Measurement of Diffusion Coefficient of Propylene Glycol in Skin Tissue

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ABSTRACT

Optical clearing of the rat skin under the action of propylene glycol was studied *ex vivo*. It was found that collimated transmittance of skin samples increased, whereas weight and thickness of the samples decreased during propylene glycol penetration in skin tissue. A mechanism of the optical clearing under the action of propylene glycol is discussed. Diffusion coefficient of propylene glycol in skin tissue *ex vivo* has been estimated as $(1.35\pm0.95)\times10^{-7}$ cm^2/s with the taking into account of kinetics of both weight and thickness of skin samples. The presented results can be useful for enhancement of many methods of laser therapy and optical diagnostics of skin diseases and localization of subcutaneous neoplasms.

Keywords: propylene glycol, skin, optical clearing, collimated transmittance, diffusion coefficient

1. INTRODUCTION

Interest in the use of optical clearing agents (OCA) for improvement of optical methods for diagnostics and therapy of various diseases constantly increases because they are safe and inexpensive[1,2]. The main criteria of the choice of OCA for the tissue clearing are: 1) the refractive index of OCA should be close to that of the main tissue scatterers (collagen and elastin fibers or cell membranes); 2) OCA should be hyperosmotic liquid; and 3) biocompatibility[3]. Among the most popular OCAs, there are glycerol, polyethylene glycols, propylene glycol, and glucose[1-7]. They are biocompatible and do not damage tissues seriously at the short-time action[5-7].

At present, the following three mechanisms of the reducing of light scattering in tissues under the action of a clearing agent have been proposed[1,2]: (i) osmotic dehydration of a tissue; (ii) partial replacement of an interstitial fluid by the clearing agent, as a result of which the refractive indices of structural components of a tissue become matched; (iii) structural modification or dissociation of collagen of a tissue. Generally, both the first and the second mechanisms work simultaneously and lead to the matching of the refractive indices of tissue scatterers and cytoplasm (for cellular structures) or interstitial fluid (for fibrous tissues). However, the diffusion of water from interstitial space into the surrounding OCA solution goes faster than the diffusion of OCA into the interstitial space because of less size of water molecules and more viscosity of OCA[8].

As it is known, collagen is soluble both in sugars (glucose, fructose, etc.) and polyhydric alcohols (ethylene glycol, glycerol, sorbitol, etc.)[9,10], and thus, these OCAs cause the dissociation of collagen fibrils into microfibrils[9,10]. This destabilization of the collagen structure can additionally reduce the scattering of light in a tissue due to a decrease in the size of the tissue scatterers[9], however, this process require of sufficiently prolonged exposure of tissue to the OCAs.

Hydrogen ion exponent (pH) also plays the important role in optical clearing of tissues. It was shown that the optical clearing can be accompanied with the swelling process, especially if the OCA has low pH in comparison with isoelectric point of collagen[11-13].

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Propylene glycol (PG) is neutral OCA and does not induce tissue swelling. PG is widely used as an OCA due to its efficiency, availability and biocompatibility[14-21]. It is a viscous colorless water-soluble hygroscopic liquid from the class of diols (chemical formula: $CH_3CH(OH)CH_2OH$) having a molecular weight 76.09[22,23]. PG is included in the composition of a large number of pharmaceutical and cosmetic preparations as a solvent, extractant and preservative[22].

In the Refs. [14, 15] was shown that under the action of 80% aqueous PG solution the relative increasing of porcine skin transmittance was 30% and the relative decreasing of diffuse reflection was 27% at the wavelength 1278 nm. At this, water loss was 30% and mass loss was 14% during 60 min. Moreover, Wang et al.[16] earlier demonstrated decrease of the thickness of rat skin *ex vivo* by 4% under action of 70-80% PG during 40 min.

The knowledge of mechanisms of optical clearing and diffusion coefficients of OCA in tissues are very important for development of the methods of optical diagnostics and therapy. We apply the optical method of evaluating the diffusion coefficient of the immersion liquid in a tissue described in Ref. [12]. However, the taking into account of the temporal dependencies of weight and volume of the tissue at the tissue - OCA interaction should allows making more accurate diffusivity evaluation.

In this paper we present the results of *ex vivo* experiments on the rat skin optical properties controlled with administration of PG. Based on temporal dependencies for optical transmittance, weight, and thickness of skin samples, the diffusion coefficient of PG in skin has been estimated.

2. MATERIALS AND METHODS

In this study we used dehydrated PG («Reagent» Corp., St.-Petersburg, Russia) as an OCA. The refractive index of PG was measured by Abbe refractometer (Atago DR-M2/1550, Japan) at some wavelengths (450, 589, 680, and 1100 nm) as 1.4384, 1.4312, 1.4285, and 1.4228 and interpolated to the spectral range 500-900 nm. pH of PG was measured by pH-meter Hanna (Germany) as 7.2.

