



DOI: 10.31643/2025/6445.07

Earth sciences



Features of obtaining composite material from hydrophobic clay with antimicrobial properties

^{1*}Ibraimova D.M-K., ^{2,3}Rozhkova O.V., ¹Musabekov K.B., ¹Tazhibayeva S.M.,
^{2,4}Rozhkov V.I., ³Yermekov M.T.

¹Al-Farabi Kazakh National University, Almaty, Kazakhstan

²Saken Seifullin Kazakh Agrotechnical University, Astana, Kazakhstan

³JSC Science and Technology Solutions, Almaty, Kazakhstan

⁴LLP Altai Geological and Ecological Institute, Ust-Kamenogorsk, Kazakhstan

* Corresponding author email: dana_kereevna@kaznu.kz

<p>Received: September 13, 2023 Peer-reviewed: December 20, 2023 Accepted: March 5, 2024</p>	<p>ABSTRACT A method for obtaining a nanocomposite from hydrophobic clays with bactericidal properties is considered, which plays the role of a drug matrix intercalated agar-agar. Such nanocomposite materials are increasingly used in medicine as matrices for medicines and vitamins using their adsorption properties and long-term exposure. It was established using TEM analysis that halloysite particles from Belaye Glinische deposit are nanoscale and have a cylindrical shape with a length from 200 nm to 1000 nm and a diameter of nanoparticles from 50 nm to 80 nm. The first stage of the bionanocomposites manufacturing process was the treatment of halloysite nanoparticles with silver ions to impart antimicrobial properties, and hydrophobization with a cationic surfactant was carried out at the next stage. It was established by the X-ray diffraction method that the interlayer space of HNT has been expanded from 9.998 Å to 17.5 Å on the result of the cationic adsorption on the HNT. FTIR spectroscopy also proved the adsorption of surfactant molecules on halloysite by the presence of an appropriate absorption band. The adsorption of silver on a nanotube made of halloysite was revealed by the method of energy-dispersive X-ray spectroscopy. Antimicrobial properties of silver-treated and organophilic halloysite have been established and proved by <i>in vitro</i> analyses in microbiological laboratories about <i>Escherichia coli</i>. Hydrophobic samples of these organophilic clays had edge angles higher than 90° and this proves that all samples are hydrophobic. The resulting organophilic clays were intercalated into an agar-agar matrix and were thrown into a solution of calcium chloride, which gave them stability in a liquid medium. It has been practically established that the most optimal bionanocomposite microsphere is a 50% ratio of organohalloysite and agar-agar.</p>
	<p>Keywords: bionanocomposite, halloysite nanoclay, silver clay, antimicrobial properties, oleophylization, microsphere, drug delivers.</p>
<p>Ibraimova Dana Mykty-Kereevna</p>	<p>Information about authors: Candidate of Chemical Sciences, Senior Lecturer, Al-Farabi Kazakh National University, 050000, al-Farabi Avenue, 71, Almaty, Kazakhstan. Email: dana_kereevna@kaznu.kz</p>
<p>Rozhkova Olga Vladimirovna</p>	<p>Doctor of Chemistry, Professor of Saken Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan, 010000, Zhenis Avenue, 62, Astana, Kazakhstan, JSC "Science and Technology Solutions", Astana, Kazakhstan, 010000, Republic Avenue, 24. Email: rozhkova.o@stsolutions.kz</p>
<p>Musabekov Kuanyshbek Bituovich</p>	<p>Doctor of Chemical Sciences, Professor, Academician of the National Academy of Natural Sciences of the Republic of Kazakhstan, Al-Farabi Kazakh National University, 050000, al-Farabi Avenue, 71, Almaty, Kazakhstan. E-mail: musabekov40@mail.ru</p>
<p>Tazhibayeva Sagdat Mederbekovna</p>	<p>Doctor of Chemical Sciences, Professor of Al-Farabi Kazakh National University, 050000, al-Farabi Avenue, 71, Almaty, Kazakhstan. E-mail: tazhibayeva_s@mail.ru</p>
<p>Rozhkov Vitaliy Igorevich</p>	<p>Candidate of Technical Sciences, Saken Seifullin Kazakh Agrotechnical University, Astana, Kazakhstan; LLP "Altai Geological and Ecological Institute", Ust-Kamenogorsk, Kazakhstan. E-mail: Vitalza1983@gmail.com</p>
<p>Yermekov Marat Teginbayevich</p>	<p>Director of the Project and Asset Management Department of Science and Technology Solutions JSC, 050000, Almaty, Kazakhstan. Email: yermekov.m@stsolutions.kz</p>

Introduction

Academician Sh.B. Battalova described in detail the chemical and physical properties of Kazakhstani clays, she showed the possibility of using bentonites from Kazakhstani deposits as catalysts for oil refining, adsorbents of dye ions and heavy metals, as well as for cleaning vegetable oils, petroleum products and wool washing [1].

Currently, Kazakhstan's clays have been sufficiently studied by scientists from foreign and neighbouring countries, but even then they do not have a wide range of applications in production facilities within Kazakhstan, as they are transported from foreign marketplaces. As a result of the work, there will be results that will determine the scope of application of various clays in Kazakhstan [[2], [3], [4], [5], [6], [7], [8], [9], [10]].

In the domestic market, there is a growing demand for organoclays, drilling fluids and additives to building mixes. In addition, the development of nanotechnology and the production of new composite materials based on nonpolar polymers implies an expansion of the range of organoclays with differentiated surface hydrophobicity [[5], [6], [7], [8]].

