# THE EFFECT OF BIORESORBABLE ADDITIVES AND MICRO-BIOOBJECTS ON GEL FORMATION, STABILIZATION AND THERMOPHYSICAL PROPERTIES

by

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*The same properties of agarose gels containing neutral bioresorbable additives and living microorganisms which are important for use in additive technologies of bioreactors creation were considered. Data on the kinetics of gel formation from the solution during cooling were obtained by spectroscopic measurement by measuring the shift of the maximum spectrum of light passing through the gel, depending on the temperature. The dynamics of aging was investigated for gels of different concentrations of agarose, bioresorbable additives and living cells. The time dependences of the decrease in the optical transparency of such gels during the aging process, characterizing the changes in their structure, were obtained. Special attention was paid to the effect of liquid evaporation from gels in the process of gel formation and during long-term storage on relaxation processes leading to their spontaneous increase in density. Experiments were performed to determine the dynamics of the temperature fields simultaneously with heat flux measurements during the formation of studied gels with different concentrations. On the basis of the obtained experimental data and previously developed method, the thermophysical coefficients of agarose gels containing an admixture of starch and living yeast cells were calculated.*

Key words: *bioprinting, agarose gel, bioresorbable additive, cells, spectroscopy, kinetics of formation, gel aging, evaporation, thermophysical properties of gel*

## Introduction

The idea of growing tissues and organs in vitro using stem cells is not new. However, in order to implement this idea, it is necessary to create special bioreactors which are capable of maintaining the required temperature, pH level, osmotic pressure, supply cells with nutrients and oxygen, removing their metabolic products as well as fulfilling many other requirements, which provides necessary physiological conditions for immobilized cells [1, 2]. The use of the method of 3-D additive manufacturing in medical purposes or for creation of different bioreactors for different applications [3, 4] seems to be very promising. It can be said that currently a separate branch of science is being formed, which is called 3-D-bioprinting [5, 6].

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Promising materials in which the cultured cells can be mobilized for usage in bioprinting are gels [7-9]. Gels may be used as bioink that forms structure for cells growth [10]. The rheological properties of gels allow forming bioreactors of complex configurations with immobilized cells based on layer-by-layer stacking of gel of various concentrations and compositions, without affecting the conditions for survival of biological microscopic objects. The gel capillary network can be used for transporting of nutrients to separate cells with culture broth and for removal products of cell metabolism. Agarose and other gels are widely used in microbiology as a medium for microorganisms growth [11, 12]. This fact proves the possibility of finding good ambient conditions of temperature and acidity for growth and reproduction of biological microscopic objects.

Contrary for example, making injection when gel is not formed enough leads to unsteadiness in a printed object, while keeping gel for too long make it lose flexibility which can lead to choking in a 3-D bioprinter nozzle [13]. Heat capacity and thermal conductivity coefficient are necessary for modeling the dynamics of temperature fields in the technology of layer-by-layer application in bioprinting. Thermal conditions determine the state of the gel, its properties, and immobilization of microbiological objects [14]. Methods for controlling the thermophysical properties of gels are the main factors that determine the final transportation properties of the gel matrix [15].

The properties of gels are determined by the composition of the gel-forming medium and by the preparation method. However, many properties of gels of different nature are similar for technological applications. For example, in mass transfer processes in gels these properties are unsteady and anisotropy, due to the structure and the behavior of the transport medium [16, 17]. Based on the assumption that many gels will exhibit similar properties when used in additive technologies, it is possible to study the fundamental features of their technological application on simple gel systems, for example, such as agarose gels.

Agarose gel is a typical gel which is formed from the solution when the temperature is lowered. It is widely used in microbiology for growing microbiological objects and in the food industry. Such gels have been thoroughly investigated [18-20], including studies by the optical methods [21]. Structure of links which are formed in the disperse phase of gel formation and physical-chemical properties of gels has been described in [22]. Nevertheless, many properties of agarose gels which determine whether they may be used for the creation of bioreactors with the help of additive manufacturing require additional studying [23]. Only recently 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 relaxation processes was obtained [24].

The extension of the field of practical applicability of gels allows us to formulate new fundamental problems of their study. In medicine and food industry the goal is to create gels with desired properties. Approaches to its solution are known, it is the use of special additives or the development of mixed gels containing two or more different gelling agents [25-28]. The situation is much more complicated if the gel is used as a frame matrix for the cultivation of living tissues. As the mass of the tissue grows, the frame matrix should disappear as a result of the bioresorbability of its material or its additives [29, 30]. Such additives can change the conditions of gel formation in comparison with pure agarose gel, peculiarities of its temporary relaxation and thermophysical properties. The effect of the properties of the gel on the behavior of microorganisms immobilized in them has been widely studied in biotechnology (see, for example [31]), the study of the reverse effect of cells on the change in the properties of the gel has begun relatively recently [32, 33].

