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AgNPs INCORPORATED IN PMMA-PVP BLENDED NANOFIBERS: SPECTROSCOPY MONITORING OF AgNP DISSOLUTION IN PMMA NANOPORES

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Background: Electrospun polymeric nanofibers incorporated with some biologically active nanoparticles have a huge range of various applications in biomedical fields. Blending several polymers with different properties allows one to obtain a new material with improved characteristics, as well as to control the incorporation and release of medical agents.

Objectives: To elaborate an approach for the preparation of biocompatible nanofibers using a blend of two polymers (polyvinylpyrrolidone (PVP) and polymethyl methacrylate (PMMA)) with incorporated silver nanoparticles (AgNPs) and to apply the absorption spectroscopy for determining of the average nanoparticle diameter and monitoring of AgNP dissolution from PMMA nanopores.

Materials and methods: A blend of hydrophilic (PVP) and water-insoluble (PMMA) polymers is proposed for nanofiber preparation with incorporated AgNPs. The absorption peak position of the band due to localized surface plasmon resonance (LSPR) and its intensity in the UV-vis spectrum were used to characterize AgNPs and to estimate the influence of the environment.

Results: A new method for fabricating nanofibers from a mixture of two polymers, one water-soluble (PVP) and the other soluble only in organic solvents (PMMA), with AgNPs, has been developed. The diameter of the nanofibers is determined to be in the range of 2-4 µm. The average nanoparticle diameter determined by the position of the absorption peak due to LSPR in the UV-vis spectrum is 35-40 nm. The peak shift of this band in different environments was determined in comparison with the spectrum obtained in an aqueous solution. A decrease in the intensity of the band was observed with an increase in the mat incubation time in the oxidizing solution, and a blue shift of the band maximum was detected, which indicates a decrease in the average size of AgNPs during their dissolution.

Conclusions: In the blended nanofibers, PVP environment of AgNPs facilitates their incorporation into PMMA and provides access of water molecules to nanoparticles, while PMMA provides the mechanical strength of the nanofibers. The appearance of AgNPs from the polymeric nanofibers soaked in water was not detected, since they are fixed in the structure of the water-insoluble polymer. Nevertheless, the experiments with the mat soaking in the oxidative solution showed that the nanopores in PMMA incorporated with AgNPs are open. This observation indicates the possibility of a gradual release of Ag^+ ions from such nanofibers.

KEYWORDS: electrospinning; nanofibers; polyvinylpyrrolidone; polymethyl methacrylate; silver nanoparticles; antibacterial agent.

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Recently, the electrospinning technique for nanofiber production has drawn a lot of attention due to different applications, including biomedical fields such as wound dressing, drug delivery, tissue engineering, and regenerative medicine. In addition, they also demonstrate high antibacterial and antimicrobial efficacy [1–6]. The nanofibers possess unique properties such as high surface area-to-volume ratio, tunable porosity, flexibility, and superior mechanical and physicochemical properties.

A wide spectrum of natural and synthetic polymers is exploited for the electrospinning technique individually or in different combinations to fabricate nanofiber mats. Hydrophilic polymers (e.g. polyvinylpyrrolidone, PVP), polyvinyl alcohol (PVA), chitosan, sodium alginate, and others) are very often employed in electrospinning as they are suitable for the incorporation of hydrophilic compounds [6, 7]. Other polymers such as poly-ε-caprolactone (PCL), polylactic acid, and others are also exploited to produce nanofibers, although they are hydrophobic [8]. Nevertheless, they are biocompatible and allow improvement in nanofibers' mechanical performance and hydrolysis resistance [6].

Blending polymers formed by biopolymers, natural polymers, and synthetic polymers have improved characteristics of nanofibers such as mechanical properties and biocompatibility [9-11]. A variety of functional agents, such as various drugs, antimicrobial compounds, or nanoparticles, can be incorporated into nanofibers, producing multifunctional nanofibers that have been applied for many medical treatments and antimicrobial purposes. In the conventional blending electrospinning method, different polymers are mixed and dissolved in a solvent in which the two polymers are soluble for subsequent electrospinning. Functional agents can be incorporated by dissolving or dispersing in the polymeric mixture before electrospinning. The agent release from nanofibers can be prolonged depending on the interaction between the polymers and the agent. The agent speed release can be controlled by balancing the hydrophilicity–hydrophobicity of the nanofiber medium.

