Chem. Chem. Technol., 2022,
Vol. 16, No. 3, pp. 404–410

Chemistry

REGULARITIES OF OBTAINING SILVER NANOPARTICLES IN THE PRESENCE OF POLYVINYLPYRROLIDONE AND THEIR APPLICATION FOR OSTEOPLASTIC COMPOSITES

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https://doi.org/10.23939/chcht16.03.404

Abstract. The regularities of obtaining silver nanoparticles in the presence of polyvinylpyrrolidone, which was both a reductant and a stabilizer of nanoparticle dispersion, have been studied. The influence of such factors as temperature, polyvinylpyrrolidone amount, concentration and nature of silver salts on the shape and size of nanoparticles has been established. The chemistry of the silver salts and polyvinylpyrrolidone reaction with the formation of vinylsuccinimide units in the structure of macromolecules has been proposed, which is confirmed by the results of IR spectroscopy. It has been established that the shape and size of silver nanoparticles are influenced by the silver salt nature. If silver nitrate is used for the reduction reaction, silver nanoparticles are formed mainly in the form of triangular prisms and polyhedra. When using silver acetate, nanoparticles of spherical shape are predominantly formed. High-quality nanoparticles are formed if the mass ratio of polyvinylpyrrolidone : silver salt is more than 20. The decrease in this ratio deteriorates the stabilization of the formed nanoparticles and increases the particle size of silver until the formation of nanocrystals several hundred nanometers in size. The kinetics of silver salts and polyvinylpyrrolidone reaction has been studied in a solution. The reaction was found to occur faster with increasing temperature and the polyvinylpyrrolidone amount. Silver reduction reaction by polyvinylpyrrolidone was used to provide fungibactericidal properties of hydroxyapatite-filled osteoplastic porous composites based on polyvinylpyrrolidone copolymers with methacrylic esters.

Keywords: polyvinylpyrrolidone, nanoparticle, nanocrystal, stabilizer, bactericidal properties, osteoplastic composites.

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1. Introduction

One of the most pressing problems of modern restorative medicine is bone regeneration after injuries and various surgeries. Therefore, in recent years, the development of new osteoplastic materials has been intensified. These materials provide rapid recovery of bone structure, have no harmful side effects and do not cause postoperative complications. A significant number of studies is dedicated to bone implants made of polylactides and inorganic materials of tricalcium phosphate and hydroxyapatite (HA).[1-4] These inorganic materials contain chemical elements in the same forms as bone in a living organism. The disadvantage of inorganic osteoplastic materials is their low strength and fragility. According to these properties, they are significantly inferior to the properties of bone.

To eliminate such specific shortcomings, inorganic materials are often combined with a polymer matrix, which binds them into a continuous porous structure, and promotes the effective germination of bone tissue. Copolymers of methacrylic esters of glycols with polyvinylpyrrolidone (PVP) are promising as polymer matrices.[5] However, long-term or lifelong presence of composite materials in the human body is often accompanied by inflammatory and repulsive processes. This, in turn, requires constant additional medication. To solve this problem and eliminate the need for drugs the composites containing silver nanoparticles are proposed to be used as the implants.

Interest in this metal is due not only to its high antimicrobial activity against a significant number of infectious agents, but also to the fact that the resistance of various microorganisms to silver nanoparticles is developing very slowly.[6] It is this property that determines the advantage of the use of silver over many modern pharmaceuticals.

The known methods for producing silver nanoparticles usually have either technological problems or problems associated with the use of toxic amine-

containing reducing agents, in particular tertiary amines.[7] Moreover, the resulting silver particles are often in the form of large fibers (up to 10 μm) and are sedimentationally unstable.

Therefore, the development of new effective methods for obtaining silver nanoparticles remains an urgent task of chemistry and chemical technology.

In this work, we used the hypothesis that the use of PVP, which contains tertiary nitrogen in its structure and is also an active component of osteoplastic composites,[8] will help to remedy the abovementioned disadvantages.

