UDC 539.5:678:539.2 doi: 10.15407/hftp16.01.090

V.L. Demchenko [1], Ye.P. Mamunya [1, 2], M.V. Iurzhenko [1, 2], S.M. Kobylinskyi [2], N.P. Rybalchenko [3], T.T. Hnatiuk [3], T.V. Rybalchenko [6], A.I. Marynin [4], D.V. Shtepa [4], I.O. Sytnyk [1], O.M. Demchenko [5], S.R. Rushkovsky [6]

STRUCTURE, THERMOPHYSICAL, ANTIMICROBIAL, AND GENOTOXIC PROPERTIES OF SILVER-CONTAINING NANOCOMPOSITES FILM, OBTAINED BY SPUTTERING DEPOSITION

[1] E.O. Paton Electric Welding Institute of National Academy of Sciences of Ukraine
11 Kazymyra Malevycha Str., Kyiv, 03680, Ukraine, E–mail: dvaleriyl@ukr.net
[2] Institute of Macromolecular Chemistry of National Academy of Sciences of Ukraine
48 Kharkivske shosse, Kyiv, 02160, Ukraine
[3] Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of Ukraine
154 Academician Zabolotny Str., Kyiv, 03143, Ukraine
[4] National University of Food Technologies
68 Volodymyrska Str., Kyiv, 01601, Ukraine
[5] State Institution "National Research Center for Radiation Medicine, Hematology and Oncology of National Academy of Medical Sciences of Ukraine"
53 Yuriia Illienka Str., Kyiv, 04050, Ukraine
[6] Taras Shevchenko National University of Kyiv
2 Academician Glushkov Avenue, Kyiv, 02000, Ukraine

To create film materials with antimicrobial properties, silver nanoparticles are widely used, which are introduced, in particular, into polymer matrices. In this work, a mixture of polylactide-polycaprolactone polymers, taken in a mass ratio of 80–20, was used as a polymer base. These studies will further contribute to the development of new safe materials, particularly in creating packaging materials for food products, which is undoubtedly an urgent problem today.

The work aimed to create silver-containing polymer composites based on a mixture of polylactide and polycaprolactone by vacuum deposition of silver nanoparticles on the surface of the polymer matrix and to study the structure, morphology, thermophysical, antimicrobial, and genotoxic properties of the obtained composites.

Silver nanoparticles were sputtered on the surface of the PLA-PCL polymer mixture using an FC-1100 ion sputtering device (JEOL, Japan) for 1, 3, and 5 min. The thickness of the films was 110 μm. The structure, morphology, thermophysical, antimicrobial, and genotoxic properties of composites formed by sputtering silver nanoparticles on the surface of the polymer were studied using wide-angle X-ray scattering on a device XRD-7000 (Shimadzu, Japan), transmission electron microscopy (TEM) (a JEM-1230 JEOL, Japan), thermogravimetric analysis (a TGA Q50) (TA Instruments, USA), differential scanning calorimetry (a DSC Q2000) (TA Instruments, USA), as well as antimicrobial and genotoxic tests.

Using X-ray structural analysis, it was found that the original polymers are characterized by a semi-crystalline structure, and the presence of metallic silver on the surface of the polymer was confirmed. It is shown that a layer of silver particles of about 425 nm is formed on the surface of the PLA-PCL mixture for a sputtering duration of 5 minutes.

According to the results of differential scanning calorimetry, it has been found that when sputtering silver particles on the surface of polymers, the degree of crystallinity increases from 35 to 39 %, and the melting temperature T_m increases from 168 to 169–170 °C. At the same time, the influence of the metallic layer of silver on the amorphous phase of the polymer was not recorded.

The antimicrobial activity of PLA-PCL-Ag samples with a spraying duration of 3 and 5 min against S. aureus and E. coli microorganisms was revealed.

It was found that the studied samples did not show a toxic effect.

***Keywords**: polylactide, polycaprolactone, silver-containing composite, structure, morphology, thermal properties, antimicrobial activity, genotoxicity*

INTRODUCTION

The development of nanotechnology contributes to the creation of several nanomaterials that differ in chemical nature and composition, shape and structure of particles, purpose, and areas of application. It should be noted that one of the most important characteristics of nanomaterials is the size of the particles included in their composition [1–4].

Recently, nanocomposite materials based on polymers of natural origin and metal nanoparticles, such as copper, silver, and zinc oxide, have attracted the most attention due to their pronounced antimicrobial, antifungal, and antiviral properties [5–7]. For the production of materials with antimicrobial and antiviral properties, one of the most promising polymers is polylactide (PLA), which is made from non-toxic renewable raw materials. PLA is an environmentally friendly polymer, biocompatible and biodegradable, endowed with high mechanical strength and elasticity [8–10]. These advantages make it possible to use PLA in biomedicine, the food industry, and for 3D printing technology [11, 12].

PLA-Ag-chitosan nanocomposites (where Ag ions have been reduced with benzoxazine as a reductant) were characterized in [13], while the size of silver nanoparticles and their antimicrobial activity were not studied.

Currently, the most common method of obtaining silver-containing nanocomposites is the reduction of Ag^+ ions in the polymer matrix, which makes it possible to control the morphology of nanoparticles [6, 14]. For the production of silver-containing nanocomposites, chemical [15] or thermochemical reduction [6] is used, as well as plant extracts are used to reduce Ag^+ ions in polymer matrices [16]. When forming nanocomposites by chemical reduction of silver ions, reducing agents such as dimethylformamide [17], hydrazine [15], and sodium borohydride [18], *etc*. are used. This is a fast, efficient, and simple way to fabricate nanocomposites with controlled morphology, but chemical impurities that remain in the samples after the reduction reaction are a problem. Thermochemical reduction of silver ions is an environmentally friendly, effective, and inexpensive method of manufacturing silver-containing nanocomposites, but sometimes this approach requires heating film materials with silver ions at rather high temperatures [6]. Recently, the "green synthesis" of silver-containing nanocomposites – the reduction of Ag^+ ions using plant extracts [16] has gained considerable popularity. this approach is effective but requires considerable time for the complete recovery of silver ions.

The analysis of the literature has shown that the results of the study of polymer film materials based on polylactide and silver nanoparticles, produced by a simple, effective, and fast method – spraying silver on the surface of the polymer, have not yet been published.

The work aims to create nanocomposite film materials based on polylactide (PLA), polycaprolactone (PCL), and silver nanoparticles by spraying silver on the surface of the polymer, as well as to study their structure, morphology, thermophysical, antimicrobial, and genotoxic properties.

EXPERIMENTAL PART

The samples were molded using the following materials: PLA filament (MonoFilament, Ukraine, M_w = 274000 g/mol), PCL granules (China, M_n = 60000–80000), chloroform (China, M = 119.38), silver foil with thickness 0.1 mm, 99.9 % trace metals basis (Sigma-Aldrich).

PLA-PCL films were obtained by mixing polylactide and polycaprolactone polymers separately in chloroform in an 80:20 mass ratio. Silver-containing nanocomposites were produced by silver sputtering deposition on the surface of the PLA-PCL film using the FC-1100 ion sputtering device (JEOL, Japan).

