

Biostable Composite Materials Filled with Silver-Containing Silica Nanocomposites with Antibacterial Properties

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Abstract: The ability of biodegradation and the expression of biological activity *in vitro* are among the most important characteristics of polymers for medical purposes, which will make it possible to predict the behavior of polymers *in vivo*. Therefore, this article is devoted to the study of the biodegradability of composite materials based on PUU filled with silver-containing silica nanocomposites of various compositions (02AgCu, AgCu, 01Ag) in different amounts (0.1, 0.5, and 1.0 wt.%) under the influence of biological medium 199 (BM 199) for 1, 3 and 6 months as potential biopolymers and the study of their antibacterial properties. The biodegradation ability was assessed by changes in structure, physical-mechanical, thermophysical and thermogravimetric characteristics. The results of IR spectroscopic studies demonstrate the absence of changes in the absorption bands of functional groups before and after incubation in BM 199, which indicates the stability of polymer materials under the influence of BM 199. According to physical-mechanical studies after 6 months of incubation in BM 199 the strength values are greater than the control values, and the values of relative elongation at break does not undergo significant changes compared to the control values. It also allows us to conclude about the biostability of composite materials for 6 months. According to the results of tests by the DSC method, after 6 months of incubation in BM 199 the values of T_g and ΔC_p at the glass-transition temperature do not undergo significant changes, which also indicates the biostability of the studied materials. Using the TGA method, it was established that after incubation in BM 199 composite materials *in vitro* remain heat-resistant materials. According to the results of microbiological studies, all filled film materials have antibacterial properties against the most common gram-positive and gram-negative bacteria, which are manifested by the formation of zones of bacterial growth retardation with a diameter of 14-31 mm. Thus, composite materials filled with silver-containing silica nanocomposites are biostable for 6 months without significant changes in their structure and properties under the influence of biological medium for a long period of time and exhibit antibacterial activity. This makes it possible to use them as biologically active polymer materials of long-term use in various branches of medicine.

Keywords: Composite Materials, Silver-Containing Silica Nanocomposites, Biodegradation, Biological Medium 199

1. Introduction

Film materials filled with silver-containing silica nanocomposites obtained based on polyurethane-urea (PUU) with fragments of poly(vinylbutyral-vinylacetate-vinyl alcohol) copolymer and macrochain extender 1,6-hexamethylenediamine in the structure [1, 2] are biocompatible and can be proposed for the manufacture of

catheters, drains and various film coatings for use in various branches of medicine [3]. Therefore, further research is required, in particular, the study of the ability to biodegradation and biological activity.

The ability to biodegradation in the conditions imitating the environment of the organism is one of the most important characteristics of polymers for medical use as this process is accompanied by changes in the structure of the polymer which cause changes of properties and it can influence on the amount

of released drug to the internal environment of an organism.

Experimental study of terms of polymer biodegradation in the body using labeled preparations is a time-consuming procedure. Estimation of effective rates of biodegradation based on polymer weight loss is also difficult due to the lack of sufficiently clear methods for separation of the implant from connective tissues and experimental errors. Therefore, predictions of the polymer biodegradability *in vivo* are based on the results of *in vitro* studies.

The biodegradation processes are influenced by such factors as the chemical composition of polymer, the hydrophilicity of polymer matrix, the presence of drug substance and the drug type etc. [4, 5]. So, the introduction of drugs into the composition of polymer materials can affect the rate of biodegradation processes [6]. For example, the introduction of tiamulin fumarate into the composition of composite materials based on polyurethane-urea accelerates the biodegradation of the polymer base [7]. At the same time, due to the presence of amizon in the composition of block-copolyurethane the polymer materials remain stable [8]. Hydrophilic drugs accelerate the polymer degradation by facilitating the water penetration in the system. In contrast, hydrophobic drugs slow down the polymer degradation by hindering the water diffusion into the matrix [5].

Therefore, there is a need to study the ability to biodegradation *in vitro*. Results of these researches will allow predicting behaviour of polymer materials *in vivo* at their further application in medical practice.

