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## Hybrid nanocarriers with different densities of silver nanoparticles formation features and antimicrobial properties

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This work presents the synthesis and characterization of silica/polyacrylamide hybrid carriers Hyb1 and Hyb2 containing different amounts and lengths of grafted PAAm chains, as well as the formation mechanism, structure, and antibacterial efficacy of their nanocomposites with silver nanoparticles (AqNPs). The main difference between Hyb1 and Hyb2 carriers, such as the thickness and permeability of the PAAm "corona", is highlighted. Using the methods of potentiometry, UV-Vis spectroscopy, TEM and viscometry, the influence of the hybrid structure and concentration of reagents on the two-stage process of reduction of Aq<sup>+</sup> ions with sodium borohydride in Hyb1-2 aqueous solutions was established. A strong binding of Ag<sup>+</sup> ions to both hybrid matrices at the first stage of reduction and a significant influence of the concentration of Aq-salt (and reducing agent) on the rate of accumulation and yield of AgNPs at the second stage were shown. The presence of two types of AgNPs (internal and external) in the resulting nanocomposites was revealed, resulting from the reduction process both in the internal space of the hybrid "corona" and on its surface. The average size of external AgNPs was larger than internal ones and increased with increasing concentration of Ag-salt (and reducing agent). The role of purification in creating more uniform AgNP/Hyb nanocomposites is demonstrated. High antibacterial effectiveness against S. aureus, E. coli, and P. aeruginosa was established using well diffusion and broth microdilution methods. The obtained MIC values ~ (1.25–2.5)·10<sup>-3</sup> kg/m<sup>3</sup>) are compared to those of potent antibiotics such as ciprofloxacin, ceftriaxone and tetracycline.

Highly dispersed composites of silver nanoparticles (AgNPs) with polymers have many areas of practical application due to the pronounced antimicrobial<sup>1-4</sup>, anticancer<sup>5</sup>, catalytic<sup>6-8</sup>, plasmonic<sup>9</sup>, and luminescent<sup>10-12</sup> properties of nanosilver and the stabilizing effect of polymer matrices. Of significant interest are not only the great functionality of these composites, but also the various methods for their preparation, which are actively discussed and compared in numerous publications<sup>13-19</sup>. Among them, the method of chemical "bottom-up" formation of metal nanoparticles in polymer solutions through in situ reduction of metal precursors (salts or oxides) still retains its rightful place. This is not surprising, since it is one of the simplest, cheapest and most effective methods for controlling the size, shape and polydispersity of nanoparticles, as well as the entire structure of composites. Recent studies have shown that the in situ reduction of Ag-salts with sodium borohydride in polymer solutions retains great advantages over many other methods, as it produces nanoparticles with a smaller size and a narrower size distribution<sup>20-22</sup>. Although the in situ reduction method in polymer solutions has been widely used to create many polymer/silver composites, its actual mechanism, in particular the processes of nucleation, growth and crystallization of metal nanoparticles in various polymer matrices, as well as the role of the nature and structure of the latter, remains poorly understood.

The situation in this field has improved significantly in recent years with the introduction of two new advances in experimental studies of these complex processes, such as in situ SAXS and in situ TEM<sup>23,24</sup>. This initiated the appearance of a series of interesting publications<sup>25–32</sup>, in which well-known concepts of the nucleation and growth of metal nanoparticles (such as the Classical theory of nucleation and growth, the La Mer mechanism,

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Ostwald ripening, coalescence, the two-stage Finke-Wacky mechanism and others<sup>13,28,29</sup>) were revised, clarified and supplemented.

The great potential of the in situ SAXS method was demonstrated in<sup>25</sup> when studying the mechanism of formation of AgNPs in aqueous-ethanol solutions of polyvinylpyrrolidone (PVP,  $M_w = 40$  kDa). Solutions of silver perchlorate (AgClO<sub>4</sub>·H<sub>2</sub>O) of various concentrations (from 5 to 40 mM) were photoinitiated by benzoin under irradiation with an ultra-high pressure mercury lamp with a power of 500 W. The kinetics of AgNP formation by photoreduction was characterized by the time evolution of nanoparticle radius, number, and volume fraction using in situ SAXS, UV–Vis spectroscopy, and TEM techniques. A three-stage process mechanism was established: rapid autocatalytic reduction/nucleation with the formation of small AgNPs (average radius ~ 2.5 nm), followed by dynamic Ostwald ripening and particle coalescence, culminating in the appearance of AgNPs with an average radius of ~ 11.5 nm<sup>25</sup>. At the initial stage (0–10 min) for all samples, the number of AgNPs increased rapidly without a noticeable change in the average radius. The duration of this period and the number of particles formed correlated with the initial concentrations of Ag-salt: the lower the metal concentration (5 mM), the shorter its duration and the greater the number of particles. In addition, at a lower concentration of Ag-salt (5 mM), the appearance of irregularly shaped particles with a diameter of d>40 nm was observed, while at a high concentration of Ag-salt (20 mM), spherical AgNPs with d < 20 nm were found.

In another work<sup>26</sup>, in situ SAXS and in situ UV-Vis spectroscopy were used to study the processes of reduction of AgClO<sub>4</sub> with sodium borohydride in an aqueous medium at a molar ratio  $[NaBH_4]/[AgClO_4] = 6$  and room temperature in two cases: without an additional stabilizer and in the presence of polyvinylpyrrolidone (PVP,  $M_w$  = 58 kDa). The results first of all confirmed the fact of a very high rate of chemical reduction of Ag<sup>+</sup> ions by NaBH<sub>4</sub>. Indeed, the reaction was completed within the first 200-400 ms after mixing the components and resulted in the formation of very small (~1 nm) AgNPs. Two different stages of nanoparticle growth were then identified, both in the absence and in presence of PVP, which were accompanied by a decrease in the number of nanoparticles. The first stage was completed in 5 s and led to the formation of AgNPs with a size of 2-3 nm. The second stage occurred after an intermediate metastable state, the duration of which in the presence of PVP was significantly longer: 30–90 min versus 5–10 min in the system without a stabilizing polymer. The final particle size in systems without a stabilizer and in the presence of PVP was 5-8 and 5-7 nm, respectively. The authors explained the presence of the second stage of AgNP growth, in contrast to a similar process for obtaining gold nanoparticles, by the chemical transformation of the surface of silver nanoparticles due to the hydrolysis of NaBH,. These studies led to the conclusion that the fundamental growth mechanism in the synthesis of metal nanoparticles with a very fast reduction reaction compared to the growth process is coalescence and hence aggregation of nanoparticles<sup>26</sup>.

Once more interesting study was published in<sup>29</sup>. In situ SAXS together with TEM, XRD and UV–Vis spectroscopy was used to monitor the reduction of  $AgNO_3$  by glucose in aqueous solutions of PVP ( $M_{wPVP}$  not specified) at 90 °C. The authors identified a system of AgNPs in the reaction mixture, consisting of two main populations: smaller nanoparticles with an average diameter of ~ 3 nm and larger nanoparticles with d from 10 to 40 nm. The fraction of smaller AgNPs had a nearly constant size distribution throughout the synthesis, while the average particle diameter in the other fraction increased over time. The results showed two modes of nanoparticle growth: a faster one during the first ~ 51 min at a rate of 0.59 nm/min and then a slower one at a rate of 0.26 nm/min. Dynamic coalescence was considered as the main mechanism of nanoparticle growth, which gradually transformed into Ostwald ripening.

A detailed analysis of the situation with understanding and interpretation of the processes of nucleation, growth, crystallization and subsequent self-assembly of metal nanoparticles, starting from aqueous solutions of salts, was presented in<sup>31</sup>. This was based on experimental in situ liquid phase TEM studies of the reduction of gold ions  $AuCl_4^-$  to the  $Au^0$  state under the action of an electron beam (due to the effect of interaction of high-energy electrons with the solution). Of particular interest here was the experimental demonstration of the stage of spinodal decomposition of an initially homogeneous solution into two gold-poor and gold-rich regions. Spinodal decomposition was observed 9.2 s after the start of the experiment and preceded the formation of the first amorphous gold clusters in the gold-rich phase by 11.3 s. Crystallization of amorphous nanoclusters occurred after 15.4 s.

A comprehensive review of studies of the nucleation, growth, and self-assembly of metal nanoparticles in solutions using in situ TEM was given in a recent study<sup>32</sup>. The authors showed that the appearance of intermediates of low symmetry or amorphous structures in the initial nucleation processes followed by crystallization and the formation of larger nanoparticles is a relatively common structural feature of such systems. As additional examples, they provided exploratory data from in situ TEM studies of amorphous phase-mediated crystallization of nickel nanoparticles. Initially, amorphous nickel nanoparticles were formed from the precursor solution, and then crystalline domains nucleated on their surface, which spread throughout the nanoparticle. The possible role of non-classical nucleation in the formation of polycrystalline nanoparticles and the important influence of the chemical environment (ligands and reducing agents) were also indicated. Regarding the growth mechanism of metal nanoparticles, several aspects such as classical monomeric attachment and non-classical particle growth have been discussed based on in situ TEM data. One of the most interesting non-classical mechanisms for the growth of crystalline nanoparticles turned out to be coalescence by oriented addition, in which, before the attachment of two nanoparticles, their crystal planes were aligned<sup>32</sup>.

Unfortunately, the real effect of the polymer matrix as a nanoreactor and regulator of the process of nanoparticle formation, and not just their stabilizer, has not been revealed even in these studies. Complex issues of competitive reactions in polymer solutions during the chemical reduction of metal ions were also not considered. However, it is necessary to clearly understand that in systems: polymer matrix—metal salt—reducing agent, metal ions interact with the active groups of the polymer, that is, we have a case of heterogeneous nucleation<sup>13</sup>. In addition,

depending on the structure of the matrix, the effect of supersaturation on the appearance of primary metal nuclei may be associated with the concentration of the metal salt not in the entire solution, but in limited cavities of the polymer matrix where ions accumulate. A significant role in this case can also be played by the diffusion of reducing agent molecules deep into the matrix to metal ions bound to the polymer. Thus, there are still many unclear aspects to achieve real control over the properties of such systems. Therefore, it is necessary to continue studying the mechanism of formation of metal nanoparticles in polymer solutions from various angles, using all available methods.

