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### Characterization of Antibacterial Polymeric Films Based on Poly(vinyl alcohol) and Zinc Nitrate for Biomedical Applications

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## Characterization of Antibacterial Polymeric Films Based on Poly(vinyl alcohol) and Zinc Nitrate for Biomedical Applications

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**Abstract:** Poly(vinyl alcohol) (PVA) was modified by zinc nitrate in the concentration range from 0 to 22.29 wt.% using the solvent cast technique. The resulting material was characterized by optical microscopy, stress-strain analysis, differential scanning calorimetry, UV-visible spectroscopy, and Fourier transform-infrared spectroscopy. In addition, the antibacterial properties of the prepared films were tested against the both gram positive and gram negative bacterial strains. The results show the uniform distribution of the modifier within the PVA matrix, enhanced mechanical properties (up to 8.24 wt.%), and good antibacterial activity against gram negative bacteria at low modifier content.

**Keywords:** Antibacterial; DSC; FT-IR; Mechanical properties; Poly(vinyl alcohol); UV-vis spectroscopy; Zinc nitrate

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## INTRODUCTION

Antibacterial polymeric systems have been widely investigated due to the damage caused by microbial pollution, which has produced many problems in industry and medicine such as degradation of materials, infections, and inadmissible changes in properties of the exposed items.<sup>[1,2]</sup> However, commonly used polymers do not have this ability. One of the possibilities for making a polymer antibacterial is its modification with an antibacterial agent to ensure desired properties. Some other characteristics, such as mechanical or thermal properties, can be also improved by to this modification.<sup>[3,4]</sup> Generally, there are two types of antibacterial agents, organic and inorganic. Organic antibacterial agents are represented by antibiotics and complex organic compounds. Inorganic modifiers, such as metallic powders and metallic salts and oxides of Ag, Cu, and Ti, have been investigated.<sup>[5-7]</sup> The latter provide the key advantage of improved safety and stability, in comparison to organic compounds.<sup>[8]</sup>

Zinc belongs into the group of metals whose ions improve antibacterial activity. Despite the fact that Zn (II) ion concentrations of  $10^{-5}$ – $10^{-7}$  M are required for optimal bacterial growth of most microorganisms in vitro, it has been found that higher content of Zn (II) has antibacterial properties.<sup>[9]</sup> Zinc nitrate is one of the most frequently reported zinc-based antibacterial agents. Its antibacterial activity is considered to be due to the generation of hydrogen peroxide from its surface.<sup>[10]</sup> Among other zinc compounds that have been investigated for the preparation of antibacterial polymeric materials are zinc acetate,<sup>[11]</sup> zinc oxide,<sup>[12]</sup> and zinc sulfate.<sup>[13]</sup>

Zinc nitrate is used as a mordant in dyeing and as the source of Zn (II) ions in organic chemistry.<sup>[14]</sup> Actually, zinc nitrate has not been studied in detail for the preparation of antibacterial polymeric materials. There are some references to the investigation of this compound as a precursor for zinc oxide production, e.g., Palms et al.<sup>[15]</sup> reported work describing the electrochemically induced growth of zinc oxide. These facts encouraged us to study zinc nitrate-based polymeric systems. Poly (vinyl alcohol) was selected as the polymer matrix.

Poly(vinyl alcohol) (PVA) is one of the commercially available synthetic polymers commonly accepted as biodegradable and biocompatible material. It is water-soluble and employed in several practical fields such as biodegradable packaging and surfactants and in medicine because of its easy preparation, good chemical resistance, and outstanding barrier properties to scents, oils, and fats.<sup>[16-18]</sup> PVA has been studied as a matrix for the preparation of antibacterial films or fibers, mostly with potential applicability in medicine as well as in agriculture after modification with silver nanoparticles, zinc oxide, or even lactic acid.<sup>[19-21]</sup>

In this work, we describe the preparation and characterization of a new biodegradable polymeric composite on the basis of PVA and zinc nitrate with potential applicability in the biomedical area. The main attention is paid to the investigation of its mechanical, thermal, and physicochemical properties as well as its structure and antibacterial activity against chosen microorganisms.

## EXPERIMENTAL SECTION

### Materials

Poly(vinyl alcohol) PVA 6–98 ( $M_w \sim 47,000$ , degree of hydrolysis 98 mol%), the chemicals required for microbiological studies, and zinc nitrate hexahydrate (analytical grade) were purchased from Fluka, Sigma Aldrich, and Penta (Czech Republic) respectively. Bacterial species *Staphylococcus aureus* (3953) and *Escherichia coli* (3954) were obtained from Czech Collection of Microorganisms, Masaryk University in Brno, Czech Republic.

