Specifics of self-organization and properties of highly dilute aqueous solutions of polyoxidonium

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Using a combination of physicochemical methods (dynamic and electrophoretic light scattering (DLS and ELS, respectively), nanoparticle tracking analysis, atomic force and transmission electron microscopy (AFM and TEM, respectively), UV spectroscopy, conductometry, pH-metry, dielcometry), it was found that dilute solutions of a multicomponent drug immuno-modulator polyoxidonium (PO) are nanoheterogeneous disperse systems, with their disperse phase undergoing considerable restructurings when diluting the solution in the range of calculated concentrations from $1 \cdot 10^{-1}$ to $1 \cdot 10^{-16}$ mg mL^{-1}, which is reflected in the non-monotonous concentration dependencies of specific electroconductivity, dielectric permittivity, and pH of the solutions. Using ELS, AFM, TEM, and UV spectroscopy methods, it was found that the disperse phase with a size of hundreds of nanometers which forms at concentrations of $\leq 1 \cdot 10^{-5}$ mg mL^{-1}, contains organized water structures substantiating the negative values of ζ-potential, which vary non-monotonously from -5 to -16 mV. Radioprotective properties of dilute solutions of PO ($1 \cdot 10^{-1}$ and $1 \cdot 10^{-9}$ mg mL^{-1}) were demonstrated for the first time when exposing the test mutant bacterial strain *Salmonella typhimurium TA 100* (Ames test) to X-ray radiation in a dose of 7.50 mGy used for medical diagnostics.

Key words: self-organization, dilute solutions, disperse system, aqueous nanoassociates, supramolecular domains, multicomponent drug immunomodulator polyoxidonium, radio-protective properties.

Recently,[1] using a combination of physicochemical methods it was found that dilute aqueous solutions of many biologically active compounds (BAC) in a wide range of calculated concentrations (10^{-20}—10^{-6} mol L^{-1}) are self-organizing disperse systems, the disperse phase of which are massive nanosized molecular ensembles (up to 400 nm, ζ-potential equal to 2—20 mV), called nanoassociates. It was found that the formation of nanoassociates and their precursors, supramolecular domains,[2] formed in the regions of higher concentrations, substantiates the appearance of non-monotonous concentration dependencies of physicochemical properties and, most importantly, correlates with the biological activity of highly dilute solutions. The extreme values of the parameters of nanoassociates, physicochemical characteristics of solutions and bioeffects are observed in virtually the same concentration intervals, which makes it possible to predict the appearance of

a bioeffect of highly dilute solutions.[3] In most cases, an external low-frequency electromagnetic field (EMF), a special procedure for the preparation of solutions and a certain structure of BAC are necessary for the formation of nanoassociates.

At the present time, when searching for the physicochemical explanation of the action of highly dilute solutions, important questions related to the determination of the nature of the nanoassociates and their role in the structure formation and functioning of complex systems, including biological ones, remain unanswered.

In order to obtain answers to these questions, we studied the self-organization, physicochemical, and biological properties of a model multicomponent system, *i.e.*, the dilute solutions of the domestic drug with a wide range of applications called immunomodulator polyoxidonium (PO, international nonproprietary name azoxymer

bromide) (NPO Petrovaksfarm).[4] The active component of the drug is the copolymer of *N*-oxy-1,4-ethylenepiperazine and (*N*-carboxy)-1,4-ethylenepiperazinium bromide (**1**) with a molecular mass of ~80 kDa. The supporting compound, which gives the drug the antioxidative properties, is β-karotin.[5] The solubility and the bioavailability of the PO are improved by a water-soluble polymer polyvinylpyrrolidone (**2**) and mannite.

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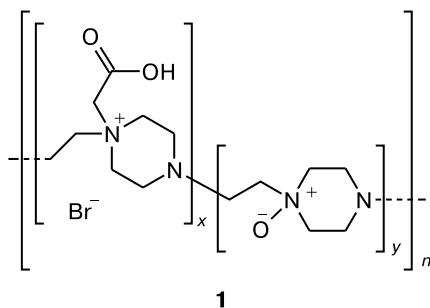
The use of solutions of PO for treatment of humans and animals is related to the appointment of allopathic doses. It is known that many pharmacological drugs can have a considerable effect on living organisms at much lower concentrations as well, however the pharmacological profiles of solutions of the drugs in the low concentration range have a complex character that is difficult to predict.[6] As it is shown in the review,[1] the prognosis of the appearance of non-monotonous dependencies of concentration—bioeffect upon treatment with highly dilute solutions of BAC can be based on the result of the study of self-organization and physicochemical properties of such solutions.

Lately, as a result of an increase in the use of ionizing radiation,[7,8] active searches for efficient radioprotectors with a possibility of application in complex therapy are being carried out. Due to the specifics of its chemical structure, polyelectrolyte **1** can be bound to DNA phosphate groups and lipids of membranes, which are among the main biological targets of radiation, stabilizing these structures,[9] while β-carotene protects the cells from damage by active forms of oxygen and free radicals formed during exposure to radiation.[5,9] Related to this, one of the possible promising directions for the application of dilute solutions of PO is radioprotective action, used at the present time in therapeutic doses in oncological practice during and after radiation and chemotherapy of tumors.[4]

The goal of the work is the study of the self-organization and the physicochemical properties of highly dilute aqueous solutions of multicomponent drug PO, as a result of which a prediction of the possibility of bioeffect appearance and its testing will be carried out, using the example of radioprotective properties of dilute solutions of PO upon treatment of microorganisms with X-ray radiation in the interval of doses used for medical diagnostics.

