



Frequency Modulation of the Raman Spectrum at the Interface DNA - ZrO₂ Nanoparticles



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THE DEVELOPMENT of modern bio-nanoelectronic technologies requires new hybrid organo-inorganic systems that have a specific functional purpose and allow work in the tissues of living organisms. The practical realization and scientific study of a hybrid two-component system in the form of DNA molecule fragments and nanoparticles of biocompatible ceramics was the goal of this work.

The interaction of a DNA molecule with nanoparticles of a solid solution of the composition ZrO₂-3mol% Y₂O₃ (YSZ) was studied using Raman scattering and transmission electron microscopy. Samples of YSZ oxide and hydroxide were investigated in triplicate at room temperature using LabRAM HR Evolution Horiba spectrometer and JEM 200A instrument.

The effect of the force field of the surface of YSZ nanoparticles on the optical properties of the DNA molecule in the TRIS buffer solution was shown. The effects of increasing the intensity of spectral lines in the long-wavelength region 250 – 660 cm⁻¹ (254 cm⁻¹, 322 cm⁻¹, 470 cm⁻¹, 663 cm⁻¹), suppressing the intensity of spectral lines in the shortwave region 600 – 3300 cm⁻¹ (604 cm⁻¹, 917 cm⁻¹, 951 cm⁻¹, 1256 cm⁻¹, 1302 cm⁻¹, 2767 cm⁻¹, 3180 cm⁻¹, 3241 cm⁻¹, 3285 cm⁻¹), as well as shifting lines 519 cm⁻¹, 917 cm⁻¹, 1048 cm⁻¹, 1547 cm⁻¹ and 1639 on 40-60 cm⁻¹ in the shortwave region are established.

From the standpoint of the theory of wave processes, a qualitative description of the detected Up-conversion effect was done. An assumption about the potential applicability of the YSZ nanoparticle surface – DNA nucleotide interfaces as a heterodyne frequency converter in molecular- and bio-electronic devices was made.

Keywords: DNA in the electric field, immobilization of DNA molecules on the surface of semiconductors and dielectrics, Raman scattering, frequency modulation, heterodyne transfer of a frequency band, molecular electronics, bio-electronic, powder nanotechnologies.

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Introduction

Molecular spectroscopic methods supported with quantum mechanical calculations are promising tools to understand many molecular phenomena, with certain focus on biological molecules [1-4].

Nanobioelectronics is rapidly developing the present time [5]. The combination of biomolecules with metal or semiconductor particles, fullerenes or carbon nanotubes led to the appearance a new class of materials, primarily for new electronic and optoelectronic systems. DNA molecules are capable of storing and translating terabytes of information by copying (a cubic centimeter of DNA can contain more information than billion CDs. [6]). In addition, DNA molecules are capable of self-assembly, self-reproducibility and have good electrical conductivity [7, 8, 9], that is, they are, if not ideal, then very suitable material for creating the elemental base of computing devices based on biological objects. The study of the interaction of DNA - molecules with solids is extremely important for new bioelectronic technologies, in particular, in the development of storage systems for large amounts of information. It is known [10] that some metal ions in the composition of metal oxides, in particular, Ca^{2+} , Mn^{2+} , Cu^{2+} , are able to interact directly with DNA, forming stable bonds with both as with negatively charged groups of the sugar – phosphate carcass as with nitrogenous bases [11]. Such interactions lead to significant changes in the secondary structure: they can contribute to the bending and unwinding of double helices, and also serve as B-Z transition triggers in DNA [12] (the Z-form was discovered for hexanucleotide d (CGCGCG) [13]). The transition of poly (dG-dC) from B- to Z-form is observed with a high concentration of sodium ions (2.4 M) or with the addition of some divalent ions [14]. The phenomenon is extremely interesting for bioelectronics, as one of the possible basic elements of computing devices of a new type. In addition, metal ions play an important role in signal transmission, being elements of complex chromatin regulatory complexes [15, 16, 17] in biological cells.

