In vitro study of control of human *dura mater* optical properties by acting of osmotical liquids

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ABSTRACT

We present experimental results and computer modeling of investigation on the optical properties of the human *dura mater* controlled by administration of osmotically active chemical, such as *mannitol* and *glucose* solutions 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 liquids (*mannitol* and *glucose* solutions) on reflectance and transmittance spectra of the human *dura mater* are presented. The significant decreasing of the reflectance and increasing of the transmittance of the human *dura mater* samples under action of osmotical solutions were demonstrated.

Keywords: optical properties, *dura mater*, light scattering, osmolytes, refractive index matching

1. INTRODUCTION

The application of cerebral diagnostics, therapy and surgery is very important for modern laser medicine. One of the problems deals with transport of the laser beam through the *dura mater* tissue. The solution of the problem is connected with control of the optical properties of *dura mater*. Such control means the change of the scattering or absorption properties of the tissue.[1-16] Various physical and chemical actions (*e.g.* compression, dehydration, coagulation and others) can change the absorption and scattering of tissue. The optical properties of tissue can be also effectively controlled by osmotically active solutions. The main idea of the use of these solutions is based on the dependence of tissue scattering properties on the refractive index mismatching between collagen fibers and extrafiber substance. In general, with increasing of *glucose* concentration in the tissue refractive index mismatching reduces and consequently the scattering coefficient decreases.

Dura mater is a typical fibrous tissue. The structure and properties of fibrous tissues (for example sclera and cornea of eye, dermis of human skin etc.) are described in detail in papers.[17-28]

In this paper we have presented the results of experimental study and computer modeling of *dura mater* tissue optical clearing under action of *mannitol* and *glucose* solutions with various concentration. *Mannitol* and *glucose* having a higher refractive index than that of tissue base (extrafiber) substance diffuse into the tissue (water diffuses from the 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.

2. MATERIALS AND METHODS

The experiments were performed *in vitro* with the samples of the human *dura mater*. Tissue samples were obtained by autopsy within a day *post mortem*. The *dura mater* tissue was kept under temperature -12°C. Before experiments the *dura mater* was cut into pieces with the area about 1 cm^2. All experiments were performed at room temperature.

The samples of *dura mater* were placed into cuvette filled by immersion liquid. As immersion liquids *glucose* solutions with different concentrations (1.5M (270 mg/ml), 40% (400 mg/ml) and 3M (540 mg/ml)) and *mannitol* solution (156 mg/ml) were used. The refractive indices of the solutions measured by Abbe refractometer (wavelength 589 nm) were 1.367, 1.391, 1.398, and 1.359, respectively. The weight and the thickness of the samples were measured before and after experiments. Measured data are presented in Table 1. Dynamics of mass change was published in our previous paper. [15]

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Table 1. Physical properties of the human *dura mater* samples.

Osmotical active substance	*Sample*	*Thickness, mm*		*Weight, mg*	
		before optical clearing	*after optical clearing*	*before optical clearing*	*after optical clearing*
Mannitol solution	sample 1*	0.524	0.51	129	124
	sample 2**	0.956	0.78	259	256
	sample 3***	0.74	0.63	192	224
1.5M-*glucose* solution	sample 4***	0.585	0.571	100	118
40%-*glucose* solution	sample 5***	0.519	0.424	108	105
3M-*glucose* solution	sample 6***	0.455	0.619	105	126

* this sample was used for measurement of total transmittance
** this sample was used for measurement of diffuse reflectance
*** this sample was used for measurement of collimated transmittance

We used a commercially available computer-controlled CARY-2415 spectrophotometer with integrating sphere for investigation of the *dura mater* optical properties and their changes under action of the *mannitol* solution. Total transmittance, diffuse reflectance and collimated transmittance were measured in the 400-700 nm wavelength range. The sequence of obtaining of the *dura mater* optical properties was the following: 1) recording of the reference for the wavelength range from 400 to 700 nm (as the reference the cuvette with *mannitol* solution was used); 2) recording of the experimental spectra during the clearing of the *dura mater* sample (the sample was fixed on a plastic plate with a square aperture 5×5 mm^2 and placed in the same cuvette filled by the *mannitol* solution); 3) subtraction of the optical density of the reference from that of the *dura mater* sample.

