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Preparation and properties of Miramistin–hyaluronic acid coatings on the nanodiamond surface

Maria G. Chernysheva, Artem V. Sinolits, Valeria S. Votyakova, Andrey G. Popov and Gennadii A. Badun

Materials

Nanodiamond produced by PlasmaChem (Germany) as a powder (DND) as well as a suspension (SDND) were used to prepare nanodiamond-Miramistin-hyaluronic acid complexes. Miramistin was a product of Infamed (Russia), and sodium hyaluronate (molecular weight of 200 kDa) was purchased from LifeCore. Note that sodium hyaluronate was subjected to additional purification by dialysis through the Spectra/Por® dialysis membrane of 8-10 kDa molecular weight cut-off against water. This procedure was required to remove low-molecular weight fractions before tritium labeling, because such fractions are competitors in the reaction with atomic tritium and will be removed from the sample during the purification stage, anyway. To reveal a direct amount of both Miramistin and hyaluronic acid in the complex, tritium labeled compounds were used. In both compounds tritium label was introduced by means of tritium thermal activation method developed previously [S1,S2].

Nanodiamond-Miramistin complex preparation

DND was suspended in water for final concentration 5 g dm^{-3} by sonication, SDND suspension was diluted for the same concentration 5 g dm^{-3} with water. Adsorption experiments were carried out according to the procedure described in Ref [S2,S3]. In brief, [^{3}H]Miramistin was dissolved in water mixed with nanodiamond suspension up to final drug concentration varied from 0.05 to 5.0 g dm^{-3} and specific radioactivity 1-1.5 μCi g cm^{-3} and nanodiamond concentration was 1 g dm^{-3}. Suspensions were incubated at 25°C for 2 days following by centrifugation at 12100×g for 15 min and radioactivity measuring of supernatant (a) and nanodiamond fraction (a_1) after washing with water. Liquid scintillation spectrometry technique was used for tritium radioactivity determination as it was described previously [S2,S3]. Equilibrium concentration (c), equilibrium adsorption value (Γ) and tightly bound with nanodiamond surface (Γ_1) of tritium labeled compound were calculated as follows

$$c = \frac{a}{Va_{SP}} \quad (1)$$

$$\Gamma = \frac{(c_0 - c)V}{ma_{SP}} \quad (2)$$

$$\Gamma_1 = \frac{a_1}{ma_{SP}} \quad (3)$$

Here a_{SP} is the molar or mass specific radioactivity of labeled compound, *m* is the mass of nanodiamonds, *V* is the volume of the suspension.

Nanodiamond-drug adsorption complexes in the aqueous suspension were analyzed by electrophoretic light scattering and FTIR after drying at room temperature using rotary evaporator [S2].

Nanodiamond-Miramistin-hyaluronic acid complex preparation

In these experiments nanodiamonds were preliminary treated by non-labeled Miramistin according to the procedure similar to the one described in the previous paragraph.

Nanodiamond-Miramistin adsorption complexes were suspended in water and mixed with tritium labeled hyaluronic acid and the following experiment and calculations were performed according to the procedure similar to the one described in the previous paragraph.

Nanodiamond-Miramistin-hyaluronic acid complexes stability

For these experiments nanodiamonds were modified with Miramistin providing DND-Miramistin and SDND-Miramistin composites with zeta-potential of 28±1 and 8±1 mV respectively.

0.16 g dm^{-3} [^{3}H]hyaluronic acid was added to nanodiamond-Miramistin complex for adsorption following by purification as it was described above. Then, nanodiamond-Miramistin-[^{3}H]hyaluronic acid composites were mixed with PBS and 40 g dm^{-3} serum albumin in PBS. Suspensions were incubated at 25ºC during 24 h. Then the suspension was centrifuged following by radioactivity measuring of both supernatant and nanodiamond phases. Amounts of tritium labeled compound in the solution and its residual amount on the nanodiamond surface were calculated according to the eq. 1 and 3 respectively.

Miramistin adsorption on nanodiamonds

In the case of SDND an equilibrium adsorption was described by Langmuir and Dubinin−Radushkevich models [S4] and their equations represented by equations 4 and 5, respectively:

$$\Gamma = \frac{\Gamma_{max} K_L c}{1 + K_L c} \quad (4)$$

$$\Gamma = \Gamma_{max} exp(-\beta \varepsilon^2) \quad (5)$$

Here Γ_{max} is maximum adsorption, K_L is the Langmuir constant, ε is the Polanyi potential:

$$\varepsilon = RT \ln\left(1 + \frac{1}{c}\right) \tag{6}$$

Parameter β is associated with free energy of adsorption

$$E = \frac{1}{\sqrt{2\beta}} \tag{7}$$

In the case of SDND an equilibrium adsorption was described by Langmuir and Dubinin−Radushkevich models (the equations are presented in the Supplementary Information).[S4,S5] Calculated parameters are summarized in Table S1. The results of fitting are shown by dashed lines in Figure S1.