Investigation of the interaction of a DNA molecule with ZrO_2 is interesting for practical applications. Zirconia ceramics are primarily biocompatible. In addition, ZrO_2 in the series of simple oxides has the lowest thermal conductivity and the highest dielectric constant. Thus, ZrO_2 -based ceramics are the best suited for creating of functional heterojunctions type biological

molecule / wide band gap dielectric due to the possibility of direct field control of the charge states and, as a result, the functional state of DNA-molecules. These heterojunctions can be used in molecular electronics, in particular, for the creation of biochips, memory arrays and computer architectures of the future.

At present, nanotechnologies have a breakthrough potential; therefore, the study of the interaction of DNA with nanoscale objects is extremely important. The study of the interaction of nanoparticles based on ZrO_2 and DNA molecules was the main aim of this work.

Experimental

As a biological object (DNA), deoxyribonucleic acid (sodium salt, high polymer) from chicken erythrocytes with a protein content of not more than 1%, water content not more than 20%, nitrogen / phosphorus ratio 1.6 - 1.7; the max wavelength is 260 nm, at pH = 12 and the extinction coefficient is 6500 - 7500.

As the model objects of the study we used suspensions of the composition of 0.1 M TRIS HCl (2-amino-2-hydroxymethyl-propane-1,3-diol) pH = 8.0 with a filler in the form of ZrO_2 -based nano-powders at a ratio of 0.1 g / 1 ml. The suspension was applied to the substrate in a volume of 0.1 ml. Lyophilization was performed under normal physical conditions during 48 hours.

Nano-powders with a particle size of 4-5 nm and 14 nm were used in the work. They were obtained by co-precipitation of zirconium and yttrium salts by ammonia with followed heat treatment. Dehydration of the precipitate in a specialized microwave oven at $T_1 = 120^\circ\text{C}$, was used for obtaining of YSZ hydroxide powder (particle size 4-5 nm). Followed heat treatment of hydroxide during 2h at a temperature $T_2 = 700^\circ\text{C}$ was used for YSZ oxide powder obtaining (particle size 14 nm). The method of obtaining powders is reviewed in detail in [14]. The morphology of bulk samples was studied by transmission electron microscopy (TEM) methods using a JEM 200A instrument. Objects for TEM were prepared from working suspensions containing DNA by the method of ultrasonic mist [19]. Raman spectra were obtained at room temperature using a LabRAM HR Evolution Horiba spectrometer. The source of excitation of Raman spectra was He-Ne laser (633 nm) and a diode-pumped laser with a wavelength of 532 nm.

Results and Discussion

It is assumed that the buffer solution does not introduce significant distortions in the DNA spectrum; however, the latter are unavoidable during the interaction of individual substances. In order to avoid errors in the analysis of the interaction of DNA with ZrO₂ nanoparticles, spectroscopic manifestations of the interaction of the elements of the DNA solution in the buffer were considered. On the obtained Raman spectra of the buffer solution, the next well-pronounced peaks were found: 224 cm⁻¹, 254 cm⁻¹, 389 cm⁻¹, 604 cm⁻¹, 764 cm⁻¹, 917 cm⁻¹, 1048 cm⁻¹, 1187 cm⁻¹, 1302 cm⁻¹, 1404 cm⁻¹, 1464 cm⁻¹, 2767 cm⁻¹, 2890 cm⁻¹, 2950 cm⁻¹, 3180 cm⁻¹ as well as peaks low intensity: 470 cm⁻¹, 663 cm⁻¹, 1547 cm⁻¹, 1639 cm⁻¹, 3241 cm⁻¹, which correspond to different vibrations of atoms that make up the buffer solution. The next peaks series appeared after adding a DNA solution to buffer: 322 cm⁻¹, 519 cm⁻¹, 799 cm⁻¹, 1256 cm⁻¹ and 3285 cm⁻¹. These peaks indicate the presence of valence and deformation vibrations of groups of atoms of nitrogenous bases: C – H, O – H, O – P – O, which are form the DNA molecule.

