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Mechanism of formation of adsorption complexes amikacin–detonation nanodiamond

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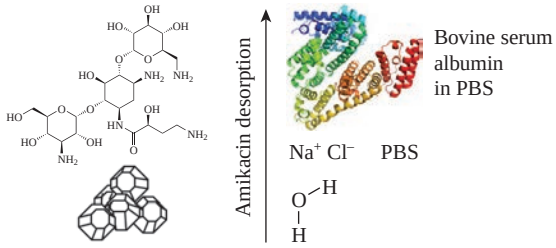
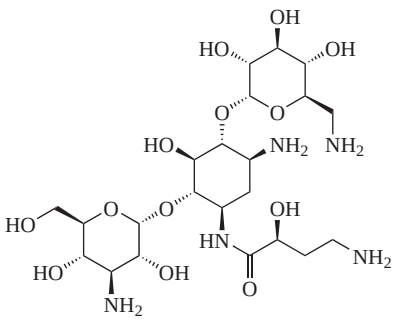
The adsorption of amikacin on the surface of detonation nanodiamonds was revealed as proceeding *via* the Coulomb or donor–acceptor interactions depending on the functional composition of the nanodiamond surface. In the case of nanodiamonds with carboxyl groups on their surface, the complexes were formed through the Coulomb interactions, while the donor–acceptor mechanism was realized in the presence of nitrate ions on the surface.



Amikacin is a broad-spectrum antibiotic used for a treatment of various diseases.[1–3] To design systems for the targeted delivery of amikacin, it is important to bear in mind many regulations, including the mechanism of its adsorption on various carriers. A reversible adsorption of this compound on polymeric materials is known.[4] It has previously been demonstrated that amikacin is capable of efficient sorption on a surface of the detonation nanodiamond (DND).[5] However, there are no reported data on the mechanism of interaction between amikacin and the DND surface. DNDs, nano-sized diamond particles (the diameter of primary particle is 4–5 nm), covered with functional groups are considered as a promising drug carrier due to their low toxicity and biocompatibility.[6,7] A quite high specific surface area and a diverse functional composition determined by a chemical treatment of the nanoparticles allow one to prepare the adsorption nanodiamonds covered with ions[8] and various substances, including drugs.[9–11]

The present work was aimed at the estimation of regularities in adsorption–desorption interactions of amikacin with detonation nanodiamonds possessing chemically different surfaces. For this purpose, the adsorption of amikacin in the form of its sulfate was carried out from an aqueous solution, and its desorption was also investigated using various desorbing solutions: water, phosphate buffered saline (PBS, pH 7.3±0.1, composition: 8 mM Na_2HPO_4, 2 mM K_2HPO_4, 0.146 M NaCl, and 8 mM NaN_3) increasing the ionic strength, and PBS solution of albumin (40 g dm^{-3}) mimic the serum.



Amikacin

Tritium-labeled amikacin prepared by the tritium thermal activation method according to the known procedure[5] was employed for the estimation of amikacin amount on the DND surface.† [^{3}H]Amikacin was adsorbed on the surface of DND washed with alkali and acid to remove possible impurities (DND-in), and

† A solution of [^{3}H]amikacin (in the form of sulfate) possessing the specific activity of 0.56 mC g^{-1} was mixed with an aqueous suspension of DND (SKTB 'Tekhnolog', St. Petersburg, Russia) containing the solid phase (5 mg) until the final amikacin concentration of 8.0±0.5 g dm^{-3}. In the case of DND-COOH, the experiment was carried out in the range of amikacin concentrations from 5 to 35 g dm^{-3}. The total volume of suspension was 1.4 cm^3. The suspensions were incubated at 25 °C for 9 days and then centrifuged, and the radioactivity of the supernatant was measured to determine the equilibrium concentration of amikacin:

$$c = I/(\varepsilon V a_{mol}), \quad (1)$$

where I is the count rate of tritium β-radiation, ε is the registration efficiency, V is the volume of supernatant solution aliquot, and a_{mol} is the molar radioactivity of amikacin.

The precipitate was washed with water and separated into four parts, each containing 1.25 mg of solid phase. An Ultima Gold scintillation liquid was added to one of them to determine the amount of sorbed amikacin, which was calculated according to equation:

$$\Gamma = I/(\varepsilon a_{mol} m), \quad (2)$$

where m is the mass of nanodiamonds in the suspension.

Desorbing agents (water, PBS, and a solution of bovine serum albumin in PBS with a concentration of 40 g dm^{-3}) were added to the three remaining parts. The suspensions were thermostated at 37 °C for 4–7 days. After that, DND was precipitated by the centrifugation, and the radioactivity of supernatant solution was measured. The supernatant was taken as completely as possible, scintillation liquid was added to the precipitate, the mixture was resuspended, and the periodic counting measurements were performed until reaching the constant values. The residual amount of amikacin was determined according to equation (2).

