The influence of glycerol on the transport of light in the skin

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ABSTRACT

We present experimental results on optical properties of the rat skin controlled by administration of osmotic chemical, such as *glycerol*. Administration of *glycerol* induces matter diffusion and as a result equalization of the refractive indices of skin scatterers and interstitial fluid. The significant increase of the skin sample transmittance under action of *glycerol* has been demonstrated. The mean value of the *glycerol* diffusion coefficient has been estimated as $5.1 \cdot 10^{-7} \pm 2.3 \cdot 10^{-7}$ cm^2/sec .

Keywords: light scattering, skin, osmolytes, refractive index matching.

1. INTRODUCTION

Absorption and scattering properties of living tissues, in particular skin, can be effectively controlled due to the matching of the refractive indices of scattering centers and ground matter by intra-tissue administration of the appropriate chemical agent.[1-18] Experimental studies on optical clearing of normal and pathological skin and its components (epidermis and dermis) and the management of reflectance and transmittance spectra using *water*, *glycerol*, *glycerol-water* solutions, *glucose*, sunscreen creams, cosmetic lotions, gels and pharmaceutical products were carried out in Refs. 5, 7, 11-22. Controlling of skin optical properties was related to the immersion of refractive indices of scatterers (keratinocytes components in epidermis, collagen and elastin fibers in dermis) and ground matter. For some tissues drying of collagen fibers and cells swelling or shrinkage should be taken into account as the secondary effects.[10,14]

A marked clearing effect through the rat skin (reduced back reflectance) was occurred for an *in vivo* tissue within a few minutes of intra-dermal injection of *glycerol*.[5] *In vivo* topical impregnation of the human skin by *glycerol*, *glucose*, *trazograph* (x-ray contrasting substance), cosmetic lotions and gels also made skin more translucent in a few minutes.[7,11,12,14,16,22] The loss of water by the skin or its impregnation by water or moisturizing substances seriously influences skin optical properties.

Knowledge of tissue optical properties in the visible range is of great importance for biomedical optics. Recently, many groups have reported the optical parameters of various tissues.[5,9,13-15] Such data are needed for laser diagnostics, surgery and therapy. In the mentioned above papers, the optical properties were defined based on rigorous or approximate solution of the radiative transfer equation for a turbid medium. In this case, the optical properties are described by phenomenological coefficients, like absorption and scattering, and anisotropy factor. For describing of light propagation in tissues using Mie theory[23] it is necessary to know the scatterer sizes and dependence of refractive index on the wavelength of both scatterers and interstitial fluid. Similar problem arises in biomedical optics when control of tissue optical properties is analysed.[1-14]

For estimation of the mean scatterers size the spectroturbidimetric method described in Ref. 28 can be used. This method allows for estimate particle size within range from 0.1 to 1 μm (typical values for many of tissues). One of the main scattering characteristics used in this version of spectroturbidimetric method is the wavelength exponent *n* in the following approximation expression for turbidity (since for many tissues scattering predominates over absorption then turbidity is characterized by a scattering coefficient):

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$$\mu_s = const\, \lambda^{-n} \tag{1}$$

where μ_s is the scattering coefficient and λ is the wavelength. Exponent n is determined on the basis of experimental dependence for the scattering coefficient

$$n = -\partial \ln \mu_s / \partial \ln \lambda \ . \tag{2}$$

Note, that n is independent on particle concentration. The substituting in Eq. (2) of the theoretical expression for μ_s obtained for the model of disperse system and comparison it with experimental value of wavelength exponent allows one to estimate the mean particle size of the disperse system under study. It is known that scattering properties of the polydisperse system are equivalent to the properties of the monodisperse system if volume fraction of the scatterers is the same for both systems.[28] As a first approximation, n can be described by the formula

$$n = -\partial \ln Q_s / \partial \ln \lambda \ , \tag{3}$$

where the scattering efficiency factor Q_s calculated for monodisperse systems of homogeneous spherical isotropic particles using Mie theory.[28]

Because of relatively big thickness of dermis (up to 1-2 mm for the human skin) and comparable scattering coefficients of epidermis and dermis the optical properties of the skin is defined by the optical properties of the dermis. Dermis is a typical fibrous tissue. The structure and properties of fibrous tissues are described in details in Refs 12-14. Unfortunately, there are only a few papers reporting the values of refractive indices of fibrils (the ground scatterers in fibrous tissues).[1,5,24-27]

In this paper, we have presented the results of experimental *in vitro* study and computer modeling of the rat skin optical properties and their changes under action of *glycerol*. The values of scatterer size of the rat skin tissue and their changes under action of *glycerol* were estimated.

