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COMPLEX SYSTEMS BIOPHYSICS

Estimation of the Glucose Diffusion Coefficient in Human Eye Sclera

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Abstract—Diffusion coefficients were estimated *in vitro* for aqueous glucose solutions in human eye sclera. The method is based on assessing the temporal dynamics of the collimated transmission of a biological tissue specimen exposed to biocompatible immersion liquids. The change in the collimated transmission is associated with the matching of the refractive indices of the tissue light scatterers and the interstitial fluid. The dynamics of the replacement of the interstitial fluid by the immersion liquid was monitored by consecutive recording of the collimated transmission spectra in the 400–800 nm range. The process was quantitatively described with a diffusion model implying constancy of the diffusion coefficient throughout the volume of the scleral specimen. Experiments were performed with glucose solutions of 0.18, 0.3, and 0.4 g/ml. The diffusion coefficients were determined by approximating the experimental data within the framework of the model proposed.

Key words: diffusion, sclera, optical immersion, control of tissue optical properties

INTRODUCTION

The optical properties of biological tissues can be efficiently controlled by impregnating them with biocompatible liquids [1–5]. Thereby the main mechanism of control is optical immersion—i.e., matching of the refractive indices of the tissue light scatterers (e.g., collagen fibers) and the interstitial fluid—taking place upon penetration of a biocompatible immersion liquid into the tissue. Such control is important both for elucidating the basic patterns of tissue metabolism and for implementing the methods of optical and laser diagnosis, therapy, and surgery [1]. In particular, clarification of the eye sclera with the aid of osmotically active liquids is essential for transscleral eye surgery, for developing noninvasive techniques of optical tomography of the eye, and for evaluating the homeostasis of tissue fluids [5].

Diffusion coefficients must be known to build mathematical models that would adequately describe the interactions of osmotic liquids with biological tissues. Although the diffusion of many biocompatible liquids in aqueous solutions has been studied well enough [6–8], their diffusion in biological tissues remains a poorly explored field [2, 5, 8–13].

The present work was aimed at estimating the diffusion coefficients for glucose in the human sclera, basing on *in vitro* experimental assessment of the optical properties of the sclera exposed to aqueous solutions of glucose.

EXPERIMENT AND MODEL

The experiments were performed using a multichannel optical analyzer LESA-6med (BioSpek, Russia). The experimental setup is schematically shown in Fig. 1. The cuvette filled with an aqueous glucose solution and containing a specimen of sclera fixed in a special plastic holder was placed between two fiber light guides (diameter 400 μm, numerical aperture 0.20). The collimated transmission spectra over the 400–800 nm range were taken every 30 s for 10–15 min after placing the sclera into the glucose solution. The measurement error did not exceed 5% of

