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DEPENDENCE OF THE SIZE AND NUMBER OF SILVER NANOPARTICLES ON THE LIGAND CONCENTRATION IN THE REACTION SYSTEM

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ЗАВИСИМОСТЬ РАЗМЕРА И КОЛИЧЕСТВА НАНОЧАСТИЦ СЕРЕБРА ОТ КОНЦЕНТРАЦИИ ЛИГАНДА В РЕАКЦИОННОЙ СИСТЕМЕ

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Technologies focusing on the synthesis of silver nanoparticles (AgNPs) are of high relevance within experimental and practical medicine, which can be accounted for by their antibacterial, antiviral, and fungicidal properties, as well as by their capacity to reveal particular antitumor activity, constitute a substantial part of several medical devices and diagnostic consumables. This study shows the effect caused by different ligand concentrations (polyvinylpyrrolidone / PVP) within the reaction system on the size and number of AgNPs obtained through cavitation-diffusion photochemical reduction. Lowering contents of the ligand (PVP) – going from 20 mg to 5 mg – have been proven to be associated with the development of mostly relatively larger nanoparticles (30+ nm of diameter). During that, a less significant decrease in the ligand used for the synthesis (within 25 % of its maximum concentration), too, leads to an increase in the number of smaller nanoparticles (under 15 nm), as well as in the AgNPs of average size (ranging from 15 to 30 nm). The results obtained through this study suggest that PVP may act not only as a stabilizing agent but also as a surfactant capable of stimulating the growth of silver nanoparticles while accelerating the rate of the Ag⁺ ion reduction reaction low-energy excitation. Generally speaking, the developed approach to AgNPs synthesis may help obtain solutions featuring a significant, reliable predominance of nanoparticles of a specific size, depending on their further use in various medical research and technology areas.

Keywords: silver nanoparticles, experimental medicine, ligand, nanoparticle synthesis, polyvinylpyrrolidone

Высокую актуальность для экспериментальной и практической медицины представляет разработка технологий синтеза наночастиц серебра (AgNPs), что обусловлено их антибактериальными, противовирусными, фунгицидными свойствами, а также способностью проявлять противоопухолевую активность. В исследовании показано влияние различных концентраций лиганда (поливинилпирролидона) в реакционной системе на размер и количество AgNPs, полученных методом кавитационно-диффузионного фотохимического восстановления. Установлено, что при снижении содержания лиганда с 20 мг до 5 мг наблюдается преимущественно образование наночастиц более крупных размеров – диаметром свыше 30 нм. При этом уменьшение используемого при синтезе лиганда (в пределах 25 % от максимальной концентрации) приводит к увеличению количества наночастиц меньших размеров (менее 15 нм), а также AgNPs среднего размера – в диапазоне от 15 до 30 нм. Результаты свидетельствуют, что поливинилпирролидон, возможно, выступает не только в качестве стабилизирующего агента, но и как поверхностно-активное вещество, способное стимулировать рост наночастиц серебра, ускоряя скорость реакции восстановления ионов Ag⁺ при низкоэнергетическом возбуждении. Разработанный подход к синтезу AgNPs позволяет получать растворы с достоверным преобладанием наночастиц определенного размера в зависимости от целей их дальнейшего использования.

Ключевые слова: наночастицы серебра, экспериментальная медицина, лиганд, синтез наночастиц, поливинилпирролидон

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Ag⁺ – silver ion
AgNPs – silver nanoparticles

nm – nanometer
PVP – polyvinylpyrrolidone

The development of advanced technologies to synthesize silver nanoparticles (AgNPs) and study their properties is one of the most relevant areas of modern scientific research. The resulting nanoparticles can be used for various purposes, e.g., in biomedicine as antimicrobial, antiviral, and antitumor agents [1, 2]. Given their photocatalytic activity, AgNPs can impart antibacterial properties to various surfaces and coatings used in other areas of human activity, such as the food industry, ventilation systems, ceramics-producing technologies, etc. [3]. Besides, a search is underway to find extra ways to use AgNPs-based compounds in test systems to detect various biologically active substances [4, 5] and identify specific pathogens of dangerous infections [6]. Depending on the potential application area, there are different requirements for silver compounds, which explains the variety of methods needed to produce them. One of the most promising fields is not just the development of methods for the «green synthesis» of nanoparticles involving several organic compounds while shaping nanoclusters [7] but the improvement of the already available technologies for producing AgNPs. Moreover, the latter can be modified by introducing additional components into the reaction system, which would enhance the efficiency of the resulting nanomaterials by transforming their physical and chemical properties, e.g., their photocatalytic activity [8, 9]. Besides, there are reasons to believe that the production of silver nanoparticles might benefit from changing the critical physical and chemical parameters of their synthesis, one of such factors being different amounts of ligand used through the process of AgNPs photochemical reduction.

