 25% in comparison with unmodified PVA. These results are in accordance with the maxi-

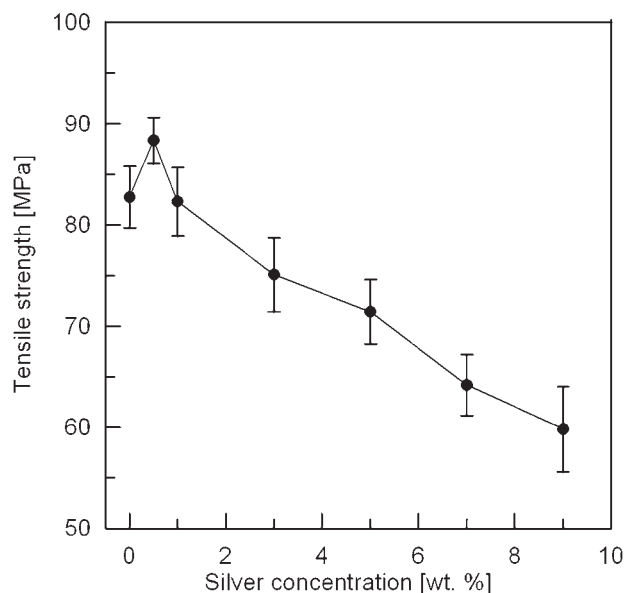


Figure 3 Tensile strength versus recalculated silver content.

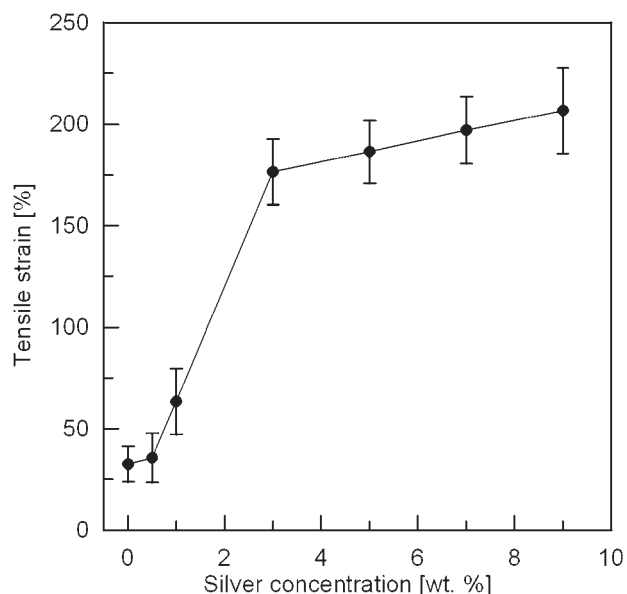


Figure 4 Tensile strain versus recalculated silver content.

mal elongations of the films prepared as depicted in Figure 4. The tensile strain of the samples is more or less constant in PVA/Ag 0 and PVA/Ag 0.5 and it is slightly raised in PVA/Ag 1. It corresponds to the increased toughness that is also revealed through the values of E modulus and tensile strength at the same concentrations. Nevertheless, a significant elevation of the tensile strain appeared starting from 3 wt % of Ag. Although the maximal elongation of unmodified PVA is 32%, PVA/Ag 9 proved to have more than 200%. These results reveal the transformations of polymer chains' physical arrangement occurred in consequence of the presence of modifier (changes of pH, ionic strength).²¹

The interactions between the polymer matrix and the modifier can be clarified through studies of thermal behavior of the samples. The differential scanning calorimetry method was used in this case to determine the values of glass transition temperature (T_g), structural properties, and thermal stability of PVA and of AgNO_3 -based polymer films. Figure 5 shows the changes of T_g as a function of AgNO_3 content. The course is similar to those in Figures 2 and 3, that is, there is a slight increase up to 1 wt % Ag (from 68.9°C, PVA to 69.7°C, PVA/Ag 1) and a gradual decrease (to 61.5°C, PVA/Ag 9). This fact is in full agreement with the data obtained from stress-strain analysis and it supports the assumption of strengthening at low contents of AgNO_3 and softening at higher concentrations.

