In vivo and *in vitro* study of control of rat skin optical properties by acting of osmotical liquid

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ABSTRACT

We present experimental results on optical properties of the rat skin controlled by administration of osmotically active chemical, such as *glycerol*. *In vivo* reflectance and *in vitro* reflectance and transmittance spectra of the rat skin were measured. Results of experimental study of influence of *glycerol* on reflectance spectra of the rat skin are presented. The significant increase of transmittance and decrease of reflectance of the rat skin under action of osmotical agent are demonstrated. Basing on mathematical modeling the optical parameters of the rat skin are determined.

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

1. INTRODUCTION

Absorption and scattering properties of living tissues, particular in skin, can be effectively controlled by matching of the refractive indices of scattering centers and ground matter by means of intra-tissue administration of the appropriate chemical agents.[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 as the secondary effect, drying of connective tissue fibers and cells swelling or shrinkage should be taken into account.[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 research groups have reported the optical parameters of various tissues.[5,9,13-15,23] Such data are very important 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[24] 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]

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).[1,5,25-28]

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For estimation of refractive indices of skin tissue components at various wavelengths, it is necessary to replace one of the components defining scattering by the substance with known refractive index. For example, the interstitial fluid can be replaced by a saline. Measuring of the scattering properties of the sample, knowing the scatterer sizes, refractive indices of the interstitial fluid under study it is possible to estimate the value of refractive index of the scatterers.

In this paper, we have presented the results of experimental *in vivo* and *in vitro* study and computer modeling of the rat skin optical properties and their changes under action of *glycerol*. The values of refractive indices of scatterers and interstitial fluid in the rat skin tissue at various wavelengths were estimated.

2. MATERIALS AND METHODS

2.1 Experimental setups

We used a commercially available computer-controlled CARY-2415 spectrophotometer with integrating sphere for investigation of the rat skin tissue optical properties. Total transmittance and diffuse reflectance were measured in the 400-800 nm wavelength range using standard technique.

The measurements of the light collimated transmittance and the reflectance 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.

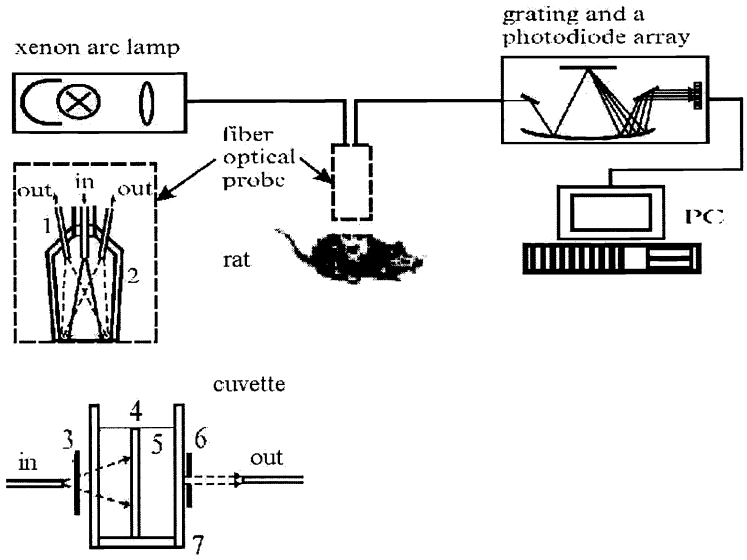

Figure 1. Experimental setup for measurements of the collimated transmittance and the reflectance spectra: 1 – optical fiber; 2 – aluminum jacket; 3 – neutral filters; 4 – rat skin sample; 5 – osmotically active solution (*glycerol*); 6 - the 0.5 mm – diaphragm; 7 – cuvette.

In the case of *in vitro* light transmission measurements the cuvette with the sample was placed between two optical fibers (400 μm core diameter). One fiber has transmitted the excitation radiation to the sample, and another fiber has 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.

In vivo reflectance measurements were performed using the fiber optical probe with seven fibers. The centrally placed fiber (400 μm core diameter) delivers incident light to the surface of the sample and used for its illumination, and the six surrounding fibers (also 400 μm core diameter) collected radiation reflected from the illuminated surface. Collecting fibers are mounted at small angles regarding the central fiber, so each fiber collects emission from the surface area a little bit

larger than the excitation light spot. All fibers are enclosed in aluminum jacket (6-mm outer diameter) to provide a fixed distance between the fibers and the sample surface. The distal ends of six collecting fibers were arranged as a vertical structure and imaged at the entrance slit of multi-channel spectrometer. The reflectance spectra of the samples were measured against $BaSO_4$ plate as a reference. The measurements were performed every 30 sec for 15 – 25 min.

