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THE STRUCTURE AND INTRAMOLECULAR MOBILITY OF MACROMOLECULES IN SOLUTION AS STUDIED BY POLARIZED LUMINESCENCE

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Abstract - The relationships of polarized luminescence (PL) of solutions of polymers containing various luminescent markers (LM) of anthracene structure in the main and side chains and also in the end groups have been considered. The relationship between the PL of solutions of labeled polymers (with anthracene- and indole-containing LM) and the relaxation spectrum of the labelled polymer was analyzed. The PL parameters and their change upon variation of intramacromolecular structure are reported for various types of anthracene-containing LM. The relationship between the intramolecular mobility (IMM) of the polymer determined by the PL method (nanosecond relaxation processes) and the main features of the chemical structure of the polymer and the formation of intramacromolecular structure is analyzed for various types of structural transitions: random coil-α-helix, random coil-compact structure and random coil-globule. The random coil-globule transition and its influence on the IMM of the polymer is considered taking as examples linear and comb-like synthetic polymers and polypeptides. The mobility of the end of the polymer chain and its middle parts is compared and the participation of the chain end in the processes of the formation of intramacromolecular structure is discussed. The extensive possibilities of using the PL method for studying multicomponent polymer systems on the basis of the investigation of IMM for each component (the component being investigated is distinguished by a LM) are shown taking as examples block copolymers, polymer-polymer complexes, stereopolycomplexes and branched and cross-linked polymers with markers in bridges or linear fragments. The possibilities of using the PL method for the solution of various problems of the chemistry of polymers are considered. The PL method was used to study the processes of the formation of branched and cross-linked polymers and polymer-polymer complexes, the relationships of competing interactions in multicomponent polymer systems and structural-kinetic heterogeneity of copolymer molecules and its effect on the course of reactions in copolymer chains.

INTRODUCTION

Polarized luminescence (PL) of labeled luminescent macromolecules or macromolecules containing covalently bonded luminescent groups (LG) is the basis of the polarized luminescence (PL) method that allows the solution of various problems of the physics and chemistry of polymers.

The PL method was found to be fruitful in the investigation of various types of polymers: homopolymers (1-3), random (4) and multicomponent compolymers (5), liquid-crystalline polymers containing mesogenic groups (6,7), comb-like (8), branched (9) and cross-linked (10) polymers, polyamic acids (11), polypeptides (12-14) and proteins (15). The PL method was used to solve a wide range of problems of polymer physics. The dependence of the intramolecular mobility (IMM) of the polymer on its chemical structure: the length, nature and position of substituents at the carbon atoms of the main chain (6) and stereoregularity (16), on micro-heterogeneity in copolymers chains with different

units and the distribution of micro-blocks along the copolymer chain has been established. The change in the IMM of the macromolecules accompanying intramolecular hydrogen bonding and the formation of α-helical parts or local structures of the "pin" type (1) has also been established. It became possible to determine the contribution of hydrophobic interactions of non-polar polymer groups in water to the change in the IMM of polymer chains (2,17). With the aid of the PL method the effect of various structural transformations in the macromolecular coil on the IMM of the polymer could be determined. The PL method is very useful for the study of coil-globule transitions (7,12-14,17). With its aid it was possible to observe for the first time the coil-globule transition in a carbon chain polymer in water on heating (18), in synthetic polypeptides upon variation of the pH of solution (12,3) and even in synthetic comb-like polymers with mesogenic groups in organic solvents (heptane and decane) upon variation of the temperature (7). The PL method was used to study the formation of internal structures in the molecules of a precipitated polymer in solvent-precipitant mixtures (18).

The PL method makes it possible to follow the formation of nuclei of the mesophase in the molecules of liquid-crystalline polymers and the subsequent stages of the formation of inner structure (7).

The use of a label permits the determination of the mobility of chain ends and in-chain parts and the study of their incorporation into elements with internal structure when internal structure of various types: α-helical parts or globular structure is formed (13,18). The labeling of various chain parts of the copolymer can be used to study the main features of its structure (4). The PL method also provides ample possibilities for the investigation of complex multicomponent systems. The labeling with a luminescent marker of the component being investigated permits the study of individual components of a multicomponent polymer system. The corresponding examples will be considered here. It is possible to establish the structure and the main features of the formation of polymer-polymer complexes by studying their individual components (19,20). The investigation of individual components of stereo-polycomplexes of iso- and syndiotactic poly(methylmethacrylate) (PMMA) makes it possible to establish the factors affecting the stereochemical PMMA structure.

