

# The human sclera dynamic spectra: in vitro and in vivo measurements

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## ABSTRACT

Experimental results on the optical properties of the human eye sclera controlled by administration of osmotically active chemicals, such as *propylene glycol* and *glucose* are presented. Administration of chemical agents induces diffusion of matter and as a result equalization of the refractive indices of collagen and ground material. Experimental study of influence of *propylene glycol* and *glucose* on reflectance and transmittance spectra of human eye sclera was performed. *In vitro* diffusion reflectance spectra of the whole human eye and transmittance spectra of the sclera samples were investigated. *In vivo* measurements were fulfilled on a rabbit eyes. The significant increase of transmittance and decrease of reflectance of human eye sclera and rabbit eye under action of osmolytic liquids was demonstrated. The matter diffusion coefficient for the scleral samples impregnated by *glucose* solution was estimated; the average value is  $1.27 \cdot 10^{-5} \pm 2.26 \cdot 10^{-6}$  cm<sup>2</sup>/sec. The results are general and can be used to describe many other fibrous tissues.

**Keywords:** light scattering, sclera, osmolytes, refractive index matching

## 1. INTRODUCTION

The possibility of application of transscleral diagnostic and therapeutic methods is one of the important tasks in the laser ophthalmology. Solution of the problem is connected with control of the optical properties of the human sclera. This control means change of the scattering or absorption properties of the media.

Absorption and scattering can be changed by using various physical and chemical reactions (e.g. compression, dehydration, coagulation and others). The optical properties of a tissue can be effectively controlled using osmotically active solutions.<sup>1</sup> Administrated chemical agent with a higher refractive index than that of tissue ground substance diffuses into a tissue, and water diffuses from a tissue to the surrounding solution, affect correspondingly changes of the interstitial substance, and equalizes the refractive indices of scatterers and the base material. As a result tissue optical clearing is observed.

The purpose of the present research is the *in vitro* and *in vivo* experimental study of scleral optical transmittance, controlled by administration of osmotically active chemicals and the estimation of the matter diffusion coefficient in a living tissue.

## 2. PHYSICAL PROPERTIES AND STRUCTURE OF THE HUMAN SCLERA

The sclera has a compound structure. 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 glycosaminoglicans, proteins, and protein-polysaccharide complexes.

The average diameter of the collagen fibrils increases gradually from about 65 nm in the innermost part to about 125 nm in the outermost part of the sclera. 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 show a wide range of widths (1 to 50 μm) and thickness (0.5 to 6 μm) and tend to be wider and thicker toward the inner layers. They cross each other in all directions but remain parallel to the scleral surface<sup>1,2</sup>. All these inhomogeneities give a high scattering of scleral tissue in normal state.

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The scattering properties of the sclera are defined not only by its structure but by the ratio of refractive indices of collagen fibers and base substance. Following Refs. 1, 2, we can estimate the refractive index of the scleral sample using the law of Gladstone and Dale

$$n = \sum_{i=1}^N n_i V_i, \quad \sum_i V_i = 1, \quad (1)$$

where  $n$  is the resulting average value of the tissue refractive index,  $n_i$  and  $V_i$  are the refractive index and volume fraction of an individual component, respectively, and  $N$  is the number of components.

For the average refractive index of the sclera the expression can be represented as

$$n_s = n_{col} V_{col} + n_{base} V_{base}, \quad (2)$$

where  $n_{col}$ ,  $n_{base}$  and  $V_{col}$ ,  $V_{base}$  are the refractive indices and volume fractions of collagen and base material, respectively.

Accounting that the average refractive index of sclera is  $n_s = 1.385$ , the volume fraction of hydrated collagen is  $V_{col} = 0.31$ , and refractive index of base substance is  $n_{base} = 1.345$ , on the basis of Eq. (2) refractive index of the scleral fibrils can be obtained,  $n_{col} = 1.474$ . All data were measured for  $\lambda = 589 \text{ nm}$ <sup>1,2</sup>.