Water-soluble PVP is one of the most commonly used polymers for the nanofiber preparation [12]. This polymer is non-toxic, biocompatible, and biodegradable. PVP is often used for the incorporation of different drugs or antimicrobial agents or as a stabilizer and capping agent in the synthesis of metallic nanoparticles, avoiding agglomeration of the colloidal particles (e.g. AgNPs [13]). Recently, AgNPs, due to attractive properties, including excellent electrical conductivity, chemical stability, antifungal, and bactericidal properties has been widely used in various fields of nanomedicine such as diagnostics, cancer therapy, pharmaceutical applications, antibacterial area, wound dressings etc. [see review [14] and references therein). The interaction between PVP and silver atoms of nanoparticles less than 50 nm in diameter occurs via an amide group, while for larger nanoparticles, the carbonyl groups of PVP are also included in the interaction [15].

Polymethyl methacrylate (PMMA) is not often applied to electrospinning, there are a few works about the production of PMMA nanofibers by electrospinning, which mainly focus on optimizing the electrospinning parameters [16–19]. Blend PMMA and hydrophilic polymers (PVA, PEO, and chitosan) were applied to nanofibers formed by electrospinning [7, 10, 11].

In the present study, we propose a new approach to prepare nanofibers incorporated with antibacterial nanoparticles (AgNPs) using a blend of two polymers: hydrophilic PVP and water-insoluble PMMA. This approach is based on using a PVP as a capping and stabilizing agent for AgNP formation, drying, and then mixing with PMMA in organic solvents. In the proposed preparation scheme, AgNPs prepared in an aqueous medium with a water-soluble polymer are surrounded by this polymer after mixing with PMMA, and this environment is retained in the nanofibers. The PVP surrounding the AgNPs facilitates their incorporation into PMMA and provides access for water molecules to the nanoparticles when the nanofiber mat is soaked in water. The release of AgNPs from the nanofiber mat by soaking in water is studied by absorption spectroscopy.

MATERIALS AND METHODS

PVP (Mw=40 000 Da), $AgNO_3$, AgCl, and glucose were purchased from Sigma-Aldrich (Europe). PMMA (M_w=996 kDa) was kindly provided by researchers from the Institute for Single Crystals of the NAS of Ukraine. Organic solvents: chloroform was purchased from BioMax (Ukraine), and acetone was purchased from Anri-Pharm Group (Ukraine). The molecular structures of PVP and PMMA are presented in Fig. 1.

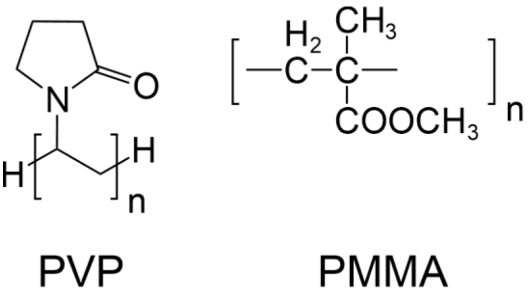

Fig. 1. The molecular structures of PVP and PMMA.

PVP:AgNPs preparation

AgNPs were synthesized in an aqueous PVP solution with AgCl as precursor material, with glucose reduction at a concentration of 8 mg/ml. AgNPs were grown at 80 ^{0}C for 30–90 min. Absorption spectra in the UV-visible spectral range were used to control the appearance of AgNPs in the suspension and to estimate the size of the nanoparticles. The PVP:AgCl concentration ratio of 240:1.6 mg/ml was determined to be optimal for the growth of AgNPs.

PMMA:PVP:AgNPs preparation

To prepare the polymer blend, in the first step, an aqueous solution of PVP:AgNPs was dried with warm air. In the next step PVP:AgNPs powder was dissolved in chloroform at a concentration of 160 mg/ml and mixed with PMMA solution in chloroform at the same concentration in a volume ratio of 1:2. Then, an equal volume of acetone was added to the mixture. After stirring, the solution became stable and was used for electrospinning. According to our calculations, the w/w ratio AgNPs:polymer (PMMA:PVP) for the nanofibers and films is 1:500.

UV-visible absorption spectroscopy

UV-visible absorption spectra observed in the range of 200–800 nm were recorded using a double-channel spectrophotometer (Hitachi M 356, Japan). The diameter of the nanofibers in the mats obtained by electrospinning was estimated from high-resolution microphotographs.

