EXPERIMENTAL STUDIES OF OPTOACOUSTIC EFFECT ON THE MODEL OF ERYTHROCYTES IN THE PRESENCE OF CARBON NANOPARTICLES

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Abstract

Experimental model has been developed to study optoacoustic signal from model blood cells presented by polystyrene microspheres with nanoparticles. It was found out that nanoparticles due to their strong absorption of light significantly affect the coefficient of cellular optical absorption, while the thermophysical parameters, namely the coefficient of thermal expansion, compressibility and isobaric specific heat of cells remain unchanged, since nanoparticles occupy a small intracellular volume compared to the cell volume. Optoacoustic signals were obtained using model solutions at various concentrations of cells and nanoparticles using 1064 nm laser. The results of experimental measurements using LIMO 100–532/1064-U system based on Nd:YAG showed that the amplitude of the optoacoustic signal increased without increasing the temperature in the laser area.

Keywords: optoacoustic signal, hematocrit, aggregation, erythrocytes, spectral power density, laser.

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ЭКСПЕРИМЕНТАЛЬНЫЕ ИССЛЕДОВАНИЯ ОПТОАКУСТИЧЕСКОГО ВОЗДЕЙСТВИЯ НА МОДЕЛЬ ЭРИТРОЦИТОВ В ПРИСУТСТВИИ УГЛЕРОДНЫХ НАНОЧАСТИЦ

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Резюме

Разработана экспериментальная модель для изучения оптико-акустического сигнала от моделей клеток крови, представляющих собой полистирольные микросферы с наночастицами. Установлено, что наночастицы из-за их сильного поглощения света существенно влияют на коэффициент клеточного оптического поглощения, при этом теплофизические параметры, а именно коэффициент теплового расширения, сжимаемость и изобарическая удельная теплоемкость клеток остаются неизменными, так как наночастицы занимают незначительный внутриклеточный объем по сравнению с объемом самой клетки. Оптоакустические сигналы были получены с использованием модельных растворов при различных концентрациях клеток и наночастиц для воздействия лазером с длиной волны 1064 нм. Экспериментальные данные, полученные с помощью лазерной установки LIMO100–532/1064-U на основе Nd:YAG, показали, что амплитуда оптоакустического сигнала возрастала без увеличения температуры в зоне воздействия лазера.

Ключевые слова: оптоакустический сигнал, гематокрит, агрегация, эритроциты, спектральная плотность мощности, лазер.

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Introduction

Optoacoustic (OA) imaging represents combined technology used for imaging in biological tissues, which is provided by recording broadband ultrasonic (US) signals generated in biological tissues illuminated by laser. Unlike ionizing imaging techniques such as X-ray, computed tomography, positron emission tomography, only low-energy photons and US waves are used in optoacoustic transformation. For example, photon energy of visible infrared light for optoacoustic imaging is only about 2 eV, while the energy of typical X-rays for radiography is about 10–100 keV. Thus, optoacoustic imaging is safe method of non-invasive studies, especially promising for frequent application. Pure optical imaging techniques such as optical coherence tomography, fluorescence imaging and various types of optical microscopy are widely used in biomedicine and are applied to study cells and biological structures. Mainly spectroscopic features of the interaction of light and tissue, internal optical contrasts (scattering, absorption, refractive index, polarization, etc.) are used in these methods. For depths about millimeter pure optical imaging techniques use short wave of coherent light and provide high-resolution imaging for biomedical research, both at the cellular scale and for individual organs and tissues. However, beyond millimeter depth photons are strongly scattered in biological tissues, which limits the spatial resolution of purely optical imaging techniques for most biomedical applications where imaging of deeper tissue layers is required while maintaining relatively high resolution [1]. The relative low spatial resolution of this method prevents further clinical application and reduces the potential of this technique in diagnostic medicine, although during the early development of malignancy and hemorrhage there is significant contrast of optical scattering/absorption along with key physiological changes (hemoglobin, oxygenation, etc.).

