Metal-Polymer Complexes as Antibacterial Agents: Characterization and Antibacterial Activity of Metal-Polymer Nanocomposite Based on Copolymers of Polyethylene(Propylene)Glycolmaleates with Acrylic Acid

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Metal-polymer complexes as antibacterial agents: characterization and antibacterial activity of metal-polymer nanocomposite based on copolymers of polyethylene(propylene)glycolmaleates with acrylic acid

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Abstract. The article presents the results of the synthesis and study of the structure of metal-polymer complexes p-EGM: AA/Ag, p-EGM: AA/Ni, p-EGM:AA/Ag-Ni, p-PGM:AA/Ag, p-PGM:AA/Ni, p-PGM:AA/Ag-Ni based on copolymers of polyethylene(propylene)glycolmaleates with acrylic acid and metals, which have been characterized using microscopy, spectroscopy and thermogravimetry. The antimicrobial activity of a new metal-polymer nanocomposite p-PGM/AA-Ag was studied, which is showing high efficiency against standard strains of *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus.*

Keywords. polyethylene glycol maleate, polypropylene glycol maleate, metal-polymer nanocomposite, antimicrobial activity, *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus*

1. Introduction

In recent years, various types of nanoscale structures have been created including metal nanoparticles in the composition of building blocks in combination with functional polymers. Such particles, possessing a significant reactivity and an increased tendency to ion exchange, upon immobilization in polymer matrices, impart new properties to the obtained nanocomposites. The stability of nanoparticles in polymer matrices is maintained for a long time.

Fundamental applied research in large-scale implementation with the aim of developing innovative materials and technologies, especially nanocomposites is an urgent task and is of great scientific and practical interest [1, 2].

Using metal nanoparticles with bactericidal properties immobilized in polymers is possible to obtain medical materials with a wide spectrum of action: implants, drug delivery systems, antibacterial coatings for biomedical devices, and antimicrobial packaging.

Previously, by immobilizing metal particles into substrates of p-EGM (PGM):AA copolymers, new mono- and bimetallic polymer complexes p-EGM: AA / Ag, p-EGM: AA/Ni, p-EGM: AA/Ag – Ni were synthesized, p-PGM: AA/Ag, p-PGM: AA/Ni, p-PGM: AA/Ag – Ni. The possibility of using copolymers of poly- (ethylene) -propylene glycol maleates (p-EGM and p-PGM) with acrylic acid (AA) as a matrix for obtaining effective metal-polymer complexes for the hydrogenation of organic compounds is shown. It has been

proven that when synthesized catalysts are used, the reaction can be carried out under mild conditions with a high yield.

Reuse of composites results in only a small loss of activity. Microscopy methods determined the average size of nanoparticles, which was 112 nm, having a spherical shape and a uniform distribution along the cross section of the polymer. The content of nickel and cobalt in the complexes is 0.52 and 0.48 wt%, respectively, in the p-EGM / AA copolymer, 0.49 and 0.51 wt% in the p-PGM / AA copolymer. On the basis of p-EGM (PGM): AA copolymers, selective catalysts can be obtained, as well as matrices for the inclusion of medical compounds to ensure delivery and prolonged action [3, 4].

In this work with the purpose of controlling the size of particles of metals changed the conditions of synthesis: 1. Selected polymer matrix with molecular weight ~ 2600, with a degree of swelling ~ 1800-2000%. 2. The conditions of the MPC synthesis have been changed: the concentration of salts of metals is 0.5 N and the concentration of the regenerator is 0.5 N.

