Methylene blue laser therapy for the treatment of chronic maxillary sinusitis

Alexey N. Bashkatov[*a)], Elina A. Genina [a)], Valery V. Tuchin [a)], Elena E. Chikina[b)], Anatoly B. Knyazev[b)], Oleg V. Mareev [b)]

[a)] Department of Optics and Biomedical Physics of Saratov State University, 410012, 83 Astrakhanskaya str., Saratov, Russia;

[b)] Department of Otolaryngology of Saratov State Medical University, 410012, 112 B. Kazachya str., Saratov, Russia

ABSTRACT

The clinical results of photodynamic therapy of chronic maxillary sinusitis have been demonstrated. Obtained results show that the photodynamic therapy is effective in comparison with conservative methods of treatment of the disease. Diffusion of Methylene Blue in the mucous tissue has been studied *in vitro* and value of the diffusion coefficient of Methylene Blue in the tissue has been estimated at 20^0C as $(4.8 \pm 2.9) \times 10^{-7}$ cm^2/sec.

Keywords: Medical optics and biotechnology, clinical applications, otolaryngology, photodynamic therapy, spectroscopy, tissue diagnostics, Methylene blue, diffusion coefficient, chronic maxillary sinusitis, He-Ne laser irradiation

1. INTRODUCTION

The problem of treatment of chronic maxillary sinusitis remains important in rhinology despite wide application of novel pharmaceutical and surgical methods of treatment of the disease. Photodynamic therapy is successfully used for treatment of cancer[1], and other inflammatory disease in gynecology[2], dermatology, as well as in cosmetology.[3-5] However, only a few reports of application of photodynamic therapy for treatment of rhinological diseases are available.[6]

Development of the novel photodynamic methods includes knowledge of photosensitizers diffusion rate, choice of laser source and optimal conditions for the laser irradiation and delivering the photosensitizers into human tissue. Methylene Blue and He-Ne laser are very appropriate as a photosensitizer and light source due to their biocompatibility and low cost. However, in spite of numerous investigations related to application of Methylene Blue in photodynamic therapy of different diseases[7-9], the Methylene Blue diffusion coefficient in epithelial tissues has not been estimated.

In this study, we investigated Methylene Blue diffusion in human mucous tissue and estimated value of its diffusion coefficient. Moreover, for the first time we shall present the clinical results of administration of Methylene Blue in combination with He-Ne laser irradiation (632.8 nm) for photodynamic therapy of patients with chronic maxillary sinusitis.

2. PHYSICAL PROPERTIES AND STRUCTURE OF THE TISSUE

The mucous membrane plays a leading role in the physiology of the nose and paranasal sinuses.[10,11] It is covered with a pseudostratified epithelium, which consists of ciliated, columbar as well as short and long inserted epithelial cells. The membrane called basic divides epithelial and proper layers of the mucous tissue and consists of reticular fibrils, which are located in the interstitial homogeneous media. The membrane has not a constant thickness. In the case of hyperplasia of the mucous membrane, the membrane considerably thickens.[12]

The proper layer of the mucous membrane is similar in structure to connective tissue, consisting of collagen and elastin

[*] bash@optics.sgu.ru; phone: +7-8452-514693

Therapeutic Laser Applications and Laser-Tissue Interactions II, edited by Hubert van den Bergh, Alfred Vogel, Proc. of SPIE-OSA Biomedical Optics, SPIE Vol. 5863 © 2005 SPIE and OSA · 1605-7422/05/$15

fibrils. The interstitial fluid of the mucous membrane contains proteins and polysaccharides and is similar in composition to the interstitial fluid of the most of connective tissues. The proper layer of the mucous membrane consists of three sublayers. A subepithelial (or lymphoid) layer contains a great amount of leukocytes. In the intermediate sublayer of the proper layer, tubuloalveolar glands are contained. In the deep sublayer of the proper layer, venous plexuses are arranged, which consist of a surface network of smaller vessels and a deeper network of larger vessels. Normally, the total thickness of the mucous membrane varies from 0.1 to 0.5 mm.[10,11] In the presence of pathology (maxillary sinusitis, rhinitis, or other rhinological disease), the thickness of the mucous membrane considerably increases and can reach 2-3 mm.[10] It should be noted, that the proper layer of the mucous membrane is the main layer protecting against microorganisms causing infectious diseases.[11] The optical properties of the mucous membranes are mainly determined by the optical properties of the proper layer since this layer is much thicker than the epithelial layer.

