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INFLUENCE OF OSMOTIC PRESSURE ON LIGHT SCATTERING BY RED BLOOD CELLS

R. Szczepanowski, T. Guszkowski and J. Mroczka
Chair of Electronic and Photonic Metrology
Wroclaw University of Technology, ul. B. Prusa 53/55, 50-317 Wroclaw, Poland

Abstract: The paper presents results of the simulation of the light scattering phenomenon by red blood cells (RBC) under reduction of the osmotic pressure in ektacytometry. The modified anomalous diffraction theory was used to describe single light scattering by RBC for small scattering angles and allowed approximation of changing shape and size of RBC. Approximation of RBC by ellipsoidal and spheroidal particles were considered. The changes in the optical properties of the blood-sodium chloride suspension induced by the osmolality reduction have been registered in a form of the intensity patterns. Shape parameters deformability (DI) and ellipticity (EI) from the simulated intensity patterns were used for deformability evaluation. The model well approximates the behaviour of RBC in hypotonic media in ektacytometry, although the deformability curves of RBC (DI) from constant observation points deviate from the real RBC ellipticity. It is caused by the increase of the RBC volume during osmolality changes. The results showed that cellular sphericity is more significant on RBC deformability than the RBC hemoglobin optical properties.
Keywords: light scattering, red blood cell, osmolality

1 INTRODUCTION

The paper presents modelling studies of light scattering by RBC due to changes of osmotic pressure for small scattering angles. Ektacytometry uses light scattering for measurements of deformation, volume, size and orientation of RBC in shear stress [1,2]. Deformability evaluation is based on the analyzis of the forward light scattering distribution. Pathologic changes of erythrocytes occur in various hemolytic diseases, which can be diagnosed by ektacytometry with enforced shear stress and osmolality in artificial environment. For example: increased internal viscosity of RBC, which can be identified as an increase RBC deformation in a hypotonic medium, is observable in desiccytes [3].

The decrease of the osmotic pressure of the medium with constant shear stress results in change of membrane properties, internal viscosity and cell surface area to volume ratio (S/V) causing RBC deformation [1,2,3]. Changes of the osmotic pressure of the medium, in which RBC are suspended *in vitro* are caused by introduced changes of concentration of the substances in the solution. Additionally, shear laminar stress forces RBC unified orientation according to the flow and prolated ellipsoid shape. In case of diluted suspension the value of the osmotic pressure is evaluated by van’t Hoff term [3]. In ektacytometry the Fraunhofer and van de Hulst [5] theories are used for light scattering description. Blood in these models is considered as a dispersive system with erythrocytes as dispersed phase suspended in a dispersion medium. Erythrocytes deform during the measurement and their shapes can be dynamically approximated by spheres or ellipsoids. In the simulations the Latimer [6] and Streekstra [5] models were used. These models are derived from modified van de Hulst’s theory for light scattering by ellipsoids. RBC deformation is a function of volume and ellipticity with corresponding osmolality solutions.

2 INFLUENCE OF OSMOTIC BLOOD PRESSURE ON RBC

Osmotic pressure p is indispensable for stopping the solvent particles flow through the semi-permeable membrane towards more concentrated solution. From two solutions, higher osmotic pressure exerts the solution with a lower relative molecular weight, or the one with a higher dissociation degree. For some water solutions cell membranes are permeable to water but not to bigger particles of the solute. In case of dilute solutions dependence between osmotic pressure and solution concentration can be approximated by an experimental equation of van’t Hoff:

$$p = c\,RT \qquad (1)$$

where *c* is the solute concentration in moles per liter, *R* - gas constant and *T* - temperature. Osmotic pressure is expressed in the osmolality term, which describes a number of free molecules in the volume unit, with no respect of either molecules or ions they are. The human organism maintains constant blood osmotic pressure, which is approximately equal to 300 mOsm. Changes of erythrocytes in anisotonic media are the functions of semi-permeable membrane properties, S/V ratio and internal RBC viscosity [1,2]. The increase of hypertonicity of the medium makes RBC to shrink and decrease its volume. Contrary, the decrease of osmotic pressure causes erythrocytes deformation, volume increase, spherocytosis [2]. RBC swells, gains water and its S/V ratio changes. In the range between 175 and 125 mOsm the area of critical volumes of RBC begins and prehemolytic lysis occurs [1]. Osmolality of the solutions in which erythrocytes are suspended is regulated usually by the concentration of certain substances in suspension (for example: dextran + phosphate-buffered saline (PBS) [1,3], PVP + PBS [7]). Final tuning of osmotic pressure is done by introducing NaCl [1,7]. The optimal number of erythrocytes in suspension must be also provided. According to Ganong [8] 0.9% solution of NaCl is isotonic relative to plasma. Healthy erythrocytes start hemolysis at concentrations below 0.48% of NaCl.

