

M.Zh. Burkeyev¹, S.R. Shibayeva^{1*}, T.O. Khamitova², Jiri Plocek³,
M.T. Nurmaganbetova¹, A.T. Kazhmuratova¹, T.S. Zhumagalieva¹

¹Karagandy University of the name of academician E.A. Buketov, Karaganda, Kazakhstan;

²S. Seifullin Kazakh Agro Technical University, Nur-Sultan, Kazakhstan;

³Institute of Inorganic Chemistry of the Czech Academy of Sciences, Husinec-Rez, Czech Republic

(*Corresponding author's e-mail: sali_r@mail.ru)

Synthesis and Investigation of the Properties of Polymer-immobilized Silver- and Gold Nanoparticles

Metal-polymer composites (MPC) based on copolymers of polypropylene glycol maleate phthalate with acrylic acid and metallic gold and silver were synthesized for the first time. The structure of nanocomposites is characterized with the help of microscopy and spectroscopy. Antimicrobial activity of novel metal-polymeric complexes based on silver and gold was studied. Metal-polymeric complexes were stabilized for the first time with polymeric matrix based on the copolymers of polypropyleneglycolmaleatephthalate with acrylic acid (p-PGMPh/AA:Ag, p-PGMPh/AA:Au). Obtained nanocomposites contain the nanoparticles of silver and gold of rhomboidal and cylindrical shapes with the sizes of 40–50 and 35–50 nm correspondingly. The size of more than 80 % of the nanoparticles are from 20 to 50 nm and has a spherical and rhombic shape. For determination of antimicrobial activity of the MPC the reference test-microorganisms such as facultative-anaerobe gram-positive *Staphylococcus aureus*, aerobic gram-positive spore-forming *Bacillus subtilis*, gram-negative facultative anaerobe of *Escherichia coli*, aerobic *Pseudomonas aeruginosa* and yeast fungi *Candida albicans* have been used. The resulting nanocomposites are stable and promising for the creation of new photochromic and nonlinear optical materials, as well as for using in medicine in the development of antiseptic and antimicrobial materials that are needed during a pandemic.

Keywords: nanocomposites, nanoparticles, silver, gold, copolymer, polypropylene glycol maleate phthalate with acrylic acid, metal-polymer complex, antimicrobial activity.

Introduction

Nanocomposite materials, containing nanoparticles of silver and gold, possess unique properties and are promising for medicine, optoelectronics, nanophotonics, and catalytic systems. The study of the processes of self-organization of hybrid nanocomposites, the regularities of constructing zero-valency metals' nanoparticles, the problems of their stabilization and accomplishment of narrow-disperse distribution are the fundamental tasks of the modern chemistry of nanomaterials [1–4].

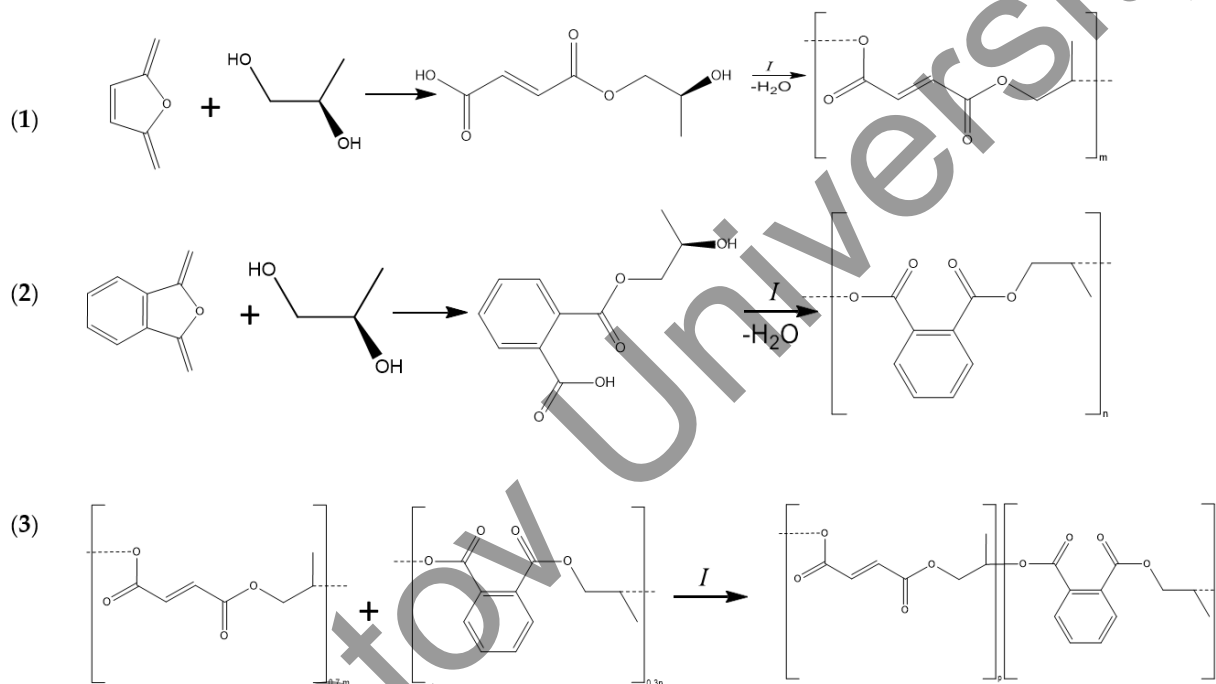
The nature of the nano-stabilizing matrix is substantially significant in designing metal-containing nanocomposites. The synthetic polymers shown previously in [5] are extensively studied as such matrices. The investigations on the development of methods of synthesizing “smart”-polymers based on UPS (unsaturated polyester resins) with unsaturated carboxylic acids have been done. Polymeric materials obtained on their basis possess unique properties which provide using them in a wide range of fields such as agricultural industry, medicine, oil chemistry, and chemical synthesis. The possibility of using the copolymers of p-PGMPh/AA as a matrix for obtaining effective catalysts for hydrogenization has been shown in [5]. Obtained metal-polymer complexes (MPC) contain isolated metal nanoparticles with diameters of 100–112 nm; they are mainly spherical and are evenly distributed within a polymer matrix. In terms of the abovesaid, it is evident, that the synthesis of smart-copolymers with the high water-absorbing volume based on available and rather cheap raw material (UPR cured with ionogenic vinyl monomers) and their study are of great importance [6–10]. The stability of nanoparticles in polymer matrices is maintained for a long time. Large-scale fundamental and applied research to develop innovative materials and technologies, especially nanocomposites, is an urgent task and of great scientific and practical interest. Using metal nanoparticles immobilized in polymers with bactericidal properties, it is possible to obtain medical materials for a wide range of applications: implants, drug delivery systems, antibacterial coatings for biomedical devices, and antimicrobial packaging.