Modern medicine needs new biologically active polymer materials with stable antimicrobial properties that will ensure suppression of infection in the product itself and the focus of inflammation. Silver-containing silica nanocomposites are composites obtained by mechanochemical modification of the surface of pyrogenic silica with Ag and Cu compounds (Ag nitrate and Cu acetate) according to the method [9, 10]. Synthesized nanocomposites with different concentrations of Ag and Cu per 1g of SiO₂ containing metal oxides and nanoparticles of metallic silver exhibit an antimicrobial effect [11]. Therefore, an important step in our work is also the study of antimicrobial properties of the composite materials.

Considering the aforesaid, the purpose of the work is to study the ability to biodegradation of composite materials filled with silver-containing silica nanocomposites based on polyurethane-urea by changing their structure, physical-mechanical, thermophysical and thermogravimetric characteristics under the influence of model medium for 1, 3 and 6 months and to study their antibacterial activity.

2. Experimental

2.1. Materials

The objects of research were polyurethane-ureas (PUU) with copolymer fragments of poly(vinyl butyral-vinyl acetate-vinyl alcohol) (PVB) and polymer chain extender 1,6-hexamethylenediamine (HMDA) in the structure synthesized at percentages ratio of HMDA:PVB as 30:70

similarly according to the method [12] and film materials on their based filled with silver-containing silica nanocomposites of various compositions (02AgCu, AgCu, 01Ag) and different amounts (0.1, 0.5, and 1.0 wt.%) [1, 2].

Biological medium 199 (BM 199) (BioTestLab, Ukraine, pH 7.4-7.7) were selected as a model medium to study the ability to biodegradation. BM 199 imitates the blood plasma and is a complex mixture of proteins, amino acids, carbohydrates, fats, salts, hormones, enzymes and soluble gases.

2.2. The Method of Incubation in BM 199

Samples were placed in sterile tubes, poured 25 ml of model medium and kept in a thermostat at a temperature of $(37 \pm 1)^{\circ}\text{C}$ for 1, 3 and 6 months. Solutions of model mediums were changed daily. After defined incubation terms in the model medium, the samples were taken out, washed with distilled water and dried to a constant mass at room temperature.

2.3. Study Methods

2.3.1. Fourier Transforms Infrared (ATR FTIR) Spectroscopy

The structure was investigated on a Tensor-37 FTIR spectrometer in the range of 650–4000 cm⁻¹ by the MATR method with the aid of a diamond crystal trapezoidal prism (a number of reflections of $N = 1$, an incidence angle of $\phi = 39^{\circ}$). The absorption bands were assigned as described in [13].

2.3.2. Mechanical Tests

The physical-mechanical properties such as tensile strength at break (σ , MPa) and relative elongation at break (ϵ , %) of PUU and film materials on their basis were determined on the tension testing machine P5 as described in [14].

2.3.3. Differential Scanning Calorimetry (DSC)

Thermophysical properties (glass-transition temperature (T_g), changes of the heat capacity at the glass-transition temperature (ΔC_p)) have been studied by the DSC method. The study has been carried out within the interval of temperature from -90 to +200°C (TA Instrument Q2000) at a heating rate 20°C/min under nitrogen atmosphere. Two heating procedures have been carried out to exclude the influence of the thermal and mechanical prehistory of the material.

2.3.4. Thermal Gravimetric Analysis (TGA)

Thermogravimetric characteristics (onset temperature of thermal decomposition (T_0), temperature of maximum decomposition rate (T_{\max}), weight loss at T_0) were studied by TGA. The study has been carried out within the interval of temperature from +20 to +700°C (TA Instrument Q50) at a heating rate 20°C/min under an air atmosphere.

