NON-STATIONARY HEAT TRANSFER IN GELS APPLIED TO BIOTECHNOLOGY

by

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Unsteady heat transfer in agarose gels of various concentrations was studied in order to make a breakthrough in the technology of 3-D additive bioprinting. Data on the kinetics of the phase transformation was obtained using spectroscopy as a function of temperature during the formation of agarose hydrogel. The dynamics of aging was investigated for gels of different densities. The time dependence of the structural changes was obtained. Particular attention was paid to the changes in the structure of the gel due to the processes of evaporation of the liquid during the gel formation and during long-term storage. Experiments were performed to determine the dynamics of the temperature fields simultaneously with heat flux measurements during the formation of agarose gels from different initial concentrations. A technique based on experimental data for the computations of the thermophysical coefficients of agarose gels was developed.

Key words: *additive bioprinting, agarose gel, spectroscopy, evaporation, phase transformations, thermophysical coefficients of gel, kinetics of formation, mechanisms of gel aging*

Introduction

For the incubation of tissues and organs from stem cells, it is necessary to create particular bioreactors. The idea of forming such bioreactors by the method of additive 3-D technologies [1-3] is considered as promising. Nowadays, 3-D technologies have already found wide application in other fields of science and industrial processes [4-6], where there is already a wide range of equipments for such technologies and appropriate software. Such research activities are carried out very actively in the development of bioprinting technologies [7, 8].

For the creation of bioreactors for the method of additive 3-D technologies the perspective material is gel, in which bioobjects are immobilized. For the cultivation of micro-organisms, agarose gels, and other gels can be used [9-11].

It is known that the properties of gels are determined by the composition of the gel-forming medium and by the preparation method. A large number of studies are dedicated to methods of gels synthesis of different chemical nature (see, for example, [12-14]). However, many properties of gels of different nature are similar in technical applications. For example, the most important features manifested in mass transfer processes in gels are unsteady and anisotropy, due to the structure and the behavior of the transport medium [15]. The properties of

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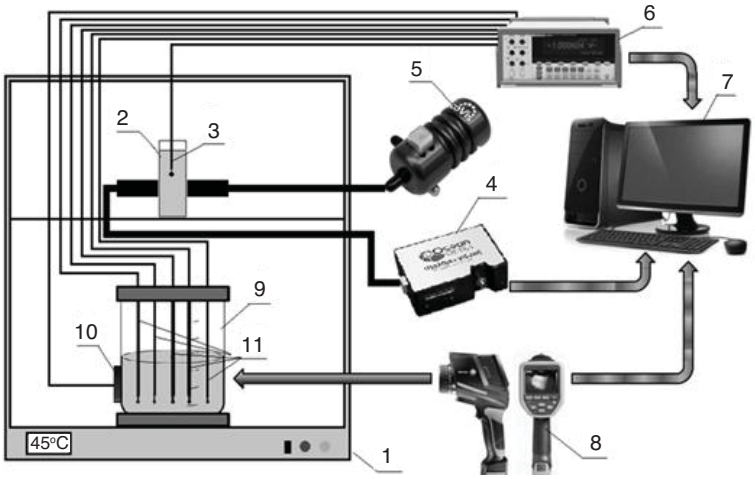
agarose gels with different compositions of sugars were investigated in [16, 17] and using optical methods in [18]. Their basic chemical composition, the composition of impurities in the gel, the structure of bonds arising in the dispersed phase during gel formation, the physico-chemical properties of gels, and the technology of their preparation for various purposes are described in [19]. Nevertheless, several properties of agarose gels that determine the possibility of their use for bioreactors are still unexplored.

Gels can be considered as a multi-phase system which consists of a liquid phase and a solid phase, where the solid phase is used as a framework that is filled by liquid. Such systems can be characterized by their complex internal structure. While regular structures of multi-phase systems and their thermophysical properties were studied previously [20], for gels such data has currently not been obtained. This data can be used for the optimization of technological processes involving gels [21]. At the moment there is no data on the thermophysical properties of the agarose gel in the temperature range of the transition from the liquid state to the gel state, about their structure, and the aging processes. The time dependence of the structural changes occurring as the liquid evaporates, has not been investigated neither for forming gels nor for ready made gels of different densities.

The article aims at studying the kinetics of agarose gel formation as a function of the concentration of the dispersed phase, of the temperature and of the aging processes. The obtained results can be used to create layered bioprinting technology using an agarose gel as a matrix with controlled transport properties for growing biomaterials.

