Study of osmotical liquids diffusion within sclera

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ABSTRACT

We present experimental results of investigation of the optical properties of the human eye sclera controlled by administration of osmotically active chemical, such as glucose solution with various concentrations. Administration of chemical agent induces diffusion of matter and as a result equalization of the refractive indices of collagen and ground material. Results of experimental study of influence of osmotical liquid (glucose solution) on reflectance and transmittance spectra of human sclera are presented. *In vitro* reflectance and transmittance spectra of the human sclera samples were investigated by commercially available spectrophotometer CARY-2415. The significant increasing of the transmittance and decreasing of the reflectance of human sclera samples under action of osmotical solutions were demonstrated. Results of our study show that the degree of the sclera samples clearing is increased with increasing of the chemical agent concentration in solution. The diffusion coefficients of glucose solution with various concentrations within scleral tissue was estimated.

Keywords: glucose; light scattering; sclera; diffusion coefficient

1. INTRODUCTION

Transscleral diagnostic, therapy and surgery are important for laser ophthalmology. The solution of the problem is connected with the success in the development of robust techniques for the control of the optical properties of the human sclera. Such control means the change of the scattering or absorption properties of a tissue [1-13]. In general, a number of laser surgery, therapy, and diagnostic technologies uses the tissue compression and stretching for a better transport of the laser beam to underlying layers of tissue [1,14]. Compression of the human eye sclera allows one to perform the transscleral laser coagulation of the ciliary body and retina/choroid [1].

Recently a number of results on noninvasive *in vivo* monitoring of glucose concentration using near infrared (NIR) light scattering techniques in application to skin surface examination were reported [15-18]. The main idea of such measurements is based on the dependence of tissue scattering properties on the refractive index mismatch between collagen fibers (and/or cellular membrane) and the extrafiber (extracellular) substance. In general, an increasing in tissue glucose concentration reduces index of mismatch and correspondingly decreases the scattering coefficient. Therefore, measurement of scattering coefficient allows to estimate the glucose concentration in the tissue. Osmotic effects play an essential role in such measurements and can dramatically change tissue optical response on glucose concentration. The possibility of scleral reflectance measurements for *in vivo* monitoring of glucose concentration was discussed in our previous papers [4-8]. It was shown that turbidity of sclera could be effectively controlled using above discussed immersion effect and that osmotic effects are very important.

In this paper we present the results of experimental study of scleral optical transmittance and reflectance controlled by administration of glucose solution with various concentrations. Glucose solution having a higher refractive index than that of tissue ground (extrafiber) substance diffuses into a tissue and water diffuses from a tissue to the surrounding solution; correspondingly the equalizing of the refractive indices of scatterers (collagen fibers) and the ground substance is observed. As a result, the tissue optical clearing is took place. *In vitro* experiments were performed using samples of the human sclera obtained from autopsy human eyes. The model of glucose diffusion is suggested and values of glucose diffusion coefficients in the human scleral tissue are estimated.

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2. PHYSICAL PROPERTIES AND STRUCTURE OF THE HUMAN SCLERA

The sclera has a compound structure. Normally it is a nontransparent fibrous tissue and mainly consists of collagen fibers (fibrils) packed in lamellar bundles that are immersed in an amorphous base substance containing glycosaminoglycans, proteins, and protein-polysaccharide complexes [1,4]. These fibrils are arranged in individual bundles in parallel fashion. Within each bundle the groups of fibers are separated from each other by large empty lacunae randomly distributed in space. Collagen bundles have a wide range of widths and thicknesses. They cross each other in all directions but remain parallel to the scleral surface. All these inhomogeneities give a high scattering of scleral tissue in normal state. The thickness of the sclera, in dependence on the age and region of the eye, is in the range 0.3 - 1.8 mm. The average value of refractive index of the scleral sample is [1,4]

$$n_s = n_{col} \cdot C_{vcol} + n_b \cdot C_{vb}, \tag{1}$$

where n_{col}, n_b and C_{vcol}, $C_{v\,b}$ are the refractive indices and volume fractions of collagen and ground material, respectively; $C_{vcol} + C_{v\,b} = 1$.

Measured the average refractive index of the human sclera, $n_s = 1.385 \pm 0.005$ at $\lambda = 589$ nm [1], for volume fraction of hydrated collagen, $C_{vcol} = 0.31$, and refractive index of ground substance, $n_b = 1.345$, allows to evaluate the refractive index of the scleral fibrils [4] as $n_{col} = 1.474$. Using a value of an refraction index of collagen fibrils and the equation (1) we can evaluate a content of water in collagen fibrils. The content of water is 0.1072 fraction of volume of a scleral sample. Content of the dry collagen in the scleral fibrils is 0.2028 fraction of volume of a scleral sample.