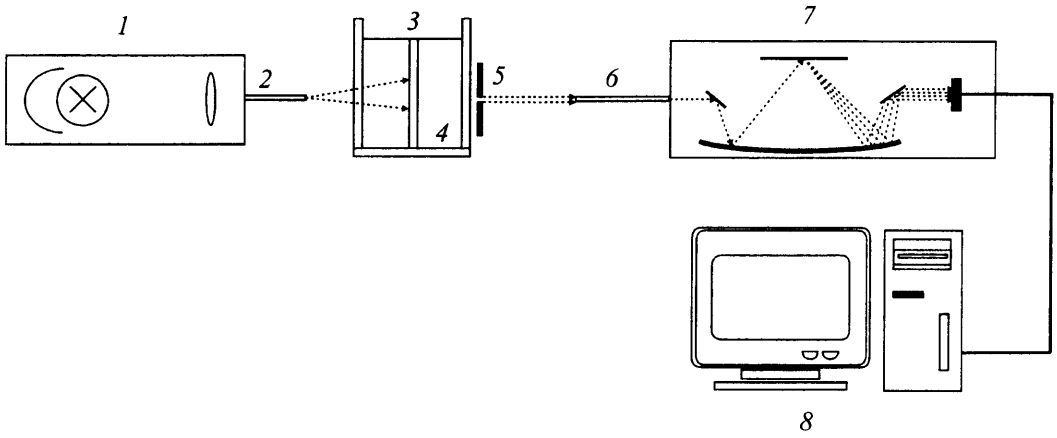

Fig. 1. Scheme of the experimental setup: (*1*) light source (250 W xenon bulb); (*2*, *6*) input and collecting light guides; (*3*) specimen of sclera; (*4*) cuvette with glucose solution; (*5*) 0.5-mm diaphragm placed 10 cm before the collecting light guide; (*7*) multichannel optical analyzer; (*8*) personal computer.

the measured value at wavelengths above 500 nm, and 10% at shorter wavelengths.

Aqueous solutions of glucose 0.18 g/ml (pH 6.32), 0.3 g/ml (pH 5.91), and 0.4 g/ml (pH 5.59) were prepared from powdered glucose monohydrate (Khim-Med, Russia). Refractive indices were measured with an Abbe refractometer at 589 nm. The pH of the solutions was determined with a HANNA pH meter (Portugal).

Scleral specimens were obtained on autopsy within 24 h post mortem, placed into 0.9% aqueous NaCl (pH 6.8) and kept at 3°C till the experiment (not longer than 24 h). Collimated transmission was measured at 20°C. The thickness of 1×1 cm specimens was determined immediately before the experiment. The results of measurements are given in the table.

The light-scattering properties of sclera are determined by its structure and by the relation of the refractive indices of the scattering inhomogeneities (collagen fibers) and of the scleral interstitial fluid that fills the space between the collagen fibers [3, 5].

Examining the interaction of aqueous glucose solutions with scleral specimens, we assumed that only the refractive index of the interstitial fluid changes in this interaction because of the diffusion of the immersion liquid into the tissue and the osmotic efflux of water from the tissue. As a substance with a refractive index higher than that of the interstitial fluid enters the tissue while water leaves it, there takes place certain matching of the indices of the scatterers and the surrounding liquid, whereby the light scattering coefficient of the tissue decreases. Assessment of the temporal dynamics of this process allows one to estimate the diffusion coefficient as a measure of the mean rate of the exchange flux of the osmotically active substance into the biological tissue and water from the tissue.

The movement of glucose into the scleral tissue was described in the framework of the diffusion theory. The following assumptions were made as regards the transfer process: (i) there is only concentration-driven diffusion, i.e., the exchange flux of glucose into the tissue and water therefrom is proportional to the glucose concentration gradient at the given point; (ii) the diffusion coefficient is the same at any point within the tissue specimen under study.

Geometrically, the scleral specimen is a plane-parallel plate of finite thickness. As the area of the upper and lower surfaces of this plate is far greater than that of its side surfaces, one may neglect the edge

Diffusion coefficients determined for glucose solutions in human sclera (averaged over 500–750 nm)

No.	Specimen thickness, cm	Glucose concentration, g/ml	Refractive index	Diffusion coefficient, cm^2/s
1	0.050 ± 0.001	0.18	1.360	$(0.57 \pm 0.09) \cdot 10^{-6}$
2	0.051 ± 0.002	0.30	1.378	$(1.47 \pm 0.36) \cdot 10^{-6}$
3	0.048 ± 0.002	0.40	1.390	$(1.52 \pm 0.05) \cdot 10^{-6}$

effects and solve a unidimensional diffusion problem, i.e.,

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

where $C(x,t)$ is glucose concentration in the sclera, g/ml; D is diffusion coefficient, cm^2/s; t is time of the diffusion process; x is the spatial coordinate along the specimen thickness, cm. As the volume of glucose solution (≈3000 mm^3) in the experiment greatly exceeded the volume of the specimen (≈50 mm^3), the corresponding boundary conditions are

$$C(0,t) = C(d,t) = C_0,$$

where C_0 is glucose concentration in solution and d is specimen thickness, cm. The initial conditions pertain to the absence of glucose at any inner point of the specimen prior to its immersion into the solution, i.e., $C(x,0) = 0$.

