

<https://doi.org/10.15407/microbiolj86.02.036>

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ANTIMICROBIAL AND ANTIVIRAL ACTIVITY OF NANOCOMPOSITES BASED ON POLYELECTROLYTE COMPLEXES WITH SILVER NANOPARTICLES

Recently, nanocomposite materials containing nanoparticles of metals such as silver, copper and zinc oxide have attracted most attention due to their pronounced pharmacological properties, such as antimicrobial, antiviral, anti-inflammatory, immunomodulatory ability and high stability in extreme conditions. Polyelectrolyte complexes based on polymers of natural origin, namely polysaccharides of chitosan and pectin, which can stabilize nanoparticles of a smaller size than individual polymers have significant potential for creation of silver-containing nanocomposites. The **aim** of this article is to study the antimicrobial and antiviral activity of silver-containing nanocomposites based on polyelectrolyte complexes. **Methods.** Peculiarities of the structural organization of silver-containing nanocomposites were investigated by the method of wide-angle scattering on an XRD-7000 diffractometer. The morphology of Ag nanoparticles in polymer matrixes were studied by transmission electron microscopy method (transmission electron microscopy JEOL 100 CXII). The antimicrobial activity of silver-containing nanocomposites was determined by agar diffusion as-

Citation: Rybalchenko N.P., Hnatiuk T.T., Artiukh L.O., Naumenko K.S., Zaremba P.Yu., Demchenko V.L., Kokhtych L.M., Iurzhenko M.V., Rybalchenko T.V., Ovsyankina V.O., Dolgoshey V.B., Sytnyk I.O., Marynin A.I. Antimicrobial and Antiviral Activity of Nanocomposites Based on Polyelectrolyte Complexes with Silver Nanoparticles. *Microbiological journal*. 2024 (2). P. 36—50. <https://doi.org/10.15407/microbiolj86.02.036>

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says against opportunistic pathogens *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans*. Cytotoxicity and antiviral activity were investigated using the MTT method and staining by gentian violet. **Results.** Analysis of wide-angle X-ray diffractograms of silver-containing nanocomposites based on polyelectrolyte complexes Na-CMC (pectin) — chitosan showed that at reduction of Ag^+ ions to metallic silver, there are two low-intensity diffraction maxima at $2\theta_m \sim 38^\circ$ and 44° in the diffractograms. These maxima correspond to the crystallographic planes of the face-centered cubic lattice of silver, are characterized by indices (111) and (200), respectively, and confirm the presence of metallic silver in the polymer system. Analysis of micrographs of silver-containing nanocomposites based on Na-CMC and chitosan showed that larger nanoparticles are formed with increasing the molecular weight of chitosan. The dependence of the size of silver nanoparticles on the wavelength of ultraviolet radiation at reduction of silver ion in polyelectrolyte-metal complexes Na-CMC— Ag^+ —chitosan of low molecular weight was also revealed. In particular, smaller particles are formed under irradiation by light with a shorter wavelength ($\lambda = 254 \text{ nm}$) than at $\lambda = 365 \text{ nm}$. Silver-containing nanocomposites Na-CMC-Ag-chitosan and pectin citrus-Ag-chitosan, obtained by reduction of Ag^+ ions under ultraviolet irradiation at a wavelength $\lambda = 365 \text{ nm}$ and $\lambda = 254 \text{ nm}$, exhibit high antimicrobial activity against the test cultures of microorganisms *S. aureus*, *E. coli*, and *P. aeruginosa* *C. albicans*. No significant dependence of antimicrobial activity on the molecular weight of the studied samples was noted: the obtained data were within close limits and had close values. In addition, no dependence of antimicrobial activity on the type of investigated test cultures of microorganisms was observed either. Nanocomposites based on Na-CMC-chitosan ($\lambda = 365 \text{ nm}$) inhibited infection titer HSV-1 by $(3.72\text{—}5.45) \text{ lgTCID}_{50}/\text{mL}$, whereas the decrease in titer during incubation with samples based on citrus pectin-chitosan was within $(2.39\text{—}2.42) \text{ lgTCID}_{50}/\text{mL}$. A dose-dependent relationship between molecular weight of chitosan and reduction of infection titer was observed. It was found that silver-containing nanocomposites formed by reduction of silver ions in polyelectrolyte-metal complexes under ultraviolet radiation of different wavelengths had no cytotoxic effect on cells of MDCK and BHK. **Conclusions.** The investigated silver-containing nanocomposites based on Na-CMC (pectin)-chitosan polyelectrolyte complexes show antimicrobial activity against test cultures of *S. aureus*, *E. coli* *P. aeruginosa*, and *C. albicans* along with antiviral activity against herpes simplex virus type 1 and influenza virus. It was established that the obtained nanocomposites did not show a cytotoxic effect on MDCK and BHK cells. The obtained data allow us to assert that investigated silver-containing nanocomposites are promising antimicrobial means for the development of new effective strategies against microorganisms and viruses and improvement of the population health.

Keywords: polyelectrolyte complexes, silver-containing nanocomposites, antimicrobial activity, antiviral activity.

In today's world, infectious diseases caused by microorganisms and viruses remain a serious problem of health protection, despite the constant progress in the creation of medical drugs and the development of pharmaceutical technologies (Sharma et al., 2023). The high risk of complications and the spread of infections in society require constantly searching for new methods to combat pathogenic microorganisms. Pathogens (bacteria, fungi, and viruses), including virulent and drug-resistant strains, are constantly found on various surfaces, survive on them quite a long time, and can be primary sources of transmission of pathogens (World Health Organization, 2022; Perlman, 2020; Setti et al., 2020). New multifunctional antimicrobial/antiviral surface coatings are needed to prevent such superficial transmission of pathogens.

Recently, polymer materials that contain silver nanoparticles have attracted the most attention (Demchenko et al., 2020a; 2020b; 2021). A peculiarity of silver nanoparticles consists in their ability to function at low concentrations, which reduces the potential toxicity to humans and environment. This makes them attractive for using in the medical industry, pharmaceuticals, packaging materials and other industries where it is important to fight infectious diseases (Flores-López et al., 2019).

Despite significant advances in silver nanoparticle research, there are still many unresolved aspects regarding their effect on microorganisms and viruses. The questions of the influence of the size and shape of silver nanoparticles on their antimicrobial activity, mechanism of action, duration of effect and potential cytotoxic-

ity, *etc.* are especially important (Carvalho et al., 2022). Currently, the most common methods for obtaining of silver-containing nanocomposites are chemical and radiation chemical reduction of silver ions in polymer films mainly of synthetic origin. However, these methods require chemical reducing agents, often toxic, and a special complex equipment that generates radiation. Polyelectrolyte complexes based on natural polymers, namely polysaccharides such as the Na-salt of carboxymethylcellulose, pectin, chitosan, *etc.* (Wang et al., 2017; Demchenko et al., 2015; 2022a; 2022b) have considerable potential for creating silver-containing nanocomposites. Polyelectrolyte complexes (PECs) are formed due to the electrostatic interaction between oppositely charged polyelectrolytes. In terms of chemical structure, they are rigid chain polymers for which, in addition to the presence of ionogenic groups, a high concentration of reactive OH groups is also characteristic. This opens up the possibility of the stabilization of nanoparticles of smaller size. Moreover, they are characterized by high biological activity and at the same time, they are non-toxic, biocompatible, and biodegradable substances.

Thus, the study of the antimicrobial activity of silver nanoparticles is an actual and promising field of research which can lead to the development of new effective strategies for combating infectious diseases.

In view of the above, the **purpose** of this work is to research the antimicrobial and antiviral activity, as well as the structure and morphology of silver-containing nanocomposites based on polyelectrolyte complexes Na—CMC (pectin)—chitosan obtained by UV irradiation of silver ions in polymer films.

Materials and Methods. In the antimicrobial research, we used the following reference strains of opportunistic pathogens: *Staphylococcus aureus* ATCC 25923 as model Gram-positive bacteria, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, as model Gram-nega-

tive bacteria, and yeast-like fungus *Candida albicans* ATCC 885-653. The bacterial strains were obtained from the Ukrainian Collection of Microorganisms at the Zabolotny Institute of Microbiology and Virology of NAS of Ukraine. The bacteria were cultured on meat-peptone agar for 24 h at 37 °C.

