Estimation of glucose diffusion coefficient in scleral tissue

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ABSTRACT

Results of experimental and theoretical study of the optical properties of the eye sclera controlled by administration of osmotically active chemical, such as glucose, are presented. Glucose administration induces the diffusion of matter and as a result the equalization of the refractive indices of collagen fibrils and base material, and corresponding changes of transmittance spectra of scleral tissue. Transmittance spectra of the human scleral samples impregnated by glucose were measured. The significant increase of transmittance under action of osmotic liquid was observed. The diffusion coefficient of glucose within scleral tissue was estimated; the average value is $3.45 \cdot 10^{-6} \pm 4.59 \cdot 10^{-7}$ $cm^2/\sec$. The results are general and can be used to describe many other fibrous tissues impregnated by osmotically active chemical agents.

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 [1-6]. In general, a number of laser surgery, therapy, and diagnostic technologies use the tissue compression and stretching for a better transport of the laser beam to underlying layers of tissue [1,7]. Compression of the human eye sclera allows us to perform the transscleral laser coagulation of the ciliary body and retina/choroid [1]. Selective clearing of the upper tissue layers should be very useful for performing of transscleral laser coagulation of the ciliary body and retina/choroid, as well as for detecting of local inhomogeneities hidden by a highly scattering medium in the human eye tomography.

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 [8-10]. 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 increase in tissue glucose concentration reduces index 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 paper [4-6]. 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 and theoretical study of scleral optical transmittance controlled by administration of glucose solution at a high concentration. Glucose having a higher refractive index than that of tissue base (extrafiber) substance diffuses into a tissue (water diffuses from a tissue to the surrounding solution) and correspondingly the equalizing of the refractive indices of scatterers (collagen fibers) and the base substance is observed. As a result, the tissue optical clearing is observed. *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 coefficient 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 thickness. 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 base material, respectively; $C_{vcol} + C_{v\,b} = 1$.

Measurement of the average refractive index of the human sclera [1], $n_s = 1.385 \pm 0.005$ at $\lambda = 589$ nm, for the estimated volume fraction of hydrated collagen, $C_{vcol} = 0.31$, and refractive index of base substance, $n_{gr} = 1.345$, 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 base substance with the refractive index $n_{gr} = 1.345$. This refractive index is a controlled parameter and can be change in the range from 1.345 to 1.474. For $n_{col} = n_{gr} = 1.474$ 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 $\mu'_s = \mu_s(1\text{-}g) \cong 25$ cm^{-1}, where g is the scattering anisotropy factor [11]. 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 [4,12-16]

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

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. 15 for a system of non-interacting thin cylinders the scattering coefficient can be estimated as $\mu_s = \sigma_s \cdot \frac{C_{v\,col}}{\pi \cdot a^2}$, where $C_{v\,col}$ 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 [17,18] we took into account the spatial ordering of scatterers.

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

To describe dynamics of the refractive index and corresponding decreasing of the scattering coefficient when glucose diffuses within the interstitial substance of the sclera we used the model of free diffusion [4,19,20]. 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 [19]

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

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 [19]. 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 [21]. Porosity coefficient we define as: $p = \frac{V - V_{c\,col}}{V}$, where V is the volume of the scleral sample, and $V_{c\,col}$ is the volume of collagen fibers. Diffusion coefficient is defined as $D=p\cdot D_0+(1-p)\cdot D_1$, where D_0 is the glucose diffusion coefficient in interstitial fluid, and D_1 is the glucose diffusion coefficient within collagen fibers. Assuming that D_1 is equal to zero we have $D=D_0/p$.

When applying the chemical change 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 [22,23].

4. MATERIALS AND METHODS

The measurements of the light 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 - 800 nm spectral range was used in these measurements.