Addition of zirconium hydroxide and zirconium oxide nano-powders to the buffer with dissolved DNA leads to a specific change in the Raman spectrum. Addition of zirconium dioxide nano-powder to the DNA buffer leads to an increasing in the intensity of the peaks: 254 cm⁻¹, 322 cm⁻¹, 470 cm⁻¹, 663 cm⁻¹ and to the suppression of peaks: 604 cm⁻¹, 917 cm⁻¹, 951 cm⁻¹, 1256 cm⁻¹, 1302 cm⁻¹, 2767 cm⁻¹, 3180 cm⁻¹, 3241 cm⁻¹, 3285 cm⁻¹.

In addition, zirconia nano-powder leads to a shifting of the peaks 1048 cm⁻¹, 1547 cm⁻¹ and 1639 cm⁻¹ to 1073 cm⁻¹, 1597 cm⁻¹ and 1679 cm⁻¹,

respectively, as well as reducing the intensity of the peaks 2890 cm⁻¹ and 2950 cm⁻¹.

Similar changes in the spectrum are observed in the case of zirconium hydroxide. The peaks: 470 cm⁻¹, 604 cm⁻¹, 663, 917 cm⁻¹, 1256 cm⁻¹, 1302 cm⁻¹, 2767 cm⁻¹, 3180 cm⁻¹, 3241 cm⁻¹, 3285 cm⁻¹ were suppressed, the peaks: 519 cm⁻¹, 917 cm⁻¹, 1048 cm⁻¹, 1547 cm⁻¹ and 1639 cm⁻¹ were shifted to 579 cm⁻¹, 951 cm⁻¹, 1073 cm⁻¹, 1597 cm⁻¹ and 1679 cm⁻¹, respectively. Taking into account the absence of the appearance of new substances in the solution after adding oxide nanoparticles due to the purely physical interaction of the liquid and solid phases, it can be concluded that the changes in the spectrum are a result of the pronounced energy interaction of DNA-fragments and functional groups / active centers which are localized on the surface of the nanoparticles.

In the author's opinion, the displacement of peaks in the frequency ranges of 519 -1639 and 1048 - 1639 in the case of hydroxide and oxide nanoparticles adding respectively has a greatest interest to the consideration, because it's indicates the appearance of an additional wave process which is lead to the transfer of spectrum to the high-frequency region (Up-conversion). Obviously, the surface of the nanoparticles serves as a frequency modulator that performs Fourier transformation of the spectrum [20, 21].

Suppose that the surface of the nanoparticles makes a certain contribution to the optical oscillatory system, which in the time coordinate can be described by the function $x(t)$. That is, the surface of the nanoparticles modulates a specific i -frequency $e^{j\omega_i t}$ of the Raman spectrum according to the law $x(t)$. Performing the Fourier transform of the signal $x(t)$, we obtain:

$$\Phi[x(t)e^{j\omega_i t}] = \int_{-\infty}^{\infty} x(t)e^{j\omega_i t} e^{-j\omega_i' t} dt = \int_{-\infty}^{\infty} x_i(t)e^{-j(\omega_i' - \omega_i)t} dt = F\{j(\omega_i' - \omega_i)\} \quad (1)$$

Where i – analyzing frequency of Raman spectrum;

ω_i - is the i - frequency of spectrum DNA + buffer without nanoparticles;

ω_i' - is the i - frequency of the spectrum after the frequency (heterodyne) transformation according to the $x(t)$ law.

Thus, the appearance of a certain factor that changes the temporal relations in the system leads to the transfer of the spectrum $F(j\omega)$ from the frequency ω to the frequency ω_i' . On this principle, in fact, the method of Raman scattering is built [22, 23].

Such a factor may be the local polarization of the medium at the interface between the solid and liquid phases. The polarization of the DNA solution with the buffer, caused by a double electric layer rigidly connected to the surface of the nanoparticles, makes an additional contribution

to the electron polarization of molecules which is induced by the action of light waves and, as a result, leads to a shift of the frequencies of Raman scattering.