The article aims to study the following features of agarose gel with bioresorbable additive of inert substance in the presence of micro-bioobjects: temperature range of gel formation, time dynamics of relaxation processes in the aging process, including the presence of evaporation of the liquid phase, thermophysical properties in the process of gel formation. Obtained results can be used for the further development of additive bioprinting technologies using agarose gels with bioresorbable additives for the formation of bioreactor frameworks in the presence of cultivated microorganisms.

## 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 microstructure of the dispersed phase, a spectroscopy method was used. It provides the opportunity to obtain indirect information (without any contact) on the dynamics of the formation of the gel structure from of their temperature in real time. The diagram of the experimental set-up and its detailed description are given in [24]. In addition, the lighting system was supplemented with a system of color filters, providing a maximum of spectrum of transmission light through distilled water at a wavelength of 550.7 nm.

This experimental set-up was used to determine the time dynamics of reducing the optical permeability of the studied agarose gels with various additives at their aging at room temperature. Since the measuring part of the set-up in addition to the optical system was equipped with thermocouples that allow measuring the temperature along the radius of the gel sample and a special heat-flow sensor, its use also makes possible to obtain experimental data for calculation by solving the inverse problem of thermal conductivity of the thermal characteristics of the studied gels with additives. In a number of experiments, the Abbe refractometer was used in order to determine the refractive index of light. The temperature of the measuring prism of the refractometer was maintained by a thermostat with an accuracy of 0.2 °C. The refractive index was measured at the wavelength of the yellow sodium line equal to 589.2 nm.

Experiments on evaporation of water from gel were performed using Petri dish and electronic scales for mass change fixation. Petri dish was 94 mm in diameter and dish walls were 16 mm in height. Petri dish was isolated from electronic scales surface using foam plastic plate in order to ensure heat isolation of a Petri dish from scales. Hygrometer was used for humidity fixation and thermometer to ensure that all experiments were performed with the same temperature. In this experiment series temperature of 23 °C and relative air humidity of 50% were maintained. Gel layer thickness was maintained at level 1 mm in order to model typical layer thickness in additive bioprinting technologies.

In experiments agarose *Chemapol* was used. Gels were obtained by mixing agarose with distilled water with subsequent heating to 90 °C and slow cooling in the thermostat to the initial temperature of the experiment. After gel temperature stabilized at 45 °C, the spectra recording began. The thermostat was slowly cooled down to 20 °C . At control temperatures, the gel sample was kept for some time to establish thermal equilibrium. Agarose gels with 0.3-1.0% of agarose were used in the experiments as control samples.

In experiments starch was used as a bioresorbable additive in agarose gels for indication purpose. During gel samples preparation with bioresorbable additives for each mass concentration of agarose, gel samples containing starch in an amount of 25, 50, 75, and 100% of the mass fraction of agarose (0.4, 0.6, and 1.0%) were also composed. Starch was diluted with distilled water to the required concentration and was added to the solution of agarose, heated up to 90 °C. In order to avoid stratification, the mixture was constantly homogenized by special

way when cooled to 50 °C. Qualitatively, the uniformity of starch distribution in the gel was controlled by the uniformity of the blue color of the gel when mixed with iodine.

Pichia polymorpha yeast related to Ascomycetes and having a single-layer cell wall was chosen as micro-biological objects for experiments. The rationale for this choice was that cell wall of the yeast Pichia polymorpha is up to 30% of the mass of the cell and consists of a homogeneous mixture of mannoproteins and beta-glucans reinforced with chitosan [34]. The reason is that mannans, glucans, like many polysaccharides, and chitosans have a high chemical affinity for agarose. In the gel of each studied sample concentration of agarose with a starch content of 25 or 100% concentration of agarose by weight, 0.5 ml of yeast suspension was sown. The yeast concentration in the suspension was 0.034 mg/cm$^3$. It was monitored by measuring the optical permeability of the suspension at a wavelength of 540 nm.

## Gel formation and stabilization

In the experiment the spectra of the samples of the studied gels of different concentrations and compositions were obtained at their cooling from 45 °C to 20 °C, *i. e.* in the temperature range in which the formation of a pure agarose gel from the solution occurs. It was found that the intensity of light passing through the sample decreases at all wavelengths with a decrease of the gel temperature and an increase of the concentration of the dispersed phase (total for agarose and starch and cells), *i. e.* the optical density of the medium increases, which reflects the formation of a new microdisperse phase. Observations prove that the initial agarose-starch solutions (with and without cells) of various concentrations as well as pure agarose gels begin to exhibit the properties of a structured disperse phase within the temperature range of 35 °C to 25 °C. Previously it was assumed that due to the change of the structure of the medium the maximum wavelength of a transmission spectrum shifts towards the red area in the course of gel formation [24].

