https://doi.org/10.15407/polymerj.45.02.153
УДК 602.4:546.57:544.77.051

T.B. ZHELTONOZHSKAYA,
Institute of Macromolecular Chemistry of the NAS of Ukraine, 48 Kharkivske shose, 02155 Kyiv, Ukraine,
ORCID: 0000-0001-5272-4244
L.V. SHEVCHENKO,
National University of Life and Environmental Sciences of Ukraine, 15 Heroyiv Oborony St., 03041 Kyiv, Ukraine,
ORCID: 0000-0001-7472-4325
N.M. PERMYAKOVA,
Institute of Macromolecular Chemistry of the NAS of Ukraine, 48 Kharkivske shose, 02155 Kyiv, Ukraine,
ORCID: 0000-0002-7622-1059
Y.Y. DOVBNIA,
National University of Life and Environmental Sciences of Ukraine, 15 Heroyiv Oborony St., 03041 Kyiv, Ukraine,
ORCID: 0000-0002-0484-6500
V.V. KLEPKO,
Institute of Macromolecular Chemistry of the NAS of Ukraine, 48 Kharkivske shose, 02155 Kyiv, Ukraine,
ORCID: 0000-0001-8089-8305
D.O. KLYMCHUK,
M.G. Kholodniy Institute of Botany of the NAS of Ukraine, 2 Tereshchenkivska St., 01601 Kyiv, Ukraine,
ORCID: 0000-0002-7076-8213

PROMISING NANOBIOTECHNOLOGY FOR POULTRY FARMING BASED ON SILVER NANOPARTICLES EMBEDDED IN POLYMER-INORGANIC HYBRID CARRIERS

A promising composite material is proposed to reduce the endogenous and exogenous contamination of chicken eggs with pathogenic microflora during their formation and storage. It is based on hybrid biocompatible and biodegradable silica/polyacrylamide nanocarriers containing small silver nanoparticles (d_{av}=2.4±1.0 nm) that are orally administered to laying hens with drinking water. The features of the formation of nanosilver in hybrid carriers by borohydride reduction of a silver salt at its various concentrations in an aqueous solution have been studied. An interesting effect of the sharp appearance of the second surface plasmon resonance band in the UV-Vis spectra of a silver salt/hybrid mixture at a high salt concentration was found. This was explained by sharp structural changes in the hybrid carriers caused by the simultaneous growth of many AgNPs in them. It was assumed that the intensive growth of many AgNPs in one hybrid particle was accompanied by detachment of the grafted PAAm chains from the SiO2 surface due to the breaking of hydrogen bonds. The change in the state of the composite material under the influence of the pH of the solution, the concentration of nanoparticles, the presence of NaCl (as in a "physiological solution"), and visible light was studied by UV-Vis spectroscopy, potentiometric titration, and TEM. Nanosilver in carriers showed high stability with respect to most of these factors. The influence of the composite material on the clinical state of laying hens and important parameters of their eggs and blood was studied when it was administered orally with drinking water three times every 10 days at doses of 0.2 and 0.4 mg per chicken per day. A striking effect of selective endogenous accumulation of silver in eggshells has been revealed. This confirmed the penetration of the nanosilver composite into the circulatory system of chickens by passing through the digestive tract, absorption through the intestinal epithelium and further transport into the tissues of the chickens, including the oviducts, where protein and eggshell are formed. Such penetration did not cause a toxic effect on the body of laying hens.

Keywords: *nanosilver composite material, silica/polyacrylamide nanocarriers, structure and stability, laying hens, parameters of chicken eggs and blood.*

Цитування: Zheltonozhskaya T.B., Shevchenko L.V., Permyakova N.M., Dovbnia Y.Y., Klepko V.V., Klymchuk D.O. Promising nanobiotechnology for poultry farming based on silver nanoparticles embedded in polymer-inorganic hybrid carriers. *Полімерний журнал.* 2023. **45**, № 2. С. 153—174. https://doi.org/10.15407/polymerj.45.02.153

Introduction

Silver nanoparticles (AgNPs) have broad antimicrobial activity and adaptability to various biological systems. Therefore, they are extensively tested in livestock [1–5], especially in poultry [3, 5, 6–8], to replace antibiotics that have been used in this area as growth promoters and drugs, but have been banned in the European Union since 2006. This is due to the emergence of a special type of bacteria that are resistant to one or more antibiotics. The spread of resistant pathogens causes huge economic losses in livestock and poultry production [3]. In addition, resistant bacteria can remain in animal products and pose a risk to humans. The use of nanosilver preparations instead of or together with antibiotics in this area allows solving the problem of bacterial resistance [3]. Today there are quite a lot of original research publications and reviews devoted to different aspects of application of AgNPs in poultry farming. However, they are not fully systematized. The focus of these studies is on the effect of AgNP administration on the development of commercially important broiler chickens [6–19] and chick embryos [6, 20–25]. There are also separate studies on laying hens [26, 27] and quails [28–31].

In groups of broiler chickens, the influence of AgNPs preparations on the bird growth performance, blood parameters, intestinal microflora and immune status was investigated at the administration of metal nanoparticles into broiler diets, drinking water, or through a tube into the crop (*per os*) [9–26]. Many of these studies provide positive examples of AgNP action in terms of chick growth performance. So, in the study [9], nanosilver at various concentrations (300, 600 and 900 ppm) was administered into the feed of chicken during 56 days, and it was found that the highest concentration of nanoparticles significantly increased the weight gain of chickens, while reducing the feed conversion ratio (FCR). In addition, different doses of silver nanoparticles did not significantly affect the level of leukocytes and a number of enzymes in the blood, such as alkaline phosphatase, ALT and AST. Significantly lower levels of AgNPs (2, 4, 6, 8 and 10 ppm) were added between 7 and 35 days to the basal diet of broiler chickens in a study [10]. The positive effect of AgNP supplementation on body weight gain, FCR and European production efficiency index (EPEI) was fixed in all treatments compared to control. But the best FCR and EPEI values (1.5 and 374, respectively) were achieved by using 4 ppm AgNPs. Serum analyzes showed significant reductions in lipid, cholesterol, and AST levels in most silver treatments compared to controls. Total serum antioxidant capacity increased significantly at all levels of dietary AgNPs, while the highest value was recorded at 4 ppm. Different doses of AgNP also reduced the number of harmful bacteria *E. coli* compared with the control and did not affect the microflora represented by lactobacilli. In another study [11], broiler chickens were fed for 12 days a basal diet supplemented with 50 ppm AgNPs or, for comparison, 100 ppm silver nitrate. Unlike $AgNO_3$, AgNP supplementation resulted in a significant improvement in chick growth performance such as weight gain, feed intake, FCR, breast and thigh muscle mass. The observed improvement in growth rates may be due to the bactericidal effect of AgNP on harmful intestinal bacteria. Another possible mechanism for the growth-stimulating effect of AgNP, according to the authors, was the stimulation of digestive enzymatic activity. This hypothesis was supported by the improved digestibility of ash and silver and the increased concentration of this metal in the muscles of broilers. A detailed analysis of blood parameters and gene expression associated with the growth of broiler chickens allowed the authors to substantiate the conclusion about the normal integrity of chicken cells without the consequences of oxidative stress. It should also be noted the study [12], where the basic diet of broiler chickens was supplemented daily for 42 days with 1% zeolite coated with 0.5% AgNPs. In this case, the FCR parameter turned out to be significantly higher in the chicken group, which received zeolite/AgNPs additive, than in the control group. However, there was no significant difference between these groups in the response of the microbial population of the gastrointestinal tract. This result was obtained by counting the number of lactic acid and anaerobic bacteria in the ileum and caecum of broilers on the 21st and 42nd day of the experiment.

At the same time, many other similar studies have shown the practical absence of any effect of AgNP on chicken growth performance. One of these is the study [13], where the daily water intake of chickens was supplemented 5, 15 and 25 ppm AgNPs for 42 days. The effect of AgNP here was expressed only in a dose-dependent change

in the indicators of oxidative stress activity and a significant decrease in the weight of the bursa of Fabricius (one of the lymphoid organs of chickens associated with the immune system). Similarly, in a study [14], drinking water with a concentration of 15 ppm (mg/l) AgNPs, which was consumed for two weeks (from 7 to 35 days of age) by broiler chickens infected with *Eimeria tenella* oocysts, did not significantly affect to their body weight gain. The silver preparation was successfully competed in this work with a registered Baycox® coccidiostat. In particular, both the groups of chickens treated with silver nanoparticles and the groups of chickens treated with coccidiostat had 50% fewer oocysts in fecal samples compared to the control group. The absence of any improvement in the productivity of chickens (live weight, feed intake, FCR and feed efficiency) in the experimental group compared to the control group within 42 days was also established in [15]. In this study, the basal diet included 20, 40, and 60 ppm AgNPs. Such relatively high doses of metal nanoparticles led mainly to negative consequences. Thus, the addition of AgNP had a negative dose-dependent stimulating effect on blood parameters, such as the content of total protein, albumin, gamma globulin, triglycerides, cholesterol, ALT, AST, and alkaline phosphatase. The activity of oxidative enzymes (glutathione peroxidase, superoxide dismutase and catalase) and malondialdehyde, the last product of lipid breakdown caused by oxidative stress, significantly increased in all experimental groups compared with the control group. In addition, the relative weight of immune organs, especially the bursa of Fabricius, decreased. Thus, in this study, AgNPs acted as factors causing oxidative stress and weakening the immune system. Some other publications [16–18] describe a similar picture.

A negative effect of AgNP administration on chick performance was detected in one study [19]. In this case, the nanosilver preparation at a concentration of 50 ppm was added for two weeks (from 15 to 30 days of age) to the drinking water of broiler chickens infected with *Campylobacter jejuni* (*C. jejuni*). The addition of AgNPs led to a decrease in the overall weight gain of chickens and the weight of individual organs. In fact, the nanosilver preparation showed a clear toxic effect.

The next large series of studies focused to the effect of colloidal AgNPs on chick embryos using *in ovo* injection of nanoparticles into fertilized eggs [20–24] and taking into account the important role of embryonic life in ensuring good health of hatched chicks. In the post-incubation period, chicks make an important transition from egg nutrition to exogenous feed, and some of them (~2–5 %) do not survive this critical period due to limited body reserves. The aim of the first mentioned study was to determine the effect of AgNPs on the developmental status of chicken embryos, especially on the bursa of Fabricius [20]. The eggs were injected 3 times with 0.2 cm^3 of AgNP dispersion at a concentration of 10 ppm on days 5, 11, and 17 of incubation. These doses did not affect the development of the embryo (according to the standard of Hamilton and Hamburger), but reduced the number and size of lymphatic follicles in the bursa of Fabricius. In another study [21], fertilized eggs were injected with 0.3 cm^3 of a 50 ppm AgNP dispersion on day 5 to evaluate their pro- or anti-inflammatory properties by examining NF-κB mRNA expression in embryonic liver. Nanosilver did not affect the survival of embryos. They were correctly developed, without any anomalies. The results also showed no effect of AgNPs on NF-κB mRNA expression, indicating that they acted as an anti-inflammatory factor. The effect of AgNPs on metabolic rate (O_2 consumption, CO_2 and heat production) and development of embryos (relative chick weight and muscle mass) from broilers and laying hens was investigated in the following study [22]. For this, eggs were injected with 0.3 cm^3 of 50 ppm AgNP dispersion on the 1st day of incubation. Nanoparticles did not suppress the growth and development of embryos of both species. The metabolic rate of the injected laying embryos was found to be significantly higher than that of the control group, indicating that AgNPs may be a potential metabolic modifier for these embryos. Studies [23] and [24] show the positive role of AgNPs in the delivery of beneficial nutrients, such as glutamine and amino acids, to chick embryo muscle cells and their subsequent development. In both cases, on the 1st day of incubation, 0.3 cm^3 of pure AgNP dispersion at a concentration of 50 ppm was injected into the eggs together with glutamine [23] or an amino acid (cysteine or threonine) [24]. It has been shown that pure AgNPs and their complexes with glutamine and amino acids do not adversely affect the development of the embryo. At the same time, the complex of glutamine with Ag NPs promoted muscle growth [30], and

the complexes of nanoparticles with amino acids acted as potential agents for enhancing innate and adaptive immunity in chickens [24].

In addition, it is necessary to note the study [25], in which the authors conducted the long series of studies on the effect of nanosilver on the development of broilers, starting with the injection of AgNP into embryos and their subsequent administration into the drinking water of broilers in the postnatal period. On the 1st day of incubation, two groups of fertilized eggs from broilers were injected with 0.3 cm^3 of an aqueous dispersion of AgNP with a concentration of 10 and 20 ppm. On the 7th day after hatching, they were given drinking water with a nanoparticle concentration of 10 and 20 ppm for 4 weeks. This treatment of broiler chickens, starting from their embryos, proved to be very strong, as feed intake and body weight decreased by about 5.0 g per day and 41.0 g, respectively. Bacterial populations in the ileum were not affected, but the number of lactose-negative enterobacteria and lactic acid bacteria decreased in the caecum. The intake of nanosilver increased the concentration of acetic acid in the caecum and did not affect the concentration of immunoglobulins (IgG and IgM) in plasma.