Our previous studies[8-9] showed the possibility of obtaining silver nanoparticles using non-toxic PVP as a reducing agent, which is widely used in medicine and pharmacy.[10-11] However, the regularities of obtaining silver nanoparticles in the presence of PVP are poor understood.

Therefore, the aim of this work was to investigate the regularities of obtaining silver nanoparticles in the presence of PVP, to establish the influence of various technological factors on the size and shape of nanoparticles and to investigate their effect on fungibactericidal properties of osteoplastic composites.

2. Experimental

2.1. Materials

2-Hydroxyethylmethacrylate (HEMA) of Bisomer brand, which was purified by distillation under vacuum (residual pressure 130 N/m^2, b.p. 351 K), silver nitrate and silver acetate of PA grade, high-purity PVP of AppliChem GmbH brand (molecular weight of $(1–3)\cdot10^4$) were used for the experiments. Hydroxyapatite (HA) $Ca_{(10-x)}(PO_4)_6(OH)_2$ with a particle size of 0.05–1.20 mm was synthesized at the Department of Silicate Technology of Lviv Polytechnic National University and met the requirements of ISO 13175-3-2015 .

2.2. Methods

Electron microscopic studies of colloidal silver dispersions were performed using a transmission electron microscope (TEM) JEOL JEM 200 CX. The average particle size of silver was determined according to the images using Atlas software.

UV spectroscopic studies were performed on a Perkin-Elmer Lambda 20 UV-VIS spectrometer (light source: deuterium and halogen lamps; monochromator: holographic curved grating; detector: photodiodes; spectral wavelength: 2 nm; software: UV WinLab v. 2.70.01).

IR spectroscopic studies were performed on a Specord M-80 spectrograph at room temperature. The spectra were recorded at equal intensities and constant scanning speed in the range of 500–4000 cm^{-1}.

Quantitative determination of $AgNO_3$ was carried out by means of a potentiometric method using digital ionometer AI-125 in the measuring electromotive force (EMF) mode, within the range from -2400.0 to 2400.0 mV. A permissible absolute measurement error was ± 0.5 mV. Working and model solutions were prepared using distilled water on the day of the experiment.

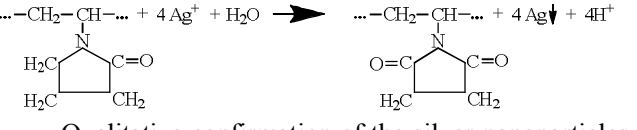
Identification of composite chemical elements was performed by energy-dispersive microanalysis using a scanning electron microscope REMMA-102-02.

Porous composites were obtained according to the method described by Semenyuk *et al.*[12]

Fungicbactericidal properties of osteoplastic composites were studied using test cultures of *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* HB 101 (*E. coli*), and *Aspergillus niger* (*A. niger*) according to the standard diffusion method on a solid nutrient medium (meat-peptone agar for bacteria and wort agar for fungi). The diameter of the composite samples was 15 mm. The microbial load was 109 CFU (colony-forming units) in 1 mL. The bacteria incubation was 24 hours at 308 K, and fungi incubation was 48–72 h at 301 K. The degree of activity was evaluated by the diameter of the inhibited zones of test cultures.

3. Results and Discussion

The regularities of silver reduction from its salts in aqueous and aqueous-alcoholic solutions of PVP under different technological conditions (nature of reducing agents, reaction time, temperature) have been studied. The reaction mixture, which consisted of silver salts and aqueous or aqueous-alcoholic solution of PVP, was stirred on a magnetic stirrer until complete dissolution of the components and kept without stirring in the dark. To our mind, under such conditions the interaction of silver ions with PVP and the formation of vinylsuccinimide units take place according to the following scheme:



Qualitative confirmation of the silver nanoparticles formation in the solution is a dark brown to gray color of the solution (color depends on the number of nanoparticles formed, as well as their size and shape), which indicates the formation of stable silver colloids (Fig. 1).