Viruses and cell culture. In the cytotoxicity research, BHK and MDCK cells were used: BHK-21 cells (ECACC N85011433) — Syrian hamster kidney, obtained from the Cell Bank of the Institute of Experimental Pathology, Oncology and Radiobiology NAS of Ukraine (Kyiv, Ukraine) and MDCK cells (ECACC N84121903) — Canine kidney, from L.V. Gromashevsky Institute of Epidemiology and Infection Diseases of NAS of Ukraine (Kyiv, Ukraine). For antiviral research, the influenza A virus (IAV) H1N1, strain A/FM/1/47, and herpes simplex virus type 1 were employed. All epithelial cells were cultured in sterile plastic falcon (Bioswisstec, Switzerland) using a growth medium consisting of 45% DMEM (Dulbecco's Modified Eagle's Medium) (Biowest, France), 45% RPMI 1640 (Roswell Park Memorial Institute medium) (Biowest, France), and 10% heat-inactivated fetal bovine serum (FBS, Biowest, France) at 56 °C, supplemented with penicillin-streptomycin antibiotics at a concentration of 100 µg/mL (Biowest, France).

To manufacture polymer systems based on polyelectrolytes complexes and silver nanoparticles, such substances were used as sodium carboxymethylcellulose salt (Na-CMC) «Aldrich», $M_w \sim 90,000$, pectin citrus «Aldrich», galacturonic acid, $\geq 74\%$, low molecular weight chitosan (LMWC) «Aldrich», degree of deacetylation $\sim 75\%$, $M_w \sim 50,000$ — $190,000$, and silver (I) nitrate (AgNO_3) (Aldrich) with $M = 169.9$. Polyelectrolyte complexes were formed by mixing of 1% aqueous solutions of anionic and cationic polyelectrolytes taken in the stoichiometric ratio of functional ionogenic groups at 20 ± 2 °C. Chitosan was dissolved in 0.1M HCl solution, and appropriate amount of 0.1M NaOH solution was added

to anionic polyelectrolyte Na-CMC (pectin). The PECs obtained in this way were poured on polytetrafluoroethylene (PTFE) plates and dried at the same temperature to constant mass. The dry PEC films were washed in distilled water until reaching the neutral pH, then were vacuumed and dried at 20 ± 2 °C to constant mass. Samples of polyelectrolyte-metal complexes (PMCs) were obtained by immersing PEC films in an aqueous solution of AgNO_3 with a concentration of 0.1 mol/L. At the same time, the transparent colorless PEC films turned dark red. Silver-containing nanocomposites were obtained by reduction of silver ions under the action of ultraviolet radiation ($\lambda=254$ and 365 nm) at room temperature.

Research methods. Features of the structure of the samples. The structure of nanocomposites was studied by wide-angle X-ray diffraction with an XRD-7000 diffractometer (Shimadzu, Japan), the X-ray optical scheme of which was performed by passing the primary beam through a sample (Galchun et al., 2015), using CuK_α radiation ($\lambda = 1.54$ Å) and a graphite monochromator at temperature 20 ± 2 °C (Matkowska et al., 2014).

Morphology of nanocomposites. The size of Ag nanoparticles and their distribution in the polymer matrix was examined using a JEM-1230 transmission electron microscope (JEOL, Japan) at the resolution of 0.2 nm (Matkowska et al., 2017).

The antimicrobial activity of silver-containing nanocomposites. The study was conducted using the disk diffusion method on a solid nutrient medium called Mueller-Hinton agar (MHA) (Case & Johnson, 1984). Petri dishes containing MHA medium were inoculated with $10 \mu\text{L}$ of test microorganism inoculum at a concentration of 2×10^5 CFU/mL. The test samples were placed on the surface of the nutrient medium that had been inoculated with the test microorganisms. The Petri dishes were then incubated for 24 h at 37 °C. The presence of a clear zone free of microorganisms around the nanocomposite samples served as an indicator of antimicrobial activity.

The antiviral assay. The studied nanocomposites were placed in the wells of a 96-well plate (darker side up), $100 \mu\text{L}$ of undiluted virus suspension was applied on the top and incubated at 37 °C for 60 minutes. The cells were infected with ten-fold serial dilutions of virus-containing material at $50 \mu\text{L}$ per well. A suspension of the virus was used as a control, which was kept in similar conditions without contact with the composites. Adsorption was performed at 37 °C for 1.5 h, after which $150 \mu\text{L}$ of support medium was added to the virus-containing material. Non-infected cells were used as cell controls. The plate was kept in an atmosphere of 5% CO_2 at 37 °C until the appearance of a pronounced cytopathic effect of the virus (3 days). The analysis was performed using the MTT method for herpes simplex virus type 1 and crystal purple staining for influenza virus. All experiments were performed in triplicate. The results were analyzed spectrophotometrically on a Multiskan FC reader (Thermo Scientific, USA) at a wavelength of 538 nm. Using the obtained optical densities was determined by % of cytopathic effect (CPE) of the virus on the cells by the formula described by Kohn et al. (Kohn et al., 2015). Virus dilution was determined, which reduces the optical density of the sample by 50% compared to that of cell control, which is the virus titer and expressed in $\text{TCID}_{50}/\text{mL}$. A decrease in the infection titer of the virus by 2 lg $\text{TCID}_{50}/\text{mL}$ or more (or 99% or more), compared to the control, indicates significant activity of the compound against the virus, 1—2 lg $\text{TCID}_{50}/\text{mL}$ (97—99%) demonstrates a moderate effect, and less than 1 lg $\text{TCID}_{50}/\text{mL}$ (97%) shows a relative activity.

The cytotoxicity assay. The cytotoxicity of nanocomposites was performed using the MTT test, which is based on determining the functioning of the dehydrogenase activity of mitochondria. The study procedure included 24 h of exposure of the plates in growth medium for cell cultures at 37 °C. The medium was then added to the monolayer of cell cultures at dilutions of 1:10, 1:100, and 1:1000. Analysis of cells was per-

formed at 48 h by adding 20 μL of MTT solution (5 mg/mL, NeoFrexx, Germany). After four h of incubation at dark, the formazan crystal of MTT reduction was dissolved in ethanol 96%, and absorbance was measured using a microtiter plate reader Multiskan FC (Thermo Scientific, USA). The effect of cytotoxicity of the samples was quantified as the percentage of control absorption of the reduced dye MTT at 538 nm. All experiments were repeated three times to test for sensitivity. Cell viability was assessed by the ability of live cells to reduce the yellow MTT dye to a blue formazan crystal (Tonder et al., 2015).

Statistical processing of results. In unraveling the results, we systematically computed the mean and standard deviation. We used Microsoft Excel to conduct a focused regression analysis, explicitly employing the linear regression to discern underlying trends. It is noteworthy that we conducted three repetitions for each experimental condition, contributing to the robustness and reliability of our statistical analysis.

Results. Analysis of wide-angle X-ray diffractograms of polyelectrolytes complexes Na-CMC — chitosan (low, medium, and high molecular mass) and nanocomposites based on them showed that all they are characterized by an amorphous structure (Fig.1). The presence of amorphous halo in all diffractograms in the region of scattering angles $(15\text{--}30)^\circ$ is shown.

The reduction of Ag^+ ions to metallic silver is evidenced by the presence of two low-intensity maxima at $2\theta_m \sim 38^\circ$ and 44° on the diffractograms of all samples. These maxima correspond to the crystallographic planes of the face-centered cubic lattice of silver and are characterized by indices (111) and (200), respectively, which confirms the presence of metallic silver in the polymer system (Fig. 1).

Analysis of the micrographs of silver-containing nanocomposites based on Na-CMC and low molecular weight chitosan (chitosan LMW) showed the dependence of the silver nanoparticle size on the wavelength of ultraviolet radia-

tion at reduction of silver ions in polyelectrolyte-metal complexes Na-CMC- Ag^+ —chitosan with low molecular weight. In particular, in the samples formed under irradiation by light with a shorter wavelength ($\lambda=254$ nm), smaller particles are formed than at $\lambda=365$ nm (Fig. 2 a, b). This may be due to the fact that the shorter wavelength of ultraviolet irradiation, the higher its frequency and quantum energy, the harder radiation. At the same time, crystallization centers of nanoparticles are formed faster.