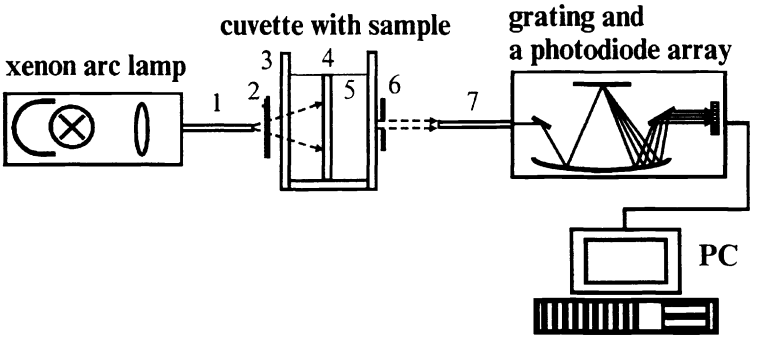

Fig. 1. Experimental setup for measurements of the collimated light transmittance spectra: 1 – optical fiber; 2 – neutral filters; 3 - cuvette; 4 – human sclera sample; 5 – osmotical active solution (glucose-40%); 6 - the 0.5 mm – diaphragm; 7 – optical fiber.

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 transmitted 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 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. 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 10×10 mm^2. The mean thickness of the human scleral samples was about 0.5 mm. The human scleral samples were fixed on a plastic plate with a square aperture 5×5 mm^2 and placed in a 5-ml cuvette filled with the glucose solution.

For scleral optical clearing the 40% aqueous solution of glucose with $n = 1.39$ ($\lambda = 589$ nm) was used. Glucose does not have strong absorbing bands within the wavelength of study, 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 cuvette filled by immersion liquid (glucose-40% solution) with volume 1,5 ml. All experiments were performed at room temperature.

5. EXPERIMENTAL RESULTS

To understand the mechanisms of the scleral tissue optical clearing we have investigated the collimated transmittance spectra and gravimetrical change of the scleral sample weight concurrently with administration of glucose solution. Figure 2 illustrates the transmittance spectra. It is easily seen that the untreated sclera is poorly transparent for the visible light. Glucose administration makes this tissue highly transparent, for example, up to 18 % at 750 nm for the sample kept in solution for $t = 8.5$ min. 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. 3. They show the dynamics of tissue clearing. Figure 3 shows that characteristic time response of human optical clearing is about 8.5 minutes. Figure 4 illustrated the time-dependent mass change of the human sclera sample measured concurrently with administration of glucose –40% solution (up triangles).

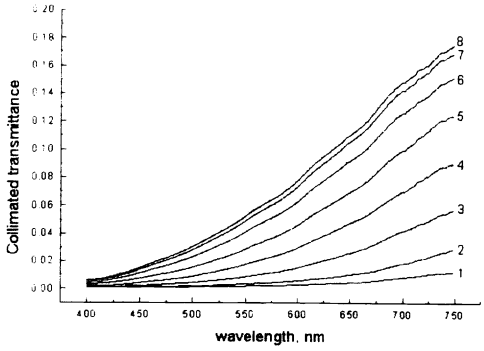

Fig. 2. The time-dependent collimated transmittance of the human sclera sample impregnated by a glucose-40%: 0 - sec; 2 – 60 sec; 3 – 120 sec; 4 – 180 sec; 5 – 240 sec; 6 – 300 sec; 7 – 390 sec; 8 – 510 sec after the sclera sample was immersed in a glucose-40%.

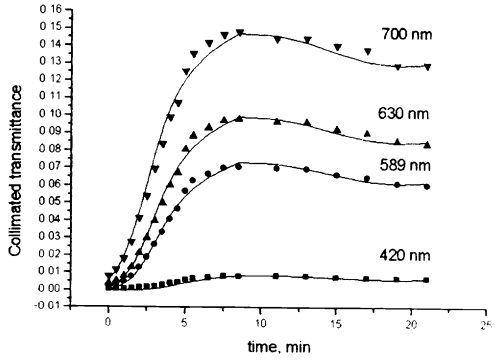

Fig. 3. The time-dependent collimated transmittance of the human sclera sample measured at 420 nm (squares); 589 nm (circles); 630 nm (up triangles); and 700 nm (down triangles) concurrently with administration of glucose-40%. Solid line corresponding to the data calculated from our model.