In the investigation of polymer networks it is possible to use the PL method for the separate study of the mobility of linear fragments (10) and bridge bonds (the label is inserted into a linear fragment or a bridge bond). On this basis the methods for the determination of the number of bridges and the fraction of the molecules of the cross-linking agent fixed at one or both ends can be developed (9). These methods are used to study the relationships of the formation of branched and cross-linked polymer systems (9). The PL method was used to study the formation of internal structure occurring in the molecules of polymers bonding biologically active substances (BAS) due to the effect of these substances, e.g. surfactants and other compounds (21,22). This method was also used to solve some problems of polymer chemistry such as an important problem of the relationship between the reactivity of the functional groups of the polymer and the mobility of the chain part bearing these groups (4). By using luminescent markers and the PL method it was possible to study the structural kinetic heterogeneity of copolymers (23) and proteins (15). The ample possibilities of the PL method that will be considered in the present paper are due to some specific features of this method.

Main features of the PL method

The PL method is based on the measurement of the polarized luminescence of the solution of a polymer containing luminescent markers attached to it by a covalent bond. Its important features is high sensitivity of the PL parameters being measured to the change in small-scale (nano-second) relaxation processes in the macromolecular coil. These small-scale relaxation processes are most profoundly affected by various changes in intra- and intermolecular contacts (18). It is possible to study various structural transformations in macromolecules because the PL parameters being measured are very sensitive to the changes in intra- and intermolecular interactions.

The second important feature of the PL method is the possibility of carrying out investigations in very dilute solutions at a polymer concentration as low as 0.003 per cent. This means that only a small amount of the polymer is needed for the experiment and, which is particularly important, intramolecular interactions are less complicated by intermacromolecular contacts.

The choice of solvents for this method is almost unlimited: it is possible to

study aqueous solutions of polymers at any pH and ionic strengths and polymer solutions in any (polar and non-polar) solvents. However, strong acids (sulphuric and dichloroacetic acids) cannot be used as solvents since they are effective luminescence quenchers.

The use of an LM for distinguishing the desired component of a multicomponent polymer system or a part of the main or side polymer chain also greatly extends the possibilities of using the PL method and obtaining new information with its aid.

At present the problem of obtaining a polymer containing a covalently bonded luminescent marker and that of introducing this marker into the desired component of a multicomponent system have been completely solved by Krakovyak and co-workers (18, section 3). They synthesized the required monomers, reagents containing luminescent anthracene (indole or naphthalene) groups and developed the methods for the bonding of luminescent groups to polymers with various chemical structures (18). It is advisable to use an anthracene containing luminescent group (LG) bonded to the polymer. It was found that when an anthracene ring was bound to the polymer chain in the position 9 it provided most information. In this case the direction of the dipole moment of transition coincides with that of the bond attaching the LG to the polymer. As a result, the mobility of the LG about the adjoining bond is not revealed in PL. The motion of the anthracene ring in a short side chain about the bond neighbouring the adjoining bond can occur only with the vibrational motion of the main polymer chain because the anthracene group is very bulky. Hence, the motion of the anthracene ring bonded to the polymer in the position 9 reflects the mobility of the main chain or the intramolecular mobility (IMM) of the polymer.

POLARIZED LUMINESCENCE OF SOLUTIONS OF POLYMERS CONTAINING LUMINESCENT GROUPS AND MICRO-BROWNIAN MOTION OF LABELED MACROMOLECULES

All the above mentioned features of the PL method result from those physical relationships on which the method is based (24,25). The theory of the relationship between the polarization of luminescence (degree of polarization) P and the relaxation spectra of macromolecules containing luminescent markers (LM), the basis of which is considered in (Ref.25) is widely developed by Gotlib (18,26,27). The degree of polarization P is equal to $(I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$ where $I_{\parallel}$ and $I_{\perp}$ are the intensities of luminescent light with vectors parallel and perpendicular to the vector of the exciting light, respectively.

Luminescence appears when a molecule that can luminescence passes from the excited electronic state into the ground state. Those molecules are excited (i.e., absorb the exciting light) the direction of whose absorbing oscillators at the moment of excitation coincides with the vector of the exciting light. Upon excitation the molecules with a certain orientation are distinguished from chaotically arranged molecules. At the moment of emission delayed from the moment of excitation by the time τ_f equal to the lifetime of the excited state this orientation may not be maintained if during the micro-Brownian motion the molecules rotate by an angle θ during the time t. The relationship between the polarization of luminescence P and the value of $\langle \cos^2\theta(t)\rangle$ where t is the time during which the rotation takes place is determined by the equation

$$[1/P+1/3]=[1/P_0+1/3]\left\{(3/2\tau_f)\int_0^{\infty}[\langle\cos^2\theta\rangle-1/3]\exp(-t/\tau_f)dt\right\}^{-1} \qquad (1)$$

Signs + and - refer to the excitation of luminescence with natural (non-polarized) or linearly polarized light and observation is carried out in the direction normal to that of the exciting light.

