

DOI: 10.1070/QEL16133

Liquid optical phantoms mimicking spectral characteristics of laboratory mouse biotissues

D.A. Loginova, E.A. Sergeeva, A.D. Krainov, P.D. Agrba, M.Yu. Kirillin

Abstract. **Optical phantoms mimicking optical properties of real biotissues in the visible and IR spectral regions are developed based on measurements of the spectral characteristics of *ex vivo* samples of laboratory mouse biotissues. The phantoms are composed of aqueous solutions of Lipofundin, Indian ink and red ink with different spectral characteristics. The deviations of the measured absorption and scattering coefficients of phantoms in the wavelength range 480–580 nm from the corresponding values for real biotissues do not exceed 25% and 2%, respectively. For phantoms in the wavelength region 580–880 nm, the deviations of the absorption coefficient do not exceed 40% and the deviations of the scattering coefficient do not exceed 25%. These values, in general, fall within the range of variations for different individual mice of one strain.**

Keywords: *mouse biotissues, scattering coefficient, absorption coefficient, optical phantom, Lipofundin.*

1. Introduction

In recent decades, optical methods have become widely used in biomedical diagnostics due to the ability of visible and IR light to non-destructively penetrate into a tissue to a depth of several centimetres, as well as to be scattered or absorbed by the tissue components.

To develop new methods of biomedical diagnostics, one needs to solve such applied problems as approbation of methods, calibration of optical devices and control of their operation, and comparison of the efficiencies of different systems [1]. To solve these problems, use is made of optical phantoms (biotissue equivalent materials), i.e., calibrated media with optical properties close to those of biological tissues and, therefore, with a similar light scattering behaviour. The possibility of designing phantoms with desired optical properties allows one to use them, for example, in fluorescence diffuse tomography [2] and diffuse optical spectroscopy [3]. These methods are applied, in particular, to monitor the development of tumour diseases of laboratory mice, to determine the oxygen status of tumours of different localisations of both laboratory animals and patients, and to test the efficiency of antitumour pharmaceutical products.

These investigations need calibration of optical spectroscopy and tomography systems, which require phantoms with known optical characteristics. Phantoms are also used for filling cells with small laboratory animals to level the strong jumps of optical characteristics at the boundary of objects with complex geometry [4, 5]. This simplifies the problem of three-dimensional reconstruction of the distribution of optical properties inside an object. Thus, the design of phantoms with the same spectral characteristics as those of real mouse biotissues is an important problem.

Most previously developed optical water-based phantoms contain Intralipid and black Indian ink in their composition (see, for example, [1, 3, 6, 7]). In [8], it was proposed to use Intralipid as a standard for diffusion media in the visible and IR regions. Investigations of several samples of different series showed that their optical diffusion properties differ only slightly and are stable for a long time. In [9], these results were generalised to other fat emulsions, in particular, to Lipofundin, which is the European analogue of Intralipid. The difference in the size of the main component of Lipofundin and Intralipid – soybean oil droplets – leads to a difference in the scattering coefficients, which, nevertheless, does not exceed 9% [9].

Absorption in fat emulsions is mainly caused by absorption by water, which is insignificant in the most part of the visible region. To achieve an absorption coefficient corresponding to real biotissues, it is necessary to introduce additional absorbing components. Since the development of phantoms is aimed at the reconstruction of the properties of real biotissues, it is natural to choose biological absorbing components (such as erythrocytes, haemoglobin, or whole blood) or non-biological but more stable substances (dyes, Indian ink, ink).

Black Indian ink is a suspension of insoluble carbon particles in water. It is a chemically stable nontoxic material. According to [10], the optical properties of Indian ink samples of different trademarks and batches are stable for a long time and linearly depend on the concentration of carbon particles but are considerably different, although the ratio of specific (normalised to the volume concentration) absorption and scattering coefficients do not change from sample to sample. When mixed with fat emulsions, Indian ink does not chemically react with them.