This is also indicated by a wider range of wavelengths in which the heterodyne transformation (or Up-conversion) occurred in the case of hydroxide, since the hydroxide nanoparticles have a significantly more developed adsorption layer compared to oxide nanoparticles. It should be noted that the particle size does not have a significant effect on the wave processes at the interface, in particular, on the parameters of frequency modulation. This follows from the fact that there is no frequency shift with a significant (more than threefold) difference in the particle size of the oxide and hydroxide of the YSZ composition. According to Fig. 2, for a given grade of nanoparticles, the average shift of the wave vector is $\Delta k = 50 \pm 10 \text{ cm}^{-1}$.

As can be seen from Fig. 2, there is a change in the magnitude of the shift of the wave number k as a function of frequency. This means that the wave process arising in the system upon the introduction of nanoparticles is not monochromatic, i.e. there is a dispersion of the wave packet $\delta\omega$. As can be seen from Fig. 2, as the frequency increases, a proportional decrease in the phase shift occurs.

Such dispersion, according to the formalism of classical optics, is called normal. As can be seen from fig. 2, in the range of wavelengths 519-1639 cm^{-1} , it is about 20 cm^{-1} .

Usually the transfer of the frequency spectrum is carried out by multiplying the signal $x(t)$ on the cosine signal $\cos\omega t$. This signal according to the Euler formula can be expressed by the sum of exponentials:

$$x(t)\cos\omega t \Rightarrow \frac{1}{2}[F(j(\omega_i - \omega_i) + F(j(\omega_i + \omega_i))] \quad (2)$$

It follows that in our case there is anti-Stokes radiation, and that the spectrum must also contain peaks: 459 cm^{-1} , 861 cm^{-1} , 998 cm^{-1} , 1599 cm^{-1} , corresponding to Stokes radiation (the first term in formula (2)). But, they are absent, moreover, there is a decreasing in the intensity of peaks 2890 cm^{-1} and 2950 cm^{-1} and suppression of a number of peaks which are characteristic of DNA, in particular, peaks 604 cm^{-1} , 917 cm^{-1} , 951 cm^{-1} , 1256 cm^{-1} , 1302 cm^{-1} , 2767 cm^{-1} , 3180 cm^{-1} , 3241 cm^{-1} , 3285 cm^{-1} - in the case of the introduction of oxide nanoparticles, and peaks 470 cm^{-1} , 604 cm^{-1} , 663 cm^{-1} , 917 cm^{-1} , 1256 cm^{-1} , 1302 cm^{-1} , 2767 cm^{-1} , 3180 cm^{-1} , 3241 cm^{-1} , 3285 cm^{-1} - in the case of the introduction of nanoparticles of YSZ hydroxide.

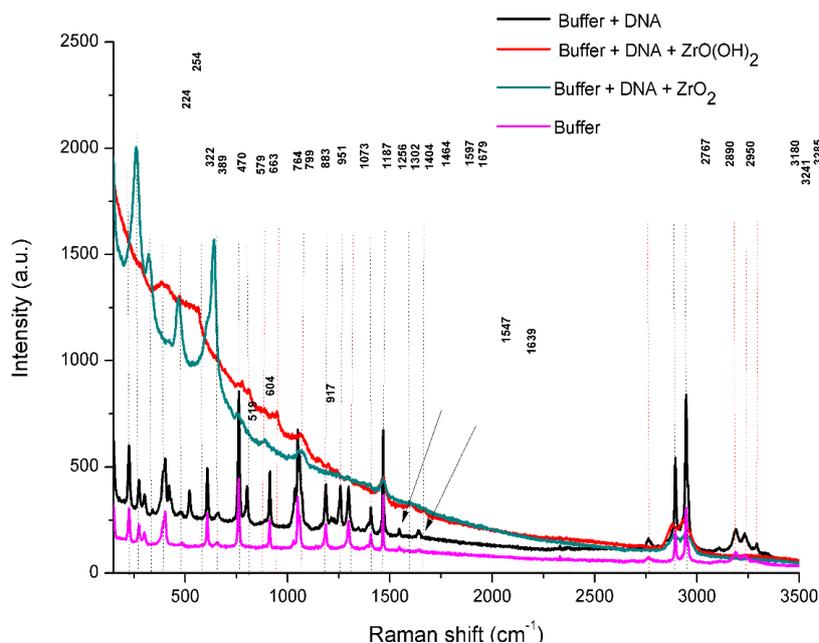


Fig. 1. Raman spectra of buffer (1), DNA in buffer solution (2) DNA in buffer solution + ($\text{ZrO}+3\text{mol}\% \text{Y}_2\text{O}_3$) (OH) $_2$ (3), DNA in buffer solution + ZrO_2 (4).