This study investigated the size range of silver nanoparticles obtained for medical purposes by cavitation-diffusion photochemical reduction at different ligand concentrations (polyvinylpyrrolidone) in the reaction system.

Material and Methods. During the experiment, the authors used the technical resources available at the «Shared Access Center for Diagnostics of Structures and Properties of Nanomaterials» of the Kuban State University. The number and the size of silver nanoparticles were assessed using electron microscopic evaluation of freshly prepared aqueous solutions [10] obtained by the cavitation-diffusion photochemical reduction method, where a decrease in the oxidation status of silver ions (Ag⁺) was achieved jointly with polyvinylpyrrolidone (PVP) [11]. Synthesizing nanoparticles implied complex exposure to ultraviolet radiation (wavelength – 280–400 nm) and ultrasonic waves (frequency – 1.7 MHz) with continuous stirring of the reaction system for 1 hour. During the experiment, the assessment was performed for silver nanoparticles synthesized while using not only the traditional amounts of the ligand (PVP), i.e., 20 mg but its smaller amounts (15, 10, and 5 mg) as well.

The electron microscopy of the obtained samples was done with a JEOL JSM-7500F scanning electron microscope (Jeol, Japan) in the mode of detecting back reflected and secondary electrons with an accelerating voltage of up to 10 kV at a magnification of up to 100000 times [12]. While interpreting the resulting outcomes, the size and the number of silver nanoparticles on the surface of each sample were assessed. The size of silver nanoparticles was measured relative to a standard marker (length – 100 nm).

The experimental data were processed employing variation statistics methods. In contrast, the reliability of the detected differences between the AgNPs indicators in different size ranges was assessed via the nonparametric Mann – Whitney U-test. Differences of $p < 0.05$ were considered statistically significant.

Results and Discussion. The experiment demonstrated that a decrease in the amount of the ligand (PVP) in the reaction system while synthesizing silver nanoparticles entailed a significant increase in the number of AgNPs of a size exceeding 30 nm. During that, the solution showed that against the backdrop of a decrease in the PVP concentration by two times or more, there was a sharp decrease to be observed in the content of nanoparticles with a diameter of 15 nm or below, down to their complete disappearance, as electron microscopy evaluation revealed (Fig. 1).

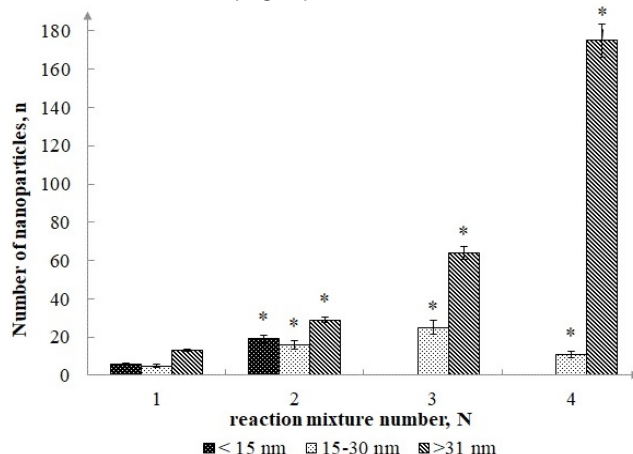


Fig. 1. Change in the size of silver nanoparticles depending on the amount of PVP used in the reaction system.

Note: * – statistically significant differences in the number of silver nanoparticles of a similar size range ($p < 0.05$) from that of the reaction mixture number 1: 1 – 20 mg of the ligand; 2 – 15 mg of the ligand; 3 – 10 mg of the ligand; 4 – 5 mg of the ligand

Notable is that even when, during AgNPs synthesis, the concentration of the ligand was brought down by 25 % (to 15 mg) from its initial amount, there was a significant increase (2.2 times, $p < 0.05$ compared with a similar indicator when using 20 mg of PVP, Fig. 1, Fig. 2) in the content of large nanoparticles (over 30 nm).