The thermal behavior of polymer films, in the form of DSC curves, is shown in Figure 6. The weak melting peak of pure PVA was recorded at 190°C. The intensity of this peak is connected with a relatively low crystallinity of the polymer matrix, PVA.

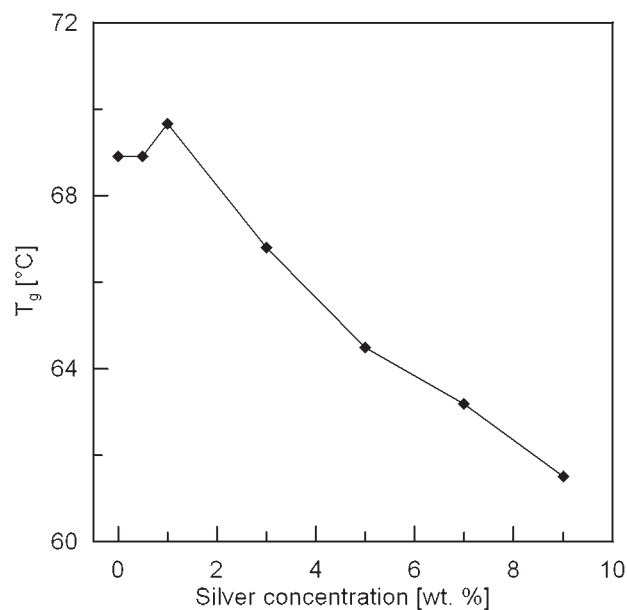


Figure 5 Glass transition temperature of PVA and PVA/silver nitrate polymer films.

However, as the concentration of AgNO_3 increased, the exothermic peak became dominant in the same temperature region. Finally, the melting peak of PVA was overlaid by this very strong peak, which could indicate that a chemical reaction of the present modifier has occurred within the melted polymer matrix. A straight line was observed in the third scan. All samples had a silver shining mirror-like surface after finishing the DSC scans. These facts could indicate that a thermally induced reduction of Ag^+ takes place, caused by organic components or

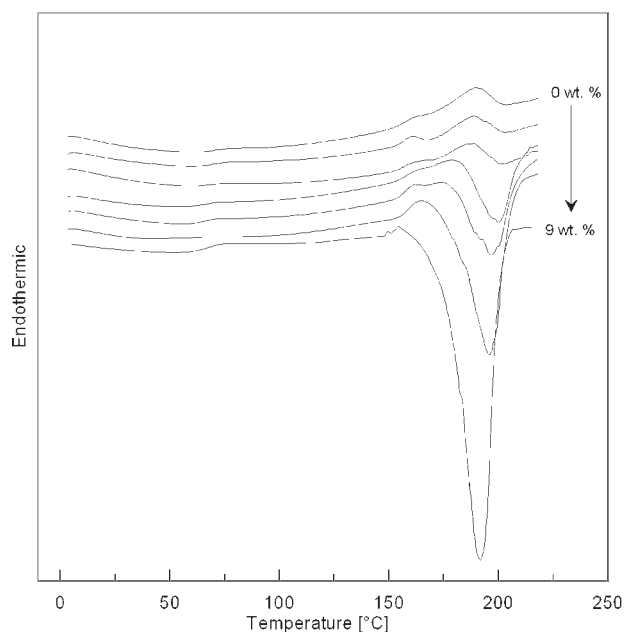


Figure 6 DSC curves of pure PVA and of PVA modified with silver nitrate (second heating scan).

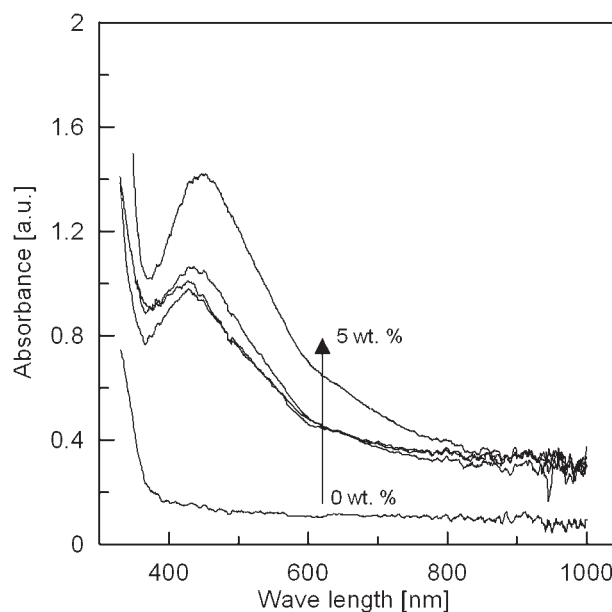


Figure 7 UV-vis spectra of PVA and PVA/silver nitrate films.

