THERMOKINETICS AND RHEOLOGY OF AGAROSE GEL APPLIED TO BIOPRINTING TECHNOLOGY

by

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The paper presents new results on the study of thermokinetics of gel system based on agarose in the process of transition from solution to gel and opposite. This issue is extremely relevant, since the stability and predictability of thermophysical and rheological properties in such transformations, especially in the presence of components of the nutrient medium and immobilized microorganisms, come to the fore in terms of design and selection of modes of operation of the printing device promising 3-D bioprinters, as well as the system of preparation and storage of the presence of the hysteresis effect, both from the point of view of the kinetics of gel formation and from the point of view of the dependence of rheological properties on temperature, at different concentrations of modifying components, is shown. The obtained results allow to draw a conclusion about the possibility of using the scheme with preliminary preparation of the initial biogel for the implementation of bioprinting technology based on agarose, and to recommend the obtained values for modeling the operating modes of devices of this type.

Key words: *bioprinting, agarose and bioresorbable gels, additive technology, spectroscopy analysis, gel hysteresis properties*

Introduction

The 3-D printing technologies are now becoming a key driver to innovation in many industries, such as engineering, industrial production, as well as educational technologies and medicine. The latest achievements of science in this field allow to realize 3-D printing on the basis of biocompatible materials, cellular structures and auxiliary substances (for example, solid frameworks) with the subsequent creation on their basis of full-functional living objects [1]. Undoubtedly, a very significant place 3-D bioprinting will take in regenerative medicine for transplantation of various tissues and organs. At the same time, in relation to the technologies of 3-D printing from inorganic materials, for bioprinting there are factors complicating the technology, such as non-trivial selection of materials, types of immobilized micrographs and cell structures, factors of their growth, as well as significant technical difficulties associated with the sensitivity of living cultures to a large number of factors both in the printing process and during further cultivation [2]. In order to solve these problems, a close integration of technologies from various fields, such as classical engineering, science of biocompatible materials, cell

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dynamics, as well as physics and regenerative medicine, is necessary. At present moment 3-D bioprinting methods are beginning to find application in the cultivation and transplantation of some tissues, including epithelial and bone tissues, elements of the vascular system, heart tissues and cartilage structures [3]. Another factor in the implementation of 3-D bioprinting technology is the need to model different tissues with realistic pharmacodynamic properties for scientific purposes, as well as to create new drugs and test them [4].

As it is known [5], one of the most promising directions in the creation of materials for 3-D bioprinting is the use of various gel systems. Recently, various groups as the main material for bioprinting are considered as gels based on a wide range of gelling materials such as: alginate gels, gels based on gelatin, hyaluronic acid, agarose, *etc*. The main advantage of these gel systems in relation to bioprinting is biocompatibility with a wide range of microbiological and cell cultures, as well as the ability to acquire and retain shape without injuring immobilized biological objects. Another important property of gel materials is the possibility of their multiple transition from liquid to gel-like state and opposite. This feature allows to transform quite far in addressing the issue of long-term storage of ink before use, as well as the issue of management and adjustment of their properties, in the process of creating a printing object.

One of the main problems that significantly inhibit the development of bioprinting technology based on hydrogels is the relatively low stability of their properties and their significant dependence on storage conditions. In this case, a special place is occupied by the so-called *aging effect*, the effect of which on the structure and some properties of the gel are described in [6]. These features of gel formation, as well as a large number of factors affecting the properties of the gel system have determined two directions in the use of gels as one of the main materials for bioprinting. The basis for the implementation of the first embodiment is the preparation of bio gel preparation immobilizing objects, *etc*. directly to the printer with the appropriate technological challenges, including the need to design complex module for preparing a multicomponent gel with the control system given concentrations of initial components. The peculiarity of the second option is the use of ready-made initial gels stored in special conditions with the possibility of their retransformation into a solution in the printing device. Both technologies have their advantages and disadvantages, but the second option, according to the authors, is the most preferable due to the simplification of the design of the printing device and the ability to use a standard certified, produced under strict quality control gel material. The disadvantages of such a scheme is that not all promising gels for bioprinting have the property of reverse transition, as well as insufficient study of the features of such a transition for a wide range of gels, especially in the presence of modifying additives.

The proposed work is devoted to the study of the behavior of gel systems based on agarose with some modifying components under conditions of both gel formation and its reverse transition into solution. The paper presents the results of the transition study for both pure 0.6% and 1% agarose gel and gel with various additives, in order to determine the main parameters and patterns, as well as to develop the technology of contactless control and diagnostics of the gel system in the technological processes of bioprinting.