The structure of composites was studied by wide-angle X-ray diffraction with an XRD-7000 diffractometer (Shimadzu, Japan), the X-ray optical scheme of which was performed by passing the primary beam through the sample, using CuK_α radiation (λ = 1.54 Å) and graphite monochromator at the temperature T = 20±2 °C.

The size of the Ag nanoparticles and their distribution in the polymer matrix was examined using a JEM-1230 transmission electron microscope (JEOL, Japan) at the resolution of 0.2 nm.

The thermal decomposition of samples was performed using a TGA Q50 (TA Instruments, USA) under an air atmosphere in the temperature interval from 20 to 600 °C at the heating rate of 20 °C/min.

Thermal transitions in the samples were analyzed by differential scanning calorimetry

(DSC) under air flow using a DSC Q2000 apparatus (TA Instruments, USA) within temperature intervals of 20–200 °C at the heating rate of 20 °C/min.

The mechanical strength at the failure of the starting materials and composites based on them was measured using an AGS-10kNX testing machine (Shimadzu, Japan) in uniaxial tension mode at $T = 20 \pm 2$ °C.

In the antimicrobial research, we used the following reference strains of opportunistic pathogens: *Staphylococcus aureus* ATCC 25923 as model Gram-positive bacteria, and *Escherichia coli* ATCC 25922 as model Gram-negative bacteria. The bacterial strains were obtained from the Ukrainian Collection of Microorganisms at the Zabolotny Institute of Microbiology and Virology of NAS of Ukraine.

The antimicrobial activity of the nanocomposites was studied using the diffusion method into agar on a solid Mueller-Hinton nutrient medium. An indicator of antimicrobial activity was the presence of a clear zone around the nanocomposite disc. The control was a nanocomposite disc without silver nanoparticles. The experiment was carried out three times. The results were interpreted by the diameter of the zones of inhibition of microbial growth around the discs according to CLSI recommendations [17, 18].

The genotoxicity of the investigated film materials was studied at the State Institution "National Scientific Center of Radiation Medicine, Hematology and Oncology of the National Academy of Medical Sciences of Ukraine" [19].

RESULTS AND DISCUSSION

The analysis of wide-angle X-ray diffractograms of the initial polymer films based on polylactide (PLA) and polycaprolactone (PCL) showed that they are characterized by a semi-crystalline structure (Fig. 1, curves 1, 2).

Analysis of wide-angle X-ray diffractograms of a polymer mixture based on PLA and PCL, taken in a mass ratio of 80 : 20, respectively, showed that this sample also has a semi-crystalline structure (Fig. 1, curve 3).

This is confirmed by the presence (Fig. 1, curve 1) of multiple diffraction maxima of discrete type on the background of an imaginary amorphous halo with a peak at $2\theta_m \sim 15.1°$ on the diffraction pattern. An average value of d period of close location of PLA macromolecular chain fragments, according to Bragg equation [20]:

$$d = \lambda(2\sin\theta_m)^{-1},$$

where λ is the wavelength of characteristic X-radiation ($\lambda = 1.54$ Å for CuK_α-radiation), equal to 5.8 Å, for PCL, this value is 4.1 Å.

Relative crystallinity level X_{cr} of PLA was calculated according to Mathews method [20]:

$$X_{cr} = Q_{cr}(Q_{cr} + Q_{am})^{-1} \cdot 100,$$

where Q_{cr} is the square of diffraction maxima which characterize the crystalline structure of polymer; $Q_{cr} + Q_{am}$ is the total square of the diffraction pattern in the interval of dispersion ($2\theta_1 \div 2\theta_2$), where the amorphous-crystalline structure of the polymer is observed. Calculations have shown that $X_{кр}$ value is approximately equal to 62.5 %, while for PCL this value is 43.5 %.

The diffractogram of the PLA-PCL polymer mixture contains diffraction maxima that characterize the crystal structure of both individual PLA and PCL polymers. Due to the higher content of PLA in the composite (80 % by mass), the diffraction maxima responsible for the crystalline structure of this polymer are more intense (curve 3 and curves 1, 2).

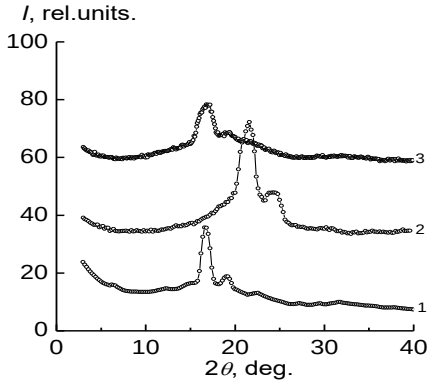

Fig. 1. Wide-angle X-ray diffractograms of (*1*) the PLA; (*2*) the PCL; and (*3*) the PLA-PCL

The presence of sprayed Ag nanoparticles for 5 min on the surface of the PLA-PCL sample (mass ratio of polymers 80 : 20) was confirmed by the method of Wide-angle X-ray diffraction (Fig. 2). This is indicated by the presence of two diffraction maxima at $2\theta_m \sim 38°$ and 44°, which characterize the crystalline structure of metallic silver.

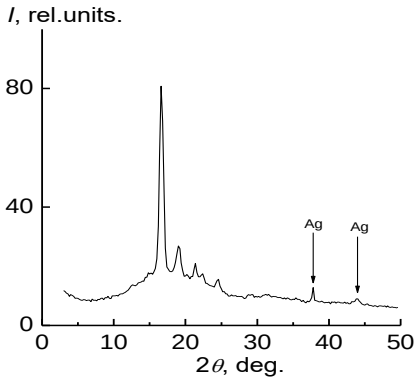

Fig. 2. Wide-angle X-ray diffractograms of the PLA-PCL-Ag (sputtering time 5 min)

Fig. 3 shows the image of the silver layer, which is formed on the surface of the PLA-PCL film as a result of sputtering Ag nanoparticles.

From the microphotographs, it can be seen that a layer of Ag nanoparticles with a thickness of ~ 425 nm is formed on the surface of the PLA-PCL film (with a sputtering time of 5 min). Analysis of the histogram of the fractional composition of nanoparticles has shown that silver particles can form small aggregates (Fig. 3). The shape of nanoparticles in the PLA-PCL-Ag samples obtained by sputtering is close to spherical.

