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COMPLEX SYSTEMS BIOPHYSICS

The Effect of Immersion Agents on the Weight and Geometric Parameters of Myocardial Tissue in vitro

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Abstract—The effect of 40%-glucose solution and 60%-glycerol solution on the weight and geometric parameters of the myocardium was studied in vitro in order to improve the accuracy of estimating the glucose and glycerol diffusion coefficients in the myocardium by including changes in the geometry and water content of a tissue sample in a mathematical algorithm. The temporal kinetics of the weight, thickness, area, and volume were measured using porcine myocardium samples during their immersion in 60%-glycerol solution or 40%-glucose solution in vitro. All parameters started to decrease immediately after placing myocardium samples in immersion agents (tissue shrinkage). The weight and geometric parameters of the sample then increased gradually (tissue swelling), leading to tissue saturation in almost all cases. By approximating the temporal kinetics, the degree of dehydration and the characteristic times of transverse and longitudinal shrinkage and swelling of the myocardium were determined. More significant and rapid dehydration of myocardial tissue was observed case of using 60%-glycerol solution, while a stronger and faster swelling of the myocardium was observed when 40%-glucose solution was applied.

Keywords: myocardium, glycerol, glucose, optical clearing, dehydration, swelling
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INTRODUCTION

Cardiovascular disorders are the main cause of mortality throughout the world, according to the World Health Organization. In addition to conventional methods of treatment and diagnostics of cardiovascular disorders, optical methods are developed rapidly and are highly likely will be employed in treatment and diagnosis of cardiovascular disorders in the nearest future [1–4]. However, despite of numerous advantages optical methods have certain limitations. One of the problems arises because radiation of the visible and near infrared spectral ranges is greatly scattered in biological tissues and the penetration depth of light is consequently limited. In the case of muscle tissue, this is due to its heterogeneity, i.e., the tissue consists of fiber structures surrounded by an amorphous basal substance and the components have different refraction indices [5]. A means to solve this problem is provided by optical clearing of biological tissues, that is, their optical properties are regulated by exposing tissues to hyperosmotic immersion agents [5]. In result of such interaction the intercellular fluid is partly replaced by the immersion agent with refraction index of the agent which is higher than that of the intercellular fluid and close to that of the structural components of the tissue. The procedure equalizes the refraction indices of the intercellular fluid and tissue structures (collagen fibers, cell organelles, etc.), thus decreasing light scattering in the tissue and increasing the light-penetration depth. Optical clearing of biological tissues is a complex process. The interaction of an immersion agent with biological tissue leads to diffusion of agent molecules into the tissue and causes tissue dehydration, which changes the packaging of tissue structures and affects the dimensions of the tissue sample [5, 6]. The changes that arise in tissue geometry upon optical clearing must be considered in order to more accurately determine the diffusion rate of the immersion agent and the light distribution within the tissue upon optical diagnosis or phototherapy of various disorders. Glucose and glycerol solutions of various concentrations are broadly used as immersion agents, along with other substances [6–10] because they are biologically compatible and have the necessary osmolality and sufficiently high refraction indices. As an example, dehydration and longitudinal and transversal shrinkage of the skin have been studied on

Table 1. The refraction index n, viscosity η, osmolality *Osm*, molecular weight *Mr*, molecular hydrodynamic radius R_M, and pH of the immersion agents

Immersion agent	$n_{589\ nm}$	η, cP	*Osm*, osmol/L	*Mr*, Da	R_M, Å	pH
60%-glycerol solution	1.414	11 (20°C) [13, 14]	12.48	92 [14]	2.6–3.1 [16]	4
40%-glucose solution	1.391	63 (60°C) [15]	2.22	180 [14]	3.6 [17]	3

exposure to glucose and glycerol solutions, which proved to be highly efficient in optical clearing of biological tissues [11, 12].

The objective of this work was to study how 40%-glucose solution and 60%-glycerol solution used as immersion agents affect the weight and geometric parameters of myocardial tissue. The changes make it possible to infer the extent of tissue dehydration and transversal and longitudinal shrinkage and swelling of the myocardium exposed to the immersion agents.

