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# Optical parameters and scattering properties of red blood cells

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FABRICE ONOFRI

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The results of investigation 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. 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.

## 1. Introduction

An erythrocyte has a round shape a biconcave disc of $7.5 \pm 0.3$ μm in diameter and thickness of $(1.4-2.1) \pm 0.4$ μm. The mean concentration of haemoglobin in an erythrocyte is $HC = 350 \pm 2.5$ g/l. Haemoglobin is responsible for the spectral absorption of RBCs. Under normal conditions the RBCs are oblate in shape and their volume equals $V_0 = 90$ μm$^3$ for the osmotic pressure $P_o = 300$ mosm (see Fig. 1a). The shape of unhealthy RBCs can significantly differ from

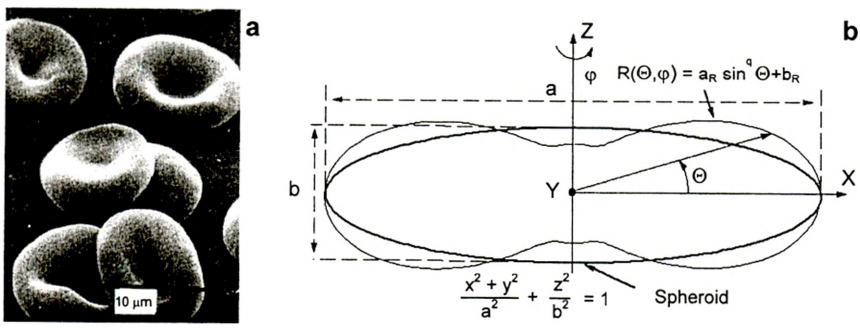


Fig. 1. View of real human RBCs for normal osmotic pressure $P_o = 300$ mosm (**a**). Models of RBC (**b**).

the one presented in the figure. It also strongly depends on the osmotic pressure. So that one can expect to diagnose RBCs oxygenation rate and the osmotic pressure from RBCs shape analysis and absorption diagnosis.

The whole blood (non-diluted and non-hemolyzed) is a disperse system in which RBCs form the major part of a dispersed phase with plasma as a dispersing medium.

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. Haemoglobin in a RBC takes mainly two forms: an oxidised form ($HbO_2$, responsible for the transport of oxygen) and unoxygenated form (Hb). Haemoglobin is responsible for light absorption properties of the erythrocyte (oxygenated blood appears to be light red and non-oxydated – dark red). 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 resistive 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 information 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" [1]

$$|m-1| \ll 1,$$
$$kd|m-1| \ll 1 \tag{1}$$

where $m$, $k$, $d$ are the RBC relative refractive index in the medium considered ($m = m_{RBC}/m_m$), 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 that is quite simple and physically obvious analytical form usually referred to as the anomalous diffraction approximation [2]–[6]. The first condition is usually fulfilled as, in the visible range, the RBCs 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 violated. For instance, for the previous parameters with $\lambda = 0.6328$ μm and $d = 4.9$ μm, we found that $kd|m-1| = 1.6$. This introduces some limitations as regards 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 recently been used in [7] to predict the light scattering properties of RBC with their shape approaching natural one (Fig. 1**b**) within the spherical co-ordinate system

$$R(\theta,\varphi) = a_R \sin^q\theta + b_R \tag{2}$$

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 $d = 7.5$ μm, corresponding to $a_R = 3$ μm and $b_R = 0.75$ μm and the exponent is estimated at $q = 5$. They have also assumed that the osmolarity of the suspension medium causes "isovolumetric sphering" of the cell, where the case $q = 0$ corresponds to the sphere. This last hypothesis seems to be invalidated by some experimental studies [8], [9].

The $T$-matrix method (or extended boundary condition method) has also been 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 method allows us to compute scattering coefficients as well as the phase function, the degree of linear or circular polarisation for arbitrary scattering angles [6], [10], [11].

## 2. Simulation strategy and procedure

To compute the light scattering properties of RBCs we have chosen the $T$-matrix method, for obtaining accurate predictions on the scattering coefficients and angular scattering dependences which are supposed 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 [10], [11] available from the web site [12]. The RBC shape will be modelled by an oblate ellipsoid (see Fig. 1**b**) with the equation: $\frac{x2+y^2}{a^2} + \frac{z^2}{b^2} = 1$, where $a$ is a symmetrical axis. This particle has the same projection area along $Z$ axis as 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 $Y$ axes.