Royston et al. [11] describe optical phantoms mimicking the optical properties of biotissues in the near-IR region, including a wavelength of 1064 nm. Intralipid with addition of polystyrene microspheres (0.804 μm in diameter) was chosen as a scattering component, and black Indian ink was used

D.A. Loginova, A.D. Krainov, P.D. Agrba N.I. Lobachevskii State University of Nizhnii Novgorod, prosp. Gagarina 23, 603950 Nizhnii Novgorod, Russia; e-mail: vekfy@inbox.ru, adkrainov@gmail.com, agrbapd@gmail.com;
E.A. Sergeeva, M.Yu. Kirillin Institute of Applied Physics, Russian Academy of Sciences, ul. Ul'yanova 46, Nizhnii Novgorod, 603950 Russia; e-mail: sea@ufp.appl.sci-nnov.ru, mkirillin@yandex.ru

Received 13 May 2016
Kvantovaya Elektronika **46** (6) 528–533 (2016)
Translated by M.N. Basieva

as an absorbing component of the phantom. The absorption and scattering coefficients of Indium ink were estimated to be 35.99 ± 4.27 and 4.64 ± 2.07 cm^{-1}/%, respectively, and the Intralipid scattering coefficient was 1.300 ± 0.047 cm^{-1}/%.

An example of a phantom based on Lipofundin MCT/LCT 10% and black Indian ink is presented in [3]. The developed phantoms modelled the properties of biotissues of internal organs of mice at wavelength of 810 and 900 nm. The Lipofundin MCT/LCT 10% scattering coefficient within the wavelength range 700–1100 nm varied from 12 to 6 cm^{-1}/%, and the absorption coefficient of Indian ink varied from 32 to 20 cm^{-1}/%. The volume concentrations of Lipofundin and Indian ink were 0.89% and 0.024% for modelling optical properties of mouse muscles at a wavelength of 810 nm and 1% and 0.03% for a wavelength of 900 nm. The authors noted that phantoms mimicking the optical properties of biotissues in a wide spectral range cannot be developed using the proposed materials due to strongly different spectral dependences of the optical properties of the biotissue and the phantom components.

Spinelli et al. [6] present the results of determination of specific absorption coefficients of Indian ink and specific scattering coefficients of Intralipid, which can be used as reference data for designing phantoms based on these components for three wavelengths, namely, 633, 750, and 830 nm.

The aim of the present work was to measure the optical properties of mouse biotissues in the visible and IR regions and to develop optical phantoms mimicking the optical properties of biotissues in a wide range. Taking into account the available results, we decided, in addition to traditionally used Lipofundin and black Indian ink, to include red ink as a phantom component. The spectral characteristics of red ink considerably differ from the characteristics of Indian ink, which ensures more accurate modelling of the optical properties of biotissues and can extend the spectral range of phantom application.

2. Materials and methods

Samples of biotissues and phantom components. As objects of the study, we chose *ex vivo* samples of laboratory mouse brain and muscles. Immediately after euthanising and preparation of mice, we performed homogenisation of biotissues in order to obtain homogeneous specimens, which were placed in 1-mm-thick plane-parallel quartz cells for subsequent spectrophotometric measurements. The spectral dependences of the transmittance and reflectance of one sample of each biotissue type were measured during 4 h after euthanisation of an animal. The small number of specimens is caused by a limited number of available laboratory animals. The measured optical characteristics can be considered as typical rather than average for mouse biotissues. However, this is sufficient for developing liquid phantoms, because the variation of these values for different individuals of one strain is larger than the expected difference between the biotissue and phantom. Experiments were performed according to the regulations of the use of experimental animals outlined by the Ministry of Health and Social Development of the Russian Federation in 'Approval of laboratory practice rules' No. 708-n dated 23.08.2010 and to the International Guide for the Care and Use of Laboratory Animals.

The optical phantom of mouse biotissues was prepared based on an aqueous solution of Lipofundin MCT/LCT 20%, Indian ink, and red ink.

Lipofundin MCT/LCT 20% 1000 mL in volume consists of 100 g of soybean oil, 100 g of medium-chain triglycerides, 25 g of glycerol, 12 g of egg lecithin, 0.2 g of α-tocopherol, and the rest volume of water for injection [12]. Hereinafter, Lipofundin means Lipofundin MCT/LCT 20%, and percentage of Lipofundin means percentage of this solution.

As absorbents, we used black Indian ink and red ink (OOO 'Kompaniya RDK', Russia, TU 2389-002-7046-4994-2004).

Spectrophotometric measurements. The spectral dependences of collimated transmittance $T_c(\lambda)$, diffuse transmittance $T_d(\lambda)$, and diffuse reflectance $R_d(\lambda)$ were measured within the range 400–1100 nm using an Analytikjena Specord 250 plus spectrophotometer equipped with an integrating sphere. The samples 1 mm thick were placed into a plane-parallel quartz cell. The refractive index of the cell walls used for reconstruction of the optical characteristics of specimens corresponded to the index determined in [13]. The Fresnel reflection was taken into account at the air–quartz interface, where the jump in the refractive index was maximal.