2.2 Tissue samples preparation

In vitro experiments were performed with the 24 samples of the rat skin tissue extracted from various rat specimens. Tissue samples were obtained by autopsy within an hour *post mortem*. Before experiments hairs were removed using tweezers. Values of tissue samples thickness are presented in Table 1. Sample thickness was measured just before experiments. To study the influence of keeping conditions on the tissue optical properties the samples 1-6 were kept with temperature about +4 ^{0}C. Experiments with samples 7-13 and 19-24 were made just after autopsy. The samples 14-18 were kept in saline solution during 20 hours. 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	Thickness, mm (standard deviation)	Sample	Thickness, mm (standard deviation)	Sample	Thickness, mm (standard deviation)
Sample 1	0.61 (0.03)	Sample 7	0.64 (0.07)	Sample 13	0.58 (0.08)	Sample 19	0.58 (0.06)
Sample 2	0.49 (0.04)	Sample 8	0.7 (0.06)	Sample 14	0.72 (0.03)	Sample 20	0.77 (0.04)
Sample 3	0.84 (0.12)	Sample 9	0.66 (0.04)	Sample 15	0.69 (0.08)	Sample 21	0.78 (0.06)
Sample 4	0.63 (0.06)	Sample 10	0.51 (0.04)	Sample 16	0.72 (0.06)	Sample 22	0.9 (0.08)
Sample 5	0.61 (0.03)	Sample 11	0.63 (0.04)	Sample 17	1.02 (0.04)	Sample 23	0.63 (0.04)
Sample 6	0.72 (0.04)	Sample 12	0.51 (0.04)	Sample 18	1.03 (0.04)	Sample 24	0.69 (0.06)

2.3 *In vivo* measurements

In vivo measurements were done for three white rat (*rat wistar*) specimens. Rats age was about 9 month and weight was about 200 g. Rat was anaesthetized by an intraperitoneal injection of 1-% solution of *natrium ethaminal* with doze 40 mg/kg of the animal weigh. Removing of the hairs was done before experiments. The measurements for rat specimen # 1 were started after 30 sec after the injection of *glycerol*. The measurements for rat specimens # 2 and 3 were started after 3 min after the injection of *glycerol*. *Glycerol* was used as a chemical agent for the skin optical clearing. Refractive index of the *glycerol* was measurement on Abbe refractometer. It is 1.454 at wavelength 589 nm. Its pH is 6.5. Recording of the rat skin tissue reflectance spectra in the spectral range from 400 to 800 nm was provided by placing the fiber optical probe on the surface of the rat skin. Two methods were used for injection of osmotically active agent: into the skin dermis and subdermis. All experiments were performed at room temperature.

2.4 Calculation of the optical properties of the rat skin

We have used the inverse adding-doubling method developed by *Prahl et al.*[29] to calculate the absorption and reduced scattering coefficients of the rat skin tissue from the measured values of the total transmittance and diffuse reflectance. To obtain optical properties of the investigated samples we have used a computer program of *S.A. Prahl* (Oregon Medical Laser Center, USA, www.omlc.ogi.edu).

The refractive index of collagen fibers (at wavelength 589 nm) can be estimated as 1.474[1]. Wavelength dependence of the refractive indices of water is described as[4]:

$$n_{H_2O}(\lambda) = 1.31848 + \frac{6.662}{\lambda[nm] - 129.2}. \quad (1)$$

For the reconstruction of scatterers refractive index, we used the method described in Refs. 23, 30. We used the values of refractive indices of water (Eq. 1) as values of refractive indices of the interstitial fluid.

3. RESULTS AND DISCUSSION

3.1 Optical properties of the rat skin

We studied three groups of the rat skin samples: 1) the samples kept 24 hours with temperature about +4 ^{0}C; 2) the samples used just after autopsy; and 3) the samples kept 20 hours in saline. Figures 2 and 3 present diffuse and total transmittance spectra of the first group of the rat skin samples. It is well-seen absorption bands of blood at 420, 547, and 578 nm (Fig. 25). Diffuse reflectance and total transmittance spectra of the rat skin samples from the second group are presented in Figs. 4 and 5. Figs. 6 and 7 show diffuse reflectance and total transmittance spectra of samples from the third group.

Using inverse adding-doubling method, we have calculated optical properties of the rat skin samples. For this calculations we have used thickness of the samples presented in Table 1. Results of this calculation are shown in Figs. 8-13. Figures 8, 10, and 12 present spectra of absorption coefficient of the rat skin from the first, second, and third group of the samples, respectively. For the same groups there were calculated reduced scattering coefficients, which are shown in Figs. 9, 11, and 13.

