

SCIENTIFIC PRINCIPLES FOR MODIFICATION OF WATER-SOLUBLE POLYMERS. FORMATION OF MACROMOLECULAR COMPLEXES*

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Abstract The study of nanosecond dynamics of macromolecules with the luminescent methods make it possible to investigate the formation and functioning of polymeric complexes, polymeric conjugates and macromolecular metal complexes, which are widely used for solving many practical tasks. The nanosecond dynamics of macromolecules are a highly sensitive indicator of interpolymer complexes (IPC) formation. It enables us to solve the problems of studying IPC formation and stability and to investigate the interpolymer reactions of exchange and substitution. The investigation of changes in the rotational mobility of globular protein molecules as a whole makes it possible to determine the complex composition and its stability, and to control the course of polymer-protein conjugate formation reaction. The nanosecond dynamics of polymers interacting with surfacants' ions (S) are the sensitive indicator of the S-polymer complex formation. A method for determining the equilibrium constants of the S-polymer complex formation was developed on the basis of the study of polymer chains mobility. It is established that nanosecond dynamics influences the course of chemical reactions in polymer chains. Moreover, the marked effect of the nanosecond dynamics is also revealed in the study of photophysical processes (the formation of excimers and energy migration of electron excitation) in polymers with photoactive groups. It was found that the efficiency of both processes increases with increasing the mobility of side chains, the carriers of photoactive groups.

Keywords Modification of water-soluble polymers; Formation of complexes

INTRODUCTION

Modification of polymers, and of their properties is an important technological problem. For the realization of this problem, some scientific principles for polymer modification are necessary. The change of the structural organization of polymer systems can be used as an indicator of polymer modification. The polymer modification can be achieved by the formation of interpolymer complexes, protein- polymer

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conjugates, macromolecular metal complexes, and complexes of polymers with low molecular weight compounds. Among them biologically active compounds are of great interest. All these polymer systems have widely been used for solving various practical problems in medicine and industry.

The formation of intermolecular structures in polymer solutions changes a nanosecond motion of polymer chains. It was established that nanosecond dynamics of macromolecules are a sensitive indicator of the changes in the structural organization of polymer systems on the molecular level^[1,2]. The results of this investigation are of great interest for developing new polymer materials, polymer compositions for technical and medico- biological purposes, and technological processes with the participation of macromolecules.

Various methods were used to study the nanosecond dynamics. We have used the luminescent method and polymers with luminescent markers^[1,2] to increase considerably the informativeness and the sensitivity of this method. At present it is possible to investigate multicomponent polymer systems and to obtain a detailed information about each system component.

MATERIALS AND METHODS

The polymers with luminescent markers were prepared according to the methods developed by M.G. Krakovyak described in Ref. [1, Section 3]. Nanosecond dynamics of the macromolecules in solution were studied by polarized luminescence in a stationary regime^[1,2]. Relaxation times τ were determined according to the well-known formula

$$\tau = (1/P'_0 + 1/3)3\tau_f / (1/P - 1/P'_0) \quad (1)$$

where P is the measured polarization of luminescence of the solution of a labelled polymer, τ_f is the duration of luminescence, and $1/P'_0$ is the parameter related to the amplitude of the high-frequency motion of luminescent groups. The values of $1/P'_0$ were obtained by extrapolating the plots of the polarization of luminescence of polymer solutions versus solvent viscosity^[1,2].

The polarization and the life time of luminescence were measured with the instrumentation described in Ref. [1, 2].

RESULTS AND DISCUSSION

Polymers can be modified by the formation of supermolecular structures. These

structures include interpolymer complexes (IPC). The nanosecond dynamics of macromolecules in a solution are a highly sensitive indicator of IPC formation. When macromolecules are inserted into IPC, the characteristic times of intramolecular motions (IMM) increase many times. This enables us to solve the problems of IPC formation and their resistance to various factors simply and clearly. For example, the relaxation time of polydimethoxyethylene (PDME) in an aqueous solution increases from 5 to 800 ns when PDME and polymethacrylic acid (PMAA) form IPC (Fig. 1).