The solutions are not layered at room temperature, and the formed nanoparticles are not agglomerated, which indicates the formation of silver stable colloids. Solutions of PVP with higher MW are of more intense color, which is an indirect confirmation of the influence of PVP molecular weight on the formation of silver nanoparticles.

IR spectroscopic studies confirm the formation of vinylsuccinimide units in the structure of the PVP macromolecule. Fig. 2 shows an increase in the number of C=O groups in the pyrrolidone cycle.

Fig. 1. Photos of solutions with silver nanoparticles. PVP:$AgNO_3$ = 10: 1(w/w); MW_{PVP}: $3 \cdot 10^4$ (I) and $1 \cdot 10^4$ (II)

The results of UV spectroscopy[13] and transmission electron microscopy also confirm the formation of silver nanoparticles in aqueous and aqueous-alcohol dispersions. The absorption spectra of the obtained dispersions in the UV region have a characteristic peak in the range of 420–435 nm. According to Serheev *et al.*[14], who compared the absorption spectra of silver aqueous dispersions and those calculated for the cluster model Ag12-30, this peak was attributed to the metallic silver.

The maximum absorption observed at 430 nm corresponds to the absorption of silver nanoparticles with a diameter of 15–35 nm[15]. So, it was concluded that PVP with a higher molecular weight is a more effective reducing agent of silver. Due to the better stabilizing ability of PVP with higher molecular weight, the predominant amount of formed silver nanoparticles has a size of 20–30 nm, in contrast to PVP with MW of 10,000, for which the share of such nanoparticles is insignificant.

The conclusions made on the basis of UV spectroscopic results are confirmed by the images obtained using electron microscopy (Fig. 3).

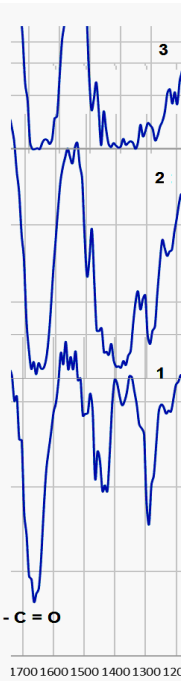
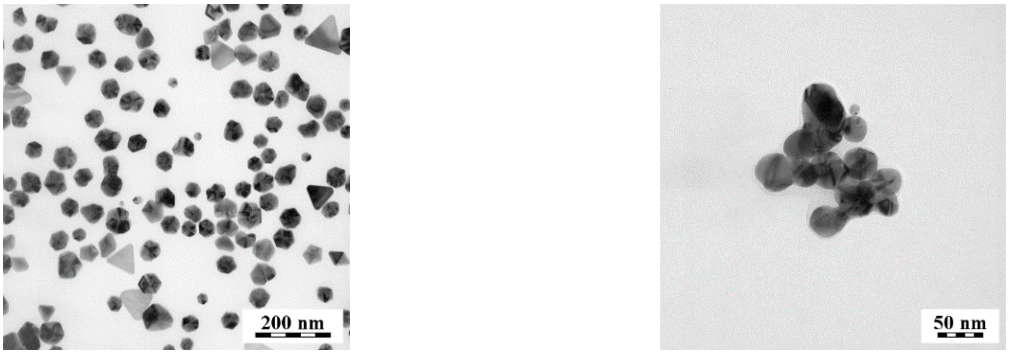


Fig. 2. Fragments of IR spectra: PVP (1); product of PVP and $AgNO_3$ reaction (1:1, w/w) in an alcoholic (2) and aqueous (3) media

In the case of using silver nitrate, the formed silver nanoparticles have a pseudospherical shape. They are obtained in the form of triangular prisms and polyhedra of different sizes (Fig. 3a). The nanoparticles formed from the silver acetate usually have a spherical shape (Fig. 3b).