Method of determination of optical properties. The optical properties of objects are characterised by the following set of parameters: absorption coefficient $\mu_a(\lambda)$, scattering coefficient $\mu_s(\lambda)$, anisotropy factor g, and refractive index n. In the general case, the first three parameters are reconstructed by spectrophotometric data, namely, by the wavelength dependences of collimated $T_c(\lambda)$ and diffuse $T_d(\lambda)$ transmittance, as well of diffuse reflectance $R_d(\lambda)$. There exist different theoretical formulas (see, for example, [13]) and numerical methods (e.g., [14–16]) for determining the relation of $\mu_a(\lambda)$, $\mu_s(\lambda)$, and g with the measured values. In the present work, we use the low-order backward scattering approximation, which allows us to derive approximate analytical formulas for subsequent reconstruction of the optical characteristics of media without using numerical methods.

In the low-order backward scattering approximation [17], we proposed a method that allows one to reconstruct the optical properties of a suspension of particles from spectrophotometric measurements [17]. The relations used in this method have a semi-empirical character and are based on the modified Bouguer–Lambert–Beer (BLB) law. This method is applicable for samples with a thickness smaller than or of the order of the transport length or for samples whose absorption coefficient is comparable with the backscattering scattering coefficient μ_{bs} (scattering to the backward hemisphere).

Let us here once again derive the main equations relating spectrophotometric data with optical characteristics according to the procedure used in [17]. The collimated transmittance for a layer with a thickness d is calculated according to the BLB law as

$$T_c = T_F^2 \exp[-(\mu_s + \mu_a) d], \quad (1)$$

where T_F is the Fresnel power transmittance for the normal incidence of radiation on the medium boundary.

The total scattering coefficient can be represented as a sum of the coefficient of scattering to the forward hemisphere μ_{fs} and the coefficient of scattering to the backward hemisphere μ_{bs}, $\mu_s = \mu_{fs} + \mu_{bs}$. The light passed through the layer is attenuated as a result of absorption in the medium and backscattering. Neglecting multiple backscattering, we can write the total transmittance T by analogy with the BLB law as

$$T = T_F^2 \exp[-(\mu_{bs} + \mu_a) d]. \quad (2)$$

The diffuse reflection coefficient R_d is also calculated in the single backscattering approximation. On the assumption of negligibly small losses at the integrating sphere opening, we have

$$R_d = T_F^2 - T - A, \tag{3}$$

where $A = T_F^2 [1 - \exp(-\mu_a d)]$ characterises the absorption in the medium. Based on these considerations, we obtained in [17] the following final formulas for the optical characteristics of a medium:

$$\mu_a = \frac{1}{d}\ln\left(\frac{T_F^2}{R_d + T}\right), \quad \mu_{bs} = \frac{1}{d}\ln\left(1 + \frac{R_d}{T}\right)$$
$$\mu_{fs} = \frac{1}{d}\ln\left(\frac{T}{T_c}\right), \quad \mu_s = \frac{1}{d}\ln\left(\frac{R_d + T}{T_c}\right). \tag{4}$$

The analytical model chosen to reconstruct the optical properties of a medium makes it possible to reconstruct three parameters (μ_a, μ_{bs} and μ_{fs}) by three measured photometric characteristics (T_c, T and R_d). Two latter parameters (μ_{bs}, μ_{fs}) are related to the basic optical scattering parameters, i.e., the scattering coefficient μ_s and the anisotropy factor g. However, the particular form of the relation between μ_{bs}, μ_{fs}, and g depends on the choice of the phase function model because, in fact, μ_{bs} and μ_{fs} include the probabilities of single scattering to the forward and backward hemispheres, respectively. The simplest relation is obtained in the case of the so-called transport model of the phase function [18], which is characterised by a sharp peak of forward scattering and isotropic scattering in the other directions. For this approximation, the μ_{bs} and μ_{fs} characteristics are related with the scattering coefficient μ_s and the factor g by the simple formulas

$$\mu_{fs} = \mu_s(1 + g)/2, \ \mu_{bs} = \mu_s(1 - g)/2 = \mu_s'/2,$$

where μ_s' is the transport scattering coefficient. The anisotropy factor within this model can be calculated by the following formula (which is absent in [17]):

$$g = \frac{\mu_{fs} - \mu_{bs}}{\mu_{fs} + \mu_{bs}}. \tag{5}$$

However, it should be noted that the transport model of the phase function differs from the real phase functions of biotissues and model media, because of which the values determined by formula (5) are approximate.

