XVIII IMEKO WORLD CONGRESS
Metrology for a Sustainable Development
September, 17 – 22, 2006, Rio de Janeiro, Brazil

SINGLE AND MULTIPLE LIGHT SCATTERING IN ERYTHROCYTE MODELLING

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Abstract: The light scattering models of red blood cell (RBC) are presented. Erythrocyte is modelled as a spheroid. Main purpose of this paper is to present single and multiple light scattering by erythrocytes in different physiological conditions. The osmotic pressure and oxygenation, as well as hematocrit influence on scattered light properties are presented.

Keywords: erythrocyte, light scattering, spheroid.

1. INTRODUCTION

The real erythrocyte has form of a biconcave disc, but the shape of a pathological RBC can significantly differ from the normal cell and it strongly depends on the osmotic pressure. Other optical parameters like refractive index, scattering and absorption cross-section depend on hematocrit and oxygenation level. Scattered light measuring enables us to estimate these haematological parameters [1,2]. There are many models of light scattering by erythrocytes phenomenon, but still there is a need to improve these models.

To solve many measurement problems (medical, biophysical, astrophysical, ocean and atmosphere optics), knowledge about properties of scattered radiation by non-spherical particles is needed [3,4,5]. One of these models is spheroid. In many applications cylinder can be used (fibres, bacteria) [6]. The change of the shape from oblate to prolate gives the many possibilities of researches.

2. METHODS

Two numerical methods were used in presented work: T-Matrix method for single scattering, and Monte Carlo method for multiple scattering. T-Matrix method was also used to obtain input parameters for Monte Carlo method.

T-Matrix method is very effective to compute scattering properties of axi-symmetrical, non-spherical particles of different size [7,8]. To compute scattered field on the base of incident field coefficients, matrix of integrals computed numerically on the surface of particle is used. These integrals include information about size, shape, refraction index and orientation of particle in the main frame of simulation. Next, the scattered light coefficients may be expressed by similar matrix where spherical Bessel functions are replaced with Hankel functions. The T-Matrix method may be used to compute properties of particle with arbitrary shape, but symmetry of particle may significantly simplify computations.

Thanks to particle symmetry, there is possibility to compute T-Matrix for arbitrary orientation of particle more effectively. In this case particle symmetry axis must agree with coordinate system axis. T-Matrix method makes possible to compute average scattering properties for set of randomly oriented particles more effective than integral methods. The scattering properties of randomly oriented particle are determined by averaging for all possible orientations. In our numerical simulations all particles in the medium were the same size and were randomly oriented.

In the Monte Carlo simulation it is possible to model full geometry of measurement system without simplifications (like infinite surfaces) [9,10]. The main idea of this approach is to consider incident and scattered light as the set of "packages of light" named "photons", emitted at the time. During simulation the path of every single "photon" is analysed. This „photon" can be emitted from the source, scattered or absorbed by erythrocyte or detected. The intensity of radiation is defined as a number of emitted or detected "photons". In the case of highly scattering media as full blood, the Monte Carlo method may be ineffective (time consuming) because of large number of treated "photons". Therefore it is necessary to limit number of emitted "photons" to value that reaches the required accuracy of the analysis. Average free-path d_f of "photon" depends on extinction cross-section C_{ext} and concentration of erythrocytes N, and is computed with use a random number r_n from range (0,1):

$$d_f = \frac{\ln(r_n)}{NC_{ext}} \tag{1}$$

In our simulations new direction of propagation of "photon" was determined with use the phase function obtained from T-Matrix method. The elevation angle θ was determined with use of distribuant of phase function $p(\theta,\phi)$ and relation:

$$r_n = \frac{\int_0^{\theta} p(\theta)d\Omega(\theta)}{\int_0^{\pi} p(\theta)d\Omega(\theta)}, \tag{2}$$

where r_n is another random number from range (0,1). Azimuth angle was randomly chosen from (0,2π) range.

"Photon" is absorbed by erythrocyte when albedo A is lower than another random number r_n from (0,1):

$$r_n > A. \tag{3}$$

3. MODELS OF ERYTHROCYTE

Two models of erythrocyte were used in computation. The spherical model was used as a reference model to compare with spheroid model.

3.1. Erythrocyte parameters

The healthy erythrocyte has form of a biconcave disc with diameter values ranges from 5.7μm to 9.35μm (mean 7.55μm). However, the diameters of most of them (about 68%) fall into the range from 7 to 8 μm [11].