Methods and materials

In order to study the kinetics of gel formation and the relationship between the physical and chemical properties of the gel and the emerging micro-structure of the dispersed phase, a spectroscopy method is used. The development of optical measurement technologies makes it possible to significantly increase its capabilities through the use of fiber optic sensors with a high degree of spatial and temporal resolution. Probing with fiber optic sensors of optically transparent gels by passing light through the sample makes it possible to obtain indirect information (without any contact) on the dynamics of the formation of the gel structure and the dependence on their temperature in real time. The experimental set-up diagram is shown on fig. 1.



1 – thermostat
2 – cuvette with gel
3 – thermocouple 1
4 – spectrophotometer
5 – light source
6 – digital multimeter
7 – personal computer
8 – thermal camera
9 – container with gel
10 – heat flow sensor
11– thermocouples 2-6

Figure 1. Scheme of the experimental set-up

The illumination system is made of a Xenon lamp with power 35 W, a color temperature of 2400 K, and emitted light waves in the range of 185-2000 nm. For illumination of the sample and receiving of the light signal optical fibers (Ocean Optics) of diameter 0.2 mm are used. These optical fibers work in the range of wavelengths of light from 220 to 1000 nm. The optical system is focused in a device for scanning the gel samples. The measure cuvette is made of quartz glass. The acquisition system is able to output and to analyze the spectra during the gel formation.

The spectrophotometer USB2000+VIS-NIR (Ocean Optics, Dunedin, Fla, USA) including Sony ILX511 2048-element linear silicon CCD array detector is the basis of the experimental set-up. These devices provide the instantaneous registration of 2048 channels in spectral range of 349-1024 nm. The optical resolution is on average 1.0 nm. The diffused light at 435 nm is less than 0.1%.

An optical cuvette of 10 × 10 mm and 45 mm in height contains the test sample of an aqueous solution of agarose and is installed in an air thermostat. The initial air temperature in the thermostat and of the aqueous agarose solution is 50 °C. With a controlled decrease in temperature in the thermostat, a gel formation from the solution occurs. The gel temperature range is chosen from 50 °C to 20 °C. The temperature is monitored by means of a thermocouple mounted in a gel cuvette.

In order to measure the thermophysical properties of agarose gels for different concentrations, the experimental set-up is equipped with a special heat flow sensor. Using a gradient heat flow sensor based on the Seebeck thermoelectric effect allows direct measurement of the amount of heat flow. The sensor is mounted on the side surface of a glass container of cylindrical shape with a diameter of 50 mm and 95 mm high. The container is filled with the gel solution at the initial temperature of 50 °C. Thermocouples are fixed to the diameter cap to control the distribution of the temperature fields in the volume during gel formation. The temperature of the outer side surface of the container is measured using the HotFind-LT thermal imager. To avoid negative effects of the convection of the ambient air that reduce the accuracy of measurements, the working area is placed in a thermostat, where a constant temperature of 20 °C is maintained.

In experiments agarose *Chemapol* is used. The gels are obtained by mixing agarose with distilled water. Agarose gels with 0.6-1.5% of agarose are used in the experiments.

Gel formation kinetic

Figure 2 shows an example of a change in the spectra of the light passing through an agarose gel of 0.8% concentration during gel formation by lowering the temperature. The spectra show qualitatively similar behavior compare to other concentrations. The spectrum of agarose gel for the mentioned concentration in distilled water is taken as the basis. To analyze the dynamics it is more convenient to use relative spectra, *i. e*. for each temperature and each wave length, the intensity of light passing through the specimen is attributed to the maximum value of spectrum, which is taken as the basis. For short wavelengths of light the spectrum is limited by the capabilities of a spectrophotometer, while for long wavelengths it is limited by the operating range of fiber optic cables.

For gels under investigation, the temperature adjustment of a gel in accordance with the environment is found to take place within 30-40 minutes. Figure 2 shows that the intensity of light passing through the specimen decreases for all wave lengths along with the gel temperature, *i. e*. the optical density of the medium increases, which reflects the process of a formation of a new micro disperse phase with a greater density than liquid. This process slows down when the temperature gets lower signifying the end of gel formation process. Visual observations

prove that the initial agarose solutions of various concentrations begin to exhibit the properties of a structured disperse phase within the temperature range of 35 °C to 25 °C. During this, the maximum wavelength of a transmission spectrum shifts towards the red area in the course of gel formation. This is probably due to the change of the structure of the medium.