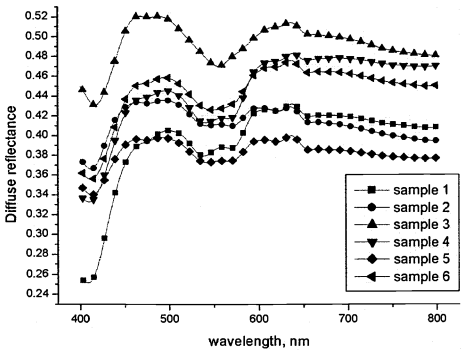

Figure 2. The diffuse reflectance spectra of the six rat skin samples kept 24 hours with temperature +4 ^{0}C.

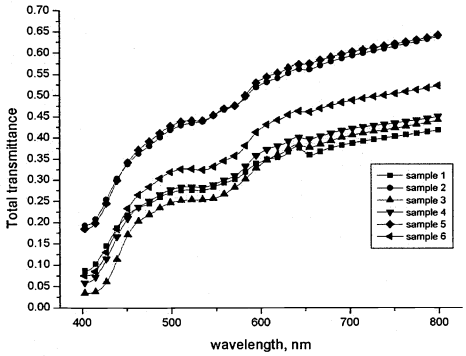

Figure 3. The total transmittance spectra of the six rat skin samples kept 24 hours with temperature +4 ^{0}C

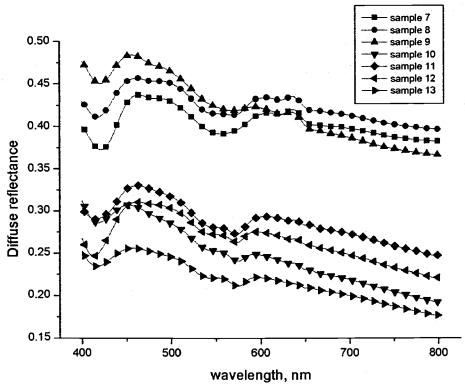

Figure 4. The diffuse reflectance spectra of the seven rat skin samples obtained just after autopsy.

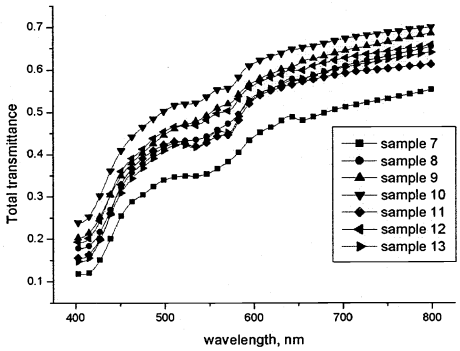

Figure 5. The total transmittance spectra of the seven rat skin samples obtained just after autopsy.

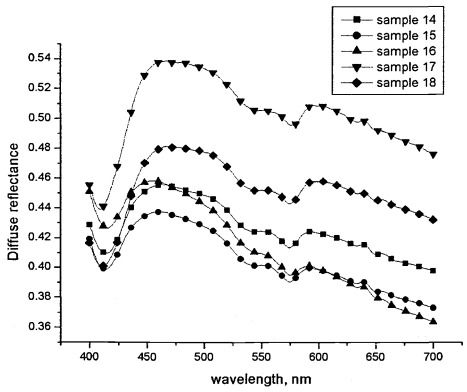

Figure 6. The diffuse reflectance spectra of the five rat skin samples kept 20 hours in saline.

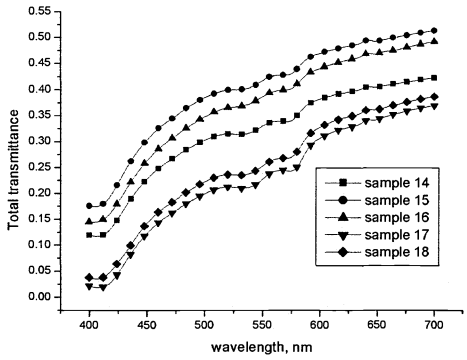

Figure 7. The total transmittance spectra of the five rat skin samples kept 20 hours in saline.

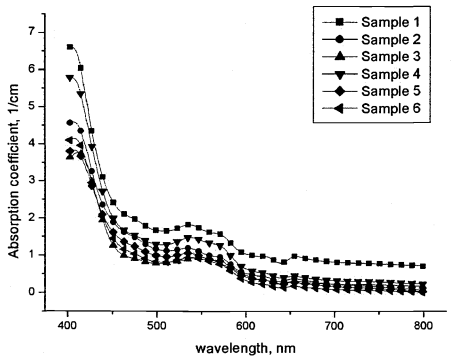

Figure 8. The absorption coefficient spectra of the six rat skin samples kept 24 hours with temperature +4 ^{0}C.

