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POLYMER, BIOORGANIC, AND HYBRID NANOMATERIALS

For the 300th anniversary of St. Petersburg State University

Hydrogels Based on Gellan and a Graft Copolymer of Pullulan with Poly(2-methyl-2-oxazoline) Side Groups

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Abstract—In this work, a three-component system is obtained based on gellan, a graft copolymer of pullulan with side chains of poly(2-methyl-2-oxazoline), and $CaCl_2$, which is capable of forming gels upon contact with an aqueous solution of NaCl. Such a composition can be used for medical purposes, in particular for the treatment of ophthalmological diseases. In this work, the molecular characteristics of the initial components of the gel are obtained and its viscoelastic properties are studied. It was established that graft copolymers of pullulan with poly(2-methyl-2-oxazoline) are integrated into the gel composition, while an increase in their proportion reduces its elastic properties. The obtained gel retain elastic properties upon heating up to 70°C.

Keywords: hydrogels, gellan, graft copolymer, pullulan, light scattering, analytical ultracentrifugation, viscometry, viscoelastic properties

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INTRODUCTION

Due to a wide range of physical and chemical properties, determined both by their composition and their structure, polysaccharides are widely used in the food chemical industry, medicine, cosmetics, and many other areas, such as gelling and film formers, coagulants, thickeners, etc. Many polysaccharides are of biological origin, which, on the one hand, facilitates their production, on the other, ensures the biodegradability of materials based on them, and also, in some cases, determines their high biocompatibility and the possibility of their use in medicine. Thanks to the film-forming properties based on pullulan and other polysaccharides, technologies for creating soluble coatings for drugs are being improved [1, 2]. On the basis of polysaccharides prone to gelation, in particular gellan, systems are obtained that ensure the delivery and dosed introduction of drugs into the body. Thus, due to the presence of charged groups in the chain, it becomes possible to control the process of gelation in gellan by introducing single- and multicharged cations into its solution. Since biological fluids contain sodium cations in significant concentrations, this makes it possible to use it as a binding agent in drug-delivery systems upon contact with them, as well as a clotting agent that limits blood loss upon contact with damaged body tissues.

One promising medical applications of gellan is drug delivery directly to the surface of the eye. Since many drugs are poorly retained, being removed along with tears from the surface of the eye when blinking, the problem of longer retention arises [3]. The binding properties of gellan, which forms a gel during interaction with positive sodium ions in lacrimal fluid, open up the possibility of creating systems for the long-term retention of drugs based on it. Gellan gum chemically modified with poly(2-ethyl-2-oxazoline) was studied in [4]. Due to the fact that the chemical modification of gellan is a rather labor-intensive procedure due to its limited solubility in water and difficulties in isolating the reaction product, an urgent task is to search for alternative options for obtaining drugs capable of gelation. One of the ways to solve this problem is to create systems containing gellan as an agent capable of controlled gelation, and a polymer compatible with it, which ensures the transport of drugs. The latter can be pullulan [2, 5], which undergoes chemical modification much more effectively due to its nonionic nature and good solubility in water.

In this work, we consider the possibility of creating systems from gellan and pullulan, chemically modified with biocompatible poly(2-methyl-2-oxazoline), suitable for the transport of drugs. The gelation processes in the studied systems are regulated by changing

the NaCl content in the presence of multiply charged Ca^{2+} ions. The modes of gel formation are studied with variations in the content of chemically modified pullulan.

REAGENTS AND MATERIALS USED

Gellan powder (Gl) (Sigma-Aldrich) according to quantitative elemental analysis using inductively coupled plasma atomic emission spectrometry obtained on an ICPE-9000 Shimadzu device (Japan), contains the following components: Ca = 0.287%, Mg = 0.032%, K = 2.56%, Na = 0.831%.

Azide-terminated polyoxazoline (pMO). 2 mL of freshly distilled methyloxazoline, 2 mL of freshly distilled sulfolane, and 40 mg of para-nitrophenylsulfonyl chloride were mixed in a vial. They were evacuated and saturated with argon (three cycles). The evacuated vial was placed in a bath at $T = 95°C$ and stirred for 30 h. At the end of the reaction, the heat was turned off and 1.5 mL of a saturated solution of sodium azide in dimethyl sulfoxide (DMSO) was added and left overnight. Purification was carried out by dialysis against water (MWCO 2000 membrane). The yield was 1.75 g. The degree of polymerization determined from the NMR ^1H spectrum, as the ratio of the integrated signal intensities of the protons of the terminal nitrophenyl group (the initiator residue) and the side methyl groups of the poly(2-methyloxazoline) chain units, turned out to be equal to 130, which corresponds to a molecular weight of MW = 11 000.

