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A.A. Toksanbay[1], Zh.B. Kubasheva[1*],
D.T. Rakhmatullaeva[1], B.E. Savdenbekova[1], A.K. Ospanova[1],
D.Zh. Batyrbayeva[2], N.F. Uvarov[3]

[1]Al-Farabi Kazakh National University, Almaty, Kazakhstan
[2] Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan
[3] Novosibirsk State Technical University, Novosibirsk, Russian Federation
*e-mail: bekisanova@gmail.com

Preparation of the antibacterial coatings based on natural mineral materials

Abstract. The purpose of the study is to obtain nanofilms based on clay minerals and to study the antibacterial activity of the obtained nanocomposite coatings. Diatomite and kaolin were pretreated with sulfuric acid. Silicon plates were used as model substrates. For the first time, multilayers of the composition diatomite/PAA and kaolin/PAA were obtained by multilayer assembly. In the multi-layer, the antiseptic chlorhexidine was introduced, which plays the role of an antibacterial agent. Scanning electron microscopy (SEM) determined the distribution and thickness of the obtained multi-layer, the elemental composition of nanofilms was determined by the SEM-EDX method. The specific surface of natural and sulfuric acid-modified diatomite and kaolin was examined by the BET method. The wetting angle was determined by the lying drop method. Obtaining coatings on the surface of implantable systems is one of the promising areas in modern medicine. In this regard, the production of nanocovers with antibacterial properties is an urgent issue in the fight against infectious diseases associated with bacterial growth. One of the modern methods for producing nanofilms currently used is the Layer by Layer (LBL) method. Nanofilms obtained by this method have found application in tissue engineering and dressings. The feature of this study is the use of clay minerals such as diatomite of the Mugodzharsky deposit and kaolin of the Alekseevsky deposit, which are concentrated in the west and north of Kazakhstan. The obtained multilayers were used as carriers for chlorhexidine and their antibacterial activity was studied. Nanofilms of diatomite/PAA/chlorhexidine and kaolin/PAA/chlorhexidine were tested against the *Escherichia coli (E.coli)* gram-negative bacterium. Thus, the obtained nanocomposite coatings based on kaolin and diatomite can be potential carriers for drugs.
Key words: nanofilms, kaolin, diatomite, antibacterial activity, LbL method, nanocoating

Introduction

Currently, there is a need to introduce nanocomposite materials into medical practice, which include antimicrobial components. In the world, there is an obvious and critical need to prevent contamination of surfaces of medical devices from sweat microorganisms, causing potential health risks [1]. Contamination of surfaces of medical materials leads to microbial colonization of a foreign body. In turn, antibacterial coatings (biofilms) can not only prevent the effects of bacteria but also increase the healing process of the resulting wounds [2].

One of the promising methods of combating infectious diseases associated with the appearance of bacterial biofilms on the surface of implanted products or external wounds is the application of nanofilms with the desired medico-biological properties [3]. Nanofilms can exhibit antibacterial, anti-inflammatory, and hemostatic properties. As a result of the content of various nanoparticles of bioactive metals, drugs, and antibacterial agents [4]. In the period of the 80s and 90s of the last century, a method of self-assembly of nanofilms was developed for bioactivation of the surface of solids, which later became known as the multilayer assembly "Layer-by-Layer" (LbL)method. The method is based on electrostatic, partially hydrogen, and in some systems on covalent or coordination bonding of polyacids and polybases [5-7]. The method allows one to obtain ultrathin films of a given thickness and composition from various systems; moreover, an assembly can be carried out on a charged surface of any geometry. The undoubted advantage of the method is the simplicity of the technology: the process can be carried out in the air and at room temperature. At present, to obtain multilayers by this method, researchers use many polymeric materials, starting with biopolymers such as proteins [8] or DNA [9-11], as well as inorganic substances such as clays [12]. Various biomedical implantable products can be taken as solids, and silicon and glass plates, as well as highly porous clay materials, can be taken as model samples. In modern practical medicine, scientists are actively studying the use of natural clay minerals in nanotechnology, for example, obtaining nanofilms based on montmorillonite for packaging materials, in tissue engineering, or using clay minerals as carriers for wound dressing and drug delivery [13-15].

