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Novel biologically active polyurethane materials containing silver and copper nanoparticles

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Silver and copper nanoparticles containing biologically active thermoplastic polyurethanes have been prepared by saturation of liquid polyether (original reactant for polyurethane synthesis) with Ag, Cu nanoparticles, followed by polyurethane synthesis. The problem encountered during the synthesis of such materials is uniform incorporation and distribution of the metal nanoparticles in the polymer matrix and at the same time retention the physico-chemical properties inherent to polyurethanes. Colloid of metal nanoparticles in a liquid polyoxytetramethylene glycol, MM 1000 was obtained by electron beam evaporation technology and vacuum deposition. Then, the metal-containing thermoplastic polyurethane materials with targeted properties and structure, depending on diisocyanates and chain extenders nature, have been produced on the basis of obtained colloid. Polyurethanes containing Cu and Ag nanometals exhibit bactericidal/bacteriostatic properties against bacteria, fungi and yeast-like fungi. Standard methods of polyurethane processing allow to produce the resulting biologically active metal-containing polyurethane materials for medical products (catheters, drains, films, and so on), scince the presence of metal nanoparticles does not affect the physical properties of the polymer.

Key words: biologically active polyurethane, copper and silver nanoparticles, bactericidal/bacteriostatic properties.

Introduction.

Polymeric materials containing metal nanoparticles (MN) are among the most versatile currently used materials [1]. Changing of the properties of metal particles at the transition from the bulk materials to the sizes smaller than 100 nm is explained mainly by two factors: a large area / volume ratio and domination of quantum effects. Composites metal nanoparticle / polymer matrix are of particular interest as they open opportunities of obtaining structural and functional materials of new generation. The silver nanoparticles exhibit biological and antibacterial activity and can be successfully used in medicine, agriculture and catalysis [2, 3, 4]. It is also known the use of copper nanoparticles as biologically active, antibacterial component [5].

Today there is a lot of research focused on polymer systems with metal nanoparticles. The main task of these works is to find ways of metal nanoparticles' synthesis in an organic medium. Methods for obtainig silver nanoparticles may be divided into chemical and physical. Chemical methods are based on the recovery of metal ions with different reducing agents [6, 7, 8]. The physical methods include spraying or mechanical grinding of bulk material [9].

One of the most promising physical methods of obtaining of MN in an organic medium is the method of pulsed laser ablation of metal, which allows to obtain MN directly in the fluid. The method consists in irradiation of the target surface with short laser impulses, leading to its destruction and emission of material, followed by the formation of nanoclusters of 5 to 100 nm. Placement of the target directly in the bulk of the liquid matrix does not exclude the chemical interaction of MN with the vapor of surrounding fluid, especially at increasing the target temperature during the laser pulse. In some cases of laser ablation the chemical interaction of MN with the liquid may lead to the modification of their physic-chemical composition, changes in the morphology and optical properties of colloids. Magnetron sputtering method presented in [10] has significantly expanded the scope of the nanoparticles production. This method also allows to obtain

MN directly in liquid, spraying the target beyond the liquid matrix bulk. An atomic flow of the sputtered particles enters on the surface of the liquid in the condensation zone, and their growth is restricted by the time of liquid flow through this zone. DC-magnetron is used as the metal spray apparatus. The disadvantages of this method are the follows: large dimensions of the magnetron cathode and the high cost of the cathode manufacture. In the case of brittle materials, they are destroyed during operation.

The above methods allow to obtain nanoparticles for studying the processes of their formation and growth, but such methods are not suitable for producing nanoparticles in an industrial scale. Method of vapor stream deposition from the vapor phase, which refers to physical methods of MN producing, enables the production of high performance colloidal solutions with different concentrations of nanoparticles of various metals (silver, copper) up to 1000 ppm. This method has a success control of the evaporation rate and density of the vapor stream. Method eliminates the need for stabilizers and reducing agents, release from foreign impurities during producing of metal nanoparticles and thus, avoid of the need for further purification of the particles. The disadvantage of this method is the impossibility of use of materials which do not remain stable in vacuum as carriers (target). The most known polymers synthesized from stable in vacuum monomers, are polyurethanes - biocompatible polymers having valuable properties such as strength, flexibility, elasticity. Due to ability of wide variation of properties they are used in virtually all fields of human activity. One of the basic components in polyurethanes synthesis is polyester/polyether. For obtaining the materials with the required properties both polyesters and polyethers are used. The nature of the polyester/ polyether used affects the strength, elasticity, crystallinity, glass transition temperature and melting point of the polyurethane material.