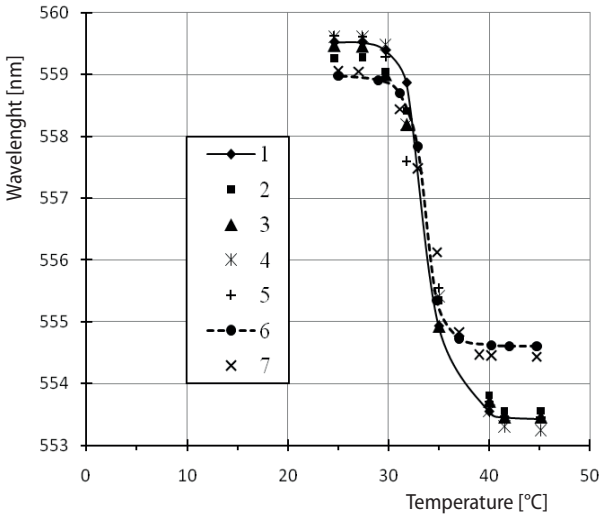


**Figure 1. The change of the maximum wavelength of the spectrums of the transmitted light depending on the temperature of formation for gels on the basis of 1.0% by weight agarose at different concentrations of starch and yeast: 1 – pure agarose gel, 2-6 – agarose gel with added starch by weight: 2 – 0.25%, 3 – 0.5%, 4 – 0.75%, 5 – 1.0%, 6 – 0.5% of starch and suspension of yeast, 7 – pure agarose gel and the suspension of yeast**

Figure 1 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 under the concentrations by weight of agarose 1.0% and different concentrations of starch and cells. 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. According to the observations, the liquid state of a gel forming medium coincides with the temperatures above 45 °C, while at the temperatures below 25 °C it forms the gel.

The obtained results show that the presence of starch in the gel in all the studied concentrations does not lead to an additional shift of the spectrum maximum in comparison with pure agarose gel. This means that the starch is not a part of the chemical compounds forming the dispersed phase of the gel. Thus, the starch itself not only does not form a micro structuring phase, but also does not affect the process of its formation from agarose. When the gel is formed, the starch acts as a bioresorbable neutral additive, the microparticles of which are located in the pores of the forming agarose gel. For gels with a constant concentration of agarose with increasing starch concentration density increases and optical permeability decreases. Starch also does not affect the formation of agarose gel in the presence of yeast culture. However, the bioresorbable properties of starch will allow it to release in gel the living space as a result of biodegradation for the growth of the number of micro-biological objects.

It is important to note that the temperature range of the gel formation from the agarose solution does not depend on the presence of starch or yeast cells in it. As follows from fig. 1, the addition of living yeast cells to the agarose gel reduces the shift of the maximum of spectrum of the transmitted light. On the one hand, during the cooling of the agarose solution containing cells with the formation of the gel the slight flocculation and sedimentation occur, which leads to the change in the spectral characteristics of the solution. On the other hand, sizes of microorganisms used in the experiment significantly exceed determined by intermolecular interaction of internal scales of the structured phase of gel. Probably, due to this reason, the dispersed phase has discontinuities on cells, which also changes the spectral characteristics of the gel. Thus, despite the chemical similarity of the substances forming the cell wall with agarose, the cells do not change the conditions of gel formation and do not strengthen its structured phase.

To finally understand the effect of starch as a neutral bioresorbable impurity on the properties of agarose gel, the refractive indices were measured at different concentrations of agarose in pure agarose gel and different concentrations of starch in a combined gel with a weight concentration of agarose 0.6%. Measurements were performed at temperatures of 25±0.2 °C. These results are presented in fig. 2 as a refractive index depending on the weight concentration of the dispersed phase. Both dependences are linear. However, they have a different angle of inclination to the horizontal axis, since the molecular masses of agarose and starch differs. The reason for this is that the refractive index at a constant temperature primarily depends on the density of the medium through which light passes. Thus, for combined agarose gels at a known weight concentration of agarose, the concentration of the known added bioresorbable impurity can be determined by measuring the refractive index.

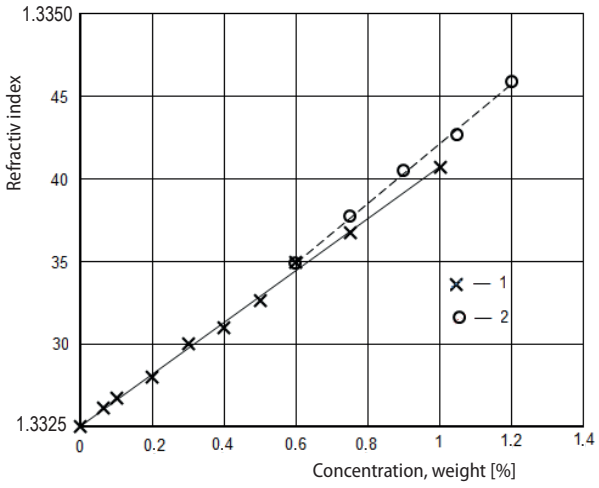


**Figure 2. The dependences of the refractive index on the weight concentration of the dispersed phase in gels: 1 – pure agarose gel, 2 – agarose gel with a weight concentration of 0.6% in the case of adding starch**

The reference point of the measured refractive index for pure agarose gel was distilled water, since in this case it corresponds to the zero concentration of agarose. For the

combined gel with addition of starch, the refractive index for pure agarose gel with a weight concentration of agarose of 0.6% was the reference point. An important observation is that in the study of pure agarose gels to a weight concentration of 0.25%, despite the fulfilment of all the conditions of their preparation, they visually did not differ in their fluidity properties from aqueous solutions of agarose of the same concentration. Probably, at such concentrations of agarose, the critical concentration of the dispersed phase required for the formation of the gel is not achieved. The distance between the agarose molecules in the solution is too large in order to form a structured phase due to chemical interactions.