2. MATERIALS AND METHODS

2.1 Experimental setups

The measurements of the light collimated transmittance spectra were performed using OMA (spectrometer LESA-6med, BioSpec, Russia). The scheme of the experimental setup is shown in Fig. 1. A 250 W xenon arc lamp with filtering of the radiation in the 400 - 700 nm spectral range was used in these measurements.

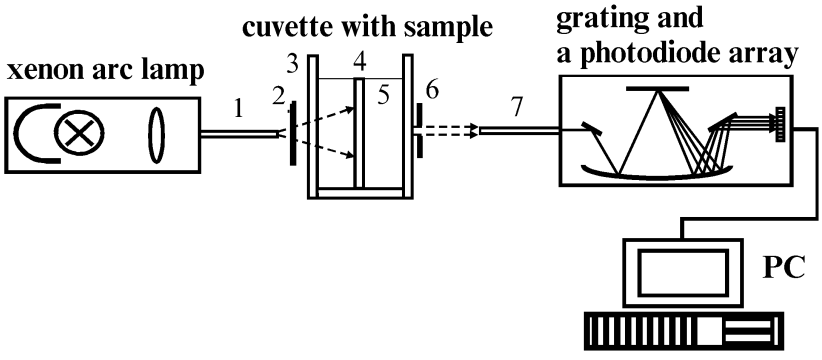

Fig. 1: Experimental setup for measurements of the collimated light transmittance spectra: 1 – optical fiber; 2 – neutral filters; 3 - cuvette; 4 – rat skin sample; 5 – osmotical active solution (*glycerol*); 6 – 0.5 mm – diaphragm; 7 – optical fiber.

In the case of *in vitro* light transmission measurements the cuvette with skin sample was placed between two optical fibers (400 μm core diameter). One fiber supply the excitation radiation to the sample, and another fiber collected the transmitted radiation. The 0.5 mm diaphragm placed 20 mm apart from the tip of the receiving fiber was used to provide collimated transmittance measurements. Neutral filters were used to attenuate the incident radiation. The measurements were performed every 30 sec for 20 – 45 min.

2.2 Tissue samples preparation

In vitro experiments were performed with 6 samples of the rat skin tissue extracted from various rat specimens. Tissue samples were obtained just *post mortem*. Before experiments hairs were removed by tweezers. Values of tissue sample thickness are presented in Table 1. Sample thickness was measured just before experiments. Experiments were made within hour *post morten*. Before experiments, the rat skin tissue was cut into pieces with the area about 1 cm^2. All experiments were performed at room temperature.

Table 1. Thickness of the rat skin tissue samples, averaged for ten measurements

Sample	Thickness, mm (standard deviation)
Sample 1	0.58 (0.06)
Sample 2	0.77 (0.04)
Sample 3	0.78 (0.06)
Sample 4	0.9 (0.08)
Sample 5	0.63 (0.04)
Sample 6	0.69 (0.06)

2.3 Estimation of *glycerol* diffusion coefficient

For estimation of *glycerol* diffusion coefficient, we used experimental data for the collimated transmittance, which are presented in Figs. 4-9. The transport of *glycerol* in tissue sample can be described in the framework of the matter diffusion model.[1] The diffusion equation for the local variation of *glycerol* concentration within a skin layer can be presented in the form

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2},$$

where C is the *glycerol* concentration, D is the diffusion coefficient $[D]=cm^2/\sec$, and x is the spatial coordinate $[x] = cm$.

