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# Spectroscopic study of bio-functionalized nanodiamonds

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**Abstract**

Nano-scale materials with attached biomolecules on the surface can be used as bioprobes in cell and tissue analysis. The conjugation of these nanoparticles with biomolecules is one of the key steps in the development of bioprobes. In this work nanometer-sized diamonds (5 and 100 nm) are functionalized and conjugated with protein lysozyme via physical adsorption. The process of creating functional groups on the nanodiamond surface is studied using Fourier Transform Infrared-spectroscopy (FTIR). The conjugation of nanodiamonds with biomolecules and nanodiamond–biomolecule interaction are analyzed with UV/VIS and FTIR spectroscopy.


*Keywords:* Nanodiamonds; Biomedical applications; Sensors; Surface characterization

## 1. Introduction

Nanocrystals with attached biomolecules on the surface are used as bioprobes in cell and tissue analysis for visualization of micro- and nano-objects and for observation of the biological processes in nano-scale [1]. The first reason for such application is that nanoparticles exist in the same size domain as many intercellular structures and large biomolecules. Initially the nanoparticles were used as carriers for bioactive molecules, but recently intrinsic physical and chemical properties of nanostructures make them promising candidates for biosensors [2]. The conjugation of nanoparticles and biomolecules is one of the key steps in the development of bioprobes. These nanoparticle–biomolecules complexes can interact specifically or non-specifically with components of investigated bio-object and hence many interactions can be studied in detail. Biomolecules can be immobilized on nanoparticles' surface through a variety of mechanisms such as physical adsorption, electrostatic binding, specific recognition and covalent coupling. The biomolecules on the nano-bio-probe allow to visualize and to distinguish some functional processes and structures of the investigated object, and to control physical and chemical treatments. Also, the attached biomolecules can prevent non-controlled adsorption of the investigated sample components. The most developed methods for applications using nanoparticles are: semiconductor nanocrystals (quantum dots) encapsulated by biomolecules to decrease toxicity and increase bio-compatibility are used for applications that utilizing their unique fluorescent properties [3–7]; metal nanoparticles are used for surface plasmon resonance microscopy and surface enhancement Raman spectroscopy [7–10]; magnetic nanoparticles are used for cell targeting both in nanobioprobing [11] and in drug delivery [12], etc.

Nanometer-sized diamond recently becomes an interesting material for the nano- and biotechnology integration. Nanodiamond–biomoleules complexes are thus studied for the purpose of using them as bioprobes [13,14] taking advantage of their chemical stability and biocompatibility. The nanodiamond particle surface can be functionalized with a large number of surface ionogenic groups (ether — C–O–C, peroxide — C–O–O–, carbonyl — C═O, and hydroxyl-type C–O–H bonding, etc.) as well as hydrocarbon fragments. The surface can also be modified with biologically active molecules by adsorption, covalent or non-covalent chemical immobilization [15,16]. The functional groups on the particle surface can interact electrostatically or chemically with bimolecular appurtenant to the investigated sample for cell specific interaction and targeting. Therefore the nanodiamond was used first of all as carrier for active molecules [2,17], but the electronic and optical properties of different nanodiamond structures render

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them attractive candidates as drug delivery agent or biosensors [12,18]. For these applications, biological molecules, such as DNA [17,19–21], different proteins [16,22], fluorescent dyes [22], etc., were immobilized on the nanodiamond.

In this work nanodiamonds (5 and 100 nm) are functionalized and conjugated with biomolecules via physical adsorption. To create the functional groups the processes of carboxylation and oxidization nanodiamond were used. The creation of functional groups on the nanodiamond surface was studied with Fourier Transform Infrared (FTIR) spectroscopy. The attachment of biomolecules via physical adsorption and nanodiamond–biomolecule interaction were analyzed with UV/VIS and FTIR spectroscopy.

## 2. Experimental

Synthetic diamond powders with diameters 100 nm (Kay Industrial Diamond, USA) and 5 nm (UltraFine Diamond, Russia) were carboxylated/oxidized in according with standard procedure [16,19]. The sample (0.5 g) was subsequently heated in a 9:1 (v/v) mixture of concentrated $H_2SO_4$ and $HNO_3$ at 75 °C for 3 days, in 0.1 M NaOH aqueous solution at 90 °C for 2 h, and in 0.1 M HCl aqueous solution at 90 °C for 2 h. The resulting carboxylated/oxidized diamonds were extensively rinsed with deionized water, separated by sedimentation with a centrifuge at 12000 rpm and dried. The IR spectra of carboxylated nanodiamond were measured using an FTIR-interferometer (Bomem MB154) with the sample in vacuum chamber or flowing nitrogen to avoid water absorption.