Fabrication of nanofibers using electrospinning

Setup for fabrication of nanofibers basically consists of a syringe with a metal needle used as a tip, a counter electrode as a ground collector at some distance from the tip, a high voltage power supply providing a positive potential applied to the tip (10–30 kV) and mechanical device that ensures uniform movement of the syringe piston. A syringe pump carries the polymer solution from the syringe to the needle. In our experiments, nanofibers were drawn at 15–20 kV, the distance to the collector was about 8 cm, solution feed rate was about 0.7 ml/hour. The collector was metal for producing thick mats and glass (on a metal base) for thin samples prepared for microphotography.

RESULTS AND DISCUSSION

Characterization of PMMA:PVP:AgNPs nanofibers

A thin disk of nanofiber mat was produced by depositing nanofibers from PMMA:PVP:AgNPs solution in chloroform/acetone onto a metallic collector. A photo of this sample is shown in Fig. 2. The diameter of the prepared ring is about 35 mm.

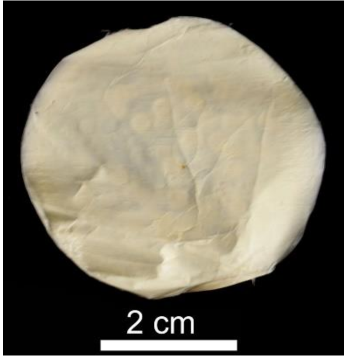

Fig. 2. Photo of samples of polymer nanofiber mat with incorporated AgNPs.

Microphotographs of two different sections of a sample of thin mat formed by PMMA:PVP:AgNPs nanofibers obtained in transmitted light are shown in Fig. 3. The diameter of the nanofibers estimated from the right part of the Figure is in the range from 2 to 4 μm.

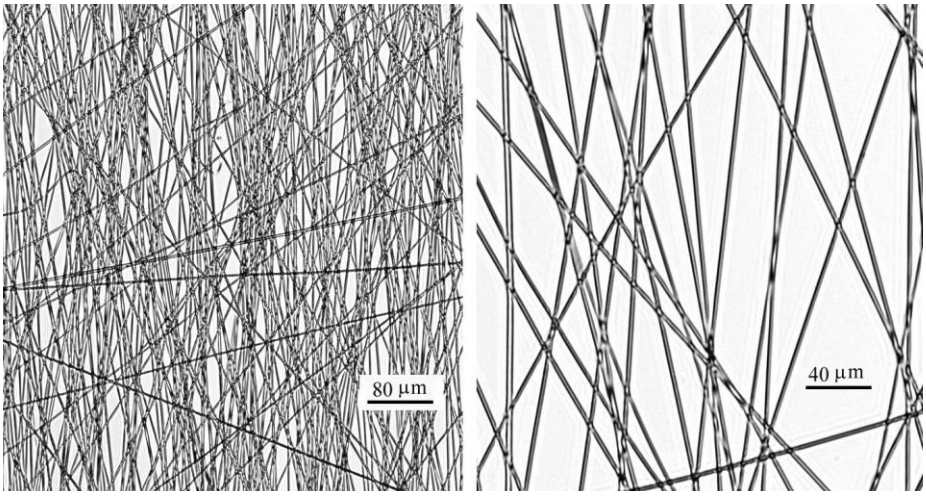

Fig. 3. Microphotographs of two different sections (middle and edge) of a thin mat formed from PMMA:PVP:AgNPs nanofibers.

Characterization of AgNPs in different environments

The appearance of AgNPs in the suspension and the estimation of the nanoparticle diameter were monitored by UV-visible absorption spectroscopy. Fig. 3a shows the absorption spectra of AgNPs during nanoparticle synthesis: 30, 60, 90, and 120 minutes. These spectra are characterized by the appearance of a band in the violet region, which is due to the phenomenon of localized surface plasmon resonance (LSPR). The increase in the absorption intensity with time shows the nanoparticle fabrication process, which slows down after 90 min. Fig. 3a shows that the band due to LSPR has a maximum at about 416 nm. Based on the position of the peak, the average diameter of the nanoparticles was determined to be 35–40 nm [20].

As can be seen from Fig. 3a, AgNPs absorption spectra after 90 and 120 minutes of growth almost coincide, indicating that the silver reduction reaction has been over. This observation makes it possible to estimate the concentration of AgNPs in the solution and polymer nanofibers.