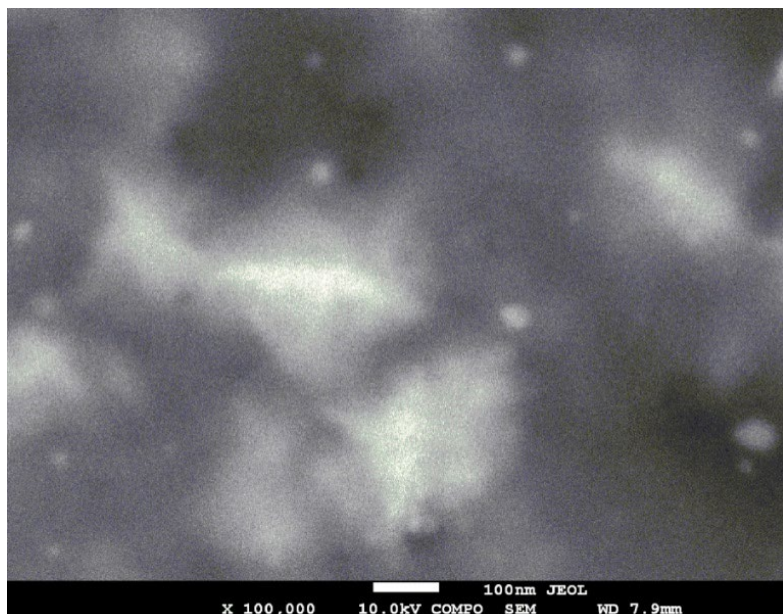


Fig. 2. Electron micrograph; solution of nanoparticles in backscattered electron detection mode («COMPO», $\times 100000$); amount of PVP – 20 mg

A further decrease in the PVP amount (down to 10 mg) was associated with an increase mainly in larger nanoparticles by 4.9 times ($p < 0.05$ compared with a similar indicator when using 20 mg of PVP), while a decrease in the ligand concentration at producing AgNPs by 75 % of its initial level (down to 5 mg), the content of large nanoclusters (exceeding the size of 30 nm) went up more than 13 times ($p < 0.05$ compared to a similar indicator at using 20 mg of PVP, Fig. 3).

The number of silver nanoparticles with a diameter of 15–30 nm increases as the concentration of the complexing agent used to stabilize them (PVP) decreases within the range of 20–10 mg. However, it would be fair

to note that further on, the dynamics are somewhat different from that described earlier for larger nanoparticles (30+ nm). First of all, it must be pointed out that the number of mid-sized nanoparticles arrives at its top value when the PVP concentration is reduced by 50 % of the maximum (to 10 mg of ligand, Fig. 1) along with a 5-fold increase in the number of AgNPs, and 1.6 times if compared with similar indicators when using 20 mg and 15 mg of PVP, respectively ($p < 0.05$). When a lower concentration of PVP (5 mg, Fig. 3) was used further in the synthesis, though, the AgNPs content dropped by 2.3 times ($p < 0.05$ compared to a similar indicator when using 10 mg of PVP). It is to be pointed that the number of the smallest nanoparticles (below 15 nm) goes up 3.1 times when the amount of PVP is reduced by 25 % (15 mg), even though later the particles falling within the said size range were not identified in the obtained micrographs, which also came along with a sharp increase in the share of larger nanoparticles with a significant predominance of nanoclusters of a diameter exceeding 30 nm (Fig. 1, Fig. 3).

As the electron microscopy data suggest, a decrease in the ligand amount (PVP) tends to predominantly promote an increase in larger nanoparticles with a diameter of over 30 nm in photomicrographs. In this case, a less significant decrease in the ligand used for the synthesis in the reaction system (within 25 % of its maximum concentration) leads, among other effects, to an increase in the number of nanoparticles of smaller sizes (<15 nm), as well as mid-sized AgNPs (15–30 nm).

As observed through the experiment, the changing amounts of AgNPs belonging to the size mentioned above ranges might indicate PVP ability to stabilize them, thus preventing, for instance, the coagulation of the resulting nanoparticles by slowing down significantly the development of their stable aggregates. This hypothesis proves that as the amount of the used ligand decreases, there is a significant increase in the number of nanoparticles of a diameter over 30 nm. Besides, respective literature claims that PVP acts as a protective shell for nanoparticles, significantly enhancing their aggregation stability under the impact of several extreme factors [13].

In addition, as facilitated by PVP, the stabilization of nanoparticles also occurs due to changes in their surface electrochemical activity, which has an even more substantial impact on their dissolution reduction than the local hydrophobic feature of their surface [14]. Also, a particular ligand shell can lead to the development of nanoparticles of a specific shape, e.g., nanocubes, nanorods, octahedrons, etc., which further affects the stability of AgNPs, their reactivity, and transportation within the body. Octahedral silver nanoparticles with dominant surface faces have been shown to dissolve faster than other AgNPs [14]. In contrast, the PVP