MATERIALS AND METHODS

Aqueous solutions of 40% glucose (NovosibKhimFarm, Novosibirsk, Russia) and 60% glycerol were used as immersion agents. To obtain an aqueous solution of 60% glycerol, dehydrated glycerol (Baza no. 1 khimreaktivov, Staraya Kupavna, Russia) was combined with distilled water. The refraction index, viscosity, osmolality, molecular weight, molecular hydrodynamic radius, and pH of the immersion agents are summarized in Table 1. The refraction indices of solutions were measured using an Abbe IRF-454B2M refractometer (LOMO, Russia) at 589 nm.

The osmolality of a glycerol solution was calculated as follows. The molar concentration was obtained as $C_M = \rho/M$ [18], where C_M is the molar concentration of the solution, ρ is the solution density, and M is the molar mass of the solute. The molar concentration (1 mol/L) is equal to osmolality (1 osmol/L) in the case of nonelectrolytes [19]. Thus, the osmolality was 12.48 osmol/L for 60% glycerol solution and 2.22 osmol/L for 40% glycose solution; i.e., the osmolality of the glycerol solution was approximately 5.5 times higher than that of the glucose solution.

A pH-400 pH meter (Akvilon, Podol'sk, Russia) was used to measure the pH of the solutions; the instrumental error was ± 0.05.

Thin myocardial samples of approximately 15 × 20 mm^2 were dissected from a porcine heart with a lancet. Intact myocardial samples were weighed, measured for thickness, imaged, and then incubated in an immersion agent for 5 min; then the weight and thickness were measured and the sample was imaged again. Measurements were continued for 2 h. The weight was measured using a SA210 electronic balance accurate to ± 1 mg. The thickness of a sample placed between two slides was measured at five points with a micrometer accurate to ± 5 μm and averaged over the five measurements.

The surface area of a sample was calculated from its images. A scale rule was used to determine the coefficient of conversion from linear dimensions in pixels to linear dimensions in millimeters and to obtain the image dimensions. To estimate the surface area, the full-color image (Fig. 1a) was firstly processed using the READ_BLUE option of MathCad (Parametric Technology, United States) to isolate the blue component (Fig. 1b). A median filter was applied to reduce noise, to remove highlights, etc. (Fig. 1c). All pixels outside the sample were set to be 255 (Fig. 1d). The pixels that corresponded to the sample (with values other than 255) were counted and converted to square millimeters by the following equation:

$$S = \frac{F(H_S)}{cols(H_S)\,rows(H_S)}\,\frac{rows(H)\,z^2}{cols(H)},$$

where F is the function that counts the pixels corresponding to the sample; *cols* and *rows* are the numbers of columns and rows in the image, respectively; H is the initial sample image; H_S is the sample image without the background; and z is the image width.

A total of 40 porcine myocardial samples were used in the study. Of these, 20 were immersed in 40% glucose solution and the other 20 were immersed in 60% glycerol solution.

The time dependences of the thickness, weight, and area of myocardial samples were obtained during their exposure to immersion agents, normalized to the initial values (measured prior to immersing the sample), and averaged over all samples. The experimental data were then approximated with a two-exponent equation, one part of which described the kinetics of tissue dehydration while the other describes the kinetics of tissue swelling:

$$B_{norm}(t) = \frac{B(t)}{B(t=0)} = A_D \exp\left(-\frac{t}{\tau_w}\right) + B_S\left(1 - \exp\left(-\frac{t}{\tau_g}\right)\right) + y_0, \quad (1)$$

where $B(t)$ and $B(t = 0)$ are the parameter values measured at the time points t and $t = 0$, respectively; A_D and B_S are the maximum extents of tissue dehydration

