# Investigation of blood cell properties using laser diffractometry and kinetic nephelometry



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**Abstract**. The portable multi-channel laser nephelometer was developed and applied for quantitative and qualitative researches of blood cells. Obtained findings were analyzed in comparison with results of laser diffractometry and microscopy.

## 1. Introduction

Coherent optical methods such as laser diffractometry, nephelometry, speckle interferometry, holographic digital microscopy allow to analyze dispersed cellular systems, individual cells and tissues [1-3]. The main advantages of these methods are the lack of contact, the ease to use, high accuracy and ability to automate the measurement.

Laser diffractometry and nephelometry are based on the analysis of light scattering patterns that are results of diffraction, refraction, reflection and absorption of laser light during the laser beam interaction with investigated objects [1-5]. The angular dependence of light scattering intensity called the indicatrix is characteristic for the particle size. Therefore, these methods are the most widely applied for determination of cell sizes and their changes. The most often the Fraunhofer approximation or the Mie theory is used for transforming the measured data to a particle size distribution [4, 5]. The exact theory of scattering (Mie theory, T-matrix method, discrete dipole approximation method and the approximate calculation methods is used to calculate the indicatrix of a single particle. Suspensions of different cells can be analyzed using the multi-mode Laplace transformation for detection and separation of multi-modal particle size distributions.

The researches of human and animal blood cells are required to the development of cellular biological and biomedical technologies. Valuable information can be obtained performing the kinetic studies of geometric and concentration parameters of cell systems. The ability to aggregate and the deformability during stimulated swelling of cells determine the key properties of normal and abnormal blood cells, in particularly, erythrocytes, platelets, neutrophils, hematopoietic progenitors and blasts cells.

The study is aimed to development of the automated multi-channel laser analyzer and its application for quantitative and qualitative researches of blood cells.

## 2. Results

The automated multi-channel laser nephelometer for researches of cells in static and kinetic regimes was developed. Semiconductor laser diode LFD635-5-3 and high-sensitive opto-electronic integrated circuits OPT301 were used in this device as the laser light source and the scattered light intensity

detectors, respectively. Microcontroller AtMega8535 provides the control of kinetic data registration and processing. Firmware for the microcontroller was written using Atmel AVR-Studio 6.0. As software UniChrom-97 ("New Analytical Systems", Belarus) was used. To support nephelometer driver DLL was developed using driver development kit UniChrom-DDK. Intensities of scattered light I(β) at the angles (β) of 7°, 90°, 187° and intensity of transmitted laser radiation (β = 0°) are measured by this nephelometer.

*2.1. Concentration of particles in suspensions*

Concentration dependencies I(β, C) for different types of human blood cells (isolated from blood and resuspended in Earle's medium or in NaCl solution) and latex particles were obtained by developed device. Such dependencies are shown in figure 1 for erythrocytes (a), "ghosts" of erythrocytes (b) and latex (c). It was revealed that the concentration dependences are described by distinct non-monotone functions for angles 7°, 90°, 187° and 0°. These dependencies distinguish for particles and cells that have different sizes. Diameter of latex particles was about 600 nm that established by atomic force microscopy. Sizes of investigated cells were determined with the use of small-angle laser diffraction analyzer detecting light scattering indicatrix from 1° to 12° angles and applying Fraunhofer approximation. The different types of investigated cells (erythrocytes and neutrophils) were characterized by sizes from 3 to 12 μm under different conditions of medium. Figure 1 (a) are represented the data for erythrocytes with the mean of diameter about 4.9 μm that were isolated from aged blood and resuspended in Earle's medium (300 mOsm, pH=7.3).