To confirm the absence of any tritium exchange between the [^{3}H]amikacin and water molecules during the experiment, a sample of the desorption solution was dried and redissolved in water. It was found that the difference in the radioactivity of the solutions did not exceed 10%.

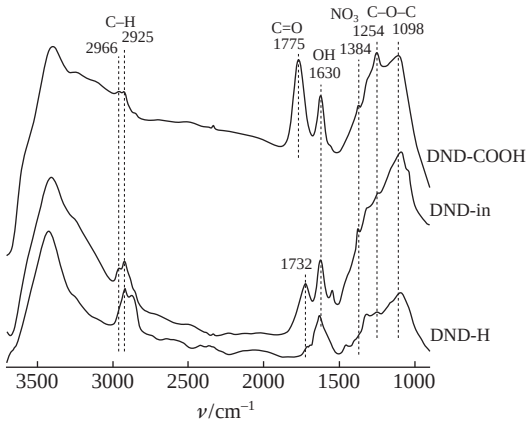


Figure 1 IR absorption spectra of the obtained nanodiamonds.

then either subjected to the liquid phase oxidation with a mixture of acids (DND-COOH)[12] or reduction in hydrogen atmosphere (DND-H).[5] Figure 1 shows IR spectra of the used DND, where signals of the major surface groups are labeled. The specific surface of the starting DND was 260 $m^2\ g^{-1}$ according to BET.

Figure 2 shows the adsorption isotherm of amikacin on DND-COOH. Note that the adsorption values exceed those observed previously.[5] This can be explained by the different functional composition of the surface of the oxidized DND. In the IR spectrum of the sample DND-COOH prepared in this work, there is an intense band in the region of 1775 cm^{-1} corresponding to vibrations of the carbonyl group C=O adjacent to a bridged carbon, while DND reported previously[5] exhibited the absorption band at 1726 cm^{-1} corresponding to the vibrations of the carbonyl group.

The topological polar area of amikacin[13] equal to 3.32 nm^2 allowed us to conclude that amikacin forms dense layers on the DND-COOH surface, wherein its molecule occupies 1.26 nm^2, while the surfaces of the initial and hydrogenated DND are covered with sparse layers, wherein the amikacin molecule occupies about 12 nm^2.

Table 1 contains data on the amikacin desorption in different media. The increased desorption in the buffer solution indicates a predominantly Coulomb interaction of amikacin with the reduced and oxidized nanodiamond surfaces. According to the FTIR spectral data, nitrate anions (absorption band at 1384 cm^{-1}) were present on the surface of DND-in in a small amount. These groups promote the amikacin binding to the surface *via* an electron transfer between the nitrate ions and amino group of amikacin. Some possible mechanisms include a protonation of the amino group of amikacin (since the amikacin in the form of its sulfate was used in adsorption experiments) and a formation of complexes, where the nitrate ion serves as a Lewis acid.[14] According to the reported computer simulation,[14] the minimum charge on the nitrogen atom is generated due to the anisotropic

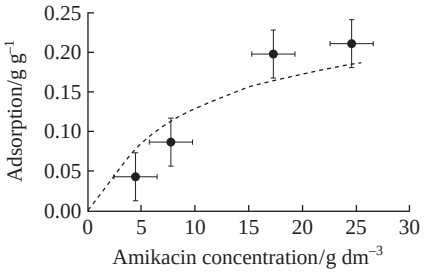


Figure 2 The amikacin adsorption on DND-COOH *vs.* the equilibrium concentration. Dotted line corresponds to the calculation according to the Langmuir model.

Table 1 Desorption of amikacin from the surface of nanodiamonds in various media.

Nanodiamond	Desorption (%)		
	Water	PBS	Albumin in PBS
DND-in	35	33	73
DND-H	32	46	83
DND-COOH	16	29	71

charge distribution in NO_3^- ion, and the nitrate ion is thus acting as the Lewis acid. The ionic strength effect on the formation of such a bond is lower than that on the electrostatic interaction.

The calcination in hydrogen atmosphere as well as the liquid phase oxidation of DND led to the release of nitrate anions from the surface,[5] while hydroxyl (after calcination in hydrogen atmosphere) and carboxyl (after wet oxidation) groups remain on the surface. These groups interact with amikacin *via* the electrostatic attraction, which is confirmed by the increased desorption in the case of PBS.

In all the cases, a significantly increased desorption of amikacin was observed in the presence of albumin in the desorbing solution, which may indicate a possible binding of amikacin to albumin,[15] and/or replacement of amikacin with albumin on the DND surface.

In conclusion, our results demonstrated that in the course of the amikacin adsorption, the bond formation in the DND–amikacin complex proceeds either due to electrostatic interactions of carboxyl groups on the DND surface with the amino groups of amikacin or *via* a donor–acceptor mechanism in the case of occurrence of nitrate ions on the DND surface.

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