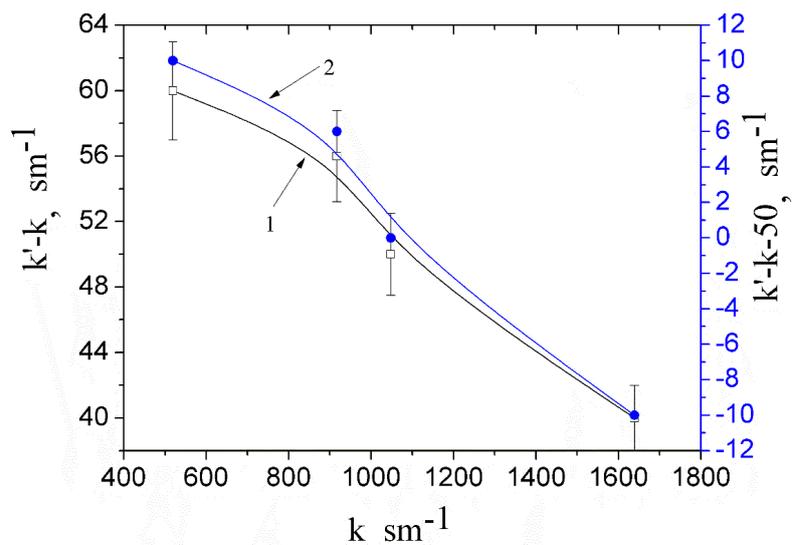


Fig. 2. The dependence of the increment of the wave number $k' - k$ on the wave number k as a result of interaction with the surface of the nanoparticles (curve 1); and the dispersion of the wave packet when the magnitude of the shift $= 5 \text{ cm}^{-1}$ (curve 2). Where k - is the wave number on the Raman spectrum of object without nanoparticles, k' - is the peak number on the Raman spectrum of object contained of nanoparticles.

This behavior of the system can be caused by blocking or screening the corresponding DNA fragments with nanoparticles. This assumption is confirmed by the results of TEM (Fig. 3). As can be seen from the pictures in Fig. 3, DNA molecules

are completely covered with a dense layer of YSZ nanoparticles (Fig. 3, d), which is probably due to the unlike charging of their surface and surfaces of nanoparticles.