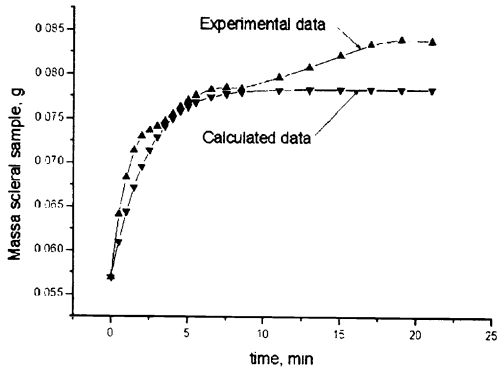

Fig. 4. The time-dependent change mass of the human sclera sample measured concurrently with administration of glucose-40%. Up triangles – experimental data; Down triangles – data calculated from our model.

6. ESTIMATION OF DIFFUSION COEFFICIENT

For estimation of diffusion coefficient we used experimental data for the collimated transmittance and gravimetrical measurements which are presented in the Fig. 2, 3 and 4. The transport of glucose in tissue sample can be described in the framework of the matter diffusion model. We assume that the following approximations are valid for glucose transport:

1. The diffusion coefficient $D(t)$ is constant over the entire scleral volume but it depends on time due to changes of porous coefficient with time;

2. The time-dependent concentration of glucose in tissue is defined as $C_{gl}(t) = C_{gl}^{0} \cdot \left(1 - \exp\left(-\frac{t}{\tau}\right)\right)$, where C_{gl}^{0} is the concentration of glucose in the external volume (expressed in g/ml), $C_{gl}^{0} = 0.38$; $\tau = \frac{d(t)^2}{\pi^2 D(t)}$ is the diffusion constant; *d(t)* is the thickness of the scleral sample, which depends on time;
3. The volume of water associated with collagen in fibrils of tissue: $V_{H_2O\_ass}(t) = V_{0H_2O\_ass} + \frac{M_{ass\_max}}{\rho_{H_2O}} \cdot \left(1 - \exp\left(-\frac{t}{\tau}\right)\right)$, where $V_{0H_2O\_ass} = 0.1072 \cdot V$ is the initial volume of water associated with collagen within fibers of the scleral sample, ρ_{H_2O} - density of water, and where M_{ass_max} - the maximum amount of water which is bounding to collagen fibrils;
4. Mass of collagen fibers: $M_c(t) = V_{0dc} \cdot \rho_{dc} + V_{H_2O\_ass}(t) \cdot \rho_{H_2O}$, where $V_{0dc} = 0.2028 \cdot V$ - volume of the dry collagen, and $\rho_{dc} = 1.27\ g/cm^3$ - density of dry collagen [24];
5. Volume of the collagen fibers is defined as $V_c(t) = V_{0dc} + V_{H_2O\_ass}(t)$;
6. Refractive index of the collagen fibers: $n_c(t) = \frac{V_{0dc}}{V_c(t)} \cdot n_{dc} + \frac{V_{H_2O\_ass}(t)}{V_c(t)} \cdot n_{H_2O}$, where $n_{dc} = 1.55$ is the refractive index of the dry collagen [4];
7. Use of equation (7) allows to define time-dependence mass of glucose dissolved in interstitial fluid of the scleral tissue sample: $M_{gl}(t) = \frac{C_{gl}(t)}{1 - \frac{C_{gl}(t)}{\rho_{gl}}} \cdot V_{0H_1O\_free} \cdot \rho_{H_1O}$, where $V_{0H_1O\_free} = 0.69 \cdot V$ - initial quantity of the water content in scleral sample; $\rho_{gl} = 1.55\ g/cm^3$ - density of glucose [24];
8. Volume of glucose dissolved in interstitial fluid: $V_{gl}(t) = \frac{M_{gl}(t)}{\rho_{gl}}$;
9. Mass of the scleral sample is equal to $M_s(t) = M_c(t) + M_{0H_2O} + M_{gl}(t)$, where $M_{0H_2O} = V_{0H_2O\_free} \cdot \rho_{H_2O}$ is the mass of water initial content of the sclera, and $M_{gl}(t)$ is the mass of glucose dissolved in interstitial fluid within scleral sample;
10. Mass of glucose solution added to mass of the scleral sample because of the swelling $M_{gl\_sol\_add}(t) = M_{exp}(t) - M_s(t)$;
11. Volume of glucose solution added to volume of the scleral sample because of the swelling: $V_{gl\_sol\_add}(t) = \frac{M_{gl\_sol\_add}(t)}{\rho_{gl\_sol}}$, where $\rho_{gl\_sol} = 1.135\ g/ml$ - density of glucose solution (it's a result of our measurement);
12. The refractive index of water is defined as [4]: $n_{H_1O} = 1.31848 + \frac{6.662}{\lambda[nm] - 129.2}$, where λ - wavelength of light;
13. Let the refractive index of glucose solution which depends on concentration and time define as[4]: $n_{gl}(t) = n_{H_1O} + 0.1515 \cdot C_{gl}(t)$;