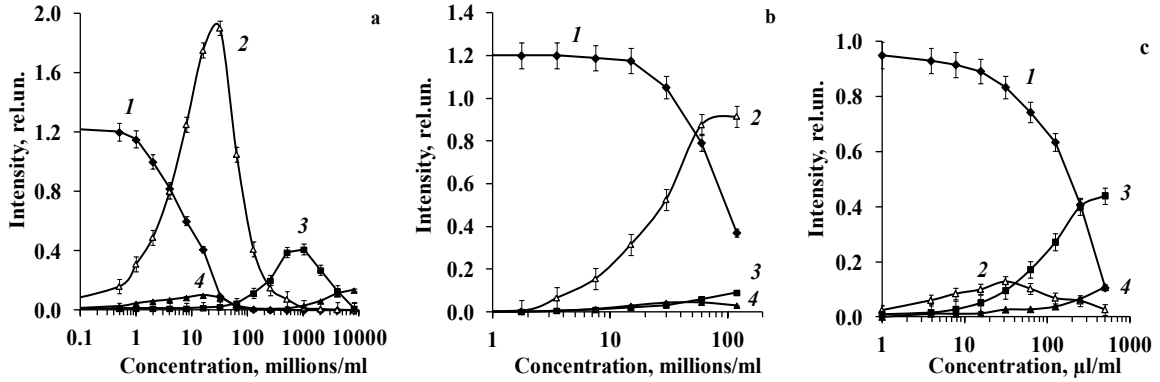


**Figure 1.** Concentration dependencies of scattered light intensity obtained for erythrocytes (a), "ghosts" of erythrocytes (b) and latex particle (c). ***1*** – light transition (at the angle of 0º), ***2*** – light scattering at the angle of 7º, ***3*** – scattering at the angle of 90º, ***4*** – scattering at the angle of 187º.

To consider the part of volume occupied by scattering particles, parameter $V_e/V_0$ was calculated as ratio of volume occupied by all cells ($V_e$) to total volume of suspension ($V_0$). The parameter $V_e/V_0$ for erythrocytes is similar to hematocrit that is partial volume of erythrocytes in whole human blood. Normal value of hematocrit is within the range of 0.35-0.48 l/l. The shape of erythrocytes from aged blood can be approximated by equivalent sphere. The mean of diameter of the cells (d) was estimated analyzing small-angle laser diffraction as described higher. Further, value d was used for calculation of volume (V), surface area (S) of the sphere and $V_e/V_0$ ratio. The data characterizing intensity of light scattering by erythrocytes as dependencies on $V_e/V_0$ are represented in figure 2. Optical density was calculated as logarithm of light transition intensities ratio of solutions without and with particles. The dependences of intensity of light scattering at small angle (7°) and transmitted light can be approximated by linear functions for narrow ranges of parameter $V_e/V_0$ and cell concentration (figure 2, a). This effect conforms to single scattering. It was revealed that the range of linearity is wider for smaller particles such as latex particles, and this range is most limited for cells with the biggest diameters. Linear function approaches the dependence describing light scattering at the angle of 90° in the range of middle cell concentrations and $V_e/V_0$ ratio from 0.005 to 0.035 l/l (figure 2, b). The

increase of $V_e/V_0$ ratio over 0.01 l/l (from 0.01 to 0.6 l/l in the figure 2, b, c) is corresponds to the rise of cell concentration over 100 millions/ml (figure 1, a), and it is accompanied by the loss of light transition and the strong decline of scattering at the angles of 7º. It is obviously that the multiple scattering and amplification of light reflection by particles take place at high values of $V_e/V_0$. Reflection of light is the main phenomenon in the range of value $V_e/V_0$ over 0.1-0.2 l/l, and in this case more information can be obtained analyzing the scattering at the large angles such as 90º and 187° (figure 2, c).

