Optical immersion of erythrocytes in blood: a theoretical modeling

Dmitry M. Zhestkov, Valery V. Tuchin, Alexey N. Bashkatov, Elina A. Genina
Saratov State University, Saratov, Russia

ABSTRACT

The human blood optical parameters have been calculated based on Mie theory. The calculations have been done in the spectral range from 400 nm to 1000 nm, which is of great interest due to usage of many therapeutic and diagnostic technologies. The influence of an immersion agent, like hemoglobin, on optical properties of blood, has been studied.

Keyword: blood, transmittance spectra, scattering coefficient, absorption coefficient, anisotropy factor, immersion agent, hemoglobin

1. INTRODUCTION

The knowledge of the optical parameters of biological tissues and blood are of great importance for models describing light propagation in human tissues. These models can be used to provide optical diagnostics and therapeutic techniques of different diseases. Besides, for the purposes of optical tomography and therapy of deep tissue tumors, arteriosclerosis plaques in blood vessels and many other applications of optical and laser methods in medicine it is essential to control the optical properties of tissues and blood *in vivo*. Consequently, such control deals with the change of scattering or absorption properties of optically inhomogeneous media. For example, the method of optical immersion, based on matching of refractive indices of scatterers and surrounding medium is well known in optics of scattering media and widely used in optics of tissues and blood.[1-11]

The goal of the paper is to calculate the optical parameters of the human blood and to study the influence of an immersion agent on these parameters. Hemoglobin has been chosen as an example of an immersion agent. The calculations have been done in the spectral range from 400 nm to 1000 nm using Mie theory.[12]

2. OPTICAL MODEL OF BLOOD

From optical point of view the whole blood is a high concentrated turbid medium. Blood consists of plasma 55% of volume and blood cells 45% (99 % erythrocytes, 1% leukocytes and thrombocytes).[13-16] The blood plasma contains 91% of water, 6.5-8% (about 70 g/l) various proteins and about 2% of low molecular compounds.[2,13] Therefore its optics under physiologic conditions depends mainly on optical properties of the erythrocytes and plasma. Distribution and scattering of light of such medium can be studied as light scattering and absorption by individual particle taking into account the interparticle correlation effects and polydispersity.

Light distribution in biological tissues and liquids can be described by the transport theory.[17] As given by the transport theory, the optical parameters are defined by the absorption coefficient μ_a, the scattering coefficient μ_s and the mean cosine of the scattering angle g (anisotropy factor). In turn, the optical parameters depend on size, shape, orientation and complex refractive index of scatterers and medium.

The normal erythrocyte has a characteristic flat biconcave form with diameter from 5.7 to 9.3 μm and thickness from 1.7 to 2.4 μm.[13, 16] Erythrocyte has a volume of 70-100 μm^3 and can be considered as consisting of 2 main components: erythrocyte membrane (thickness from 7 to 25 nm[18], and refractive index is about 1.46[19-20]) and cytoplasm (refractive index is about 1.40). As it has been shown in Ref. 21 due to small thickness, the influence of erythrocyte membrane on the scattering properties of blood is less then 1%.

The phase function and scattering cross-section of individual erythrocyte depend on orientation.[17] However, light scattering of large number of random distributed non-spherical particles is the same with light scattering of system of

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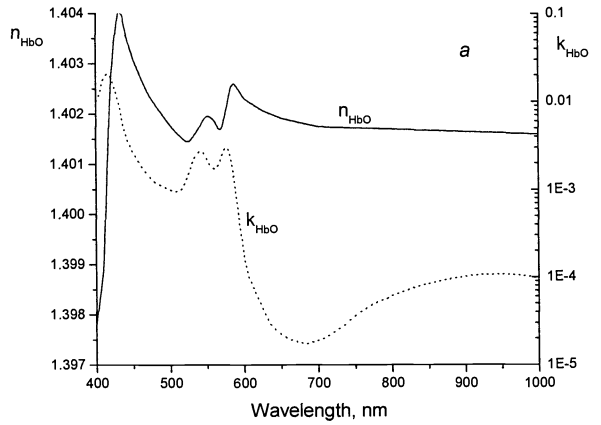
randomly distributed spherical equivalent size particles.[22-23] Therefore, for the calculations we used homogeneous spheres with the volume equal to the volume of real erythrocytes.