Much less attention has been paid to the effect of AgNPs on laying hens and their egg quality. One of such experiments is presented in [26]. The authors characterized the quality of eggs of laying hens under the influence of AgNPs included in the nanocomposite together with Cu, Fe, and Mn dioxide nanoparticles of different sizes in an aliquot ratio. Two groups of laying hens received nanocomposite supplements daily for 30 days at doses of 0.3 and 4.0 ppm ($mg \cdot kg^{-1}$ of body weight). Another group was treated with a mixture of salts of the corresponding metals at a dose of 0.3 ppm for comparison. After the cessation of supplementation, the hens were observed for 15 days. The influence of the nanocomposite on the productivity of laying hens was shown, which exceeded the effect of metal salts. This was expressed in an increase in the level of egg production during the experiment by an average of 8.2% and 36.1% (at doses of 0.3 and 4.0 ppm, respectively) and egg weight by 24.7% (at a dose of 4.0 ppm), as well as in changing the pH level of egg white and yolk, but within acceptable values. A fundamental study of the effect of AgNPs of two sizes (13 and 50 nm) and three concentrations (1, 10 and 100 ppm) on the function of the ovaries of laying hens was carried out in [27]. Birds were administered *per os* daily at 1 $cm^3 \cdot kg^{-1}$ of each dispersion for 14 days. The concentrations of sex steroids and thyroid hormones in blood plasma were found. In addition, the expression of messenger ribonucleic acid (mRNA) and proteins of three important enzymes and the concentration of steroid hormones in ovarian follicles of chickens were determined. The results showed that the chicken ovary is the target of AgNPs and that their effect on ovarian function is related to the regulation of steroidogenesis.

Particular attention should be paid to the effect of nanosilver on growth performance, blood counts, intestinal microflora and the condition of quail eggs, which are considered model animals for poultry farming and whose eggs are of significant commercial interest. These issues are discussed in [28–31]. Four groups of quails were treated for 12 days with drinking water containing 0, 5, 15, and 25 ppm ($mg \cdot kg^{-1}$) AgNPs with a predominant size of 3–4 nm, and the weight of the birds, the microbial profile of the caecum, and the morphology of enterocytes in the duodenum were monitored [28]. Nanosilver did not have a noticeable effect on the weight status and microflora of the caecum of quails, but at the highest concentration of AgNPs in drinking water, the population of lactobacilli increased significantly. In addition, AgNPs did not show any damaging properties on enterocytes of the duodenal villi. The effect of treatment of laying quails with drinking water, including 0, 4, 8, and 12 ppm AgNPs in a titanium dioxide matrix at a weight ratio of 1:99, on egg quality was studied in [29]. The treatment was carried out for 5 weeks and the egg production of the hens was determined along with egg parameters including egg and yolk weight, egg length and width, and eggshell thickness. The results showed that AgNPs at all concentrations caused a significant decrease in laying quail productivity and egg yolk weight compared to the control group, while they had no significant effect on egg weight, egg length and width, and eggshell thickness. One of the longest (30 weeks) studies of the effect of nanosilver on the weight of organs of laying quails, their biochemical, haematological and coagulation parameters of blood, the activity of antioxidant enzymes, as well as histopathological changes and the concentration of silver in liver and kidney tissues was presented in work [30]. Silver nanoparticles with a z-average

size of 59 nm were administrated daily to laying quails with drinking water at AgNP concentrations 0, 4, 8, and 12 ppm ($mg \cdot L^{-1}$). The most negative results were noted for the last concentration of nanoparticles. Indeed, the concentration of 12 ppm AgNP in drinking water reduced the relative weight of the liver, ileum, colon and serum AST activity. This concentration of AgNPs also caused an increase in lipid peroxidation and vacuolization in the liver of quails. Nanoparticles accumulated in the liver caused oxidative stress associated with possible hepatic dysfunction. Thus, the main organ affected by chronic administration of AgNPs was the laying liver.

An alternative method of using AgNPs in quail breeding was proposed in [31]. The authors sprayed four groups of embryonated quail eggs with either three AgNP solutions (30, 40, and 50 ppm) or a commercial disinfectant (TH_4), given that clean, fertilized eggs with minimal microbial contamination are required for a successful hatching process and further development of the chicks. The effect of AgNP on quail egg shell microbial population, embryonic mortality, hatchability, chick quality and postnatal performance was assessed. It has been shown that the bacterial load on the eggshell decreases with increasing AgNP concentration. This resulted in a consistent increase in hatchability in the treated groups and a decrease in embryonic mortality. Weight, length and quality of chickens in all treated groups were higher compared to the control. However, after hatching quails in all studied groups, similar indicators of live weight, live weight gain, feed intake and feed conversion rate were observed. Moreover, the treatments did not adversely affect the structure of the liver.

Unfortunately, these studies are difficult to generalize completely. The first problem is the expression of AgNP doses in different ppm units: mg of nanoparticles per 1 kg of feed, 1 liter of drinking water, or 1 kg of bird, embryo or egg weight. The second problem is lack important characteristics of AgNPs, such as size, shape, physical state, and the presence or absence of a stabilizing agent on the surface of the nanoparticles. At the same time, all the functional properties of these nanoparticles, including their antimicrobial activity, which is mainly used in poultry farming, is determined by the marked parameters of the nanoparticles. In this context, a study [32] should be noted, which revealed a difference in the antibacterial activity *in vitro* and *in vivo* of uncoated AgNPs and nanoparticles coated with citrate (Cit) and poly(vinylpyrrolidone) (PVP) at comparable average sizes of 75, 82, and 86 nm, respectively. This was due to the strong interaction of uncoated AgNPs with blood serum proteins. As a result, their antimicrobial activity against *Salmonella* infection was significantly reduced in contrast to coated AgNPs. In addition, PVP-AgNPs had better antibacterial properties *in vitro* and *in vivo* compared to Cit-AgNPs due to their better stability and higher uptake by microbial cells. An alternative "green" covering of AgNPs 2–20 nm in size with ovalbumin, ovotransferrin, and ovomucoid of egg white was performed in [33]. Thus, a high antibacterial activity of the nanoparticles against *Salmonella typhimurium* and *Escherichia coli* was achieved in combination with their excellent biocompatible and non-toxic properties. Coated AgNPs did not cause *in vitro* haemolytic effects or structural damage to the cell membranes of chicken erythrocytes up to a concentration of 12 ppm ($mg \cdot L^{-1}$).

In our previous studies, efficient nanoreactors and nanocarriers were proposed for the synthesis and delivery of AgNPs [34, 35]. They had a hybrid structure and contained biocompatible and biodegradable components: an inorganic "core" of silica and a "corona" of grafted polyacrylamide chains (SiO_2-*g*-PAAm). These hybrids made it possible to obtain and retain in the "corona" of PAAm very small silver nanoparticles (d_{AgNPs}<10 nm), which exhibited antimicrobial properties at very low concentrations [34]. This material was nontoxic and could be used in fish farming [35]. The objectives of this work included studying the features of the formation and structure of the composite material AgNPs/SiO_2-*g*-PAAm with different content of AgNPs, assessing its resistance to the action of important factors acting in a living organism, as well as its effect on laying hens and their eggs when administered orally. Thus, this work combined a detailed physical and chemical study of the process of obtaining a nanosilver preparation, its structure, particle size and use for oral treatment of laying hens. The main attention in the biological part was paid to the accumulation of silver nanoparticles in various parts of chicken eggs, since such data were absent in the literature. In addition, their influence on a number of biochemical parameters of the blood of laying hens is considered. Taking

into account that the toxicity of AgNPs is lower at a lower dose and when administered not daily, but after a certain period, a special experimental protocol was developed.

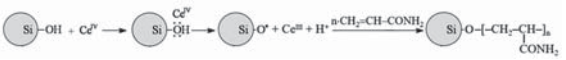
Experimental section

Materials

Aerosil A-175 from Orisil (Ukraine) with a specific surface area of 1.82×10^5 $m^2\cdot kg^{-1}$, cerium (IV) ammonium nitrate (CAN) from Aldrich (USA), and acrylamide (AAm) from Merck (Germany), recrystallized from chloroform were used to synthesize the SiO_2-*g*-PAAm hybrid. A silica sol with a concentration of C_{SiO_2}=20.2 $kg\cdot m^{-3}$ was prepared as in [35]. Silver salt $AgNO_3$ of analytical grade (Ukraine) and sodium borohydride ($NaBH_4$) from Merck (Germany) were used to prepare a nanosilver in hybrid carriers. Deionized water was used as a solvent.

Synthesis of silica/polyacrylamide hybrid

The SiO_2-*g*-PAAm sample was obtained by free-radical surface-initiated AAm polymerization (reaction {1}).



{1}

The synthesis was carried out in an inert (argon) atmosphere at *T*=20 °C at the following reagent concentrations: C_{SiO_2}=1.35 $kg\cdot m^{-3}$, C_{CAN}=0.55 $kg\cdot m^{-3}$, C_{AAm}=71 $kg\cdot m^{-3}$. A detailed synthesis protocol and purification methods and characterization of the resulting product are described elsewhere [35]. Thus, the chemical composition, the average size of silica sol nanoparticles (r_{avSiO_2}), and important parameters of the hybrid, namely the number (N) and length (molecular weight) of PAAm grafts, were determined by the methods of elemental analysis, dynamic thermogravimetric analysis, static light scattering, and viscometry. They are shown in Table 1. This hybrid sample had a smaller number of grafted chains compared to the previously studied one [34,35].

Determination of structure of hybrid carriers in aqueous solutions

The morphology and size of hybrid particles were determined using transmission electron microscopy (TEM). TEM images were obtained on a JEMI230 device (JEOL, Japan) at an accelerating voltage of 80 kV. Small droplets (~1×10^{-4} cm^3) of a hybrid dispersion in deionized water with *C*= 1 $kg\cdot m^{-3}$ were placed on copper grids coated with Formvar films and carbon. They were dried in air for ~1–2 minutes and in a vacuum desiccator for 24 hours. The computer program "ImageJ" was used to calculate the average particle size and size distribution using TEM images.

The surface charge of SiO_2 nanoparticles was determined by potentiometric titration as the number of charged silanol groups in sol and hybrid particles. Aqueous dispersions of the SiO_2 sol and the SiO_2-*g*-PAAm hybrid with a concentration of *C*=0.7 and 1.0 $kg\cdot m^{-3}$, respectively, and deionized water were titrated with a 0.2 N NaOH solution in a thermostated cuvette and argon atmosphere at *T*=25±0.1 °C. For this, a 1-160M digital ion meter (Belarus) calibrated with five standard buffer solutions was used. The accuracy of pH values was 0.02 units. Each subsequent volume of titrant was added to the cuvette after 2 minutes to achieve ionic equilibrium. The dependences of the absorption of hydroxyl ions on pH were calculated using the relation (1) [36]:

$$\sigma_{OH^-} = \frac{C}{g}(1-10^{pH-pH_0}). \qquad (1)$$

Here σ_{OH}- is the absorption of hydroxyl ions ($mol\cdot kg^{-1}$), *C* is the titrant concentration ($mol\cdot m^{-3}$), g is the weight (kg) of silica sol or hybrid sample in 1 m^3 of solution, pH and pH_0 are the negative logarithms of the concentration of hydroxyl ions in the sol or hybrid solution and pure water, respectively.

Formation of nanosilver in hybrid carriers

Silver nanoparticles were incorporated into SiO_2-*g*-PAAm carriers by *in situ* reduction of silver nitrate with sodium borohydride in hybrid aqueous solutions. This process can be described by several main and side chemical reactions [35]. Taking into account side reactions, an eightfold molar excess of $NaBH_4$ was used with respect to silver nitrate. To obtain a composite material with different content

Table 1. **Chemical composition and structural parameters of hybrid**

Hybrid	w_{PAAm}, wt %	w_{H2O}, wt %	w_{SiO2}, wt %	r_{avSiO2}, nm	M_{vPAAm}, kDa	N
SiO_2-*g*-ПАА	72.9	12.6	14.5	7.7	1513	8

of AgNPs, the concentrations of the hybrid C_{Hyb}= 1.0 kg·m^{-3} and Ag-salt C_{AgNO_3}=1.82×10^{-2} and 3.64·10^{-2} kg·m^{-3} were chosen. *In situ* reduction was carried out in two stages. At the 1-st stage, the hybrid solution was mixed with Ag-salt at *T*=20 °C and kept in a dark box for 30 minutes. At the 2-nd stage, the reducing agent was added and the appearance of a thin yellow dispersion of AgNPs was observed.

