Influence of physiological conditions on optical parameters and scattering properties of red blood cells

by

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ABSTRACT

The results of investigations of the scattering properties of Red Blood Cells (RBC or erythrocyte) modelled as a spheroid for fixed and random orientations versus osmotic pressure and oxygenation have been presented. An erythrocyte has a round form of a biconcave disc of a diameter of 7.5±0.3µm and thickness of (1.4-2.1)±0.4µm. The mean concentration of haemoglobin in an erythrocyte is HC=350±2.5g/l. Haemoglobin is responsible for the spectral absorption of RBCs (oxygenated blood appears to be sharp red or dark red when not). Under normal conditions the RBCs have an oblate shape with a volume equals \( V_0 = 90\text{µm}^3 \) for the osmotic pressure \( P_o = 300\text{mosm} \), see figure 1 a). The shape of unhealthy RBCs can significantly differ from the one presented in the previous figure. It also strongly depends on the osmotic pressure. So that one can expect to diagnosis RBCs oxygenation rate and the osmotic pressure from RBCs shape analysis and absorption diagnosis.

In the present work we investigate the scattering properties of a single RBC for fixed and random orientations versus the osmotic pressure and oxygenation. The final goal of this study is to determine whether it is possible to infer the previous blood characteristics from a whole blood sample (under single or multiple scattering). To compute the light scattering properties of RBCs we have chosen the T-Matrix methods, to obtain accurate predictions on the scattering coefficients and angular scattering dependencies which are expected to be necessary to infer statistical properties of a RBC from a blood sample and not only from single RBCs. From the obtained results it appears that in contrary to fixed oriented RBCs the diagnosis of the osmotic pressure of randomly oriented RBC is expected to be rather difficult. The osmotic pressure has some influence on the phase function and on the extinction scattering coefficient however it is not a one-to-one relation. The diagnosis of the oxygenation rate of RBCs seems to be even more difficult task. The influence of the haemoglobin absorption on the basic scattering parameters and functions, which is rather large, seems to be too small to be detected experimentally. Finally, one can expect that in case of a whole blood sample with a polydisperse size distribution for RBCs and obviously under multiple scattering conditions, it would not be possible to infer reliable statistical properties of a RBC. According to the authors one solution to this problem would be to fix the orientation of all RBCs present in the blood sample as done for a single RBC in flow-cytometric methods.

Fig.1. a) View of real human Red Blood Cells (RBCs or "erythrocytes") for normal osmotic pressure \( (P_o=300\text{ mosm}) \)  b) Models of RBC
1. INTRODUCTION

Whole blood (non-diluted and non-hemolyzed) is a disperse system in which Red Blood Cells (RBCs or "erythrocytes"), form the major part of a dispersed phase with plasma as a dispersing medium. An erythrocyte has a round form of a biconcave disc of a diameter of $7.5\pm0.3\mu m$ and thickness of $(1.4-2.1)\pm0.4\mu m$. The volume of a normal erythrocyte equals $V_0=90\mu m^3$ for the osmotic pressure $P_0=300mosm$, see figure 1 a). The mean concentration of haemoglobin in an erythrocyte is $HC=350\pm2.5g/l$.

In clinical haematology, there is a need for accurate and precise measurement of the RBC geometrical (size and shape) and mechanical (deformability) properties, as well as oxygenation. The shape of unhealthy RBCs can significantly differ from the one presented in the previous. In addition the shape of RBCs strongly depends on the osmotic pressure. In fact low osmotic pressure may cause severe problems for the gas transfer through capillary vessels by decreasing the deformability of RBCs and then increasing the whole blood viscosity. Haemoglobin in a RBC takes mainly two forms: an oxidised form (HbO2, responsible for the transport of oxygen) and un oxygenated form (Hb). Haemoglobin is responsible for light absorption properties of the erythrocyte (Oxygenated blood appears to be sharp red or dark red when not). Thus the measurement of the RBC light absorption may be a measurement of the blood cell haemoglobin concentration and oxygenation and thus, its efficiency in oxygen transport.

There exist several clinical laboratory instruments, which employ flow-cytometric methods in counting RBCs and measuring their volume distribution. Most of them use forward light scattering at one or two angles or an electronic res istive pulse sizing method for the RBCs volume distribution measurements. There is nevertheless a need to improve the techniques which estimate the RBC geometrical properties only from effective volume measurements and give no informations about the oxygenation and shape of RBCs.

