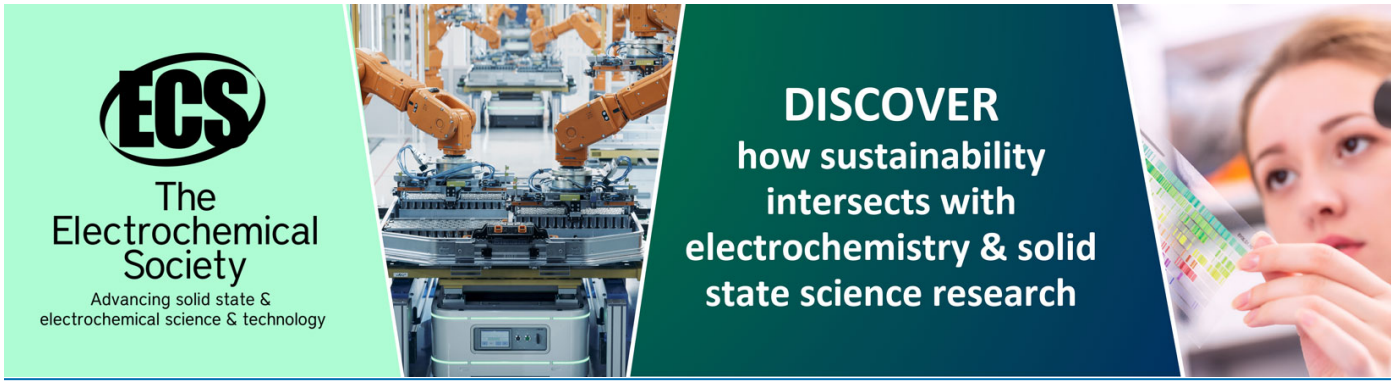
# Power law relation between particle concentrations and their sizes in the blood plasma





# Power law relation between particle concentrations and their sizes in the blood plasma

**M N Kirichenko**[1], **L L Chaikov**[1,2], **A R Zaritskii**[1]

[1] Lebedev Physical Institute of the Russian Academy of Sciences, Moscow 119991, Russia
[2] National Research Nuclear University "MEPhI", Moscow 115409, Russia

**Abstract.** This work is devoted to the investigation of sizes and concentrations of particles in blood plasma by dynamic light scattering (DLS). Blood plasma contains many different proteins and their aggregates, microparticles and vesicles. Their sizes, concentrations and shapes can give information about donor's health. Our DLS study of blood plasma reveals unexpected dependence: with increasing of the particle sizes r (from 1 nm up to 1 μm), their concentrations decrease as $r^{-4}$ (almost by 12 orders). We found also that such dependence was repeated for model solution of fibrinogen and thrombin with power coefficient is -3,6. We believe that this relation is a fundamental law of nature that shows interaction of proteins (and other substances) in biological liquids.

## 1. Introduction

Study of sizes and concentrations of proteins and their aggregates in biological liquids as well as development of methods of their measurement is an important problem of modern analytical biophysics and medicine. Measurement of particle sizes in the range from one to hundreds of nm, in which the traditional microscopy fails, is of great interest. Dynamic light scattering takes an important place among different methods because of its noninvasive nature and the possibility of particle measurements in a wide range of sizes (from 1 to 3000 nm) without disrupting of sample integrity. However, processing of DLS data obtained for complex liquids, which contains more than three groups of scattering particles, sometimes demonstrates non-reproducible results.

**The aim** of the study was to investigate sizes and concentrations of scattering particles (proteins, their aggregates and vesicles) in blood plasma and model proteins solutions and to adopt DLS method to the case of liquid with wide distribution of particle sizes.

## 2. Methods and materials

For our study we used the 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 [1].

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. Fresh blood was taken from the donor's ulnar vein to 10-ml syringes with heparin (anticoagulant). The blood plasma samples were obtained by centrifuging whole blood for 15 minutes on the 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 2 mg/ml. After sample preparation solution was filtered through 0.2 μm "Superpure PVDF" filter.

