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ADSORPTION COMPLEXES OF VANCOMYCIN WITH NANODIAMONDS: FORMATION KINETICS, COMPOSITION, AND ANTIMICROBIAL PROPERTIES

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Abstract. Adsorption complexes of vancomycin with detonation nanodiamonds having positive and negative surface charges are obtained. The kinetics of vancomycin adsorption on nanodiamonds is described by a pseudo-second-order equation with close parameters for both types of nanodiamonds. The kinetics of vancomycin-nanodiamond complex formation is described by a pseudo-first order equation. Methods of radioactive indicators and IR spectroscopy are used to find that a part of vancomycin is firmly bound to the surface of nanodiamonds and is not removed by washing. The amount of firmly bound matter is found to be three times greater for the complexes with negative nanodiamonds. However, the retention strength of vancomycin on positive nanodiamonds was higher and its content practically did not change during desorption for 10 days. Both types of complexes have the same antimicrobial properties against *Staphylococcus aureus*. The totality of the obtained data confirms the assumption that the formation of hydrogen bonds with water molecules plays a key role in the adsorption and retention of vancomycin on the surface of nanodiamonds.

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INTRODUCTION

Coatings containing nanodiamonds can be used to improve the mechanical characteristics of materials used for the fabrication of heart valve prostheses [1]. An important advantage of such coatings is the presence of an antiseptic agent on the surface of nanodiamonds to prevent the development of bacterial infections in the post-surgery period. One knows that the dose of drugs can be reduced when they are deposited on the functionally developed surface of nanodiamonds, and the desired therapeutic result will be achieved [2–6].

We have previously shown that both positive and negative nanodiamonds improve the mechanical performance of collagen matrices to an approximately equal extent, although the amount of positive nanodiamonds sorbed onto the matrix surface tends to be greater than that of negative nanodiamonds [1]. The stability of such coatings on the material surface during their use is also worth noting. The resulting coatings contain an antibiotic on the surface of nanodiamonds to prevent the development of infections and a layer that prevents calcium deposition, such as made of chitosan [7–10]. Note that the antibiotic included in the coating should retain antimicrobial activity when adsorbed on the surface of nanodiamonds.

The purpose of this work was to obtain adsorption complexes of vancomycin with nanodiamonds for them to be used in obtaining antimicrobial coatings of collagen matrices of bovine pericardium. Vancomycin is a drug belonging to the class of antimicrobial agents that inhibit cell wall synthesis and exhibit high activity against Gram-positive bacterial pathogens [11]. We have previously shown that vancomycin can be adsorbed on the surface of nanodiamonds [12]. When obtaining adsorption complexes, it is necessary to take into account the complexity of working with this compound, viz. its instability in aqueous solutions at neutral pH-conditions necessary for the formation of adsorption complexes with nanodiamonds of detonation synthesis, which have a functionally developed surface and are able to aggregate at a certain pH value and ionic strength of the solution. In this work, we study the adsorption kinetics of vancomycin on detonation synthesis nanodiamonds whose surface is positively (DND) and negatively (SDND) charged.

The amount of vancomycin in the solution and on the surface was determined using tritium-labeled vancomycin by radioactivity. The obtained complexes were characterized by IR spectrometry, and the antimicrobial activity of adsorption complexes against *Staphylococcus aureus*, one of the most common pathogens of bacterial infections, was determined.

EXPERIMENTAL PART

Studying the Adsorption Kinetics of Vancomycin on Nanodiamonds

In this work, we used [^{3}H]vancomycin obtained by the method of thermal activation of tritium [12]. Nanodiamonds (SDND, PlasmaChem), which represent an aqueous suspension of 100 mg/mL, were diluted with water to the required concentration. From the powder of nanodiamonds (PlasmaChem), an aqueous suspension (5 mg/mL) was prepared by ultrasonic treatment using a Grad ultrasonic bath (Russia). According to the low-temperature nitrogen adsorption method, the specific surface area of nanodiamonds is 390 and 250 m^2/g for SDND and DND, respectively. The IR spectra of the nanodiamonds used are summarized below. The electrokinetic potential values of the aqueous suspensions were $+21 \pm 2$ and -41 ± 5 mV for DND and SDND, respectively. For DND, p*I* is 11.4 while there is no isoelectric point for SDND in the p*H* range of 3 to 13.