Natural clay minerals are used as objects to obtain highly porous nanofilm substrates. As a substrate, montmorillonite [16; 17], kaolin [18], diatomite [19] are often used.

The healing properties of clay minerals, passed from ancient cultures, continue to be used in modern life to treat various diseases. With the advent of modern technology, the benefits of using clay minerals in several industries have also been explored. Clay minerals have specific physicochemical characteristics, such as high surface reactivity (adsorption and cation exchange capacity), colloidal and swelling capacity, optimal rheological behavior, and high water dispersibility, which makes them suitable for various biological applications, including pharmaceuticals [20], cosmetics [21], veterinary medicine [22], biomaterials [23], and biosensors [24].

Nanofilms based on clay materials containing ions of biocidal metals are widely used in modern pharmaceutical chemistry. Composite materials such as ions of biocidal metals / clay objects will exhibit antibacterial and anti-inflammatory properties [25].

As well as the potential of the application of polyelectrolyte multilayers for biomedical purposes was discussed in reviews [6-26]. Clay particles are very often used as one of the components of LbL films [27]. LbL films were obtained by the authors from positively charged polyelectrolytes such as polydiallyl dimethyl ammonium chloride, polyallyl amine hydrochloride, polyethyleneimine, and copolymers containing quaternary monomers of acrylic ammonium. The components were applied in a specific sequence to negatively charged clay particles, and it was found that after applying each layer of the surface, the charge changes from positive to negative.

Hybrid organic-inorganic nanocomposites based on polymers and clay plates make it possible to prepare modern advanced materials with new applied properties in pharmaceuticals [28] for obtaining nanocapsules with controlled release of drugs, biosensors, [29], as well as for the preparation of fuel cells for fire-resistant materials [30].

Clay-containing LbL films are known for their high strength, [31; 32] fire resistance, and magnetic properties [33]. The potential of layered clays/polymer nanocomposites as antimicrobial coatings has been investigated by many researchers. Podsiadlo et al. showed that clay containing nanostructured LbL coatings have not only high strength but also strong antimicrobial properties after the introduction of silver (AgNPs) nanoparticles stabilized with starch [34].

The purpose of this study is to obtain nanofilms based on natural clay minerals and study their antibacterial activity. The advantage of clay materials is that they are environmentally clean and economically profitable, as well as huge deposits are concentrated in Kazakhstan.

Materials and methods

Characteristics of materials. Minerals of Kazakhstan deposits were used in this study. The source of natural diatomite was the Mugodzhar deposit (Kazakhstan, Aktobe region). The source of kaolin was the Alekseevskoye deposit (Kokshetau region, Kazakhstan). Phosphoric acid H_3PO_4 was used for acid modification of kaolin diatomite. As substrates were used silicon wafers (Sigma Aldrich Saint Louis, MO 63103, USA) polished on one side (< 100 >) of the N-type with an oxide layer of ~ 2 nm. Linear polyethyleneimine (PEI powder, MW=10 kDa, Sigma Aldrich Saint Louis, MO 63103, USA) was applied to treat silicon wafers. Polyacrylic acid (PAA powder, MW = 17.7 kDa, Sigma Aldrich Saint Louis, MO 63103, USA) was used for obtaining multi-layers.