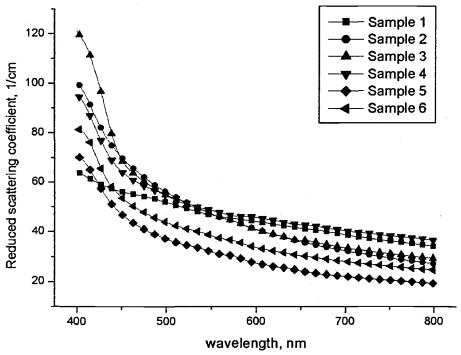

Figure 9. The reduced scattering coefficient spectra of the six rat skin samples kept 24 hours with temperature +4 ^{0}C.

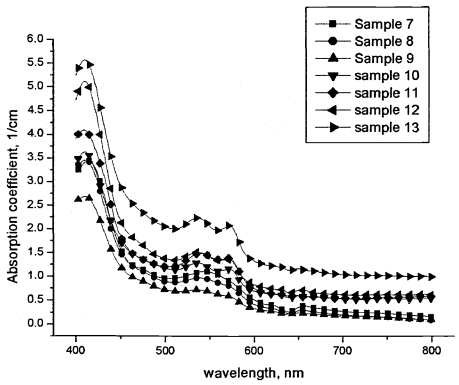

Figure 10. The absorption coefficient spectra of the seven rat skin samples obtained just after autopsy.

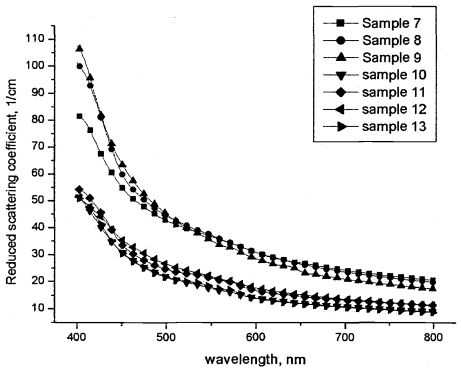

Figure 11. The reduced scattering coefficient spectra of the seven rat skin samples obtained just after autopsy.

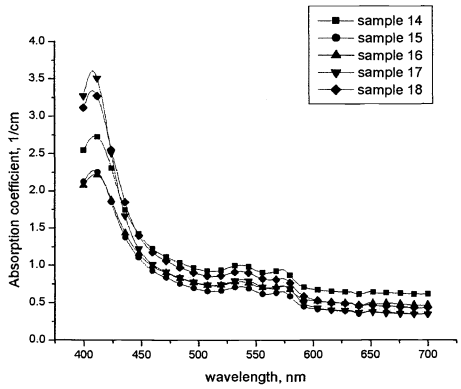

Figure 12. The spectra of absorption coefficient for the five rat skin samples kept 20 hours in saline.

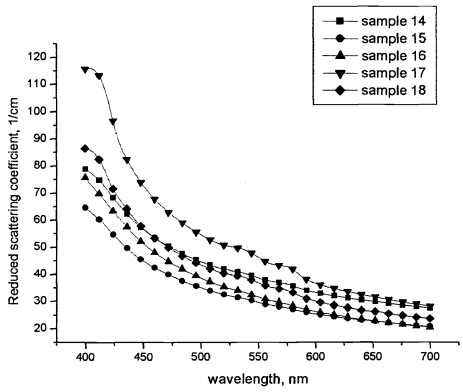

Figure 13. The spectra of reduced scattering coefficient for the five rat skin samples kept 20 hours in saline.

Obtained data were averaged for all samples of the rat skin. These data are presented in Figs. 14 and 15 together with optical parameters of another tissues[9,30] for comparison. From Fig. 14, it is seen that the absorption coefficients of the both *dura mater* and rat skin tissue are close to each other, but they are less than absorption coefficient of the human scleral tissue. It is apparently bounding with the layer of pigmented epithelium of scleral tissue. The considerably major differences of data are seen in Fig. 15. The samples of the *dura mater* have the lowest value of the reduced scattering coefficient. The scleral tissue put in saline in 24 hours has the largest value of the reduced scattering coefficient. The skin samples from the first group have the largest scattering properties.