The value of P_0, the limiting polarization of luminescence for completely immobile oscillators, is determined by the chemical structure of the luminescent molecule and the conditions of excitation of luminescence. The relationship between the polarization of luminescence P of the luminescent group bonded to the polymer chain and the relaxation spectrum of the labelled polymer is determined by the equation

$$[1/P + 1/3] / [1/P_0 + 1/3] = \left\{\sum_j f_j/(1 + 3\tau_f/\tau_j)\right\}^{-1} \qquad (2)$$

where τ_i and f_i are the times and weights of single relaxation processes in which the luminescent oscillator participates, respectively.

Polarized luminescence of macromolecules with anthracene containing luminescent groups (LG)

The change in the polarization of luminescence of a solution of a polymer with covalently bonded anthracene containing LG can also be described in a two-time approximation

$$[1/P + 1/3]/[1/P_0 + 1/3] = \left\{f_1/(1 + 3\tau_f/\tau_w) + f_2/(1 + 3\tau_f/\tau_{LG})\right\}^{-1} \quad (3)$$

where τ_{LG} is the relaxation time characterizing the motion of LG in the polymer and τ_w is the time characterizing the IMM of the polymer (18). Eq.(3) shows that the mobility of LG or the IMM of the polymer change the polarization of luminescence and can be determined by the PL method if τ_{LG} and τ_w do not differ from τ_f, the lifetime of luminescence, by a factor greater than 100. For anthracene containing LG τ_f is 5 to 10 nanoseconds and this means that for these macromolecules the times determined by the PL method are in the nanosecond range: 10^{-7} to 10^{-10} sec.

Relaxation times of small-scale relaxation processes are proportional to solvent viscosity η : $\tau_w \sim \eta$ (28). Rapid motions of anthracene containing LG about the bonds that do not ajoin the anthracene-group affect the polarization of luminescence in viscous solvents at $(T/\eta) < 2.10^2$ K/cP where T is the temperature in °K. The values of τ_{LG} in viscous solvents become comparable to τ_f. The motion of the anthracene LG group about the bond adjoining the anthracene ring at C9 is not reflected in PL. Relaxation times of slower motions of the chain in which LG takes part become comparable to τ_f in less viscous solvents, $(T/\eta) > 2.10^2$ K/cP. Hence, for the determination of the times of fast and slow relaxation processes in polymer molecules containing LG usually the dependence of P for a solution of a polymer containing LG in mixed solvents on the viscosity of a mixed solvent is measured at a constant temperature T. Relaxation times τ_w characterizing the intramolecular mobility (IMM) of the polymer are determined by the equation

$$\tau_w = [(1/P_0' + 1/3)3\tau_f]/[1/P - 1/P_0'] \quad (4)$$

where $1/P_0'$ is the parameter characterizing the contribution of high frequency motions of the marker to the relaxation spectrum of the labelled luminescent polymer. The parameter 1/P' is determined with the aid of the dependence of 1/P on (T/η); at constant T the linear part of the curve $(T/\eta > 2.10^2$ K/cP) may be extrapolated to $(T/\eta) = 0$ to give the value of 1/P'. The value of 1/P' for LG of a definite chemical structure depends on the structure (type) of the luminescent marker (LM) containing LG and on the interaction between LG (LM) and the macromolecular environment. For anthracene-containing LG these interactions are the steric interactions between the anthracene ring and the macromolecular environment when the macromolecular coil becomes more compact or the interactions between other groups of side chains bearing LG and the macromolecular environment as well as hydrophobic interactions between the antracene group and non-polar groups of water soluble polymers in water. For macromolecular coils in organic solvents, the value of 1/P' for anthracene-containing LG is determined only by the type of LM (in the absence of specific interactions between other LM groups and macromolecular environment) (Table 1).

Data for other types of LM, LM_2, LM_7, LM_8, LM_9 and LM_{11}, have been reported proviously (18).

For the determination of relaxation times τ_w characterizing the IMM of macromolecules without internal structure it is sufficient to measure P (or the inverse value 1/P) of the luminescent light of the polymer solution investigated.

The relationship of changes in the parameter $1/P_0'$ for macromolecules with internal structure in water and organic solvents can be easily studied. To determine the values of $1/P_0'$ the dependence 1/P (T/η) is obtained for a solution of labelled polymer in solvent mixtures containing a viscous additive and is plotted taking into account the effect of this additive on the lifetime of luminescence τ_f and the thermodynamic strength of the solvent. It has been shown (28) that $(1/P - 1/P_0')$ changes proportionally to the intrinsic viscosi-

ty of a polymer solution $[\eta]$ or $\tau_w \sim (1/[\eta])$.