### 3. OPTICAL AND MATTER DIFFUSION MODELS OF THE HUMAN SCLERA

The transmission of collimated light by a tissue layer of thickness  $l$  is defined as<sup>1</sup>

$$T_c = I/I_0 = \exp(-\mu_t l), \quad (3)$$

where  $I_0$  and  $I$  are the intensities of the collimated incident and detected light, respectively;  $\mu_t = \mu_a + \mu_s$  is the extinction coefficient, where  $\mu_a$  and  $\mu_s$  are the absorption and scattering coefficients, respectively. For the human sclera the absorption and scattering coefficients are  $\mu_a \cong 0.008 \text{ mm}^{-1}$  and  $\mu_s \cong 25 \text{ mm}^{-1}$  at the wavelength  $\lambda = 650 \text{ nm}$ <sup>3</sup>.

Optical model of the sclera in a local region can be represented as a slab with a thickness  $l$  that is filled by thin and long dielectric cylinders (collagen fibers) with average diameter  $\sim 100 \text{ nm}$  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 base substance with the refractive index  $n_{base} = 1.345$ . This refractive index is a controlled parameter and can be changed from 1.345 to 1.474. For  $n_c = n_{base} = 1.474$  the medium becomes totally homogeneous and optically transparent<sup>1</sup>.

In an approximation of noninteracting dielectric thin cylinders the ratio of two values of scattering coefficients  $\mu_s$  which correspond to the definite wavelength and different values of  $n_{base}$  is given by<sup>1</sup>

$$\mu_{s2} \cong \mu_{s1} [(m_2 - 1)(m_1 - 1)]^2, \quad (4)$$

where  $m = n_{col}/n_{base}$  is the ratio of refractive indices of the cylinders and base materials. The value  $m_1 = n_{col}/n_{base1} \cong 1.096$  is corresponded to the normal sclera in this model. It is expected the matching degree up to  $m_2 \cong 1.001$ ,  $\mu_{s2} \sim 10^{-4} \mu_{s1}$ .

To estimate the diffusion coefficient of chemical agent when it diffuses within the interstitial substance of the sclera we may consider it as a diffusion through a membrane. The following model was used for solution of the task. There are two spaces separated by a partially permeable membrane. Internal volume is filled by interstitial substance of the sclera and external one is filled by agent solution. We accounted the fluxes of the chemical agent and water containing in interstitial substance through a thin membrane. Membranes degree of permeability, accounts the structure of tissue collagen fibrils arrangement, spaces between fibrils and etc., and defines the value of the diffusion coefficient.

Assuming that both water and agent have the same paths for diffusion we can find the diffusion coefficient. From Refs. 1, 3 follows that for a large external volume, when concentration  $C_0$  of matter which surrounds tissue can be considered as a constant, the time dependent concentration of substance under study  $C(t)$  can be expressed by

$$C(t) \cong C_0 [1 - \exp(-t/\tau)], \quad (5)$$

where  $\tau = l^2/D$ ,  $D$  is the diffusion coefficient,  $l$  is the thickness of the membrane (thickness of the scleral sample).

#### 4. METHODS AND MATERIALS

Our experiments were performed *in vitro* with the human sclera and *in vivo* with the rabbit eye. During *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  $10 \times 10 \text{ mm}^2$ . The thickness of the human scleral sample was  $\sim 0.5 \text{ mm}$ .

The human scleral samples were fixed on a plastic plate with square hole  $5 \times 5 \text{ mm}^2$  and placed in a 5-ml cuvette filled with the chemical agent.

Two types of chemical agents were used for scleral optical clearing, such as *glucose-40%*, *72%* and *propylene glycol*. For the present study it was important to know the refractive indices of osmolytes. Experimental values of the refractive indices measured by Abbe refractometer ( $\lambda = 589 \text{ nm}$ ) were equal to  $n = 1.39$ ,  $1.442$  and  $1.431$  for *glucose-40%*, *glucose-72%* and *propylene glycol*, respectively.

