STUDY OF AGAROSE GEL STRUCTURE BY DYNAMIC LIGHT SCATTERING METHOD

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The microstructure (particles size distribution of) of the agarose gel was investigated by the dynamic light scattering method. The results of measurements showed that there are two types of particles in the gel structure: large particles, the sizes of which depend on the agarose concentration, temperature and retention time of holding at a given temperature; small particles, the sizes of which do not depend on the above-mentioned factors. Analysis shows that small particles are individual bispirals, while large particles are associations consisting of a large number of bispirals.

Keywords: polymer gel, agarose, double spiral, suprafiber, dynamic light scattering
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INTRODUCTION

Some polymer solutions and colloids transist into a gel state below some critical temperature and at a certain concentration interval of polymer (or colloidal particles). The main feature of gels is that they retain their shape (or lose their fluidity) under small mechanical stresses. Gels, as a special state of matter, attract great scientific interest. This is confirmed by modern monographs devoted to this subject [1-4]. Due to their unique mechanical properties, gels are widely used in materials science, food technology, biotechnology, cosmetology, etc. These include ceramic technology (manufacturing of thin parts using a 3D printer), pharmacology (storage, prolongation of action and targeted transport of drugs), organ engineering (replacement of some organs such as bones, teeth, cartilage and soft tissue with appropriate biocompatibleartificial organs) etc. [5,6].

Many famous scientific centers and research groups in the world are conducting important research on the properties and practical applications of biopolymer gels because of their biocompatibility with the human body. These works can be divided into two groups.

Works of purely research nature. For example, works [7-9] investigate the influence of associates of different levels on the dynamic rheological properties of an agar gel. The works [10, 11] experimentally investigated the dependences of storage and loss moduli of such polysaccharide gels as rhamsan, wellan gum on temperature and concentration of the polymer. A group of researchers from Yale University studied the effect of inorganic salts on the viscoelastic properties of an alginate gel [12]. In [13] the effect of concentration and size ofair bubbles on the rheological properties of an agar gel was investigated. In [14], the kinetics of the gel formation process was studied using the methods of dynamic light scattering and shear oscillation rheology. In the work of Richtering et al [15], by using original rheomechanical and rheooptical methods, it was possible to measure the rheological properties and acquire valuable information about the microstructure of polysaccharide gels. Another group [16] investigated the dependence of the rheological properties of agar gel on the origin, molecular weight, and concentration of the agar.

The following works can be noted as bright examples of purely applied research. A biomaterial made on the basis of alginate hydrogel makes it possible to successfully replace the damaged part of the heart in case of a massive infarction [17]. In recent years, "smart hydrogels" based on polyelectrolytes can claim to be in the list of promising materials in terms of developing artificial muscles [18].

This list could go on and on. When solving a specific problem associated with the application of the gel, the latter must have relevant thermal and rheological properties: the temperature interval of existence, heat capacity, thermal conductivity, yield stress, elastic moduli, viscosity, etc.

Therefore, the management of these properties is very important in terms of practical application and one of the options for solving this problem is the introduction of substances of different nature into the gel. The effect of these additives on gel properties is associated with changes in the gel microstructure. In this sense, determining the microstructure of the gel is important for predicting the basic properties of the gel.

In this work, dynamic light scattering was used to study the microstructure of the agarose gel, or rather the size distribution of particles (associates of different levels) in the agarose gel structure.

EXPERIMENT

High-purity powdered agarose used in the experiment was purchased from the firm HISPANAGAR and acquires. Agarose has a high gel-forming ability in aqueous medium. According to our estimates, the critical concentration of agarose gel formation is 0.14 %.

The main structural unit of agarose is the agarobiose disaccharide, the chemical structure of which is shown in Figure 1.

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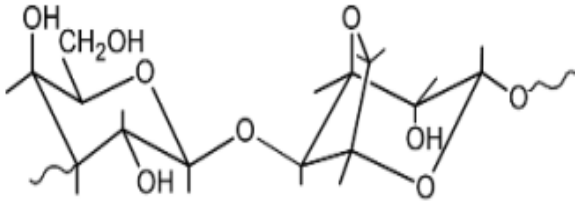

Fig. 1.