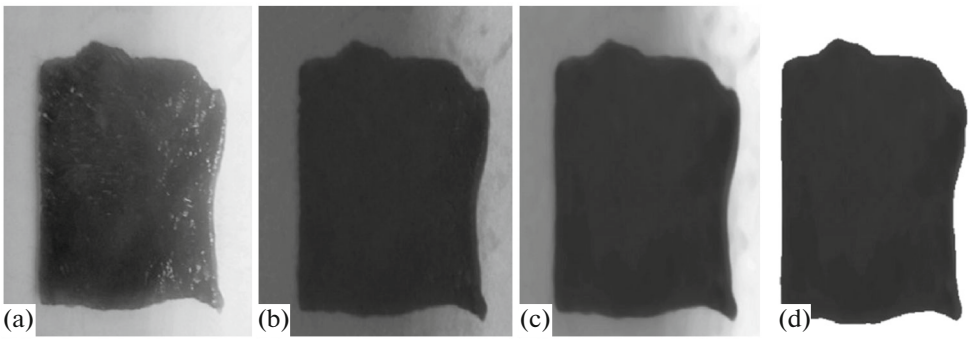

Fig. 1. (a) A digital image of a myocardial sample, (b) the blue component of the image, (c) the blue component processed with a median filter, and (d) the result of the digital processing of the image.

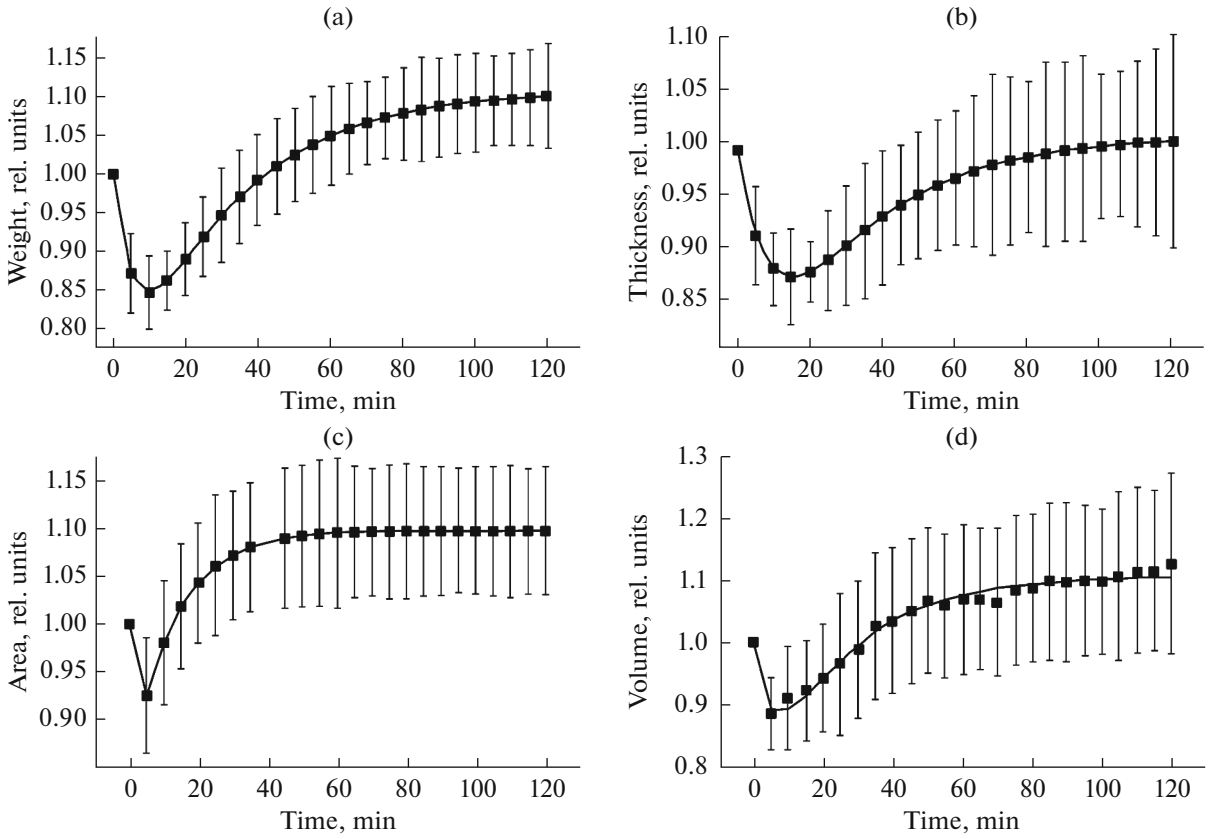

Fig. 2. The temporal kinetics of in the (a) weight, (b) thickness, (c) area, and (d) volume of myocardial samples in the course of their immersion in 40% glucose.

