Influence of clearing solutions osmolarity on the optical properties of RBC

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ABSTRACT

The possibility of immersion clearing of human blood in visible and NIR spectral range has been discussed. Based on presented model the spectral behavior of scattering and absorption characteristics of blood caused by immersion properties of glucose solution has been analyzed. The influence of osmotic properties of glucose solution on blood erythrocytes has been shown.

Keyword: blood, scattering coefficient, absorption coefficient, anisotropy factor, immersion agent, glucose, osmolarity

1. INTRODUCTION

Due to significant progress, achieved recently in development of noninvasive systems of multifunctional clinical monitoring of different disease, optical methods have become widely used as a technique in modern experimental and clinical medicine[1-6]. For the optical probing of tissue most of the existing methods are used as a diagnostic window in spectral range from 650 nm to 1200 nm[6]. One of the main problem of the use of optical methods in medicine deals with high scattering and absorption of light by different tissue chromophores, like hemoglobin. Optical properties of blood in the above mentioned spectral range are mainly determined by the hemoglobin content. Thus, the possibility of *in vivo* control of blood optical properties significantly increases the efficiency of existing methods of optical tissue diagnostic in visible and NIR spectral range.

The optical properties control implies the change of the scattering or absorption properties of optically turbid media, including tissues and blood. Optical immersion is a rather effective method to decrease the scattering. This method is based on matching the refractive indices of scatterers and surrounding medium by administration of corresponding liquids in tissue. The light scattering of tissues can be decreased by administration of osmotically active immersion liquids[4, 5, 7-26]. In this way it has recently been shows, that using such immersion liquids as the aqueous glucose solution, propylene glycol, trazograph, glycerol, allows one to decrease significantly (up to several folds) the scattering properties of tissues and blood[5, 6]. The aqueous glucose solution with different concentration is used more widely in contrast to other substances. Such a choice of the clearing agent is caused by its biological compatibility and permission to clinical application[27], as well as by its accessibility. The increasing glucose concentration in a tissue leads to matching the refractive indices of scatterers and interstitial liquid and consequently to decreasing of tissue light scattering. The same results were obtained for the whole blood[3]. Besides that, due to its osmotic properties, the immersion liquids can induce local dehydration that also leads to matching the refractive index of different components of tissues. The changes in tissue due to osmotic properties of clearing liquids have complex nature and mainly depend on the acid properties of clearing liquids. It is well known, that the action of hypotonic solutions on the tissues, having cell structure, for example liver, leads to osmotic swelling of cells, and action of hypertensive solutions leads to its shrinkage[28]. In turn, the change of volume of scatterers in a tissue leads to the change of its scattering properties[29].

Computer modeling shows that using aqueous glucose solution as clearing agent leads to sufficient decreasing light scattering and increasing probing depth of light in tissue[12, 30]. At the same time, the influence of osmotic properties of immersion liquids on optical, and, in particular, scattering characteristics of blood remains poorly investigated.

The goal of the present paper is investigation of the optical and osmotic changes in blood under action of immersion agents such as glucose solution, in the spectral range from 400 nm to 1000 nm.

Saratov Fall Meeting 2003: Optical Technologies in Biophysics and Medicine V, edited by Valery V. Tuchin, Proceedings of SPIE Vol. 5474 (SPIE, Bellingham, WA, 2004)
1605-7422/04/$15 · doi: 10.1117/12.578999

2. MATERIALS AND METHODS

As it was shown experimentally[9-11], the use of the osmotic immersion liquids (including aqueous glucose solution of different concentrations) leads to the sufficient decrease of scattering properties of blood in visible and NIR spectral range. The refractive index of blood plasma at administration of the glucose solution becomes close to refractive index of erythrocytes, and thus decreasing scattering coefficient and increasing anisotropy factor of blood is seen. The clinical method of optical immersion of blood erythrocytes will not be discussed in the present paper. At the same time, for the optical clearing of blood the method of intravenous injection of aqueous glucose solution seems more reliable, because such technique had already been applicated in clinical practice[27].