For centuries, silver has been known for its antimicrobial activity, but only recently has the biomedical use of silver become a rapidly developing area of research. One of the current trends in biomedical research is the development of silver-based antimicrobial medicine for the treatment of infectious diseases<sup>33</sup>. While soluble ionic forms of silver are highly toxic to cells and their vital biological functions, metallic colloidal silver exhibits much less toxicity and can be used directly in living organisms. In addition, although "naked" silver nanoparticles has low resistance to aggregation and sedimentation due to its high activity, the inclusion of AgNPs in polymer matrices of different chemical compositions provides greater variability in the chemical modification of their surface properties, which facilitates their interaction with microorganisms. Therefore, various polymers, copolymers, dendrimers, polymer complexes, and polymer-inorganic hybrids have been successfully used to stabilize and functionalize AgNPs<sup>21,34-36</sup>.

Polymer-inorganic SiO<sub>2</sub>-g-PAAm hybrids based on a silica "core" and a grafted polyacrylamide "corona" were previously chosen as matrices for the in situ synthesis of AgNPs due to their ability to bind metal ions both by the negatively charged surface of SiO<sub>2</sub> and by the active amide groups of PAAm<sup>21</sup>. An additional interaction of grafted PAAm chains with a chemically complementary SiO<sub>2</sub> surface through a system of hydrogen bonds has also been established<sup>37</sup>, which can be considered as a special factor in regulating the size of metal nanoparticles. These hybrid matrices/nanocarriers with biocompatible and biodegradable components showed high efficiency in the synthesis and long-term stabilization of AgNPs in aqueous solutions, which made it possible to develop new biocidal nanocomposites for wide practical use. In particular, it was shown that these nanocomposites can become the basis of new nanotechnologies for the disinfection of hygienic materials and fishponds, wound healing, pre-sowing treatment of agricultural seeds, as well as extending the shelf life of chicken eggs without losing their sanitary and hygienic properties<sup>21,38–41</sup>.

The number and molecular weight (length) of grafted chains are among the most important factors influencing the structure and properties of hybrids of the inorganic "core"/polymer "corona" type, but their role in the in situ synthesis of metal nanoparticles has not been sufficiently studied. Therefore, the main goals of this work were obtaining SiO<sub>2</sub>-g-PAAm hybrids with different numbers and lengths of PAAm grafts, studying their main parameters and influence on the formation processes of AgNPs and the structure of the resulting nanocomposites, as well as studying their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* as the most studied gram-negative and gram-positive bacterial pathogens.

#### Experimental section Materials

Sample Aerosil A-175 from Orisil (Ukraine) with a specific surface area of  $1.82 \cdot 10^5 \text{ m}^2/\text{kg}$ , Red/Ox initiator cerium (IV) ammonium nitrate (CAN) from Aldrich (USA) and monomer acrylamide (AAm) from Merck (Germany), recrystallized from chloroform, were used to synthesize hydrophilic SiO<sub>2</sub>-g-PAAm hybrids of various structures. The silica sol was prepared by stirring a suspension of Aerosil with a concentration of C=50 kg/m<sup>3</sup> in deionized water for 24 h, followed by double centrifugation at 6000 rpm. The concentration of silica sol in the resulting supernatant was determined by the gravimetric method. To prepare AgNPs in hybrid solutions by the in situ method, silver salt AgNO<sub>3</sub> (analytical grade, Ukraine) and sodium borohydride (NaBH<sub>4</sub>) from Merck (Germany) were used. Deionized water was used as a solvent in all experiments.

#### Synthesis of hybrids with variable number and length of grafted chains

Two samples of hybrids were obtained by free radical graft polymerization of AAm from the surface of a silica sol. The synthesis of SiO<sub>2</sub>-g-PAAm1 (Hyb1) and SiO<sub>2</sub>-g-PAAm2 (Hyb2) was carried out in an inert (argon) atmosphere at T = 20 °C. A constant concentration of silica sol (2.7 kg/m<sup>3</sup>) and the following weight ratios were used:  $[Ce^{IV}]/[SiO_2]=0.4$  and  $[Ce^{IV}]/[AAm]=7.72\cdot10^{-3}$  for Hyb1 and  $[Ce^{IV}]/[SiO_2]=0.2$  and  $[Ce^{IV}]/[AAm]=3.86\cdot10^{-3}$  for Hyb2. The detailed synthesis protocol and purification methods and characterization of the resulting products have been described elsewhere<sup>21</sup>. Thus, an average size of silica sol nanoparticles ( $r_{avSiO2}$ ) and important parameters of the hybrids, such as the number (N) and molecular weight ( $M_{vPAAm}$ ) of grafted PAAm chains, were determined using static light scattering, elemental analysis, dynamic thermogravimetric analysis, and viscometry<sup>21</sup>. The found parameters are collected on the left side of Table 1. Hybrid samples had significantly different numbers and molecular weights of grafts.

| Sample | r <sub>avSiO2</sub><br>nm | M <sub>vPAAm</sub><br>kDa | N  | d <sub>avHyb</sub><br>nm | h <sub>avPAAm</sub><br>nm | σ <sub>OH</sub> - <sub>lim</sub><br>mol/kg | [η]<br>m <sup>3</sup> /kg | k    |
|--------|---------------------------|---------------------------|----|--------------------------|---------------------------|--|---------------------------|------|
| Hyb1   | 7.7                       | 822                       | 72 | $21.0\pm4.7$             | 2.8                       | 1.10                                       | 0.39                      | 0.11 |
| Hyb2   | 7.7                       | 5084                      | 5  | $30.1\pm9.0$             | 7.3                       | 2.29                                       | 0.89                      | 0.25 |

Table 1. Molecular, structural and hydrodynamic parameters of hybrids.

#### Studying the properties of hybrid matrices in aqueous solutions

In this section, the influence of the main parameters of the hybrids on the morphology, size and hydrodynamic volume of their nanoparticles, as well as the surface charge of the  $SiO_2$  "cores" was determined using transmission electron microscopy (TEM), viscometry and potentiometric titration. TEM images of the hybrid particles were obtained using a JEM1230 instrument (JEOL, Japan) operating at an accelerating voltage of 80 kV. Small drops (volume ~  $1 \cdot 10^{-10}$  m<sup>3</sup>) of hybrid solutions in deionized water with C = 1 kg/m<sup>3</sup> were applied to copper grids coated with a Formvar film and carbon; then, they were dried in air for ~ 1-2 min and in a vacuum desiccator for 24 h. Average particle sizes and size distributions were calculated from TEM images using the ImageJ computer program.

The viscosity of hybrid solutions of various concentrations was measured at  $T=25\pm0.1$  °C on an Ostwald viscometer. The outflow time of deionized water was  $\tau_0=94.4$  s. The accuracy of determining the outflow time was  $\pm 0.1$  s. The incubation time of every solution prior to measurement was 10 min. The intrinsic viscosity [ $\eta$ ] of solutions was found by extrapolating the linear parts of the concentration dependences of the reduced viscosity  $\eta_{red} = f(C)$  to C=0 and the Haggins constant k was calculated using the relation<sup>42</sup>:

$$\eta_{red} = [\eta] + k \cdot [\eta]^2 \cdot C_{Hyb}$$

where  $C_{Hyb}$  is the concentration of hybrid matrices.

The surface charge of SiO<sub>2</sub> "cores" was found by potentiometric titration as the number of charged silanol groups in hybrid particles depending on the pH of the solution. For this, aqueous solutions of hybrids (C=1.0 kg/m<sup>3</sup>), as well as pure deionized water, were titrated with 0.2 N NaOH. Titrations were carried out in a thermostated cuvette and an inert (argon) atmosphere at T=25±0.1 °C using a digital 1–160 M Ion meter (Belarus), calibrated with five standard buffer solutions. The pH measurement accuracy was 0.02 units. Each subsequent volume of titrant was added to the cuvette after 2 min to achieve ionic equilibrium. Then, the dependence of the absorption of hydroxyl ions on pH was calculated using the known method<sup>37</sup> and the following relationship:

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

Here  $\sigma_{OH}^{-}$  is the absorption value of hydroxyl ions (mol/kg), C is the titrant concentration (mol/m<sup>3</sup>), g is the mass (kg) of the hybrid in m<sup>3</sup> of solution, pH and pH<sub>0</sub> are the negative logarithms of the concentration of hydroxyl ions in the solution of the hybrid and pure water, respectively.

#### Preparation of silver nanoparticles in hybrid matrices using in situ method

Silver nanoparticles were prepared in hybrid aqueous solutions with pH=5.7-6.4 by in situ reduction of silver nitrate with sodium borohydride. This process can be described by several main and side chemical reactions<sup>43</sup>:

$$\begin{split} 2AgNO_3 + \ 2NaBH_4 &= \ 2Ag + \ H_2 + \ B_2H_6 + \ NaNO_3 \\ B_2H_6 + \ H_2O &= \ 6H_2 + \ 2H_3BO_3 \\ NaBH_4 + \ 2H_2O &= \ NaBO_2 + \ 4H_2 \\ BH_4^- + \ H_3O^+ + \ 2H_2O &= \ H_3BO_3 + \ 4H_2 \end{split}$$

Taking into account the side reactions, in particular the active hydrolysis of NaBH<sub>4</sub> in the acidic pH region with a high exothermic effect<sup>44</sup>, an eight-fold molar excess of NaBH<sub>4</sub> relative to AgNO<sub>3</sub> was used. Three concentrations of hybrid carriers ( $C_{Hyb}$ =0.5, 1.0 and 2.0 kg/m<sup>3</sup>) and three concentrations of Ag-salt ( $C_{AgNO3}$ =0.91·10<sup>-2</sup>, 1.82·10<sup>-2</sup>, and 7.1·10<sup>-2</sup> kg/m<sup>3</sup>) were used to study the formation mechanism of AgNPs/Hyb1-2 nanocomposites. The in situ reduction process was carried out in two stages. At the 1st stage, hybrid solutions were mixed with Ag-salt solutions at T = 20 °C and stored in a dark box for 1 h. Then, in the 2nd step, a reducing agent was added. The appearance of yellow color indicated the formation of AgNPs.