Propargyl-pullulan (Pu). A solution was prepared containing 4.43 g of KOH, 6.1 mL of water, and 5.5 mL of DMSO. 1.6 g of pullulan was added to this solution. After 10 min of stirring with a magnetic stirrer, but without waiting for complete dissolution, a 4.0 cm^3 solution of 80 wt % propargyl bromide in toluene (Acros Organics) was added in 1-mL portions every 2 min. Stirring of the reaction mixture continued for 24 h. After this, the reaction mixture was washed with alcohol twice, 40 mL each time, then with water until neutral on indicator paper (at least four portions of 30 mL each) and again with alcohol twice, 20 mL each. The yield was 1.08 g. Before adding the side chains, the propargyl pullulan was fractionated by fractional precipitation from water with ethanol. Three fractions with intrinsic viscosities of 0.7, 0.52, and 0.26 g/dL were isolated. For further modification and research, a sample with the highest intrinsic viscosity of 0.7 g/dL was used.

Graft copolymers of pullullan with poly(2-methyl-2-oxazoline) side chains (Pu-pMO). Azide-terminated poly(2-methyl-2-oxazoline) (300–700 mg), sodium ascorbate, and copper(II) acetate were added to a solution of propargyl-pullulan (20–50 mg) in water or DMSO, and the solution was stirred using a magnetic stirrer at room temperature for several days. The product was then dialyzed against water (MWCO 12000 membrane) and lyophilized. The dialysis product contained some unreacted polyoxazoline, which was removed by reprecipitation from methanol into ethyl acetate.

According to the NMR data, every tenth to eleventh maltotriose residue contained one pMO macromolecule. The synthesis of graft copolymers of pullullan with poly(2-methyl-2-oxazoline) side chains is described in more detail in [6].

EXPERIMENTAL

Studies of the initial polymers by viscometry were carried out on a Lovis 2000 M microviscometer (Anton Paar GmbH, Graz, Austria). Device operation is based on the Heppler principle. For measurements, we used a capillary with an internal diameter of 1.59 mm, inside of which a steel ball coated with gold was placed. The diameter of the ball was 1.5 mm. The rolling time of the ball in the capillary was measured when it contained solutions (t_c) or solvents (t_0), the angle of inclination of the capillary was 45°. Values of the characteristic viscosity [η] of the studied polymers was determined from the ratio $\lim\limits_{c\to0}\frac{t_c - t_0}{t_0 c} = \lim\limits_{c\to0}\frac{\eta - \eta_0}{\eta_0 c} =$ $[\eta] + [\eta]^2 k_H$, where k_H is the Huggins constant [7], which is determined by the thermodynamic quality of the solvent.

Research using the dynamic-light-scattering method (DLS) was carried out on a PhotoCor Complex installation (Photocor, Russia) with a 288-channel correlator (minimum delay time of 10 ns) and a single-mode solid-state laser ($\lambda_0 = 654$ nm).

Autocorrelation functions of the scattered light intensity were processed using the DynaLS program using the inverse Laplace transform method, which made it possible to obtain distribution functions of the studied compounds in solution according to relaxation times τ. For all studied systems, the dependence of the inverse relaxation time on the square of the scattering wave vector $q^2 = (4\pi n_0/\lambda\sin(\theta/2))^2$ passed through the origin in accordance with the expression: $\tau^{-1} = Dq^2$ (Fig. 1), which indicates the diffusion nature of the observed process [8]. The scattering angles θ varied from 30° to 130°. The translational diffusion coefficients D_0 of the studied macromolecules were determined from the concentration dependences $D(c)$ when extrapolated to infinite dilution in accordance with the expression $D(c) = D_0(1 + 2A_2cM + ...)$, here A_2 is the second virial coefficient. The refractive indices of the solvents n_0 were measured in a Mettler Toledo automatic refractometer (RM40, Switzerland). To calculate hydrodynamic radii, the Stokes–Einstein relation was used: $R_h = \frac{kT}{6\pi\eta_0 D_0}$, here k is the Boltzmann constant, T is the absolute temperature, and η_0 is the solvent viscosity.