3. OPTICAL AND MATTER DIFFUSION MODELS OF THE HUMAN SCLERA

Model of the sclera in a local region can be represented as a slab with a thickness d that is filled by thin and long dielectric cylinders (collagen fibers) with average diameter ~100 nm and refractive index $n_{col} = 1.474$. The cylinders are located in planes that are parallel to the slab surface, but within each plane their orientations are random. The interstitial space is filled by homogeneous ground substance with the refractive index $n_b = 1.345$. This refractive index is a controlled parameter and can be change in the range from 1.345 to 1.474. For $n_{col} = n_b = 1.474$ the medium becomes totally homogeneous and optically transparent [4]. The transmission of collimated light by a tissue layer of thickness d is defined as

$$T_C = I / I_0 = exp(-\mu_t \cdot d), \tag{2}$$

where I_0 and I are the intensities of the incident and detected light, respectively; $\mu_t = \mu_a + \mu_s$ is the extinction coefficient, μ_a and μ_s are the absorption and scattering coefficients, respectively. For the human sclera at the wavelength $\lambda = 650$ nm the absorption coefficient $\mu_a \cong 0.08$ cm^{-1} and reduced scattering coefficient $\mu'_s = \mu_s(1-g) \cong 25$ cm^{-1}, where g is the scattering anisotropy factor [19]. For g=0.9 $\mu_s \cong 250$ cm^{-1}.

For computer modeling of the scattered light distribution in the space around an individual thin cylinder, the scattering cross section σ_s for non-polarized incident light is given by [4,20-24]

$$\sigma_s \cong \left(\pi^2 a^4 k^3 / 8\right) \cdot \left(m^2 - 1\right)^2 \cdot \left(1 + 2 / \left(m^2 + 1\right)^2\right), \tag{3}$$

where k is the wave number of light in the sclera; $m = n_{col} / n_b$ is the ratio of the refractive indices of the cylinders and base materials, and a is the radius of the scatterers.

As shown in Ref. 23 for a system of non-interacting thin cylinders the scattering coefficient can be estimated as $\mu_s = \sigma_s \cdot \frac{C_{vcol}}{\pi \cdot a^2}$, where C_{vcol} is the volume fraction of collagen fibers of scleral tissue. Tissues like sclera are densily packed

systems, so spatial ordering of scatterers should be taken into account. Following papers [25, 26] we took into account the spatial ordering of scatterers.

$$\mu_S=\sigma_S \frac{C_{v\,col}}{\pi \cdot a^2} \frac{\left(1-C_{v\,col}\right)^3}{1+C_{v\,col}}, \tag{4}$$

To describe dynamics of the refractive index change and corresponding decreasing of the scattering coefficient when glucose diffuses within the interstitial substance of the sclera we used the model of free diffusion [4,27,28]. The diffusion equation for the local variation of glucose concentration within a layer can be presented in the form

$$\frac{\partial c}{\partial t}=D\frac{\partial^2 c}{\partial x^2}, \tag{5}$$

where c is the glucose concentration $[c] = g/ml$, D is the diffusion coefficient $[D]=cm^2/\sec$, and x is the spatial coordinate $[x] = cm$.

The solution of Eq. (5) for a plane slab with a thickness d, at the moment $t = 0$ and concentration c_0 (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 [27]

$$c=c_0\left\{1-\frac{4}{\pi}\left[\exp(-t/\tau)\cdot\sin(\pi x/d)+\frac{1}{3}\exp(-9t/\tau)\cdot\sin(3\pi x/d)+\frac{1}{5}\exp(-25t/\tau)\cdot\sin(5\pi x/d)+...\right]\right\}, \tag{6}$$

where $\tau = \frac{d^2}{\pi^2 D}$ is the diffusion constant, D is the diffusion coefficient, d is the thickness of the scleral sample. As a first approximation Eq. (6) can be reduced to

$$C=C_0\cdot\left(1-\exp\left(-\frac{t}{\tau}\right)\right), \tag{7}$$

that is very close to the equation describing diffusion through a partially permeable membrane [27]. Eq. (7) is written for diffusion through a homogeneous slab. Due to fibrous structure we can present tissue as a porous material, and have to correct Eq. (7) using the coefficient of porosity [29]. Porosity coefficient we define as: $p = \frac{V - V_{col}}{V}$, where V is the volume of the scleral sample, and V_{col} is the volume of collagen fibers. Diffusion coefficient is defined as $D=D_0/p$, where D_0 is the glucose diffusion coefficient in interstitial fluid.

When applying the chemical agent the change of pH of the environment is very important for tissue swelling. The swelling of fibrous tissue is caused not only by the increasing of collagen fibril size but also by the increasing of the sample volume due to rising of the mean distance between fibrils [30-32].

4. MATERIALS AND METHODS

In this study we used a commercially available computer-controlled CARY-2415 spectrophotometer with integrating sphere to make total transmittance, diffuse reflectance and collimated transmittance measurements in the 400-800 nm wavelength range for human eye sclera. The sequence of obtaining of the scleral optical properties was the following: 1) recording of the reference for the wavelength range from 400 to 800 nm (as the reference 5-ml cuvette with glucose solution was used); 2) recording of the experimental spectra during the clearing of the scleral sample (the sample was fixed on a plastic plate with a square aperture 5×5 mm^2 and placed in the same cuvette filled with the glucose solution); 3) subtraction of optical density of the reference from that of the scleral sample. For each tissue sample the thickness and initial weight were measured. For each scleral sample diffuse reflectance R_d, or total transmittance T_d, or collimated transmittance T_c were measured. The diffuse reflectance were calibrated on the basis of reflectance value from standard reflectance plate (BaF_2).