3. METHOD FOR ESTIMATION OF METHYLENE BLUE DIFFUSION COEFFICIENT

Method of estimation of Methylene Blue (MB) diffusion coefficient is based on the time-dependent measurement of the tissue absorbance in the spectral range from 600 to 700 nm, which correspond to absorption bands of the dye. The transport of MB within the mucous tissue is described in the framework of free diffusion model. We assume that the following approximations are valid for the transport process:

1. Only concentration diffusion takes place; i.e., the flux of the dye into the tissue, at a certain point within the tissue sample, is proportional to the MB concentration at this point;
2. The diffusion coefficient is constant over the entire sample volume.

Geometrically the tissue sample is presented as a plane-parallel slab with a finite thickness. Since lateral sides of the experimental samples were fixed, the one-dimensional diffusion problem has been solved. The one-dimensional diffusion equation of the dye transport has the form

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2}, \tag{1}$$

where $C(x,t)$ is the MB concentration, g/ml; D is the MB diffusion coefficient, cm^2/s; t is time, in second, sec; and x is the spatial coordinate, cm.

We also suppose that penetration of the MB into a tissue sample does not change the dye concentration in the external volume. Besides, due to geometry of the measurements, penetration of the MB into a tissue sample takes place from top surface of the tissue sample only. The corresponding boundary conditions are

$$C(0,t) = C_0 \quad \text{and} \quad \frac{\partial C(l,t)}{\partial x} = 0, \tag{2}$$

where C_0 is MB concentration in external solution, g/ml, and l is tissue sample thickness, cm.

The initial condition corresponds to the absence of MB inside the mucous tissue before the measurements,

$$C(x,0) = 0, \tag{3}$$

for all inner points of the tissue sample.

Solution of Eq. 1 for a slab with a thickness l at the moment t with boundary (Eq. 2) end initial (Eq. 3) conditions has the form

$$C(t) = C_0\left(1 - \frac{8}{\pi^2}\sum_{i=0}^{\infty}\frac{1}{(2i+1)^2}\exp\left(-(2i+1)^2\, t\frac{\pi^2}{4}D\Big/l^2\right)\right), \tag{4}$$

where $C(t)$ is the volume-averaged concentration of MB within tissue sample.

In a first-order approximation, Eq. 4 is reduced to the form

$$C(t) \approx C_0\left(1 - \exp\left(-t\pi D/l^2\right)\right). \tag{5}$$

For determination of MB diffusion coefficient in the mucous tissue the approach suggested by Mourant et al.[13] has been used. The method is based on the use of modified Lambert-Beer law and in this case, tissue absorbance can be determined as

$$A = \mu_a \sigma\rho + G, \tag{6}$$

where μ_a is absorption coefficient, ρ is source-detector distance, σ is differential factor of photon path-length, taking into account the lengthening of photon trajectories due to multiple scattering, and G is constant, defined by geometry of the experiment. To simplify calculations, $\rho\sigma$ can be replaced by parameter L which is defined by both absorption and scattering tissue properties, and source-detector distance. Since in this study the distance (290 μm) is commensurable with photon free path-length, parameter L is defined by tissue scattering properties only.[14,15]

Penetration of MB into tissue is increasing the tissue absorbance in spectral range corresponding to absorption bands of the dye. Thus, the tissue absorbance measured in different time intervals can be determined as

$$A(t,\lambda) = A(t=0,\lambda) + \Delta\mu_a(t,\lambda)L, \tag{7}$$

where t is the time interval, λ is the wavelength, $\Delta\mu_a(t,\lambda) = \varepsilon(\lambda)C(t)$ is the absorption coefficient of MB within tissue, $\varepsilon(\lambda)$ is MB molar absorption coefficient, $C(t)$ is MB concentration in tissue, and $A(t=0,\lambda)$ is the tissue absorbance, measured in the initial moment.

Thus, the equation

$$\Delta A(t,\lambda) = A(t,\lambda) - A(t=0,\lambda) = \Delta\mu_a(t,\lambda)L = \varepsilon(\lambda)C_0\left(1 - \exp\left(-t\pi D/l^2\right)\right)L \tag{8}$$

can be used for calculation of the MB diffusion coefficient.

