Effect of iron oxide nanoparticles on the concentration-versus-sizes relation of proteins in the blood plasma and serum, and in model solutions

M. N. Kirichenko, N. A. Bulychev, L. L. Chaikov, M. A. Kazaryan, A. V. Masalov

M. N. Kirichenko, N. A. Bulychev, L. L. Chaikov, M. A. Kazaryan, A. V. Masalov, "Effect of iron oxide nanoparticles on the concentration-versus-sizes relation of proteins in the blood plasma and serum, and in model solutions," Proc. SPIE 10614, International Conference on Atomic and Molecular Pulsed Lasers XIII, 106140M (16 April 2018); doi: 10.1117/12.2303471

SPIE.

Event: XIII International Conference on Atomic and Molecular Pulsed Lasers, 2017, Tomsk, Russia

Effect of iron oxide nanoparticles on the concentration-versus-sizes relation of proteins in the blood plasma and serum, and in model solutions

M.N. Kirichenko*[a], N.A. Bulychev[a,b], L.L.Chaikov[a,c], M.A. Kazaryan[a], A.V. Masalov[a,c]
[a]Lebedev Physical Institute of the Russian Academy of Sciences, Moscow 119991, Russia; [b]Moscow Aviation Institute, Moscow125993, Russia; [c]National Research Nuclear University "MEPhI", Moscow 115409, Russia

ABSTRACT

The effect of iron (III) oxide nanoparticles produced in acoustoplasma discharge with cavitation on the concentration and the sizes of particles in model protein solutions, human blood serum and plasma samples is studied. Dynamic and static light scattering data on size and concentration of particles show that the nanoparticles addition to fibrinogen-thrombin system affects the course of enzymatic reaction. Interaction of nanoparticles with fibrinogen solution (before thrombin addition) does not significantly change the distribution of scattered light intensity on particle sizes. Comparison of the relations of particle sizes and their concentration for fibrinogen solution with and without nanoparticles shows an increase of the slope of size-concentration relation in a log-log scale, which indicates an increase in the concentration of small particles and decrease of big ones. For model solution of fibrinogen with thrombin, initially incubated with iron oxide nanoparticles, the slopes of the size-concentration relation equals to $k = -(4.62\pm0.33)$ and slightly differs from the slope of the relation for fibrinogen-thrombin system without nanoparticles $k=-(4.23\pm0.28)$. We believe that changes in the size-concentration relation indicate the interaction of nanoparticles with proteins, which results in gelation rate change.

Keywords: dynamic light scattering, blood plasma, particle sizes fibrinogen-thrombin reaction, concentration-size relation, power law, iron oxide nanoparticles, acoustoplasma discharge with cavitation.

1. INTRODUCTION

The study of the effect of new nanoparticles on biological enzyme processes is of great interest because of the rapid introduction of nanotechnology into medical practice. Nanoparticles being injected to a blood stream can interact with proteins and other substances there, which lead to different (positive or negative) effects for the human body.[1,2] To study the interaction of nanoparticles with blood plasma proteins, scientists carry out experiments on model protein solutions by means of different optical methods.[3,4] In our previous experiment [5], we studied the effect of iron oxide nanoparticles obtained in acoustoplasma discharge with cavitation on the reaction of fibrin gel formation caused by enzymatic cleavage of fibrinogen by thrombin (one of the stages of blood coagulation) in the model solution. By means of dynamic light scattering technique we found out that in dependence of sequence of the nanoparticles injection to the protein solution, the course of the reaction of gelation is dramatically changed. In this paper, we estimate the concentrations of the proteins and their complexes (Rayleigh-Gans-Debay approximation) in model protein solution and human blood plasma when interacting with nanoparticles. Previously we have shown [6-8] that in the samples of human blood plasma relation between concentrations Np of particles and their sizes r obey power law dependence with the exponent –4,0±0,3. For model fibrinogen-thrombin system, the relation Np–r was close to that obtained for blood plasma. In this paper we show how iron oxide nanoparticles affect the concentrations of particles of different sizes in fibrinogen-thrombin system with various sequence of adding them to the solution.

The aim of the paper is to measure the intensity of light scattering on protein particles and their radii, to obtain concentrations of scattering particles (proteins and their aggregates) in Rayleigh-Gans-Debay approximation and to obtain the concentration-sizes relation in model fibrinogen-thrombin system with various sequence of addition of iron oxide nanoparticles produced in acoustoplasma discharge to the solution.