The spectral bandwidth of spectrophotometer was set at 1 nm. Scan rate was 2 nm/sec. The time period for registration of one spectra was about 5 min. For each wavelength we obtained high-degree polynomial time-dependence approximation to correct measured spectra (i.e. to obtain value in each moment of the time).

In the case of collimated transmittance measurements we used the system consisted of three diaphragms. Diameter of each diaphragm was 2 mm. Distance between these diaphragms were 20 mm (between first and second) and 110 mm (between second and third). This system allows to obtain collimated beam of light. Cuvette with investigated sample was placed between first and second diaphragms. The measurements of the each sample were performed continuously during 25 min.

The samples of the sclera were extracted from the human eye. The dissection and measurements on the eye were performed within 24 h *postmortem*. After enucleation, the eye was placed in saline. Before *in vitro* measurements, the conjunctiva and the ciliary body as well as the retina with choroid were removed. The human scleral samples were cleaned and cut into pieces of about 10×10 mm^2. The mean thickness of the human scleral samples was about 0.5 mm.

Aqueous solutions of glucose with concentrations 180 mg/ml, 300 mg/ml, and 400 mg/ml and refractive indices n = 1.36, 1.378, and 1.39 (λ = 589 nm), respectively, was used for scleral optical clearing. Glucose does not have absorbing bands within the wavelength of study, from 400 to 800 nm. That is why changes in scleral transmittance due to the administration of glucose can be described only in the term of scattering coefficient, μ_s.

The gravimetrical measurements were performed using torsion scales. Precision of scale measurements was 1 mg. The samples of sclera were placed into 1.5-ml cuvette filled by immersion liquids (glucose solution with concentration 180 mg/ml, 300 mg/ml, 400 mg/ml, and 540 mg/ml, respectively). All experiments were performed at room temperature.

5. RESULTS AND DISCUSSION

To understand the mechanisms of the scleral tissue optical clearing we have investigated the collimated transmittance, total transmittance, diffuse reflectance spectra and weight change of the scleral sample concurrently with administration of glucose solutions with various concentrations (Fig. 1-19).

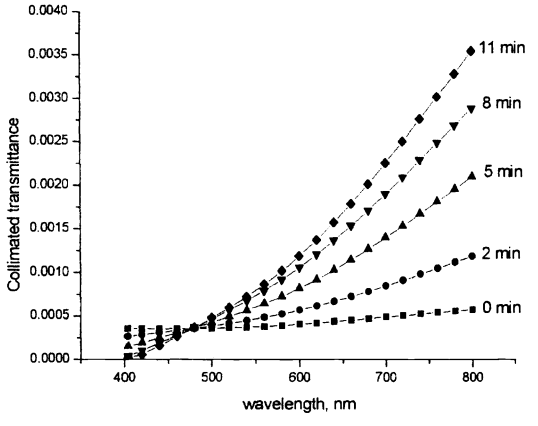

Figure 1. Collimated transmittance spectra of the human sclera sample measured concurrently with administration of glucose solution (180 mg/ml) at different time intervals.

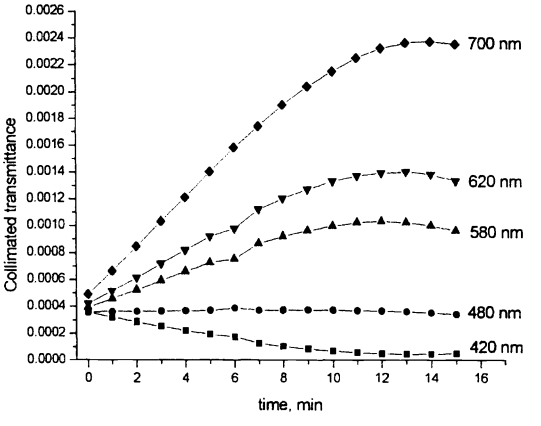

Figure 2. The time-dependent collimated transmittance of the human sclera sample measured at different wavelength concurrently with administration of glucose solution (180 mg/ml).

Figure 1 illustrates the collimated transmittance spectra measured concurrently with administration of relatively low concentrated glucose solution (180 mg/ml). It is easily seen that the untreated sclera is poorly transparent for the visible light. Glucose administration makes this tissue transparent, increasing collimated transmittance (for wavelength 589 nm) in to 2.62 time for the sample kept in solution during t = 12 min (Fig. 2 and Table 1). These spectra are well match to spectra presented in Refs. 1 and 4, respectively, for untreated and treated samples. The corresponding plots for time-dependent collimated transmittance at different wavelengths are presented in Fig. 2. They show the dynamics of tissue clearing. Figure

2 shows that characteristic time response of human optical clearing (at use glucose solution with low concentration, 180 mg/ml) is about 12 minutes.