The purpose of this paper is to obtain the biologically active polyurethane materials containing silver and copper nanoparticles on the basis of colloidal solutions of metal nanoparticles in polyether and characterize their structure and properties.

Experimental.

Materials.

Polyether polyoxytetramethylene glycol POTMG 1000 [Aldrich], 4, 4'-diphenylmethane diisocyanate (MDI) [Aldrich] as diisocyanate constituent and 1,4-butanediol (BD) [Aldrich] as chain extender were used for the synthesis of metal-containing polyurethane materials.

Selection of polyoxytetramethylene glycol with molecular weight of 1000 (POTMG-1000) [Aldrich] as the polyether to create biologically active polyurethane materials has been specified by necessity of obtaining of flexible and durable material for medicine. Furthermore this polyether has a melting point of 24 – 26 °C that allows to condense the metal vapors in liquid polyether under normal conditions.

Methods.

Preparation of colloidal solutions of metals in POTMG -1000.

Electron-beam equipment UE-142 was used in this study. POTMG-1000 monomer with molecular weight of 1000 g/mol was taken as target. During the experiment POTMG-1000 was loaded into the working chamber and constantly stirred in a specially equipped vessel to prevent formation of a continuous of metal film on the monomer surface. The electron-beam gun was used as a heating source metal (silver and copper). Formed by the vapor stream was directed on the target surface. Arriving at the monomer surface, atomic vapor stream of a metal was deposited, whereby metal particles were formed, which are then distributed throughout the volume of POTMG-1000. The entire process was carried out in a vacuum of 10^{-4} mm of mercury. A constant temperature of POTMG-1000 not exceed 50 °C was maintained during the experiment. All details of this process are described in [11].

Determination of metal content in the colloid composition.

Determination of metal content in the colloid POTMG 1000 - Ag, Cu was carried out by atomic absorption analysis [12] on the atomic absorption spectrophotometer with flame atomization AAS-1N (Carl Zeiss Jena)

S*tudy of colloids by laser correlation microscopy (LCS).*

The average size and range of particle sizes was determined by dynamic scattering of light on the instrument “Zeta Sizer-3” (Malvern, Great Britain). The device is equipped with a helium-neon (He-Ne) laser with a wavelength of 633 nm and a power of 25 mW. The measuring range of the instrument from 1 nm to 20 microns [13, 14].

Samples of monomer with metal particles (polytetrahydrofuran POTMG-1000 + Ag, Cu) were measured six times during 60 seconds at a constant temperature of 30 °C. This temperature is caused by two factors: the first is the melting point of POTMG-1000 (MW 1000 g / mol) Tm = 26 °C, and the second factor is due to relatively high dynamic viscosity of POTMG-1000; increase of the temperature reduces this parameter that contributes to a more accurate determination of particle size by this method. The value of dynamic viscosity of POTMG-1000 h= 440 mPaЧs, the scattering angle was 90°. The autocorrelation function was processed using standard computer program PCS-Size mode of the v 1.61.

Study of colloids by transmission electron microscopy (TEM).

The method of TEM in transmission mode was used for study of sediment after separation of the liquid POTMG 1000 matrix from the POTMG 100 - Ag, Cu colloid. Transmission electron microscope JEM-1200 EX (JEOL, Japan) was used. TEM micrographs were processed using specialized software complex computer image analysis «Media cybernetics image analysis program» Image-Pro Plus version 6.0 with following statistical analysis.

Obtained data reflect the 95% confidence interval of

average particle size values. The mathematical processing of the results was performed using the methods of variation statistics by means of statistical analysis software Microsoft Excel, Statgraphics.

Tensile tests of polyurethane materials.

Tensile tests of polymer systems were performed on a tensile machine FU-1000 (Germany) at a tensile rate of 100 mm/min and 25 °C.

Study of the biological activity of polyurethane materials.

The biological activity of metal comprising polyurethane materials was studied in relation to a number of pathogenic microorganisms: Bacteria*: S.aureus, E.aerogenes, P.mirabilis, E.coli, K.pneumoniae, P.aeruginosa*; Yeast-like fungi: *Candida albicans* and *Candida non-albicans*;

Mould fungi: (micromycetes) *Aspergillus flavus, Aspergillus niger, Alternaria alternate, Penicillium spp., Paecilomyces lilacinus.*

The following nutrient solutions were used: blood agar, nutrient agar (for bacteria), agar Saburo - for candida and micromycetes. The nutrient solutions (20 mL of each) were overflowed per Petri dish of 90 mm diameter.