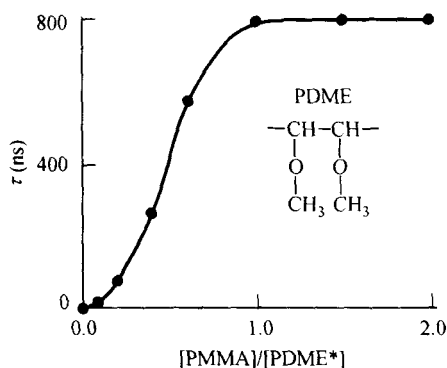


Fig. 1 The formation of interpolymer complex PDME* PMAA in water; 25°C; τ —the relaxation time of PDME* vs. $[PMAA]/[PDME^*]$; $C_{PDME^*} = 0.5 \text{ mg/mL}$

It was found that homopolymers can form IPC with heteropolymers of different chemical structures^[3]. The fact of IPC formation is manifested in an increase of relaxation times interacting polymer chains (Table 1). The heteropolymer molecules with a low content of groups active in IPC formation can form IPC with homopolymers with the aid of both hydrogen and salt bonds even if they contain only 10~20% of units with active groups. For example, during the formation of interpolymer contacts between polyvinylcaprolactam (PVC) and methacrylic acid

(MAA) units in vinylpyrrolidone (VP)-MAA (co)polymers with different compositions in aqueous solution, the relaxation time of PVC increases from 40 to 220 or even 800 ns. The factor which prevents the participation of active heteropolymer groups in the formation of intermolecular contacts was found to be the inclusion of these groups into intramacromolecular contacts with the comonomer units adjacent along the chain^[4]. This is indicated by the complete absence of intermolecular contacts of PVC units with crotonic acid (CA) units in the VP-CA copolymer or by the almost complete absence of intermolecular contacts between PMAA and VP units in a VP-CA (co)polymer with a regular alternation of units, whereas at a slight excess of VP units (in a (co)polymer which contains 60% of VP units and 40% of CA units) the complex is formed and the relaxation time of the (co)polymer in an aqueous solution increases from 12 up to 200 ns.

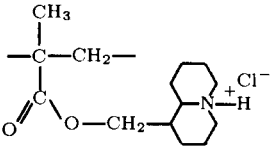
The molecular mechanisms of IPC formation and functioning are related to the interpolymer reaction of exchange and that of substitution (Fig. 2). The kinetics of

interpolymer exchange reactions reveal the duration of interpolymer contacts. Using luminescently labelled polymers we can study these reactions^[5,6]. Our analysis showed that in most cases the exchange reaction in a solution is carried out in two stages (Fig. 3). Characteristic times of these stages differ by several orders of magnitude. This fact indicates that IPC has heterogenous structure.

Table 1 The formation of complexes of heteropolymers with homopolymers in solutions

System $P_1^* + P_2$	Solvent	τ^{red} (nd)	
		P_1^*	$P_1^* + P_2$
The interaction of proton-donor and proton-acceptor groups			
PVC*+VP- <u>MAA</u> (10%)	wate, pH3	40	220
PVC*+VP- <u>MAA</u> (19%)		40	800
PVC*+VP- <u>CA</u> (19%)		40	40
PVC*+VP- <u>CA</u> (50%)		40	40
<u>VP</u> - CA* (50%) +PMAA	methanol	10	25
<u>VP</u> - CA* (40%) +PMAA		12	200
The interaction of ionized groups			
(PML ⁺)*+VP-CA ⁻ (19%)	water, $\alpha_{\text{CA}} = 0.8$	51	170
(PML ⁺)*+VP-MAA ⁻ (19%)	water, $\alpha_{\text{MAA}} = 0.8$	60	260

P^* – the luminescent labelled polymers; P_- – comonomers content of the group active in IPC formation; PVC–polyvinylcaprolactam; VP–vinylpyrrolidone; MAA–methacrylic acid; CA–crotonic acid; PML⁺–polymethacryloyl lupinine hydrochloride



τ^{red} is the value of τ reduced to viscosity 0.89 sP according to the equation $\tau^{\text{red}} = \tau \eta_{\text{red}} / \eta$ where η and η_{red} are the solvent viscosities

Table 2 Parameters of exchange reaction IPC (PMAA PVP)+PMAA* → IPC (PMAA PVP) + IPC (PMAA* PVP) Water, 25°C

α_{PMAA}	L_1	T_1 (s)	T_2 (s)
0	0.16	330	$> 10^6$
0.05	0.30	80	$> 10^6$
0.11	0.64	< 80	$> 10^6$
0.20	0.80	< 80	$> 10^6$
0.30	1.00	< 80	

L_1 –the fraction of PVP molecules taking part in the fast exchange; T_1 –characteristic time of the fast exchange stage; T_2 –characteristic time of the slow exchange stage