Solution of thrombin was prepared using thrombin from human plasma (Sigma Aldrich, lyophilized powder, ≥2,000 NIH units/mg protein) that was diluted in Tris-buffer. Final activity of thrombin after adding it to fibrinogen solution was 0.00125 NIH/ml.

## 3. Results of plasma study with DLS method.

The DLS study of fresh blood plasma showed that intensity size distributions obtained by processing of autocorrelation functions with conventional DynaLS software were unstable from measurement to measurement. Adaptation of the DLS method is presented in our paper [2] where we demonstrated that the storage of a big array of DLS data (intensity size distributions) with their subsequent information compression allows obtaining stable particle sizes. Figure 1a demonstrates data array where every point corresponds to the particle size obtained at the certain time. The frequencies of registration of each size and their distribution are shown in figure 1b.

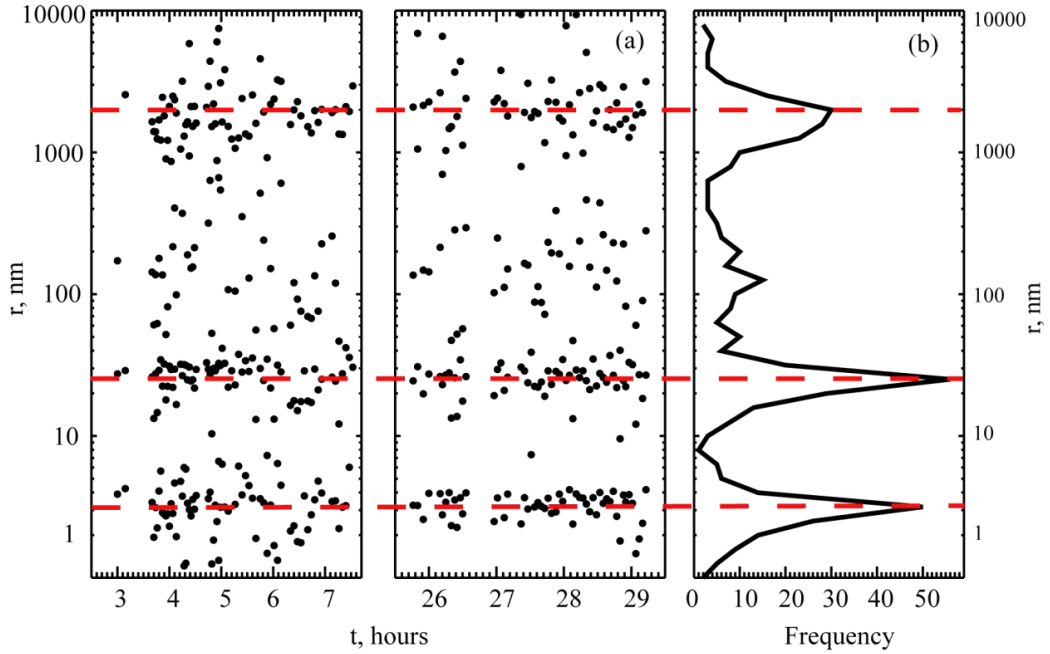



**Figure 1 (a, b). (a)** Array of data presented as the dependence of particle sizes (from intensity size distribution) on time of measurements. **(b)** Resulting frequency distribution of particle sizes.