Note, when E is lower than 8 kJ mol^{-1}, the adsorption process belongs to physical adsorption, and when E is between 8 kJ mol^{-1} and 16 kJ mol^{-1}, the process belongs chemical adsorption [S5,S6]. So, we can conclude that in the case of SDND chemical adsorption takes place. After purification of nanodiamond-Miramistin complexes from free and loosely bonded Miramistin we determined the amount of drug that is tightly bonded to the nanodiamond surface (Γ_1). Such complexes were analyzed using electrophoretic light scattering and FTIR (Figure S3), and used for hyaluronic acid adsorption.

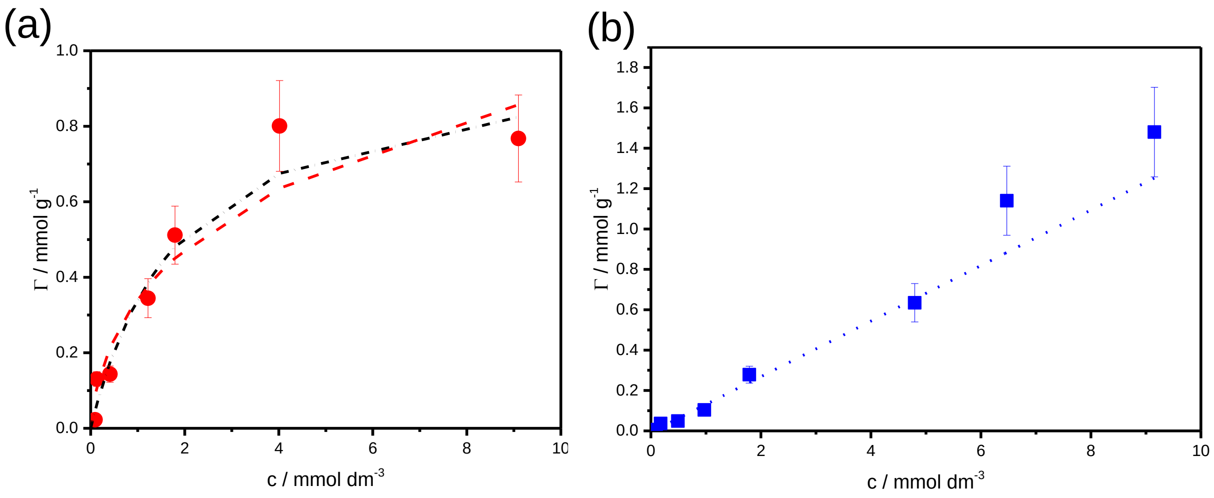

Figure S1 Adsorption isotherm of Miramistin on (*a*) SDND and (*b*) DND. Dash dot line is simulations of the adsorption on SDND by Langmuir equation (black) and dash line is simulations of the adsorption on SDND by Dubinin− Radushkevich equation (rad). In the case of DND dot line illustrates fitting by linear function.