*maslovamarina87@gmail.com; phone number +7 499 132 6309.

XIII International Conference on Atomic and Molecular Pulsed Lasers, edited by Victor F. Tarasenko, Andrey M. Kabanov, Proc. of SPIE Vol. 10614, 106140M

2. METHODS AND MATERIALS

For our study we used traditional DLS setup with a He-Ne laser (the wavelength of 633 nm, the power of 5 to 10 mV), an optical system ensuring spatial coherence of recorded light, a goniometer allowing measurements at various angles, a special photomultiplier, a Photocor-FCm correlator (in the «Multiple tau» regime), and a PC with Photocor-FCm and DynaLS programs. For a more detailed description of the setup see paper.[9] Blood plasma and protein solution samples 3 to 4 ml in volume were placed in a dust-free cylindrical cuvette 15 mm in diameter and an immersion cuvette 34 mm in diameter to ease the setup adjustment. The cuvette was thermostated at 37ºC.

Fresh blood was taken from the donor's ulnar vein to 10-ml syringes with anticoagulant K2-EDTA. The blood plasma samples were obtained by centrifuging of whole blood for 15 minutes on a lab centrifuge CLC-1 with the rate 3000 rev/min.

For model fibrinogen solution preparation we used fibrinogen from human plasma (Sigma Aldrich, ≥80% of protein is clottable) diluted in Tris-HCl buffer (7.2 pH). Final concentration of fibrinogen was about 3 mg/ml. Fibrinogen solution was filtered through 0.2 μm "Superpure PVDF" filter in order to remove essential aggregates.

Solution of thrombin was prepared using thrombin from human plasma (Sigma Aldrich, lyophilized powder, specific activity≥2,000 NIH units/mg protein) that was diluted in Tris-buffer. Final activities of thrombin in fibrinogen-thrombin solution were 0.000155 and 0.125 NIH/ml.

Iron oxide nanoparticles were obtained by unique acoustoplasma method with cavitation that combines effect of the elastic oscillations of high intensity ultrasound and pulsed or steady electric fields in a liquid medium. As a result relatively narrow size distribution of the synthesized nanoparticles with specific surface properties was obtained. Detailed description of the setup for the nanoparticles production is presented in [10, 11]. In our experiments, final volume concentration of Fe_2O_3 nanoparticles in the solution was Cv ≈ $0.5 \cdot 10^{-7}$ (np ≈ 4·10-7 μ^{-3}). Mean hydrodynamic radii of nanoparticles aggregates in the solution were of about 183 nm and 7900 nm.

3. THEORETICAL CONSIDERATIONS

The DLS technique allows us to obtain scattered light intensity distribution on particle sizes by processing of autocorrelation functions with DynaLS software. For some biological liquids (human blood plasma and serum), the distributions occur to be unstable from measurement to measurement. Paper [6] presents an adaptation of DLS method to such solutions, which consists in the accumulation of a large array of the distributions with subsequent statistical processing. For each size in the array, the particle concentrations N were calculated from Rayleigh-Gans-Debay approximation:

$$N = (I_{tot} A_i r_i (\sum_i A_i \cdot r_i)^{-1} (\eta_e \frac{V_{sc}}{R^2} |n_1 / n_2 - 1|^2 G^2(\theta) r_i^6)^{-1} \quad (1)$$

where I_{tot} is the total light intensity registered in each measurement, A_i is logarithmic part of intensity of scattering on particles with radius r_i, $A_i r_i (\sum_i A_i r_i)^{-1}$ is the fraction of the light intensity scattered by particles of hydrodynamic radii r_i, n_1 is the refractive index of particle (proteins refractive index is 1.34), n_2 is the refractive index of water; $G^2(\theta)$ is the form-factor of particles for cylindrical shapes. Unknown parameters (such as quantum efficiency of photomultiplier cathode η_e, scattering volume V_{sc}, distance between the point of observation and scattering volume R) were determined by comparing our signal with a signal of scattered light intensity in toluene, whose scattering coefficient is known. The relations of the particle sizes and their concentrations for the blood plasma and the model solution of fibrinogen with thrombin (in concentration 0.000155 NIH/ml) obey to power law dependence with an exponent close to 4.0±0.3.