The suspension of microorganisms was prepared in saline (0,95% NaCl) using the instrument DENSI LA METR II and it was adjusted to a concentration of $1x10^6$ CFU / ml. *Candida species* were used in a concentration $1x10^3$ CFU/ ml. Micromycetes – $1x10^4$ CFU / ml [15].

During the time of experiment preparation the test tubes containing the suspension of bacteria and fungi were placed in a shaker. Next, 1 ml of each sample was transferred to the agar surface and with the Drigalski spatula was uniformly distributed on the surface. An excess of suspension was removed with disposable pipette. The dishes were left near the burner for 30 minutes for drying. Then the polyurethane discs, comprising the stated concentrations of nanometals were applied to the surface and placed to a thermostat at 37 °C for bacteria and at 28 °C for micromycetes. The results were primary fixed in 24 hours (for bacteria), 48 hours (for *Candida* fungi) and 10 days (for micromycetes). To eliminate the secondary growth of pathogens all dishes after the growth fixation were kept for a month. Then a second record of results was performed. After the primary and second registration the scrapings from the surface of each polyurethane disc containing various concentrations of nanometals were made, and inoculations on appropriate media were carried out.

Results and discussion.

Results of (TEM) of POTMG-1000/metal nanoparticles colloid solutions.

Microphotographs of colloid solutions of metals in POTMG-1000 (fig. 1) display that the copper particles in POTMG-1000 matrix mainly have a spherical shape. According to the TEM data the histograms of Cu nanoparticles' distribution in POTMG-1000 volume were built (fig. 2), which represent the dependence of the particle

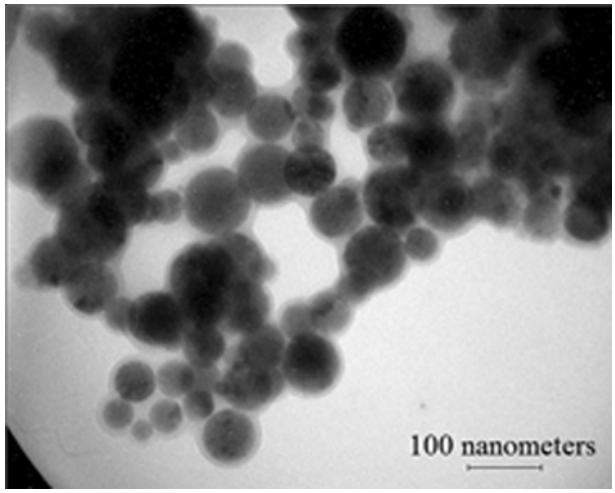


Fig. 1. Microphotographs of Ag-POTMG-1000 colloid

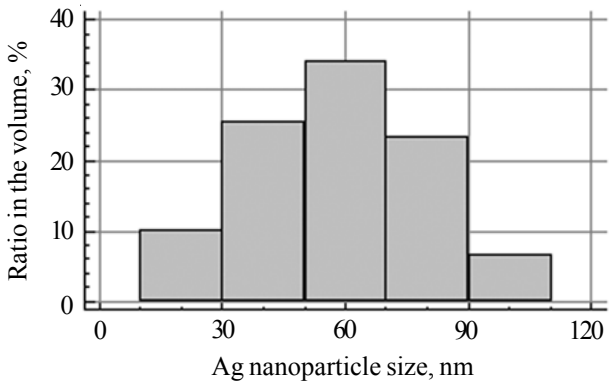


Fig. 2. Histogram of Ag nanoparticles distribution in POTMG-1000 volume

size of Cu (nm) on their ratio in POTMG-1000 (%) volume.

According to histogram's data the copper particles are distributed in the size range of 10 - 110 nm, an average particle size amounts to 58 nm. A fraction ranged in 30–90 nm amounts to 86 % from the total particles quantity. The area occupied by Cu particles amounts to 42 % of the total area.