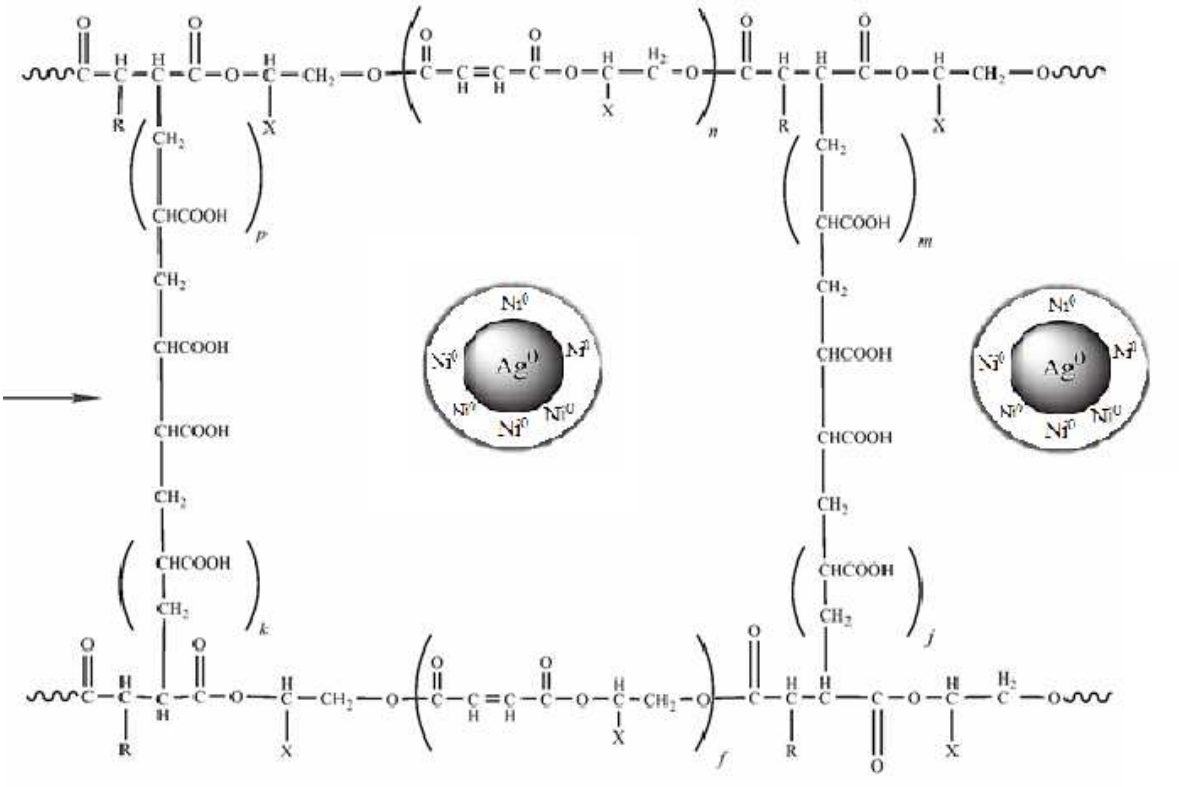
The main purpose of this work is to study the physicochemical properties and antibacterial activity of nanocomposites based on the "Smart" polymer matrix p-EGM (p-PGM)/AA with metals (Ag, Ni).

2. Experimental

2.1 Materials and Methodology

Synthesis of metal–polymer composites p-EGM: AA/Ag, p-EGM: AA/Ni, p-EGM: AA/Ag-Ni, p-PGM:AA/Ag, p-PGM: AA /Ni, p-EGM: AA /Ag -Ni, p-PGM: AA/Ag-Ni. Immobilization of metal particles into substrates of p-EGM:AA copolymers with a composition of 14.8: 85.2 wt% and p-PGM: AA 15.1: 84. 9 wt% was carried out by the reduction of Ni^{+2}, Ag^{+1} to Ni^0, Ag^0 with sodium hypophosphite (0.5 N) in a 0.5 N solution of their nitrates, in the presence of a catalyst - an ammonia solution of silver chloride. Reduction of Ni^{+2}, Ag^{+1} in the volume of the polymer matrix p-EGM: AA, p-PHM: AA is carried out in several stages: the introduction of Ni^{+2}, Ag^{+1} ions into the polymer matrix; diffusion of reagents inside the polymer matrix; the reaction between nitrates of nickel, silver and sodium hypophosphite, which proceeds with the formation of nanosized metal particles (NMP). The main disadvantage of this method is the free migration of nanoparticles in the volume of the gel and their leaching into the environment due to the large pore size of the network. When pH- and thermosensitive hydrogels p-EGM (PGM): AA are used as a hydrogel matrix, which can change their volume-phase properties depending on external stimulating factors, it will be possible to finely regulate the morphology and pore size of the

spatial network, which ultimately leads to a targeted controlling the behavior of metal nanoparticles. Figure 1 shows the main structural fragments of p-EGM: AA/Ag-Ni (a), p-EGM: AA/Ag (b).