The kinetics of silver reduction was studied by potentiometric method regarding the decrease of the silver ions concentration in solution. The effect of temperature and reagents ratio on the reduction reaction rate was studied. Kinetic curves of changes in Ag^+ concentration depending on the temperature and nature of medium are shown in Fig. 4.



a) b)

Fig. 3. TEM images of silver nanoparticles obtained in PVP aqueous-alcoholic (1:1, w/w) solution from silver nitrate (a) and silver acetate (b). [$AgNO_3$]: [PVP]: [H_2O] = 1:10:10 (w/w/w), $MW_{PVP} = 1 \cdot 10^4$, T = 348 K, reaction time 60 min

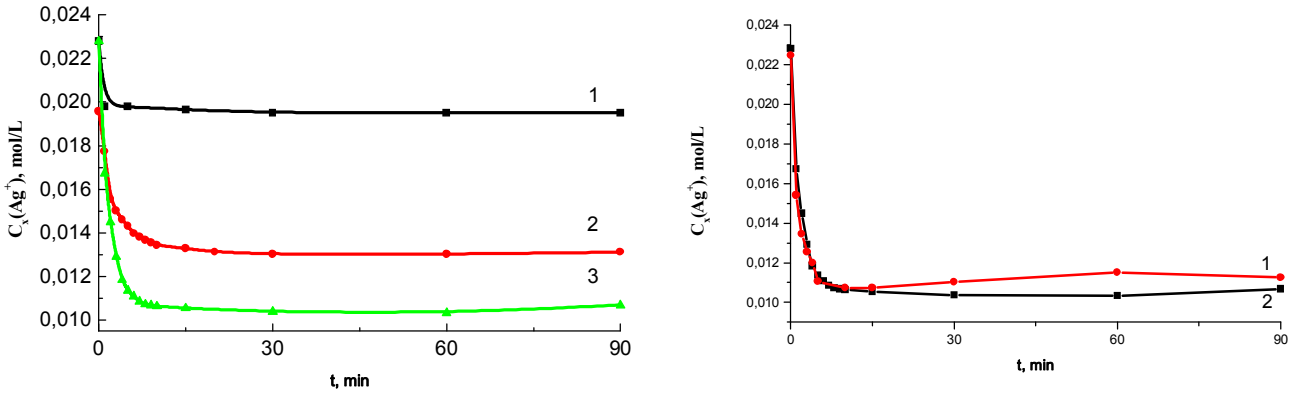


Fig. 4. Potentiometric curves of changes in Ag^+ concentration depending on temperature (a) and nature of medium (b). Temperatures: 293 K (1a); 323 K (2b) and 343 K (3a, 1b, 2b). Media: aqueous (1a, 2a, 3a, 1b) and water:ethanol (w/w) = 1:1 (2a). $[AgNO_3]$:[PVP] = 1:1, w/w

The formation of silver nanoparticles occurs quite quickly at the initial stages – the reduction reaction mainly occurs in the first 10–15 min and is almost complete after about 30 min. A sharp decrease in the concentration of silver ions corresponds to the stage of nanoparticles growth and significantly depends on the temperature of the reaction medium. At room temperature there is only a slight decrease in the concentration of silver ions, and at the temperature of 343 K the concentration decreases twice after 15 min. The nature of the medium slightly affects the reaction rate (Fig. 4b). With the increase in the PVP amount in the reaction mixture, the reduction reaction occurs faster and higher conversions are achieved (Fig. 5).

It is known that the biological, chemical, thermodynamic and electrical properties of silver nanosystems depend on their dimensional characteristics. For a complete description of the nanoscale system, in addition to the direct particle size, it is necessary to have information about their size distribution (polydispersity).