The aim of this work is to synthesize new hybrid nanocomposites with a uniform stable distribution of silver and gold nanoparticles based on copolymers of polypropylene glycol maleate phthalate with acrylic acid (p-PGMP/AA) and evaluate their antibacterial properties.

Experimental

Synthesis of polypropylene glycol maleate phthalate. For obtaining the nanoparticles of metal silver and gold, a chemical way of dispersing these metals by reduction of Argentum nitrate and aurichlorohydric acid with sodium borohydride in an aqueous medium in the presence of polypropylene glycol maleate phthalate with the acrylic acid (p-PGMPh:AA) was used [5].

Polypropylene glycol maleate phthalate (p-PGMPh) was obtained by condensation polymerization of propyleneglycol with maleic, phthalic anhydride at a temperature of 423–433 K in a four-neck flask supplied with a reverse condenser, stirrer (from the top), thermometer, Dean-Stark trap in a nitrogen air. Polycondensation was carried out according to the standard procedure [11] at a constant stirring in the presence of catalyst zinc chloride in nitrogen air to avoid the processes of gelation (Fig. 1).



(1) the formation of an acidic ester of maleic anhydride; (2) the formation of acidic ester of phthalic anhydride; (3) obtaining polypropylene glycol maleate phthalate

Figure 1. Synthesis of polypropylene glycol maleate phthalate

Synthesis of nanocomposites of silver and gold. Immobilization of silver particles in p-PGMP:AA copolymer substrates (1 g) was added to 2 ml of an aqueous solution containing 0.034 g – 0.340 g of AgNO_3 and containing 0.510 g – 1.019 g of HAuCl_4 . The reduction was carried out for 5 h at room temperature. Then 0.012 g – 0.120 g of $\text{Na}(\text{PH}_2\text{O}_2)$ was added in small portions and 0.8 ml of NH_4OH was continued to be vigorously stirred for 12 h at room temperature. Dark gray powders (60–87 % yield) with 20–22 % silver content and dark purple powder (65–80 % yield) with 12–15 % gold content were obtained.

Figure 2 presents the schematic structure of p-PGMPh/AA: Au^0 .

Physicochemical methods of investigation of MPC. The samples for the IR-spectroscopy were prepared by long-termed triturating 2 ± 0.1 mg sample with 200 ± 0.1 mg anhydrous KBr (KBr was a background sample). The samples were pressed under the pressure of 200 atm. IR spectra of obtained materials were recorded on a device FSM1201 (RF, OOO “Infraspec”) in the range of $450\text{--}4000\text{ cm}^{-1}$ with the best possible resolution in a regimen of measuring a relative emission. The number of repeated scanning was increased to maximum – 100 (Fig. 3).