In the method of [17] based on numerical simulation of spectrophotometric data by the Monte-Carlo method, the systematic error of calculations using relations (4) was estimated for a large set of scattering and absorption coefficients in order to compare the reconstructed data with initial. For a 1-mm-thick cell at g = 0.4–0.6, the relative reconstruction error varies from 10% to 30% for μ_s within the range 2–5 mm^{-1} and from 7% to 15% for μ_a within the range 0.2–2 mm^{-1}.

3. Results and discussion

Using the method from paper [17], the spectral dependences of the optical properties of mouse brain and muscle biotissues *ex vivo* were determined from the measured spectral characteristics $T_c(\lambda)$, $T_d(\lambda)$, and $R_d(\lambda)$ (see Fig. 1). These results were compared with independent measurements performed in [14, 15]. Paper [14] presents the absorption coefficient and the transport scattering coefficient of a native specimen of a rat brain biotissue. The differences in the values of μ_a lie within the error related to the variations in the properties of biotissues of different individuals of one strain, which reaches 30% (according to the results of [14]). The estimated μ_s' turned out to be lower than the result for rats in [14] but was in agreement with the results obtained in [15] for native samples of mouse brain [15]. It should be noted that the method of preparation of biotissue samples used in [14, 15] was different from ours. According to [16], the difference in the absorption coefficients of cryogenically homogenised and native samples on average does not exceed 5.9%, and the scattering coefficient decreases after cryogenic homogenisation by approximately 26%, but this process is accompanied by cooling of the biotissue to 77 K. In the absence of preliminary freezing, the deviation of T_d and A of the homogenised sample with respect to the native sample does not exceed 3% [19]. Thus, the reconstructed optical characteristics in general agree with the values from the literature and can be used as basic data for creating phantoms.

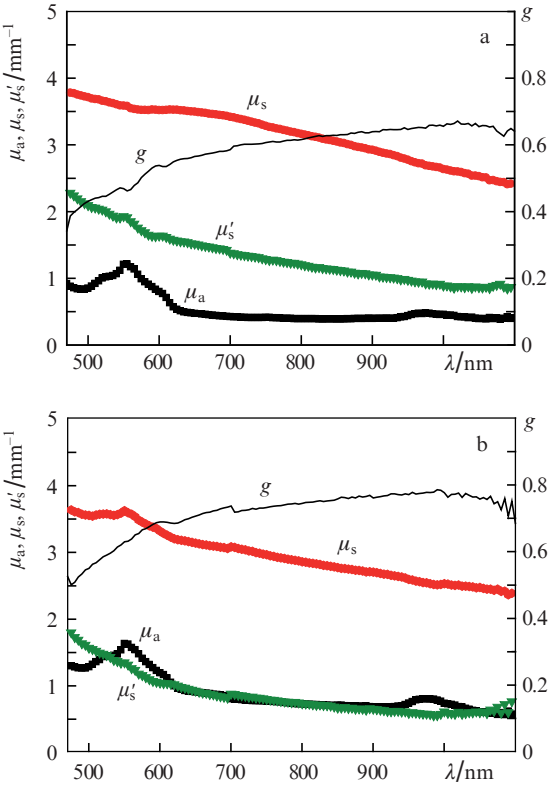

Figure 1. Spectral dependences of the absorption coefficient, scattering coefficient, anisotropy factor, and transport scattering coefficient of mouse (a) brain and (b) muscle biotissues *ex vivo*.

The absorption spectra of brain biotissues exhibit specific features typical for the absorption spectrum of haemoglobin, while the shape of the muscle absorption spectra is determined by the absorption spectra of haemoglobin and myoglobin, which have similar characters. Oxygenated haemoglobin and myoglobin exhibit two characteristic peaks with maxima at λ = 542 and 576 nm; deoxygenated haemoglobin and myoglobin have one maximum at a wavelength of 556 nm [20–22]. Thus, the shape of the obtained spectral dependences in the range 540–580 nm is determined by the concentration ratio

of oxygenated and deoxygenated haemoglobin in blood, as well as of oxygenated and deoxygenated haemoglobin and myoglobin in muscle tissue.

Separately, we studied the spectral characteristics of model media chosen as phantom components. The optical properties of black Indian ink were reconstructed in the low-order backscattering approximation because the absorption coefficient for the considered concentrations significantly exceeded not only the backscattering coefficient but even the total scattering coefficient. Since the diffuse reflectance and the difference between the collimated and diffuse transmittances for red ink did not exceed the measurement error, the optical characteristics were determined on the assumption that the scattering coefficient of red ink is negligibly small and the collimated beam is attenuated only due to absorption. The optical characteristics of Indian ink and red ink were measured using their aqueous solutions with a volume concentration of 0.1%.