#### Characterization of the formation process of nanosilver

The degree of binding of  $Ag^+$  ions by hybrids at the 1st stage of AgNP formation was determined by the potentiometric method. The amount of unbound  $Ag^+$  ions in Hyb1-2 solutions was measured using a 1–160 M Ion meter (Belarus) with an  $Ag^+$ -selective electrode (Alice-131 Ag) after adding AgNO<sub>3</sub> and separating hybrid particles with bound  $Ag^+$  ions by reprecipitation with ethanol and subsequent centrifugation of the solution. All measurements of electromotive force in supernatants after ethanol removal were carried out at  $T = 25 \pm 0.1$  °C in a 50 cm<sup>3</sup> polypropylene cup (placed in a thermostated glass cuvette) to avoid unwanted adsorption of  $Ag^+$  ions on the glass surface. A silver chloride electrode was used as a reference electrode. The  $Ag^+$ -selective electrode was calibrated using a series of aqueous solutions of  $AgNO_3$  of known concentration, to which a certain mass of KNO<sub>3</sub> was added to maintain a constant ionic strength. The resulting calibration graph is shown in Fig. 1.

The kinetics of nanoparticle formation in hybrid aqueous solutions was monitored by the time change in the position ( $\lambda_{max}$ ) and integrated intensity (S) of the surface plasmon resonance band (SPRB) arising in the visible region of the spectrum. To do this, the NaBH<sub>4</sub> solution was added to the Hyb solution with Ag-salt, the mixture was shaken for ~ 5 s, poured into a measuring cell and inserted into the device. Extinction spectra were recorded every 3 min for 90 min in the range of 200–1000 nm using a Cary 50 Scan UV–Vis spectrometer from Varian



Fig. 1. Calibration plot for determining the concentration of unbound Ag<sup>+</sup> ions in supernatants.

(USA). To determine the integral intensity of SPRB, this band was graphically integrated in the corresponding spectrum using the Origin program, as  $in^{21}$ .

#### Determination of the structure of silver/hybrid nanocomposites

The morphology of AgNPs/Hyb nanocomposites with various concentration (density) of silver nanoparticles was studied using ex situ TEM. Microphotographs of the composites were taken 3–4 days after their preparation. They were then processed according to the method described above. Thus, the average sizes and size distributions of hybrid matrices with AgNPs and separately metal nanoparticles were established. Composites for all biological and individual TEM experiments were first purified from in situ reduction byproducts by re-precipitating the reaction mixtures with ethanol, centrifuging at 4000 rpm for 30 min, and re-dissolving in deionized water.

The structure of AgNPs/Hyb compositions in the bulk state was studied by wide-angle X-ray scattering (WAXS) in a 2-mm cuvette on a DRON-2.0 X-ray diffractometer with a Ni filter in the primary beam<sup>21</sup>. For this purpose, metal/hybrid composites ( $C_{Hyb} = 2.0 \text{ kg/m}^3$  and  $C_{AgNO3} = 3.64 \cdot 10^{-2} \text{ kg/m}^3$ ) were cast from aqueous solutions after in situ synthesis (within 90 min) into special Teflon molds placed in a dark box, after which they were dried in air and in a vacuum desiccator. Monochromatic Cu-Ka radiation with  $\lambda = 0.154$  nm, filtered by Ni, was provided by an IRIS M7 generator at an operating voltage of 30 kV and a current of 30 mA. The scattering intensities were measured by a scintillation detector scanning with a step of 0.2° in the range of scattering angles  $2\theta = 3-45^\circ$ . The diffraction curves were normalized to equal primary beam intensities and equal scattering volumes<sup>21</sup>.

Based on the positions of the diffraction maxima on the WAXS profiles, the average interplane distances in the paracrystalline and crystalline lattices of the amorphous and crystalline regions of the nanocomposites were calculated using the Bragg relationship:

$$d = \frac{\lambda}{2 \cdot \sin \theta}$$

Here  $\lambda$  is the wavelength of X-ray radiation,  $\theta$  is the half of scattering angle.

#### The study of antibacterial properties of nanocomposites

Antibacterial properties of AgNPs/Hyb composites were studied on gram-positive *Staphylococcus aureus* ATCC 6538, gram-negative *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Serratia marcescens* KM-4. Standard quality control strains of *S. aureus*, *E. coli*, and *P. aeruginosa* were obtained from LV Gromashevsky Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine. *S. marcescens* KM-4 was from the collection of the National Taras Shevchenko University of Kiev, Ukraine. The study was conducted by well diffusion and broth microdilution methods commonly applied for in vitro evaluating antimicrobial activity<sup>45</sup>.

To assess the effects of AgNPs/Hyb1 and AgNPs/Hyb2 composites on bacterial growth inhibition, well diffusion method was applied<sup>45</sup>. Two sets of experiment were conducted. First, cell concentration, i.e. the number of colony forming units per ml (CFU/cm<sup>3</sup>) was varied at constant concentration of AgNPs/Hyb by metal ( $C_{AgNPs}$ ). For this purpose, freshly grown bacterial suspensions cultured in LB (Luria Bertani) medium were spread on the nutrient agar plates to reach cell concentrations 1.5·10<sup>4</sup>, 1.5·10<sup>5</sup>, 1.5·10<sup>6</sup>, and 1.5·10<sup>7</sup> CFU/cm<sup>3</sup>. Equal aliquots of AgNPs at concentration of  $5\cdot10^{-3}$  kg/m<sup>3</sup> were applied to each of 6 wells (7 mm holes cut in agar layer). In other series of experiments, aliquots of AgNPs with increasing  $C_{AgNPs}$  were added to each well, while bacteria concentration was kept constant. Plates were incubated for 24 h at 36 °C, under aerobic conditions. After

incubation, confluent bacterial growth and zones of growth inhibition were observed, which diameters  $(D_i)$  were evaluated in mm.

To examine antimicrobial activity of AgNPs/Hyb composites, minimum inhibitory concentrations (MIC) were found by broth microdilution method<sup>45</sup>. Samples with 1:2 serial dilutions were prepared by addition of culture broth to reach concentrations  $C_{AgNPs}$  ranging  $(2.5-0.31)\cdot10^{-3}$  kg/m<sup>3</sup>. Aliquots of each dilution were distributed in 96-well plates, as well as a sterility control (pure AgNPs/Hyb systems) and a growth control (cells without AgNPs). Each dilution test and growth control well was inoculated with an aliquot  $(0.1 \text{ cm}^3)$  of a bacterial suspension  $(10^4 \text{ CFU/cm}^3 \text{ or } 10^5 \text{ CFU/cm}^3)$ . All experiments were performed in triplicate and the microdilution trays were incubated at 36 °C for 18 h. Bacterial growth was detected by optical density (Adsorbance Microplate Reader ELx, BioTek Instruments). MIC values were defined as the lowest concentration of AgNPs, which completely inhibited microbial growth.

Data were presented as mean  $\pm$  SD. To find reliable differences between groups, one-way ANOVA was performed followed by post hoc Tukey test. P < 0.05 was considered to be statistically significant.

#### Results and discussion

#### Parameters of hybrid matrices of various structures in an aqueous medium

The state and size of hybrid particles in aqueous solutions can be observed in the TEM images shown in Fig. 2a– d. The general picture is similar to that described in our previous studies for other samples of these hybrids<sup>21,40,41</sup>. In particular, both individual small hybrid particles and their aggregates can be identified in these images. The shape of individual hybrid particles was close to spherical, and their aggregates resembled various bunches of grapes. Strong aggregation of hybrid particles in an aqueous medium was apparently due to the interaction between amide groups of the PAAm "coronas" via hydrogen bonds.

The ImageJ computer program was used to determine the average diameter  $(d_{avHyb})$  of individual hybrid particles and their size distribution, as well as to calculate the average height (thickness) of the PAAm "corona":  $h_{avPAAm} = d_{avHyb}/2 - r_{avSiO2}$ . According to the results obtained (insets in Fig. 2 and central part of Table 1),



**Fig. 2.** Lower and higher magnification TEM images obtained using aqueous solutions of (**a**,**b**) Hyb1 and (**c**,**d**) Hyb2, and the corresponding particle size distributions as insets.  $C_{Hyb} = 1.0 \text{ kg/m}^3$ ; T = 20 °C.

individual Hyb1 particles, in contrast to Hyb2 particles, had a smaller size, a narrower size distribution and a thinner polymer "corona".

It was previously shown that in silica/polyacrylamide hybrids, grafted PAAm chains additionally interacted with the surface of the inorganic "core" via hydrogen bonds, forming a dense hydrophilic polymer layer<sup>21</sup>. This interaction should influence the surface charge of SiO<sub>2</sub> nanoparticles due to the participation of their surface groups  $\equiv$ Si–OH simultaneously in two equilibria, such as dissociation and hydrogen bonding:

$$s_i$$
  $OH \neq s_i$   $O^- + H^+$ 

$$\begin{array}{c} \text{Si} \xrightarrow{\text{OH}} \text{OH} + \underset{H}{\overset{\text{O}}{\overset{\text{Si}}{\xrightarrow{\text{OH}}}} C \xrightarrow{i} \not \rightleftharpoons \begin{array}{c} \text{Si} \xrightarrow{\text{OH}} \text{OH} \cdots \text{O} \\ \text{Si} \xrightarrow{\text{OH}} \text{OH} \xrightarrow{i} \begin{array}{c} \text{OH} \\ \text{Si} \xrightarrow{\text{OH}} \\ H \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \\ \text{H} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \\ \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \\ \text{OH} \\ \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \\ \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \\ \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \\ \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{c} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \begin{array}{i} \text{OH} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \end{array} \xrightarrow{i} \end{array}$$

In this regard, it was of interest to determine and compare the total number of  $\equiv$ Si-OH groups in the hybrids that participated in the dissociation equilibrium and created a negative surface charge. The results of potentiometric titration of aqueous solutions of Hyb1-2 with NaOH are presented in Fig. 3a in the form of dependences of the absorption value of hydroxyl ions on pH.