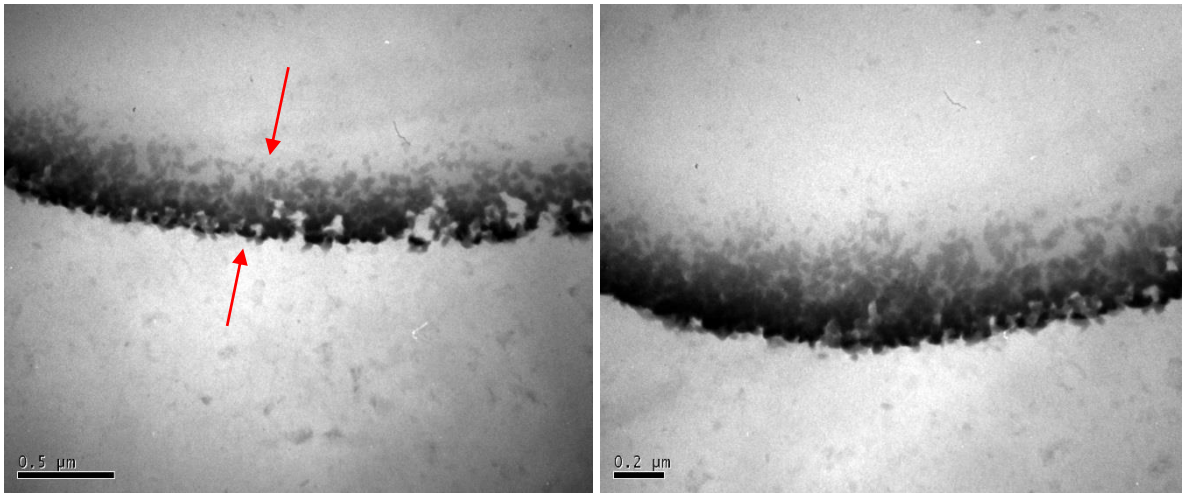

Fig. 3. Photomicrographs of transmission electron microscopy of silver-containing nanocomposites based on polylactide and polycaprolactone with silver nanoparticles formed by sputtering deposition (sputtering time 5 min)

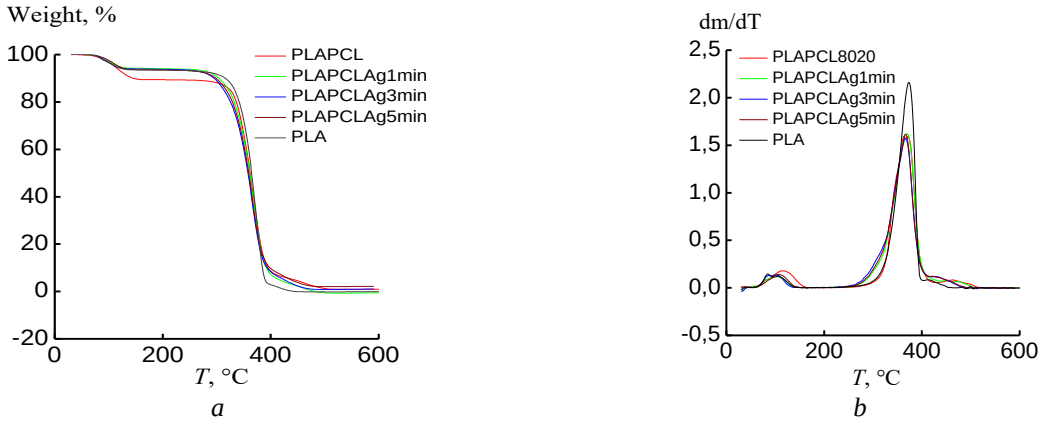

Fig. 4. Curves of (*a*) TGA and (*b*) DTGA of the PLA, the PCL, the PLA-PCL, and PLA-PCL-Ag composites with sputtering times of 1, 3, and 5 min

Fig. 4 shows the TGA and DTGA curves of polylactide (PLA), its PLA-PCL mixture, and the PLA-PCL-Ag sample. All curves show a mass loss in the temperature range of 70–130 °C in the amount of 6.5 % for PLA and 10.5 % for the PLA-PCL mixture.

The weight loss of the films in this temperature range can be explained by the removal of solvent residues - chloroform during heating. The mass loss process of composites begins at 70 °C, which is slightly higher than the boiling point of chloroform (61.1 °C), i.e. solvent residues in this temperature range change to a gaseous state and diffuse from the films. As can be seen, there are more chloroform residues in the PLA-PCL mixture, which is due to the looser molecular structure in mixtures of incompatible polymers [21]. The temperature of the onset of thermal destruction for PLA is 332.6 °C, for the PLA-PCL mixture it is 3 °C lower and 329.4 °C, which may be due to the less dense molecular structure of the mixture. The maximum temperature of DTGA (that is, the highest rate of thermal destruction) is the same for all films except PLA and is ~ 371 °C. For PLA, this value is somewhat higher 373.4 °C.

As for the coke residue (that is, the polymer residue that did not burn at 500 °C), it is 0.96 % for the PLA-PCL mixture and close to zero for pure PLA. The parameters of the composites are given in Table 1.

Table 1. TGA data for polylactide, PLA-PCL mixture, and PLA-PCL-Ag composites obtained during different times of silver sputtering

Sample	Loss of mass at 70–130 °C, %	The beginning of thermal destruction, °C	The maximum rate of thermal destruction, °C	Coke residue at 500 °C, %
PLA	6.5	332.6	373.4	0,00
PLA-PCL	10.5	329.4	371.1	0.96
PLA-PCL-Ag (1 min)	6.4	322.9	371.3	0.08
PLA-PCL-Ag (3 min)	6.4	322.9	371.3	0.96
PLA-PCL-Ag (5 min)	6.4	322.9	371,3	2.10

As can be seen from Fig. 4, in the temperature range of 70–130 °C, the mass loss of PLA-PCL-Ag composites with different amounts of silver (that is, with different sputtering times) is equal to each other and amounts to 6.4 %, while the mass loss of the mixture PLA-PCL is 10.5 %. This result can be explained by the partial evaporation of the remaining chloroform at room temperature when placing the PLA-PCL film in a vacuum chamber for sputtering, which reduced its content in the polymer to 6.4 %. The difference in sputtering time did not affect this process, since the bulk of the remaining solvent evaporated immediately after pumping out the vacuum chamber.

The temperature of the onset of thermal destruction for the PLA-PCL sample is 329.4 °C, while for metal-containing compositions it is 6.5 °C lower and equals 322.9 °C on average. The sprayed metallic layer of silver contributes to better heat transfer from the device chamber to the polymer part of the sample and the temperature of the onset of thermal destruction decreases. At the same time, the maximum on the DTGA curves, which corresponds to the highest rate of thermal destruction, is the same for all composites and equals 371.3 °C. The amount of coke residue is insignificant and ranges from 0.077 to 2.1 %, depending on the spraying time.

Fig. 5 shows the DSC curve of the PLA film. As can be seen, three temperature transitions are observed on the curve, namely, glass transition in the temperature range of 58–62 °C, cold crystallization at 100–150 °C, and melting in the temperature range of 155–180 °C. The parameters of these three transitions are presented in Table 2. The presence of a large peak of cold crystallization indicates that during the heating process, some part of the amorphous phase in the polymer is ordered and transformed into a structured crystalline phase. Upon further heating, the crystalline phase formed during cold crystallization melts and gives an endothermic peak on the DSC curve.

The degree of crystallinity of the composites χ was calculated from the equation

$$\chi = \frac{\Delta H_m}{m\Delta H_m^0} 100\%$$

where ΔH_m is the heat of fusion of the composite, ΔH^0_m is the heat of fusion of PLA with 100 % crystallinity (93 J/g) [22], m is the mass fraction of PLA in the composite. From the data in Table 2, it follows that the heat of crystallization ΔH_{cr} and the heat of melting ΔH_m of polylactide differ significantly, namely $\Delta H_{cr}/\Delta H_m = 0.63$. It follows that cold crystallization gives a share of 0.63 of the degree of crystallinity, the rest is the initial crystallinity of PLA, i.e. $\chi = 10.5$ % for PLA, which was obtained after the first run in the equilibrium state, and $\chi = 17.9$ % caused by cold crystallization.