3 MODELLING OF A LIGHT SCATTERING DISPERSIVE SYSTEM

3.1 Erythrocyte

The simulations were performed for erythrocytes suspended in a medium with given osmolality and shear stress. In these conditions RBC can be considered as a homogenous particle with large size compared to the length of the incident light wave, size parameter $a >> 1$. Single light scattering by RBC was assumed. RBC were modelled by ellipsoids. The geometry of ellipsoid is described by $q = a / b$, size parameter $a = k \cdot A$, $A = \sqrt{a \cdot b}$, where *a, b, c* are characteristic radii of ellipsoid. If $a = b > c$, the particle has circular dissection - it is a spheroid, if $a > b > c$ the particle is an ellipsoid. Erythrocyte can be modelled as a homogenous solution of hemoglobin. Hemoglobin's content in RBC is about 95% of the cell volume. Refraction index of RBC (2) depends on the concentration of hemoglobin. According to Tycko [9] imaginary part of the refractive index was neglected.

$$n = n_0 + a \cdot HC \qquad (2)$$

where $n_0 = 1.34$, $\alpha = 0.0019$ dl/g, *HC* concentration of hemoglobin in g/dl.

3.2 Medium

In our simulations constant properties of the optical medium were evaluated theoretically. Usually the constant, measured value of the refractive index is assumed. A simplified medium (water solution of NaCl) was used for our calculation.

Light scattering in fluids occurs due to fluctuations of elementary volume scatterers composed of many medium molecules. If the medium is nonabsorbent and nonmagnetic ($\mu = \mu_0$) the refractive index can be obtained from Maxwell's dependence (5) [10]:

$$e = n^2 \qquad (3)$$

For solutions light scattering increases due to additional fluctuations of mass concentration of the solute.

The evaluation of the refractive index for a fluid demands a definition of the polarizability of the entire multi-component system and external electromagnetic field $\overline{E}$, because particles form a local field $\overline{E_i}$ in dense media, which influences the external field. This relation is described by Lorentz-Lorenz equation:

$$\overline{E_i} = \frac{n^2 + 2}{3}\overline{E} \qquad (4)$$

For a multicomponent solution, due to the rule of linear additivity of polarizability the refractive index for a fluid is obtained from a modified Lorentz-Lorenz equation [10]:

$$\left[\frac{n^2 - 1}{n^2 + 2}\right] = \frac{4p}{3} N_A r \sum_{k=1} \frac{c_k a_k}{M_k}, \qquad (5)$$

where c_k is the mass fraction of the k-th substance, M_k is the molar mass of the k-th component, a_k - partial polarizability of the k-th compound, r - density and N_A is the Avogadro's number.

4 SCATTERING MODEL

The paper considers the light scattering by RBC as the case of single scattering on a single particle. Approximating model of Latimer [6] and Streekstra et al. [5], based on the anomalous diffraction theory of van de Hulst [11] was used. The model can be used to describe light scattering on ellipsoids. This theory assumes that a part of light radiation is transmitted through the RBC particle and the part is diffracted. The transmitted and diffracted part interfere with each other. Spatial distribution of light intensity in point *P(x,y,z)* can be obtained from eq. (6) [5].

$$S(v) = a^2 \int_0^{2p} \left[1 - \exp(-i f_{\max} \sin t)\right] J_0(av \cos t) \sin t \cos t dt, \tag{6}$$

where

$$n = 1/r((x^2/q) + (q y^2)) \tag{7}$$

$$a = k A = (2p n_{med} / l_0) A, \qquad f_{\max} = 2kc|m-1| \tag{8),(9}$$

where q denotes scattering angle, $J_0(u)$ the zeroth-order Bessel function, f_{max} phase shift, a particle size parameter, l_0 laser wavelength in vacuum, n_{med} medium refractive index, m relative refractive index and c is the length of the third axis of the ellipsoid, parallel to the direction of the incident light.
Assuming that m and $r = f_{max}$ are real, the expression (6) can be solved with Sonin's integrals [11]. Let's assume that

$$A(r,z) = a^{-2} S(n) \tag{10}$$

where $z = an$; $A = \mathrm{Re}A + \mathrm{Im}A$, then the solution of (11) for a real part is the Sonin's first integral expressed as a series convergent for each z and r:

