Effect of hemoglobin localization in erythrocytes on optical absorption by human blood

Vladimir V. Barun and Arkady P. Ivanov

B.I. Stepanov Institute of Physics, National Academy of Sciences of Belarus
68 Nezavisimosti Pr., Minsk 220072, Belarus
Tel: +375(17)284-0796, fax: +375(17)284-0879; e-mail barun@dragon.bas-net.by

Abstract

Hemoglobin localization in erythrocytes is shown to lead to a decrease of their apparent absorption coefficient in the blue spectral range. This effect is quantitatively evaluated by using analytical relations. The replacement of erythrocytes by equivalent spheres is considered to simplify the final calculation formulas. The spheres with the same volume as that of erythrocytes is shown can be used for single red blood cells or for their rouleau with small number of erythrocytes. For rather a long rouleau, the spheres with the same ratio of their volume to surface are applicable. The asymptotic behavior of the absorption decrease and its influence on absorption coefficient of skin dermis are studied.

1 Introduction

There are known and widely used a lot of procedures to study human blood by optical means. Among them are diffuse reflectance and transmittance spectroscopy [1], time- and frequency-domain investigations [1], optical measurements of blood samples in a cell [2], etc. The two first procedures are usually used under multiple light scattering by *in vivo* tissues. The latter one is applicable for both multiple and single scattering conditions. All the above procedures are highly affected by optical blood absorption coefficient that itself is often a subject of the studies to indicate various blood pathologies. The main absorbing components of blood are well known to be hemoglobins in different forms, the major of which are oxy- and deoxyhemoglobins. Their optical absorption is reliably referenced [2 – 4]. How does hemoglobin, which is localized in erythrocytes, affect the optical absorption coefficient of blood? At the first glance, one could average the hemoglobin absorption over a blood unit volume according to its volume fraction $f_{\mathrm{H}}H$ in blood, where f_{H} is the volume fraction of hemoglobin in an erythrocyte and H is the hematocrit (volume fraction of erythrocytes in blood). Let $f_{\mathrm{H}} = 0.25$ and $H = 0.4$ [1] below. However, such an averaging is not correct always. Really, let, for a moment, hemoglobin have infinitely large absorption. Then the averaged absorption coefficient will be infinitely large too. It is clear, however, that some light portion will pass through "holes" that do not contain any absorbing substance. So averaging weight $f_{\mathrm{H}}H$ is not applicable here. What will be the consequences of using another weight for calculating the absorption coefficient of a biological tissue as a whole? The paper will answer these questions.

The effect of light transmission through the above "holes" is sometimes called by the "sieve" effect [5]. We proposed earlier [6] an analytical procedure to treat the localized absorption of light by chaotically or regularly oriented cylindrical capillaries. Hemoglobin absorption was uniformly spread over blood volume there. On the other hand, the "sieve" effect of hemoglobin in erythrocytes was already evaluated [5] by replacing an erythrocyte with a spherical particle. It is shown that the hemoglobin localization leads effectively to some decrease in the volume fraction of the absorbing substance. The effect is physically understood, because internal portions of an erythrocyte, where light comes highly attenuated, participate in the absorption to a less degree that the periphery. The decrease has the clearest manifestation in the blue spectral range or in the Soret band, where blood absorbs light strongly. Meanwhile, an erythrocyte is a disk-shaped particle, not a spherical one as is validly mentioned in [5]. How one can replace an erythrocyte by an equivalent sphere? What will be its radius? How does erythrocyte aggregation in a rouleau affect the absorption? These topics are considered in the paper.