The solution of the diffusion equation for a plane slab with a thickness d, at the moment t (the initial concentration of this substance within the slab is equal to 0, i.e., $t = 0; 0 \le x \le d; c = 0$) has the form

$$c=c_0\left\{1-\frac{4}{\pi}\left[\exp(-t/\tau)\cdot\sin(\pi x/d)+\frac{1}{3}\exp(-9t/\tau)\cdot\sin(3\pi x/d)+\frac{1}{5}\exp(-25t/\tau)\sin(5\pi x/d)+...\right]\right\},$$

where $\tau = \frac{d^2}{D}$ is the diffusion constant, D is the diffusion coefficient, d is the thickness of the skin sample. The first approximation of this equation can be presented as

$$C=C_0\cdot\left(1-\exp\left(-\frac{t}{\tau}\right)\right),$$

that is very close to the equation describing diffusion through a partially permeable membrane.

We assume that the following approximations are valid for description of *glycerol* transport:

a) The diffusion constant is constant over the entire skin volume.

b) Volume fraction of *glycerol* in interstitial fluid is defined as $C_{gl}(t) = C_{gl}^{0} \cdot \left(1 - \exp\left(-\frac{t}{\tau_{gl}}\right)\right)$, where C_{gl}^{0} is the concentration of *glycerol* in the external volume (expressed in volume fractions); $\tau_{gl} = d(t)^2/D_{gl}$ is the diffusion constant; D_{gl} is the diffusion coefficient; $d(t)$ is the time-dependent thickness of the skin sample. *Glycerol* is very strong osmotic liquid. Therefore, we took into account decreasing thickness of skin tissue sample. For estimation of the time dependence of the skin tissue thickness we have offered the following phenomenological expression: $d(t) = d(t=0) - A \cdot \left(1 - \exp\left(\frac{t}{\tau_{sh}}\right)\right)$, where A and τ_{sh} are some phenomenological constants for describing of shrinkage process caused by *glycerol* action.

c) The refractive index of the interstitial fluid during the *glycerol* action can be estimated as $n_1(t) = (1 - C_{gl}(t)) \cdot n_{base} + C_{gl}(t) \cdot n_{gl}$, where n_{base} is initial refractive index of the interstitial fluid and n_{gl} is refractive index of *glycerol*. We have measured the refractive index of *glycerol* with the standard method. It is equal 1.47, that is very close to the refractive index of collagen fibers.

d) The scattering coefficient dependence on the refractive index is defined by the following relationship: $\mu_s(t) = N \cdot \sigma_s(t)$, where N is the number particles in the volume unit and σ_s is the cross-section of scattering, which is determined by the Mie theory. N can be estimated as $N = \frac{\mu_s(\text{experimental value for } \lambda = 589\,\text{nm})}{\sigma_s(\text{calculated value for } \lambda = 589\,\text{nm})}$ in the initial moment. The values of refractive indices of collagen particles and interstitial fluid are 1.47 and 1.36, respectively.[14] These values are necessary for calculation σ_s at wavelength 589 nm. For calculation of the time-dependent scattering cross-section $\sigma_s(t)$, we have used the Mie theory. Algorithm of this calculation was described in Ref. 23. We assume that the refractive index of collagen particles don't changes during *glycerol* action and the time dependence of scattering cross-section is connected with the change of refractive index of interstitial fluid.

e) The collimated transmittance of the skin sample impregnated by *glycerol* can be defined as: $T_c(t) = (1 - R_s)^2 \cdot \exp(-(\mu_a + \mu_s) \cdot d(t))$, where R_s is the specular reflectance and μ_a and μ_s are the absorption and the scattering coefficients, respectively.

This set of relations describing the *glycerol* concentration in dependence on time represents the direct problem. The reconstruction of the diffusion coefficient of the skin sample were carried out on the basis of measured time evolution of the collimated transmittance. The solving of the inverse problem were done by minimization of the target function: $F(t) = (T_c(t) - T_c^*(t))^2$, where $T_c(t)$ and $T_c^*(t)$ are respectively the theoretical and experimental values of the time-dependent collimated transmittance.