In medical laboratories a sample of blood is characterized, by such parameters as:

- mean RBC volume (MCV = 75 – 100μm^3),
- RBC concentration (3.5 – 5.5x10^6/mm^3),
- mean concentration of haemoglobin in the RBC (MCHC = HC = 31.0 - 38.0 g/dlitr)
- hematocrit HCT defined as the ratio of the RBC volume to the total volume of blood (HCT = 32 – 46 % for females, 38-49.5% for males, and 30-40% for children).

The diameter of erythrocyte is a typical parameter characterizing the erythrocyte during diagnosis. Thus a shift of the mean diameter outside the typical range indicates pathology [11].

Fung [12] has made precise interferometric measurements of a size of 1581 erythrocytes originating from 14 donors in case of normocytosis (osmolarity 298-307 mosm). Hochmuth [13] announces the erythrocyte parameters obtained from microscopic measurements performed in isotonic solutions (300 mosm) and for osmolarity of 217 and 131 mosm. He makes also a reference to the measurements done by Fung – see table 1. The erythrocyte shape coefficient ξ_{eryt} is defined as:

$$\xi_{eryt} = \frac{d_{eryt}}{h_{eryt}}. \tag{4}$$

Table 1. Size parameters of erythrocytes by Hochmuth.

osmotic pressure	P_o [mosm]	300	217	131
diameter	d_{eryt} [μm]	7.82	7.59	6.78
minimum central thickness	u_{eryt} [μm]	0.81	2.10	-
thickness	h_{eryt} [μm]	2.58	3.30	-
volume	V_{eryt} [μm^3]	94	116	164
surface area	S_{eryt} [μm^2]	135	135	145
shape coefficient	ξ_{eryt} [-]	3.031	2.300	~1.0

The erythrocyte can be modeled as a homogeneous water solution of haemoglobin (about 34 g/dliter), salt (about 0.7 g/dliter) and other organic components (about 0.2 g/dliter) surrounded by a transparent membrane of a negligible thickness.

The real part of the refraction index depends mainly on the content of haemoglobin *HC* [1]:

$$n_R = n_0 + \alpha HC, \tag{5}$$

where n_0=1.335, α=0.001942 [dliter/g].

The imaginary part of the refraction index, related to absorption, is given as follows:

$$n_I = \frac{\ln 10}{\pi M} \lambda \varepsilon_{\mu M} HC, \tag{6}$$

where M=65500 [g/mol] is a molecular weight of haemoglobin, λ is a wavelength in μm, $\varepsilon_{\mu M}$ is a micromolar coefficient of extinction at a given wavelength expressed in cm^2/micromole [14].

3.2. Models

The erythrocyte was modeled as an oblate spheroid. The spheroid is obtained by turning an ellipse of a large semi-axis a and a small semi-axis b (where a>b) around an axis covering both b and the axis OZ of the Cartesian system of coordinates. The spheroid intersection in the XY plane is a circle of the radius 2a. The shape coefficient ξ of the spheroid is defined as a ratio of the major semi-axis to the minor one. Alike in case of the spherical model, a spheroid of the same volume and the shape coefficient as the erythrocyte was chosen:

$$a = b\xi, \qquad b = \sqrt[3]{\frac{3V_e}{4\pi\xi^2}}. \tag{7}$$

The main parameters of spherical model are: the sphere radius r_k, volume V_k, and surface area S_k. In our computations the model with a sphere of the same volume as the erythrocyte was used, so

$$r_k = \sqrt[3]{\frac{3V_e}{4\pi}}. \tag{8}$$

4. METHODOLOGY

In case of single light scattering the T-Matrix Method was used. The main optical parameters like phase function $p(\theta,\phi)$, extinction cross-section C_{ext}, and albedo were computed for the single erythrocyte modeled as a sphere and as an oblate spheroid placed at all possible orientations. The results were averaged. The erythrocyte modeled as a sphere was used as a reference.

The parameters obtained in single scattering simulations were used as input parameters for multiple light scattering simulations carried out with use of Monte Carlo Method. So the monodisperse distribution of randomly oriented erythrocytes were erythrocytes were concerned. The concentration of erythrocytes N was extra input parameter in

these simulations. Hematocrit depends on erythrocyte concentration:

$$HCT = \frac{NV_{eryt}}{m^3} \cdot 100\% . \quad (9)$$

In our simulations the blood sample thickness was 0.5 mm, and the apertures of light source and detector were the same. The number of analyzed beam was 10^5. The computed optical density *OD* (*T* – transmittance) defined as:

$$OD = -\log_{10}(T) \quad (10)$$

was analyzed.