TABLE 1. Values of $1/P_0'$ for various types of anthracene-containing markers (LM). The anthracene group is bonded to the polymer chain in the position 9,25°C, excitation by non-polarized light, λ_{exc} = 365 nm.

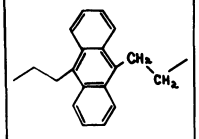
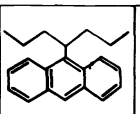
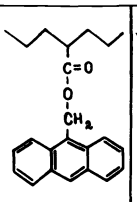
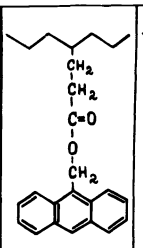
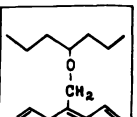
Structure of LM					
Types of LM	LM_5	LM_6	LM_1	LM_3	LM_4
$1/P_0'$	7,0	8,3	9,0	16	20

TABLE 2. Effect of a viscous additive on the lifetime of luminescence τ_f and $[\eta]$ for a PMMA solution in methyl acetate-triacetin, LM_1 marker, 25°C.

Triacetin content of methyl acetate (weight %)	τ_f, ns	$[\eta]$, dl/g
0	4,4	0.60
5	4.7	-
20	4.7	-
35	5.1	-
40	5.8	0.54

The data in Table 2 show only a slight change in τ_f and $[\eta]$ upon variation of the composition of the mixed solvent but this change should be taken into account for a precise determination of the parameter $1/P_0'$.

For anthracene containing LG the examples of changes in the contribution of high frequency motions of LG (parameter $1/P_0'$) upon variation of intramacromolecular structure are shown in Fig.1.

When the solution of a polymer containing LM is heated, the amplitude of high frequency motions of LM increases (18).

In our experiments luminescence was excited with non-polarized light. The luminescence time τ_f was measured with a phase fluorometer for all the investigated polymers LM types and solvents. Some values of τ_f for the labeled PMMA in methylacetate are given in Table 3 as examples. The wavelength of the exciting light for anthracene-containing polymers λ_{exc} is 365 nm.

Polarized luminescence of macromolecules containing luminescent indole groups (IG).

The PL method was applied to the investigation of macromolecules containing luminescent indole groups in tryptophan residues. The value of parameter $1/P_0'$ was estimated for macromolecules without internal structure and its change was studied upon variation of intramacromolecular structure (Table 4). The data in Table 4 show that the amplitude of high frequency motions of indole groups (parameter $1/P_0'$) changes when both the groups capable of hydrogen

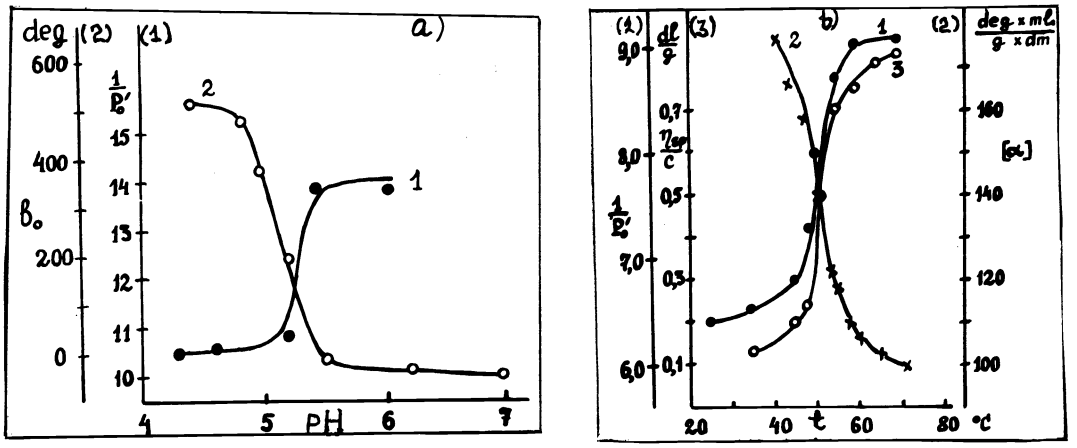


Fig.1. Dependence a) 1) $1/P_0'$ (pH) and 2) b_0 (pH) and b) 1) $1/P_0'$ (t) 2)[α](t) and 3) (η_{sp}/c) (t), where b_0 is the Moffit-Young constant, [α] is the optical activity and t is the temperature a) the random coil-α-helix transition, polyglutamic acid in 0.2 M NaCl, marker-LM_3, 25°C (12). b) random coil-globule transition, cholesterol ester of poly-N-methacryloyl-ω-oxycarboxylic acid, PCh MOA-10 in heptane, LM_1 marker.