2 Calculation scheme

Let an erythrocyte be a disk with diameter D_e = 8.8 μm and thickness L = 2 μm [1, 7]. Although an erythrocyte is really a concavo-concave disk, we will approximate it by a cylinder with flat bases for the estimations below. Absorption coefficient $\mu_{a,e}$ of erythrocytes occupying volume fraction H is

$$\mu_{a,e} = C_e(\mu_H) H \mu_H = C_e(\mu_H) H f_H [S\mu_{HbO} + (1-S)\mu_{Hb}], \quad (1)$$

where C_e is the correction factor due to the hemoglobin localization; μ_H is the absorption coefficient of hemoglobins uniformly spread over an erythrocyte volume; S is the blood oxygenation degree (fraction of oxyhemoglobin with respect to total hemoglobin); μ_{HbO} and μ_{Hb} are the oxy- and deoxyhemoglobin absorption coefficients, respectively. Our aim is to find the correction factor for chaotically oriented erythrocytes. The following analytical relation [6] will be used for this purpose:

$$C_e(\mu_H) = \frac{0.25\pi L_{ag} + 0.5a_{ef} - \int_0^{\pi/2} \sin\vartheta d\vartheta \int_0^{L_{ag}\sin\vartheta + a_{ef}\cos\vartheta} \tau(x,\vartheta) dx}{0.25\mu_H L_{ag} \pi D_e}, \quad (2)$$

where $L_{ag} = NL$ is the length of an erythrocyte rouleau, N is the number of erythrocytes in the aggregate,

$$a_{ef} = (1/\mu_H) \ln[\int_0^1 \exp(-\mu_H D_e \sqrt{1-x^2}) dx], \quad (3)$$

$\tau(x,\vartheta)$ is the transmission coefficient of erythrocytes with their generatrix (of length L_{ag}) set at angle ϑ to light propagation direction. While deriving Eq.(3), an erythrocyte disk or cylinder was replaced by a parallelepiped with the same generatrix L_{ag} and two bases with sides a_{ef} and D_e. Its light transmission is strictly the same as that of the cylinder [6]. The first two terms in the numerator and integration of Eq.(2) correspond to the averaged light transmission over all possible erythrocyte orientations. The integral over x of Eq.(2) can be calculated analytically [6]. We do not give its explicit, but cumbersome form here.

We will also consider the correction factor for equivalent spheres of two kinds. The first one is a sphere with the same volume as an erythrocyte. Its diameter is $D_s = [1.5(D_e)^2 L]^{1/3}$. Another sphere kind has the same ratio of its volume to its surface as that of an erythrocyte. In this case, the sphere diameter is $D_s = 1.5D_e$. The light transmission coefficient and the correction factor for a sphere are, respectively, $T_s(\mu_H) = [1-(1+D_s\mu_H)\exp(-D_s\mu_H)]/[0.5(D_s\mu_H)^2]$ and $C_s(\mu_H) = 1.5[1 - T_s(\mu_H)]/(D_s\mu_H)$.

3 Sample results

Figure 1 shows correction factors C_e (solid lines) or C_s (marks) as a function of optical diameter $\mu_H D_e$ of the erythrocyte base. Points on the abscissa axis indicate wavelengths, where the corresponding values of $\mu_H D_e$ occur. Different curves (1 to 4) give the C_e values for N = 1, 2, 8, and ∞, respectively. One can see that, due to the "sieve" effect, the decrease in the apparent absorption coefficient of erythrocytes can be up to about 3 times within the real range of $\mu_H D_e$ variations. The data of Fig.1 answer the question on possible approximation of erythrocyte aggregates by equivalent spherical particles. When number N of erythrocytes in a rouleau is small (N = 1 ÷ 4), the aggregate can be represented by a sphere with the same volume (■ and ▲). When N > 8 (+), one can use the spheres with the same ratio of their volume to surface for calculating the correction factor. The more erythrocytes are in an aggregate (rouleau), the more pronounced is the "sieve" effect.

As one can see from Fig.1, absorption coefficient $\mu_{a,e}$ depends on how many erythrocytes form an aggregate. This dependence is explicitly shown in Fig.2. The more erythrocytes are in a rouleau, the smaller the correction factor and, hence, the smaller the absorption coefficient. The problem on the influence of erythrocyte aggregation on blood optical characteristics has been studied in [7] too. However, wavelength λ = 632 nm selected for the investigations there shows no "sieve" effect (C_e = 1), as follows from Figs.1 and 2 (see also Fig.4 below). So the dependence of C_e on N was omitted in [7].