The spectral dependence of the refractive index of the aqueous glucose solution is described as[18]: $n_{gl}(\lambda) = n_w(\lambda) + 0.1515 C_{gl}$, where $n_w(\lambda)$ is refractive index of water[31] and C_{gl} is glucose concentration in solution, g/ml. By analogy with this equation the refractive index of the glucose solution in plasma $\left(n_p^{gl}\right)$ can be described by:

$$n_p^{gl}(\lambda) = n_p(\lambda) + 0.1515 C_{gl} \tag{1}$$

Due to the absence of glucose absorption bands in the investigated spectral range, it is suggested that changes of blood absorption caused by glucose self-absorption at its penetration in blood does not occur. In this study, as a first approximation, it was suggested, that glucose molecules and proteins of blood plasma do not bind.

Change of plasma osmolarity leads to changes of size and complex refractive index of blood scatterers, due to their osmotic dehydration[32, 33] and thus to changes of their scattering and absorption properties. Under physiological conditions the blood osmolarity is 280-300 mOsm/l[32, 34]. The glucose injection into the blood plasma leads to linear increasing osmolarity, up to 3000 mOsm/l when glucose concentration in blood plasma is 0.5 g/ml[32].

Based on the data presented in Ref. 32, for accounting of erythrocytes volume changes the phenomenological equation have been used:

$$V(osm) = V_0\left(0.515 + 1.177\exp\left(-osm/337\right)\right), \tag{2}$$

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. During penetration of glucose solution into plasma the blood haematocrit is decreased and is equal to 45% for $osm = 300$ mOsm/l $\left(C_{gl} = 0 \quad g/ml\right)$, 32% for $osm = 590$ mOsm/l $\left(C_{gl} = 0.05 \quad g/ml\right)$, 25% for $osm = 1100$ mOsm/l $\left(C_{gl} = 0.15 \quad g/ml\right)$, 24% for $osm = 1700$ mOsm/l $\left(C_{gl} = 0.25 \quad g/ml\right)$. For further increasing glucose concentration in plasma $\left(C_{gl} = 0.3 \div 0.5 \quad g/ml\right)$ the value of haematocrit is about 23%, and, despite the blood osmolarity increasing $\left(osm = 2000 \div 3000 \quad mOsm/l\right)$, decreasing of haematocrit does not occur. In the present modeling it was taken, that the shape of function of erythrocytes size distribution does not change.

Osmotic dehydration leads to increasing hemoglobin concentration in blood erythrocytes, and thus leads to increasing real and imaginary part of erythrocytes refractive index. The estimation of change of real part of erythrocytes refractive index have been made based on Gladstone-Dale law[35]:

$$n_s = n_w C_w + \left(1 - C_w\right) n_h, \tag{3}$$

where C_w is the volume fraction of water in erythrocyte (under physiological condition C_w is about 0.7) and n_h is a mean refractive index of hemoglobin, various proteins and other components of blood erythrocytes. Since the change of the volume of erythrocytes at osmotic dehydration is only due to the change of water content in erythrocyte, the use of equation (2) and (3) allows to estimate the change in real part of erythrocytes refractive index during the change of blood osmolarity (Fig. 1).

The imaginary part of refractive index of erythrocytes is proportional to hemoglobin concentration in erythrocyte[33], i.e.:

$$\chi_s = \beta C_{Hb}, \tag{4}$$

where C_{Hb} is hemoglobin concentration in erythrocyte, g/ml. Spectral dependence coefficient β has been estimated according to the data presented in Ref. 33 for hemoglobin concentration 0.3 g/ml. The calculations of χ_s with the change of blood osmolarity having been done, taking into account that hemoglobin mass at osmotic dehydration of erythrocytes does not change. The result of the calculation with using the equations (2) and (4) is presented in Fig. 2.

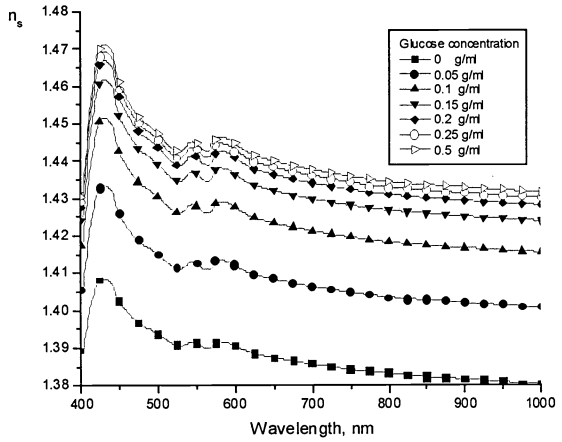

Fig. 1. Spectral dependence of real part of the refractive index of blood erythrocytes for different concentration of glucose solution into the blood plasma.