Experimental

The self-organization and the physicochemical properties of solutions of PO for a wide range of dilutions, corresponding to calculated concentrations from $1 \cdot 10^{-16}$ to 3 mg mL^{-1}, were studied by dynamic and electrophoretic light scattering (DLS and ELS, respectively) methods (Zetasizer Nano ZS analyzer, Malvern Instruments, Great Britain), nanoparticle tracking analysis (NTA) (Nanosight LM 10 analyzer, NanoSight, Great Britain), atomic force microscopy (AFM, MultiMode V scanning probe microscope, Veeco, USA), transmission electron microscopy (TEM, HT 7700 transmission electron microscope with an accelerating voltage of 80.0 kV, Hitachi, Japan), UV spectroscopy (UV/Vis Spectrometer Lambda 35, Perkin—Elmer, USA), conductometry (inoLab Cond Level 1 conductometer, WTW), dielkometry (BI-870 dielkometer, Brookhaven Instruments), and pH-metry (inoLab pH 720 ionometer, WTW). When studying solutions of PO by AFM and TEM methods, 10 μL of the solution was applied to the substrate surface, which was either mica (AFM) or a copper grid (200 mesh) coated with the polymer formvar (TEM), and dried for 4 h at 80 and 60 °C, respectively. Analysis by the AFM method was carried out in a tapping mode using a RTESP silicon rectangular cantilever (Veeco, USA) with a rigidity of 40 N m^{-1} and resonant frequency of 300 kHz, the radius of needle curvature was 10—13 nm.

The term "concentration" used in this work to describe highly dilute solutions ($1 \cdot 10^{-16}$—$1 \cdot 10^{-1}$ mg mL^{-1}) refers to the calculated concentration. For the preparation of solutions, only freshly prepared bidistilled water was used, in which a complete absence of particles was recorded by a Zetasizer Nano ZS analyzer (Malvern Instruments, Great Britain) as described earlier.[1] The specific electroconductivity of such water was ≤1.5 μS cm^{-1}. Initially the drug PO (lyophilisate), diluted with bidistilled water according to the procedure[4] was used for the preparation of a solution for injections with a PO concentration of 3 mg mL^{-1}, which was a transparent colorless solution. For the preparation of highly dilute solutions, a solution with a concentration of $1 \cdot 10^{-1}$ mg mL^{-1} was used.

Working solutions with a volume of 10 mL were prepared by a method of sequential decimal dilutions with solutions of each concentration being allowed to stand for 20 h. The procedure for the analysis of highly dilute solutions developed earlier[1] involves the study of self-organization and properties of such solutions in two parallel series. The only difference between the first and the second series was that in the first series the working solutions were allowed to stand under ambient conditions (on the laboratory bench) before studying them by physicochemical methods, whereas in the second series, the conditions were hypoelectromagnetic, *i.e.*, in a cylindrical three-layer permalloy container, which protected its contents from external EMF with a screening coefficient of ~1000. The use of such a procedure allows one to establish a threshold concentration (c_{thr}), with nanoassociates being formed in solutions below it, and supramolecular domains being formed above it.[1]

The procedure for the preparation of the samples for the study of sizes and ζ-potential provided the necessary "dedusting" of solutions (disposable Iso-Disc N-25-4 Nylon filters were used, Supelco, USA). The solutions were stirred for 10 s using the IKA Lab Dancer minishaker. Before measurement, the working solutions were thermostated for 1 h at 25±0.1 °C.

In order to determine the influence of dilute solutions of PO on the genotoxic effects of irradiation, the toxicity and mutagenicity of irradiation of a test mutant bacterial strain *Salmonella typhimurium TA 100* (Ames test) were studied on an X-ray computer tomograph and digital fluorograph with a total dose per sample from 0.67 to 22.79 mGy in the absence and in the presence of solutions of PO as described earlier.[10] A solution of PO in the ratio of 1 : 10 (v/v) was added to the bacterial culture, stirred and applied onto a 2% LB agar in a Petri dish before irradiation, after which the culture was incubated for 24 h at 37 °C, and then the number of grown colonies in the test and control samples was determined.

The statistical analysis of the results was carried out by the parametric statistics method using the Microsoft Excel computer program with a statistical reliability of 95%. The errors in the measurements of physicochemical properties of solutions and of toxicity of irradiation were in the 2—20% range.

Results and Discussion

The study of aqueous solutions of multicomponent drug PO by DLS and ELS methods showed that the dilute solutions of PO are complex dispersed systems, which, when highly diluted, similar to one-component systems based on many low-molecular-weight BAC,[11] have a tendency to simplification and monomodal distribution of particle by size.

In the solution for injection with a concentration of 3 mg mL^{-1} we observed three separate maxima, which are responsible for the formation of particles, the size of which is in the range of several, tens, and hundreds of nanometers (Fig. 1, *a*). According to the data presented in the works,[12,13] consistently recorded separate maxima on the DLS curves indicate the coexistence of particles of different nature in the solution.