The content of metals in the nanocomposites was determined on an Agilent 4210 atomic emission spectrometer with microwave plasma, in which the flame was acetylene-air, a lamp with a full cathode, a wave-

length of 328 nm, and a slit width of 0.5 nm Agilent 4210 (MP-AES). The study of the structure of samples' surfaces was made on an SEM MIRA3 (TESCAN, Czech Republic). Before the study, the samples were covered with a carbon layer on a sputtering apparatus Quorum Q150R ES (Quorum Technology, Great Britain). The images (Fig. 4) were obtained by using the detector of secondary electrons (SE detector) at an accelerating tension of (HV) 20 kV. Thermal stability of the composites was studied thermogravimetrically on a synchronic TGA/DTA/DC analyzer LabSYSEvo (2014) within the temperature interval of 30–1000 °C in the aluminum oxide crucibles at a heating rate of 5 °C/min in air with the expense of 30 ml/min by putting the weighing of 20 mg (Fig. 5).

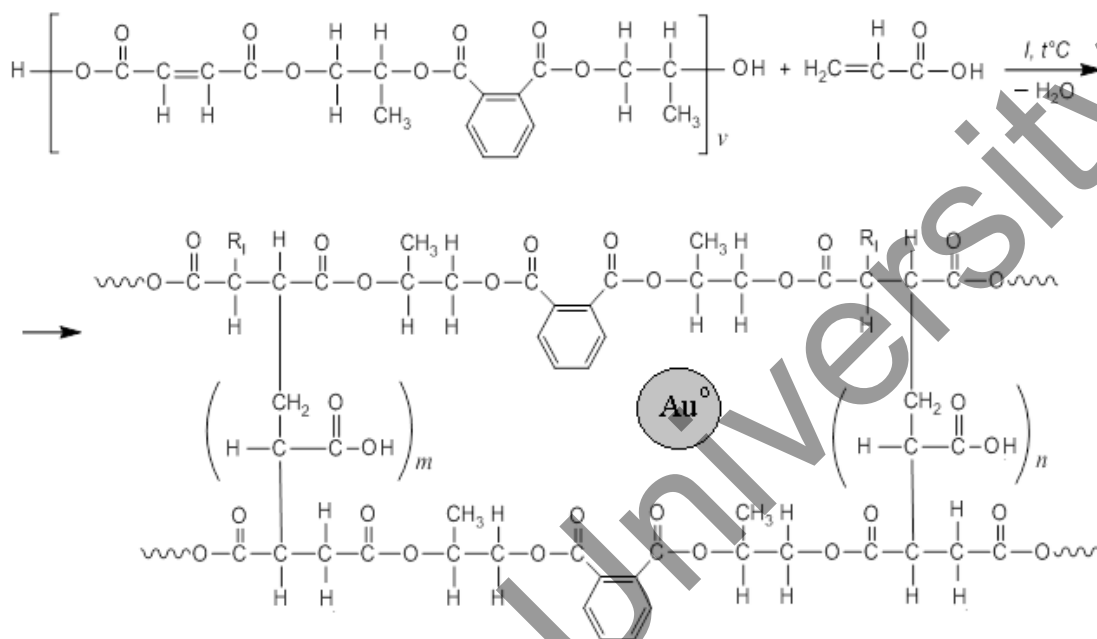


Figure 2. Schematic structure of p-PGMPh/AA:Au⁰

Antimicrobial activity. For determination of the antimicrobial activity of the metal-polymer complexes of p-PGMPh/AA:Ag and p-PGMPh/AA:Au, the reference test microorganisms, such as facultative-anaerobe gram-positive *Staphylococcus aureus* ATCC 6538, aerobic gram-positive spore-forming *Bacillus subtilis* ATCC 6633, gram-negative facultative anaerobe of *Escherichia coli* ATCC 25922, aerobic *Pseudomonas aeruginosa* ATCC 27853, and yeast fungi *Candida albicans* ATCC 10231, were used. Test bacteria of microorganisms used in this study were obtained from the American collection of type cultures. For the antimicrobial studies of complexes of the polymers, the suspensions of test bacteria at concentrations 10⁶ CFU/ml were used. Standard bacterial suspensions were prepared from daily cultures which were grown on an agar slant at a temperature of 37 °C within 24 hours by breeding the culture 1:1000 in a sterile isotonic 0.9 % solution of sodium chloride. 1 mkg of particles' complexes of the polymers p-PGMPh/AA:Au and p-PGMPh/AA:Ag were added to the testing tubes separately. The test tubes were incubated within 1 hour at room temperature [12]. Then the content of the test tubes was poured into the Petri dish with Muller-Khilton agar and they were incubated for 24 hours at a temperature of 37 °C. The results were estimated by defining the presence or absence of growth of microorganisms in the Petri dish which contains testing polymeric complexes in comparison with control.

Results and Discussion

Showing a plasma resonance (PR) is characteristic of gold and silver nanoparticles, which occurs when the frequency of the incident radiation coincides with the frequency of the collective vibration of excited electrons in metal nanoparticles. As a result, an absorption band appears in the visible region of the spectrum, its position significantly depends on the size, shape, and state of the surface of the nanoparticles, as well as on the presence of stabilizing substances and other compounds. The resistance of gold to oxidation and high sensitivity of plasma resonance peak to the state of the surface of nanoparticles allow using them in

the creation of sensors for selective determination of individual substances solving a wide range of analytical problems.