In the *ex vivo* experiments twenty samples of skin of laboratory albino autbred rats with size about 10×20 mm^2 were used. The hair was carefully removed from the surface of the *post mortem* rat bodies using depilatory cream "Veet" (Reckitt Benckiser, France). Fat sublayer was carefully removed from the skin samples.

To clarify the mechanism of skin optical clearing, the time-dependent measurements of thickness and weight of ten skin samples during optical clearing were performed. The measurements of the weight and thickness were carried out sequentially on the each of the studied sample. The weight measurements were performed using electronic balance (SA210, Scientech, USA). The accuracy of the measurements was ±1 mg. The thickness of the samples was measured by micrometer with accuracy ± 5 μm. The sample was placed between two glass slides. The thickness was measured at five different points and averaged.

The thickness and the weight of the skin samples were measured before the optical clearing and during 2 hours every 10-15 minutes after placing the samples into a Petri dish with PG. For this, the skin samples were taken out from the Petri dish; the excess of the PG was removed from the surface of the sample using a filter paper.

The collimated transmittance was measured with the spectrometer USB4000-Vis-NIR (Ocean Optics, USA). As a light source halogen lamp HL-2000 (Ocean Optics, USA) was used. Optical fiber cables P400-1-UV-VIS (Ocean Optics, USA) with inner diameter 400 μm and collimators 74-ACR (Ocean Optics, USA) on its ends were used for delivery of light to the skin sample and collection of the light passed through the sample. To measure the collimated transmittance, the skin samples were fixed on the plastic holder with size 38×17 mm^2 with a hole with size 8×8 mm^2 and were placed into the glass cuvette with volume 5 ml between two optical fiber cables. Ten skin samples were included in this study.

Kinetics of the collimated transmittance of ten skin samples in PG was registered by sequential recording of spectra of the collimated transmittance in the range of 500-900 nm every 10-15 minutes for 2 hours. Before measurements the reference signal from cuvette with PG and the holder without skin sample was registered. All measurements were carried out at room temperature (about 20°C).

The method of diffusivity evaluation was based on the time-dependent measurement of collimated transmittance of tissue samples during the optical clearing. It was supposed that only refractive index of interstitial liquid of the sample changed. This process is associated with diffusion of the immersion agent into the tissue and osmotic outflow of the water from the tissue, i.e. dehydration. These processes have different rates; diffusion of water from tissue to an external volume of PG goes faster than the diffusion of PG into the interfibrillar space. It was shown in Ref. [8] by the example of glycerol solution. At the evaluation of the diffusion coefficient, the average rate of the exchanging flow of the PG into the tissue and the water from the tissue was taken into account.

The penetration of the immersion agent occurs only from the inner side of the skin sample (from the dermis). This fact is explained by the protective properties of the epidermis, which prevents from the penetration of the immersion agent into the skin.

The transport process of immersion liquids in fibrous tissues can be described with the model of free diffusion. We use the following assumptions: 1) only concentration diffusion takes place; i.e., the exchange flow of PG into the tissue and water from the tissue, at a certain point within the tissue sample, is proportional to PG concentration at this point; 2) the diffusion coefficient is constant over the entire sample volume.

Geometrically the sample of skin is presented in the form of the plane-parallel plate of finite thickness. The areas of the upper and bottom surfaces of this plate are much larger than the areas of the lateral sides, so we can ignore the boundary effects and solve a one-dimensional problem, i.e. the equation (the second Fick's law):

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2}, \tag{1}$$

where $C(x, t)$ is the concentration of PG in the skin; D is the diffusion coefficient, cm^2/s; t is the time of the diffusion process, s; x is the spatial coordinate in thickness of the skin sample, cm. In our experiments the volume of the immersion liquid is much more then the volume of the skin sample, so the boundary conditions are:

$$C(0,t) = C_0 \qquad \text{and} \qquad \frac{\partial C(l,t)}{\partial x} = 0, \tag{2}$$

where C_0 is the PG concentration; l is the thickness of the skin sample, cm. The second boundary condition reflects the fact that diffusion of the immersion liquid into the skin sample occurs only on the one surface of the sample, i.e. on the surface of the dermis. The initial conditions reflect an absence of PG at all internal points of the skin sample before its incubation into the OCA, i.e.

$$C(x,0) = 0. \tag{3}$$

The solution of the equation (1) with boundary (2) and initial (3) conditions is:

$$C(x,t) = C_0\left(1 - \sum_{i=0}^{\infty}\frac{4}{\pi(2i+1)}\sin\left(\frac{(2i+1)\pi x}{2l}\right)\exp\left(-\frac{(2i+1)^2 D\pi^2 t}{4l^2}\right)\right).$$