Fig. 6 presents DSC thermograms for mixtures of polylactide with polycaprolactone (PLA-PCL) in a mass ratio of 80–20. It should be noted that the DSC curves do not show their exo- and endothermic peaks of PCL, in particular, the melting temperature of PCL is about 60 °C, but the corresponding endothermic peak is absent.

That is, it can be assumed that the polymeric component of PCL in the mixture affects only the crystallization and melting of polylactide. Indeed, the heat of crystallization in the mixture increases dramatically compared to PLA (24.25 compared to 16.56 J/g), and the temperature of crystallization decreases significantly (118.3 compared to 135.3 °C). This indicates that the second component of the mixture contributes to the intensive flow of cold crystallization. As a result, the resulting degree of crystallinity of PLA-PCL mixtures is significantly higher than that of the original PLA (χ is 35.0 compared to 28.4 %).

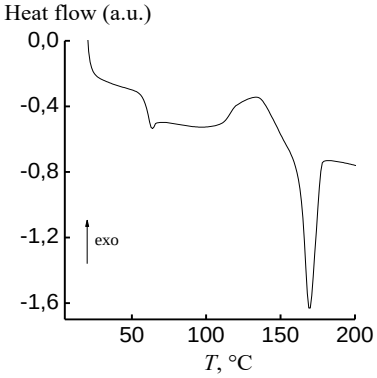

Fig. 5. DSC thermogram of the initial polylactide film material

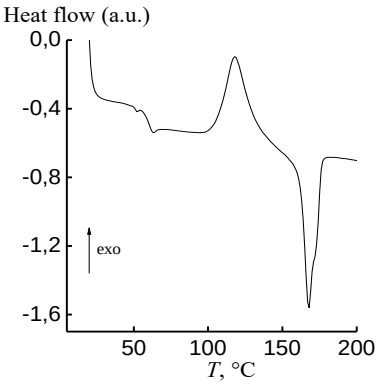

Fig. 6. DSC thermogram of the PLA-PCL mixture, taken in a mass ratio of 80–20

Table 2. Parameters of DSC thermograms of polylactide, its PLA-PCL mixture, and silver-containing composites based on the PLA-PCL mixture

Sample	T_g, °C	T_{cr}, °C	ΔH_{cr}, J/g	T_m, °C	ΔH_m, J/g	χ, %
PLA	61.6	135.3	16.56	169.5	26.44	28.4
PLA-PCL	60.7	118.3	24.25	167.7	26.06	35.0
PLA-PCL-Ag (1 min)	60.5	119.2	21.88	169.0	22,95	30.8
PLA-PCL-Ag (3 min)	60.6	118.8	26.76	169.0	29.12	39.1
PLA-PCL-Ag (5 min)	60.9	120.6	23.10	170.5	28.94	38.9

The melting temperature of the mixture is ~ 2 °C lower than that of pure PLA and an additional high-temperature shoulder appears at the endothermic melting peak, indicating a complex structure of the crystalline phase that is formed during cold crystallization. As for the glass transition temperature, it decreases by ~ 1 °C in the mixture compared to PLA.

Fig. 7 shows DSC thermograms of the PLA-PCL mixture with silver sputtering for 1, 3, and 5 min.

As can be seen, the thermograms are similar to each other, but there is an increase in the

degree of crystallinity χ from 35 to 39 % (excluding PLA-PCL-Ag (1 min) and the melting temperature T_m from 168 to 169–170 °C. This is evidence of the effect of silver particles penetrating the polymer film to a certain depth and causing a nucleating effect during polymer crystallization. At the same time, there is no effect of the metallic silver layer on the amorphous phase, since the glass transition temperature T_g of the composites practically does not change compared to the pure PLA-PCL mixture (Table 2).

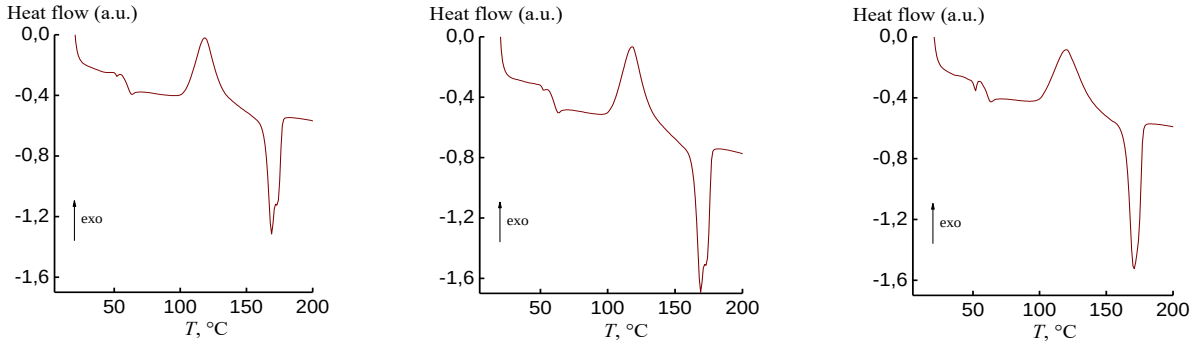

Fig. 7. DSC thermograms of composites based on a PLA-PCL mixture with silver nanoparticles sprayed for 1 (*a*), 3 (*b*), and 5 min (*c*)

At the next stage, studies of the tensile strength of film materials based on polylactide and polycaprolactone, as well as samples with silver sprayed on their surface, were conducted.

Polylactide exhibits moderate mechanical properties with a maximum elongation at a break of less than 2 %. When adding 20 % by weight of polycaprolactone to polylactide, the elongation at break increases to 22.4 %. PLA-based films showed the highest tensile strength, namely 33.3 MPa. The tensile strength of the sample based on polylactide and polycaprolactone is 26.7 MPa (Fig. 8).

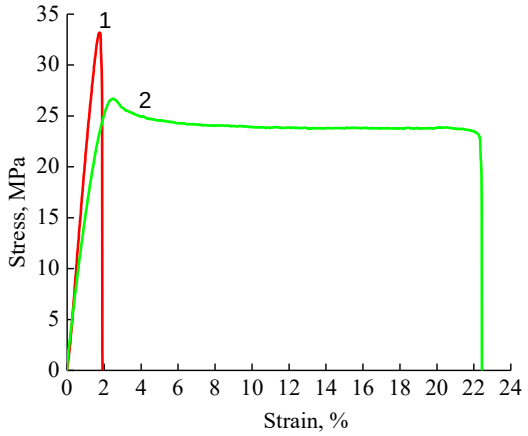

Fig. 8. Stress-strain curves of film materials based on polylactide (*1*) and its mixture with polycaprolactone (*2*)

When sputtering PLA-PCL films with silver, the tensile strength and elongation to break the samples decrease. As the sputtering time increases, the elongation to break in the samples decreases (Fig. 9).