The value of  $\sigma_{OH^-}$  at a certain pH corresponded to the number of charged silanol groups on the surface of silica nanoparticles in the structures of Hyb1 (Fig. 3a, curve 1) and Hyb2 (Fig. 3a, curve 2). Noticeable charging of silica "cores" in hybrids Hyb1 and Hyb2 began at pH > 10 and pH > 9, respectively, and ended at pH slightly above and below 11. The limiting values of  $\sigma_{OH^-lim}$ , corresponding to the total numbers of surface groups  $\equiv$ Si-OH of both hybrids and found from these absorption curves are given in Table 1. The smaller value of  $\sigma_{OH^-lim}$  in Table 1 was typical for the Hyb1 sample with a larger number of grafts and a shorter length. This indicated a stronger interaction of the grafted PAAm chains with the inorganic "core" and with each other in Hyb1 particles and a more dense structure of the polymer layer. A decrease in the number of grafts along with an increase in their length in Hyb2 led to a weakening of the interaction between the PAAm chains and the surface of the "core" and an increase in the permeability of the grafted polymer layer. This conclusion was confirmed by the higher value of  $\sigma_{OH^-lim}$  for Hyb2 in Table 1, which reflected the greater number of  $\equiv$ SiOH groups on the surface of its "core".

Viscometry studies of hybrid aqueous solutions over a wide range of their concentrations are shown in Fig. 3b. As can be seen, in the region  $C_{Hyb} = 0.1-0.2 \text{ kg/m}^3$  the dependences  $\eta_{red} = f(C_{Hyb})$  were linear for both hybrids and indicated a slight increase in reduced viscosity with concentration. At lower concentrations, both dependences initially exhibited a small polyelectrolyte effect in the form of a weak maximum at C=0.5 kg/m<sup>3</sup>, apparently due to the presence of individual hydrolyzed amide groups on the PAAm chains. However, at  $C_{Hyb} < 0.5 \text{ kr} \cdot \text{m}^{-3}$  the value of  $\eta_{red}$  decreased. This indicated an increase in the interaction of PAAm chains with the SiO<sub>2</sub> surface in the hybrid particles, exceeding the effect of swelling of the polyelectrolyte due to the electrostatic repulsion of some  $-COO^-$  groups.

The intrinsic viscosity  $[\eta]$  of hybrid solutions and the Huggins constant k are presented on the right side of Table 1. It should be noted that the  $[\eta]$  values of hybrid solutions in water were low, which was not consistent with the large sizes of their aggregates recorded by TEM (Fig. 2). This discrepancy could only be explained by



**Fig. 3**. (a) Absorption curves of hydroxyl ions and (b) concentration dependences of the reduced viscosity obtained in aqueous solutions of Hyb1 -1 and Hyb2 -2.  $C_{Hyb} = 1.0 \text{ kg/m}^3$ ; T = 25 °C.

the labile nature of such structures, which were easily destroyed in the capillary of the viscometer under the influence of a hydrodynamic shear field. This meant that the parameters  $[\eta]$  and k in Table 1 related to individual hybrid particles and not to their aggregates. A comparison of these parameters for two hybrid samples showed: (i) a significantly larger hydrodynamic volume of Hyb2 particles in solution compared to Hyb1 particles and (ii) relatively good water quality as a solvent for hybrid particles (k < 0.3 in Table 1).

#### Influence of hybrid structure and reagent concentrations on synthesis of silver nanoparticles

In this part, the main attention was paid to the kinetic aspects of the formation of AgNPs and the yield of metal nanoparticles depending on the number and length of grafted PAAm chains, as well as the concentrations of Ag-salt (and reducing agent) and hybrid matrices. The kinetic features of the formation of AgNPs in solutions of various polymer matrices are relatively poorly covered in the literature. Figure 4 shows the time evolution of extinction spectra recorded in the UV–Vis region for aqueous mixtures of Hyb1-2 with silver nitrate after the addition of NaBH<sub>4</sub>. In these experiments, three concentrations of each hybrid and one lowest concentration of Ag-salt (0.91·10<sup>-2</sup> kg/m<sup>3</sup>) were used. The key parameters for further analysis were the position ( $\lambda_{max}$ ) and integrated intensity (S) of SPRBs in the UV–Vis spectra. The first parameter is determined by the size of the AgNPs<sup>21,46</sup>, whereas the second in the case of very small silver nanoparticles (d<sub>AgNPs</sub> < 30 nm) depends on the number of nanoparticles, but not on their size<sup>21</sup>. The latter became the basis for assessing the yield of AgNPs under specific reaction conditions as the value of the integral intensity of SPRB achieved in a certain time (in our systems in 60 min).

Looking at these figures, several interesting conclusions can be drawn. The formation of AgNPs in all reaction mixtures was confirmed by the appearance of weak SPRBs in the UV–Vis spectra, the position of which ( $\lambda_{max}$ ) was about 400 nm. However, the yield of nanoparticles after 60 min was relatively small. The parameters  $\lambda_{max}$  and S for SPRB in most cases increased over time to some almost constant values. The yield of metal nanoparticles was higher at lower concentrations of hybrid matrices, such as  $C_{Hyb} = 0.5 \text{ kg/m}^3$ . The reason for this may be a slight polyelectrolyte swelling of the hybrid particles at this concentration (Fig. 3b). Finally, the yield of AgNPs in Hyb2 solutions was lower than that in Hyb1 solutions. These effects appeared more clearly in Fig. 5. It should be noted here that most of the time dependences of  $\lambda_{max}$  and S contained a short induction period, during which the position of SPRB changed little (Fig. 5a,c), and the yield of silver nanoparticles increased only slightly over time. This period apparently corresponded mainly to the nucleation of primary small AgNPs. The subsequent increase in S was accompanied by a sharp increase in  $\lambda_{max}$ , which can be attributed to a sharp increase in the yield and size of nanoparticles. The described changes were completed within the first 10–15 min.

According to the kinetic curves S = f(t) in Fig. 5b,d, the main kinetic parameters of the reduction process were additionally calculated, such as the induction period ( $\tau_0$ ), the maximum accumulation rate of AgNPs ( $v_{max}$ ) and the yield of nanoparticles after 60 min ( $S_{60}$ ). They showed (Table 2) that the rate of AgNP formation and the



**Fig. 4.** Time evolution of extinction spectra in aqueous mixtures of  $(\mathbf{a}-\mathbf{c})$  Hyb1 and  $(\mathbf{d}-\mathbf{f})$  Hyb2 with AgNO<sub>3</sub> through 3 -1, 6 -2, 9 -3, 21 -4, 36 -5, 60 -6, 72 -7 and 90 min -8 after the introduction of NaBH<sub>4</sub>. C<sub>Hyb</sub> = 0.5 (**a,d**), 1.0 (**b,e**) and 2.0 kg/m<sup>3</sup> (**c,f**); C<sub>AgNO3</sub> = 0.9 · 10<sup>-2</sup> kg/m<sup>3</sup>; T = 20 °C.



**Fig. 5.** Time changes in the position  $(\lambda_{max})$  and integrated intensity (S) of the SPRB of AgNPs in the UV–Vis spectra of (**a**,**b**) Hyb1 and (**c**,**d**) Hyb2 solutions of different concentrations.  $C_{AgNO3} = 0.9 \cdot 10^{-2} \text{ kg/m}^3$ .

| Sample | C <sub>Hyb</sub> , kg/m <sup>3</sup> | $C_{AgNO3}$ ·10 <sup>2</sup> , kg/m <sup>3</sup> | $\tau_0^{}$ , min | $v_{max} \cdot 10^2$ , nm/s | S <sub>60</sub> , nm |
|--------|--------------------------------------|--|-------------------|-----------------------------|----------------------|
|        | 0.5                                  |  | 3.4               | 7.5                         | 25.3                 |
|        | 1.0                                  | 0.9  | 0.6               | 6.8                         | 11.1                 |
| Hyb1   | 2.0                                  |  | 0                 | 5.0                         | 15.8                 |
| 11901  | 0.5                                  |  | 1.6               | 14.7                        | 89.1                 |
|        | 1.0                                  | 1.8  | 1.2               | 19.2                        | 83.2                 |
|        | 2.0                                  |  | 0                 | 17.3                        | 79.5                 |
|        | 0.5                                  |  | 3.3               | 3.8                         | 12.2                 |
|        | 1.0                                  | 0.9  | 0.6               | 2.0                         | 6.5                  |
| Hyb2   | 2.0                                  |  | 1.4               | 5.0                         | 5.6                  |
| 11902  | 0.5                                  |  | 3.3               | 16.2                        | 84.5                 |
|        | 1.0                                  | 1.8  | 7.1               | 17.0                        | 79.6                 |
|        | 2.0                                  |  | 3.1               | 16.0                        | 84.9                 |



yield of nanoparticles after 60 min were noticeably higher in Hyb1 solutions, especially at low concentrations of this hybrid.

The situation in the reaction mixtures changed dramatically when the initial concentration of Ag-salt (and reducing agent) was doubled. Indeed, at all Hyb1-2 concentrations studied, the appearance of intense SPRBs in the UV–Vis spectra was observed (Fig. 6). The integrated intensity of SPRB, reflecting the yield of metal nanoparticles, rapidly increased with time up to a certain limiting value. In this case, the position of this band sharply decreased during the first 10–15 min, in contrast to the behavior of the same reaction mixtures with half the concentration of the original Ag-salt and reducing agent (Figs. 4, 5).