However, according to data from [11], the Lipofundin absorption is considerably weaker than scattering, and the Lipofundin parameters were reconstructed using another analytical model, which is based on the diffusion approximation of the radiation transfer theory. This model, which was proposed in [3], makes it possible to reconstruct the absorption coefficient and the transport scattering coefficient and is applicable for weakly absorbing objects with a thickness larger than the transport length. In the experiment, the volume concentration of Lipofundin in a cell was chosen to be 5% to satisfy the latter condition.

Figure 2 shows the spectra of specific scattering and absorption coefficients of aqueous solutions of Lipofundin MCT/LCT 20% and absorbing components. It should be noted that the absorption spectra in Fig. 2a are plotted with preliminary subtraction of the water absorption spectra, which explains the absence of the characteristic water absorption peak in the region 970–980 nm [23].

Lipofundin possesses pronounced scattering properties with its transport scattering coefficient μ_s' varying from 0.1 to 0.3 mm^{-1}/%, which well agrees with the results of [3, 11], while the absorption coefficient is as low as ~0.008 mm^{-1}/%. The absorption coefficient of black Indian ink monotonically decreases from 4.7 to 2 mm^{-1}/% within the range 400 – 1100 nm.

The absorption spectrum of red ink has two peaks at wavelengths of 520 and 560 nm. The spectra of oxyhaemoglobin and oxymyoglobin have typical bands peaking at 542 and 576 nm, while deoxyhaemoglobin and deoxymyoglobin exhibit a peak at 556 nm [20–22]. From the viewpoint of the development of phantoms, these differences are insignificant, because of which these features can be used for modelling the spectral characteristics of haemoglobin. However, in addition to absorption, these model media are also characterised by scattering, which must be taken into account when developing phantoms.

On the assumption of linear concentration dependences of the spectral characteristics of model media [3, 8, 9], based on the plotted spectra of absorption and scattering coefficients, we determined the concentrations of oil emulsion, black Indian ink, and red ink such that the optical properties of their combination were close to the properties of modelled biotissues. Due to a strong wavelength dependence of the absorption coefficient of mouse brain, we decided to develop two phantoms mimicking the properties of biotissues in the spectral ranges 480–580 and 580–880 nm. The volume concentrations of the components for each phantom are given in Table 1. The spectral characteristics of the developed phantoms and the optical characteristic of mouse brain biotissue are presented in Fig. 3.

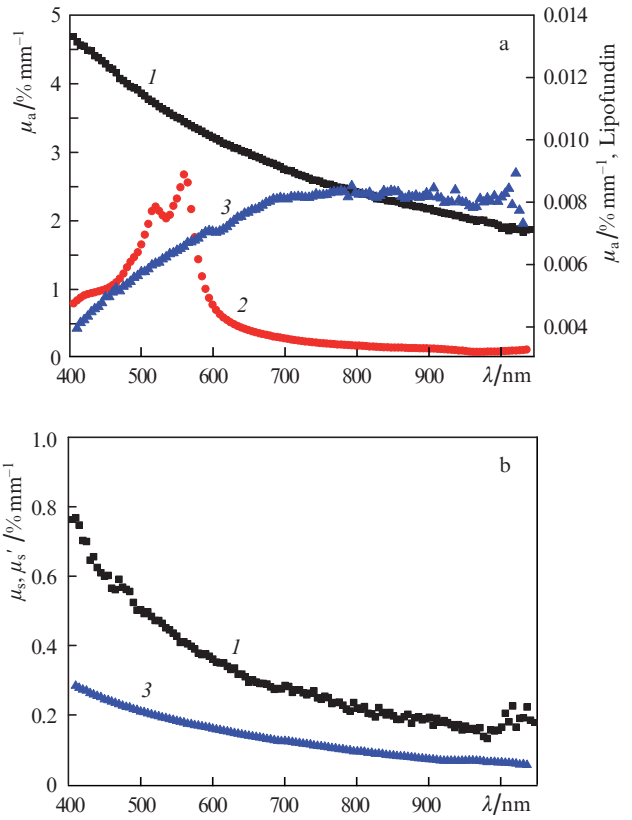

Figure 2. Specific (normalised to the volume concentration in percentage) spectral dependences of (a) the absorption coefficients of (*1*) black Indian ink, (*2*) red ink, and (*3*) Lipofundin, as well as of (b) the scattering coefficient of black Indian ink and the transport scattering coefficient of Lipofundin.