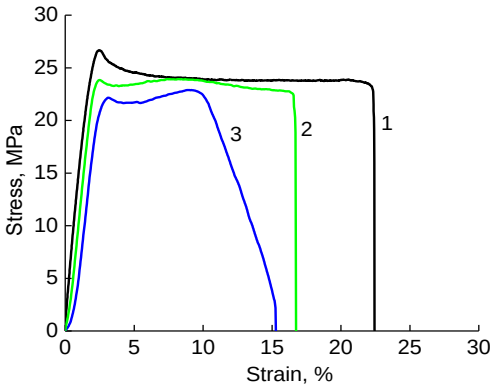

Fig. 9. Stress-strain curves of film composite materials of pure PLA-PCL sample (*1*) and PLA-PCL samples with silver sputtered for 3 (*2*) and 5 min (*3*)

The study examined the antimicrobial activity of film materials made from PLA and PCL polymers with Ag nanoparticles deposited on the polymer surface for 1, 3, and 5 minutes.

It was shown that the formed PLA-PCL polymer materials with Ag nanoparticles, which were sprayed for 1 min, did not exhibit antimicrobial activity against any of the test cultures of microorganisms (Table 3). The

studied films with Ag nanoparticles sputtered for 3 and 5 min showed antimicrobial activity against *S. aureus* and *E. coli*. Thus, after 24 hours of incubation at 37 °C, a clear zone was observed around the disks of the PLA-PCL-Ag samples with sputtering times of 3 and 5 min, indicating an inhibition of microbial growth. The diameters of the growth retardation zones for *S. aureus* were 13.80±0.50 mm in the sample where silver sputtering was carried out for 3 min and 14.82±0.60 mm in the sample where silver sputtering was carried out for 5 min (Table 3, Fig. 10). The diameter of the growth retardation zone for *E. coli* was 16.44±0.70 mm for the sample sputtered for 5 min (Fig. 10). The PLA-PCL-Ag film sample was sputtered with silver for 3 min and showed a slight antimicrobial effect. The diameter of the growth retardation zone for *E. coli* measured 11.02±0.40 mm. In the control samples (without Ag nanoparticles) of PLA and PLA-PCL, active growth of test microorganisms and the absence of growth retardation zones were observed (Fig. 10).

Table 3. Antimicrobial activity of PLA-PCL-Ag films against conditionally pathogenic microorganisms

Spraying time, min	Polymer systems	Diameter of growth retardation zones, mm	
		S. aureus	*E. coli*
0	PLA	0	0
0	PLA-PCL	0	0
1	PLA-PCL-Ag	0	0
3	PLA-PCL-Ag	13.80±0.50	11.02±0.40
5	PLA-PCL-Ag	14.82±0.60	16.44±0.70

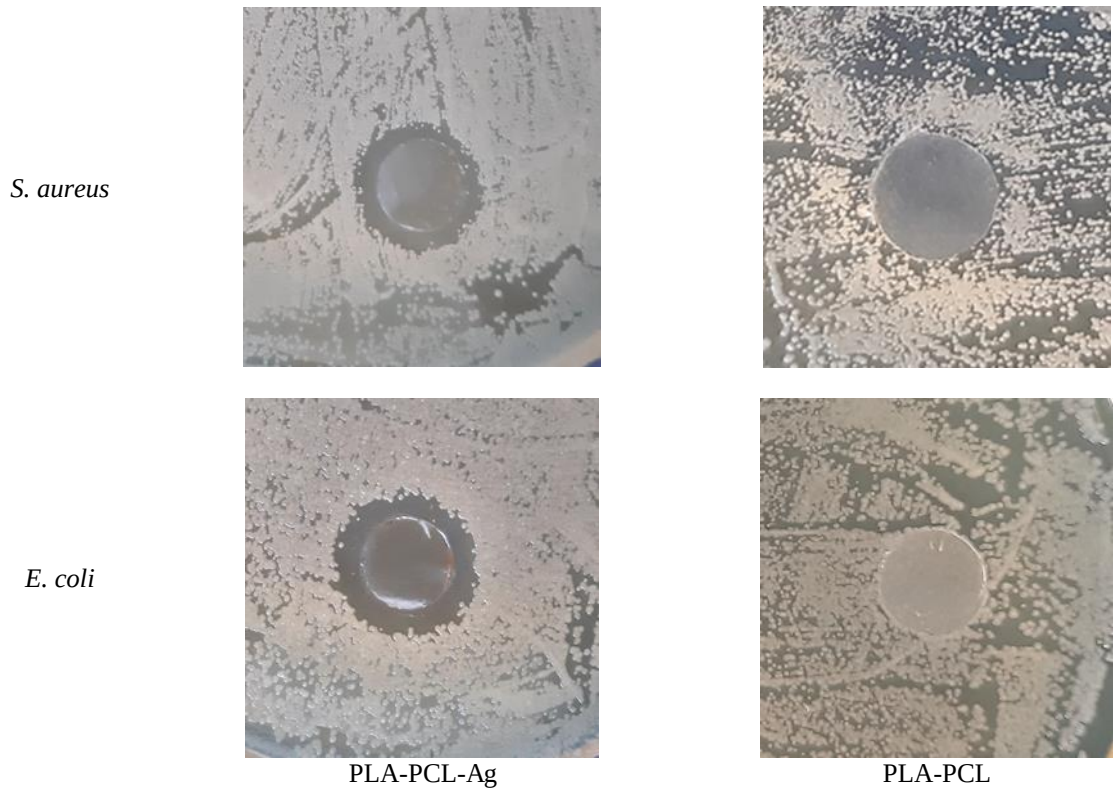

Fig. 10. Antimicrobial activity of PLA-PCL films with Ag nanoparticles deposited on the polymer surface for 5 minutes

Thus, it was shown that the polymeric materials PLA and PLA-PCL, with Ag nanoparticles, sputtered for 5 minutes and exhibited the highest antimicrobial effect against *S. aureus* and *E. coli.*

Nanoparticles of various metals can penetrate living cells, bind to nucleic acids, form DNA adducts, cause DNA damage, integrate into biological membranes, lead to cell death or oncotransformation, reduce proliferative activity,

penetrate cell organelles, and variously change the functions of biological structures. Therefore, it is necessary to ensure the biosafety of the investigated composites. The genotoxicity of samples is an important indicator of biosafety.

The genotoxicity (mutagenicity) of polymer films was studied on the culture of peripheral blood lymphocytes of healthy volunteers using cytogenetic and molecular genetic methods.

To determine the mutagenicity of polymer films PLA, PLA-PCL, PLA-PCL-Ag (sputtering time 5 min) using the cytogenetic research method, it is necessary to study the spontaneous level of chromosome aberrations, which is an important quantitative characteristic of the mutation process.

Therefore, we conducted our assessment of the spontaneous level of chromosome aberrations of volunteer donors. It was found that the value of the spontaneous level of chromosome aberrations for volunteer donors was 2.0 % ± 1.2 and it is represented by aberrations of the chromatid type – single fragments and chromosome type – paired fragments. All other metaphase plates 98 % contained normal chromosomes that did not have structural rearrangements (Fig. 11). It is known that the most common type of spontaneous aberrations, which is characteristic of healthy individuals, are single and paired fragments (acentric aberrations), that is, the simplest structural damage to chromosomes, which, according to various authors, account for almost 90 % of the total number of aberrations (Fig. 12, 13).