Characterization of instrumental methods. The physicochemical characteristics of the obtained nanofilms were examined by various methods. Surface morphology and elemental composition of natural and modified minerals (kaolin and diatomite) were determined by using scanning electron microscopy (SEM-EDX Quanta 3D 200i Dual system, FEI, USA).The specific surface area of the natural and modified diatomite and kaolin was studied on a Sorbtometer-M (VCJSC, KATAKON, Novosibirsk) by BET (Brunauer-Emmett-Teller) method. The specific surface area analyzer and the specific surface area was determined by thermal desorption of nitrogen. Determination of silicon wafer-water contact angle was performed by the method of the lying drop at the 25 ° C temperature and normal pressure by a Drop Shape Analyzer DSA100 (KRUSS GmbH, Germany). The average droplet diameter was 2-5 mm. The contact angle was measured at five points to obtain an average wetting angle.

Preparation of the nanofilms based on diatomite/PAA and kaolin/PAA. Natural kaolin and diatomite were subjected to acid activation to increase the specific surface area and remove impurities. The acid treatment was carried out with 10% phosphoric acid (H_3PO_4) for 5 hours. Then the precipitate was washed with distilled water to neutral pH=7 and dried for 5 hours at 100 °C.

Silicon wafers were used as standard solid support. The surface of the plates was pre-treated with a mixture of sulfuric acid and hydrogen peroxide ("piranha") for 1 hour and washed with a large amount of distilled water to remove residues of impurities.

The preparation of multi-layers on the surface of substrates was carried out by the method of multi-layer assembly [35], which is based on the sequential adsorption of two solutions. The substrates were previously treated with a PEI solution, the molecular layer of which created a uniform positive charge on the hydrophilic surface of the flint plate to enhance bonding with negatively charged PAA molecules, as shown on Figure 1.

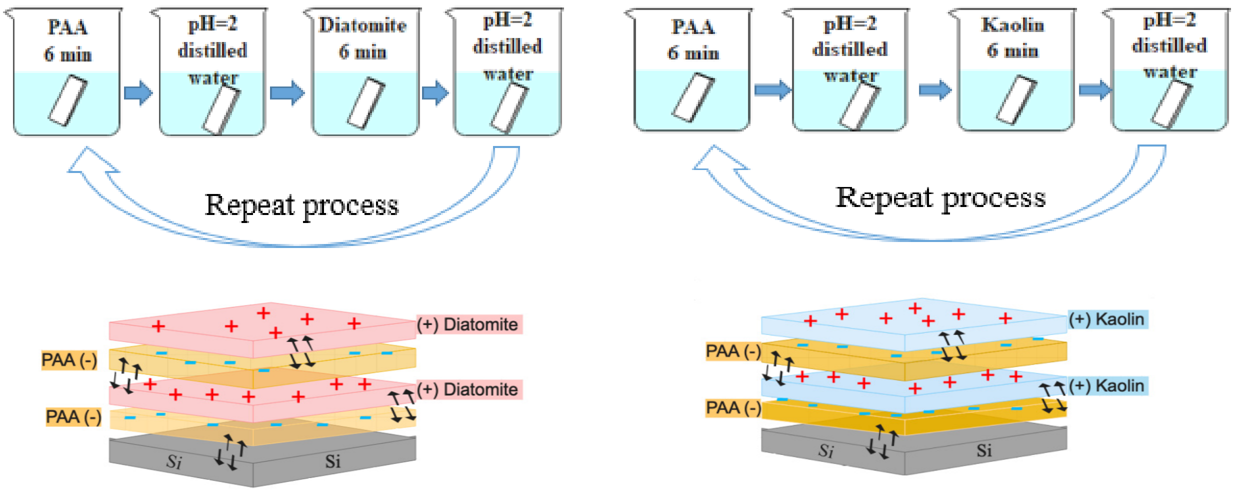

Figure 1 – Diatomite/PAA and kaolin/PAA multilayer assembly scheme

A layer of diatomite or kaolin that has a positive charge is then applied to the negatively charged surface. An acid-base bond is formed between the layers by electrostatic interactions, as schematically shown on Figure 1. The process of assembling multi-layers was as follows: a purified silicon plate was lowered into a polyethyleneimine solution at a concentration 0.001 mg/ml for 30 min to strengthen the bonding of multi-layers with the surface. The polyacrylic acid solution at a concentration of 0.02 mg/ml and a clay mineral suspension of 0.04 mg/ml, respectively, were used as the polyanion and cation, which were previously sonicated for 72 hours. The wafer was then lowered into a PAA solution for 6 min, washed at pH=2, then lowered into a solution of clay minerals (diatomite or kaolin).