The deviation of the absorption coefficient of the phantom in the range 480–580 nm from the absorption coefficient of real biotissues varies from 0 to 25%; the corresponding deviation of the scattering coefficient does not exceed 2%. For the phantom in the wavelength range 580–880 nm, the deviations of the absorption and scattering coefficients do not exceed 40% and 25%, respectively. For clarity, the spectral dependence of the modulus of the relative deviation of the optical characteristics of the developed phantoms from the characteristics of the biotissue is presented in Fig. 4.

We studied the optical stability of the developed phantoms, i.e., their ability to retain the optical properties in time. The samples of phantoms were stored at a temperature of +4 °C. The optical characteristics of the samples were mea-

Table 1. Volume concentration of the components of phantoms for different spectral ranges.

Component	Phantom for 480–580 nm	Phantom for 580–880 nm
Lipofundin MCT/LCT 20%	12.23%	16.59%
Red ink	0.35%	0.41%
Indian ink	0.03%	0.04%
Water	87.39%	82.96%

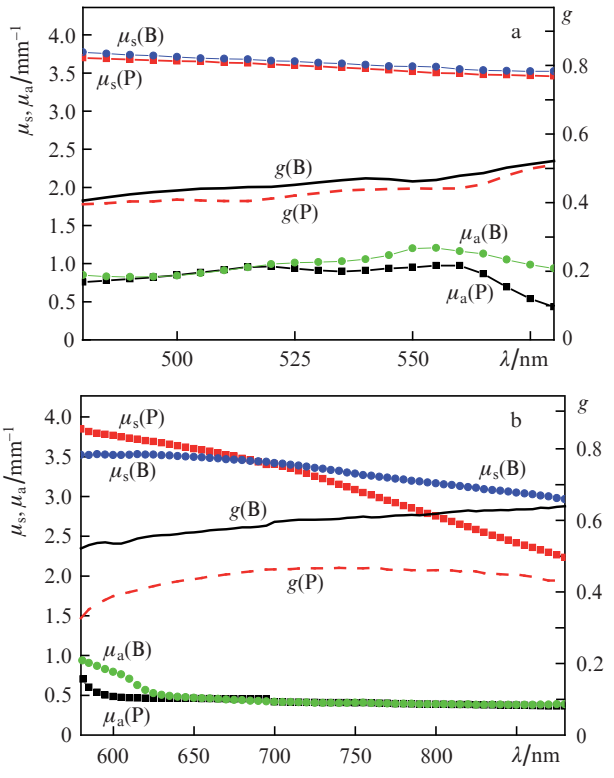

Figure 3. Comparison of the absorption and scattering coefficients and anisotropy factors of phantoms for the spectral ranges (a) 480–580 and (b) 580–880 nm with the optical characteristics of laboratory mouse brain. (P) phantom, (B) brain.

sured on the first, second, and seventh day after their preparation before and after exposure to ultrasound in an ultrasonic cleaning bath (CT-400 °C, CT Brand) with a power of 60 W for 20 min. Potentially, a long storage of samples may lead to sedimentation of Indian ink particles and aggregation of Lipofundin vesicles, which, in turn, may cause a change in the optical properties, while the action of ultrasound allows one to eliminate these effects. Comparison of the optical properties measured immediately after phantom preparation and after chosen time intervals showed that these properties differ by no more than 5%, which characterises phantoms as stable optical media.

4. Conclusions

The spectral characteristics of laboratory mouse biotissues (brain and muscles) are measured in the visible and near-IR ranges (480–1100 nm). The absorption coefficients of mouse brain and muscles vary from 0.38 to 1.21 mm^{-1} and from 0.6 to 1.6 mm^{-1}, respectively; the spectra have typical maxima belonging to the absorption by haemoglobin; the scattering coefficients of brain and muscle vary from 2.4 to 3.8 mm^{-1}and from 2.3 to 3.7 mm^{-1}, respectively. Using the obtained data, water-based optical phantoms of mouse brain for the wavelength ranges 480–580 and 580–880 nm are developed. The first phantom contains Lipofundin (12.23%), black Indian ink (0.03%), and red ink (0.35%). The phantom for the second wavelength range contains 16.59% of Lipofundin, 0.04% of black Indian ink, and 0.4% of red ink. The difference between the optical properties of phantoms and biotissue samples in the larger part of the considered spectral range