possible crystallization of amorphous silver nanoparticles formed during the film preparation.¹¹

The presence of Ag nanoparticles was confirmed by UV-vis spectra of the samples (Fig. 7) where a strong characteristic absorption peak was located at around 435 nm. The peak maximum occurs at 430 nm in the PVA/Ag 0.5 sample and shifts with increasing silver concentration to 450 nm in the PVA/Ag 5. The transparency of higher concentrated samples was too low to be investigated in the transmission mode. The absorption peak has an asymmetric shape with a broad shoulder in the range from 500 to 700 nm which may indicate the presence of nanoparticles with a nonspherical shape. The increase in optical density of the spectra baseline from 1.1 to 3.2 cm^{-1} may be ascribed to an increased reflectivity of the sample surface or of any other induced surface phenomena rather than to the increase in light dispersion in the sample volume due to heterogeneity, as the baseline position does not show any dependence on silver concentration.

TEM images (Fig. 8) show the presence of ovoid nanoparticles of various morphologies. Single nanoparticles appeared in size of about 50–100 nm [Fig. 8(a)]. A cluster of several nanoparticles with clearly observable grain boundaries can be seen in Figure 8(b) showing a step-by-step growth of the particle. The Figure 8(c) demonstrates a bunch of particles, more clearly shown when exemplified through a large agglomerate of particles shaped like a bunch of grapes composed of nanoparticles varying in size from 20 to 100 nm in Figure 8(d). Every single particle, cluster, or multimer bunch exists as an isolated island in the polymer matrix. The occurrence

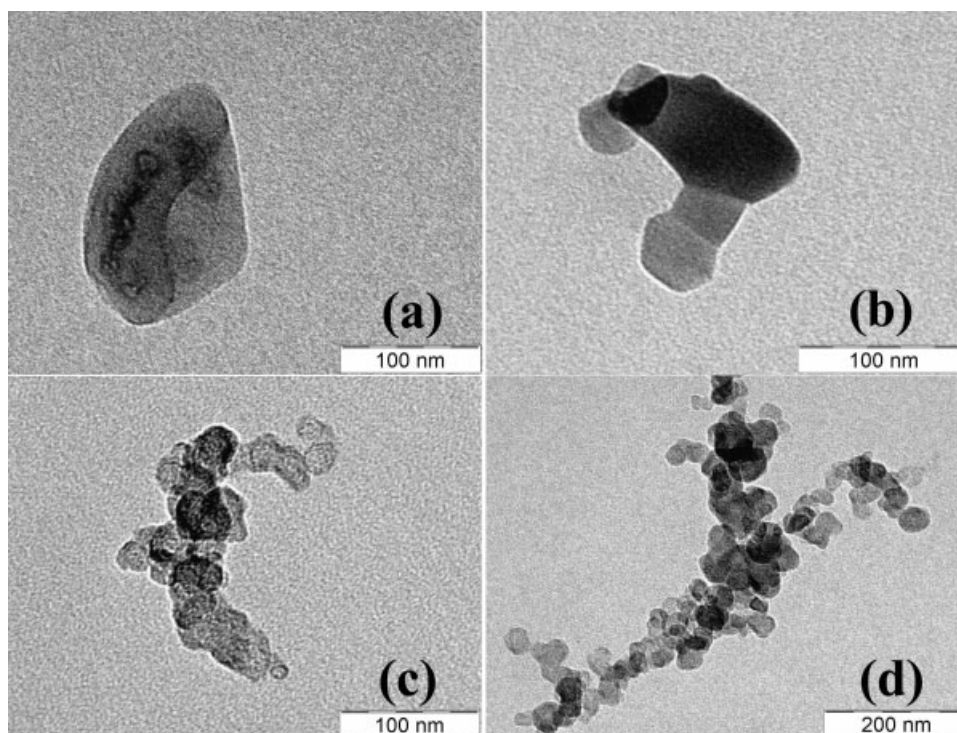


Figure 8 TEM of silver nanoparticles within PVA matrix.