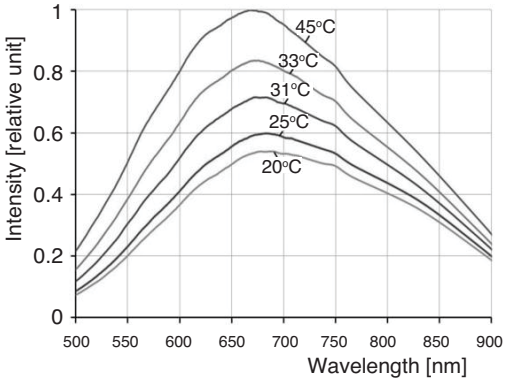


Figure 2. Changes in the relative intensity of light transmission *vs.* wavelength in the agarose gel with a 0.8% mass concentration

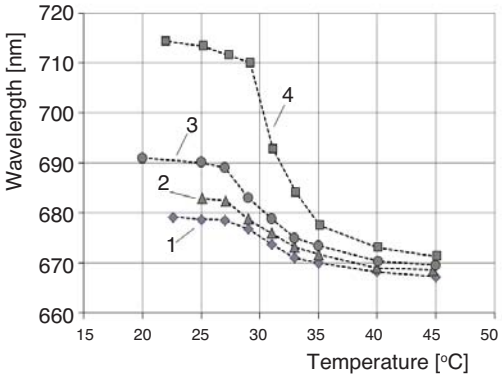


Figure 3. The change in maximum wavelength of the transmitted light, depending on the temperature of agarose gels formation and concentration; (1) 0.6%, (2) 0.8%, (3) 1.0%, and (4) 1.5%

Figure 3 shows the experimental data on the change of wavelength, which coincides with the maximum of the light transmission spectrum, depending on the temperatures of gels formation and the concentrations of agarose 0.6-1.5%. Along with the decrease of the temperature and gel formation, the wavelengths coinciding with the maximum of spectra suddenly increase at high temperatures, as compared to constant values, and then again take new constant values at low temperatures. This is caused by the increase of dispersion and light absorption of the selected wavelength when non-uniformities of less than a wavelength occur in the process of gel formation. In other word this is due to the change of the structure of the transmission medium. According to the visual observations, the liquid state of a gel forming medium coincides with the temperatures above 45 °C, while at the temperatures below 20 °C it is a formed gel.

The dependencies shown in fig. 3 are not equidistant for various concentrations of agarose. This means that while the solution structure shows weak dependency on the concentration of agarose at high temperatures (when the mediums are liquid), in the gel state, the structure of micro disperse medium significantly depends on the concentration of agarose at the temperatures below the necessary one to form gel. Moreover, along with the increase of this concentration, the changes in the structure of micro disperse medium are clearer. It should be noted that these properties are not unique for the agarose gels. A similar phenomenon was found earlier for silica gels [22].

Gels aging

Due to their rheological properties, gels refer to an intermediate state between liquid and solid. The common definition of gels is a dispersive system with liquid dispersing medium, and the dispersion phase makes up a spatial structured mesh due to intermolecular interaction in the contact areas [23]. Gels are capable of displaying both elasticity and plasticity. Effect of hysteresis related to optical properties of a gel can also be observed during the process of gel

heating and subsequent cooling. The change of internal structure of a gel during aging process can affect evaporation dynamics by changing pores configuration. For practical use it would be important to investigate the behavior of agarose gels in case of a long-term storing (ageing) under the conditions which prevent water evaporating from the surface.

It is important to investigate this problem since the phenomenon of syneresis occurs during the aging of the gel. It consists in the spontaneous consolidation of the gel phase and the formation of a layer of pure dispersive phase. For the agarose gel, the dispersion phase is water. It forms a layer above the surface of the spontaneously condensing agarose gel and evaporates during storage. Thus, the density of the gel, from which bioreactors are supposed to be build using the method of layer-by-layer printing, will increase in the process of bioprinting in comparison with the initial one. Such a change in the density of the structuring medium due to syneresis and evaporation of the liquid phase require prediction in the development of the bioreactor design and taking into account for the creation of additive technologies.

