ABSTRACT
of the dissertation for the degree of Doctor of Philosophy (PhD)
in the specialty "6D072000 – Chemical technology
of inorganic substances"
Bekissanova Zhanar Bolatovna
"Synthesis, characterization and antibacterial activity study of composites based on natural adsorbents and silver nanoparticles"

General job description

In this work, methods for the synthesis of new micro-nanostructured composites with antibacterial properties containing silver nanoparticles were developed using natural mineral materials: diatomite from the Mugodzhar deposit (Aktobe region), kaolinite from the Alekseev deposit (Kokshetau region), as well as biosilica obtained from laboratory-grown diatoms. Mineral matrices were used as a carrier and stabilizer for silver nanoparticles. Hydrogen peroxide was used as an environmentally friendly reducing agent. The potential for antibacterial activity of the resulting composites was investigated.

Relevance of the work

Currently, an urgent problem for practical medicine is the search for new approaches and methods in obtaining accessible antibacterial materials using biocompatible and non-toxic carriers that provide prolonged action of antibacterial drugs. Such carriers should also be low cost and environmentally friendly. Therefore, the dissertation work is devoted to the synthesis of nanocomposites with antibacterial properties based on natural mineral carriers that have the above properties. An original method for the synthesis of antibacterial composite materials based on diatomite, kaolinite and biosilica has been developed. Metallic silver nanoparticles, as well as hybrid complexes of metallic silver nanoparticles and silver chlorides, were used as an antibacterial agent.

The purpose of the dissertation work is: Synthesis, characterization of micro-nanostructured composites based on diatomite, kaolinite, biosilica modified with nanoparticles of metallic silver and silver chlorides and study of the antibacterial activity of the synthesized composites.

Research objectives:

– study of the physicochemical properties of Kazakhstan kaolinite, diatomite and biosilica obtained from laboratory-grown diatoms, justification of their use as carriers for antibacterial agents (silver nanoparticles);

– development of methods for producing environmentally friendly composites based on kaolinite, diatomite, biosilica containing silver nanoparticles and silver chloride nanoparticles;

– establishing optimal conditions for the synthesis of composites (L:S ratio, initial concentration of silver ions in solutions, temperature, pH, time of contact with the surface of the carrier);

– synthesis of new micro-nanostructured composites with antibacterial properties;

– study of the physicochemical properties of synthesized new micro-nanostructured composites with antibacterial properties;

– study of the antibacterial activity of the resulting nanocomposites using the minimum inhibitory concentration (MIC) method against gram-positive pathogenic bacteria *Staphylococcus aureus* and gram-negative *Klebsiella pneumoniae*, *Escherichia coli*.

Research methods.

Characterization of the physicochemical properties of the resulting composites using a set of instrumental methods: transmission electron microscopy (TEM), thermogravimetric analysis (TG), energy dispersive X-ray spectroscopy (EDX), X-ray diffraction analysis (XRD), IR spectroscopy, scanning electron microscopy (SEM), photoluminescence spectroscopy (PL) and zeta potential (ZP). Study of the antibacterial activity of the synthesized composites using the minimum inhibitory concentration (MIC) method.

Objects of research: The object of the study is the synthesis of micro-nanostructured composites with antibacterial properties using natural matrices: diatomite from the Mugodzhar deposit (Aktobe region), kaolinite from the Alekseev deposit (Kokshetau region), biosilica obtained from cultivated diatoms.

Subject of scientific research: Development of methods for the synthesis of new composites with antibacterial properties using natural mineral matrices. Study of the physicochemical properties of starting materials and synthesized micro-nanostructured composites using instrumental methods, study of the antibacterial activity of the resulting composite materials.