### Sample Preparation

Sample of PVA with various concentrations of zinc nitrate were prepared using a 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 polyvinyl alcohol, a relevant portion of zinc nitrate was added (see Table I) to the polymer solution and stirring of this mixture continued for another 30 min. Then the samples were poured into an acrylic mold and dried at 35°C for 48 h in a temperature-controlled incubator. The thickness of the final product was about 200  $\mu\text{m}$ .

**Table I.** Designations and compositions of samples

Sample index	Concentration of $\text{Zn}(\text{NO}_3)_2$ [wt.%]	Calculated content of Zn [wt.%]
PVA	0.00	0
PVA/Zn 0.5	1.42	0.5
PVA/Zn 1	2.82	1
PVA/Zn 3	8.24	3
PVA/Zn 5	13.10	5
PVA/Zn 7	17.86	7
PVA/Zn 9	22.29	9

## Methods

### Optical Microscopy

The mixing nature of the samples was investigated by using the Optech Model B4 optical microscope equipped with a polarized light accessory. The images of the pure zinc nitrate hexahydrate, PVA, and modified polymer films were taken by a Nikon Coolpix E8400 digital camera.

### Mechanical Properties Measurements

To determine the mechanical behavior of the prepared polymer films, stress-strain analysis was used. Mechanical properties (E modulus, tensile strength, and strain at break) of the samples were investigated on an Instron 8871 tensile testing machine, at 25°C and 40% relative humidity. The initial size of the samples was length 40 mm, width 15 mm, and thickness about 150–200  $\mu\text{m}$ . The speed of the moving clamp was 50  $\text{mm}\cdot\text{min}^{-1}$ . Thereafter, the conditioned specimens were studied in a dessicator in each case. The moisture content in the samples was measured before the analysis of mechanical properties by using the gravimetric method described in our previous work.<sup>[17]</sup> The results are shown in Table II.

### Differential Scanning Calorimetry (DSC)

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

**Table II.** Water content in the polymer films determined by using gravimetric method

Sample index	Concentration of $\text{Zn}(\text{NO}_3)_2$ [wt. %]	W [%]
PVA	0.00	$6.185 \pm 0.098$
PVA/Zn 0.5	1.42	$6.298 \pm 0.100$
PVA/Zn 1	2.82	$6.587 \pm 0.146$
PVA/Zn 3	8.24	$6.423 \pm 0.086$
PVA/Zn 5	13.10	$6.290 \pm 0.109$
PVA/Zn 7	17.86	$6.481 \pm 0.106$
PVA/Zn 9	22.29	$6.364 \pm 0.098$

- Initial temperature 0°C
- First heating cycle, heating to 150°C (30°C·min<sup>-1</sup>)
- Holding at 150°C for 1 min
- Cooling to 0°C (30°C·min<sup>-1</sup>)
- Holding at 0°C for 1 min
- Second heating cycle, heating to 220°C (20°C·min<sup>-1</sup>)

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

### Spectroscopic Measurements

To characterize the structure of the samples, UV-visible spectrometry was used. UV-vis spectra of the polymer films were taken using the AvaSpec 2048 (Avantes) fiber optic UV-vis spectrometer in a transmission mode. In addition, the spectra of zinc nitrate hexahydrate in ethanol solution (1 M) were measured to obtain the absorption range of this pure component.

To identify the physicochemical structure of pure PVA and PVA/zinc nitrate, Fourier transform-infrared (FT-IR) spectroscopy analysis was carried out. The experiments were conducted on crystals (pure zinc nitrate) and thin polymer films by a Nicolet 320 FT-IR, equipped with the attenuated total reflectance (ATR) accessory utilizing the Zn-Se crystal and the OMNIC software package over the wavelength range 4000–750 cm<sup>-1</sup> at room temperature. The uniform resolution 2 cm<sup>-1</sup> was maintained in all cases.