2.3.5. Microbiological Tests

Microbiological researchers studied the bactericidal activity of film materials in relation to the most widespread gram-positive and gram-negative bacteria that contaminated the surface of the wound, causing its infectious lesion. The researches were conducted using the disco-diffusion method

according to the Order of the Ministry of Health of Ukraine No. 167 dated April 5, 2007 “On Approval of Training Guidance “Assessment of the sensitivity of microorganisms to antibiotics” and the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Disks (samples of the studied film materials) placed in the Petri dish with nutrient medium, and the microbial loading is $5 \cdot 10^7$ colony forming units in 1 ml. Calculations carried out after an incubation of crops in the thermostat at a temperature of $+36.6^\circ\text{C}$ from 1 to 48 hours. The bactericidal action of samples was evaluated by the presence or absence of microbial growth under and around the disks that is result of the diffusion of fillers. At measuring zone of inhibition of growth around the disks were guided by the zone of full inhibition of growth of microorganisms that is determined by the naked eye. The diameter of the zones of growth inhibition was measured with accuracy to 1 mm.

3. Results and Discussion

To study the biodegradability, PUU and composite materials based on PUU filled with silver-containing silica nanocomposites of various composition (0.2AgCu, AgCu, 0.1Ag) and different amounts (0.1, 0.5 and 1.0 wt.%) were incubated in model medium (BM 199) for periods of 1, 3 and 6 months. The influence of the model medium was evaluated by changes in the structure, physical-mechanical, thermogravimetric and thermophysical properties of polymer materials by comparing the samples before (control) and after their incubation.

3.1. FTIR Spectroscopy

According to IR spectroscopy, in the frequency interval of valence vibrations of NH groups $3000\text{--}3700\text{ cm}^{-1}$ on the spectra of PUU (Figure 1) and composite materials filled with silver-containing silica nanocomposites (Figure 2, Figure 3, Figure 4) there are characteristic absorption bands of $\nu_{\text{NH-bond}}$ – 3304 cm^{-1} and $\nu_{\text{NH-free}}$ with an approximate maximum of 3508 cm^{-1} in both control and samples incubated in BM 199.

In the range of $1500\text{--}1800\text{ cm}^{-1}$ of IR spectra of PUU (Figure 1) and filled composite materials (Figure 2, Figure 3, Figure 4) there is a characteristic absorption band with a maximum at 1725 cm^{-1} , which corresponds to vibrations of $\nu_{\text{C=O}}$ of the copolymer PVB and $\nu_{\text{C=O}}$ of urethane fragment, an absorption band with a maximum at 1630 cm^{-1} , which refers to the vibrations of $\nu_{\text{C=O}}$ and δ_{NH} urea groups and an absorption band with a maximum at 1536 cm^{-1} , which corresponds to the δ_{NH} vibrations of the urethane fragment. These absorption bands are similar for both control and samples incubated in BM 199.

In the IR spectra interval of $1000\text{--}1300\text{ cm}^{-1}$, which is responsible for vibrations of $\nu_{\text{C-O}}$ of the urethane fragment for PUU (Figure 1) and filled composite materials (Figure 2, Figure 3, Figure 4) there are characteristic absorption bands with a maximum of 1226 cm^{-1} , 1090 cm^{-1} , 1001 cm^{-1} in both control and samples incubated in BM 199.

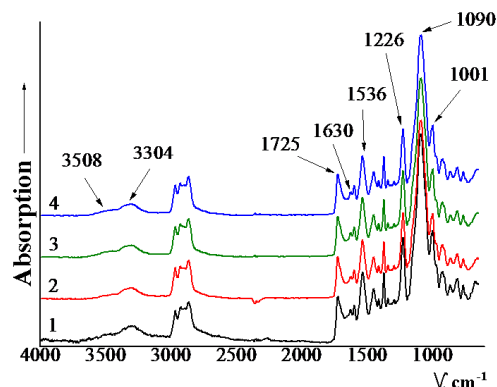


Figure 1. IR spectra of PUU before (1) and after incubation in BM 199 for 1 month (2), 3 months (3), 6 months (4).