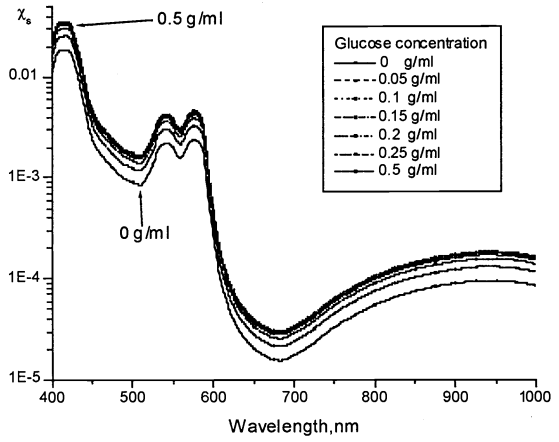

Fig. 2. Spectral dependence of imaginary part of the refractive index of blood erythrocytes for different concentration of glucose solution into the blood plasma.

The calculations of the absorption coefficient, the scattering coefficient and anisotropy factor of whole blood (haematocrit is 45%) both for normal blood and the one at administration of glucose solution into plasma have been performed based on equations:

$$\mu_s = W_s \sum_{i=0}^{M} N_i \sigma_{s_i}, \tag{5}$$

$$\mu_a = \sum_{i=1}^{M} N_i \sigma_{a_i}, \tag{6}$$

$$g = \frac{\sum_{i=1}^{M} \mu_{s_i} g_i}{\sum_{i=1}^{M} \mu_{s_i}}, \tag{7}$$

where $W_s = (1-H)(1.4-H)$ is the packing factor of scatterers[36], which account the interparticle correlation effects; H – haematocrit; M is a number of particle diameters (in this case is 6); $N_i = C_i / v_{ei}$ is a number of particles in a volume unit of medium; C_i is the volume fraction of scatterers with diameter d_i (see Tab. 1); $v_{ei} = 4\pi a_i^3 / 3$ is the volume of individual erythrocyte.

The calculations of the scattering and absorption cross-sections, and anisotropy factor of individual erythrocyte have been performed using equations[37]:

$$\sigma_s = \left(\frac{\lambda^2}{2\pi n_p^2}\right)\sum_{n=1}^{\infty}(2n+1)\left(|a_n|^2+|b_n|^2\right), \tag{8}$$

$$\sigma_a = \left(\frac{\lambda^2}{2\pi n_p^2}\right)\sum_{n=1}^{\infty}(2n+1)\left[\mathrm{Re}(a_n+b_n)-\left(|a_n|^2+|b_n|^2\right)\right], \tag{9}$$

$$g = \frac{\lambda^2}{\pi n_p^2\sigma_s}\left[\sum_{n=1}^{\infty}\frac{n(n+2)}{n+1}\mathrm{Re}\left\{a_n a_{n+1}^* + b_n b_{n+1}^*\right\} + \sum_{n=1}^{\infty}\frac{2n+1}{n(n+1)}\mathrm{Re}\left\{a_n b_n^*\right\}\right], \tag{10}$$

where an asterisk indicates that the complex conjugate is taken; a_n and b_n are the Mie coefficients.

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

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

Where $m = n_e/n_p$ is relative refractive index of the erythrocyte; $x = 2\pi n_p a/\lambda$ is diffraction parameter of erythrocyte; a is radius of erythrocyte; $\psi_n(\rho) = \rho J_n(\rho)$, $\xi_n(\rho) = \rho H_n^{(1)}(\rho)$ are Ricatti-Bessel functions; and $J_n(\rho)$ and $H_n^{(1)}(\rho)$ are Bessel functions.

Table 1 Size distribution of spherical particles, which models the blood erythrocytes. [38]

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

The spectral dependence of the real part of refractive index of the blood erythrocytes under physiological condition has been taken according to the data presented in Ref. 33. The spectral dependence of the refractive index of blood plasma versus the glucose concentration has been calculated using equation (1). The spectral dependence of refractive index of blood plasma before administration of glucose solution have been calculated according to[39]:

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

where λ is the wavelength in μm.