The aim of this work was the synthesis of a biodegradable nanocomposite based on halloysite nanotubes (HNT), with prolonged bactericidal properties and high capacity, capable of absorbing harmful ions in the body and releasing vitamins contained in it during ion exchange.

Experimental

Materials

The halloysite clay of the White Clay deposit, which has the shape of a hollow cylinder with a length of 200-1000 nm and a diameter of 50-80 nm, was chosen as a matrix for obtaining a biodegradable nanocomposite [[4], [5]]. The mineral is characterized by a 1:1 crystal structure and a high water content. The colour is white with a hardness of 1-2.5 on the mineralogical scale and a density of 2.6 g/cm³.

Octadecylamine (CAS No: 124-30-1, Sigma Aldrich) A solution of a cationic surfactant was used to produce organoclay [[4], [6], [11]]. The chemical formula is C₁₈H₃₉N, a white crystal with a molecular weight of 269,5 g/mol.

To impart bactericidal properties to halloysite nanotubes, silver nitrate (CAS: 7761-88-8, Sigma Aldrich) was used, which is a salt of the transition

metal of silver and inorganic nitric acid with the formula AgNO₃.

Agar-agar (C₁₂H₁₈O₉)_n (CAS No: 9002-18-0, Sigma Aldrich) was used as a polymer biodegradable matrix and for the absorption of drugs

Methods

For X-ray diffraction analysis, an automated Drone-3 diffractometer with CuK_α radiation and a β filter was used (U=35 kV; I=20 mA; capture θ-2θ; detector speed 2 degrees/min.). X-ray phase analysis of the diffractogram of powder samples was carried out on a semi-quantitative basis by the method of equal suspensions and artificial mixtures.

The scanning electron microscope belongs to the most modern devices for conducting research work of the highest automated processes in the direction of nanotechnology. It is an apparatus that is necessary for obtaining permissible shots of at least 2.5 nm and conducting quantitative and qualitative analyses of nanomaterial objects.

Transmission electron microscopy (TEM) was used to describe the structure of the material both in terms of sample size and surface.

Infrared spectroscopy is a method that studies the interaction of infrared light with matter.

The goniometer is a device for measuring the contact angle by the method of measuring the drop lying down.

Results and Discussion

In the process of carrying out the work, it was necessary to prepare the first halloysite, then silvered clay and after the silvered organohalloysite

Halloysite deposits in Kazakhstan are located in two places [[6], [7], [8], [9], [10], [11], [12], [13], [14], [15], [16], [17]], these are the halloysite of the Beloye Glinishche deposit near Karaganda and the Aizin-Tamara halloysite deposit in the Akmola region. The choice of halloysite from the Beloye Glinishche deposit is justified by the fineness of the mineral and the basic composition, which is represented by halloysite with chemical formula Al₄[Si₄O₁₀][OH]₈·4H₂O or Al₂O₃·2SiO₂·4H₂O. It has been proven that the mineral halloysite practically never occurs in its pure form, most often together with kaolinite and quartz or a mixture with other aluminosilicate minerals. To separate the mineral from quartz particles, Karaganda halloysite was crushed, sieved and washed by decantation in laboratory conditions. X-ray diffraction analysis was performed to confirm the resulting powder's composition.

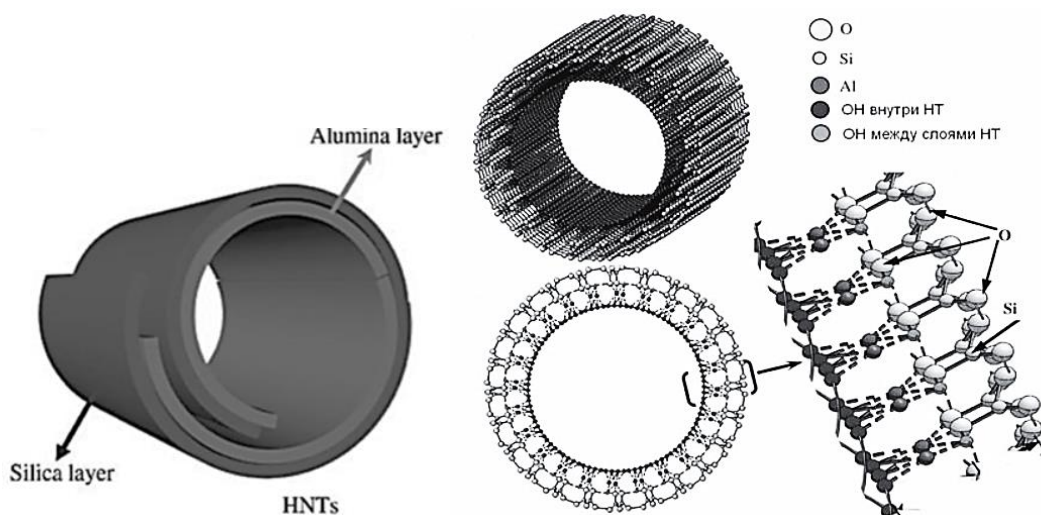


Figure 1 - 3D dimensional structure of halloysites [[4], [17]]

Table 1 below shows the mineral composition of the sample by the quantitative indications of a semi-digital X-ray phase analysis.