Each application step was followed by washing with water at a pH of 2.0. In this manner, the desired amount of bilayers was obtained. The intercalation of the antibacterial agent was carried out by impregnation of the obtained multi-layers in a chlorhexidine solution with a concentration of 0.3 g/l for 48 hours.

Determination of antibacterial activity. The determination was carried out by diffusion into agar-agar on a dense nutrient medium by comparing the size of growth inhibition zones of test microbes formed by testing solutions of certain concentrations. The *Escherichia coli (E.coli)* was used as a reference strain for testing antibacterial activity.

The nutrient agar volume of 20 ml was poured into sterile Petri dishes. The thickness of the agar layer affects the determination results, so the specified amount of nutrient medium was strictly observed. Mueller Hinton and Sabouraud agar media were used as the nutrient medium.

To obtain lawns, a homogeneous suspension of bacterial cells was prepared in physiological saline, corresponding to the McFarland turbidity standard of 0.5 Units. The bacterial suspension was applied with a sterile tampon on the surface of the agar in three different directions. A titanium implant with antimicrobial properties was applied to the dried surface of the agar 5-10 min after incubation.

The cups were left at room temperature for 30 min and then incubated in a thermostat at 28-37°C for 24 hours without reversing. The formation of a clear zone around the sample is an indicator of antibacterial activity for the obtained materials. Microbial growth inhibition zones were measured with a millimetre line.

Results and discussion

Brunauer-Emmett-Teller method. Modification of natural kaolin and diatomite with phosphoric acid was carried out to increase the specific surface area. As a result, the specific surface area of diatomite and kaolin increased almost 2 times, data presented in Table 1.

Table 1 – Specific surface area of natural and modified diatomite and kaolin

Sample	Specific surface area, m^2/g	Specific pore volume, cm^3/g	Average pore size, nm
Natural diatomite	32.689	0.018	1.713
Diatomite +H_3PO_4	64.226	0.028	1.713
Natural kaolin	13.453	0.006	1.713
Kaolinite + H_3PO_4	33.166	0.012	1.713

The specific surface area of the natural diatomite after modification with phosphoric acid increased from 32.689 m^2/g to 64.226 m^2/g, and in kaolinite from 13.453 m^2/g to 33.166 m^2/g. The specific volume also increases almost 2 times, and the average pore size remains unchanged. After modification, the porous structure and physicochemical properties of diatomite and kaolin are expected to improve.

Plate cleaning and ultrasonic treatment. In modern medicine, solid substrates are used that differ from each other in chemical nature, physical characteristics, and therefore various activation methods are used for them. However, during storage, the surface of such substrates becomes highly contaminated as a result of the adsorption of dust and water from the air, as well as organic and inorganic impurities. Therefore, an important requirement for obtaining a coating with a developed surface is the preliminary treatment of solid substrates [36].

Silicon wafers were used as model substrates. As a result of the analysis of chemical and physical methods of surface cleaning, etching with a piranha solution based on organic and inorganic substances was chosen [37]. Figure 2 shows the results obtained before and after processing the silicon wafers. As can be seen from Figure 2, a, it is possible to observe the presence of certain impurities and an oxide film. This film has formed as a result of long storage in the open air. After processing, you can see the complete removal of the oxide film and clean from physical contaminants (Figure 2, b). Perhaps this is due to the fact that sulfuric acid, which is part of the piranha solution, dissolves the oxide film and thereby excludes the process of surface polishing. As a result of oxide film removal and etching, a change in surface wettability can also be observed (Figure 2, c).