We used a rabbit for the *in vivo* measurements. The rabbits age was 5 month and its weight was 1.5 kg. The rabbit was anaesthetized by an injection of 0.1% natrium etaminal solution. The doze was 4 ml. The measurements were started in a 30 min after the injection. *Glucose-40%* was used as a chemical agent for the scleral optical clearing. Volume of the solution was 0,003 ml, each drop.

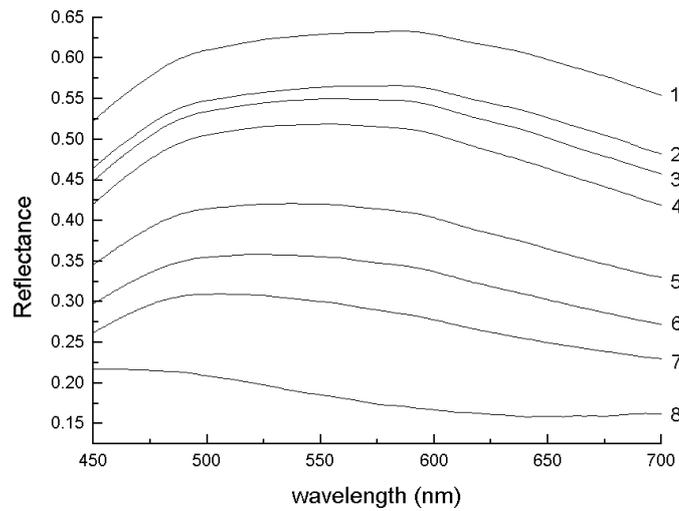
The measurements of the collimated light transmittance and the reflectance spectra were performed using OMA (fiber optic spectrometer LESA-6med, BioSpec, Russia). A 250 W xenon arc lamp with filtering of the radiation in the 400 - 800 nm spectral range was used in these measurements.

In the case of *in vitro* collimated light transmission measurements the sample was placed between two fiber optical (400  $\mu\text{m}$  core diameter). One fiber transmitted the excitation radiation to the sample, and other fiber collected the radiation transmitted by the sample. 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.

*In vitro* and *in vivo* reflectance measurements were performed using the fiber optical probe with seven fiber holders. The centrally placed fiber (400  $\mu\text{m}$  core diameter) delivers incident light to the surface of the sample and used for illumination of one, and the six surroundly fibers (400  $\mu\text{m}$  core diameter) collect reflected from the illuminated surface radiation. Fibers for collection of reflected light are mounted at small angles regarding the central fiber, so each fiber collects emission from the surface area some larger than the excitation light spot. All fibers are enclosed in aluminium 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 multichannel fiber optic spectrometer. The reflectance spectra of the samples were measured against  $\text{BaSO}_4$  as a reference. The measurements were performed every 30 sec for 30 – 60 min.

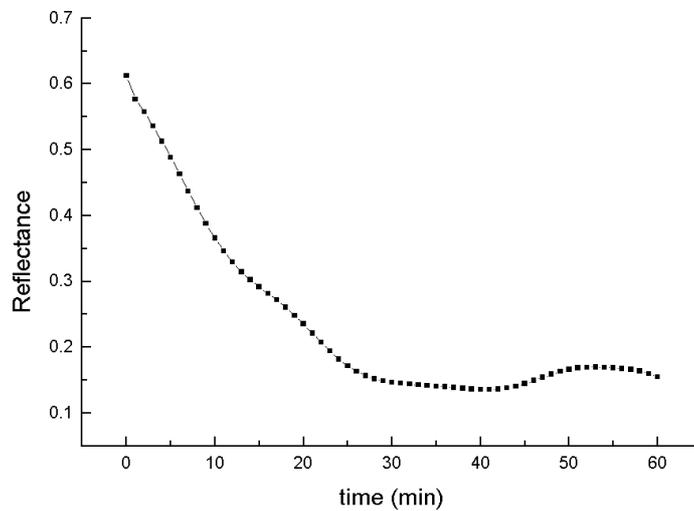
#### 5. RESULTS AND DISCUSSION

The whole human eye reflectance spectra measured by spectrophotometer for different periods of *propylene glycol* administration are presented in Figure 1. *Propylene glycol* administration makes this tissue reduced down reflectance to 15% at 630 nm. These spectra show that the reflectance of the human eye for different wavelengths can be controlled.



