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OPTICS AND SPECTROSCOPY IN BIOMEDICAL INVESTIGATIONS

Effect of Storage Conditions of Skin Samples on Their Optical Characteristics

E. A. Genina, A. N. Bashkatov, V. I. Kochubey, and V. V. Tuchin

Chernyshevsky State University, Saratov, 410012 Russia

e-mail: eagenina@yandex.ru

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Abstract—The optical characteristics of skin samples are experimentally studied *ex vivo* and *in vitro* at different storage conditions. The experiments are performed on a Cary-2415 spectrophotometer in the spectral range 400–700 nm. Based on the measured diffuse reflectance and total transmittance spectra, the spectra of the absorption and reduced scattering coefficients are calculated in terms of the inverse adding–doubling method. It is shown that the method of storage of samples mainly affects the reduced scattering coefficient of biotissue. Thus, upon storage of skin in an isotonic solution and in its absence, the reduced scattering coefficient increases ~1.5 and ~2 times, respectively, compared to the value of this parameter for the intact sample. The differences in the absorption spectra of *ex vivo* samples and samples stored under different conditions are the most noticeable in the absorption range of blood and are significant above 600 nm.

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INTRODUCTION

Knowledge of the optical characteristics of biotissues is the necessary condition for obtaining adequate information on biotissues in the course of optical diagnostics and radiation dosimetry in photodynamic therapy and laser surgery, as well as upon developing adequate mathematical models of propagation of light in biotissues [1–3].

At present, spectral methods of investigating the optical parameters of biological tissues are well recognized and the results of these studies are widely presented [1–12]. One of the most frequently used methods of determining the optical parameters of biotissues is spectrophotometry using integrating spheres, which makes it possible to measure the reflection and transmission coefficients. Experimental data are mainly processed by the inverse Monte Carlo method [13, 14] and inverse adding–doubling method [15], as well as methods based on the diffusion approximation of the theory of radiation transfer [1, 3, 16–19], which make it possible to calculate the absorption coefficient and the reduced scattering coefficient of samples of biotissue under study. The advantage of the inverse adding–doubling method is that it allows one to rather rapidly process the data of spectral measurements with a satisfactory accuracy, whereas the application of the inverse Monte Carlo method reactors considerable amounts of computing time, and methods based on the diffusion approach are not free from intrinsic limitations [1, 3].

Clearly, the optical parameters of a biotissue measured *in vitro* should be maximally close in value to the parameters of this biotissue *in vivo*. This requirement causes one to use complicated methods of preparation of samples to measurements. These methods should ensure that the constant geometry of samples prevent significant changes in their structure during experiments [20–23]. An important factor of obtaining reliable measurement results of the optical parameters of a biotissue *in vitro* is also adherence to storage conditions under which the spectral pattern either does not change at all or is maximally close to the spectral pattern obtained in *in vivo* studies.

In a series of studies, it was shown that homogenization [21, 22]; rapid freezing and unfreezing [20–22]; and storage in an isotonic solution [20, 22, 23], buffer solutions [23], and preservatives [23] affect the optical properties of biotissues, such as skin with cartilaginous tissue [20], the liver [23], the aorta wall and jejunum wall [21], and bone marrow and blood [23]. The authors of [24] compared the optical properties of derma of the human skin *in vivo* and *in vitro*, obtained by different researchers.

The objective of this work is to quantitatively compare the optical parameters of *ex vivo* samples of skin with the optical parameters of the same biotissue that was stored for 24 h under different conditions, namely, in an isotonic solution and in its absence.