The haemoglobin, Hb (0% oxygenation) and $HbO_2$ (100% oxygenation), complex refractive index dependence on wavelength [13], [14] is presented in Fig. 2**a**. At the first sight, it appears from the figure that the major difference in the spectral absorption between Hb and $HbO_2$ is in the range of 600−750 nm. Nevertheless, if we consider a simple intensity ratio experiment to distinguish the two types of haemoglobin from the intensity transmission through blood samples (width $L$), the relevant parameter is the difference in the two spectral absorptions: $\frac{I_{Hb}}{I_{HbO_2}} = \exp[-(k_{Hb} - k_{HbO_2})L]$. Figure 2**b** presents the difference in the spectral absorption of the two types of haemoglobin. The difference in the blood sample transmission is

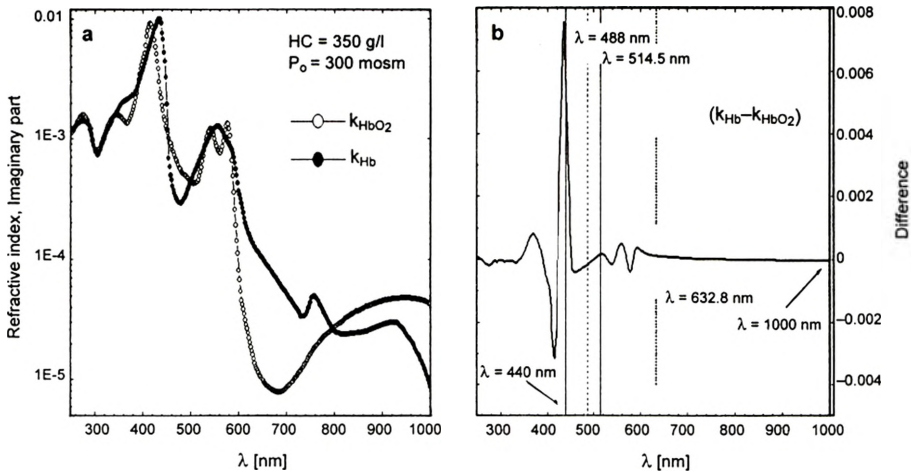


Fig. 2. Spectral absorption of RBC for oxygenated ($HbO_2$) and non-oxygenated haemoglobin (Hb): a — imaginary part of refractive index *vs.* wavelength, b — difference between the imaginary parts of refractive index *vs.* wavelength for non-oxygenated and oxygenated blood.

expected to be a maximum for wavelength about 380-450 nm. The refractive index inside a RBC is

$$\tilde{m}_{\mathrm{RBC}} = m_{\mathrm{RBC}} + ik, \tag{3}$$

$m_{\mathrm{RBC}}$ is expected to depend on the RBC haemoglobin concentration, the concentration being varied for different people (typical value: HC = 350 g/dm$^3$). The linear dependence upon HC is known [1]

$$m_{\mathrm{RBC}}(\mathrm{HC}) = m^0_{\mathrm{RBC}} + \alpha \mathrm{HC} \tag{4}$$

where for the constant we use: $\alpha = 0.0019$ g/dm$^3$ and $m^0_{\mathrm{RBC}} = 1.335$.

The real part of the refractive index dependence on the wavelength is neglected. This is justified by the following arguments:

i) in the light scattering calculations we use the relative refractive index $m = m_{\mathrm{RBC}}/m_{\mathrm{m}}$ so that for some part the wavelength dependence is cancelled out,

ii) as the best of authors' knowledge, there are no reliable data in the literature on this dependence.

There are also very scarce 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 [8] giving the evolution of the volume of RBCs in terms of a cylinder with the length $2b$ and radius $a$. Note that this way of defining the RBCs volume is common in the clinical practice.