In the range of concentrations from $1 \cdot 10^{-1}$ to $1 \cdot 10^{-4}$ mg mL^{-1} (Fig. 1, *b*), the distribution of particle sizes is also trimodal. The particles with a size of tens of nanometers can be supramolecular associates, including polymeric micelles **1** of the type core—shell,[14,15] whereas particles with a size of hundreds of nanometers can be domains[2,13] formed by molecules or associates of the dissolved compounds and water structures. The "cut-off"
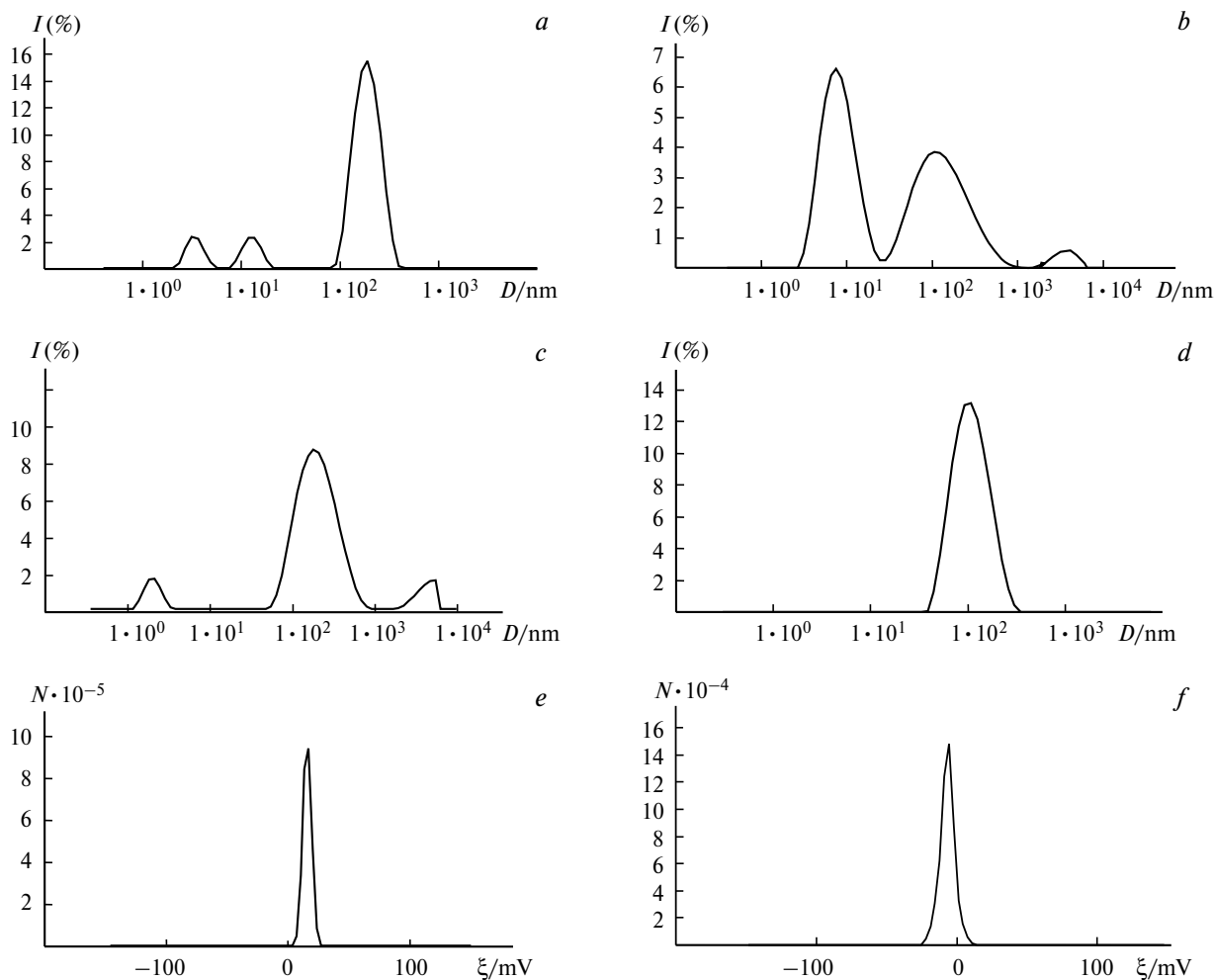

Fig. 1. Distribution of particles by size (*a*—*d*) and ζ-potential (*e*, *f*) in aqueous systems of PO with a concentration of 3 (*a*), $1 \cdot 10^{-1}$ (*b*), $1 \cdot 10^{-4}$ (*c*, *e*), $1 \cdot 10^{-14}$ (*d*), and $1 \cdot 10^{-9}$ mg mL^{-1} (*f*); 25 °C.

maximum in the range of >1000 nm, located beyond the working capabilities of the analyzer, indicates the formation of much more scaled particles with a size of >10 μm in the solution.

At concentrations of $\leq 1 \cdot 10^{-3}$ mg mL^{-1}, the domains with a size of hundreds of nanometers, composing 70—100% based on the intensity of light scattering, predominate in solutions of PO (Fig. 1, *c*, *d*), which is typical of dilute aqueous solutions of BAC.[1,11] When diluting the solution and decreasing the concentration from $1 \cdot 10^{-1}$ to $1 \cdot 10^{-4}$ mg mL^{-1}, the size of supramolecular domains changes non-monotonously from 120 to 300 nm, whereas the ζ-potential determined, probably, by cationic micelles **1** decreases non-linearly from +70 to +2 mV (Fig. 1, *e*).