TABLE 3. Values of the luminescence time τ_f, PMMA in methyl acetate

Types of LM, (see Table 1)	LM_5	LM_6	LM_1	LM_{10}	PDME-LM_4 in methyl acetate
τ_f, ns	8.3	7.6	4.4	3.6	3.4

PDME - Poly (1,2-dimethoxyethylene).

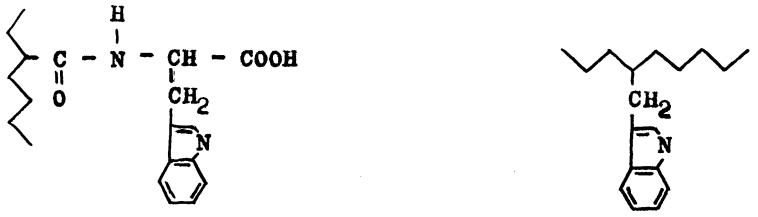
bonding (Table 4, lines 6-8) and non-polar groups (Table 4, lines 4-5) are present in macromolecular environment. Indole containing LG in labeled polymers and, hence, in proteins as well, provide information on the mobility of LG rather than on that of the main polymer chain in contrast to anthracene-containing LG (29,30). This follows from a comparison of the reduced values of relaxation times $\tau_{m.ch.}^{red}$ and $\tau^{red}(IG)$ obtained for polymers containing the anthracene groups in the main chain (m.ch.) and those containing the indole groups, respectively (Table 4) (29). Anthracene-containing LG (anthracene ring is bonded to the polymer chain in the position 9) provide information about the mobility of the main chain of the polymer even if the anthracene ring is bonded to a short side chain (e.g., LM_1 in Table 1), since in this case also the mobility of the main chain is distinctly reflected in the change in the PL of anthracene-containing polymers owing to the large bulk of the anthracene group. The difference in the amount of information provided by indole- and anthracene-containing LG is due to different structural features of these LG. In contrast to anthracene-containing LG, in indole-containing LG the dipole moment of the transition of the indole group does not coincide with the direction of the bond attaching the LG to the polymer. Hence, the low frequency motion of the indole group itself (less bulky than the anthracene ring) about the adjoining bond is manifested in PL. On the basis of the PL of indole groups it is possible to obtain information about the mobility of tryptophan residues in proteins containing indole groups. This information is of great interest to the solution of various problems of molecular biology.

INVESTIGATION OF THE INTRAMOLECULAR MOBILITY (IMM) OF POLYMERS WITH ANTHRACENE-CONTAINING LUMINESCENT MARKERS BY THE PL METHOD

This investigation made it possible to establish the changes in the IMM of polymers upon variation of their chemical and intramacromolecular structure and intra- and intermolecular interactions of various types. If relaxation times characterizing the IMM of polymers are known, this characteristic can be used

for the study of complex multicomponent polymer systems, their structure, and for the determination of the effect of various structure-forming factors. The following part of the present work deals with the analysis of these data.

TABLE 4. Comparison of relaxation times characterizing the mobility of the main polymer chain, $\tau^{red}_{m.ch.}$ and relaxation times of the relaxation process manifested in the PL of indole-containing polymers, τ^{red}_{IG}. The values of τ are reduced to one value of solvent viscosity η_{red} = 0.38 cp. Excitation with non-polarized light, λ_{exc} = 296 nm. Location of IG in PMMA (PMAA)-Trp and in Glu-Trp, Glu-Leu-Trp, in proteins



N	Polymer	Solvent	pH,	$\tau^{red}_{m.ch.}$ ns	$\tau^{red}_{(IG)}$ ns	$1/P'_0$ (IG)	τ_f ns. (IG)
1	PMMA	DMF		8.5	1.4	13.5	3.0
2	PMMA	Methyl acetate		8.3	2.2	11	3.4
3	Glu-Trp (200:1)mol.	0.2 M NaCl	pH 7.0	13	1.6	13.5	3.0
4	- " -	0.2 M NaCl	pH 4.3	38	1.8	13	1.9
5	Glu-Leu-Trp (83:17;0.8)%	0.2 M NaCl	pH 4.8	>60	9	11.5	2.5
6	PMAA	water-NaOH	α=0.3	10	2.7	13.5	5.4
7	PMAA	water-NaOH	α=0.12	52	4.8	11	4.7
8	PMAA	water-HCl	α = 0	52	10.8	9	3.5
9	α-lactal-bumin	water	pH= 7	-	19	10.3	2.1

PMAA - polymethacrylic acid, (Glu-Trp) and (Glu-Leu-Trp) are copolymers

When relaxation times τ_w for polymer solutions in solvents of various viscosities η are compared, all the values of τ_w are reduced to one value, the solvent viscosity, η_{red} = 0.38 cP and $\tau_w^{red} = \tau_w (\eta_{red}/\eta)$.