Main provisions submitted for defense:

1. A new composite material with antibacterial properties based on natural diatomite containing (0.71, 4.65, 7.21%) hybrid nanoparticles (1–10 nm) of silver chlorides and metallic silver, (AgNPs/AgClNPs/diatomite) is obtained by formation/immobilization of silver nanoparticles on the surface of a mineral carrier using ion exchange reactions and chemical reduction with an environmentally friendly reducing agent (hydrogen peroxide). The number of silver chloride nanoparticles in the composite is limited by the amount of halite impurity in the diatomite. The synthesized composite exhibits high antibacterial activity against *S.aureus* and *K.pneumoniae* with a minimum inhibitory concentration of the composite of 5 mg/ml and 10 mg/ml, respectively, with a silver content of 0.71% in the composite.
2. Composite materials based on natural kaolinite containing (0.60, 2.05 and 7.0%) nanoparticles of metallic silver ranging in size from 20 to 50 nm, are obtained by chemical reduction using an environmentally friendly reducing agent - hydrogen peroxide, exhibit antibacterial activity against gram-positive *S.aureus* and gram-negative *K.pneumoniae*, *E.coli* strains of bacteria at a minimum inhibitory concentration of the composite of 10 mg/ml and a silver content of 7% in the composite.
3. A new 3D micro-nanostructured composite material based on biosilica (AgCl,Ag)NPs/biosilica contains epitaxial hybrid intergrowths of metallic silver and silver chloride in the form of AgCl-Ag hybrid nanoparticles (4.61 and 8.49% silver)

with a predominant size from 5 to 25 nm in the form of crystalline twins Axialy Twined (AT) and Layer Twinned (LT). The composites exhibit high antibacterial activity against gram-positive *S.aureus* and gram-negative *K.pneumoniae*, *E.coli* strains of bacteria with a minimum inhibitory concentration of the composite of 1.25 mg/ml and a silver content of 4.61% in the composite.

4. The antibacterial activity of hybrid silver nanoparticles is due to the synergistic effect of two types of nanoparticles, while composites with a predominant content of silver chloride nanoparticles have a greater inhibitory effect compared to metallic silver nanoparticles, both against gram-positive *S.aureus* and gram-negative *K.pneumoniae*, *E.coli* strains of bacteria from the typical collection cultures and clinical isolates (diabetic foot infection and wound isolates).

Main results of the study

1. An original method has been developed for the production of new hybrid composites (AgCl,Ag)NPs/diatomite based on natural diatomite with a mineral admixture of halite and various silver contents of 0.71, 4.65, 7.21%. The presence of metallic silver nanoparticles and silver chloride nanoparticles in the synthesized composite was confirmed by X-ray diffraction analysis. The TEM method revealed that silver nanoparticles are uniformly distributed over the surface of the matrix mineral, and their sizes range from 1 nm to 10 nm with predominant sizes from 3 nm to 6 nm. Synthesized hybrid composites (AgCl, Ag)NPs/diatomite with a dominant content of AgClNPs exhibit higher antibacterial activity against *S.aureus* and *K.pneumoniae*.

2. AgNPs/kaolinite nanocomposites with different silver contents (0.60, 2.05, and 7.00%) were synthesized by chemical reduction of silver with hydrogen peroxide as an environmentally friendly reducing agent. SEM-EDA spectral analysis demonstrates a uniform distribution of doped silver in the kaolinite. The presence of metallic silver nanoparticles in the composite was confirmed by XRD. Metallic silver nanoparticles of quasi-spherical shape with predominant sizes from 20 to 50 nm are immobilized on the surface of kaolinite nanoplates. Also, multiple effects of the formation of crystalline twins occur. The synthesized AgNPs/kaolinite nanocomposites exhibit antibacterial activity against collection of type cultures and clinical isolates (diabetic foot infection and wound isolates).

3. A new (AgCl, Ag)NPs/biosilica composite containing epitaxially growing metallic silver nanoparticles on top of silver chloride nanoparticles was synthesized. The resulting composites contain hybrid AgCl-Ag nanoparticles with a predominant size of 5 to 25 nm. With the percentage ratio of AgClNPs/AgNPs being 86/14 and 51/49, respectively, the content of nanoparticles in the two synthesized composites is 4.61 and 8.49%. Silver nanoparticles are often found in the quasi-spherical crystalline twins Axialy Twined (AT) and Layer Twinned (LT). The synthesized AgCl-Ag(NPs)/biosilica composites showed high (1.25 mg/ml MIC of the composite with a silver amount of 0.058 mg/ml) antibacterial activity against collections of type cultures and clinical isolates (diabetic foot infection and wound isolates).