(shrinkage) and swelling, respectively; τ_w is the characteristic dehydration time; τ_g is the characteristic swelling time; and y_0 is the minimum achievable value of the parameter. The volume of a sample was calculated as a product of its area and thickness.

RESULTS AND DISCUSSION

Figures 2 and 3 show the (a) weight, (b) thickness, (c) area, and (d) volume of myocardial samples as functions of the time of sample immersion in 40% glucose solution and 60% glycerol solution, respectively. All measurements were normalized to the initial value and averaged over all samples. As is seen from Figs. 2 and 3, each parameter started to decrease immediately after samples were placed in the immersion agents and continued decreasing for approximately 5–15 min. The decrease in sample weight was explained by dehydration of the myocardium as a result of its interaction with the immersion agent.

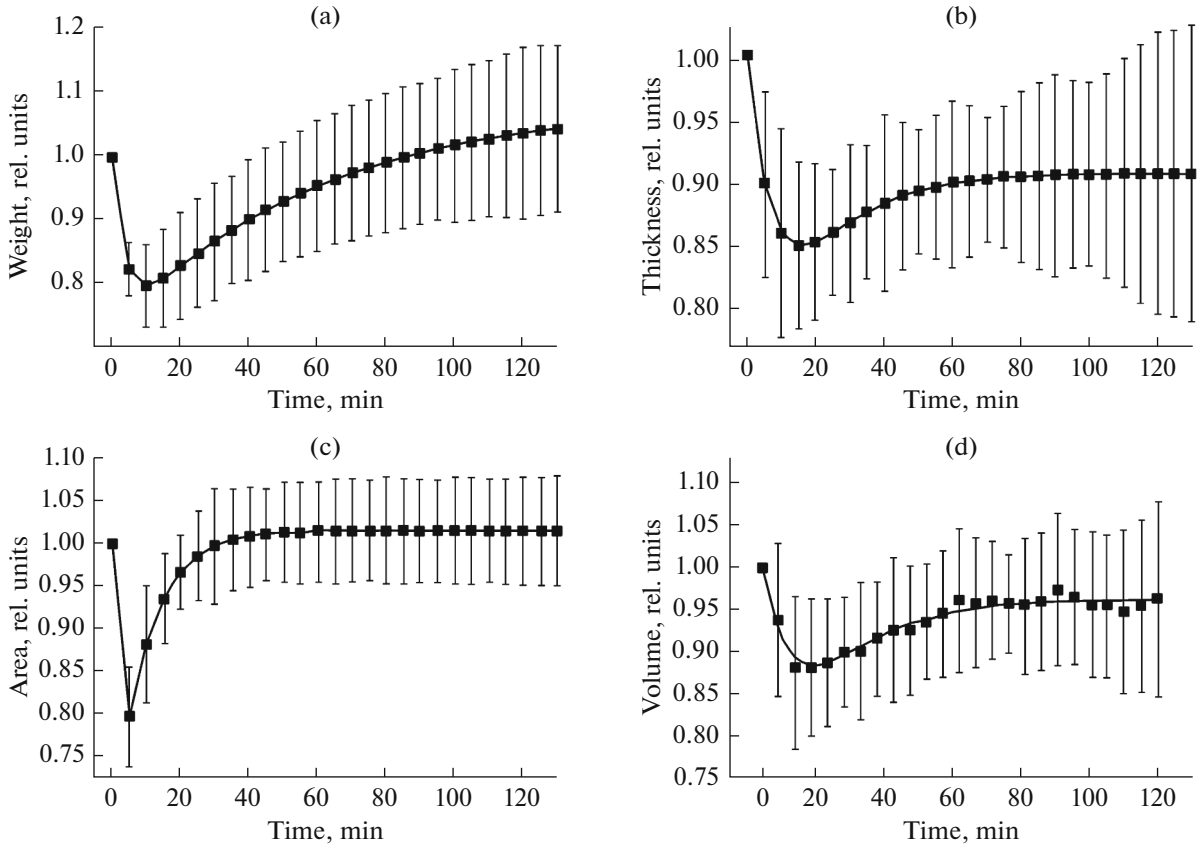

Fig. 3. The temporal kinetics of the (a) weight, (b) thickness, (c) area, and (d) volume of myocardial samples in the course of their immersion in 60% glycerol solution.