In the experimental study an agarose gel with concentration of 0.6 and 1.0% is used. At the beginning of the experiment, a prepared sample of the gel at a temperature of 50 °C is applied to the surface of the Petri dish. Then a Petri dish was mounted on a heat insulating plate and installed at the scale. Further thermocouples are injected into the gel. The ambient temperature is maintained in the range of 20-22 °C. The measurements begin when the cooling sample reached a temperature of 32 °C. During the experiment, the dependencies of the gel mass in the Petri dish and its temperature on time were determined. The time interval of measurements was chosen to be 30 min, based on the characteristic time of reaching the gel temperature of a practically stationary value of 24 °C. At this temperature, according to the results shown in fig. 3, the agarose gel is already fully formed. For additive technologies, after this characteristic time, it is possible to apply the next layer of gel.

The results of the previously described above measurements are shown in fig. 4. For the chosen range of concentrations (corresponding to the best conditions for the immobilization of microorganisms), the dependencies of the samples mass loss on time are practically linear. This confirms the assumption that evaporation of the liquid takes place under syneresis conditions from the surface of the liquid layer located above the gel. The rate of loss of mass by gel due to evaporation (the tangent of the angle of the inclination of curves 1 and 2 to the abscissa in fig. 4) depends on the initial concentration of agarose in the gel. Increase in initial agarose concentration decreases the rate of mass loss due to evaporation. It is likely caused by slower flow of syneresis in gels of higher density.

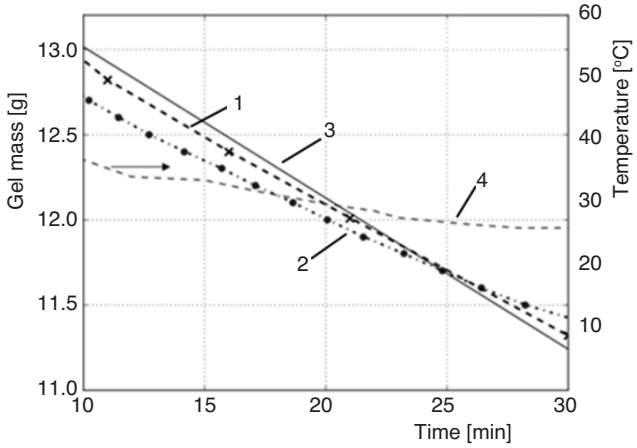


Figure 4. Dependency of gel mass and temperature on time during liquid evaporation from gel surface: *(1) mass concentration of agarose 0.6%, (2) mass concentration of agarose 1.0%, (3) computations, (4) temperature in the gel volume*

Theoretical analysis shows that at the initial stage the evaporation occurs from the liquid surface film under conditions of external diffusion resistance. It is assumed that the temperature

of the liquid layer on the surface of the gel is constant and there is no convective mixing in the gas phase. In this case, the mass transfer from the liquid to the gas phase occurs solely by the diffusion mechanism. Absolute moisture content of the gaseous medium near the liquid layer and away from it under constant temperature conditions is constant. Hence, the vapor flow from the liquid to the gas phase in the time interval under consideration remains constant. Therefore, with a constant evaporation, the mass of the gel decreases linearly with time.

It is important to emphasize that at times exceeding the time interval of interest for bioprinting technology, the process of evaporation of liquid from the gel drastically changes. Gel contains pores [24] which supply liquid to gel surface due to capillary forces and enable intense evaporation from the whole surface. The dependency demonstrates more non-linearity, which can correspond to a case where evaporation depends not only on the evaporation of the liquid from the surface but also on the gel shrinkage due to lack of liquid. Finally, approximately after 100 minutes, evaporation intensity significantly slows down. This effect can be related to the lower volume of the liquid after a long evaporation period, when there is not enough liquid to supply the gel surface with capillary forces. In this case the evaporation continues inside the gel pores which lowers area of evaporation and hence reduces its intensity. Basically, the change of gel humidity rate over evaporation is similar to dehydration of wet porous matter having pore-diffusion resistance to the process of moisture mass transfer.

Thermal properties of the gel during gelation

For the device calibration of bioprinting using layer-by-layer gel technology, data on the thermophysical characteristics of the gel during gel formation is required. The heat capacity and the thermal conductivity are essential coefficients for calculations of the temperature fields in layer-by-layer deposition of heated gels during the *printing* of the bioreactor. Temperature governs the state of the gel and its rheological properties. Forecasting, control and governing methods of the thermophysical properties of the gel are the most important factors affecting the final properties of the gel matrix, including mass transfer.

