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Research paper

Biological activity and sorption ability of synthetic montmorillonite modified by silver/lysozyme nanoparticles

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ABSTRACT

New bioactive materials based on synthetic montmorillonite (Mt) of different chemical compositions ($Na_{2x}(Al_{2(1-x)},Mg_{2x})Si_4O_{10}(OH)_2{\cdot}nH_2O$, where x = 1, 0.75, 0.5 and 0.1) modified by silver/lysozyme nanoparticles (Ag/Lys) have been synthesized. Antimicrobial and hemolytic activities, as well as sorption ability of composite materials towards the simulated toxic agent of medium molecular weight (methylene blue) in environments, similar to those found in human body (pH 1.8 and 6.9) has been studied. It was found that Ag/Lys-Mt materials are active against Gram-negative and Gram-positive bacteria (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* SG-511), while maintaining a low hemolytic effect. The hemolytic activities of synthetic Mt with specified characteristics are presented for the first time. The study of synthetic Mt of different chemical compositions made it possible to establish the main regularities of the effect of Mt composition on the interaction with Ag/Lys nanoparticles. An increase in the aluminium oxide content in Mt leads to an increase in the negative zeta potential of the surface, an increase in the hemolytic activity (toxicity) of the samples, a decrease in silver content at the Mt surface, and consequently, to a decrease in antimicrobial activity with respect to aluminium-free samples. At the same time, the adsorption capacity of samples of all the compositions remains sufficiently high. This makes it possible to talk about the possibility of using them as antiseptic substances for external application, as well as new enterosorbents with antibiotic properties.

1. Introduction

The universal antimicrobial properties of silver nanoparticles can considerably increase the effectiveness of antiseptic substances and other medical drugs and materials for medical use (for example, Rai et al., 2009; Lara et al., 2011; Egorova, 2010; Rizello and Pompa, 2014; Kumar et al., 2016). Of particular interest are silver nanoparticles that have broad spectrum of antimicrobial properties and possesses low toxicity to human cells. Bioconjugates synthesis on the basis of inorganic nanoparticles and biological molecules can be considered as one of the method to produce the antibiotics with optimized properties (Golubeva et al., 2011; Panáček et al., 2016; Jiang et al., 2016).

Stabilization of silver nanoparticles covered by biological active shell on porous matrixes allows extending the fields of their application in the development of ointments, sorbents with prominent oncotic properties for wound surgery and enterosorbents. From the point of view of further medical application layered silicates with montmorillonite structure (Mt) are some of the best matrix materials. The main advantages of these materials are their considerable biological inactivity and ease of their removing form the human body (Lee et al., 2005; Carretero et al., 2013; Petushkov et al., 2010; Joshi et al., 2009). Another advantage is their capacity to intercalate the biologically active molecules and nanoparticles (Joshi et al., 2009; Kollar et al., 2003; Block et al., 2015; Corma et al., 2002; Amorim et al., 2012; Nagy et al., 2011; Roy et al., 2017; Borrego-Sánchez et al., 2018; De Mattos Amadio et al., 2017).

This work aimed to synthesis and investigation of the bioactive sorbents, based on montmorillonite matrixes of different chemical compositions and silver nanoparticles, stabilized by natural antibiotic lysozyme molecules (Ag/Lyz).

Lysozyme (muramidase, mucopeptide glycohydrolase) is a cationic protein with a molecular weight of approximately 14 kDa. It is contained in protective cells of humans and animals (neutrophils, monocytes etc.), and in different biological fluids (milk, saliva, tears, urine etc.). Lysozyme is also present in great quantities in the protein of chicken eggs. Lysozyme is a highly active antimicrobial agent towards

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Table 1
Chemical composition, porous characteristics and hemolytic activity of the initial Mt matrices.

Denotation	Composition by synthesis	Al_2O_3 content, wt%[a]	Specific surface area[b], m^2/g	Mean pores diameters, nm	zeta-potential, mV	Hemolytic activity, %[c]
Al0	$Mg_3Si_4O_{10}(OH)_2\ H_2O$[*]	0	232	8.0	−34 ± 2	4 ± 1
Al0.5	$Na_{1.5}Al_{0.5}Mg_{1.5}Si_4O_{10}(OH)_2\ H_2O$	12.1	220	3.7	−36 ± 1	34 ± 4
Al1.0	$Na_{1.0}Al_{1.0}Mg_{1.0}Si_4O_{10}(OH)_2{\cdot}H_2O$	22.8	190	3.9	−34 ± 2	87 ± 6
Al1.8	$Na_{0.2}Al_{1.8}Mg_{0.2}Si_4O_{10}(OH)_2{\cdot}H_2O$	24.8	120	3.9	−39 ± 2	71 ± 10

[*] Analogue of natural saponite.
[a] By chemical analysis.
[b] Relative error in specific surface area value was 1%, in mean pores diameters value −1%.
[c] The data are presented as means ± SD, n = 9.