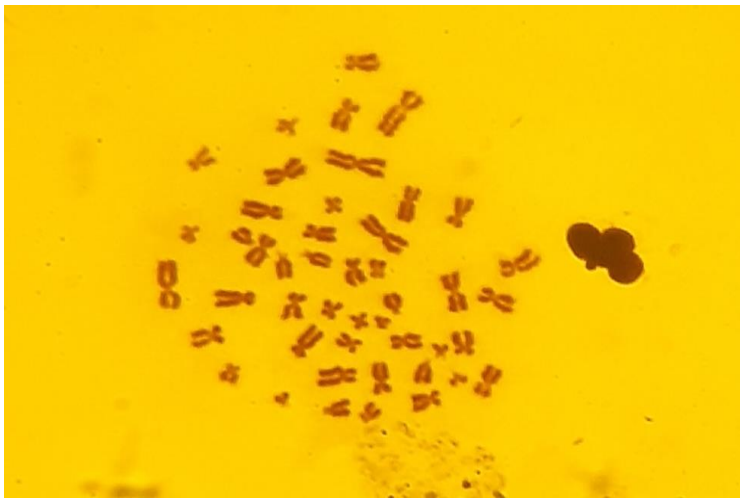

Fig. 11. Metaphase plate 46 XX. Norm

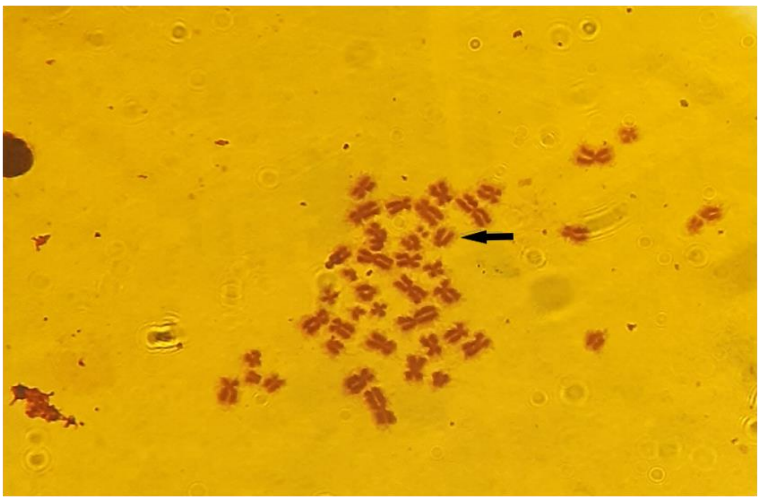

Fig. 12. Metaphase plate 46 XX with a paired fragment (indicated by arrows)

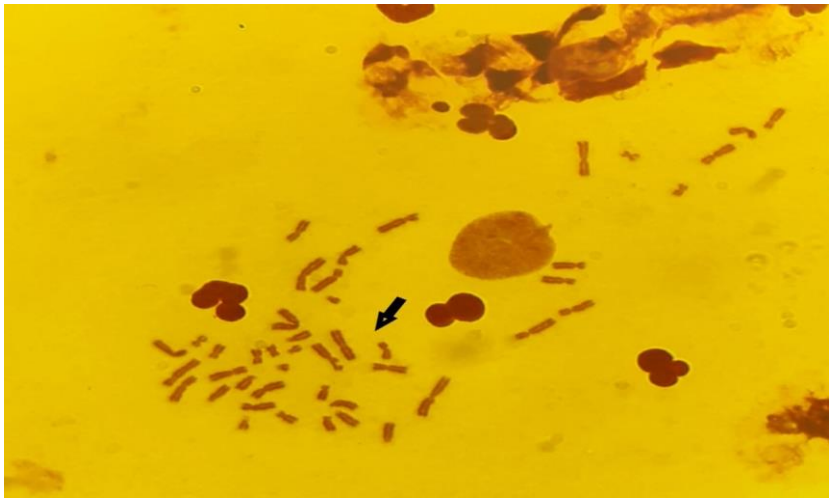

Fig. 13. Metaphase plate 46 XX with a single break (indicated by arrows)

During co-cultivation of the original polymer films PLA, PLA-PCL, PLA-PCL-Ag (sputtering time 5 min), formed by the irrigation method from a solution with peripheral blood lymphocytes, no statistically significant $p \geq 0.05$ increase in the frequency of chromosome aberrations was observed, by the control values (Fig. 14). The spectrum of chromosome aberrations during co-cultivation of peripheral blood lymphocytes and polymer films is represented by chromatid breaks and paired fragments.

Analyzing the obtained values of the frequency of chromosome aberrations during the cultivation of peripheral blood lymphocytes with polymer films PLA, PLA-PCL, PLA-PCL-Ag (sputtering time 5 min), no mutagenic effect of these nanocomposites was noted, but this is possible due to the effective work of the body's reparative systems.

A molecular genetic method was also used to determine the genotoxicity of polymer films.

First, using the classical Somet assay method, the value of background individual indicators of DNA damage of individual cells in a volunteer donor, which served as a control, was determined.

It was found that in the volunteer donor, the control of % DNA yield was 20.4±0.7 %. During the co-cultivation of LPK with PLA, PLA-PCL, PLA-PCL-Ag (sputtering time 5 min) films, the % DNA yield was 18.92±0.51 %, 14.38±0.37 %, and 16.2±0.61 %, respectively, which was within the control (Fig. 15).

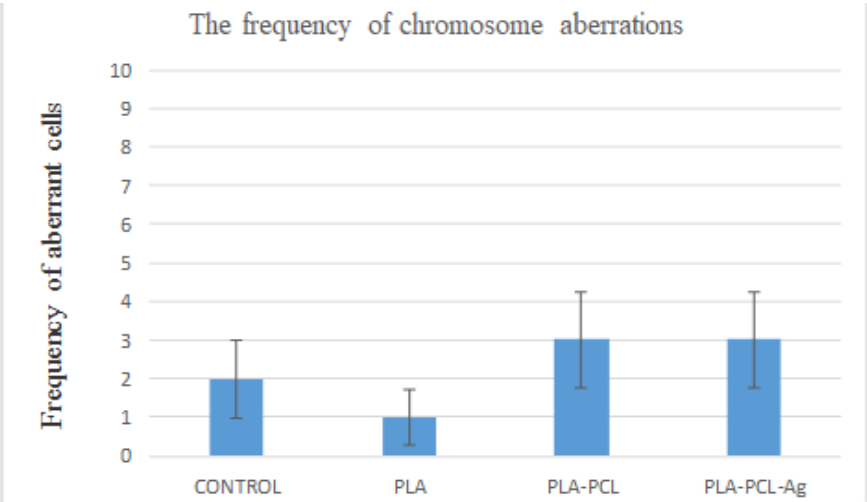

Fig. 14. The frequency of chromosome aberrations during co-cultivation of polymer films PLA, PLA-PCL, PLA-PCL-Ag (sputtering time 5 min) with peripheral blood lymphocytes