3. RESULTS AND DISCUSSION

Fig. 3 shows the spectral dependence of the absorption coefficient of the whole blood at administration of glucose solution in plasma. From Fig. 3(a), which shows absorption spectra of blood calculated without accounting osmotic properties of glucose solution, it is clearly seen that glucose solution changes the absorption coefficient of blood insignificantly, and there 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 oxyhemoglobin. At the same time, increasing of concentration of the immersion agent leads to linear decreasing of the absorption coefficient. Such changes of absorption spectrum deal with the change of relative refractive index of blood erythrocytes $m = \frac{n_e}{n_p} + i\frac{\chi_e}{n_p}$, where the first term is connected with scattering, and second one with absorption of blood erythrocytes. During administration of glucose solution into plasma this equation has to be rewritten, taking into account (1), as:

$$m = \frac{n_e}{n_p + 0.1515C_{gl}} + i\frac{\chi_e}{n_p + 0.1515C_{gl}}.$$

So, glucose solution increases the refractive index of plasma, that leads to decreasing of imaginary part of relative refractive index of blood erythrocytes.

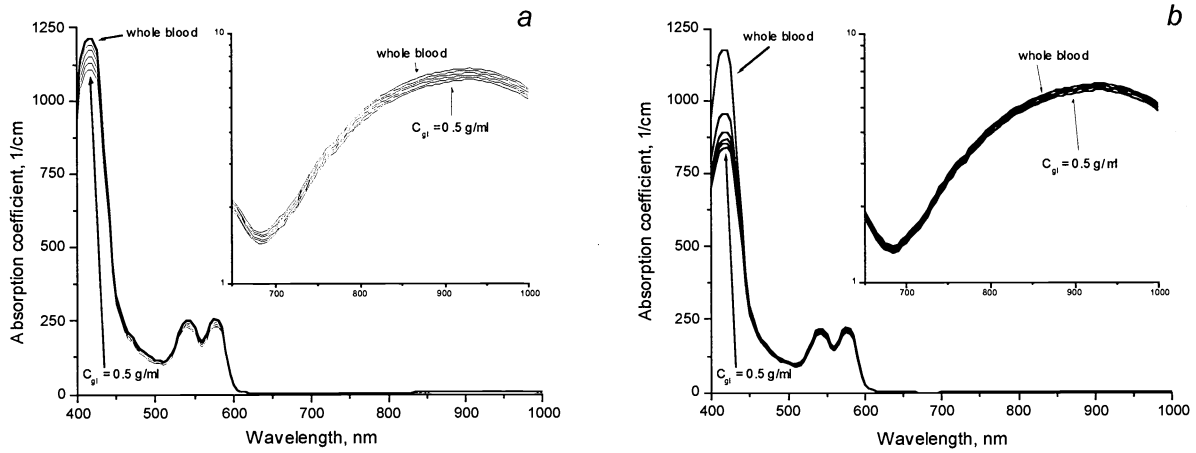

Fig. 3. The calculated absorption spectra of whole blood (H=45%) for optical clearing of blood by glucose solution.
a – without accounting the osmotic properties of glucose solution;
b – taking into account the osmotic properties of glucose solution.

When accounting of osmotic activity of glucose solution the dynamics of decreasing of absorption coefficient with increasing of glucose concentration in blood plasma acquires exponential character. Most significantly it is seen in the Soret band with maximum at 420 nm (Fig. 3(b)). Decreasing of blood absorption, in this case, deals with decreasing of erythrocytes size during osmotic dehydration.

In contrast with the change of absorption coefficient, more significant changes of the scattering properties of blood have been observed at administration of the glucose solution into plasma (Figures 4-7). Fig. 4 shows the scattering spectra of blood for different concentration of the glucose solution in plasma. The spectra, calculated without accounting of osmotic properties of glucose solution (Fig. 4(a)), shows that the depth of minimum of 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 decreasing with increasing of the concentration of the glucose solution. The spectral dependence of the scattering coefficient becomes more monotonous. As a whole, the scattering coefficient in the investigated spectral range decreases down to the minimum at administration of the glucose solution with concentration 0.25 g/ml. Further increase of concentration of the immersion agent leads to increasing of the scattering coefficient. Besides, it is worth noting, that maximum of scattering, which in initial time is localized at wavelength 450 nm, is shifted to violet range of spectra. At administration of glucose solution with concentration 0,1 g/ml maximum of scattering shifts to wavelength 440 nm, and administration of glucose solution with concentration 0,2 g/ml shifts this maximum to wavelength 418 nm. Similar behavior is in good agreement with general character of behavior of the system of scattering particles at changing of refractive index of surrounding medium. Fig. 4(b) shows the scattering spectra of blood calculated taking into account the osmotic properties of the glucose solution. It is seen, that changes of spectrum of scattering deal with boss immersion and osmotic properties of glucose solution. Osmotic dehydration leads to shrinkage of erythrocytes, resulting in significant increase of refractive index and blood scattering coefficient. As was mentioned above, starting from glucose concentration 0.25-0.3 g/ml with osmolarity increasing, further shrinkage of erythrocytes dos not occur. The changes of scattering spectrum are determined by immersion properties of glucose solution. Therefore, the scattering coefficient in the investigated spectral range decreases down to the minimum at administration of the glucose solution with concentration 0.5-0.6 g/ml.