The erythrocyte is a biological object characterized by a high intersubject variability. In our simulations the erythrocyte parameters from experimental measurements published in the literature [13] were used – see table 1.

Two wavelengths were selected for simulations. For 632.8 nm the absorption (micromolar extinction coefficient $\varepsilon_{\mu M}$ spectrum relation [14]) is relatively small and relative change between saturated and non-saturated haemoglobin reaches 80%. At the 800 nm this change is close to zero.

The table 2 presents the relative complex refraction index computed for two wavelength with equations (5) and (6). The haemoglobin concentration *HC*=34g/dlitr and refraction index of medium n_s=1.33.

Table 2. Relative complex refraction index of erythrocyte for used wavelength and saturated and non-saturated haemoglobin.

λ [nm]	m_R		m_I	
	HbO2	*Hb*	*HbO2*	*Hb*
632.8	1.0534		0.0001	0.0009
800			0.0002	

5. RESULTS

The numerical simulations were carried out at normal conditions (normocytosis), in case of hematocrit changes, osmotic pressure and oxygenation variations. In case of single scattering (averaged for all orientations of erythrocyte) the phase function, and for multiple light scattering the optical density were analyzed.

5.1. Normocytosis

In case of normal conditions the erythrocytes keep their basic shape (the solution is isotonic). The dimentions of erythrocyte are given in table 1 for osmotic pressure P_o=300 mosm. The haemoglobin concentration was *HC*=34g/dlitr. In case of multiple scattering hematocrit values was assumed as *HCT*=40%. The other input parameters for two concerned models of erythrocytes are presented in table 3.

Table 4 presents normalized phase functions at small scattering angle (in practice, the forward scattering is concerned by overexpose a sample) for the case of single light scattering by erythrocyte at random orientation (integration over all locations). The reference for normalization is the value of phase function for erythrocyte modelled by a sphere at scattering angle θ=0° and incident wavelength 800 nm for non-saturated haemoglobin. Note, that the differences between phase function for saturated and non-saturated haemoglobin at this wavelength are negligible a cause of no different between the imagine parts of refractive indices (table 2).

Table 3. Parameters of erythrocyte models at normal conditions.

Parameter	Erythrocyte	Sphere	Spheroid
d [μm]	7.82	5.642	8.164
h [μm]	2.52	5.642	2.694
V [μm^3]	94.0	94.0	94.0
ξ [-]	3.031	1.000	3.031

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Table 4. Normalized phase functions for small scattering angles at normal conditions – two models of erythrocyte

θ	800nm, Hb		632.8nm, Hb		632.8nm, HbO2	
[deg]	sphere	spheroid	sphere	spheroid	sphere	spheroid
0	1.0000	1.1766	1.5745	1.9318	1.5680	1.9181
1	0.9682	1.1224	1.4931	1.7870	1.4872	1.7748
2	0.8778	0.9739	1.2703	1.4123	1.2659	1.4038
3	0.7432	0.7678	0.9620	0.9503	0.9596	0.9461
4	0.5847	0.5498	0.6389	0.5450	0.6384	0.5441
5	0.4239	0.3588	0.3626	0.2740	0.3634	0.2747
6	0.2796	0.2166	0.1688	0.1340	0.1701	0.1350
7	0.1645	0.1257	0.0616	0.0759	0.0627	0.0767
8	0.0838	0.0752	0.0210	0.0509	0.0217	0.0513
9	0.0354	0.0494	0.0171	0.0340	0.0173	0.0343
10	0.0127	0.0352	0.0236	0.0208	0.0235	0.0211

The distinct differences between phase function (λ=800 nm) for sphere and spheroid models at scattering angle close to zero are observed. The change of the wavelength to 632.8 nm results in increased forward scattering (a change of a size parameter), however the relative differences between the models nearly do not alter.

Table 5. Output parameters of erythrocyte for single and multiple light scattering simulations at normal conditions.