The results of the study of the rheological properties of agarose gel are also presented. Despite the fact that there is a large number of works devoted to the study of agarose solutions and the processes of gelation in them, the rheological properties of agarose solutions were not studied thoroughly [7]. At the same time, most of the works are devoted to the study of the ability of solutions to gelation and the properties of the resulting films. And, nevertheless, the important question is the nature of not only the high viscosity of agarose solutions, but also the dependence of rheological properties on concentration and temperature [8].

Kinetics of the solution-gel-solution transition

In order to study the hysteresis effect of agarose gel, the experimental stand used earlier to determine the thermophysical properties [9] was modernized and based on the optical spectroscopy method, with the implementation of measuring the maximum intensity of the light passing through the sample was used. The scheme of the experimental set-up is shown in fig.1.

Samples of agarose gel without additives with a mass concentration of 0.6% and 1% were used to test the technique. Liquid gel volume of 3.5 mL at a temperature of 50 °C was poured into a quartz cell with 10 mm optical path of the beam. Further, the sample was placed in a preheated to 45 °C thermal volume and kept there until the temperature equilibrium. The temperature of the gel was controlled by a thermocouple placed in the center of the sample cell. Registration of the spectra of light passing through the gel sample was carried out in the process of cooling the agarose gel to complete gelation from 45 °C to 25 °C with a step of 3 °C. Further, the sample was heated at full melting from 25 °C to 90 °C. After complete melting of the gel, the sample was cooled again to a temperature of 25 °C. Throughout the experiment, the spectrum of light passing through the gel sample was recorded at control points (every 3 °C).

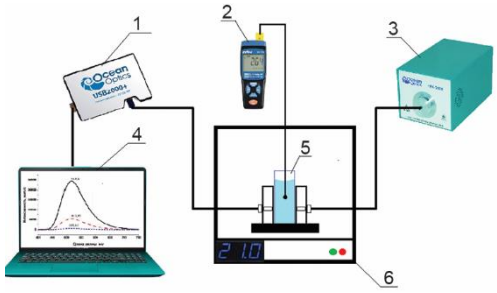

Figure 1. Scheme of the experimental set-up for the study of gel-solution-gel transition processes; *1 – spectrometer, 2 – electronic thermometer, 3 – stabilized light source, 4 – computer, 5 – quartz cell with the test sample, 6 – air thermostat*

On the basis of the obtained data, the graphs of the dependence of the wavelength at the maximum intensity on the temperature in the process of *cooling-heating-cooling* of the agarose gel were constructed, fig. 2.

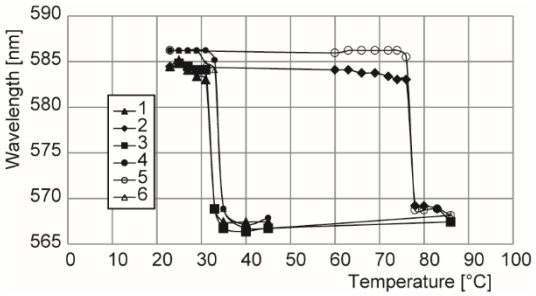

Figure 2. Variation of the maximum wavelength of the transmitted light spectrum as a function of temperature for pure gels 0.6% and 1.0% concentration; *1 – gel 0.6% (cooling), 2 – gel 0.6% (heating), 3 – gel 0.6% (re-cooling), 4 – gel 1% (cooling), 5 – gel 1% (heating), 6 – gel 1% (re-cooling)*

It follows from the graphs that for both concentrations of the studied system the transition from the liquid state to the gel state and in the opposite direction were realized. Moreover, the dependence on the concentration of the initial component, both in wavelength and temperature, were clearly identified. It was shown that with an increase in the concentration of agarose in the initial solution, the curves shift towards higher values both in wavelength and in the temperature of gelation.

Influence of modifying components

In order to study the effect of the presence of various additives that can be included in the original gel, their effect on the hysteresis effect was investigated. Modified starch was used as an additive simulating a bioresorbable component, for example, to stabilize the gel, create a

frame structure, as well as to fill the volume and then release it for the growth and development of immobilized cell cultures.