In the case of more highly dilute solutions of PO for virtually all the calculated concentrations and, in particular, in the region from $1 \cdot 10^{-13}$ to $1 \cdot 10^{-16}$ mg mL^{-1}, a monomodal distribution of the size of particles with a diameter of hundreds of nanometers is observed (see Fig. 1, *d*), and, less often, a bimodal distribution with the appearance of an additional "cut-off" maximum in the >1 μm region. The particle size is in the range from 100 to 250 nm, depending on dilution, whereas the ζ-potential changes its sign to the opposite, changing non-monotonously from −5 to −16 mV (Fig. 1, *f*), which is a feature characteristic of the formation of nanoassociates in the dilute solution,[1,6] which are formed mainly by water structures.

The study of solutions of PO with calculated concentrations $1 \cdot 10^{-1}$—$1 \cdot 10^{-16}$ mg mL^{-1} maintained in a permalloy container by the DLS method showed that in con-

trast to one-component solutions of low-molecular-weight BAC,[1] for which it is normally possible to determine a threshold concentration (c_{thr}), *i.e.*, a concentration, below which nanoassociates are formed in dilute solutions, in solutions of PO there is a range of such concentrations from $1 \cdot 10^{-4}$ to $1 \cdot 10^{-6}$ mg mL^{-1} determined in several repeated experiments. Most likely, the obtained result, similarly to the one described earlier,[12] is related to the unstability of a complex disperse system containing disperse phases of different nature, which makes the system quite sensitive to external influences. This means that in such systems, the formation and restructuring of nanostructures, as well as the transformation of domains into nanoassociates, can be dependent on a wider complex of factors than it was found[1] for highly dilute one-component solutions of low-molecular-weight BAC.

When studying the specific electroconductivity (χ), the dielectric permittivity ($\Delta\varepsilon = \varepsilon_S - \varepsilon_{solv}$, where ε_S and ε_{solv} are the dielectric permittivities of the solution and the solvent, respectively), and the pH of aqueous systems of PO in the region of calculated concentrations of $1 \cdot 10^{-1}$—$1 \cdot 10^{-16}$ mg mL^{-1} (Fig. 2), it was found that when diluting the solutions, the χ values change in a non-monotonous manner in the 12—2 μS cm^{-1} range, $\Delta\varepsilon$ changes from −1 to +2, and the pH changes from 7 to 6 pH units, which is typical of the systems based on dilute solutions of BAC.[1,6,13] For dilutions corresponding to the calculated concentrations of $1 \cdot 10^{-6}$, $1 \cdot 10^{-8}$—$1 \cdot 10^{-9}$, $1 \cdot 10^{-11}$—$1 \cdot 10^{-12}$, and $1 \cdot 10^{-14}$ mg mL^{-1}, the concentration dependencies of χ, $\Delta\varepsilon$, and pH exhibit maxima, caused, as
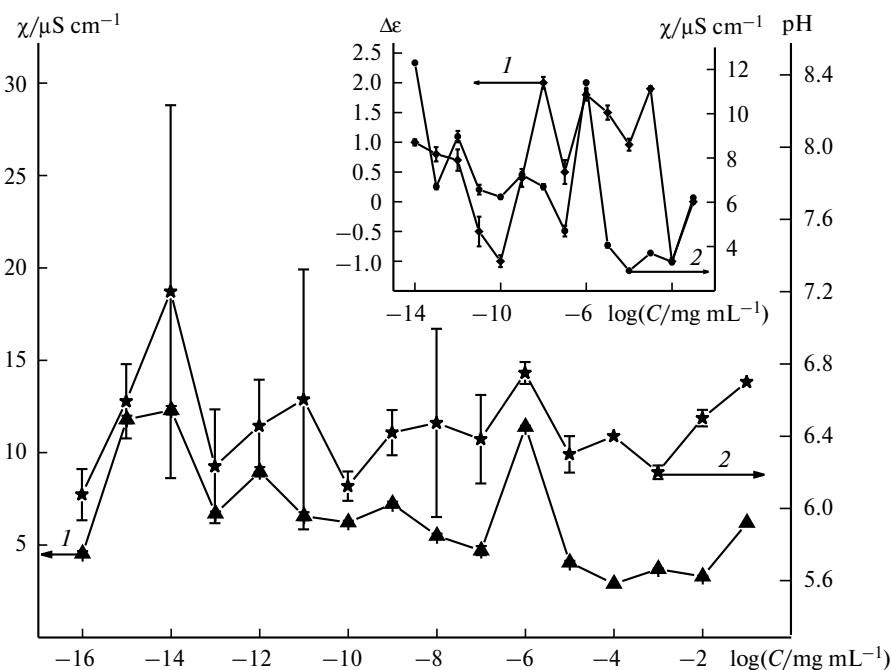
Fig. 2. A dependence of specific electroconductivity χ (*1*) and pH (*2*) of aqueous solutions of PO on concentration; 25 °C. The inset shows the dependence of dielectric permittivity $\Delta\varepsilon$ (*1*) and specific electroconductivity (*2*) of aqueous solutions of PO on concentration; 25 °C.

was demonstrated earlier,[1,6,16] by the formation and the restructuring of domains and nanoassociates. In such a case, it is possible to predict the biological activity of the aqueous systems based on dilute solutions of PO, the concentration—bioeffect dependence curve can have a non-monotonous profile and extreme effects in the vicinities of the indicated concentrations.[1,3]