The Levenberg-Marquardt method has been used for the minimization of the target function. The Levenberg-Marquardt method is a quasi-Newton method (a variation of the gradient method). At each step, we estimate the first partial derivatives of the errors with respect to the variables to be solved to create a Jacobian matrix. Ordinarily, we can determine the next estimate to make by computing the Gauss-Newton step s for each variable. In matrix notation, we solve the matrix equation: $J \cdot s = -f(x)$. In this equation, J is the Jacobian matrix, s is the step to take, and x is the vector of current estimates for unknown variables. For the first step, x is the vector of guesses; at each subsequent step, the new x is the previous x plus s, the vector of steps. Notice, that computing of this step involves inverting the Jacobian matrix J.

Computing of this step is not always possible. It is fail when the Jacobian matrix can not be inverted or when there are more constraints than variables to be solved. In these cases, we add the additional condition that the following quantity be reduced to a minimum: $\sum_j (D_j)^2 (s_j)^2$. Here D is a vector of weight factors computed from the norms of the columns of the Jacobian matrix. In these cases, s is computed to satisfy this minimization criteria as well as solving the Newton equation with the Jacobian.

3. RESULTS AND DISCUSSION

3.1 Estimation of the mean scatterers size of the rat skin

For estimation of the mean scatterers size we used spectroturbidimetry method described in Ref. 28 and experimental data presented in Figs. 3-9. To obtain dependence of wavelength exponent on diameters of the scatterers (see Eq. 3) we have used the Mie theory with the following parameters: 1) the spectral range is from 600 to 700 nm, 2) refractive index of the scatterers is 1.47 and refractive index of the interstitial fluid is estimated from Gladstone-Dale law. The range of the change of the scatterer's diameters has been set from 0.1 to 1 μm. At the calculations, we have used the following additional condition: anisotropy factor must be in the range from 0.8 to 0.9. This condition allows one to avoid oscillating character of the spectra. Results of this calculation are presented in Fig. 2. We can see, that scatterer's size decrease during the time clearing. It is caused by osmotical nature of the clearing substance (*glycerol*).

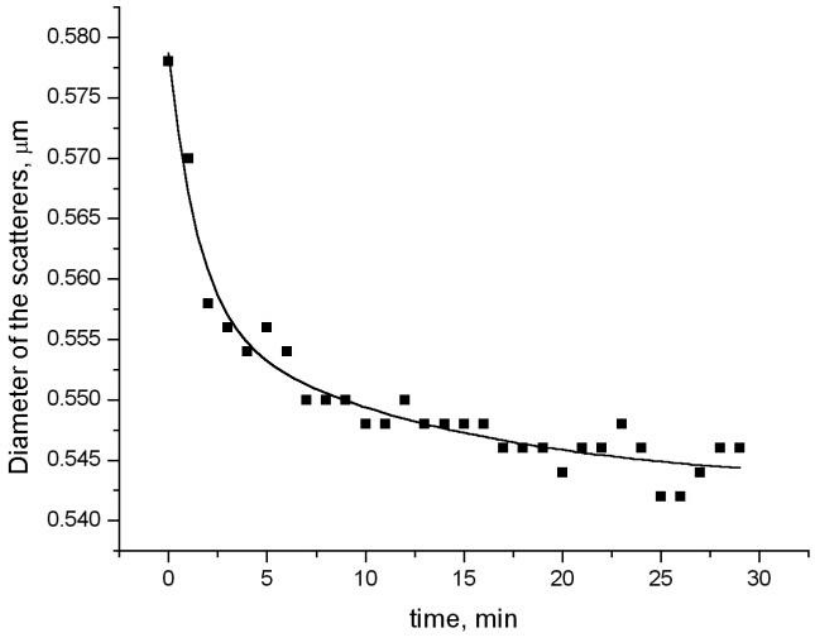

Fig. 2: The time-dependent "effective" diameter of the rat skin scatterers (sample 2). Symbols correspond to experimental data and solid line corresponds to fitting of the experimental data.

3.2 *In vitro* study of the optical clearing of the rat skin

To understand the mechanisms of the rat skin tissue optical clearing we have studied the collimated transmittance spectra concurrently with administration of *glycerol*. Figure 3 illustrates the typical collimated transmittance spectra (sample 4). It is well seen that the untreated rat skin is poorly transparent for the visible light. *Glycerol* administration makes this tissue highly transparent, for example, increasing transmittance up to 10 times at 700 nm for the sample kept

in *glycerol* for t = 45 min. Figures 4-9 present the time-dependent collimated transmittance at different wavelengths. They show the dynamics of tissue clearing for various skin samples.