The evolution of parameter $a$ is deduced from a linear fit of a few pieces of experimental data on the shape of RBCs [9]. As a result, using these data we derive shape dependence on osmotic pressure for the RBCs:

$$V_{cyl}(P_o) = 339 - 1.88P_o + 0.00491P_o^2 - 4.46 \cdot 10^{-6}P_o^3,$$
$$a = [0.00310(P_o - 150) + 3.210],$$
$$b = \frac{V_{cyl}(P_o)}{2\pi a^2}. \tag{5}$$

In terms of volume, the RBC is equivalent to a sphere with radius $r_{sph} = \left(\frac{V_{cyl}(P_o)}{2\pi}\right)^{1/3}$. When the osmotic pressure decreases from 300 to 111 mosm the RBC geometrical aspect ratio decreases from $\xi = a/b = 3.28$ (oblate shape) to $\xi = 1.002$ (spherical shape), see Fig. 3. In the opposite case, the RBC volume

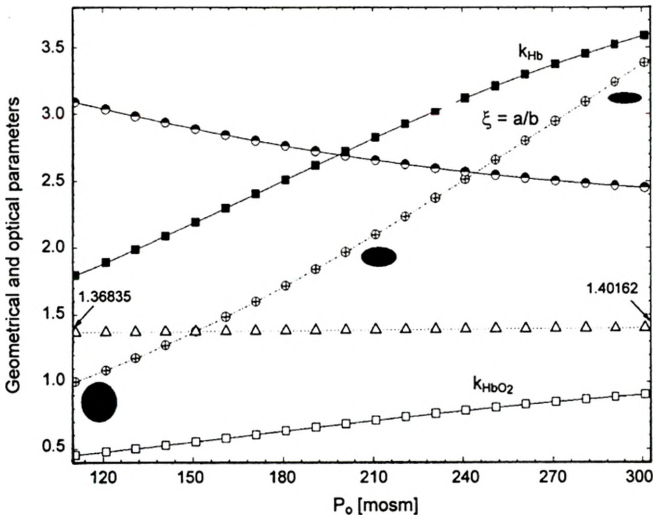


Fig. 3. Evolution of the RBC geometrical and optical properties *vs.* the osmotic pressure: ○ – spheroidal aspect ratio, $\zeta = a/b$, -○- – radius volume equivalent sphere, -△- – refractive index, real part, ■- – refractive index, imaginary part $\times 500$/Hb, -□- – refractive index, imaginary part $\times 500/HbO_2$.

increases (the equivalent sphere radius increases). This causes a dilution of the haemoglobin inside the RBC: $HC(P_0) = HC(300)V_{cyl}(300)/V_{cyl}(P_o)$ and thus a change in the RBC refractive index $m_{RBC}(HC)$ and absorption $k(P_o) = k(300)V_{cyl}(300)/V_{cyl}(P_o)$. In Figure 3 the evolution of the spectral absorption and refractive index with the osmotic pressure is given for $\lambda = 100$ nm and $HC(300) = 350$ g/l.

## 3. Numerical results and discussion

First, we consider the case where the RBC is in fixed orientation: the incident laser beam propagates along the RBC symmetrical axis, and we analyse the scattering in the azimuthal plane. Under such geometry, Fig. 4 presents an iso-level map for the

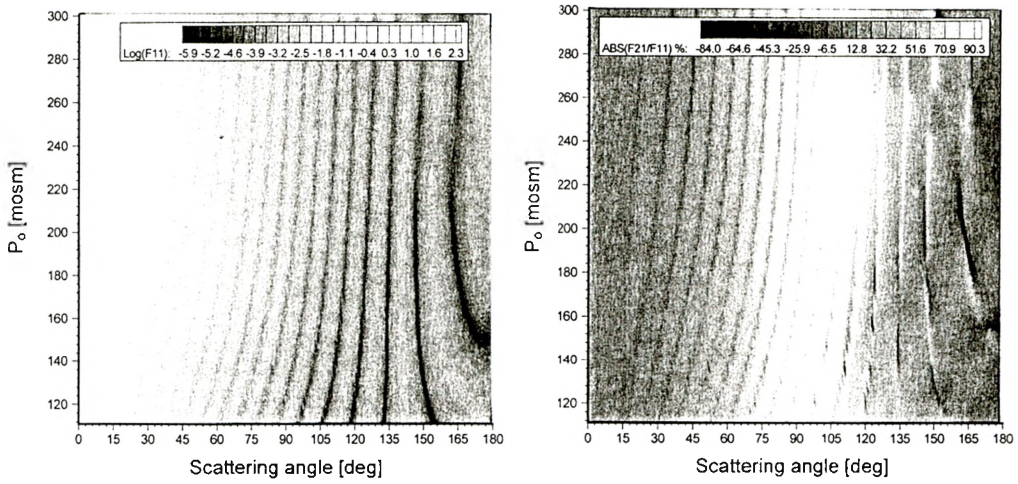


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Fig. 4. Iso-level map of the scattering phase function *vs.* the scattering angle and the osmotic pressure, RBC in fixed orientation.