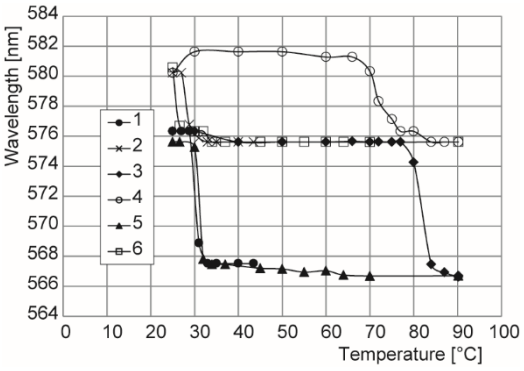

Figure 3. Variation of the maximum wavelength of the transmitted light spectrum as a function of temperature for agarose gel 0.4% concentration with the addition of modified starch; *1 – gel 0.4% + starch 0.4% (cooling), 2 – gel 0.4% + starch 0.2% (cooling), 3 – gel 0.4% + starch 0.4% (heating), 4 – gel 0.4% + starch 0.2% (heat), 5 – gel 0.4% + starch 0.4% (re-cooled), 6 – gel 0.4% + starch 0.2% (re-cooling)*

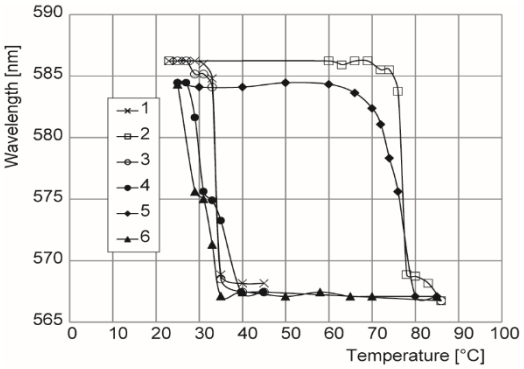

Figure 4. Variation of the maximum wavelength of the transmitted light spectrum as a function of temperature for agarose gel 1% concentration with addition of ethyl alcohol; *1 – gel 1% + alcohol 1% (cooling), 2 – gel 1% + alcohol 1% (heating), 3 – gel 1% + alcohol 1% (re-cooling), 4 – gel 1% + alcohol 10% (cooling), 5 – gel 1% + alcohol 10% (heating), 6 – gel 1% + alcohol 10% (re-cooling)*

It is also known that when 3-D printing using somatic cells of animal or plant origin, the question of preserving aseptic conditions arises sharply. With contamination of nutrient media and gels by foreign microflora, somatic cells do not withstand competition for the main sources of nutrition, having significantly lower growth rates than wild species of foreign microflora. In this case, for somatic cells, the growth rate does not exceed 0.02 g/L per hour, while bacteria can multiply at a rate of 0.2-0.3 g/L per hour. [10]. The use of thermal sterilization of nutrient media is possible only at the stage of their preparation, before use in a 3-D printer. To ensure asepsis directly during printing, it remains to use only chemical sterilization.

Among the various chemical methods for maintaining aseptic conditions, it is necessary to choose a sterilizing agent that provides either decay or suppression of the growth of foreign microflora, which will not damage the somatic cells.

As a model agent for maintaining aseptic conditions in 3-D printing, ethyl alcohol was considered as the most common and simple agent that provides both partial death and suppression of the growth of foreign microflora.

The experiments used modified starch with concentrations of 0.2% and 0.4%, as well as ethyl alcohol with concentrations of 1% and 10%. The results are presented in figs. 3 and 4.

It can be seen from the graphs that, despite quite significant concentrations of modifying components, the ability of the agarose gel to reverse transition remains, as well as a slight influence of the considered components on the temperature range. This result is important from the point of view of selection of modes of operation of perspective printing devices.

Rheological properties

The rheological properties of biogels, especially the influence of additives and modifying components on them, are of no less interest from the point of view of bioprinting technology [11].

There are studies on the rheology of agarose gels with various modifying additives, including bioresorbable. In work [12] samples of agarose gels with additives of glycerol, sorbitol, citric acid, sodium citrate, and sodium chloride in various concentrations were investigated. It was found that citric acid reduces the viscosity and sodium citrate increases the viscosity of gels. In the case of mutual influence of these additives at low concentrations of sodium citrate, an increase in the concentration of citric acid leads to a decrease in viscosity, and at high concentrations to an increase. The influence of temperature shift rate and gel concentration was studied in [13]. The results obtained by the authors indicate a significant dependence of the structure of gels on these parameters, which is due to the redistribution of hydrogen bonds. In the cited papers due to the non-Newtonian properties of the gel, measurement of viscosity and shear stresses was performed with the method of rotational viscometry.