Table 1 - Findings of semi-digital X-ray phase analysis

1	2
Halloysite $Al_2(Si_2O_5)(OH)_4$	SiO_2 and others
81.2. %	18.8. %

The mineral halloysite has the form of rolled sheets [[4], [12]], the diagram below shows the 3D dimensional structure of the halloysite, Figure 1.

As the results of X-ray diffractometric analysis showed, the bulk of the mineral, i.e. 81.2%, is halloysite. Two types of halloysite 10A and halloysite 7A are known in nature, since the interlayer space is 9.997 Å, it is determined that halloysite White Clay belongs to the type - halloysite 10A. In the generalization of halloysite [[7], [12]], the authors described that the studied composition of halloysite corresponds to the chemical formula $Al_2Si_2O_5(OH)_4 nH_2O$, where $n=0-2$. The structure of halloysite - (7Å) is described by a hexagonal unit cell with edge lengths $a=5.14$, $b=8.9$, $c=17.7$ Å; the values of angles $\alpha=97-104$, $\beta=90-91.8$, $\gamma=90^\circ$ differ slightly from different authors [[8], [13]]. In tubular structures of halloysite, its long axis is often associated with the crystallographic axis. To identify halloysite-(10 A), X-ray phase analysis uses a reflex with d001, which is absent in the spectrum of several polymorphs of the kaolins. This corresponds to the sum of the

thicknesses of one mineral layer of 7.14 Å and a monolayer of water of 3 Å. The authors [[4], [5], [6], [7], [8], [9], [10], [11], [12]] determined that the outer surface of aluminosilicate nanotubes consists mainly of siloxane groups (Si-O-Si). Aluminol (Al-OH) functional groups are also placed on the inner surface of the cavity and between the layers. The inner type is Al-IT is directed towards the tetrahedral silica mesh. As a result of structural defects, silanol (Si-OH) and additional Al-OH functional groups are formed on the fractures of silicate particles (Fig. 1 and 2) [12].

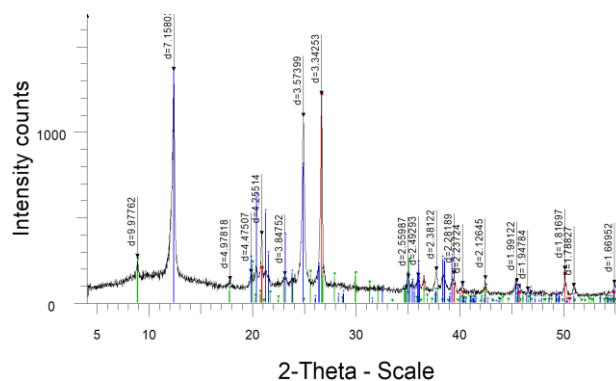


Figure 2 - Diffractogram of the halloysite (a) mineral

Also of interest is the demonstration of the presence of halloysite nanotubes determined by scanning electron microscopy (SEM), this analysis was conducted through the "National Open-type Nanotechnology Laboratory" at the Al-Farabi Kazakh National University. The results are shown in Figure 3.

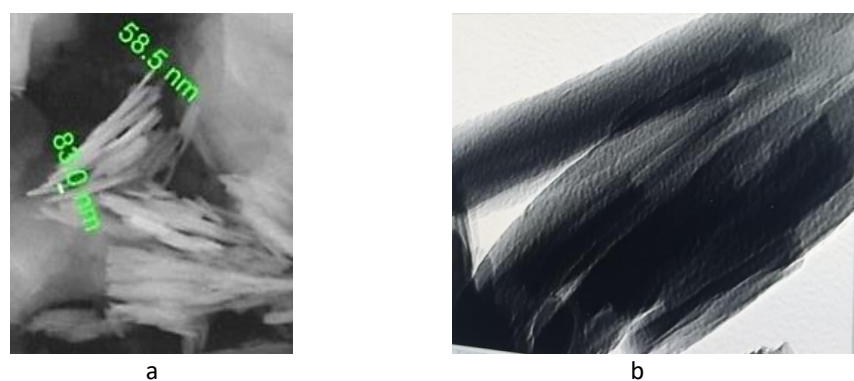


Figure 3 - Morphology of mineral halloysite. a – SEM figures and b – TEM figures

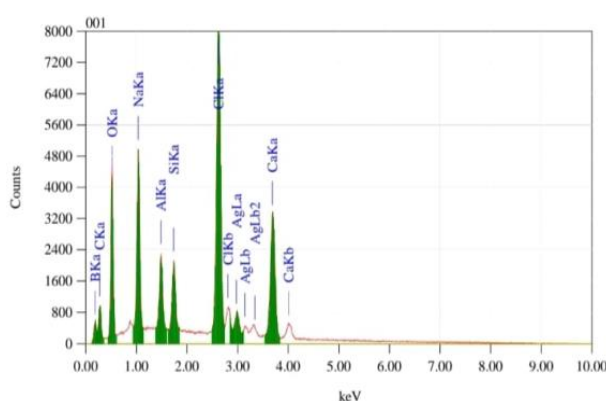


Figure 4 - Determination of silver ions by energy dispersion X-ray spectroscopy

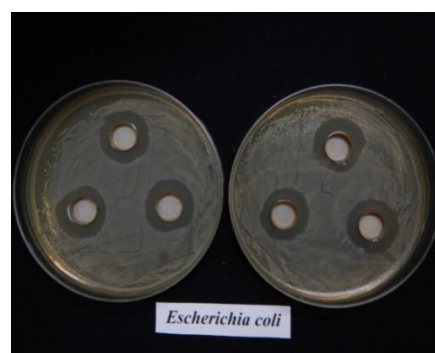


Figure 5 - Zones of inhibition of the growth of *Escherichia coli* shoots of silver ion planted clay (21-23mm).