4. EXPERIMENTAL

4.1 Model fibrinogen-thrombin systems

To understand the nature of the power law relation of particle sizes and their concentrations in blood plasma we carried out the model experiments on protein solution. After taking blood from the body, a cascade of clotting reactions is triggered in it, which, although slowed down by anticoagulants, but continues.[12] Therefore, we decided to investigate the reaction of fibrinogen degradation by thrombin followed by gelation, as one of the stages of a clotting mechnism. The

reaction was modeled at concentration of fibrinogen (3 mg/ml) and two concentrations of thrombin (0.000155 and 0.125 NIH/ml). Change of the distribution of scattered light intensity on particle sizes in the solution because of the reaction passing is presented in [5]. Processing of the obtained data in accordance with the description above has made it possible to obtain a relation of particle sizes and their concentrations for model solutions in two concentrations of thrombin (figure 1). The data were obtained during 1.5 hours after fibrinogen-thrombin mixing. One can see that the slope of straight lines approximating the data are practically the same for two concentrations: for high concentration (red line) the slope is –4.23±0.28 and for low concentration (black line) is –4.12±0.24. Apparently, the slope does not depend on the thrombin concentration and, consequently, reaction rate (since the reaction rate depends on the concentration of the enzyme), but is determined by the ratio of concentrations of protein monomers and their aggregates.

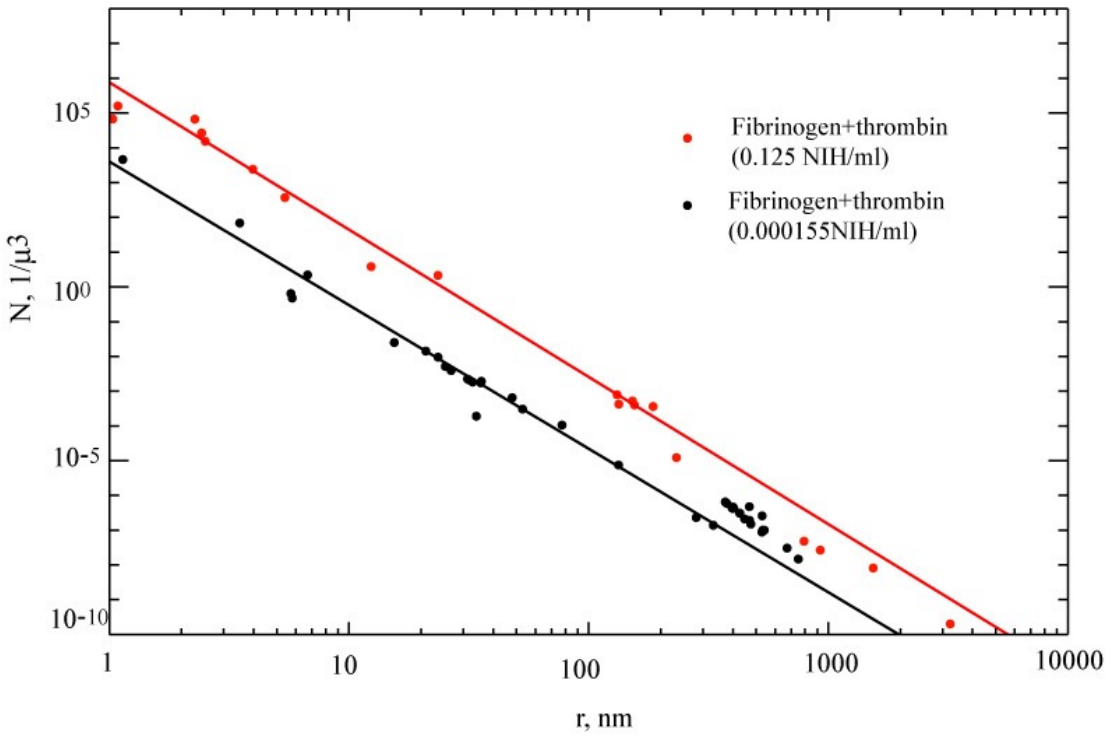

Figure 1. Relations between particles sizes and corresponding concentrations in a log-log scale for fibrinogen solution with thrombin in high concentration of 0.125 NIH/ml (red dots and red line) and in low concentration of 0.000155 NIH/ml (red dots and red dashed line. The data were obtained during 1.5 hours.