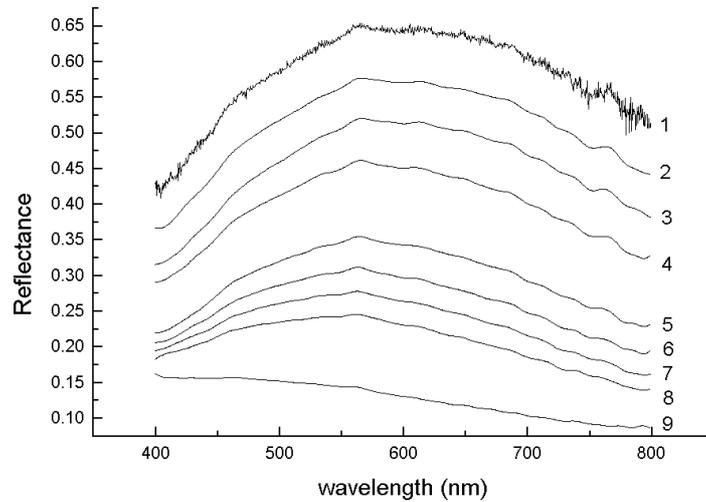
**Figure 1.** The time-dependent reflectance of the whole human eye impregnated by a *propylene glycol* solution. The represented graphs correspond to 1 - 5 sec, 2 - 120 sec, 3 - 240 sec, 4 - 360 sec, 5 - 480 sec, 6 - 600 sec, 7 - 1020 sec, 8 - 3600 sec after human eye was immersed in a *propylene glycol* solution.

The time-dependent reflectance spectra of the whole human eye sample impregnated by a *propylene glycol* solution was measured at 630 nm. The results are presented in Figure 2. It show the dynamics of tissue clearing.

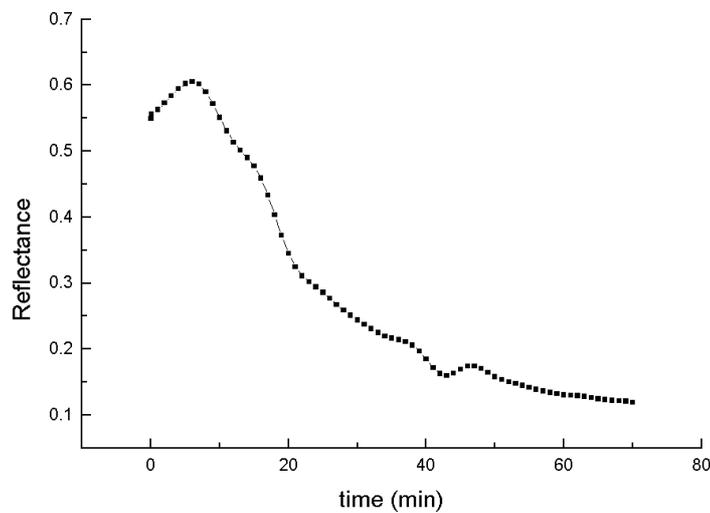


**Figure 2.** The time-dependent reflectance of the whole human eye sample measured at 630 nm concurrently with administration of *propylene glycol* solution.

For comparison the measurements of the reflectance of the human sclera sample immersed in a *propylene glycol* solution was done. The results are presented in Figure 3 and 4.