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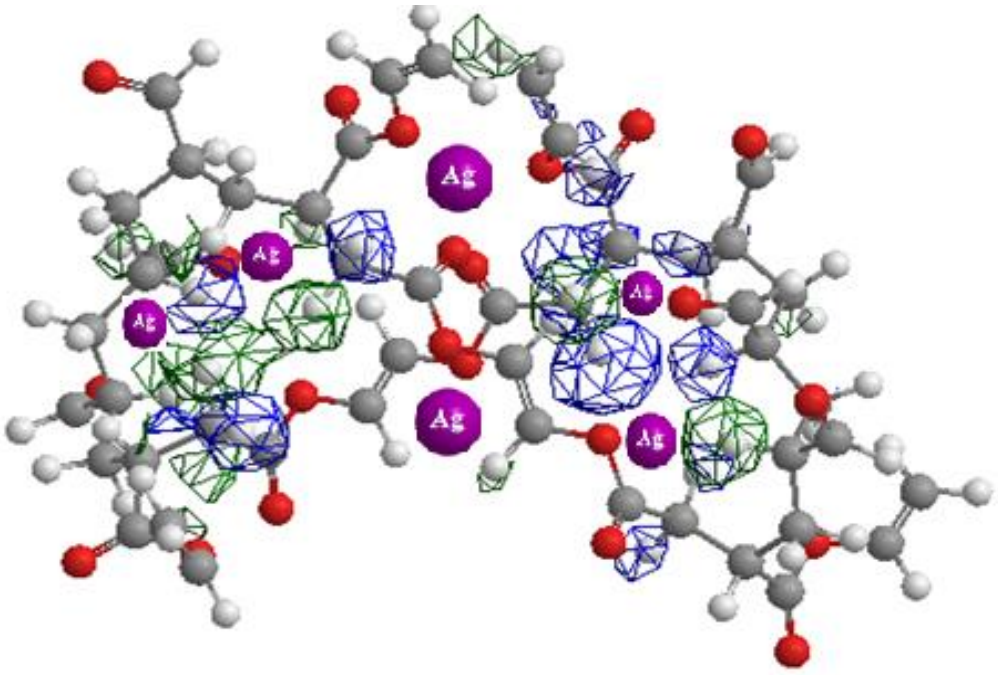

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Figure 1. Structural fragments of the bi-, monometallic polymer complex p-EGM: AA/Ag-Ni (a), p-EGM: AA/Ag (b)

2.2 Study of the structure of polymer and nanocomposites

An image of an ultrathin sample was obtained using a universal thermal-field transmission electron microscope (TEM) JEOL JEM-2100 200 kV (Japan); the studies were carried out on the basis of the Department of Analytical and Inorganic Chemistry of Karlov University (Czech Republic). The resolution at the optimal working distance is 0.8 nm at 2 kV, at the convergence point - 1.2 nm at 1 kV (Fig. 2).

The analysis of the molecular weight characteristics of the polymers was carried out by gel permeation chromatography in dioxane on a PolymerLabs GPC-120 chromatograph with two PLgel columns. Molecular weight (MW) was calculated by calibration against standards.

The study of the structure, morphology, and elemental composition of the synthesized complexes was established by microscopy on a SEM MIRA 3TESCAN "Oxford Instruments" (2012) with a highly efficient silicon-drift detector X-Act for elemental analysis at an accelerating voltage of 20 kV (Fig. 3.4).

The thermal stability of the composites was investigated thermogravimetrically on a synchronous TGA/DTA/DSC analyzer LabSYSEvo (2014) in the temperature range of 30–1000 ° C in an aluminum oxide crucible at a heating rate of 5°C / min, in air with a flow rate of 30 ml / min, by decomposing a sample with weighing 20 mg (Fig. 5).

The amount of adsorbed metal in the complex was determined on a 4210 MP-AES atomic emission spectrometer (Agilent Technologies Bayan Lepas Free, Malaysia), based on the determination of the elemental composition of the substance from the optical emission spectra of atoms and ions of the analyzed sample, excited by nitrogen plasma.

2.3 Study of antibacterial activity

The antibacterial effect of the initial polymers and synthesized nanocomposites was studied using standard strains of microorganisms - *Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922),* obtained from the National Collection of Microorganisms RSE (Nursultan). The research was carried out on the basis of the Scientific Research Center of the Karaganda Medical University. A modified Kirby-Bauer disk diffusion method was used under standard conditions, Mueller-Hinton agar culture medium. The base concentration of polymer solutions was 10% m/m, which were subsequently diluted with two-fold dilutions to a concentration of 0.001% m/m.

100 mdm^3 of test bacteria were grown in 10 cm^3 of fresh medium until the concentration reached 105-108 cells/cm^3. Next, the specified volume of microbial suspension was

distributed in a Petri dish with agar medium, according to the calculated concentrations in a volume of 100 µl. Gram negative bacteria were incubated at 35-37°C for 48 hours, the surrounding sample was calculated as% inhibition i.e. zones of sample inhibition [5, 6].

The results were evaluated according to the size and purity of the zone of inhibition of bacterial growth according to a 5-point system. Where "0" is the absence of inhibition of bacterial growth, and "5" is the complete absence of growth at the site of application of the solution with the expansion of the growth inhibition zone beyond the area of application of the sample.