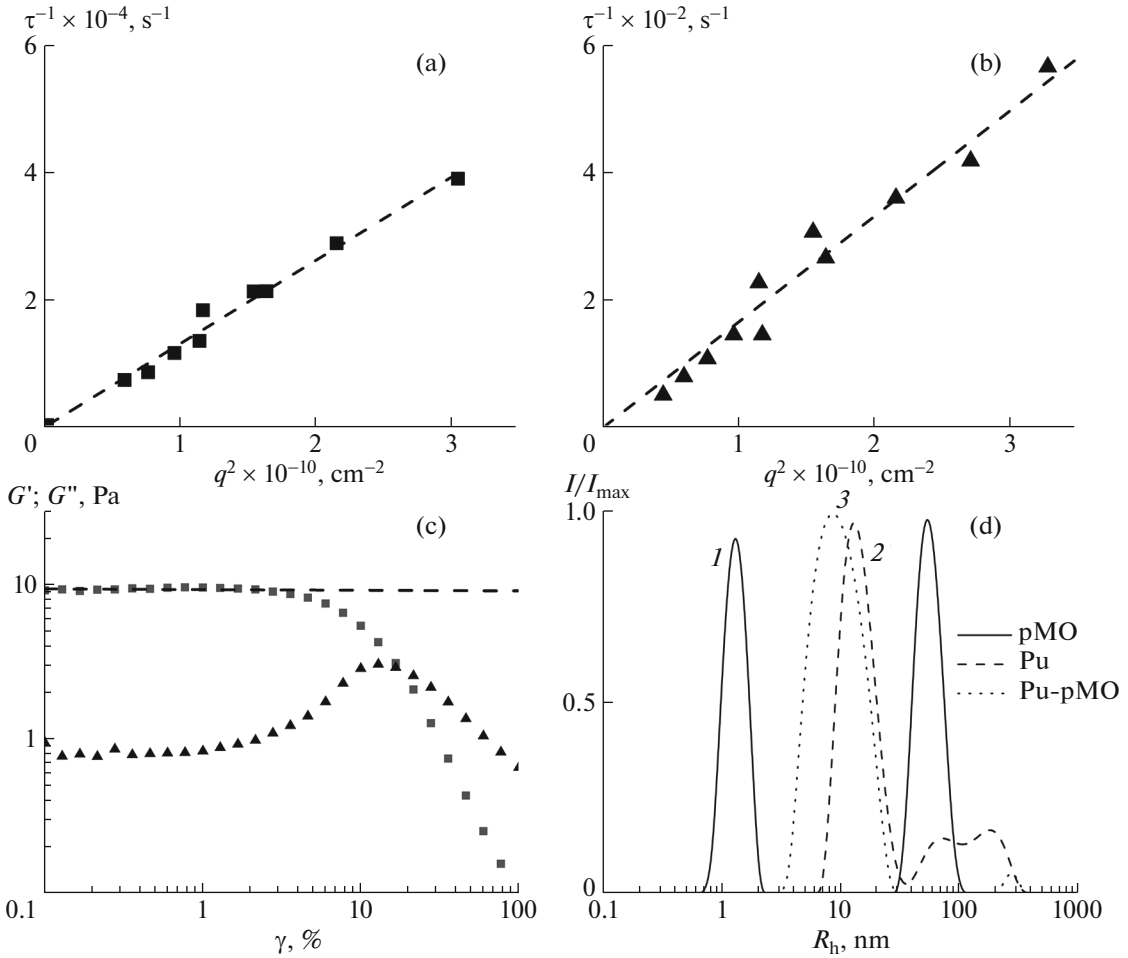

Fig. 1. Dependences of the inverse relaxation time (τ^{-1}) on the square of the scattering wave vector q^2, obtained for fast (a) and slow (b) scattering modes in an aqueous solution of pMO at a concentration of 1.06 g/dL; amplitude dependence of the elastic modulus G' (■) and loss modulus G'' (▲) of Gl in water at a concentration of 0.1 g/dL with the addition of $CaCl_2$ at a concentration of 5 mM/L (c); normalized distributions of the scattered-light intensity over the hydrodynamic radii of Pu, Pu-pMO, and pMO samples obtained in water by the DLS method at a scattering angle of $\theta = 90°$ (d).