The average concentration of immersion solution inside the skin sample $C(t)$ in every time moment is:

$$C(t) = C_0\left(1 - \frac{8}{\pi^2}\sum_{i=0}^{\infty}\frac{1}{(2i+1)^2}\exp\left(-(2i+1)^2 t\frac{\pi^2}{4}D\Big/l^2\right)\right). \tag{4}$$

During the penetration of PG into the tissue, the refractive index of the interstitial liquid increased. The time dependence of refractive index of the interstitial liquid can be evaluated using the law of Gladstone and Dale, which states that, for a

multicomponent system, the resulting value of the refractive index represents an average of the refractive indices of the components related to their volume fractions. In the case of two-component solution the law of Gladstone-Dale is:

$$n_1(t) = (1 - C(t))\, n_{base} + C(t)\, n_{PG}, \tag{5}$$

where n_{base} is the refractive index of the interstitial fluid of the skin at the initial moment; n_{PG} is the refractive index of PG.

Spectral dependence of the refraction index of the interstitial fluid of the skin is[24]:

$$n_1(\lambda) = 1.351 + \frac{2134.2}{\lambda^2} + \frac{5.79 \times 10^8}{\lambda^4} - \frac{8.15 \times 10^{13}}{\lambda^6}, \tag{6}$$

where λ is a wavelength, nm. Collagen fibers are a basic type of scatterers in fibrous tissues and determine their scattering characteristics. Spectral dependence of the refractive index of the collagen fibrils is[12]:

$$n_s(\lambda) = 1.439 + \frac{15880.4}{\lambda^2} - \frac{1.48 \times 10^9}{\lambda^4} + \frac{4.39 \times 10^{13}}{\lambda^6}. \tag{7}$$

Optical characteristics of the skin are determined by optical properties of the dermis, as a thickest layer in the skin. Therefore the optical model of the skin can be presented as a slab with a thickness l containing scatterers (collagen fibrils) in the form of infinitely long thin dielectric cylinders with the average diameter of 60-100 nm. They are located parallel to the surface of the sample. Spectral dependence of the refraction index of these scatterers is described by the Eq. (7).

For quantitative evaluation of scattering in the skin we used the Eq. (8):

$$\mu_s = \frac{\varphi}{a} \frac{\pi x^3}{8} \left(m^2 - 1\right)^2 \left(1 + \frac{2}{\left(m^2 + 1\right)^2}\right) \frac{(1 - \varphi)^3}{1 + \varphi}, \tag{8}$$

where φ is the volume fraction of the scatterers; $m = n_s / n_1(t)$ is the relative refractive index of the scattering particles that are determined with the Eqs. (7) and (8); $x = 2\pi n_1(t)\, a / \lambda$ is the relative size of the scatterers; a is the radius of the scatterers.

Time dependence of the collimated transmittance of the skin sample in the hyperosmotic liquid solution has the form:

$$T_c(t) \approx \exp\left(-\left(\mu_a + \mu_s(t)\right) \times l(t)\right), \tag{9}$$

where μ_a is the absorption coefficient of the skin sample. During calculations, we assumed that the absorption coefficient was constant. Since at the optical clearing the thickness of skin samples changed, the changes were taken into account.

To analyze the kinetic of weight and thickness changing owing to dehydration the following empirical equation was used:

$$\frac{y(t)}{y(t=0)} = A \exp\left(-\frac{t}{\tau}\right) + y_0, \tag{10}$$

where $y(t)$ is the measured value of weight or thickness. Here, the parameter A shows quantity of water outflow from tissue to surrounding solution; τ is the characteristic time constant, which describes the dehydration rate; y_0 is the residual deal with quantity of collagen and bound water. The parameters A, τ, and y_0 were determined by the least square method.

The Eqs. (1)-(10) determine the dependence of collimated transmittance on the PG concentration inside the skin sample, i.e. they form the direct problem. On the basis of measurement of the temporal evolution of the collimated transmittance, the reconstruction of the diffusion coefficient of PG in skin was carried out. The inverse problem solution was obtained by minimization of the target function:

$$f(D) = \sum_{i=1}^{N_t} \left(T_c(D, t_i) - T_c^*(t_i) \right)^2 , \tag{11}$$

where N_t is the number of time points obtained at registration of the temporal dynamics of the collimated transmittance at the fixed wavelength; $T_c(D, t)$ is the transmittance value, that was calculated with the equation (9) at the time moment t for a given value D; $T_c^*(t)$ is the experimentally measured value of the transmittance at the moment t.

3. RESULTS AND DISCUSSION

In Figures 1 and 2 collimated transmittance spectra of one sample and the kinetic of the collimated transmittance of this sample for 140 minutes are presented. In the figures we can see, that the skin is poorly transparent for the visible and near IR radiation at the initial moment. PG administration makes this tissue more transparent, increasing the collimated transmittance at the presented wavelengths on average, by 19-20-fold.