We prepared 1 mL of nanodiamond suspensions in the [^{3}H]vancomycin solution with the concentration 0.7 g/L, the specific radioactivity 1 mCi/g, and the nanodiamond concentration 1 mg/mL. The suspensions were incubated at 25°C. At intervals, the suspensions were centrifuged for 20 min at 12100 g, and the radioactivity of the supernatant liquid was measured, which was then sampled completely. The nanodiamond precipitate was washed of unbound vancomycin and poured by the UltimaGold scintillation liquid (PerkinElmer) to measure radioactivity and determine the concentration of vancomycin on the nanodiamond surface using the equation

$$A = \frac{I}{\varepsilon a_{sp} m_{ND}}, \qquad (1)$$

where I is the count rate of tritium beta radiation, ε is the recording efficiency, a_{sp} is the specific (mass) radioactivity of vancomycin, and m_{ND} is the mass of nanodiamonds in the suspension.

Analyzing Complexes of Nanodiamonds with Vancomycin by IR Spectroscopy

1 mg of the nanodiamond complex with vancomycin was mixed with potassium bromide. The spectra were recorded using a Nicolet Protege 460 spectrometer in the wave number range from 400 to 4000 cm^{-1} at an optical resolution of 4 cm^{-1}.

Determining the Amount of Nanodiamond-Vancomycin Complex on the Surface of Collagen Matrices

In this work, we used collagen matrices of bovine pericardium stabilized and devitalized by glutaric aldehyde. We prepared the adsorption complexes of vancomycin with nanodiamonds according to the technique described in Section 2.1. In these experiments, we used tritium-labeled nanodiamonds obtained using the method of thermal activation of tritium [13]. After a day of incubation of nanodiamonds in the vancomycin solution, the suspension was centrifuged, the precipitate was decanted, washed with water, and suspended in water to a nanodiamond concentration of 1 mg/ml. Collagen matrices from bovine pericardium were placed in the suspension and stirred during 24h. After stirring, the matrices were placed in 0.9% sodium chloride solution. The matrices were then dried to constant weight and dissolved by boiling in concentrated nitric acid. The solution was added with water, centrifuged, and the radioactivity of the nanodiamond precipitate was measured as described in Section 2.1. The amount of nanodiamonds on the surface of the matrices (Γ_{ND}) was determined by the equation

$$\Gamma_{ND} = \frac{I}{\varepsilon a_{NDsp} m_{matrix}}, \qquad (2)$$

where a_{NDsp} is the specific radioactivity of nanodiamonds, m_{matrix} is the mass of the matrix.

Determining Antimicrobial Properties of Complexes of Nanodiamonds with Vancomycin

We prepared coatings from complexes of nanodiamonds with vancomycin on the surface of bovine pericardium collagen matrices by the method described in Section 2.3. Bacterial adhesion to modified and control samples of bovine pericardium collagen matrices was assessed in compliance with GOST R ISO 11737-1-2000 [14]. Clinical isolates of *Staphylococcus aureus* bacteria causing complications after valve replacement surgery were chosen as test cultures. All preparations and studies were performed under sterile conditions at an ambient temperature of 20–22°C.