Density measurements were carried out on a DM40 laboratory density meter (Mettler Toledo, Switzerland).

Analytical ultracentrifugation (AUC) experiments were carried out using a "ProteomeLab XL-I Protein Characterization System" analytical ultracentrifuge (Beckman Coulter, Inc., Brea, USA). The rotor speed was 40000 rpm. Sedimentation of the studied samples was observed using a Rayleigh interference optical system equipped with a red laser (wavelength of 655 nm) as a light source. Processing of the experimental data was carried out using the Sedfit program, using a model of continuous distribution of the sedimentation coefficient $c(s)$ [9]. The sample sedimentation coefficients were determined s; from the concentration dependence $s^{-1} = s_0^{-1}(1 + k_s c + \ldots)$, the Gralen coefficient was determined k_s; and we also found the sedimentation coefficient at infinite dilution s_0. The molecular weights of the samples M_{sD} were calculated from the sedimentation coefficient values s_0 and diffusion coefficient D_0 according to the Svedberg equation: $M_{sD} = \frac{s_0}{D_0}\frac{kTN_A}{(1-\bar{\nu}\rho_0)}$, where $\bar{\nu}$ is the specific partial volume of the studied macromolecule. It was found that it is equal to 0.650 cm^3/g for Pu, 0.804 cm^3/g for pMO and Pu-pMO, and 0.597 cm^3/g for Gl. The diffusion coefficient of gellan was calculated from the AUC data using the relation $D = \frac{kT(1-\bar{\nu}\rho_0)^{1/2}}{\eta_0^{3/2}(9\pi\sqrt{2})(f/f_0)^{3/2}(s_0\bar{\nu})^{1/2}}$, here (f/f_0) is the determined ratio of the coefficient of translational friction of the macromolecule under study to the coefficient of translational friction of the equivalent sphere.

