CHEMICAL ENGINEERING TRANSACTIONS
VOL. 57, 2017
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Sauro Pierucci, Jiří Jaromír Klemeš, Laura Piazza, Serafim Bakalis

ISBN 978-88-95608- 48-8; **ISSN** 2283-9216
DOI: 10.3303/CET1757222

Agar Gels: Kinetics of Formation and Structure

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The idea that gels can be used as a carrier during the formation of ordered bio-structured elements for additive medical technology is promising. For this experimental study we use gels based on agarose. Such gels are appropriate for additive technologies used in 3D printing for the creation of matrix of bioreactors. The kinetics of formation and structure of different density gels are investigated using an optical method. The decomposition of the radiation spectrum allows us to get detailed information about the nature of the gel.

1. 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 particular bioreactors capable of maintaining the required temperature, pH level, osmotic pressure, supplying cells with nutrients and oxygen, removing their metabolic products and fulfilling many other requirements, providing necessary physiological conditions for immobilized cells (Rodrigues et al., 2011). The method of 3D additive manufacturing to create such tissues and organs from stem cells (Placzek et al., 2009; Wang et al., 2015) seems to be very promising.

Currently gels are considered as a high-potential structure-forming material for a formation of artificial tissues from immobilized cells using methods of additive manufacturing. The usual definition of a gel refers to a dispersive system with liquid dispersing medium, where the dispersion forms a spatial structured mesh due to intermolecular interaction in the contact sites. Gels are capable of displaying both elasticity and plasticity, and while exposed to a high shear stress, they exhibit fluidity. One of the important characteristic of gels is a property of thixotropy that is an ability of restoring the gel original structure after mechanical destruction under isothermal conditions. All these properties, including the loss of fluidity, are attained at low contents of dispersed phase in a gel from fractions of a percent to several percents only. Gels are subject to aging, i.e. their physical and chemical properties significantly change due to recondensation and recrystallization, including the process of releasing of liquid phase (Scherer, 1999).

All these features create interesting applications of gels in industries and technologies. Gels are widely used in the production of polymers, catalysts, sorbents, membrane filters, service wellbore fluids, cosmetics and pharmacy compositions (Kajiwara and Osada, 2000). Agarose and other gels are widely used in microbiology to grow microorganisms (Hitchens and Leikind, 1939; Tuson et al., 2012). This fact proves the possibility of finding good ambient conditions of temperature and acidity for growth and reproduction of biological microscopic objects. The gel capillary network can be used for transport of nutrients to separate cells and for removal of cell metabolism. The properties of gels allow to form artificial tissues of complex configurations from immobilized cells, based on the layer-by-layer mapping of gels of various concentrations and compositions.

The properties of gels are known to depend on the composition of the gel-forming medium and on the preparation method. There are many publications related to methods of gel synthesis from various chemicals, see for instance (Weiss and Terech, 2006; Shabanova and Sarkisov, 2012). However, many gels share common properties in terms of application technology. For instance, the most significant peculiarities manifesting in the processes of mass transfer in gels are their instability and anisotropy due to the structure and behavior of a transfer medium (Amsden, 1998). Here, the critical aspect is the size of the transferred particles with respect to the scale of inner microchannels (Yankov, 2004).

Please cite this article as: Pokusaev B., Vyazmin A., Karlov S., Zakharov N., Reznik V., Nekrasov D., 2017, Agar gels: kinetics of formation and structure, Chemical Engineering Transactions, 57, 1327-1332 DOI: 10.3303/CET1757222