From Figures 1 and 2 it is also seen, that for wavelength range from 400 to 480 nm glucose solution with low concentration makes unclearing of the sample. Possible cause of this fact is bounded with large swelling of the sample (Fig. 19). It is possible that there is other interpretation of this phenomenon. In this case refractive index of glucose solution for wavelength 480 nm equals to the refractive index of interstitial fluid scleral sample. By this cause the refractive index of ground substance of the scleral sample does not change and so the scattering coefficient of the sclera does not change as well. The plot presented on Figure 2 serves by implication confirmation of this fact. It corresponds to the time-dependence collimated transmittance for wavelength 480 nm.

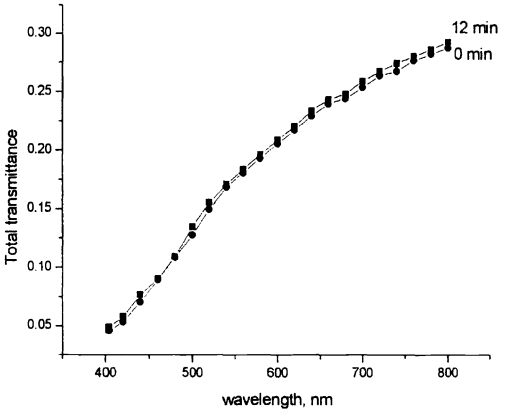

Figure 3. Total transmittance spectra of the human sclera sample measured concurrently with administration of glucose solution (180 mg/ml) at different time intervals.

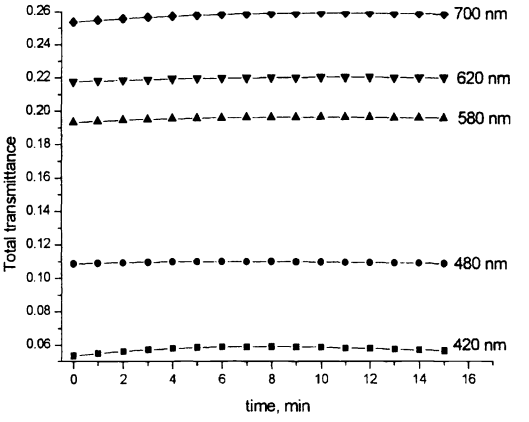

Figure 4. The time-dependent total transmittance of the human sclera sample measured at different wavelength concurrently with administration of glucose solution (180 mg/ml).

Figure 3 presents total transmittance spectra of the human sclera sample measured concurrently with administration of relatively low concentrated glucose solution (180 mg/ml) at different time intervals. Figure 4 illustrates the time-dependent total transmittance of the human sclera sample measured concurrently with administration of the same glucose solution. From these figures it is seen that total transmittance is not changed upon administration of glucose solution with low concentration in otherness from collimated transmittance.

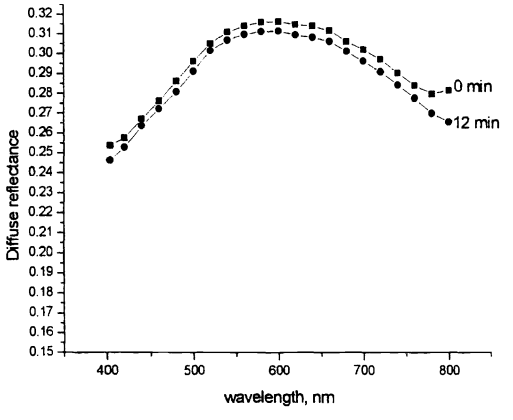

Figure 5. Diffuse reflectance spectra of the human sclera sample measured concurrently with administration of glucose solution (180 mg/ml) at different time intervals.

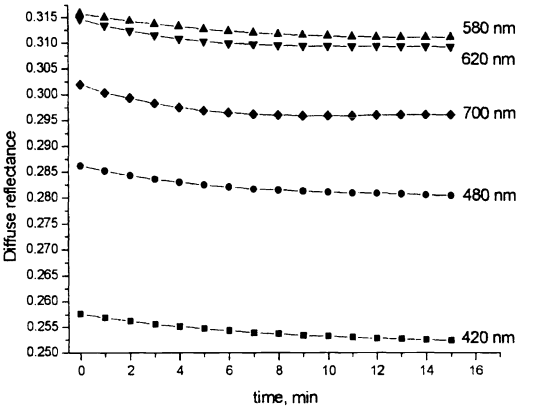

Figure 6. The time-dependent diffuse reflectance of the human sclera sample measured at different wavelength concurrently with administration of glucose solution (180 mg/ml).

Figure 5 presents diffuse reflectance spectra of the human sclera sample measured concurrently with administration of low concentrated glucose solution at different time intervals. Figure 6 illustrates the time-dependent diffuse reflectance of the human sclera sample measured concurrently with administration of this glucose solution. From these figures it is seen that the change of the diffuse reflectance upon administration of low concentrated glucose solution is small.