Also, the antimicrobial activity was studied for silver-containing nanocomposites obtained by reduction of Ag^+ ions under ultraviolet radiation ($\lambda = 365$ and 254 nm) in polymer systems Na-CMC— Ag^+ —chitosan and citrus pectin— Ag^+ —chitosan of low, medium, and high molecular weight relative to reference strains of opportunistic microorganisms *S. aureus*, *E. coli*, *P. aeruginosa* and yeast-like fungi *C. albicans*.

Silver-containing nanocomposites Na-CMC— Ag —chitosan of low, medium, and high molecular weight, obtained by reduction of Ag^+ ions under ultraviolet radiation at wavelengths $\lambda = 254$ nm and $\lambda = 365$ nm, showed antimicrobial activity against the test microorganisms (Table 1). After 24 h of incubation at 37°C , a clear zone around the samples obtained by reduction of Ag^+ ions under ultraviolet irradiation at a wavelength $\lambda = 365$ nm was observed. It indicates the inhibition of the growth of microorganisms (Fig. 3). Its diameter was established within $(21.79\text{--}22.71)$ mm for *S. aureus*; $(20.00\text{--}21.62)$ mm — *E. coli*; $(22.90\text{--}24.42)$ mm — *P. aeruginosa*, and $(18.93\text{--}21.52)$ mm — *C. albicans*. There was no significant dependence of the antimicrobial activity on the tested samples' molecular weight. The obtained data fell within close ranges and had similar values, which were assessed based on the zones of growth inhibition of microorganism test cultures on Petri dishes. Additionally, there was no dependence of antimicrobial activity on the type of tested microorganism cultures. All samples of silver-containing nanocomposites Na-CMC— Ag -chitosan of low, medium,

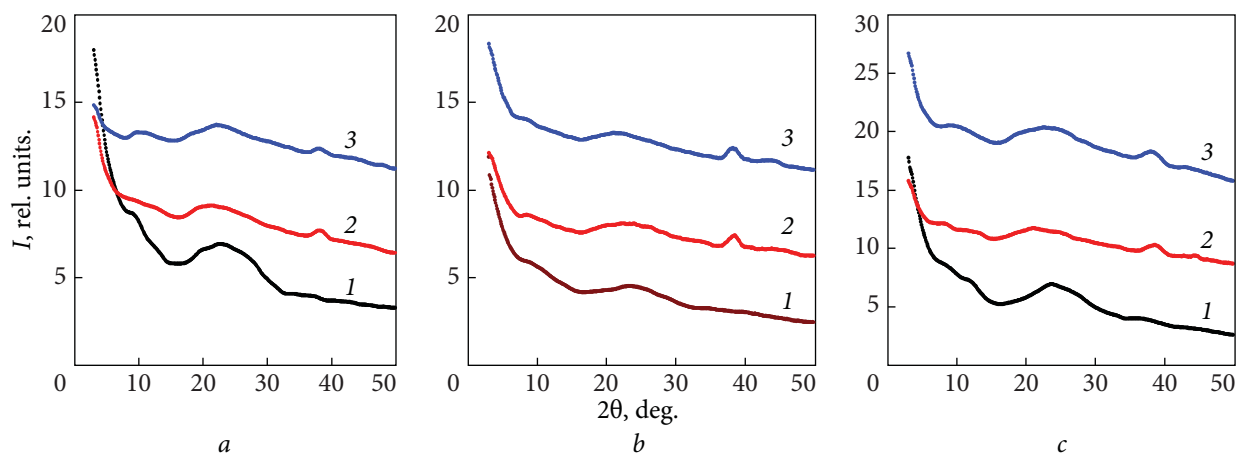


Fig. 1. Wide-angle X-ray diffractograms of the initial polyelectrolyte complexes (1): Na-CMC—chitosan LMW (a), Na-CMC—chitosan MMW (b), Na-CMC—chitosan HMW (c) and silver-containing nanocomposites based on them, obtained by reduction of Ag^+ ions in polyelectrolyte-metal complexes under ultraviolet radiation of different wavelengths: 254 nm (2) and 365 nm (3).

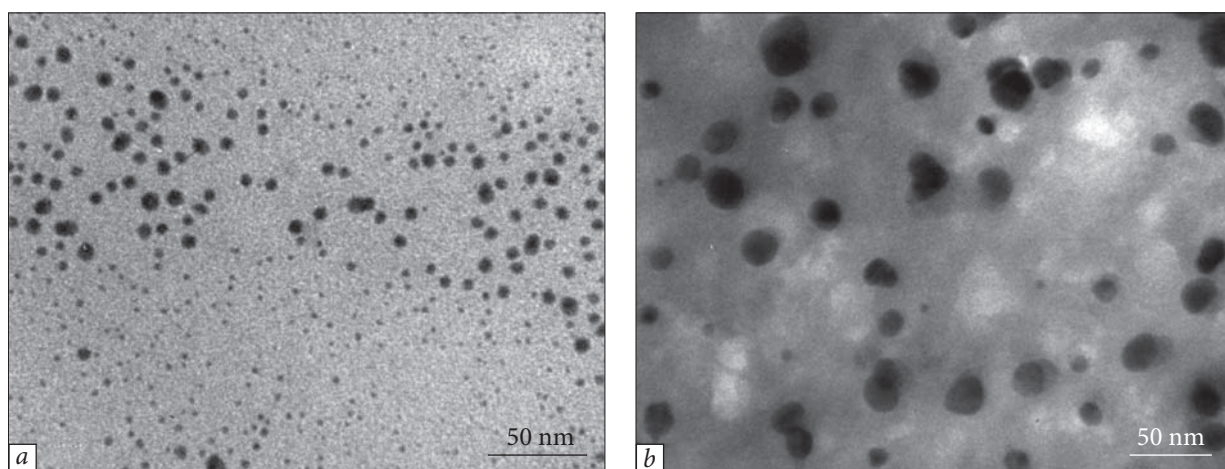


Fig. 2. Photomicrographs of transmission electron microscopy of silver-containing nanocomposites based on polyelectrolyte complexes Na—CMC—chitosan LMW obtained by reduction of silver ions under ultraviolet radiation of different wavelengths: (a) $\lambda = 254$ nm; (b) $\lambda = 365$ nm.

and high molecular weight obtained by reduction of Ag^+ ions under ultraviolet irradiation have demonstrated high antimicrobial activity against gram-positive *S. aureus*, gram-negative *E. coli*, *P. aeruginosa* bacteria and yeast-like fungi *C. albicans*.

The studied silver-containing nanocomposites Na-CMC-Ag-chitosan of low, medium and high molecular weight obtained by reduction of ions Ag^+ under radiation at a wavelength of

$\lambda = 254$ nm, demonstrated high antimicrobial activity as well (Table 1). The growth inhibition zone diameter was established to be within (21.49—23.06) mm for *S. aureus*; (19.15—22.17) mm for *E. coli*; (23.77—24.71) mm for *P. aeruginosa*, and (20.04—23.93) mm for *C. albicans*. The antimicrobial activity data were close to the values obtained for the samples synthesized under irradiation at a wavelength of $\lambda = 365$ nm.

In the control samples Na-CMC-chitosan of low, medium, and high molecular weight, which did not contain silver nanoparticles, active growth of test microorganisms was observed, and there were no zones of the growth inhibition.

The following samples in researching antimicrobial activity were silver-containing nanocomposites citrus pectin—Ag—chitosan of low, medium, and high molecular weight obtained by reduction of ions Ag^+ under the action of ultraviolet radiation at wavelengths of $\lambda = 365$ nm and 254 nm. The research revealed that all of them show high antimicrobial activity against test-cultures of microorganisms *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* (Table 2). So, after 24 h of incubation at 37 °C, clear growth inhibition zones were observed around the disks of the investigated silver-containing nanocomposites obtained by the reduction of Ag^+ ions under ultraviolet irradiation at a wavelength of $\lambda = 365$ nm, indicating the inhibition of microbial growth (Fig. 4).