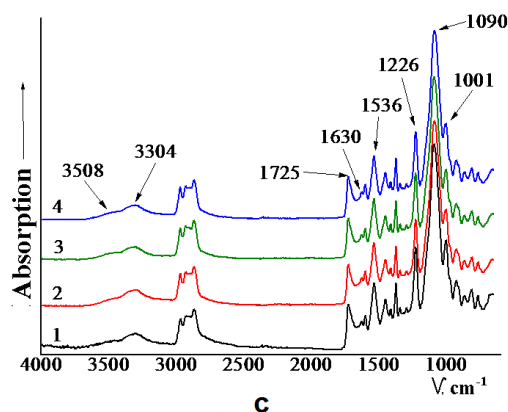
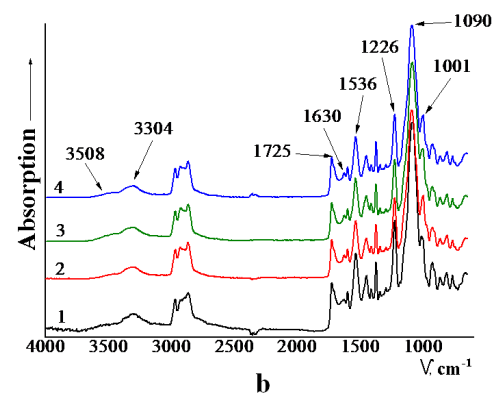
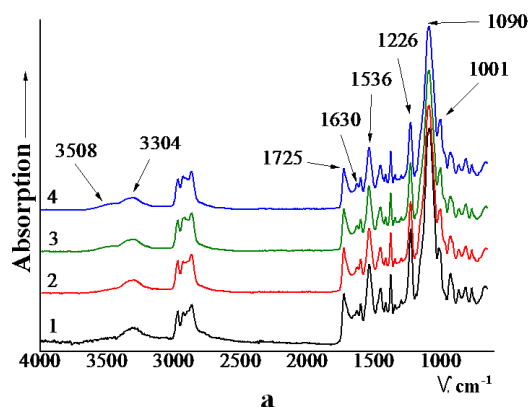


Figure 2. IR spectra of composite materials with different 0.2AgCu content: 0.1 (a), 0.5 (b) and 1.0 wt.% (c) before (1) and after incubation in BM 199 for 1 month (2), 3 months (3), 6 months (4).

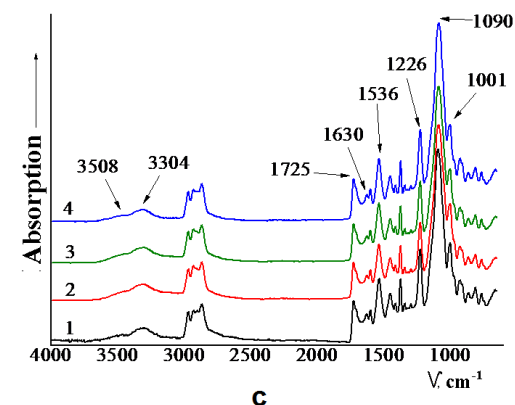
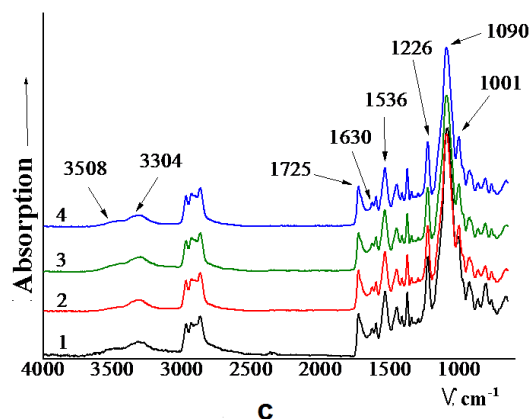
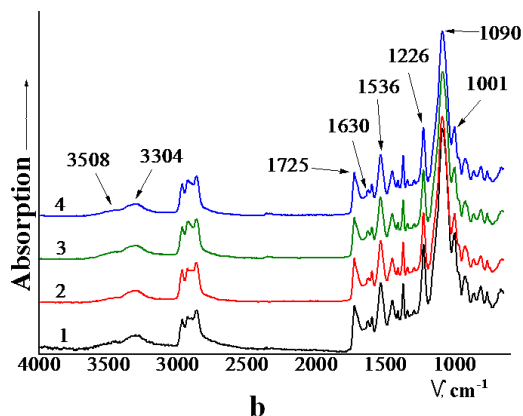
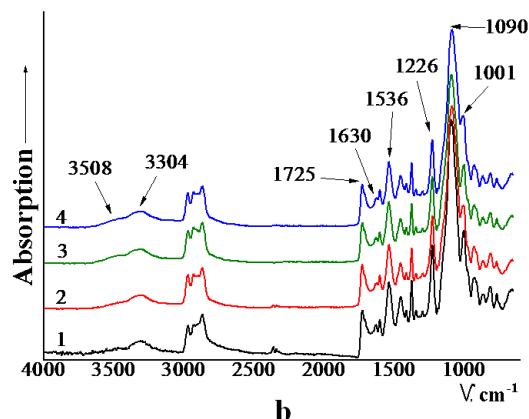
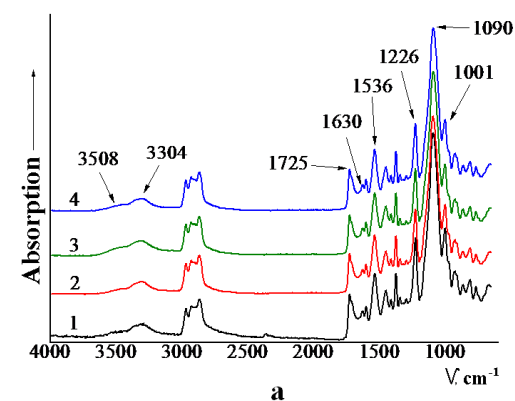