The structure of synthesized monometallic polymeric complexes was confirmed by IR-spectroscopy (Fig. 3) (cm^{-1}).

- Polypropylene glycole maleate phthalate /AA, white powder: 3449 (O–H); 2936 (C–H_{st}); 2361 (C–H_{st}, CH₂–N_{st}); 1724 [C=O bond with OH(COOH)]; 1269 (CH₂ δ); 1169 (CH₃ δ);
- Polypropylene glycole maleate phthalate /AA: Au⁰, dark purple powder: 3425 (O–H); 2939 (C–H_{st}); 2365 (C–H_{st}, CH₂–N_{st}); 1610 [C=O bond with OH(COOH)]; 1624 (CH₂ δ); 1277 (CH₃ δ); 659 (Au);
- Polypropylene glycole maleate phthalate /AA: Ag⁰, dark gray powder: 3429 (O–H); 2928 (C–H_{st}); 2372 (C–H_{st}, CH₂–N_{st}); 1604 [C=O bond with OH(COOH)]; 1454 (CH₂ δ); 1161 (CH₃ δ); 817 (Ag).

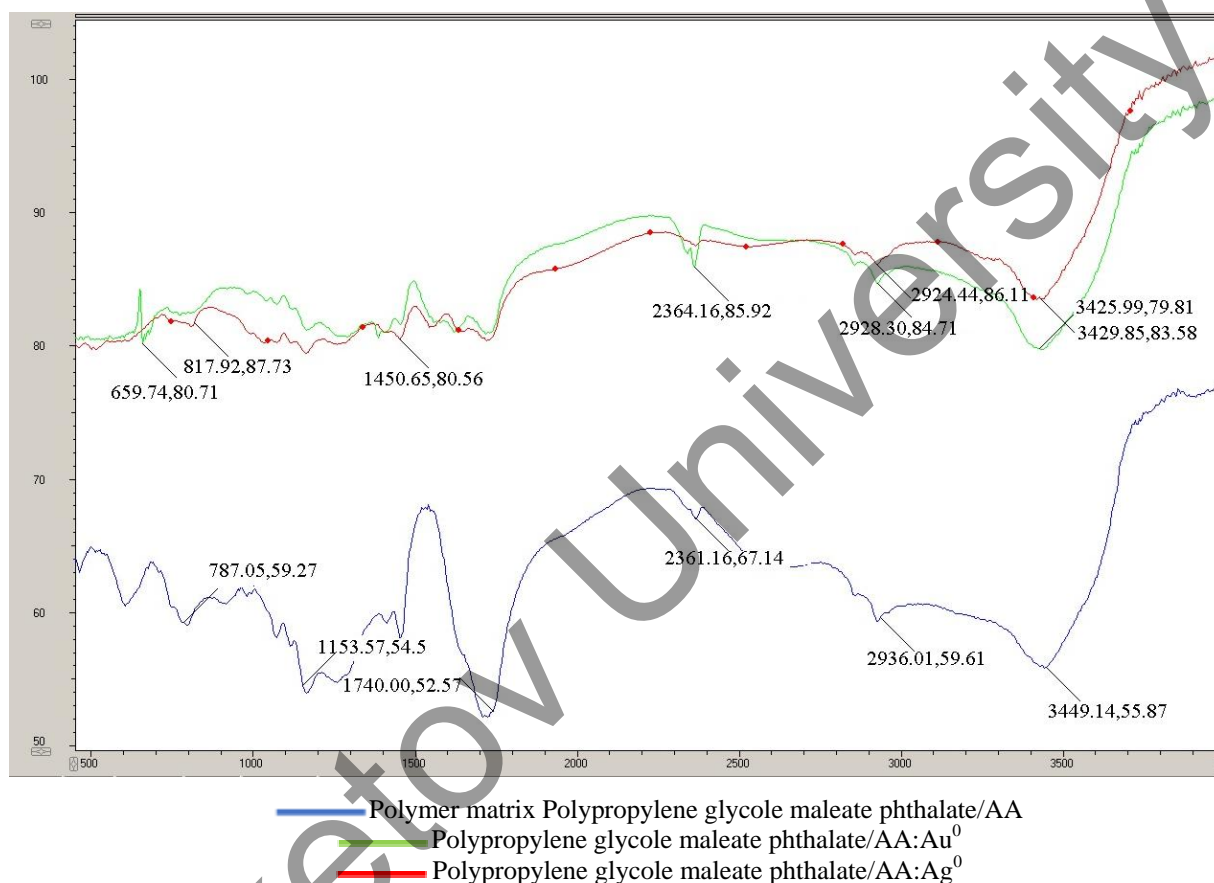


Figure 3. IR-spectra of the samples

In the IR-spectra of obtained composites, the corresponding PR bands appear with maxima in the region of 648–659 and 806–817 nm respectively which is characteristic for gold and silver of nano-sized zero-valency state; consequently, the original properties of polymer matrix are not changed.