3. Results and Discussion

For the synthesis of the polymer matrix, polyethylene (propylene) glycolmaleate with a molecular weight of ~ 2600 c.u. was used as the initial monomer. The molecular mass of the polymer affects the degree of swelling of the copolymer, which in turn determines the pore size of the polymer matrix. The degree of swelling of the polymers was ~ 1800-2000%. Since the structure of the polymer matrix has a significant effect on the crystal structure and morphology of the resulting metal NPs, we present electron micrographs of copolymers in Figure 2.

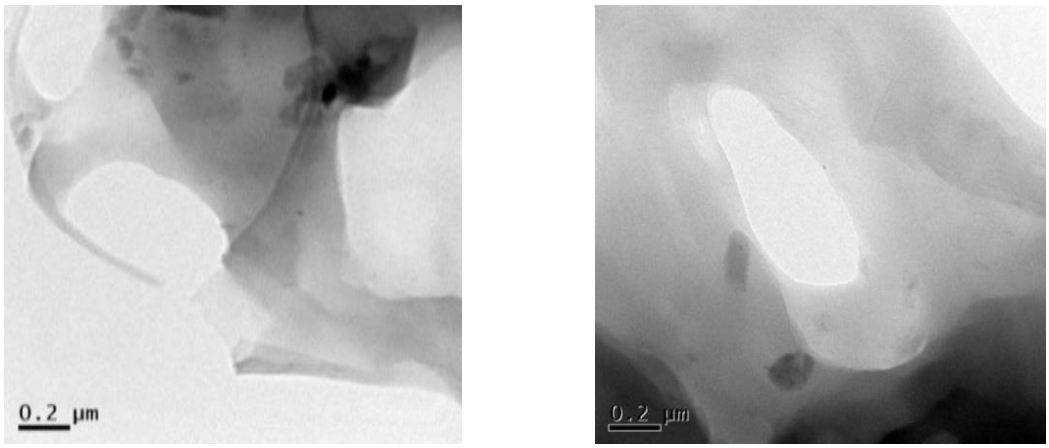

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Figure 2. Micrographs of polymer matrices: 1-p-EGM / AA, 2-p-PGM / AA on TEM

The pore size of network copolymers according to the TEM results for p-EGM / AA with composite data 14.8: 85.2 wt% (Fig.2.1) is from 0.4 μm to 1 μm, and for p-PGM / AA (15.1: 84.9 wt%) (Fig. 2.2) ranged from 0.5 μm to 1.2 μm. Uniform pores of the polymer matrix promote the formation and growth of individual particles in the volume of the polymer, preventing their aggregation.

X-ray powder diffraction (X-ray phase analysis) was used to determine the phase and crystalline nature of the synthesized MPC. The diffraction spectra of Ni (1) and Ni-Ad (2) fixed on the polymer matrix P-EGM/white are shown in figures. The bands 853 EV and 870 EV from RF Spectra relate to Ni (0) 2 p3/2 and Ni (II) 2 p1/2 . At the same time, the intensity bands of 850.5 and 872.3 EV in RF spectra are characteristic of the saturated form of NI (II) nickel oxide. According to the X-ray, (111), (200), (220) according to the lattice planes, nickel-specific bands 2θ 54º, 44º and 77º are observed. From X-rays, it can be seen that nickel

has a cubic structure of NB. The absence of stripes characteristic of nickel chloride at an angle of 2θ=15º is evidence of complete reduction of nickel and proves the nanoscale nature of the metal.