$$\mathrm{Re}\,A = r^2 + \frac{1}{z^2} J_2(z) - r^4 \frac{1}{1 \cdot 3 \cdot z^3} J_3(z) + r^6 \frac{1}{1 \cdot 3 \cdot 5 \cdot z^4} J_4(z) + \ldots \tag{11}$$

whereas the solution for imaginary part of (12) is the Sonin's second integral for n=1/2 and m=0 and assumes the shape of:

$$\mathrm{Im}\,A = \frac{r}{y^2} \left(\frac{p y}{2} \right)^{1/2} J_{3/2}(y) = \frac{r}{y^2} y_1(y) \tag{12}$$

where $y^2 = z^2 + r^2$, and y_1 is a Riccati-Bessel function [11].

5 SIMULATION RESULTS

Based on the aforementioned theory, the simulations of light scattering by RBC under decreased osmotic pressure were performed for scattering angles in range from 0 to 15 deg. A light detector was placed in a large distance compared to the particle size. Scattered light intensity for an ellipsoid at point *P(x,y,z)* with the distance to scattering particle larger than the particle size is given by the following expression [5]:

$$I = I_0 \left(\frac{1}{k^2 r^2} \right) |S(n)|^2, \; r = \left(x^2 + y^2 + z^2 \right)^{1/2} \tag{13}$$

where k is a wave number, I_0 is intensity of incident lightwave. Isointensity curves for scattered light for $x, y << z$ for erythrocytes are ellipses given by equation [5].

$$\frac{x^2}{q z^2 n^2} + \frac{y^2}{z^2 n^2 / q} = 1 \qquad (14)$$

Deformability parameters *DI* [1] and ellipticity *EI* of RBC are evaluated by light scattering analyzis.

$$DI = \frac{A - B}{A + B} \qquad (15)$$

where *A*, *B* are light intensity for points A and B for different shapes of the same particle. It is determined on the major and minor axes of ellipsoid respectively.

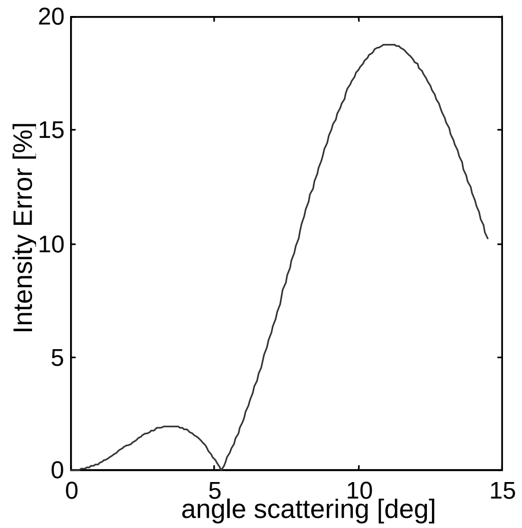

Figure 1. The model errors curve from a difference of the normalized intensity by the latex microsphere n_{int} =1.593; osmolalities: $\pi 1$ = 130, $\pi 0$ = 300 mOsm/kg and refraction indices n_{med}= 1.334, 1.337, respectively.

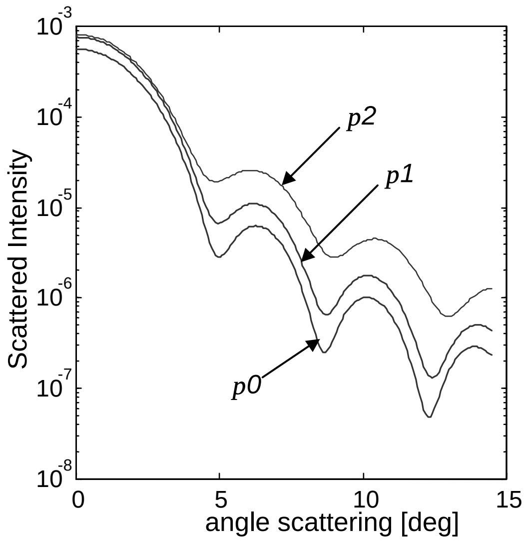

Figure 2. The scattered intensity patterns by the spheroid for three osmolalities: *p0* = 290, *p1* = 220, *p2* = 150 mOsm/kg respectively; *HC* concentration 36.3 g/dl; shear stress τ = 0 dyn/cm^2