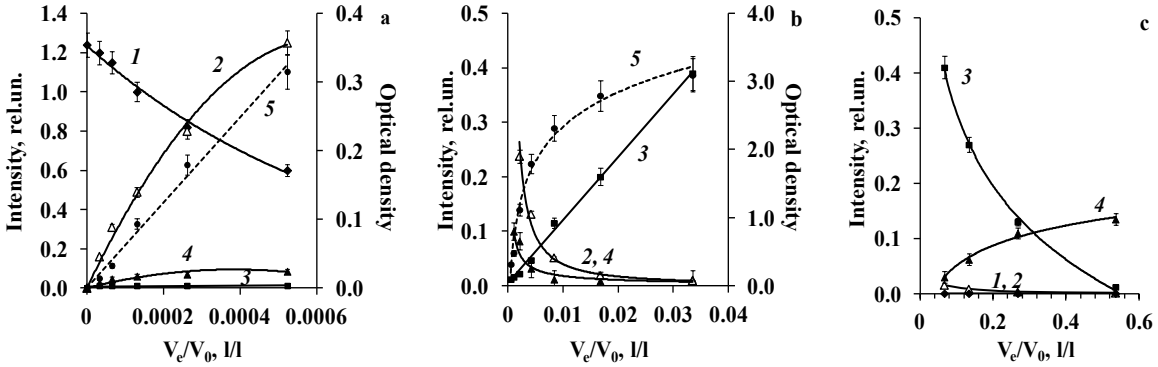


**Figure 2.** Dependencies of scattered light intensity on parameter $V_e/V_0$ characterizing the ranges of low (a), middle (b) and high (c) concentrations of erythrocytes. ***1*** – light transition (0º), ***2*** – light scattering in angle 7º, ***3*** – light scattering in angle 90º, ***4*** – light scattering in angle 187º, ***5*** – optical density (dashed line)

*2.2. Deformation of cells*

To avoid the influence of multiple scattering, the study of cell deformation is performed with the use of low concentration of cells. The concentrations have not to exceed of approximately 5-8 and 2-3 millions/ml for erythrocytes and neutrophils, respectively. If ratio $V_e/V_0$ is not greater than of 0.0005, that analysis of small-angle scattering and light transition intensities provides the highest both sensitivity and linearity.

Deformation of cells and the increase of their membrane surface area without the loss of cell integrity or aggregation are accompanied by variations that not exceeding of approximately 40 % for erythrocytes and 250 % for neutrophils with respect to normal value. The normal cell surface area in isotonic media with respect to blood plasma (300 mOsm, 0.150±0.004 M NaCl,) is 151±3 $\mu m^2$ for discoid blood erythrocytes and 214±30 $\mu m^2$ for spherical neutrophils. Surface of the cells can be smooth or rough with folds. Ability of cell membrane to extension depends on the number of factors including initial elastic stress of the membrane, the presence or the absence of folding, membrane lipids and proteins qualitative content, cytoskeleton and other. Membranes of discoid erythrocytes from fresh blood is smooth in isotonic medium (figure 3, a), and their volume is about 98±2 $\mu m^3$. In hypertonic media volume of the cells is decreased, and their membrane becomes folding (figure 3, b). In hypotonic media deformation and swelling of the cells are observed (figure 3, c).

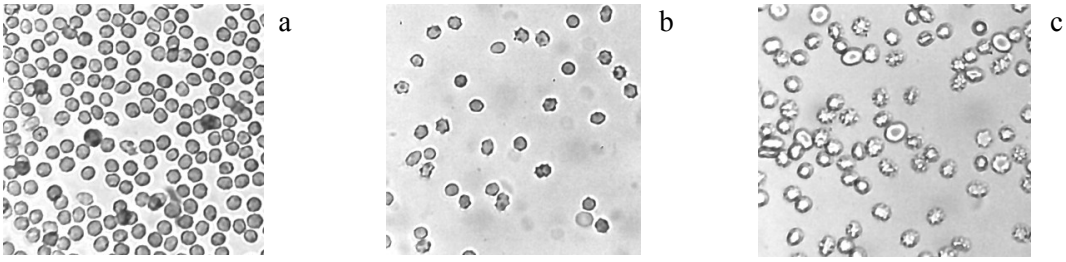


**Figure 3**. Light microscopy of erythrocytes from fresh blood in isotonic (a, 0.15 M NaCl), hypertonic (b, 0.20 M NaCl) and hypotonic (c, 0.12 M NaCl) media.