Study of the process of formation, stability and structure of a composite material

The kinetics of the formation of nanoparticles in hybrid solutions was controlled by the change in time of the position (λ_{max}) and the integrated intensity (*S*) of the surface plasmon resonance band (SPRB) in the UV-Vis spectrum [35]. Extinction spectra were recorded every 3 min for 90 min on a Cary 50 Scan UV-Vis spectrometer from Varian (USA). To determine the integrated intensity of SPRB, we performed its graphical integration in spectra using the Origin program. This approach was also used to characterize the stability of AgNPs in hybrid carriers under various conditions: i) in a "physiological solution", ii) at various pH values, iii) upon dilution of the composite, and iv) during its long-term storage in a dark box and in the light.

The morphology of the AgNPs/SiO_2-*g*-PAAm composite was studied by TEM. The composite was preliminarily purified from reduction by-products by reprecipitation of the reaction mixture with ethanol, centrifugation at 6000 rpm, and subsequent dissolution in deionized water. TEM images were obtained and processed as described above.

Testing the effect of the nanosilver composite on laying hens and their eggs

Biological experiments were carried out on 45 laying hens of the High Line W36 cross at the age of 38 weeks. Three groups of laying hens (*n*=15) such as Control and Research 1–2 were created. Hens of the experimental groups additionally received a dispersion of nanosilver in hybrid carriers with drinking water 3 times a month with an interval of 10 days. In these cases, concentrations of C_{AgNPs}=1.0·10^{-3} (Research 1) and 2.0·10^{-3} kg·m^{-3} (Research 2) were used, which corresponded to nanosilver doses of 0.2 and 0.4 mg per hen, respectively. All hens were fed commercial compound feed, the composition of which corresponded to the needs of the bird in nutrients and biologically active substances. Chickens were kept in cages of 5 animals in a room with controlled ventilation, a temperature in the range of 21–22 °C and a relative humidity of 60–62 %. All experiments were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Scientific Experiments or for other Scientific Purposes of 1986, as well as the Law of Ukraine "On the Protection of Animals from Cruelty" of February 21, 2006 No. 3447-IV in revision dated 04.08.2017.

During the experiment, the consumption of feed and water was monitored, as well as the safety and productivity of laying hens in groups. On the tenth, twentieth and thirtieth days, 5 freshly laid eggs were taken from each group of hens to study the content of various metals in the shell, white and yolk of eggs. Biochemical parameters of blood serum of chickens of different groups were also determined on the 30th day of the experiment. For this, blood was taken from the axillary vein in 5 chickens of each group in the morning before feeding.

Evaluation of metal content in various parts of eggs

The metal content was determined in individual components of the eggs, such as shell, white and yolk, by atomic emission spectrometry. Each shell was homogenized on a Retech GM 200 instrument (Germany) and 6×10^{-4} kg were taken for analysis. Protein and yolk samples weighing 6×10^{-4} kg were used in the native state. The samples were mineralized using a Milestone Ethnos Easy microwave mineralizer (Italy). To do this, each weighed portion of the sample was placed in a Teflon beaker of a microwave mineralizer and 5 cm^3 of concentrated HNO_3 was added. The resulting solution was filtered into a 50-cm^3 flask and made up to the mark with deionized water. The content of various metals (Ag, Cu, Zn, Fe and Pb) in the resulting solutions was determined using a PlasmaQuantPQ 9000 ICPOES plasma-optical emission spectrometer (Analytik Jena, Germany) in accordance with the method based on ISO 11885:2005. The following plasma parameters were used: sputtering rate 0.68 dm^3·min^{-1}; power supplied to the plasma 1200-1700 W; solution feed rate 19 rpm; aerosol gas 0.5 dm^3·min^{-1}; auxiliary flow 0.5 dm^3·min^{-1}; plasma gas 12 dm^3·min^{-1}. The spectrometer was calibrated using multicomponent standard solutions for atomic emission spectrometry (Merck, Germany).

Measurements were started by flushing the sample injection system with a background solution with a reagent, and this operation was repeated after each sample. Quality control of reagents

and calibration standards was carried out every 10 samples. First, a blank solution was introduced into the plasma, and then calibration and test solutions in order of increasing concentration, and the intensities of the analytical lines of the elements were measured. Analytical signals were processed automatically using the software of the spectrometer according to calibration dependences and taking into account the background, and, if necessary, the mutual influence of the elements under study.

Determination of biochemical parameters of the blood serum of laying hens

Serum samples for analyses were separated from the collected volumes of chicken blood by centrifugation at 2000 rpm for 10 minutes; then they were stored at -20 °C. The content of total protein, albumin, creatinine, glucose, cholesterol, total calcium, inorganic phosphorus, potassium, magnesium, as well as a number of enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and gamma-glutamyl transpeptidase (GGT) were determined using standard methods, reagent kits from Pointe Scientific Inc. (USA), and semi-automatic analyzer Pointe 180 (Poland). Statistical processing of the results of biological studies was carried out using the ANOVA program. The difference between the parameter values in individual groups was analyzed using Tukey's test; it was considered significant at $p<0.05$.

Results and discussion

Structure and charge of synthesized hybrid carriers in solution

The state and size of hybrid particles in aqueous

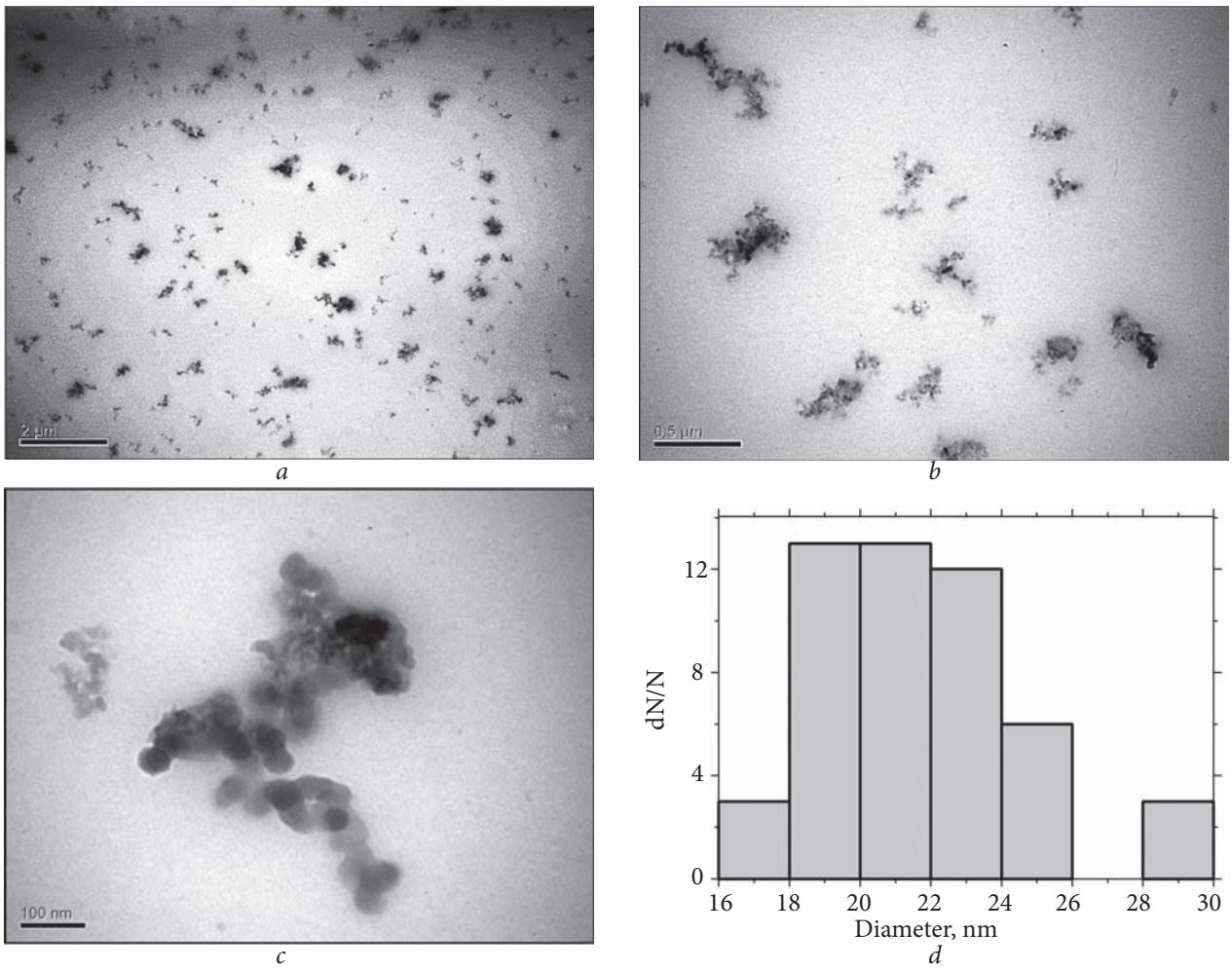


Figure 1. TEM images at (*a*) lower and (*b*, *c*) higher magnifications obtained using an aqueous solution of SiO_2-*g*-PAAm; (*d*) particle size distribution calculated from image (*c*). C_{Hyb}=1.0 kg·м$^{-3}$, *T*=20 °C

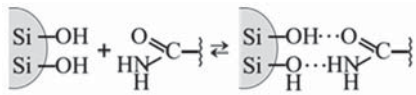
solutions can be seen in the TEM images in Fig. 1 (*a–c*).

The overall picture is similar to that described in our previous studies for other samples of SiO_2-*g*-PAAm hybrids [34, 35]. In particular, both individual and aggregated hybrid particles were observed in aqueous solutions. The shape of individual hybrid particles was close to spherical. The hybrid aggregates had a fractal structure and consisted of individual spherical hybrid particles. The strong aggregation of hybrid particles in an aqueous medium was apparently associated with the interaction of the amide groups of the PAAm "coronas" through hydrogen bonds. The sizes and size distribution of individual hybrid carriers in an aqueous solution were quite small (Fig. 1*d*, Table 2), which makes them promising for use in biomedicine. The average height (thickness) of the PAAm "corona" was also determined from the d_{avHyb} value and relation (2) (Table 2):

$$h_{avPAAm} = \frac{d_{avHyb}}{2} - r_{avSiO2} \quad . \qquad (2)$$

Previously, it was shown that the grafted PAA chains additionally interact with the surface of the inorganic "core" via hydrogen bonds [35]. The smooth and dense surface of hybrid particles in the TEM image in Fig. 1*c* confirms this conclusion. This interaction should affect the surface charge of SiO_2 nanoparticles due to the participation of their surface groups Si-OH in two equilibria: dissociation {2} and hydrogen bonding {3}:

{2}



{3}

Therefore, it was of interest to compare the total number of surface groups ºSi-OH in silica sol and hybrids, which participate in the dissociation equilibrium {2} and create a negative surface charge. The results of potentiometric titration of aqueous dispersions of pure SiO_2 sol and a hybrid sample with NaOH are shown in Fig. 2 as dependences of the absorption of hydroxyl ions on pH.