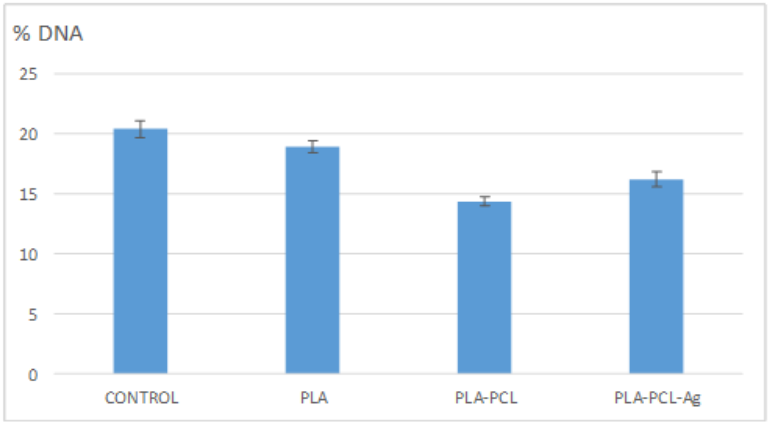

Fig. 15. Level of % DNA during cultivation of polymer films PLA, PLA-PCL, PLA-PCL-Ag (sputtering time 5 min) with peripheral blood lymphocytes

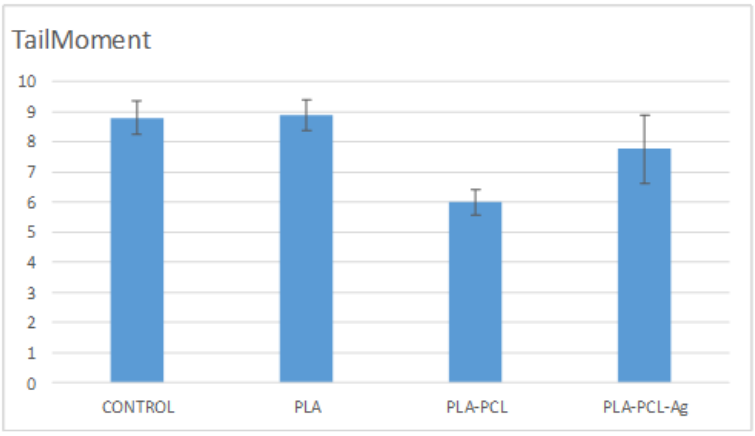

Fig. 16. TM level during the cultivation of polymer films PLA, PLA-PCL, PLA-PCL-Ag (sputtering time 5 min) with peripheral blood lymphocytes

The next histogram (Fig. 16) shows the data of the Tail Moment indicator. It was shown that the values of Tail Moment ranged from 5.99±0.42 to 8.9±0.5 in the studied samples during the simultaneous cultivation of polymer films and peripheral blood lymphocytes.

It should be noted that there is no statistically significant difference between the minimum and maximum individual TM values in the presented group.

CONCLUSIONS

In this article, film composite materials based on polylactide polymer, its mixture with polycaprolactone, and PLA-PCL mixture with sputtered silver nanoparticles were investigated at different times.

Using the method of wide-angle X-ray scattering, it has been found that the investigated film polymers are characterized by a semi-crystalline structure, and the presence of metallic Ag particles on their surface was confirmed. The analysis of the morphology of the samples showed that a layer of silver nanoparticles with a thickness of ~425 nm is formed on the surface of the PLA-PCL polymers (with a sputtering time of 5 min).

According to DSC data, it was established that when sprinkling silver on the PLA-PCL mixture, there was an increase in the degree of crystallinity χ from 35 to 39 % and the melting temperature T_m from 168 to 169–170 °C of the polymer, which is evidence of the influence of silver particles, which penetrate the polymer film to a certain depth and have a nucleating effect during polymer crystallization. At the same time, the effect of the metal layer on the amorphous phase of the polymer

is not observed, since the glass transition temperature *T*c does not change.

It was established that the best mechanical indicators, in particular mechanical elongation, were shown by the original film based on PLA-PCL.

It was shown that the polymeric materials PLA and PLA-PCL, with Ag nanoparticles, sputtered for 5 min and exhibited the highest antimicrobial effect against *S. aureus* and *E. coli*.

When studying the genotoxicity of biopolymer films using cytogenetic and molecular genetic methods, it has been found that the films do not exhibit a toxic effect.

This work was partially realized in the frame of the project from the National Research Foundation of Ukraine (Development of antimicrobial packaging biopolymer materials and technology of their welding for long-term food storage). Application ID 2022.01/0019).

Структура, теплофізичні, антимікробні та генотоксичні властивості срібловмісних плівкових нанокомпозитів, отриманих шляхом напилення

В.Л. Демченко, Є.П. Мамуня, М.В.Юрженко, С.М. Кобилінський, Н.П. Рибальченко, Т.Т. Гнатюк, Т.В. Рибальченко, А.І. Маринін, Д.В. Штепа, І.О. Ситник, О.М. Демченко, С.Р. Рушковський

Інститут електрозварювання ім. Є.О. Патона Національної академії наук України
вул. Казимира Малевича, 11, Київ, 03680, Україна, dvaleriyl@ukr.net
Інститут хімії високомолекулярних сполук Національної академії наук України
Харківське шосе, 48, Київ, 02160, Україна
Інститут мікробіології і вірусології ім. Д.К. Заболотного Національної академії наук України
вул. Академіка Заболотного, 154, Київ, 03143, Україна
Національний університет харчових технологій
вул. Володимирська, 68, Київ, 01601, Україна
Державна установа «Національний науковий центр радіаційної медицини, гематології та онкології Національної академії медичних наук України»
вул. Юрія Іллєнка, 53, Київ, 04050, Україна
Київський національний університет імені Тараса Шевченка
вул. Академіка Глушкова, 2, Київ, 02000, Україна

Для створення плівкових матеріалів з антимікробними властивостями широко використовуються наночастинки срібла, які вводяться, зокрема, в полімерні матриці. У цій роботі як полімерну основу використовували суміш полімерів полілактид-полікапролактон, взятих у масовому співвідношенні 80÷20. Ці дослідження в подальшому сприятимуть розробці нових безпечних матеріалів, зокрема при створенні пакувальних матеріалів для харчових продуктів, що на сьогодні, безперечно, є актуальною проблемою.

Метою роботи було створення срібловмісних полімерних композитів на основі суміші полілактиду і полікапролактону шляхом вакуумного напилення наночастинок срібла на поверхню полімерної матриці та вивчення структури, морфології, теплофізичних, антимікробних та генотоксичних властивостей одержаних композитів.

Напилення наночастинок срібла на поверхню суміші біополімерів ПЛА-ПКЛ виконували за допомогою приладу FC-1100 ion sputtering device (JEOL, Japan) протягом 1, 3 та 5 хв. Товщина плівок становила 110 мкм. Структура, морфологія, теплофізичні, антимікробні та генотоксичні властивості композитів, сформованих шляхом напилення наночастинок срібла на поверхню полімера, досліджено за допомогою ширококутного розсіювання рентгенівських променів на приладі XRD-7000 (Shimadzu, Японія), трансмісійної електронної мікроскопії (ТЕМ) (JEM-1230 JEOL, Японія), термогравіметричного аналізу (TGA Q50) (TA Instruments, USA), диференціальної сканувальної калориметрії (DSC Q2000) (TA Instruments, USA), а також антимікробних та генотоксичних випробувань.

Методом рентгеноструктурного аналізу було встановлено, що вихідні біополімери характеризуються напівкристалічною структурою та підтверджено наявність металічного срібла на поверхні полімера. Показано, що на поверхні суміші ПЛА-ПКЛ утворюється шар частинок срібла товщиною близько 425 нм за час напилення 5 хв.