Experimental study of rheological properties of agarose solutions in the concentration range from solutions to gels formed in this work were based on measurements of the coefficient of viscosity by Stokes method (falling ball method), which provides an absolute measurement and calibration liquid with a known viscosity value. The Geppler device was used, which provided greater accuracy in determining the viscosity in the field of gelation. The experimental scheme is shown in fig. 5.

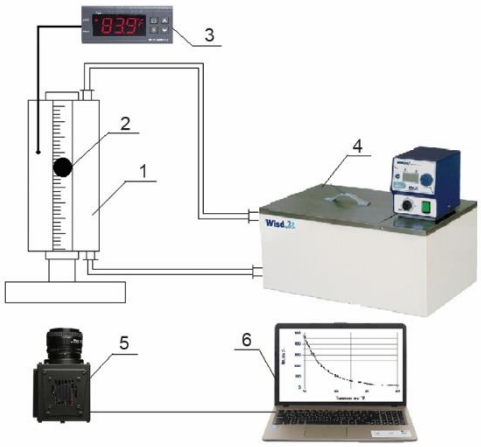

Figure 5. Experimental aparature; *1 – Geppler viscometer, 2 – steel ball, 3 – electronic thermometer, 4 – water thermostat, 5 – high-speed camera, 6 – computer*

Solutions of different concentrations in the range from 0.4% to 0.8% were prepared according to the standard method. The required amount of dry powder was poured with distilled water at room temperature and left to swell. In the future, the samples were heated in a water bath with continuous stirring, and then brought to a boil. Cooling of the obtained solutions was carried out under natural conditions for several hours.

Figure 6 shows the dependence of the kinematic viscosity of agarose gels of different concentrations on temperature.

In fig. 6, in addition to the temperature dependence of the viscosity of gels, such a dependence for water is presented.

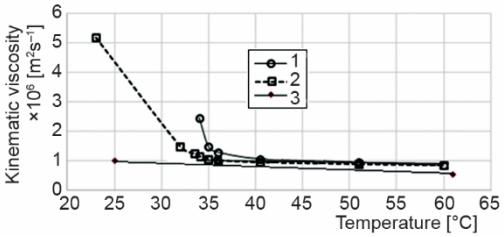

Figure 6. Dependence of viscosity on temperature; *1 – gel 0.8%, 2 – gel 0.4%, 3 – water*

Earlier [14] the authors investigated the effect of bioresorbable starch additive on the properties of agarose gels. For all samples of gels in the temperature range from 40 °C to 30 °C there is a transition region from the solution to the actual gel with non-Newtonian properties, which provides results with accordance to prevous obtaines results related to non-Newtonian physics [15], particularly applied for gels [16]. The vertical arrow shows the temperature at which the agarose gel concentration of 0.8% at a given size and material of the ball passes

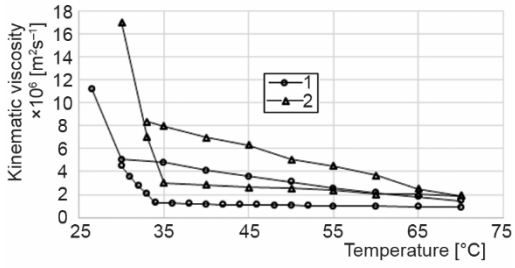

Figure 7. Dependence of agarose gel viscosity on temperature in the presence of a modifying component during the *solution-gel-solution* transition; *1 – gel 0.4% + starch 0.2%, 2 – gel 0.4% + starch 0.4%*

from a state characteristic of *soft* matter to a conditionally *rigid* state with an elastic frame, which was fixed by the hovering of the ball.

Also, an experiment was conducted to study the viscosity with a decrease in the temperature of the sample with the addition of starch. The results of which are shown in fig. 7.

Conclusions

Thermokinetics and rheology of agarose-based gel systems were studied by spectroscopy and Stokes methods. The study of the transition process *cooling-heating-cooling* showed the presence of hysteresis of the wavelength dependence at the maximum transmission intensity on the temperature and the influence on it (hysteresis) of both the concentration of agarose and the presence of bioresorbable and aseptic components.

The obtained results allow to draw a conclusion about the possibility of using the scheme with preliminary preparation of the initial biogel for the implementation of agarose-based bioprinting technology, including taking into account the previously studied effect of aging, and also to recommend the obtained values for modeling the operating modes of devices of this type.

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