The formation in disperse systems based on highly dilute aqueous solutions of PO of particle with sizes of hundreds of nanometers was confirmed by the NTA method, which makes it possible[2,13,17] to determine the size and the number of particles per 1 mL of a solution, as well as visualize them. For the study of the systems by the NTA method, the PO concentrations of $1 \cdot 10^{-1}$, $1 \cdot 10^{-6}$, $1 \cdot 10^{-8}$, and $1 \cdot 10^{-9}$ mg mL^{-1} were chosen, at which domains and nanoassociates are formed. It was found that in all the studied solutions, the particles with sizes of tens and hundreds of nanometers are visualized, their number per 1 mL of the solution is $4.2 \cdot 10^9$, $7.5 \cdot 10^7$, $2.5 \cdot 10^8$, and $1.5 \cdot 10^8$, respectively, and the average diameter is 100 ± 9, 208 ± 12, 104 ± 0.7, and 76 ± 9 nm. The number of particles in bidistilled water is $\leq 1 \cdot 10^6$ per 1 mL, i.e., two to three orders of magnitude lower than in the studied solutions.

It is important to note the following fact, which was observed for the first time: when decreasing the calculated concentrations of PO by five orders of magnitude from $1 \cdot 10^{-1}$ to $1 \cdot 10^{-6}$ mg mL^{-1}, within the bounds of which domains and nanoassociates exist, the number of particles decreases by a factor of 55 from $4.2 \cdot 10^9$ to $7.5 \cdot 10^7$ per 1 mL. A further decrease of the concentration of PO by three orders of magnitude from $1 \cdot 10^{-6}$ to $1 \cdot 10^{-9}$ mg mL^{-1} is accompanied by a non-monotonous 2—3-fold increase in the number of particles. The obtained result, on one hand, confirms that the self-organization of highly dilute solutions is a complex non-linear process, not directly related to the concentration of the dissolved compound, and, on the other hand, indicates that the formation of an unusual disperse phase, i.e., the nanoassociates with sizes of tens and hundreds of nanometers, is caused, first of all, by water structures with a minimal involvement of compound molecules or even without them.

Lately, AFM and TEM methods are successfully used for the visualization and study of particularities of structures formed in highly dilute aqueous solutions. These methods are used for analyzing the surface of the substrates after the aqueous solutions applied to them are dried.[12,14,16,18—20] For the purpose of investigating by AFM and TEM methods the processes, which occur during the dilution of solutions of PO, we have chosen the calculated concentrations $1 \cdot 10^{-2}$, $1 \cdot 10^{-5}$, and $1 \cdot 10^{-6}$ mg mL^{-1}, at which the sharp changes of physicochemical properties of the solution occur (see Fig. 2). For the solution with a concentration of $1 \cdot 10^{-2}$ mg mL^{-1}, AFM-images of the structure with a complex topology were obtained (Fig. 3, a—d). The substrate is covered by particles with a non-uniform

composition and a size of 200—1000 nm composed of one to four "dense" globules, formed by the aggregates of micelles 1, with a diameter of 100—200 nm and a height of 10—15 nm, which are surrounded by a "soft" film with a height of 1—4 nm and a width of 100—300 nm. As it was shown in the works,[16,18,19] the film is, quite possibly, the ordered structured water remaining not dried up on the substrate. The branched thick web of threads formed by polymer 2 with globules strung together on them, piercing the "soft" film of the particles and conglomerates, is visible virtually throughout the whole substrate (see Fig. 3, c). The conglomerates with a size of ~6 μm, similarly to particles, consist of a dense core with a size of 1—2 μm formed by a large congestion of globules, and the film surrounding it. On the conglomerate core, the "noise" in the image (the "scales") is visible, caused by the excitation of the AFM probe as a result of its interaction with a more viscous region of the surface. This effect is thought to be caused by the adsorbed water,[12] which indicates the ability of polymers 1 and 2, forming the globules and the threads, to efficiently bind and retain water, similar to some proteins.[21] On the substrate, are also visible AFM-images typical of polyelectrolytes of the dendrite fractal structures[14] with a size of tens of micrometers (see Fig. 3, d), consisting of granules with a diameter of 500—700 nm and a height of 20—40 nm.

When the dilution of solutions is accompanied by a decrease in the calculated concentration by several orders of magnitude, the observed AFM image is considerably simpler. Figure 3, e, f shows AFM-images obtained for solutions of PO with concentrations of $1 \cdot 10^{-5}$ and $1 \cdot 10^{-6}$ mg mL^{-1}. In both cases, a thin film with a branched form is visualized on the substrate, which has a height of 1.3 nm with lateral sizes of 100—1000 nm. At an even lower concentration of the solution, for example, $1 \cdot 10^{-6}$ mg mL^{-1} (see Fig. 3, f), the lateral sizes of the film become larger, and the number of single particles with a height of 1.3 nm and a diameter of 20—60 nm increases considerably.