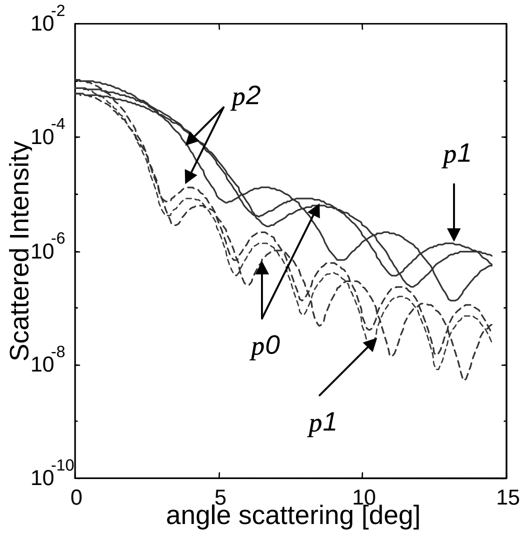

Figure 3. The scattered intensity patterns by the ellipsoid for three osmolalities: *p0* = 290, *p1* = 220, *p2* = 150 mOsm/kg, respectively at X,Y major and minor axis; shear stress τ =100 dyn/cm^2; *HC* =36.3 g/dl.

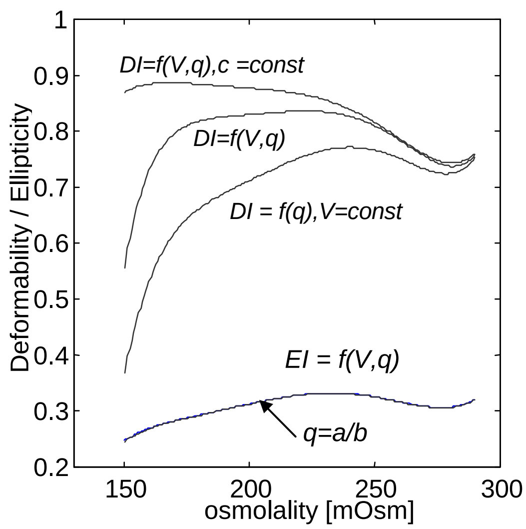

Figure 4. Deformability (*DI*) / ellipicity *EI* of erythrocyte vs. osmolality. *EI* covers with the ellipticity *q* of the particle. HC concentration 36.3 g/dl; shear stress τ = 100 dyn/cm^2

$$EI = \frac{L - W}{L + W} \tag{16}$$

where *L, W* are major and minor axes of diffraction patterns respectively for the isointensity curve.
In simulation the wavelength of 632.8 nm was assumed (laser He-Ne). RBC refraction indices were determined for erythrocytes for a hemoglobin concentration *HC* from 31.4 to 39.5 g/dl [5,7]. Simulations for higher *HC* > 40 g/dl appearing in some hemolytic diseases were also performed. To evaluate the refractive index of the water solution of NaCl the value of polarizability was calculated. Polarizability of water was taken on the basis of the real refractive index measurements for $l = 632.8$ *nm* [12]. Polarizabilities of ions Na^+, Cl^- were taken from [13]. Refraction index n_{med} depends on NaCl concentration corresponding to fixed osmolalities of the solution. In the range of osmolality *p* from 131 to 300 mOsm the simulation of calibration of the refractive index with latex particles with constant volume, diameter 3.18 μm and n_{int} = 1.593 was performed. Model error due to a difference of normalized intensities for the changes of the refractive indices medium was equal to about 2% for the low-scattering angles smaller than 5 deg (Fig.1).

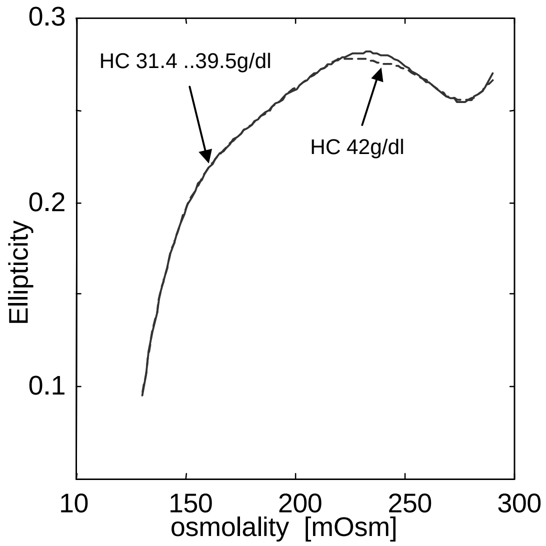

Figure 5. Ellipticity curves of the erythrocyte vs. osmolality at different hemoglobin concentrations. Dashed line marks curve for HC 42 g/dl. Shear stress τ = 100 dyn/cm^2

