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Experimental Studies of the Potentialities of Backscattering Dynamical Spectroscopy for Investigating the Characteristics of Biological Particles with Different Shape and Surface Relief

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Abstract

The attempt is made to extent the applicability of dynamic spectroscopy methods, that are commonly used to investigate media with relatively low concentrations of light scattering particles, for analyzing media with high volume content of scatters where there is established the so-called multiple scattering regime. The whole blood of a person under normal and pathological state is chosen as a model medium. The dependence of characteristics of the spectrum of the temporal fluctuations of light scattered intensity on parameters of particles (the concentration, their shape, absorption coefficient, aggregation degree) is studied. It was found that the fluctuation spectrum of multiply scattered light under high concentrations of scattering particles is mainly influenced by their optical characteristics, rather than by geometrical ones.

1. Introduction

In various fields of science and technology there arise problems associated with the determination or control of the characteristics of small particles: their sizes, shapes, degree of aggregation, concentration.

Applications of methods of the light scattering media optics for solving these problems is based on their high accuracy as well as on the fact that they are not time consuming and do not exert any action on the investigated object. Thus, these methods are most actively used to investigate the characteristics of biological objects in medicine and biophysics.

One of the most important optical methods is the method of the correlation spectroscopy [1]. In contrast to other optical methods, this method is based not on the analysis of the spatial distribution of the scattered radiation field but on the analysis of its temporal fluctuations. In other words, this method is based on the fact that the statistic characteristics of scattered radiation are related to the dynamic characteristics of particles: their sizes, shape, relief of the surface.

In modern practice the method of correlation spectroscopy is successfully used for solving various problems: determination of the diffusion coefficient, drift velocity, dynamics of muscle contractions, sizes and asphericity of particles. However, it should be noted that in all applications of this method the determination of the characteristics of scatters is possible only in the case that the distance between the particles is much greater than their sizes.

There is a sufficiently great number of media with a high concentration of particles. Because of this, the necessity arose for extending the method of correlation spectroscopy to determine the

characteristics of scatters at high concentrations. The advantages of the correlation spectroscopy method make it possible to determine the characteristics of particles without the dilution of the medium, i.e., in their natural state. For a number of media, such as biological, this is the matter of principle.

For the model medium we took the whole blood, i.e., the undiluted blood. First, this is the most important biological medium. Second, its all characteristics are clearly understood. Third, the shape of the main blood elements, erythrocytes, can be relatively easily changed.

2. Experimental Results

Since erythrocytes are in the aggregate state in the whole blood, the influence of aggregation on the experimentally measured spectrum of temporal fluctuations of scattered radiation $S(\omega)$ can be investigated.

The diameter of the healthy erythrocyte in the natural condition is 7-8 μm , that of the spheroidal one (i.e. its shape was changed from the disc-like to spherical) was 10-20 μm . The use of special methods was made it possible to change the erythrocyte shape without affecting its volume. The sphericity coefficient is 3-4, the erythrocyte concentration in 1 mm^3 – $5 \cdot 10^6$, and the relative volume concentration is 46%.

Thus, using one and the same object, we managed to investigate the potentialities of the method of correlation spectroscopy for determining various characteristics of scattering particles: size, shape, surface microrelief, of aggregation degree.

And it should be noted that this has been done in the conditions of "nontraditional" use of the method of correlation spectroscopy, at multiple scattering of light.

We investigated the influence of the change in the erythrocyte characteristics on the spectrum line width of intensity fluctuations of multiple scattered radiation $\Delta\omega$.

The scattered radiation spectrum was measured according to the scheme of self-pulsings [1]. The laser radiation wavelength is 0.63 μm . Intensity fluctuations of the speckle-pattern were recorded in the direction of 170 degree from the direction of laser radiation incidence. The spectrum width $\Delta\omega$ was analyzed by a spectrum analyzer.

In the Table 1 the results of the measurements of the spectrum width $\Delta\omega$ depending on the shape of the erythrocytes in the human blood are given.

Table 1 The spectrum width $\Delta\omega$ for different shapes of particles

shape	discs	discs with relief	spheroids	spheres
$\Delta\omega$, Hz	323 ± 23	429 ± 30	375 ± 26	273 ± 19

Since the particle size remained constant, the change in the spectrum width is due to the change in the shape of the particle.

This, solving the inverse problem, we can determine the shape of scattering particles at multiple scattering.

It is evident that the change in the erythrocyte shape leads to a change in the width of the recorded spectrum $S(\omega)$. Thus, it was shown, that, as in the case of small concentrations, the correlation spectroscopy methods could be used for determining the form of scatters. And in this case the method is sensitive not only to the change in the shape of particles, but to the relief of their surface as well.

Theoretical and experimental investigations of the concentration dependence of the coefficient of diffusion D show that in the region of relatively small concentrations ($C < 10\%$) marked changes are observed for spherical particles and relatively small changes for disk-shaped particles. It was

shown that value of D spheroidal erythrocytes is greater than for disk-shaped ones with the same volume (Fig. 1).