The observed picture reflects the considerable changes occurring in the solution when it is being diluted and confirms the conclusion made based on the NTA data that the self-organization of a highly dilute solution is a complex non-linear process, not directly related to the concentration of the dissolved compound. The works[16,18,19] show that a similar film found on the AFM-images of dilute solutions of calixarene and sodium chloride is a result of the formation of aqueous clusters such as domains or nanoassociates in the solutions, which can also be visualized by the TEM method.[18—20]

The results of the study of a solution of PO with a concentration of $1 \cdot 10^{-5}$ mg mL^{-1} (Fig. 4, a) using the TEM method indicate the formation of structures-"containers" on the substrate, which are blurred on the periphery of ellipses and circles with a size of 200—1000 nm, and containing in the center cationic micelles 1 with a size of
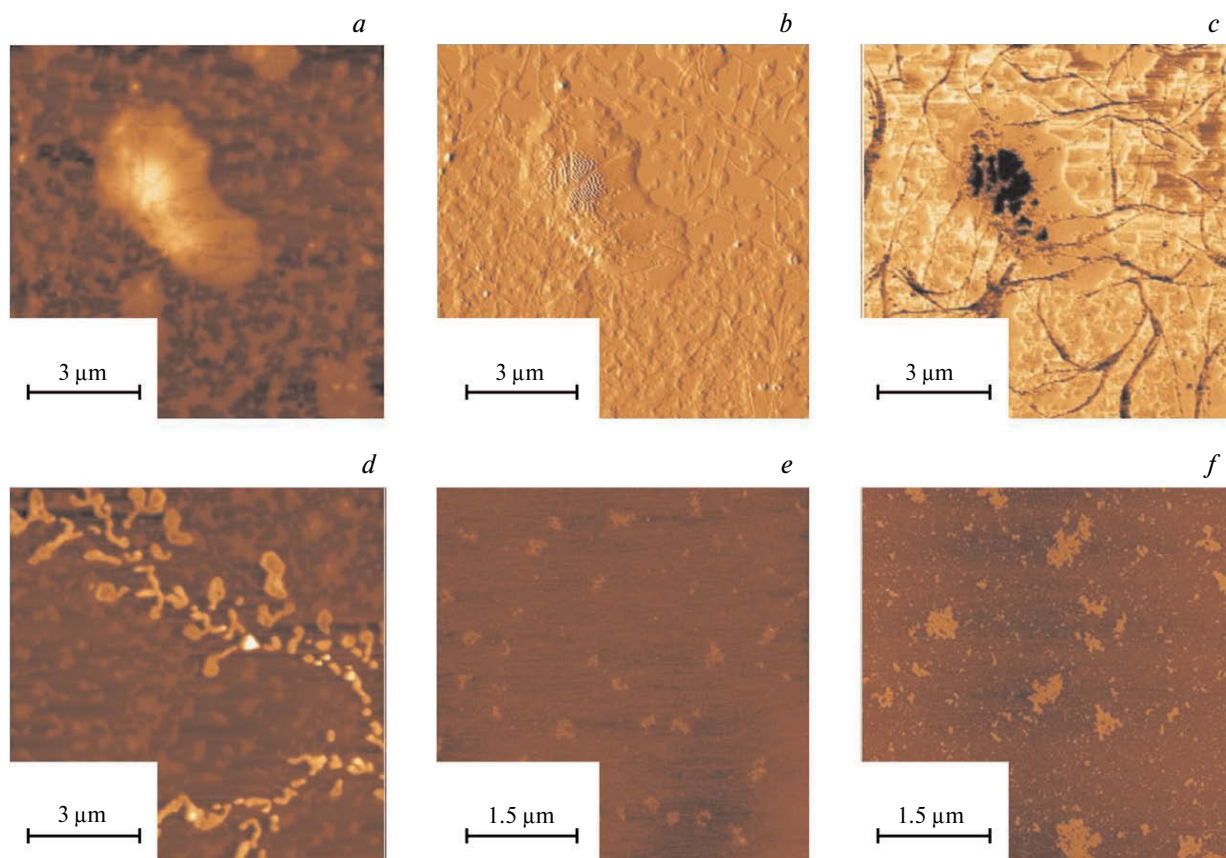
Fig. 3. Images, obtained using the AFM method, of an aqueous solution of PO precipitated on mica in different concentrations: (*a*) $1 \cdot 10^{-2}$ mg mL^{-1}, *z*-axis scale is 100 nm; (*b*) $1 \cdot 10^{-2}$ mg mL^{-1}, derivative; (*c*) $1 \cdot 10^{-2}$ mg mL^{-1}, phase contrast; (*d*) $1 \cdot 10^{-2}$ mg mL^{-1}, *z*-axis scale is 100 nm; (*e*) $1 \cdot 10^{-5}$ mg mL^{-1}, *z*-axis scale is 5 nm; (*f*) $1 \cdot 10^{-6}$ mg mL^{-1}, *z*-axis scale is 5 nm.
Note. Fig. 3 is available in full color on the web page of the journal (http://www.link.springer.com).

20—50 nm in a concentrated form.[15] A characteristic feature of this structures is a clear dark dense circle bordering the central part and becoming progressively lighter when moving away from the center. It is known that the dark areas of TEM-images indicate that electrons, when passing these areas, are more strongly scattered.[18,19] Most likely, the dark circles bordering positively charged micelles **1** are the threads of polymer **2** with adsorbed negatively charged layers of the so-called surface (interfacial) water,[21,22] which are a matrix for the formation of subsequent less charged layers. The obtained data is in agreement with the results of the study of solutions of PO by the ELS method, since approximately in this range of concentrations the ζ-potential of particles becomes negative, which indicates the formation of negatively charged surfaces in the structure of domains or nanoassociates in the solution.