There was no significant dependence of antimicrobial activity on the molecular weight of chitosan in the composition of PEC. The diameter of growth retardation zones was (29.12; 29.02; 26.17) mm for *S. aureus*; (21.36; 22.46; 21.35) mm for *E. coli*; and (24.06; 24.32; 24.69) mm for *P. aeruginosa* in samples citrus pectin—Ag—chitosan of low, medium, and high molecular weight, respectively. Slightly lower values of

antimicrobial activity were observed for yeast-like fungi *C. albicans* — (16.88; 20.46; 20.29) mm, respectively.

The researched silver-containing nanocomposites citrus pectin—Ag—chitosan of low, medium, and high molecular weight obtained by reduction of Ag^+ ions under ultraviolet irradiation at $\lambda = 254$ nm, also demonstrated high antimicrobial activity against researched test cultures of microorganisms *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* (Table 2). The samples showed the highest antimicrobial activity against gram-positive bacteria *S. aureus*. The diameters of growth inhibition zones were (29.74; 28.92; 27.58) mm. Somewhat lower activity was noted for the other test microorganism cultures: *E. coli* — (21.61; 20.87; 22.05) mm; *P. aeruginosa* — (22.11; 23.63; 23.35) mm; and *C. albicans* — (20.65; 21.61; 25.08) mm.

In the control samples citrus pectin—chitosan of low, medium, and high molecular weight that did not contain silver nanoparticles, an active growth of test-microorganisms was observed with no zone of the growth inhibition.

The antiviral activity of nanocomposites based on polyelectrolyte-complexes and silver nanoparticles obtained by the reduction of silver ions under ultraviolet irradiation is shown in Figs. 5 and 6. It was established that silver-containing nanocomposites based on Na-CMC—

Table 1. Antimicrobial activity of silver-containing nanocomposites Na-CMC—Ag—chitosan of low, medium, and high molecular weight (LMW, MMW, and HMW)

Polymer system	Diameter of the growth inhibition zone, mm			
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Control sample Na-CMC—chitosan LMW	0	0	0	0
Na-CMC—Ag—chitosan LMW ($\lambda = 365$ nm)	22.26 ± 1.40	20.00 ± 0.80	22.77 ± 1.50	18.93 ± 0.40
Na-CMC—Ag—chitosan MMW ($\lambda = 365$ nm)	21.76 ± 1.10	21.62 ± 1.00	22.90 ± 1.60	21.52 ± 0.90
Na-CMC—Ag—chitosan HMW ($\lambda = 365$ nm)	22.71 ± 1.60	21.33 ± 1.10	24.42 ± 1.80	20.45 ± 0.70
Na-CMC—Ag—chitosan LMW ($\lambda = 254$ nm)	23.06 ± 1.70	21.45 ± 1.00	24.8 ± 1.60	23.00 ± 0.80
Na-CMC—Ag—chitosan MMW ($\lambda = 254$ nm)	22.50 ± 1.20	21.17 ± 1.40	23.77 ± 1.80	23.00 ± 1.80
Na-CMC—Ag—chitosan HMW ($\lambda = 254$ nm)	22.84 ± 1.60	19.15 ± 0.50	24.00 ± 1.90	20.04 ± 0.60

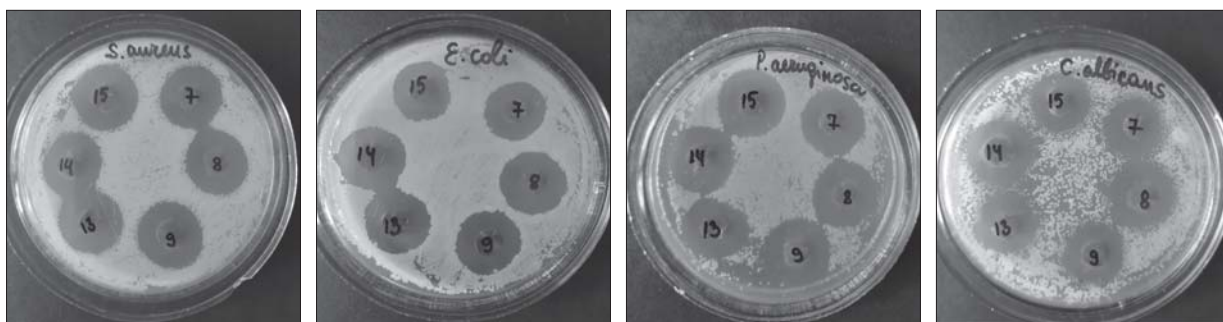


Fig. 3. Antimicrobial activity of silver-containing nanocomposites based on Na-CMC and chitosan of low, medium, and high molecular weight, obtained by reduction of Ag^+ ions under ultraviolet radiation: (7) Na-CMC—Ag—chitosan LMW ($\lambda = 365$ nm), (8) Na-CMC—Ag—chitosan MMW ($\lambda = 365$ nm), 9 — Na-CMC—Ag—chitosan HMW ($\lambda = 365$ nm), 13 — Na-CMC—Ag—chitosan LMW ($\lambda = 254$ nm), 14 — Na-CMC—Ag—chitosan MMW ($\lambda = 254$ nm), 15 — Na-CMC—Ag—chitosan HMW ($\lambda = 254$ nm)

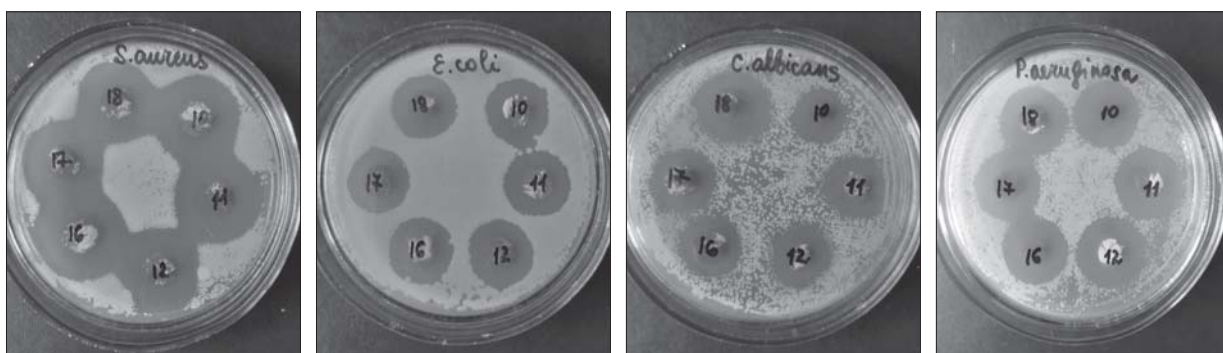


Fig. 4. Antimicrobial activity of silver-containing nanocomposites based on citrus pectin and chitosan of low, medium, and high molecular weight, obtained by reduction of Ag^+ ions under ultraviolet radiation: 10 — citrus pectin—Ag—chitosan LMW ($\lambda = 365$ nm); 11 — citrus pectin—Ag—chitosan MMW ($\lambda = 365$ nm); 12 — citrus pectin—Ag—chitosan HMW ($\lambda = 365$ nm); 16 — citrus pectin—Ag—chitosan LMW ($\lambda = 254$ nm); 17 — citrus pectin—Ag—chitosan MMW ($\lambda = 254$ nm); 18 — citrus pectin—Ag—chitosan HMW ($\lambda = 254$ nm)

Table 2. Antimicrobial activity of silver-containing nanocomposites citrus pectin—Ag—chitosan of low, medium, and high molecular weight (LMW, MMW, and HMW)

Polymer system	Diameter of the growth inhibition zone, mm			
	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
Control sample citrus pectin—chitosan LMW	0	0	0	0
Citrus pectin—Ag—chitosan LMW ($\lambda = 365$ nm)	29.12 ± 2.20	21.36 ± 1.20	24.06 ± 1.50	18.88 ± 0.50
Citrus pectin—Ag—chitosan MMW ($\lambda = 365$ nm)	29.02 ± 2.10	22.46 ± 1.00	24.32 ± 1.60	20.46 ± 0.80
Citrus pectin—Ag—chitosan HMW ($\lambda = 365$ nm)	28.17 ± 1.80	21.35 ± 1.20	24.69 ± 1.80	20.29 ± 0.70
Citrus pectin—Ag—chitosan LMW ($\lambda = 254$ nm)	29.74 ± 2.40	21.61 ± 1.30	22.11 ± 0.90	20.65 ± 0.60
Citrus pectin—Ag—chitosan MMW ($\lambda = 254$ nm)	28.92 ± 2.00	20.87 ± 0.80	23.63 ± 1.20	21.61 ± 0.90
Citrus pectin—Ag—chitosan HMW ($\lambda = 254$ nm)	27.58 ± 1.90	22.05 ± 1.10	23.35 ± 1.20	25.08 ± 1.40