### Antibacterial Properties

Antibacterial properties of the poly (vinyl alcohol)/zinc nitrate based films were assessed by an agar diffusion test. A piece of sample (circular shape, diameter 8 mm) was put on the surface of an individual nutrient agar plate, where the bacterial solutions ( $4 \times 10^8$  CFU·ml<sup>-1</sup>) of chosen microorganisms (*Escherichia coli* 3954 and *Staphylococcus aureus* 3953) had been swabbed uniformly. After 24 h of incubation at 37°C, the dimensions of inhibition zones around the samples were measured in five directions and the average values were used to calculate the circle inhibition zone area.

## RESULTS AND DISCUSSION

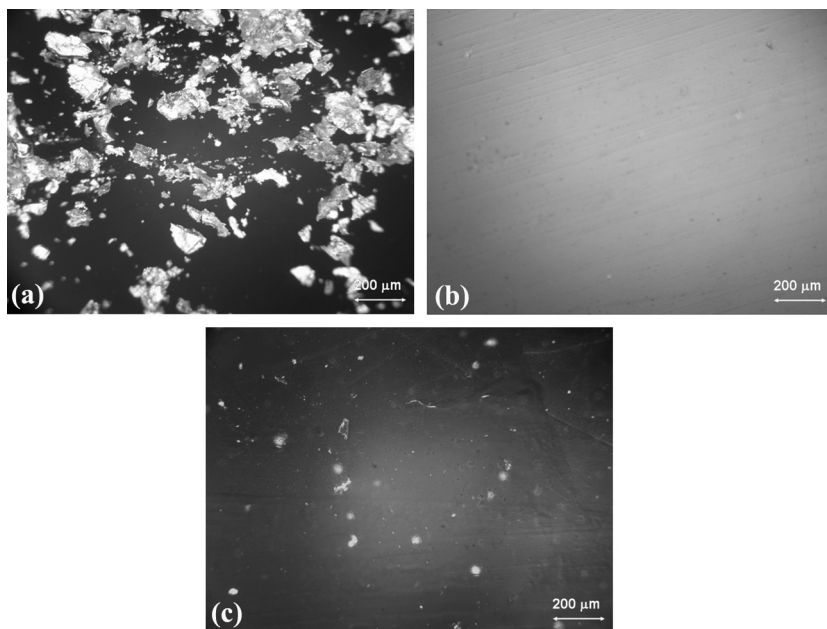
### Optical Microscopy

The films of pure as well as zinc nitrate containing PVA were transparent and colorless even at higher concentrations of the modifier. The optical

micrographs of zinc nitrate crystals, PVA, and PVA/Zn 9 films taken in polarized light are shown in Figure 1. The morphology of zinc nitrate within the polymer matrix was expected to be crystalline, following the analogy of dissolving and repeated exclusion of such material from water solutions. It can be seen in Figure 1, where the crystals (about  $50\ \mu\text{m}$  in size) of the modifier are relatively uniformly distributed within the polymer matrix. On the other hand, pure PVA showed no interaction with the polarized light, unlike the pure zinc nitrate crystals, which showed visible response in polarized light.

### Mechanical Properties

The influence of the PVA modification with zinc nitrate on mechanical properties of the resulting material is expressed as the zinc nitrate concentration dependences on E modulus, tensile strength, and tensile strain in Table III. It can be noted that the values of E modulus increase with the increasing modifier concentration up to 3 wt.% (PVA/Zn 3) of the recalculated zinc content, where the enhancement of E modulus is almost 300 MPa. The further addition of the modifier causes gradual decreasing



**Figure 1.** Optical micrographs of zinc nitrate crystals (a), pure PVA (b), and PVA/Zn 9 polymer films (c) taken under polarized light.

**Table III.** Mechanical properties of pure PVA and PVA-zinc nitrate polymeric films

Sample index	E modulus [MPa]	Tensile strength [MPa]	Tensile strain [%]
PVA	2686.5 ± 182.2	50.5 ± 3.4	159.2 ± 8.5
PVA/Zn 0.5	2754.0 ± 160.3	54.4 ± 3.9	146.5 ± 5.7
PVA/Zn 1	2880.6 ± 117.5	56.8 ± 4.1	137.2 ± 6.2
PVA/Zn 3	2952.9 ± 159.9	57.8 ± 5.6	127.5 ± 10.0
PVA/Zn 5	2452.0 ± 105.5	51.4 ± 2.4	124.1 ± 7.1
PVA/Zn 7	1889.6 ± 168.5	42.6 ± 5.2	106.5 ± 12.5
PVA/Zn 9	1245.5 ± 140.3	38.0 ± 5.3	80.5 ± 12.5