Figure 3. IR spectra of composite materials with different AgCu content: 0.1 (a), 0.5 (b) and 1.0 wt.% (c) before (1) and after incubation in BM 199 for 1 month (2), 3 months (3), 6 months (4).

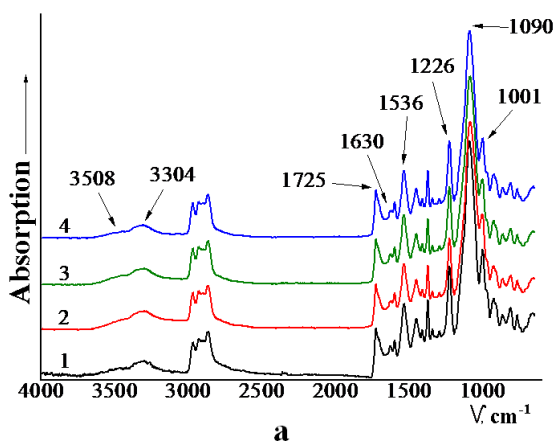


Figure 4. IR spectra of composite materials with different O1Ag content: 0.1 (a), 0.5 (b) and 1.0 wt.% (c) before (1) and after incubation in BM 199 for 1 month (2), 3 months (3), 6 months (4).

Therefore, significant changes in the absorption bands of valence or deformation vibrations of functional groups before and after incubation in BM 199 were not observed on the spectra of all samples. Therefore, the results of IR spectroscopic studies indicate the stability of composite materials under the influence of model biological medium 199 at all study periods.

3.2. Mechanical Tests

According to the results of physical-mechanical studies, the tensile strength of PUU before incubation is 0.49 MPa, while after 6 months of incubation in BM 199 it is 5.91 MPa. The tensile strength of composite materials filled with silver-containing silica nanocomposites before incubation is 0.51–0.81 MPa, while after 6 months of incubation in BM 199 it is in the range from 4.59 MPa to 6.24 MPa. Therefore, after incubation in BM 199 there is an increase in the strength characteristics of both PUU and filled composite materials.

It should be noted that the increase in tensile strength at break occurs already after 1 month of incubation. Further stay in the model medium for up to 6 months indicates a gradual decrease in strength compared to the previous period (Figure 5a). Considering that BM 199 is a complex multicomponent biological system, the increase in tensile strength after 1 month of incubation can be explained by the specific interaction of enzyme molecules with urethane groups of the polymer matrix [15].