IR-spectra of the samples of p-PGMPh:AA/Au and p-PGMPh:AA/Ag are rather close according to their place to maximums of absorption and they are characterized by low contrast which is due to non-selective absorption of emission of metal particles. In comparison with the spectra of the initial polymer, there are no significant shifts in absorption frequencies in the spectra.

Figure 4 presents the composition and the structure of synthesized samples, and the sizes of metal-NPs. The images are obtained using the SE detector at an accelerating voltage (HV) of 5 kV. From all sides of the pictures, there are observed the porous; the layer (porous) structure is mostly dominated. It is established that the pore sizes are 0.9–1.1 μm in these samples, which is characteristic of three-dimensional network polymers.

In electron microscopy images (Fig. 4), obtained metal-polymer composites consist of the nanoparticles of silver and gold of rhomboidal and cylindrical shapes with the sizes of 40–50 and 35–50 nm correspondingly.

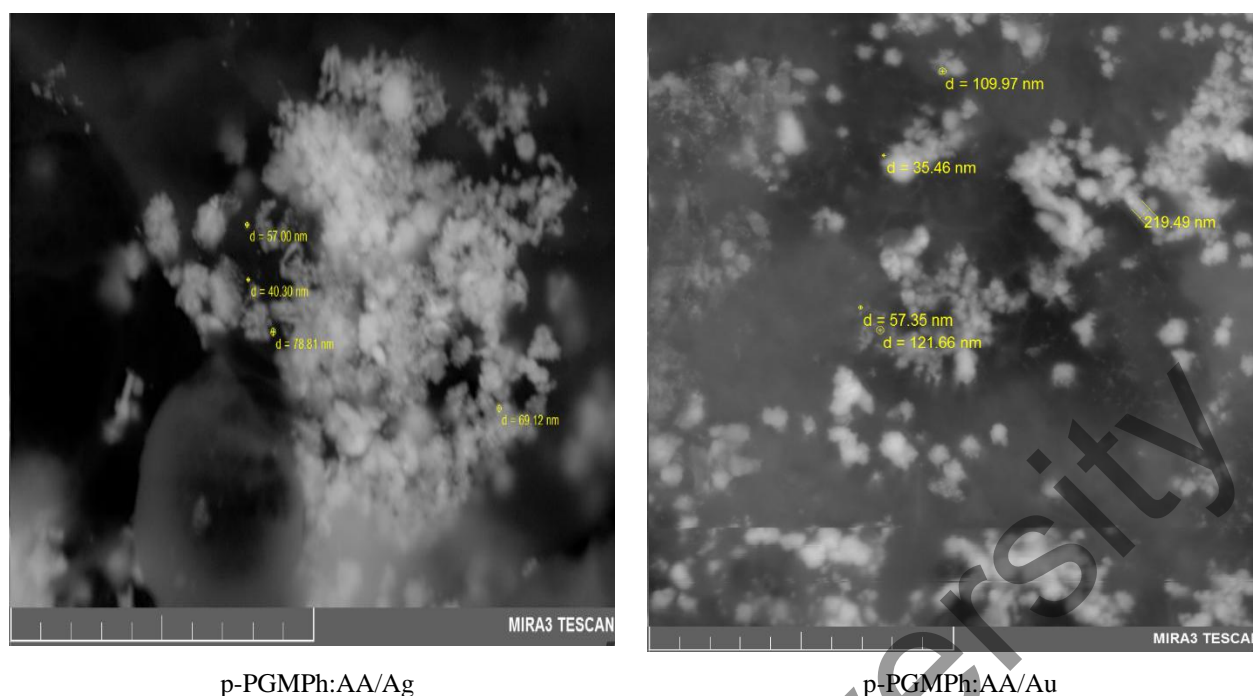


Figure 4. Electron microscopic images

According to data of atomic-adsorption spectroscopy, the content of the particles in nanocomposites is around 20–22 % for Ag and 12–15 % for Au. Dioxane was used as a solvent during sample preparation. Copolymers were dissolved in dioxane for 60–90 min. The solution is filtered with an autosampler and introduced into the column of the gas analyzer.

The nature of functional groups in the polymer structure, the molecular mass, and in the case of copolymers — their composition and distribution in a polymer matrix affect the size of forming particles (Table 1).

Compared to the results of a previous study [5], with metal nanoparticles of spherical (silver) and rhomboidal (nickel) forms distributed in the matrix of gel, the particle sizes are smaller. The main part of nanoparticles (about 80 % of total mass) are the particles with sizes within 20–60 nm and the less part is much bigger formations with sizes of 80–90 nm. The aggregates with sizes more than 200 nm ($\approx 10\%$) formed during the process of coalescence of nanoparticles which can be seen on the surface of the polymer. The content of Ag and Ni in nanocomplexes of p-PGMPh/AA:Ag⁰, p-PGMPh/AA:Ni⁰ is $\sim 23,21$ mass. % from the total mass of the complex correspondingly.

