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Agarose gel structure study using dynamic light scattering

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The microstructure (particle size distribution) of the agarose hydrogel was investigated using the dynamic light scattering method. The results of measurements showed that there are two types of particles in the gel structure: 1) large particles, the sizes of which depend on the agarose concentration, temperature, and holding time at a given temperature; 2) small particles, the sizes of which weakly dependent on the above-mentioned factors. Analysis shows that small particles are free bispirals (double helices), while large particles are associations consisting of a large number of bispirals. The effect of hydrophobic and hydrophilic additives on the particle size distribution was also studied. Hydrophobic additive increases the number of free bispirals and they disappear when a hyrophilic substance is added to the gel.
Key words: polymer, hydrogel, agarose, bispiral, suprafiber, dynamic light scattering

Вивчення структури агарозного геля методом динамічного розсія світу. *А. Р. Імамаліев, І. І. Аббасов, Р. Ш. Рагімов, Ч. А. Імамалізаде*

Мікроструктура (розподіл часток за розмірами) агарозного гідрогелю досліджувалась методом динамічного розсіювання світла. Результати вимірювань показали, що в структурі гелю присутні два типи частинок: 1) великі частинки, розміри яких залежать від концентрації агарози, температури та часу витримки за даної температури; 2) дрібні частинки, розміри яких слабо залежать від перерахованих вище факторів. Аналіз показує, що дрібні частинки є вільні біспіралі (подвійні спіралі), а великі – асоціації, що складаються з великої кількості біспіралей. Також було вивчено вплив гідрофобних та гідрофільних добавок на розподіл частинок за розмірами. Гідрофобна добавка збільшує кількість вільних біспіралей і вони зникають при додаванні в гель гірофільного речовини.

1. Introduction

Some polymer solutions and colloids transist into a gel state below some critical temperature and at above certain concentration of polymer. 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 and reviews devoted to this subject [1-5]. 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 biocompatible artificial organs), etc. [6,7]. The use of gel allows maintaining high efficiency of zinc-ion battery under any climatic conditions [8].

Many well-known scientific centers and groups around the world are engaged in research

into the properties and practical applications of biopolymer hydrogels, taking into account their biocompatibility with the human body. These works can be divided into two groups.

The first group includes works of a research character. For example, in works [9-11], the influence of associates of different levels on the dynamic rheological properties of agar gel is investigated. In works [12,13], the dependences of storage and loss moduli of such polysaccharide gels as rhamsan, and wellan gum on the temperature and concentration of the polymer were experimentally studied. The authors of [14] studied the effect of inorganic salts on the viscoelastic properties of an alginate gel. In [15], the effect of concentration and size of air bubbles on the rheological properties of an agar gel was studied. The kinetics of the gel formation process by using the methods of time resolved dynamic light scattering and shear oscillation rheology was studied in [16]. In works [17, 18], the dependence of the rheological properties of agarose gel on the origin, molecular weight, and concentration were investigated. The microstructure and rheological properties of gels can also be significantly changed by mechanical processing [19]. In a recently published paper [20], the main reason for the strong gelation in the agarose-water system was elucidated. It turns out that agarose molecules involved in the formation of associates play a major role in the center of tetrahedral cavities, which, due to the hydrophobic effect, are occupied by water molecules bound by hydrogen bonds (as in the case of ice formation).

The other group of works can be classified as applied research. A biomaterial based on alginate hydrogel allows for the successful replacement of a damaged area of the heart in the event of an extensive infarction [21]. In recent years, "smart hydrogels" based on polyelectrolytes may be among the most promising materials for the creation of artificial muscles [22]. Among the works devoted to the practical application of polysaccharide gels, DNA fractionation using gel filtration (agarose gel electrophoresis) should be particularly highlighted. [23, 24].

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, pore size, 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 various natures 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 is important for predicting the basic properties of the gel.

In this work, the dynamic light scattering method was used to study the microstructure of agarose gel, more precisely, the distribution of particle sizes at different levels in the agarose gel structure. The effect of agarose concentration, temperature, hydrophobic and hydrophilic additives on this distribution was also investigated.

2. Experiment

High-purity powdered agarose used in the experiment was purchased from the firm CONDA. This sufficiently monodisperse linear polymer has an average molecular mass of M=120,000 g/mol. The main structural unit of agarose is the agarobiose disaccharide, the chemical structure of which is shown in Figure 1. Agarose has a high gel-forming ability in aqueous medium. According to our estimation, the critical concentration of agarose gel formation is of order 0.1 %.

The agarose gels were prepared according to the following scheme. Agarose powder was weighed in a balance (ADAM PW 124 with 0.1 mg accuracy), added to bidistilled water, and kept for 24 hour until swelling. The mixture is then heated up to 95 ^{o}C and the resulting homogeneous solution is cooled to room temperature. Transition to the gel state is accompanied by a loss of fluidity and the appearance of turbidity in the solution [25].