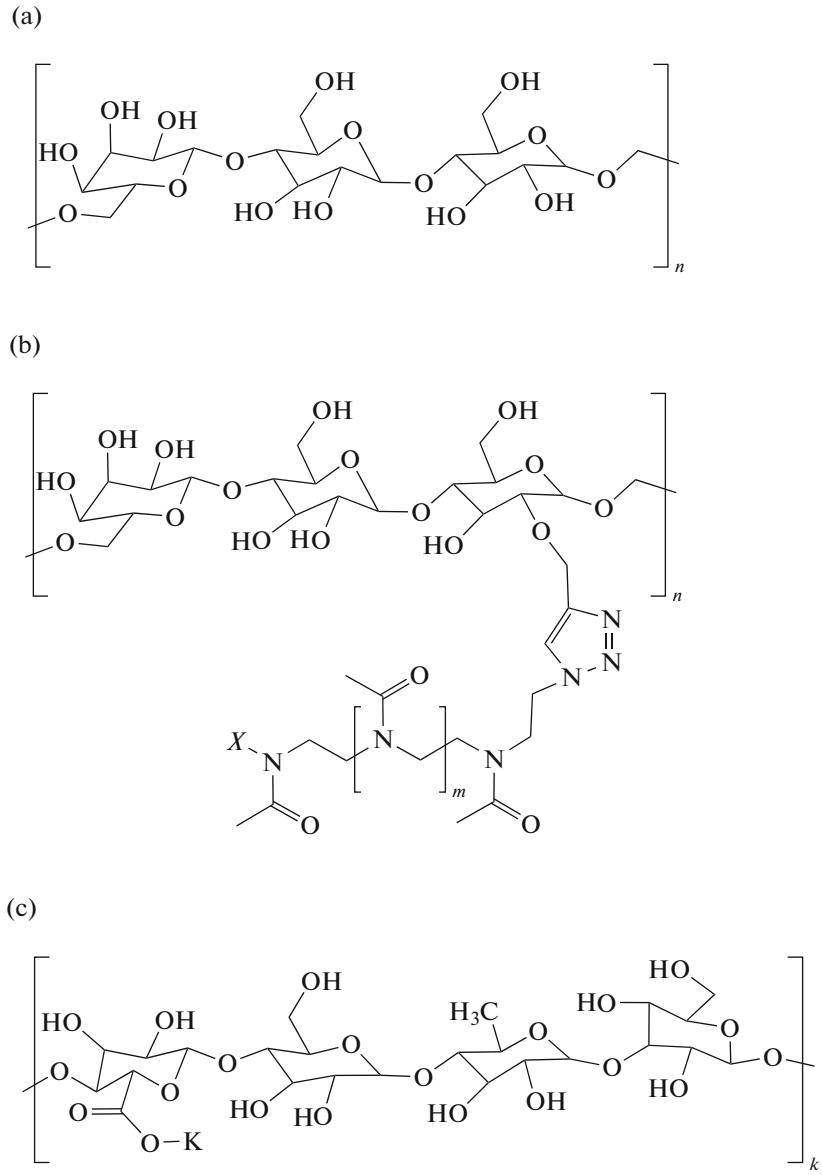

Fig. 2. Chemical structure of pullulan (a), pullulan modified with poly(2-methyl-2-oxazoline) (b), and gellan (c); *X* is the initiator remainder ($-SO_2-Ph-NO_2$).

The rheological properties of the gels were studied using an MCR 702 TwinDrive rheometer (Anton Paar, Austria). The measurements were carried out in the "cone–plate" geometry, the diameters of the upper and lower plates were 50 mm, the cone angle was 2°. The volume of the analyzed sample was 1.2 mL and the studied samples were thermostatted with an accuracy of 0.01 K.

All studies were carried out at a temperature of 25°C.

RESULTS

The samples for the formation of biocompatible gels were studied by DLS, AUC, and viscometry. Figure 2 shows the chemical structures of pullulan with side groups of poly(2-methyl-2-oxazoline) (pMO) and gellan.

It was found in [5] that Pu and Gl have good affinity, which allows these polymers to be mixed in a wide range of mass ratios. Pu is highly soluble in water, as was established in [10], water is a good solvent for this polymer. Gl tends to swell in water, and the solution becomes homogeneous only after heating. In water, Gl forms a system of hydrogen and other bonds [11], which makes it impossible to determine the molecular characteristics of Gl in this solvent. In this regard, in [12] DMSO with the addition of $NaNO_3$ was used as a solvent for Gl. In this work, we used a similar solvent

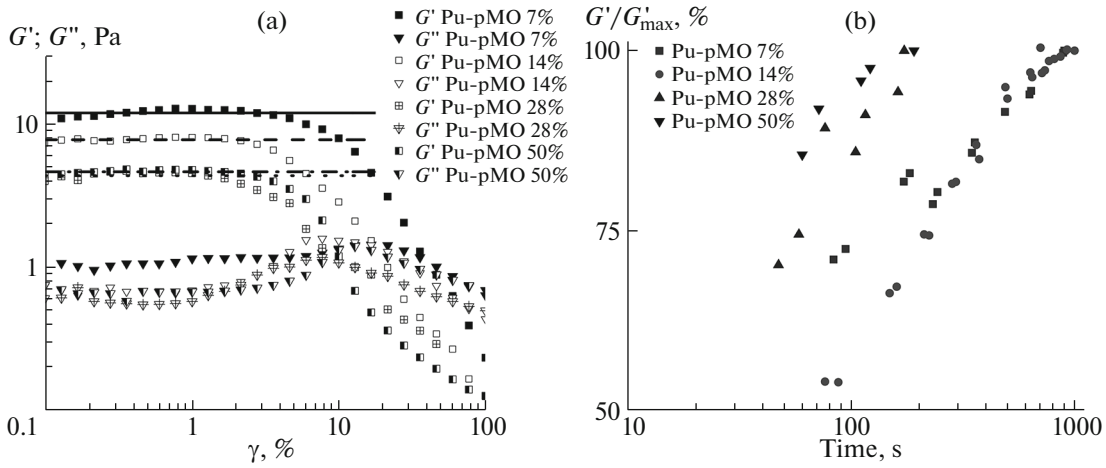

Fig. 3. Dependences of the elasticity modulus G' and loss modulus G'' on the deformation amplitude γ, obtained for Gl systems with the addition of Pu-pMO (a). Dependences G' on the time obtained at $\gamma = 1\%$ immediately after 100% deformation for the studied systems (b).