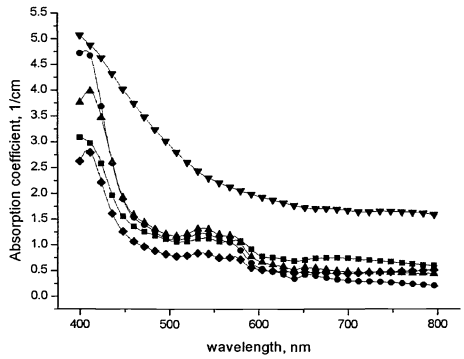

Figure 14. Comparison of the absorption coefficient spectra for various types of tissue samples (squares – the human *dura mater* samples[9]; circles - the rat skin samples kept 24 hours with temperature +4 ^{0}C (averaging for 1-6 samples); up triangles - the rat skin samples obtained just after autopsy (averaging for 7-13 samples); down triangles – the human scleral samples kept 24 hours in saline[30]; diamond - the rat skin samples kept 20 hours in saline.

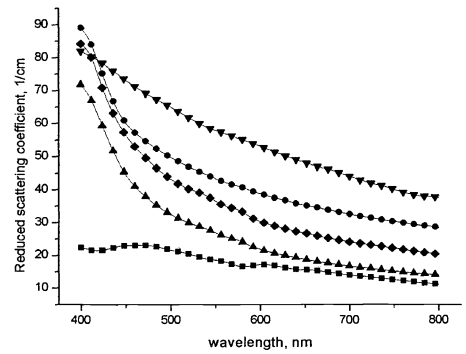

Figure 15. Comparison of the reduced scattering coefficient spectra for various types of tissue samples (squares – the human *dura mater* samples[9]; circles - the rat skin samples kept 24 hours with temperature +4 ^{0}C (averaging for 1-6 samples); up triangles – the rat skin samples obtained just after autopsy (averaging for 7-13 samples); down triangles – the human scleral samples kept 24 hours in saline[30]; diamond - the rat skin samples kept 20 hours in saline.

For explanation of these results, the following hypothesis is suggested. The human *dura mater* samples were kept with temperature about –12 ^{0}C. In these condition water was freeze out from interstitial fluid and lost. Thus, the matching of refractive indices of both scatterers and interstitial fluid took place and as consequence, the reduced scattering coefficient is decreased. Scleral samples were kept in saline. Ratio of refractive indices of scatterers and interstitial fluid is increased and therefore scattering of tissue is also increased. The results obtained for rat skin confirm this hypothesis. It is seen that the values of the reduced scattering coefficient of the samples put into saline is larger in average than that of the samples

measured just after autopsy. In addition, the values of reduced scattering coefficient of samples kept with low temperature are larger than that of the samples measured just after autopsy and the samples in saline.

We have calculated the wavelength dependence of refractive index of scatterers of the rat skin (Fig. 16).[23,30] Knowing of the wavelength dependence of refractive index the scatterers we have calculated the wavelength dependence of refractive index of the interstitial fluid of the rat skin (Fig. 17) using the same calculation technique as for scatterers refractive index evaluation.. In this, calculations we used as a tissue model ensemble of spherical scatterers with diameter from 0.8 to 2.5 μm. It is interesting to say that obtained wavelength dependencies of refractive indices of scatterers and interstitial fluid do not depend on scatterers sizes. Analogous results can be obtained if the scatterers are presented as infinite cylinders with diameters about 200 nm. It must be noted that in this calculations we used "effective" scatterers and they do not show polydisperse nature of real skin scatterers.

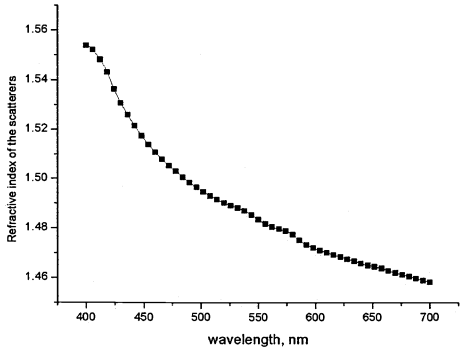

Figure 16. Wavelength dependence of refractive index of the scatterers of the rat skin.

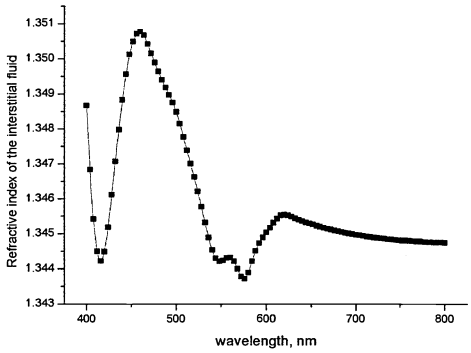

Figure 17. Wavelength dependence of refractive index of the interstitial fluid of the rat skin.