Deformation of aged blood erythrocytes (8 millions cells per ml, $V_e/V_0$ = 0.0005) in hypotonic media was studied during the decrease of NaCl concentration in extracellular medium from 0.150 to 0.015 mM. Dependencies of light scattering intensities on NaCl concentration are shown in figure 4. It was established that light scattering intensity at 7º rises during NaCl concentration decrease from 0.150 to 0.105 mM. Using laser diffractometry analyzer the means of the cell diameters in these media were determined as 4.9 μm in 0.150 mM NaCl, 6.2 μm in 0.135 mM NaCl, 6.7 μm in 0.120 mM NaCl and 7.4 μm in 0.105 mM NaCl. The calculated V and S of equivalent spheres were 62 $\mu m^3$ and 76 $\mu m^2$ in 0.150 mM NaCl, 122 $\mu m^3$ and 119 $\mu m^2$ in 0.135 mM NaCl, 159 $\mu m^3$ and 142 $\mu m^2$ in 0.120 mM NaCl and 211 $\mu m^3$ and 171 $\mu m^2$ in 0.105 mM NaCl, respectively. In these cases, the means of V correlate linearly with NaCl concentration outside the cells (confidence probability p=0.99). Correlation between V and intensities of light scattering at different angles are illustrated in figure 5 for swelling of the cells. The best linear correlation (p=0.99) is revealed between V and light scattering intensity at 7º (figure 5, ***2***). In concentration range of NaCl from 0.090 to 0.015 mM and osmolarity from 180 to 30 mOsm the transition and scattering of light are reduced in result of lysis of the cells (figure 4, ***1-3***). Extension of cellular membrane during swelling in such conditions exceeds the limit of S about 210 $\mu m^2$, and erythrocytes destruct that are confirmed by microscopy.

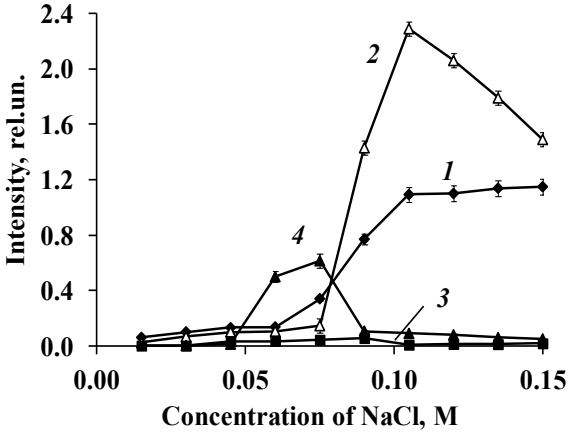


**Figure 4**. Dependencies of intensities of light transition (***1***) and light scattering at 7º (***2***), 90º (***3***), 187º (***4***) on NaCl concentration for aged blood erythrocytes.

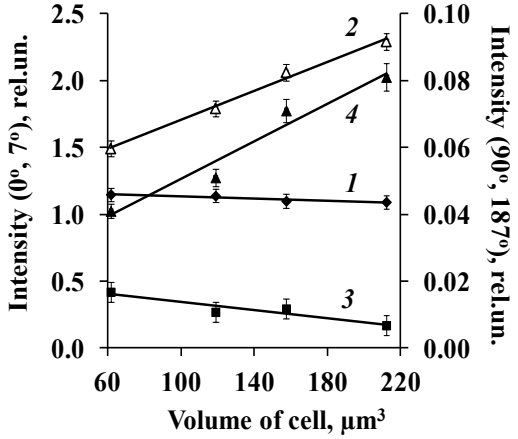


**Figure 5**. Correlation between volume of the erythrocytes and intensities of light transition (***1***) and light scattering at 7º (***2***), 90º (***3***), 187º (***4***).

One of the methods for evaluation of erythrocyte ability to deform is kinetic analysis of erythrocyte swelling and lysis induced by HCl. We determined the basic characteristic parameters of the processes as shown in figure 6.