of a Ag crystalline phase was ruled out according to the results obtained from the XRD analysis (Fig. 9), where no peak typical of Ag was observed. Khanna et al. have reported similar results regarding the systems containing silver nanoparticles with dimensions of about 30–50 nm.²² The peak of PVA crystals appeared at $20^\circ 2\theta$ only. Its intensity is reduced due to a decreasing RTG-transparency of the samples. The position of the UV–vis absorption maximum and the amorphous phase structure of observed nanoparticles are comparable with the features observed in silver nanocolloids prepared from ethanol solutions.²³

Possibilities of further thermal modification of prepared films (due to rest of Ag^+ ions) were examined through heat treatment. Specimens of 2 cm^2 in size, cut from conditioned samples were placed into an oven and annealed at 220°C in air atmosphere for 2 h. Optical micrographs of the materials taken before and after thermal treatment are shown in Figure 10. In case of nontreated samples, no visible response was found. On the other hand, needle-like structures (PVA/Ag 0.5) and even large plates of Ag crystals (PVA/Ag 1) can be seen in the optical micrographs of annealed samples containing AgNO_3 . A uniform silver shining film with a mirror-like reflectance from its surface was obtained for the samples containing higher amounts of modifier. This proves the presence of metallic Ag in crystal form after annealing and thus a thermally mediated metallic Ag formation in the polymer matrix.

The inhibition of bacterial growth occurs in all cases where AgNO_3 is present. It is confirmed by circular inhibition zones which appeared due to diffusion of silver ions (Ag^+) when an antimicrobial ingredient had been released from the sample into the surrounding medium in the presence of both *E. coli* and *S. aureus*. On the other hand, pure PVA does not have any antibacterial action. The dependence of the observed inhibition areas on the modifier concentration in polymer films are shown in Figure 11.

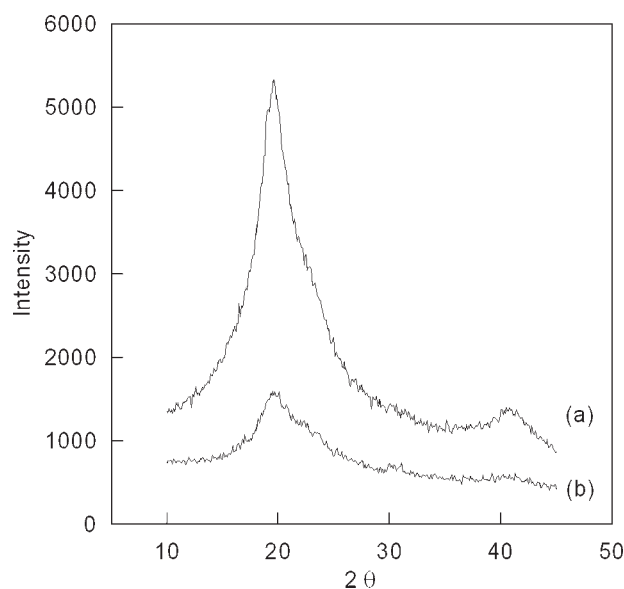


Figure 9 XRD spectra of PVA (a) and PVA/Ag9 (b).