The rapid exchange stage increases with increasing number of "defective" units which do not participate in the interpolymer contacts formation. For example that effect is revealed by the increase of the degree of PMAA ionization in complex with PVP (e.g. with increasing number of ionized PMAA units which do not form hydrogen bonds with PVP units) (Table 2). Hence, the method of variation the times of interpolymer contacts is found. This is very important because the change in this time makes it possible to control IPC functioning. For example, we can change the course of substitution reactions in a multicomponent polymer system. The data in Fig. 4 show the complete substitution in IPC or that the complete substitution is not achieved not only during the time of component mixing, but also during several hours and days.

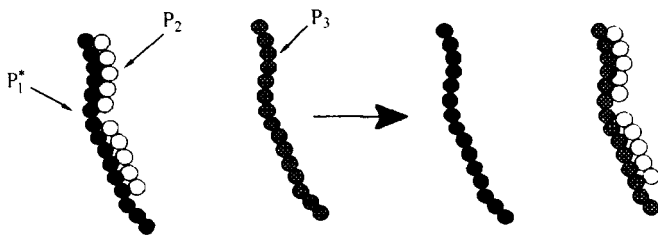
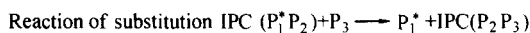
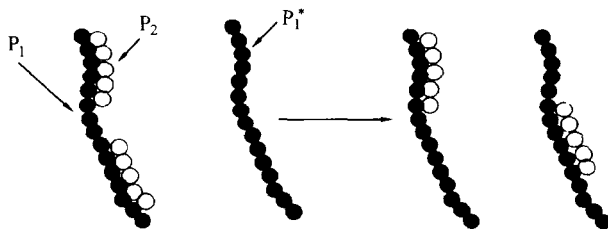
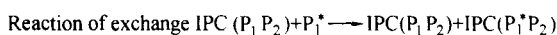


Fig. 2 Schematic representation of reaction of exchange and reaction of substitution

The development of new protein- polymer preparations for medical purposes is based on the formation of complexes of synthetic polymers with proteins. These complexes are used for the immobilization of protein molecules or for the stabilization of their biological activity with the purpose of decreasing the harmful side effects. The rotational mobility of globular protein molecules as a whole, just as the intramolecular mobility of polymer chains, is a sensitive indicator of intermolecular contacts formation. It enables us to determine quantitatively the fraction of protein molecules inserted in the complex, to establish the complex composition and its stability when conditions changes^[7,8]. For example, it is established, that in aqueous-

salt solutions when electrostatic contacts are destroyed, the protein- polyelectrolyte complex is formed with a decreasing degree of polyelectrolyte ionization (Fig. 5).

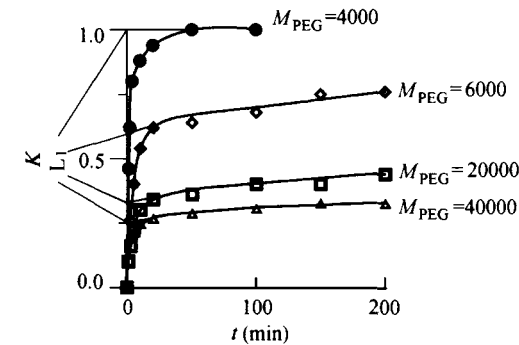


Fig. 3 Conversion κ of the exchange reaction $IPC (PMAA\ PEG)+PMAA^* \rightarrow IPC (PMAA\ PEG) +IPC (PMAA^*\ PEG)$; $[PEG]/[PMAA]=0.5$ $[PEG]/[PMAA+PMAA^*]=0.25$ $M_{PMAA} = 3.510^5$; water pH 4; 25°C; $C_{PMAA^*} = 0.5\text{ mg/mL}$; L_1 -the fraction of PEG molecules taking part in the fast exchange

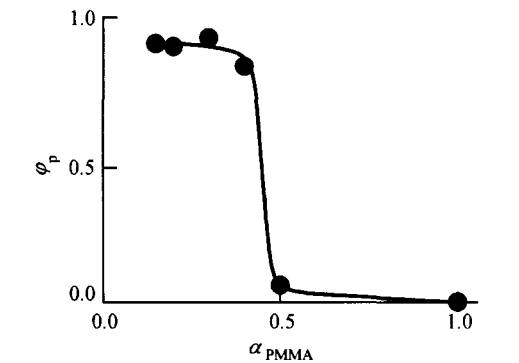


Fig. 5 Complex protein (lysozim)-PMAA stability vs. α of PMAA in aqueous-salt solutions (0.4 M NaCl) φ_p -fraction of protein molecules in the complex $[PMAA]/[\text{protein}] = 170$; 25°C; $C_{\text{protein}} = 0.5\text{ mg/mL}$