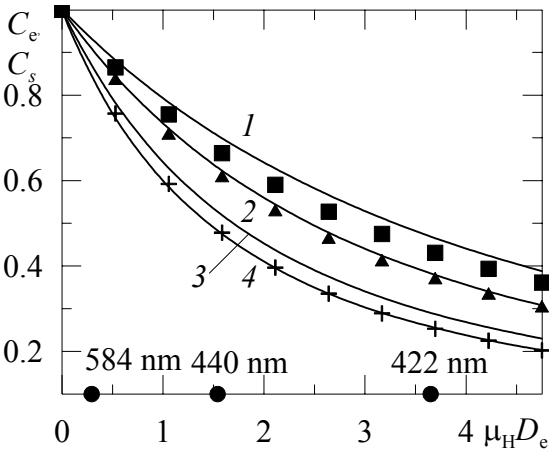

Figure 1: Correction factor as a function of $\mu_H D_e$ for single erythrocyte (curve 1), rouleau of two (2) and eight (3) erythrocytes, and for $L_{ag} = \infty$. Marks ■ ($N = 1$) and ▲ ($N = 2$) correspond to spheres of the same volume, **+'s** correspond to spheres with the same ratio of their volume to surface.

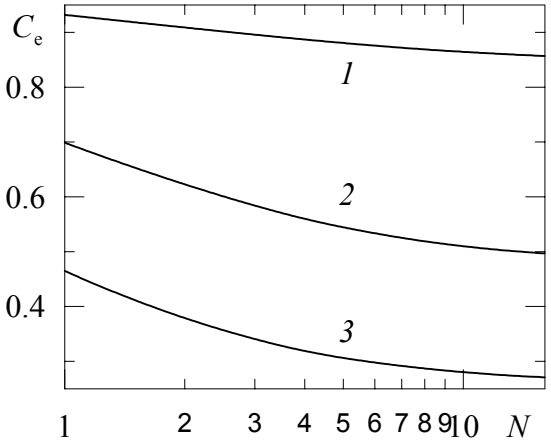

Figure 2: Correction factor as a function of erythrocyte number N in a rouleau for $\mu_H D_e = 0.29$ (curve 1), 1.5 (2), and 3.7 (3).

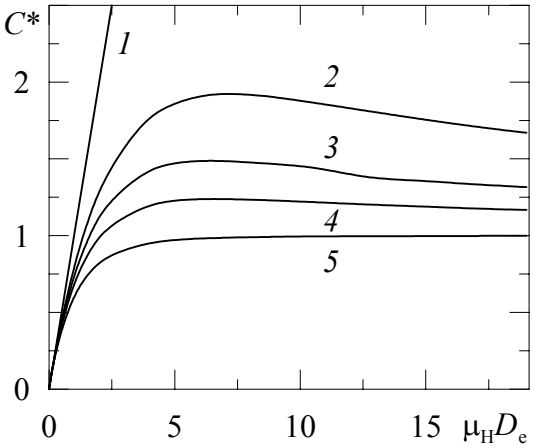

Figure 3: Normalized correction factor C^* as a function of $\mu_H D_e$ for uniform distribution of absorbing substance (curve 1), single erythrocyte (2), aggregates of two (3) and four (4) erythrocytes (4), and for $L_{ag} = \infty$ (5).

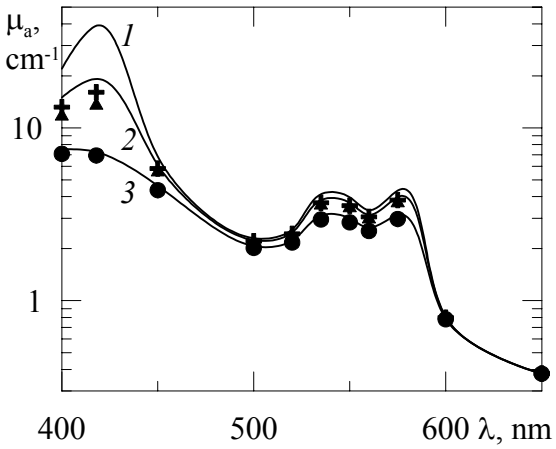

Figure 4: Absorption spectra of dermis without (curve 1) and with "sieve" effect by capillaries only (2 and 3, $D_c = 10$ and 40 μm, respectively). Marks show the effect of erythrocyte aggregates additional to that of capillaries for $N = 1$, $D_c = 10$ μm (+) and for $N = 8$, $D_c = 10$ (▲) and 40 μm (●).