The results shown in fig. 2 allowed us to study the composition of the dispersion phase in pure agarose gel. When storing the gel in isolated container for several days as a result of syneresis, a layer of liquid appears above the gel surface, which is a dispersion phase. The presence of this liquid is enough to determine its composition by refractometric method. It was found that the dispersion phase is an aqueous solution of agarose (possibly very low concentration agarose gel), in which the concentration of agarose is an order of magnitude less than its mass concentration in the gel from which samples were taken. It was found that the concentration of agarose in the dispersion phase increased with increasing its concentration in the gel and the duration of its storage. Note that in all the studied samples of pure agarose gel (including those exceeding the weight concentration of agarose in gels suitable for practical application in microbiology and any duration of their storage in an isolated container), the weight concentration of agarose in the dispersion phase exceeding 0.25% was not found.

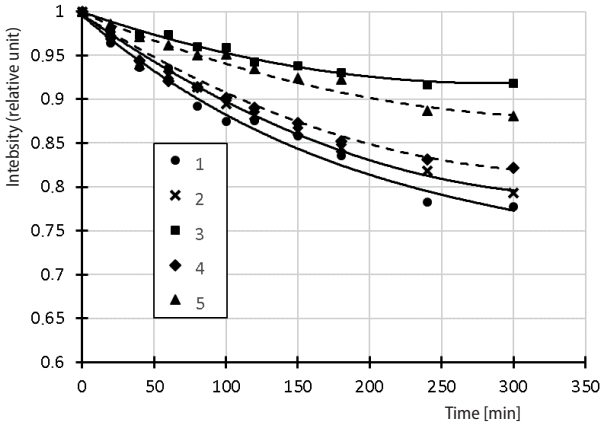


**Figure 3. The dependence of the relative intensity of light passing through the samples of agarose gels weight concentration of 1.0% agarose with starch and yeast cells additives on the stabilization time: 1 – pure agarose gel, 2-3 – agarose gel with added starch by weight: 2 – 0.25%, 3 – 1.0%, 4 – pure agarose gel and the suspension of yeast, 5 – agarose gel with added starch by weight 0.5% and the suspension of yeast**

Temporary stabilization of gels during their storage in the isolated container was studied by measuring the relative intensity of light transmission in an agarose gel with weight concentration of 1.0% depending on the time (as well as adding starch and yeast cells). The results are shown in fig. 3 as a function of the time of the relative intensity of the transmitted light at the wavelength corresponding to the maximum transmission spectrum. The relative intensity of the transmitted light through the gel is determined with respect to a similar value at the time taken as the starting point of observations.

As can be seen from the type of dependencies in fig. 3, even at large times, the intensity of the transmitted light from gels continues to decrease. At the same time, the appearance of free liquid on the gel surface is also visually detected. Thus, the presence of the phenomenon of syneresis characterise both pure agarose gels and gels with the addition of starch and living yeast cells. However, for the combined gels, the addition of starch slows the syneresis structured dispersed phase. In this case it is denser than for the pure gel because the starch prevents the change of structure. Cells also decreased spontaneous compaction of gels during stabilization.

### Gels stabilization with evaporation

The change in the internal structure of the gel during its use may be due to a change in the configuration of the pores as a result of evaporation of water from the dispersion phase. For practical application in additive technologies, it is important to investigate the behavior of agarose gels when used under conditions of evaporation of water from the surface. For combined gels or gels with cells, it is necessary to understand their influence on the dynamics of evaporation and, as a consequence, on the dynamics of changes in the internal structure of the gel. This information can help to determine the optimal time range when the layer of gel after the injection from the nozzle of the bio-printer is most suitable for overlaying the next layer of gel.

The dynamics of change in the mass of samples as pure agarose gel with a weight agarose concentration of 0.4%, and combined gels with different concentrations of starch due to evaporation of water from its surface at the initial stage of stabilization are shown in fig. 4. Evaporation from water free surface was used as a benchmark. If we consider the process of evaporation from the surface of a thin layer of water in isothermal and stationary conditions, the decrease in the mass of the sample will depend linearly on time. Comparison with experiment demonstrates that linear model of evaporation does not describe precisely water evaporation in the experiment. The decrease in water mass has non-linear dependence from time. This can be related to non-stationarity conditions of evaporation, influence Petri dish borders and formation of micro dry spots due to thinness of water layer.