Methods for investigation of the properties of disperse systems, including gels, are diverse. For gels optical tools help visualizing the transfer process in real time, and are useful for the understanding of features of the process. They are useful for obtaining direct data on the macrokinetic formation and on the transfer coefficients of gels. In some cases these coefficients can be related to parameters describing the intrinsic structure of the transport medium. There are a lot of optical methods used in the investigation of gels including simple visualization (Helseth, 2011), spectral sounding (Pokusaev et al., 2013; Pokusaev et al., 2015), light dispersion (Kuz'min et al., 2014), fluorescence (Michelman-Ribeiro et al., 2007). The major advantage of optical methods is the lack of contact with the investigation media, i.e. they do not interfere with the natural development of the process. Modern optical sounding equipments have high optical time/space resolution, the equipment allows for quick processing and analyzing of large arrays of experimental information.
Based on the assumption that physical-chemical mechanisms are similar among many processes of mass transfer in gels, we have previously investigated thoroughly silica gels using optical methods. The silica gels are easy to prepare, are non-toxic, biologically neutral, stable enough and optically transparent. Gels with different densities and lifetimes have been studied. The investigation suggested critical differences for diffusion process in gels compared with true solutions and with solutions with nanoparticles (Pokusaev et al., 2016a). It was shown that layers of gels are spatially inhomogeneous and anisotropic. It was later found that the gel-forming layers have low adhesion to each other, due to the volume between different layers of gels filled with liquid. The presence of liquid in the interlayer volume has been visually observed and confirmed by light scattering of a laser beam (Pokusaev et al., 2016b).
Agarose gel is a typical gel formed from a solution as the temperature is lowered. Such gels were widely investigated (Rees, 1981), including using optical methods (Rees et al., 1970). The following aspects were studied: structure of links which are formed in the disperse phase of gel formation, and physical-chemical properties of gels. Nevertheless, many properties of agarose gels determining whether it is possible to use this gel to create bioreactors require additional study. The aim of this work is to investigate the kinetic mechanism of agarose gel formation depending on the concentration of the disperse phase and, using methods of additive manufacturing, to study the "thin" structure and adhesion between gel layers applied each other.

2. Methods and materials

For the investigation of the kinetics of gel formation a spectroscopic method is used. This method analyses the shape of the spectrum of transparent and/or reflected light of a sample. The experimental set-up diagram is shown in Figure 1. The illumination system (1) is a light source HXP-2000 35 W xenon lamp. Xenon lamps emit a continuous spectrum of stable power from ultraviolet to near infrared light waves in the range of 185-2000 nm. For illumination of the sample a collimator is used (2), which provides a narrow probe beam with a cross section about 0.1 mm^2 on the front surface of the cell. For reception of the light signal, an optical fiber of 0.1 mm and 0.4 mm diameters (5) are used. These optical fibers work in the range of light wavelengths from 220 to 1000 nm. The optical system is focused for scanning the gel samples (3). This cuvette (3) is made of quartz glass; it is transparent to the full operating range of light. The experimental cuvette is vertically moved relative to the focused optical system with an accuracy of 0.1 mm, allowing one to determine the intensity of the transmitted light depending on the distance from the boundary between gel layers (4). The registration system (7) is able to output and to analyze the spectra during the scanning of the gel. The spectrophotometer (6) USB2000+ (Ocean Optics) including Sony ILX511 2048-element linear silicon CCD array detector is the basis of the experimental setup. This device provides the registration in 2048 channels in the spectral range of 200–1100 nm. The optical resolution is in average 1.0 nm. The diffused light at 435 nm is less than 0.1 %.
A single mode gas helium–neon laser with a wavelength of 632.8 nm and output of 5 mW is used. The beam cross-section has a Gaussian intensity distribution; the size at half of maximum intensity on the front surface of the sample holder is approximately 1 mm. A silicon photodiode-based photodetector of FD7K type includes a pre-amplifier; the electrical signal from the amplifier, proportional to the laser light on the sensitive surface, is transmitted to a processing unit connected to a PC.
An agarose-based gel "Chemapol" is used as a main gel during the experimental campaign. The gels used in the experiments are obtained by mixing agarose with distilled water and heating up to 90 °C via convectional and UHF methods. Agarose gels with 0.6 – 1.5 % of agarose are used in the experiments.
Consider an example of the change of light spectrum passing through an agarose gel specimen with a concentration of 0.8 % during gel formation with decreasing of temperature, as shown in Figure 2. The spectrum of agarose gel with the aforementioned concentration in distilled water is taken as the base. To simplify of the analysis of the process dynamics it is more convenient to use spectra in relative values, i.e. for each temperature and each wavelength the intensity of light passing through the specimen is attributed to the maximum value in the spectrum, taken as the base.