The value of σ_{OH^-} at a certain pH corresponds to the number of charged silanol groups on the surface of silica nanoparticles in the free state (Fig. 2*a*) and in SiO_2-*g*-PAAm structures (Fig. 2*b*). A noticeable increase in the value of σ_{OH^-} (i.e. surface charge) for nanoparticles of pure silica sol begins at pH>8 and does not end at pH=10.5. The limit value $\sigma_{OH^-_{lim}}$, corresponding to the total number of surface groups Si-OH, is not reached in this case. Obviously, it exceeds the value σ_{OH^-}=7.33 mol·kg^{-1}, achieved at pH=10.5. Noticeable charging of silica "cores" in SiO_2-*g*-PAAm particles begins at a higher pH>10 and ends at a pH above 11 (Fig. 2*b*). The limiting value of $\sigma_{OH^-_{lim}}$ (Table 2) was significantly lower than 7.33 mol·kg^{-1}, which indicated a sharp decrease in the amount of free silanol groups on the surface of SiO_2 "cores" in hybrid particles. This result confirmed the interaction of the grafted polymer

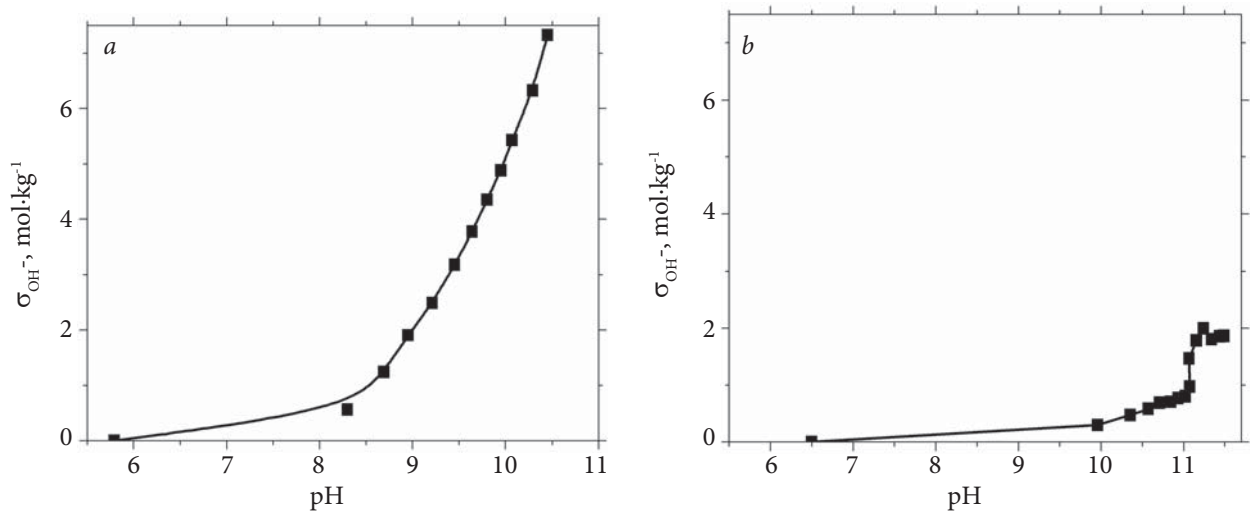


Figure 2. Absorption curves of hydroxyl ions calculated from the data of potentiometric titration of aqueous dispersions: (*a*) pure SiO_2 sol, (*b*) SiO_2-*g*-PAAm hybrid. C_{SiO_2}=0.7 kg·m^{-3}, C_{Hyb}=1.0 kg·m^{-3}, T=25±0.1 °C

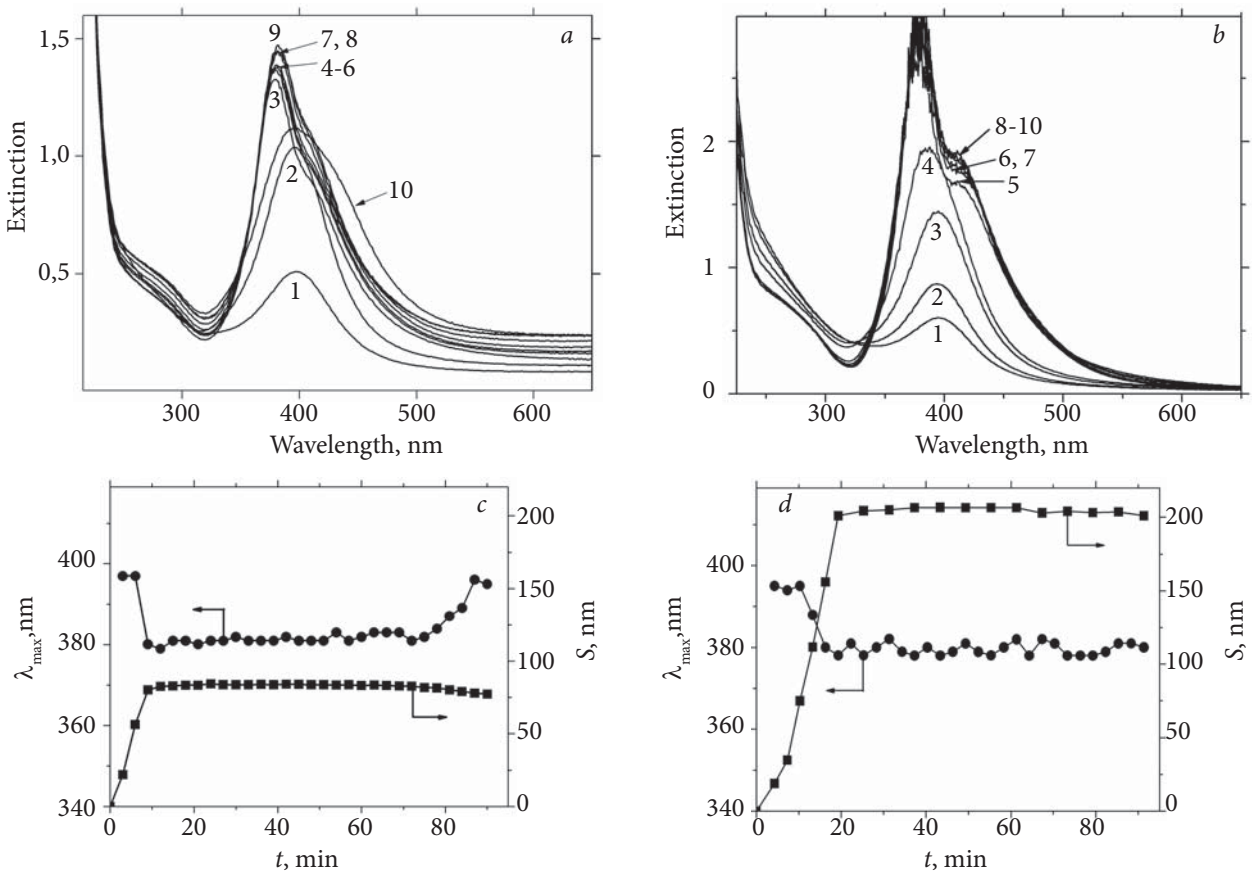


Figure 3. (*a*, *b*) Time evolution of the extinction spectra of the $AgNO_3/SiO_2$-*g*-PAAm mixture through 3 – *1*, 6 – *2*, 9 – *3*, 12 – *4*, 15 – *5*, 21 – *6*, 36 – *7*, 60 – *8*, 72 – *9* and 90 min – *10* after the introduction of the reducing agent. (*c*, *d*) Time dependences of the position (λ_{max}) and integrated intensity (*S*) of SPRB of AgNPs formed in SiO_2-*g*-PAAm carriers. C_{Hyb}=1.0 kg·m^{-3}, C_{AgNO_3}=1.82·10^{-2} (*a*, *c*) and 3.64·10^{-2} kg·m^{-3} (*b*, *d*), *T*=20 °C

"corona" with the inorganic "core" according to the {3} equilibrium.

Features of the formation of nanoparticles in hybrid carriers at different silver salt concentration

Kinetic studies performed by UV-Vis spectroscopy are shown in Fig. 3.

At both studied concentrations of Ag-salt, intense SPRBs appeared in the UV-Vis spectra (Fig. 3*a*, *b*). The integrated intensity of SPRBs, which reflects the yield of metal nanoparticles [35], rapidly increased with time up to a certain limiting value (Fig. 3*c*, *d*). The S_{lim} value for C_{AgNO_3}=3.64·10^{-2} kg·m^{-3} was significantly higher than the same value for half the silver salt concentration C_{AgNO3}=1.82·10^{-2} kg·m^{-3}. This indicated an increase in the yield of AgNPs with increasing salt concentration. In addition, the position of SPRB sharply decreased in the first 10–15 min, which indicated the process of ordering or crystallization of primary AgNPs [35].

At the same time, the shape of very intense SPRBs for silver nanoparticles obtained at the maximum concentration of the Ag-salt (Fig. 3*b*) differed from those observed in the same system at half the concentration of the initial Ag-salt (Fig. 3*a*). The main difference was the appearance of the second SPRB with λ_{max}~412 nm and a lower intensity in a narrow time interval from 12 to 15 minutes (Fig. 3*b*), coinciding with the ordering period of primary AgNPs (Fig. 3*d*). During the next time interval up to 90 min, the position of both maxima and their total integrated intensity S, which reflects the yield of Ag NPs, remained almost unchanged (Fig. 3*b*, *d*). The unexpected appearance of an additional SPRB in the UV-Vis spectra can be explained by a sharp transition of some of the formed and ordered AgNPs to an aggregated state or a sharp increase in their size [37, 38]. To elucidate the reason for such a sharp transition, TEM images of the final purified

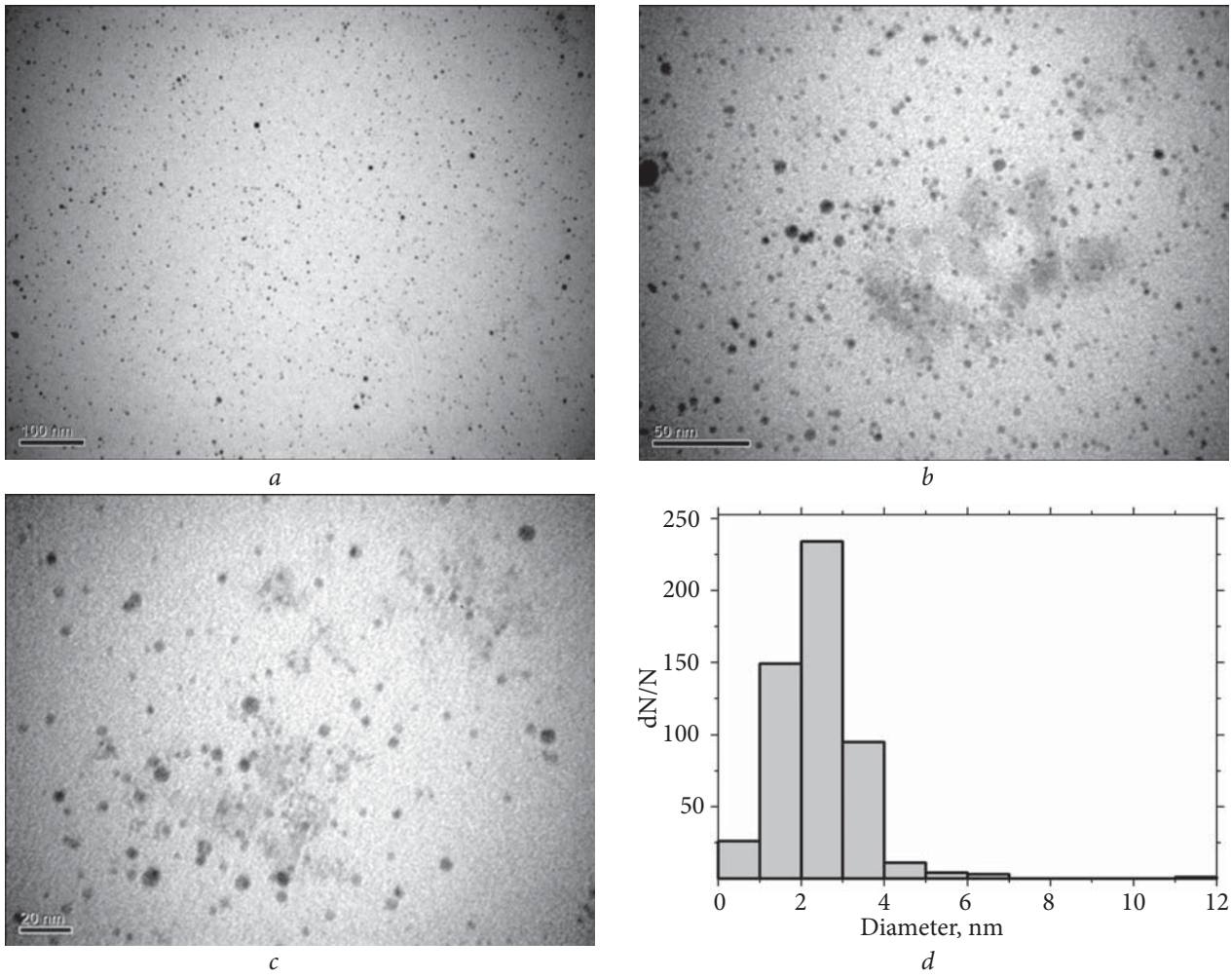


Figure 4. TEM images of the AgNPs/SiO_2-*g*-PAAm composite obtained at lower (*a*) and higher (*b*, *c*) magnifications; (*d*) size distribution of AgNPs calculated from image (*b*). C_{Hyb}=1.0 kg·m^{-3}, C_{AgNO_3}=3.64·10^{-2} kg·m^{-3}, T=20 °C

AgNPs/SiO_2-*g*-PAAm composite were obtained and analysed (Fig. 4).