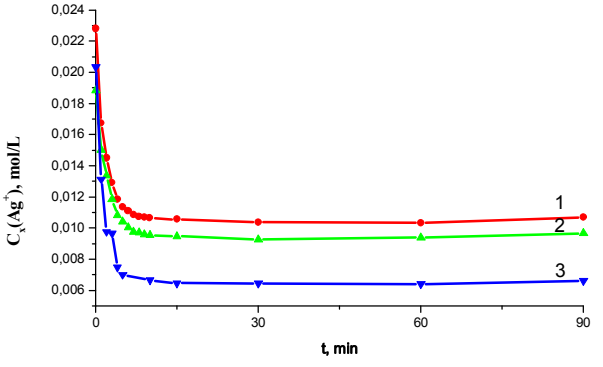


Fig. 5. Potentiometric curves of changes in Ag^+ concentration at T = 343 K. Depending on $[AgNO_3]$:[PVP] ratio (w/w): 1:1.2 (1); 1:1.5 (2) and 1:2 (3)

The results of studies on the effect of temperature, nature of the medium and silver salts, as well as the reagents ratio on the size of silver nanoparticles are presented in Table 1.

Table 1. Effect of obtaining conditions on the size of silver nanoparticles

Conditions			Average diameter, nm
Solvent	T, K	[PVP]:[$AgNO_3$], w/w	
water	313	40	7.9
ethanol	313	40	6.5
ethanol + water (1:1)	313	40	$\frac{7.1}{4.2*}$
ethanol + water (1:1)	333	40	13.8
ethanol + water (1:1)	313	20	10.8
ethanol + water (1:1)	313	5	31.7

Note: * for silver acetate

The increase in temperature has the greatest effect on the size of the obtained particles in the case of aqueous-ethanol solutions. The increase in particle size with increasing temperature is apparently due to the higher product yield at the same ratio of PVP:silver salt. This leads to the fact that the adsorbed polymer shell on the formed silver particles is insufficient for their steric stabilization. Increasing the particle size with decreasing the polymer: salt ratio from 40 to 5 confirms that the shell formed by PVP macromolecules is permeable and is not able to limit the growth, coagulation and aggregation of particles.

Thus, by changing the temperature, selecting the nature of the reagents and the medium, we can directly change the size of silver nanoparticles.

The results of studies of the effect of PVP molecular weight on the particle size distribution of the obtained silver particles are graphically presented in the form of distribution curves, which show the share of individual fractions in the material (Fig. 6).

The graphs show a certain fraction of silver particles, which corresponds to their average diameter. Larger diameter particles are formed when using PVP with MW 10,000. Such silver particles are also characterized by a wide size distribution.

If PVP with MW 30,000 was used, the distribution curve is shifted to a region of smaller particles, and a narrower size distribution is observed. This effect of PVP molecular weight on the particle size distribution can be explained as following: PVP with larger MW reduces silver nanoparticles, and also acts as a stabilizer, which forms a structural-mechanical barrier that prevents particles aggregation and promotes their better stabilization.

Well-shaped silver nanoparticles are obtained when PVP:$AgNO_3$ > 5:1 (w/w). With the decrease in this ratio the particles become less stabilized, resulting in the change of the silver particles shape due to their growth in unprotected areas of the polymer surface. In addition, aggregation of some particles and the formation of nanocrystals with a size of several hundred nanometers can occur (Fig. 7).

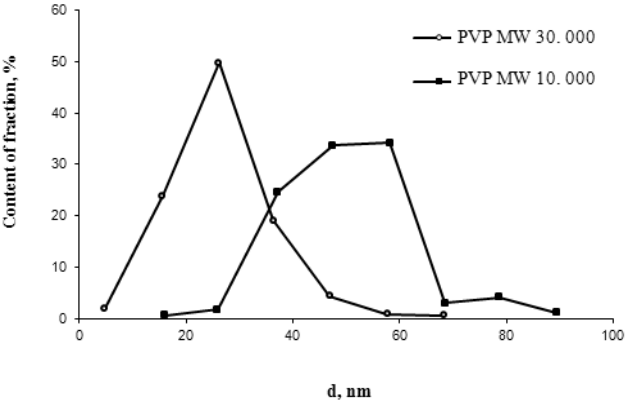


Fig. 6. Distribution curves for silver nanoparticles obtained at different molecular weights of PVP

Another factor that affects the formation of silver nanoparticles is the use of different in nature solvents. In particular, using aqueous-ethanol and aqueous solutions of PVP, it was possible to obtain nanoparticles with different dimensional characteristics (Fig. 8).