For each tissue sample the thickness and initial weight were measured. For each sample diffuse reflectance, or total transmittance, or collimated transmittance were measured. The diffuse reflectance were calibrated on the basis of the reflectance value from standard reflectance plate (BaF_2). The time period for registration of one spectrum was about 3 min. For each wavelength we have obtained high-degree polynomial time-dependence approximation to correct measured spectra (i.e. to obtain value of transmittance and reflectance in the each moment of time). In the case of collimated transmittance measurements we have used the system consisted from three diaphragms. Diameter of each diaphragm was 2 mm. Distances 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 the investigated sample was placed between the first and the second diaphragms. The measurements of the each sample were performed continuously during 16 min.

The measurements of the collimated light transmittance spectra of *dura mater* samples under action of the *glucose* solutions were performed using OMA (fiber optic spectrometer LESA-6med, BioSpec, Russia). A 250 W xenon arc lamp with filtering of the radiation in the 400-700 nm spectral range was used in these measurements.

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

3. RESULTS AND DISCUSSION

To understand of the *dura mater* tissue optical properties and their changes under action osmotical active chemical we have investigated the diffuse reflectance, total transmittance, and collimated transmittance spectra of the *dura mater* samples concurrently with administration of *mannitol* solution (Figs. 1-4). Figures 1 and 2 illustrate the diffuse reflectance and the total transmittance spectra of human *dura mater* samples measured at different time intervals. From figure 1 it is seen that diffuse reflectance decreases with increasing of time of interaction of the *mannitol* solution and the *dura mater* sample. The total transmittance practically does not vary. Figure 3 presents the collimated transmittance spectra of the *dura mater* sample measured at different time intervals. Figure 4 presents the time-dependent collimated transmittance of the human *dura mater* sample measured at different wavelengths concurrently with administration of *mannitol* solution. In these figures it is seen that untreated *dura mater* poorly transparent for the visible light. *Mannitol* administration makes this tissue more transparent. Collimated transmittance of the sample at wavelength 700 nm increases in 2.38 time during 6 min (Fig. 4). In this figure also seen that collimated transmittance decreases after about 6 minutes of *mannitol* administration.

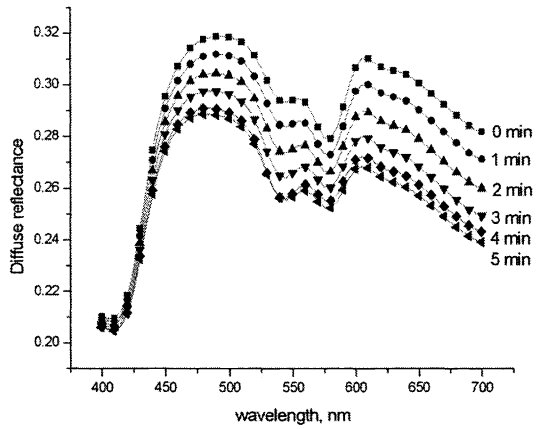

Figure 1. The diffuse reflectance of the human *dura mater* sample measured concurrently with administration of *mannitol* solution at different time intervals.

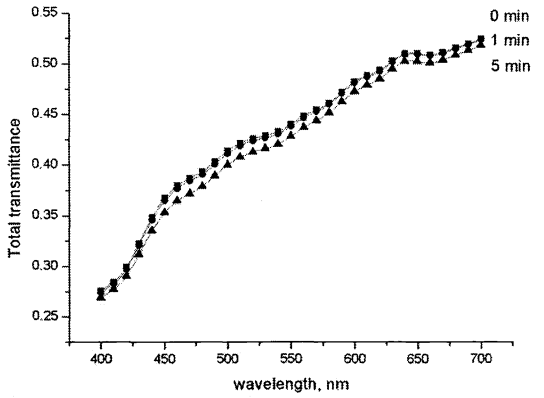

Figure 2. The total transmittance of the human *dura mater* sample measured concurrently with administration of *mannitol* solution at different time intervals.