All noted differences are clearly visible in Fig. 7 compared to Fig. 5. Kinetic parameters of AgNP formation, calculated from Fig. 7b,d and collected in Table 2, provided additional information about the important role of



**Fig. 6.** Time evolution of extinction spectra in aqueous mixtures of  $(\mathbf{a}-\mathbf{c})$  Hyb1 and  $(\mathbf{d}-\mathbf{f})$  Hyb2 with AgNO<sub>3</sub> through 3 -1, 6 -2, 9 -3, 21 -4, 36 -5, 60 -6, 72 -7 and 90 min -8 after the introduction of NaBH<sub>4</sub>. C<sub>Hyb</sub> = 0.5 (**a**,**d**), 1.0 (**b**,**e**) and 2.0 kg/m<sup>3</sup> (**c**,**f**); C<sub>AgNO3</sub> = 1.8  $\cdot 10^{-2}$  kg/m<sup>3</sup>; T = 20 °C.

the initial Ag-salt (and NaBH<sub>4</sub>) concentration. In particular, doubling the Ag-salt concentration led to a sharp increase in the rate of accumulation of nanoparticles: in Hyb1 solutions by 2.0–3.5 times, in Hyb2 solutions by 3.2–8.5 times. At the same time, the yield of nanoparticles increased by 3.5–7.5 times in the case of Hyb1 and by 6.9–15.2 times in the case of Hyb2. As a result, at such a high concentration of Ag-salt, the influence of the structure and concentration of hybrid matrices was practically not manifested (Table 2).

#### Mechanism of formation of silver nanoparticles in hybrid matrices

To gain a more detailed understanding of the processes occurring in hybrid solutions at each stage of Ag-salt reduction, several additional studies were carried out. As noted earlier<sup>21</sup>, the addition of Ag-salt to hybrid solutions led to the complex formation of Ag<sup>+</sup>-ions with the primary amide groups of the PAAm "corona" and, probably, with charged  $\equiv$ SiO<sup>-</sup> groups on the surface of the silica "core". In this regard, the first important question was how completely did Ag<sup>+</sup> ions bind to the hybrid particles in the 1st stage of the reduction process? This information was obtained by potentiometric method using an Ag-selective electrode and a special protocol described in the Experimental section. Here, a constant rather high concentration (1.82·10<sup>-2</sup> kg/m<sup>3</sup>) of Ag-salt and a variable concentration of hybrid matrices (C<sub>Hyb</sub>) were used. The results of these experiments are summarized in Table 3.

A very high degree of binding ( $X_{Ag+}$ ) of silver ions to hybrid particles, close to 100% (within the experimental error), was found for all studied molar ratios ( $n_{AgNO3}/n_{PAAm}$ ) between the Ag-salt and PAAm units of the hybrids, regardless of their different structures. This fact was not unexpected, taking into account: (i) the formation of stable monodentate complexes of Ag<sup>+</sup> ions with primary amide groups<sup>47</sup> and (ii) the low relative amount of Ag<sup>+</sup> ions per PAAm unit in the initial reaction mixtures with hybrids (Table 3).

The next two important questions were: what changes in the state of individual hybrid particles occurred during interaction with  $Ag^+$  ions and did this cause the destruction of particle aggregates already at the 1st stage of the reduction process? To clarify this aspect, TEM studies were carried out on a solution of one of the hybrids before and after the addition of silver nitrate. No changes in the aggregated state of Hyb1 particles under the influence of interaction with  $Ag^+$  ions were recorded. However, in this case, compaction of individual hybrid particles occurred in the aggregates. This conclusion was based on calculations of the average diameters and size distributions of individual hybrid particles in two similar aggregates identified in TEM images of the pure hybrid and its mixture with Ag-salt (Fig. 8a,b). A decrease in the  $d_{av}$  value for individual hybrid particles from  $22 \pm 4$  nm to  $16 \pm 3$  nm due to interaction with  $Ag^+$  ions and characteristic changes in the size distribution, shown in the insets of Fig. 8a,b, confirmed this assumption. Thus, it became clear that the processes of swelling and disaggregation of hybrid particles during the synthesis of silver nanoparticles, which were noticed in our previous works<sup>21,38,41</sup>, developed only at the 2nd stage of the reduction reaction due to the formation of AgNPs.

It should be clearly understood that the hydrogen bonding of PAAm with the  $SiO_2$  surface, the complexation of Ag<sup>+</sup> ions with the active groups of the hybrids, the reduction of ions to nanoparticles by NaBH<sub>4</sub> additives, and, finally, the hydrolysis of the reducing agent are four competing reactions. Therefore, their development





| Sample | C <sub>Hyb</sub><br>kg/m <sup>3</sup> | C <sub>AgNQ3</sub> ·10 <sup>2</sup><br>kg/m <sup>3</sup> (initial) | n <sub>AgNO3</sub> /n <sub>PAAm</sub> ·10 <sup>2</sup><br>mol/base-mol | C <sub>AgNQ3</sub> ·10 <sup>4</sup><br>kg/m <sup>3</sup> (supernatant) | ${f X}_{Ag^+}$ |
|--------|---------------------------------------|--|--|--|----------------|
| Hyb1   | 1.0<br>2.0                            | 1.82   | 1.04<br>0.52   | 4.0<br>2.9   | 97.8<br>98.4   |
| Llub 2 | 1.0                                   | 1.92   | 0.96   | 4.3  | 97.6           |
| 11902  | 2.0                                   | 1.02   | 0.48   | 4.2  | 97.7           |

Table 3. The degree of binding of Ag<sup>+</sup> ions by hybrids at the 1-st stage of the reduction process.

(speed and efficiency) in one direction or another is determined by the total and relative concentrations of the Ag-salt, reducing agent and hybrids, as well as the structure of the hybrid matrices. It has been shown that the Ag-salt concentration is one of the key factors affecting the borohydride reduction of Ag<sup>+</sup> ions in Hyb solutions. It determined not only the rate of AgNP accumulation and the yield of nanoparticles, but also the positive or negative shift of the SPRB position ( $\lambda_{max}$ ) along the wavelength axis in the first minutes of the reduction reaction. The last interesting feature requires a separate discussion.

A clear relationship between the position of SPRB and the size of the resulting AgNPs has been established and substantiated in many studies<sup>46</sup>. In accordance with it, the positive shift  $\lambda_{max}$  along the wavelength axis, observed at a lower concentration of Ag-salt (Fig. 5a,c), can be interpreted as an increase in the size of primary nanoparticles with a disordered atomic structure and/or their aggregation. However, the negative shift  $\lambda_{max}$ turned out to be more interesting, as was shown in this work (Fig. 7a,c) and our previous studies<sup>21,40,41</sup>. A decrease in  $\lambda_{max}$  was observed at a high concentration of Ag-salt, in particular 1.82·10<sup>-2</sup> kg/m<sup>3</sup>, and was explained by the ordering or crystallization of primary AgNPs during their rapid accumulation in polymer matrices. The ordering or crystallization of metal nanoparticles led to compaction of the particle structure and a decrease in their size (and  $\lambda_{max}$  value). The presence of the opposite effect at half the concentration of Ag-salt (Fig. 6a,c)



Fig. 8. TEM images of Hyb1 aggregates (a) in the initial aqueous solution and (b) in the same solution 1 h after adding AgNO<sub>3</sub>. The size distributions of individual hybrid particles in the corresponding aggregates are shown as insets.  $C_{Hyb} = 1.0 \text{ kg/m}^3$ ;  $C_{AgNO3} = 1.82 \cdot 10^{-2} \text{ kg/m}^3$ ; T = 20 °C.

suggested that the number of primary AgNPs formed in cavities of hybrid matrices was not enough to form highly ordered (or crystalline) metal nanoparticles. However, due to their high activity, primary AgNPs could combine (coalesce) inside hybrid matrices, forming amorphous metal nanoparticles<sup>30</sup>, which was expressed in

an increase in  $\lambda_{max}$ . The structural features of Hyb1-2 particles in aqueous solutions played a significant role in the reduction process only at a low concentration of Ag-salt, such as  $0.91 \cdot 10^{-2}$  kg/m<sup>3</sup> (Table 3). In this case, the lower rate of formation and lower yield of AgNPs in Hyb2 solutions at all hybrid concentrations studied can be explained by the deeper penetration of Ag<sup>+</sup> ions into the larger and looser "corona" of these hybrid particles at the 1st stage of the reduction process. As a result, at the 2nd stage, the diffusion time of the reducing agent to the bound Ag<sup>+</sup> ions and the competition between the complexation and reduction reactions increased. Obviously, the higher viscosity of the Hyb2 solution compared to Hyb1 (Fig. 3b) also facilitated this. However, the structure factor stopped working at high concentration of Ag-salt (and reducing agent).

Similar kinetic studies of AgNP formation in Hib1-2 solutions were not possible at the maximum Ag-salt concentration  $(7.1 \cdot 10^{-2} \text{ kg/m}^3)$  due to the very high intensity of the corresponding SPRBs in the UV–Vis spectra. Therefore, this Ag-salt concentration was only used to prepare AgNP composites with Hyb1 and Hyb2 for selected TEM and biological studies.

**Structural features of hybrid composites with different densities of silver nanoparticles** In our previous works<sup>21,38–41</sup>, TEM studies of AgNPs/Hyb composites were not systematic and were carried out mainly after purification of the composites from excess reagents and reduction byproducts, as described in the Experimental section. Therefore, the following detailed information on the actual synthesis products in Hyb1 and Hyb2 solutions with different concentrations of Ag-salt (and reducing agent) before and after purification of the composites is presented for the first time.