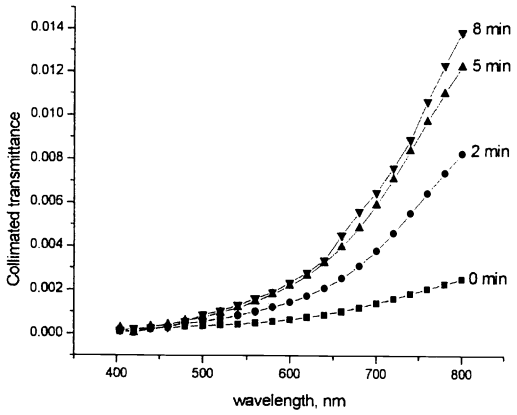

Figure 7. Collimated transmittance spectra of the human sclera sample measured concurrently with administration of glucose solution (300 mg/ml) at different time intervals.

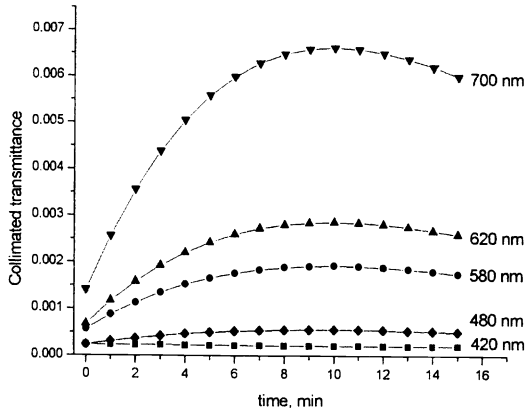

Figure 8. The time-dependent collimated transmittance of the human sclera sample measured at different wavelength concurrently with administration of glucose solution (300 mg/ml).

Figure 7 illustrates the collimated transmittance spectra measured concurrently with administration of glucose solution with medium value of concentration (300 mg/ml). Glucose administration makes this tissue transparent, increasing collimated transmittance (for wavelength 589 nm) in 3.34 times for the sample kept in solution during t = 10 min (Table 1). The corresponding plots for time-dependent collimated transmittance at different wavelengths are presented in Fig. 8. They show the dynamics of tissue clearing. Characteristic time response of human optical clearing (at used glucose solution) is about 10 minutes.

From Figure 8 it is seen, that for wavelength 420 nm glucose solution with medium value of concentration makes the sample less transparent. This effect is the same with that described above for more low concentrated solution of glucose.

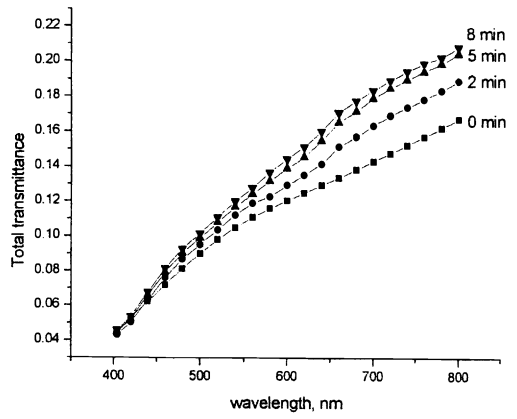

Figure 9. Total transmittance spectra of the human sclera sample measured concurrently with administration of glucose solution (300 mg/ml) at different time intervals.

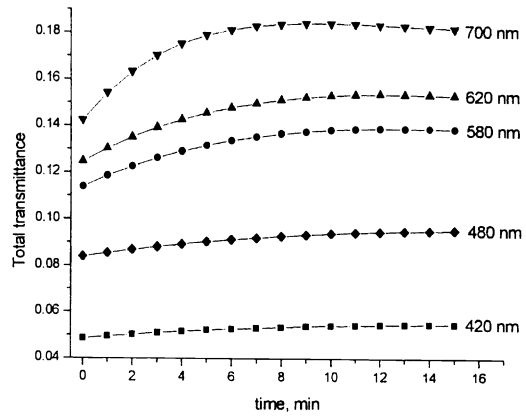

Figure 10. The time-dependent total transmittance of the human sclera sample measured at different wavelength concurrently with administration of glucose solution (300 mg/ml).

Figure 9 presents total transmittance spectra of the human sclera sample measured concurrently with administration of glucose solution with 300 mg/ml concentration at different time intervals. Figure 10 illustrates the time-dependent total transmittance of the human sclera sample measured concurrently with administration of the same glucose solution. From these Figures it is seen that total transmittance is small changed upon administration of glucose solution with that concentration in otherness from collimated transmittance as in previous case.

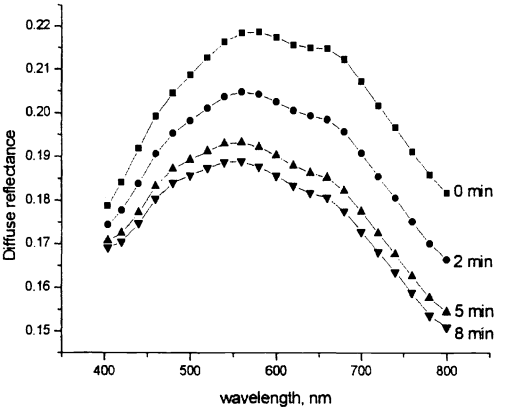

Figure 11. Diffuse reflectance spectra of the human sclera sample measured concurrently with administration of glucose solution (300 mg/ml) at different time intervals.