Table 1

Morphology of metal-containing copolymers

Sample	Content of metal, % (mass.)	Particle size, nm	Morphology of NPs, nm
Ag	20–22	40 \pm 10	sphere
Au	12–15	35 \pm 10	Rhomb

Figure 5 illustrates the thermograms of metal-polymer complexes of the composition of p-PGMPh/AA-Au and p-PGMPh/AA-Ag. The first thermogram (a) shows that the metal-polymer complex p-PGMPh/AA-Au is stable till 800.0 °C; 10 % mass loss at 200.0 °C with the maximum value of endothermal effect at 297.0 °C on DTG curve is observed. When thermal decomposition of the metal-polymer complex of p-PGMPh/AA-Au, the DTG curve (Fig. 5b) with three peaks at maximums $T_{\max} = 134.0, 292.5,$ and 410.0 °C is obtained. Low-temperature peak is due to the elimination of crystalline water, whereas the second and third peaks are explained by the destruction of the main chains which takes place as zipping. Metal-polymer complex p-PGMPh/AA-Ag (Fig. 5c) is stable till 800.0 °C; there is a 12 % mass loss at 190.0 °C and the residual mass is 58 %. As can be seen from Figure 5b, the process of thermal decomposition of the p-PGMPh/AA-Ag metal-polymer complex is a multiple-stage and, for many studied compounds, includes four stages of thermal decomposition, in which the bends on the curves correspond to the mass loss point.

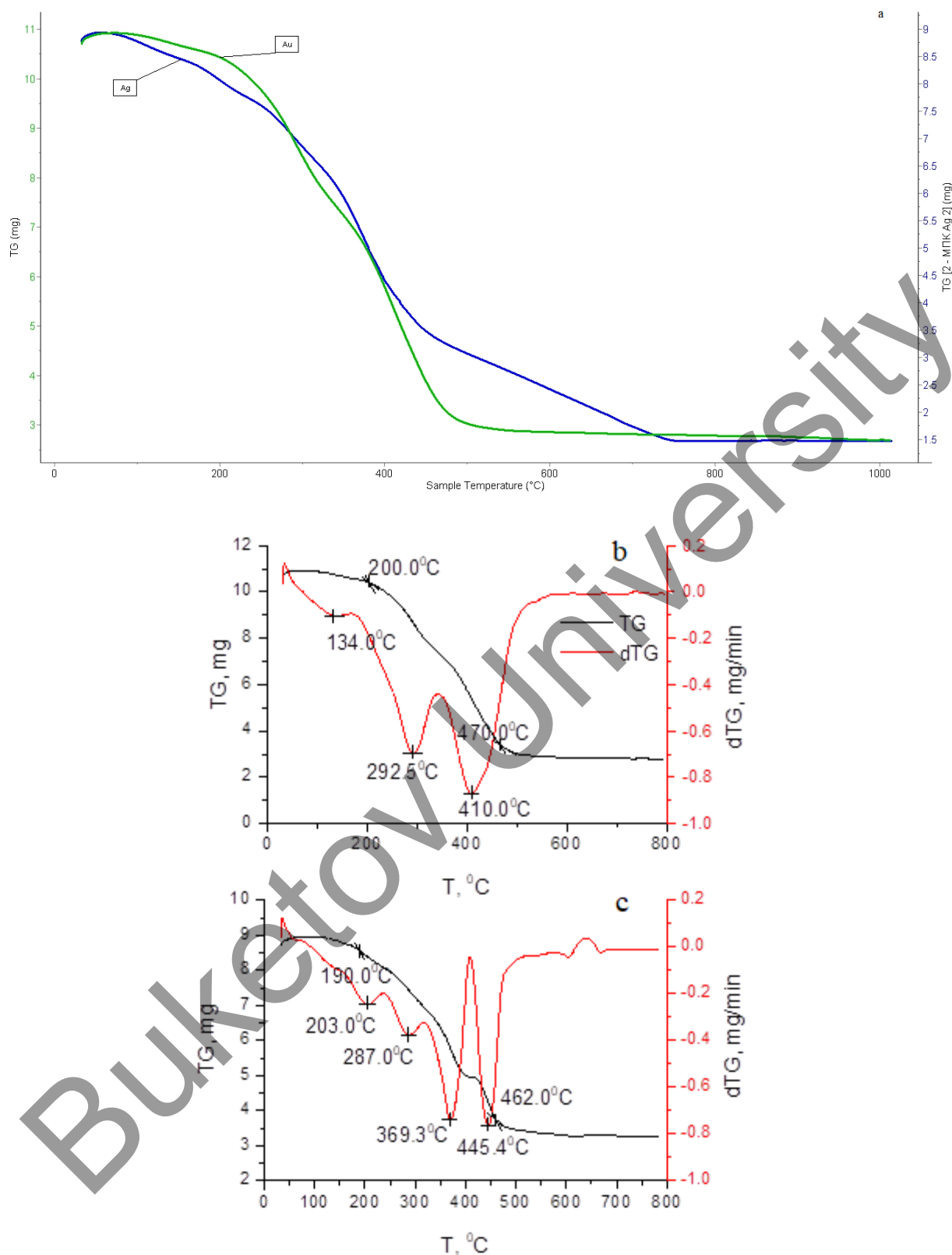


Figure 5. Temperature dependences of change of the mass (TG curve) (a) and the rate of change of the mass (DTG curve) for metal-polymer complexes: p-PGMPh/AA-Au (b) and p-PGMPh/AA-Ag (c)