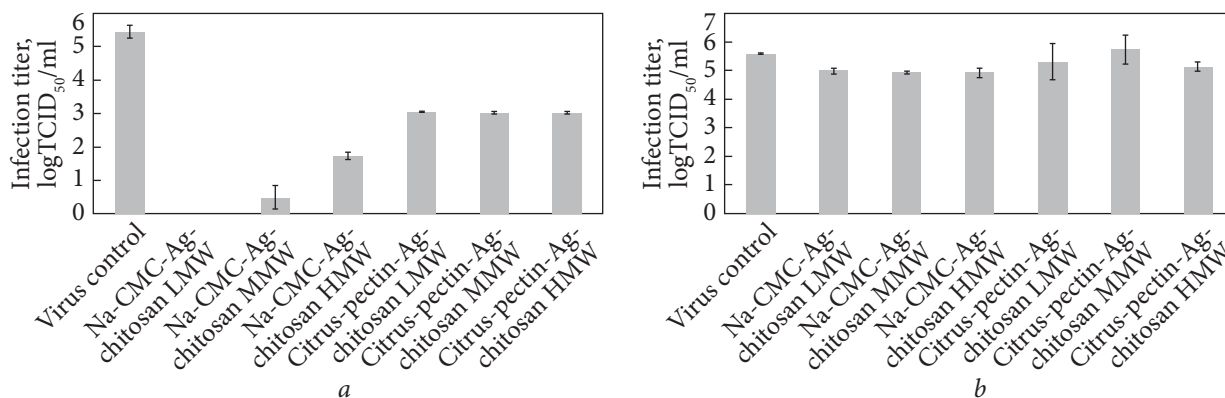


Fig. 5. Infection titer of viruses: (a) herpes simplex virus and (b) influenza virus type A after incubation with silver-containing nanocomposites obtained by reduction of silver ions under ultraviolet radiation ($\lambda = 365$ nm). Statistical significance $p < 0.05$

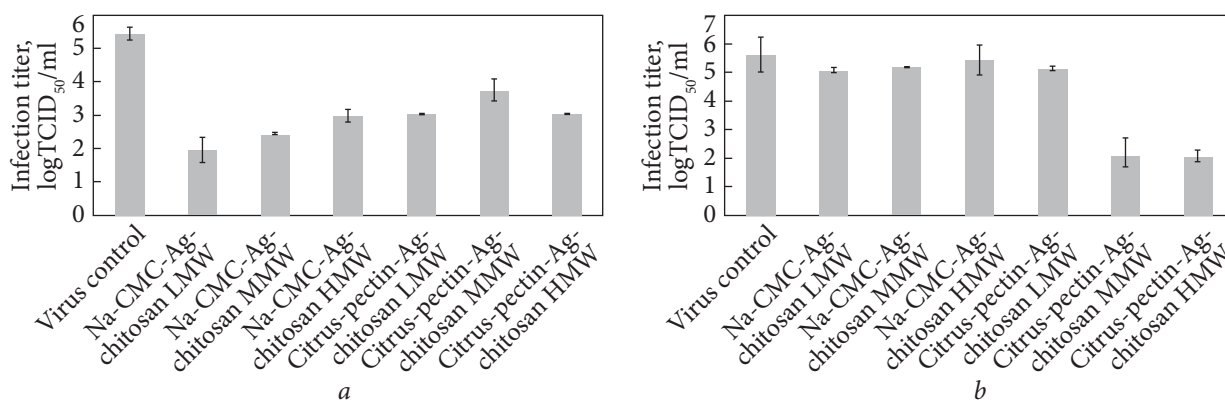


Fig. 6. Infection titer of viruses: (a) simplex virus herpes and (b) influenza virus type A after incubation with silver-containing nanocomposites obtained by reduction of silver ions under ultraviolet radiation ($\lambda = 254$ nm). Statistical significance $p < 0.05$

chitosan obtained by the reduction of silver ions under ultraviolet radiation ($\lambda = 365$ nm) inhibited the infection titer HSV-1 by 3.72–5.45 lgTCID₅₀/mL. A dose-dependent relationship between the molecular weight of chitosan in the composition of PEC and the reduction of the infection titer HSV-1 was observed. Silver-containing nanocomposites based on Na-CMC—chitosan of low molecular weight completely inhibited the reproduction of the herpes simplex virus, and samples with chitosan of medium and high molecular weight reduced infection titer of the virus by 4.96 and 3.72 lgTCID₅₀/mL, respectively. Instead, samples of pectin—Ag—chitosan

($\lambda = 365$ nm) decreased of the titer in the range from 2.39 to 2.42 lgTCID₅₀/mL (Fig. 5, a), compared to the control of HSV-1.

On the model of the influenza virus in the range from 0.61 to 0.68 lgTCID₅₀/mL for nanocomposites Na-CMC—Ag—chitosan, inhibition of the infection titer and even lower efficiency for samples pectin—Ag—chitosan at $\lambda = 365$ nm (Fig. 5, b) were noted.

Silver-containing nanocomposites based on polyelectrolyte complexes Na-CMC—chitosan and pectin—chitosan obtained by reduction of silver ions under ultraviolet radiation ($\lambda = 254$ nm) showed a similar effect to the above de-

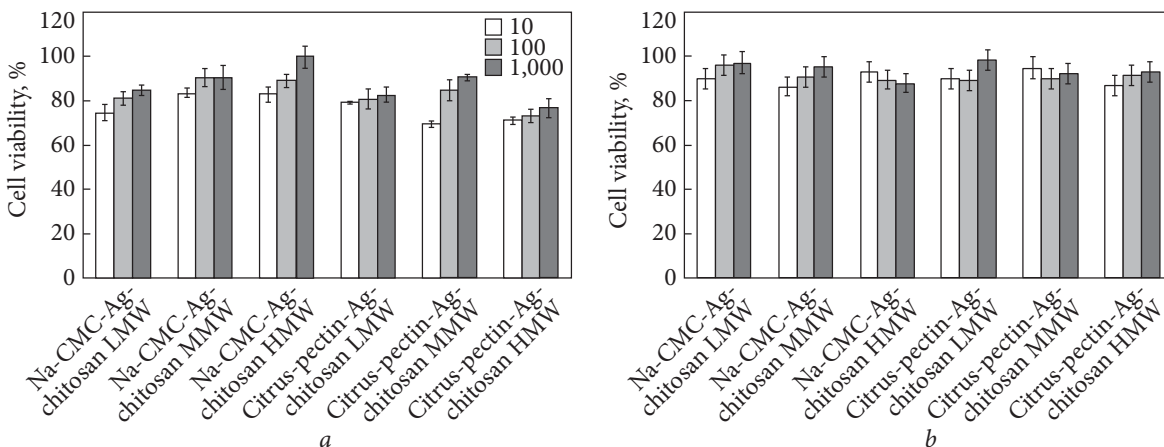


Fig. 7. The influence of nanocomposites Na-CMC—Ag—chitosan and pectin—Ag—chitosan, obtained by reduction of Ag^+ ions under ultraviolet radiation ($\lambda = 365 \text{ nm}$) on the viability of epithelial cultures of (a) BHK and (b) MDCK. Statistical significance $p < 0.05$.