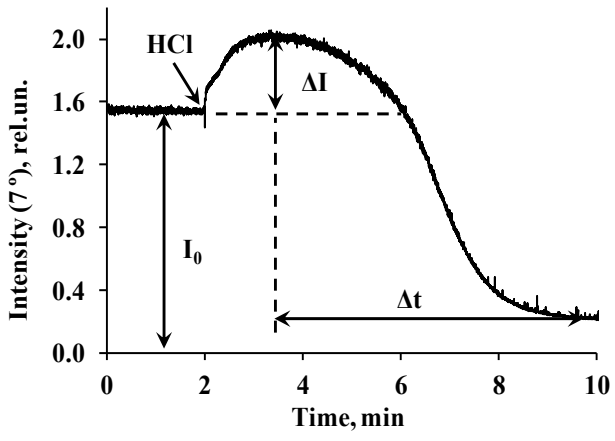


**Figure 6.** Determination of parameters that describe erythrocyte swelling and destruction using kinetic dependencies of light scattering intensity:
$I_0$ – intensity concerned by initial volume of cells,
ΔI – the changes of light scattering intensity that characterized the additional swelling of erythrocytes under HCl action before cell disruption,
Δt – duration of cell disruption process.

Kinetics of HCl-induced lysis of erythrocytes swelled in hypotonic conditions are represented in figure 7. It was established that $I_0$ as well as the initial volume of erythrocytes before addition of HCl correlate linearly with the changes of light scattering intensity ΔI characterizing the additional cell swelling under HCl action before disruption and the duration of cell destruction Δt (figure 8).

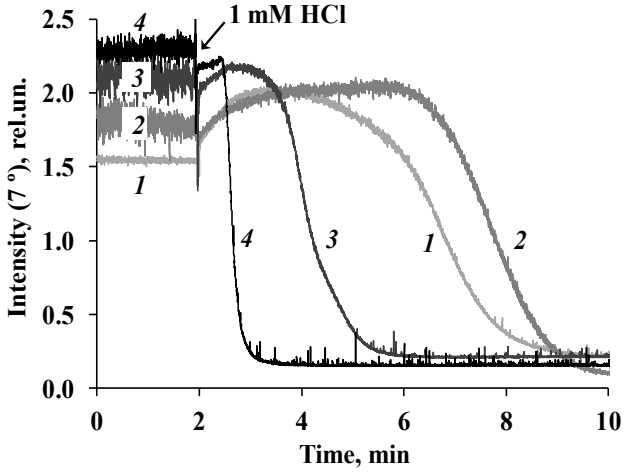


**Figure** 7. Kinetic dependencies of scattered light intensity (in angle 7º) during HCl-induced lysis of erythrocytes in isotonic (***1***) and hypotonic conditions (***2*** – ***4***). ***1*** – 0.150 M NaCl, ***2*** – 0.135 M NaCl, ***3*** – 0.120 M NaCl, ***4*** – 0.105 M NaCl.

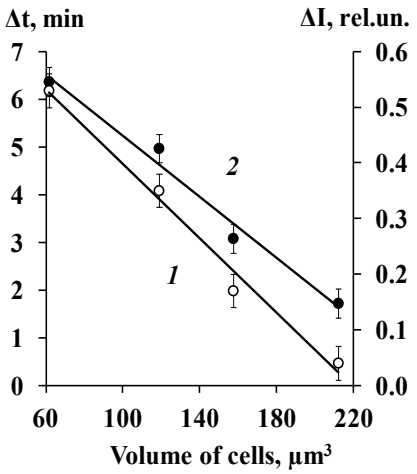


**Figure 8.** Linear correlation between initial volume of cells and parameters of the following HCl-induced lysis of erythrocytes.
***1*** – Δt, ***2*** – ΔI.