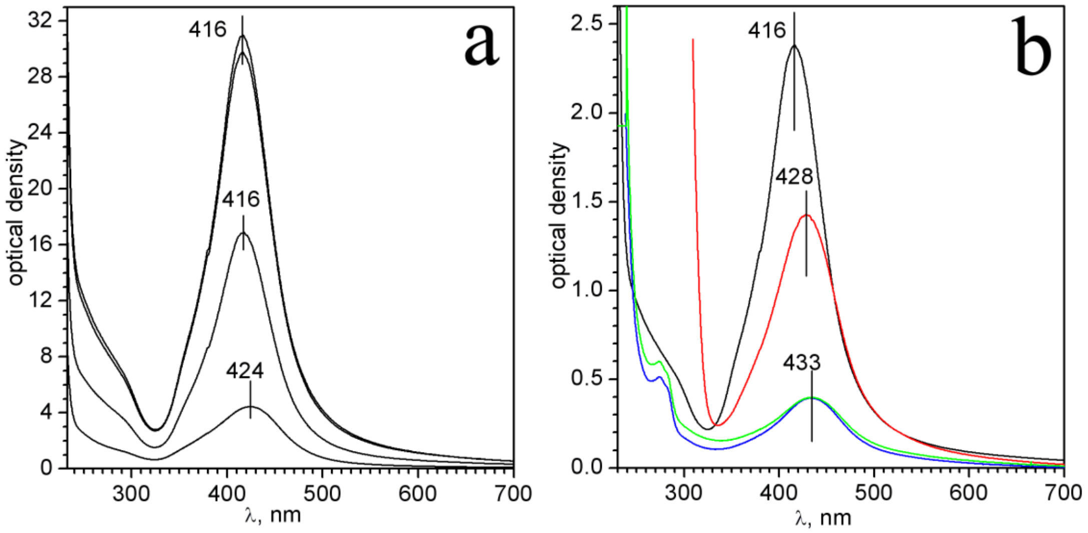

Fig. 3. Absorption spectra of AgNPs: a – enhancement of the intensity of the band due to LSPR obtained by increasing the silver reduction time: 30, 60, 90 and 120 minutes; b –change of AgNP spectrum in different conditions: in aqueous solution (black), in the solution of PMMA:PVP in chloroform-acetone mixture (red); in dry polymer film prepared from fresh solution (blue) and after one week (green). The numbers shown in the figure indicate the peak position in nm.

Nanofibers obtained from PMMA:PVP:AgNPs had a diameter of about 2–4 μm estimated from high-resolution photos by visual analysis. Fig. 3a shows the absorption spectra of AgNPs during the synthesis of nanoparticles and their change after the incorporation into PMMA:PVP mixture (Fig. 3b).

Fig. 3b shows that the peak of the band due to LSPR of AgNPs in a chloroform-acetone mixture of PMMA:PVP is red-shifted by about 12 nm compared to the spectrum obtained in aqueous solution. It could be explained by a change in the nanoparticle environment. The small red shift of the band peak in the PMMA:PVP:AgNPs film (about 5 nm) compared to the spectrum of this mixture in chloroform-acetone may also be due to a change in the interaction of the nanoparticles with the polymer in the solid state. It can be assumed that, in this case, the main factor influencing the band position is the change in the dielectric constant of the nearest environment of AgNPs [21]. For this frequency range, the value of the dielectric coefficient can be assumed to be equal to the square of the refractive index, which increases with the transition from the PVP aqueous solution to the PMMA:PVP chloroform-acetone solution and then to the solid phase of the polymer blend. This assumption is confirmed experimentally when spectra obtained by soaking a nanofiber mat containing AgNPs in various immersion liquids are compared (Fig. 4a). When soaking the mat in vaseline oil, the absorption spectrum of AgNPs has the same characteristics as in the polymer mixture film (Fig. 3b). When the mat is wetted with water in an amount minimally necessary to fill the space between the nanofibers, the maximum of the AgNPs absorption band is blue-shifted by 15 nm compared to the film or the mat in vaseline oil. It means that in the surroundings of AgNPs, molecules of water appear. Note that the spectral analysis of the water solution after the mat wetting showed the availability of PVP that demonstrates water-soluble polymer release from nanofibers. Finally, when the sample is washed with a large amount of water and the PVP is almost completely removed from the fiber material, the absorption band (Fig. 4a) is positioned in a spectrum (at 417 nm) similar to the spectrum of AgNPs in the aqueous PVP solution (Fig. 3b).