As the thermal analysis of the studied p-PGMPh/AA-Ag and p-PGMPh/AA-Au, metal-polymer complexes containing the elements Ag and Au showed (Fig. 5b, c) that the complexes are stable up to 800.0 °C and gradually decompose in the range from 0.0 °C to 800.0 °C. In all cases, there is an exothermal effect $T_{\text{beginning}} = 350.0 \pm 0.1$ °C (at 410.0 °C) for p-PGMPh/AA-Au and $T_{\text{beginning}} = 400.0 \pm 0.1$ °C (at 445.0 °C) which

is absent in initial polymeric matrix which confirms the presence of the elements Ag and Au in the network of the polymer complex.

Antimicrobial activity. In the next step of the work, the antimicrobial activity of metal-polymer composites of the samples of p-PGMPh/AA: Au, p-PGMPh/AA: Ag to the strains of gram-positive bacteria of *Staphylococcus aureus*, *Bacillus subtilis*, gram-negative bacteria of *Escherichia coli*, *Pseudomonas aeruginosa* and yeast fungi of *Candida albicans* was studied. To determinate the antimicrobial effect of metal-polymer complexes of p-PGMPh/AA: Au and p-PGMPh/AA: Ag, a comparative experiment using several pharmacopeia testing bacteria was done. Table 2 presents the results of the study of antimicrobial activity metal-polymer complexes of PGMPh/AA: Au and p-PGMPh/AA: Ag.

Table 2

Antimicrobial activity of the samples

Bacteria/Sample	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>
MPC Au	+	+	+	+	+
MPC Ag	–	–	–	+	–
Control	+	+	+	+	+

Conventional signs: “–” — no growth, “+” — significant growth.

As a result of antimicrobial analysis, it was found that in the sample of MPC-Ag there was no growth of presented testing bacteria except *Pseudomonas aeruginosa*. This points to the antimicrobial effect of the sample — MPC Ag; in this case the activity is higher than in the other testing samples. The sample of MPC-Au did not show an antimicrobial effect related to the presented test microorganisms.

Conclusions

Thus, the use of the polymer as a stabilizing matrix allowed obtaining hybrid nanocomposites with an equally stable distribution of nanoparticles of silver and gold. Synthesized nanoparticles were characterized (size and particle size distribution) with the help of electron microscopy and showed that the nanocomposites contain nanoparticles of silver and gold of rhomboidal and cylindrical shapes with the sizes 40–50 and 35–50 nm accordingly. Metal–polymer composites based on copolymers of polypropylene glycolmaleate-phthalate with acrylic acid and metallic gold and silver were synthesized for the first time. The results demonstrated an equal distribution of nano-sized particles in the volume of polymeric matrix; the sizes of more than 80% of metal nanoparticles are in the range of 20 to 50 nm and have the shape of a sphere and rhomboidal shape.

Obtained nanocomposites are stable and promising for the creation of novel photochromic and non-linear-optical materials, and the use in medicine in the creation of antiseptic and antimicrobial materials which are necessary during pandemic.

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М.Ж. Буркеев, С.Р. Шибаетова, Т.О. Хамитова, Иржи Плоцек,
М.Т. Нурмаганбетова, А.Т. Кажмуратова, Т.С. Жумагалиева

Күміс пен алтын полимер-иммобилизацияланған нанобөлшектерінің синтезі және олардың қасиеттерін зерттеу

Полипропиленгликольмалеинатфталаттың акрил қышқылымен және алтын мен күміспен сополимерлері негізіндегі металл-полимерлі композиттері алғаш рет синтезделді. Нанокөпозиттердің құрылымы микроскопия мен спектроскопияның көмегімен сипатталды. Күміс пен алтын негізіндегі жаңа металл-полимерлі кешендердің микробқақарсы белсенділігі зерттелді. Металл-полимерлі кешендер алғаш рет акрил қышқылымен полипропиленгликольмалеинатфталаттың сополимерлеріне негізделген полимерлі матрицамен тұрақтандырылды (п-ПГМФ/АК:Ag, п-ПГМФ/АК:Au). Алынған нанокөпозиттердің құрамында сәйкесінше 40–50 және 35–50 нм өлшемдегі ромб тәрізді және цилиндрлік пішіндегі күміс пен алтынның нанобөлшектері бар. Нанобөлшектердің 80 %-дан астамының өлшемі 20-дан 50 нм-ге дейінгі аралықта жатыр және сфералық және ромб тәрізді пішінге ие. Металл-полимерлі композиттердің микробқақарсы белсенділігін анықтау үшін факультативті-анаэробты грамаң *Staphylococcus aureus*, аэробты грамаң спора түзетін *Bacillus subtilis*, *Escherichia coli-idaa*-ға қарсы грамагеріс факультативті анаэробтар қолданылған. Алынған нанокөпозиттер тұрақты және жаңа фотохромды және сызықты емес оптикалық материалдарды жасау үшін, сондай-ақ пандемия кезінде қажет антисептикалық және микробқақарсы материалдарды әзірлеуде медицинада қолдану үшін перспективті болып табылады.

