Research of gel systems stability in relation to additive technologies

B G Pokusaev¹, A V Vyazmin², S P Karlov¹, N S Zakharov¹, O A Sulyagina¹

¹ Moscow Polytechnical University, Bolshaya Semenovskaya st. 38, Moscow, 107023, Russia
² MIREA – Russian Technological University, Vernadsky Avenue 78, Moscow, 119454, Russia

E-mail: Schev_olga@mail.ru, NicolaZaharov@yandex.ru

Abstract. The study of thermokinetics and rheology of gel systems based on agarose gel was performed. The dependencies of the spectral characteristics of both pure gels and gels with special additives in the conditions of the "cooling-heating-cooling" transition process are obtained. The effect of hysteresis of the studied samples during the transition from a liquid solution to a gel and back was found. The presented results can be useful for the development of a promising 3D-bioprinting technology, for the design and selection of operating modes of printing devices.

1. Introduction

The technology of additive (layered) three-dimensional printing is currently widely used in various fields of activities, such as construction engineering, industry and small-scale production, educational technologies, as well as medicine, bioengineering, etc. to create elements of complex geometric shapes, plastics, powders and other composite materials are used, in contrast to the developing direction like bioprinting. The possibility of growing biological tissue structures and organs from stem cells using additive 3D technologies has laid the foundation for numerous studies aimed at developing 3D bioprinting technology and creating new biomaterials [1, 2]. Compared to 3D printing from inorganic materials, there are complicating factors in bioprinting associated with the choice of a material with the required properties, in which it would be possible to grow living tissue structures and biological objects. Gels are promising materials for bioprinting. [3, 4]

Gel systems have special physicochemical and rheological properties capable of forming objects (scaffolds) of complex configuration by layer-by-layer deposition of gels layers of various concentrations and compositions [5]. The listed parameters of gel materials make it possible to manufacture and maintain the complex shape of the developed biological objects without disrupting their vital processes. However, one of the problems in the implementation of 3D bioprinting technology is to ensure the stability of the properties of the frame structure of biological objects both during printing and during storage. The technological process of printing involves heating gels, in which they can repeatedly pass from a liquid state to a gel-like state and vice versa. For a wide practical application of bioprinting, gel materials with desired stable properties are required, which can
be obtained using both mixed gels and gels with the addition of modifying components. The presence of such components can significantly change the conditions for gel formation, the peculiarities of its temporary stabilization, and other technological properties. The implementation of the technology of layer-by-layer 3D-bioprinting using multicomponent gel systems involves the formulation and solution of new fundamental problems aimed at studying the properties of such materials and the processes occurring in them.

2. Study of the kinetics of agarose gel during the transition of solution-gel-solution

Experimental study and diagnostics of gel systems under the conditions of their formation from solution to gel and reverse transition from gel to solution were carried out using optical and heat-measuring techniques. Probing optically transparent gels with fiber-optic elements by transmitting light through the sample under study made it possible to measure spectral characteristics during the stabilization of gel systems. The features of the experimental setup and the measurement technique were previously described in detail in [6].

In the experiment, we used samples of both pure agarose gel with a mass concentration of 0.6% and 1.0%, and a gel with the addition of a modifying additive. To work out the experimental technique, experiments were first performed with pure gels. At the beginning of the operation, a 3.5 ml liquid gel at a temperature of 50 °C was poured into an optical quartz cuvette with an optical beam path of 10 mm. Then the sample was placed in a heating cabinet preheated to 45 °C and kept there until the temperatures were completely equalized. The gel temperature was monitored with a thermocouple installed in the center of the cuvette with the sample. The registration of the spectra of light passing through the sample of the gel was carried out during the cooling of the agarose gel until complete solidification from 45 to 25 °C with an interval of 3 degrees. Then the sample was heated to complete melting in the temperature range from 25 to 90 °C. After complete melting of the gel, the sample was cooled again to a temperature of 25 °C. Throughout the experiment, at the control points (every 3 degrees), the spectrum of light passing through the gel sample was recorded.