4.2 Model fibrinogen solution with iron oxide nanoparticles

As mentioned above, the question about interaction of novel nanoparticles with plasma proteins is of great interest now. In paper [5], we showed that iron oxide nanoparticles obtained in acoustoplasma discharge with cavitation affect the course of the enzymatic reaction fibrinogen-thrombin. Incubation of nanoparticles with fibrinogen before thrombin addition to the solution completely stops the reaction. Here we show changes in sizes and concentrations of the particles in fibrinogen solution after nanoparticle addition (figure 2). The slope of straight line (fig.2, red line) approximating the concentration-size relation in the pure fibrinogen solution is –5.08±0.29, which differs from the slope for blood plasma (k=–4.0±0.3) and model fibrinogen-thrombin solutions. Addition of nanoparticles does not practically change of the slope of the concentration-size relation (fig.2, black line) k = –5.06±0.25. But one can see the absence of large particles (more 1000 nm) after nanoparticle addition and appearence of the particles of intermediate sizes (20-100 nm). Such a redistribution seems to be associated with the interaction of nanoparticles with proteins, which leads to an inactivation of fibrinogen and the impossibility of further interaction with thrombin (which we observed in [5]). The mechanism of such inactivation is the subject of another study and will be considered elsewhere.

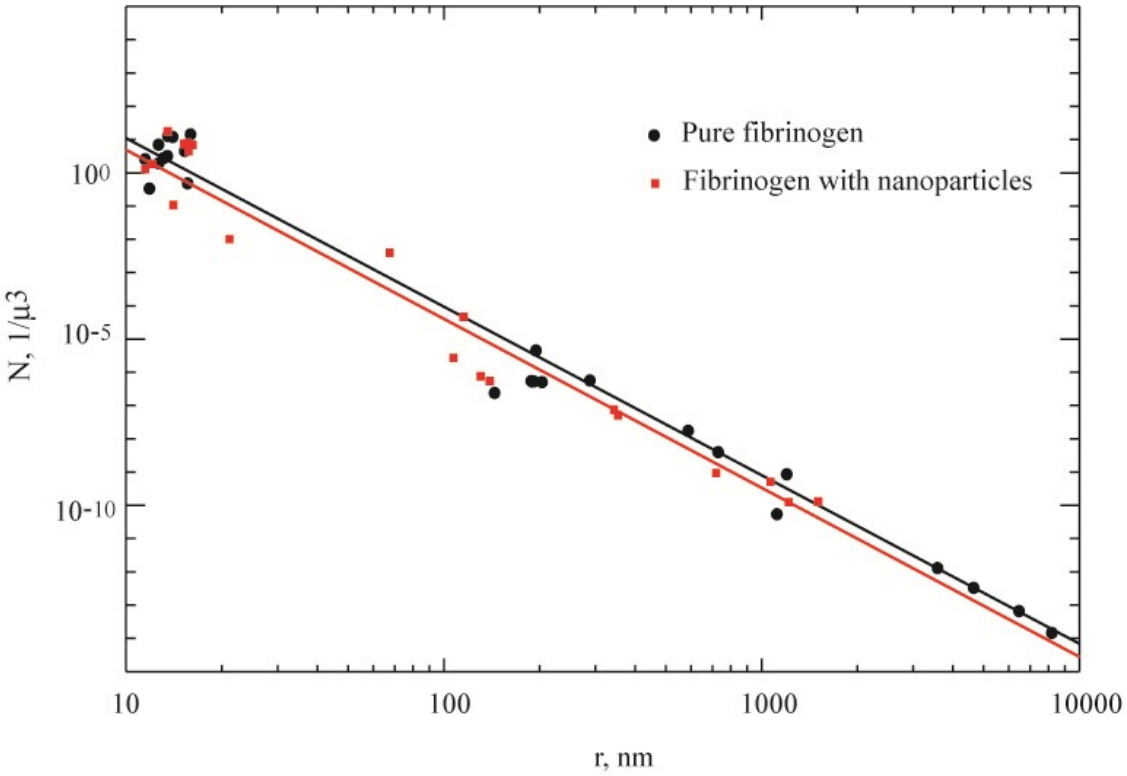

Figure 2. Relations between particles sizes and corresponding concentrations in a log-log scale for pure fibrinogen solution (black dots and black line) and for fibrinogen solution with iron oxide nanoparticles (red dots and red dashed line).