Halloysite particles consist of fibrous particles, with a particle length of the order of 200-1000 nm, tube diameters range from 50-80 nm, SEM and TEM pictures (Fig. 3a and 3b). It is known that halloysite exists in the form of nanotubes, and these nanotubes are present in a hollow form [3], although due to the limitations of the SEM method, this cannot be confirmed. However, SEM analysis made it possible to determine the size of the nanotubes. In this regard, a TEM study was undertaken to verify the hollow structure of the nanotube. TEM analysis was carried out in the nanolaboratory of the Kazakh-Japanese Innovation Center of the Kazakh National Agrarian University. The obtained result is presented in Figure 3.

From Figure 3, it is evident that HNTs are in the shape of spherical tubes, with diameters ranging from 20 to 50 nm. Halloysite belongs to the colinite group and, unlike kaolinite, is located in two layers and contains a small number of water molecules. The critical grid scheme has a 1:1 ratio of clay types, one of which consists of aluminium oxide, and the

other of silicon oxide [13]. The plates curl into tubes due to the sharp difference in the amount of silicon and aluminium ions. In this case, a folded tube is formed, inside of which there is a layer of aluminium oxide, and outside - a layer of silicon oxide. With different ionization of silicon by aluminium oxide, the dielectric properties are different. Aluminium oxide, if for example placed in a solution with pH = 8.5, will have a positive charge. The different charges on the outer and inner surfaces of halloysite tubes are associated with the possible filling of the inner part of the tubes with negatively charged molecules. Another attractive feature of Halloysite in comparison with other clays is that it has a large specific surface area. During the work, the halloysite was previously modified with silver ions. The bactericidal and antiseptic properties of silver ions and the ability to resist 650 types of bacteria are known [[14], [15]]. When modifying halloysite with silver ions, it was important not to change the morphological nature of the HNT, because the

introduction of a drug into the hollow part of the tube is implied.

The modification process was carried out simply, using a prepared solution of silver ions $C(AgNO_3) = 0.4 \text{ mg/l}$

The result of energy dispersive spectroscopy, proving that halloysite contains silver ions, is presented in Figure 4. To determine the presence of silver ions, an SEM image is first created, then by randomly placing a point on an area of the sample, an area of interest is selected (point 001 in Figure A) and the spectra of this region B. In Figure 4, the spectra coloured green are responsible for the clay mineral and silver ion. There is no need to pay attention to the content of carbon and chlorine ions revealed by the analysis since they are present in the composition of the substrate used.

Since silver ions can quickly oxidize in the sun and turn into silver oxide, all the samples obtained were stored in tinted glass containers, away from sunlight. The bactericidal properties of the resulting silver halloysite were tested. Testing of bactericidal properties was carried out by order in the microbiological laboratories INVITRO in Almaty.

The bactericidal properties were studied on the bacterium *Escherichia coli* (intestinal worm Rod),

clay in an amount of 0.4 g was placed in a test tube, and then 10 ml of distilled water was added and thoroughly mixed. The test culture of *Escherichia coli* was sown in Petri dishes on top of the nutrient Agar. Then grooves were made on the agar, as shown in Figure 5, and 0.2 ml of the test suspension was poured. It was grown for 3 days in a Petri dish at 37 °C in a thermostat. As a result, the bactericidal property of the clay on which silver ions were applied was confirmed, in particular, to suppress the growth of *Escherichia coli* bacteria.

If the interlayer gallery expands, this may indicate the ingress of large molecules into it, that is, intercalation or detachment, therefore, to exclude changes in the structure of the sample under study after modification with silver, a repeated X-ray diffraction analysis was performed.

The analysis confirmed the change in the interlayer gaps from $d = 9.9 \text{ \AA}$ (Figure 6) to $d = 17.50 \text{ \AA}$ showing that octadecylamine (ODA) adsorption occurs. After organomodification with octadecylamine (ODA), the excess ODA was washed in three stages for complete removal. In this connection, ODA molecules were studied by IR spectroscopy to confirm their presence on the surface of the halloysite.

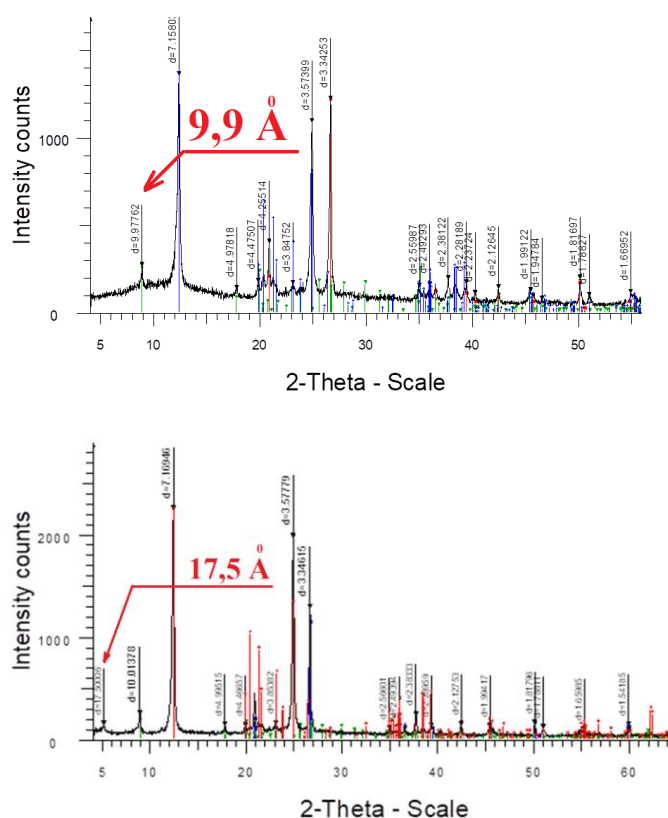


Figure 6 - Diffractogram of the halloysite (a) and organomodified halloysite (b) minerals