We assume that distortion in wavelength dependence of refractive index of the interstitial fluid (Fig. 17) connected with anomalous behavior of refractive indices within absorption band of the blood. As seen from Fig. 14 absorption bands of the blood correspond to 410, 538, and 572 nm, respectively. Minimums of wavelength dependence of refractive index of the interstitial fluid of the rat skin correspond to 416, 549, and 576 nm that confirm our hypothesis.

In Ref. 30 the wavelength dependence of refractive index of the scatterers of the sclera is presented. The values of the refractive indices of the scleral fibers will be very close to values of the refractive indices of the skin (Fig. 16) within wavelength range 600-700 nm. Differences observable between wavelength dependencies of the scatterers in general deals with differences of hydration between scleral and skin scatterers. For scleral tissue main scatterers are collagen fibers, which have a degree of hydration about 30%.[8] In the case of skin tissue in addition to collagen and elastin fibers (which are the main scatterers within of dermis) there are scatterers (within epidermis and stratum corneum) which have smaller degree of hydration and as a consequence more higher refractive index. Since in our calculation we used "effective" scatterers then refractive indices of the scatterers are more higher.

The wavelength dependence of the refractive index of the skin scatterers, presented in Fig. 16, is well described by Cauchy's formula. This formula is prevailing for approximation of wavelength dependence of refractive index for various substances including tissues. We have obtained the following dispersion formula using the least-squares method:

$$n_{scatterers}(\lambda) = 1.438 + \frac{7000}{\lambda^2[nm]} + \frac{1.884 \cdot 10^9}{\lambda^4[nm]} \tag{2}$$

The wavelength dependence of the refractive index of the interstitial fluid within the wavelength range 460-500 and 620-800 nm was also obtained:

$$n_{interstitial\ fluid}(\lambda) = 1.346 - \frac{1521}{\lambda^2[nm]} + \frac{5.356 \cdot 10^8}{\lambda^4[nm]} \tag{3}$$

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

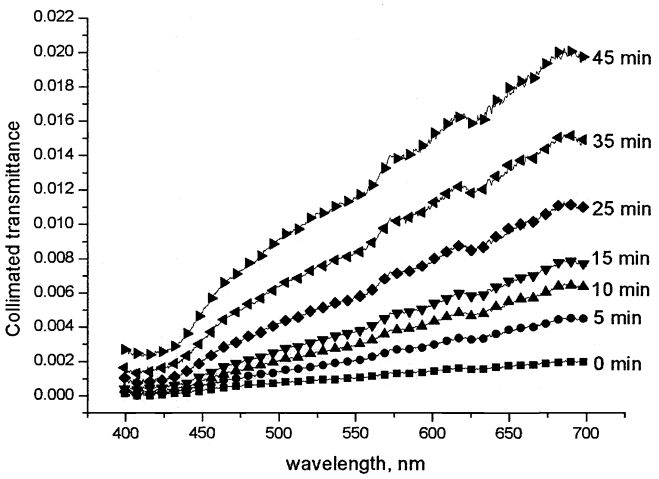

Figure 18. Collimated transmittance spectra of the rat skin sample measured concurrently with administration of *glycerol* at different time intervals (sample 22).

To understand the mechanisms of the rat skin tissue optical clearing we have investigated the collimated transmittance spectra concurrently with administration of *glycerol*. Figure 18 illustrates the typical collimated transmittance spectra (sample 22). 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 19-24 present the time-dependent collimated transmittance at different wavelengths. They show the dynamics of tissue clearing for various skin samples.

Presented experimental results well show that the rat skin transmittance spectra can be substantially changed by administration of osmotically active chemical agent (*glycerol*). Analyzing the data presented in a Figs. 19-24 and comparing their with data obtained by us 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 the absence of saturation of clearing with protecting properties of epidermis and fat layer, which prevent from penetration of osmotically active agent into dermis. In this case, action of osmotic stress allows describing the mechanism of clearing of the rat skin tissue, as *glycerol* is a high 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 other hand, the long-time clearing of the samples can be explained by high viscosity of *glycerol* that prevents from rapid penetration of this matter into tissue. In Refs. 6-9 we used aqueous solutions of *glucose* as a clearing agent. They have less viscosity and less time of clearing. Degree of clearing of various skin samples is presented in Table 2. Degree of clearing is a ratio of transmittance value measured in a fixed moment to that measured in an initial moment.