There are many reports in the literature on the computation of the scattering properties of a RBC. The earlier works were based on the Lorenz-Mie theory assuming a RBC as homogenous spherical particles. Nevertheless, due to the complex shape of these particles, this approach cannot be of real practical use. Latter works were based on the assumption of an oblate form (axi-symmetric ellipsoid with symmetrical axis radius, $a$, like $a/b>1$) for the RBC and their optically soft nature, Tycko et al (1985) i.e.:

$$|m−1|<<1$$
$$kd|m−1|<<1$$

where $m, k, d$ are the RBC relative refractive index in the considered medium ($m=m_{RBC}/m_{m}$), the wave vector ($k=2\pi m_{m}/\lambda$, where $\lambda$ is the laser wavelength in free space) and an equivalent diameter of the RBC, respectively. The previous conditions make it possible to obtain a solution of the scattering problem quite simple and physically obvious analytical form usually referred as the Anomalous Diffraction Approximation (e.g. Van de Hulst, 1957; Latimer, 1972; Latimer, 1980; Streekstra et al., 1983; Barber and Hill, 1990). The first condition is usually fulfilled as, in the visible range, the RBC have a proper refractive index about $m_{RBC}=1.4$ and the surrounding medium (plasma) about $m_m=1.335$, i.e. $m=1.4/1.335=1.05$. Nevertheless, the second condition is always broken. For instance, for the previous parameters with $\lambda=0.6328\mu m$ and $d=4.9\mu m$, we found that $kd|m−1|=1.6$. This introduces some limitations in the accuracy of this approach and it also limits the prediction of the RBC scattering properties in the small angle range. In spite of the strong limitations of this approach it has been recently used by Borovoi et al. (1998) to predict the light scattering properties of RBC with a shape approaching their natural shape, see figure 1 b) within the spherical co-ordinate system:

$$R(\theta,\varphi) = a_R * \sin \theta + b_R,$$

where $d=(2a_R+b_R)$ is the diameter and $2b_R$ is the thickness in the centre. As typical parameters for erythrocytes they have chosen $d=7.5\mu m$, corresponding to $a_R=3\mu m$ and $b_R=0.75\mu m$ and the exponent is estimated as $q=5$. They have also assumed that the osmolarity of the suspension medium causes isovolumetric s phering of the cell, where the case $q=0$ corresponds to the sphere. This last hypothesis seems to be invalidated by some experimental studies (e.g. Fung, 1972; Azouzi, 1995).
The T-Matrix methods (or Extended Boundary Condition methods) has been also previously used to compute the scattering properties of a RBC for the fixed orientation. This accurate method does not require any particular assumption on the optical parameters of the scattering particles, i.e. RBC in the present case. It is nevertheless limited to simple geometrical shapes and also, due to numerical instabilities, in the maximum size parameter that can be calculated. The T-Matrix allows to compute scattering coefficients as well as the phase function, the degree of linear or circular polarisation for arbitrary scattering angles (e.g. Barber and Hill, 1990; Mishchenko, 1991; Mishchenko et al., 1999).

In the present work we investigate the scattering properties of a RBC for fixed and random orientations versus the osmotic pressure and oxygenation. The final goal of this study is to determine whether it may be possible to infer the previous blood characteristics from a whole blood sample, under single or multiple scattering (e.g. Onofri et al., 1999). In the following, as a preliminary step, simulation results on the scattering properties of a single RBC are presented.

2. SIMULATION STRATEGY AND PROCEDURE

To compute the light scattering properties of RBCs we have chosen the T-Matrix methods, to obtain accurate predictions on the scattering coefficients and angular scattering dependencies which are expect to be necessary to infer statistical properties of RBC from a blood sample and not only from single RBCs. For this purpose we used the T-Matrix code from Mishchenko (1991), Mishchenko et al. (1999) available from the web site of Mishchenko and Travis (Mischenko and Travis web site).

The RBC shape will be modelled by an oblate ellipsoid (see figure 1 b)) with the equation: \((x^2+y^2)/a^2+z^2/b^2=1\), where \(a\) is a symmetrical axis. This particle has the same projection area along Z that the form given in eq. (2), when \(a=a_R+b_R\) and \(b=b_R\). It has a comparable volume but a smaller projected surface along X or Y.