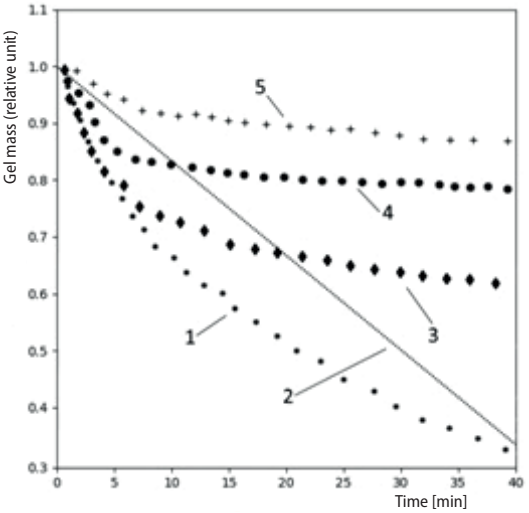


**Figure 4. Intensity of liquid evaporation during formation and initial stabilization of agarose gels weight concentration of 0.4% agarose with added starch; pure water evaporation is used as a benchmark: 1 – water, experiment, 2 – water, model, 3 – pure agarose gel, 4 – agarose gel with added starch by weight 0.1%, 5 – agarose gel with added starch by weight 0.3%**

During the first 5-7 minutes from the beginning of the evaporation process, the mass loss of the pure gel sample does not differ from the mass loss in the water layer. This is probably related to the fact that the initial mass loss of the gel used by evaporation of water from the dispersion phase, released on its surface as a result of syneresis. Since the dispersion phase on the gel surface is an aqueous solution of agarose, the evaporation of water from it forms a thin dense film of agarose after some period of time. Theoretical analysis shows that at the initial stage the evaporation occurs from the liquid surface film under conditions of external diffusion resistance. Mass transfer from the liquid to the gas phase occurs solely by the diffusion mechanism. Hence, the vapor flow from the liquid to the gas phase in the initial time interval under consideration remains constant. Therefore, with a constant evaporation, originally the mass of the gel decreases linearly with time.

The change in the moisture content of the gel due to evaporation with some differences is similar to the drying process of porous wet material in the presence of intra-diffusion resistance to moisture mass transfer. After evaporation of water from the dispersion phase on the gel surface, the intra-diffusion regime initiates. After about 10 minutes from the beginning

of the process, the dependence of moisture loss on time slows down and becomes non-linear. The cause here is not only the capillary rise of the dispersion phase to the surface, but also the simultaneous compaction of the dispersed phase by reducing the amount of moisture inside the gel that could take place. Obtained data shows that agarose gels with starch additives demonstrate lower evaporation intensity and this intensity lowers with increasing starch concentration, fig. 4. Starch increases the intra-diffusion resistance as its molecules clog the gel pores and prevent rise of the liquid to surface. Additionally all mixtures demonstrate the same behavior in evaporation intensity with more intense evaporation during the first 10 minutes of experiment with consecutive flattening of mass reduction intensity.

Two hypotheses were formed in order to explain change in evaporation intensity after first 10 minutes. First hypothesis stated that samples of gels on based on pure agarose or agarose with starch additive forms double layers systems where upper layer inhibits evaporation from the deeper layer. The top layer is a compacted thin film, which arose as a result of syneresis due to the appearance of the dispersion phase on the surface and evaporation of water from it. The bottom layer is a regular gel. A photo of the cross-section of such a two-layer system in the gel is shown in fig. 5. The film was very thin and it was not possible to measure its thickness by the available means. It can be assumed that its thickness increases with time and with agarose concentration increase. It is important that such a film was identified on samples of gels of all compositions and if they were stored for a long time in isolated containers without evaporation. The film in the near-surface layer was observed as an object with a sharp gradient of changes in optical properties in contrast to the previously studied near-surface layers between layers in multilayer gels, which confirms the model of its formation.

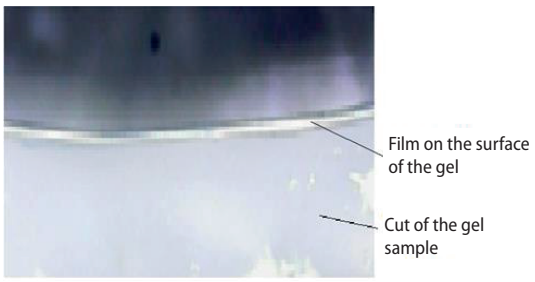


**Figure 5. Photo of cross-section of agarose gel**

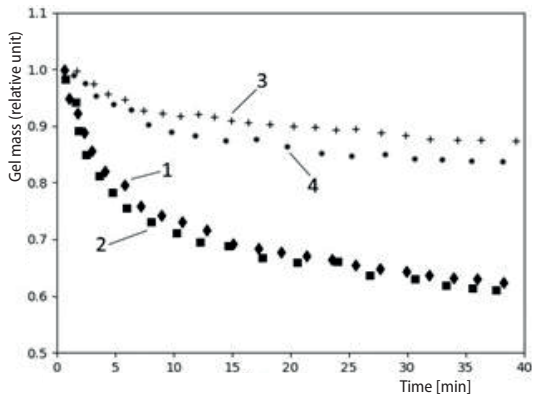


**Figure 6. Intensity of liquid evaporation of agarose gels weight concentration of 0.4% agarose and with added starch by perforating the surface: 1 – pure agarose gel, 2 – pure agarose gel with perforation, 3 – agarose gel with added starch by weight 0.3%, 4 – agarose gel with added starch by weight 0.3% with perforation**