λ [nm]	parameter	Hb		HbO2	
		sphere	spheroid	sphere	spheroid
632.8	C_{ext} [μm^2]	69.557	61.959	70.377	62.306
	A	0.9743	0.9710	0.9971	0.9967
	OD	1.8904	1.8517	0.3526	0.3396
	OD_{rel} [%]	0.00	-2.05	0.00	-3.69
	OD_{norm}	3.801	3.724	0.709	0.683
800	C_{ext} [μm^2]	53.36	47.04	53.36	47.04
	A	0.9939	0.9931	0.9939	0.9931
	OD	**0.4973**	0.4763	0.4973	0.4763
	OD_{rel} [%]	0.00	-4.22	0.00	-4.22
	OD_{norm}	**1.000**	0.958	1.000	0.958

The other output parameters for single and multiple light scattering simulations are presented in table 5. The optical density of sphere model is bigger, but the normalized OD_{norm} and relative OD_{rel} optical density show that there are no significant differences between the models.

5.2. Osmotic pressure

The simulation with osmotic pressure variations was carried on at incident wavelength 800 nm. There are no influence of haemoglobin saturation in this case.

Table 6. Parameters of erythrocyte versus osmotic pressure at λ = 800nm

P_o [msom]	300	217	131
d [μm]	7.82	7.59	6,78
h [μm]	2.58	3.30	-
V [μm^3]	94	116	164
ξ [-]	3.031	2.3	~1,0
HC [g/dlitr]	34,0	26.4	18,6
m_R	1.0534	1.0440	1,0322
m_I	0.0002	0.0001	0,0001

Table 7. Parameters of erythrocyte models versus osmotic pressure for λ = 800nm

	Sphere			Spheroid		
P_o [msom]	300	217	131	300	217	131
d [μm]	5,642	6,050	6,792	8,164	7,987	6,791
h [μm]	5,642	6,050	6,792	2,694	3,473	6,791
V [μm^3]	94	116	164	94	116	164
ξ [-]	1,000	1,000	1,000	3,031	2,300	1,000

The changes of osmotic pressure strongly influence the erythrocyte shape and volume. The haemoglobin concentration *HC* changes with erythrocyte volume because the haemoglobin mass in the erythrocyte stays unchanged [15]. This effect influences the value of the complex refraction index, as shown in Table 6. The shape parameter shows than erythrocyte changes from oblate biconcave disc to almost spherical particle.

The parameters of two considered erythrocyte models versus osmotic pressure are shown in Table 7.

Table 8 presents the normalised phase functions for two models under consideration. The erythrocyte modelled by spheroid scatter light more effectively than the sphere model, especially into scattering angle close to zero (θ <5), but the nature of presented functions are not significantly differ.

Table 8. Normalized phase functions at small scattering angles for different osmotic pressures, λ = 800nm

θ	Sphere			Spheroid		
	300 osm	217 osm	131 osm	300 osm	217 osm	131 osm
0	1.0000	1.1484	1.4432	1.1766	1.2532	1.4432
1	0.9682	1.1071	1.3790	1.1224	1.1964	1.3790
2	0.8778	0.9905	1.2009	0.9739	1.0403	1.2009
3	0.7432	0.8196	0.9478	0.7678	0.8221	0.9478
4	0.5847	0.6229	0.6704	0.5498	0.5888	0.6704
5	0.4239	0.4301	0.4171	0.3588	0.3816	0.4171
6	0.2796	0.2650	0.2206	0.2166	0.2248	0.2206
7	0.1645	0.1414	0.0933	0.1257	0.1232	0.0933
8	0.0838	0.0623	0.0281	0.0752	0.0668	0.0281
9	0.0354	0.0213	0.0062	0.0494	0.0394	0.0062
10	0.0127	0.0068	0.0064	0.0352	0.0265	0.0064

Table 9. Results of single and multiple analysis versus osmotic pressure for λ = 800nm, non-saturated haemoglobin .

	Sphere			Spheroid		
P_o [mso]	300	217	131	300	217	131
C_{ext} [μm^2]	53.36	51.02	46.63	47.04	47.52	46.63
A	0.9939	0.9961	0.9942	0.9931	0.9958	0.9942
OD	**0.4973**	0.2708	0.2165	0.4763	0.2661	0.2180
OD_{norm}	**1.000**	0.544	0.435	0.958	0.535	0.438
OD_{rel} [%]	0.00	0.00	0.00	-4.20	-1.74	-0.70

The other results of single and multiple light scattering simulations are mentioned in table 9. The relative changes of the optical density caused by the osmotic pressure (from 300 mosm to 131 mosm) are comparable for two models:

–56.5% for sphere and -54.3% for spheroid. The normalized optical density OD_{norm} shows that erythrocytes modelled by spheroid are more transparent, but the optical density changes versus osmotic pressure are the same character for both models. The differences between the models do not excide 5% (relative optical density OD_{rel}). These differences are smallest for lower osmotic pressure when the erythrocyte shape is close to the sphere (ξ~1.0).