Fig. 5. Iso-level map of the scattering degree of linear polarisation *vs.* the scattering angle and the osmotic pressure, RBC in fixed oriantation.

evolution of the phase function (scattering element $F_{11}$) for a non-oxygenated RBC *vs.* 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: $-F_{21}/F_{11}$).

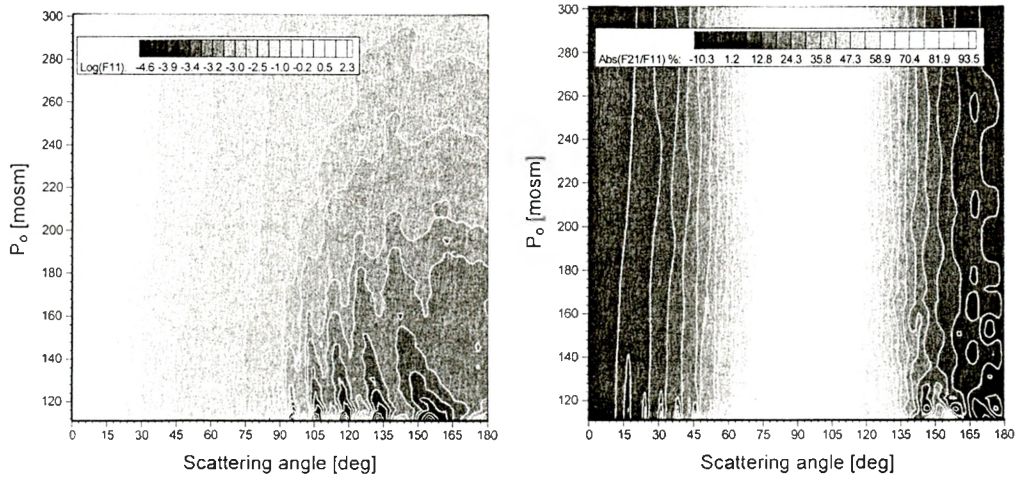


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Fig. 6. Iso-level map of the scattering phase function *vs.* the scattering angle and the osomotic pressure, RBC in random orientation (integration over all directions).

Fig. 7. Iso-level map of the scattering degree of linear polarisation *vs.* the scattering angle and the osmotic pressure, RBC in random orientation.

In both cases there is the evolution of the scattering elements *vs.* 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 Figs. 4 and 5 but for randomly oriented RBC. It appears from Fig. 6 that there is still the evolution of the phase function *vs.* the osmotic pressure, however, it is more confusing. From Fig. 7, it appears, surprisingly to some extent, that the degree of linear polarisation is no more a good parameter for the diagnosis of RBCs osmotic pressure or shape.