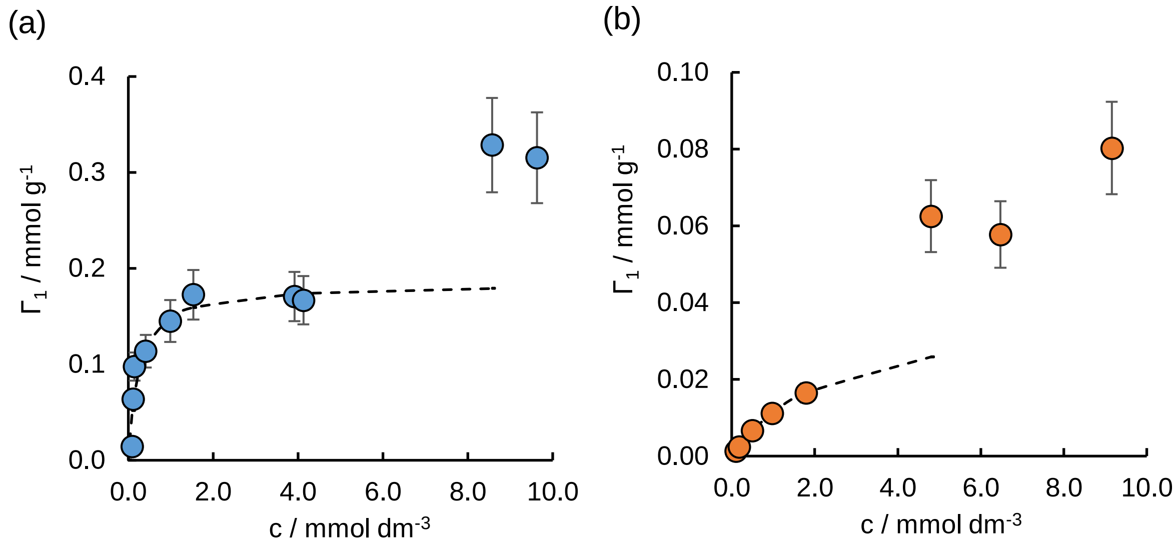

Figure S2 The dependence of surface coverage (Γ_1) of nanodiamond surface by Miramistin on its equilibrium concentration: (*a*) SDND and (*b*) DND. Dash line is the result of fitting by eq. 4.

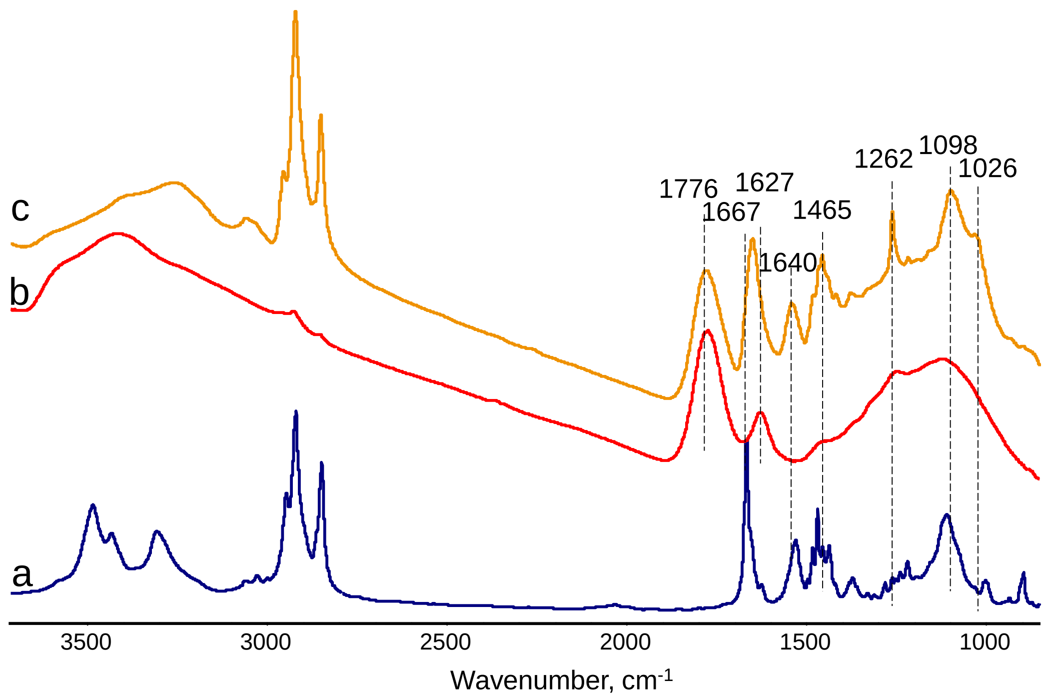

Figure S3 FTIR spectra of (*a*) Miramistin, (*b*) SDND and (*c*) SDND-Miramistin adsorption complex.

FTIR spectra were decoded according to the data summarized in the reference [S7,S8]. The signal at 1776 cm^{-1} resulted from C=O bonds vibrations in anhydrides remains without shift in nanodiamond-Miramistin as well as in nanodiamond. However, several signals appear in the complex, which relate to Miramistin and are observed in its FTIR spectrum. Namely, the signal at 1627 cm^{-1} results from vibrations of C=O of amide bond, the signal at 1530 cm^{-1} follows from

Amide II, vibrations of aromatic C=C and CH_3 groups are observed at 1465 cm^{-1}, and the band at 1261 cm^{-1} results from Amide III as well as skeletal vibrations of C-C bonds.

Table S1 Adsorption parameters for Miramistin adsorption on SDND evaluated from the simulation by Langmuir and Dubinin−Radushkevich models with correlation coefficient (*r*).

Adsorption model	Langmuir model			Dubinin−Radushkevich model			
Parameter	Γ_{max}, mmol g^{-1}	K_L, dm^3 mol^{-1}	*r*	Γ_{max}, mmol g^{-1}	*β*, mol^2 kJ^{-2}	*E*, kJ mol^{-1}	*r*
Value	1.0	518	0.962	1.9	0.006	9.2	0.951

References

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