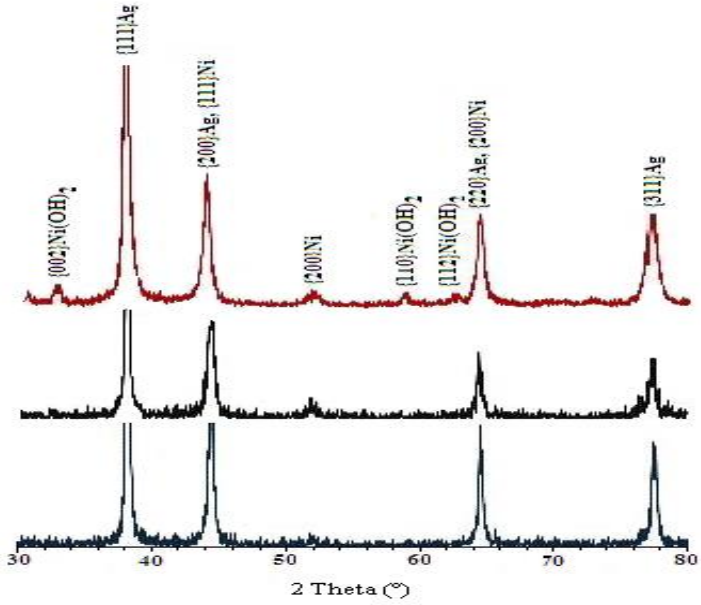

Figure 2. Ni-Ag diffraction Spectra fixed on the polymer matrix P-EGM/AA

The resulting nanocomposites contain isolated Ag^0 NPs with a diameter of 60 ± 10 nm, predominantly spherical, and NPs of metallic Ni^0, 70±10 nm in size, cubic (Fig. 3.1), uniformly distributed in the polymer matrix. In some cases, they form agglomerates on the surface of the polymer matrix with sizes in the range of 150-200 nm (Fig. 3.2).

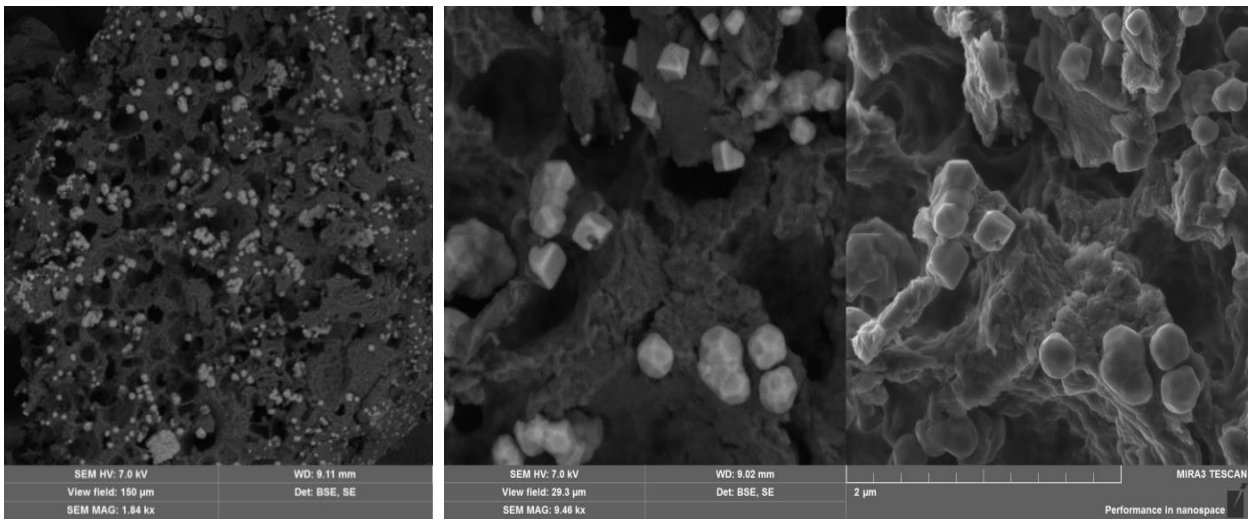

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Figure 3. Electron microscopic images of the n-EGM:AA/Ni nanocomposite

The main part of NPs (about 80%) of the total mass is made up of particles with sizes ranging from 20 to 70 nm. A smaller part is accounted for by larger formations, 80-100 nm in size. Aggregates larger than 200 nm (about 10%) are formed as a result of adhesion of small particles that are marked on the polymer surface. Copolymers p-EGM (PGM) / AA allow achieving high uniformity of distribution of metal particles in the volume of the polymer matrix, as demonstrated by the results of SEM with EMF in Figure 4. According to the results of energy dispersive spectrometry, the average number of metal particles per 10 μm is ~ 1300 ± 100 units. particles for Ag0 and ~ 1000 ± 100 units. for Ni. The Ag: Ni metal ratio is 63: 37% in the p-EGM: AA copolymer and 61: 39% in the p-PGM: AA copolymer (Fig. 5). Thus, it is possible to synthesize metal NPs with a smaller size. The content of Ag: Ni metals in p-EGM (PGM): AA / Ag0-Ni0 composites is ~ 20 wt% of the total mass.