In contrast to the strong scattering of the optical beam, the scattering of US waves in biological tissues is two or three orders weaker [2, 3], as a result US wave provides improved signal-to-noise ratio and higher spatial resolution compared to the diffuse photon wave for deeply located objects in biological tissues. Independent image contrast, which is absent in other imaging methods such as ultrasonography, radiography and magnetic resonance imaging (MRI), is used as one of the key optical biomarkers for tumor detection by optical absorption provided by OA imaging [4–7].

Various metallic and non-metallic nanoparticles are widely used as contrast agents in OA imaging techniques to improve its sensitivity. Biomedical imaging uses various nanoscale structures such as nanospheres, nanorods, silver, gold nanosystems, and carbon nanotubes (CNTs) as contrast enhancers.

Analysis of a lot of works published mainly in the last three years, concerning the problem of toxicity of nanotubes in living organisms and environment showed: in [8] it is noted that nanotubes are widely used in biomedicine and in conjunction with proteins play important role in the potential cytotoxicity of nanomaterials. The effect of fibrinogen shells on the biodegradation and cytotoxicity of single-wall CNTs was studied. Investigations have shown that fibrinogen binding reduces the toxicity of CNTs without affecting their biodegradation in activated cells, which opens up new opportunities in the development of safe nanotubes for biomedical applications; [9] provides critical review of available data on the effects of CNTs on human health to assess the risks associated with the use of CNTs. The CNTs parameters most likely to control toxicity, namely length, metal content, tendency to aggregation/agglomeration and chemical composition of the surface, were determined. It is noted that since CNTs have a great useful potential, it is necessary to carefully select their parameters to avoid harmful effects. In [10] it is noted that CNTs have shown promising potential in various biomedical applications. Two groups of mice were studied, which were injected with different CNTs. As a result, no significant toxicity was detected for the administered doses in any of the groups. The paper [11] discusses aspects of the use of CNTs in the treatment of melanoma, such as reducing toxicity and increasing biocompatibility. The authors propose methods to solve the problem and talk about the prospects of CNTs as a means of drug's delivery to the tumor.

Carbon nanoparticles are most suitable for diagnosis and therapy due to their simple and rapid preparation, tunable light scattering and absorption properties, ability to bind to target cells, and lack of toxicity.

Summarizing the data of the studies conducted by numerous authors, the following can be noted. The toxicity of nanotubes is not fully understood, but there is evidence that functionalized (associated with any material) nanotubes exhibit low toxicity. Toxicity also depends on the parameters of the nanotubes, such as the length and number of walls. In general, CNTs represent effective tool in therapy and diagnosis, which is noted by all authors.

The presented experimental work is based on previous studies by both the authors and other scientists [9–14]. The aim of the work is experimental verification of the theoretical model of the optoacoustic effect in the moving medium in the presence of nanoscale particles [15]. In comparison with the studies of other authors [16, 17], acoustic signal from nano objects in the moving liquid which is placed in the tube and connected to pump was registered in experiment.