In average the increase of the collimated transmittance is observed during the first 80 minutes. We can assume that the diffusion of PG into the tissue and the diffusion of water from the interstitial space into the surrounding solution along the concentration gradient go simultaneously. The both processes contribute to the increase of the collimated transmittance during the clearing.

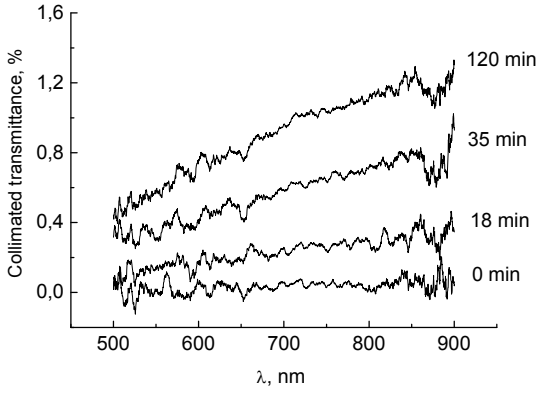

Figure 1. Typical spectra of the collimated transmittance of the rat skin sample *ex vivo* measured at different moments after placing into the propylene glycol

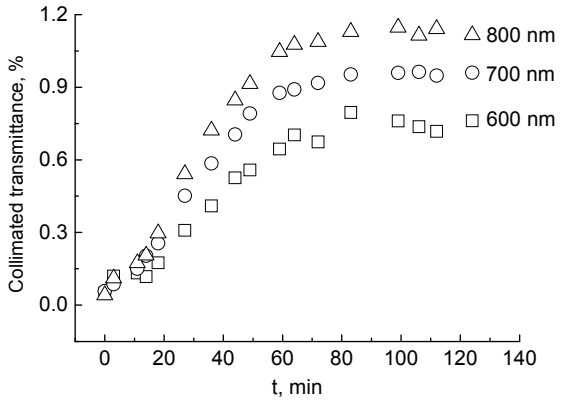

Figure 2. Typical kinetics of the collimated transmittance of the rat skin sample *ex vivo* under the action of the propylene glycol measured at different wavelengths

In Figure 3 the experimentally measured and averaged temporal dependences of weight and thickness of the investigated samples are presented. The values of weight and thickness were normalized on their initial values. It is easily seen that the weight of the samples decreased by 30±6%, and the thickness decreased by 32±17%. The experimental dependencies were approximated in accordance with Eq. 10. From Fig. 3 a good matching is seen between experimental data (symbols) and approximating dependence (solid line) calculated with Eq. 10. Parameters of the approximation show

clearly that propylene glycol induces ~30% dehydration of the skin tissue. Moreover, we can assume that area of the skin samples will change insignificantly or not change.

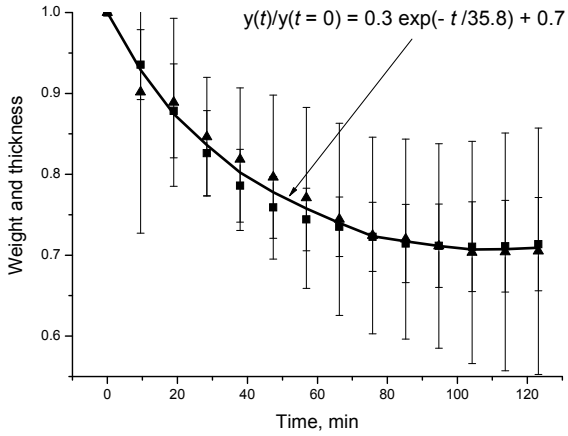

Figure 3. Kinetics of thickness (up triangles) and weight (squares) of the rat *ex vivo* skin samples under the action of the propylene glycol. The thickness and weight were normalized on initial values. Symbols correspond to experimental data and solid line corresponds to approximation (Eq. 10). $y(t)$ is value of weight or thickness measured in different moment and $y(t = 0)$ is value of weight or thickness measured in initial moment.

The diffusion coefficient of PG in skin was evaluated on the base of the analysis of the collimated transmittance kinetics with the taking into account the dehydration kinetics of the rat skin sample thickness. The value of the diffusion coefficient is $(1.35\pm0.95)\times10^{-7}$ cm^2/s.

4. CONCLUSION

The results of the experiments show the effectiveness of propylene glycol as an optical clearing agent for the control of the scattering characteristics of the skin. Particularly, there is about 20-fold increase of the collimated transmittance in the spectral range of 500-900 nm during 2 hours. The measured value of propylene glycol diffusion coefficient in the rat skin *ex vivo* is $(1.35\pm0.95)\times10^{-7}$ sm^2/sec.

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