The lysozyme (AMRESCO, USA), concentration 180–200 μM, was dissolved in double-distilled deionized water (pH 4.4–4.5) or in phosphate buffer saline (PBS), pH=6.5. The sample preparation by both media followed by spectral analysis was estimated. The initial protein concentration was checked with UV/VIS spectrometer (Jasco V-550) by the solution absorption at 280 nm. A molar absorbance of lysozyme at 280 nm ($3.7547\times10^4$ $M^{-1}$ $cm^{-1}$) served to calibrate the protein concentration by the measured absorbance at the Soret band maximum. The concentration of lysozyme in solution was measured before adsorption; then carboxylated nanodiamond, concentration 4–10 mg/ml, was added to the solution. To ensure maximum adsorption, the protein solution and the diamond powder were thoroughly mixed using a shaker for 2 h, after that the mixture was centrifuged and washed several times with deionized water. After first separation of nanodiamond with adsorbed protein, the residual concentration of protein in supernatant was measured. The quantity of protein adsorbed by nanodiamond was estimated by the difference between initial and residual protein concentrations. FTIR spectra were measured in ambient condition and in vacuum chamber at $\sim10^{-6}$ Torr pressure.

## 3. Results and discussions

Infrared spectra of carboxylated/oxidized nanodiamonds are plotted in Fig. 1. The acid treatment of nanodiamond creates the functional groups on the diamond surface and can be observed

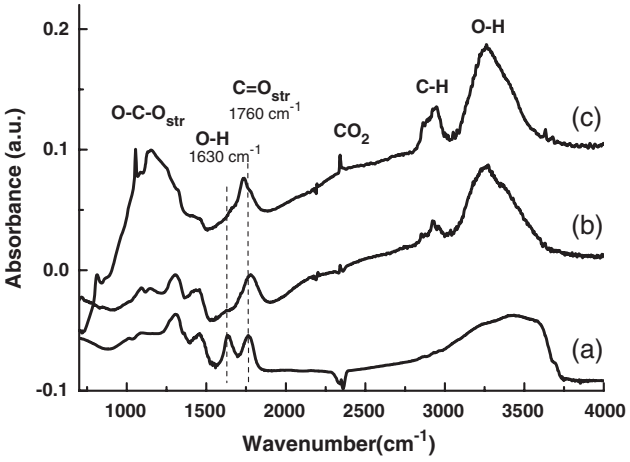


Fig. 1. IR spectra of carboxylated/oxidized nanodiamonds: (a) 100 nm; initial concentration in suspension 20 mg/60 μl, spectrum taken in ambient air; (b) concentration 1.4 mg/200 μl, spectrum taken in vacuum; (c) 5 nm; concentration 1 mg/200 μl, spectrum taken in vacuum.

using FTIR spectroscopy. Fig. 1(a) and (b) are from the same carboxylated 100 nm diamonds, except Fig. 1(a) was measured in air with higher diamonds concentration (20 mg/60 μl) while (b) was taken in vacuum chamber at lower concentration (1.4 mg/60 μl). The band near 1630 $cm^{-1}$ can be attributed to the O–H bending of physically adsorbed water and the hydrogen bonding, which arise between neighboring carboxylated nanoparticles at high concentration [16]. The assignment is evidenced from the disappearing of this band when the spectrum was taken in the vacuum ($\sim10^{-6}$ Torr) as shown in Fig 1(b). For smaller 5 nm diamonds, this band shifted to higher wavenumbers that presumably arise from the intermolecular interaction of the neighboring bonding due to small particle size. The C═O bond of carboxylic acid usually appears around 1775 $cm^{-1}$ and that of carboxylic anhydrite at about 1800 $cm^{-1}$. In Fig. 1, the IR bands at 1750–1760 $cm^{-1}$ can be assigned as C═O stretching mode for the carboxylic acid. The shoulder, especially visible in the 5 nm carboxylated diamonds, in the ~1800 $cm^{-1}$ may associate with the carboxylic anhydrite. In addition, O–H stretching from surface –COOH group appears near 3710 $cm^{-1}$; the broad band at 3000–3600 $cm^{-1}$ can be attributed to the hydrogen-bonded-OH of physisorbed water on the surface, and when in vacuum it may desorb from the surface which results in the narrowing of the band as seen in Fig. 1(b), (c); the broad band near 3000 $cm^{-1}$ can be caused by the CH stretching on the sample surfaces. The bands in the range 700–1450 $cm^{-1}$ also (in particular, 1275 $cm^{-1}$) have been ascribed to ether-like groups on the diamond powders [23–25].

The carboxylated/oxidized surface of nanodiamond in water solution (including PBS etc.) is negatively charged due to dissociation of –COOH and the forming of $-COO^-$ ion groups. This is essential for modification of the nanodiamond surfaces with biomolecules. We analyze here physical adsorption of protein lysozyme. It is used as test protein for its well-known properties and structure. Lysozyme is a small globular protein widely used for researches in biophysics, molecular biology, etc. Particularly, lysozyme has been used for diamond film–lysozyme biochip construction [26]. In Fig. 2, typical