The first feature of this nanocomposite prepared at a high Ag-salt concentration was the strong disaggregation of hybrid carriers in solution (Fig. 4*a*). Indeed, TEM images showed only single diffuse aggregates of swollen hybrid particles containing AgNPs (Fig. 4*b*, *c*). In fact, the aggregates of hybrids seemed to "dissolve" during the *in situ* synthesis of nanoparticles at a high concentration of the Ag-salt. The second feature of this composite was the presence of two separate sets of spherical AgNPs: small up to 7 nm and large ~11–12 nm (Fig. 4*c*). The average diameter of all these particles was d_{av}=2.4±1.0 nm. Based on these facts, the appearance of the second SPRB in the spectra of the $AgNO_3$/SiO_2-*g*-PAAm mixture 15 min after the addition of $NaBH_4$ (Fig. 3*a*) can be explained by sharp structural changes in some hybrid particles, which were caused by the simultaneous growth of many AgNPs in them. It is reasonable to assume that the intensive growth of many AgNPs in one hybrid particle was accompanied by detachment of the grafted PAAm chains from the SiO_2 surface due to the breaking of hydrogen bonds. As a result, the grafted chains were stretched in solution, facilitating the interaction (coalescence) of a certain part of the formed active AgNPs.

To study the effect of a nanosilver on laying hens, an experimental batch (27 dm^3) of an aqueous dispersion of the AgNPs/SiO_2-*g*-PAAm composite was obtained using a high concentration of Ag-salt (3.64·10^{-2} kg·m^{-3}). The resulting dispersion was purified from reduction by-products as described

above. Thus, a basic concentrated composite material for biological testing was created. In addition, its stability under various special conditions was characterized.

Resistance of nanosilver composite to various external factors

Considering the possible use of the nanosilver composite as an oral form for laying hens, it was important to check the changes in its state in the "saline solution" and at various pH values that may occur in the digestive system of their organisms. The results of temporary control of the UV-visible spectra of the nanosilver preparation after adding NaCL to C=9.0 kg·m^{-3}, sodium hydroxide to pH=9 and hydrochloric acid to pH=2 are shown in Fig. 5. They showed the absence of any changes in the position and integrated intensity of the SPPR of silver nanoparticles in hybrid carriers during the experiment (90 min) in the case of the addition of NaCl and NaOH (Fig. 5*a*, *b*). Therefore, under these conditions, the nanosilver preparation remained completely stable. A somewhat different situation was observed with the addition of HCl (Fig. 5*c*). A gradual decrease in the integrated intensity of SPRB was observed (Fig. 5*c*, *d*), apparently caused by the slow dissolution of AgNPs inside the nanocarriers at this pH value. At the same time, the position of this band (l_{max}=405 nm) remained constant for about 45 minutes, and then began to decrease, which indicated a slow decrease in the size of AgNPs (Fig. 5*d*).

A similar effect of the dissolution of AgNPs stabilized with polyvinylpyrrolidone under the action of hydrochloric acid was also found in [39]. This process developed as a chemical reaction {4}:

$$2\,Ag + 2\,HCl = 2\,AgCl + H_2 \{4\}$$

Attention should be paid to the relatively low dissolution rate of AgNPs in hybrid carriers. In fact, the dissolution did not end even after 1.5 hours (Fig. 5*d*). This fact allows us to conclude that

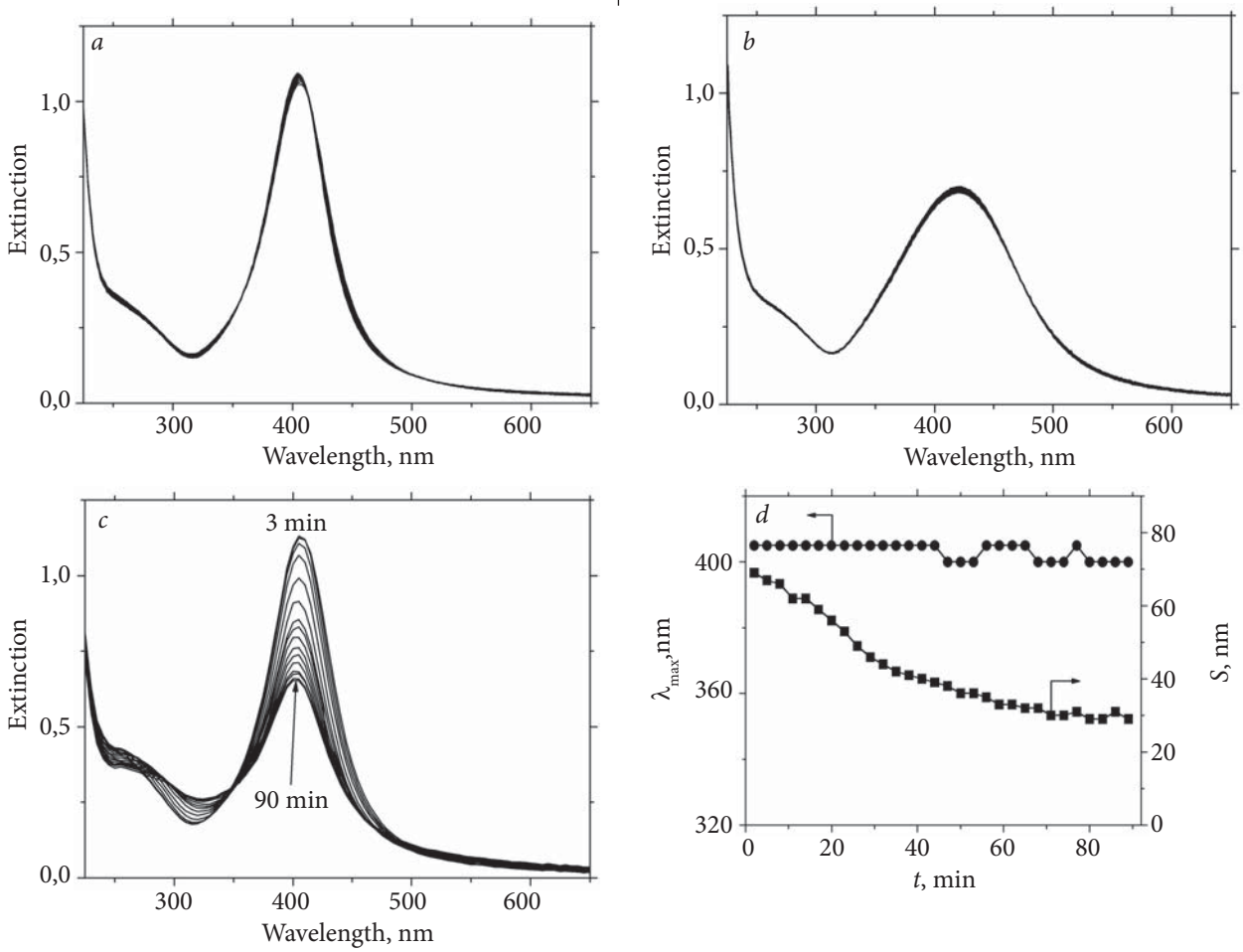


Figure 5. Extinction spectra of the composition AgNPs/SiO_2-*g*-PAAm recorded for 90 minutes under various conditions: (*a*) with the addition of NaCl, (*b*) at pH=9, and (*c*) at pH=2. (*d*) Time dependences of the position and integrated intensity of SPRB of AgNPs calculated based on figure (*c*). C_{Hyb}=1.0 kg·m^{-3}; C_{AgNO_3}=1.82·10^{-2} kg·m-3; C_{NaCl}=9.0 kg·m^{-3}; *T*=22 °C

this nanosilver preparation can successfully "pass through" the stomach (ventricle and stomach) of laying hens with low pH values [40,41] without significant damage to metal nanoparticles. As for the chemical resistance of the hybrid carriers themselves to the action of these factors, it is based on the known properties of SiO_2 and PAAm. Thus, silica nanoparticles can slowly dissolve in water at low temperatures only at high pH values [42]. In principle, PAAm chains can be hydrolysed in acidic and alkaline media, but the rate of this process at low temperatures is insignificant [43].

A special experiment concerned stability of this preparation to dilution, at which principally possible evacuation of a part of AgNPs from hybrid carriers under the effect of the gradient of particle concentration inside carriers and surrounding medium. For this, the aqueous dispersion of initial preparation was diluted 2 times, kept for 24 hours, and then reprecipitated with ethanol and centrifuged. The absence of SPRB, characteristic for AgNPs, in UV-Vis spectrum of supernatant became a criterion of full stability of studied preparation to action of this factor due to strong retention of metal nanoparticles in hybrid carriers.

The last test was devoted to studying the long-term stability of the purified nanosilver preparation during storage under various conditions, including light. It was found that the resistance of this preparation to the action of time and light depended significantly on the concentration of AgNPs in the hybrid carriers (Fig. 6). With a relatively smaller amount of AgNPs in the carriers, the preparation remained stable for a long time regardless of its storage conditions: in a dark box (Fig. 6*a*) or in the light (Fig. 6*b*). This conclusion is confirmed only by a slight increase in the position (from 405 to 416 nm) and integrated intensity (from 73 to 83 nm) of the corresponding SPRB of AgNPs in the UV-visible spectrum (Figs. 6*a*, *b*; spectra 1). In contrast, the light resistance of the preparation containing a large amount of AgNPs turned out to be significantly lower (Fig. 6*a*, *b*; spectra *2*). Obviously, in this case, hybrid carriers with extended PAAm chains (see the discussion above) provided less protection for AgNPs against their aggregation under the action of light.

Accumulation of nanosilver in various parts of chicken eggs

Drinking water for laying hens was obtained by dilution of the base preparation of nanosilver with C_{AgNPs}=2.4·10^{-2} kg·m^{-3} to reach C_{AgNPs}=1·10^{-3} and 2·10^{-3} kg·m^{-3}. Obviously, at such a strong dilution (24 and 12 times), the process of complete disaggregation of hybrid particles occurred, and the final drinking water contained mainly individual swollen particles of the SiO_2-*g*-PAAm carrier filled with AgNPs. These diluted formulations were used for laying hens in Research 1 and Research 2 groups. Taking into account the average volume of consumed drinking water per hen per day ~200 cm^3, the doses of nanosilver in these research groups can be determined as 0.2 and 0.4 mg/hen, respectively. Note, that the concentration of AgNPs in the base preparation was calculated using the concentration of the initial Ag-salt (3.64·10^{-2} kg·m^{-3})

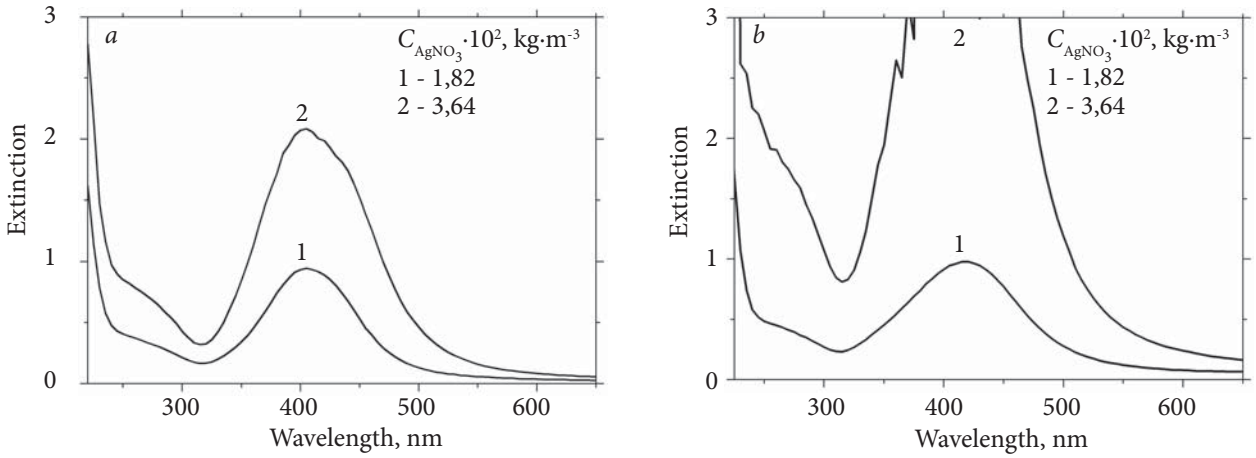


Figure 6. Extinction spectra of the AgNPs/SiO_2-*g*-PAAm preparations obtained at various concentrations of Ag-salt after storage: (*a*) within 3 months in a dark box and (*b*) within the next 4 months in the light. *T*=22 °C

Table 3. **Influence of the nanosilver composite on the metal content in the shell of chicken eggs (x±SD, n=5)**

Metal	Metal content, mg·kg^{-1}		
	Groups of laying hens		
	Control	Research 1	Research 2
	After 10 days		
Ag	0.006 ± 0.002	0.008 ± 0.001	0.008 ± 0.001
Cu	0.5 ± 0.1	0.5 ± 0.1	0.9 ± 0.1
Zn	2.0 ± 0.2	1.5 ± 0.6	2.8 ± 0.9
Fe	0.6 ± 0.3	0.5 ± 0.2	0.6 ± 0.2
Pb	0.009 ± 0.004	0.004 ± 0.001	0.008 ± 0.004
	After 20 days		
Ag	0.007 ± 0.004	0.063 ± 0.020	0.080 ± 0.006
Cu	2.7 ± 0.9	0.8 ± 0.2	1.1 ± 0.4
Zn	3.0 ± 1.0	2.0 ± 0.2	3.0 ± 2.0
Fe	0.5 ± 0.2	0.7 ± 0.1	0.9 ± 0.2
Pb	0.024 ± 0.009	0.007 ± 0.001	0.010 ± 0.002
	After 30 days		
Ag	0.006 ± 0.006	0.038 ± 0.007	0.060 ± 0.020
Cu	0.7 ± 0.2	0.6 ± 0.2	0.6 ± 0.2
Zn	2.3 ± 0.5	1.1 ± 0.3	2.5 ± 0.9
Fe	0.2 ± 0.1	0.17 ± 0.03	0.1 ± 0.1
Pb	0.006 ± 0.002	0.005 ± 0.001	0.006 ± 0.001

and taking into account almost 100 % binding of Ag^+-ions with hybrid carriers and the subsequent complete reduction of these ions to zero-valence state by an eightfold excess reducing agent [35].