Chemical structure and the IMM of the polymer

Polymethylene, polyoxyethylene and polymethylacrylate, polymers without bulky substituents at the α and β carbon atoms of the main chain, exhibit high IMM and $\tau_w < 1$ ns. When substituents appear and increase in length and bulk, the IMM of the polymer decreases and the values of τ_w increases (Table 5, lines 1 and 2-8).

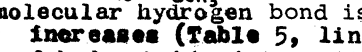
The IMM of the polymer is sensitive to its structural stereoregularity for a polymer with bulky substituents at each carbon atom, such as poly (1,2-dimethoxyethylene). The value of τ_w increases from 4.5 nsec to 10.3 nsec (methyl acetate, 25°C) even if the fraction of R of regular triad segnences "mm" of the structure  decreases as little as from 86 to 74%. If an intramolecular hydrogen bond is formed in polymer molecules, IMM decreases and τ_w increases (Table 5, lines 10 and 9). There are many examples of the effect of hydrophobic interactions between the non-polar groups of the polymer on its IMM in aqueous solutions. The interaction of methyl groups in non-ionized PMAA molecules in water leads to a considerable increase in intramolecular hindrance (Table 5, lines 10 and 11). This is caused not only by the appearance of new intrachain contacts, hydrophobic interactions of non-

TABLE 5. Effect of chemical structure and intramolecular interactions on the IMM (τ_w^{red}) of the polymer. η_{red}=0.38 cP, 25°C

N	Polymer	Solvent	τ_w^{red}, ns
1.	Polymethylene-polymethyl acrylate	toluene	<1
2.	Polymethyl methacrylate-PMA-1	toluene	4.3
3.	PMA-1	chloroform	2.5
4.	PMA-4	- " -	4.1
5.	PMA-6	- " -	5.3
6.	PMA-10	- " -	6.5
7.	PMA-16	- " -	8.8
8.	PMA-22	- " -	10.5
9.	PAA	water-HCl α = 0	10
10.	PAA	water-NaOH α = 0.1	4.9
11.	PMAA	water-HCl α =0;25°C	33
12.	PMAA	- " - α =0;60°C	52

α-degree of ionization, PAA - polyacrylic acid.

polar methyl groups, but also by the processes of intramacromolecular structurization (1,2). The increase in hydrophobic interactions of non-polar groups in water when aqueous solutions are heated to 60°C is also reflected in an increase in intramolecular hindrance (Table 5, lines 11 and 12). By using the PL method it is possible to observe even fine changes in intramacromolecular contacts, such as the change in the polymer-polymer and polymer-solvent interactions upon variation of the thermodynamic strength of the solvent. When it decreases, relaxation times describing the IMM of the polymer increase inversely proportionally to the change in the intrinsic viscosity $[\eta]$ of a polymer solution. Table 6 gives as an example data for poly (1,2 dimethoxyethylene) (PDME) although the ratio $\tau_w \sim (1/[\eta])$ is valid for all the polymers investigated over a wide range of changes in the thermodynamic strength of the solvent (28).

TABLE 6. Effect of the thermodynamic strength of the solvent on the IMM of poly (1,2-dimethoxyethylene) molecules (τ_w^{red}), η_{red} = 0.38 cP, 25°C

Solvent	$[\eta]$, dl/g	τ_w^{red}, ns
Water	0.70	8.0
Methanol	0.51	9.5
Methyl acetate	0.46	10.3
Toluene	0.40	14

Electrostatic interactions of polymer groups bearing charges of the same sign (of cationic or anionic nature) do not appreciably affect the IMM of the polymer.

Fine structure of copolymer molecules. The application of the PL method to the investigation of copolymers makes it possible to establish such features of their fine structure as the occurrence of micro-blocks, micro-heterogeneity in unit distribution, the size of micro-blocks and even the details of micro-block distribution along the chain. These data were obtained for water-

soluble copolymers of vinyl alcohol and vinyl acetate (VAl-Vac) with different compositions and prepared in different ways and for copolymers of vinylpyrrolydone and methacrylic acid. It was found that the dependence of IMM on the composition of the VAl-VAc copolymer is determined not only by the micro-heterogeneity but also by the micro-block sequence (Fig.2 and Table 7).