MATERIALS AND METHODS

Objects of study were 18 samples of skin of laboratory albino rat, seven of which were taken *ex vivo*, i.e., immediately after autopsy (group I), six samples were

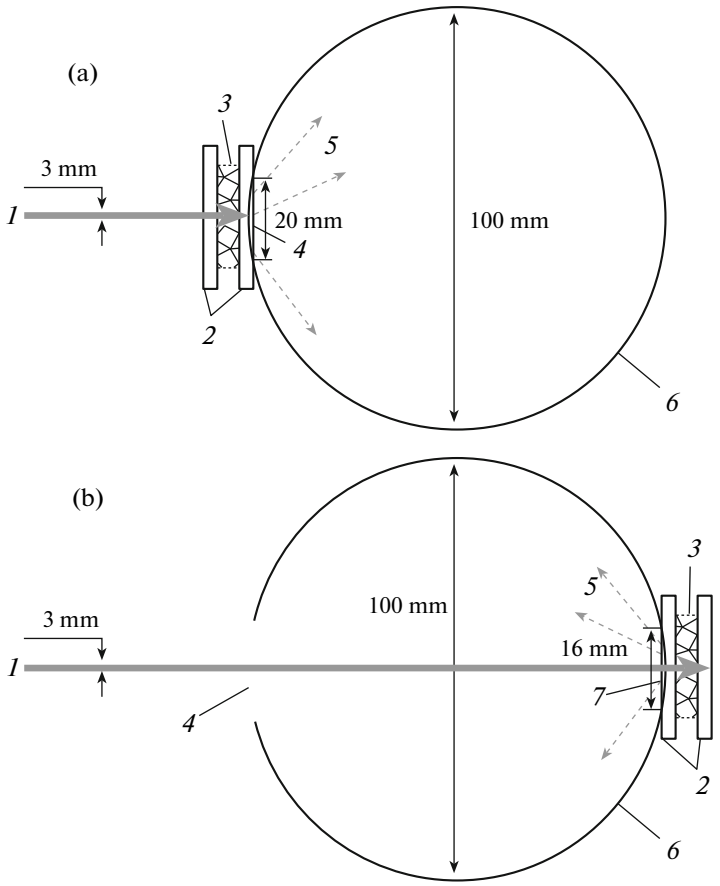

Fig. 1. Geometry of measurements of (a) total transmittance and (b) diffuse reflectance of a sample of skin: (*1*) incident beam (with a diameter of 3 mm), (*2*) slides, (*3*) skin sample, (*4*) entrance hole, (*5*) transmitted (or diffusely reflected) light, (*6*) integrating sphere, and (*7*) exit hole.

stored for 24 h at 4°C(group II), wrapped in a polyethylene film to protect from drying, and five samples were stored for 24 h at 4°C in an isotonic 0.9% solution of NaCl (pH 6.0) (group III).

Prior to experiments, hair coverings and subcutaneous fat layers were carefully removed from the surfaces of samples. The skin was cut into samples with an area of about 20 × 20 mm.

To measure the thickness of the samples, they were placed between two slides; the measurements were performed with a micrometer at several points of each sample. The accuracy of each measurement was ±50 μm. The obtained values were averaged. The average thickness of the samples was 0.73 ± 0.08 mm. The thickness of samples of each group was also measured immediately prior to spectrophotometric measurements. The standard deviation (*SD*) was calculated by the formula

$$SD = \sqrt{\frac{\sum_{i=1}^{N}(\bar{A} - A_i)^2}{N(N-1)}},$$

where $N = 10$ is the number of the measured samples, A_i is the thickness of the ith sample of the biotissue, and $\bar{A}$ is the average thickness determined by the formula $\sum_{i=1}^{N} A_i/N$.

The spectra of the total transmittance T_t and diffuse reflectance R_d of the skin samples were measured in the spectral range 400–700 nm on an Cary-2415 double-beam spectrophotometer (Varian, Australia) equipped with an integrating sphere (Fig. 1). The inner diameter of the sphere is 100 mm, the size of the entrance hole is 20 × 20 mm, and the diameter of the exit hole is 16 mm. A halogen lamp served as a source of radiation, which was filtered in the studied spectral range. The diameter of the light beam incident on the sample of biotissue was 3 mm. The scanning rate was 2 nm/s. All the experiments were performed at room temperature (about 20°C).