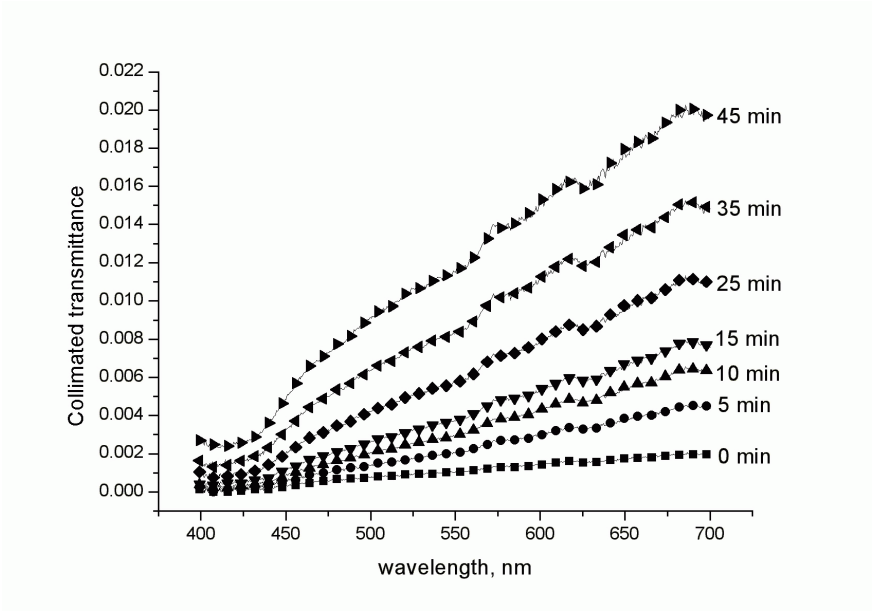

Fig. 3: The collimated transmittance spectra of the rat skin sample measured concurrently with administration of *glycerol* at different time intervals (sample 4).

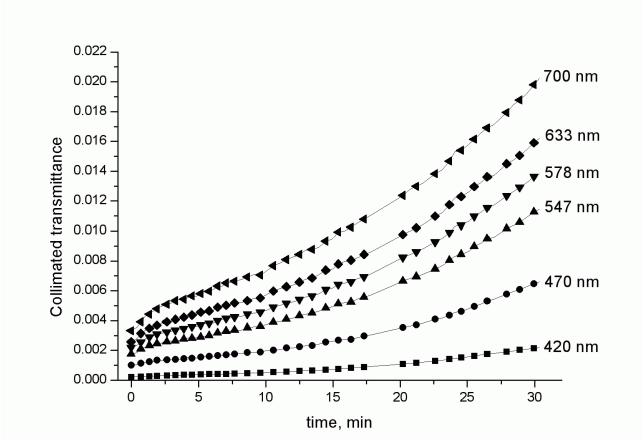

Fig. 4: The time-dependent collimated transmittance of the rat skin sample (sample 1) measured at different wavelength concurrently with administration of *glycerol*.

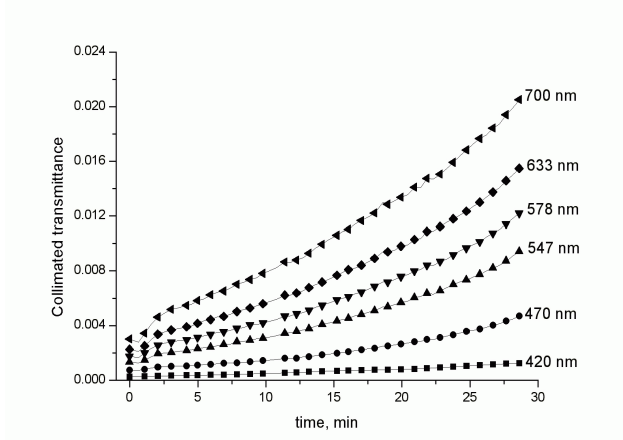

Fig. 5: The time-dependent collimated transmittance of the rat skin sample (sample 2) measured at different wavelength concurrently with administration of *glycerol*.