TABLE 7. Effect of conditions for obtaining a copolymer of vinyl alcohol and vinyl acetate (VAl-VAc) on the inhomogeneity of distribution of the VAc units (parameter L) and the IMM of the copolymer (τ_w) in water, 25°C.

Conditions of the preparation of a VAl-VAc copolymer (solvent for PVA saponification)	L-inhomogeneity parameter Content of VAc units, (mole %)		τ_w, ns (marker LM4)	
	8	20	8	20
Methanol	1.1	1.3	5.6	7.3
Ethanol	1.5	3.5	10.5	9.2
Water-acetone mixture	5		30	

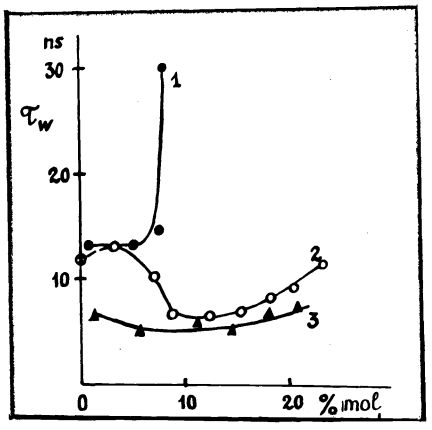


Fig.2. IMM (τ_w) of the VAl-VAc (vinyl alcohol-vinyl acetate) copolymer vs content of Vac units. Method of the copolymer preparation - saponification of polyvinyl acetate 1. in a water-acetone mixture, 2. in ethanol, 3. in methanol.

Formation of intramolecular structure and IMM of the polymer.
The formation of internal structure of any type in polymer molecules leads to a considerable intramolecular hindrance.

When secondary structure in polyglutamic acid molecules is formed, i.e., after the random coil-α-helix transition, relaxation times τ_w increase by a factor of three (12). The same changes are observed in the molecules of polymethacrylic acid when local structures are formed (1). If elements of tertiary structure appear as a result of the interaction of local structures both in polymer molecules with mesogenic groups (7) and in the molecules of the copolymer of glutamic acid and leucine (83:17), Glu-Leu, relaxation times increase by one order of magnitude (12).

Times τ_w characterizing the IMM of the polymer increase by one order of magnitude or more when structures of globular type are formed in macromolecules. The PL method was used to detect and study the formation of a structure of globular type in the molecules of synthetic polypeptides in aqueous solutions upon variation of pH (12), in molecules of a water-soluble carbon chain polymer, poly (1,2-dimethoxyethylene) (PDME) in water on heating (17) and in molecules of comb-like polymers containing mesogenic groups in long side chains in organic solvents when the temperature was varied (Fig.3) (6,7). The relationships for the formation of globular structure are established: the dependence of the range of random coil-globule transition on the molecular weight

of the polymer and the absence of this dependence on polymer concentration when it is varied by one order of magnitude over the range from 0.003 to 0.03%, which shows the intramolecular character of the transition. These relationships are also observed when internal structure in polymer molecules is formed due to the action of a precipitant, naturally, when dilute polymer solutions are investigated (with dilution as low as a few thousandths of per cent).

The PL method allows a direct observation of the formation of a compact globule: the increase in the mobility of the macromolecule, as a whole on the background of increasing intramolecular hindrance (Fig.3). As the coil becomes more compact, the mobility of the macromolecule as a whole increases and the times τ_{whole} decrease. This leads to a characteristic bell-shaped plot of changes in τ observed for various polymers when a globular type of structure is formed in macromolecules (Fig.3).

Mobility of chain end and its change caused by the formation of intramacromolecular structure.

The bonding of LG to the end of the chains of polymethylmethacrylate, polystyrene and the copolymer of glutamic acid and leucine makes it possible to observe that the mobility of a chain end is higher than that of the in-chain units (18,13) (Table 8).

TABLE 8. Relaxation times characterizing the mobility of the in-chain, τ_w(I), and end, τ_w(II), units, 25°C.

Polymer	τ_w(I), ns	Type of LM	τ_w(II), ns
PMMA in methyl acetate	8.3	LM_5	3.1
Polystyrene in toluene	8.9	"	4.1
Glu-Leu (83:17) copolymer in 0.2 M NaCl, pH 6.5, coil	15	LM_3	5
Glu-Leu (83:17) copolymer in 0.2 M NaCl, α- helix	48	"	12

By using the PL method it was possible to study the effect of the formation of intramacromolecular structure on the mobility of the main chain and its end taking as an example Glu-Leu (83:17) (12,13). It was found that not only the in-chain part of the main-chain but also its mobile end is incorporated in the parts of the macromolecule internal structure in all the stages of the formation of this intramacromolecular structure (12,13) (Fig.4).