DMSO with $NaNO_3$ at a concentration of 0.2 M. Gl dissolves well in this solvent, and the concentration dependence of the reduced viscosity is linear. The presence of low-molecular-weight salt $NaNO_3$ screens the electrostatic interaction in Gl chains.

At a Gl concentration of 0.1% in water, this solution has good fluidity at room temperature. In [11], Gl-based gels formed using low-molecular-weight salts NaCl and $CaCl_2$ were studied. Adding NaCl or $CaCl_2$ to the Gl solution led to the solution losing its fluidity and forming a homogeneous elastic mass. In this work, a combination of these two salts was used to obtain gels. At the first stage, $CaCl_2$ was added to the Gl solution in such a concentration that at a temperature of 25°C the resulting solution remains liquid for a long period (more than 2 days), but loses its fluidity with a further increase in the concentration of $CaCl_2$. The concentration of calcium ions in the resulting system was such that there was approximately one calcium ion per two Gl monomer units; it was 3.8 mM. The NaCl solution was then added to the solution, which resulted in the formation of a gel.

The values of the elastic moduli G' and losses G'' of the gel on the deformation amplitude γ at small amplitudes are constant, which indicates the linearity of the mechanical behavior. This region of constant values is quite extensive, but when the critical value is reached γ^* there was a fairly sharp drop in G' and an increase in G'', which indicates a transition from a solid to a fluid state of the gel (Fig. 3). This behavior is typical for systems in a gel state [13].

Noticeable changes in the properties of solutions of Pu and graft-copolymer Pu-pMO in the presence of the divalent salt $CaCl_2$ in water was not observed. According to the DLS data, the pMO sample in water had a bimodal distribution of the scattering intensity over hydrodynamic radii (Fig. 1); the first peak corresponding to the diffusion of individual molecules. The mass fraction of the second component in the solution was insignificant; the contributions of both components were compared according to the relation $I \sim cR_h^{\alpha}$ [13], here I is the scattering intensity, c is the mass concentration of particles, and α is the exponent connecting the size of a particle and its volume. To assess the contribution of the second component, we selected $\alpha = 3$, which corresponds to close spherical packing, and $\alpha = 5/3$, which corresponds to a polymer in a good solvent. An estimate based on the dense sphere model gives a contribution of large particles, close to zero, and for the polymer model in a good solvent, it is approximately 0.2%. Apparently, the partial aggregation of Pu chains occurs, but their mass fraction in the solution is insignificant.

The Pu sample and the grafted Pu-pMO copolymer, according to the DLS data, exhibited a unimodal distribution of hydrodynamic radii in the solution. It was found that after modification of the Pu macromolecules with pMO side chains, the molar mass of the resulting Pu-pMO graft copolymer noticeably decreases (Table 1). This is probably due to the fact that the partial degradation of Pu occurs during the click reaction [6].

For all studied polymers, the values of the hydrodynamic invariant were calculated according to the relation $A_0 = (M_{sD}[\eta])^{\frac{1}{3}}\eta_0 D_0/T$. The A_0 values range from 3.0×10^{-10} up to 3.6×10^{-10} erg/(K mol$^{1/3}$), which is typical for flexible chain polymers in a good or θ solvent.

Gels based on gellan and pullulan modified with poly(2-methyl-2-oxazoline). The preparation of Gl-based gels was carried out as follows: an aqueous solu-

Table 1. Molecular characteristics of the polymers studied in this work

Sample	Solvent	[η], dL/g	$D_0 \times 10^7$, cm^2/s	$s_0 \times 10^{13}$, s^{-1}	$M_{sD} \times 10^{-3}$, g/mol	$A_0 \times 10^{10}$, erg/(K $mol^{1/3}$)
Pu	Water	0.7	1.8	7.24	280	3.1
Pu-pMO	Water	0.3	2.8	4.46	200	3.3
pMO	Water	0.1	14.4	0.68	6	3.6
Gl	DMSO 0.2 $NaNO_3$	16.1	0.19*	1.38	520	3.0