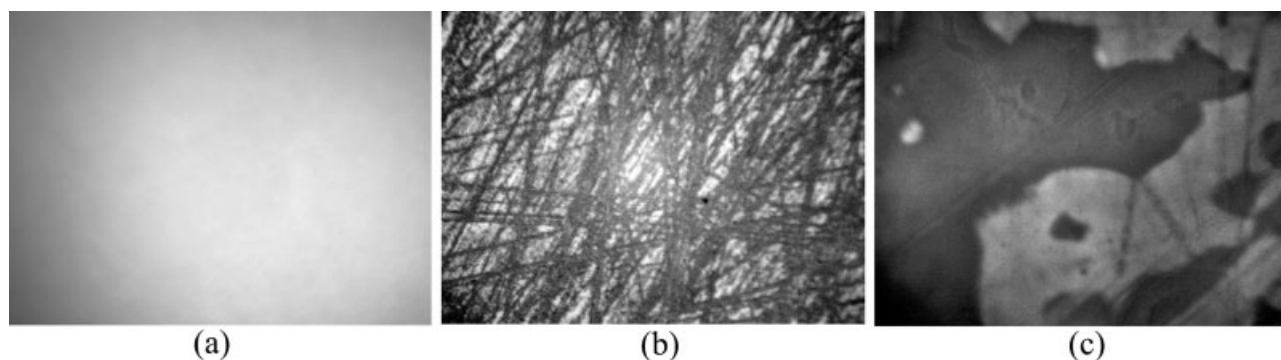


Figure 10 Optical micrographs of annealed films: pure PVA (a), PVA/Ag0.5 (b), and PVA/Ag1 (c).

In case of the former bacterial strain tested, *E. coli*, the antibacterial activity is significantly more intensive when compared with the latter one showing a concentration of Ag higher than 3 wt %. In this concentration range, the sensitivity of *E. coli* to Ag^+ seems to be doubled in comparison to *S. aureus*. This is in disagreement with the results published by Xu et al., who describe approximately the same antibacterial activity of polylactic acid and AgNO_3 -based nanofibers.²⁴ The discrepancies may be ascribed to the differences in the matrix used as well as to the methodology for evaluation of antibacterial action. Nevertheless, the antibacterial activity against both bacterial strains investigated is more or less constantly identical at low Ag concentrations. This could indicate the possible limits of the method used for the assessment of antibacterial activity. The distribution of Ag^+ through the solid agar medium can be restricted due to their low concentration. In

spite of that, the antibacterial effect found even at these low concentrations (below 3 wt % Ag) can be regarded as sufficient for certain applications.

CONCLUSIONS

This work was aimed at the preparation and characterization of the structure, mechanical, and thermal properties of antibacterial polymer films based on PVA and silver nitrate. The results reveal a formation of nanoparticles within the polymer matrix which occurs due to the partial reduction of the present silver ions. This assumption was confirmed by DSC measurements and additional investigation by using XRD and UV-vis spectroscopy and optical microscopy. The level of silver reduction depends on the thermal history of the sample.

The mechanical properties of the polymer films are enhanced up to 1 wt % (in case of Young's modulus) and 0.5 wt % (in case of tensile strength) of the recalculated silver content. We suppose that the modification of the PVA matrix affects the physical arrangements and it increases the ductility of the systems. The DSC measurements are in accordance with these results.

The investigated polymer nanocomposites containing PVA-based films demonstrated strong antibacterial activity against *E. coli* and *S. aureus* starting from the lowest level of addition of silver nitrate already and increasing with the modifier content. This fact, together with the result indicated above, may enable these materials properties to be adjusted for their potential use in selected practical applications mentioned above.

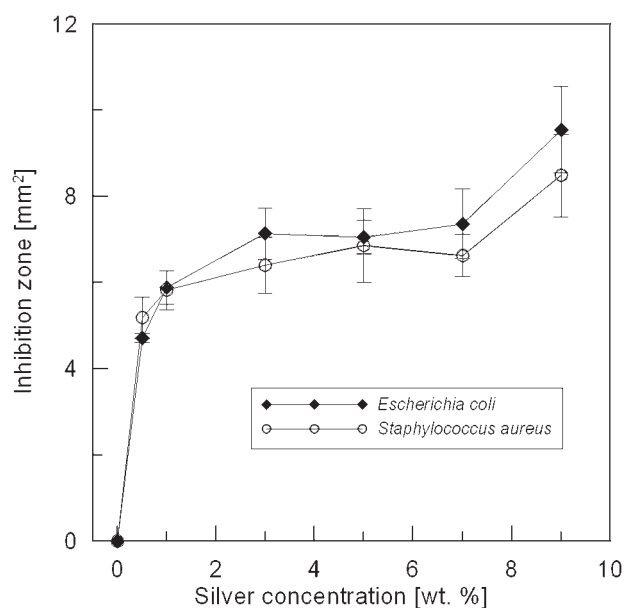


Figure 11 Antimicrobial activity of PVA/silver nitrate polymeric films against *Escherichia coli* and *Staphylococcus aureus*.

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