Longitudinal and transverse shrinkage of tissue samples is due to changes in the packaging of fibers, which become closer together as tissue is dehydrated. Tissue swelling occurred in parallel with dehydration; all of the parameters started to increase and the kinetic curves reached saturation in almost all of the cases. A polyelectrolyte gel model can be used to explain the swelling of myocardial samples [20–23]. When tissue pH corresponds to the isoelectric point, then the attraction forces that arise between equal positive and negative charges (zwitterion pairs) [24] render the tissue sample in its densest state and the extent of swelling is minimal in this case [20]. As an example, corneal collagen has been shown to swell to the highest extent at pH 4.0 and to the lowest extent at pH 7.0 [25]. Similar results have been obtained for the undried ox cornea [26]. Thus, as pH shifts from the isoelectric point, tissue hydration increases because the number of zwitterion pairs decreases and, consequently, the resulting static charge grows. The resulting static charge is negative at pH values higher than the isoelectric point and positive at pH values lower than the isoelectric point and can affect tissue swelling via two mechanisms. First, to maintain a neutral tissue sample, the static charge will attract many oppositely charged ions and small ions will consequently accumulate in the within-tissue space. Their accumulation will lead to an excess internal osmotic pressure to facilitate further swelling. Second, a decrease in the number of zwitterion pairs will decrease the attraction forces and, therefore, the fiber package density, thus also contributing to the tissue swelling [25].

To obtain qualitative characteristics of dehydration (shrinkage) and swelling, the time dependences of the weight, thickness, and area of myocardial samples immersed in the agents were approximated by Eq. (1). Table 2 summarizes the approximation parameters obtained for the time dependences of the weight, thickness, area, and volume of myocardial samples immersed in 40% glucose solution or 60% glycerol solution.

An analysis of the approximation parameters showed that the glycerol solution caused greater (A^{W} and y_0, Table 2) and faster (τ_w) dehydration of myocardial tissue. This trend was observed for all of the parameters under study, including the weight, thickness, and area. Myocardial tissue dehydration in the glycerol solution was greater than in the glucose solution because the glycerol osmolality is higher.

More-efficient optical clearing of the myocardium was achieved with an aqueous 60% gycerol solution

Table 2. The approximation parameters for dehydration (shrinkage) and swelling of myocardial samples immersed in 40% glucose solution or 60% glycerol solution

Approximation parameter		Immersion agent	
		40% glucose solution	60% glycerol solution
Dehydration/swelling (weight)	A^W	0.28 ± 0.04	0.33 ± 0.09
	τ_w^W, min	4.66 ± 2.32	3.83 ± 2.24
	B^W	0.41 ± 0.07	0.40 ± 0.12
	τ_g^W	42.20 ± 11.02	48.59 ± 14.54
	y_0^W	0.71 ± 0.05	0.67 ± 0.08
Transverse shrinkage/swelling (thickness)	A^l	0.24 ± 0.10	0.28 ± 0.07
	τ_w^l, min	9.45 ± 8.14	8.70 ± 7.79
	B^l	0.31 ± 0.14	0.26 ± 0.12
	τ_g^l	63.84 ± 58.79	61.75 ± 59.76
	y_0^l	0.76 ± 0.10	0.73 ± 0.07
Longitudinal shrinkage/ swelling (area)	A^S	0.23 ± 0.07	0.34 ± 0.05
	τ_w^S, min	1.50 ± 0.59	0.18 ± 0.06
	B^S	0.36 ± 0.14	0.34 ± 0.07
	τ_g^S	9.23 ± 5.40	11.01 ± 3.43
	y_0^S	0.76 ± 0.07	0.66 ± 0.05
Volumetric shrinkage/swelling (volume)	A^V	0.34 ± 0.13	0.35 ± 0.03
	τ_w^V, min	5.53 ± 4.69	6.30 ± 4.36
	B^V	0.45 ± 0.16	0.31 ± 0.12
	τ_g^V	24.73 ± 9.87	15.29 ± 12.88
	y_0^V	0.66 ± 0.12	0.65 ± 0.03

compared with an aqueous 40% glucose solution [7]. The finding can be explained, in particular, by greater and faster dehydration of myocardial tissue in 60% glycerol solution compared with 40% glucose solution.