This set of equation represents the direct problem, i.e., describes the temporal evaluation of the absorbance of a tissue sample dependent on MB concentration within the tissue sample. Based on measurement of the evolution of the tissue absorbance, the reconstruction of the MB diffusion coefficient in mucous tissue has been carried out. The inverse problem solution has been obtained by minimization of the target function as

$$F(D) = \sum_{i=1}^{N_t}\left(A(D,t_i) - A^*(t_i)\right)^2, \tag{9}$$

where $A(D,t)$ and $A^*(t)$ are the calculated and experimental values of the time-dependent absorbance, respectively, and N_t is the number of time points obtained at registration of the temporal dynamics of the absorbance. To minimize the target function the Levenberg-Marquardt nonlinear least-squares-fitting algorithm described in detail by Press et al.[16] has been used. Iteration procedure repeats until experimental and calculated data are matched.

4. MATERIALS AND METHODS

4.1 Estimation of Methylene Blue diffusion coefficient

In this study, aqueous solution of Methylene Blue with concentration 1 g/L has been used. The measurements of MB diffusion coefficient have been performed *in vitro* with ten samples of human mucous tissue obtained from ten patients with chronic maxillary sinusitis. All tissue samples were kept in saline at room temperature about 20°C until spectroscopic measurements. The thickness of each tissue sample has been measured with a micrometer in several points over the sample surface and averaged. Precision of the single measurement was ±50 μm. The mucous tissue samples were measured during 2-3 hours after biopsy. The measurements have been performed in the spectral range 500-1000 nm using a commercially available optical multichannel spectrometer LESA-5 (BioSpec, Russia) with fiber-optical probe at room temperature about 20°C. The scheme of the experimental setup is shown in Fig. 1.

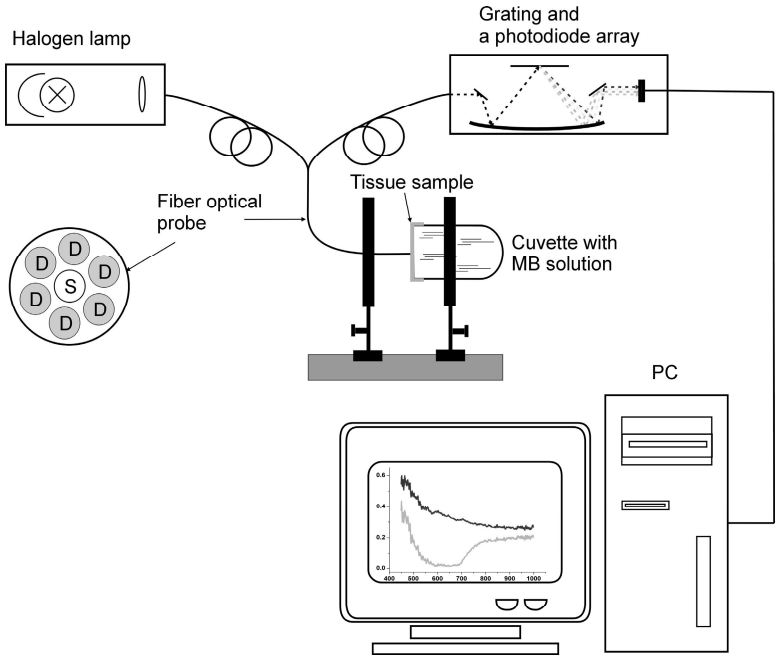

Fig. 1. Experimental setup for measurements of the mucous tissue reflectance spectra

The fiber-optical probe consists of the seven optical fibers. All fibers had 200 μm core diameter and a numerical aperture of 0.22. The central fiber delivers incident light to the tissue surface and the six fibers (the fibers are placed around the central fiber) collected reflected light. Distance between the delivering and receiving fibers is 290 μm. As a reference a white slab $BaSO_4$ with a smooth surface has been used. For the spectrophotometric measurements each tissue sample is fixed on the special cuvette with solution of MB. Penetration of the dye solution into a tissue sample was provided only through the outward surface of the sample that modeled real conditions of photodynamic procedure. Measured reflectance spectra have been recalculated in absorbance spectra with the relation

$$A(\lambda) = -\ln\left(R(\lambda)\right). \tag{10}$$

For processing of the experimental data and estimation of MB diffusion coefficient the algorithm, presented in the Section 3, has been used.

4.2 Patient selection

Seventy-five patients of both sexes (ages from 20 to 41 years) with chronic maxillary sinusitis were enrolled for the study and separated in two groups in dependence of treatment protocol: I group - the patients were treated with photodynamic therapy, and II group (the control group) - the patients were treated by traditional (pharmaceutical) therapy. The first group included 35 patients. Control group included 40 patients.