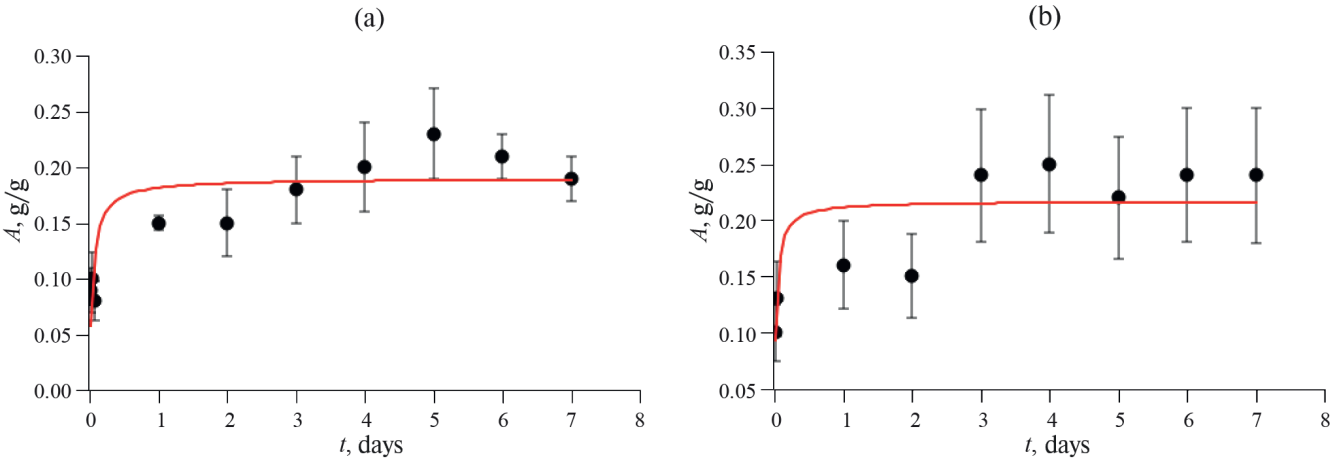

Fig. 1. Time dependences of the vancomycin adsorption on DND (a) and SDND (b) nanodiamonds.

Table 1. Parameters of the pseudo-second-order adsorption kinetics equation

Type of nanodiamonds	A_{eq}, g/g	k_2, day^{-1}	R^2
DND	0.19 ± 0.01	114.7 ± 51.0	0.974
SDND	0.22 ± 0.01	174.4 ± 97.2	0.973

From daily cultures, we prepared suspensions according to the McFarland method with a concentration of 10^6 cells/mL. The studied samples of collagen matrices with diamond-containing coating were immersed in the prepared culture suspensions and incubated in the thermostat at 37°C for 4 hours. At the end of the exposure, the samples were removed and washed from unadhered cells with sterile 0.9% NaCl and dried on sterile filter paper. Further, prints were made from the experimental and control samples by sequentially applying the smooth and villous sides of collagen plates to the surface of Hinton-Mueller nutrient medium followed by rubbing the obtained prints dry.

After the performed application, each plate was rubbed in 1 ml of the sterile physiological solution with sterile glass flakes. The obtained homogenates of 0.5 ml without dilution and with 10-fold dilution were placed on the dense Hinton-Mueller nutrient medium and rubbed dry with a sterile spreading rod. Seeds of experimental and control samples on the Hinton–Muller medium were incubated in the thermostat for 24 h at 37°C. At the end of incubation, the number of colonies of viable cells of test microorganisms on the test and control samples was counted.

DISCUSSION OF THE RESULTS

Figure 1 shows the adsorption kinetics of vancomycin on the surface of nanodiamonds found from the variation of vancomycin concentration in the solution.

The kinetic dependences of adsorption on nanodiamonds of both types were described by the pseudo-second-order equation [15]

$$A = \frac{A_{\text{равн}}^2 k_2 t}{1 + A_{\text{eq}} k_2 t}. \quad (3)$$

Parameters were selected using the SciDAVis software package with the Levenberg–Marquardt algorithm applied with a tolerance of 0.0001. The result of approximation is shown in Fig. 1 by a line. Table 1 gives the parameters of the pseudo-second-order equation.

During the first 24 h, adsorption reaches its constant value of about 0.20 g/g. It is important to emphasize that the parameters of adsorption kinetics for both types of nanodiamonds were close. This confirms the previously proposed assumption that the adsorption of vancomycin on the surface of nanodiamonds occurs through the formation of bonds between sorbate and water molecules bound to the surface [12]. Note that serious changes occur with vancomycin in water at concentrations greater than 20 g/L and storage times greater than 24 h [16].