14. The refractive index of the interstitial fluid (base substance): $n_b(t) = \frac{V_{0_{H_2O\_free}} + V_{gl}(t)}{V_{0_{H_2O\_free}} + V_{gl}(t) + V_{gl\_sol\_add}(t)} \cdot n_{gl}(t) + \frac{V_{gl\_sol\_add}(t)}{V_{0_{H_2O\_free}} + V_{gl}(t) + V_{gl\_sol\_add}(t)} \cdot n_{gl\_sol}$, where $n_{gl\_sol} = 1.39$ - refractive index of glucose solution (it's a result of our measurement);
15. Wave number of light in the sclera: $k(t) = \frac{2 \cdot \pi \cdot n_b(t)}{\lambda}$;
16. Time-dependence of the sclera volume: $V_{scl}(t) = V_c(t) + V_{0_{H_2O\_free}} + V_{gl}(t) + V_{gl\_sol\_add}(t)$;
17. Time-dependence of the scleral sample thickness: $d(t) = \frac{V_{scl}(t)}{S}$, where $S = 1$ cm^2 – area of the scleral sample. The time-dependent change of area scleral sample was neglected;
18. As we apparent the change of volume of scatterers, we calculate the new radius of scatterers using the formula: $a(t) = \sqrt{\frac{V_c(t) \cdot a_0^2}{V \cdot 0.2028}}$, where a_0 = 50 nm - initial radius of the scatterers, and $V \cdot 0.2028$ is volume collagen fibers in initial time;
19. Time-dependent volume fraction of the scatterers: $\eta(t) = \frac{V_c(t)}{V_{scl}(t)}$;
20. The scattering coefficient dependence on refraction index is defined by Eq. 4;
21. The refractive index of the scleral sample: $n_{scl}(t) = \frac{V_c(t)}{V_{scl}(t)} \cdot n_c(t) + \left(1 - \frac{V_c(t)}{V_{scl}(t)}\right) \cdot n_b(t)$, where $n_c(t)$ is the time-dependent refractive index of collagen fibers, $n_b(t)$ is the time-dependent refractive index of base substance (interstitial fluid);
22. The specular reflectance can be estimated from the collimated transmittance of the scleral sample impregnated by a glucose solution: $T_c(t) = (1 - R_s(t))^2 \cdot \exp(-(\mu_a + \mu_s(t)) \cdot d(t))$, where $R_s(t)$ is the specular reflectance and μ_a and μ_s are the absorption and the scattering coefficients, respectively.