For the experimental set-up shown in fig. 1, the gel formation process that occurs with decreasing temperature, the temperature dependence of the gel at the center and near the side wall of the experimental cuvette, and the heat flux through its outer side wall were measured. The obtained experimental data for gel forming solutions with a mass concentration of agarose 0.6% and 1.0% is shown on fig. 5.

Figure 5 demonstrates that the experimentally measured values of temperatures and heat flux decrease with time, which corresponds to the generally accepted idea of sample cooling. In accordance with this, the temperature in the center of the cuvette is higher than near its wall for all concentrations of agarose. The temperatures in the gel samples decrease with time and tend to the temperature inside the thermostat, and the heat flux tends to zero. Within the experimental error, the heat fluxes on the outer wall of the cuvette coincide for different concentrations of agarose. Note that the temperatures at all points in the sample for gels with a mass concentration of 1.0% agarose are always lower than at the corresponding points for a 0.6% gel sample.

Based on data from experiments similar to those presented in fig. 5, but with a larger number of thermocouples inside the gel, by solving the inverse heat conduction problem, the average thermophysical coefficients for agarose gels of different densities in the temperature range of their formation are calculated. A mathematical model of non-stationary cooling of an 1-D infinite in length cylindrical sample with constant thermophysical properties (heat capacity and thermal conductivity) is considered for calculations.

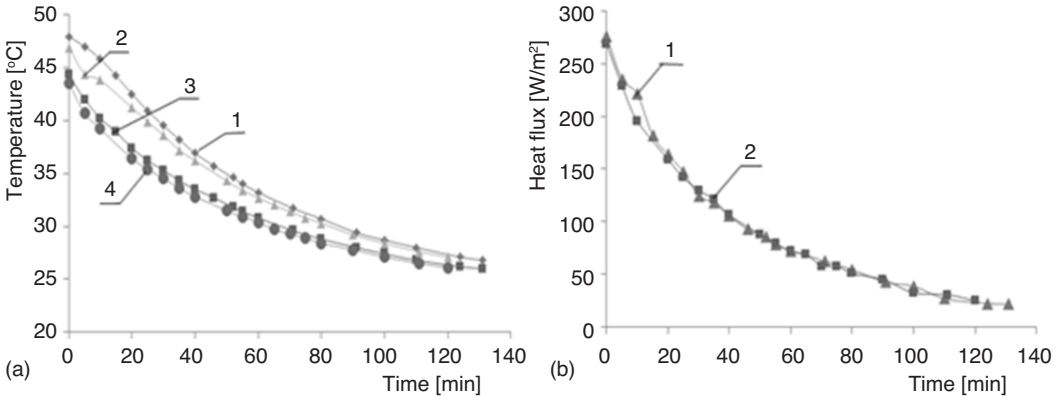


Figure 5. Dependence of the temperature inside gel (a) and the heat flux for the outside wall of the experimental cuvette (b) on time during the gel formation: (1), (3) – mass concentration of agarose 0.6% (2), (4) mass concentration of agarose – 1.0%. On fig (a) the temperature is measured (1), (2) – in the center of the experimental cuvette filled with gel, (3), (4) – close to the inner wall

It is based on the unsteady heat equation in a cylindrical co-ordinate system, with initial and boundary conditions corresponding to the experimental conditions:

$$C\frac{\partial T}{\partial t}=\lambda\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial T}{\partial r}\right),\quad t>0,\quad 0<r<R \tag{1}$$

$$t=0:\ \ T(r,0)=T_0,\qquad r=0:\ \ \frac{\partial T}{\partial r}=0,\qquad r=R:\ \ -\lambda\frac{\partial T}{\partial r}=q(t) \tag{2}$$

where r is the radial co-ordinate, t – the time, R – the radius of the external surface of the sample, T_0 – the initial temperature, C – the coefficient of volumetric heat capacity, λ – the coefficient of thermal conductivity, and $q(t)$ – the heat flux density from the surface of the sample.

The initial boundary value problem described by eqs. (1) and (2), has an exact solution [25] represented in the form:

$$T(r,t)=T_0-\frac{2}{CR}\int_0^t q(\tau)\,\mathrm{d}\tau+\sum_{n=1}^{\infty}\exp\left(-\frac{\lambda\mu_n^2 t}{CR^2}\right)J_0\left(\frac{\mu_n r}{R}\right)H_n(t) \tag{3}$$

where

$$H_n(t)=\frac{1}{J_0^2(\mu_n)}\left[\frac{2T_0}{R^2}\int_0^R rJ_0\left(\frac{\mu_n r}{R}\right)\mathrm{d}r-\frac{2}{CR}\int_0^t q(\tau)\exp\left(\frac{\lambda\mu_n^2\tau}{CR^2}\right)\mathrm{d}\tau\right]$$

where J_n is Bessel function of the first order n and μ_n is the root of the equation $J_1(\mu_n)=0$.