Since nanodiamond-vancomycin complexes are supposed to be used as antibacterial coatings, it is necessary to determine the amount of vancomycin firmly bound to the surface of nanodiamonds. Therefore, after determining the adsorption of vancomycin by the difference between the initial concentration and the concentration in the solution at the current time, the

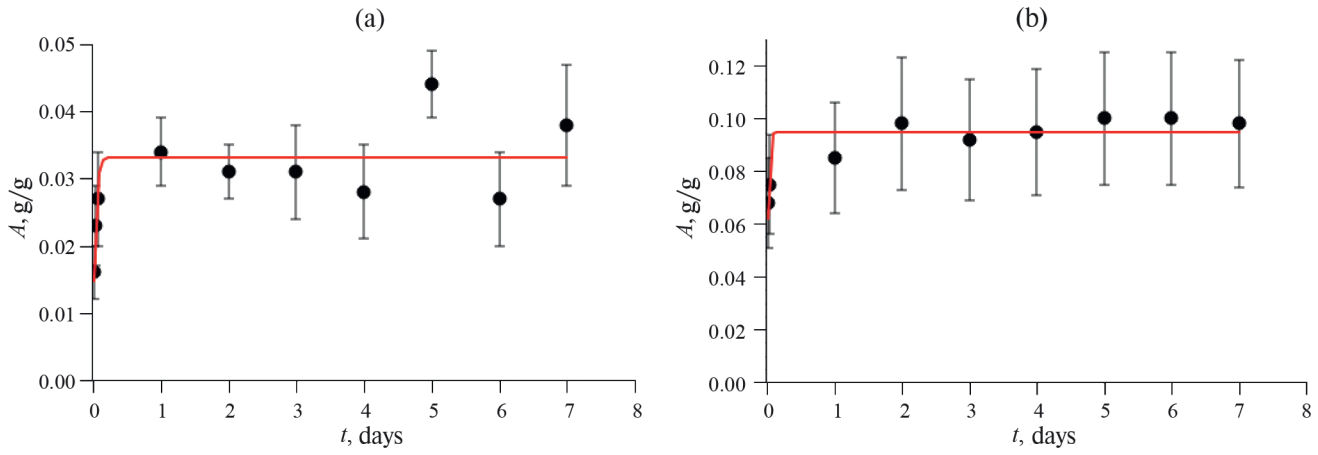

Fig. 2. Time dependences of firmly bound vancomycin on DND (a) and SDND (b) nanodiamonds.

nanodiamond precipitate was washed from the loosely bound vancomycin, its radioactivity was measured, and the remaining amount of vancomycin was determined by radioactivity (1). The dependence of vancomycin adsorption on nanodiamonds after washing the sample and measuring directly the radioactivity of the precipitate of the nanodiamond complex with vancomycin is shown in Fig. 2.

The kinetic dependences for both types of nanodiamonds had a similar form and were described by the pseudo-first order equation

$$A = A_{eq}\left(1 - e^{-k_1 t}\right). \quad (4)$$

The parameters of the pseudo-first-order equation are given in Table 2; the approximation results are shown in Fig. 2 by a line.

The limiting value of the amount of vancomycin firmly bound to the surface of both types of nanodiamonds is reached during the first day of adsorption. Taking into account the size of vancomycin molecule (3.2 × 2.2 nm) [17], the value of the vancomycin residual amount on SDND is close to the surface completely filled with densely packed vancomycin molecules. While the coverage of DND decreases by 4.5-5 times and three times less than on SDND.

The desorption of vancomycin from the surface of nanodiamonds was studied in water, in 0.9% sodium chloride solution, and in 40g/L bovine serum albumin solution in 0.9% NaCl for 10 days at 25°C. Table 3 summarizes the results.

The results in Tables 2 and 3 show that vancomycin is not removed from DND nanodiamonds in the presence of desorbing agents while vancomycin is easily desorbed from the SDND surface. One can assume

Table 2. Parameters of the pseudo-first-order kinetic equation for firmly bound vancomycin with nanodiamonds

Type of nanodiamonds	A_{eq}, g/g	k_1, day^{-1}	R^2
DND	0.033 ± 0.002	29.2 ± 9.1	0.977
SDND	0.095 ± 0.002	53.1 ± 8.6	0.996

Table 3. Desorption of vancomycin from the surface of nanodiamonds

Desorbing solution	Amount of vancomycin on the surface of nanodiamonds after desorption, g/g	
	DND	SDND
Water	0.030	0.009
0.9% NaCl	0.033	0.010
Serum albumin 40 g/L in 0.9% NaCl	0.022	0.003

that the retention of vancomycin on DND occurs due to the electrostatic attraction of the carboxyl group of vancomycin and positive groups on the DND surface.