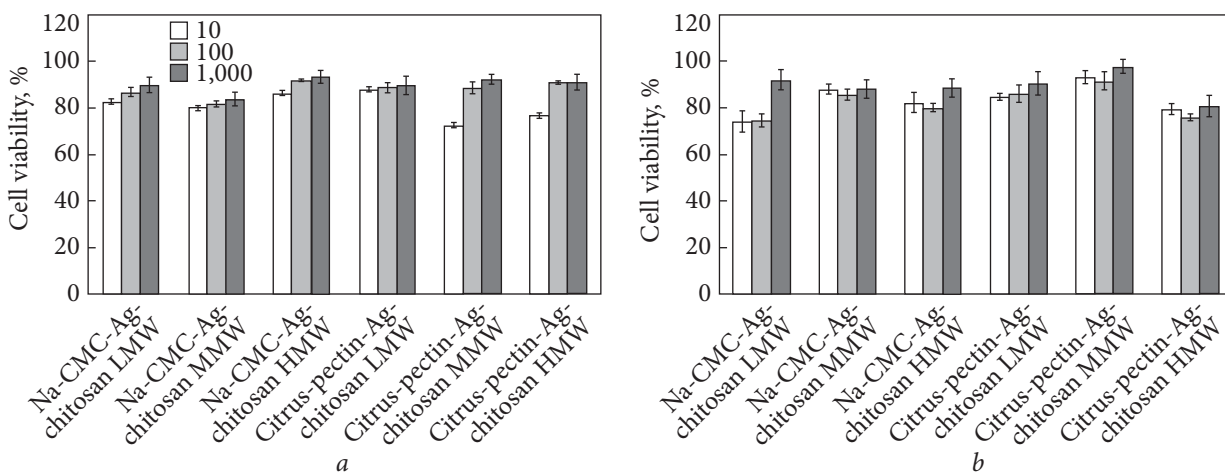


Fig. 8. Viability of epithelial cultures under the influence of nanocomposites Na-CMC—Ag—chitosan and pectin—Ag—chitosan obtained by reduction of Ag^+ ions under ultraviolet radiation ($\lambda = 254 \text{ nm}$) on the viability of epithelial cultures of (a) BHK and (b) MDCK. Statistical significance $p < 0.05$.

scribed samples, obtained at $\lambda = 365 \text{ nm}$, but were less efficient. Among the silver-containing nanocomposites based on Na-CMC—chitosan, samples with low and medium molecular weight of chitosan showed activity (titer reduction, respectively) 3.49 and 3.01 $\text{lgTCID}_{50}/\text{mL}$, while samples of pectin—Ag—chitosan inhibited the infection titer HSV-1 in the range from 1.71 to 2.42 $\text{lgTCID}_{50}/\text{mL}$ (Fig. 6, a). It was established that samples Na-CMC—Ag—chitosan had dose-

dependent inhibitory influence on the level of the infection titer of the virus depending on the molecular weight of chitosan.

In relation to the influenza virus, silver-containing nanocomposites based on polyelectrolyte complexes pectin-chitosan of medium and high molecular weight showed a pronounced antiviral effect: inhibition of the virus titer was from 3.54 to 3.56 $\text{lgTCID}_{50}/\text{mL}$, respectively (Fig. 6, b).

At the same time, nanocomposites should have antimicrobial and antiviral activity, be non-toxic, environmentally friendly, and stable for a long time. Therefore, we investigated the cytotoxic effect of nanocomposites on cultures of mammalian cells. As shown in Fig. 7, the viability indicators for MDCK cells in the studied samples of Na-CMC—Ag—chitosan and pectin—Ag—chitosan were in the range from 87 to 99%, while for BHK cells, it was somewhat smaller (70—99%). So, nanocomposites Na-CMC—Ag—chitosan and pectin—Ag—chitosan obtained by UV irradiation at $\lambda = 365$ nm do not have a toxic effect on both cultures of cells.

Silver-containing nanocomposites Na-CMC—Ag—chitosan and pectin—Ag—chitosan obtained by reduction of Ag^+ ions under ultraviolet radiation ($\lambda = 254$ nm) did not have a cytotoxic impact on epithelial cultures of the cells. The indicator of the vitality of cells was in the range of 74—98% in MDCK cells, whereas for the model of the BHK cell, it was in the range from 72 to 93% (Fig. 8).

Discussion. One of the main ways of spreading of viruses and microorganisms in the environment is contamination of objects, in particular surfaces. Surface contact can lead to the spreading of many types of pathogenic microorganisms that are capable of being stored in the environment for a certain time (Gralinski & Menachery, 2020). Research of the antiviral surface is often limited by the complexity of the viral structure, the type of viruses and the lack of understanding of their influence at the molecular level. However, in recent years, nanoparticles and nanomaterials have gained great popularity.

This allowed us to take a big step in developing new antimicrobial materials (Pemmada et al., 2020; Sassi et al., 2015). AgNPs are known for their antibacterial and antifungal activity. There are also numerous studies of antiviral activity of AgNPs against a wide range of viruses, and they have demonstrated virucidal activity and/or changes in binding of virus with host cells (Luceri et al., 2023).

In this work, new silver-containing nanocomposites based on Na-CMC and pectin with chitosan of different molecular weight were investigated. According to the literature, both components have a wide range of biological properties (Tan et al., 2022; Chaika et al., 2022). In our work, it was established that the researched silver-containing nanocomposites Na-CMC—Ag—chitosan and citrus pectin Ag-chitosan of low, medium, and high molecular weight, obtained by reduction of Ag^+ ions under ultraviolet irradiation at wavelengths $\lambda = 365$ nm and $\lambda = 254$ nm, exhibit high antimicrobial activity against test cultures of microorganisms. No significant dependence of antimicrobial activity on the molecular weight of the studied samples was noted. The obtained data were within close limits and had close values. In addition, no dependence of antimicrobial activity from the type of investigated test cultures of microorganisms was observed either. All samples of silver-containing nanocomposites Na-CMC—Ag—chitosan showed a high antimicrobial activity against test cultures of microorganisms *S. aureus*, *E. coli*, *P. aeruginosa* *C. albicans*.

The studies show that the bactericidal effect of nanoparticles of silver (AgNP) depends on their size and morphology: smaller AgNPs or octahedral and decahedral particles are more potent antibacterial agents due to the fact that AgNPs can directly damage cell walls and penetrate inside through plasma membranes (Sánchez-Salcedo et al., 2023). Antibacterial mechanism of nanoparticles of silver ions is not clearly defined. Three main ways of their action are proposed: nanoparticles release positively charged silver ions, which causes cell lysis through their interaction with cellular walls of peptidoglycan or plasma membrane; prevents DNA replication through their interaction with bacterial cytoplasmic DNA; disrupts protein synthesis by interacting with bacterial proteins (Yamanaka et al., 2005; Jung et al., 2008; Shrivastava et al., 2007; Sarviya et al., 2023).

While studying the antiviral activity of silver-containing nanocomposites, it was established that

nanocomposites Na-CMC—Ag—chitosan are the most effective against the virus of herpes simplex of type 1. A dose-dependent effect was observed between the molecular weight of chitosan as part of polyelectrolyte complexes and a decrease in the infection titer. Nanocomposites with high molecular weight completely suppressed titer HSV-1. Relative to the flu virus nanocomposites pectin—Ag—chitosan expressed antiviral effect. It should be noted that nanocomposites Na-CMC—Ag—chitosan of medium and low molecular weights had an antiviral effect. The studied nanocomposites contained chitosan and silver nanoparticles. As described above, one of the mechanisms of the action of silver nanoparticles is an interaction with an extracellular virus, so it is possible to assume that the nanoparticles immobilized on the surface of the composites had the same mechanism of action (Boroumand et al., 2021).

Conclusions. It was established that the investigated samples of silver-containing nanocomposites Na-CMC—Ag—chitosan and citrus pectin—Ag—chitosan of low, medium, and high molecular weight, obtained by reduction of Ag⁺ ions under ultraviolet radiation at wavelengths $\lambda = 365$ and 254 nm for 60 min, exhibit high antimicrobial activity against test cultures of microorganisms *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*. The values of antimicrobial activity estimated by the diameter of zone

of the retention growth of test cultures of microorganisms were close and did not show significance dependence on the method of preparation and the molecular weight of the samples studied. Samples of Na-CMC—Ag—chitosan and citrus pectin—Ag—chitosan, obtained at $\lambda = 365$ nm had a pronounced anti-HSV-1 efficiency among the silver-containing nanocomposites. On the other hand, samples of pectin—Ag—chitosan obtained by UV irradiation at $\lambda = 254$ nm were effective against the influenza virus.

The use of silver-containing nanocomposites as antimicrobial agents opens new perspectives in the fight against infectious diseases. Further research and development of this field of science can contribute to the development of new effective strategies against microorganisms and viruses and improve the general state of health of the population.