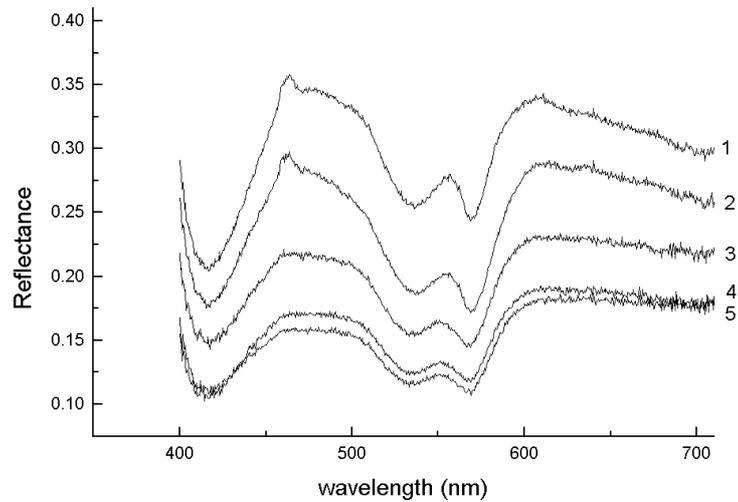
**Figure 3.** The time-dependent reflectance of the 0.5-mm-thick human scleral sample impregnated by a *propylene glycol* solution. The represented graphs correspond to 1 - 300 sec, 2 - 540 sec, 3 - 780 sec, 4 - 1020 sec, 5 - 1200 sec, 6 - 1500 sec, 7 - 1740 sec, 8 - 2040 sec, 9 - 4200 sec after human sclera sample was immersed in a *propylene glycol* solution.



**Figure 4.** The time-dependent reflectance of the 0.5-mm-thick human scleral sample measured at 630 nm concurrently with administration of *propylene glycol* solution.

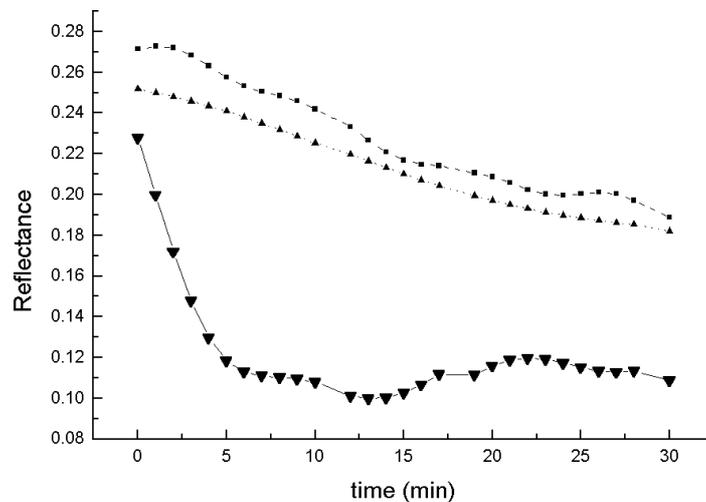
Figures 2 and 4 show that characteristic time response of human scleral optical clearing in the range 10 to 25 min was determined.

The results of the *in vivo* measurements are shown in the Figure 5. Valleys of the spectrs are caused by influence blood vessels in the rabbit eye.



**Figure 5.** The *in vivo* time-dependent reflectance spectra of the rabbit eye sclera concurrently with administration of *glucose-40%* solution. The represented graphs correspond to 1 - 60 sec, 2 - 240 sec, 3 - 1260 sec, 4 - 1500 sec, 5 - 1800 sec after the drop of *glucose-40%* solution was put into the rabbit eye.

Plots for the time-dependent reflectance of the rabbit eye sclera at three wavelength, are presented in Figure 6.