This set of relations describing the dependence of glucose concentration on time represents the direct problem. The reconstruction of the diffusion constant and the maximal quantity of the water additionally associated with collagen in fibers of the scleral sample was carried out based on measured time evolution of the collimated transmittance. The solving of the inverse problem were fulfilled by minimization of the target function: $F(t) = (T_c(t) - T_c^*(t))^2$, where $T_c(t)$ and $T_c^*(t)$ are the theoretical and experimental values of the time-dependent collimated transmittance respectively. The Levenberg-Marquardt method described in Ref.25 was used for the minimization of the target function.

This method allows us to calculate time-dependent volume (and consequently the thickness) of the scleral sample. Knowing it we can estimate the glucose diffusion coefficient in scleral tissue. The results of such calculations are presented in Fig. 4-8. We have estimated the average value of the diffusion coefficient of glucose transport in the scleral sample as $3.45 \cdot 10^{-6} \pm 4.59 \cdot 10^{-7}$ cm^2/sec. Maximal mass of water additionally associated with collagen of fibers scleral sample is equal to $2.56 \cdot 10^{-3}$ g.

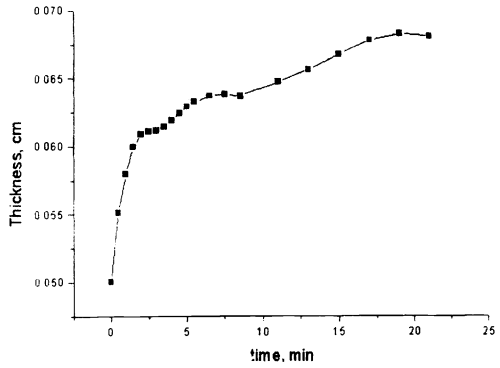

Fig. 5. The calculated time-dependent thickness of the human sclera sample.

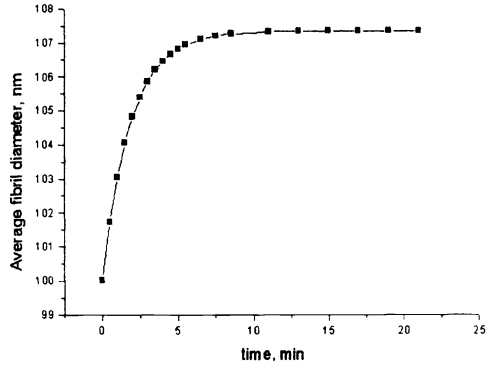

Fig. 6. The calculated time-dependent change of the average fibril diameter of the human sclera sample.

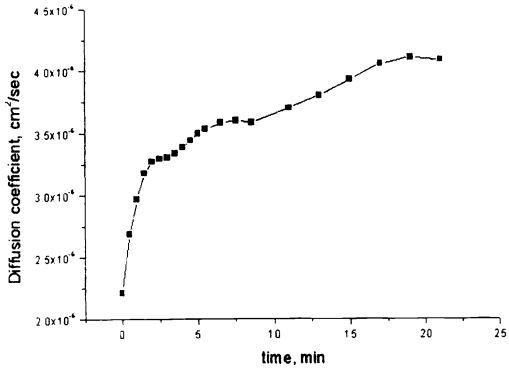

Fig. 7. The calculated time-dependent change of glucose diffusion coefficient in the human sclera sample.

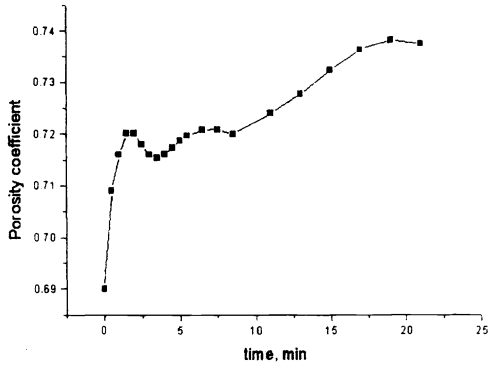

Fig. 8. The calculated time-dependent change of porosity coefficient of the human sclera sample.