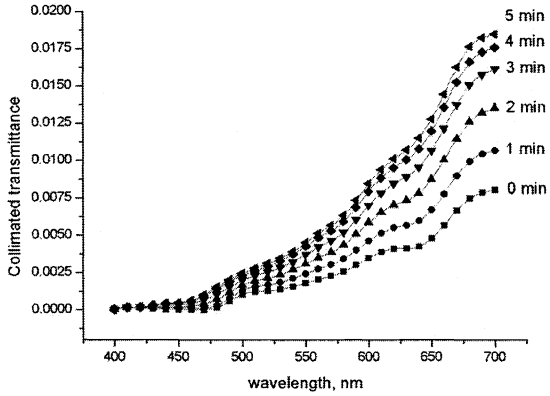

Figure 3. The collimated transmittance of the human *dura mater* sample measured concurrently with administration of *mannitol* solution at different time intervals.

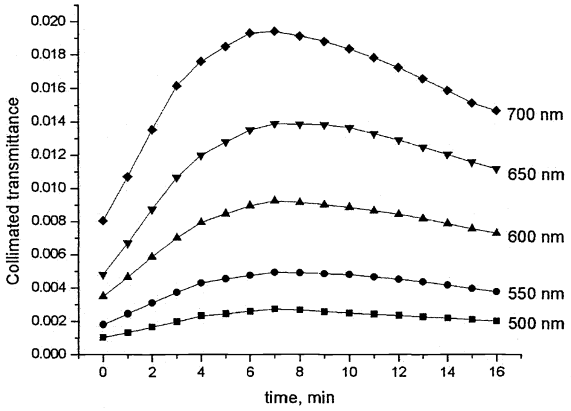

Figure 4. The time-dependent collimated transmittance of the human *dura mater* sample measured at different wavelengths concurrently with administration of *mannitol* solution.

Using inverse adding-doubling method we have calculated optical properties of the *dura mater* sample. In the all calculations we have assumed that thickness of the sample is constant and equal 0.74 mm. Results of this calculation are presented in Figs. 5-8. Figure 5 presents absorption coefficient of the untreated and treated by *mannitol* solution *dura mater* sample at different time intervals. Figure 6 illustrates the time-dependent absorption coefficient of the tissue sample calculated at different wavelengths. In this figure it is seen that the absorption coefficient of the *dura mater* increases with increasing of time of *mannitol* administration. This increasing of the absorption coefficient saturates during about 10 min. In Ref. 1 it was shown that the similar results is caused by tissue compression. In Table 1 it is seen that during optical clearing the thickness of the tissue samples decreases. Increasing of the absorption coefficient is connected with increasing of local chromaphore concentration.

Figures 7 and 8 present reduced scattering coefficients of the untreated and treated human *dura mater* at different time intervals and the time-dependent reduced scattering coefficient of the tissue sample calculated at different wavelengths, respectively. In figure 8 it is seen that under action of osmotical active liquid (*mannitol* solution) the reduced scattering coefficient decreases at the first and then (after 4-6 minutes of *mannitol* solution action) begins to increase. In this case we

have two process. From one hand, we see refractive indices matching process under action of the *mannitol* solution, and consequent decreasing of the reduced scattering coefficient. From other hand, we have increasing of the packing factor of the scatterers and consequent increasing of the reduced scattering coefficient with decreasing of the sample thickness.[30]

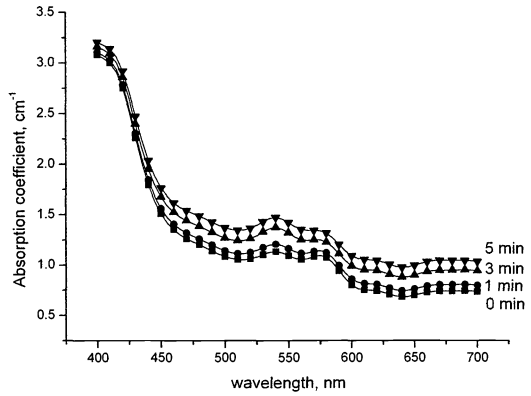

Figure 5. The calculated absorption coefficient of the human *dura mater* sample at different time intervals.

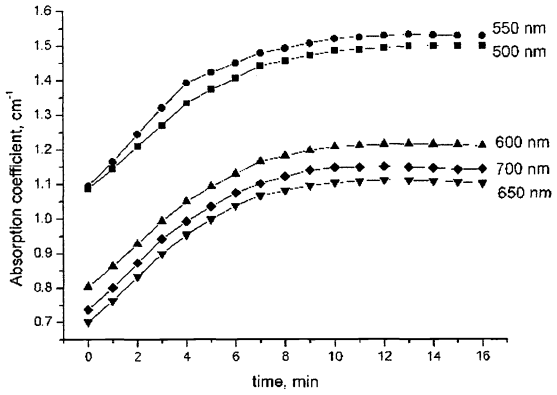

Figure 6. The time-dependent absorption coefficient of the human *dura mater* sample calculated at different wavelengths.