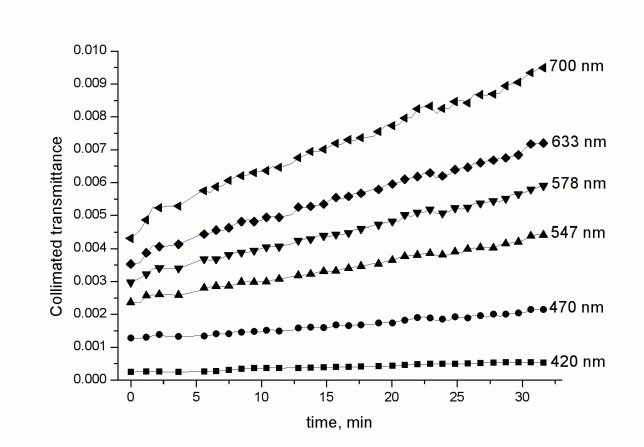

Fig. 6: The time-dependent collimated transmittance of the rat skin sample (sample 3) measured at different wavelength concurrently with administration of *glycerol*.

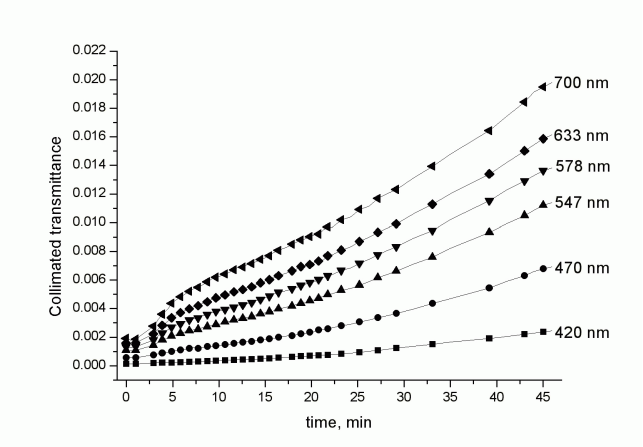

Fig. 7: The time-dependent collimated transmittance of the rat skin sample (sample 4) measured at different wavelength concurrently with administration of *glycerol*.

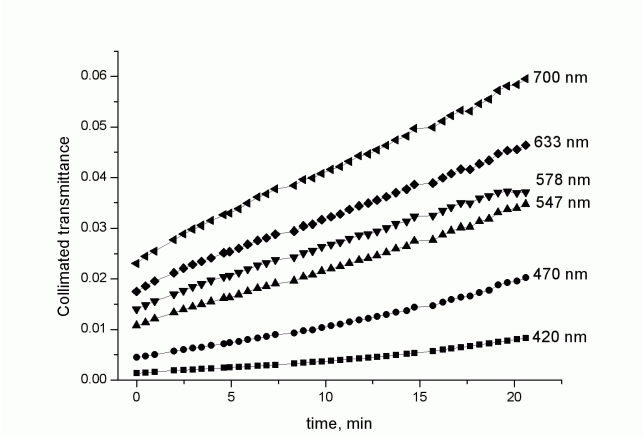

Fig. 8: The time-dependent collimated transmittance of the rat skin sample (sample 5) measured at different wavelength concurrently with administration of *glycerol*.

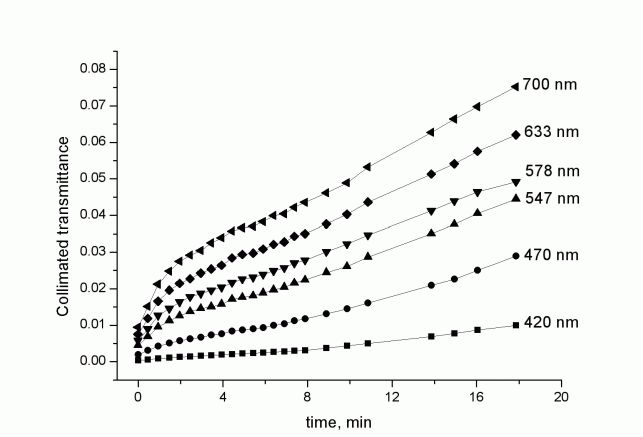

Fig. 9: The time-dependent collimated transmittance of the rat skin sample (sample 6) measured at different wavelength concurrently with administration of *glycerol*.

