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APPLICATIONS OF NANODIAMONDS

Nanodiamonds for Biological Investigations

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Abstract—Nanoparticles with a modified surface are prepared from nanodiamonds produced in Russia. The properties of modified nanodiamonds and their hydrosols and organosols are investigated by biophysicists with the aim of preparing nanoparticles with controlled properties for solving biological problems. © *2004 MAIK "Nauka/Interperiodica".*

1. INTRODUCTION

In recent years, we have analyzed the possibility of using nanodiamonds in biological investigations [1–3] and reached the conclusion that, despite undeniable advantages, detonation nanodiamonds have a number of disadvantages from the biological and biochemical standpoints. These are the impossibility of preparing stable hydrosols without ultrasonic treatment, the difficulties encountered in preparing hydrosols with specified concentrations of nanodiamonds, the formation of nanodiamond aggregates upon autoclaving of hydrosols (which is a widely accepted method of sterilization in biology and medicine), and the formation of nanodiamond aggregates in the course of freezing of hydrosols and subsequent thawing of ice (this technique is frequently used for stabilizing the properties of biological materials during long-term storage). In this respect, we attempted to modify the surface of nanoparticles in order to render their properties desirable for biological investigations.

2. SAMPLE PREPARATION AND EXPERIMENTAL TECHNIQUE

The nanodiamonds used in our experiments were synthesized at the Department of Physics of Nanodispersed Materials, Krasnoyarsk Research Center, and the Federal Research and Production Center Altaĭ (Biĭsk). The nanodiamonds were chosen because there are two types of powders that, after addition of water and treatment with an ultrasonic disperser, form or do not form hydrosols that are stable over a long period of time. The nanodiamonds synthesized at the Department of Physics of Nanodispersed Materials (Krasnoyarsk Research Center) according to the procedure described in [4] and chemically purified using the technique proposed in [5] possess the ability to form stable hydrosols. The Federal Research and Production Center Altaĭ has manufactured several types of nanodiamonds with different properties. We performed the experiments with samples **1-3/91** and **30/92**, which are incapable of forming stable hydrosols.

As is known, many physicochemical properties of nanodiamonds substantially depend on the composition and properties of the particle surface formed in the course of synthesis and chemical purification [6]. Apparently, it is almost impossible to affect the physicochemical properties of nanodiamonds through modification of the diamond core. Therefore, the properties of nanodiamonds can be changed only by modifying the nanoparticle surface. We used this approach and succeeded in preparing modified nanodiamonds free of the above disadvantages.

In this work, we compared the characteristics of the initial and modified nanodiamonds and did not dwell on the technique of their modification. It should be noted that all modified nanodiamonds possess similar characteristics that do not depend on the properties of the initial nanodiamonds, which can differ significantly in terms of the colloidal stability of the nanoparticles. For this reason, we do not duplicate results of the same type and present data for only one type of modified nanodiamond sample, with the manufacturer indicated.

An important parameter characterizing the nanoparticle stability in hydrosols is the electrostatic repulsion energy (ξ potential). Depending on the pH of hydrosols and the purification procedure, the ξ potentials for the nanodiamonds synthesized at the Department of Physics of Nanodispersed Materials (Krasnoyarsk Research Center) vary from –30 to –38 mV [6, 7]. The ξ potential for the modified nanodiamonds is shifted to the range from –50 to –52 mV. According to the calculations carried out by Chiganova [7], these changes lead to a considerable increase in the electrostatic repulsion energy of particles. This energy is proportional to the square of the potential of a diffuse layer, which is taken equal to the electrokinetic potential in dilute electrolyte solutions: $U_e \sim \Psi\delta^2$. Probably, the increase in the electrostatic stability of a disperse system provides aggregative stability for hydrosols formed by modified nanodiamonds. It is quite possible that a decrease in the

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surface impurity concentration, which is characteristic of modified nanodiamonds (Table 1), has a specific effect. Although the factors responsible for the observed changes are as yet not entirely clear, we managed to produce modified nanodiamonds with radically new properties.