The reason for the turbidity of gels is the scattering of light by the particles (associates) contained in the gel structure. 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) [26]. DLS measurements were performed on Horiba Nano Partica SZ-100 device in heating mode.

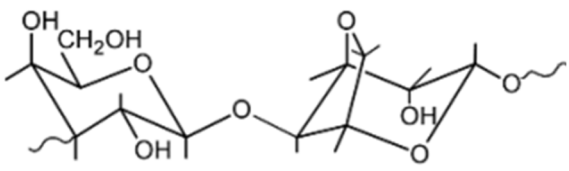

Fig. 1. The main structural unit of agarose - agarobiose disaccharide

Table 1. Concentration dependences of particle sizes distribution in agarose gel

Agarose concentration c, %wt	Minimal size d_{min}, nm	Maximal size d_{max}, nm	Most common size d_c, nm	Percentage fraction, %
0.2	19	48	33	100
0.5	2	10.5	5.2	89
	45	106	75	11
1	3.5	9.5	6.3	32
	120	300	210	68

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

Based on general considerations, it can be concluded that the introduction of hydrophobic and hydrophilic additives into the gel should affect the number and size of particles in the gel by changing the structure of water (the ratio of free and bound molecules in water). To test this idea, isoamyl alcohol $C_4H_4O_6Na_2 \cdot 2H_2O$ (hydrophobic additive) and sodium salt of tartaric acid $C_4H_4O_6Na_2 \cdot 2H_2O$ (hydrophilic additive) were added to 1% agarose hydrogel in a molar amount of 0.5%.

3. Results and discussion

The measurement results are shown in Figures 2-7. Figures 2, 3, and 4 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 it for 0.5 hours. The effect of temperature on the particle size distribution for 1% agarose gel in heating mode is shown in Figure 5. The results of the effect of hydrophobic and hydrophilic additives on the particle distributions in agarose gel are presented in Figures 6 and 7, respectively.

A summary of the most interesting data on the concentration dependence of particle size distributions from the plots of Figures 2-4 is presented in Table 1. As can be seen from the Table, for 0.2% agarose gel (the case of weak gelation), only large associates with sizes from 20 nm to 50 nm are observed, and the distribution maximum corresponds to 33 nm. At other concentrations (0.5% and 1%), in addition to large particles (associates) in addition to large particles (associates), the gel structure also contains small particles of about 5–6 nm in size. As the concentration increases, the size of these associates increases (becoming 75 nm and 210 nm, respectively) while the size of the small ones changes insignificantly.

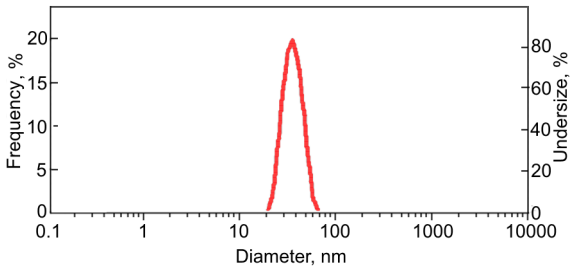

Fig.2. Particle size distributions in the 0.2% agarose hydrogel

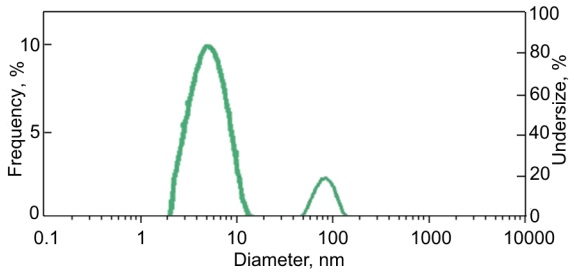

Fig 3. Particle size distributions in the 0.5% agarose hydrogel

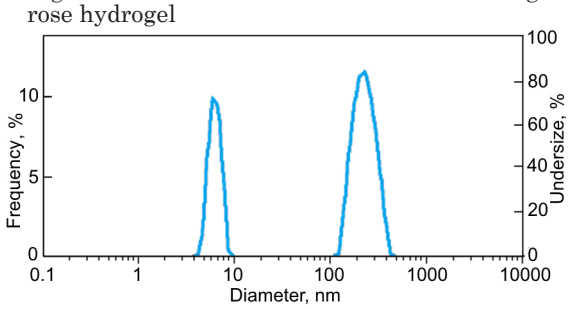

Fig.4. Particle size distributions in the gel for agarose concentrations of 1%,

The absence of small particles in the case of gels with an agarose concentration of 0.2% is explained by the high value of the diffusion coefficient. Under such conditions, moving small particles join the associates. 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

Table 2. Temperature dependence of particle sizes distribution for 1% agarose gel