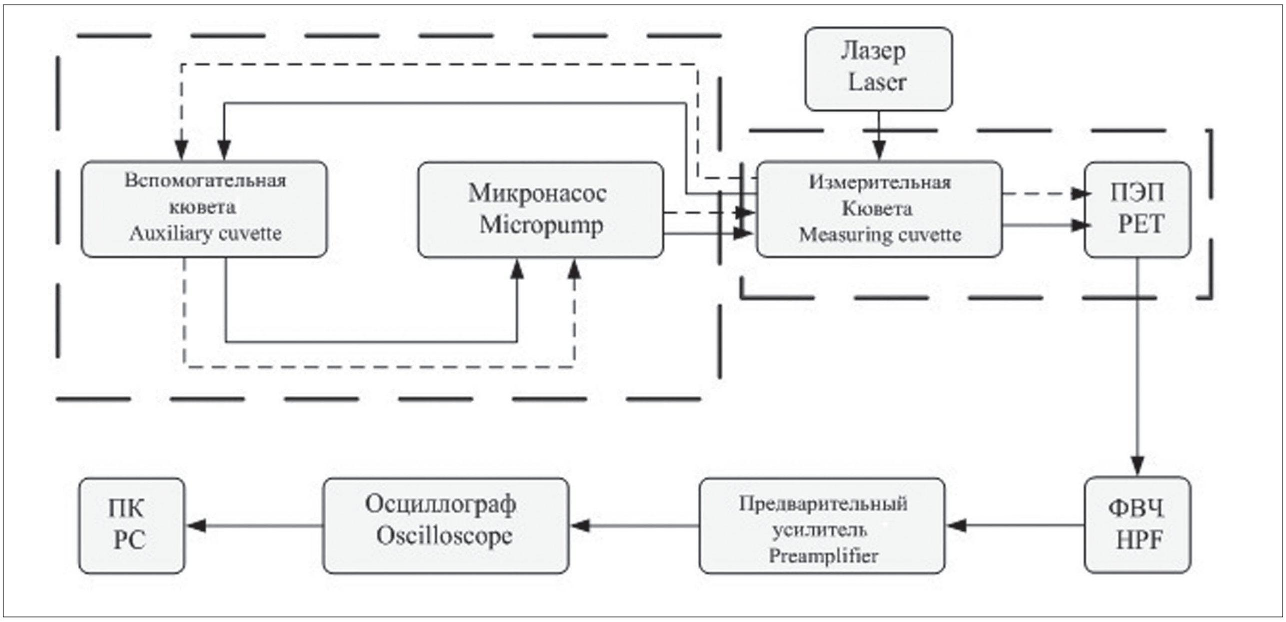


Рис. 1. Структурная схема экспериментальной установки для исследования формирования акустического сигнала в суспензиях при оптоакустическом преобразовании
Fig. 1. Block diagram of the experimental setup for the study of the formation of an acoustic signal in suspensions during optoacoustic conversion

Materials and methods

The block diagram of measurement of amplitude time realization and acoustic waves spectrum as a result of OA transformation in model liquid is shown in Fig. 1. The model fluid contains erythrocyte models and nanoscale objects (carbon nanotubes), the fluid velocity was constant, the temperature was 37±1 °C.

The pump makes it possible to form volumetric fluid velocity identical to the volumetric blood flow velocity in the human body (4 liter/min) [18–23]. When converted into linear velocity for a tube with a diameter 7 mm, we obtain 2.89 cm/s, this corresponds to the size of the arteriole in the human body and the speed of blood flow in it.

The laser beam was directed to the surface of a moving model liquid located in a measuring thermostatic cell [24]. As a result of the optoacoustic transformation, acoustic waves are formed in the model liquid, which interact with the models of erythrocytes and the conglomerate of nanoscale particles and liquid flow, amplitude and profile of the acoustic signal were changed. The acoustic signal was detected by piezoceramic transducer (probe), fed to the high-pass filter to isolate the useful signal and suppress the low-frequency noise of the laser.

Digital oscilloscope based on LabView platform (National Instruments, USA) records experimental data values. The oscilloscope is connected to personal computer (PC), where data was processing in Matlab software (MathWorks, USA), that allows to compare theoretical calculations and experimental data.

Pulses with duration 84 ns and repetition period 10 kHz was formed by LIMO 100–532/1064-U [12, 24] by single-mode Nd: YAG laser with variable power level 0.1–100 W, the installation parameters are given in table. 1.

The laser pulse repetition rate, which determines the fundamental harmonic frequency of the generated optoacoustic signal, was set programmatically in Labview as 10 kHz [12–14].

When exposed to laser beam (with the laser parameters given in table 1) acoustic waves were formed on the model liquid in cuvette as a result of OA transformation. Experiments were carried out for different types of model solutions with models of erythrocytes and carbon nanotubes.

In the experiment a number of model fluids with fillers were used to model blood and red blood cells. Next, consider the types of liquids and their characteristics.