In order to test this hypothesis the following experiment was performed: two samples of agarose and agarose-starch mixture were prepared with the same properties as were used in the previous experiment. After gel from a mixture was formed, its surface was perforated by needle in order to form an array of holes in an upper thin layer of gel. If upper layer inhibits water evaporation this procedure should release water from the lower layer and eliminate reduction in evaporation intensity that happens after first 10 minutes. Obtained data demonstrates that evaporation in perforated samples has approximately the same pattern of evaporation with intensity reduction (see fig. 6). Probably, a slight increase in the rate of weight loss of perforated gel samples compared to normal is associated with an increase in the total evaporation surface due

to perforation channels in the bottom layer of the gel. These results makes possible to conclude that presence of a second layer on the top of the sample does not affect evaporation intensity.

The second hypothesis is that the decrease in the evaporation intensity is caused by drying of a thin film of the dispersion phase, which is formed after the preparation of the gel as a result of syneresis. This liquid film cause more intensive evaporation in the beginning of the experiment and reduction happens after film is completely evaporated and further evaporation happens from the gel pores. There appears intra-diffusion resistance, which significantly inhibits the evaporation. To test this hypothesis, a newly prepared sample of agarose solution with the addition of starch was placed in the thermostat that maintained a constant temperature of 35 °C inside. This is the temperature of the beginning of gel formation from solution. This level of temperature allowed the sample to be kept in a liquid state during the entire period of the experiment. Such experimental conditions ensure the evaporation of water directly from the liquid phase throughout the experiment. In this case, the intra-diffusion resistance to water evaporation is concentrated in the liquid phase, not the gel.

Experiment demonstrates that in the thermostat the dynamics of evaporation of water from agarose solution with adding starch significantly differs from experiment in normal conditions. The results of the experiment are shown in fig. 7. Moreover intensity of the process under 35 °C overpasses evaporation intensity of pure gel sample in normal conditions. This occurs despite the presence of starch, which creates additional intra-diffusion resistance to water transfer in the solution. Of course, the experiment is of an extremely qualitative nature, since it does not take into account that at high temperature chemical hydrate relations of agarose with water are not formed, creating a structured dispersed phase. However, the data obtained showed that the hypothesis of the evaporation of water from the film of the dispersion phase on the surface of the gel may be a promising concept for describing the behavior of such a system by evaporation of liquid.

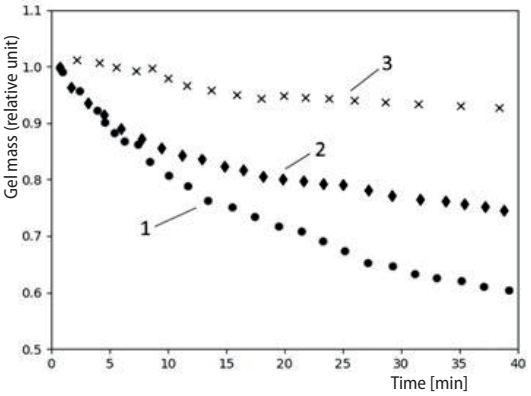


**Figure 7. Intensity of liquid evaporation of agarose gels (solution) weight concentration of 0.6% agarose and with added starch with weight concentration 0.2% under different temperature: 1 – combined gel, 35°C; 2 – pure agarose gel, normal conditions; 3 – combined gel, normal conditions**

Since the ultimate goal of the research is to find new gel compositions containing bioresorbable additives suitable for the immobilization of living cells in bioreactors created using additive technologies, the next step was to determine the effect of living cells on the process of evaporation of liquid from samples of agarose gel and agarose gel with the addition of starch. This means that it is necessary to understand not only how the gel affects the living cells, but how the cells affect the gel and change its properties. For this purpose a sample of yeast cells was added into gel. During the experiments, the dependencies of the relative loss of mass of samples on time during evaporation of water from pure agarose gel and agarose gel with the addition of starch were obtained. In the samples of both types, the previously described culture of living yeast cells was added in an amount of 15% of the weight concentration of agarose. The results of the experiment on evaporation of water from the described samples in comparison with the corresponding samples without yeast cells are shown in fig. 8.