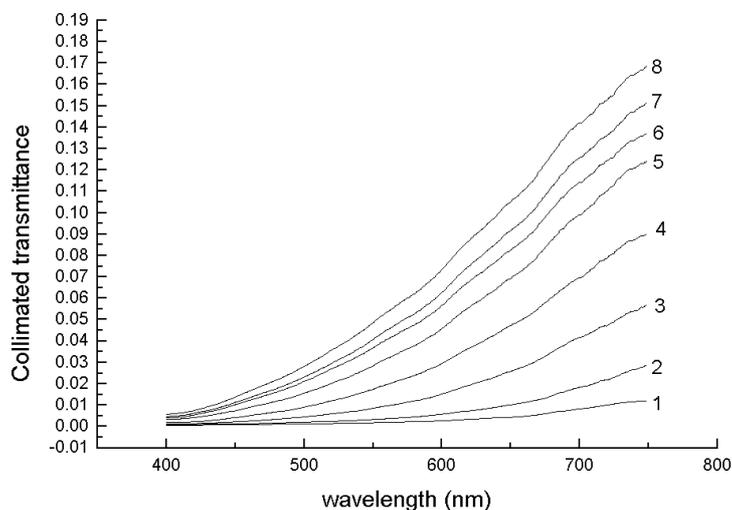


**Figure 6.** The *in vivo* time-dependent reflectance of the rabbit eye measured at 420 nm (solid line, down triangles), 630 nm (dash line, squares) and 700 nm (dot line, up triangles) concurrently with administration of *glucose-40%* solution.

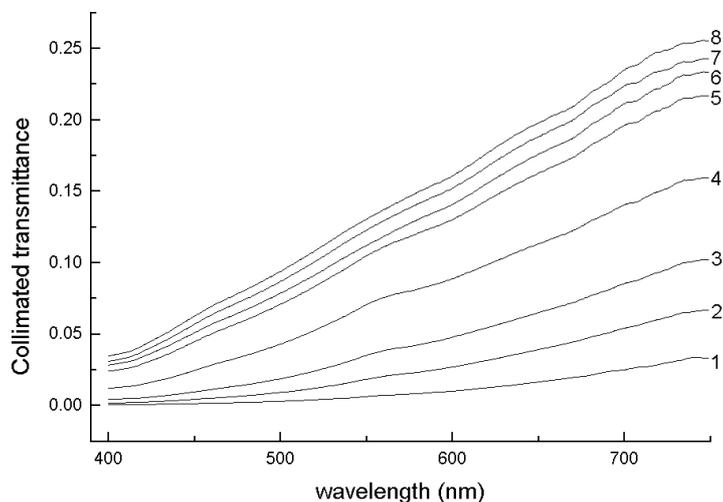
The lower reflectance at 420 nm is caused by the strong absorption of the blood, more fast decay probably reflects blood dynamics due to eye conjunctiva inflammation.

To understand the mechanisms of scleral tissue optical clearing we have investigated the collimated transmittance spectra of the human scleral samples concurrently with administration of *glucose-40%* and *glucose-72%*.

Figures 7 and 8 illustrate the transmittance spectra of the human scleral samples immersed in a *glucose-40%* and *glucose-72%* solutions, respectively.



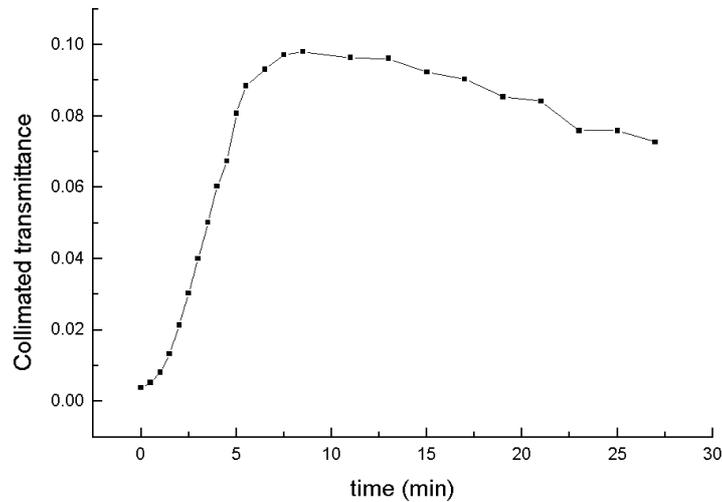
**Figure 7.** The time-dependent collimated transmittance of the human sclera sample impregnated by a *glucose-40%* solution. The represented graphs correspond to 1- 10 sec, 2 – 60 sec, 3 – 120 sec, 4 – 180 sec, 5 – 240 sec, 6 – 300 sec, 7 – 390 sec, 8 – 1620 sec after the scleral sample was immersed in a *glucose-40%* solution.