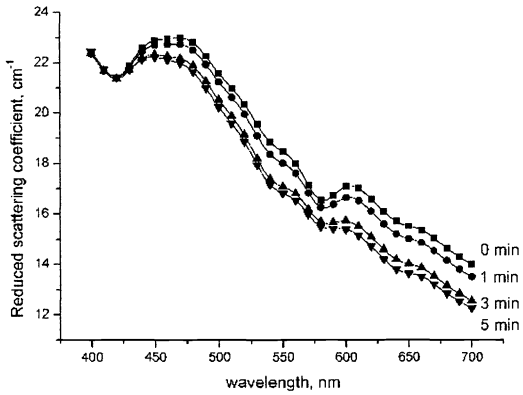

Figure 7. The calculated reduced scattering coefficient of the human *dura mater* sample at different time intervals.

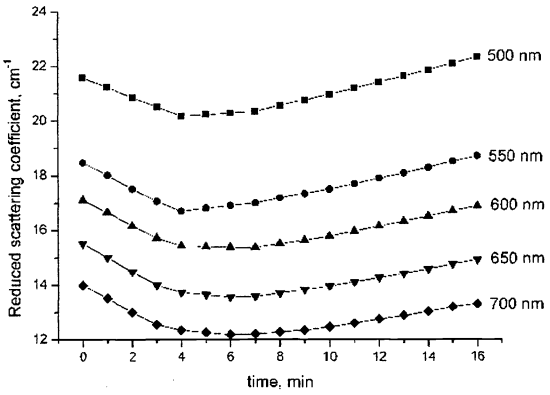

Figure 8. The time-dependent reduced scattering coefficient of the human *dura mater* sample calculated at different wavelengths.

Using collimated transmittance measurements (Fig. 3, 4) we have calculated the attenuation coefficients of the *dura mater* sample impregnated by the *mannitol* solution at different time intervals and wavelengths. These data and values of the absorption coefficient allow to calculate the scattering coefficient spectra (Figs. 9, 10). Using the values of the reduced scattering coefficient and the scattering coefficient we have calculated the anisotropy factor of untreated and treated by the *mannitol* solution *dura mater* sample at different time intervals and different wavelengths (Figs. 11-12). In figure 10 it is seen that the scattering coefficient decreases during the first 6-7 minutes of osmotical active liquid influence. Then the scattering coefficient increases. In Fig. 12 it is seen that the anisotropy factor decreases under action of the *mannitol* solution. Spectral behavior of the scattering coefficient spectra we connect with influence of blood absorption band at 420 nm. Bloodless of the *dura mater* sample due to the blood comes out from the tissue into surrounding substance (*mannitol* solution) causes of decreasing of distortion of the scattering coefficient spectra. Increasing of the anisotropy factor in range from 500 nm to 700 nm spectral range well matches with data reported by S. Jacques in Ref. 21.

To understand the mechanisms of the fibrous tissue optical clearing we have investigated the collimated transmittance spectra of the human *dura mater* samples concurrently with administration of *glucose* solution with various concentrations. Figures 13-18 illustrate the these transmittance spectra. Figures 13, 15, and 17 present the collimated transmittance of the human *dura mater* samples. Figures 14, 16, and 18 present of the dynamic changes of the collimated transmittance under action of *glucose* solution with various concentrations at different wavelengths.

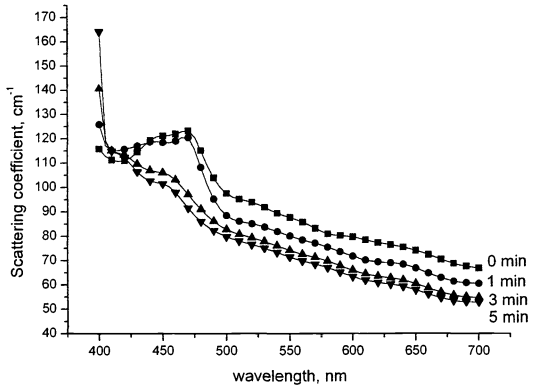

Figure 9. The calculated scattering coefficient of the human *dura mater* sample at different time intervals.