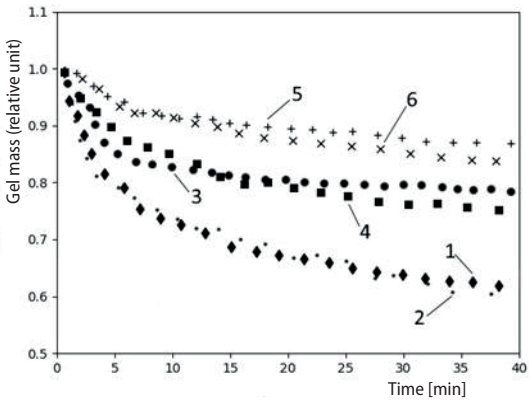


**Figure 8. Intensity of liquid evaporation of pure agarose gels weight concentration of 0.4% agarose and with added starch in the presence and absence of living yeast cells: 1 – pure agarose gel, 2 – pure agarose gel with yeast cells, 3 – agarose gel with added starch by weight 0.1%, 4 – agarose gel with added starch by weight 0.1% and yeast cells, 5 – agarose gel with added starch by weight 0.3%, 6 – agarose gel with added starch by weight 0.3% and yeast cells**

Experimental data demonstrate that the addition of yeast cells in the combined gels of different composition of agarose and starch practically does not affect the intensity of evaporation of water from the gel. The obtained result was expected, since the yeast cell sizes significantly exceed the typical size of the capillary channels of the structured dispersed phase of the gel, through which the liquid rises to the gel surface in the intra-diffusion evaporation mode, so cells do not affect the mass conductivity of the gels. As shown earlier in this paper, the chemical interaction of cells with the components of the gels used is also absent. This allows us to conclude that the influence of microbiological objects on the transport properties of the gel matrix can be ignored in the development of additive technologies for the creation of bioreactors.

An experiment was performed to determine the effect of liquid evaporation on the dynamics of mass loss of the gel sample for a long storage time in order to study the behavior of gels in the process of their practical application. The available method was used to test a sample of agarose gel with a weight concentration of 0.6%. Mass loss due to evaporation of the gel sample was determined 40 minutes after the start of the experiment and after 15 days of storage under normal conditions. It was found that after 40 minutes of stabilization with evaporation, gel lost 22% of its mass, and after 15 days of storage under normal conditions, the total weight loss was 36%. Much faster evaporation rate during the first 40 minutes of the experiment proves that the process of syneresis in the gel slow down over the time, and the structured dispersed phase tends to its equilibrium state.

It is important to emphasize that of the 64% of the remaining mass of the gel after its storage with evaporation under normal conditions for 15 days, the solid (agarose) has a mass of only 0.6%. The rest of the gel mass is chemically water. At the same time water in the form of liquid was not observed in the gel sample. The reason for that can be due to chemically bound state in the form of hydrate compounds. To release it, additional energy is needed, which significantly slows down the evaporation process.

## Thermal properties of the gel

The need for data on the thermophysical characteristics of gels arises when analyzing the technology of their use by the additive method. Previously, such data was obtained for agarose gel [24]. However, since the usage of combined gels (including those with bioresorbable additives) and gels that include microbioobjects is considered more promising for technological purposes, it is also necessary to understand their effect on the thermal properties of gels.

The technique of the experiment described in [24] was significantly changed, despite the fact that we used the same experimental equipment. The reason is the following: previously, the experiments were carried out by cooling the agarose solution to form a gel in the tempera-

ture range from 40 °C to 25 °C. Since the experimental data processing technique assumes that the calculated thermophysical values are averaged over the specified temperature range, it is not unambiguously determined whether the values relate to an aqueous solution of agarose or to an agarose gel. Moreover, the process of gel formation may be accompanied by thermal effects associated with the phase transition. Since the phase state of the system was uncertain, such effects were simply neglected.

If gel is heated, the aforementioned issues do not arise. Due to the phenomenon of hysteresis in the formation of the gel, in the temperature range from 25 °C to 40 °C phase transition from gel to solution is absent. It takes place at higher temperatures, which is known from the experience of using agarose gels in microbiology.

The main difference in the technique was that the studied gel sample was initially formed by mixing the initial components. Then the process of gel formation was carried out until the temperature stabilizes (25 °C) and the spectral pattern corresponds to the formed gel. Next, the gel was heated to the temperature of 45 °C and at specified intervals of time the temperature values were recorded along the radius of the sample and the heat-flow on the wall. The thermophysical properties of both pure agarose gel and agarose gel with addition of starch and yeast cells in different concentrations were investigated. Figure 9 shows some new experimental data on the temperature increase at the center of the samples, fig. 9(a), depending on the time and heat flux dynamics, fig. 9(b), received by the improved technique.