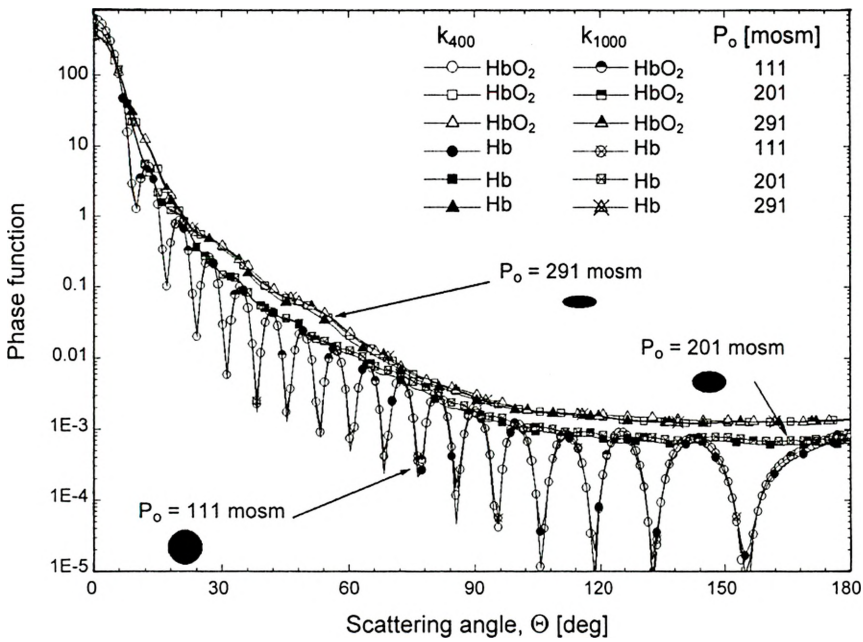


Fig. 8. Phase function *vs.* the scattering angle for various osmotic pressures, two types of spectral absorption (at 440 and 1000 nm) and for oxygenated and non-oxygenated haemoglobin.

Figures 8 and 9 show the evolution of the phase function and the degree of linear polarisation *vs.* the scattering angle for two cases of spectral absorption (corresponding to a laser wavelength of $\lambda = 440$ nm and $\lambda = 1000$ nm), for oxygenated (100%) and non oxygenated (0%) haemoglobin, and for three osmotic pressures ($P_o = 111$, 201 and 291 mosm). For low osmotic pressure the evolution of the phase function and the degree of linear polarisation are characteristic for those obtained with the Lorenz–Mie theory 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 Fig. 8 increasing osmotic

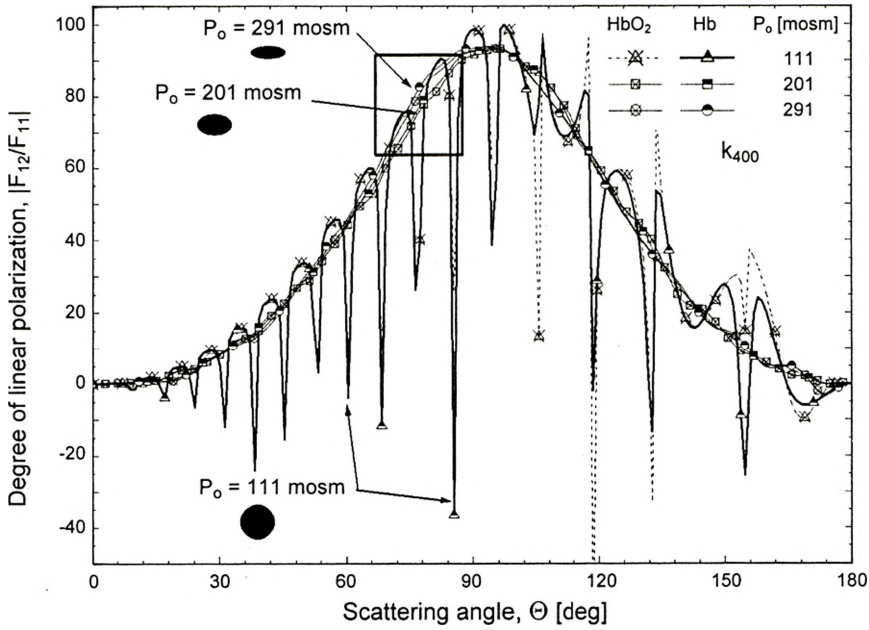


Fig. 9. Degree of linear polarisation *vs.* the scattering angle for various osmotic pressures and spectral absorption at 400 nm.