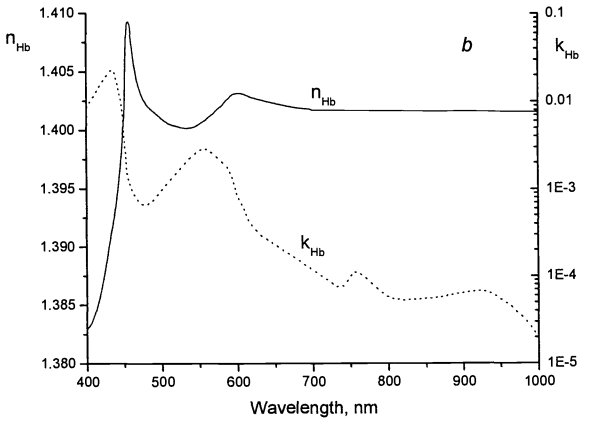
The polydispersity of blood erythrocytes has been taken into account on the basis of the data presented in Ref. 24. Table 1 shows the distribution of scattering particles used in the calculations. The total volume fraction of the blood erythrocytes (blood haematocrit) is 45 %, which corresponds to the value of haematocrit of adult man's venous blood.[13,25] The presence of the particles with the volume exceeding significantly the volume of real erythrocytes is connected with the aggregation of erythrocytes into clusters contributing into scattering spectrum of whole blood.[24]

Table 1

Radius of particles, μm	Volume fraction, %	Radius of particles, μm	Volume fraction, %
1.2 ± 0.2	1.8	2.7 ± 0.3	14.8
1.7 ± 0.3	6.5	3.4 ± 0.4	6.2
2.2 ± 0.2	13.3	4.3 ± 0.5	2.4

Figure 1(a,b) shows the complex refractive index of blood erythrocytes versus the wavelengths according to Refs.16 and 26. The hemoglobin concentration is 2.036 mmol/liter.





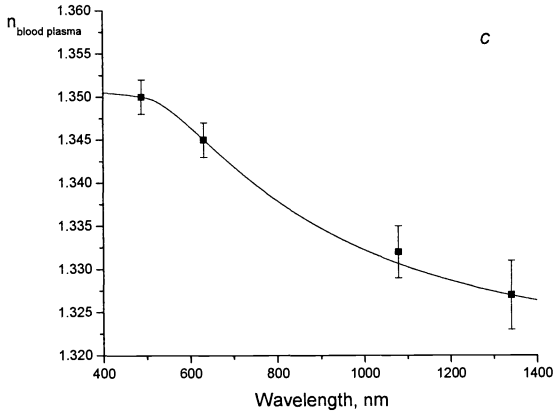


Fig.1. The spectral dependencies for:
(a) real and imaginary part of an erythrocyte. Oxygenation is 100%, the hemoglobin concentration is 2.036 mmol/liter.
(b) real and imaginary part of an erythrocyte. Non-oxygenated form, the hemoglobin concentration is 2.036 mmol/liter.
(c) real part of refractive index of human blood plasma

Blood plasma contains up to 91% of water, 6.5-8% (about 70 g/l) of various proteins, and about 2% of low molecular compounds. The spectral dependence of the refractive index of the human blood plasma in spectral range from 488 to 1341 nm is described by the equation[27]:

$$n_p = 1.3194 + \frac{1.4578\times10^{-2}}{\lambda^2} - \frac{1.7383\times10^{-3}}{\lambda^4}, \quad (1)$$

where λ is wavelength, μm. For the calculation, the spectral dependence of refractive index of blood plasma has been extrapolated into the spectral range from 400 to 1000 nm:

$$n_p = 1.3254 + \frac{8.4052\times10^{3}}{\lambda^2} - \frac{3.9572\times10^{8}}{\lambda^4} - \frac{2.3617\times10^{13}}{\lambda^6}, \quad (2)$$

where λ is wavelength, nm (Fig. 1(c)). Since blood plasma in the present spectral range does not have absorption bands, the imaginary part of refractive index of plasma has been neglected in our calculation.