The haemoglobin, Hb (0% oxygenation) and HbO2 (100% oxygenation), complex refractive index dependence on wavelength (Pahl web site), Steinke and Shepherd (1988) is presented in figure 2 a). At the first view it appears from this figure that the major difference in the spectral absorption between Hb and HbO2 is in the range 600-750nm. Nevertheless, if we consider a simple intensity ratio experiment to differentiate the two types of haemoglobin from the intensity transmission through blood samples (width \(L\)), the relevant parameter is the difference in the two spectral absorption: \(I_{Hb}/I_{HbO2}=\exp[-(k_{Hb}-k_{HbO2})L]\). Figure 2 b) presents the difference in the spectral absorption of the two types of haemoglobin. The difference in the blood sample transmission is expected to be a maximum for wavelength about 380-450nm.

![Fig. 2. Spectral absorption of RBC for oxygenated (HbO2) and non oxygenated haemoglobin (Hb).](image)
The refractive index inside a RBC is,

\[ \hat{m}_{\text{RBC}} = m_{\text{RBC}} + ik \]  

(3)

\( m_{\text{RBC}} \) is expected to depend on the RBC haemoglobin concentration (HC), the concentration being varied for different people (typical value: HC=350 g/l). The linear dependence upon HC is known, Tycko et al. (1985):

\[ m_{\text{RBC}}(HC) = m_{\text{RBC}}^0 + \alpha HC \]  

(4)

where the constant we use: \( \alpha = 0.0019 \) g/l and \( m_{\text{RBC}}^0 = 1.335 \).

The real part of the refractive index dependence on the wavelength is neglected. The proving arguments are that (i) in the light scattering calculations we use the relative refractive index \( m = m_{\text{RBC}}/m_m \) so that for some part the wavelength dependence is cancelled out, (ii) as known by the authors there are no reliable data in the literature on this dependence.

There are also very few reliable data in the literature about the RBC shape dependence on osmotic pressure, so we use the following experimental results and procedure.

The aspect ratio \( a/b \) is deduced from the experimental data (Fung, 1972) giving the evolution of the volume of RBCs given in term of a cylinder with the length \( 2b \) and radius \( a \). Note that this way to define the RBCs volume is common in the clinical field.

The evolution of \( a \) is deduced from a linear fit of few experimental data on the shape of RBCs (Azouzi, 1995).

So that from these data we derive shape dependence on osmotic pressure for the RBCs:

\[ V_{\text{cyl}}(P_o) = 339-1.88P_o + 0.00491P_o^2 - 4.46 \times 10^{-4}P_o^3 \]

\[ a = \left[ 0.00310(P_o-150) + 3.210 \right] \]

\[ b = V_{\text{cyl}}(P_o)/(2\pi a^2) \]  

(5)

![Fig. 3. Evolution of the RBC geometrical and optical properties versus the osmotic pressure.](image)

In terms of volume, the RBC is equivalent to a sphere with radius \( r_{\text{sph}} = (V_{\text{cyl}}(P_o)/2\pi)^{1/3} \). When the osmotic pressure decreases from 300mosm to 111mosm the RBC aspect ratio decreases from \( a/b=3.28 \) to \( a/b=1.002 \), see figure 3. In an opposite way the RBC volume increases (the equivalent sphere radius increases). This cause a
dilution of the haemoglobin inside the RBC: $H(Po)=H(300)\frac{V_{cyt}(300)}{V_{cyt}(Po)}$ and thus a change in the RBC refractive index $n_{RBC}(H)$ and absorption $k(Po)=k(300)\frac{V_{cyt}(300)}{V_{cyt}(Po)}$. From figure 3 the evolution of the spectral absorption and refractive index with the osmotic pressure is given for $\lambda=1000\text{nm}$ and $H(300)=350 \text{ g/l}$. Note that for an osmotic pressure decreasing from 300mosm to 111osm the RBC geometrical aspect ratio $\xi=a/b$ evolves from 3.28 (oblate shape) to $\xi=1$ (spherical shape).