4. For all new antibacterial composites obtained based on natural carriers of diatomite, kaolinite and biosilica, original new methods for their production were developed.

Justification of the novelty and importance of the obtained results

– for the first time, domestic natural minerals diatomite from the Mugodzhar deposit (Aktobe region), kaolin from the Alekseev deposit (Kokshetau region) were used as natural carrier matrices for nanoparticles of metallic silver and nanoparticles of silver chloride;

– for the first time, biosilica obtained from diatoms was used as a mineral carrier for hybrid metallic silver nanoparticles and silver chloride nanoparticles; the possibility of the formation of epitaxial intergrowths of silver chlorides and metallic silver on the surface of biosilica was revealed;

– the antibacterial activity of biosilica obtained from diatoms was discovered for the first time, as well as the synergistic effect of biosilica as a carrier and immobilized hybrid nanoparticles of metallic silver and silver chlorides;

– the study of the antibacterial activity of the resulting composites was carried out using not only bacterial strains from the American Type Culture Collection, but also clinical isolates (diabetic foot infection and wound isolates).

Theoretical significance of the study. The results of the study expanded the known knowledge in the field of synthesis of composite materials based on natural mineral matrices and silver nanoparticles with antibacterial properties and the possibility of their use as an effective antibacterial agent for various biomedical applications. The developed methodology for obtaining medicines based on inert clay carriers can be used in lecture material for chemical and chemical-pharmaceutical specialties.

Practical value.

The developed methods for the synthesis of composite materials based on natural mineral matrices and silver nanoparticles with antibacterial properties can be used for the production of inexpensive, non-toxic, stable, environmentally friendly products for various biomedical applications, antimicrobial water purification, for the production of antibacterial biofilms and biopolymer matrices for packaging materials and in pharmaceutical technologies.

Communication with scientific research and government programs.

Research within the framework of the dissertation work was carried out within the framework of grant funding from the Ministry of Education and Science of the Republic of Kazakhstan: GF 2021 AP09561734, GF for postdoctoral students under the project “Zhas Galym-2” AR14972864. Program “PROM - International scholarship exchange of doctoral students and teachers” (December-2022). As part of scientific cooperation between the Nicolaus Copernicus University in Toruń and the Al-Farabi Kazakh National University in Almaty.

The personal contribution of the author of the study lies in the preparation and analysis of literary data on the topic of the dissertation, conducting experiments on the synthesis of composites, processing and interpretation of the obtained scientific results, and presentation in the form of scientific papers. The results obtained were discussed jointly with a foreign scientific supervisor and a local

scientific supervisor, as well as with employees of the Nicolaus Copernicus University in Toruń.

Description of the doctoral student's contribution to the preparation of each publication

1. **Kubasheva, Z.,** Sprynskyy, M., Railean-Plugaru, V., Pomastowski, P., Ospanova, A., & Buszewski, B. (2020). Synthesis and Antibacterial Activity of (AgCl, Ag) NPs/Diatomite Hybrid Composite. Materials, 13(15), 2020 , 3409., https://doi:10.3390/ma13153409 (Investigation, Writing – original text. Visualization)
2. **Bekissanova, Z.,** Railean, V., Brzozowska, W., Wojtczak, I., Ospanova, A., Buszewski, B., & Sprynskyy, M. (2022). Synthesis, characterization of silver/kaolinite nanocomposite and studying its antibacterial activity. *Colloids and Surfaces B: Biointerfaces*, 112908. https://doi.org/10.1016/j.colsurfb.2022.112908 (Investigation, Writing – original text. Visualization)
3. **Bekissanova, Z.,** Railean, V., Wojtczak, I., Brzozowska, W., Trykowski, G., Ospanova, A., & Sprynskyy, M. (2023). Synthesis and Antimicrobial Activity of 3D Micro–Nanostructured Diatom Biosilica Coated by Epitaxially Growing Ag-AgCl Hybrid Nanoparticles. Biomimetics, 9(1), 5. https://doi.org/10.3390/biomimetics9010005 (Investigation, Writing – original text. Visualization)

The dissertation work is presented in the form of a series of articles published by the doctoral student.