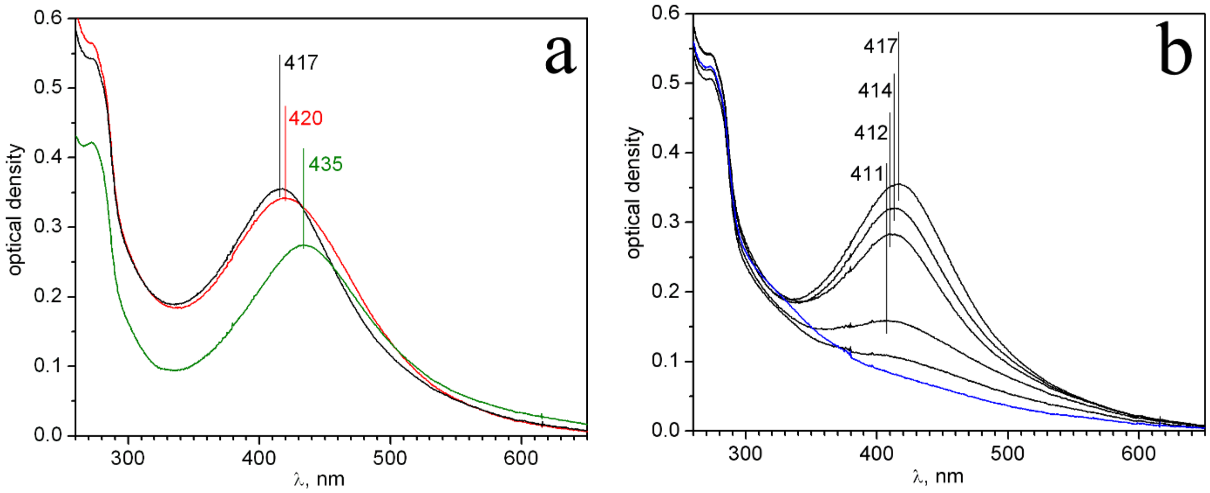

Fig. 4. Absorption spectra of AgNPs in different environments (a): in solid polymer mixture when the mat is immersed in Vaseline oil (green), in PMMA fibers surrounded by an aqueous solution of PVP (red), in PMMA fibers surrounded by water (black); or (b) at treating of the mat by the oxidative solution during different time intervals: 0, 0.5, 1.5, 4.5, and 7.5 min (black spectra, the intensity of the band decreases with increasing time), the blue line is corresponding to the spectrum after the AgNP treatment for 18 min (when AgNPs are completely dissolved).

We attempted to detect the release of AgNPs after an incubation of the PMMA:PVP:AgNPs mat in water. The rinse water spectrum shows the presence of PVP, no band characteristic of AgNPs was detected. It is assumed that AgNPs are retained in the nanopores of PMMA in nanofibers. Thus, a relatively slow release of silver ions in an aqueous medium from such nanofibers with the incorporation of AgNPs is expected. A prerequisite for this is the availability of the surrounding aqueous medium to the AgNPs fixed in the PMMA fibers after the PVP dissolution.

To check whether the nanopores in PMMA containing AgNPs are open, we placed the nanofiber mat in a weak oxidative solution prepared by adding H_2SO_4 (25 µL, density 1.8 g/ml) and $K_2Cr_2O_7$ (25 mg) in 1 L of water. In such a solution, metallic silver is converted to a soluble salt according to the following reaction:

$$6Ag + K_2Cr_2O_7 + 7H_2SO_4 = 3Ag_2SO_4 + Cr_2(SO_4)_3 + K_2SO_4 + 7H_2O.$$

It is assumed that AgNPs located in open pores should dissolve in this oxidative solution. We washed the mats in this solution for different time intervals and monitored the dissolution of AgNPs by the intensity of the band due to LSPR after each time interval. For spectroscopy measurement, each washed mat was fixed between two quartz plates. To reduce the effect of light scattering, the samples were mounted near the detector (photomultiplier) as soon as possible.

A decrease in the intensity of the band due to LSPR was observed with an increase in the mat incubation time in the oxidizing solution. In addition, a slight blue shift of the band maximum is observed, which indicates a decrease in the average size of AgNPs during their dissolution (Fig. 4b). The spectra were recorded after the following time intervals: 0.5, 1.5, 4.5, 7.5, and 18 minutes. After 18 min, the band due to LSPR disappeared in the spectrum almost completely, indicating the dissolution of AgNPs. The gradual decrease in the band intensity with increasing time of mat soaking in the oxidative solution indicates that the nanopores with AgNPs are open and can be successfully used for the release of Ag^+ ions. Thus, a relatively slow release of Ag^+ ions in an aqueous medium from such nanofibers with the incorporation of AgNPs can be obtained.