The extent of myocardial swelling (B^W) was approximately the same in both cases, but the time to achieve it (τ_g^W) was shorter with a glucose solution. Biological tissues swell because immersion-agent molecules diffuse into the interfiber space. Because the molecular weight of glucose is approximately twice as high as that of glycerol, diffusion of glucose molecules into a tissue sample causes a greater increase in sample weight within a shorter period of time. It is also possible that myocardial tissue swelled faster in 40% glucose solution because glycerol caused greater sample shrinkage and a greater weight loss at the dehydration step, so that it took more time for tissue samples to recover from the dehydrated state.

It is of interest to note that the characteristic times of voluminous swelling of the myocardial samples ($\tau_g^V = 24.73 \pm 9.87$ min for 40% glucose solution and $\tau_g^V = 15.29 \pm 12.88$ min for 60% glycerol solution) were relatively close to the characteristic times of diffusion of glucose and glycerol molecules ($\tau = 20 \pm 12$ in for glucose and 12 ± 10 min for glycerol [7, 27]), which have been obtained by analyzing the collimated transmission kinetics with the same immersion agents.

A greater transverse swelling (B^l) of myocardial tissue in a glucose solution can be explained by the difference in hygroscopic properties between glucose and glycerol. A glycerol molecule can absorb approximately six water molecules [28], while a glucose molecule can absorb approximately ten water molecules [29]. Thus, a glucose molecule is capable of holding more remaining water molecules in a tissue sample,

causing greater tissue swelling. The temporal kinetics of the total volume similarly showed greater and slower swelling.

Another cause of myocardial tissue swelling was a pH of approximately 3.0 or 4.0 in the case of glucose and glycerol solutions, respectively (Table 1), while the pH of myocardial tissue is approximately 5.5 [22].

It should be noted that myocardial tissue swelling after dehydration during immersion in the solutions under study did not reduce the collimated transmission of samples [7], causing only a minor, if any, decrease in light transmission. Similar effects have been observed upon optical clarification of the sclerotic coat of the eye in vitro with 40%-glucose solution [30]; i.e., substantial optical clarification of a sclerotic coat sample was accompanied by a decrease in its thickness. As the duration of exposure to a glucose solution increased, optical clarification reached saturation and the sample thickness increased. Although the tissue sample thickness displays a complex behavior in 40% glucose, the variation of sample thickness does not considerably impair optical clearing [30].

The regularities observed in our experiments are of a general character and can be used to qualitatively describe the behavior of many fibrous tissues, such as skeletal muscle, derma, eye sclera, dura mater, and other tissues. The study is important for developing optical methods to diagnose and treat cardiovascular disorders in terms of a greater optical transparency of myocardial tissue and the results may additionally be used to develop new techniques of organ preservation for transplantation in cryoprotective solutions, which include glycerol and glucose [31–33].

CONCLUSIONS

The temporal kinetics of the weight, thickness, area, and volume was studied for porcine myocardial samples immersed in 60%-glycerol solution or 40%-glucose solution. Approximation of the parameter kinetics was performed to estimate the extents of dehydration and transverse and longitudinal shrinkage and swelling of myocardial tissue and the characteristic times of tissue dehydration and swelling. Greater and faster dehydration of myocardial tissue was observed in 60% glycerol solution, while its swelling was greater and faster in 40% glucose solution. The results can be used to further study myocardial tissue, to develop optical methods of diagnosing and treating cardiovascular disorders, and to design new methods of organ preservation for transplantation in cryoprotective solutions, including those of glycerin and glucose.