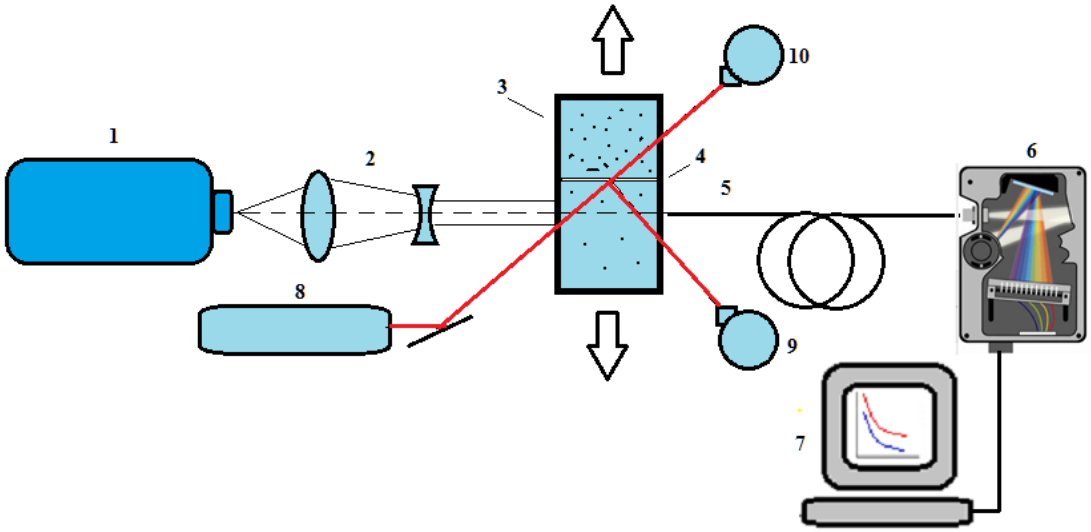

Figure 1: 1 – xenon lamp light source; 2 – collimator for probe beam generation; 3 – cuvette with gel sample transferred by micrometer mechanism; 4 –interface of double-layer gel; 5 – fiber cable; 6 – spectrophotometer USB2000+; 7 – computer; 8 – helium–neon laser; 9 – photodetector for recording of laser emission transmitted through the capacity and reflected from gel interface; 10 – photodetector for recording of laser emission transmitted through capacities and gel interface

3. Results and discussion

Considering the concentrations of gels of interest, the temperature adjustment of a gel according to 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 wavelengths with the gel temperature, i.e. the optical density of the medium increases, which reflects the process of formation of a new microdisperse phase with a higher density than liquid. This process slows down when the temperature gets lower, indicating the end of the gel formation process. Visual observations demonstrate that the initial agarose solutions of various concentrations begin to exhibit the properties of a structured disperse phase within the temperature range of 35 to 25 °C. At this stage, the wavelength of the maximum of the transmission spectrum shifts towards the red area in the course of gel formation. It can be explained by a change of the structure of the media.

Figure 3 shows the experimental data for the change of wavelength, which coincides with the maximum of the light transmission spectrum, depending on the temperature of gel formation and for the concentrations of agarose of 0.8 and 1.5 %. The process of gel formation occurs as the temperature of the medium decreases. In this case, the maximum wavelength of the spectrum of the light passing through the gel increases in the temperature range in which gel formation occurs. Furthermore, it turns to be a constant at temperatures when the gel has already formed. From an optical point of view, this is due to an increase in dispersion and light absorption of a selected wavelength when non-uniformities in the process of gel formation occur. According to visual observations, the liquid state of a gel-forming medium is found at temperatures above 45^{o}C, while at temperatures below 20 ^{o}C it forms a gel.

The dependencies shown in Figure 3 are not equidistant at various concentrations of agarose. This means that the structure of the solution depends weakly on the concentration of agarose when the medium is liquid (at high temperatures). If the medium turns to the gel state (at low temperatures), its structure is strongly dependent on the concentration of agarose. Moreover, along with the increase of this concentration, the changes in the structure of microdisperse medium are clearer. It should be noted that these properties are not unique for the agarose gels. A similar phenomenon was found earlier investigating the silica gels (Pokusaev et al., 2016a).