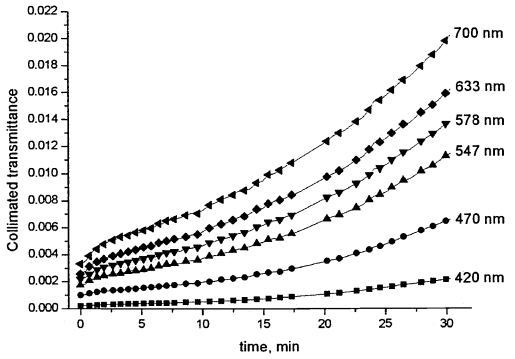

Figure 19. The time-dependent collimated transmittance of the rat skin sample (sample 19) measured at different wavelength concurrently with administration of *glycerol*.

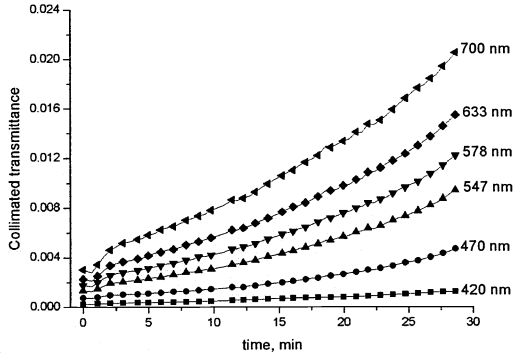

Figure 20. The time-dependent collimated transmittance of the rat skin sample (sample 20) measured at different wavelength concurrently with administration of *glycerol*.

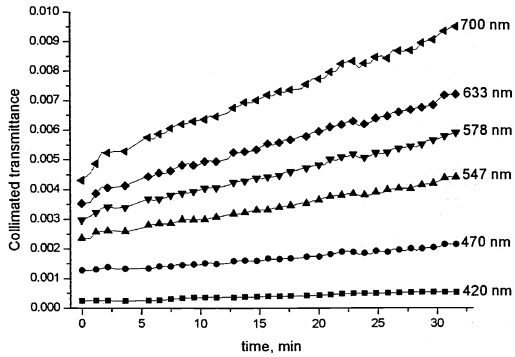

Figure 21. The time-dependent collimated transmittance of the rat skin sample (sample 21) measured at different wavelength concurrently with administration of *glycerol*.

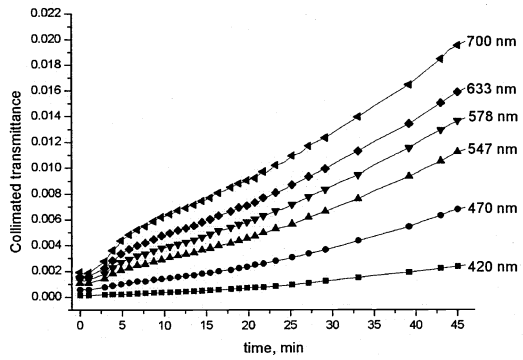

Figure 22. The time-dependent collimated transmittance of the rat skin sample (sample 22) measured at different wavelength concurrently with administration of *glycerol*.

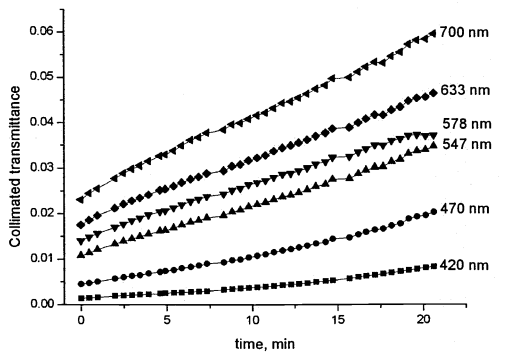

Figure 23. The time-dependent collimated transmittance of the rat skin sample (sample 23) measured at different wavelength concurrently with administration of *glycerol*.

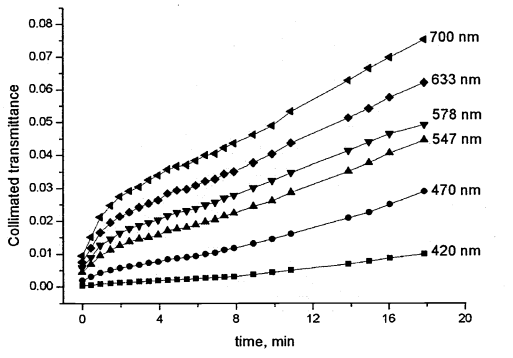

Figure 24. The time-dependent collimated transmittance of the rat skin sample (sample 24) measured at different wavelength concurrently with administration of *glycerol*.

Table 2. Degree of rat skin clearing at different wavelengths.