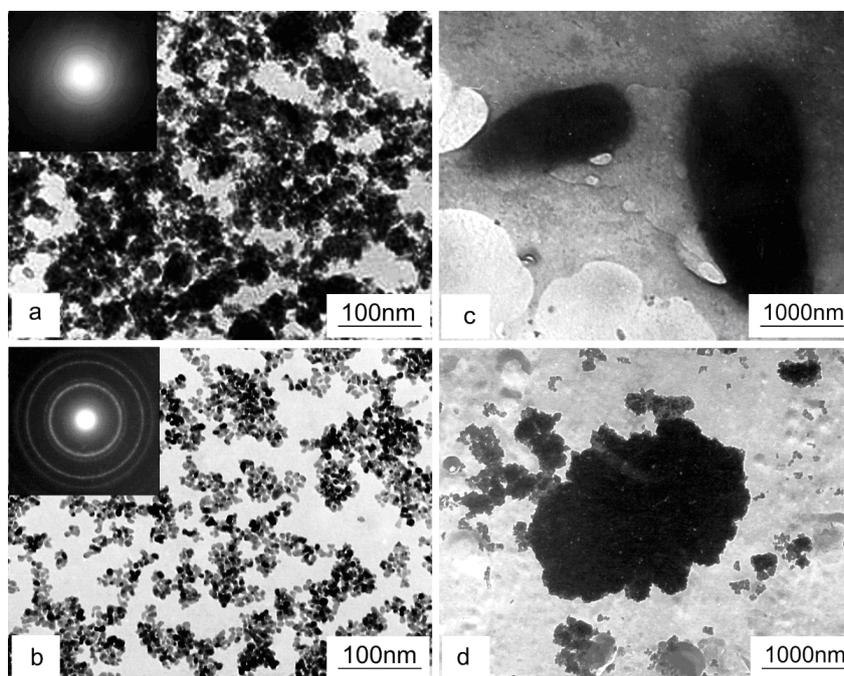


Fig. 3. TEM — images of hydroxide (a) and oxide (b) YSZ-nanoparticles, DNA in the form of a cocoon (c), DNA in a nanoparticle envelope (d). The insets are shows electron diffraction patterns of the corresponding powders.

Such spatial structure resembles a “core-shell” type structure. The functional heterojunction between biological and solid base objects opens up prospects for using of the obtained systems in the development of biocompatible electronic devices with field control and sensors of the chemical composition of the medium.

Conclusion

Using the method of Raman scattering a stable interaction of DNA with nanoscale ZrO₂ crystals is shown. The effects of increasing of the intensity of spectral lines in the long-wavelength range 250 – 660 cm⁻¹ (254 cm⁻¹, 322 cm⁻¹, 470 cm⁻¹, 663 cm⁻¹), suppressing of the intensity of spectral lines in the short-wave region 600 – 3300 cm⁻¹ (604 cm⁻¹, 917 cm⁻¹, 951 cm⁻¹, 1256 cm⁻¹, 1302 cm⁻¹, 2767 cm⁻¹, 3180 cm⁻¹, 3241 cm⁻¹, 3285 cm⁻¹) as well as shifting of lines: 519 cm⁻¹, 917 cm⁻¹, 1048 cm⁻¹, 1547 cm⁻¹ and 1639 to 40-60 cm⁻¹ to the shortwave region have been found. From the standpoint of the theory of wave processes, a qualitative description of the last established effect was given, as a heterodyne frequency spectrum transformation, due to the formation of an optically active heterogeneous transition between DNA molecules and YSZ nanoparticles.

The conclusion was made about the possibility of using the surface of YSZ nanoparticles as a heterodyne frequency converter in bio-molecular electronics operating at frequencies of the optical range.

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Ethics approval and consent to participate

The authors declare that they:

- consent for publication,
- have no conflict of interest.

Availability of data.

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توليف الترددات لاطياف رامان عند الحد الفاصل بين جزئ الحمض النووي وثاني اكسيد الزركونيوم النانومتري

نظرا للتطور المذهل في العلوم الحيوية والتي ادت الي التطور في هندسة المواد الحيوية خصوصا تلك التي تستخدم في علاج الخلايا حتي الخلايا الحية منها. ادي هذا التطور الي استحداث وتطور تقنيات نانومترية الكترونية تعمل بناء علي مواد ذكية يدخل في بنيتها مواد عضوية واخري غير عضوية بطريقة مهجنه. وفي هذا المجال يمكن لتقنية رامان وكذلك الميكروسكوب الالكتروني النافذ ان يؤديا أدورا كبيرة في دراسة ما يحدث بين جزء الحمض النووي DNA وكذلك سطح ثاني اكسيد الزركونيوم النانومتري. ويمكن القول ان فهم سلوك الحمض النووي عند الحد الفاصل مع الاكاسيد النانومترية من الامور المهمة للتحكم في كثير من الصفات التي من شأنها ان تطور اجهزة الكترونية حيوية. والمنتبع للاطياف التي تم قياسها سوف يلاحظ عدد من الامور الطيفية المهمة وعدد من الظواهر التي تم رصدها. ومن اهم الظواهر التي تم رصدها هو ازدياد شدة الخطوط الطيفية في الاطوال الموجية الطويلة في مدي 250 – 660 cm^{-1} . بينما علي العكس يحدث انتقاص في شدة الخطوط الطيفية في الاطوال الموجية القصيرة في مدي 600 – 3300 cm^{-1} كما لوحظ حدوث ازاحة للخطوط الطيفية في الاطوال الموجية القصيرة.