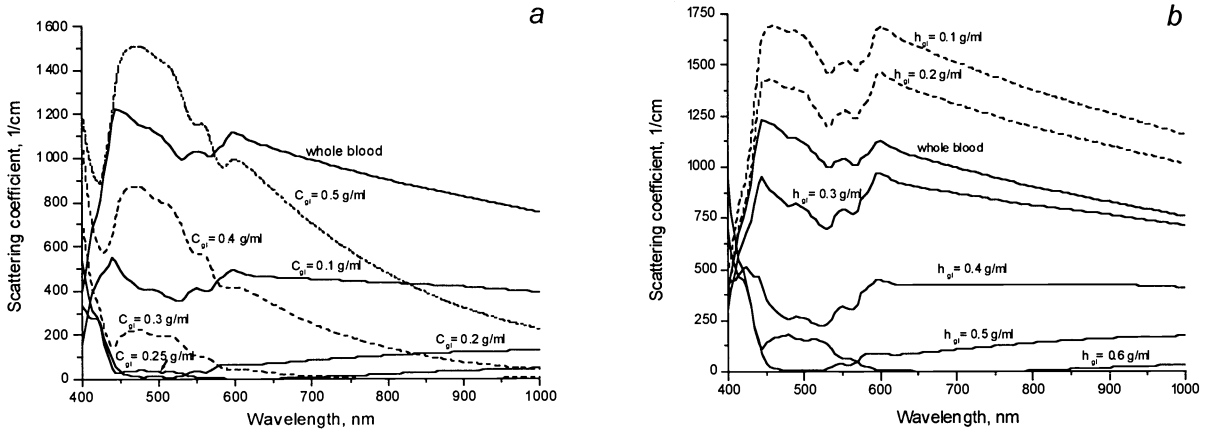

Fig. 4. The calculated scattering spectra of whole blood (Ht=45%) for optical clearing of blood by glucose solution.
a – without accounting the osmotic properties of glucose solution;
b – taking into account the osmotic properties of glucose solution.

Fig. 5 shows the scattering coefficient versus concentration of the glucose solution in blood plasma. The presented data were obtained both without account of osmotic properties of glucose solution (Fig. 5(a)) and with osmotic activity of glucose solution taking into account (Fig. 5(b)). Fig. 5 shows that the decreasing of the scattering coefficient for different spectral bands, which were calculated using both methods, is nonuniform. It deals with the properties of spectral dependence of real part of refractive index of erythrocytes. The local minimum of real part of refractive index at wavelength 420 nm (Soret band of absorption) leads to more significant optical clearing of blood in the spectral range at administration of glucose solution with lower concentration. The most essential clearing is seen in the spectral range from 500 nm to1000 nm at administration of the glucose solution 0.25 g/ml, while in the Soret band (420 nm) the maximum is achieved at concentration of glucose solution 0.2 g/ml (Fig. 5(a)). At the same time, it is worth noting that sufficient difference between calculation without accounting of osmotic properties of glucose solution and calculation taking into account osmotic properties of glucose solution occurs.