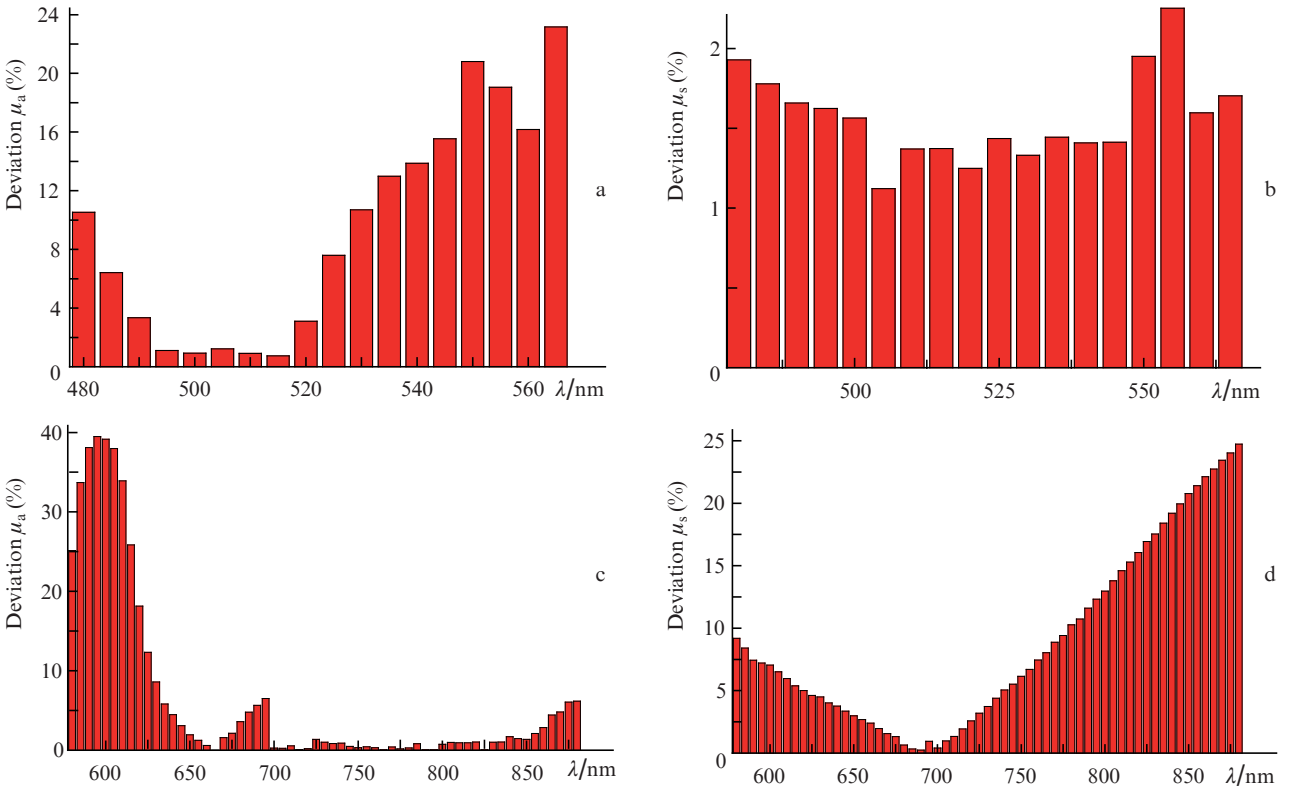

Figure 4. Moduli of the relative deviations of the (a, c) absorption and (b, d) scattering coefficients for phantoms for the spectral ranges (a, b) 480–580 and (c, d) 580–880 nm from the corresponding coefficients of mouse brain biotissues.

does not exceed 20%. Comparison of the spectral characteristics of the brain biotissue of four mice in work [14] showed that the variation in the coefficients for different individuals can reach 30%. Thus, we reconstructed the optical properties with an error no larger than the variations of optical properties from object to object. The presented phantoms are optically stable and can be used as both calibration objects and immersion media in the development and application of optical (including spectral) methods of biomedical diagnostics. Since the methods of diffuse optical tomography most frequently use the wavelength range 650–1100 nm [24] (in [25], the measurements were performed in the wavelength range 661–849 nm, while the spectral range from 650 to 930 nm was considered in [26]), the imitation of the optical properties of a biotissue in this wavelength range by a phantom makes it possible, in particular, to use it in diffuse optical tomography systems.

Acknowledgements. The authors thank A.N. Balashova and E.A. Agrba for their help in the preparation of samples. This work was supported by the Russian Foundation for Basic Research (Grant Nos 15-32-20227 and 15-02-04270).