The results of the study of a solution of PO with a concentration of $1 \cdot 10^{-6}$ mg mL^{-1} by the TEM method (Fig. 4, *b*, *c*) in an even more persuasive manner than that of the AFM-images indicate the considerable restructuring of the structures when diluting the solution, which in its turn is reflected in the TEM-images. Branched planar

fractals of micelles **1** with a length of >1000 nm are visible on the substrate. They resemble dendrites, which are formed at a concentration of $1 \cdot 10^{-2}$ mg mL^{-1}, as well as bulky structures similar to those presented in the works,[18,19] which are formed by slightly bent dark threads of polymer **2** and clusters of structured water adsorbed on them. Clearly, as a result of the solution dilution procedure a stratification of the "containers" occurred (see Fig. 4, *a*), leading to the concentrates of micelle **1**, located in the center, relocating to the solution bulk. As the solution was drying after being applied on the substrate, a further concentration of micelles **1** occurred, during the course of which dendrite fractals were formed (see Fig. 4, *a*, *b*). Hydrophilic threads **2** and structured layers of interfacial water bound to them were transformed into the bulky, flexible, elastic spherical forms, consisting of dark curving threads and lighter clusters surrounding them. In Fig. 4, *c*, the clusters of structured water, which "sit" on the dendrite branches of micelle **1**, are clearly visible.

Earlier it was found[19,22] that the formation of "water clusters" and "interfacial water" with a size of hundreds and thousands of nanometers, which possess a number of
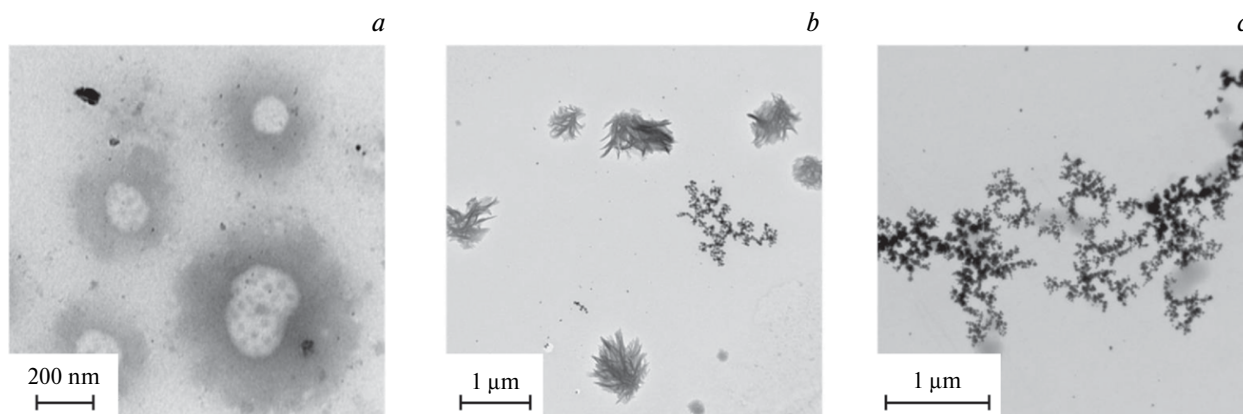
Fig. 4. Images, obtained using the TEM method, of an aqueous solution of PO precipitated on formvar in a concentration of $1 \cdot 10^{-5}$ (*a*), $1 \cdot 10^{-6}$ mg mL^{-1} (*b* and *c*); *b* and *c* are TEM-images of different areas of the substrate surface.

specific properties of "soft" nanomaterials, is accompanied by the appearance of an absorption band with a maximum in the region of 270 nm in the UV spectra of solutions. The study of solutions of PO using UV spectroscopy has shown that at a concentration of $\leq 1 \cdot 10^{-5}$ mg mL^{-1}, a band with a maximum at 270 nm appeared in the spectra of the solutions. This can be an indication that "water clusters", "interfacial water", domains, and nanoassociates have the same nature.

The observed picture made it possible to demonstrate and explain for the first time how when undergoing treatment with external factors, such as the solution dilution procedure, for example, a restructuring of the disperse phase formed in dilute solutions can occur, which determines the change of its parameters and the appearance of abnormal physicochemical properties and bioeffect of solutions related to it. The phenomenon of concentration of the dissolved compound ("concentration factor") in the "container", which is a domain or a nanoassociate, and the subsequent emergence of this concentrate in the solution

bulk is quite capable of causing a sharp change of physico-chemical properties of a highly dilute solution and of creating conditions to cause an effect on the biosystem, which can be virtually adequate to the interaction of the solution with a common concentration of the compound.

Earlier,[23] when studying the influence of highly dilute solutions of PO on the organism of laboratory male rats, it was shown that injecting the rats with solutions of PO with calculated concentrations of 10^{-6}, 10^{-9}, and 10^{-14} mg mL^{-1} can be used for preventative purposes in order to improve the immune status of the animal organisms.