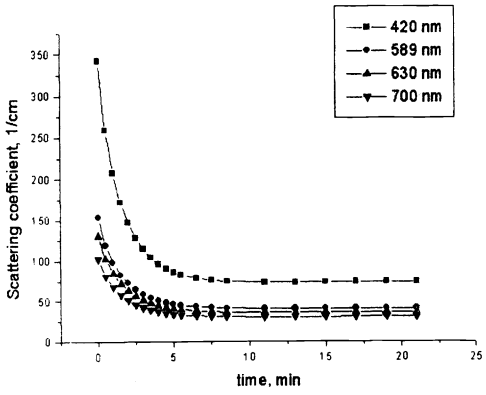

Fig. 9. The calculated time-dependent change of scattering coefficients of collagen fibrils in the human sclera sample at different wavelength..

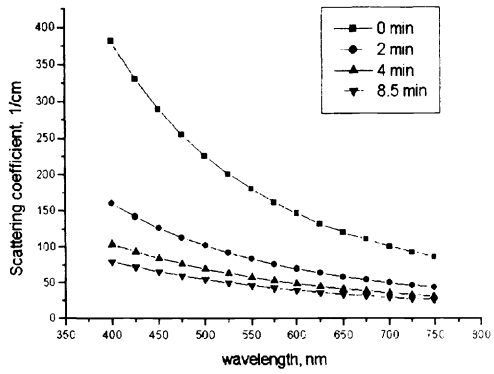

Fig. 10. The calculated scattering coefficient of sclera sample at different time: 0 min – squares; 2 min – circles; 4 min – up triangles; 8.5 min – down triangles.

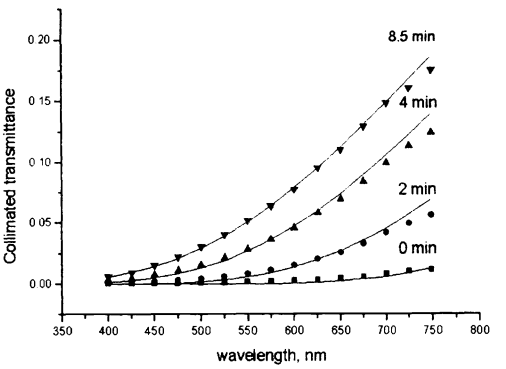

Fig. 11. The collimated transmittance of the human sclera sample measured concurrently with administration of glucose-40% from different time. Squares – 0 min; circles – 2 min; up triangles – 4 min; down triangles – 8.5 min. Solid lines correspond to data calculated from our model.

7. DISCUSSION

Presented experimental and theoretical results show well that the human scleral transmittance spectra can be substantially changed by administration of osmotically active chemical agent (glucose solution). Analysing the data presented in a fig. 2-4 we offer the following model, which describes the apparented results. We suggest to divide the description of osmotically active liquid action on a fibrous tissue into two stages. At the first stage, after location of a sample in an osmotically active solution, we apparent considerable increasing of the sample mass (fig. 4) and its considerable enlightenment (fig. 3). This stage is finished through the 8.5 min after beginning of the process. It is because of considerable swelling of collagen fibers in glucose solution with pH 3.5 (our measurements). Therefore, the magnification of water content within collagen fibrils and because of this the decreasing of refractive index of scatterers takes place. Simultaneously the increasing of the refractive index base substance of a sclera is accrued. Thus, at the first stage we apparent two competing processes: on the one hand the magnification of the sizes of scatterers gives increase of a scattering coefficient and on the other hand we apparent the matching effect. This effect caused by both the magnification of an refractive index of base substance of a sclera and the decreasing of the refractive index of the scatterers. As a result, we apparent a considerable enlightenment of a scleral sample. At the following stage, which occurs through the 8.5 minutes, the swelling of the collagen fibrils is finished. However, the solution of glucose continue to act in space between fibrils. This process is presented in figures 3 and 4. The figure 4 shows that the weight of a sample prolongs to be incremented, and the transmittance is decreased (figure 3). From this discussion we conclude, that the glucose diffusion has stopped, and the only process of the sample swelling is continued. It result in volume sample magnification mainly due to the increasing of the thickness and consequently its unclearing is occurred. The curves presented in a figure 4 well illustrate this process. The upper curve shows the experimentally measured change of scleral sample mass at the first stage. It is closed to the lower curve, which characterizes the process of glucose diffusion inside the scleral sample.