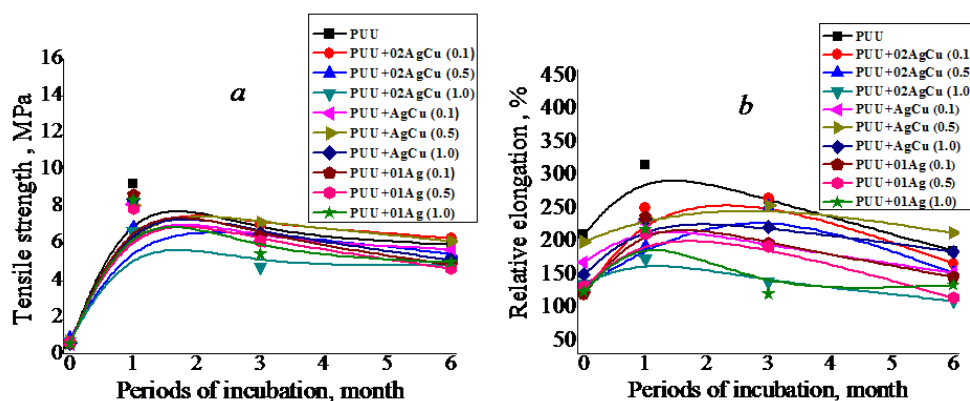


Figure 5. Dependence of tensile strength (a) and relative elongation (b) at break of PUU and filled composite materials on the term of their incubation in BM 199.

The relative elongation at break of PUU before incubation is 210%, while after 6 months of incubation in BM 199 it is 185%. For composite materials filled with silver-containing silica nanocomposites relative elongation before incubation is 118-199%, while after 6 months of incubation in BM 199 it is in the range from 110 to 212%.

After incubation in BM 199 an increase in the relative elongation at break is observed already after 1 month of incubation (Figure 5b). This can also be explained by the specific interaction of enzyme molecules with urethanegroups of the polymer matrix [15]. Further stay in the model medium for up to 6 months indicates a gradual decrease in values compared to the previous period.

Thus, according to the results of physical-mechanical studies of composite materials an increase in strength and relative elongation at break is observed already after 1 month of incubation, followed by their decrease compared to the

previous period. After 6 months of incubation in BM 199 the strength values are greater than the control values, and the values of relative elongation at break does not undergo significant changes compared to the control values. Thus, composite materials filled with silver-containing silica nanocomposites remain biostable for 6 months.

3.3. Thermal Gravimetric Analysis

According to TGA, onset temperature of thermal decomposition (T_0) of PUU before incubation is 197.34°C, while after 6 months of incubation in BM 199 it is 198.78°C. For filled composite materials based on PUU before incubation T_0 is 166.16-194.32°C, after 6 months of incubation it is 192.23-206.60°C (Table 1). Consequently, under the influence of BM 199 an increase in T_0 is observed for all studied samples.

Table 1. Thermogravimetric characteristics of PUU and composite materials filled with silver-containing silica nanocomposites after incubation in BM 199.

Samples	Periods of incubation	T_0 , °C	T_{max} , °C	Weight loss at T_0 , %
PUU	control	197.34	346.91	0.86
	1 month	211.60	358.73	1.34
	3 months	195.88	326.65	0.97
	6 months	198.78	324.64	5.43
PUU +02AgCu (0.1)	control	166.16	318.27	0.52
	1 month	191.35	319.22	0.77
	3 months	184.01	325.01	0.97
	6 months	192.23	322.97	1.07
PUU +02AgCu (0.5)	control	194.28	327.46	0.52
	1 month	189.29	323.38	0.92
	3 months	193.60	315.88	0.77
	6 months	206.12	327.79	1.14
PUU +02AgCu (1.0)	control	194.04	315.20	0.46
	1 month	194.61	328.05	0.65
	3 months	189.30	315.7	0.93
	6 months	195.82	325.72	1.37
PUU +AgCu (0.1)	control	181.24	331.21	0.60
	1 month	192.77	328.31	1.11
	3 months	191.62	317.64	0.96
	6 months	198.73	336.18	1.07
PUU +AgCu (0.5)	control	166.63	386.35	0.59
	1 month	178.86	319.73	0.73
	3 months	190.23	318.73	0.89
	6 months	196.25	322.23	0.76