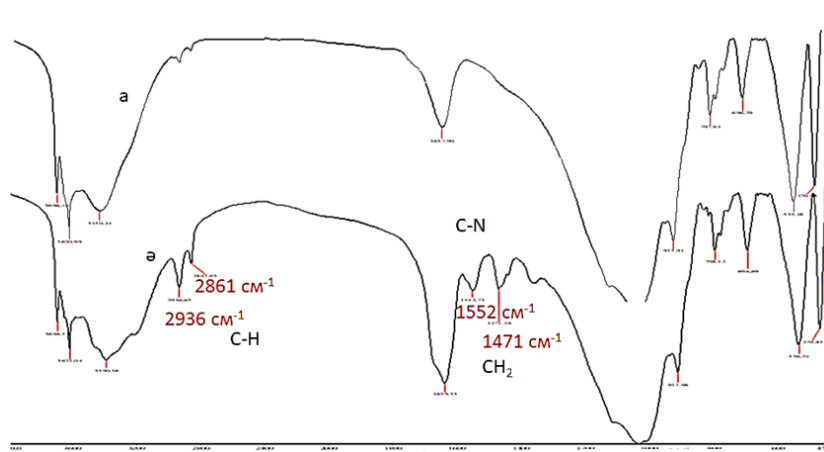


Figure 7 - IR-spectroscopic analysis results; a-halloysite; b-organohalloysite

Figure 8 shows the IR spectra of the halloysite clay of the White Clay deposit (a) and its modified ODA (b) hydrophobic clay. The values of 3699.06 cm^{-1} , 3698.64 cm^{-1} , 3696.52 cm^{-1} , and 3698.48 cm^{-1} in halloysite determine the presence of an OH group with which absorption bands are associated. The absorption of the free OH group in the spectrum of modified ODA halloysite corresponds to a frequency of $3550\text{--}3150\text{ cm}^{-1}$ and in the enriched natural spectrum of halloysite a short band of medium intensity $3620.99\text{--}3621.38\text{ cm}^{-1}$. These bands indicate a strong association of the OH group with Halloysite. In the spectrum of modified ODA halloysite, the absorption of the trans-N-H band of the secondary amine is determined by bands 3317.80 and 3323.97 cm^{-1} with moderate intensity, and these bands manifest themselves at a very higher frequency compared to the spectrum of modified ODA (B) halloysite, whereas in halloysite these bonds manifest themselves at a lower frequency (a). The absorption of the spectrum in question in this area occurs at a very high intensity. The sample is presented in the form of a film (or tablets with KBr), and the absorption region of various symmetric C-H bonds in the spectrum of ODA-modified halloysite is observed in the regions of 2937 and 2860 cm^{-1} . Absorption bands of deformation vibrations of the hydroxyl group in the spectrum of natural halloysite and its modified form with ODA bands are manifested at frequencies 1639.33 , 1639.76 , and 1637.90 cm^{-1} . The CH_2 -group in deformation oscillations is in the region of $1500\text{--}1300\text{ cm}^{-1}$ of the research spectrum. In this area, high-frequency components are associated with

antisymmetric deformation and low-frequency components with symmetric deformation of these groups.

The absorption bands 1129 cm^{-1} and 1150 cm^{-1} of the spectra of modified ODA halloysite reflect valence vibrations of the natural and antisymmetric C-N bond associated with various particles in the molecule. This region is not observed in the spectrum of enriched natural halloysite. Thus, from the results of the IR spectra of natural halloysite and modified ODA halloysite, the following conclusion is drawn: in modified ODA halloysite, there is a significant decrease in free O-H bonds, which indicates a decrease in the number of bands in the corresponding spectral region and also indicates mixing of the remaining bands at a lower frequency. The spectrum pattern changes accordingly in the region of $1120\text{--}1000\text{ cm}^{-1}$.

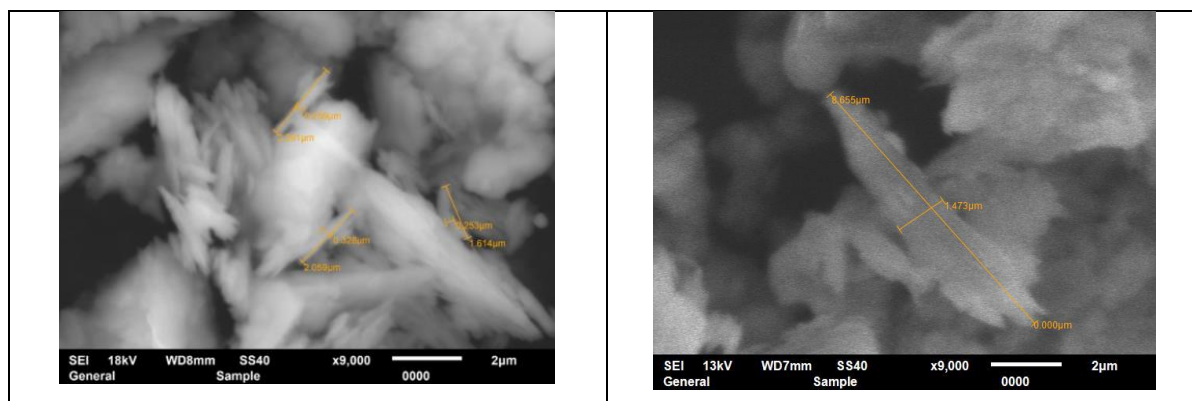
Organomodified halloysite can float on the surface of water, Figure 8 (the picture was taken after $t=150$ days). That is, organophilic halloysite is hydrophobic because it has a constant chemical composition