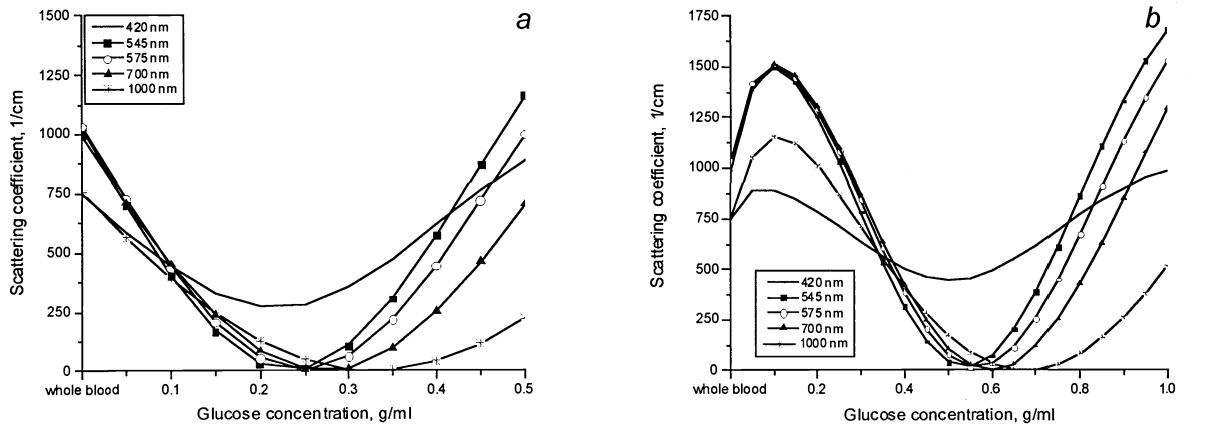

Fig. 5. The calculated dependences of scattering coefficient of whole blood (Ht=45%) versus the concentration of glucose solution.
a – without accounting the osmotic properties of glucose solution;
b – taking into account the osmotic properties of glucose solution.

From Fig. 5(b) it is clearly seen that osmotic properties of glucose solution have major role, producing the dehydration of blood erythrocytes that leads to increasing of refractive index of erythrocytes and consequently their scattering cross-section. The scattering coefficient increases up to the maximum at administration of the glucose solution with concentration 0.1 g/ml. Further increasing of glucose concentration leads to immersion clearing up to the maximum clearing at administration of the glucose solution with concentration 0.5-0.6 g/ml.

Figs. 6 and 7 show the anisotropy factor versus the wavelength and concentration of the glucose solution in blood plasma. From Figs. 6(a) and 7(a), which show the result of calculation without taking the osmotic properties of glucose solution into account, it is seen, that increasing the glucose concentration in blood plasma leads to increasing of anisotropy factor in investigated spectral range up to the maximum at administration of glucose solution with concentration 0.3-0.35 g/ml. Further increase of glucose concentration in blood plasma leads to decreasing of the anisotropy factor. This clearing action of the glucose solution is very important to provide optical diagnostics and therapeutic techniques, because it makes possible to increase the depth of the optical probing.

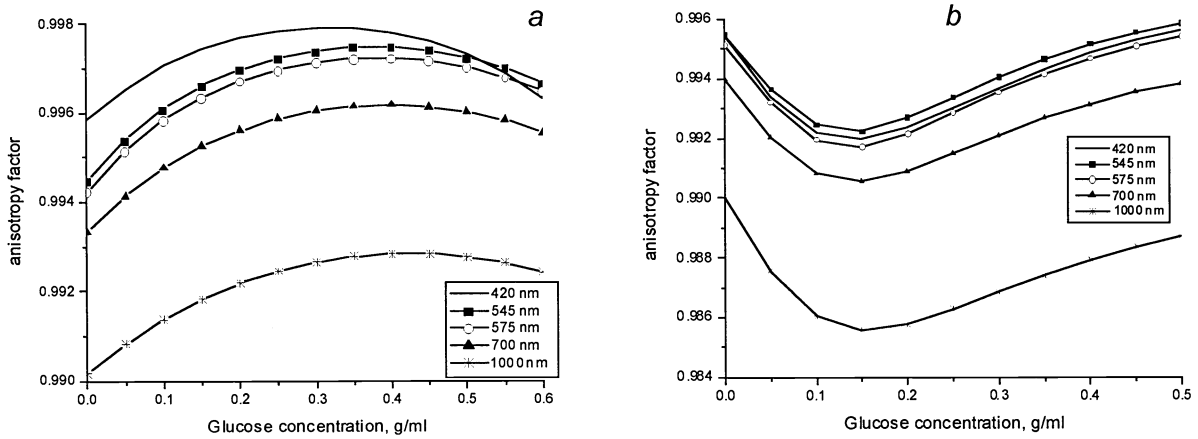

Fig. 6. The calculated dependences of anisotropy factor of whole blood (Ht=45%) versus the concentration of glucose solution.
a – without accounting the osmotic properties of glucose solution;
b – taking into account the osmotic properties of glucose solution.