To obtain nanofilms based on kaolin and diatomite, it is necessary that the solution contains nanosized particles of clay minerals. Therefore, an aqueous mixture of diatomite (Figure 3, No. 1, 2) and kaolinite (Figure 3, No. 3, 4) was subjected to dispersion for 72 hours in an ultrasonic bath to a clay-water suspension.

After treatment, turbidity of the solution was observed, which indicates the appearance of nanosized particles of diatomite and kaolin in the solution.

Scanning electron microscopy and SEM EDX study. The elemental composition of multilayers based on diatomite and kaolin before and after introduction of chlorhexidine is presented in Table 2.

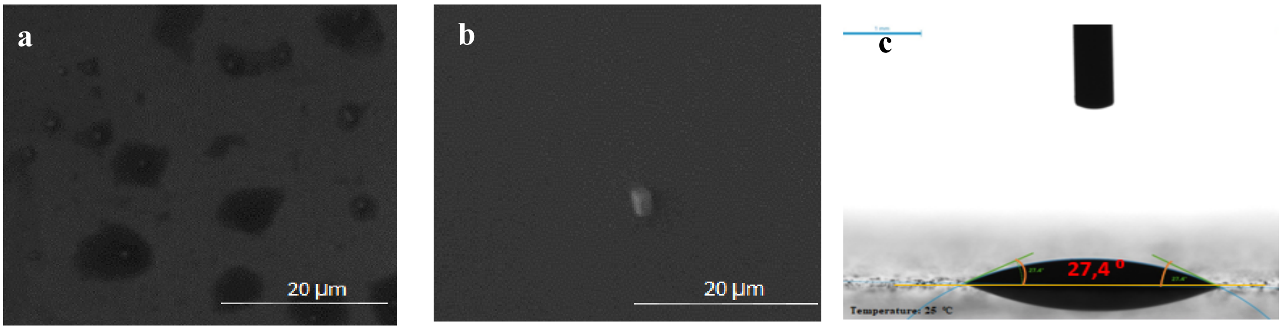

Figure 2 – Topographic images (SEM) of the surfaces of silicon substrates (a) before processing, (b) after processing, and the contact angle after processing (c)

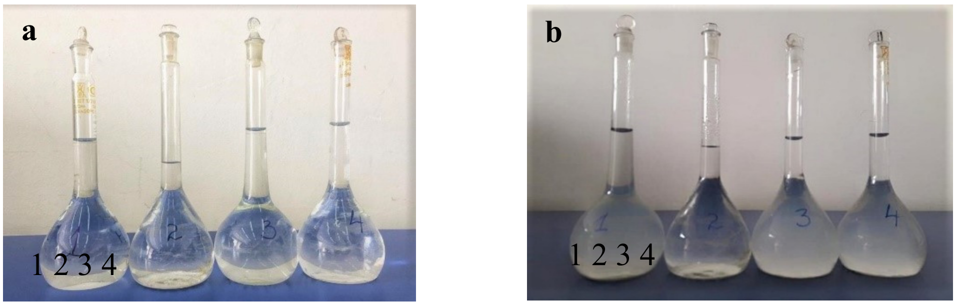

Figure 3 – Ultrasonic treatment of diatomite and kaolin suspensions before (a) and after (b) ultrasonic treatment of clay-containing solutions

Table 2 – Elemental composition of diatomite and kaolin nanofilms

Sample	Chemical element					
	C	O	Al	Si	Na	Cl
PAA/diatomite	5.30	5.80	1.15	87.75	-	-
PAA/diatomite+chlorhexidine	17.56	2.12	0.61	72.03	3.11	4.56
PAA/kaolin	6.42	13.66	1.62	76.78	1.52	-
PAA/kaolin + chlorhexidine	12.26	1.15	-	83.27	1.58	2.64

This method was used to obtain 100 bilayers consisting of 100 PAA monolayers and 100 diatomite or kaolin monolayers. Based on the results of SEM, the morphology and thickness of nanofilms of clay minerals on the substrate surface were studied. Figure 4 shows topographic images of 100 diatomite/ PAA-based bilayers on the surface of a silicon wafer before coating (a), after coating (b), and the thickness of the multilayer (c), which was measured at 815 nm, 320.5 nm, and 1.75 µm.

