Theoretical study of immersion optical clearing of blood in vessels at local hemolysis

Valery V. Tuchin, Dmitry M. Zhestkov, Alexey N. Bashkatov, and Elina A. Genina

Division of Optics, Department of Physics, Saratov State University
83 Astrahanskaya str., Saratov, 410012 Russia
tuchin@sgu.ru

Abstract: Mie based theoretical analysis has been performed for investigation of the possibility of application of the plasma hemoglobin releasing due to local hemolysis for optical clearing of blood. The 30-40% reduction of the scattering coefficient of blood in the spectral range from 400 to 1000 nm with increase of degree of hemolysis (up to 20%) was shown. At the same time, the reduction of absorption coefficient of blood is localized mainly within the Soret band with maximum at 415 nm (~15%), the α-band at 540 nm and the β-band at 577 nm (~10%) of oxyhemoglobin. In the spectral range from 700 to 1000 nm the decrease of absorption coefficient is less than 8%.

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References and links

1. M. Brezinski, K. Saunders, C. Jesser, X. Li, J. Fujimoto, "Index matching to improve optical coherence tomography imaging through blood," Circulation **10**, 1999-2003 (2001).
2. V.V. Tuchin, *Tissue Optics: Light Scattering Methods and Instruments for Medical Diagnosis* (SPIE Press, TT38, Bellingham, Washington, 2000)
3. V.V. Tuchin (ed.), *Handbook of Optical Biomedical Diagnostics* (**PM107**, SPIE Press, Bellingham, WA, 2002).
4. Tuan Vo-Dinh (ed.), *Biomedical Photonics Handbook* (CRC Press, Boca Raton, 2003).
5. B.E. Bouma and G.J. Tearney (eds.), *Handbook of Optical Coherence Tomography* (Marcel-Dekker, New York, 2002).
6. V.V. Tuchin, X. Xu, R. K. Wang, "Dynamic optical coherence tomography in studies of optical clearing, sedimentation, and aggregation of immersed blood," Appl. Opt. **47**, 258-271 (2002).
7. X. Xu, R.K. Wang, J.B. Elder, V.V. Tuchin, "Effect of dextran-induced changes in refractive index and aggregation on optical properties of whole blood," Phys. Med. Biol. **48**, 1205-1221 (2003).
8. A. Roggan, M. Friebel, K. Dorschel, A. Hahn, and G. Muller, "Optical Properties of Circulating Human Blood in the Wavelength Range 400-2500 nm," J. Biomed. Opt. **4**, 36-43 (1999).
9. D.M. Zhestkov, A.N. Bashkatov, E.A. Genina, V.V. Tuchin, "Influence of clearing solutions osmolarity on the optical properties of RBC," Proc. SPIE **5474**, (2004 to be published).
10. A.G. Borovoi, E.I. Naats, and U.G. Oppel,"Scattering of Light by a Red Blood Cell," J. Biomed. Opt. **3**, 364-372 (1998).
11. D.H. Tycko, M.G. Metz, E.A. Epstein, A. Grinbaum, "Flow cytometric light-scattering measurement of red cell volume and hemoglobin concentration," Appl. Opt. **24**, 1355-1365 (1985).
12. N.G. Khlebtsov, S.Yu. Shchyogolev, "Account of particle nonsphericity at determination of parameters of dispersion systems by a turbidity spectrum method. 1. Characteristic functions of light scattering by nonspherical particle systems in Rayleigh-Gans approximation," Opt. Spectrosc. **42**, 956-962 (1977)
13. A.Ya. Khairullina and S.F. Shumilina, "Determination of the distribution function of erythrocytes according to size by the spectral transparency method," J. Appl. Spectrosc. **19,** 1078-1083 (1973).
14. J.M. Steinke, A.P. Shepherd, "Diffusion model of the optical absorbance of whole blood," J. Opt. Soc. Am. A. **5**, 813-822 (1988).
15. J.M. Schmitt, G. Kumar, "Optical scattering properties of soft tissue: a discrete particle model," Appl. Opt. **37,** 2788-2797 (1998).
16. C. Bohren, D. Huffman, *Absorption and Scattering of Light by Small Particles* (John Wiley & Sons, New York, 1983).
17. S. Cheng, H.Y. Shen, G. Zhang, C.H. Huang, X.J. Huang, "Measurement of the refractive index of biotissue at four laser wavelengths," in *Optics in Health Care and Biomedical Optics: Diagnostics and Treatment*, B. Chance, M. Chen and G. Yoon , eds., Proc. SPIE **4916,** 172-176 (2002).

