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ScienceDirect
Mendeleev Commun., 2012, **22**, 290–291
Mendeleev Communications

Radionuclide diagnostics of the adsorption of biologically active substances on detonated nanodiamonds

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DOI: 10.1016/j.mencom.2012.11.002

The adsorption of lysozyme, albumin and calcium homopantothenate on detonated nanodiamond powder was quantitatively characterised by a radiotracer method.

Carbon-based nanomaterials are of interest in bioscience and biotechnology. Because of their low toxicity to different types of cells,[1] diamonds 2–10 nm in diameter may be promising for medical applications.[2–4] To provide biological compatibility to carbon-based nanomaterials, the covalent[5–7] and noncovalent[8–10] binding of organic compounds is widely used. The formation of stable suspensions in a buffer solution is critically important for biochemical applications. However, detonated nanodiamonds exist in the form of strongly bound agglomerates in aqueous systems. Thus, the stabilization of the colloidal solutions of detonation diamond primary particles remains a challenging task. The stability of colloids is controlled by the surface energy, ζ-potential, pH and ionic strength of solution, the presence of surfactants *etc*.[4] In this work we studied the noncovalent functionalization of nanodiamonds with physiologically active low-molecular-weight compounds and biopolymers by a radiochemical approach.†

Adsorption experiments were carried out according to a published procedure.[13,14,‡] Adsorption (Γ) was calculated according to the equation

$$\Gamma = \frac{(c_0 - I/\varepsilon a_{sp} V_a)V}{m}. \quad (1)$$

Here, I is the counting rate, ε is the detection efficiency of tritium β-radiation (from 40 to 50%), V_a is the volume of an aliquot portion, $V = 0.8$ cm^3, a_{sp} is the specific radioactivity of the compound, c_0 is the initial concentration of the compound solution, and m is the mass of nanodiamonds.

To control that there was no nanodiamond conjugate in the supernatant, the experiments with tritium-labeled nanodiamonds were performed according to the above procedure, where a tritium label was bound to nanodiamonds and the organic compound was not labeled. The tritium-labeled nanodiamonds were sonicated in the solution of a biologically active compound followed by centrifugation and supernatant radioactivity measurement. Since radioactivity in such experiments was on the background level we can conclude that the supernatant was free from nanodiamonds or nanodiamond conjugates.

When the concentration of the test compounds did not significantly change after the adsorption (HPA), the precipitate after ultracentrifugation was decanted and washed with water at least three times controlled by radioactivity measuring. Then, 1.5 cm^3 of the OptiPhase HiSafe 3 scintillation cocktail was added and counting rate was measured as described above. The measurement was carried out immediately and three to five days after the addition of the scintillator. HPA was completely desorbed from nanodiamonds by surfactants presented in the scintillation cocktail. In this case, Γ was calculated as

$$\Gamma = \frac{I/\varepsilon - a_{res}}{m a_{sp}}. \quad (2)$$

Here, a_{res} is the radioactivity of the residual solution after washing.

Figures 1 and 2 show the adsorption isotherms of proteins and HPA, respectively. Analogously to humic substances,[13] the isotherms were described by a Langmuir equation with a relative error of 15% (solid lines in Figures 1 and 2).

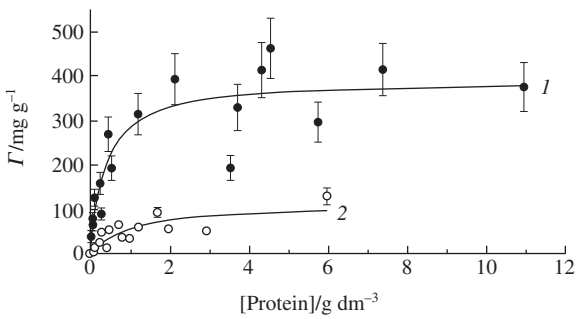

Figure 1 Adsorption of (*1*) human serum albumin and (*2*) lysozyme on detonated nanodiamonds. Solid lines obtained by calculations according to equation (3) with parameters presented in Table 1.

† Nanodiamonds from Sinta (Belarus) were used without further purification. The specific surface area determined by a continuous flow method[11] was 350 m^2 g^{-1}. The adsorption of two globular proteins (lysozyme and human serum albumin from MP Biomedicals) and homopantothenic acid (HPA) as a calcium salt was studied using tritium-labeled compounds and the liquid scintillation spectrometry of tritium. All labeled compounds were obtained by a tritium thermal activation technique.[12] The specific radioactivities of the labeled compounds were 1.7, 3.3 and 0.28 TBq mmol^{-1} for lysozyme, albumin and HPA, respectively.

‡ A 5–12 mg sample of nanodiamond powder was placed in an Eppendorf tube, and 0.8 cm^3 of an ^{3}H-compound solution in a phosphate buffer (for proteins) or in Milli-Q water (for HPA) was added. The initial concentrations of proteins and HPA were 0.1–14 and 0.1–10 g dm^{-3}, respectively. The bulk radioactivity of the solutions was about 55 MBq dm^{-3}. In each series, one sample of nanodiamonds in 0.8 cm^3 of a buffer (water) was used as a control. The dispersion was ultrasonicated for 2 h using a 110-W GRAD Model 28-35 bath sonicator (Russia) followed by incubation at room temperature for 24 h. The solutions thus obtained were centrifuged at 2400g (Vortex) for 60 min. The upper 75% of the supernatant after ultracentrifugation was filtered through a 13 mm syringe filter with a 0.2 μm PVDF membrane (Acrodisc LC, Life Sciences). A filtered solution sample was stirred in 7 cm^3 of OptiPhase HiSafe 3 scintillation cocktail (PerkinElmer). Counting rate was measured by a RackBeta 1215 liquid scintillation spectrometer (Finland).