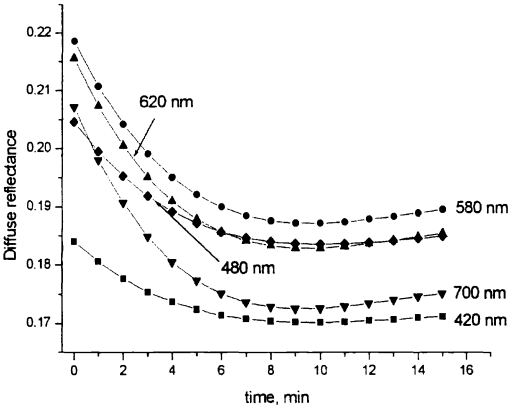

Figure 12. The time-dependent diffuse reflectance of the human sclera sample measured at different wavelength concurrently with administration of glucose solution (300 mg/ml).

Figure 11 presentes diffuse reflectance spectra of the human sclera sample measured concurrently with administration of glucose solution with medium value of concentration (300 mg/ml) at different time intervals. Figure 12 illustrates the time-dependent diffuse reflectance of the human sclera sample measured concurrently with administration of that glucose solution. From these figures it is seen that diffuse reflectance is small changed upon administration glucose solution.

Thus, the clearing of the scleral tissue by action of previous described glucose solutions is more visible for the collimated transmittance that for the total transmittance and diffuse reflectance measurements.

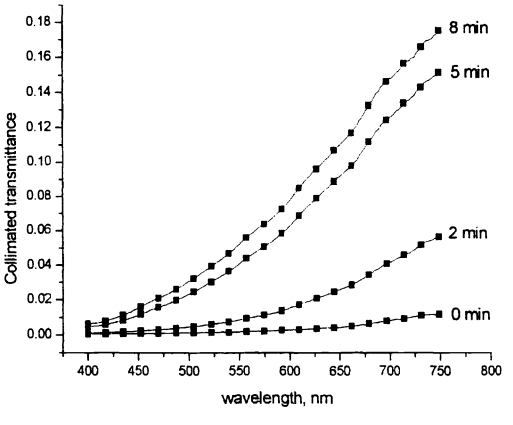

Figure 13. Collimated transmittance spectra of the human sclera sample measured concurrently with administration of glucose solution (400 mg/ml) at different time intervals.

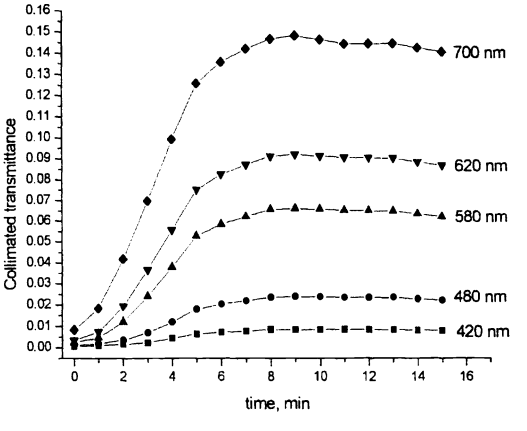

Figure 14. The time-dependent collimated transmittance of the human sclera sample measured at different wavelength concurrently with administration of glucose solution (400 mg/ml).

Figure 13 illustrates the collimated transmittance spectra measurement concurrently with administration of glucose solution with high concentration (400 mg/ml). Glucose administration makes this tissue highly transparent, increasing collimated

transmittance (for wavelength 589 nm) in to 28.7 time for the sample kept in solution for t = 8 min (Table 1). The corresponding plots for time-dependent collimated transmittance at different wavelengths are presented in Fig. 14. They show the dynamics of tissue clearing. Figure 14 shows that characteristic time response of human optical clearing (at use of glucose solution with high concentration, 400 mg/ml) is about 8 minutes.

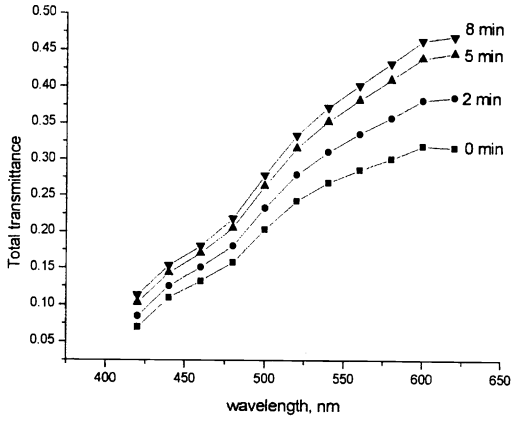

Figure 15. Total transmittance spectra of the human sclera sample measured concurrently with administration of glucose solution (400 mg/ml) at different time intervals.

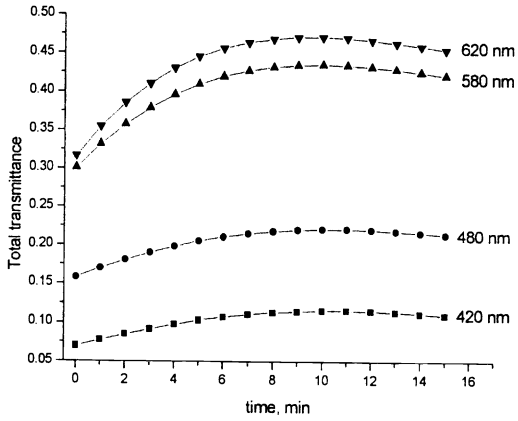

Figure 16. The time-dependent total transmittance of the human sclera sample measured at different wavelength concurrently with administration of glucose solution (400 mg/ml).