Presented experimental results well show that the rat skin transmittance spectra can be substantially changed by administration of osmotic chemical agent (*glycerol*). Analyzing the data presented in a Figs. 4-9 and comparison with data obtained for dynamics of optical clearing of another fibrous tissue, it can be concluded that permeability of agent into the skin less than that into the sclera or into the *dura mater*. Representative clearing time for scleral tissue is about 8 min. Thus, we can connect rather prolonged clearing time and the absence of saturation of the clearing curve with protecting properties of epidermis and fat layer, which preventing fast penetration of agent into dermis and backward flow of interstitial fluid (water) from the sample into surrounding liquid (glycerol). In this case, action of osmotic stress allows for describing the mechanism of clearing of the skin tissue, as *glycerol* is a highly osmotic substance. Dehydration of the samples leads to high clearing. The shrinkage process takes place in this case. After action of *glycerol* on the tissue, it becomes more dense and rigid. From the other hand, the long-time clearing of the samples can be explained by a high viscosity of *glycerol* that prevents rapid penetration of this matter into a tissue.

We have calculated *glycerol* diffusion coefficients using the method presented in Sec. 2.3. The values are presented in Table 2.

Table 2. The *glycerol* diffusion coefficients obtained from *in vitro* measurements time-dependent collimated transmittance of the rat skin samples under action of *glycerol*.

Sample	The *glycerol* diffusion coefficients, cm^2/sec ± standard deviation
Sample 1	$3.5 \cdot 10^{-7} \pm 5.8 \cdot 10^{-10}$
Sample 2	$6.4 \cdot 10^{-7} \pm 1.8 \cdot 10^{-9}$
Sample 3	$2.5 \cdot 10^{-7} \pm 1.2 \cdot 10^{-10}$
Sample 4	$5.4 \cdot 10^{-7} \pm 3.7 \cdot 10^{-9}$
Sample 5	$4.1 \cdot 10^{-7} \pm 1.8 \cdot 10^{-9}$
Sample 6	$8.8 \cdot 10^{-7} \pm 1.5 \cdot 10^{-8}$

From data of Table 2 the mean value of the *glycerol* diffusion coefficient can be found as $5.1 \cdot 10^{-7} \pm 2.3 \cdot 10^{-7}$ cm^2/sec. From the Handbook on Physical Values (see, Ref. 29) it follows that diffusion coefficient of *glycerol* in water at 15^0C is $7.2 \cdot 10^{-6}$ cm^2/sec, water in water at 20^0C is $1.0 \cdot 10^{-5}$ cm^2/sec, and water in glycerol at 27^0C is $8.3 \cdot 10^{-8}$ cm^2/sec. Supposing that interstitial liquid within the space between collagen and elastin fiber of dermis is mostly water it can be stated that for tissue clearing at *glycerol* application both flows of water directed outside tissue and glycerol inside tissue are important. More than one order difference between diffusion coefficients of glycerol in water and water in water and measured value for skin can be referred to interaction of mentioned flows with tissue structures and between each other. It should be also noted that for physiological temperatures (*in vivo* conditions) diffusion coefficients increased on about 13% per each 5 degrees.[29]

CONCLUSION

Experimental data on optical properties of the rat skin controlled by topical administration of *glycerol* are presented. The significant increase of the skin sample transmittance under action of *glycerol* has been demonstrated. It was shown that administration of *glycerol* induces matter diffusion (glycerol inside tissue and water outside) and as a result equalization of the refractive indices of skin scatterers and interstitial fluid. The mean value of the *glycerol* diffusion coefficient has been estimated as $5.1 \cdot 10^{-7} \pm 2.3 \cdot 10^{-7}$ cm^2/sec.

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