3. HYDROSOLS

As a rule, hydrosols of nanodiamonds can be prepared only after ultrasonic treatment of a mixture of a powder with water. In this case, hydrosols that are reliably stable over a long period of time can be produced at a content of no higher than 1 wt % [8]. Despite the "rigid" preparation procedure with the use of ultrasound, part of the nanodiamond particles form a sediment, which leads to a change in their content in the suspension. With the aim of determining the true content of nanodiamonds, it is necessary to dry the hydrosol aliquot and to measure the weight of the particles. As a rule, nanodiamonds can be used only once to prepare a hydrosol. Already after the first removal of the dispersive medium and drying of the sample, in the majority of cases, it is impossible to reproduce the hydrosol without additional mechanical dispersion even with ultrasonic treatment.

The powders of the modified nanodiamonds are characterized by a high colloidal stability of the particles and form stable hydrosols even when water is simply added without ultrasonic dispersion. After repeated removal of the dispersive medium and subsequent addition of water to the dry modified nanodiamond powder, we once again obtain a stable hydrosol (Fig. 1).

Investigations into the properties of nanoparticles revealed that the most significant difference in the stability of hydrosols with time is observed between the initial nanodiamonds manufactured at the Federal Research and Production Center Altaĭ and the modified nanodiamonds. As was noted above, these nanodiamonds do not form stable hydrosols even after ultrasonic dispersion. The modified nanodiamonds prepared from these particles form hydrosols upon simple addition of water. Particles (clusters) in hydrosols formed by the modified nanodiamonds cannot be completely sedimented even through centrifugation at 16000 *g* for 10 min (Fig. 2). This indicates a high colloidal stability of the particles (clusters). It should be noted that the ultrasonic treatment favors a further increase in the colloidal stability of the modified nanodiamonds in hydrosols.

From the biological viewpoint, the advantages of hydrosols formed by modified nanodiamonds are the high colloidal stability in solutions used in biological investigations (buffer solutions, culture media, physiological liquids) and the possibility of achieving a uniform distribution of nanoparticles in an agar gel.

The hydrosols of the initial and modified nanodiamonds substantially differ in behavior in the course of freezing–thawing cycles. During the formation of ice crystals upon freezing of nanodiamond hydrosols, nanoparticles are displaced from the aqueous phase and

Table 1. Percentage of impurities in powders of the initial and modified nanodiamonds (prepared at the Department of Physics of Nanodispersed Materials, Krasnoyarsk Research Center)

Chemical element	Initial nanodiamond powder	Modified nanodiamond powder
Fe	5.7	1.20
B	1	1
Na	0.216	0.417
Ca	0.396	0.291
K	0.076	0.075
Cu	0.1	0.08
Al	0.03	0.02
Sr	0.055	0.02
Ti	0.2	0.1
Mg	0.034	0.002
Ni	0.006	0.004
Cr	0.0046	0.002
Sn	0.0016	0.0014
Pb	0.0015	0.0013
Mo	0.0004	0.0003
Mn	0.00033	0.00025
V	0.00012	0.000044
Ag	0.0000093	0.000003

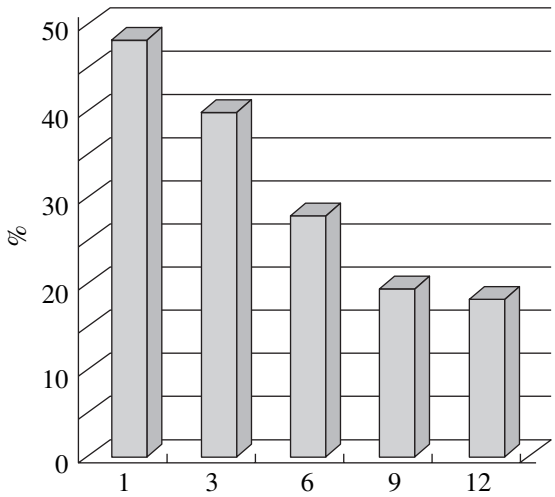

Fig. 1. Variations in the content of modified nanodiamonds in liquid supernatants of hydrosols for 12 cycles of removal and addition of the dispersive medium. The data are calculated from the optical densities of the hydrosols formed by modified nanodiamonds (prepared at the Federal Research and Production Center Altaĭ) after their centrifugation at 16000 *g* for 10 min. The optical density of the initial hydrosol (the first addition of water to the modified nanodiamond powder) without centrifugation is taken as 100%.