Figure 15 presents total transmittance spectra of the human sclera sample measured concurrently with administration of glucose solution with high concentration (400 mg/ml) at different time intervals. Figure 16 illustrates the time-dependent total transmittance of the human sclera sample measured concurrently with administration of glucose solution (with this concentration). From these figures it is seen that total transmittance is changed upon administration of glucose solution with high concentration.

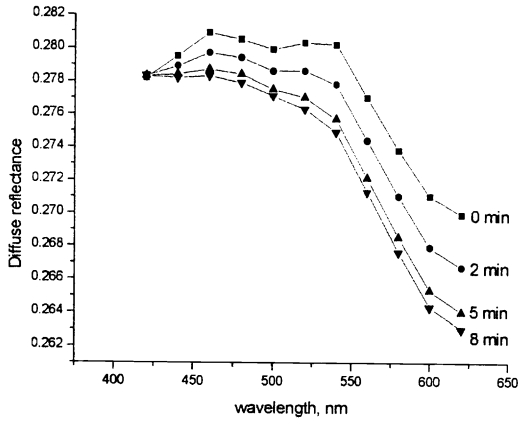

Figure 17. Diffuse reflectance spectra of the human sclera sample measured concurrently with administration of glucose solution (400 mg/ml) at different time intervals.

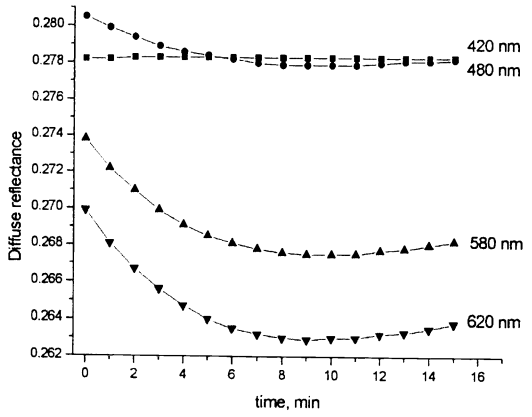

Figure 18. The time-dependent diffuse reflectance of the human sclera sample measured at different wavelength concurrently with administration of glucose solution (400 mg/ml).

Figure 17 presents diffuse reflectance spectra of the human sclera sample measured concurrently with administration of glucose solution with the same concentration at different time intervals. Figure 18 illustrates the time-dependent diffuse reflectance of the human sclera sample measured concurrently with administration of this glucose solution. From these Figures it is seen that diffuse reflectance is changed upon administration of glucose solution with high concentration.

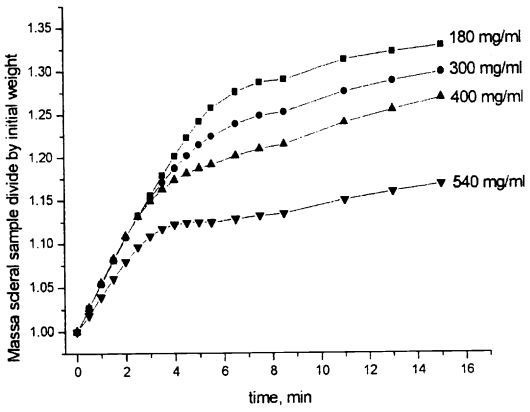

Figure 19. The time-dependent changes of normalized mass (weight of the scleral sample divided by its initial weight) of the scleral sample measured concurrently with administration of glucose at different concentration 180 mg/ml, 300 mg/ml, 400 mg/ml, and 540 mg/ml, respectively.

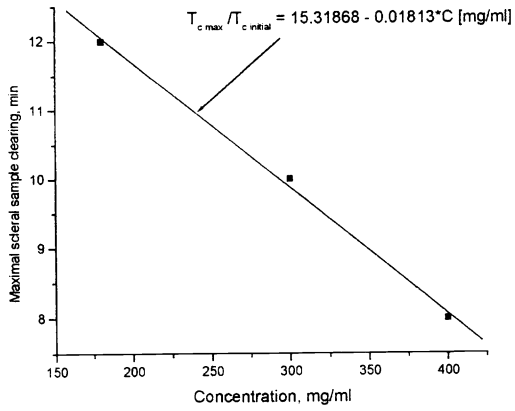

Figure 20. Maximal scleral sample clearing (T_{cmax}/ $T_{cinitial}$) versus concentration (mg/ml) glucose solution. Squares corresponded experimental data and solid line – offer our approximation, respectively. This data presented for wavelength 589 nm.