Thus, the size of the formed silver nanoparticles can be controlled by the selection of the medium and the reagents nature.

The reduction reaction of silver nanoparticles with polyvinylpyrrolidone during the formation of composites was used to develop porous osteoplastic fungibactericidal composite materials based on PVP/HEMA copolymers filled with hydroxyapatite (HA) (Fig. 9).

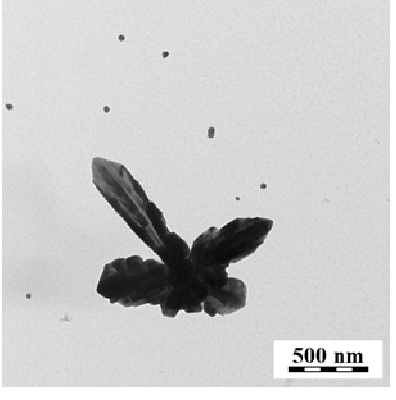
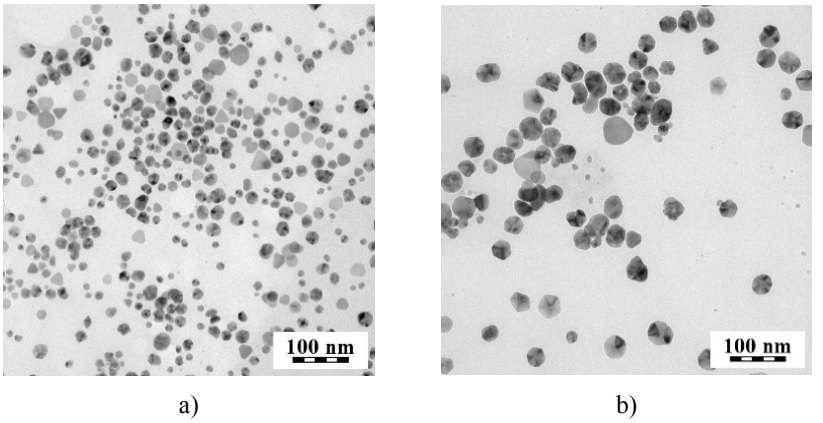
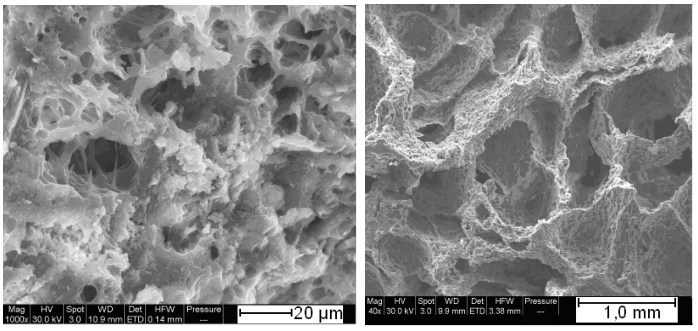


Fig. 7. TEM image of nanoparticles and silver nanocrystal. [$AgNO_3$]:[PVP] = 1:2 (w/w); $MW_{PVP} = 3 \cdot 10^4$



a) b)

Fig. 8. TEM images of silver nanoparticles from aqueous-alcoholic (a) and aqueous (b) solution (1:1). $T = 333$ K



а) б)

Fig. 9. TEM images of micro- (a) and macroporous (b) structure of HA-filled silver-containing composite based on PVP-HEMA