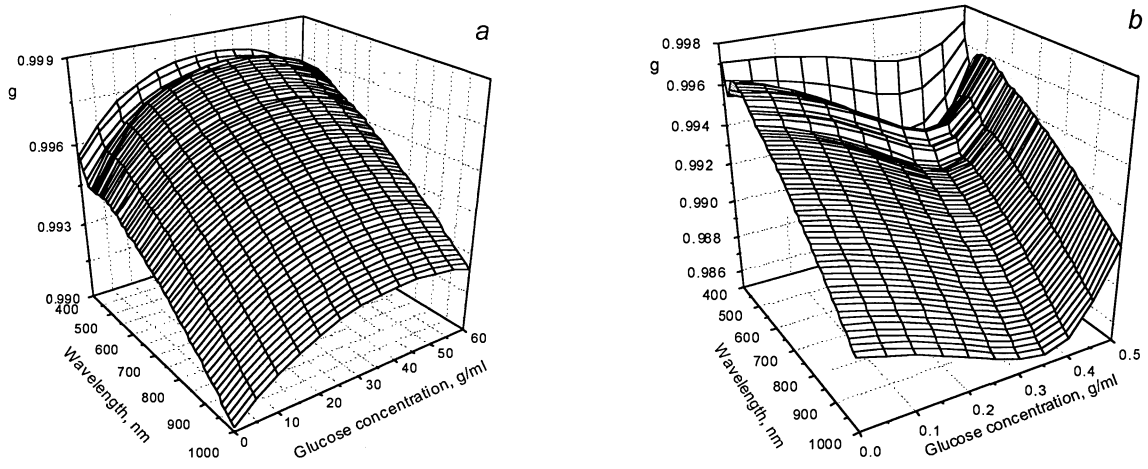

Fig. 7. The calculated anisotropy factor spectrum of whole blood (Ht=45%) versus the concentration of glucose solution.
a – without accounting the osmotic properties of glucose solution;
b – taking into account the osmotic properties of glucose solution.

Taking the osmotic properties of glucose solution into account leads to change of the dynamics of anisotropy factor (Fig. 6(b) and 7(b)). Due to the osmotic shrinkage of erythrocytes and increasing of its refractive index the anisotropy factor decreases down to the minimum at administration of the glucose solution with concentration 0.15 g/ml. With further increase of the glucose concentration the behavior of the anisotropy factor is determined by the immersion properties of the glucose solution. Increasing the concentration of glucose solution leads to increasing the anisotropy factor up to the maximum at administration of the glucose solution with concentration 0.5-0.6 g/ml. Greater concentrations of glucose solution result in decreasing the anisotropy factor and increasing scattering coefficient.

It is worth noting that the presented result could differ from observations *in vivo*, because in the calculations the influence of blood shear stress has not been accounted for. The change of erythrocytes volume and blood haematocrit, probably, could have difference with the optical model of blood, because shear stress will decrease the glucose concentration, and thus significant changes of erythrocytes volume and blood haematocrit will not occur.

4. CONCLUSIONS

Calculations have been performed for investigation of possibility of application of the aqueous glucose solution for immersion clearing of blood.

Based on the presented model the spectral behavior of the absorption and scattering properties of blood under action of aqueous glucose solution has been analyzed.

It is shown that the administration of the glucose solution into the blood 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 of the refractive indices of scatterers (blood erythrocytes) and surrounding medium (blood plasma). At the same time, osmotic properties of immersion agent have to be taken into account.

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.

ACKNOWLEDGMENTS

The research described in this publication has been made possible in part by Award No. REC-006 of the U.S. Civilian Research & Development Foundation for the Independent States of the Former Soviet Union (CRDF) and Ministry of Education of Russian Federation; by Award of President of Russian Federation "Leading Scientific Schools" No 25.2003.2 and Contract No. 40.018.1.1.1314 of the Ministry of Industry, Science and Technologies of Russian Federation. The authors thank Dr. S.V. Eremina (Department of English and Intercultural Communication of Saratov State University) for help in manuscript translation to English.

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