4.3 Nanoparticle-thrombin interaction, effect on the rate of fibrinogen-thrombin reaction

As noted above, the sequence of addition of iron oxide nanoparticles affects the course and the result of enzymatic reaction. If thrombin in high concentration (0.125 NIH/ml) is incubated with nanoparticles during 1 hour before its adding to fibrinogen solution, the reaction of gel formation is accelerated. Relation of particle sizes and their concentrations in this case is presented in figure 3 (blue dots and dashed line for approximation). One can see that in comparison with relations for fibrinogen-thrombin system without nanoparticles, the slope of straight line changes and equals to –4.62±0.33. Such a change of the slope seems to be related with a change of the sample state (transition to gel).

4.4 Human blood plasma and nanoparticles addition

In the work [6], we showed that for blood plasma samples the relation between particles sizes and their concentrations demonstrates power law dependence with the exponent equals to –4.0±0.3. Such a form of the dependence and the exponent do not change significantly under plasma dilution 10 and 100 times.[8] Here we show results of measurements of particles sizes and their concentrations for human blood plasma sample diluted 10 times with and without iron oxide nanoparticles. Figure 4 presents relations between particles sizes and their concentrations for the blood plasma sample (red dots and line) and for the same plasma with nanoparticles (black dots and line). One can see that the slopes of straight line are very close to each other: for diluted blood plasma –4.33±0.25 and for plasma with nanoparticles –4.10±0.23. This indicates that, although insignificant, but perhaps an increase in the concentration of large particles (200-300 nm) due to the interaction of blood plasma particles with iron oxide nanoparticles.