Data in Fig. 9 and Table 4 reflected the real situation in the AgNPs/Hyb1 system after syntheses. These TEM images clearly demonstrated the presence of three type of particles. The first of them were predominantly individual hybrid particles of spherical or ellipsoidal shape, the average size of which increased with increasing Ag-salt concentration because of swelling. The second were larger silver nanoparticles formed on the surface of the hybrid matrices, some of which, as a result of growth, may have been detached from the matrices and found themselves in the bulk of the solution (they were called external AgNPs). Still others were tiny spherical nanoparticles embedded in hybrid matrices (called internal AgNPs). Interestingly, the average size of both types of AgNPs increased with increasing Ag-salt concentration (Table 4). At the lowest concentration of Ag-salt (and NaBH<sub>4</sub>), the AgNPs/Hyb1 composite contained small metal nanoparticles formed predominantly on the surface of the hybrid matrices (Fig. 9a,b). The absence of noticeable swelling of Hyb1 particles in this system (Table 4) compared to their initial state (Table 1) confirmed this conclusion.

A different picture was observed in the same system with a twofold increase in the concentration of Ag-salt and reducing agent (Fig. 9c,d; Table 4). The matrix particles turned out to be highly swollen and included many small internal AgNPs. At the same time, the system also contained many external AgNPs, which apparently formed on the surface of the matrix particles. The average size of external metal particles was significantly larger than internal ones (Table 4). A further increase in the Ag-salt concentration was accompanied by maximum swelling of Hyb1 particles, so that the grafted PAAm chains were completely extended deep into the solution from the "core" and resembled stars (Fig. 9e,f). In addition, the relative amount and size of external AgNPs also increased in the system (Table 4).



**Fig. 9.** TEM images with different magnifications of the uncleaned AgNPs/Hyb1 composites obtained at  $C_{AgNO3} = 0.9 \cdot 10^{-2}$  (**a**,**b**),  $1.8 \cdot 10^{-2}$  (**c**,**d**) and 7.1 kg/m<sup>3</sup> (**e**,**f**). Size distributions calculated separately for hybrid particles -1, external AgNPs -2 and internal AgNPs -3 (see text) are shown as insets.  $C_{Hyb} = 1.0 \text{ kg/m}^3$ ; T = 20 °C.

A slightly different situation was recorded in TEM images after the synthesis of AgNPs in Hyb2 solutions (Fig. 10, Table 4), which was associated with the higher permeability of the grafted "corona" of PAAm in these hybrid matrices, as discussed above.

hybrid matrices, as discussed above. Indeed, at  $C_{A_{gNO3}} = 0.91 \cdot 10^{-2} \text{ kg/m}^3$ , the AgNPs/Hyb2 composite contained predominantly small internal AgNPs embedded in hybrid particles (Fig. 10a,b). In this case, external AgNPs were practically not observed in

|   |     | Average diameter of nanoparticles ( $d_{av} \pm SD$ ), nm |                |                |             |  |  |
|---|-----|---|----------------|----------------|-------------|--|--|
| Composite C <sub>AgNO3</sub> ·10 <sup>2</sup> kg/m <sup>3</sup> |     | Hybrid particles  | External AgNPs | Internal AgNPs | Final AgNPs |  |  |
|   | 0.9 | $19.2 \pm 5.3$  | $1.4 \pm 0.8$  | -              |             |  |  |
| AgNPs/Hyb1  | 1.8 | $40.2 \pm 10.7$   | $6.2 \pm 2.4$  | $1.5 \pm 0.7$  | $6.1\pm2.8$ |  |  |
|   | 7.1 | 57.8±21.6   | 7.2±2.7        | $2.7 \pm 1.0$  | 1           |  |  |
|   | 0.9 | 31.8±9.8  | -              | $1.4 \pm 0.5$  |             |  |  |
| AgNPs/Hyb2  | 1.8 | $27.2 \pm 10.1$   | 3.4±2.5        | $1.0 \pm 0.4$  | 6.2±2.7     |  |  |
|   | 7.1 | 29.9±10.9   | 8.3±2.8        | $1.1 \pm 0.4$  | ]           |  |  |

Table 4. Type and size of nanoparticles in silver/hybrids composites.

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TEM images. The next difference was the virtual absence of the effect of swelling of Hyb2 particles during the formation of AgNPs at all studied concentrations of Ag-salt (Table 4). However, external AgNPs formed on the surface of Hyb2 particles also appeared in this system at  $C_{AgNO3} \ge 1.82 \cdot 10^{-2} \text{ kg/m}^3$  (Fig. 10c–f). The average size and relative number of these AgNPs also increased with increasing Ag-salt concentration, as in the AgNPs/Hyb1 system (Fig. 10, Table 4).

The results showed that the initial binding of  $Ag^+$  ions by Hyb1-2 matrices (1st stage of AgNP formation) and their subsequent reduction by NaBH<sub>4</sub> (2nd stage) occurred both in the internal space of the "corona" of the hybrids and on its surface. The relationship between both processes depended, among other things, on the structure of the polymer layer. When the PAAm "corona" was denser and thinner, as in the Hyb1 sample, which had a significantly higher number of grafts and shorter their lengths, both stages of in situ synthesis developed more actively on the surface of the "corona". However, when the polymer "corona" was thicker and had significant permeability, as in Hyb2 matrices, the formation of many smaller AgNPs deep in the "corona" could occur even without its swelling.

In this regard, it was of interest to monitor the final purified products of in situ synthesis in Hyb1-2 solutions at all Ag-salt (and reducing agent) concentrations studied. Unfortunately, this proved difficult due to incomplete precipitation of the composites by ethanol when using low concentrations of Ag-salt (including  $C_{AgNO3} = 1.82 \cdot 10^{-2} \text{ kg/m}^3$ ) and the centrifugation procedure at 4000 rpm. Therefore, this operation was carried out only for AgNPs/Hyb1-2 composites at a maximum concentration of  $C_{AgNO3} = 7.1 \cdot 10^{-2} \text{ kg/m}^3$ , which were subsequently used in biological experiments. TEM images of the resulting purified composites are shown in Fig. 11.

As can be seen, both composites became more homogeneous after their precipitation by ethanol, centrifugation and following re-dissolution in deionized water. They contained swollen particles of Hyb1 and Hyb2, which created a network of entanglements that stabilized AgNPs. It should be noted the complete (quantitative) precipitation of metal nanoparticles with hybrids at the composite cleaning. This fact was confirmed by the absence of AgNPs in both supernatants that was established by UV–Vis spectroscopy. The average diameter of final AgNPs was slightly higher in the composite with Hyb2 (Table 4).

It should be noted that both obtained nanocomposites retained their stability over time in a dark box for at least six months. Special studies of this aspect for a similar AgNPs/Hyb nanocomposite were published earlier<sup>41</sup>. However, this fact contradicted the known data on the possible reversibility of the reduction of AgNO<sub>3</sub> using NaBH<sub>4</sub>, leading to the dissolution of metal nanoparticles<sup>48</sup>. In this regard, the high stability of AgNPs/Hyb composites over time was explained by: (i) the high stabilizing ability of the studied polymer/inorganic matrices to silver nanoparticles, (ii) rapid ordering/crystallization of the resulting primary AgNPs (at a sufficiently high concentration of the initial Ag salt) and (iii) purification of the nanocomposites from excess reagents and by-products. The last factor, namely the development of a simple, fast, eco-friendly and efficient method for purifying AgNPs/Hyb nanocomposites immediately after synthesis (by ethanol reprecipitation), also opened up the prospect of their use in biomedicine<sup>21,38-41</sup>.

#### Composites of hybrid carriers with silver nanoparticles in a bulk state

The structure of AgNPs/Hyb1-2 composites in dry state was characterized by wide-angle X-ray scattering (WAXS). Figure 12 shows two experimental and smoothed WAXS diffraction patterns.

The bulk structure of the composites contained: amorphous regions of hybrids, which appeared as three diffuse overlapping maxima ("halos") at  $2\theta \sim 15^\circ$ ,  $21^\circ$  and  $29^\circ$ , and crystalline regions of AgNPs, which were confirmed by characteristic crystalline peaks of silver {111} at  $2\theta \sim 38^{\circ 49}$ . The appearance of the last peaks indicated the formation of crystalline AgNPs with a tetragonal face-centered cubic lattice<sup>49</sup>. Three diffuse overlapping maxima in Fig. 12 can be explained by the presence of three systems of paracrystalline lattice planes in the amorphous regions of nanocomposites containing predominantly PAAm chains. Amorphous maxima at  $2\theta \sim 23^\circ$ , characteristic of SiO<sub>2</sub> nanoparticles<sup>50</sup>, did not appear in the diffraction patterns of Fig. 12 due to the low mass fraction of SiO<sub>2</sub> in Hyb1 and Hyb2 (9.5 and 7.2 wt. %, respectively). The first maximum with lower intensity at  $2\theta_1 \sim 15^\circ$  (Fig. 12) characterized the lateral periodicity in the arrangement of PAAm chains<sup>21,51</sup>. The second maximum with highest intensity at  $2\theta_2 \sim 21^\circ$  reflected the periodic arrangement of planar hydrogenbonded *cis*-dimers of amide groups in the structures of *cis-trans*-multimers<sup>21,51</sup>. The third maximum at  $2\theta_3 \sim 29^\circ$  was also observed in WAXS profile of pure PAAm in<sup>52</sup> (at  $2\theta = 27.4^\circ$ ), but was not discussed. Table 5 shows more accurate positions of the diffraction maxima and average interplane distances (d<sub>1</sub>-d<sub>4</sub>) calculated using the Bragg ratio in the paracrystalline lattice of the amorphous regions of PAAm and the crystal lattice of AgNP.



**Fig. 10**. TEM images with different magnifications of the uncleaned AgNPs/Hyb2 composites formed at  $C_{AgNO3} = 0.9 \cdot 10^{-2}$  (**a**,**b**),  $1.8 \cdot 10^{-2}$  (**c**,**d**) and 7.1 kg/m<sup>3</sup> (**e**,**f**). Corresponding size distributions calculated for hybrid particles -1, external AgNPs -2 and internal AgNPs -3 (see text) are represented as insets.  $C_{Hyb} = 1.0$  kg/m<sup>3</sup>; T = 20 °C.