### *3.1. Calculation of particles concentration from scattering intensity in blood plasma.*

For each size presented on figure 1a we calculate the particle concentrations N from the Rayleigh-Gans-Debay approximation [3]:

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

where $I_{tot}$ is the total light intensity registered in experiment, $A_i \cdot r_i \cdot \left(\sum A_i \cdot r_i\right)^{-1}$ is the fraction of intensity of light scattered by particles of hydrodynamic radii $r_i$ (recalculated from the logarithmic scale of sizes), $n_1$ is the refractive index of particle (for all groups of particle sizes one and the same refractive index of 1.34 was used), $n_2$ is the refractive index of water; $G^2(\theta)$ is the form-factor of particles for cylindrical shapes [4]. Unknown parameters (such as quantum efficiency of photomultiplier cathode $\eta_e$, scattering volume $V_{sc}$, distance between the point of observation and scattering volume $R$) we determined by comparing of our signal with light scattering intensity signal of toluene, scattering coefficient of which is known.

The relation between particle concentrations and corresponding sizes in a double logarithmic scale is plotted in figure 2. Approximation of this relation with straight line is presented with red line. Particle concentrations N turned out to depend on the sizes with a power law with an index of -4, i.e. $N=a \cdot r^{-4}$. Dilution of blood plasma in 10 and 100 times does not disturb such power law dependence [5].

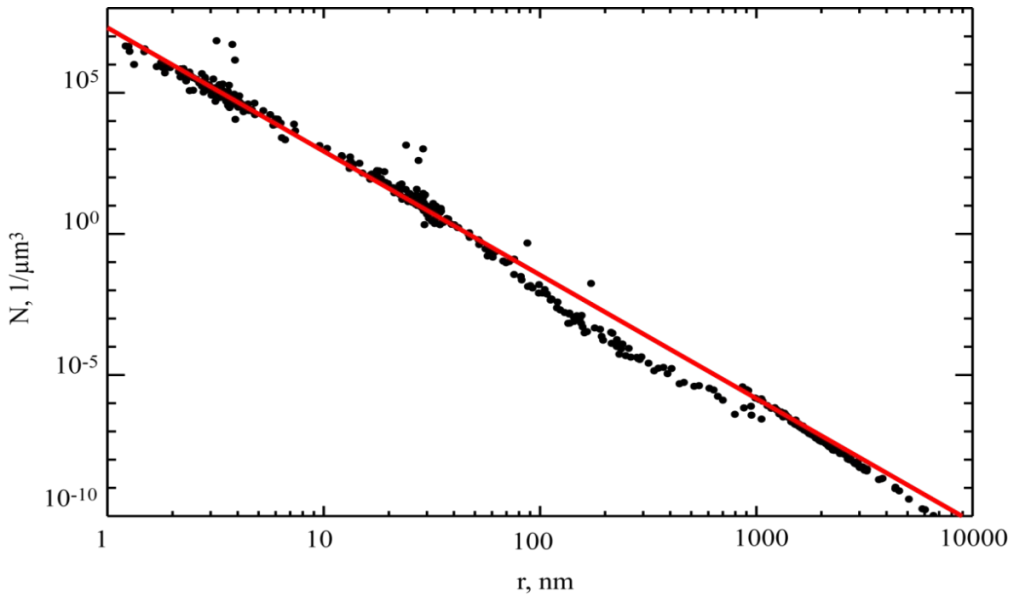



**Figure 2.** Relation between particles sizes and corresponding concentrations in a double logarithmic scale for nondiluted plasma (black points are concentration of particles with certain sizes and red line is power law approximation of these data).

## 4. Results of model experiments.

### *4.1. Model fibrinogen solution.*

To understand the nature of the power law relation of concentrations and sizes in blood plasma we carried out the model experiments. It is known that blood plasma consists of different proteins: albumins, globulins, fibrinogen, etc. The processes of enzymatic degradation of proteins and their further aggregation take place there [6]. For our model experiment we chose fibrinogen from blood plasma and thrombin because these proteins are main components of blood cloating system.