of E modulus. The significant drop (from 2686.5 MPa for pure PVA to 1245.5 MPa) was observed in the case of PVA/Zn 9. Another property, tensile strength, shows a trend similar to that for E modulus: first, the increase from 50.5 MPa (PVA) to 57.8 MPa (PVA/Zn 3) and second, the drop of the tensile strength to 38.0 MPa (PVA/Zn 9). On the other hand, tensile strain has a gradually decreasing trend over the concentration range. Whereas the maximal elongation of the unmodified PVA film is almost 160%, a drop to 127.5% and 80.5% was noted for PVA/Zn 3 and PVA/Zn 9, respectively. These facts reveal that low additions of zinc nitrate enhance the toughness (PVA/Zn 0.5, 1, and 3). Further additions cause the overall weakening of mechanical properties of the investigated polymer films. The reason for the observed phenomenon may be found in the physical reorganization of polymeric chains, which is due to the presence of low concentrations of the modifier, which can change the conditions (e.g., ionic strength) during the PVA-based polymeric film formations.

### Thermal Properties

Differential scanning calorimetry (DSC) could be useful for explaining the results presented above. The observed values of glass transition temperatures ( $T_g$ ), detected from the second heating scan to avoid the effect of moisture presence, of PVA and PVA/Zn samples are given in Table IV. As can be noted, the unmodified PVA shows  $T_g$  at 69.14°C. A trend similar to the concentration dependences of E modulus and tensile strength was found for  $T_g$  as well, i.e., slight increase of  $T_g$  up to 71.9°C for PVA/Zn 3 and further decrease with increasing content of zinc nitrate even up to 54.8°C for PVA/Zn 9. These interactions can be explained by changes of the polymeric chain mobility. It is obvious that  $T_g$  of the modifier, zinc nitrate, can be rarely detected due to its



**Table IV.** Glass transition temperature of the pure PVA and PVA/zinc nitrate films

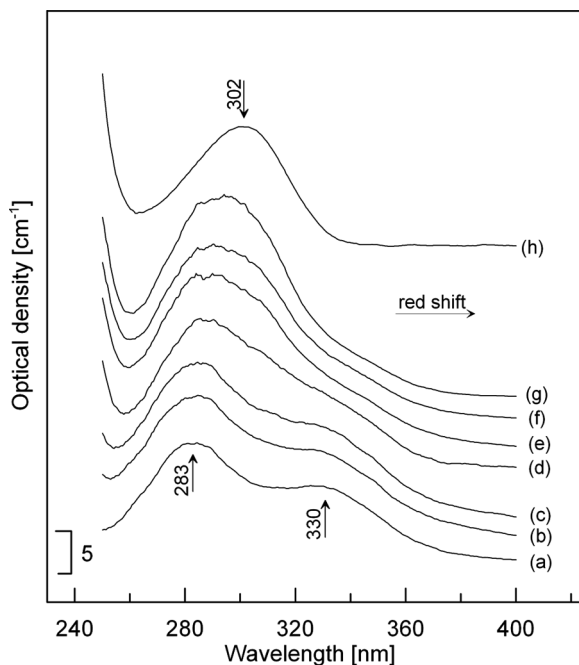
Sample index	Concentration of Zn(NO <sub>3</sub> ) <sub>2</sub> [wt.%]	Glass transition temperature [°C]
PVA	0.00	69.14
PVA/Zn 0.5	1.42	69.82
PVA/Zn 1	2.82	71.65
PVA/Zn 3	8.24	71.91
PVA/Zn 5	13.10	68.37
PVA/Zn 7	17.86	65.67
PVA/Zn 9	22.94	54.82

crystallinity. Thus, the results obtained from DSC measurements represent only the movements of the polymeric chains. This fact could support the assumption of the rearrangements of PVA chains. The lower contents of zinc nitrate lead to the reduction of the chain's mobility and improvement of mechanical properties. Nevertheless, concentrations over 5 wt.% (related to zinc) cause the reduction of  $T_g$  as well as mechanical properties, as is usual for the systems polymer versus low molecular compound.