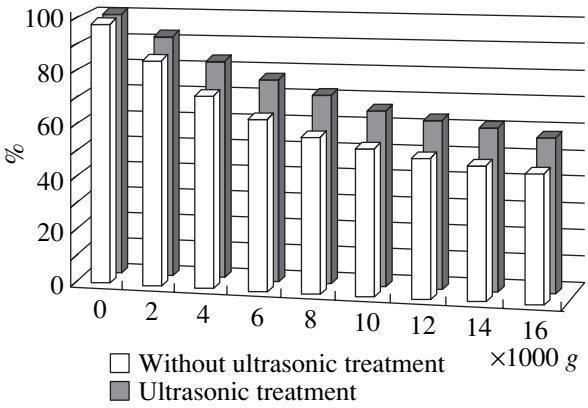

Fig. 2. Contents of modified nanodiamonds (prepared at the Federal Research and Production Center Altaĭ) in ultrasound-treated and untreated hydrosols after centrifugation. The data are calculated from the optical densities of the initial hydrosols and liquid supernatants after centrifugation at different accelerations for 10 min. The optical densities of the initial hydrosols without centrifugation are taken as 100%.

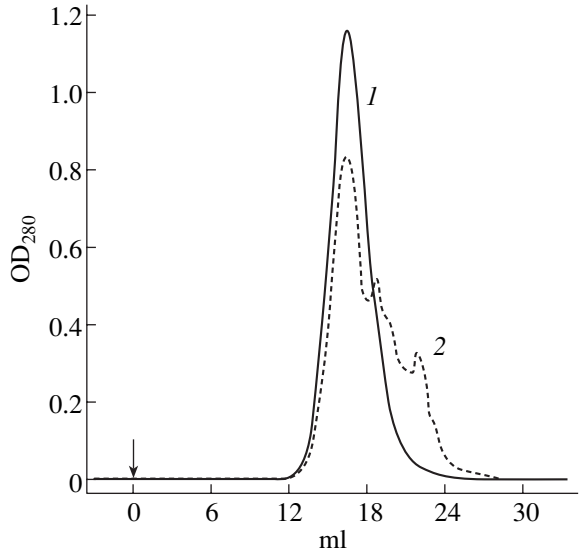

Fig. 3. Chromatograms of samples on the column with an AcA 22 gel: (*1*) the hydrosol of modified nanodiamonds (prepared at the Department of Physics of Nanodispersed Materials, Krasnoyarsk Research Center) and (*2*) ferritin.

form large-sized aggregates. Upon thawing of ice, the nanodiamond aggregates precipitate and their repeated transformation into the colloidal state becomes problematic. The formation of ice in hydrosols of modified nanodiamonds is not accompanied by the precipitation of large-sized nanoparticle aggregates, and, hence, modified nanodiamonds retain the colloidal stability after the thawing of ice.

Column chromatography (gel filtration), without changing the properties of the sorbent matrix, can be performed with hydrosols of modified nanodiamonds. In the chromatogram measured upon gel filtration with a column 1×46.5 cm in size at a flow rate of 5 ml/h, the emergence of particles is characterized by one peak (Fig. 3). We can assume that the modified nanodiamond particles pass through a free volume of columns without penetrating into the pores of gel sorbent particles. The asymmetry of the peak most likely indicates a possible weak interaction of particles with the surface of gel spherules. Chromatography of nanodiamond hydrosols under the same conditions leads to their irreversible location in the upper part of the gel into which the sample is injected.

The use of modified nanodiamonds substantially simplifies the preparation of hydrosols with a specified concentration of nanoparticles. The high colloidal stability of the modified nanodiamonds permits us to remove the restriction on a particle concentration of 1% in stable hydrosols.

4. ORGANOSOLS

Stable colloidal systems of modified nanodiamonds can be prepared not only in water but also with other dispersive media. Table 2 presents the dispersive media (the parameters of solvents were taken from [9]) in which it is possible to obtain colloidal systems of modified nanodiamonds.