We obtained intensity size distribution for fibrinogen solution and found out that the size of main peak that corresponds to fibrinogen monomers was approximately 13 nm (very close to data that were obtained by other authors). Besides the main peak we also identified another peak with bigger size (approximately 200-300 nm) despite of solution filtration before measurements. Apparently, this size coresponds to aggregates of fibrinogen. Calculation of concentrations of the proteins and their aggregates and matching of sizes and concentrations allow us to obtain the correlation size-concentration diagram (figure 3). One can see that the points on the diagram group near the mean value and form the spot (not like in the blood plasma). The diagram is not linear but for simplicity of

comparison we fitted this one with linear dependence. Its index is 5.9 that is not very close to that one obtained for blood plasma.

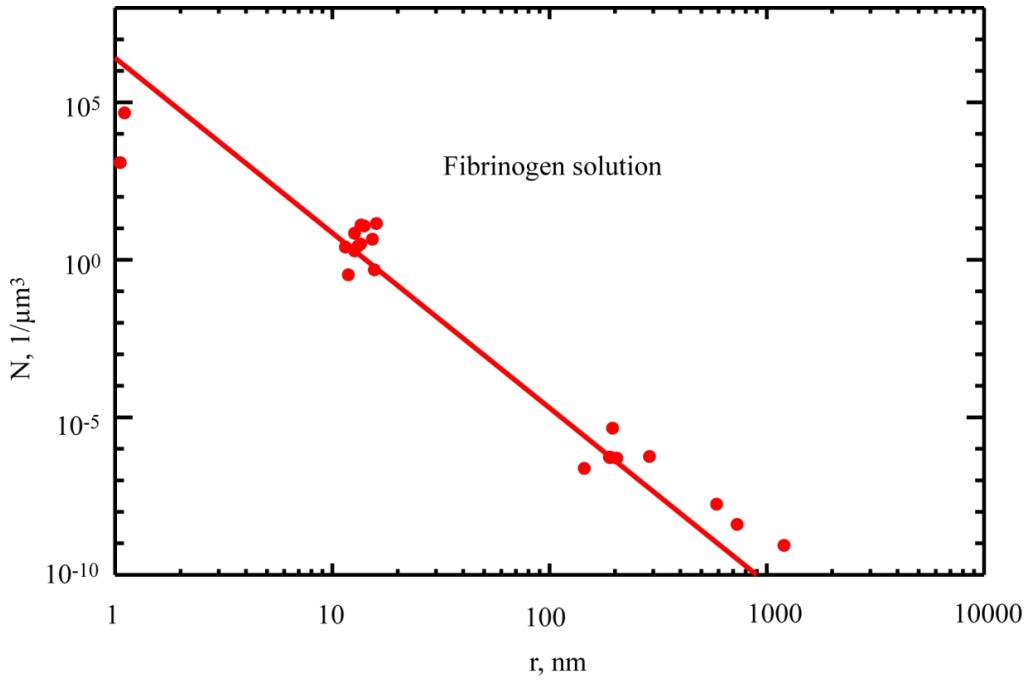



**Figure 3.** Relation between particles sizes and corresponding concentrations in a double logarithmic scale for model fibrinogen solution.