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1. Introduction

Myocardial infarction is the leading cause of death in the industrialized world. Most myocardial infarction results from the rupture of small, thin-walled plaques in the coronary arteries. Most of these plaques are below the detection limit of currently available imaging technologies. Therefore, a true clinical need exists for an imaging technology capable of identifying these plaques [1].

Over the last decade, non-invasive imaging techniques have witnessed widespread and exciting application in biomedical diagnostics, for example, optical coherence tomography (OCT), back reflectance spectroscopy, fluorescence spectroscopy, etc. [2-5].

OCT has demonstrated considerable potential as a method for static and dynamic imaging of various kinds of tissue and blood [1-7]. *In vitro* studies performed on the human aorta have shown that OCT is able to identify structural features such as lipid collections, thin intimal caps, and fissures that are characteristic of vulnerability to plaques [1]. In *in vitro* OCT imaging of the rabbit aorta through a catheter, a vascular structure was defined, but saline infusion was required during imaging because blood led to significant attenuation of imaging.

Light attenuation in blood results from both scattering and absorption. OCT imaging in blood is typically performed at 820 nm or 1300 nm, where absorption is low [1-7].

Normal human whole blood is a scattering system that consists of about 45 vol% of scattering particles (99% red blood cells (RBC or "erythrocytes"), 1% leukocytes and thrombocytes) and the about 55 vol % plasma [1,3,8]. The refractive index mismatch between erythrocyte cytoplasm and blood plasma is the major source of light scattering in blood that dramatically limits the light penetration depth. The scattering properties of blood depend on erythrocytes volume, shape, oxygenation, and hematocrit. Under normal physiological condition hematocrit ranges from 36.8% to 49.2% [8]. Propagation of light in such medium can be studied within the model of light scattering and absorption by an individual particle taking into account the interparticle correlation effects and polydispersity.

Recently, dextrans [1,7] and intravenous contrast agent [1,6] have been used to increase the refractive index of plasma to achieve an index matching with RBC that led to significant improvement in light penetration through circulating or steady-state blood measured by OCT. Similar effects of increase in transmittance and decrease in scattering were demonstrated by use of glucose, glycerol, and propylene glycol at various concentrations [6].

It should be noted that the blood plasma osmolarity is also an important factor in changes in the scattering properties of blood [6,8-9]. The effects of glucose, glycerol, trazograph, and propylene glycol, which are hyperosmotic agents, led to significant change of plasma osmolarity [6]. The change in osmolarity induces a variation of the RBC volume due to water exchange and therefore has an impact on the hemoglobin concentration within the RBC and consequently on their refractive index. Our previous work also demonstrated that glucose solution with concentration less than 20% led to increase of blood scattering due to the osmotic dehydration of erythrocytes [9]. The significant optical clearing was obtained at glucose concentration higher than 40%, but such concentration can cause erythrocytes aggregation [6].

In the present study, the feasibility of index matching caused by a local hemolysis with production of some amount of free hemoglobin in the vicinity of optical head of endoscopic OCT or other endoscopy-spectroscopic system is demonstrated for the potential improvement of images of vessel wall lesions due to increase of light penetration through blood. The local hemolysis can be obtained by intravenous injection of an hypoosmotic solution, for example, deionized water. Hemolysis is accompanied by the damage of RBC membrane by mechanical or chemical impact with consecutive hemoglobin into the plasma. Local increase of hemoglobin concentration, with refractive index greater than that of the plasma would decrease the difference in refractive indices between the scatterers (erythrocytes, leukocytes and thrombocytes) and their environment (blood plasma). This refractive index matching is

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accompanied by decrease of scattering coefficient and increase of anisotropy factor of blood.