![Iso-level map of the scattering phase function versus the scattering angle and the osmotic pressure, RBC in fixed orientation.](image1)

![Iso-level map of the scattering degree of linear polarisation versus the scattering angle and the osmotic pressure, RBC in fixed orientation.](image2)

3. NUMERICAL RESULTS AND DISCUSSION

At the first step we consider the case where the RBC is in fixed orientation: the incident laser beam propagates along the RBC symmetrical axis, we analyse the scattering in the azimuthal plane.
Under such geometry, figure 4 presents an iso-level map for the evolution of the phase function (scattering element $F_{11}$) for a non-oxygenated RBC versus the osmotic pressure (change in absorption, refractive index and shape) and the scattering angle. Figure 5 presents the same kind of map but for the degree of linear polarisation of the scattered light (ratio of two scattering elements: $F_{21}/F_{11}$). In both cases there is the evolution of the scattering elements versus the osmotic pressure and obviously with the scattering angle. This dependence seems nevertheless to be weaker and weaker as the RBC osmotic pressure tends to 300 mosm (spherical shape).

We now consider the case of randomly oriented RBC. This case is expected to be of greater interest to infer RBCs properties from a whole blood sample.

Figures 6 and 7 are equivalent to figures 4 and 5 but for randomly oriented RBC. It appears from figure 6 that there is still the evolution of the phase function versus the osmotic pressure, however it is more confusing. From figure 7, it appears, surprisingly to some extend, that the degree of linear polarisation is no more a good parameter for the diagnosis of RBCs osmotic pressure or shape.
Figures 8 and 9 show the evolution of the phase function and the degree of linear polarisation versus the scattering angle for two spectral absorption (corresponding to a laser wavelength of $\lambda=440\text{nm}$ and $\lambda=1000\text{nm}$), for oxygenated (100%) and non oxygenated (0%) haemoglobin, and for three osmotic pressures ($P_\text{o}=111$, 201 and 291 mosm). For low osmotic pressure the evolution of the phase function and the degree of linear polarisation are characteristic for these obtained with the Lorenz-Mie Theory (LMT) for spherical particles: strong resonance structures are observed. When the osmotic pressure increases, i.e. the RBC geometrical aspect ratio increases, the previous evolution tends to be smoothed. In figure 8 increasing osmotic pressure from 202 to 291 induces an onset of the phase function, it has nevertheless no significant influence on the linear degree of polarisation, see figure 9. The influence of the spectral absorption in figures 8 and 9 is extremely weak, so we may have some doubt about the possibility to diagnose the RBC oxygenation from the analyses of the phase function and the degree of linear polarisation.

Fig. 8. Phase function versus the scattering angle for various osmotic pressures, two spectral absorption (at 440nm and 1000 nm) and for oxygenated and non-oxygenated haemoglobin.
Figure 10 presents the evolution of the corresponding extinction scattering coefficients, $Q_{\text{ext}}$. For wavelength $\lambda = 1000\text{nm}$ the extinction coefficient dependence on RBC oxygenation is almost null. For $\lambda = 440\text{nm}$ this dependence is not negligible, the ratio $Q_{\text{ext}}(\text{Hb})/Q_{\text{ext}}(\text{HbO}_2)$, $k_{440}$ evolves of 4-5% which could be measurable, it is nevertheless not a one-to-one relation with the osmotic pressure, see curve in figure 10. This behaviour can be explained from figure 11, where the evolution of the scattering coefficients (extinction, scattering, and absorption) versus the wavelength is given for the two types of haemoglobin. Note that in these calculations the size parameter is kept constant (independent of the laser wavelength) only the absorption coefficient dependence on wavelength is taken into account. This procedure was found to be convenient to reduce our analysis of the influence of the absorption coefficient on the scattering pattern and coefficients, to one parameter analysis.

Fig. 9. Like in figure 8, but for degree of linear polarisation.

Fig. 10. Extinction coefficient versus osmotic pressures for two spectral absorption (at 440 and 1000 nm) and for oxygenated and non-oxygenated haemoglobin.
It appears from figures 6-11 that in contrary to fixed oriented RBCs the diagnosis of the osmotic pressure of randomly oriented RBC is expected to be rather difficult. The osmotic pressure has some influence on the phase function and on the extinction scattering coefficient which is not a one-to-one relation. The diagnosis of the oxygenation rate of RBCs seems to be even more difficult task. The influence of the haemoglobin absorption on the basic scattering parameters and functions, which is rather large, seems to be too small to be detected experimentally.

Finally, one can expect that in case of a whole blood sample with a polydisperse size distribution for RBCs and obviously under multiple scattering regime, it would not be possible to infer reliable statistical properties of a RBCs. According to the authors one solution to this problem would be to fix the orientation of all RBCs present in the blood sample as done for a single RBC in flow-cytometric methods.

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