Throughout the experiment, the safety of the number of chickens in all groups was 100 %, and their egg productivity did not differ between groups. In addition, three-time feeding of laying hens with a solution of nanosilver preparation in both doses did not affect the morphological parameters of chicken eggs: the mass of the eggs themselves and their individual components (proteins, yolks and shells).

Single feeding to laying hens of Research 1 and Research 2 groups of an aqueous dispersion of the drug in doses of AgNPs 0.2 and 0.4 mg per hen per day led to a relatively small (by 33.3 %) increase in the silver content in eggshell (Table 3). At the same time, the content of zinc, iron and lead did not change within the error, and the content of copper increased (by 80.0 %), but only at a high dose of the preparation. After two-fold feeding of laying hens with a solution of the preparation, the silver content in the eggshells of the Research 1 and Research 2 groups increased significantly (by an average of 9.0 and 11.4 times) compared with the control. However, this did not affect the content of copper, zinc, iron and lead in the shell (Table 3). After a three-time exposure of the preparation to the body of laying hens, the amount of silver accumulated in the shell slightly decreased. Indeed, in the Research 1 and Research 2 groups, the silver content exceeded the control group by only 6.3 and 10.0 times, respectively.

Similar data on the accumulation of silver in egg white are presented in Table 4. After a single feeding of hens with the preparation in the Research 1 and Research 2 groups, there was a slight increase in the content of silver in the protein (by 33.3 and 16.7 %, respectively) compared with the control group. After a double exposure to the preparation, an increase in the content of silver in the protein (by 50 %) was observed only in the Research 2 group, whose hens received a large dose of nanosilver (0.4 mg per hen). But the most significant increase in the content of silver in the egg white of

Table 4. **Influence of the nanosilver composite on the metal content in the protein of chicken eggs (x±SD, n=5)**

Metal	Metal content, mg·kg^{-1}		
	Groups of laying hens		
	Control	Research 1	Research 2
	After 10 days		
Ag	0.0006 ± 0.0001	0.0008 ± 0.0001	0.0007 ± 0.0001
Cu	0.30 ± 0.10	0.28 ± 0.08	0.30 ± 0.05
Zn	0.0020 ± 0.0010	0.0018 ± 0.0004	0.0020 ± 0.0003
Fe	0.027 ± 0.004	0.030 ± 0.007	0.030 ± 0.008
Pb	0.033 ± 0.003	0.049 ± 0.004	0.061 ± 0.005
	After 20 days		
Ag	0.0006 ± 0.0001	0.0006 ± 0.0003	0.0009 ± 0.0002
Cu	0.15 ± 0.01	0.21 ± 0.07	0.19 ± 0.02
Zn	0.0020 ± 0.0003	0.0017 ± 0.0004	0.0019 ± 0.0001
Fe	0.010 ± 0.010	0.014 ± 0.009	0.010 ± 0.002
Pb	0.040 ± 0.010	0.039 ± 0.008	0.036 ± 0.003
	After 30 days		
Ag	0.0005 ± 0.0001	0.0011 ± 0.0001	0.0011 ± 0.0001
Cu	0.30 ± 0.10	0.30 ± 0.10	0.20 ± 0.07
Zn	0.0020 ± 0.0001	0.0017 ± 0.0004	0.0021 ± 0.0002
Fe	0.025 ± 0.002	0.030 ± 0.003	0.030 ± 0.010
Pb	0.088 ± 0.008	0.083 ± 0.004	0.093 ± 0.003

both Research 1 and Research 2 groups compared to the control group (2.2 times on average) occurred 10 days after the third feeding of chickens with nanosilver preparation (Table 4). The content of other metals (Cu, Zn, Fe, and Pb) in the protein practically did not change.

The pattern of silver accumulation in egg yolk differed from the previous two. Indeed, the largest and the same increase in silver in the yolk of eggs of Research 1 and Research 2 groups compared to the control group (5.7 times on average) occurred 10 days after the first feeding of laying hens with the preparation (Table 5). Ten days after the second feeding, this level of silver accumulation remained only in the yolk of eggs obtained from hens of the 2-nd group, which were given a large dose of the drug (0.4 mg per hen). At a lower dose of nanosilver (0.2 mg per hen), which was given to chickens of the 1-st group, the increase in the silver content in the yolk was less significant: only 1.4 times compared to the control group. After the third treatment of laying hens with the preparation in the Research 1 group, the silver content in the yolk remained practically at the same level compared to the control group (it was 1.8 times higher). Unlike this, in the Research 2 group, the silver content decreased compared to the data obtained after the first and second treatment of chickens, and exceeded the control group by only 2.2 times (Table 5). As for the content of other metals in the yolk of the eggs of the experimental groups, it practically did not change compared to the control.

The accumulation of silver in various parts of eggs after oral administration of the drug to laying hens proved the fact that the drug penetrates into the circulatory system of chickens. This occurs by passing the drug through the digestive tract and absorption through the intestinal epithelium, as well as further transport to the tissues of the chickens, including the oviduct, where protein and eggshells are formed. Another interesting fact was established by analysing the changes in the distribution of silver between different parts of the eggs after single, double and triple treatment of chickens with the drug. In particular, in the control group of chickens, the proportion of silver that entered the

Table 5. **Influence of the nanosilver composite on the content of metals in the yolk of chicken eggs (x±SD, n=5)**

Metal	Metal content, mg·kg^{-1}		
	Groups of laying hens		
	Control	Research 1	Research 2
	After 10 days		
Ag	0.0007 ± 0.0001	0.0040 ± 0.0020	0.0040 ± 0.0040
Cu	1.4 ± 0.1	0.9 ± 0.4	1.0 ± 0.3
Zn	22 ± 8	20 ± 8	19 ± 5
Fe	27 ± 8	35 ± 10	23 ± 10
Pb	0.035 ± 0.020	0.053 ± 0.030	0.025 ± 0.010
	After 20 days		
Ag	0.0007 ± 0.0001	0.0010 ± 0.0001	0.0040 ± 0.0001
Cu	1.6 ± 0.1	1.4 ± 0.5	1.2 ± 0.5
Zn	29 ± 3	26 ± 10	26 ± 10
Fe	45 ± 4	39 ± 20	40 ± 20
Pb	0.013 ± 0.004	0.020 ± 0.020	0.017 ± 0.003
	After 30 days		
Ag	0.0006 ± 0.0001	0.0011 ± 0.0002	0.0013 ± 0.0001
Cu	1.4 ± 0.4	1.6 ± 0.3	1.2 ± 0.5
Zn	26 ± 10	28 ± 5	17 ± 6
Fe	38 ± 20	41 ± 10	29 ± 10
Pb	0.029 ± 0.010	0.047 ± 0.009	0.030 ± 0.002

shell, protein and yolk of eggs with food after the first 10 days of the experiment was 82.2, 8.2 and 9.6 %, respectively, and changed little after 20 and 30 days. In contrast, in the Research 1 group, after the first treatment of chickens with the drug, the proportion of silver in the indicated parts of the eggs differed significantly: 62.5, 6.25, and 31.25 %. A similar distribution of silver in individual parts of the eggs was observed after the first feeding of the hens and in the Research 2 group: 63.0, 5.5 and 31.5 %. After double treatment of chickens with the drug, the proportion of silver in individual components of the eggs of the experimental groups changed again and amounted to: 97.2, 1.2, 1.5 % in the Research 1 group and 94.4, 0.8 and 4.7 % in the Research 2 group. However, after three times the treatment of chickens, these parameters remained at the same level: 94.6, 2.7, 2.7 % in the Research 1 group and 96.2, 1.8 and 2.1 % in the Research 2 group. Thus, an important effect of silver accumulation, mainly in the eggshell, was found with a sharp decrease in the proportion of silver in the protein and yolk (in the edible part of the eggs), which manifested itself after two times the treatment of chickens with the drug. In this regard, the sharp difference in the data on the distribution of silver between different parts of the eggs 10 days after the first dose of the drug can be explained simply by the primary reaction of the body of chickens to the introduction of a new substance. Subsequently (after the second and third doses) there was obviously an adaptation of the body of chickens to external influences and a transition to a new state of equilibrium.

The selective endogenous accumulation of silver in the shell of chicken eggs, established in the experiment, can be of great practical importance, due to its wide antimicrobial properties. It will significantly extend the shelf life of chicken eggs without compromising their sanitary and hygienic indicators. This assumption is supported by the results of studies by a number of authors who used exogenous treatment of bird eggshells with nanosilver. For example, the use in [44] colloidal spray and composite film containing nanosilver at concentrations of 500, 1000 and 2000 ppm for 28 days

led to an improvement in the sanitary indicators of food eggshells. In other studies, hatching eggs were treated with nanosilver-based disinfectants, which contributed to a decrease in mortality and an increase in body weight of quails [45] and partridges [46].

It should be noted a slight total intake of silver in the protein and yolk of eggs after two- and three-time oral administration of the drug into the body of chickens (2.2 and 2.6 μg·kg^{-1} after three-time administration at doses of 0.2 and 0.4 mg per hen, respectively) and practically unchanged content of other metals in them (Tables 7-8). This suggests that there is no toxic threat to laying hens and hen egg consumers. A risk assessment of chicken consumption in studies of the effect of nanosilver on domestic chickens at a concentration of 5×10^{-2} kg·m^{-3} of drinking water showed that 1.2 mg of silver contained in 1 kg of chicken does not pose a danger to humans [47]. According to the World Health Organization (2008), the average consumption of silver by a modern person is approximately 5–8 mg per day, while the recommended daily intake of silver (essential or vital dose) is 50–100 mg, that is, an order of magnitude more. Thus, even in the case of daily consumption of 10 eggs from chickens treated two or three times (with an interval of 10 days) with our nanosilver preparation in the indicated doses, the human body will receive a significantly lower dose of silver than recommended per day.

Effect of nanosilver preparation on biochemical parameters of blood serum of laying hens

The results of biochemical studies of the blood serum of laying hens of two experimental groups compared with the control, which were carried out at the end of the experiment (on the 10th day after the administration of the third dose of the drug), are presented in Table 6.

One of the indicators of chicken liver function was the content of total protein and albumin in blood serum. As can be seen from the table, both of these parameters did not change in the blood serum of hens of the Research 1 group, which received a lower dose of the nanosilver preparation (0.2 mg per hen) three times. The content of albumin did not change within the error in the blood serum of chickens of the Research 2 group, which were treated three times with a higher dose of the drug (0.4 mg per hen). However, the content of total protein in the blood of hens of this group increased slightly, by an average of 34.1% ($p<0.05$) compared with the control group, which can be considered as a positive result. On the contrary, in the cited study [15], where nanosilver was added to the feed of broiler chickens for 6 weeks at doses of 10, 40 and 60 mg·kg^{-1}, significant dose-dependent negative changes were observed in the blood

Table 6. **Biochemical parameters of blood serum of laying hens after 30 days of the experiment (x±SD, n=5)**

Parameter	Value		
	Groups of laying hens		
	Control	Research 1	Research 2
Total protein, g·dm^{-3}	44 ± 3	46 ± 4	59 ± 3
Albumin, g·dm^{-3}	7.2 ± 0.5	7.1 ± 0.4	6.8 ± 0.6
Cholesterol, g·dm^{-3}	1.9 ± 0.3	2.2 ± 0.1	1.3 ± 0.1
Creatinine, μmol·dm^{-3}	70 ± 6	76 ± 3	64 ± 4
Glucose, mmol·dm^{-3}	9.9 ± 1.0	11.9 ± 0.5	8.7 ± 0.7
ALT, U·dm^{-3}	12 ± 2	14 ± 1	11 ± 1
AST, U·dm^{-3}	130 ± 20	109 ± 8	140 ± 10
ALP, U·dm^{-3}	190 ± 20	205 ± 5	190 ± 20
GGT, U·dm^{-3}	5.8 ± 0.9	6.5 ± 0.5	3.7 ± 0.5
Ca, mmol·dm^{-3}	2.8 ± 0.5	3.2 ± 0.4	3.0 ± 0.2
P, mmol·dm^{-3}	1.8 ± 0.5	1.2 ± 0.1	2.3 ± 0.3
K, mmol·dm^{-3}	4.4 ± 0.3	5.2 ± 0.2	4.3 ± 0.4
Mg, mmol·dm^{-3}	1.0 ± 0.2	1.0 ± 0.1	1.1 ± 0.2

serum at the end of the experiment, namely: decrease in the content of total protein, albumin and gamma globulins.