In the framework of Mie theory, the expressions for the scattering and the absorption cross-sections can be written in a form[12]:

$$\sigma_s = \left(\frac{\lambda^2}{2\pi n_p^2}\right)\sum_{n=1}^{\infty}(2n+1)\left(|a_n|^2 + |b_n|^2\right), \quad (3)$$

$$\sigma_a = \left(\frac{\lambda^2}{2\pi n_p^2}\right)\sum_{n=1}^{\infty}(2n+1)\left[\mathrm{Re}\left(a_n + b_n\right) - \left(|a_n|^2 + |b_n|^2\right)\right], \quad (4)$$

$$g = \frac{\lambda^2}{\pi n_p^2 \sigma_s}\left[\sum_{n=1}^{\infty}\frac{n(n+2)}{n+1}\mathrm{Re}\left\{a_n a_{n+1}^* + b_n b_{n+1}^*\right\} + \sum_{n=1}^{\infty}\frac{2n+1}{n(n+1)}\mathrm{Re}\left\{a_n b_n^*\right\}\right], \quad (5)$$

where an asterisk indicates that the complex conjugate is taken; a_n and b_n are Mie coefficients, which are functions of the relative complex refractive index of particles (m) and parameter $x = 2\pi n_p a/\lambda_0$;

$$a_n = \frac{m\psi_n(mx)\psi_n'(x) - \psi_n(x)\psi_n'(mx)}{m\psi_n(mx)\xi_n'(x) - \xi_n(x)\psi_n'(mx)}, \quad (6)$$

$$b_n = \frac{\psi_n(mx)\psi_n'(x) - m\psi_n(x)\psi_n'(mx)}{\psi_n(mx)\xi_n'(x) - m\xi_n(x)\psi_n'(mx)}. \quad (7)$$

The relevant parameters are the size (radius a) and shape of the particles, their complex refractive index $n_e(\lambda_0) = n_e'(\lambda_0) + in_e''(\lambda_0)$, the refractive index of the dielectric host (ground material), $n_p(\lambda_0)$, and the relative refractive index of the scatterers and the ground materials, $m = n_e/n_p$; λ_0 is the wavelength in vacuum. The imaginary part of the complex refractive index of scatterer material is responsible for light losses due to absorption.

The absorption, scattering coefficients and the anisotropy factor g of close-packed polydisperse system of particles, such as the human whole blood, is described by[28]:

$$\mu_s = \sum_{i=1}^{M} W_{s_i} N_i \sigma_{s_i}, \quad (8)$$

$$\mu_a = \sum_{i=1}^{M} N_i \sigma_{a_i}, \tag{9}$$

$$g = \frac{\sum_{i=1}^{M} \mu_{s_i} g_i}{\sum_{i=1}^{M} \mu_{s_i}}, \tag{10}$$

where $W_{s_i} = (1 - C_i)(1.4 - C_i)$ is the packing factor of scatterers with diameter d_i [29], which account the interparticle correlation effects; C_i is the volume fraction of scatterers with diameter d_i; M is a number of particle diameters; $N_i = C_i / v_{ei}$ is a number of particles in a volume unit of medium; $v_{ei} = 4\pi a_i^3 / 3$ is the volume of individual erythrocyte.

3. MODELING OF OPTICAL IMMERSION OF BLOOD BY PENETRATION OF HEMOGLOBIN IN PLASMA

As it was mentioned above, the possibility of *in vivo* control of optical parameters of tissues and blood are of great importance for many applications of laser diagnostic and therapy. The sufficient decrease of light scattering of blood can be achieved by matching the refractive indices of scatterers and surrounding medium via inserting the corresponding immersion agent. Hemoglobin has been chosen as an example of an immersion agent. The refractive index of blood plasma at administration of the hemoglobin solution becomes close to refractive index of erythrocytes.