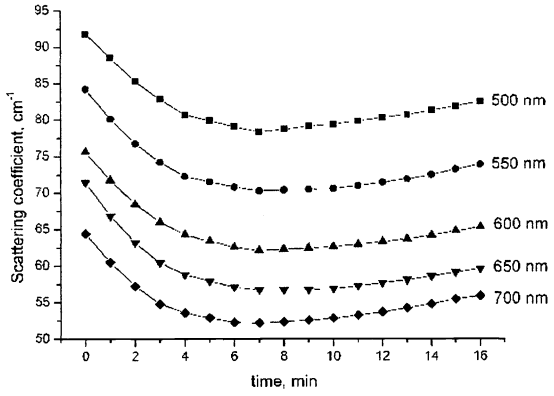

Figure 10. The time-dependent scattering coefficient of the human *dura mater* sample calculated at different wavelengths.

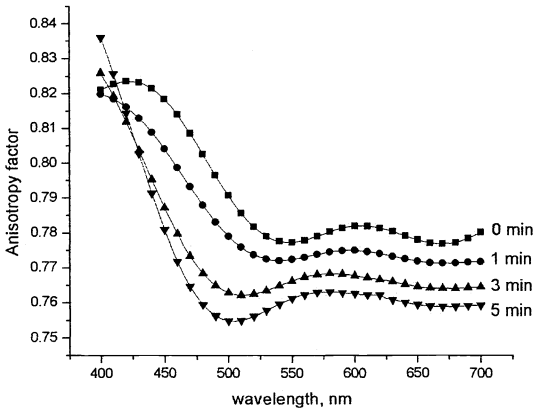

Figure 11. The calculated anisotropy factor of the human *dura mater* sample at different time intervals.

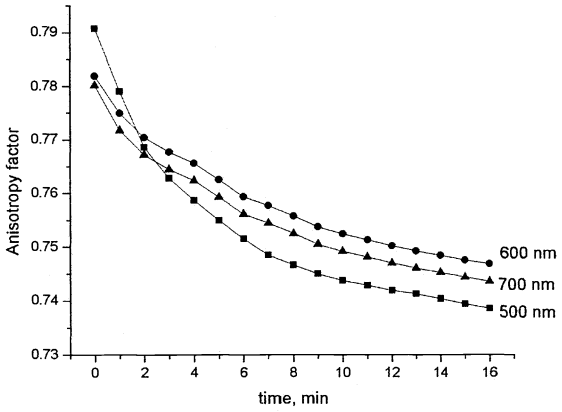

Figure 12. The time-dependent anisotropy factor of the human *dura mater* sample calculated at different wavelengths.

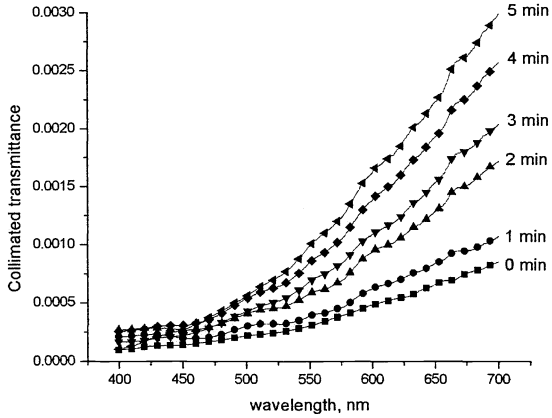

Figure 13. The collimated transmittance of the human *dura mater* sample measured concurrently with administration of 1.5M-*glucose* solution at different time intervals.

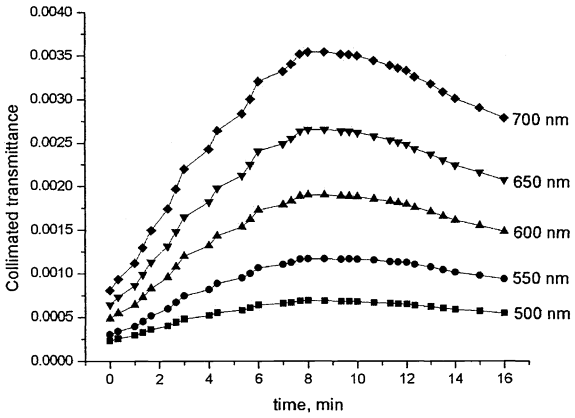

Figure 14. The time-dependent collimated transmittance of the human *dura mater* sample measured at different wavelengths concurrently with administration of 1.5M-*glucose* solution.