Таблица 1
Параметры измерительной установки LIMO 100–532/1064–4
Table 1
LIMO 100–532/1064–4 measurement system parameters

Длительность импульса, нс Pulse duration, ns	84
Однородность пучка лазерного излучения, % Laser beam homogeneity, %	98,5
Энергия в импульсе (E), мДж Pulse energy (E), mJ	11
Диаметр лазерного луча (d), мм Laser beam diameter (d), mm	3,5

Sodium phosphate solution (sodium chloride solution, sodium hydrophosphate Na_2HPO_4, potassium chloride KCl and potassium dihydrophosphate KH_2PO_4) was prepared as a homogeneous absorbing medium for the experiment. Osmotic concentration and pH (7.32) of the solution are identical to blood plasma.

Polystyrene microspheres (PST) with diameter 5, 8, 15 and 20 microns (Fig. 2), produced in "Diapharm" LCC (Russia) were used for simulation. The size of polystyrene spheres was chosen to match the size of red blood cells, which are normally biconvex discs with a diameter of about 5–6 microns and average thickness of 2.0 microns. The optical absorption coefficient of the spheres is in good agreement with the data for erythrocytes in equivalent concentrations. Erythrocytes were experimentally modeled with spherical polystyrene spheres to test the theoretical model, where scattering objects were modeled as spheres in the first order approximation. Currently, the authors have moved to the next stage: theoretical modeling of real forms of red blood cells and calculation of optoacoustic response. To confirm the theoretical results, the production of polystyrene "erythrocytes", i.e. biconvex discs, will be provided.

To count the number of microspheres, the technique of counting red blood cells in the Goryaev chamber was used: it is necessary to count microspheres in five large squares located in different places of the solution sample, for example, diagonally. Thus, knowing the sum of the microspheres in five large squares (80 small), we found the arithmetic mean number of microspheres in one small square. Multiplying the found number by 4000 (the volume of the chamber space over one small square is 1/4000) we obtained the number of microspheres in 1 mm^3 of diluted blood. As a result, we got the number in terms of 1 liter of blood, i.e. the number of millions of microspheres.

The suspension was further diluted with deionized water to obtain lower concentrations (100 % = $5 \cdot 10^6$ particles/μliter).

Nanoparticles are known to be used in medicine as contrast enhancers in various optical imaging techniques such as optical coherence tomography, fluorescence imaging, and optical reflection microscopy. CNTs are cylindrical molecules that consist of rolled sheets of single-layer carbon atoms (graphene). CNTs with average length 5 μm and diameter 20 nm, which were manufactured in the Scientific Educational Center "Nanotechnology" of Southern Federal University, were used in experiment. Nanotubes are structured particles that do not dissolve in water or organic liquids. Mixing in ultrasonic bath was applied for their suspension. The output result was closer to carbon nanofibers with an average length 70–100 μm and diameter 30–50 nm.

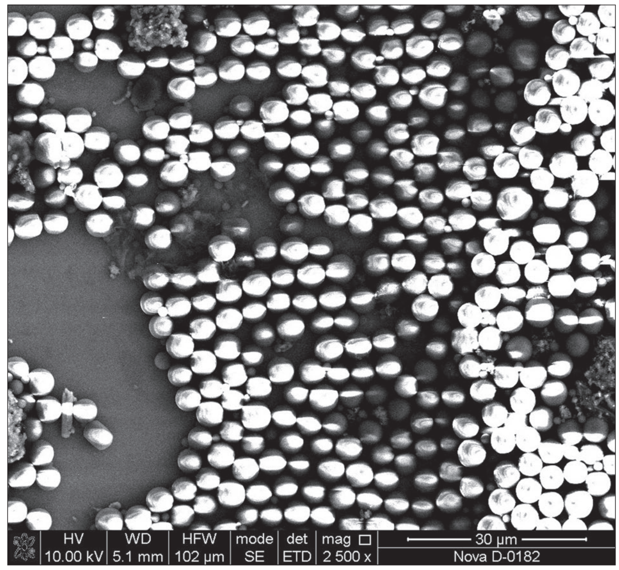


Рис. 2. Полистирольные микросферы в модельном растворе (70% микросфер) (Nova Nanolab 600)
Fig. 2. Polystyrene microspheres in a model solution (70% microspheres) (Nova Nanolab 600)

Results

During the experiments on investigation of acoustic signal generation in model suspensions, control images of the solutions were taken (Fig. 2) using the scanning electron microscope Nova Nanolab 600 (FEI Company, the Netherlands) and microscope Olympus X-71 (NTEGRA Vita, Russia).