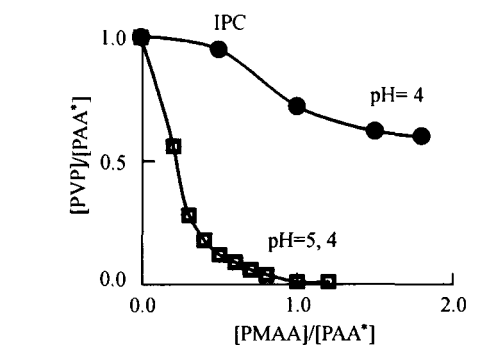


Fig. 4 Reaction of substitution $IPC (PVP\ PAA^*)+PMAA \rightarrow PAA^*+IPC (PVP\ PMAA)$ $[PVP]/[PAA^*]=1$ $M_{PAA} = 1.5 \times 10^5$; $M_{PMAA} = 3.5 \times 10^5$ $M_{PVP} = 1.2 \times 10^4$; water; 25°C; $C_{PAA^*} = 0.5\text{ mg/mL}$

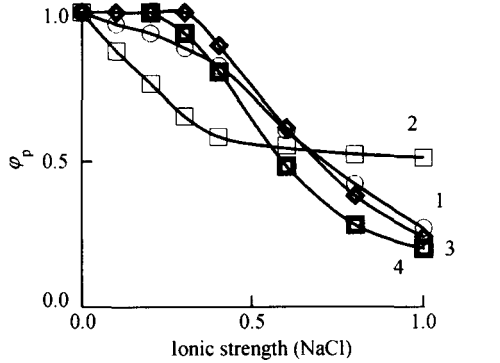


Fig. 6 Complexes protein (lysozyme)-PMAA (1) and protein-network PMAA (2,3,4) stability vs. ionic strength in aqueous-salt solutions. $\alpha_{PMAA} = 0.4$, φ_p -fraction of protein molecules in the complex. $[PMAA]/[\text{protein}] = 170$; 25°C; 2, 3-network PMAA with 2.5 mol% and 4 mol% of the sewing agent accordingly; 4-microgranules PMAA with 2.5 mol% of the sewing agent. $C_{\text{protein}} = 0.5\text{ mg/mL}$

The stability of protein-network polyelectrolyte complexes depends also on the structural features of network polyelectrolyte (PMAA)^[8] (Fig. 6). These features are related to the electrochemical heterogeneity of network polyelectrolyte.

It is known that new protein- polymer preparations can be obtained by covalent binding of protein molecules to polymer chains. The nanosecond dynamics of protein molecules make it possible to control the course of binding reaction and to evaluate quantitatively the composition of the resulting conjugate^[9]. For example, Fig. 7 shows the data reflecting the course of the attachment of protein molecules to dialdehydedextrane chains at different protein-dextrane ratios in the reaction mixture. In the course of this reaction, the first protein molecule is attached during the first minutes whereas the bonding of subsequent protein molecules to the same chain (which is hindered as a result of interpolymer contacts) requires a much longer time. In other words, polymer chains mobility influences the course of the chemical reaction with the participation of a macroreagent.

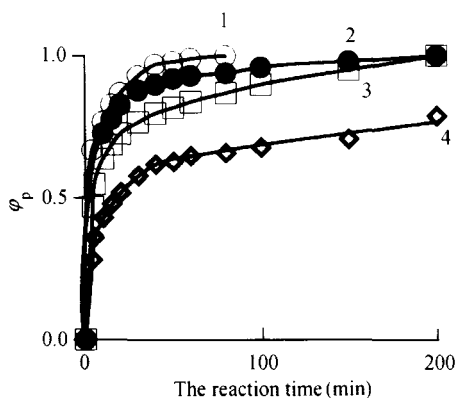


Fig. 7 The formation of protein- dialdehydedextrane (DAD) conjugate at different ratios (molecules)/(molecules DAD)=0, 62 (1); 1, 8 (2); 3, 5 (3); 7, 3 (4). φ_p - fraction of protein molecules in the conjugate. $M_{DAD} = 80000$; pH 9; 25°C