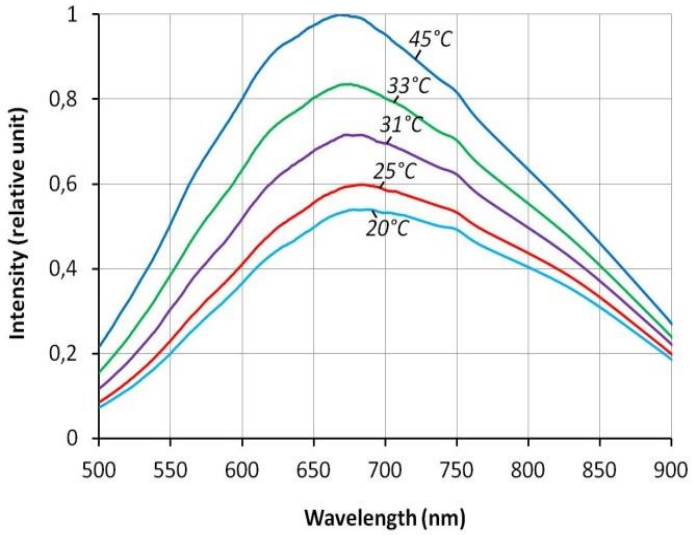

Figure 2: Changes in the relative intensity of light transmission with temperature in the agarose gel with a 0.8% concentration by mass

The kinetics of the formation of the agarose gel is fundamentally different from the kinetics of the silicate gel formation. The silicate gels form due to a chemical reaction. Hence a kinetic law giving the dependence of the the solution conversion rate on time, can be defined from experimental data. For the agarose gel, its conversion rate into a gel is regulated by its temperature, which can vary in time. For the agarose gel, the kinetic law of gel formation depends on temperature

Two-layer gel systems are formed by application layer-by-layer by means of a syringe of gel-forming solutions prepared in advance. Wherein, the top layer is applied after the completion of the gel formation in the lower layer. This is done to avoid the occurrence of a spontaneous convection. Using additive technologies to create bioreactors implies the layer-by-layer application of gel of various concentrations and compositions. It is hence of concern to investigate the structure of such layers in near-border areas adjacent to their interface. Gels are very promising for additive technologies, since they easily form matrixes of different shapes and densities. It is possible to apply a denser layer of agaroze gel on the lesser dense one without any mixing corresponding to liquid media (for example, see Figure 4). Such bi-layer systems are hydrodynamic stable. The same figure displays the potential of layer-by-layer application of organic agarose gel on nonorganic silica gel. For the layered gel structures presented in Figure 4 the border between the layers is clearly visible. The structure of the experimental facility enabling to fix the spectrum with a sampling resolution of 0.2 mm allows us to investigate the intensity of light transmitted depending on the distance from the gel interface.

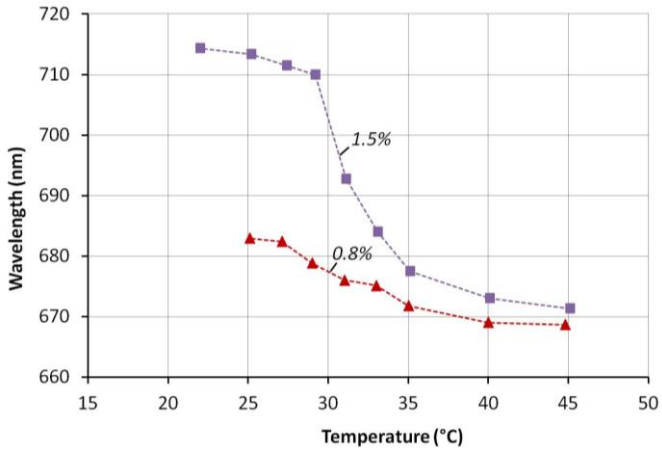

Figure 3: The change in wave-length corresponding to the maximum of the spectrum of the transmitted light, depending on the temperature in the process of the formation of agarose gels

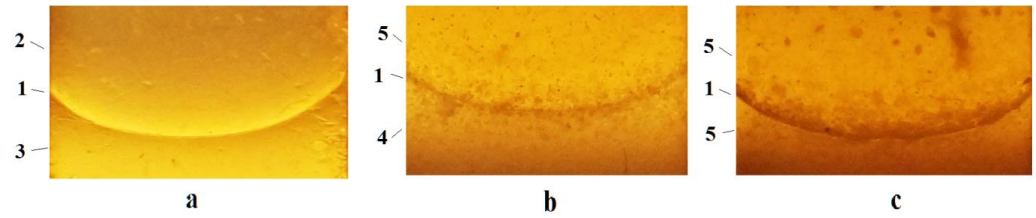

Figure 4: Double-layer gels of different composition with the interface plainly observable by eye (1): a) above is the agarose gel with a 1.0 % mass concentration (2) and silica gel of 1.04 g/cm^3 density is below (3); b) below is the agarose gel with a 0.6 % mass concentration (4) and agarose gel with a 0.8% concentration by mass is above (5); c) similar agarose gels with a 0.8% mass concentration are above and below