The agarose gels were prepared according to the following scheme. Agarose powder was weighed in ADAM PW 124 scales (0.1 mg accuracy), added to bidiistilled water, and kept for 24 h to swell the agarose. The mixture is then heated to 95 °C and the resulting homogeneous solution is cooled to room temperature. Transition to the gel state is accompanied by a loss of fluidity and turbidity of the solution.

The reason for the turbidity of gels is the scattering of light by the particles (associates) contained in the gel. By analyzing this scattering, it is possible to determine the size and number of these particles, which is the basis of the method of dynamic light scattering (DLS) [19].

DLS-measurements were performed in Horiba Nano Partica SZ-100 device in heating mode.

In this work, agarose gel was considered in three weight concentrations: 0.2 % - case of weak gelation; 0.5 % - case of medium gelation; 1 % - case of strong gelation.

RESULTS AND DISCUSSIONS

The measurement results are shown in Figures 2-5. Figures 1, 2, and 3 show the particle size distributions in the gel for agarose concentrations of 0.2%, 0.5%, and 1%, respectively. These measurements were performed at 25 °C after obtaining the gels by cooling the solution from the sol-phase and holding for 0.5 hour.

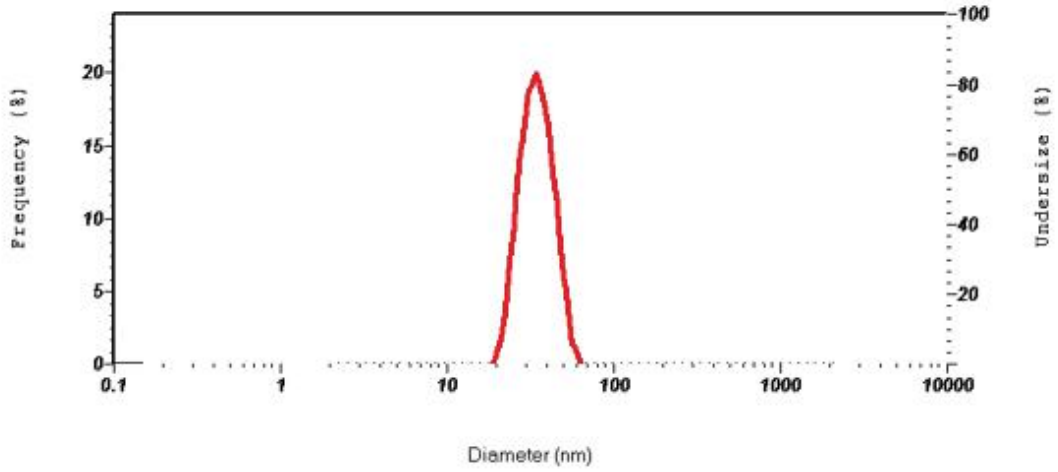

Fig.2. Particle size distributions in the gel for agarose concentrations of 0.2%

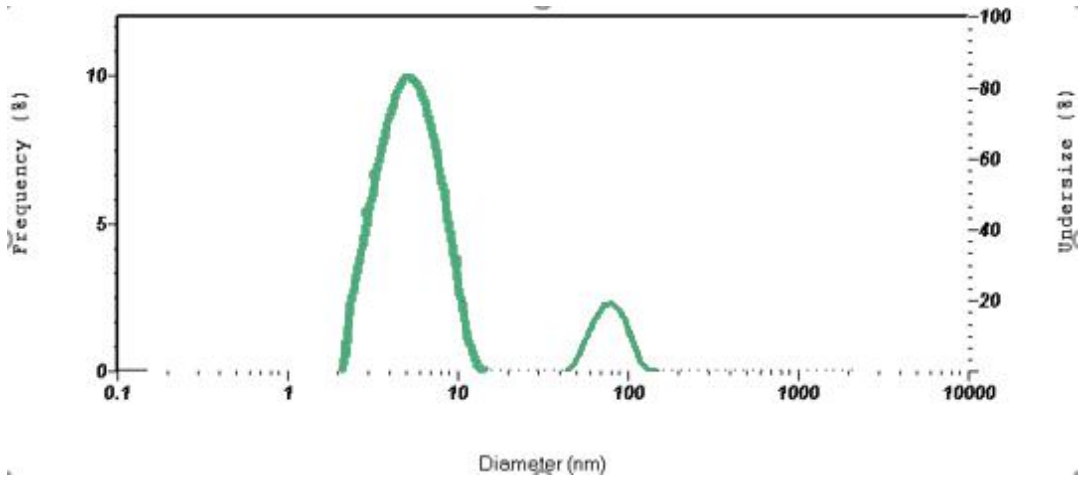

Fig.3. Particle size distributions in the gel for agarose concentrations of 0.5%

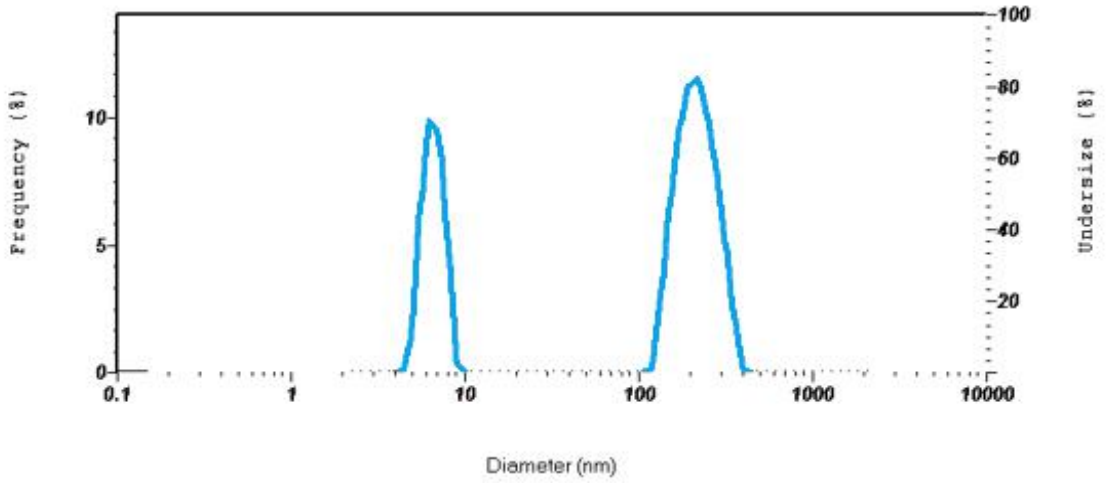

Fig.4. Particle size distributions in the gel for agarose concentrations of 1%,

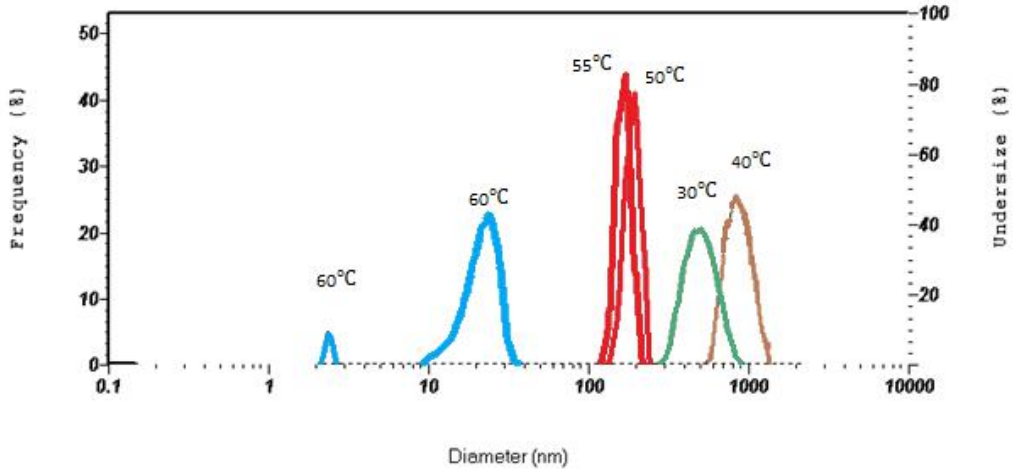

Fig.5. Particle size distribution in a 1% agarose gel at different temperatures in the heating mode after the gel was held for 24 h at 30 °C.