Samples of nanodiamonds with the highest vancomycin content (adsorption for 1 day, washed with water) were analyzed by IR spectroscopy, and their antimicrobial properties were tested. Figures 3 and 4 show the IR spectra of vancomycin complexes with nanodiamonds.

In the IR spectrum of DND nanodiamonds (Fig. 3), the band at 1795 cm^{-1} refers to the vibrations of C=O-groups in anhydride and lactone groups on the nanodiamond surface [18]. The band at 1723 cm^{-1}

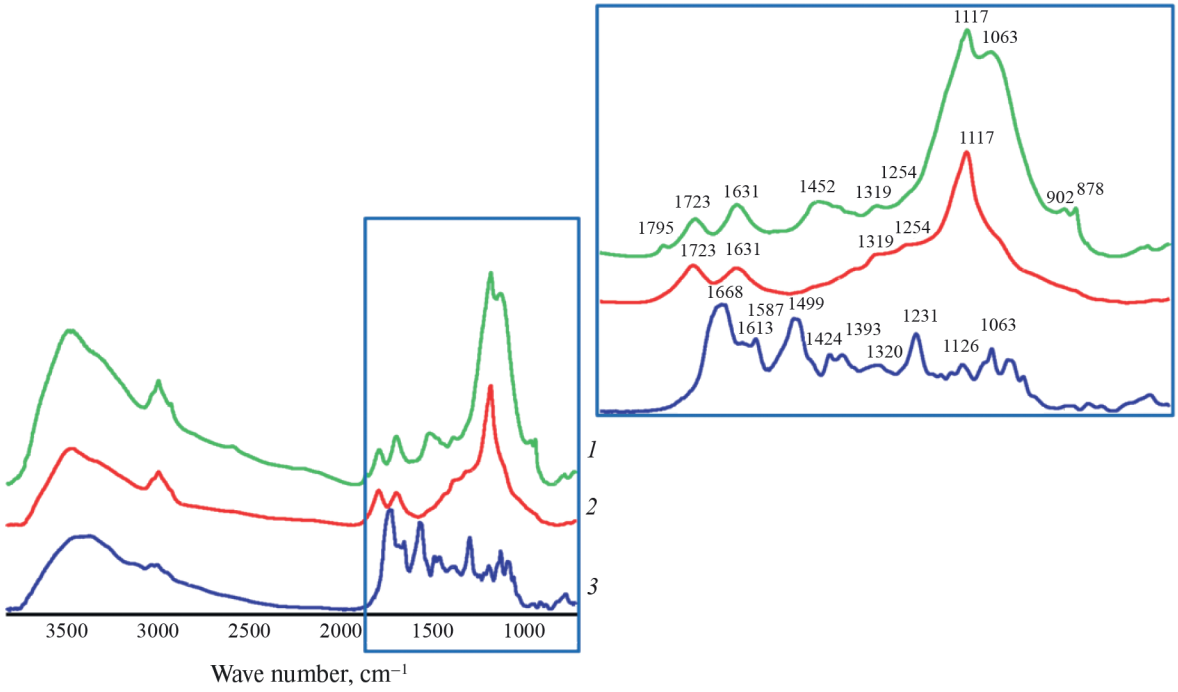

Fig. 3. IR spectra of DND (1), DND-vancomycin complex (2), and vancomycin (3).

refers to the vibrations of C=O-groups in carbonyls. The signal at 1630 cm^{-1} is explained by the presence of water bound to the surface. The band at 1452 cm^{-1} refers to the strain vibrations of CH bonds in CH_2-groups (sp^2 and sp^3) [19]. The signals at 1319 and 1254 cm^{-1} refer to valence vibrations of C-C bonds in sp^2- and sp^3- states. The signal at 1117 cm^{-1} refers to asymmetric vibrations of C-O-C bonds, and the signal at 1063 cm^{-1} refers to valence vibrations of the C-O bond in hydroxyl or ether groups [18]. The signals at 902 cm^{-1} and 878 cm^{-1} refer to the vibrations of CH bonds in $CH_2(sp^2)$-groups and the graphitized part of the surface, respectively [19].