In the process of layer-by-layer probing of double-layer gel formation the intensity of light transmitting through the gel refers to the value corresponding to the optical system without gel. The measurements are processed for the optical wavelength of 580 nm representing the minimum transmission of silica gel. Figure 5 shows the results of measurements of the dimensionless intensity of transmitted light through two-layer gels as a function of the distance from the layers boundary. The experiments are carried out on two-layer agarose gels with the same and different layer densities, as well as two-layer gel systems of different chemical natures (agarose – silicate gel). Prior to the measurement, gels are conditioned within an hour to insure that they are fully-formed. The boundary between the layers of the gel is taken to be the zero point for the thickness (zero point in figure 5). In Figure 5 the distance is measured below the border using as negative values, and above as positive.
The numbering of the curves in Figure 5 corresponds to the order of gels (from left to right) depicted in Figure 4. The silica gel has the lower optical density compared to agarose. The amount of light transmission of agarose gels decreases with increasing concentration of agarose. This is observed for both upper and the lower layers of the gel. However it is important that the level of light transmission at the bottom of the upper layer is smaller than for the bottom layer. It can be explained by the spontaneous sedimentation of the dispersed phase inside the gel layer due to the gravity, in which the dispersed phase is squeezed out from its volume in its upper layers, i.e. the concentration of the dispersed phase inside of the gel layer increases towards the bottom. The independence of the process above from the external pressure, created by layers located at the top, points towards a spontaneous process. Hence the layered gels are inhomogeneous and anisotropic in density of the dispersed phase.
It is worth emphasizing that all curves shown in Figure 5 possess a characteristic minimum of the relative transmittance of light near the border between gel layers. As noted above, the border between gel layers is a thin layer filled with a dispersion phase (water). Although its light-passing ability is significantly higher than for gels, the upper and the lower borders of such a thin layer have significant internal reflections that lead to a light scattering and a decrease in its intensity at the exit of the cuvettes.

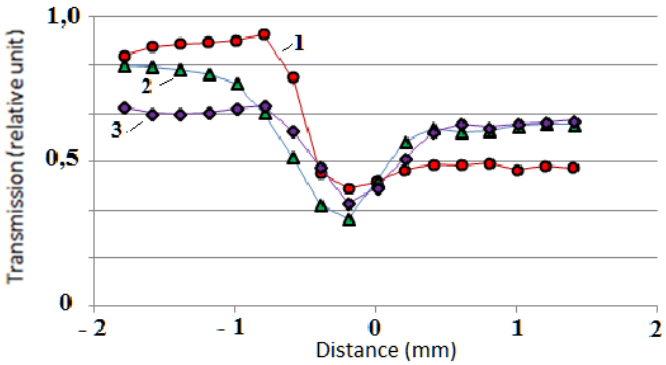

Figure 5: Changes in the relative intensity of light transmittance of different layered gels in the border areas depending on the distance to the border. Sensing was performed from the bottom to the top. Visually observe the interface between the layers of the gels corresponds to the initial value. 1 – silica gel density of 1.04 g/cm^2 from below, agarose gel concentration of 1.0 % on the top; 2 – agarose gel of 0.6 % concentration on the bottom and agarose gel concentration of 0.8%on the top; 3 - agarose gel of 0.8% concentration from below and from above

4. Conclusions

Characteristic shapes of spectra during gel formation show that the light intensity passing during the process decreases as the temperature decreases, meaning that the optical density of the media increases. This indicates the formation of a new dispersed phase. The maximum wavelength of the spectrum of the passing light shifts towards the red range during the gel formation. The extent of such an alteration increases along with the concentration of agarose. This phenomenon seems to be connected not only to the density variation, but also to the structure of the gel. The temperature ranges of phase transition from liquid state to a structured gel for various concentrations of agarose have been determined.

It was shown that the layering of gels is possible even if they have different chemical structures or different concentrations. Such a layered system is hydrodynamically stable, i.e. denser gels can be stable over less dense gels. Between the layers of gels with identical or different chemical nature, a thin border filled with the dispersion phase is formed. Due to the spontaneous compaction of gels by deposition of a dispersed phase, gel layers are heterogeneous and anisotropic in density.

Acknowledgments

This work was supported by the Russian Science Foundation (project no. 15-19-00177).

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