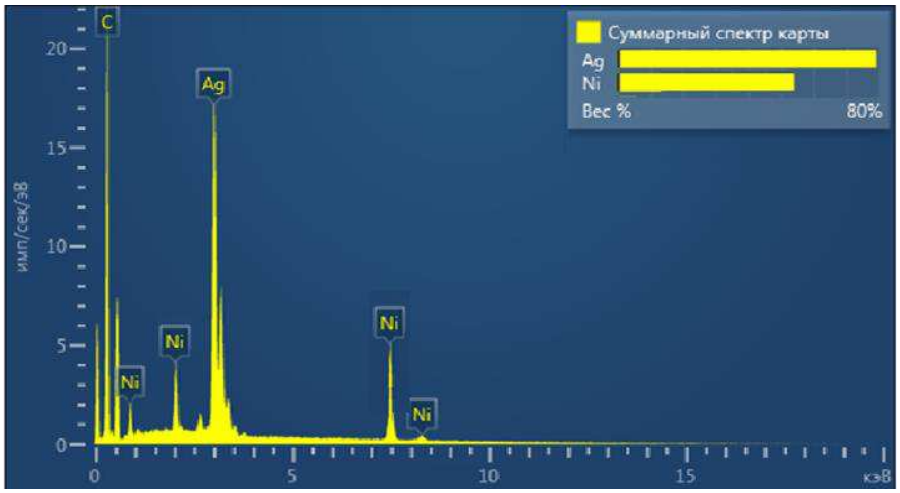

Figure 4. Micrographs: the distribution of individual elements in the volume of the matrix

To estimate the temperature of decomposition of metal-polymer complexes, thermal analysis of samples of these polymer complexes (p-EGM / AA-Ni-Co (a) and p-PHM / AA-Ni-Co) was carried out. Figure 5 shows thermograms of p-EGM (PGM) / AA copolymers with a constant heating rate of 10 deg / min in the temperature range of 30-1500 ° C in air.

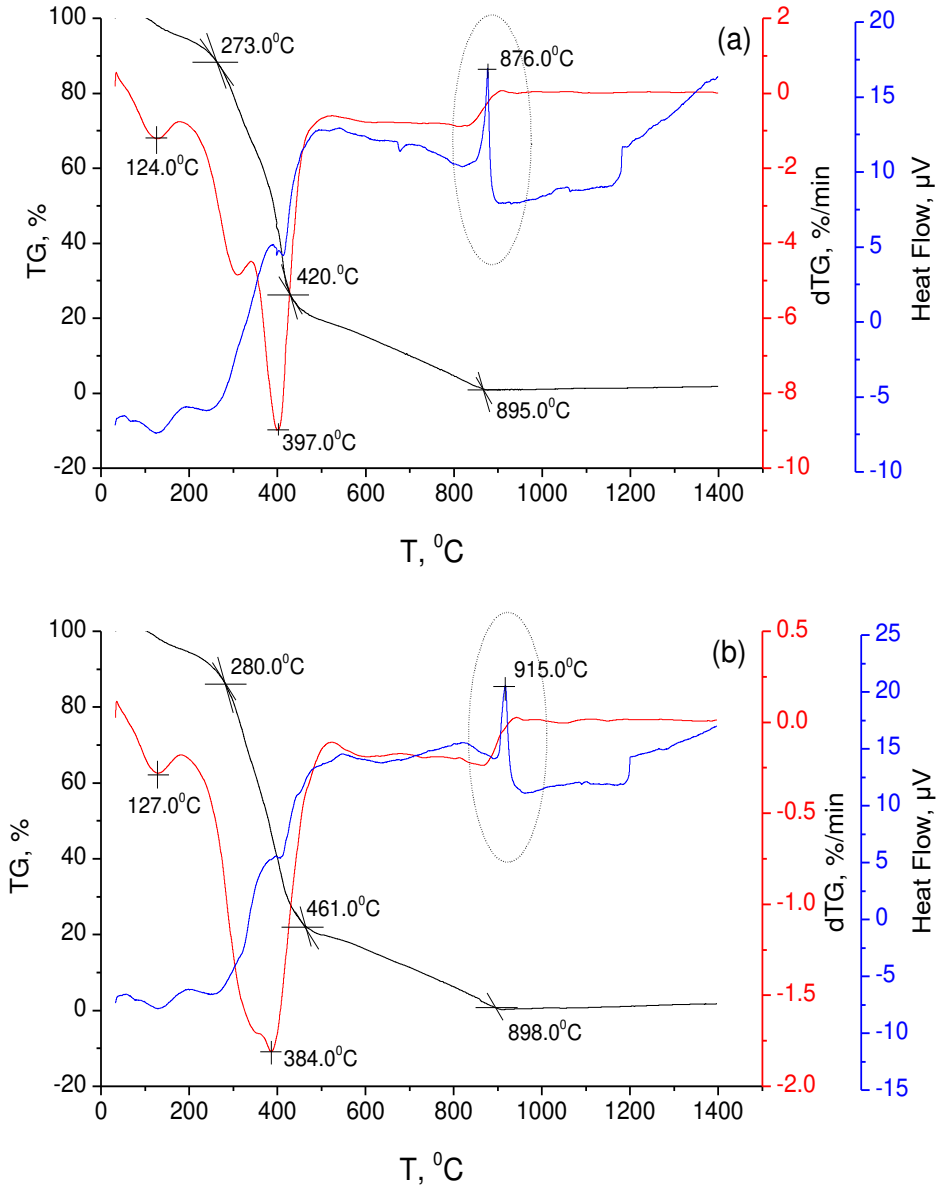

Figure 5. Temperature dependences of mass change (TG curve), rate of mass change (dTG curve) and heat flow (Heat Flow) for metal-polymer complexes: p-EGM / AA-Ni-Co (a) and p-PGM / AA- Ni-Co (b)