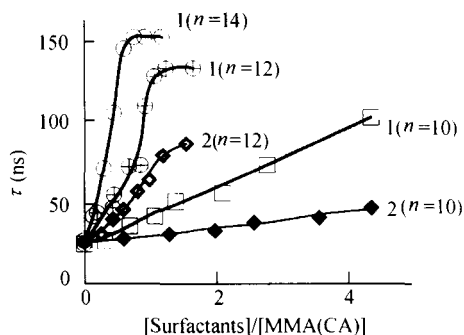


Fig. 8 Surfactant (DMBAAC-*n*)-polymer complex formation; τ vs. $[S]/[MAA(CA)]$ 1-VP-MAA (15%); 2-VP-CA (19%); water, pH 9; 25°C; $C_{copolymer} = 0.5$ mg/mL

The use of polymers as the carriers of low molecular weight compounds gives. The possibility of solving many problems related to their practical application. For instance, the formation of complexes of biological active surfactants (S) with polymers makes it possible to decrease considerably their harmful side effects^[10]. The investigation of the nanosecond dynamics of polymers interacting with S ions revealed the effect of the ratio of interacting components, their chemical structure, and

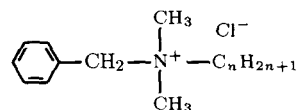
even the character of ionogenic group's distribution in polymer chains (microblock or random distribution) on the formation of the polymer- S complexes^[10~12] (Fig. 8).

A new method for determining the equilibrium constants of polymer- S ions complex formation was developed^[13,14]. This method is based on the study of polymer chains mobility. It is possible to obtain the information about the stability of S-polyelectrolyte (PE1) complexes quantitatively. For instance, it was found that when the length of alkyl chains in S increases by only four carbon atoms, from 10 to 14, the constant for the formation of the S-PE1 complex increases by almost two orders of magnitude (Table 3). The microblock character of ionogenic group's distribution in PE1 chains favours the stabilization of this complex. When a copolymer with a random distribution of ionogenic groups (VP-CA) is replaced by a copolymer with their microblock distribution (VP-MAA copolymer), the constant for complex formation increases several times (Table 3).

Table 3 The parameters for the stability of surfactant- polyelectrolyte complexes.

System	Solvent	Constant for complex formation K_f (l/mol) $\Theta = 0.2$
VP-CA (19%) + DMBAAC-10	water pH9	$3.7 \cdot 10^2$
+DMBAAC-12		$11.5 \cdot 10^2$
+DMBAAC-14		$125.0 \cdot 10^2$
VP-CA (15%) +DMBAAC-12	water pH9	$5.9 \cdot 10^2$
VP-MAA (15%) +DMBAAC-12		$25.0 \cdot 10^2$
VP-CA (19%) + DMBAAC-12	water pH9	$11.5 \cdot 10^2$
	0.1 M NaCl	$2.2 \cdot 10^2$
VP-VA (13%) +DDS	water pH3	$35.0 \cdot 10^2$
	0.1 M NaCl	$7.7 \cdot 10^2$
VP-DMAEM (13%) + DDS	water	$1.1 \cdot 10^2$
	0.1 M NaCl	$3.2 \cdot 10^2$

Θ -degree of polyelectrolyte ionogenic group's filling by surfactants ions. DMBAAC-*n*-dimethylbenzylalkylammonium chloride



DDS-dodecylsulphate sodium; VA-vinylamine; DMAEM-dimethylaminoethylmethacrylate chloride ethylate

The data in the same Table demonstrating the effect of the ionic strength of solution on the stability of S- PE1 complexes. On passing from aqueous to aqueous- salt solutions, the complex is destabilized and its formation constant decreases markedly. However, when ionogenic PE1 groups are shielded by alkyl substituents, i.e. when

the contribution of electrovalent interactions to the formation of intermolecular contact becomes smaller, the increase in the ionic strength of the solution leads to the higher complex stability as the hydrophobic interactions of the alkyl groups become stronger. Thus we can choose the optimum parameters of these systems.