It can be seen from the results of these data that coatings based on diatomite are not uniformly formed over the surface compared to multilayers based on kaolin-PAA, for which the average film thickness over the entire surface is about 13.36 µm. This distribution of multilayers is likely associated with the chemical structures of diatomite and kaolin. Diatomite has an amorphous structure, which consists of fragments of frustull, while kaolin is a layered structure of a tetrahedral sheet (SiO_4) and an octahedral sheet (AlO_6), which are linked by oxygen atoms [11].

The resulting multilayers were used as carriers for drugs. Chlorhexidine was used as an antiseptic, which was applied by impregnation to the resulting films for 30-36 hours at a temperature of 370 °C. The presence of chlorhexidine in nanofilms based on diatomite and kaolin was confirmed using the SEM-EDX method. It was revealed that in multilayers based on diatomite, chlorhexidine content is 4.56%, and based on kaolin 2.64%

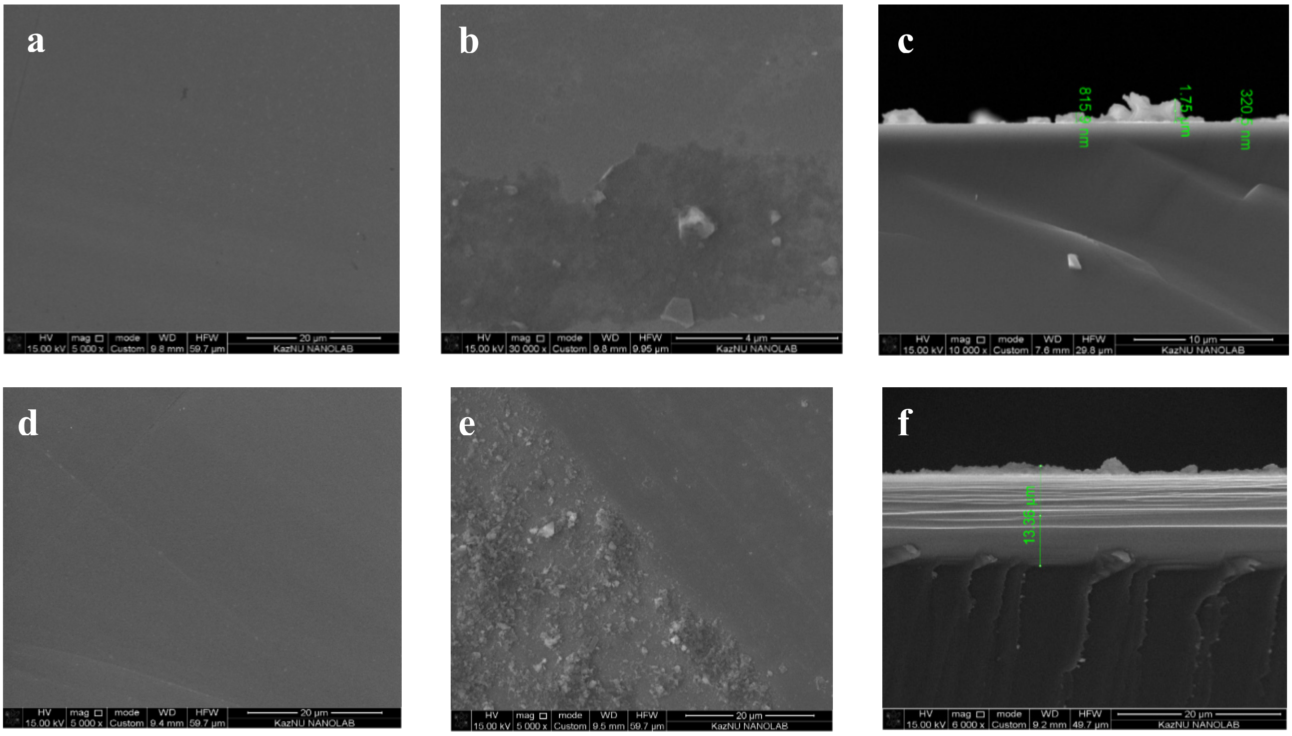

Figure 4 – Topographic images of 100 bilayers of diatomite/PAA (a, b, c) and kaolin / PAA (d, e, f)

Determination of antibacterial activity. Preliminary studies of the antibacterial activity of the obtained multilayers containing chlorhexidine by the disk-diffusion method at S.Zh. Asfendiyarov Kazakh National Medical University are shown on Figure 5. Antibacterial activity was established against gram-negative bacteria *E. coli*. Nanofilms based on kaolin/PAA/chlorhexidine showed better antibacterial activity than diatomite/PAA/chlorhexidine films. Preliminary studies of the obtained multilayers based on diatomite and kaolin as carriers of drugs indicate that such studies are promising.