In fig. 5. shows thermograms of metal-polymer complexes of compositions: p-EGM / AA-Ni-Co and p-PGM / AA-Ni-Co. Figure 1, (a) shows that the metal-polymer complex p-EGM / AA-Ni-Co is stable up to 900.00C, 10.0% weight loss at 124.00C, with the maximum endothermic effect on the dTG curve at 397.0^{0}C. The metal-polymer complex p-PGM / AA-Ni-Co (Fig. 1, b) is stable up to 1000.0^{0}C, 52% weight loss at 384.0^{0}C. Residual weight 48%. As can be seen from Figure 5, the process of thermal decomposition of metal-polymer complexes (p-EGM / AA-Ni-Co and p-PGM / AA-Ni-Co) is a one-stage process, and for most of the studied compounds includes one stage of thermal destruction, as evidenced by

inflection point on weight loss curves. Based on the obtained dependences (Fig. 5), it can be concluded that the least thermally stable is p-EGM / AA-Ni-Co, which is stable up to 900.0^{0}C and decomposes in one stage with exothermic maxima on the heat flux curve at 876.0^{0}C.

The most thermally stable are p-PGM / AA-Ni-Co - stable up to 1000.0^{0}C, with maxima on the heat flow curve at 915.00C, respectively. For all studied compounds, weight loss curves from 10.0% of the weight are observed in the temperature range from 0.0^{0}C to 150.0^{0}C, which indicates that crystallization water is eliminated, which is present in all metal-polymer complexes. As shown by thermal analysis (Fig. 5) of the investigated metal-polymer complexes p-EGM / AA-Ni-Co and p-PHM / AA-Ni-Co, including the elements Ni and Co, they are stable up to 1000.00C and gradually decompose in the range from 0.00 From to 1000.0^{0}C, while in all cases there is an exothermic effect Tinit. = 850.0 ± 0.1^{0}C (at 876.0^{0}C) for p-EGM / AA-Ni-Co and Tinit. = 890.0 ± 0.1^{0}C (at 915.0^{0}C), which are absent in the initial polymer matrix, which indicates the presence of Ni and Co elements in the network of the polymer complex. Thus, the synthesized nanocomposites have thermal stability up to ~ 900 ° C and are promising for the development of biocompatible medical materials.

Gel-immobilized silver nanoparticles have a wound-healing effect due to the antibacterial nanocluster silver. Hydrogel membranes containing silver nanoparticles are used to treat burns, trophic ulcers, wounds and cuts. The hydrogel layer has through pores of submicron sizes and provides gas exchange and, at the same time, protection of the wound from external infection [7]. In the course of determining the antimicrobial activity, it was found that the polymer itself in the established concentration did not show antimicrobial activity. The minimum inhibitory concentration for MIC is presented in Table 1.

Table 1.

Antimicrobial activity of MPS

Substances	Substance concentration,%	Microorganisms		
		Pseudomonas aeruginosa	*Escherichia coli*	*Staphylococcus aureus*
Initial polymeric matrix p-PGM / AA	More than 10%	-	-	-
p-PGM / AA: Ag	0,00375%*	+	+	+
p-PGM / AA: Ni	0,25%*	+	+	+
p-PGM / AA: Ag-Ni	0,0075%*	+	+	+

* The base concentration of polymer solutions was 10% m / m, which were subsequently diluted with two-fold dilutions to a concentration of 0.001% m / m.

The study of the metal-polymer nanocomposite p-PGM/AA-Ag showed that the test sample has antibacterial activity against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa (Fig. 6, Table 2). Growth processes in gram-positive bacteria - Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa depend on the diffusion and concentration of the antimicrobial agent (activity increases with increasing concentration).