However, since the exact solution described by eq. (3) is expressed in terms of infinite series, it is not appropriate for determining the heat capacity and thermal conductivity. Finite truncation additionally requires an estimate of the accuracy of the simplification solution. Hence it seems more suitable to use numerical methods for solving the problem eqs. (1) and (2).

The values of the temperatures inside the sample and the heat fluxes on its lateral surface, obtained experimentally, are represented in the form of numerical arrays: $\tilde{T}(r_m,t_n)$

and $\tilde{q}(t_n)$, where r_m $(1 \le m \le M)$ is the radii of thermocouple locations within the samples under study, and t_n $(1 \le n \le N)$ is the instant of time of measurement. Knowing the values of temperatures and the heat flux it is possible to compute coefficients C and λ using the inverse problem of hear transfer. To solve this problem we need to minimize the difference in norm between the experimental temperatures $\tilde{T}(r_m, t_n)$ and the computed one $T(r_m, t_n)$, as well as the difference between the experimental values of heat flux $\tilde{q}(t_n)$ and the computed values $q(t_n)$ at all times t_n and all thermocouple locations r_m used in the processing of experimental data:

$$\sum_{m=1}^{M}\sum_{n=1}^{N}\left[\tilde{T}(r_m,t_n)-T(r_m,t_n)\right]^2 \to \min$$
$$\sum_{n=1}^{N}\left[\tilde{q}(t_n)-q(t_n)\right]^2 \to \min \tag{4}$$

To solve the inverse heat conduction problem, we used the extreme formulation and the principle of iterative regularization. The direct heat conduction problem, described by eqs. (1) and (2), is solved using an implicit difference scheme. A solution of a system of difference equations with a tridiagonal matrix of coefficients is obtained by the sweep method. The minimization problem, see eqs. (4), is solved by the method of co-ordinate wise descent. For the numerical implementation based on the previously developed model [26], new algorithms were written in Visual Fortran language.

Table 1. Averaged during gel formation thermophysical characteristics of gels for different mass concentrations of agarose

Mass concentration of agarose, [%]	Average thermal conductivity of the gel, λ, [$Wm^{-1}K^{-1}$]	Average heat capacity of the gel, C, [$kJm^{-3}K^{-1}$]
0.6	0.538	2129.7
1.0	0.501	2121.8

Using the experimental data obtained and the developed calculation model, the values of the thermal conductivity and bulk heat capacities averaged over the gel formation time for gels with different initial concentrations of agarose were obtained. Their values are given in tab. 1. The results show that within the experimental accuracy, the average heat capacity of gels of different concentrations in the range of values most suitable for immobilization of microorganisms is constant. Under the same conditions, the average thermal conductivity of gels decreases with increasing concentration of agarose. The obtained results will be used for the modeling of the gel behavior with respect to additive technologies of *bioprinting* and for the analysis of thermal effects on the formed gel.

Conclusions

Experimental results on the kinetics of the phase transformation of an aqueous solution of agarose in a gel of different concentrations were obtained using optical absorption spectroscopy. It is demonstrated that the wavelength of the maximum in the transmission spectrum shifts towards the red in the course of gel formation. It is found that the solution structure shows a weak dependency on the concentration of agarose at high temperatures (when the mediums are liquid), in the gel state, the structure of micro disperse medium significantly depends on the concentration of agarose at the temperatures below the necessary one to form gel.

With respect to bioprinting technology, it is shown that during the aging of the gel, it is essential to take into account the multistage nature of this process which consists of several steps, including evaporation from a surface, gel layer shrinkage and evaporation from gel pores. For the first stage of liquid evaporation from the surface, the dependence of the mass loss of the gel samples on time is linear. The rate of mass loss by gel due to evaporation decreases with an increase of the concentration of agarose in the gel.

Based on highly-equipped experiments recording the dynamics of the temperature fields in the volume of the gel and the simultaneous change of the heat flux during the gel formation process at different concentrations of agarose in the gel, a method for the determination of the thermophysical coefficients was developed. The values of the thermal conductivity and bulk heat capacities averaged over the gel formation time for gels with different initial concentrations of agarose were found.

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