The complexes of metal ions with polymers are of great interest and are used widely. In the study of complexes of metal ions with polymers, the use of a competing polymer and the study of nanosecond dynamics of each polymer in solution provide information about the stability of the polymer-metal ion bond^[15,16]. To study these complexes, an original method was developed for characterizing quantitatively the ability of a polymer to bind Fe ions^[16], i.e. a solution polymer- Fe ions is inserted into the water cavity of the surfactant micelle in a nonpolar solvent. This method is interesting as it permit us to characterize the metal-bonding ability of individual macromolecules and the stability of the metal- polymer complexes (Table 4).

Table 4 The ability of polymers to bind Fe ions

(CO)POLYMER	Content of COOH-groups (mol %)	I_0/I	x^*
PVP	0	2.8	1.5
P (VP-MAA)	15	20.0	12.0
P (VP-AA)	15	15.0	8.8
P (VP-CA)	15	8.0	4.7
P (VP-MG)	15	12.0	7.0

* $\chi = (I_0/I)_{\text{polymer}} / (I_0/I)_{\text{model of LG}}$ I -intensity of luminescence of the anthracene containing polymers in aqueous solution (decreases owing to suppression by Fe ions) PVP-poly-(*n*-vinylpyrrolidone); MAA-methacrylic acid; AA-acrylic acid; CA-crotonic acid; MG-methacryloylglycine

The nanosecond dynamics are useful in the polymer investigation not only in studying the structural changes in macromolecules but also in studying chemical reactions in polymer chains. The nanosecond dynamics affect the course of chemical reactions in polymer chains. The data in Fig. 9^[17] demonstrate a good agreement between the reactivity of the functional groups of the polymer and the mobility of the corresponding chain parts.

A considerable effect of the nanosecond dynamics is also manifested in the study of photophysical processes in polymers with photoactive groups. These data were obtained for both synthetic and natural polymers (protein molecules)^[18,19]. In macromolecules with a high content of photoactive groups, processes of the formation of excimers (complexes of excited and unexcited photoactive groups) and energy migration (nonirradiative energy transfer of electron excitation to non-excited photoactive

groups) take place. It was found that the efficiency of both processes increases with increasing the mobility of side chains, the carriers of photoactive groups (Fig. 10), naturally, if the average distances between these groups in the macromolecules do not change.

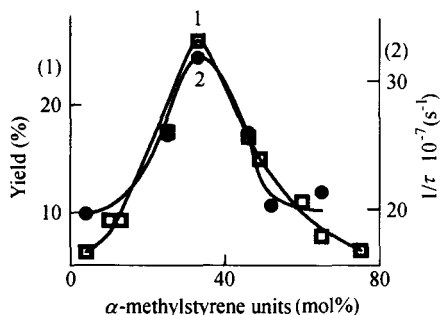


Fig. 9 Reactivity of the functional groups and the mobility of the corresponding chain parts. Degree of transformation of 9-chloromethylanthracene in the reaction with styrene- α -methylstyrene copolymers (1) and local dynamic characteristics ($1/\tau$) of the copolymer molecules vs. content of α -methylstyrene units in the copolymers; toluene; 25°C; $C_{\text{copolymers}} \approx 0.5 \text{ mg/mL}$ (2)

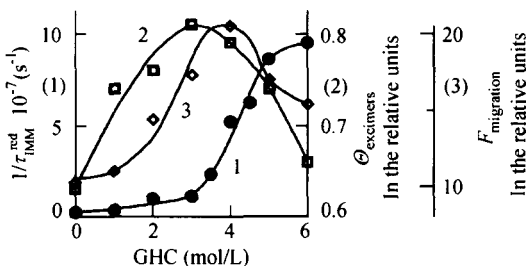


Fig. 10 Photophysical processes and nanosecond dynamics of protein molecules. $\tau_{\text{IMM}}^{\text{red}}$ - the relaxation times of the intramolecule mobility of lysozyme molecules reduced to the solvent viscosity 0.89 sP; Θ_{excimers} and $F_{\text{migration}}$ - the characteristics of photophysical processes efficiency in the luminescently labelled lysozyme molecules vs. C guanidinehydrochloride (GHC1) in aqueous solution. $C_{\text{protein}} = 1.0 \text{ mg/mL}$ (1) and 0.2 mg/mL (2,3). The content of the luminescent labels in protein molecules 0.2 (1) and 1.5 (2, 3)

Consequently, the study of nanosecond dynamics of macromolecules is a promising research field for solving a wide range of problems. They are structural organization of multicomponent polymer systems in solutions, and their stability. The results of these investigations will make it possible to predict the functional characteristics of polymer systems under different conditions and their changes caused by various factors.

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