### Spectroscopic Measurements

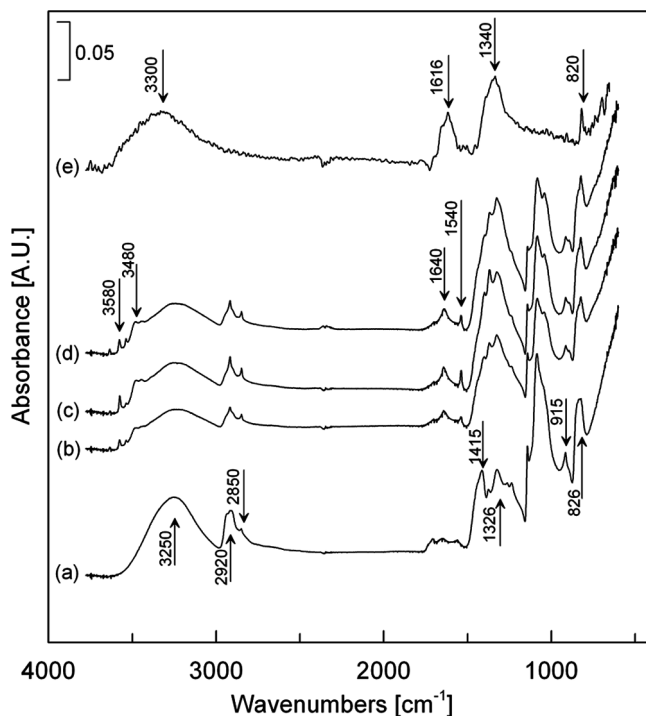
The structure of PVA/zinc nitrate polymeric films could be revealed by spectroscopic measurements in the UV and visible range. This method can provide evidence of the changes in structural arrangements due to modification of PVA with the modifier. The UV-vis spectra of pure components and modified PVA films are shown in Figure 2. As can be seen, pure PVA (Figure 2, curve a) is characterized by two peaks, at 283 and 330 nm. On the other hand, pure zinc nitrate has one dominant peak with a maximum at 302 nm (Figure 2, curve h). The increasing content of zinc nitrate in PVA causes the slight disappearance of PVA's second peak (situated at 330 nm) to the exclusion of increasing absorbance of the present modifier. It is also confirmed by the redshift of the first PVA's peak (Figure 2, curves b-g).

The FT-IR-attenuated total reflectance (ATR) spectra of the pure components of zinc nitrate as well as their combinations can be seen in Figure 3. The polymeric matrix, PVA, exhibits a broad absorption characteristic peak at  $3250\text{ cm}^{-1}$ , indicating the presence of moisture. The peaks corresponding to C-H groups may be noticed at  $2920$  and  $2850\text{ cm}^{-1}$  (stretching),  $1415$  and  $1326\text{ cm}^{-1}$  (rocking), and  $915$ ,  $848$ , and  $826\text{ cm}^{-1}$  (bending).<sup>[22]</sup> The high hydrolysis degree of the PVA



**Figure 2.** UV-vis spectra of the pure PVA (a), PVA/Zn 0.5 (b), PVA/Zn 1 (c), PVA/Zn 3 (d), PVA/Zn 5 (e), PVA/Zn 7 (f), PVA/Zn 9 (g) polymeric films, and zinc nitrate (1 M) ethanol solution (h).

(98 mol.%) corresponds to the strong absorption around the wavenumber  $1000\text{ cm}^{-1}$ , which can be assigned to the presence of hydroxyl groups (Figure 3, curve a). The spectrum of zinc nitrate hexahydrate is plotted as curve e in Figure 3. The effect of the presence of moisture is distinguishable by the absorption peak appearing above  $3000\text{ cm}^{-1}$  as well as in the case of pure PVA. Further, the peaks of the nitrate group can be noticed at  $1340$  and  $820\text{ cm}^{-1}$ . Zinc nitrate exhibits the absorption of average intensity over the region of wavenumbers from  $1700$  to  $1520\text{ cm}^{-1}$ , with the maximum situated at  $1620\text{ cm}^{-1}$ . The spectra of the selected polymer films containing zinc nitrate (PVA/Zn 5, PVA/Zn 7, and PVA/Zn 9) are shown in Figure 3, curves b–d. Concentration variations of the modifier caused new peaks to appear at  $1640$  and  $1540\text{ cm}^{-1}$ . Another set of new peaks can be noticed at  $3580$  to  $3480\text{ cm}^{-1}$ . Their height increases with increasing concentration of the modifier in the samples. Furthermore, the peaks of nitrate groups occur in these spectra. Their evidence increases with rising content of zinc nitrate in the systems. These results reveal that zinc nitrate is distributed uniformly within the PVA matrix. In addition, optical microscopy