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REFERENCES

1. S. Alali, M. Ahmad, A. Kim, et al., J. Biomed. Opt. **17** (4), 045004 (2012).
2. T. Lindbergh, E. Haggblad, H. Ahn, et al., J. Biophotonics **4** (4), 268 (2011).
3. Y. Wang, K. Zhang, D. Duan, and G. Yao, Biomed. Opt. Exp. **8** (3), (2017).
4. Y. Xinwen, G. Yu, C. M. Charles, and P. H. Christine, J. Biomed. Opt. **21** (6), 061006 (2016).
5. *Handbook of Optical Biomedical Diagnostics*, 2nd ed., Ed. by V. V. Tuchin (SPIE Press, Bellingham, WA, 2016).
6. X. Wen, Z. Mao, Z. Han, et al., J. Biophotonics **3** (1), 44 (2010).
7. D. K. Tuchina, A. N. Bashkatov, E. A. Genina, and V. V. Tuchin, J. Innovat. Optic. Health Sci. **8** (3), 1541006 (2015).
8. J. Wang, N. Ma, R. Shi, et al., IEEE J. Sel. Top. Quant. Electron. **20** (2), 7101007 (2014).
9. L. M. Oliveira, M. I. Carvalho, E. Nogueira, and V. V. Tuchin, Laser Phys. **23** (7), 075606 (2013).
10. X. Guo, Z. Guo, H. Wei, et al., Laser Phys. **20** (9), 1849 (2010).
11. E. A. Genina, A. N. Bashkatov, A. A. Korobko, et al., J. Biomed. Opt. **13** (2), 021102 (2008).
12. D. K. Tuchina, R. Shi, A. N. Bashkatov, et al., J. Biophotonics **8** (4), 273 (2015).
13. *Physical Properties of Glycerine and Its Solutions* (Glycerine Producers' Association, New York, 1963).
14. R. C. Rowe, P. J. Sheskey, and M. E. Quinn, *Handbook of Pharmaceutical Excipients* (Pharmaceutical Press and American Pharmacists Association, 2009).
15. L. A. Alves, J. B. A. Silva, and M. Giulietti, J. Chem. Eng. Data **52** (6), 2166 (2007).
16. S. G. Schultz and A. K. Solomon, J. Gen. Physiol. **44**, 1189 (1961).
17. B. Amsden, Macromolecules **31** (23), 8382 (1998).
18. N. F. Stas' and L. D. Svintsova, *Chemistry of Solutions* (Izd. TPU, Tomsk, 2006) [in Russian].
19. A. A. Ragimov and G. N. Shcherbakova, *Infusion–Transfusion Therapy* (GEOTAR_Media, Moscow, 2010) [in Russian].
20. Y. Huang and K. M. Meek, Biophys. J. **77**, 1655 (1999).
21. T. T. Berezov and B. F. Korovkin, *Biological Chemistry* (Meditsina, Moscow, 1998) [in Russian].
22. M. I. Ravich-Shcherbo and V. V. Novikov, *Physical and Colloid Chemistry* (Vysshaya Shkola, Moscow, 1975) [in Russian].
23. E. M. Culav, C. H. Clark, and M. J. Merrilees, Phys. Ther. **79**, 308 (1999).
24. A. Katchalsky, Prog. Biophys. Chem. **4**, 1 (1954).
25. A. Pirie, Biochem. J. **41**, 185 (1947).
26. A. Pirie and R. van Heyningen, *Biochemistry of the Eye* (Oxford: Blackwell, 1956; Meditsina, Moscow, 1968).

27. D. K. Tuchina, Cndidate's Dissertation in Physics and Mathematics (Saratov, 2016).

28. J. W. Wiechers, J. C. Dederen, and A. V. Rawlings, in *Skin Moisturization*, 2nd ed., Ed. by A. V. R. Rawlings and J. Leyden (Informa Healthcare, Taylor & Francis Group, New York, 2009), pp. 309–321.

29. C. Molteni and M. Parrinello, J. Am. Chem. Soc. **120**, 2168 (1998).

30. A. N. Bashkatov, E. A. Genina, Yu. P. Sinichkin, et al., Biophysics (Moscow) **48** (2), 292 (2003).

31. S. Giwa, J. K. Lewis, L. Alvarez, et al., Nat. Biotechnol. **35** (6), 530 (2017).

32. J. Choi and J. C. Bischof, Cryobiology **60**, 52 (2010).

33. M. P. Longinotti, J. A. T. Gonzalez, and H. R. Corti, Cryobiology **69**, 84 (2014).

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