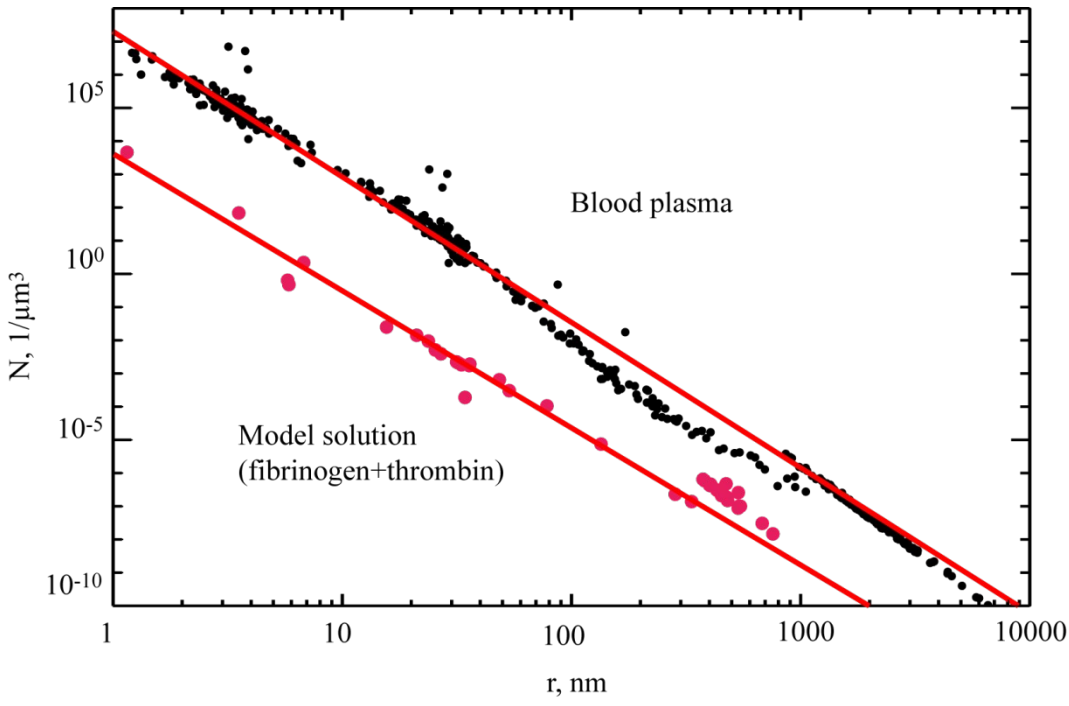



**Figure 4.** Relation between particles sizes and corresponding concentrations in a double logarithmic scale for nondiluted plasma (black points) and for model solution of fibrinogen with thrombin (red points).

*4.2. Model fibrinogen-thrombin solution.*

We also investigated intensisty size distribution in the fibrinogen-thrombin solution. All obtained intensity size distributions were treated in the same way like previous data on fibrinogen and blood palsma. Results of calculation of concentrations and matching them to corresponding sizes presented on the figure 4. Red points corresponds to fibrinogen-thrombin system and black points – to blood plasma. One can see that the slope of straight line in a double logarithmic scale is very close to this one in blood plasma (the index is 3.6±0.3), and one can not see the spot of points like in the case of fibrinogen solution.

## Conclusions

There are a lot of empirical relations in nature that do not have a rigorous theoretical justification. Widely known Moore's law (Gordon E. Moore), according to which the number of transistors placed on an integrated circuit chip doubles every 24 months. It is also known Damuth's law (John Damuth), which shows that the density of population of mammalian N corresponds with a body mass m as $N = a \cdot m^{-3/4}$.We believe that empirical law $N=a \cdot r^{-4}$, which we found in our investigation, which is also the fundamental law of nature of biological liquids, but now there are no mathematical models to explain such dependence. We believe that relation between particle concentrations and sizes not only shows the distribution of particles in biological solution but also reflects the process of enzymatic degradation of proteins and their further aggregation in biological liquid.

## References

[1] Kovalenko K V, Krivokhizha S V, Masalov A V and Chaikov L L 2009 *Bull. Lebedev Phys. Inst.* **36**(4), 65
[2] Chaikov L L, Kirichenko M N, Krivokhizha S V and Zaritskii A R 2015 *J Biomed Opt*, **20** (5), 057003.
[3] Barber P W and Wang D S 1987 *Appl. Opt.* **17**(5), 797.
[4] Neugebauer T 1943 *Ann. Phys.* **42**(7–8), 509.
[5] Kirichenko M N, Masalov A V, Chaikov L L and Zaritskii A R 2015 *Bull. Lebedev Phys. Inst.*, **42**(2), 3.
[6] Yi J, Liu Z, Craft D, O'Mullan P, Ju G and Gelfand C A 2008 *J. Proteome Res.* **7**, 5112.