The refractive index of the aqueous hemoglobin solution depends on hemoglobin concentration. Linear dependence upon C is described by the equation[13,16,26]:

$$n_{Hb}(\lambda) = n_{water}(\lambda) + \alpha C, \tag{11}$$

$$k_{Hb}(\lambda) = \beta C, \tag{12}$$

where α and β are constants depending on the wavelengths, $n_{water}(\lambda)$ is the refractive index of water and C is the hemoglobin concentration, that was changing in our calculations from 0.05 g/ml to 0.5 g/ml. By analogy with equation (11), the refractive index of the hemoglobin solution in plasma was taken to be describe by the equation:

$$n_{Hb}(\lambda) = n_{pl}(\lambda) + \alpha C, \tag{13}$$

where $n_{pl}(\lambda)$ is the refractive index of blood plasma.

The calculations of the optical parameters of whole blood as normal (haematocrit is 45 %), as at administration of hemoglobin solution into plasma have been performed in the spectral range from 400 nm to 1000 nm. The absorption coefficient, the scattering coefficient and anisotropy factor have been calculated based on equations (8)-(10). The calculations of the scattering, absorption cross-sections and anisotropy factor of individual erythrocyte have been performed using equations (3)-(5). For the calculation, the spectral dependence of refractive index of erythrocytes has been taken from Ref. 26(Fig.1(a)), and the spectral dependence of refractive index of hemoglobin solution in plasma has been taken using equation (13). The refractive index of the blood plasma has been calculated based on equation (2).

3. RESULTS AND DISCUSSION

The scattering and absorption coefficients of blood have been determined by double integrating sphere measurements and inverse Monte Carlo simulation (Roggan *et al.* [13] and Yaroslavsky *et al.* [14]). For the testing our model, the

calculations of the absorption and the scattering coefficient of blood have been performed for the value of haematocrit equal to 5 %. Fig.2 shows calculated scattering (a) and absorption (b) coefficients of whole blood. Fig.2(a-b) shows good agreement between the scattering and absorption coefficients calculated by us and reported by Roggan *et al.* [13] and Yaroslavsky *et al.* [14] Comparison of calculated and experimental data shows that the presented model can be used for modeling of the optical parameters of blood in the investigated spectral range including optical immersion.

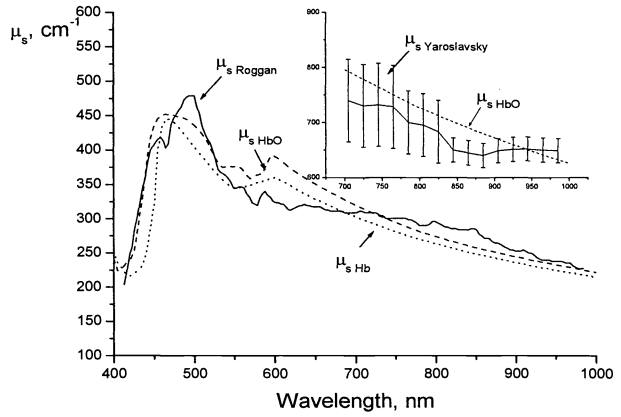


Fig.2(a) The calculated spectral dependencies for scattering coefficients.

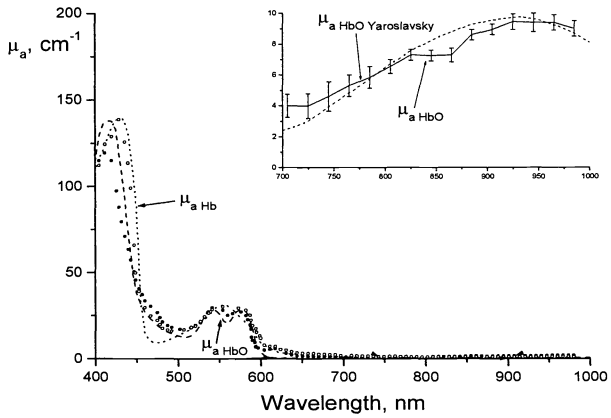


Fig.2 (b). The calculated spectral dependencies for absorption coefficient.