As can be seen, the parameters of the paracrystalline and crystalline lattices in the amorphous and crystalline regions of both nanocomposites are close (Table 5). At the same time, the relative contribution of the second diffraction maximum to the overall WAXS profile is smaller in the case of the AgNPs/Hyb2 nanocomposite (Fig. 12b). This indicates a looser structure of the amorphous regions of PAAm in this composite.



**Fig. 11.** TEM micrographs of (**a**,**b**) AgNPs/Hyb1 and (**c**,**d**) AgNPs/Hyb2 composites created at  $C_{AgNO3} = 7.1 \cdot 10^{-2} \text{ kg/m}^3$  and purified from reaction byproducts. The insets show the size distributions of AgNPs in the final composites.  $C_{Hyb} = 1.0 \text{ kg/m}^3$ ; T = 20 °C.



**Fig. 12.** Wide-angle X-ray scattering intensity as a function of scattering angle for (**a**) AgNPs/Hyb1 and (**b**) AgNPs/Hyb2 nanocomposites. Smoothed dependencies are shown as insets.  $C_{AgNO3} = 3.64 \cdot 10^{-2} \text{ kg/m}^3$ ;  $C_{Hyb} = 1.0 \text{ kg/m}^3$ ; T = 20 °C.

|                | Positions of the diffraction maxima |                     |                     |                      |                                   | The average interplane distances, nm |                |                         |  |  |
|----------------|-------------------------------------|---------------------|---------------------|----------------------|-----------------------------------|--------------------------------------|----------------|-------------------------|--|--|
|                | Amorphous halos<br>(PAAm)           |                     | alos                | Crystal peak (AgNPs) | Paracrystalline<br>lattice (PAAm) |                                      | ie<br>1)       | Crystal lattice (AgNPs) |  |  |
| Composite      | $2\theta_1^{\circ}$                 | $2\theta_2^{\circ}$ | $2\theta_3^{\circ}$ | $2\theta_4^{\circ}$  | d <sub>1</sub> ,                  | d_2                                  | d <sub>3</sub> | <b>d</b> <sub>4</sub>   |  |  |
| AgNPs/<br>Hyb1 | ~14.8                               | 21.4                | ~ 29.0              | 37.8                 | ~ 0.60                            | 0.41                                 | ~ 0.31         | 0.24                    |  |  |
| AgNPs/<br>Hyb2 | ~15.9                               | 21.1                | 28.8                | 37.8                 | ~ 0.56                            | 0.42                                 | 0.31           | 0.24                    |  |  |

Table 5. Parameters of paracrystalline and crystalline lattices in nanocomposite structures.

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#### Antimicrobial properties of silver nanocomposites with different hybrid structures

In accordance with the objectives of the study, AgNPs/Hyb1 and AgNPs/Hyb2 composites, obtained at the highest concentration of Ag-salt (7.1·10<sup>-2</sup> kg/m<sup>3</sup>) and purified of synthesis by-products (Table 4), were chosen to study their activity against gram positive and gram negative bacteria pathogens. To find antimicrobial efficiency of AgNPs/Hyb and possible differences in bacterial sensitivity towards these systems, two series of experiments were carried out. First, cell concentration (CFU/cm<sup>3</sup>) was varied at constant concentration of AgNPs/Hyb by metal ( $C_{AgNPs}$ ). In other series of experiments,  $C_{AgNPs}$  was varied at constant concentration of colony forming units.

As showed the experiments, antibacterial efficiency increased with lessening the size of bacterial population, i.e. growth inhibition zones became smaller with increasing bacteria concentration (Fig. 13). Unlike AgNPs/Hyb systems, matrices Hyb1 and Hyb2 were inactive against bacteria (Fig. 14). Alternatively, when  $C_{AgNPs}$  was varied at constant cell concentration, inhibition zones increased with increasing  $C_{AgNPs}$ , showing that antibacterial activity was strongly dependent on AgNPs concentration in nanocomposites (Fig. 14, upper panel). Obtained results were summarized on the plots (Fig. 15). However, we failed to find reliable difference in antibacterial activity between AgNPs/Hyb1 and AgNPs/Hyb2 systems: both exhibited similar efficiency against studied microorganisms, and growth inhibition zones at the same  $C_{AgNPs}$  did not much differ between bacteria strains (Fig. 15).

Based on the obtained dependences (Fig. 15a,b), bacteria populations at  $1.5 \cdot 10^4$  and  $1.5 \cdot 10^5$  CFU/cm<sup>3</sup> were further examined for their sensitivity to AgNPs. As we have found (Table 6), obtained minimal inhibition concentrations (MICs) showed high sensitivity of studied bacteria strains to both AgNPs composites, which MICs was at the level of ~  $1.25 \cdot 10^{-3}$  kg·m<sup>-3</sup> for *S. aureus* and *E. coli* and  $2.5 \cdot 10^{-3}$  kg·m<sup>-3</sup> for *P. aeruginosa* that agreed with the measurements of growth inhibition zones (Fig. 15c,d). However, similar to well diffusion technique, we were unable to find reliable difference in antibacterial efficiency between two nanosystems (Table 6).

Based on AgNPs/Hyb susceptibility testing and the published data<sup>53,54</sup>, we compared obtained MIC values with those of commonly used antibiotics to the same bacterial pathogens. As we have found, the antibacterial activity of AgNPs was slightly weaker than that of potent antibiotic ciprofloxacin (CF). Still, it was at the widely used tetracycline (TC) and ceftriaxone (CT) levels, dependent on the bacteria species (Table 6). The antibacterial activity of AgNPs/Hyb systems could be placed in following raws of MIC values in kg/m<sup>3</sup>.

S. aureus ATCC 6538: CF  $(0.25-1.0)\cdot 10^{-3}$  > CT = TC  $(0.25-4.0)\cdot 10^{-3}$  = AgNPs/Hyb1-2.

E. coli ATCC 25922: CF (0.8–1.6)·10<sup>-5</sup> > >CT (0.6–1.25)·10<sup>-4</sup> >TC (1.0–8.0)·10<sup>-3</sup> = AgNPs/Hyb1-2.

*P. aeruginosa* ATCC 27853: CF (0.25–2.0) 10<sup>-3</sup> > CT (2.0–32.0) 10<sup>-3</sup> > AgNP/Hyb1-2 > TC (8.0–64.0) 10<sup>-3</sup>.

Considering different bacteria resistance mechanisms, AgNPs showed high effectiveness against all studied strains, however, dependent on bacteria species.

It is worth mentioning that the antibacterial efficiency of AgNPs is highly dependent on the particle size, shape, synthesis method, structure, chemical properties of the polymer carrier for polymer-coated AgNPs, and bacteria species<sup>34</sup>. This explains why the MIC diapason of AgNPs found in the literature against *S. aureus*, *E. coli*, and *P. aeruginosa* was too wide-ranging—(~0.5–100)·10<sup>-3</sup> kg/m<sup>334</sup>. The MIC values found in our work for the studied nanocomposites are close to the lower limit of this concentration range, which indicates their good efficiency against gram-positive and gram-negative bacteria. Compared to the literature<sup>20,22,34</sup>, growth inhibition zones also showed high effectiveness of our AgNPs/Hyb composites.

Regarding bacteria susceptibility to AgNPs/Hyb1-2, gram-positive *S. aureus* and gram-negative *E. coli* were similarly sensitive to AgNPs. This could indicate common mechanisms of antibacterial action of AgNPs/Hyb composites described in the literature: the adhesion on bacteria outer membrane with following permeabilization, cell penetration and release of Ag<sup>+</sup> ions, stimulation of ROS overproduction, inactivation of vital bacteria enzymes (such as nitrate reductases), impairment of bacterial DNA, targeting bacterial genome and signaling pathways resulting in cell death<sup>33,34</sup>. In contrast to *E. coli* and *S. aureus, P. aeruginosa* showed much higher resistance to the AgNP/Hyb composites, which was reflected in a higher MIC of  $2.5 \cdot 10^{-3}$  kg/m<sup>3</sup> (Table 6) and much smaller inhibition zones of  $14.0 \pm 1.0$  mm. This agreed with the published data<sup>53</sup> and could be explained by strong antibacterial resistance mechanisms inherent to this bacteria species, first of all, the much lower permeability of its outer membrane, which can be 10-100 times lower than that of *E. coli*<sup>55</sup>.

As we mentioned above, we failed to find the impact of structural features of the polymer matrices on their antibacterial efficiency against all studied bacteria species (Figs. 13, 14). To explain this phenomenon, we need to consider that despite structural differences of hybrid carriers, similar mean diameters of the formed AgNPs, and equal loading of the matrices with metal AgNP were obtained, which could reflect similar mechanisms working



**Fig. 13**. The effect of AgNPs/Hyb1 (Ag/Hyb1) and AgNPs/Hyb2 (Ag/Hyb2) composites on bacterial growth inhibition: the dependence of inhibition zones on *Escherichia coli* cell concentration at  $C_{AgNPs} = 5 \cdot 10^{-3} \text{ kg/m}^3$ .

at the stage of AgNP formation. So, we suppose that the "likeness" of the size, shape, and loading of metallic AgNP in a hybrid carrier was primarily responsible for their similar antibacterial activity.

Based on our observations that antibacterial efficiency was critically dependent on the proportion of AgNPs to bacteria concentration (Figs. 12, 14), we assume that increasing hybrid "loading" by metal Ag nanoparticles could improve their antibacterial properties. In addition, as shown in our studies on different bacterial pathogens, obtained AgNPs/Hyb nanocomposites may be promising for further design of effective antibacterial preparations, including highly resistant bacteria strains, such as *P. aeruginosa*. However, to resolve these issues, detailed future research is required.