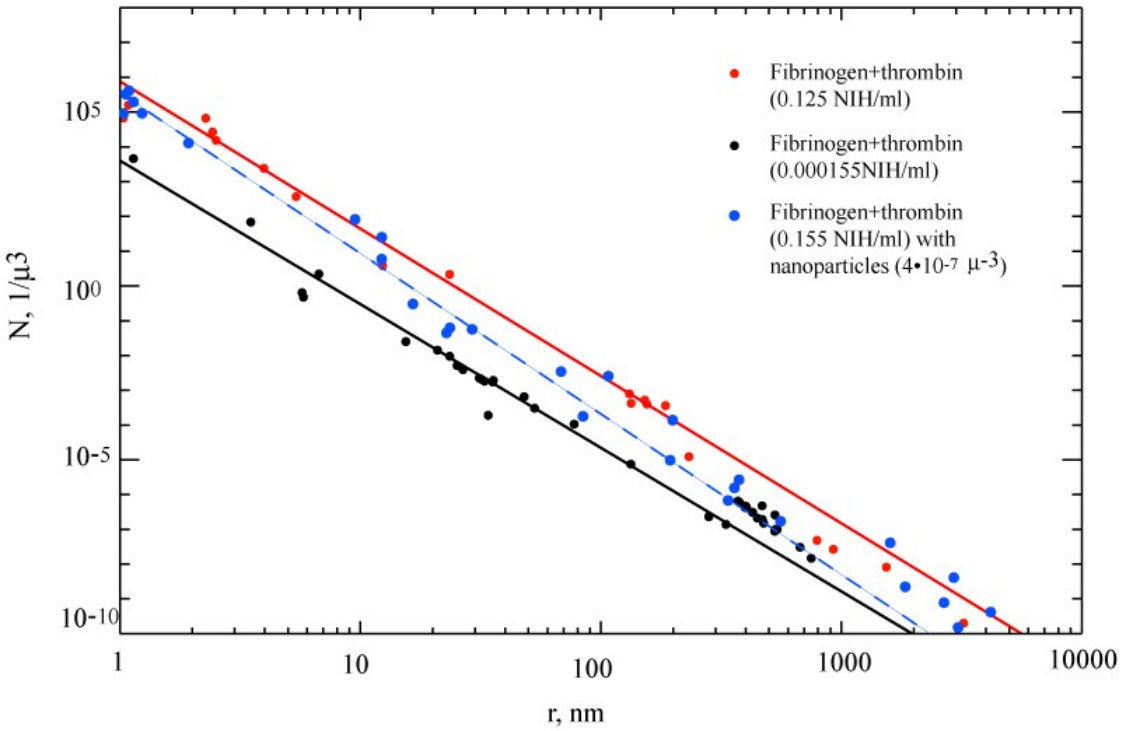

Figure 3. Relations between particles sizes and corresponding concentrations in a log-log scale for fibrinogen solution with thrombin in high concentration of 0.125 NIH/ml (red dots and red line) and in low concentration of 0.000155 NIH/ml (red dots and red dashed line), and for fibrinogen with thrombin in high concentration initially incubated with iron oxide nanoparticles (blue dots and dashed line).

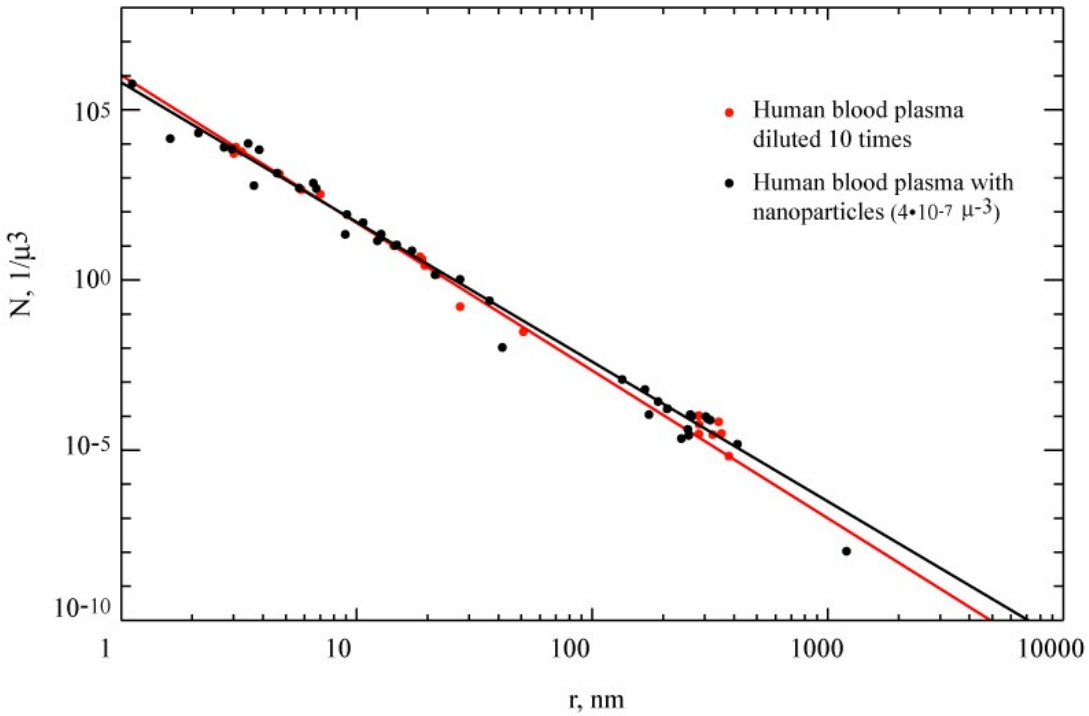

Figure 4. Relation between particles sizes and corresponding concentrations in a log-log scale for human blood plasma diluted 10 times (red dots and line) and for human blood plasma with iron oxide nanoparticles (black dots and line).

5. CONCLUSIONS

In the paper, we showed that the addition of iron oxide nanoparticles (obtained in acoustoplasma discharge with cavitation) to the model fibrinogen-thrombin system changes the exponent of the power law concentration-sizes relation. Such changes seem to be connected with the processes of interaction of nanoparticles with proteins with further redistribution of particles number between existing sizes. It is interesting that despite of obvious biological effect of iron oxide nanoparticles on the course of the enzymatic reaction fibrinogen-thrombin (that was shown previously), the formation of big complexes of nanoparticles with proteins was not detected in our samples. For example, Bychkova et al. [13] registered the formation of large aggregates of nanoparticles with fibrinogen (with 20-30 layers of fibrinogen molecules on the particle) for model fibrinogen solution with magnetite (Fe_3O_4) nanoparticles. We observed neither a significant increase in the size of aggregates nor in their concentration. Perhaps this is due to the morphology of the surface of our nanoparticles and they do not cause the formation of large aggregates, but have obvious biological effect which can be useful from the point of view of their use in medical practice. However, this problem requires further study.