Financial support. The work was performed with the financial support by a project of the research team of young scientists of the National Academy of Sciences of Ukraine No. 22/02-2023(4), as well as within the grant from the National Research Foundation of Ukraine (Development of antimicrobial packaging biopolymer materials and technology of their welding for long-term food storage. Application ID 2022.01/0019).

Conflict of interest. The authors declare that there are no conflicts of interest.

REFERENCES

- Boroumand, H., Badie, F., Mazaheri, S., Seyedi, Z. S., Nahand, J. S., Nejati, M., Baghi, H. B., Abbasi-Kolli, M., Bادهنووش, B., Ghandali, M., Hamblin, M. R., & Mirzaei, H. (2021). Chitosan-Based Nanoparticles Against Viral Infections. *Front Cell Infect Microbiol*, 11, Article 643953. <https://doi.org/10.3389/fcimb.2021.643953>
- Carvalho, I., Lima, M. J., Nobre, D., Marques, S. M., Castro, D., Leite, T. R., Henriques, M., Duarte, F., Ramalho, A., & Carvalho, S. (2022). Silver oxide coatings deposited on leathers to prevent diabetic foot infections. *Surf Coat Technol*, 442, Article 128338. <https://doi.org/10.1016/j.surfcoat.2022.128338>
- Case, C. L., & Johnson, T. R. (1984). *Laboratory experiments in microbiology*. (San Francisco, CA: Benjamin Cummings), 126.
- Chaika, M., Zahorodnya, S., Naumenko, K., Pankivska, Yu., Povnitsa, O., Mukha, Iu., Vityuk, N., Dorovskih, A., Lokshyn, M., Lysenko, V., Lozovski, V., & Rusinchuk, N. (2022). Hide full author list Virus deformation or destruction: size-dependence of antiviral and virucidal activities of gold nanoparticles. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 13 (3), Article 035008. <https://iopscience.iop.org/article/10.1088/2043-6262/ac879a/meta>
- Demchenko, V., Kobylinskyi, S., Iurzhenko, M., Riabov, S., Vashchuk, A., Rybalchenko, N., Zahorodnia, S., Naumenko, K., Demchenko, O., Adamus, G., & Kowalczyk M. (2021). Nanocomposites based on polylactide and

- silver nanoparticles and their antimicrobial and antiviral applications. *React and Funct Polym*, 170, Article105096. <https://doi.org/10.1016/j.reactfunctpolym.2021.105096>
- Demchenko, V., Riabov, S., Kobylinskiy, S., Goncharenko, L., Rybalchenko, N., Kruk, A., Moskalenko, O., & Shut M. (2020). Effect of the type of reducing agents of silver ions in interpolyelectrolyte-metal complexes on the structure, morphology and properties of silver containing nanocomposites. *Sci Rep*, 28, 10(1), 7126. <https://doi.org/10.1038/s41598-020-64079-0>
- Demchenko, V., Riabov, S., Sinelnikov, S., Radchenko, O., Kobylinskiy, S., & Rybalchenko, N. (2020). Novel approach to synthesis of silver nanoparticles in interpolyelectrolyte complexes based on pectin, chitosan, starch and their derivatives. *Carbohydrate Polymers*, 242, Article 116431. <https://doi.org/10.1016/j.carbpol.2020.116431>
- Demchenko, V., Rybalchenko, N., Zahorodnia, S., Naumenko, K., Riabov, S., Kobylinskiy, S., Vashchuk, A., Mamunya, Ye., Iurzhenko, M., Demchenko, O., Adamus, G., & Kowalczyk, M. (2022). Preparation, Characterization, Antimicrobial and Antiviral Properties of Silver-containing Nanocomposites based on Poly(lactic Acid)—Chitosan. *ACS Applied Bio Materials*, 5 (6), 2576—2585. <https://doi.org/10.1021/acsabm.2c00034>
- Demchenko, V., Shtompel, V., Riabov, S., & Lysenkov, E. (2015). Constant electric and magnetic fields effect on the structuring and thermomechanical and thermophysical properties of nanocomposites formed from pectin—Cu²⁺—polyethyleneimine interpolyelectrolyte—metal complexes. *Nanoscale Research Letters*, 10, 479—485. <https://doi.org/10.1186%2Fs11671-015-1181-z>
- Demchenko, V., Mamunya, Ye., Kobylinskiy, S., Riabov, S., Naumenko, K., Zahorodnia, S., Povnitsa, O., Rybalchenko, N., Iurzhenko, M., Adamus, G., & Kowalczyk M. (2022). Structure-Morphology-Antimicrobial and Antiviral Activity Relationship in Silver-Containing Nanocomposites Based on Polylactide. *Molecules*, 27 (12), Article 3769. <https://doi.org/10.3390/molecules27123769>
- Flores-López, L. Z., Espinoza-Gómez, H., & Somanathan, R. (2019). Silver nanoparticles: Electron transfer, reactive oxygen species, oxidative stress, beneficial and toxicological effects. *Mini Rev. J Appl Toxicol*, 39 (1), 16—26. <https://doi.org/10.1002/jat.3654>
- Galchun, A., Korab, N., Kondratenko, V., Demchenko, V., Shadrin, A., Anistratenko, V., & Iurzhenko, M. (2015). Nanostructurization and thermal properties of polyethylenes' weld. *Nanoscale Research Letters*, 10, Article 138. <https://doi.org/10.1186/s11671-015-0832-4>
- Gralinski, L. E., & Menachery, V. D. (2020). Return of the Coronavirus: 2019-nCoV. *Viruses*, 12 (2), Article 135. <https://doi.org/10.3390/v12020135>
- Jung, W. K., Koo, H. C., Kim, K.W., Shin, S., Kim, S. H., & Park, Y. H. (2008). Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. *Appl Environ Microbiol*, 74 (7), 2171–2178. <https://doi.org/10.1128/aem.02001-07>
- Kohn, L. K., Foglio, M. A., Rodrigues, R. A., Sousa, I. M., Martini, M. C., Padilla, M. A., Lima Neto, D. F., & Arns, C.W. (2015). In-Vitro Antiviral Activities of Extracts of Plants of The Brazilian Cerrado against the Avian Metapneumovirus (aMPV). *Brazilian Journal of Poultry Science*, 17(3), 275–280. <http://dx.doi.org/10.1590/1516-635X1703275-280>
- Luceri, A., Francese, R., Lembo, D., Ferraris, M., & Balagna, C. (2023). Silver Nanoparticles: Review of Antiviral Properties, Mechanism of Action and Applications. *Microorganisms*, 11(3), Article 629. <https://doi.org/10.3390/microorganisms11030629>
- Matkovska, L., Iurzhenko, M., Mamunya, Y., Matkovska, O., Demchenko, V., Lebedev, E., Boiteux, G., & Serghei, A. (2014). Electrophysical behavior of ion-conductive organic-inorganic polymer system based on aliphatic epoxy resin and salt of lithium perchlorate. *Nanoscale Research Letters*, 9, Article 674. <https://link.springer.com/article/10.1186/1556-276X-9-674>
- Matkovska, L., Iurzhenko, M., Mamunya, Ye., Tkachenko, I., Demchenko, V., Synyuk, V., Shadrin, A., & Boiteux, G. (2017). Structural Peculiarities of Ion-Conductive Organic-Inorganic Polymer Composites Based on Aliphatic Epoxy Resin and Salt of Lithium Perchlorate. *Nanoscale Research Letters*, 12, Article 423. <https://link.springer.com/article/10.1186/s11671-017-2195-5>
- Pemmada, R., Zhu, X., Dash, M., Zhou, Y., Ramakrishna, S., Peng, X., Thomas, V., Jain, S., & Nanda, H.S. (2020). Science-Based Strategies of Antiviral Coatings with Viricidal Properties for the COVID-19 Like Pandemics. *Materials*, 13(18), Article 4041. <https://doi.org/10.3390/ma13184041>
- Perlman, S. (2020). Another Decade, Another Coronavirus. *N Engl J Med*, 382, 760—762. <https://www.nejm.org/doi/full/10.1056/nejme2001126>
- Sánchez-Salcedo, S., García, A., González Jiménez, A., & Vallet-Regí, M. (2023). Antibacterial effect of 3D printed mesoporous bioactive glass scaffolds doped with metallic silver nanoparticles. *Acta Biomaterialia*, 155, 654–666. <https://doi.org/10.1016/j.actbio.2022.10.045>