Within the framework of this work, we carried out a check of the radioprotective action of the dilute solutions of PO in concentrations of 10^{-1} and 10^{-9} mg mL^{-1} when exposing the culture of the test mutant bacterial strain (*Salmonella typhimurium TA 100*) to radiation on an X-ray computer tomograph in the "Brain native standart" (1) and the "Abdomen native" (2) regimes and on a digital fluorograph (3) with an absorbed X-ray radiation dose of 22.79, 7.5, and 0.67 mGy, respectively (Fig. 5).
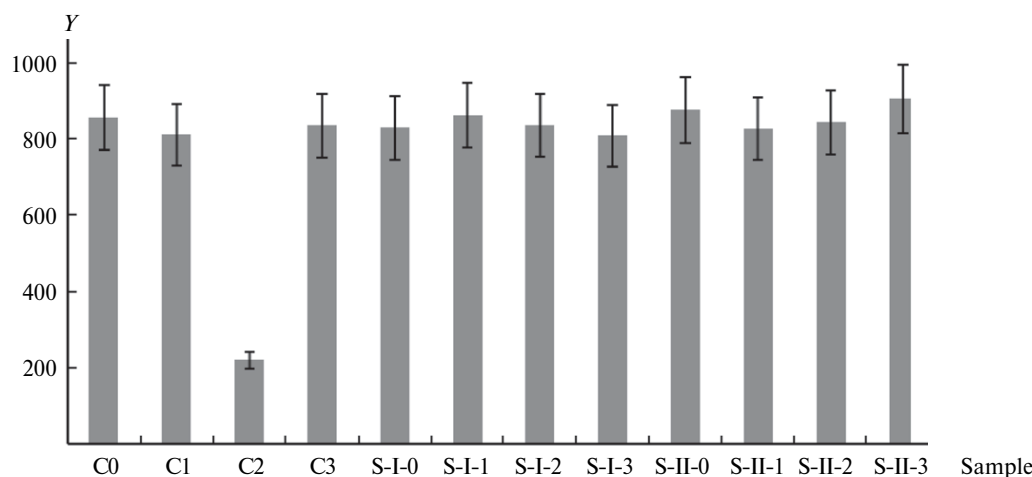

Fig. 5. An estimate of the toxicity of X-ray radiation against the test microorganisms *Salmonella typhimurium TA 100* without and with the addition of solutions of PO: C is control; 0 is without irradiation; 1—3 are irradiation regimes (see text); S-I and S-II are samples with a concentration of PO equal to $1 \cdot 10^{-9}$ and $1 \cdot 10^{-1}$ mg mL^{-1}, respectively; *Y* is the number of CFU on the Petri dish.

When studying[10] the influence of X-ray irradiation (a total dose on the sample equal to 0.67—74.5 mGy) on the cultures of test mutant gram-negative bacterial strains (*Salmonella typhimurium TA 100*), it was found that the X-ray diagnostics, with the exception of the procedures carried out on the digital small-dose apparatuses, has a toxic and weakly mutagenic (Ames test) effect on the bacteria, the degree of severity of which is dependent on the dose in a non-monotonous manner.

In the course of the work, it was found that regimes 1 and 3 do not exhibit an antimicrobial action. Only for irradiation regime 2 (7.5 mGy) a decrease of the number of colony-forming units (CFU) by a factor of 4 was demonstrated for the version that underwent exposure to radiation as compared to the one that did not, *i.e.*, the culture partially dies when treated with the radiation in this regime or loses the ability to reproduce. The obtained data is in agreement with the results of the study[24,25] of the phenomenon of hyper-radiosensitivity in the range of small doses, in particular, for the dose of ~10 mGy,[25] after which a further increase of the dose led to an increased radioresistivity.

Adding to the bacterial culture solutions of PO in concentrations of 10^{-1} and 10^{-9} mg mL^{-1} before irradiation protects the cells from the damaging effect of radiation, which is indicated by the equal amount of CFU in the versions with an addition of a solution of PO during irradiation, without irradiation, and in the control sample (see Fig. 5). When determining the mutagenic effects of the indicated irradiation doses and solutions of PO in the Ames test, it was found that the studied irradiation regimes and solutions of PO do not possess mutagenic activity. Thus, it was found that dilute solutions of PO exhibit radioprotective properties in concentrations of 10^{-1} and 10^{-9} mg mL^{-1}, eliminating the negative effects of treatment with X-ray radiation of the model biological system.

The results of the study of self-organization of dilute solutions of PO make it possible to suggest that their radioprotective action is related to the formation of domains and nanoassociates, consisting of molecules of the dissolved compound and ordered water structures, which are able, as it was convincingly demonstrated using dilute solutions of hydrated fullerene as an example,[20] to manifest clearly pronounced antioxidant and radioprotective actions, related to the ability of the structures of bordered water to recombine the active forms of oxygen and free radicals formed upon exposure to radiation.[9,25]

In conclusion, as a result of the study by a complex of physicochemical methods (DLS, ELS, NTA, AFM, TEM, UV spectroscopy, conductometry, pH-metry, dielcometry) of dilute solutions of a multicomponent drug immunomodulator polyoxidonium, it was found that the solutions are nanoheterogeneous disperse systems, the disperse phase of which, when diluting the solution in the range of calculated concentrations from $1\cdot10^{-1}$ to $1\cdot10^{-16}$ mg mL^{-1},

undergoes a considerable restructuring that is reflected on the non-monotonous dependencies of physicochemical properties of the solutions. Using ELS, AFM, TEM methods, and UV spectroscopy, it was shown that the disperse phase with a size of hundreds of nanometers contains the ordered water structures, which are responsible for the negative values of the ζ-potential, changing non-monotonously from −5 to −16 mV, and the appearance of a band at 270 nm in the UV spectrum. Radioprotective properties of dilute solutions of PO ($1\cdot10^{-1}$ and $1\cdot10^{-9}$ mg mL^{-1}) were identified for the first time, when the test mutant bacterial strain *Salmonella typhimurium TA 100* (Ames test) was treated with X-ray radiation in a 7.50 mGy dose used in medical diagnostics.

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