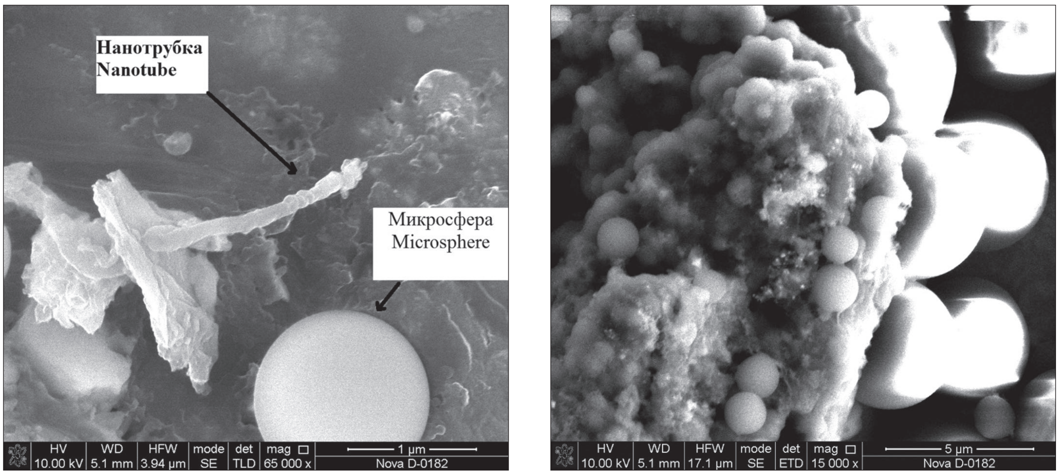
As seen in Fig. 2, carbon nanotubes assemble into conglomerates, while "capturing" microspheres. This process can be viewed using images obtained with the scanning microscope Nova Nanolab 600 (Fig. 3).

The ability of nanoparticles and their conglomerates to encapsulate substances is demonstrated in Fig. 4.

The time of laser exposure was about 80 ns (time of signal peak growth). The signal amplitude increased by 28% with the increase of laser power by 15%. At the same time, we note that the relaxation time after the peak of heating changed, so at the power 0.085 W, the relaxation time was 9.2 μs, and at 0.1 W – 18.5 μs. This is further illustrated in Fig. 5.

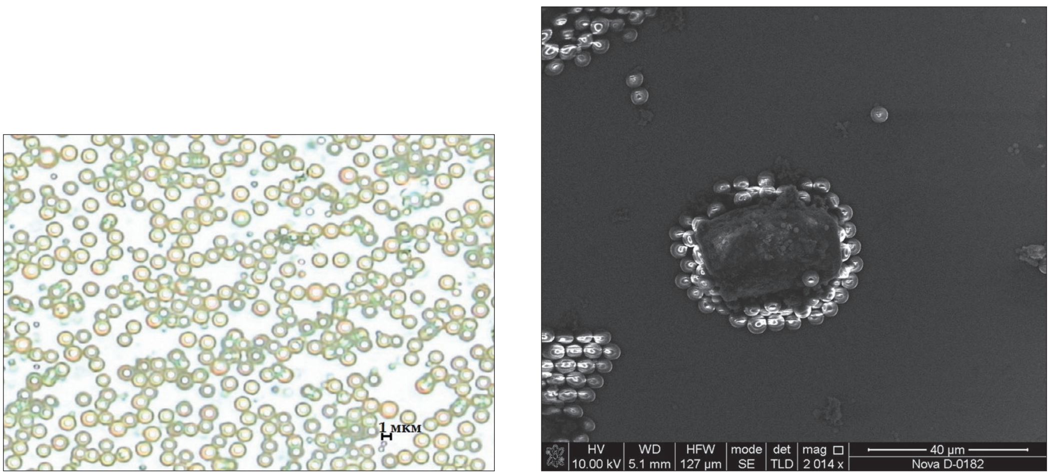
On Fig. 6 the profiles of acoustic signal generated in sodium-phosphate solution and sodium-phosphate solution with 52% of microspheres are given, which corresponds to hematocrit parameters [8–10].