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	35	20	92
	2	10	5	8

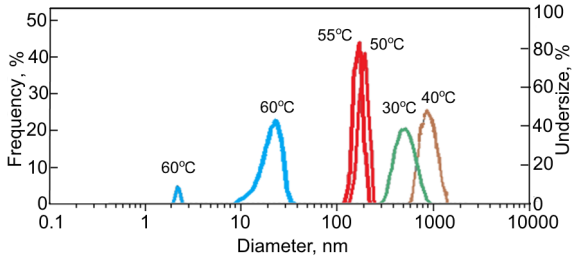

Fig.5. Particle size distribution in a 1% agarose gel at different temperatures in heating mode after keeping the gel for 24 h at 30 °C

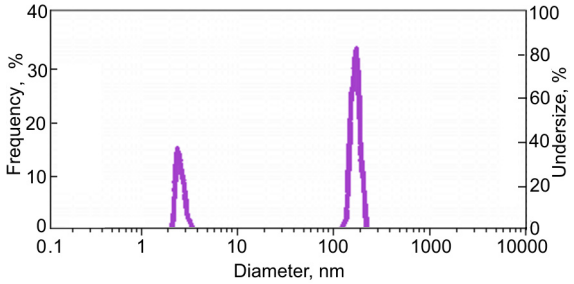

Fig. 6. Particle size distribution in the system 1 % agarose gel+isoamyl alcohol (hydrophobic additive),T=25°C.

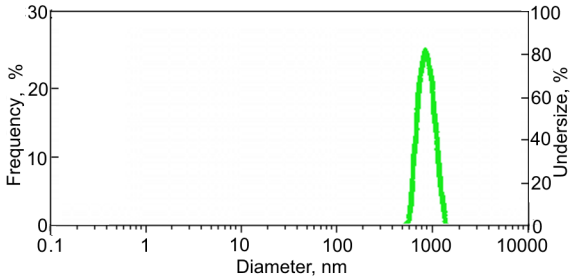

Fig. 7. Particle size distribution in the system 1 % agarose gel+sodium salt of tartaric acid (hydrophilic additive), T=25 °C.

heating mode starting from 30 °C. As expected, the size of the associates decreases with increasing temperature. This indicates that the structural elements that give the gel its hardness, i.e. large associates, disintegrate into relatively small ones when heated. Beginning from 60 °C, along with large particles, small 5 nm particles also appear in the gel structure.

All observed patterns can be explained on the basis of the 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 (double helix) conformational transition above the sol-gel transition. The bispiral pitch is 1.9 nm and consists of two helices shifted by 0.95 nm relative to each other [9, 10]. The small particles observed in the experiment are most likely these solitary bispirals.

In agarobiose, three of the four carboxyl groups are directed outward and interact with the water molecules in the volume via hydrogen bonding. These hydrogen bonds allow the bispirals to link together to form supramolecular assemblies, sometimes called suprafibres. An atomic force microscope image shows that for 1% agarose gel these particles are on the order of 0.1 μm in size [27].

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 and temperature (as well as 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 pores in the gel network decreases. This leads to difficulty for penetration of particles and from one area to another, i.e., a decrease in the diffusion coefficient.

The results shown in Figures 4, 6, and 7 are presented in Table 3 in a more accessible (detailed) form for analysis. It is immediately obvious that the hydrophobic additive somewhat reduces the size of large associates. On the contrary, a hydrophilic additive sharply increases the size of associates, while small particles in the gel structure disappear. This means that bispirals are fully incorporated into the associates in the case of hydrophilic additives,

Table 3. Influence of hydrophobic and hydrophilic additives on particle sizes distribution for 1% agarose gel

gel composition	Minimal size d_{min}, nm	Maximal size d_{max}, nm	Most probable size, d_c, nm	Percentage fraction, %
1%agarose gel	3.5	9.5	6.3	32
	120	300	210	68
1 % agarose gel + isoamyl alcohol	3.5	5.5	4.2	28
	120	250	200	72
1% agarose gel+sodium salt of tartaric acid	400	1200	850	100

4. Conclusion

Dynamic light scattering clearly shows that there are two types of particles in the structure of agarose gels:

1) large particles (0.1 - 1 μm) consisting of a huge number of bispirals;

2) small particles (5 - 10 nm) representing free (not participating in the formation of associates) bispirals. Free bispirals exist only in gels with a high concentration of agarose, where, due to the low value of the diffusion coefficient, they cannot join associates. The state of water, where the gel network is formed, plays a significant role in the number and size of particles in the gels. This is proved by the effect of hydrophobic or hydrophilic additives. Hydrophobic additive increases the number of small particles in the gel. And when a hyrophilic substance is added to the gel, the small particles disappear, i.e. they are completely involved in the associate-forming process.

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