5.3. Hematocrit

The normalized relations between optical density and hematocrit for normal conditions are presented in table 10. For incident wavelength 800nm optical density rises about 100% (hematocrit changes from 30% to 60%). The change of the wavelength to 632.8 nm decreases sensibility of the models (maximum about 20% for non-saturated haemoglobin), but in the same way for both models. The differences of sensibility between the models do not exceed 2% The differences between the models are smaller than 5.1 %.

Table 10. Normalized optical density versus hematocrit at normocytosis.

	HCT [%]	*N* [mm^{-3}] x10^6	OD_{norm} 800nm Hb	OD_{norm} 632.8 nm Hb	OD_{norm} 632.8 nm HbO2
sphere	30	3.1915	0.742	2.876	0.540
	40	4.2553	**1.000**	3.801	0.709
	50	5.3191	1.236	4.575	0.888
	60	6.3830	1.486	5.168	1.071
total changes			101 %	80 %	98 %
spheroid	30	3.1915	0.724	2.846	0.516
	40	4.2553	0.958	3.724	0.683
	50	5.3191	1.195	4.566	0.843
	60	6.3830	1.441	5.175	1.017
total changes			100 %	81 %	96 %

Table 11. Normalized density versus hematocrit for different values of osmotic pressure at 800nm.

	HCT [%]	OD_{norm} P_o=300mosm	OD_{norm} P_o=217mosm	OD_{norm} P_o=131mosm
sphere	30	0.742	0.768	0.783
	40	**1.000**	**1.000**	**1.000**
	50	1.236	1.257	1.237
	60	1.486	1.496	1.474
total changes		101 %	95 %	88 %
spheroid	30	0.724	0.755	0.776
	40	0.958	0.983	1.007
	50	1.195	1.231	1.225
	60	1.441	1.473	1.477
total changes		100 %	93 %	90%

The effect of osmotic pressure variation on the optical density as a function of hematocrit at the wavelength of 800 nm is presented in table 11. The changes of osmotic pressure (the shape of erythrocyte) cause the same effect than mentioned above – small sensibility decreasing of models (maximum about 10% for minimum osmotic pressure). The differences between the sensibilities are smaller than 2% and between the models do not exceed 4.3%.

5.4. Oxygenation

The optical density changes for saturated and non-saturated haemoglobin reach about -82% for both models (difference smaller than 0.5%) – table 12. The differences between absolute values of optical density for sphere model and spheroid model do not excide 3.7% for both saturated and non-saturated haemoglobin.

Table 12. Optical density for saturated and non-saturated haemoglobin, HCT=40%, λ=632.8 nm.

OD_{HbO2}		OD_{Hb}		$100*(OD_{HbO2}-OD_{Hb})/OD_{Hb}$ [%]	
sphere	spheroid	sphere	spheroid	sphere	spheroid
0.3526	0.3396	1.8904	1.8517	-81.3	-81.7
differences: (spheroid-sphere)/sphere [%]					
-3.7		--2.0		0.5	

6. CONCLUSION

The presented work concerned simulations of single and multiple light scattering on the erythrocytes. Two models of erythrocyte were used in computation: erythrocyte modelled by an oblate spheroid and erythrocyte modelled by a sphere. The monodisperse distribution of random located erythrocytes was assumed. The numerical simulations of osmotic pressure, hematocrit level and haemoglobin saturation influence on the phase function and optical density were analysed. The optical parameters like a phase function, extinction cross-section and albedo of the single erythrocyte were computed using the T-Matrix method, whereas the optical density was determined by the Monte Carlo simulations. The erythrocyte parameters measured experimentally in the conditions of normocytosis were selected from the literature as the reference.

The obtained results show that the erythrocyte shapes used in the models do not have a significant impact on the character of the analysed dependences especially in case of multiple scattering. The differences between the "behaviour" of the models were on the level of several percent.

ACKNOWLEDGMENTS

Article published with the support of the Foundation for Polish Science.

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