Samples	Periods of incubation	T_0 , °C	T_{max} , °C	Weight loss at T_0 , %
PUU +AgCu (1.0)	control	171.77	330.92	0.35
	1 month	189.94	323.24	0.63
	3 months	186.57	312.75	0.54
	6 months	206.60	321.62	0.94
PUU +01Ag (0.1)	control	194.32	357.78	0.48
	1 month	199.90	350.75	1.22
	3 months	181.02	313.35	0.90
	6 months	197.79	325.12	0.94
PUU +01Ag (0.5)	control	185.44	357.72	0.41
	1 month	194.59	324.09	1.24
	3 months	189.15	397.13	0.85
	6 months	194.3	333.93	0.90
PUU +01Ag (1.0)	control	182.03	313.12	0.97
	1 month	179.46	314.88	0.81
	3 months	186.63	369.54	0.93
	6 months	194.50	331.89	1.00

T_0 accompanied by a slight weight loss for all samples. For PUU before incubation the weight loss is 0.86%, after 6 months of incubation in BM 199 it is 5.43%. For filled composite materials the weight loss before incubation is 0.35-0.97%, after 6 months it is 0.76-1.37%. Therefore, after incubation in BM 199 there is an increase in weight loss for all the studied samples, while for filled composite materials this is a rather insignificant increase.

The temperature of maximum decomposition rate (T_{max}) after incubation in BM 199 for PUU before incubation is 346.91°C, after incubation it ranges from 324.64 to 358.73°C at different study periods. For filled composite materials T_{max} before incubation is 313.12-386.35°C, after incubation it is in the range from 313.35 to 397.13°C (Table 1). T_{max} depends on the incubation period and changes non-linearly.

Consequently, according to the results of TGA after incubation in model medium an increase in the values of T_0 is observed for both PUU and filled composite materials. Thus, after incubation in BM 199 the composite materials remain heat-resistant materials.

3.4. Differential Scanning Calorimetry

According to DSC, the T_g of the 2nd heating procedure for PUU before incubation in BM 199 is -23.39°C, while after 6 months of incubation it is -24.49°C. For filled composite materials the T_g before incubation is in the range from -23.41°C to -27.43°C, after 6 months of incubation it is in the range from -24.00°C to -25.27°C (Figure 6a). Therefore, T_g changes non-linearly depending on the incubation period, and after 6 months it does not undergo significant changes.