Solving the diffusion equation, one can estimate the mean concentration of glucose within the specimen at any moment [8]:

$$C(t) = $$
$$= C_0\left(1 - \frac{8}{\pi^2}\sum_{i=0}^{\infty}\frac{1}{(2i+1)^2}\exp\left(-\frac{(2i+1)^2\pi^2 D}{d^2}\right)\right); \quad (1)$$

further, using the relationship $n_{gl} = n_{H_2O} + 0.1515C$ [14] for aqueous glucose solutions, where n_{H_2O} is refractive index of water [1] and C is glucose concentration, g/ml, one can assess the time dependence of the refractive index $n_I(t)$ of the liquid in the tissue:

$$n_I(t) = n_{I_0} + \frac{0.1515C(t)}{1-\varphi},$$

where $n_I(0)$ is the refractive index at the initial moment; φ is the volume fraction of scatterers in the tissue (for sclera, $\varphi = 0.3$ [5]). The $(1 - \varphi)$ term was introduced to account for the porosity. Since the specimens were kept for about 24 h in 0.9% aq. NaCl, it was assumed that by the time of the experiment the interstitial fluid had been replaced with the NaCl solution, which has practically the same refractive index as water.

The change in n_I leads to a decrease in the scattering coefficient μ_s, which can be described [5, 15] as

$$\mu_s(t) = N\sigma_s(t) = N\frac{\pi^2 a x^3}{8}(m^2-1)^2\left(1 + \frac{2}{(m^2+1)^2}\right), \quad (2)$$

where N is the number of scatterers per unit volume; σ_s is the scattering cross-section; $x = 2\pi a n_I/\lambda$ is the diffraction parameter; $m = n_C/n_I$ is the relative refractive index of the scatterers (n_C is the refractive index of collagen in sclera [16]); a is the scatterer radius.

For a scleral specimen, at 589 nm, at the initial moment $n_C = 1.474$ [5, 16], $n_I = 1.333$ [1], and $a \approx 50$ nm [5]; having calculated the scattering cross-section for the given wavelength, one can estimate N as the ratio of the experimentally measured refractive index for sclera at zero time to the scattering cross-section at the same wavelength.

The time dependence of the collimated transmission coefficient for a specimen of sclera placed in glucose solution appears as

$$T_c(t) = \exp(-(\mu_a + \mu_s(t))d), \quad (3)$$

where μ_a is the sclera absorption coefficient. In calculations we used the absorption coefficients obtained by other authors [17]. Since the decline in acidity caused by displacement of the interstitial fluid by the glucose solution during the experiment causes only insignificant tissue swelling [18], the change in specimen thickness was disregarded.

Equations (1)–(3) define the dependence of the collimated transmission coefficient on glucose concentration within the scleral specimen, i.e., set a direct problem. The reverse problem in this case is to deduce the diffusion coefficient from the dynamics of collimated transmission. This problem was solved by minimization of the target function

$$f(D) = \sum_{i=1}^{N_t}(T_c(D,t_i) - T_c^*(t_i))^2, \quad (4)$$

where N_t is the overall number of experimental points obtained by registering the temporal dynamics of collimated transmission at fixed wavelength; $T_c(D,t)$ is the transmission coefficient calculated with equation (3) at moment t for given D; $T_c^*(t)$ is the experimentally measured transmission coefficient at moment t.