2. Modeling of optical immersion of blood due to hemolysis

The real $n_{Hb}(\lambda)$ and imaginary $\chi_{Hb}(\lambda)$ part of refractive index of the aqueous hemoglobin solution depends on hemoglobin concentration. Linear dependence upon C is described by the equation [8, 10]

$$n_{Hb}(\lambda) = n_{water}(\lambda) + \alpha C \,, \tag{1}$$

$$\chi_{Hb}(\lambda) = \beta C \,, \tag{2}$$

where α and β are constants depending on the wavelength, $n_{water}(\lambda)$ is the refractive index of water and C is the hemoglobin concentration in grams per 100 milliliters. By analogy with equation (1), the real part of refractive index of the hemoglobin solution in plasma was taken as to describe by the equation:

$$n_{p}^{Hb}(\lambda) = n_{pl}(\lambda) + \alpha C \,, \tag{3}$$

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

The local blood hemolysis is accompanied with decrease of local blood hematocrit. To provide the level of blood hematocrit for the whole body not lower than physiological values the level of hemolysis should not be higher than 15-20%. At the same time according to the fact that blood volume in the body of adult human is equal to 5000 см^3, the local blood hemolysis in the measurement volume of 1 см^3 results in change of total blood hemolysis not higher then 0.02%. This value is in the limits of physiological fluctuations of hemolysis.

Table 1 shows the calculated values of local changes of blood hematocrit and hemoglobin concentration in plasma at increase of blood hemolysis percentage. The hemoglobin concentration within an erythrocyte under physiological condition is 33 g/dl [11].

Table 1. The calculated values of local blood hematocrit and hemoglobin concentration in plasma at different degree of hemolysis.

Local blood hemolysis, %	Local blood hematocrit, %	Local hemoglobin concentration in plasma, g/dl
0	42	0
5	39.9	1.15
10	37.8	2.23
15	35.7	3.23
20	33.6	4.91

The local increase of hemoglobin concentration in plasma can lead to local change of plasma osmolarity [9]:

$$osm'_{pl} = osm_{pl} + \frac{C_{FHb}}{M_{Hb}} \,, \tag{4}$$

where osm_{pl} is plasma osmolarity under physiological condition (280-300 mOsm/l), C_{FHb} is concentration of plasma hemoglobin, g/l, M_{Hb} - molar mass of hemoglobin (M_{Hb} = 66500 g/M).

Based on the data for erythrocyte volume change [8], the phenomenological equation has been suggested [9]:

$$V(osm) = V_0\left(0.515 + 1.177\exp\left(-osm/337\right)\right) , \tag{5}$$

where osm is the blood osmolarity, mOsm/l, V is the erythrocyte volume versus the osmolarity, and V_0 is the erythrocyte volume at the osmolarity 300 mOsm/l. The calculations of osmolarity and volume change show that in average erythrocyte volume is 0.1% increased with increase of hemolysis up to 20%.

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The phase function and scattering cross section of individual erythrocyte depend on its orientation [10]. However, light scattering characteristics of a large number of randomly distributed non-spherical particles is very close to light scattering characteristics of a system of randomly distributed spherical particles with the equal volume [8,12]. Therefore, for simplicity, calculations were done for a model of homogeneous spheres with the volume equal to the volume of real erythrocytes. The polydispersity of RBC has been taken into account on the basis of the data presented in Table 2. The total volume fraction of RBC (blood haematocrit) was taken as 42% [2].