From Fig. 6 it is seen that the relaxation time in the solution without microspheres is 0.52 ms. In the presence of microspheres, the relaxation time of the acoustic signal decreases (up to 0.45 ms) due to absorption and scattering of the optical signal by the spheres, while an increase in the signal amplitude and shift in the signal spectrum towards lower frequency is observed (Fig. 7), which also indicates on the greater absorption capac-



a b

Рис. 3. Раствор полистирольных микросфер:
a – полистирольная микросфера с нанотрубкой;
b – конгломерат наночастиц с микросферами в модельном растворе (Nova Nanolab 600)
Fig. 3. Polystyrene microspheres solution:
a – polystyrene microsphere with nanotube;
b – conglomerate of nanoparticles with microspheres in a model solution (Nova Nanolab 600)



a b

Рис. 4. Натрий-фосфатный раствор с микросферами и углеродными наночастицами:
a – изображение, полученное на оптическом микроскопе Olympus X-71
b – изображение, полученное на растровом микроскопе Nova Nanolab 600
Fig. 4. Sodium phosphate solution with microspheres and carbon nanoparticles:
a – image from Olympus X-71 optical microscope;
b – image from scanning electron microscope Nova Nanolab 600

ity of solution. Fluctuations of the relaxation part of the signal due to multiple reflections from microspheres are also observed in the profile of the acoustic signal with microspheres.

Conclusion

The authors of this work theoretically [13, 14, 24] and experimentally [12, 15] investigated the OA signal in a moving liquid in the presence of CNTs. Since it is supposed to diagnose erythrocytes by blood flow, experimental installation was designed and tested that allows to simulate the movement of blood in a vessel. It is shown that at low velocities (in medium vessels) the influence of the flow can be neglected.

Experimental studies of the effect of contrast agents based on nanoparticles on the formation of the opto-

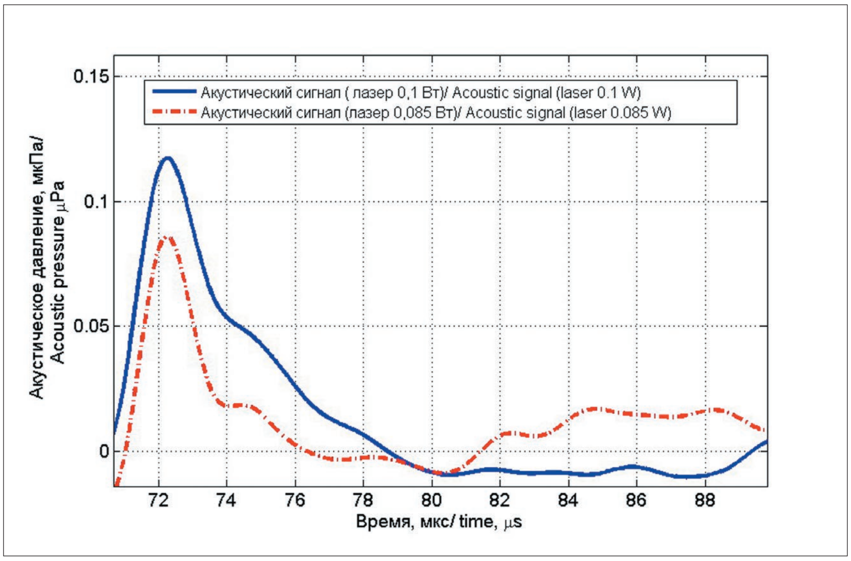


Рис. 5. Акустический сигнал, зарегистрированный в водном растворе при различных используемых мощностях
Fig. 5. Acoustic signal registered in aqueous solution at various used laser power