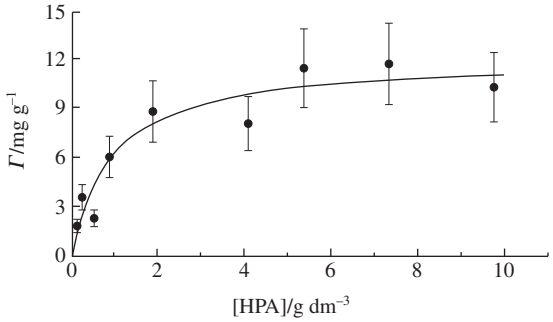

Figure 2 Adsorption of homopantothenic acid on detonated nanodiamonds. Solid line obtained by calculations according to equation (3) with parameters presented in Table 1.

$$\Gamma = \Gamma_{max} \frac{Ac_{eq}}{1 + Ac_{eq}}. \quad (3)$$

Here, A is the ratio between the rate constants of adsorption and desorption, and Γ_{max} is the maximum adsorption. Table 1 summarizes the adsorption parameters and correlation coefficients (R^2). An available surface for a high-molecular-weight compound could be significantly smaller than that for nitrogen in BET. Therefore, the adsorption data in Figures 1 and 2 and Table 1 are given in mg g^{-1}.

The reversibility of protein adsorption was studied in a pure buffer, 3 M NaOH and 10.5 mM SDS. In the case of HPA, desorption was studied in pure water only. The desorption was calculated as a ratio between the sorbate amount in the supernatant and the initial adsorbed on nanodiamonds (Table 2).

The nanodiamond-conjugates suspensions were subjected to the dynamic light scattering (DLS) analysis according to a published procedure.[15] Nanodiamonds modified by HPA form sediment and aggregative stable sols, with average particles diameter *ca.* 120 nm, while in control systems aggregation to 1 μm particles was observed less than in 24 h. Supposed that nanodiamond-HPA particles have a spherical shape, the adsorption monolayer should be 0.84 mg m^{-2}. Surface coverage is 0.46 nm^2 per HPA molecule, which corresponds to a dense adsorption monolayer. Compared the DLS data obtained for nanodiamonds modification with biopolymers (see below for proteins and humic acids[13]) and HPA, we can suggest that the sonication of nanodiamond powder in the presence of a low-molecular-weight surfactant that can be adsorbed only in monolayer on its surface leads to the formation of stable nanodiamond-surfactant hydrosols in the aqueous solutions.

The data obtained for lysozyme in a concentration range from 0.7 to 2 mg cm^{-3} are close to published data.[16] It was shown that 1 g of carboxylated nanodiamonds with a particle size of 100 nm can adsorb 80±10 mg of lysozyme. Our results suggest that the adsorption of lysozyme varied from 60 to 90 mg g^{-1} in this concentration range. However, the maximum adsorption value is higher than the reported one.[16] We also observed by DLS that nanodiamond modification by biopolymers (proteins and humic materials[13]) results in an increase of coagulation in the aqueous solutions. The sedimentation was more rapid than that in pure nanodiamonds. An average size of nanodiamonds modified by polymers directly after sonication was ~100 nm, it was increased to 2 μm in 24 h. Extra ultracentrifugation provides the formation of solid phase that sometimes was impossible to break even by long time sonication. Note that the adsorption and the aggregation stability of nanodiamond-conjugate particles depend on the preliminary preparation of nanodiamonds. It probably explains the difference between our results and published data.[16,17] In reported procedures, nanodiamonds were subjected to acids wash and sonication before the adsorption experiments. Such nanodiamond preparation can significantly change the surface properties of solid particles. Thus, we first determined the quantitative characteristics of the binding of nanodiamonds with biologically active compounds in a wide concentration range.

Table 1 Adsorption parameters in equation (3) and correlation coefficients (R^2) for the adsorption of biologically active compounds on detonated nanodiamonds.

Biologically active compound	Γ_{max}/mg g^{-1}	A/cm^3 mg^{-1}	R^2
Human serum albumin	392	2.7	0.92
Lysozyme	115	1.0	0.80
Homopantothenic acid	12	1.1	0.96

Table 2 Desorption of biologically active compounds from detonated nanodiamonds.

Biologically active compound	Desorptive agent		
	Buffer or water	3 N NaOH	10.5 mM SDS
Human serum albumin	< 10% buffer	64±3%	15%
Lysozyme	< 10% buffer	85±5%	2%
Homopantothenic acid	< 10% water	–	–

We are grateful to Corresponding Member of the National Academy of Sciences of Belarus A. G. Moiseenok for providing us with homopantothenic acid.

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Received: 3rd July 2012; Com. 12/3951