References

1. Pogue B.W., Patterson M.S. *J. Biomed. Opt.*, **11** (4), 041102 (2006).
2. Turchin I.V., Plehanov V.I., Orlova A.G., Kamenskiy V.A., Kleshnin M.S., Shirmanova M.V., Shakhova N.M., Balalaeva I.V., Savitskiy A.P. *Laser Phys.*, **16** (5), 741(2006).
3. Krainov A.D., Mokeeva A.M., Sergeeva E.A., Agrba P.D., Kirillin M.Yu. *Opt. Spektrosk.*, **115** (2), 47 (2013).
4. Hervé L., Koenig A., Da Silva A., Berger M., Boutet J., Dinten J.M., Peltié P., Rizo P. *Appl. Opt.*, **46**, 4896 (2007).
5. Mo W., Rohrbach D., Sunar U. *J. Biomed. Opt.*, **17**, 071306 (2012).
6. Spinelli L., Botwicz M., Zolek N., Kacprzak M., Milej D., Sawosz P., Liebert A., Weigel U., Durduran T., Foschum F., Kienle A., Baribeau F., Leclair S., Bouchard J.-P., Noiseux I., Gallant P., Mermut O., Farina A., Pifferi A., Torricelli A., Cubeddu R., Ho H.-C., Mazurenka M., Wabnitz H., Klauenberg K., Bodnar O., Elster C., Bénazech-Lavoué M., Bérubé-Lauzière Y., Lesage F., Khoptyar D., Subash A.A., Andersson-Engels S., Di Ninni P., Martelli F., Zaccanti G. *Biomed. Opt. Express*, **5** (7), 2037 (2014).
7. Flock S.T., Jacques S.L., Wilson B.C., Star W.M., Gemert M.J.C. *Lasers Surg. Med.*, **12**, 510 (1992).
8. Di Ninni P., Martelli F., Zaccanti G. *Phys. Med. Biol.*, **56**, 21(2011).
9. Di Ninni P., Bérubé-Lauzière Y., Mercatelli L., Sani E., Martelli F. *Appl. Opt.*, **51**, 7176 (2012).
10. Di Ninni P., Martelli F., Zaccanti G. *Opt. Express*, **18**, 26854 (2010).
11. Royston D.D., Poston R.S., Prahl S.A. *J. Biomed. Opt.*, **1**, 110 (1996).
12. *Instruktsiya po meditsinskomy primeneniyu lekarstvennogo sredstva Lipofundin MCT/LCT 20%* (Lipofundin MCT/LCT 20% Instruction For Use).
13. Malitson H. *J. Opt. Soc. Am.*, **55**, 1205 (1965).
14. Mesradi M., Genoux A., Cuplov V., Haidar D.A., Jan S., Buvat I., Pain F. *J. Biomed. Opt*, **18** (11), 117010 (2013).
15. Moy A.J., Capulong B.V., Saager R.B., Wiersma M.P., Lo P.C., Durkin A.J., Choi B. *J. Biomed. Opt.*, **20** (9), 095010 (2015).
16. Roggan A., Schadel D., Netz U., Ritz J.-P., Germer C.T., Muller G. *Appl. Phys. B: Lasers and Optics*, **69**, 445 (1999).
17. Sergeeva E.A., Krainov A.D., Agrba P.D., Kirillin M.Yu. Metodika GSSSD ME 217-2014 (FGUP Standartinform, 26.03.2014, No. 911a – 2014).
18. Devison B. *Neutron Transfer Theory* (Oxford: Clarendon, 1958).
19. Peters V.G., Wyman D.R., Patterson M.S., Frank G.L. *Phys. Med. Biol.*, **35**, 1317 (1990).
20. http://omlc.ogi.edu/spectra/hemoglobin.
21. Schenkman K.A., Marble D.R., Burns D.H., Feigl E.O. *J. Appl. Phys.*, **82** (1), 86 (1997).
22. Bosschaart N., Edelman G.J., Aalders M.C.G., van Leeuwen T.G., Faber D.J. *Lasers Med. Sci.*, **29**, 453 (2014).
23. Hale G., Querry M.R. *Appl. Opt.*, **12** (3), 555 (1973).
24. Tuchin V.V. *Lazery i volokonnaya optika v biomeditsinskikh issledovaniyakh* (Lasers and Fibre Optics in Biomedical Studies) (Moscow: Fizmatlit, 2010).
25. Dehghani H., Eames M.E., Yalavarthy P.K., Davis S.C., Srinivasan S., Carpenter C., Pogue B.W., Paulsen K.D. *Appl. Opt.*, **42** (1), 135 (2003).
26. Eames M.E., Wang J., Pogue B.W., Dehghani H. *J. Biomed. Opt.*, **13** (5), 054037 (2008).