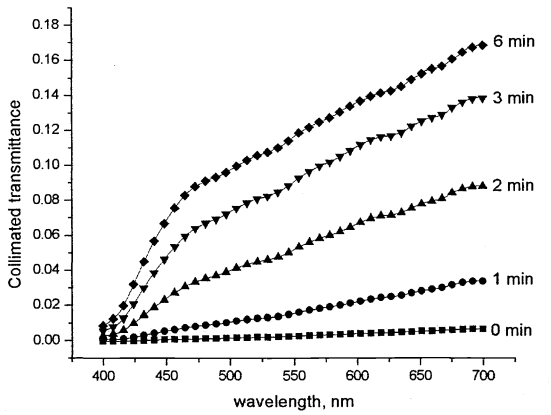

Figure 15. The collimated transmittance of the human *dura mater* sample measured concurrently with administration of 40%-*glucose* solution at different time intervals.

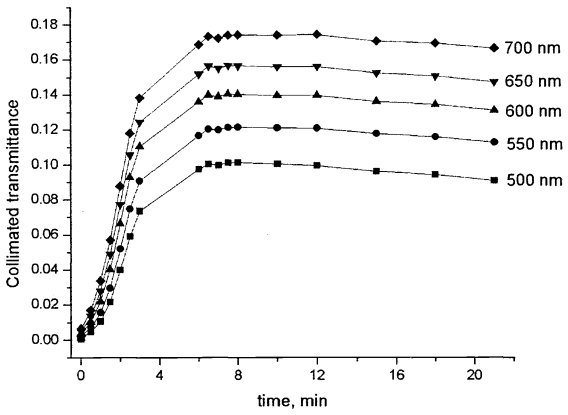

Figure 16. The time-dependent collimated transmittance of the human *dura mater* sample measured at different wavelengths concurrently with administration of 40%-*glucose* solution.

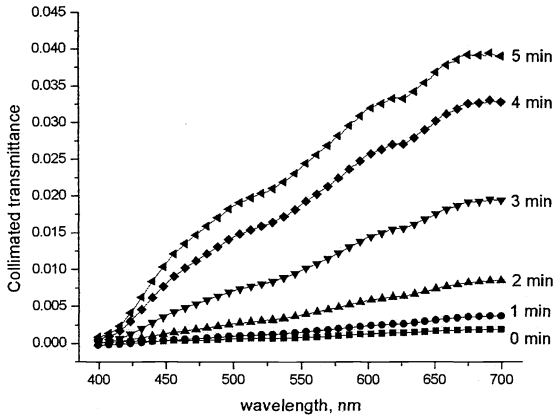

Figure 17. The collimated transmittance of the human *dura mater* sample measured concurrently with administration of 3M-*glucose* solution at different time intervals.

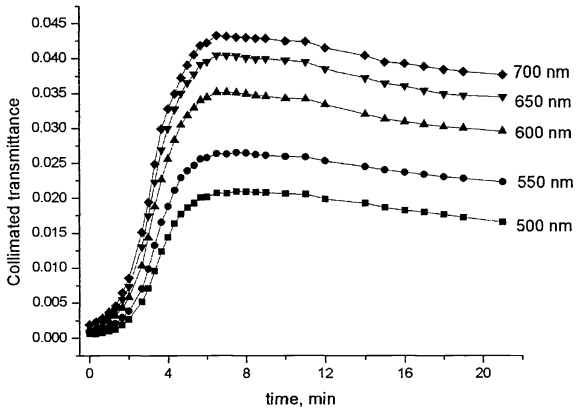

Figure 18. The time-dependent collimated transmittance of the human *dura mater* sample measured at different wavelengths concurrently with administration of 3M-*glucose* solution.

4. CONCLUSION

In this study we have measured and calculated the optical properties of the *dura mater* tissue and its changes under action of osmotical active chemical such as *mannitol* and *glucose* solution with various concentrations. The results of this paper show that administration of osmolytes to a fibrous tissue allows to control its optical characteristics effectively.

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