- Sarviya, N., Mahanta, U., Dart, A., Giri, J., Deshpande, A., Khandelwal, M., Bhawe, M., & Kingshott, P. (2023). Biocompatible and antimicrobial multilayer fibrous polymeric wound dressing with optimally embedded silver nanoparticles. *Applied Surface Science*, 612, Article 155799. <https://doi.org/10.1016/j.apsusc.2022.155799>
- Sassi, H. P., Sifuentes, L. Y., Koenig, D. W., Nichols, E., Clark-Greuel, J., Wong, L. F., McGrath, K., Gerba, C. P., & Reynolds, K. A. (2015). Control of the spread of viruses in a long-term care facility using hygiene protocols. *Am J Infect Control*, 43(7), 702–706. <https://doi.org/10.1016/j.ajic.2015.03.012>
- Setti, L., Passarini, F., De Gennaro, G., Barbieri, P., Perrone, M. G., Borelli, M., Palmisani, J., Di Gilio, A., Piscitelli, P., & Miani A. (2020). Airborne transmission route of COVID-19: Why 2 meters/6 feet of inter-personal distance could not be enough. *Int J Environ Res Public Health*, 17, 2932. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7215485/>
- Sharma, P., Fialho, L., Figueiredo, N. M., Serra, R., Cavaleiro, A., & Carvalho, S. (2023). Antimicrobial Polymeric Surfaces Using Embedded Silver Nanoparticles. *Antibiotics*, 12 (2), 207. <https://doi.org/10.3390/antibiotics12020207>
- Shrivastava, S., Bera, T., Roy, A., Singh, G., Ramachandrarao, P., & Dash, D. (2007). Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology*, 18 (22), Article 225103. <https://doi.org/10.1088/0957-4484/18/22/225103>
- Tan, R. S. L., Hassandarvish, P., Chee, C. F., Chan, L.W., & Wong, T.W. (2022). Chitosan and its derivatives as polymeric anti-viral therapeutics and potential anti-SARS-CoV-2 nanomedicine. *Carbohydr Polym*, 15, 290, Article 119500. <https://doi.org/10.1016/j.carbpol.2022.119500>
- Tonder, A., Joubert, A. M., & Cromarty, D. (2015). Limitation of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. *BMC Research Notes*, 8, Article 47. <https://doi.org/10.1186/s13104-015-1000-8>
- Wang, H., Yang, B., & Sun, H. (2017). Pectin-Chitosan Polyelectrolyte Complex Nanoparticles for Encapsulation and Controlled Release of Nisin. *American Journal of Polymer Science and Technology*, 3(5), 82-88. <https://doi.org/10.11648/j.ajpst.20170305.11>
- World Health Organization. (2022, December 24). *Coronavirus Disease (COVID-19)*. <https://www.emro.who.int/pandemic-epidemic-diseases/covid-19/covid-19-situation-updates-for-week-51-1824-december-2022.html>
- Yamanaka, M., Hara, K., & Kudo, J. (2005). Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. *Appl Environ Microbiol*, 71 (11), 7589-7593. <https://doi.org/10.1128/aem.71.11.7589-7593.2005>

Received 9.10.2023

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АНТИМІКРОБНА ТА ПРОТИВІРУСНА АКТИВНІСТЬ НАНОКОМПОЗИТІВ НА ОСНОВІ ПОЛІЕЛЕКТРОЛІТНИХ КОМПЛЕКСІВ З НАНОЧАСТИНКАМИ СРІБЛА

Останнім часом нанокompозитні матеріали, що містять наночастинки таких металів, як срібло, мідь та оксид цинку, привертають велику увагу завдяки своїм вираженим фармакологічним властивостям, таким як антимікробна, противірусна, протизапальна, імуномодуюча здатність та висока стабільність в екстремальних умовах. Значний потенціал для створення срібловмісних нанокompозитів мають поліелектролітні

комплекси на основі полімерів природного походження, а саме полісахаридів хітозану та пектину, які здатні стабілізувати наночастинки меншого розміру, ніж індивідуальні полімери. **Метою** статті було дослідити антимікробну та противірусну активність срібловмісних наноконкомпозитів, сформованих на основі поліелектролітних комплексів. **Методи.** Особливості структурної організації срібловмісних наноконкомпозитів досліджували методом ширококутного розсіювання на дифрактометрі XRD-7000. Морфологію наночастинок Ag в полімерних матрицях вивчали методом трансмісійної електронної мікроскопії (трансмісійний електронний мікроскоп JEOL 100 CXII). Антимікробну активність срібловмісних наноконкомпозитів щодо умовно-патогенних мікроорганізмів *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* та *Candida albicans* визначали диско-дифузійним методом. Цитотоксичність та противірусну активність досліджували методом МТТ та за забарвленням генціановим фіолетовим. **Результати.** Аналіз ширококутових рентгенівських дифрактограм срібловмісних наноконкомпозитів на основі поліелектролітних комплексів Na-КМЦ (пектин)-хітозан показав, що при відновленні іонів Ag^+ до металевого срібла на дифрактограмах спостерігаються два низькоінтенсивні дифракційні максимуми при $2\theta_m \sim 38^\circ$ і 44° . Ці максимуми відповідають кристалграфічним площинам гранецентрованої кубічної решітки срібла, характеризуються відповідно індексами (111) і (200) та підтверджують наявність металевого срібла в полімерній системі. Аналіз мікрофотографій срібловмісних наноконкомпозитів на основі Na-КМЦ та хітозану показав, що зі збільшенням молекулярної маси хітозану утворюються наночастинки більшого розміру. Також виявлено залежність розміру наночастинок срібла від довжини хвилі ультрафіолетового випромінювання при відновленні іонів срібла в поліелектроліт-металевих комплексах Na-КМЦ- Ag^+ -хітозан низької молекулярної маси. Зокрема, у зразках, сформованих під опроміненням світлом з коротшою довжиною хвилі ($\lambda = 254$ нм), утворюються дрібніші частинки, ніж при $\lambda = 365$ нм. Срібловмісні наноконкомпозити Na-СМС- Ag -хітозан та пектин цитрусовий- Ag -хітозан, отримані шляхом відновлення іонів Ag^+ під дією ультрафіолетового опромінення з довжиною хвилі 365 та 254 нм, проявляють високу антимікробну активність по відношенню до тест-культур мікроорганізмів *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*. Суттєвої залежності антимікробної активності від молекулярної маси досліджуваних зразків не відмічено, отримані дані знаходяться у вузьких межах і мають близькі значення. Крім того, не виявлено залежності антимікробної активності від виду досліджуваних тест-культур мікроорганізмів. Наноконкомпозити Na-СМС-хітозану- Ag ($\lambda = 365$ нм) інгібували інфекційний титр HSV-1 на (3.72—5.45) IgTCID₅₀/мл, натомість зниження титру при інкубації зі зразками цитрусового пектину-хітозану- Ag було в межах (2.39—2.42) IgTCID₅₀/мл. Спостерігався дозозалежний ефект між молекулярною масою хітозану та зниженням інфекційного титру. Встановлено, що срібловмісні наноконкомпозити, утворені шляхом відновлення іонів срібла в поліелектроліт-металевих комплексах за допомогою ультрафіолетового випромінювання різної довжини хвилі не чинять цитотоксичної дії на клітини MDCK і ВНК. **Висновки.** Досліджені срібловмісні наноконкомпозити на основі поліелектролітних комплексів Na-КМЦ (пектин)-хітозан проявляють антимікробну активність щодо тест-культур *S. aureus*, *E. coli*, *P. aeruginosa* та *C. albicans*, а також противірусну активність щодо вірусу простого герпесу 1 типу та вірусу грипу. Встановлено, що отримані наноконкомпозити не проявляють цитотоксичної дії щодо клітин MDCK та ВНК. Отримані дані дозволяють стверджувати, що досліджені срібловмісні наноконкомпозити є перспективними антимікробними засобами для розробки нових ефективних стратегій боротьби з мікроорганізмами і вірусами та покращення здоров'я населення.

Ключові слова: поліелектролітні комплекси, срібловмісні наноконкомпозити, антимікробна активність, противірусна активність.