We have calculated the change of the investigated sample thickness (figure 5). Under solution of an inverse problem, the change of the average diameter of scattering fibrils was found. Figure 6 shows that during the first 8.5 minutes the swelling of scatterers is observed, that completely confirm our model. The other results obtained by us are presented in figures 7 and 8. The time-dependent change of glucose diffusion coefficient in scleral tissue is shown in the figure 7. In a figure 8 the change of a porosity coefficient of an investigated sample is presented. The direct correlation between these figures is exist. As was mentioned above, the diffusion coefficient of glucose in porous bodies is a ratio of a diffusion coefficient of the glucose through the tissue's liquid to the porosity coefficient. The porosity coefficient is a ratio of lacuna volume in a tissue (sclera) to total volume of the sample. In a limit, with increasing of a porosity coefficient, the diffusion coefficient of glucose in a sclera is close to a diffusion coefficient of glucose into free fluid (water). Usually in the literature the value of a diffusion coefficient of glucose into water is $D_0 = 5.2 \cdot 10^{-6}\ cm^2/\sec$. At the same time it is well known that with the increasing of concentration the quantity of diffusion coefficient is decreasing. It is connected with increasing of diffusing

solution viscosity [26,27]. We have estimated a diffusion coefficient of glucose in interstitial fluid as $2.5 \cdot 10^{-6} \pm 3.62 \cdot 10^{-7}$ cm^2/sec. This value is closed to data presented in Ref. 26 and 27. Average value of glucose diffusion coefficient in scleral tissue is equal to $3.45 \cdot 10^{-6} \pm 4.59 \cdot 10^{-7}$ cm^2/sec. Difference between our results and values reported by us in previous works [5,6] is explained by the following reasons: we did not take into account the sample swelling and the change of scatterer sizes. Using data obtained under solution of an inverse problem (diffusion coefficient of glucose, size and refraction index of the fibrils) we have calculated a time dependence of change of scleral sample scattering coefficient (figure 9). We can see that although with magnification of the scatterer sizes (see figure 6) the scattering cross-section will increase (see eq.3) the explicit and monotonic decreasing of the scattering coefficient is observed. We also have calculated the transmittance of the scleral sample depending on wavelengths in different periods of enlightenment. The results are represented in a figure 11. The different symbols and solid lines correspond to experimental data and data obtained from our calculations, respectively. The good matching between model and experiment for transmittance dependence on a wavelength is observed. In figure 10 the scattering coefficient of the sclera dependence on light wavelength in different periods of an enlightenment of a sample are shown. With increasing of the wavelength the scattering coefficient decrease. It is well matched with known experimental data and theoretical representation [28].

8. CONCLUSION

The results of this paper show that administration of osmolytes to a fibrous tissue allows to control its optical characteristics effectively. The dynamics of tissue optical clearing by 40%-glucose solution is defined by a characteristic time response of about 8.5 min. Besides, the tissue swelling plays an important role in tissue clearing. For more continued administration of the osmolyte (from 8 to 20 min for 40%-glucose solution) tissue swelling saturates and even slightly reduces the tissue transparency.

Dynamic optical characteristics can be used for determination of diffusion coefficient of endogenous (metabolic) and exogenous (chemical agent) fluids in the human sclera and other tissues. Obtained value for diffusion coefficient of 40%-glucose in the human scleral tissue is well matched with values of diffusion coefficient of small molecules diffusion in water [4,19,29].

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