The traditional therapy included antibiotic therapy in combination with paracentesis and lavage of maxillary sinus in addition to desensitizing therapy and physiotherapy. The antibiotic therapy included intramuscular injection of lincomycin (0.6-0.9 g/day). Course of the treatment was 12-13 days.

Patients were excluded if they had surgery treatment of maxillary sinuses before the photodynamic or pharmaceutical therapy and all patients gave their informed consent for participation and the study protocol.

4.3 Laser system and irradiating protocol

For photodynamic treatment of chronic maxillary sinusitis the AFL-1 laser system (Russia) was used. The GN-80 He-Ne laser (λ = 632.8 nm) (Russia) with an originally designed fiber-optic diffuser. The power of the laser system at the fiber tip was 40 mW. Since area of one maxillary sinus varied from 14.6 cm^2 to 29.2 cm^2 then the power density varied from 1.4 to 2.7 mW/cm^2 within the sinus. Dose of one irradiation was 1.3–2.4 J/cm^2. Total dose of the irradiation was 1.3–19.2 J/cm^2 in dependence from number of the PDT treatments.

4.4 PDT protocol for treatment of sinusitis

The multiple (up to 3-8) treatments were provided. For PDT the following protocol has been used. Before the treatment the lavage of maxillary sinus of a patient was performed by physiological solution and after that 1.5 ml of the aqueous 0.1%-Methylene Blue solution was administered into the maxillary sinus. The staining time was 30 min. Before irradiation the lavage of maxillary sinus of a patient by physiological solution was performed again. Laser radiation was delivered into the maxillary sinus by optical fiber with the fiber-optic diffuser. The exposition time was 15 min ones per treatment. Course of the treatment was 10-12 days.

Efficacy of the PDT was estimated with the use of the Student test (with $p<0.05$) by the following criteria: the state of respiration, olfaction, duration of purulent discharge, reconstruction of transport function of ciliary epithelium, etc.

5. RESULTS AND DISCUSSION

5.1 Estimation of Methylene Blue diffusion coefficient

Figures 2 and 3 show the wavelength and the temporal dependencies of MB absorbance. Figure 2 presents the dye absorbance measured for different time intervals. From the figure it is well seen that in the spectral range from 600 to 700 nm, corresponding to absorption bands of MB, absorbance of the mucous tissue is increased during MB penetration into the tissue. In the figure it is also seen, that shape of the spectrum is different for different moments. At the initial moment only one peak with maximum at 669 nm is seen, that corresponds to monomer form of the Methylene Blue molecules. During the penetration of the MB solution into the tissue the interaction between MB molecules and tissue components are taking place. As a sequence of the interaction the MB molecules form dimmers, and two peaks with maximums at 609 and 669 nm in the spectrum presented in figure 2 are observed.

In the figure it is also seen that the penetration of the dye into the tissue does not change scattering properties of the skin. It is caused from the absence of the change in the absorbance spectra in the range 750-1000 nm, where MB has not the absorption bands, and the form of the absorbance spectra is determined by spectral dependence of scattering coefficient.

Figure 3 presents the measured and calculated dynamics of dye absorbance for different wavelengths. From the figure it is seen, that the optimal staining time was about 30 min. After that time the change of the absorbance was not observed. On the basis of the measurement of the temporal evolution of the absorbance, the reconstruction of the MB diffusion coefficient in the mucous tissue was carried out. Taking into account thickness of the skin samples the diffusion coefficient of MB was estimated from analysis of experimental curves. To minimize a target function the Levenberg-Marquardt nonlinear least-squares-fitting algorithm was used. Calculations were made for ten wavelengths in the range 600-700 nm for each samples and obtained values were averaged. Table 1 shows values of the MB diffusion coefficient calculated for different tissue samples.