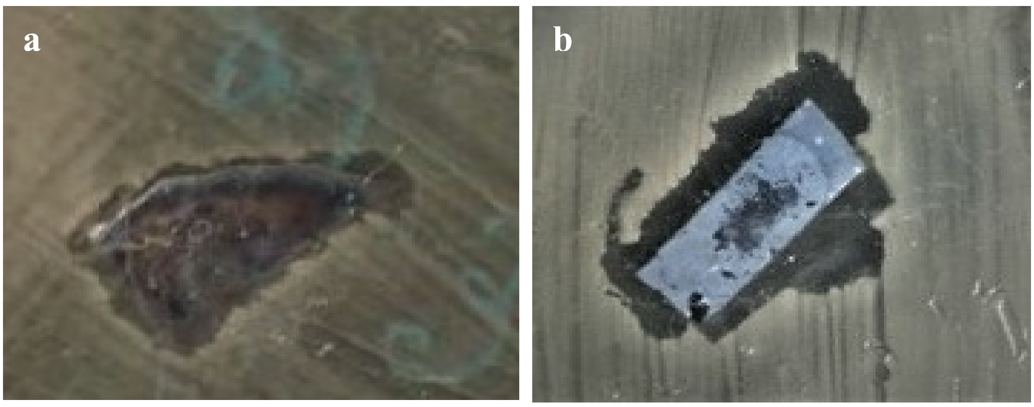

Figure 5 – Zone of suppression of bacteria *E. coli:*
a) a sample of diatomite, b) a sample of kaolin

Conclusion

For the first time the specific surface area of the natural diatomite after modification with phosphoric acid increased from 32.689 to 64.226, and in kaolinite from 13.453 to 33.166. The specific volume also increases almost 2 times, and the average pore size remains unchanged. After modification, the porous structure and physicochemical properties of diatomite and kaolin are expected to improve, the potential possibility of obtaining multilayers of diatomite-PAA and kaolin-PAA by the method of multilayer assembly on model flint plates has been established. The distribution and thickness of the films were characterized by various physicochemical methods. After impregnation of chlorhexidine in multilayers based on diatomite, the content of chlorhexidine is 4.56%, and on the basis of kaolin 2.64%.

The resulting multilayers were used as carriers for the antiseptic, chlorhexidine, and their antibacterial activity was studied. Multilayers based on kaolin/PAA/chlorhexidine showed better antibacterial activity against gram-negative bacteria *E.Coli* than multilayers based on diatomite/PAA/chlorhexidine.

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