### **Concluding remarks**

Two synthesized and well-characterized silica/polyacrylamide hybrids Hyb1 and Hyb2 with chemically complementary components were used to study the role of their structure in the synthesis of AgNPs by sodium borohydride reduction. These hybrids had the same inorganic "core" but different polymer "coronas" due to significantly different number and length of grafted PAAm chains. Individual Hyb1 particles contained many (72) relatively short PAAm grafts, which interacted strongly with the SiO<sub>2</sub> surface and with each other and formed a dense and thin polymer layer in solution. In contrast, Hyb2 particles included only 5 very long PAAm grafts, which formed a 2.6-fold thicker and more permeable polymer layer. Small particles of Hyb1 and Hyb2 were highly aggregated in aqueous solutions due to the interaction of PAAm "coronas" through hydrogen bonds.

A study of the mechanism of the two-stage reduction process confirmed the high binding ability of both hybrids with respect to  $Ag^+$  ions at the 1st stage. Complexation with silver ions led to compaction of individual Hyb particles, but was not accompanied by noticeable destruction of their aggregates. The process of their



**Fig. 14.** Effect of Hyb1 and Hyb2 matrices and AgNPs/Hyb1 (Ag/Hyb1) and AgNPs/Hyb2 (Ag/Hyb2) composites on bacterial growth inhibition. Upper panel: inhibition zones and their dependence on  $C_{AgNPs}$  (5·10<sup>-3</sup> kg/m<sup>3</sup> –1; 2.5·10<sup>-3</sup> kg/m<sup>3</sup> –2; 1.25·10<sup>-3</sup> kg/m<sup>3</sup> –3) for *S. aureus*; bottom panel: inhibition zones at  $C_{AgNPs}$  5·10<sup>-3</sup> kg/m<sup>3</sup> for *P. aeruginosa* and *S. marcescens*.

destruction actively occurred only at the 2nd stage of borohydride reduction under the influence of AgNP growth.

Kinetic studies revealed a short induction period for the nucleation of primary silver nanoparticles and a strong dependence of the rate of accumulation and yield of AgNPs in hybrid solutions on the concentration of the original Ag-salt (and reducing agent). Thus, with an increase in the Ag-salt concentration from  $0.91 \cdot 10^{-2}$  to  $1.82 \cdot 10^{-2}$  kg/m<sup>3</sup>, the rate of accumulation of nanoparticles in Hyb1 and Hyb2 solutions increased by 2.0-3.5 and 3.2-8.5 times, and the yield of nanoparticles increased by 3.5-7.5 and 6.9-15.2 times, respectively. Kinetic studies also showed an interesting fact of the opposite change in the position of SPRB (nanoparticle size) at the indicated concentrations of Ag-salt in Hyb1-2 solutions during the first 10-15 min of reduction, when intensive accumulation of AgNPs occurred. A sharp decrease in  $\lambda_{max}$  at higher  $C_{Ag-salt} = 1.82 \cdot 10^{-2}$  kg/m<sup>3</sup> was explained by a decrease in the size of nanoparticles caused by their ordering or crystallization. At the same time, a sharp increase in  $\lambda_{max}$  at a lower  $C_{Ag-salt} = 0.91 \cdot 10^{-2}$  kg/m<sup>3</sup> was interpreted as an increase in the size of primary AgNPs with a disordered atomic structure due to their association (coalescence). The difference in the structure of the hybrid matrices was clearly manifested at the lowest concentration of Ag-salt, namely: in Hyb2 solutions, lower values of the rate of accumulation and yield of nanoparticles were observed compared to Hyb1.

A more detailed mechanism of AgNP formation in hybrid matrices, depending on their structure and reagent concentration, was revealed during systematic morphological studies of the resulting AgNP/Hyb1-2 composites using ex situ TEM. One of the most important results here was the presence in the final nanocomposites of two types of AgNPs (internal and external), which arose due to the primary connection of Ag<sup>+</sup> ions and their subsequent reduction both in the internal space of the hybrid "corona" and on its surface. The average size of the external AgNPs was larger than the internal nanoparticles in both composites and increased markedly with increasing Ag-salt (and reducing agent) concentration. The structure of the polymer layer in the hybrid particles



**Fig. 15.** Effect of AgNPs/Hyb1 (Ag/Hyb1) and AgNPs/Hyb2 (Ag/Hyb2) on growth inhibition zones of *Staphylococcus aureus* and *Escherichia coli*. The effect of composites on inhibition zones at (**a**,**b**) a constant value of  $C_{AgNPs} = 5 \cdot 10^{-3} \text{ kg/m}^3$  and a variable concentration of bacteria (from  $1.5 \cdot 10^4$  to  $1.5 \cdot 10^7$  CFU/cm<sup>3</sup>) and (**c**,**d**) a constant cell concentration of  $1.5 \cdot 10^4$  CFU/cm<sup>3</sup>, but different concentrations of AgNPs. M±m, n = 3; \*, #—p < 0.05 as compared to control:  $C_{cell} = 1.5 \cdot 10^4$  CFU/cm<sup>3</sup> (**a**,**b**) and  $C_{AgNPs} = 1.25 \cdot 10^{-3}$  kg/m<sup>3</sup> (**c**,**d**) for *S. aureus* and *E. coli*, respectively.

|                       |                              |                                     | Serial dilutions (C <sub>AgNPs</sub> 10 <sup>3</sup> , kg/m <sup>3</sup> ) |               |                |                |                  |  |
|-----------------------|------------------------------|-------------------------------------|--|---------------|----------------|----------------|------------------|--|
| System                | Bacteria strains             | Bacteria count, CFU/cm <sup>3</sup> | 1:2<br>(2.5)   | 1:4<br>(1.25) | 1:8<br>(0.625) | 1:16<br>(0.31) | Positive control |  |
|                       | 6                            | 104                                 | -  | -             | +              | +              | +                |  |
|                       | 5. uureus, ATCC 0558         | 105                                 | -  | -             | +              | +              | +                |  |
| AgNDo/Hyb1 (Ag/Hyb1)  | E coli ATCC 25922            | 104                                 | -  | -             | +              | +              | +                |  |
| Agines/hybi (Ag/hybi) | <i>E. cou</i> , AICC 23922   | 105                                 | -  | -             | +              | +              | +                |  |
|                       | D                            | 10 <sup>4</sup><br>10 <sup>5</sup>  | -  | +             | +              | +              | +                |  |
|                       | P. aeruginosa, AICC 27855    |                                     | -  | +             | +              | +              | +                |  |
|                       | ATCC (520                    | 10 <sup>4</sup><br>10 <sup>5</sup>  | -  | -             | +              | +              | +                |  |
|                       | <i>S. aureus</i> , AICC 6558 |                                     | -  | -             | +              | +              | +                |  |
| AgNPs/Hyb2 (Ag/Hyb2)  |                              | 10 <sup>4</sup><br>10 <sup>5</sup>  | -  | -             | +              | +              | +                |  |
|                       | <i>E. coll</i> , ATCC 25922  |                                     | -  | -             | +              | +              | +                |  |
|                       | D somusiums ATCC 27852       | 10 <sup>4</sup><br>10 <sup>5</sup>  | -  | +             | +              | +              | +                |  |
|                       | P. aeruginosa, AICC 27855    |                                     | -  | +             | +              | +              | +                |  |

**Table 6**. Sensitivity of bacteria to silver/hybrid nanocomposites (symbols "–" and "+" indicate the absence and presence of bacterial growth, respectively).

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influenced the relationship between both reduction processes. Thus, in the case of a denser and thinner "corona" of PAAm in Hyb1 particles and the lowest  $C_{Ag-salt}$ , the process of binding Ag<sup>+</sup> ions and their reduction on the surface of the polymer layer prevailed. However, the situation changed with increasing  $C_{Ag-salt}$  due to the strong swelling of Hyb1 particles. In contrast, with a thicker and more permeable PAAm "corona" in Hyb2 particles, the formation of many small AgNPs within the polymer layer was preferential at a minimum Ag-salt concentration and could occur with increasing  $C_{Ag-salt}$  even without matrix swelling. The bulk structures of both composites prepared at high concentrations of hybrid matrices and Ag-salt contained amorphous regions of PAAm with three systems of paracrystalline lattice planes and embedded crystalline AgNPs with a tetragonal face-centered cubic lattice.

Purification of nanocomposites from excess reagents and byproducts transformed them into homogeneous systems with small AgNPs ( $d_{av}$ =6.1±2.8 nm in Hyb1 and 6.2±2.7 nm in Hyb2) and highly swollen hybrid particles. The latter formed networks of entanglements in the aqueous medium, which stabilized silver nanoparticles for a long time.

AgNPs/Hyb1 and AgNPs/Hyb1 composites showed high antibacterial efficiency against *S. aureus*, *E. coli*, and *P. aeruginosa*. Obtained MIC values were ~  $(1.25-2.5)\cdot10^{-3}$  kg/m<sup>3</sup>, which was lower as compared to potent antibiotics such as ciprofloxacin, but comparable with those of ceftriaxone or tetracycline, dependent on bacteria strain. A strong relationship was obtained between antibacterial efficiency and the concentration of AgNPs in the composites: growth inhibition zones strongly increased with increasing C<sub>AgNPs</sub> in the range of (1.25-5.0)\cdot10^{-3} kg/m<sup>3</sup>. This indicated that increasing the silver loading in AgNPs/Hyb composites is a promising way to improve their antibacterial properties.

#### Data availability

Data are provided within the manuscript.

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#### Author contributions

T.Z. contributed to the concept and methodology of the study, prepared the starting silver/hybrid nanocomposites for TEM and biological experiments, analyzed the data, and wrote the physicochemical part of the manuscript. O.A. conceived the microbiological experiments, analyzed the data, and wrote relevant parts of the manuscript. I.D. conducted a study of the antimicrobial properties of silver/hybrid nanocomposites. N.P. synthesized silica/polyacrylamide hybrids and studied some of their properties, including their functions as templates in the formation of AgNPs. V.K. calculated the average sizes and size distributions of nanoparticles in composites using the ImageJ program and analyzed the results. D.K. performed and discussed TEM studies on the morphology of individual hybrids and their nanocomposites with AgNPs.

#### Declarations

#### **Competing interests**

The authors declare no competing interests.

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