The content of creatinine and potassium in the blood serum reflected the functional state of the kidneys of laying hens. Creatinine is a product of protein metabolism that is produced in the muscles, enters the bloodstream and is excreted by the kidneys. The content of creatinine in the blood serum of chickens of Research 1 and Research 2 groups, which received nanosilver preparation with drinking water three times at doses of 0.2 and 0.4 mg per hen, remained at the level of the Control group (Table 6). However, the content of potassium slightly increased (by 18.2 % at $p<0.05$) only in the blood serum of chickens from the Research 1 group.

The content (activity) of enzymes such as ALT, AST, ALP and GGT characterized the state of the liver and other vital organs of laying hens. In our experiment, no serious and systemic changes in the activity of these enzymes were observed in laying hens after a triple exposure of the nanosilver preparation in hybrid carriers (Table 6). Only a slight increase (on average by 16.7 %) in ALT activity in the Research 1 group ($p<0.05$) compared with the Control group and a decrease in this indicator by 8.3 % in the Research 2 group can be noted. There was also a decrease by 16.2 % in AST activity in the Research 1 group ($p<0.05$) and an increase in the activity of this enzyme by 7.7 % in the blood of Research 2 chickens. The greatest effect was a decrease by an average of 36.2 % ($p<0.05$) of GGT activity in the blood serum of hens of the Research 2 group, which received nanosilver at a dose of 0.4 mg per hen three times (Table 6). A decrease in the activity of ALT, AST and ALP enzymes in the blood serum of broiler chickens after exposure to nanosilver was also observed in [10,15].

The content of glucose and cholesterol in the blood serum of laying hens were indicators of carbohydrate and lipid metabolism in their bodies. As can be seen from Table 6, both parameters changed little in the Research 1 and Research 2 groups compared to the Control group after three times treatment of chickens with nanosilver preparation in hybrid carriers. In the 1st group, where the chickens were given a smaller dose of nanosilver, the content of glucose and cholesterol increased slightly (on average by 20.2 and 15.8 %, respectively, at $p<0.05$), and in the 2nd group, where the chickens received a large dose of the drug, these figures decreased by 12.1 and 31.6 %, respectively. This can be regarded as a positive effect. In this case, a stronger (and unfavorable) effect of a lower dose of the drug on the carbohydrate and lipid metabolism of laying hens was manifested. A decrease in the level of cholesterol in the blood serum of broiler chickens under the action of nanosilver was also observed in [10,11].

The indicators of the content of total calcium, inorganic phosphorus, potassium and magnesium in Table 6 characterized the mineral metabolism in the blood serum of chickens. For Research 2 chickens, which were treated three times with a higher dose of the drug, these indicators, within the error, remained at the level of the Control group. In contrast, in the blood serum of Research 1 chickens, compared with the Control group, there was a slight increase in the content of calcium (by 14.3 %), a decrease in the content of phosphorus (by 33.3 %) and an increase in the content of potassium, which has already been discussed above. As can be seen, a smaller threefold dose of the nanosilver preparation had a greater effect on the mineral metabolism in the organisms of laying hens. The practical absence of the effect of nanosilver on the content of most macro- and microelements (except iron) in the blood serum of broiler chickens was also noted in [16] after oral administration to chickens of uncoated and lipid-coated silver nanoparticles with a size of 22 and 5 nm, respectively, and a dose of 5 mg/kg of body weight.

The results of the studies indicate the absence of a toxic effect in the organisms of laying hens after three oral administration of nanosilver in hybrid carriers at doses of 0.2 and 0.4 mg per hen. This, obviously, is a manifestation of their high adaptive capabilities in relation to this preparation.

Concluding remarks

Thus, our studies have revealed a number of important results that can become the basis of a new promising nanobiotechnology in the field of poultry farming. First, hybrids of SiO_2-*g*-PAAm with biocompatible and biodegradable components have shown that they are suitable matrices for the formation, stabilization, and retention of very small AgNPs in their structure. The yield of metal nanoparticles in hybrid carriers increased significantly with an increase in the initial Ag-salt

concentration. At a high concentration of the Ag-salt, an unusual effect of a sharp appearance of the second SPRB in the UV-Vis spectra of the $AgNO_3$/SiO_2-*g*-PAAm mixture was detected already a few minutes after the addition of the reducing agent. This was explained by the intensive growth of many AgNPs in one hybrid particle, which was accompanied by detachment of the grafted PAAm chains from the SiO_2 surface due to the breaking of hydrogen bonds. The obtained nanocomposite was easily purified from by-products of *in situ* synthesis of metal nanoparticles. It was easily diluted and had significant time stability in an alkaline medium and in "physiological solution". In the strongly acidic pH region (pH~2), AgNPs in hybrid particles began to dissolve, but the rate of this process was rather low. This fact led to the conclusion that our nanosilver preparation, when taken orally, will "pass" through the stomach of laying hens without significant damage to AgNPs.

The next important result was the accumulation of silver in various parts of the egg after oral administration of the drug to laying hens. This fact testified to the penetration of the nanosilver preparation into the circulatory system of chickens by passing through the digestive tract, absorption through the intestinal epithelium and further transport into the tissues of the chickens, including the oviducts, where the formation of protein and eggshell occurs. At the same time, it was shown that such penetration, which occurred three times after 10 days at doses of 0.2 and 0.4 mg per chicken, did not cause a toxic effect on the body of laying hens.

However, the most striking result was the selective endogenous accumulation of silver in the shell of chicken eggs compared to their protein and yolk. The reasons for this are not yet clear and require further research. But it is this fact that opens up the prospect of creating a simple and effective nanobiotechnology based on the developed nanosilver preparation for obtaining chicken eggs with their own long-term protection from endogenous and exogenous pollution.

REFERENCES

1. *Fondevila M.* Potential use of silver nanoparticles as an additive in animal feeding. Chapter 17. In book: Silver nanoparticles / Ed. D.P. Perez. InTech: Mountain View, CA, USA, 2010, 325–334. DOI: 10.5772/8509, ISBN 978-953-307-028-5.
2. *Fondevila M., Herrer M., Casallas C., Abecia L., Ducha J.J.* Silver nanoparticles as a potential antimicrobial additive for weaned pigs. Animal Feed Science and Technology, 2009, **150**, no. 3–4: 259–269. https://doi.org/10.1016/j.anifeedsci.2008.09.003.
3. *Da Costa Junior S.D., de Almeida Campos L.A., Palácio S.B., Ferro Cavalcanti I.M.* Silver nanoparticles as a promising therapeutic strategy for infections caused by resistant bacteria in cattle and birds. Approaches in Poultry, Dairy & Veterinary Sciences, 2018, **4**, no. 3: 348–352. DOI:10.31031/APDV.2018.04.000592.
4. *Abdelsalam M., Al-Homidan I., Ebeid T., Abou-Emera O., Mostafa M., Abd El-Razik M., Shehab-El-Deen M., Abdel Ghani S., Fathi M.* Effect of silver nanoparticle administration on productive performance, blood parameters, antioxidative status, and silver residues in growing rabbits under hot climate. Animals, 2019, **9**, no. 10: 845. https://doi.org/10.3390/ani9100845.
5. *Michalak I., Dziergowska K., Alagawany M., Farag M.R., El-Shall N.A., Tuli H.S., Emran T.B., Dhama K.* The effect of metal-containing nanoparticles on the health, performance and production of livestock animals and poultry. Veterinary Quarterly, 2022, **42**, no. 1:68–94.
https://doi.org/10.1080/01652176.2022.2073399.
6. *Patra A., Lalhriatpuii M.* Progress and prospect of essential mineral nanoparticles in poultry nutrition and feeding – a review. Biological Trace Element Research, 2020, **197**: 233–253. https://doi.org/10.1007/s12011-019-01959-1.
7. *Abd El-Ghany W.A.* Nanotechnology and its considerations in poultry field: an overview. Journal of the Hellenic Veterinary Medical Society, 2019, **70**, no. 3: 1611–1616. http://dx.doi.org/10.12681/jhvms.21783.
8. *Anwar M.I., Awais M.M., Akhtar M., Navid M.T., Muhammad F.* Nutritional and immunological effects of nano-particles in commercial poultry birds. World's Poultry Science Journal, 2019, **75**, no. 2: 261–272. https://doi.org/10.1017/S0043933919000199.
9. *Ahmadi J.* Application of different levels of silver nanoparticles in food on the performance and some blood parameters of broiler chickens. World Applied Sciences Journal, 2009, **7**(Suppl 1): 24–27. ISSN 1818-4952. https://www.researchgate.net/publication/228484573.
10. *Elkloub K., Moustafa M.E.l., Ghazalah A.A., Rehan A.A.A.* Effect of dietary nanosilver on broiler performance. Inter-

national Journal of Poultry Science, 2015, **14**, no. 3: 177–182. DOI:10.3923/ijps.2015.177.182.
11. *Saleh A.A., El-Magd M.A.* Beneficial effects of dietary silver nanoparticles and silver nitrate on broiler nutrition. Environmental Science and Pollution Research, 2018, **25**, no. 27: 27031–27038. https://doi.org/10.1007/s11356-018-2730-7.
12. *Abbasi A., Hashemi S.R., Hassani S., Ebrahimi M.* Gastrointestinal microbial population response and performance of broiler chickens fed with organic acids and silver nanoparticles coated on zeolite under heat stress condition. Iranian Journal of Applied Animal Science, 2018, **8**, no. 4: 685–691. ISSN (print) 2251-628X; ISSN (online) 2251-631X.
13. *Ahmadi F., Kurdestany A.H.* The impact of silver nanoparticles on growth performance, lymphoid organs and oxidative stress indicators in broiler chicks. Global Veterinaria, 2010, **5**, no. 6: 366–370. ISSN 1992-6197.
14. *Chauke N., Siebrits F.K.* Evaluation of silver nanoparticles as a possible coccidiostat in broiler production. South African Journal of Animal Science, 2012, **42**, no. 5: 493–497. DOI:10.4314/SAJAS.V42I5.10.
15. *Ahmadi F.* Impact of different levels of silver nanoparticles (Ag-NPs) on performance, oxidative enzymes and blood parameters in broiler chicks. Pakistan Veterinary Journal, 2012, **32**, no. 3: 325–328. ISSN: 0253-8318 (print), 2074-7764 (online).
16. *Ognik K., Sembratowicz I., Cholewińska E., Wlazło L., Nowakowicz-Dębek B., Szlązak R., Tutaj K.* The effect of chemically synthesized silver nanoparticles on performance and the histology and microbiological profile of the jejunum in chickens. Annals of Animal Science, 2016, **16**, no. 2: 439–450. http://dx.doi.org/10.1515/aoas-2015-0067.
17. *Kulak E., Ognik K., Stępniowska A., Sembratowicz I.* The effect of administration of silver nanoparticles on silver accumulation in tissues and immune and antioxidant status of chickens. Journal of Animal and Feed Science, 2018, **27**, no. 1: 44–54. https://doi.org/10.22358/jafs/84978/2018.
18. *Dosoky W.M., Fouda M.M.G., Alwan A.B., Abdelsalam N.R., Taha A.E., Ghareeb R.Y., El-Aassar M.R., Khafaga A.F.* Dietary supplementation of silver-silica nanoparticles promotes histological, immunological, ultrastructural, and performance parameters of broiler chickens. Scientific Reports, 2021, **11**: 4166. https://doi.org/10.1038/s41598-021-83753-5.
19. *Vadalasetty K.P., Lauridsen C., Engberg R.M., Vadalasetty R., Kutwin M., Chwalibog A., Sawosz E.* Influence of silver nanoparticles on growth and health of broiler chickens after infection with Campylobacter jejuni. BMC Veterinary Research, 2018, **14**:1. https://doi.org/10.1186/s12917-017-1323-x.
20. *Grodzik M., Sawosz E.* The influence of silver nanoparticles on chicken embryo development and bursa of Fabricius morphology. Journal of Animal and Feed Science, 2006, **15**, suppl. 1: 111–114. https://doi.org/10.22358/jafs/70155/2006.
21. *Sawosz E., Grodzik M., Lisowski P., Zwierzchowski L., Niemiec T., Zielinska M., Szmidt M., Chwalibog A.* Influence of hydrocolloids of Ag, Au, and Ag/Cu alloy nanoparticles on the inflammatory state at transcriptional level. Bulletin of the Veterinary Institute in Pulawy, 2010, **54**, no. 1: 81–85. ISSN: 0042-4870.
22. *Pineda L., Sawosz E., Hotowy A., Elnif J., Sawosz J., Ali A., Chwalibog A.* Effect of nanoparticles of silver and gold on metabolic rate and development of broiler and layer embryos. Comparative Biochemical Physiology, Part A, 2012, **161**, no. 3: 315–319. DOI:10.1016/j.cbpa.2011.11.013.
23. *Sawosz F., Pineda L.M., Hotowy A.M., Hyttel P., Sawosz E., Szmidt M., Niemiec T., Chwalibog A.* Nanonutrition of chicken embryos. The effect of silver nanoparticles and glutamine on molecular responses, and the morphology of pectoral muscle. Baltic Journal of Comparative & Clinical Systems Biology, 2012, **2**: 29–45. https://doi.org/10.7136/bjccsb.2012.2.0029.
24. *Bhanja S.K., Hotowy A., Mehra M., Sawosz E., Pineda L., Vadalasetty K.P., Kurantowicz N., Chwalibog A.* In ovo administration of silver nanoparticles and/or amino acids influence metabolism and immune gene expression in chicken embryos. International Journal of Molecular Sciences, 2015, **16**, no. 5: 9484–9503. https://doi.org/10.3390/ijms16059484.
25. *Pineda L., Sawosz E., Lauridsen C., Engberg R.M., Elnif J., Hotowy A., Sawosz F., Chwalibog A.* Influence of in ovo injection and subsequent provision of silver nanoparticles on growth performance, microbial profile, and immune status of broiler chickens. Open Access Animal Physiology, 2012, **4**: 1–8. http://dx.doi.org/10.2147/OAAP.S35100.
26. *Orobchenko O.L., Roman'ko M.Y., Kutsan O.T.* The impact of potential feed additive nanocomposite (Ag, Cu, Fe, Mn dioxide) on eggs' quality parameters of laying hens compared with metal salts. Journal for Veterinary Medicine, Biotechnology and Biosafety, 2015, **1**: 10–13. ISSN / EISSN: 2411-3174 / 2411-0388, http://nbuv.gov.ua/UJRN/jvmbb_2015_1_3_4.
27. *Katarzyńska-Banasik D., Grzesiak M., Kowalik K., Sechman A.* Administration of silver nanoparticles affects ovarian steroidogenesis and may influence thyroid hormone metabolism in hens (Gallus domesticus). Ecotoxicology and Environmental Safety, 2021, **208**: 111427. https://doi.org/10.1016/j.ecoenv.2020.111427.
28. *Sawosza E., Bineka M., Grodzika M., Zielińskaa M., Sysaa P., Szmidt M., Niemiec T., Chwalibog A.* Influence of hydrocolloidal silver nanoparticles on gastrointestinal microflora and morphology of enterocytes of quails. Archives of