Figure 3 shows the spectral dependence of the absorption coefficient of the whole blood at administration of hemoglobin solution in plasma. It is clearly seen that hemoglobin solution changes insignificantly the absorption coefficient of blood, and this changes are localized mainly in the Soret band with maximum at 420 nm, the α - band with maximum at 545 nm and the β - band with maximum at 580 nm of oxihemoglobin absorption. At that, increasing concentration of the immersion agent leads to linear decreasing the absorption coefficient.

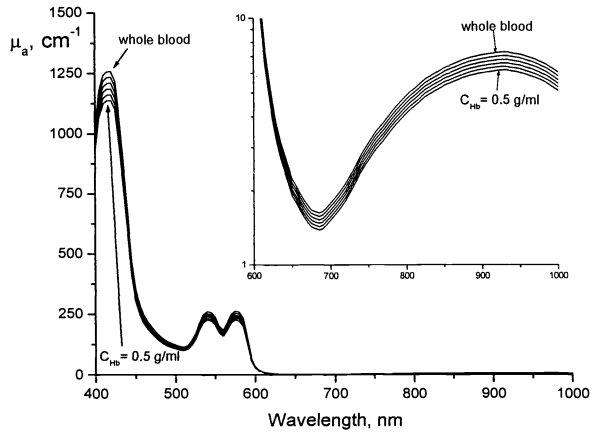


Fig.3 The absorption spectra of whole blood (Ht=45%) for optical clearing of blood by hemoglobin solution

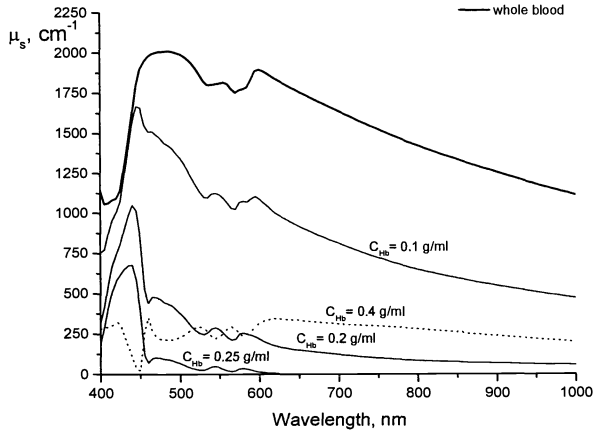


Fig.4 The scattering spectra of whole blood (Ht=45%) for optical clearing of blood by hemoglobin solution.

More significant changes of the scattering properties of blood have been observed at administration of the hemoglobin solution into plasma (Fig.4-6). Figure 4 shows the scattering spectra of blood for different concentration of the hemoglobin solution in plasma. The spectra shows that the depth of minimum of the scattering at wavelength 420 nm, 545 nm and 580 nm connected with the influence of the imaginary part of the refractive index of erythrocytes is decrease

with increasing of the concentration of the hemoglobin solution. The spectral dependence of the scattering coefficient becomes more monotonous. Generally, the scattering coefficient decreases up to the minimum at administration of the hemoglobin solution with concentration 0.25 g/ml. Further increase of concentration of the immersion agent leads to increasing of the scattering coefficient.

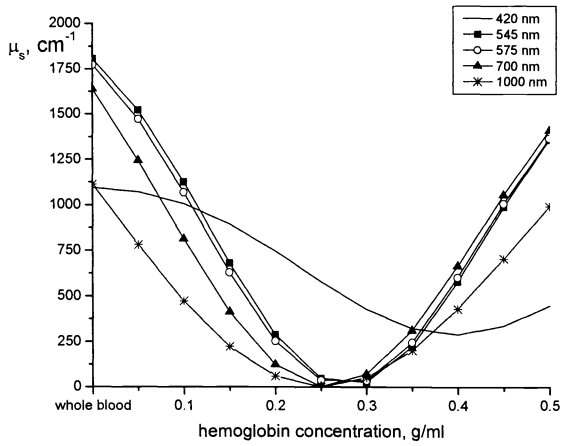


Fig.5 Scattering coefficient versus concentration of the hemoglobin solution in blood plasma

Figure 5 shows the scattering coefficient versus concentration of the hemoglobin solution in blood plasma. From Fig. 5 it is seen that decreasing of the scattering coefficient for different spectral bands is nonuniform. The most essential clearing is seen in the spectral range from 500 nm to1000 nm at administration of the hemoglobin solution 0.25 g/ml, while in the Soret band (420 nm) the maximum is achieved at concentration of hemoglobin solution 0.4 g/ml. This is due to the fact that the absorption bands of the immersion agent (hemoglobin solution) and the erythrocytes coincide.