The entrance and exit holes of the sphere were located on the same axis. To measure the total transmittance spectrum, the exit hole was closed by a scattering $BaSO_4$ standard. The sample of skin was fixed

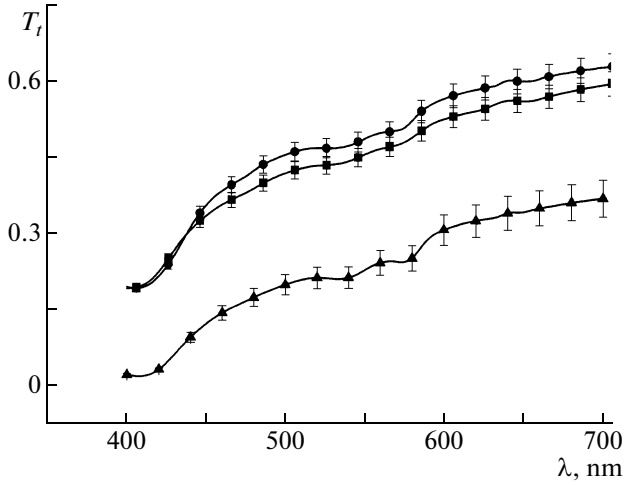

Fig. 2. Total transmittance spectra of skin samples of (squares) group I, (circles) group II, and (triangles) group III. Vertical lines correspond to the standard deviation.

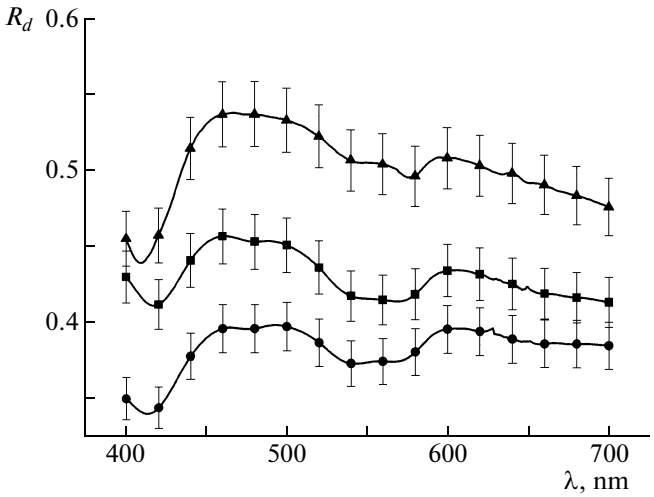

Fig. 3. Diffuse reflectance spectra of skin samples of (squares) group I, (circles) group II, and (triangles) group III. Vertical lines correspond to the standard deviation.

between two slides with a thickness of 1 mm and was placed in front of the entrance hole. To measure the diffuse reflectance spectrum, the standard was replaced by the sample under study, which was arranged immediately behind the exit hole.

To process the experimental data and to determine the optical parameters of biotissues, the inverse adding–doubling method was used [15]. This method makes it possible to determine the absorption coefficient and the reduced scattering coefficient one biotissue (i.e., μ_a and $\mu_s' = \mu_s(1-g)$, where μ_s is the scattering coefficient and g is the scattering anisotropy factor) from the values of the coefficients of the total transmission T_t and diffuse reflection R_d. In all calculations, the anisotropy factor was assumed to be 0.8, since this value is typical for the most biotissues in the visible wavelength range [3]. The used algorithm includes the following steps:

(i) setting the assumed optical parameters;

(ii) calculating the diffuse reflection and total transmission coefficients by the adding–doubling method [15];

(iii) comparing the calculated and experimentally measured values of these parameters;

(iv) performing the iterative procedure until the calculated and experimental data match each other within a specified accuracy.

RESULTS AND DISCUSSION

Figures 2 and 3 present the averaged total transmittance and diffuse reflectance spectra of skin samples for each of the three groups. Certain differences between the spectra within each group were primarily caused by differences in the sample thickness and by individual peculiarities of the structure (thickenings of collagen fibers, blood vessels, etc.).