One can also see anhydride groups on the SDND surface (Fig. 4) (the C=O-bond signal at 1776 cm^{-1}). The signal at 1465 cm^{-1} is related to the strain vibrations of the CH-bond in the CH_3-groups [19]. The band at 1254 cm^{-1} corresponds to the C-O bond vibrations in epoxy and ether groups [18].

The interpretation of the IR spectrum of vancomycin is given in detail in [20-22]. The signal at 1668 cm^{-1} is related to the C=O bond vibrations of amide I, the signals in the region of 1587 and 1499 cm^{-1} are related to the bond vibrations in the aromatic rings. Also, the vibrations of C=C bonds in aryl fragments are indicated by the band at 1613 cm^{-1} [23]. The signals at 1424 and 1393 cm^{-1} are related to the symmetric vibrations of the CO_2^--group [20, 23]. The signal at 1320 cm^{-1} is typical of amino acids, but its origin is uncertain [23]. The signal at 1231 cm^{-1} is due to vibrations of phenolic groups [22].

When complexes with SDND nanodiamonds are formed, the IR spectrum of the complex retains signals characteristic of vancomycin associated with vibrations of amide I and aromatic fragments, which are absent in the spectrum of the original diamond and confirm the presence of the substance on its surface. In contrast, the IR spectrum of the DND-vancomycin complex contains signals characteristic of nanodiamonds. The bands 1063, 902, and 878 cm^{-1} disappear, which suggests that a small amount of vancomycin binds to graphitized parts of positively charged nanodiamonds.

Thus, the IR spectroscopy data confirmed the data of radiochemical method about the significantly lower amount of vancomycin on DND as compared to SDND, the surface of which is completely covered with the vancomycin layer.

To test the antimicrobial properties of the complex, it was necessary to obtain coatings of bovine pericardium collagen matrices with a layer of nanodiamond-vancomycin complexes [24]. Therefore, tritium-labeled nanodiamonds were used to determine the amount of the complex that adsorbed onto the surface of the collagen matrix. It was shown that 5.6 ± 1.7 mg/g of the complex with SDND and 8.6 ± 1.1 mg/g of the complex with DND could be deposited on the surface of the matrix. These values are larger than those previously obtained for positively and negatively