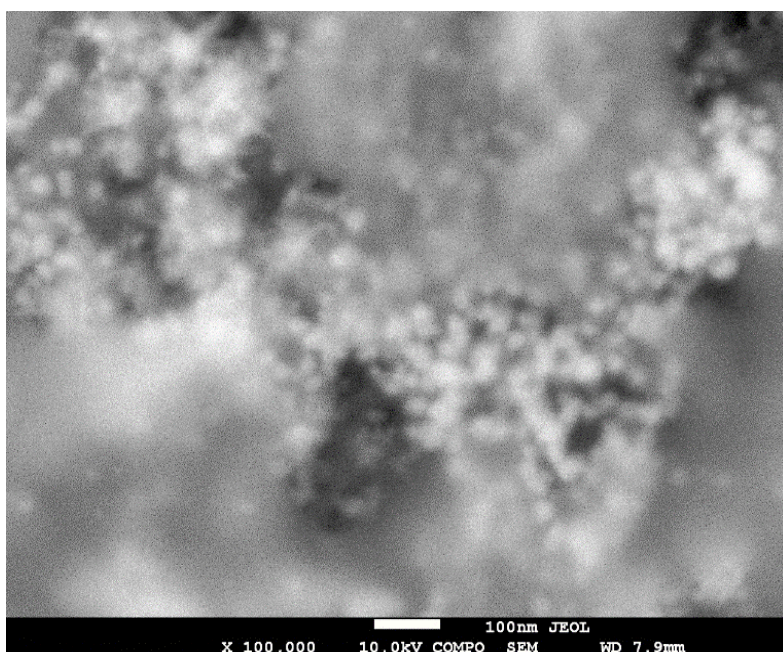


Fig. 3. Electron micrograph; solution of nanoparticles in backscattered electron detection mode («COMPO», $\times 100.000$); amount of PVP – 5 mg

coating on the face is critical for stabilizing and preventing its dissolution. The latter is especially relevant when using the said nanoparticles in veterinary and human medicine since the slow dissolution of silver-containing nanoclusters when treating purulent and contaminated wounds allows increasing significantly the exposure time of Ag^+ in the wound fluid, reaching their effective microbicidal concentration, which notably reduces the risk of infectious complications [15, 16]. Such silver nanoparticles were found to have an antiseptic effect and a regenerative one in treating wounds, thus reducing the exudate microbial contamination by 24 times within ten days, comparable to the similar impacts of using chlorhexidine [17].

The resulting changes in the quantitative and rate content of nanoparticles of different sizes, however, not only prove that PVP plays a role as a stabilizing agent in the reaction system but also is a surfactant capable of simulating the growth of silver nanoparticles, acting as a so-called photochemical relay. Certain studies have demonstrated that at shorter wavelengths with higher energy, Ag^+ reduction occurs faster, leading to the development of structures with more twin planes. In contrast, excitation wavelength overlap for Ag_2S clusters developing in the early synthesis stages accelerates the reaction rate under low-energy excitation [18]. This assumption is also confirmed by the fact that when the amount of PVP goes down from 20 mg to 15 mg, the number of nanoclusters of the smallest studied size goes up to 15 nm.

Conclusion. Given the above, the study showed that a decreasing PVP concentration in the reaction system significantly increases the number of larger AgNPs (over 30 nm). At the same time, the content of this ligand is within the range of 20–15 mg, and there is an increase in the development of nanoparticles sized below 15 nm and falling within the diameter range of 15–30 nm. This potentially can be explained by the direct impact that the ligand has on the nanoparticle synthesis process, as well as by its surface-active properties, such as stimulating the growth of anisotropic silver nanoparticles through kinetic control [18]. Therefore, the developed approach for AgNPs synthesis, including various concentrations of the ligand, will allow for obtaining solutions with a

notable predominance of nanoparticles of a specific size, depending on their potential use in multiple areas of medical research and technology [4, 5]. In surgical practice, for instance, nanoparticles with a diameter of up to 15 nm are effective as an antibacterial agent in wound dressings when eliminating gram-negative microorganisms [2], whereas nanoparticles with a diameter of 15 to 30 nm feature microbicidal properties mostly against gram-positive bacteria only [19]. Also, AgNPs with a diameter of over 30 nm have proven helpful in the case of their sorption on suture material [20] and within compound gels in combustiology, as well as in endoprosthetics. The research literature also notes that, in case of similar shape, electrical charge, and ligand features, nanoparticles exceeding 30 nm in size have higher biocompatibility and lower cytotoxicity than AgNPs of a smaller diameter. Another point to be mentioned is that there is data on the antitumor activity of nanoparticles with a diameter below 30 nm in case of having them cultivated with the MDA-MB-231 breast cancer cells [1]. In dentistry, AgNPs (typically up to 15 nm) can be used for disinfection and to prevent oral infections [21].

They also have a pronounced antiviral inhibitory capacity, reproduce and spread viruses, and can also be used as a post-infectious virostatic agent [22]. Smaller negatively charged AgNPs (<15 nm), in the meantime, are known to possess powerful fungicidal properties [23], whereas for laboratory diagnostics, effective are nanoparticles sized 15–30 nm, which will act as an element of biosensors, and which have a reasonably high photocatalytic activity explained by the development of oxygen active forms [24]. In general, all of the above is a clear indication of a high demand for silver nanoparticles of a specific size range to be employed in various areas of medicine, and this, in turn, further confirms the relevance of the technology developed for producing such particles.

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