Figure 8 - Organoclay powder newly placed on the surface of the water after $t=150$ days

Table 3 - Values of water absorption angles for samples of organoclay powders obtained at different concentrations of ODA

№	Consumption of ODA spent on modification, mole/litre	Angles of incidence, degrees	Names of organoclay of various modifications (ODA-modified and bactericidal)
1	Silvered halloysite	9°	BOMC0
2	0.000625	122°	BOMC1
3	0.00125	129°	BOMC2
4	0.0025	130°	BOMC3
5	0.005	131°	BOMC4
6	0.01	132°	BOMC5

**Figure 9** - SEM images of organomodified silvered halloysite mineral

Thus, bactericidal organoclays of 6 types were obtained (Table 3) by modifying silver-plated halloysite with octadecylamine, labelled from BOMG0 to BOMG5. Since the organomodification process is carried out at a certain temperature mode of operation, it was important to make a morphological analysis of the particles of the resulting hydrophobic organohalloysite since it was necessary to preserve the organohalloysite spatially intact in the form of a nanotube during the process. For verification, an SEM picture of the organohalloysite was taken using the SEM method (Figure 9).

Using SEM analysis, the width and length of organophilic nanotubes made of halloysite were measured, it was found that the width ranges from 10 to 100 nm, and the length reaches ~1000 nm. It has been proven that this does not affect the type of rolled sheet.

At the next stage, the resulting silver sample of organophilic halloysite BOMC4 was embedded in a polymer matrix (agar-agar) to obtain samples of bionanocomposite. To create compactness, several pieces of samples were placed in a calcium solution. At the next stage, the masses, sizes and swollen and dried samples of bionanocomposite were studied. The data is shown in Table 4.

According to the data in the table, a spherical bionanocomposite formed from silvered organoclay and agar-agar in a calcium shell shows water resistance and shows good swelling, although they were left in water for 72 hours to swell. This shows that this bionanocomposite with antimicrobial properties is a good enough result to be introduced into its drugs [[16], [17], [18], [19], [20]].

Table 4 - Composition of the bionanocomposite and their parameters

№ №	Percentag es of the compositi on of the OHNT in agar-agar	Mass of micropar ticles in swollen form, g	The mass of micropa rticles in dried form, g	Percent age of reducti on of microp articles	The average diameter of the micropartic les in the swollen form, g	The average diameter of micropartic les in dried form, g	The proportion of absorbed water, dried samples, after swelling in water from its size	The "lifetime" of microspher es, the frequency of swelling / drying
1	0,1%	0.560	0.059	89.46	~2	~0.8	320%	42 time
2	1%	0.623	0.068	89.09	~2	~0.83	303%	45 time
3	10%	0.681	0.0998	85.31	~2	~0.85	273%	53 time
4	50%	0.710	0.132	81.42	~2	~0.9	233%	63 time
5	90%	0.740	0.149	79.86	~2	~1.6	143%	15 time

As can be seen from Table 4, in microspheres with an increase in the amount of organoclay, the absorption capacity of the microspheres of the bionanocomposite decreases. According to Table 4, a 50% microsphere with an oragnohalloysite nanotube (OHNT) absorbs liquid (water) almost 1.6 times worse than with their 0.1% sample, but the lifetime is better than other samples. Hence, it was concluded that the most optimal bionanocomposite microsphere is a 50% ratio of OHNT and agar-agar since it was necessary to introduce the maximum value of OHNT into these microspheres. The need to introduce OHNT into the agar-agar microsphere is that the bionanocomposite we have obtained should participate in the metabolism of nonpolar substances in the human body.

Conclusion

Thus, attention to the creation of bionanocomposites has been increasing recently. The results obtained by us significantly affect the methods of obtaining new types of medicines using clay minerals, expanding the scope of application of organomodified clays. Nanotechnology seems to be a serious scientific field capable of creating various nanobiocomposites with the expansion of their scope of application. Thanks to scientific research in

this direction, the prospects for the creation and application of various types of nanoglines are significantly expanding. Research in this direction is promising and can expand the possibilities of creating various forms of medicines due to the synthesis of halloysite with oleophilic and bactericidal properties, structuring and microcapsulation of a bionanocomposite based on halloysite.

Acknowledgements

This scientific research was carried out within the framework of grant financing of the AP19674742 (IRN) project "Technology for obtaining a new organomineral composite material based on natural bentonite of East Kazakhstan" (IRN AP19674742 "Technology for obtaining a new organomineral composite material"). based on the natural bentonite of East Kazakhstan"). The source of funding is the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan. The authors express their gratitude for the allocated grant funding.