Dry powders of the initial and modified nanodiamonds are incapable of forming colloidal solutions (organosols) in pure organic solvents. However, unlike the initial nanodiamonds, the modified nanodiamonds can form organosols in the case when organic solvent–water mixtures are added to a dry powder. For example, the addition of the modified nanodiamond powder to a 35–40% ethanol solution leads to the formation of an organosol without ultrasonic treatment.

The high colloidal stability of the modified nanodiamonds ensures the stability of nanoparticles in mixtures formed upon addition of their hydrosols to organic solvents. In a number of cases, it is possible to produce stable organosols with a high content of organic solvents (for example, a 80% ethanol solution). When the content of an added organic solution exceeds the threshold value, a finely dispersed precipitate is formed in the solution. The precipitate can be transformed into the colloidal state by decreasing the organic solution concentration through the addition of water.

The high stability of modified nanodiamond hydrosols, which is virtually independent of the particle concentration, enables one to prepare organosols with a high content of the dispersed phase.

As a rule, modified nanodiamond organosols possess high colloidal stability over a wide range of temperatures, namely, from the boiling point to the freezing point of the dispersive medium. After the thawing of ice, the organosol retains the colloidal stability. In particular, the modified nanodiamond–ethanol organosol

Table 2. Solvents forming dispersive mixtures used for preparing stable colloidal solutions of modified nanodiamonds (prepared at the Department of Physics of Nanodispersed Materials, Krasnoyarsk Research Center)

Solvent	δ	δ_d	δ_o	δ_a	δ_h	η
Acetone	9.4	6.8	5	2.5	0	
1,2-Dichloroethane	9.7	8.2	4	0	0	0.79
Ethanol	11.2	6.8	4	5	5	1.2
Dimethylformamide	11.5	7.9				
Acetic acid	12.4	7.0				1.26
Dimethyl sulfoxide	12.8	8.4	7.5	5	0	2.2
Water	21	6.3	Large			

Designations: δ is the solubility parameter (calculated from the boiling point), δ_d is the dispersion solubility parameter, δ_o is the orientational (polar) solubility parameter (approximate values), δ_a is the proton-acceptor solubility parameter (approximate values), δ_h is the proton-donor solubility parameter (approximate values), and η is the viscosity at 20°C.

is stable to a temperature of –82.5°C [the temperature was specified with a ScienTemp kelvinator (USA)] and also after the thawing of ice obtained by freezing in liquid nitrogen.

Earlier, we demonstrated that nanodiamonds can be used in biotechnologies, namely, in the rapid separation of a recombinant protein from a crude protein extract [1] and in the creation of planar luminescent biochips [2]. These applications are based on the ability of nanodiamonds to adsorb protein molecules. The results of investigations into the adsorption of marker protein cytochrome C demonstrated that modification of the nanoparticle surface does not result in a change in their sorption properties.

It should be noted that modified nanodiamonds are not ideal objects. This is indicated by the decrease in their colloidal stability upon multiple drying of hydrosols, which is apparently associated with the aggregation of nanoparticles (Fig. 1). However, despite this fact, modified nanodiamonds offer a number of considerable advantages over nanodiamonds in biological investigations. The use of modified nanodiamonds allows one to prepare stable hydrosols with a controlled weight concentration of particles. The autoclaving of modified nanodiamond hydrosols does not lead to precipitation or aggregation, which permits their use in research that requires aseptic (sterile) conditions. The retention of the colloidal stability by modified nanodiamond hydrosols after freezing–thawing cycles, in addition to the aforementioned properties, allows us to consider them to be similar to other reactants used in biological investigations. In some cases, this makes it possible to retain techniques and stereotypes established in biology.

5. CONCLUSIONS

Thus, the results obtained in this work can be summarized as follows.

(1) Modified nanodiamonds with useful properties that are lacking in the initial materials were prepared from nanodiamonds fabricated in Russia.

(2) The modification of the properties of nanodiamonds was aimed at adapting them to biological investigations. Moreover, the modified nanodiamonds can most likely be used in all fields of application of nanodiamond sols with a high colloidal stability, minimum sizes of the nanoparticle clusters, and a specified concentration of nanoparticles.

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