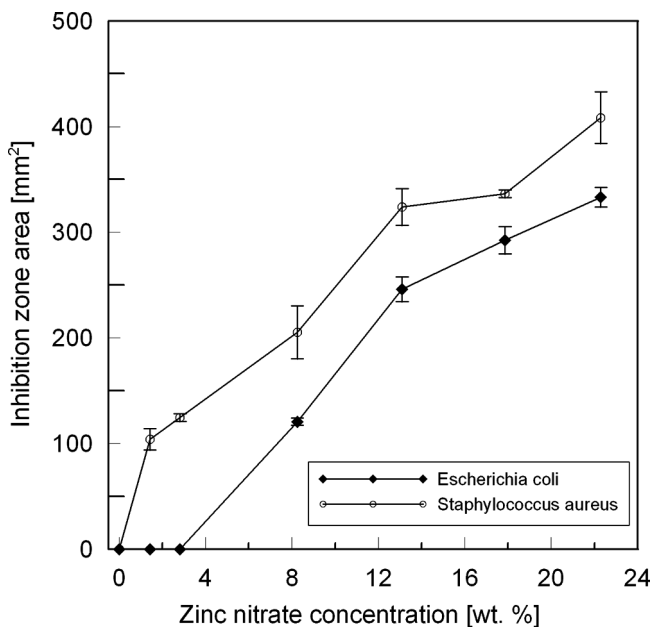


**Figure 3.** FT-IR-ATR spectra of pure PVA (a), PVA/Zn 5 (b), PVA/Zn 7 (c), PVA/Zn 9 (d), and pure zinc nitrate (e).

showed that the crystalline zinc nitrate domains, excluded from the aqueous solution, are also present in the investigated system.

### Antibacterial Properties

The inhibition of bacterial growth occurs in all cases where zinc nitrate is present. It was confirmed by the circular inhibition zones that appeared due to the diffusion of the antibacterial agent from the sample through the surrounding agar medium in the presence of *Escherichia coli* and *Staphylococcus aureus*. The dependence of inhibition area on the concentration of modifier can be found in Figure 4. The polymer film made of pure PVA does not inhibit bacterial growth. *Escherichia coli* shows resistance against the antibacterial ingredient for lower modified samples PVA/Zn 0.5 and PVA/Zn 1. The inhibition zone appeared only for PVA/Zn 3 and higher modified samples. In addition, the inhibition zones gradually increase with rising content of zinc nitrate in the samples.



**Figure 4.** Antibacterial activity of the zinc nitrate-based polymer films against *Escherichia coli* and *Staphylococcus aureus*.

On the other hand, *Staphylococcus aureus* seems to be much more sensitive towards the modifier and a noticeable inhibition occurred already in the case of PVA/Zn 0.5. Further increase of zinc nitrate level in the samples markedly improves the antibacterial activity of the films.

The differences in the growth inhibitions are based on the variance of the chosen bacterial strains. *Escherichia coli* belongs to the group of gram positive bacteria, whereas *Staphylococcus aureus* is gram negative. These two groups differ in the structure of their cell walls since the cell walls of gram positive bacteria contain more peptidoglycan layers than gram negative bacteria.<sup>[14]</sup> It can explain the higher resistance of *Escherichia coli* against the lower concentrations of the modifier.

## CONCLUSIONS

The aim of this work was the preparation and characterization of polymeric films based on PVA and zinc nitrate. The modification was done in an aqueous solution of polymer and the samples were prepared by the casting technique. Optical microscopy in polarized light revealed relatively uniform distribution of zinc nitrate crystalline domains in the

PVA matrix. In addition, this process yields a transparent, colorless material with enhanced E modulus and tensile strength up to 8.24 wt.% of zinc nitrate content. On the other hand, ductility of the material was reduced. These results correspond to the data obtained from differential scanning calorimetry.

The antibacterial activity assessment of the modified PVA films showed higher antimicrobial capability against gram negative bacteria at lower contents of zinc nitrate in the systems. On the other hand, the films with higher concentrations of zinc nitrate have relatively strong activity against both types of bacteria.

The spectroscopic characterizations of the samples are in agreement with the optical microscopy and confirm the assumption that the modifier, zinc nitrate, is uniformly distributed within its carrier, PVA. This arrangement of the investigated system can ensure the sufficient transport of the active substance into the environment where it is needed.

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