Gram-positive and (to a considerably lesser degree) Gram-negative bacteria. Furthermore, some data has been obtained indicating that lysozyme can be effective in the case of the human immunodeficiency virus (HIV) (Yang et al., 2007; Reinhofer et al., 2014). It was found (Eby et al., 2009a, 2009b) that the application of lysozyme as a stabilizer in the synthesis of silver nanoparticles by chemical reduction method extends the range of antimicrobial activity of both lysozyme and silver nanoparticles. So, it is known that although silver nanoparticles are efficient in inactivation both Gramm-positive and Gramm-negative bacteria, there were pronounced results showing that it tended to inhibit the growth of Gram-negative bacteria (Siddhartha et al., 2007; Park et al., 2016.). The combination of silver nanoparticles and lysozyme allows the development of broad-spectrum antibiotics. The modification of porous matrices by Ag/Lyz nanoparticles provides an opportunity to produce the materials with optimized complexes of properties, such as good adsorption capacity, combined with antimicrobial activity.

Previously, the results of the study of bioinorganic complexes based on silver nanoparticles covered by lysozyme and natural Mt has been presented (Golubeva et al., 2016; Jiang et al., 2016). It has been shown that materials of broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria can be obtained. Furthermore, it was found that the basis of the antimicrobial effect of these complexes is the destruction of the bacterial membrane (Jiang et al., 2016).

In the above-mentioned works devoted to the study of Ag/Lyz nanoparticles interaction with the surface of layered silicates, K10 grade Mt was used. As is known (Jiang and Zeng, 2003) this type of Mt is not a single phase material. It contains different admixtures, such as muscovite, and has a rather low cationic exchange capacity, due to the absence of isomorphic substitution in octahedral layers. These features do not allow determining the main patterns of the Ag/Lyz nanoparticles interactions with Mt surface.

In present study the synthetic Mt matrixes with different degrees of isomorphic substitution of magnesium atoms in the octahedral layers by aluminium were used. Synthetic Mt are characterized by a certain set of characteristics, such as chemical composition, particle size, surface properties and porosity. Constancy of these characteristics and the possibility of their control (Golubeva, 2016) make the synthetic Mt promising for the development of new materials for medical applications, particularly in the field of medical sorbents with antibiotic activity.

2. Materials and methods

2.1. Reagents

For the ag/Lyz nanoparticles synthesis chicken egg protein lysozyme (LYS, ≈70,000 units/mg, sigma-Aldrich, USA), sodium borohydride ($NaBH_4$, 99%, sigma-Aldrich, USA), and silver nitrate ($AgNO_3$, 99.9%, Khimmedsintez, Russia) were used without additional purification. Mt samples were prepared using tetraethoxysilane ($(C_2H_5O)_4Si$, special purity grade, EKOS-1, Russia), $mg(NO_3)_2{\cdot}6H_2O$ (reagent grade, Vekton, Russia), $Al(NO_3)_3{\cdot}9H_2O$ (reagent grade, Neva-reactive, Russia), HNO_3 (reagent grade, 65 wt%, Vekton, Russia), NH_4OH (special purity grade, Vekton, Russia) and ethanol

2.2. Synthesis of Ag/Lys nanoparticles

Ag/Lys nanoparticles were synthesized in aqueous solutions using a chemical reduction of silver nitrate by sodium borohydride in the presence of lysozyme. The precursor solutions were incubated at 2 °C. After mixing the silver nitrate and lysozyme solutions, cooled sodium borohydride was added with vortex stirring. Mixing was carried out at the weight ratio $NaBH_4$: LYZ: $AgNO_3$ = 0.22: 0.77: 1. The hydrosols obtained were kept at room temperature in sealed plastic containers. Such reagent ratios were selected based on available data (Yakovlev and Golubeva, 2014) on the properties of silver nanoparticles dispersions in lysozyme medium. According to these data, hydrosols of silver nanoparticles that ate stable over time with the average particle size of 10–20 nm and ionic silver content not higher than 50 μmol/l can be obtained.

2.3. Mt synthesis

Samples were synthesized under hydrothermal conditions in steel autoclaves with platinum crucibles. Synthesis was carried out in an aqueous medium using dried hydrogels of the appropriate composition as starting reagents. Mt samples were synthesized 72 h at 350 °C and 70 MPa. Samples were obtained corresponding to the chemical formula $Na_{2x}(Al_{2(1-x)},Mg_{2x})Si_4O_{10}(OH)_2{\cdot}nH_2O$, where x = 1, 0.75, 0.5 and 0.1, with varying degrees of isomorphous substitution of magnesium for aluminium in the octahedral layers. Subsequently, sample designations corresponding to the degree of substitution of magnesium for aluminium will be used (see Table 1).

2.4. Ag/Lyz-Mt synthesis

In order to obtain Ag/Lyz-Mt complexes 1 g of Mt were mixed with 30 ml of nanoparticle hydrosol and then centrifuged. To increase the concentration of nanoparticles in the complexes, Mt treatment according to the above described procedure was carried out several times. The resulting material was placed in a Petri dish and dried for 12 h at room temperature. The presence of nanoparticles in samples obtained were confirmed by UV-spectroscopy.

2.5. Characterizations

X-ray diffraction patterns of Mt powders and Ag/Lyz-Mt complexes were performed on a Bruker D8-Advantage powder diffractometer with CuKα-radiation range 2ϑ = 5-70°.

The UV adsorption spectra were carried out on a UV–vis spectrometer with an integrating sphere (Shimadzu UV-2600, Germany). Silver nanoparticle sols were preliminary diluted 10 times and measured in quartz cuvettes with the optical path length of 5 mm. The absorption spectra of Ag/Lyz-Mt powders were out using tableted samples mixed with $BaSO_4$.