A summary of the most interesting data extracted from these plots is presented in Tables 1 and 2. As can be seen from Table 1 for 0.2% agarose gel (the case of weak gel) there are only large associates with sizes from 20 nm to 50 nm, and the maximum of distribution is equal to 33 nm, i.e., in the gel structure most of all there are particles with approximately this size.

At other concentrations (0.5% and 1%), in addition to such associates (large particles) in the gel structure there are also small particles with a size of about 5 – 6 nm. As the concentration increases, the size of these associates increases (become 75 nm and 210 nm, respectively), while the size of the small ones does not change.

The last column shows the values of the diffusion coefficient at 25 °C. As can be seen, with increasing agarose concentration, the diffusion coefficient decreases, i.e., the movement of particles in the gels becomes more difficult.

Similar data are shown in Table 2 for 1% agarose gel at different temperatures in the heating mode starting from 30 °C. As expected, the size of the associates decreases with increasing ща temperature. This suggests that the structural elements that gives the gel hardness, i.e., the large associates break up into relatively small ones, when heated. Beginning from 60 °C, along with large particles, small 5 nm particles also appear in the gel structure.

Table 1

Agarose concentration, (c), %wt	Minimal size d_{min}, nm	Maximal size d_{max}, nm	Most common size d_c, nm	Percentage fraction, %	Diffusion coefficient, 10^{-12} m^2/s
0.2	19	48	33	100	14.8
0.5	2 45	10.5 106	5.2 75	89 11	10.4
1	3.5 120	9.5 300	6.3 210	32 68	2.4

Table 2

Temperature, °C	Minimal size d_{min}, nm	Maximal size d_{max}, nm	Most probable size, d_c, nm	Percentage fraction, %
30	500	1400	850	100
40	200	900	400	100
50	120	220	150	100
55	110	200	130	100
60	9 2	35 10	20 5	92 8

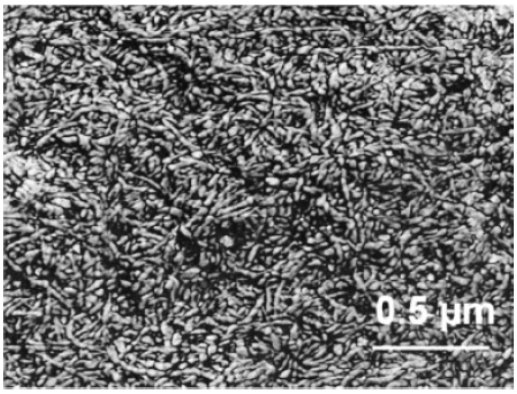

Fig. 6. View of a 2% agar gel under an atomic force microscope.

All observed patterns can be explained on the basis of existing agarose gel model. As in the case of many biopolymers, the presence of some functional hydrophilic and hydrophobic groups in agarobiose, leads to the random coil - bispiral conformational transition above the sol-gel transition. The bispiral (or double helix) pitch is 1.9 nm and consists of two helices shifted by 0.95 nm relative to each other [7, 8]. The small particles observed in the experiment are most likely these separate bispirals.

In agarobiose, three of the four carboxyl groups point outward and interact with the water molecules in the volume via hydrogen bonding. With these hydrogen bonds, the bispirals bond together to form supramolecular associations, sometimes referred to as suprafibers. A photo taken with an atomic force microscope (Fig.6) shows that these particles are on the order of 0.1 μm in size [20].

CONCLUSION

Comparing the described model with the obtained experimental data, it is easy to understand that the large particles are suprafibers and the small particles are bispirals. Therefore, the size of small particles (bispirals) almost does not change with changing of agarose concentration and temperature. On the contrary, the size of associates (suprafibers) changes greatly with changes in agarose concentration, temperature, and the time the gel is held at a given temperature. All of this is confirmed by the data given in Tables 1 and 2.

As the agarose concentration increases, the size of the associates increases and the size of the cells in the gel netwrk decreases. This leads to difficulty for penetration of particles and from one area to another, i.e., a decrease in the diffusion coefficient.

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