Кілт сөздер: нанокөпозиттер, нанобөлшектер, күміс, алтын, сополимер, акрил қышқылымен полипропиленгликольмалеинатфталат, металл-полимер кешені, микробқақарсы белсенділік.

М.Ж. Буркеев, С.Р. Шибаетова, Т.О. Хамитова, Иржи Плоцек,
М.Т. Нурмаганбетова, А.Т. Кажмуратова, Т.С. Жумагалиева

Синтез и исследование свойств полимер-иммобилизованных наночастиц серебра и золота

Впервые синтезированы металл-полимерные композиты на основе сополимеров полипропиленгликольмалеинатафталата с акриловой кислотой и металлического золота и серебра. Структура нанокөпозитов охарактеризована с помощью микроскопии и спектроскопии. Изучена антимикробная активность новых металл-полимерных комплексов на основе серебра и золота. Металл-полимерные комплексы впервые стабилизированы полимерной матрицей на основе сополимеров полипропиленгликольмалеинатафталата с акриловой кислотой (п-ПГМФ/АК:Ag, п-ПГМФ/АК:Au). Полученные нанокөпозиты содержат наночастицы серебра и золота ромбовидной и цилиндрической форм размерами 40–50 и 35–50 нм соответственно. Размер более 80 % наночастиц лежит в пределах от 20 до 50 нм и имеет сферическую и ромбическую формы. Для определения антимикробной активности МПК референс-тест-микроорганизмы были использованы факультативно-анаэробные грамположительные

Staphylococcus aureus, аэробные грамположительные спорообразующие *Bacillus subtilis*, грамотрицательные факультативные анаэробы *Escherichia coli*, аэробные *Pseudomonas aeruginosa* и дрожжевые грибы *Candida albicans*. Полученные наноконпозиты стабильны и перспективны для создания новых фотохромных и нелинейно-оптических материалов, а также для применения в медицине при разработке антисептических и антимикробных материалов, необходимых в условиях пандемии.

Ключевые слова: наноконпозиты, наночастицы, серебро, золото, сополимер, полипропиленгликоль-малеинатфталат с акриловой кислотой, металл-полимерный комплекс, антимикробная активность.

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Information about authors*

Burkeyev, Meyram Zhunusovich — Full Professor, Doctor of Chemical Sciences, Karagandy University of the name of academician E.A. Buketov, Universitetskaya street, 28, 100024, Karaganda, Kazakhstan; e-mail: m_burkeyev@mail.ru; <https://orcid.org/0000-0001-8084-4825>;

Shibayeva, Saliman Ramazanovna (*corresponding author*) — 2nd year PhD student, Karagandy University of the name of academician E.A. Buketov, Universitetskaya street, 28, 100024, Karaganda, Kazakhstan; e-mail: Sali_r@mail.ru; <https://orcid.org/0000-0002-3520-3962>

Khamitova, Tolkyn Ondirisovna — PhD, Department of Soil Science and Agrochemistry, Agronomic Faculty, S. Seifullin Kazakh Agro Technical University, Nur-Sultan 010000, Kazakhstan; e-mail: khamitova.t@inbox.ru <https://orcid.org/0000-0002-4691-3732>

Plocek, Jiri — PhD., CSc. Institute of Inorganic Chemistry of the Czech Academy of Sciences, Czech Republic, Husinec-Rez 1001 25068 Rez; e-mail: plocek@iic.cas.cz; <https://orcid.org/0000-0001-6082-5766>;

Nurmaganbetova, Margarita Taukenovna — Candidate of Chemical Sciences, Karagandy University of the name of academician E.A. Buketov, Universitetskaya street, 28, 100024, Karaganda, Kazakhstan; e-mail: ritunur@mail.ru <https://orcid.org/0000-0002-1785-1069>

Kazhmuratova, Akerke Temirgalievna — Candidate of Chemical Sciences, Karagandy University of the name of academician E.A. Buketov, Universitetskaya street, 28, 100024, Karaganda, Kazakhstan; e-mail: kazhmuratova@mail.ru; <https://orcid.org/0000-0003-4044-8419>;

Zhumagalieva, Tolkyng Sergazyevna — Candidate of Chemical Sciences, Professor, Karagandy University of the name of academician E.A. Buketov, Universitetskaya street, 28, 100024, Karaganda, Kazakhstan; e-mail: Zhumagalieva79@mail.ru; <https://orcid.org/0000-0003-1765-752X>

*The author's name is presented in the order: *Last Name, First and Middle Names*

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Buketov University