*2.3. Aggregation of cells*

The aggregation processes of erythrocytes and neutrophils from fresh blood under different influences were investigated in kinetic regime. Figure 9 are shown the kinetic dependences of scattered light intensity during erythrocyte aggregation ($5 \cdot 10^6$ cells per ml, $V_e/V_0 = 0.0005$) induced by addition of $La^{3+}$. The corresponding data from light microscopy are represented in figure 10. During aggregation a quantity of light-scattering centers and total scattering surface are reduced, therefore the intensity of light transition rises (figure 9, ***1***) and intensity of light scattering at 7º decreases (figure 9, 2). It is necessary to note that the erythrocytes in aggregates destruct in the presence of $La^{3+}$, and hemoglobin is released in extracellular medium. Thus, the registering kinetic dependencies are the results of aggregation and destruction of the cells.

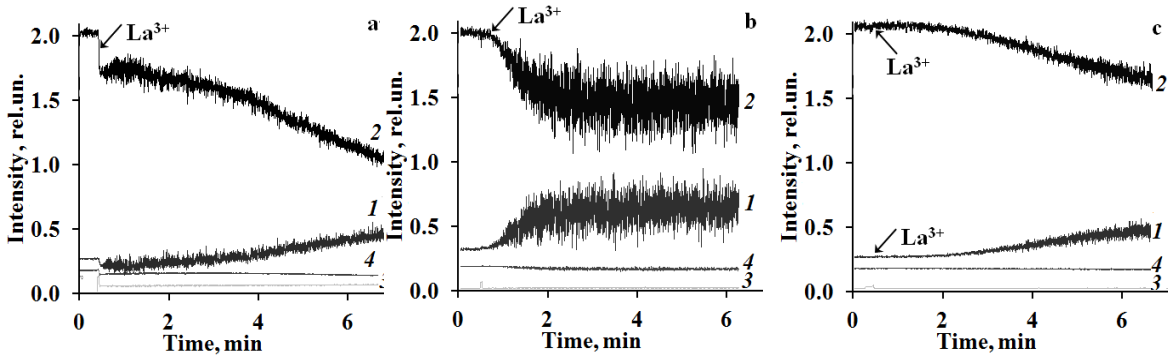


**Figure 9.** Kinetic dependences of scattered light intensity during erythrocyte aggregation induced by addition of $La^{3+}$ in concentration of 2.5 mM (a), 0.5 mM (b) and 0.125 mM (c). ***1*** – light transition (0º), ***2*** – light scattering in angle 7º, ***3*** – light scattering in angle 90º, ***4*** – light scattering in angle 187º

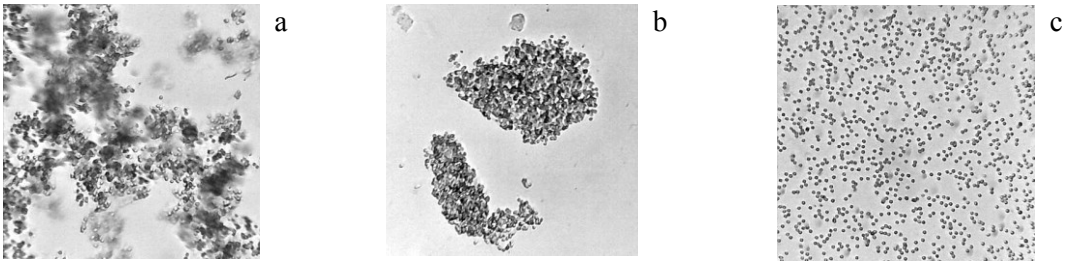


**Figure 10.** Light microscopy of erythrocyte aggregates. a – 2.5 mM $La^{3+}$, b – 0.5 mM $La^{3+}$, c – 0.125 mM $La^{3+}$.

## 3. Conclusion

The portable laser nephelometer was developed. This device supports automatic multi-channel registration of kinetic nephelometric data and permits to carry out quantitative and qualitative researches of blood cells. Data about structure of blood cells obtained by nephelometry correlate with data from laser diffractometry and microscopy.

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