It can be seen from these figures that the spectra of total transmittance and diffuse reflectance of samples from all the three groups are similar in shape, but the values of the coefficients are different. Thus, the total transmission coefficients of the samples of group II (i.e., the samples that were stored in the absence of the isotonic solution) somewhat exceed the values of T_t for the intact skin. This fact can be explained by an insignificant clearing of skin as a result of partial dehydration of the sample that occurs despite the protective action of the polyethylene film. As is known, the removal of water from the interstitial space results in matching between the refractive indices of collagen fibers and interstitial fluid because the concentrations of proteins and salts in the interstitial fluid increase, which increases the transparence of the biotissue [25]. The dehydration of the skin also leads to a decrease in the sample thickness. The thickness of the samples of this group measured immediately prior to spectrophotometric experiments was 0.65 ± 0.12 mm, which is smaller than the initial thickness by approximately 10%.

The coefficient of the total transmission of the samples of skin stored in the isotonic solution is lower by a factor of 1.5–2 compared to the value of T_t for the intact skin. This decrease is related to the replacement of the interstitial fluid by the isotonic solution, which causes an increase in light scattering [25], and to certain swelling of the biotissue during storage, which results in an increase in the sample thickness. The average thickness of the samples of group III measured prior to spectrophotometry was 0.84 ± 0.17 mm,

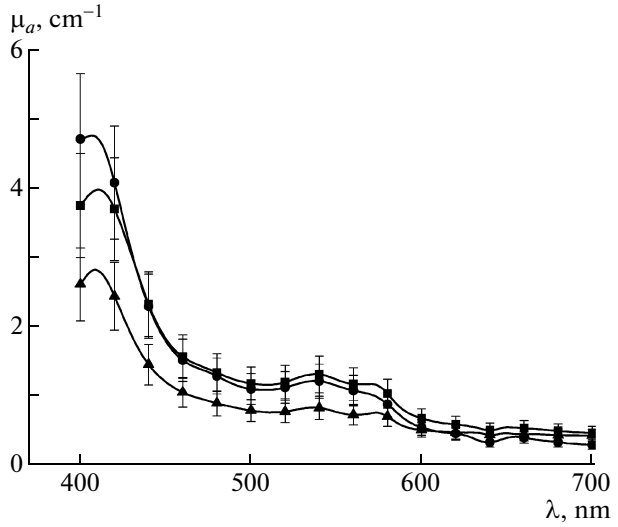

Fig. 4. Wavelength dependences of the absorption coefficient of skin samples stored under different conditions: (squares) group I, (circles) group II, and (triangles) group III. Vertical lines correspond to the standard deviation.

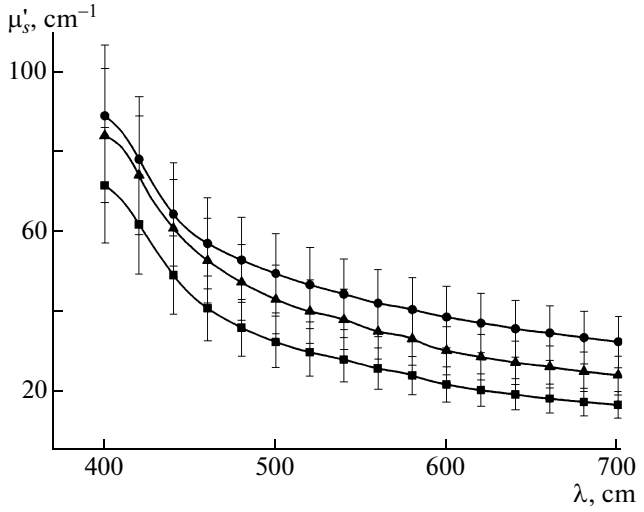

Fig. 5. Wavelength dependences of the reduced scattering coefficient of skin samples stored under different conditions: (squares) group I, (circles) group II, and (triangles) group III. Vertical lines correspond to the standard deviation.

which is higher by 15% than the average thickness of these samples measured prior to the experiment.

The diffuse reflectance spectra of the skin samples from different groups also exhibit differences related to changes in the optical parameters of skin during its storage. These differences directly correlate to the differences observed in the total transmittance spectra. The coefficient R_d is the largest for the samples of group III (the storage in the isotonic solution) and is the smallest for the samples of group II (the storage in the absence of the isotonic solution).