*Diffusion coefficient was determined from the AUC data.

tion of Pu-pMO warmed up to 60° was added to a solution of Gl in water with constant stirring. The Gl concentration in the final mixture was 0.1 g/dL. The concentration of Pu-pMO in the total mixture was varied from 7 to 50% by weight of Gl. Next, an aqueous solution of $CaCl_2$ was added to the mixture, so that its final concentration is 5 mM. The result was a mixture with a volume of 2 cm^3; visually it had high fluidity. After adding 1 cm^3 of the aqueous solution of NaCl with a concentration of 0.2 M to the mixture, a gel formed. It has been established that when heated to 70°C, the samples become fluid and, upon cooling, form a gel again. This property was used when applying gels to the measuring surfaces of the rheometer.

The nature of the dependences G' and G'' obtained for Gl systems with the addition of Pu-pMO was similar to that observed for pure Gl. It was found that an increase in the concentration of Pu-pMO in the gel composition leads to a decrease in the value G', measured on the horizontal section of the amplitude dependence (Fig. 3). The decrease in G' is apparently due to a reduction in the number of network nodes formed by divalent calcium ions and Gl molecules. It was also found that the gel strength decreased with an increase in the proportion of Pu-pMO in its composition, the value γ^* for the studied systems decreased from 4.6 to 1.7% with an increase in the proportion of Pu-pMO from 7 to 28% (Table 2).

A drop in the elasticity of gels in the presence of Pu-pMO in the system from 7 to 28% probably indicates that this polymer is integrated into the gel structure and prevents the formation of calcium bridges between Gl monomers. In this case, an increase in the concentration of Pu-pMO in the gel composition should lead to an increase in the degree of gel hydration. As can be seen from the experimental data, the elasticity ceases to change with a further increase in the proportion of Pu-pMO; for systems with 28 and 50% PU-pMO in the gel composition, the amplitude dependences of the moduli G' and G'' turned out to be close. This may be due to the fact that in this system a stable system of bonds is formed between Gl macromolecules of constant concentration, when an increase in the concentration of Pu-pMO in the considered range is no longer able to affect its physical and chemical properties.

Table 2. Limiting strains and recovery times of Gl gels with Pu-pMO

Proportion of Pu-pMO in the gel, % Gl mass content	g^*, %	$t_{recover}$, s
7	4.6	880
14	2.8	930
28	1.7	170
50	2.2	190

Estimation of the recovery rate of the gel after exposure to deformation $\gamma = 100\%$ showed that gels with Pu-pMO concentrations of 28 and 50% recover their structure most quickly, this happens over the time $t_{recover}$, approximately equal to 3 min. Gels with 7 and 14% Pu-pMO concentrations recovered in approximately 15 min. This increase in the rate of gel recovery with increasing proportion of Pu-pMO in the mixture may be due to the fact that this polymer with its hydration environment acts as a plasticizer in the gel composition. Thus, it facilitates tuning of the system to form a network of intermolecular bonds. On the other hand, the absolute values of the elastic modulus for systems with a high content of Pu-pMO (28 and 50%) are on average 2 times lower than for those with a low Pu-pMO content (7 and 14%) (Table 2), which also affects the rate of gel recovery.

CONCLUSIONS

Three-component systems based on Gl and Pu-pMO with the addition of $CaCl_2$ were obtained, which form gels upon contact with a solution of NaCl. This opens up the prospect of their use as bioactive agents capable of transforming into a gel state upon contact with biological fluids. In particular, such systems are applicable in ophthalmology.

It has been shown that at such concentrations of Ca^{2+} ions, when their total charge in the solution is

close to that of gellan chains (assuming complete dissociation), gelation occurs after adding NaCl solution. The presence in the solution of Pu-pMO leads to noticeable changes in the elastic properties of the gels, indicating that Pu-pMO is integrated into the gel structure. The resulting gels were not destroyed when heated to temperatures of ~70°C, despite the fact that, according to [11], in the presence of only Na^+ ions with an increase in temperature, the gel structure is destroyed even at temperatures below 30°C. This behavior of the studied gels demonstrates the possibility of their practical use for the transport of drugs to body tissues in the presence of biological fluids in a wide temperature range.

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CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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