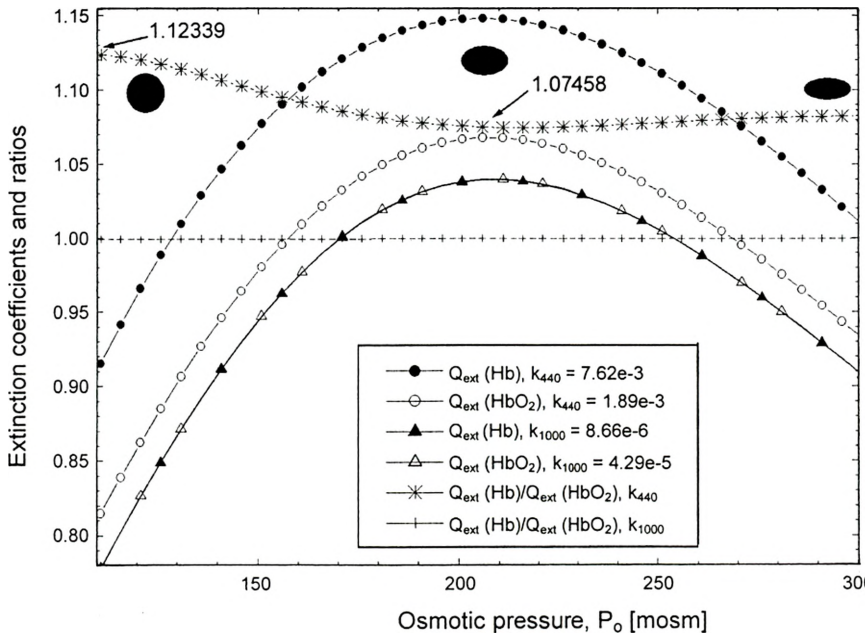


Fig. 10. Extinction coefficient *vs.* osmotic pressures for two types of spectral absorption (at 400 and 1000 nm) and for oxygenated and non-oxygenated haemoglobin.

pressure from 202 to 291 induces the onset of the phase function, it has nevertheless no significant influence on the linear degree of polarisation, see Fig. 9. The influence of the spectral absorption in Figs. 8 and 9 is extremely weak, so we may have some doubt about the possibility of diagnosing the RBC oxygenation from the analyses of the phase function and the degree of linear polarisation.

Figure 10 presents the evolution of the corresponding extinction scattering coefficients $Q_{ext}$. For wavelength $\lambda = 1000$ nm the extinction coefficient dependence on RBC oxygenation is almost null. For $\lambda = 440$ nm this dependence is not negligible, the ratio $Q_{ext}(Hb)/Q_{ext}(HbO_2)$, $k_{440}$ evolves about $4-5\%$ which could be measurable, it is nevertheless not a one-to-one relation with the osmotic pressure, see the curve in Fig. 10. This behaviour can be explained based on Fig. 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 $\alpha$ 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 for reducing our analysis of the influence of the absorption coefficient on the scattering pattern and coefficients to one parameter analysis.

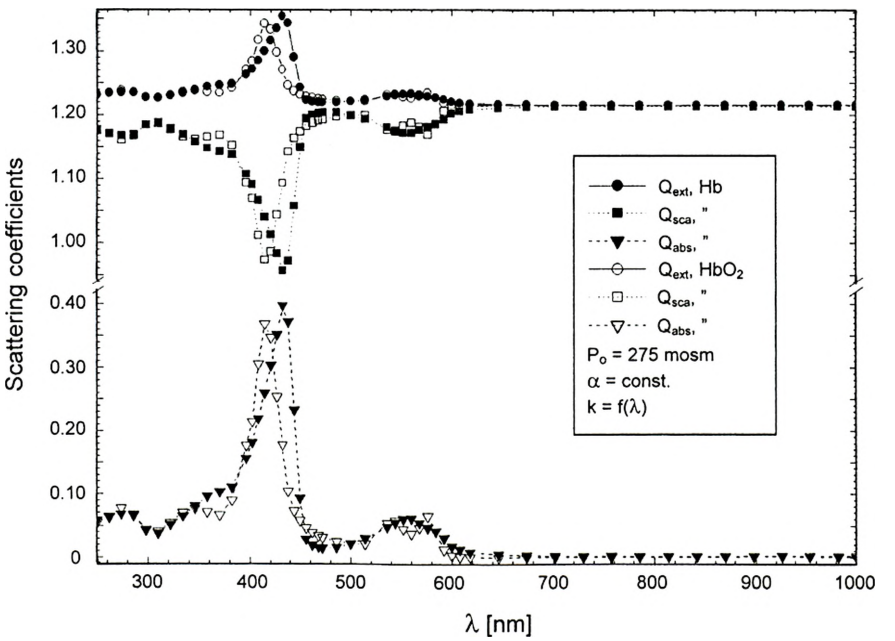


Fig. 11. Scattering coefficients (extinction, scattering and absorption) *vs.* the incident light wavelength and for oxygenated and non-oxygenated haemoglobin. Note that the RBC size parameter is taken independent of the wavelength.

It appears from Figs. 6–11 that 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 an 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.

## 4. Conclusions

Finally, one can expect that in the 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. We claim that one solution to this problem would be to fix the orientation of all RBCs present in the blood sample (as is done for a single RBC in flow-cytometric methods) under single or multiple light scattering conditions [15]–[17].

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