LCS results (fig. 3) shows the presence of particles in the range of 10 - 160 nm. The most probable particle size is

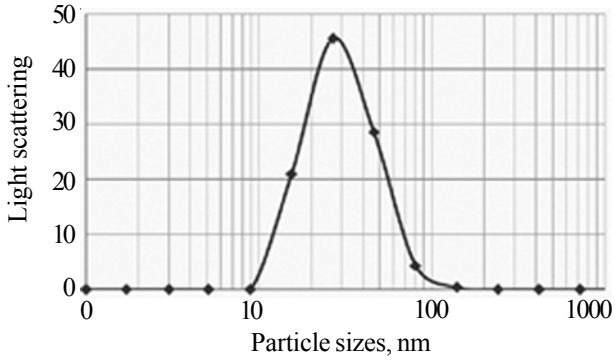
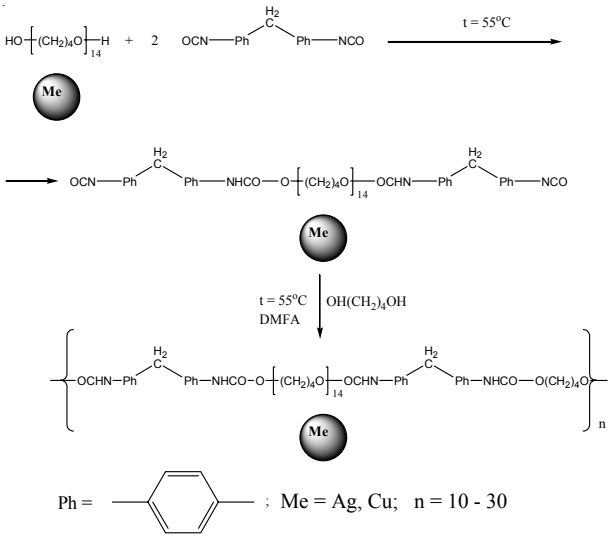


Fig. 3. Nanoparticle size distribution of Ag-POTMG by LCS

52 nm.

Synthesis of metal-containing polyurethane nanomaterials based on Ag-POTMG and Cu – POTMG colloids.

Synthesis of Ag-POTMG and Cu-POTMG colloids based metal-comprising polyurethane nanomaterials exhibits a process of obtaining of linear polyurethane based on POTMG- 1000, 4,4'-MDI and butanediol (BD). The synthesis includes two stages: synthesis of MDI and extension of macromolecule by polycondensation reaction of MDI and BD. The synthesis can be presented as follows:



Elongation process takes place in an aprotic polar solvent (DMF). The presence of metal nanoparticles does not affect the chemical reactions at all stages of the synthesis, which allows to obtain a metal comprising polyurethane of given structure. This method is versatile and allows to use the colloids of metals in POTMG-1000 to produce metal comprising polyurethanes of different structures and properties [16]. As the results of the synthesis a polyurethane solutions with metal nanoparticles in an organic solvent DMF have been obtained. After gradual evaporation of the solvent the samples of polyurethane metal comprising film materials have been formed. The composition and properties of the obtained polyurethane materials are presented in table.

Nanoparticles of silver and copper do not affect the thermoplastic nature and strength of the polyurethane nanocomposite material, which allows to process the obtained polyurethane material by extrusion.

Biological activity.

Results of mycological and microbiological study of polyurethane metal comprising nanomaterials show that bacteria and fungi of the genus *Candida* were highly sensitive to the action of all the studied concentrations of copper and combinations Cu (200 ppm) and Ag (69 ppm). It should be noted that growth inhibition zone of bacteria and *Candida* around the disk was not observed; however, the inoculation of scraping/smear from the surface of the disk was sterile as at the primary registration, and in a month.

Study of the effect of different concentrations of introduced into the polyurethane nanometals on micromycetes has shown that during the initial registration in 10 days from the start of the experiment, the surface of the disc remained sterile, but in a month there was a secondary growth of fungi on the contour of the disk. At that the disks with Cu concentration (666,7 ppm and 308 ppm), and combined Cu and Ag content (Cu - 200 ppm; Ag - 69 ppm) were the most active against micromycetes cultures polyresistant to antibiotics. The results revealed that the sensitivity of fungi to copper and silver is genus-dependent.

Biological study has shown that polyurethanes containing nanometal particles of Cu and Ag exhibit bactericidal properties in relation to both gram-positive and gram-negative bacteria, and yeast-like fungi. The same polyurethanes demonstrate bactericidal and bacteriostatic effect in relation to fungi - micromycetes.

Conclusions.