Minimization was performed by the "complex" method [19]. The iterative procedure was repeated until reconciling the calculated and experimental data. The criterion for ceasing the iteration was the condition

$$\frac{1}{N_t}\sum_{i=1}^{N_t}\frac{|T_c(D,t_i) - T_c^*(t_i)|}{T_c(t_i)} \le 0.01$$

RESULTS AND DISCUSSION

Figures 2 and 3 present respectively the spectra of collimated transmission and the temporal dynamics of collimated transmission at particular wavelength, which characterize the changes in the optical properties of the scleral specimen immersed in a 0.4 g/ml glucose solution. Similar behavior of the optical properties was observed in 0.3 and 0.18 g/ml glucose. As evident from Fig. 2, at the initial moment the scleral specimen is only slightly transparent for optical radiation. Upon immersion into a glucose solution, the interstitial liquid is gradually replaced with the glucose solution and, as the result, light scattering decreases and the collimated transmission rises. One can see that optical clarification takes place throughout the visible range, being most pronounced in the red region.

Figure 3 demonstrates a nice fit between the experimental data (points) and the approximating curves (solid lines) generated within the framework of the proposed model. Minor discrepancies between the experimental and theoretical data may be partly explained by measurement errors and simplification of the model, because the diffusion coefficient may actially change somewhat in the course of glucose permeation into the tissue, which is inhomogeneous through its volume.

The data on the dynamics of transmission enabled us to estimate the diffusion coefficients for glucose in the sclera. Calculations were run for four wavelengths (500, 600, 650, and 700 nm), and the wavelength-averaged values are given in the table. The results thus obtained indicate that the diffusion coefficient increases with the concentration of the diffusing substance.

These results agree nicely with the initial assumption of the prevalence of concentration-driven diffusion. The deduced diffusion are lower than those in water [20]; this is obviously due to the complex inner structure of sclera, which hinders diffusion. One can expect that the diffusion coefficients for glucose in sclera *in vivo* will be somewhat higher, as they should increase with temperature [6, 7].

Thus, we can conclude that the proposed method of estimating the diffusion coefficients from the changes in the optical properties of biological tissues is a promising tool for studying the diffusion processes in tissues.

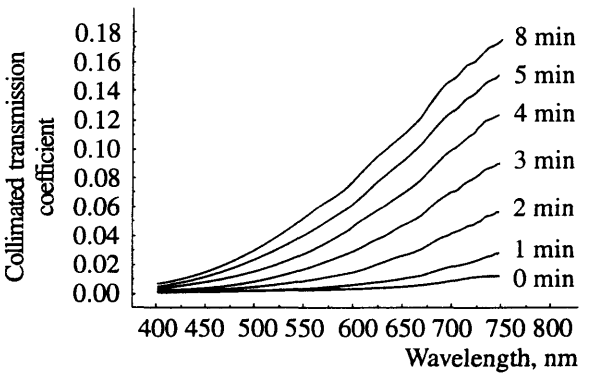

Fig. 2. Collimated transmission spectra of human sclera after specified times of incubation in 0.4 mg/ml glucose.

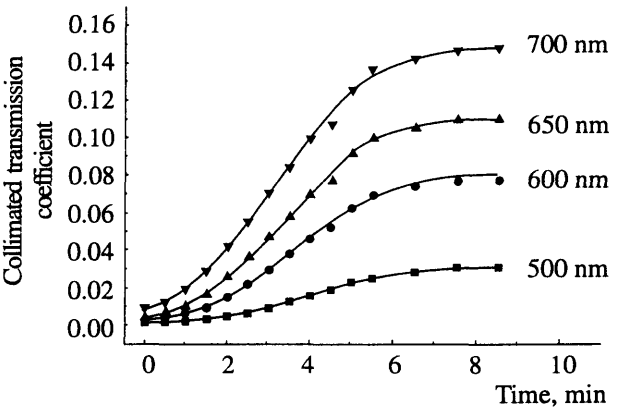

Fig. 3. Temporal dynamics of collimated transmission spectra of human sclera at specified wavelengths during incubation in 0.4 mg/ml glucose. Points are experimental data, curves are model approximations.

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