This work was partially supported by the MEPhI Academic Excellence Project (agreement with the Ministry of Education and Science of the Russian Federation of August 27, 2013, project no. 02.a03.21.0005).

REFERENCES

[1] Ilinskaya, A., Dobrovolskaia M., "Nanoparticles and the blood coagulation system. Part I: Benefits of nanotechnology," Nanomedicine. 8(5), 773-784 (2013).
[2] Ilinskaya, A., Dobrovolskaia, M., "Nanoparticles and the blood coagulation system. Part II: safety concerns," Nanomedicine. 8(6), 969-981 (2013).
[3] Zhang, H., Wu, P., Zhu, Z., and Wang, Y., "Interaction of γ-Fe_2O_3 nanoparticles with fibrinogen," Spectrochim Acta A Mol Biomol Spectrosc. 151, 40-47 (2015).
[4] Canoa, P., Simon-Vazquez R., Popplewell, J., and Gonzalez-Fernandez, A., "A quantitative binding study of fibrinogen and human serum albumin to metal oxide nanoparticles by surface plasmon resonance," Biosens. Bioelectron. 74, 376–383, (2015)
[5] Kirichenko, M.N., Krivokhiza S.V., Chaikov L.L., and Bulychev, N.N., "The influence of the sequence of nanoparticles injection to solution on the rate of fibrinogen-thrombin reaction," J. Phys.: Conf. Ser. 784, 012025 (2017).
[6] Chaikov, L.L., Kirichenko M.N., Krivokhizha S.V., and Zaritskii A.R., "Dynamics of statistically confident particle sizes and concentrations in blood plasma obtained by the dynamic light scattering method," J Biomed Opt, 20 (5), 057003 (2015).
[7] Kirichenko M.N., Masalov A.V., Chaikov L.L., and Zaritskii A.R., "Relation between particle sizes and concentration in undiluted and diluted blood plasma according to light scattering data," Bull. Lebedev Phys. Inst., 42(2), 3 (2015).
[8] Kirichenko, M.N., Chaikov, L.L., Zaritskiy, A.R., "Power law relation between particle concentrations and their sizes in the blood plasma," J. Phys.: Conf. Ser. 741 012055 (2016).
[9] Kovalenko, K.V., Krivokhizha, S.V., Masalov, A.V., and Chaikov, L.L., "Correlation spectroscopy measurements of particle size using an optical fiber probe," Bull. Lebedev Phys. Inst. 36(4), 65 (2009).
[10] Bulychev, N.A, Kazaryan, M.A., Chaikov, L.L., Ivashkin, P.I., Zakharyan, R.A., Averyushkin, A. S., and Chernov A.A., "Effect of ultrasonic cavitation on dynamics and characteristics of electric discharge in liquid," Bull. Lebedev Physics Ins. 41(9), 264 (2014).
[11] Feofanov, I.N., Bulychev, N.A., Kazaryan, M.A., Chaikov, L.L., Burkhanov, I.S., Averyushkin, A.S., Krasovskii, V. I., Rasmagin, S.I., Zadorin, D.A., Chenet, C.H., "Nanoscale metal oxide particles produced in the plasma discharge in the liquid phase upon exposure to ultrasonic cavitation. 3. Optical nonlinearities of particle suspensions," Bull. Lebedev Physics Ins. 43(5), 37–44 (2016).
[12] Yi, J, Liu, Z., Craft, D., O'Mullan, P., Ju, G., and Gelfand, C.A., "Intrinsic peptidase activity causes a sequential multi-step reaction (SMSR) in digestion of human plasma peptides," J. Proteome Res. 7, 5112 (2008).
[13] Bychkova, A.V.; Sorokina, O.N.; Kovarskii, A.L.; Leonova, V.B.; Rozenfel'd, M.A. "Interaction between blood plasma proteins and magnetite nanoparticles," Colloid Journal. 72(5), 696-702 (2010).