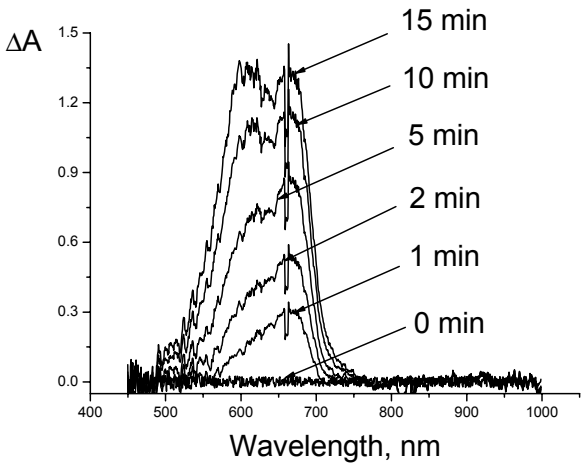

Fig. 2. The dye absorbance measured for different time intervals

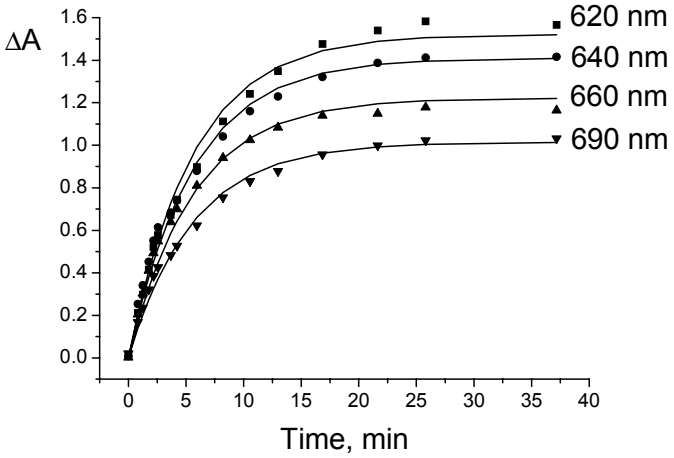

Fig. 3. Time-dynamics of the dye absorbance calculated for different wavelengths(solid lines) in comparison with experimental data (symbols)

Table 1. The MB diffusion coefficient values estimated for different tissue samples

Sample	*Thickness, mm*	*Diffusion coefficient, cm²/sec*	*Sample*	*Thickness, mm*	*Diffusion coefficient, cm²/sec*
1	0.60	$(1.26 \pm 0.3) \times 10^{-7}$	6	0.35	$(4.77 \pm 0.5) \times 10^{-7}$
2	0.50	$(5.61 \pm 0.2) \times 10^{-7}$	7	0.37	$(6.78 \pm 0.2) \times 10^{-7}$
3	0.51	$(2.83 \pm 0.4) \times 10^{-7}$	8	0.41	$(2.76 \pm 0.7) \times 10^{-7}$
4	0.55	$(2.63 \pm 0.1) \times 10^{-7}$	9	0.44	$(3.79 \pm 0.6) \times 10^{-7}$
5	0.63	$(1.15 \pm 0.1) \times 10^{-6}$	10	0.54	$(5.75 \pm 0.8) \times 10^{-7}$

The mean value of the diffusion coefficient is $(4.8 \pm 2.9) \times 10^{-7}$ cm^2/sec.

5.2 Clinical result of Methylene blue laser therapy of chronic maxillary sinusitis

Efficacy of the PDT was estimated with the following criteria: the state of respiration, olfaction, duration of purulent discharge, reconstruction of transport function of ciliary epithelium, etc. Besides, suppression of microflora in maxillary sinus was observed. Recovery of nasal breathing was observed after 7.3 ± 0.2 days of the PDT treatment. In control group recovery of nasal breathing was observed after 8.7 ± 0.3 days of the pharmaceutical treatment. In both groups treated by photodynamic therapy and control one, recovery of olfaction was observed after 6.1 ± 0.3 days of the treatments. Presence of purulent discharge was not observed after 7.1 ± 0.3 days of the PDT treatment and after 8.1 ± 0.3 days of the pharmaceutical treatment. Recovery of transport function of ciliary epithelium was observed after 6.2 ± 0.2 days of the PDT therapy. In control group recovery of the transport function of ciliary epithelium was observed after 7.1 ± 0.2 days of the pharmaceutical treatment. Hospital stay was 13.4 ± 0.3 days for patients treated with PDT therapy and 15.7 ± 0.4 days for patients treated with pharmaceutical therapy.

For application of the photodynamic protocol for treatment of patients with chronic maxillary sinusitis the full recovery was observed for 54.3% of patients. In control group the full recovery was observed for 50% patients. Satisfactory results (insignificant difficulty at respiration etc) were observed for 31.4% of patients from the I group. In control group the satisfactory results were observed for 27.5% of patients. Non-satisfactory results were obtained for 14.3% of patients from the I group, and for 22.5% patients from the control group. Course of the PDT treatment was 10-12 days. Course of the pharmaceutical therapy was 12-13 days. The observations during two years after the treatment showed efficacy of PDT for treatment of patients with chronic maxillary sinusitis.