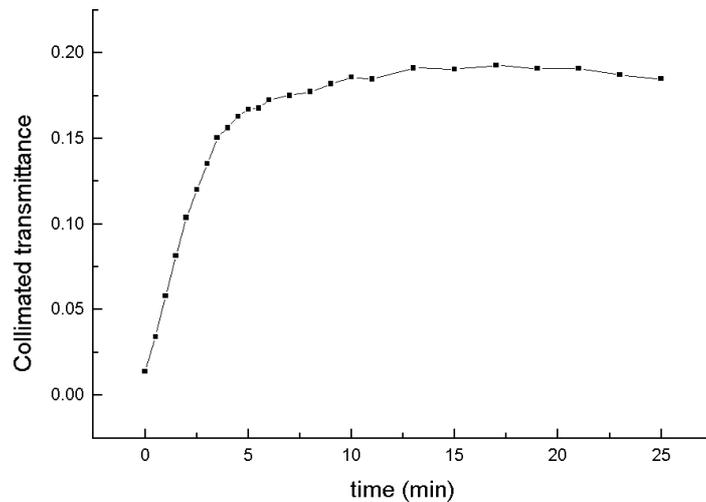
**Figure 8.** The time-dependent collimated transmittance of the human sclera sample impregnated by a *glucose-72%* solution. The represented graphs correspond to 1- 0 sec, 2 – 30 sec, 3 – 60 sec, 4 – 120 sec, 5 – 210 sec, 6 – 270 sec, 7 – 420 sec, 8 – 1500 sec after scleral sample was immersed in a *glucose-72%* solution.

The time-dependent collimated transmittance of the human scleral samples impregnated by a *glucose-40%* and *glucose-72%*

solutions was measured at 630 nm. The results are presented in Figures 9 and 10, respectively.



**Figure 9.** The time-dependent collimated transmittance of the 0.5-mm-thick human scleral sample measured at 630 nm concurrently with administration of *glucose-40%* solution.



**Figure 10.** The time-dependent collimated transmittance of the 0.5-mm-thick human scleral sample measured at 630 nm concurrently with administration of *glucose-72%* solution.

Measurements of the time-dependent collimated transmittance allowed us, basing on presented tissue refractive index matching model, to estimate the diffusion coefficients of chemical agents [see Eqs. (1) – (4)]. The refractive indices of *glucose-40%* and *glucose-72%* were obtained experimentally. The mean value of the diffusion coefficient for *40% glucose* solution transport in the human scleral sample is equal to  $1.27 \cdot 10^{-5} \pm 2.26 \cdot 10^{-6}$  cm<sup>2</sup>/sec and mean value of the diffusion

coefficient for 72%-glucose solution transport in the human scleral sample is equal to  $1.48 \cdot 10^{-5} \pm 5.23 \cdot 10^{-6}$  cm<sup>2</sup>/sec. This result are illustrated by Figures 9 and 10. It is well shown that the clearance is more effectively in the case of higher concentration of the osmolyte. The degree of clearance of the sclera administrated by 72%-glucose solution is 5 time higher than that under influence of 40%-glucose solution. The results of the experiments confirm the correctness of the refractive index matching model <sup>1</sup> since the refractive index of the 72%-glucose solution is higher than that of the 40%-glucose solution.

## 6. CONCLUSION

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 sclera are effectively reduced by the refractive indices matching of the collagen fibrils and interstitial substance.

The dynamics of tissue optical clearing using osmolytes is defined by a characteristic time response of about 10 – 20 min in dependence on of the used tissue and osmolyte. Dynamic characteristics were successfully used for estimation of diffusion coefficients of studied chemicals (40%-glucose and 72%-glucose solutions) in the human sclera. These values are well match to values of diffusion coefficient of small molecules diffusion in water <sup>4</sup>.

## 7. ACKNOWLEDGEMENTS

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