Biologically active polyurethanes, comprising the silver and copper nanoparticles were prepared by saturation of liquid POTMG – 1000 with Ag, Cu nanoparticles followed by synthesis of polyurethane. The colloidal solutions of Ag and Cu nanoparticles with diameter ranging from 10 to 160 nm in the liquid POTMG – 1000 matrices was obtained using technology of electron-beam evaporation and vacuum deposition of inorganic materials. The average particle size Cu amounts 52 nm, the fraction, located in the range of 30-90 nm amounts 86% of the total number of particles, the average particle size Ag amounts 30 nm. The method applied for preparation of biologically

Table. Composition and properties of biologically active polyurethane materials

Composition and properties	Cu:Ag [200:69]	Cu [666,7]	Cu [308]	Cu [200]	Ag [98]	Ag [27]	Ag [15]
Ag, ppm	69	-	-	-	98	27	15
Cu, ppm	200	666,7	308	200	-	-	-
Melting point, *T*m.°C	136–138						
Degradation temperature*T*d.°C	175–176						
Tensile strength, MPa	32,5	33,5	35	34	35,5	36	35

active metal-containing polyurethane nanomaterials is versatile and allows to obtain biologically active materials with targeted properties. Biological study has shown that polyurethanes containing nanometal particles of Cu and Ag exhibit bactericidal properties in relation to both gram-positive and gram-negative bacteria, and yeast-like fungi. The same polyurethanes demonstrate bactericidal and bacteriostatic effect in relation to fungi - micromycetes. Resulting biologically active metal-containing polyurethane nanomaterials can be processed into products for medical purposes (catheters, drains, film, mesh and so on) by standard methods of polyurethane processing, scince the presence of metal nanoparticles in their structure does not affect the physical properties of the polymer.

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Нові біологічно активні поліуретанові матеріали, що містять наночастинки срібла і міді

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Біологічно-активні термопластичні поліуретани, що містять наночастинки срібла і міді, були отримані насиченням вихідного для синтезу поліуретанів компонента, рідкого поліефіру, наночастинками срібла і міді з подальшим синтезом поліуретану. Головною проблемою при створенні полімерних матеріалів, що містять наночастинки металів, є рівномірне введення і розподіл наночастинок металів у полімерній матриці без зміни фізико-хімічних властивостей полімеру. Отримання колоїду наночастинок металів у рідкому поліефірі здійснювалося технологією електронно-променевого випаровування і осадження у вакуумі. Цей метод дає змогу створювати на основі отриманих колоїдів металвмісні поліуретанові матеріали з різними властивостями і структурою, залежно від вибору діізоціанатів і подовжувачів ланцюга. Поліуретани, що містять нанометали срібла і міді, мають бактерицидні/бактеріостатичні властивості щодо бактерій, грибів і дріжджоподібних грибів. Стандартні методи обробки поліуретанів дають можливість виробляти біологічно активні металвмісні поліуретанові матеріали для медичних виробів (катетерів, дренажних трубок, плівок і т.д), оскільки наявність наночастинок металів не впливає на фізичні властивості полімеру.

Ключові слова: біологічно активні поліуретани, наночастинки срібла і міді, бактерицидні/бактеріостатичні властивості.

Новые биологически активные полиуретановые материалы, содержащие наночастицы серебра и меди

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Биологически-активные термопластичные полиуретаны, содержащие наночастички серебра и меди, были получены насыщением исходного для синтеза полиуретанов компонента, жидкого полиэфира, наночастицами серебра и меди с последующим синтезом полиуретана. Главной проблемой при создании полимерных материалов, содержащих наночастички металлов, является равномерное введение и распределение наночастиц металлов в полимерной матрице без изменения физико-химических свойств полимера. Получение коллоида наночастиц металлов в жидком полиэфире осуществлялось технологией электронно-лучевого испарения и осаждения в вакууме. Данный метод позволяет создавать на основе полученных коллоидов металлосодержащие полиуретановые материалы с различными свойствами и структурой, в зависимости от выбора диизоцианатов и удлинителей цепи. Полиуретаны, содержащие нанометаллы серебра и меди, обладают бактерицидными/бактериостатическими свойствами по отношению к бактериям, грибам и дрожжеподобным грибам. Стандартные методы обработки полиуретанов позволяют производить биологически активные металл-содержащие полиуретановые материалы для медицинских изделий (катетеров, дренажных трубок, пленок и т.д), поскольку присутствие наночастиц металлов не влияет на физические свойства полимера.

Ключевые слова: биологически активные полиуретаны, наночастицы серебра и меди, бактерицидные/ бактериостатические свойства.