CONCLUSIONS

A new approach to preparing a blend of two polymers incorporated with antimicrobial agents for electrospinning: water-soluble PVP and soluble only in organic solvents PMMA is developed. In the obtained nanofibers, PMMA provides the mechanical strength of the nanofibers and can facilitate the gradual release of antimicrobial agents. This approach is based on using a water-soluble PVP as a capping and stabilizing agent for the growth of AgNPs in an aqueous medium, preventing their aggregation, drying, and then mixing with PMMA in an organic solvent. The PVP surrounding the AgNPs facilitates their incorporation into PMMA and provides access for water molecules to the nanoparticles when the nanofiber mat is soaked in water. This approach allows the preparation of nanofibers with the gradual release of silver ions from the robust PMMA environment. The average nanoparticle diameter determined by the position of the absorption peak of the band due to LSPR in the UV-vis spectrum is 35-40 nm. The diameter of the nanofibers is determined to be in the range of 2-4 μm. Although the appearance of AgNPs in water from the obtained polymeric nanofibers was not detected, nevertheless, the experiments with the mat soaking in the oxidative solution showed that the nanopores in PMMA incorporated with AgNPs are open. This observation indicates the possibility of a gradual release of silver ions from such nanofibers. Thus, the membrane prepared from these nanofibers can provide a gradual release of the antimicrobial agent. Nanofibers loaded with antimicrobial nanoparticle AgNPs can be used in various biomedical fields, including the fabrication of effective wound dressing materials due to their similarity to the extracellular matrix and excellent protection from microorganisms, and can be used for drug delivery.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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AgNPs ІНКОРПОРОВАНІ ДО НАНОВОЛОКОН З СУМІШИ ПММА-ПВП: СПЕКТРОСКОПІЧНИЙ МОНІТОРИНГ РОЗЧИНЕННЯ AgNPs В НАНОПОРАХ ПММА

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Актуальність. Електропрядене полімерне нановолокно, об’єднане з деякими біологічно активними наночастинками, має величезний спектр різноманітних застосувань у біомедичних галузях. Змішування декількох полімерів з різними властивостями дозволяє отримати новий матеріал з поліпшеними характеристиками, а також контролювати введення і вивільнення лікарських речовин. **Мета роботи.** Розробити методику отримання біосумісних нановолокон з використанням суміші двох полімерів (полівінілпіролідону (ПВП) і поліметилметакрилату (ПММА)) з вбудованими наночастинками срібла (AgNPs) і застосувати спектроскопію поглинання для визначення середнього діаметра наночастинок і моніторингу розчинення AgNPs з нанопор ПММА.

Матеріали та методи. Запропоновано суміш гідрофільних (ПВП) та водонерозчинних (ПММА) полімерів для отримання нановолокна з включеними AgNPs. Положення піку поглинання смуги, яка викликана локалізованим поверхневим плазмонним резонансом (LSPR)) та її інтенсивність в УФ-спектрі були використані для характеристики AgNPs та оцінки впливу навколишнього середовища.

Результати. Розроблено новий метод виготовлення нановолокон, утворених сумішшю двох полімерів: водорозчинного (ПВП) і розчинного лише в органічних розчинниках (ПММА) з AgNPs. Діаметр нановолокон розташований в діапазон і 2–4 мкм. Середній діаметр наночастинок, визначений з положення піку поглинання смуги, яка обумовлена LSPR в УФ-спектрі, становить 35–40 нм. Визначено зсув піку смуги у різних середовищах порівняно зі спектром, отриманим у водному розчині. Спостерігалося зменшення інтенсивності смуги зі збільшенням часу інкубації мату в окислювальному розчині, а також виявлено зміщення максимуму смуги в синю область, що свідчить про зменшення середнього розміру AgNPs під час їх розчинення.

Висновки. У нановолокнах ПВП середовище AgNPs полегшує їхнє включення в ПММА та забезпечує доступ молекул води до наночастинок, тоді як ПММА забезпечує механічну міцність нановолокон. Поява AgNPs із полімерних нановолокон, змочених водою, не виявлена, оскільки вони закріплені в структурі водонерозчинного полімеру. Тим не менш, експерименти із замочуванням мата в окислювальному розчині показали, що нанопори в ПММА зі включенням AgNPs відкриті. Це спостереження вказує на можливість поступового вивільнення іонів Ag^+ з таких нановолокон.

КЛЮЧОВІ СЛОВА: електропрядіння; полімерні нановолокна; полівінілпіролідон; поліметилметакрилат; наночастинки срібла; антибактеріальний засіб.