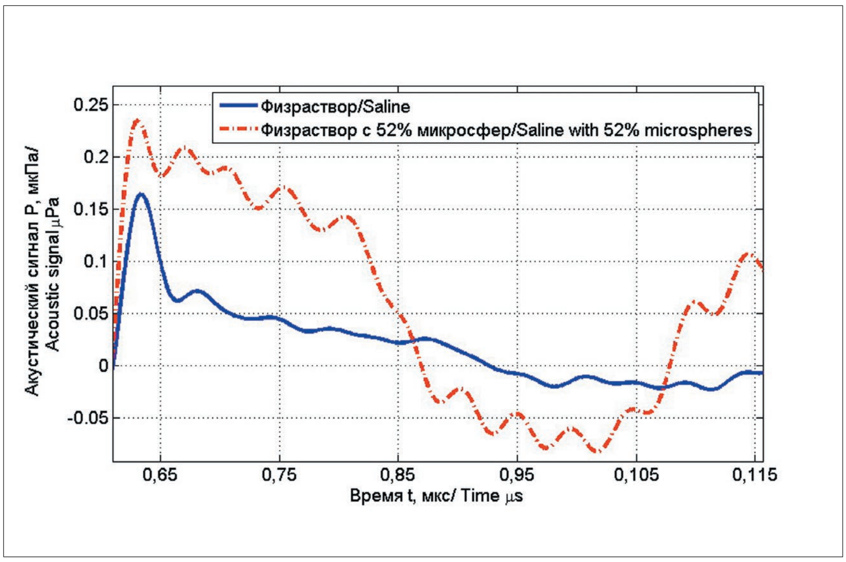


Рис. 6. Регистрируемые опытные значения оптоакустического сигнала в чистом физрастворе и физрастворе, содержащем микросферы
Fig. 6. Recorded experimental optoacoustic signal in pure saline and saline containing microspheres

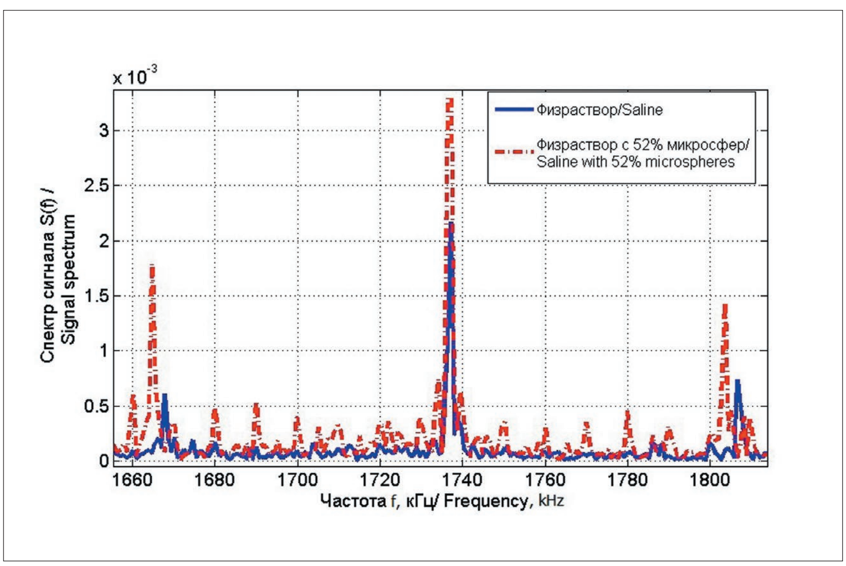


Рис. 7. Спектр оптоакустического сигнала в чистом физрастворе и физрастворе, содержащем микросферы
Fig. 7. Optoacustic signal spectrum in pure saline and saline containing microspheres

acoustic signal showed an increase in the amplitude of the OA signal due to increased absorption by CNTs. The OA signal form at high concentration of nanoparticles confirms the theoretical calculations of the forms of the optoacoustic signal obtained in previously published works [13, 14, 24].

These results can be used in the design of systems for rapid diagnosis of blood for the presence of bacterial, cancer cells, the degree of aggregation of red blood cells.

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