It follows from Eqs.(2) and (3) that product $C_e \mu_H D_e \to 1$ as $\mu_H D_e$ or simply μ_H (D_e is fixed) $\to \infty$. Although the optical diameter of an erythrocyte cannot be infinitely large in the optical wavelength range, it is interesting to study the asymptotic behavior of the correction factor. Fig.3 shows the dependence of normalized correction factor $C^* = C_e \mu_H D_e$ on $\mu_H D_e$ for a single erythrocyte and its aggregation. Note first that for usual uniform distribution of hemoglobins over blood volume one has the linear dependence (curve 1). This corresponds to the weakly absorbing substance for $\lambda > 600$ nm. With increasing optical erythrocyte diameter, one observes deviations from the linearity that leads to the decrease in the blood absorption coefficient as compared to the homogeneous distribution of hemoglobins over blood volume. It is worthwhile to note the non-monotonic behavior (with a maximum) of the curves for single erythrocytes (curve 2) or for rouleaux with small number N (curves 3 and 4). The more number of erythrocytes in a rouleau, the less pronounced is the maximum. It disappears for large enough N (curve 5).

The maximum can be explained by follows. As μ_H increases, a_{ef} and $t(x,J)$ are obviously to decrease. So the competition between these two terms entering Eq.(2) with opposite signs provides the maximum.

How does the hemoglobin localization in erythrocytes affect the optical absorption of biotissue? The absorption spectra of dermis containing blood vessels are illustrated in Fig.4 without and with accounting for the "sieve" effect by both the capillaries and erythrocytes. Eqs.(2) and (3) with $L_{ag} = \infty$ and D_e replaced with vessel diameter D_c were used for the calculations of the correction factor for capillaries. The absorption coefficient of dermis is

$$m_a = C_c(m_{a,e})f_c m_{a,e} + (1 - f_c)m_{a,t}, \quad (4)$$

where $f_c = 0.02$ is the volume fraction of blood vessels in dermis and $\mu_{a,t}$ is the absorption coefficient of bloodless tissue. One can see from Fig.4 that the "sieve" effect of the vessels only gives rise to a considerable decrease in μ_a of the blue spectral range (up to 5 times). As for erythrocytes too, the larger the optical diameter $\mu_{a,e}D_c$ of a vessel, the more pronounced the effect is. Additional accounting for the hemoglobin localization in erythrocytes leads to a further decrease in the absorption coefficient in the blue for small capillary diameter ($D_c = 10$ μm, + and ▲) and is practically inessential for large vessels ($D_c = 40$ μm, ●). Such a behavior is physically transparent. Really, the "sieve" effect by erythrocytes decreases absorption coefficient $\mu_{a,e}$ of blood inside a vessel. In its own turn, this makes the "sieve" effect by capillaries less significant. In other words, the two effects somewhat compensate each other for large vessels. The compensation for small capillaries occurs to a less degree.

4 Conclusion

The decrease in absorption coefficient $\mu_{a,e}$ illustrated in Figs.1 and 2 can be observed for a suspension of intact erythrocytes in a cell. This effect opens a new opportunity to essentially detect the erythrocyte aggregation degree in a blood sample by optical means. For example, comparing the transmittance values of the erythrocyte suspension and of the solution with the same quantity of broken erythrocytes at several wavelengths in the blue can directly give the correction factor. The latter is analytically related with the aggregation degree and other unknown parameters of erythrocytes. The solution to a set of equations for used wavelengths can, in principle, simplify the unknown characteristics. As to the determination of erythrocyte parameters *in vivo*, this topic requires additional investigations. In any case, the screening of the "sieve" effect in skin by melanin, which strongly absorbs blue light too, should be taken into consideration. The screening can hide the hemoglobin localization effect.

References

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