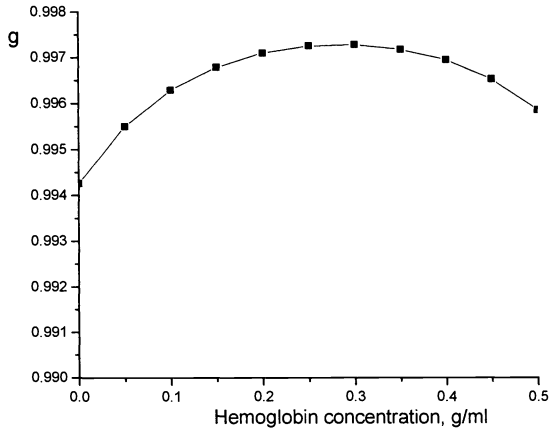


Fig.6. Anisotropy factor of blood as function of hemoglobin concentration in plasma; λ=630 nm

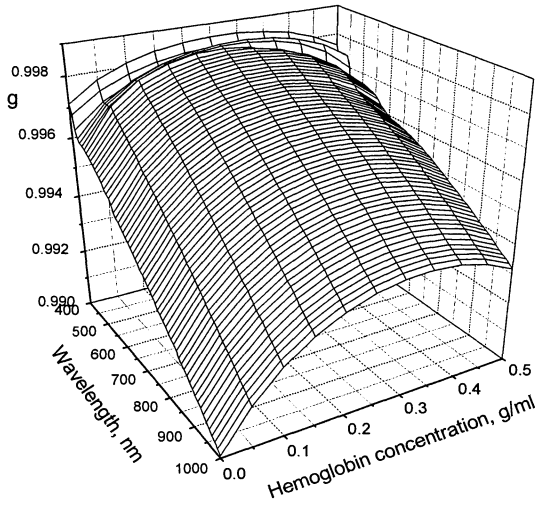


Fig.7 Anisotropy factor of blood versus the wavelength and concentration of the hemoglobin solution in plasma.

Figures 6 and 7 shows the anisotropy factor versus the wavelength and concentration of the hemoglobin solution in blood plasma. It is seen, that with increasing of wavelength the value of the anisotropy factor is decreasing insignificantly. At that, the increasing concentration of the hemoglobin solution to 0.3 g/ml leads to increasing of the

anisotropy factor. This clearing action of the hemoglobin solution is very important to provide optical diagnostics and therapeutic techniques, because it makes possible to increase of depth of the probing. As in the previous case, the concentration of hemoglobin solution equal to 0.25-0.30 g/ml is optimal, and further increasing of the concentration of hemoglobin solution leads to decreasing of the anisotropy factor.

4. CONCLUSIONS

The possibility of immersion optical clearing of blood by addition of hemoglobin solution to a whole blood was considered theoretically on the basis of Mie theory accounting for polydispersity of whole blood.

It is shown that the administration of the hemoglobin solution in plasma allows one to control the absorption and scattering properties of blood in wide spectral range. The efficiency of such control is based on matching the refractive indices of scatterers (erythrocytes) and surrounding medium (plasma) of blood. From the point of view of matching the refraction indices and controlling the scattering properties of the medium the results presented here are general for numerous scattering biological objects.

The optical model of blood presented shows good agreement with well-known experimental data and allows one to model the variation of the absorption and scattering characteristics of blood under the administration of immersion agent, like hemoglobin.

Spectral behavior of the absorption and scattering characteristic of blood under immersion clearing using hemoglobin solution is analyzed. It is shown, that the optimal concentration of the hemoglobin solution in plasma is equal to 0.25 g/ml.

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