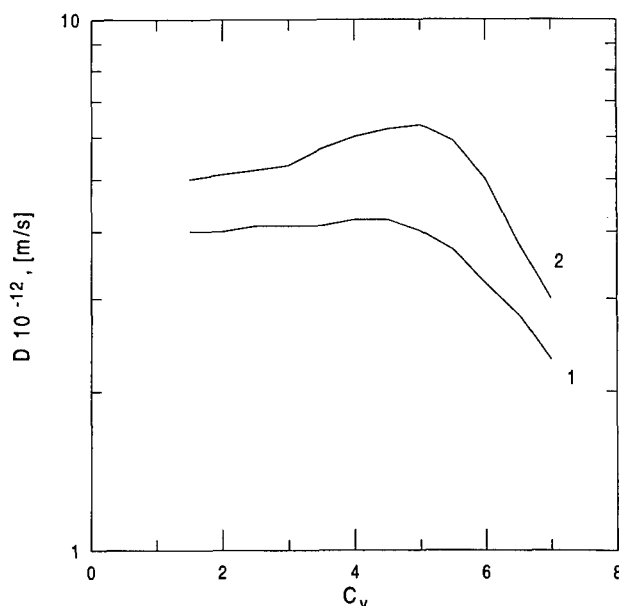


Fig. 1 Dependence of the diffusion coefficient D of disk-shaped (curve 1) and spherical particles (curve 2) on the volume concentration C_v at a constant volume. The dashed lines denote the theoretical calculation for a diluted medium.

Analogous measurements of $\Delta\omega$ have been made for erythrocytes at large concentrations. The results are diametrically opposite. The spectrum width for disc-shaped erythrocytes is smaller than for spheroidal ones. The explanation of this result is given below where the influence of optical characteristics of particles on the fluctuation spectrum of radiation scattered by them is investigated.

The result of the investigation of the influence of the optical characteristics of scattering particles (absorption and scattering coefficients) on temporal fluctuations of scattered radiation $S(\omega)$ was unexpected. It was found that in the region of high concentrations of weakly absorbing biological particles, the function $S(\omega)$ is dependent not only on their geometrical characteristics, but on the optical ones as well.

It is seen from the table (Table 2) that if the value of ϵ/κ is great (absorption coefficient is small).

Table 2 Dependence of the halfwidth of the scattered radiation spectrum on the form of scattering particles at different values of ϵ/k .

Shape of particle	$\Delta\omega, Hz$	ϵ/k
Spheres	429	870
Disk with relief	716	870
Disk	665	2330
Disk	377	28

The broadening of the spectrum for nonspherical particles is observed. If the absorption of the disperse phase is significant, on additional broadening of the spectrum for nonspherical particles is not observed as compared with spherical particles.

Thus, this offers possibilities of investigating not only the dynamics, but also the optical parameters of biological particles.

In this connection the question arises about the possible influence of the close packed media effects on the characteristics of scattered radiation. To solve, this problem, theoretical and experimental

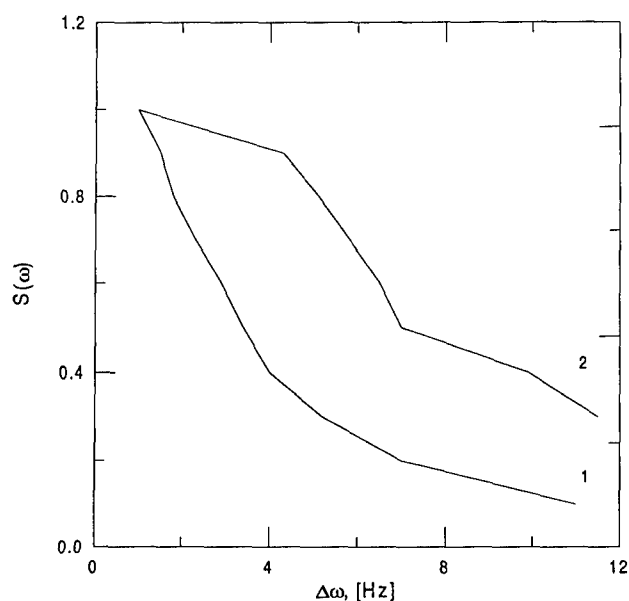


Fig.2. Spectra of fluctuations of the intensity $S(\omega)$ of the radiation scattered by the blood samples with a great (curve 1) and small (curve 2) degree of aggregation.

investigations of the influence of the volume concentrations of disk-shaped and spheroidal particles on the coefficient of the diffuse reflection R were performed at the wavelength $\lambda = 0.63\mu\text{m}$. It was found that this value practically does not depend on the volume concentration of particles for optically thick layers (semi - infinite media).

The possibility of the application of the correlation spectroscopy method to the estimation of the degree of aggregation of particles was investigated for two cases: weak aggregation - the sizes of the aggregates were $< 50\mu\text{m}$ and strong aggregation - sizes of the aggregates were $< 300\mu\text{m}$. The width of the measured spectrum $S(\omega)$ in this case was two times less, than in the case of weak aggregation [2] (Fig. 2).

Thus, the possibility of the application of the correlation spectroscopy method to the investigation not only of sizes, shape, surface of particles and the degree of aggregation, but also their optical characteristics was demonstrated.

References

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