For light scattering in ektacytometry the results of examination of RBC behaviour in hypotonic media were used [1,2,3,7]. In our model RBC are suspended in a water solution of NaCl and for this system curves volume vs. osmolality were calculated. Similarly, ellipticity *q* as a function of osmolality was designated basing on the literature data [1]. In our work two cases for τ = 0 and τ = 100 dyn/cm^2 were simulated. Examinations were performed up to the limit when osmotic lysis starts (150 mOsm).
For τ = 0 the erythrocyte is a spheroid (Fig. 2). Our simulation results showed that erythrocyte scatters light more with the decreased osmotic pressure. For τ = 100 dyn/cm^2 intensity distributions and deformability vs. osmolality curves were simulated (Fig. 4). Ellipsoidal particle scatters light more under decreasing osmolality. Deformability curves *DI* were obtained from the constant observation points for changing particle sizes and ellipticity *EI* from the isointensity level. When the *q* parameter approaches zero the decrease of osmolality makes deformability curve (DI) fall more rapidly (Fig. 4). The influence of the RBC volume of the ellipsoid on deformability simulation results was tested with the assumed constant or changing volume as an osmolality function. In our examination the dependence deformability vs. hemoglobin concentration was considered (Fig.5), but the model was not sensitive with the HC changes falling into a range from 31.4 g/dl to 39.5 d/dl.

6 DISCUSSION

The changes which were registered along the *x* and *y* axes for intensity patterns may point to common existence of two types of particles: spheroids and ellipsoids. Spheroids were examined without flow, and ellipsoids at shear stress. Deformability curves of RBC (*DI*) are dependent on RBC volume during osmolalities changes and therefore deviate from the real RBC ellipticity. Contrary ellipticity curves from isointensity level is a function of RBC shape and well reflect the RBC ellipticity, if the third axis *c* parallel to the incident light is uniform. *DI* is the better parameter for small scattering angles. It is more convenient to establish fixed observation points, for which the errors from the refractive index are small enough, but *EI* may demand a measurement of a broader range of scattering angles, and in this case detector would need continuos calibration of the medium refractive index. In the range hemoglobin concentrations from 31.4 g/dl to 39.5 g/dl no significant changes of ellipticity curves were detected and over *HC* 42 g/dl a deformability level reduction occurs. This phenomenon testifies that changes of RBC cellular sphericity affects deformability more than a modification the RBC optical properties due to changes of the hemoglobin concentrations. The simulation results agreed with the experimentally measured deformability patterns [1,2,7]. It should be emphasized that values of

volume and ellipticity are compiled literature data, which introduces model uncertainty. Model errors for the small light scattering angles (<5 deg) caused by the difference of normalized light intensities agrees with McGann's experimental results [14], where calibrated refractive index medium by microspheres in the different osmolalities saline solutions were performed.
Additional cause of the model errors are theoretical values of the refraction index. In the range of p from 130 to 300 mOsm our results of the refraction index n_{med} are from 1.334 to 1.337 respectively. In contrary, measured refraction index is about 1.345 for 290 mOsm [5]. Such differences between these values arise firstly from the assumption that a medium is nonabstorptive and an exact estimation would demand determination of the real and imaginary parts of the medium refractive index, because of the existence of infrared bands in the NaCl solution. These bands are shifted due to the monatomic ions Na^{+} and Cl^{-} content and their amplitudes depend on the solution concentration. In this case the better method to estimate optical constants of a medium is Kramers-Kroning analyze [15]. Secondly, differences are caused by errors of estimation of polarizabilities of the solution due to the use of the linear polarizability additivity rule in mixture which introduces about 5% error to the experimental results of the refraction index [10]. Thirdly, main reason of these differences is simplification of medium components - influence of dextran was neglected. Theoretically, according to eq.(1), dependence of osmotic pressure, dextran does not significantly change osmotic pressure, especially for a large molecular weight dextran. Dextran in blood suspension maintains a constant viscosity, securing the shape of RBC during fluctuation of the hemoglobin concentration while measurements.
The model well approximates the behaviour of RBC in hypotonic media during ektacytometry measurements. Further model development should respect more detailed analyzis of dielectric constant in mixtures, broader extent of mixture component and the influence of viscosity on RBC membrane.

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AUTHORS: Chair of Electronic and Photonic Metrology, Wroclaw University of Technology, ul. B. Prusa 53/55, 50-370, Wroclaw, Poland, Phone Int. +48 71 320 6571, Fax Int. +48 71 321 4277, E-mail: rszczepanowski@kmeif.pwr.wroc.pl