Table 2. Size distribution of spherical particles modeling RBC[a]

Radius of particles, μm	Volume fraction, %	Radius of particles, μm	Volume fraction, %
1.2 ± 0.2	4	2.7 ± 0.3	32
1.7 ± 0.3	14	3.4 ± 0.4	14
2.2 ± 0.2	30	4.3 ± 0.5	6

[a]From J. Pricl. Spectr. **19**, 340-347 (1973). [13]

Calculations of the absorption coefficient, the scattering coefficient and anisotropy factor of whole blood at normal conditions and at local hemolysis have been performed using equations [9,14]:

$$\mu_a = \sum_{i=1}^{M} N_i \sigma_{a_i}\ ; \quad \mu_s = W_s \sum_{i=0}^{M} N_i \sigma_{s_i}\ ; \quad g = \sum_{i=1}^{M} \mu_{s_i} g_i \Big/ \sum_{i=1}^{M} \mu_{s_i}\ ,$$

where $W_s=(1-H)(1.4-H)$ is the packing factor of scatterers [15], which accounts for the interparticle correlation effects; H is the hematocrit; M is the number of particle diameters; $N_i=C_i/v_{ei}$ is the number of particles in a volume unit of medium; C_i is the volume fraction of scatterers with radius a_i (see Tab. 1); $v_{ei}=4\pi a_i^3/3$ is the volume of an individual erythrocyte. The calculations of the scattering and absorption cross sections and anisotropy factor of individual erythrocyte have been performed using Mie theory [16].

The spectral dependence of the real part of the refractive index of RBC under physiological condition has been taken according to the data presented in Ref. [10] for blood oxygenation equal to 100%. The spectral dependence of refractive index of blood plasma was calculated according to [9,17]:

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

where λ is the wavelength in nm.

3. Results and discussion

Figure 1 shows the spectral dependence of the absorption coefficient of the whole blood at increase of hemoglobin in blood plasma. As seen from the figure, the changes (reduction) of the absorption coefficient of blood are stronger in the vicinity of the absorption bands: the Soret band with maximum at 415 nm, the α-band at 540 nm, and the β-band at 577 nm. The modification of absorption spectrum is caused by the change of relative refractive index of RBC $m=n_e/n_p+i\chi_e/n_p$. At increase of hemoglobin in plasma this equation has to be rewritten, taking into account (3), as:

$$m_{im} = \frac{n_e n'_p + \chi_e \beta C_{FHb}}{n'^2_p + \beta^2 C^2_{FHb}} + i\frac{\chi_e n'_p - n_e \beta C_{FHb}}{n'^2_p + \beta^2 C^2_{FHb}}, \tag{6}$$

where $n'_p = n_p + \alpha C_{FHb}$ is the real part of the refractive index of hemoglobin in plasma; values of C_{FHb} are given in Table1.

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In the imaginary part of the relative refractive index of RBC the value of n'_p is close to n_e, however χ_e is significantly greater than βC_{FHb} (for degree of hemolysis equal to 15%, in 10 folds). Therefore, the numerator of the imaginary part of the Eq. (6) changes insignificantly. At the same time, the denominator increases more significantly due to both real and imaginary parts of the refractive index of hemoglobin in plasma. So, the appearance of free hemoglobin in plasma decreases the relative imaginary part of the refractive index of RBC that leads to decrease of absorption coefficient of blood.

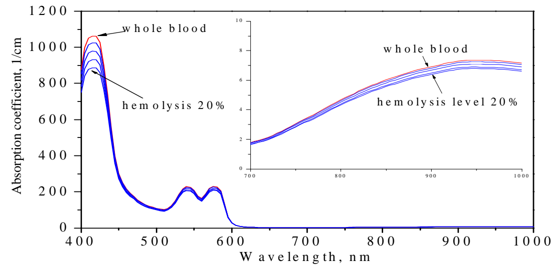

Fig. 1. The absorption spectra at different degree of whole blood hemolysis.

In contrast with the change of absorption coefficient, more significant changes of the scattering properties of blood have been observed at increase of free hemoglobin concentration in plasma (Figs. 2 and 3). Figure 2 shows the evolution of scattering spectra of blood at different degree of hemolysis. The spectra show, that as a whole, the scattering coefficient in the investigated spectral range decreases with increase of the concentration of free hemoglobin in plasma. The scattering coefficient decreases non-uniformly in different spectral ranges. At spectral range from 400 to 500 nm it goes down to about 30% with raise of degree of hemolysis up to 20%. At the same time, in the range from 500 to 1000 nm 40% decrease is seen. Observed spectral difference is connected with behavior of the imaginary part of hemoglobin refractive index. According to Eq. (6), the imaginary part of hemoglobin refractive index belongs to both numerator and denominator of the real part of this equation. One can conclude that in spectral range with significant absorption of hemoglobin, the imaginary part of hemoglobin refractive index influences the real part of erythrocyte relative refractive index significantly and decreases the immersion action of plasma hemoglobin.