Figures 4 and 5 show the absorption and reduced scattering coefficients calculated by the inverse adding–doubling method. The calculations were performed for each sample, and then the results were averaged for each group.

It can be clearly seen that the absorption coefficients of all the groups of samples are rather close to each other. Differences (up to 40%) are only observed in the absorption range of hemoglobin near 405 nm (the Soret band) and in the range 540–570 nm. The minimal value of μ_a in this wavelength range shows the skin stored in the isotonic solution, which is related to the washing-out of blood from the sample. In the absence of the isotonic solution, changes in the absorption spectrum of the skin can be attributed to a weak dehydration of the sample which leads to an increase in the concentration of hemoglobin in capillaries and, consequently, to an increase in the absorption coefficient of the skin in the range of the absorption bands of hemoglobin. A decrease in the resolution of absorption bands in the range 540–570 nm can be attributed to deoxygenation of blood during the storage of samples without access for air [23]. In the spectral range above 600 nm, differences between the absorption spectra of *ex vivo* samples and samples stored under different conditions are insignificant. It is seen that the absorption spectra of different samples are partially overlapped; consequently, we can state that the storage of samples for 24 h does not significantly affect the coefficient μ_a of biotissue.

Figure 5 clearly demonstrates that the coefficient μ_s' of the native skin is the smallest in the range 400–700 nm. An increase in μ_s' of the samples that were stored in the isotonic solution is explained by an increase in the ratio between the refractive index n_c of skin scatterers (mainly, collagen fibers, for which $n_c = 1.411$ [3]) and the refractive index of the interstitial fluid n_b (for native skin, $n_b = 1.365$ [3]). In the samples that were stored in the isotonic solution, the replacement of the interstitial fluid by the isotonic solution ($n_w = 1.333$) leads to an increase in the scattering compared to the native skin. On average, the reduced scattering coefficient of the tissue increases by a factor of 1.5. However, the storage of the skin in the absence of the isotonic solution changes the value of μ_s' in the visible wavelength range even more. In this case, the reduced scattering coefficient of the tissue increases approximately two times. On one hand, due to the dehydration, the refractive index in the interstitial fluid increases, which causes an increase in the degree of matching between the refractive index of collagen fibers and the base substance and, in turn, optical clearing, i.e., a reduction in the light scattering. On the other hand, the dehydration of the biotissue leads to an

increase in the density of packing of scatterers and, as a consequence, to an increase in the light scattering [25]. It is likely that this process prevails over the clearing, which manifests itself in a general increase in the reduced scattering coefficient.

Therefore, in our opinion, the storage of skin samples in isotonic solution is the most favorable method of the two studied, since it distorts least the spectra of absorption and reduced scattering of biotissue.

A comparison of our results with the data available in the literature showed that, under similar storage conditions, various biotissues show similar behavior of their absorption and scattering. Our results qualitatively correlate with the data of [22]. In the wavelength range 440–690 nm, the absorption coefficient and the reduced scattering coefficient of samples of pig liver stored for 24 h at 4°C and kept for 5 min in an isotonic solution exhibit similar behavior.

The authors of [20] observed that the absorption coefficient of samples of skin on a cartilaginous tissue (rat ear) also decreases upon storage in an isotonic solution. However, the behavior of the scattering coefficient of these samples differs from the behavior of the reduced scattering coefficient of skin observed in this work. In [20], the scattering coefficient decreases during the storage. It is likely that this distinction can be explained both by differences in the methods of storage (in that work, the samples were not immersed into the isotonic solution but were wetted by it) and by different anatomic properties of the investigated objects (skin samples from the body of the animal and from its ear).

CONCLUSIONS

We experimentally studied the optical characteristics of *ex vivo* skin samples and samples stored for 24 h at 4°C in a polyethylene film or in an isotonic 0.9% solution of NaCl. We showed that, although it is preferable to store samples of biotissue in the isotonic solution, it should nevertheless be taken into account that, in this case, the scattering of the biotissue increases and its absorption decreases compared to *ex vivo* tissue samples.

Our results can be used in analysis of data of optical measurements of optical parameters of skin, which is important in development of methods of optical diagnostics and therapy of various diseases.

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