Wavelength, nm	420				470				547				578				633				700			
Time, min	15	20	30	45	15	20	30	45	15	20	30	45	15	20	30	45	15	20	30	45	15	20	30	45
Sample 19	2.3	4.7	10	-	2.6	3.5	6.6	-	2.8	3.8	6.5	-	2.8	3.7	6.3	-	2.9	3.8	6.3	-	2.9	3.7	6.1	-
Sample 20	3.2	3.5	5.3	-	2.7	3.6	6.4	-	3.3	4.3	7.1	-	3.4	4.3	7	-	3.4	4.3	6.9	-	3.5	4.4	6.8	-
Sample 21	1.9	1.9	2.2	-	1.3	1.4	1.7	-	1.4	1.5	1.9	-	1.5	1.6	2	-	1.6	1.7	2	-	1.7	1.8	2.2	-
Sample 22	3.8	5	9-	19	3.3	4.2	6.5	12	3.4	4.2	6.1	11	3.6	4.4	6.3	10	3.8	4.5	3.7	10	3.9	4.7	6.4	10
Sample 23	3.6	5.9	-	-	3.2	4.5	-	-	2.6	3.2	-	-	2.3	2.6	-	-	2.2	2.7	-	-	2.2	2.6	-	-
Sample 24	32	45	-	-	11	15	-	-	8.3	9.8	-	-	7.6	8.5	-	-	7.2	8.2	-	-	7	8	-	-

3.3 *In vivo* investigation of the optical clearing of the rat skin

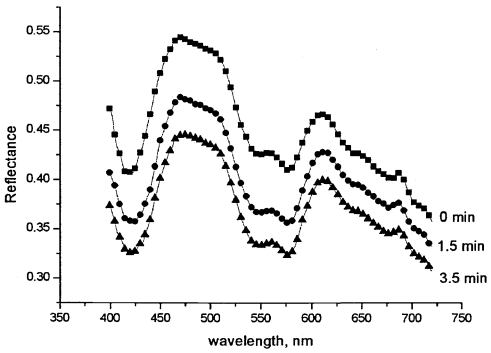

Figure 25. The *in vivo* time-dependent reflectance spectra of the rat skin measured concurrently with administration of *glycerol* at different time intervals (Rat #1).

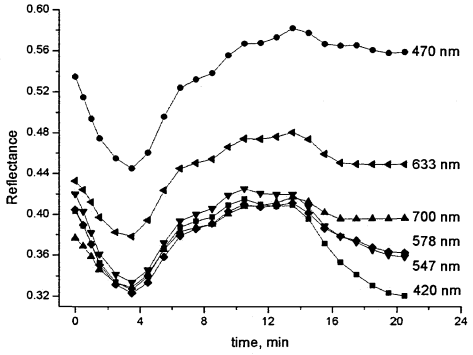

Figure 26. The *in vivo* time-dependent reflectance of the rat skin measured at different wavelengths concurrently with administration of *glycerol* (Rat #1).

Figures 25 and 26 show the results of *in vivo* back reflectance experiments. Figure 25 illustrate typical reflectance spectra of the rat skin recorded by fiber-optical probe in different moments. Figure 26 are presented the dynamics of the change of reflectance at different wavelengths.

Analyzing the results of the first experiment (*glycerol* was impregnated under skin into connective tissue) it can be seen that dynamics of reflectance change has non-monotone character (Fig. 26). We underline three stages of *glycerol* action on the skin and hypodermic tissues of the rat.

1. The first 3.5 minutes. Decreasing of the reflectance due to decreasing of the scattering coefficient of the tissue under action of *glycerol* is seen.
2. The second stage is from 3.5 to 13-13.5 min. The diffusion of *glycerol* into the ambient tissue, decreasing of *glycerol* concentration, increasing of scattering coefficient, and increasing of the reflectance take place.
3. The third stage begins from about 13 minute of influence of *glycerol* on hypodermic tissues of the rat. The rush of blood to the place of interaction osmotically active substances with hypodermic tissues arises. Therefore, we observe decreasing of the reflectance in the absorption bands of blood (420, 547 and 578 nm).

Observable decreasing of the reflectance after 13 minutes of *glycerol* action (Fig. 26) is explained by the following way. In spite of the fact that *glycerol* often apply in a cosmetology, probably, he renders irritant action on hypodermic tissues of the rat, as apparently after 13 minutes of *glycerol* action.

4. CONCLUSION

In this study we have measured and calculated the optical properties of the rat skin tissue. Using Mie theory we have estimated the wavelength dependence of refractive indices of skin scatterers and interstitial fluid. The corresponding dispersion formula was derived. The results of this paper show that administration of osmolytes to a fibrous tissue allows for effectively control of its optical characteristics. The scattering properties of the skin are effectively reduced by the refractive indices matching of the scatterers and interstitial substance.

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