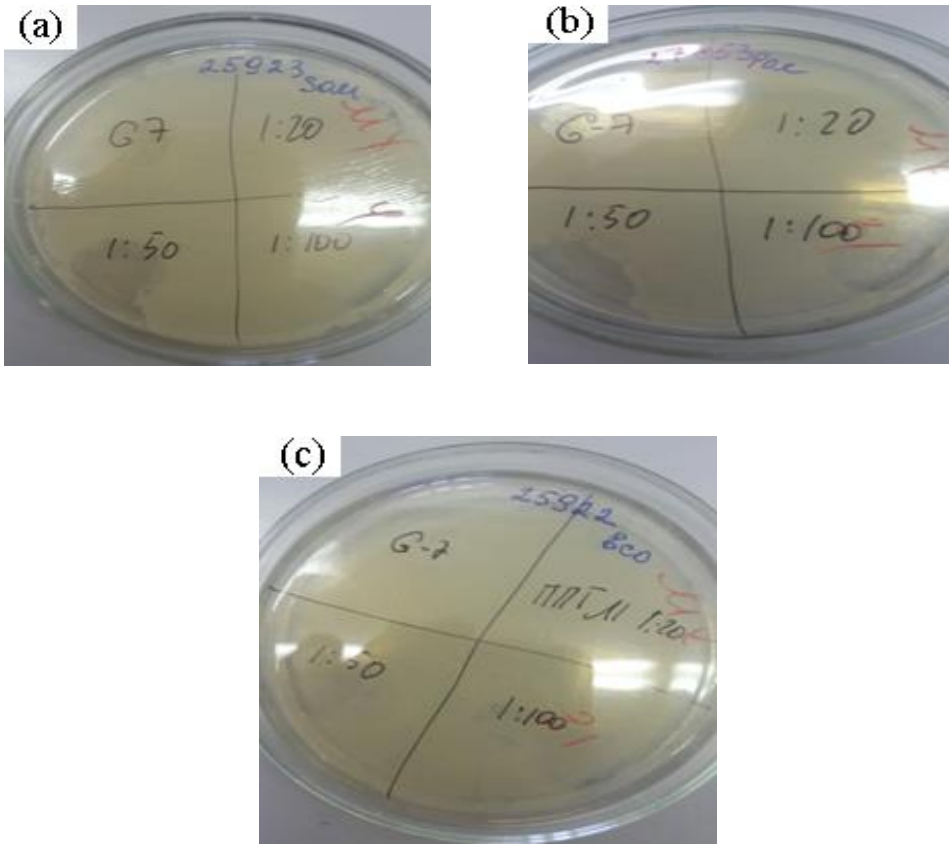

Figure 6. Inhibition of Staphylococcus aureus (a); Pseudomonas aeruginosa (b) and Escherichia coli (c)

Table 2.

Antibacterial activity of the tested metal-polymer nanocomposite p-PGM / AA-Ag

Microorganism	Concentration of metal-polymer complex p-PGM / AA-Ag

	1:100	1:50	1:20	1:1
Pseudomonas aeruginosa	5	4	2	2
Escherichia coli	4	4	4	2
Staphylococcus aureus	4	3	2	2

Note: "0" - no growth; "5" - complete lack of growth

It was found that for all strains of standard microorganisms, a similar picture is observed: the presence of a zone of suppression of the same size, but at the same time the growth of rare colonies is observed within the zone, and an increase in the effect of suppressing growth is observed from a concentration from 1: 1 to 1: 100.

The high activity of this metal-polymer nanocomposite may be due to the presence of [Ag +] in the system, which has a bactericidal, antifungal and antiseptic effect [7].

4. Conclusions

The obtained nanocomposites under new synthesis conditions contain isolated Ag^0 NPs with a diameter of 60±10 nm, predominantly spherical in shape and NPs of metallic Ni^0, 70 ± 10 nm in size, cubic shape, uniformly distributed in the polymer matrix. In some cases, they form agglomerates on the surface of the polymer matrix with sizes ranging from 150-200 nm. The synthesized nanocomposites have thermal stability up to 500°C and are promising for the development of biocompatible medical materials.

Thus, for the first time, the biological activity of the initial polymer and the synthesized metal-polymer complex, including silver nanoparticles - p-PGM/AA-Ag was studied. It was found that the original polymer does not have antibacterial activity against standard strains, while the metal-polymer complex with p-PGM/AA-Ag silver showed high efficiency against all strains of the studied bacteria at a concentration from 1:50 to 1:100.

The work was carried out at the Scientific Research Institute of Chemical Problems of the KarU named after acad. E.A. Buketova (Karaganda, KazAAhstan), in the biotechnology laboratory of the KarU named after acad. E.A. Buketova (Karaganda) and on the basis of the analytical and inorganic department of the Karlov University (Czech Republic), the National Collection of Microorganisms RSE (Nursultan), the Scientific Research Center of the Karaganda Medical University.

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