Figure 3 (red lines) shows the scattering coefficient versus the local hemolysis rate in blood. It is seen, that the increase of the hemoglobin concentration in blood plasma results in significant decrease of the blood scattering coefficient at the wavelengths 633 and 820 nm. The blood hemolysis degree of approximately 10% allows one to reduce the scattering coefficient up to 15%. With increasing of blood hemolysis degree up to 20% the scattering coefficient decreases up to 40%.

It should be noted that with the change of hematocrit from the 33% to 42% the scattering coefficient changes insignificantly [8], thus one can conclude that decrease of scattering coefficient is mainly caused by the immersion action of plasma hemoglobin.

Figure 3 (blue lines) shows the anisotropy factor versus the hemolysis rate of blood. It is seen, that increase of the hemoglobin concentration in blood plasma causes a slight increase of anisotropy factor, from 0.9940 to 0.9952 at the wavelength 633 nm, and from 0.9919 to 0.9929 at 820 nm, while degree of hemolysis increases up to 20%. Thus, besides strong reduction of scattering free hemoglobin local immersion directness of single scattering is also improved, both effects are important for coherent methods of diagnostics, especially for OCT.

In general, this optical clearing technology at local action of free hemoglobin in blood plasma is of great importance for a variety of optical diagnostic and therapeutic techniques, because it makes possible to increase the depth of the optical probing.

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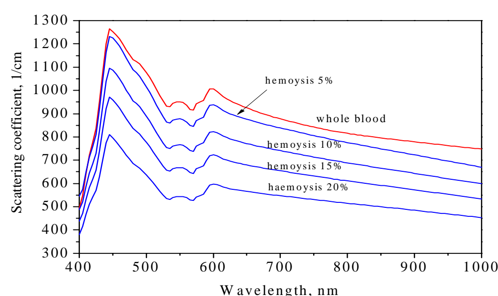

Fig. 2. The change of scattering spectrum at different degree of blood hemolysis

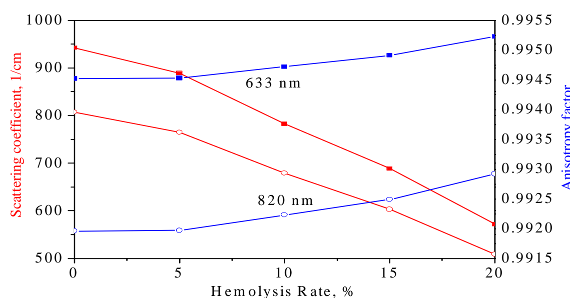

Fig. 3. Scattering coefficient (red) and anisotropy factor (blue) versus the degree of blood hemolysis.

4. Summary

Mie based theoretical analysis has been performed to study the possibility of application of plasma hemoglobin releasing due to local hemolysis for optical clearing of blood.

It is shown that the local increase of hemoglobin concentration in blood plasma allows one to control the absorption and scattering properties of blood in a wide spectral range. The efficiency of such control is caused by the high refractive index of hemoglobin, which provides effective matching of the refractive indices of scatterers (blood erythrocytes) and surrounding medium (blood plasma) at rather small increase of its concentration in plasma.

Based on the presented model the spectral behavior of the absorption and scattering properties of blood under action of hemoglobin has been analyzed. It was shown that the scattering coefficient of blood in the spectral range from 400 nm to 500 nm decreases to about 30% and in the spectral range from 500 nm to 1000 nm up to 40% with increase of degree of hemolysis up to 20%. At the same time, 10-15% - reduction of absorption coefficient is localized mainly within the Soret band (415 nm) and α- and β-bands (540 and 577 nm) of oxyhemoglobin; in the spectral range from 700 to 1000 nm the decrease is less than 8%.

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