As a result, the dependences of the wavelength at the maximum intensity of the transmitted light on the temperature were obtained, obtained in the study of the process of "cooling-heating-cooling" of an agarose gel.

Figure 1 shows the experimental results with pure agarose gels. The graphs show that for both concentrations of the system under study, a transition from a liquid to a gel state and vice versa occurs. In this case, the dependence on the concentration of the initial component is clearly identified, both in terms of wavelength and temperature. It can be seen that with an increase in the concentration of agarose in the initial solution, the curves shift towards higher values both in wavelength and in gelation temperature.

![Figure 1](image-url)
Figure 2 shows the results of similar studies with samples from agarose gel with the addition of a modifying additive. In this work, modified starch with a mass concentration of 0.2% and 0.4% was used as a special additive to create the framework structure of the gel. The use of such an additive is explained by the fact that starch in relation to agarose is a neutral biodegradable mixture that can be used as a nutrient for the growth and development of cell cultures [7].

Figure 2. 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-cooling); 6 - Gel 0.4% + starch 0.2% (re-cooling).

As can be seen from the graphs, despite the rather significant concentrations of modifying components, the agarose gel retains the ability to reverse transition, as well as the insignificant effect of the considered components on the temperature range. This result is important in relation to selection of the operating modes for promising printing devices.

3. Study of agarose gels rheological properties
An experimental study of the rheological properties of agarose solutions in the concentration range from solutions to formed gels in this work was carried out on the basis of measuring the viscosity by the Stokes method (falling ball method), which provides both absolute measurement and calibration against a liquid with a known viscosity value. In the work, we used the Heppler device, which provided a high accuracy in determining the viscosity in the gelation region. The experimental setup is shown in figure 3.

Figure 3. Experimental setup:
1 - Heppler Viscometer;
2 - steel ball;
3 - electronic thermometer;
4 - water thermostat;
5 - high-speed camera;
6 - computer.
Agarose gel solutions of mass concentration from 0.4% to 0.8% were prepared according to the standard procedure. The required amount of dry powder was poured with distilled water at a temperature of 25 °C and left to swell. Subsequently, the obtained samples were heated in a water bath with continuous stirring and then brought to a boil. Cooling of the resulting solutions was carried out under natural conditions for several hours.

Figure 4 shows the dependences of the kinematic viscosity of pure agarose gels of various concentrations on temperature. The figure, in addition to the temperature dependence of the viscosity of the gels, shows the same dependence for water. For all gel samples in the temperature range from 40 to 30 °C, there is a transition region from solution to gel with non-Newtonian properties.

After testing the viscosity measurement on pure gels, an experiment was carried out to study the viscosity with decreasing temperature for samples with an addition of starch. The results are shown in figure 5. Plot demonstrates that despite the addition of modifying components, the agarose gel retains the ability to reverse transition, and the addition of starch has a slight effect on the temperature range.
4. Summary
The obtained data on the kinetic properties of gel systems in the "cooling-heating-cooling" transient process, studied by the spectroscopic method and the Stokes method, demonstrated the presence of hysteresis. Cyclicity is observed in gel samples both from pure agarose and in samples with the addition of a modifying component. A change in the concentration of agarose and the addition of a modifying component do not affect the presence of hysteresis. In this case, the nature of the dependence of the wavelength at the maximum intensity of light transmission on temperature remains unchanged (characteristic transitions are clearly traced during gelation and melting of the gel upon heating). Thus, the stability of properties is ensured under conditions of transition from a liquid state to a gel state and vice versa. It was found that with an increase in the mass concentration of agarose in the composition of the sample under study, the kinetic curves of gelation mixture move in the direction of increasing temperatures. The kinetic curves of gel systems are identified by temperature. Consequently, due to the usage of agarose-based gels in bioprinting, it becomes possible to control the process of creating a printed object during the implementation of additive 3D bioprinting.

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