ACKNOWLEDGMENTS

The research described in this publication has been made possible, in part, by grant REC-006/SA-006-00, Annex N. 07 “Nonlinear Dynamics and Biophysics” of U.S. Civilian Research and Development Foundation for the Independent States of the Former Soviet Union (CRDF) and the Russian Ministry of Science and Education; the Russian Federation President’s grant N 25.2003.2 “Supporting of Scientific Schools” and grant “Leading Research-Educational Teams” N 2.11.03 of the Russian Ministry of Science and Education.

REFERENCES

1. F.S. de Rosa, M.V.L.B. Bentley, "Photodynamic therapy of skin cancers: sensitizers, clinical studies and future directives," *Pharmaceutical Research,* **17**, 1447-1455, 2000.
2. M.K. Fehr, R. Hornung, A. Degen, V.A. Schwarz, D. Fink, U. Haller, P. Wyss, "Photodynamic therapy of vulgar and vaginal condyloma and intraepithelial neoplasia using topically applied 5-aminolevulinic acid," *Lasers Surg. Med.,* **30**, 273-279, 2002.
3. T. Kormeili, P.S. Yamauchi, N.J. Lowe, "Topical photodynamic therapy in clinical dermatology," *Br. J. Dermatol.,* **150**, 1061-1069, 2004.
4. V.V. Tuchin, E.A. Genina, A.N. Bashkatov, G.V. Simonenko, O.D. Odoevskaya, G.B. Altshuler, "A pilot study of ICG laser therapy of *acne vulgaris*: photodynamic and photothermolysis treatment," *Lasers Surg. Med.*, **33**, 296-310, 2003.
5. E.A. Genina, A.N. Bashkatov, G.V. Simonenko, O.D. Odoevskaya, V.V. Tuchin, G.B. Altshuler, "Low-intensity indocyanine-green laser phototherapy of acne vulgaris: pilot study," *J. Biomed. Opt.*, **9**, 828-834, 2004.
6. A.N. Nasedkin, V.G. Zenger, S.V. Grachev, V.M. Isaev, E.I. Prokof'eva, V.N. Selin, I.V. Leskov, A.V. Reshetnikov, I.D. Zalevskiy, S.E. Goncharov, Yu.V. Kemov, "The experience of application of photodynamic therapy for treatment acute and chronic purulent antritis," *Russian Rhinology*, **2**, 116, 2002.
7. K. Orth, G. Beck, F. Genze, A. Ruck, "Methylene blue mediated photodynamic therapy in experimental colorectal tumors in mice," *J. Photochem. Photobiol. B.*, **57**, 186-192, 2000.
8. I.D. Botterill, P.M. Sagar, "Intra-dermal methylene blue, hydrocortisone and lignocaine for chronic, intractable pruritus ani," *Colorectal Disease,* **4**, 144-146, 2002.

9. M.C. Teichert, J.W. Jones, M.N. Usacheva, M.A. Biel, "Treatment of oral candidiasis with methylene blue-mediated photodynamic therapy in an immunodeficient murine model," *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics*, **93**, 155-160, 2002.
10. G.Z. Piskunov, S.Z. Piskunov, *Clinical Rhinology,* Moscow: Miklon, 2002 (in Russian).
11. G.Z. Piskunov, S.Z. Piskunov, *Diagnostics and medical treatment of inflammatory processes of the mucous membrane of nose and maxillary sinuses,* Voronezh: Voronezh State University Press, 1991 (in Russian).
12. M.A. Zavaliy, A.G. Balabantsev, A.K. Zagorul'ko, T.G. Filonenko, "State of ciliary epithelium of patients with the chronic purulent sinusitis," *Russian Rhinology,* **2**, 19-22, 2002 (in Russian).
13. J.R. Mourant, T.M. Johnson, G. Los, I.J. Bigio, "Non-invasive measurement of chemotherapy drug concentrations in tissue: preliminary demonstrations of *in vivo* measurements," *Phys. Med. Biol.*, **44**, 1397-1417, 1999.
14. J.R. Mourant, I.J. Bigio, D.A. Jack, T.M. Johnson, H.D. Miller, "Measuring absorption coefficients in small volumes of highly scattering media: source-detector separations for which path lengths do not depend on scattering properties," *Appl. Opt.*, **36**, 5655-5661, 1997.
15. F. Bevilacqua, C. Depeursinge, "Monte Carlo study of diffuse reflectance at source-detector separations close to one transport mean free path," *J. Opt. Soc. Am. A*, **16**, 2935-2945, 1999.
16. W.H. Press, S.A. Tuekolsky, W.T. Vettering, B.P. Flannery, *Numerical Recipes in C: The Art of Scientific Computing.* Cambridge University Press, Cambridge, UK, 1992.