Animal Nutrition, 2007, **61**, no. 6: 444–451. https://doi.org/10.1080/17450390701664314.
29. *Farzinpour A., Karashi N.* The effects of nanosilver on egg quality traits in laying Japanese quail. Applied Nanoscience, 2013, **3**: 95–99. https://doi.org/10.1007/s13204-012-0097-5.
30. *Rezaei A., Farzinpour A., Vaziry A., Jalili A.* Effects of silver nanoparticles on hematological parameters and hepatorenal functions in laying Japanese quails. Biological trace element research, 2018, **185**, no. 2: 475–485. https://doi.org/10.1007/s12011-018-1267-4.
31. *Ibrahim F.A., Elkloub K., El Mousafa M., El Sabry M.I., Badr J.M., Hassan A.S.I.* Effect of egg disinfection by silver nanoparticles on eggshell microbial load, hatchability and post-hatch performance of quail chicks. International Journal of Poultry Science, 2018, **17**, no. 5: 234–242. https://scialert.net/abstract/?doi=ijps.2018.234.242.
32. *Gnanadhas D.P., Thomas M.B., Thomas R., Raichur A.M., Chakravorttya D.* Interaction of silver nanoparticles with serum proteins affects their antimicrobial activity in vivo. Antimicrobial Agents and Chemotherapy, 2013, **57**, no. 10: 4945–4055. https://doi.org/10.1128/AAC.00152-13.
33. *Thiyagarajan K., Bharti V.K., Tyagi S., Tyagi P.K., Ahuja A., Kumar K., Raja T., Kumar B.* Synthesis of non-toxic, biocompatible, and colloidal stable silver nanoparticle using egg-white protein as capping and reducing agents for sustainable antibacterial application. RSC Advances, 2018, **8**: 23213–23229. https://doi.org/10.1039/C8RA03649G.
34. *Zheltonozhskaya T.B., Permyakova N.M., Kondratiuk T.O., Beregova T.V., Klepko V.V., Melnik B.S.* Hybrid-stabilized silver nanoparticles and their biological impact on hospital infections, healing wounds, and wheat cultivation. French-Ukrainian Journal of Chemistry, 2019, **7**, no. 2: 20–39. https://doi.org/10.17721/fujcV7I2P20-39.
35. *Zheltonozhskaya T.B., Permyakova N.M., Kravchenko O.O., Maksin V.I., Nessin S.D., Klepko V.V., Klymchuk D.O.* Polymer/inorganic hybrids containing silver nanoparticles and their activity in the disinfection of fish aquariums/ponds. Polymer-Plastic Technology and Material, 2021, **60**, no. 4: 369–391. https://doi.org/10.1080/25740881.2020.1811318.
36. *Zheltonozhskaya T., Permyakova N., Eremenko B.* Inter- and intramolecular polycomplexes in polydispersed colloidal systems, Chapter 8. In book: Hydrogen-bonded interpolymer complexes: formation, structure and application / Eds. V. Khutoryanskiy, G. Staikos. World Scientific: New Jersey-London-Singapore etc, 2009, 201–234. ISBN-10: 9812707859; ISBN-13: 978-9812707857.
37. *Kelly K.L., Coronado E., Zhao L.L., Schatz G.C.* The optical properties of metal nanoparticles: The influence of size, shape, and dielectric environment. Journal of Physical Chemistry, Part B, 2003, **107**, no. 3: 668–677. https://doi.org/10.1021/jp026731y.
38. *Liz-Marzan L.M.* Tailoring surface plasmons through the morphology and assembly of metal nanoparticles. Langmuir, 2006, **22**, no. 1: 32–41. https://doi.org/10.1021/la0513353.
39. *Li L., Zhu Y.-J.* High chemical reactivity of silver nanoparticles toward hydrochloric acid. Journal of Colloid and Interface Science, 2006, **303**, no. 2: 415–418. https://doi.org/10.1016/j.jcis.2006.07.059.
40. *Ghişe A.* The pH of the digesta in the gastrointestinal tract, in laying hens. Bulletin of UASVM, Veterinary Medicine, 2009, **66**: 491–492. ISSN 1843-5270; Electronic ISSN 1843-5378.
41. *Ghişe A., Olariu L., Trif A., Zehan R., Cărpinisan L.* The determination of the mean retention time (MRT) of the dry matter in the segments of the gastrointestinal tract in laying hens. Bulletin of UASVM, Veterinary Medicine, 2009, **66**: 378–383. ISSN 1843-5270; Electronic ISSN 1843-5378.
42. SCCS (Scientific Committee on Consumer Safety), Opinion on solubility of synthetic amorphous silica (SAS), 20-21 June 2019, SCCS/1606/2019. Corrigendum of 6 December 2019. https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_228.pdf
43. *Huang S.-Y., Lipp D.W., Farinato R.S.* Acrylamide Polymers. In book: Encyclopedia of Polymer Science and Technology. Vol 1. / John Wiley & Sons, 2002, 41–79. https://doi.org/10.1002/0471440264.pst004.
44. *Farrokhi S., Ahari H., Abedini M.R.* Comparative effects of colloidal silver nanoparticles used in packaging film and spray in inactivating bacteria experimentally added to chicken eggshells. International Journal of Food Properties, 2017, **20**, no. 10: 2314–2322. https://doi.org/10.1080/10942912.2016.1236274.
45. *Batkowska J., Al-Shammari K.I.A., Gryzinska M.M., Brodacki A., Wlazlo L., Nowakowicz-Debek B.* Effect of using colloidal silver in the disinfection of hatching eggs on some microbial, hatchability and performance traits in Japanese quail. European Poultry Science, 2017, **81**. **ISSN 1612-9199, DOI**:10.1399/eps.2017.211.
46. *Chmielowiec-Korzeniowska A., Tymczyna L., Dobrowolska M., Banach M., Nowakowicz-Dębek B., Bryl M., Drabik A., Tymczyna-Sobotka M., Kolejko M.* Silver (Ag) in tissues and eggshells, biochemical parameters and oxidative stress in chickens. Open Chemistry, 2015, **13**: 1269–1274. https://doi.org/10.1515/chem-2015-0140.
47. *Kumar I., Bhattacharya J.* Assessment of the role of silver nanoparticles in reducing poultry mortality, risk and economic benefits. Applied Nanoscience, 2019, **9**: 1293–1307. https://doi.org/10.1007/s13204-018-00942-x.

Received 15.05.2023

Т.Б. Желтоножська,
Інститут хімії високомолекулярних сполук НАН України, Харківське шосе, 48,
02155, Київ, Україна
Л.В. Шевченко,
Національний університет біоресурсів та природокористування, вул. Героїв Оборони, 15, 03041, Київ, Україна
Н.М. Пермякова,
Інститут хімії високомолекулярних сполук НАН України, Харківське шосе, 48,
02155, Київ, Україна
Ю.Ю. Довбня,
Національний університет біоресурсів та природокористування, вул. Героїв Оборони, 15, 03041, Київ, Україна
В.В. Клепко,
Інститут хімії високомолекулярних сполук НАН України, Харківське шосе, 48, 02155, Київ, Україна
Д.О. Климчук,
Інститут ботаніки імені М.Г. Холодного НАН України, вул. Терещенківська, 2, 01601, Київ, Україна

ПЕРСПЕКТИВНА НАНОБІОТЕХНОЛОГІЯ ДЛЯ ПТАХІВНИЦТВА НА ОСНОВІ НАНОЧАСТИНОК СРІБЛА, ВБУДОВАНИХ У ПОЛІМЕР-НЕОРГАНІЧНІ ГИБРИДНІ НОСІЇ

Запропоновано перспективний композиційний матеріал для зниження ендогенного та екзогенного забруднення курячих яєць патогенною мікрофлорою в процесі їх утворення та зберігання. Його основу становлять біосумісні та біодеградабельні гібридні наноносії кремнезем/поліакриламід, що містять малі наночастинки срібла (d_{av}=2,4±1,0 нм), які перорально вводять курям-несучкам з питною водою. Досліджено особливості формування наночастинок срібла в гібридних носіях шляхом борогідридного відновлення солі срібла за різних її концентрацій у водному розчині. Виявлено цікавий ефект різкої появи другої смуги поверхневого плазмонного резонансу в УФ-видимих спектрах суміші сіль срібла/гібрид за високої концентрації солі. Він був пояснений різкими структурними змінами в гібридних носіях, що викликані одночасним зростанням у них великої кількості AgНЧ. Передбачалось, що інтенсивне зростання багатьох AgНЧ в одній гібридній частинці супроводжувалось відокремленням прищеплених ланцюгів ПАА від поверхні SiO_2 завдяки руйнуванню водневих зв'язків. Методами УФ-видимої спектроскопії, потенціометричного титрування та ТЕМ досліджено зміну стану композиційного матеріалу під впливом pH розчину, концентрації наночастинок, наявності NaCl (як у "фізіологічному розчині") та видимого світла. Наносрібло в носіях показало високу стабільність щодо більшості з цих факторів. Досліджено вплив композиційного матеріалу на клінічний стан курей-несушок і важливі параметри їхніх яєць і крові у разі триразового (через 10 днів) перорального введення з питною водою дозою 0,2 та 0,4 мг на курицю на день. Виявлено вражаючий ефект селективної ендогенної акумуляції срібла в шкаралупі яєць. Це підтвердило проникнення наносрібного композиту в кровоносну систему курей шляхом проходження через травний тракт, всмоктування через кишковий епітелій і подальше транспортування в тканини курей, включаючи яйцепровід, де утворюються білок і яєчна шкаралупа. Показано, що проникнення композиту не викликало токсичний ефект в організмах курей-несушок.

Ключові слова: наносрібний композиційний матеріал, кремнезем/поліакриламідні наноносії, структура і стабільність, кури-несушки, параметри курячих яєць і крові.