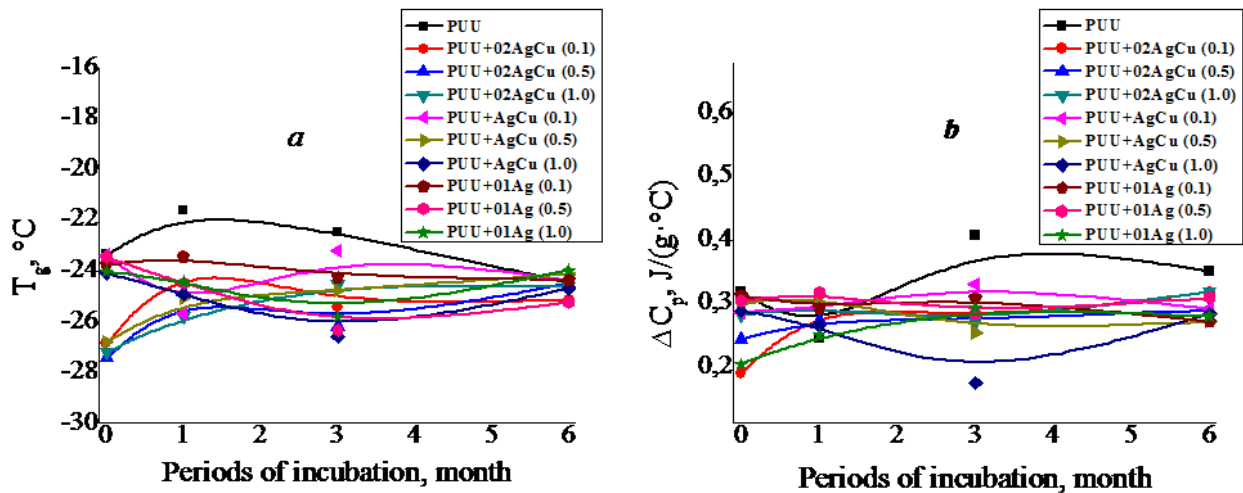


Figure 6. Dependence of T_g (a) and ΔC_p (b) at the glass-transition of PUU and filled composite materials on the term of their incubation in BM 199.

The value of ΔC_p at the glass-transition at the second heating procedure for PUU before incubation is 0.3165, while after incubation it is in the range of 0.2435-0.4054 at different study periods. For filled composite materials before incubation ΔC_p is 0.1876-0.3107, after incubation it is 0.1728-0.3287 (Figure 6b). Therefore, ΔC_p at the glass-transition temperature also changes non-linearly depending on the incubation period, and after 6 months it does not

undergo significant changes.

Therefore, according to DSC, after 6 months of incubations in BM 199 the values of T_g and ΔC_p at the glass-transition temperature do not undergo significant changes compared to the control values. Thus, composite materials filled with silver-containing silica nanocomposites remain biostable for 6 months.

3.5. Microbiological Tests

Microbiological studies of bactericidal activity against the most common gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus flavus*) and gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica*, *Proteus vulgaris*) were

carried out. PUU which do not contain silver-containing silica nanocomposites were used as a control.

It was established that the polymer film without filler does not affect the proliferation of bacteria, while studies of filled composite materials showed the formation of zones of growth retardation of bacteria with a diameter of 14-31 mm (Table 2).

Table 2. Bactericidal activity of composite materials filled with silver-containing silica nanocomposites.

Test cultures	Zones of growth inhibition, mm			
	composite materials filled with nanocomposite			control
	02AgCu	AgCu	01Ag	
gram-negative bacteria				
<i>Pseudomonas aeruginosa</i> B-900	19	14	14	0
<i>Escherichia coli</i> B-906	30	27	27	0
<i>Salmonella enterica</i> B-921	28	22	23	0
<i>Proteus vulgaris</i> B-905	31	30	31	0
gram-positive bacteria				
<i>Bacillus subtilis</i> B-901	24	22	19	0
<i>Staphylococcus aureus</i> B-918	22	18	18	0
<i>S. epidermidis</i> B-919	28	20	19	0
<i>Micrococcus flavus</i> Ac-634	29	24	25	0

The most effective in the composition of film materials is the silica filler 02AgCu. For composite materials filled with nanocomposite 02AgCu the formation of zones of bacterial growth retardation with a diameter of 19-31 mm is observed.

Considering the obtained results, composite materials filled with silver-containing silica nanocomposites exhibit antibacterial activity, so they can be proposed for the manufacture of catheters, drains and various film coatings for use in various branches of medicine.

Considering the obtained results, composite materials filled with silver-containing silica nanocomposites can be proposed for follow-up biomedical research.

4. Conclusions

The biodegradation ability of composite materials based on PUU filled with silver-containing silica nanocomposites of different compositions (02AgCu, AgCu, 01Ag) in different amounts (0.1, 0.5, and 1.0 wt.%) *in vitro* was investigated. The influence of BM 199 during 1, 3, and 6 months was evaluated by changes in structure, physical-mechanical, thermophysical, and thermogravimetric characteristics. According to the results of studies, after incubation in BM 199 composite materials *in vitro* remain heat-resistant materials. The test results allow concluding the biostability of composite materials for 6 months without significant changes in their structure and properties under the influence of biological medium, which makes their long-term use possible. Microbiological studies have established that all composite materials filled with silver-containing silica nanocomposites have antibacterial properties, manifested by the formation of zones of growth retardation of bacteria with a diameter of 14-31 mm. Therefore, they can be proposed for the manufacture of catheters, drains and various film coatings for long-term use in various branches of medicine.

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