Conflict of interest

The authors of this study do not cooperate with other publishers on this topic.

Cite this article as: Ibraimova D M-K, Rozhkova OV, Musabekov KB, Tazhibayeva SM, Rozhkov VI, Yermekov MT. Features of obtaining composite material from hydrophobic clay with antimicrobial properties. *Kompleksnoe Ispolzovanie Mineralnogo Syra* = Complex Use of Mineral Resources. 2025; 332(1):79-89. <https://doi.org/10.31643/2025/6445.07>

Антимикробтық қасиетке ие гидрофобты галлуазиттен композициялық материал алу ерекшеліктері

¹Ибраимова Д.М.-К., ^{2,3}Рожкова О.В., ¹Мұсабеков Қ.Б., ¹Тәжібаева С.М.,
^{2,4}Рожков В.И., ³Ермеков М.Т.

¹Эл-Фараби атындағы Қазақ ұлттық университеті, Алматы, Қазақстан

²Сакен Сейфуллин атындағы Қазақ агротехникалық зерттеу университеті, Астана, Қазақстан

³"Science and Technology Solutions" АҚ, Алматы, Қазақстан

⁴«Алтай геологиялық-экологиялық институты» ЖШС, Өскемен, Қазақстан

<p>Мақала келді: 13 қыркүйек 2023 Сараптамадан өтті: 20 желтоқсан 2023 Қабылданды: 5 наурыз 2024</p>	<p>ТҮЙІНДЕМЕ</p> <p>Мақалада дәрілік матрица рөлін атқаратын бактерицидтік қасиеті бар гидрофобты саздардан бионанокомпозит алу әдісі қарастырылған. Мұндай дәрілік бионанокомпозиттік материалдар жоғары адсорбциялық қасиетке және ұзартылған әрекетке ие болғандықтан, әртүрлі дәрумендер мен дәрі-дәрмектерді тасымалдаушы матрица ретінде пайдалану үшін ұсынылады. ПЭМ талдауы арқылы Белое Глинище кенорнының галлуазиті нанобөлшектің диаметрі 200 нм-ден 1000 нм-ге дейін цилиндр тәрізді және нанобөлшектердің диаметрі 50 нм-ден 80 нм-ге дейін болатындығы анықталған. Бионанокомпозитті өндіру процесінің бірінші кезеңінде микробқа қарсы қасиеттер беру үшін галлуазит нанобөлшектері күміс иондарымен өңделді, ал келесі кезеңде катионды беттік белсенді затпен гидрофобталады. Катионды БАЗ адсорбциясы жүргендігі рентгендіфракциялық әдіспен анықталды, мұны пакетаралық кеңістік 9,998 Å-дан 17.5 Å-ға дейін ығысқанынан білуге болады. Сондай-ақ, Фурье инфрақызыл спектроскопиясына сәйкес адсорбциялық жолақтардың болуы БАЗ молекулаларының галлуазитте адсорбцияланатынын дәлелдеді. Энергетикалық дисперсиялық рентгендік спектроскопияда күміс иондарының сәйкес жұту жолақтарының болуы галлуазит нанотүтікшесінде күмістің адсорбцияланатынын дәлелдейді. Күміспен өңделген және органофильді галлуазиттің микробқа қарсы қасиеттері ішек таяқшасына қарсы invitro нәтижелері бойынша микробиологиялық зертханада анықталды. Гидрофобталған органогаллуазит үлгілерінің жиік бұрыштары 90 градустан жоғары болды және бұл барлық үлгілердің гидрофобты екенін дәлелдейді. Алынған органофильді саздар агар-агар матрицасына енгізіліп, сұйық ортада тұрақтылық беретін.</p> <p>Түйін сөздер: Бионанокомпозит, галлуазиттің наносаз, күмістелген саз, бактерицидтік қасиет, олеофилизация, микросфера, дәрі тасымалдағыштар.</p>
<p>Ибраимова Дана Мыкты-Кереевна</p>	<p>Авторлар туралы ақпарат: химия ғылымдарының кандидаты, аға оқытушы, Эл-Фараби атындағы Қазақ ұлттық университеті, 050000, Эл-Фараби даңғылы, 71, Алматы, Қазақстан. E-mail: dana_kereevna@kaznu.kz</p>
<p>Рожкова Ольга Владимировна</p>	<p>химия ғылымдарының докторы, «С.Сейфуллин атындағы Қазақ агротехникалық зерттеу университеті» КЕАҚ профессоры, Астана, Қазақстан, 010000, Жеңіс даңғылы, 62, Астана қ., Қазақстан, «Science and Technology Solutions» АҚ, Астана, Қазақстан, 010000, Республика даңғылы, 24. E-mail: rozhkova.o@stsolutions.kz</p>
<p>Мұсабеков Қуанышбек Битұулы</p>	<p>химия ғылымдарының докторы, профессор, Қазақстан Республикасы Ұлттық жаратылыстану ғылымдары академиясының академигі, атындағы Қазақ ұлттық университеті. Эл-Фараби, 050000, Эл-Фараби даңғылы, 71, Алматы, Қазақстан. E-mail: musabekov40@mail.ru</p>
<p>Тәжібаева Сағдат Медербекқызы</p>	<p>химия ғылымдарының докторы, Эл-Фараби атындағы ҚазҰУ профессоры, 050000, Эл-Фараби даңғылы, 71, Алматы, Қазақстан. E-mail: tazhibayeva_s@mail.ru</p>
<p>Рожков Виталий Игоревич</p>	<p>т.ғ.к., «Сакен Сейфуллин атындағы Қазақ агротехникалық университеті» ҰБ, Астана, Қазақстан; «Алтай геологиялық-экологиялық институты» ЖШС, Өскемен, Қазақстан. E-mail: Vitalrza1983@gmail.com</p>
<p>Ермеков Марат Тегінбайұлы</p>	<p>«Science and Technology Solutions» АҚ Жоба және активтерді басқару департаментінің директоры, 050000, Алматы, Қазақстан. E-mail: yermekov.m@stsolutions.kz</p>