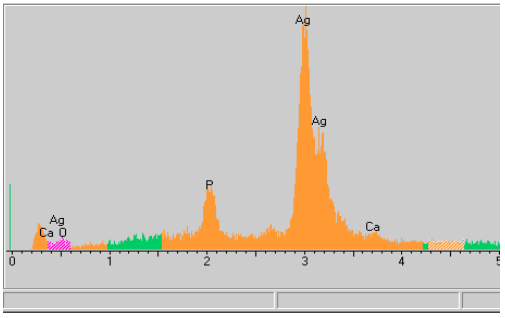


Fig. 10. The spectrum of the surface characteristic radiation of silver-containing composite with HA

The results of energy dispersion analysis (Fig. 10) confirm the formation of silver in the composite structure, providing the composites with fungicidal and bactericidal properties. It was found that for the composites obtained by polymerization of HEMA:PVP:HA = 70:30:70 (w/w/w) in the presence of 6 wt% of $AgNO_3$ the growth inhibition for *E. coli*, *S. aureus* and *A. niger* is 60%, 73% and 44 %, respectively.[16]

The composites without silver nanoparticles did not show fungicidal properties. The developed porous silver-containing composites have the potential to be used in osteogenesis to replace and restore bone tissue.

4. Conclusions

The effect of temperature, concentration of silver and PVP salts, nature of medium and temperature on the shape and size of silver nanoparticles has been established. It was found that PVP is not only a good stabilizer, but also an efficient reducing agent in the creation of silver nanoparticles. With the ratio of PVP:$AgNO_3$ > 20:1 small particles of silver are formed. With the decrease in this ratio, the formed nanoparticles are getting less stabilized, and thus the shape of silver particles is changed. It is possible to change the size of silver nanoparticles by selecting the nature of reagents and medium, as well as by changing the temperature.

Silver-containing composites in the form of porous blocks were synthesized and their bactericidal and fungicidal properties were confirmed.

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Received: November 03, 2021 / Revised: January 14, 2022 / Accepted: January 27, 2022

ЗАКОНОМІРНОСТІ ОДЕРЖАННЯ НАНОЧАСТИНОК СРІБЛА В ПРИСУТНОСТІ ПОЛІВІНІЛПІРОЛІДОНУ ТА ВИКОРИСТАННЯ ЇХ В ОСТЕОПЛАСТИЧНИХ КОМПОЗИТАХ

Анотація. *Досліджено закономірності одержання наночастинок срібла в присутності полівінілпіролідону, який був одночасно відновником та стабілізатором дисперсії наночастинок. Встановлено вплив таких факторів як температура, кількість полівінілпіролідону, концентрація та природа солей аргентуму на форму та розміри наночастинок. Запропоновано хімізм реакції взаємодії солей аргентуму з полівінілпіролідоном з утворенням у структурі макромолекул вінілсукцинімідних ланок, що підверджено результатами ІЧ спектроскопічних досліджень. Встановлено, що на форму та розмір наночастинок срібла впливає природа солі аргентуму. Якщо для реакції відновлення використовувати аргентуму нітрат, то утворюються наночастинки срібла переважно у формі трикутних призм і багатогранників. У випадку використання аргентуму ацетату утворюються наночастинки, які мають переважно сферичну форму. Якісні наночастинки утворюються, якщо масове співвідношення полівінілпіролідон:сіль аргентуму більше 20. Якщо це співвідношення зменшується, то стабілізація утворених наночастинок погіршується і розмір частинок срібла збільшується аж до утворення нанокристалів, розмір яких сягає декілька сотень нанометрів. Досліджено кінетику взаємодії солей аргентуму з полівінілпіролідоном у розчині і встановлено, що з підвищенням температури та кількості полівінілпіролідону реакція відбувається швидше. Реакцію відновлення срібла полівінілпіролідоном було використано для надання фунгібактерицидних властивостей наповнених гідроксіапатитом остеопластичних пористих композитів на основі кополімерів полівінілпіролідону з метакриловими естерами.*

Ключові слова: *полівінілпіролідон, наночастинка, нанокристал, стабілізатор, бактерицидні властивості, остеопластичні композити.*