To understand the mechanisms of the scleral tissue optical clearing we have investigated change of the scleral sample weight concurrently with administration of glucose solution at different concentrations (180 mg/ml, 300 mg/ml, 400 mg/ml, and 540 mg/ml) (Fig. 19). To provide this measurements we took four scleral samples (weight of the each sample was about 70 mg) and placed them into glucose solutions with different concentration. Each 1 minute time interval we balanced these samples, using the torsion balance. Results of our study have shown that for sample placed into glucose solution with 180 mg/ml concentration the most degree of swelling took place. Last its weight up to 33% from initial one (Fig. 19) after 15 minutes of administration of glucose solution. For other concentrations we looked on less degree of the swelling. Thus, for concentration 300 mg/ml the increasing of weight consisted 30%, for 400 mg/ml – 28%, and for 540 mg/ml – 15% from initial weight, respectively. We conclude that degree of the swelling of scleral sample have inverse dependence from concentration of the glucose solution. It is seen that time period of sharp increasing of the sclera sample weight is differed for various glucose solutions. Thus, for glucose solution with concentration 540 mg/ml this time period consisted about 4 min, for glucose solution with concentration 300-400 mg/ml it was about 6 minutes, and for glucose solution with concentration 180 mg/ml it was about 9 minutes.

Figure 20 presents dependence of time of maximal scleral sample clearing on glucose solution concentration (mg/ml). Here the maximal scleral sample clearing is the ratio of maximal value of collimated transmittance to value of collimated transmittance registrated at initial time. As a first approximation we offer the following dependence of time of maximal scleral sample clearing on glucose solution concentration:

$$t_{T_c}[\min] = \frac{T_{c\max}}{T_{c\,initial}} = 15.31868 - 0.01813 \cdot C[mg/ml] \tag{8}$$

Figure 21 presents dependence of degree of maximal scleral sample clearing on glucose solution concentration (mg/ml). In otherness from dependence presented in previous figure dependence of degree of maximal scleral sample clearing on glucose solution concentration has non-linear character. From this figure we can see that at high concentration of glucose solution (400 mg/ml) sharp increasing of degree of maximal scleral clearing takes place.

Using equations (1-7) and algorithm describing in Refs. 7, 8 we calculated glucose diffusion coefficient within of the scleral tissue for various glucose solution concentrations. We have obtained next values of the glucose diffusion coefficient: $1.69 \cdot 10^{-7}\ cm^2/\text{sec}$ for 180 mg/ml glucose solution concentration, $2.78 \cdot 10^{-7}\ cm^2/sec$ for 300 mg/ml glucose solution concentration, and $2.32 \cdot 10^{-6}\ cm^2/\text{sec}$ for 400 mg/ml, respectively. This results are very close to results, which reported in Refs. 4-8, 27, and 33. Obtained results is presented on Figure 22. From this figure we can see that glucose diffusion

coefficient is increased with increasing of concentration of used for tissue clearing glucose solution. We offer that this increasing is bounded with increasing of porosity coefficient, that is confirmed by implication by graphics from Figure 19, because with increasing of porosity coefficient the diffusion coefficient of liquid in fibrous porous tissue is decreased.

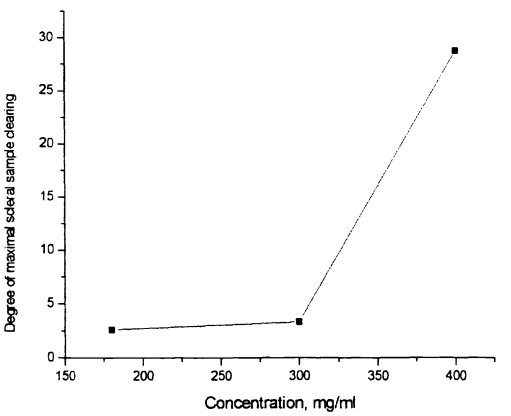

Figure 21. Degree of maximal scleral sample clearing ($T_{cmax}/T_{cinitial}$) at 589 nm versus concentration (mg/ml) of glucose solution.

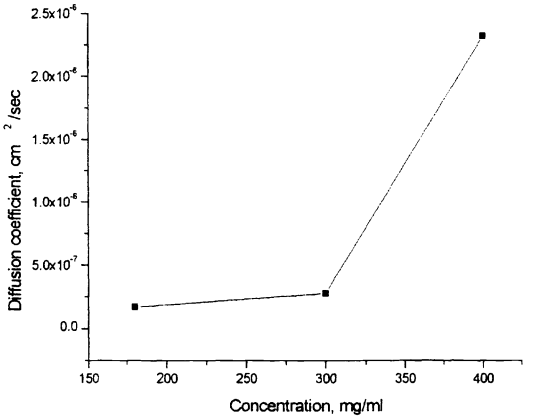

Figure 22. The calculated concentration-dependent changes of glucose diffusion coefficient for its diffusion within the human scleral sample.

Table 1.

Concentration, mg/ml	Time of maximal scleral tissue clearing, min	Degree of maximal scleral tissue clearing, time (wavelength = 589 nm)	Glucose diffusion coefficient, cm^2/sec
180	12	2.62	$1.69 \cdot 10^{-7}$
300	10	3.34	$2.78 \cdot 10^{-7}$
400	8	28.7	$2.32 \cdot 10^{-6}$

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