За результатами диференціальної сканувальної калориметрії було виявлено, що при напиленні частинок срібла на поверхню біополімерів підвищується ступінь кристалічності з 35 до 39 % та температура

плавлення $T_{пл}$ з 168 до 169–170 °С. При цьому впливу металевого шару срібла на аморфну фазу полімера не зафіксовано. Виявлено антимікробну активність зразків ПЛА-ПКЛ-Ag з тривалістю напилення 3 та 5 хв щодо мікроорганізмів S. aureus та E. coli. Було встановлено, що досліджувані зразки не проявляли токсичного ефекту.

***Ключові слова**: полілактид, полікапролактон, срібловмісний композит, структура, морфологія, термічні властивості, антимікробна активність, генотоксичність*

REFERENCES

1. Demchenko V.L., Shtompel V.I. Structuring, Morphology, and Thermomechanical Properties of Nanocomposites Formed from Ternary Polyelectrolyte-Metal Complexes Based on Pectin, Polyethyleneimine, and $CuSO_4$. *Polym. Sci. Ser. B.* 2014. **56**: 927.
2. Demchenko V.L., Shtompel V.I., Riabov S.V. DC Field Effect on the Structuring and Thermomechanical and Electric Properties of Nanocomposites Formed from Pectin–Cu^{2+}–Polyethyleneimine Ternary Polyelectrolyte–Metal Complexes. *Polym. Sci. Ser. A.* 2015. **57**: 635.
3. Pavoski G., Kalikoski R., Souza G., Brum L.F.W., dos Santos C., Markeb A.A., dos Santos J.H.Z., Font X., Dell'erba I., Galland G.B. Synthesis of polyethylene/silica-silver nanocomposites with antibacterial properties by*in situ* polymerization. *Eur. Polym. J.* 2018. **106**: 92.
4. Oliveira M.M., Ugarte D., Zanchet D., Zarbin A.J. Influence of synthetic parameters on the size, structure, and stability of dodecanethiol-stabilized silver nanoparticles. *J. Colloid Interface Sci*. 2005. **292**(2): 429.
5. Rakowska P.D., Tiddia M., Faruqui N., Bankier C., Pei Y., Pollard A.J., Zhang J., Gilmore I.S. Antiviral surfaces and coatings and their mechanisms of action. *Commun. Mater*. 2021. **2**: 53.
6. Li J., Zhuang S. Antibacterial activity of chitosan and its derivatives and their interaction mechanism with bacteria: current state and perspectives. *Eur. Polym. J.* 2020. **138**: 109984.
7. Abbas M., Buntinx M., Deferme W., Peeters R. (Bio)polymer/ZnO Nanocomposites for Packaging Applications: A Review of Gas Barrier and Mechanical Properties. *Nanomaterials*. 2019. **9**(10): 1494.
8. Li G., Zhao M., Xu F., Yang B., Li X., Meng X., Teng L., Sun F., Li Y. Synthesis and Biological Application of Polylactic Acid. *Molecules*. 2020. **25**(21): 5023.
9. Singhvi M.S., Zinjarde S.S., Gokhale D.V. Polylactic acid: synthesis and biomedical applications. *J. Appl. Microbiol*. 2019. **127**(6): 1612.
10. Sikorska W., Zięba M., Musioł M., Kowalczuk M., Janeczek H., Chaber P., Masiuchok O., Demchenko V., Talanyuk V., Iurzhenko M., Puskas J.E., Adamus G. Forensic Engineering of Advanced Polymeric Materials–Part VII: Degradation of Biopolymer Welded Joints. *Polymers*. 2020. **12**(5): 1167.
11. Masiuchok O., Iurzhenko M., Kolisnyk R., Mamunya Y., Godzierz M., Demchenko V., Yermolenko D., Shadrin A. Polylactide/carbon black segregated composites for 3D printing of conductive products. *Polymers*. 2022. **14**(19): 4022.
12. Beltrán F.R., Arrieta M.P., Moreno E., Gaspar G., Muneta L.M., Carrasco-Gallego R., Yáñez S., Hidalgo-Carvajal D., de la Orden María U., Urreaga J.M. Evaluation of the Technical Viability of Distributed Mechanical Recycling of PLA 3D Printing Wastes. *Polymers*. 2021. **13**(8)*:* 1247.
13. Nootsuwan N., Sukthavorn K., Wattanathana W., Jongrungruangchok S., Veranitisagul C., Koonsaeng N., Laobuthee A. Development of Antimicrobial Hybrid Materials from Polylactic Acid and Nano-silver Coated Chitosan. *Orient. J. Chem*. 2018. **34**(2): 683.
14. Velgosova O., Mačák L., Lisnichuk M., Vojtko M. Synthesis and Analysis of Polymorphic Silver Nanoparticles and Their Incorporation into the Polymer Matrix. *Polymers*. 2022. **14**(13): 2666.
15. García B.O., Kharissova O.V., Dias R., Aguirre-Tostado F.S., Leyva C., González L.T., Kharisov B.I. A Comparison of Different Methods of MWCNTs Metalation in a Single Step Using Three Different Silver-containing Compounds. *Recent Pat. Nanotechnol*. 2019. **13**(1): 59.
16. Tatarchuk V.V., Sergievskaya A.P., Korda T.M., Druzhinina I.A., Zaikovsky V.I. Kinetic Factors in the Synthesis of Silver Nanoparticles by Reduction of Ag^+ with Hydrazine in Reverse Micelles of Triton N-42. *Chem. Mater.* 2013. **25**(18): 3570.
17. Gupta A., Briffa S.M., Swingler S., Gibson H., Kannappan V., Adamus G., Kowalczuk M., Martin C., Radecka I. Synthesis of Silver Nanoparticles Using Curcumin-Cyclodextrins Loaded into Bacterial Cellulose-Based Hydrogels for Wound Dressing Applications. *Biomacromolecules*. 2020. **21**(5): 1802.

18. Clinical and Laboratory Standards Institute NCCLS. Performance standards for antimicrobial susceptibility testing; fifteenths informatonal supplement. CLSI/NCCLS document M100-S15. Clinical and Laboratory Standards Institute. Wayne. PA. 2005.
19. Brouillard J.E., Terriff C.M., Tofan A., Garrison M.W. Antibiotic selection and resistance issues with fluoroquinolones and doxycycline against bioterrorism agents. *Pharmacotherapy*. 2006. **26**(1): 3.
20. Rodriguez-Garraus A., Azqueta A., Vettorazzi A., López de Cerain A. Genotoxicity of Silver Nanoparticles. *Nanomaterials*. 2020. **10**(2): 251.
21. Shtompel' V.I., Kercha Yu.Yu. *Structure of Linear Polyurethanes*. (Kiyv: Naukova dumka, 2008). [in Russian].
22. Borukaev T.A., Shaov A.Kh., Kharaev A.M., Borodulin A.S. Influence of the compatibilizer on the properties of composites based on low density polyethylene and polybutylene terephthalate. *E3S Web of Conferences*. 2023. **413**: 1.
23. Körber S., Moser K., Diemert J. Development of high temperature resistant stereocomplex PLA for injection moulding. *Polymers*. 2022. **14**(3): 384.

Received 30.07.2024, accepted 05.03.2025