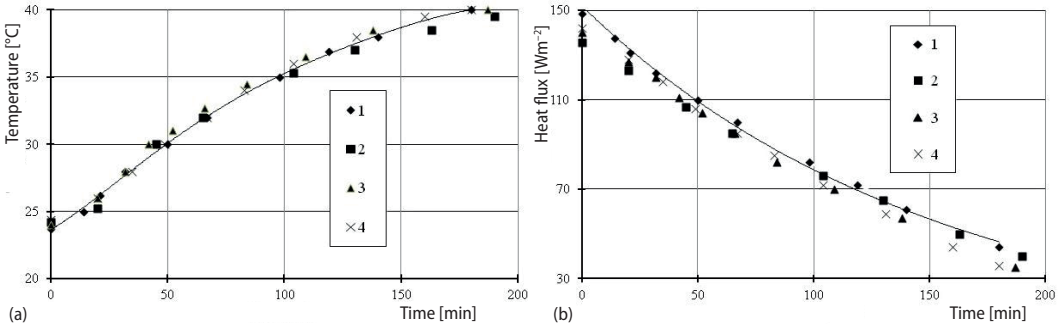


**Figure 9. Dependence of the temperature inside gel (a) and the heat flux for the outside wall of the experimental cuvette (b) on time during of gel heating from 25 °C to 40 °C; all gels have a concentration of agarose by weight 1.0%: 1 – pure agarose gel; 2-4 – agarose gel with addition of: 2 – starch with concentration by weight 0.25%, 3 – starch with concentration by weight 0.25% and 15 ml of a suspension of live yeast cells, 4 – starch with concentration by weight 1.0%; an (a) the temperature is measured in the center of the experimental cuvette filled with gel**

Based on data from experiments similar to presented in fig. 9 by solving the inverse heat conduction problem, the average thermophysical coefficients for pure agarose gel and gels with added starch and yeast cells were calculated. A mathematical model of non-stationary heat transfer of the 1-D infinite in length cylindrical sample with constant thermophysical properties (heat capacity and thermal conductivity) was considered for calculations. It is based on the unsteady heat equation in a cylindrical co-ordinate system, with initial and boundary conditions corresponding to the experimental conditions:

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

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

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, $\lambda$ – the coefficient of thermal conductivity, the $q(t)$ – heat flux density from the surface of the sample.

Knowing the values of temperatures and the heat flux it is possible to compute coefficients $C$ and $\lambda$ using the inverse problem of heat transfer. To solve this problem we need to minimize the difference in norm between the experimental temperatures and the computed ones, as well as the difference between the experimental values of heat flux and the computed values at all times and all thermocouple locations used in the processing of experimental data from fig. 9. 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 is solved by the method of co-ordinate-wise descent.

The basis of the obtained experimental data and with aid of the computational model, the effective values of thermal conductivity and volumetric heat capacity for gels with different initial concentrations of different initial components were obtained. Their values are presented in tab. 1. The results show that within the accuracy of the experiment, the average effective values of the thermal conductivity and volumetric heat capacity of agarose gel samples with the addition of starch and/or yeast cells in the range of values most suitable for the immobilization of microorganisms practically do not change. Thus, the presence of additives in agarose gel in the investigated range of concentrations does not affect the thermal regimes of the bioprinter that uses this gel as a bioink.

**Table 1. Average thermal properties of agarose gel with concentration of agarose by weight of 1.0% and with the addition of starch and yeast cells**

| Gel composition | Average thermal conductivity of the gel, $\lambda$, [$Wm^{-1}K^{-1}$] | Average heat capacity of the gel, $C$, [$kJm^{-3}K^{-1}$] |
|---|---|---|
| Pure agarose gel | 0.509 | 2087 |
| Agarose gel with addition of starch with concentration by weight 0.25% | 0.498 | 2071 |
| Agarose gel with addition of starch with concentration by weight 1.0% | 0.492 | 2067 |
| Agarose gel with addition of starch with concentration by weight 0.25% and 15 ml of a suspension of live yeast cells | 0.485 | 2064 |

## Conclusions

The addition of starch as bioresorbable additive and/or yeast cells do not affect conditions of formation and structure of the agarose gel. As a constituent of the gel, they show themselves as neutral additives. However, such additives inhibit relaxation processes during the stabilization of the gel and lead to a slower change in its initial structure.

It is established that the dispersion phase of the agarose gel is not water, but an aqueous solution of agarose with a concentration less than the critical concentration of gel formation.

According to measurements, this concentration increases with the concentration of agarose in the gel and the duration of its storage, probably aiming for critical value.

With regard to bioprinting technology, it is shown that during gel stabilization, it is necessary to take into account the multi-stage nature of this process, which consists of several stages, including evaporation of the liquid film from the surface and evaporation from the pores of the gel in the presence of intra-diffusion inhibition. For the first stage of evaporation of the liquid from the surface, which appears on it as a result of the gel syneresis, the dependence of the mass loss of the gel samples on time is linear. The presence of starch in the agarose gel and/or live yeast cells reduces the intensity of evaporation, as it prevents the mass transfer of water from the gel volume to the surface.

On the basis of experiments that record the dynamics of temperature fields in the gel volume and the simultaneous change in the heat-flow during its heating, the method for determining the thermophysical coefficients was improved. The values of thermal conductivity and volumetric heat capacity for both pure agarose gel and agarose gel with addition of starch and/or yeast cells are found. These values were close to each other. The reason is that the composite gels and pure agarose gel have similar chemical composition and the same structure of the dispersed phase. Thus, during the evaluation calculations of bioprinting processes without loss of accuracy, the thermal characteristics of composite gels can be replaced by the corresponding values for pure agarose gel.

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