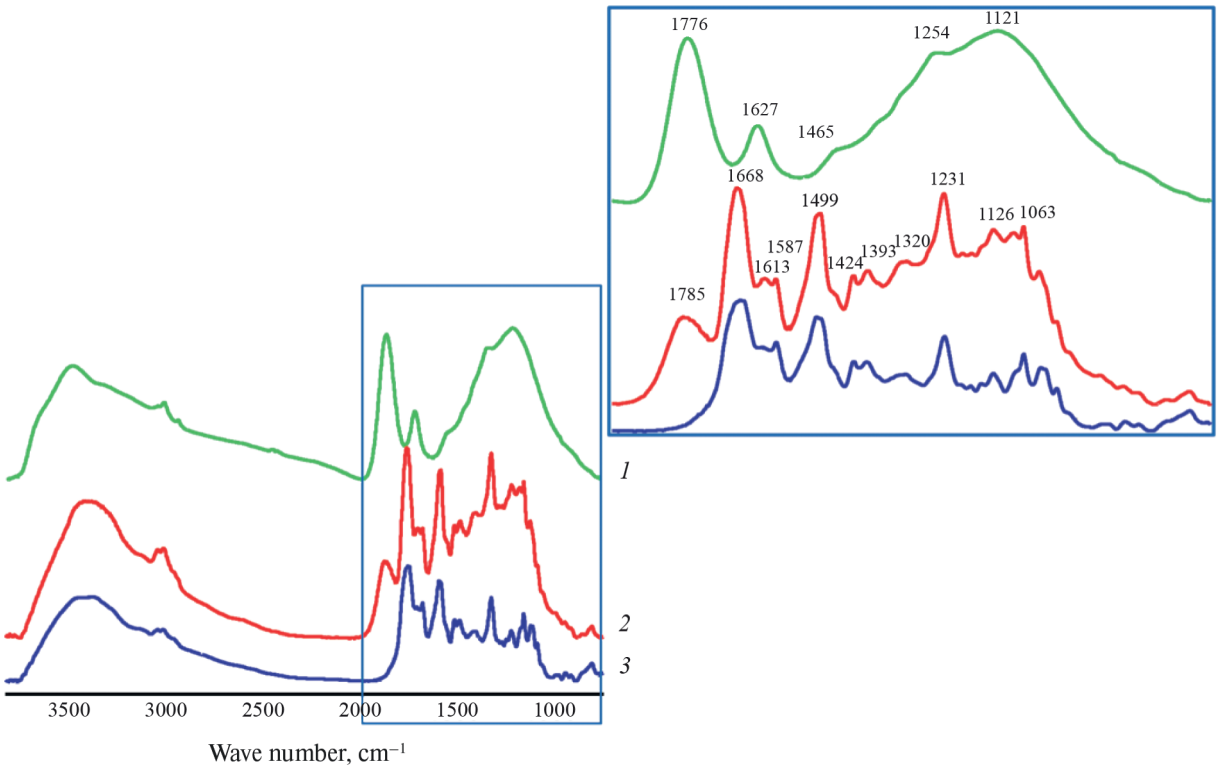

Fig. 4. IR spectra of SDND (1), SDND-vancomycin complex (2), and vancomycin (3).

charged nanodiamonds because thinner matrices were used, apparently better sorbing nanodiamonds. Thus, we concluded that the vancomycin content on the surface of the matrices is 0.27 and 0.56 mg vancomycin per 1 g collagen matrix for the complexes with DND and SDND, respectively. This value is lower than when vancomycin is applied from a pressurized solution in carbonic acid [25]. However, the results of studying antimicrobial properties against *Staphylococcus aureus* showed similar results for adhesion (Table 4). The survival rate was higher than in the case of vancomycin as part of coatings with hyaluronic acid and chitosan, but three orders of magnitude lower than in the case of the control sample.

Thus, nanodiamonds allows using less antibiotic to achieve the desired result.

Table 4. Antimicrobial characteristics of adsorption complexes of nanodiamonds with vancomycin

Type of nanodiamonds	Adhesion, lg CFU	Survival rate, lg CFU
Control sample without nanodiamonds	≈ 5	≈ 5
DND	2.4	2.1
SDND	2.4	2.2

CONCLUSIONS

As a result of this work, we showed that the adsorption kinetics of vancomycin is similar for two types of nanodiamonds and has two stages, viz. a layer with dense packing of molecules (the complex composition is 0.2 g/g) is formed during 1 day, and with increasing contact of the components there is a further slight decrease in the concentration of vancomycin in the solution, but the additional adsorption of vancomycin is reversible and the substance is easily washed out with water. The amount of vancomycin firmly bound to nanodiamonds was smaller and depended on the surface charge — it was 0.10 g/g for negatively charged SDND nanodiamonds and 0.03 g/g for positively charged DND. Further desorption of vancomycin into water and 0.9% NaCl for 10 days reduced its content to 0.01 g/g in the complex with SDND, but vancomycin was not extracted from the complex with DND under these conditions. In the presence of serum albumin, additional release of vancomycin from the complexes was observed. The antimicrobial properties of both complexes were similar, viz. adhesion and survival of bacteria on coatings from

both types of nanodiamonds was lower by about 10^3 times than for the control samples. Thus, the obtained complexes can be considered as potential components of heart valve prostheses.

The totality of the obtained data confirms the assumption that the formation of hydrogen bonds with water molecules plays a key role in the adsorption and retention of vancomycin on the surface of nanodiamonds.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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