Особенности получения композиционного материала из гидрофобного галлуазита, обладающего антимикробными свойствами

¹Ибраимова Д.М.-К., ^{2,3}Рожкова О.В., ¹Мұсабеков К.Б.,
¹Тәжібаева С.М., ^{2,4}Рожков В.И., ³Ермеков М.Т.

¹Казахский национальный университет имени аль-Фараби, Алматы, Казахстан

²Казахский агротехнический исследовательский университет имени Сакена Сейфуллина, Астана, Казахстан

³АО «Science and Technology Solutions», Алматы, Казахстан

⁴ТОО «Алтайский геолого-экологический институт», Усть-Каменогорск, Казахстан

<p>Поступила: 13 сентября 2023 Рецензирование: 20 декабря 2023 Принята в печать: 5 марта 2024</p>	<p>АННОТАЦИЯ</p> <p>Рассмотрен способ получения бионанокompозита из гидрофобных глин с бактерицидными свойствами, который играет роль лекарственной матрицы. Такие лекарственные бионанокompозитные материалы рекомендуются для использования в качестве носителей различных витаминов и лекарственных препаратов благодаря их высоким адсорбционным свойствам и пролонгированному действию. Установлено с помощью ПЭМ-анализа, что частицы галлуазита месторождения Белое Глинище являются наноразмерными и имеют цилиндрическую форму длиной от 200 нм до 1000 нм и диаметр наночастиц от 50 нм до 80 нм. Первой стадией процесса изготовления бионанокompозита являлась обработка наночастиц галлуазита ионами серебра для придания антимикробных свойств, а на следующем этапе была проведена гидрофобизация катионным поверхностно-активным веществом. Рентгендифракционным методом установлена адсорбция катионным ПАВ, так как межпакетное пространство расширена с 9,998 Å до 17.5 Å. Также ИК-спектроскопия Фурье наличием соответствующих полос поглощения доказала адсорбцию молекул КПАВ на галлуазите. Наличие соответствующих полос поглощения ионов серебра на энергодисперсионной рентгеновской спектроскопии доказывает адсорбцию серебра на галлуазитовой нанотрубке. Установлены антимикробные свойства обработанного серебром и органофильного галлуазита микробиологическими лабораторными результатами инвитуру по отношению кишечной палочки <i>Escherichia coli</i>. Гидрофобизированные образцы органогаллуазита имели краевые углы выше, чем 90 градусов и это доказывает, что все образцы являются гидрофобными. Полученные органофильные глины были внесены в агар-агаровую матрицу и были брошены в раствор кальция хлорида, которые придают им устойчивость в жидкой среде. Практически установлено, что наиболее оптимальной бионанокompозитной микросферой является 50% соотношение органогаллуазита и агар-агар.</p>
	<p>Ключевые слова: Бионанокompозит, галлуазитовая наноглина, серебряная глина, бактерицидные свойства, олеофилизация, микросфера, носители лекарств.</p>
<p>Ибраимова Дана Мыкты-Кереевна</p>	<p>Информация об авторах: кандидат химических наук, старший преподаватель, КазНУ им. аль-Фараби, 050000, проспект аль-Фараби, 71, Алматы, Казахстан. E-mail: dana_kereevna@kaznu.kz</p>
<p>Рожкова Ольга Владимировна</p>	<p>доктор химических наук, профессор НАО «Казахский агротехнический исследовательский университет имени С.Сейфуллина», Астана, Казахстан, 010000, проспект Женис, 62, г. Астана, Казахстан, АО «Science and Technology Solutions», Астана, Казахстан, 010000, проспект Республики, 24. E-mail: rozhkova.o@stsolutions.kz</p>
<p>Мусабеков Куанышбек Битуович</p>	<p>доктор химических наук, профессор, академик Национальной академии естественных наук РК, КазНУ им. аль-Фараби, 050000, проспект аль-Фараби, 71, Алматы, Казахстан. E-mail: musabekov40@mail.ru</p>
<p>Тажибаяева Сагдат Медербекевна</p>	<p>доктор химических наук, профессор Казахского национального университета имени аль-Фараби, 050000, проспект аль-Фараби, 71, Алматы, Казахстан. E-mail: tazhibayeva_s@mail.ru</p>
<p>Рожков Виталий Игоревич</p>	<p>кандидат технических наук, НАО «Казахский агротехнический университет имени Сакена Сейфуллина», Астана, Казахстан; ТОО «Алтайский геолого-экологический институт», Усть-Каменогорск, Казахстан. E-mail: Vitalrza1983@gmail.com</p>
<p>Ермеков Марат Тегинбаевич</p>	<p>директор департамента Управления проектами и активами АО «Science and Technology Solutions», 050000, Алматы, Казахстан. E-mail: yermekov.m@stsolutions.kz</p>

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