STUDY OF MULTICOMPONENT POLYMER SYSTEMS BY THE PL METHOD

Block copolymers

The investigation of multi-component copolymers consisting of macro-blocks having a molecular weight of tens and even hundreds of thousands, such as three-block copolymers based on polystyrene (PS) and polymethylmethacrylate (PMMA) or polymethacrylic acid (PMAA) by the PL method made it possible to reveal structural transformations in the block copolymer molecules due to the action of selective solvents and to study their dependence on the chemical structure and the molecular weight of blocks and their sequence in the polymer chain (5). In these experiments block copolymers of the ABA and BAB types were investigated. The study of the IMM of individual blocks shows the structural changes that occur in each block in different solvents. The comparison of the IMM of the block with that of the homopolymer of the corresponding chemical structure and molecular weight also makes it possible to observe the changes in inter-block interactions upon variation of the solvent. For the PMMA-PS-PMMA, PMAA-PS-PMAA and PS-PMMA-PS block copolymers the conditions for the formation of various structures; a) globular, b) dumbell- and c) ring structure were established (Fig.5) (5). Table 9 gives as an example the data describing the change in the IMM of the PMMA and PS homopolymers and the PMMA* and PS* blocks in three-block copolymers; PMMA*-PS-PMMA*, PS-PMMA*-PS and PMMA-PS*-PMMA in toluene-octane when solvent composition is varied. Sym-

bol* refers to the labelled block copolymer component.

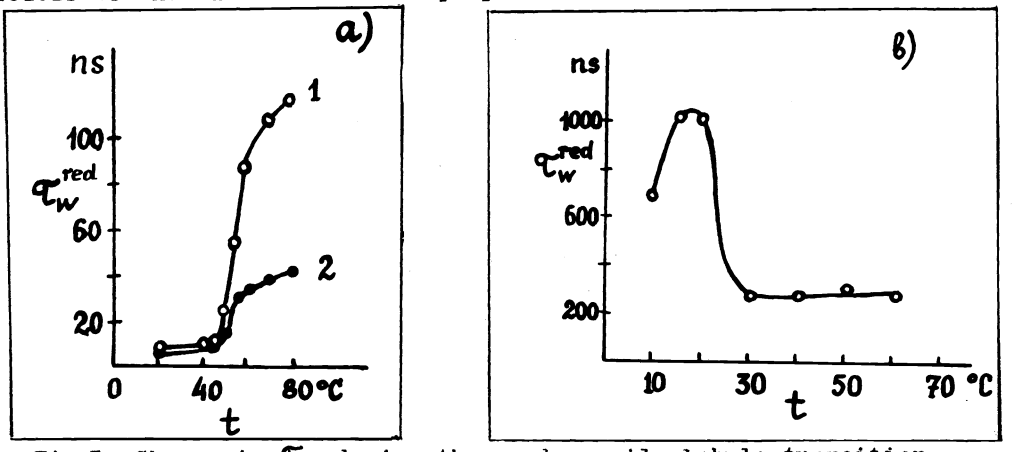


Fig.3. Change in τ_w during the random coil-globule transition in the following polymer molecules.
a) poly (1,2-dimethoxyethylene) in water on heating, M = 1) 80000 and 2) 18000 (17), b) cholesterol ester of poly-N-methacryloyl-ω-aminolauric acid (PChMALA) (M=280000) in heptane on cooling (6,7).

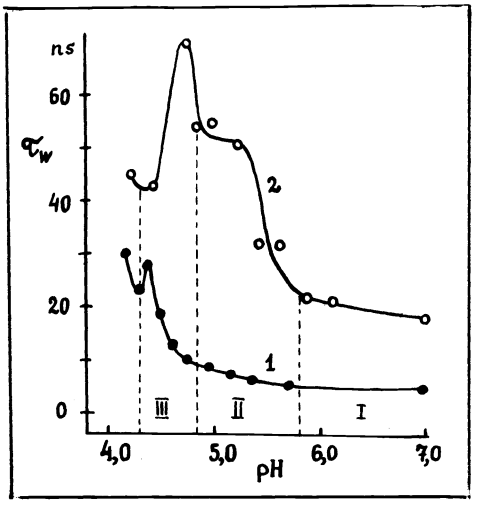


Fig.4. Change in the mobility of 1) the chain end (τ_e) and 2) in-chain units ($\tau_{m.ch.}$) in the molecules of a copolymer of glutamic acid and leucine (83:17%) in aqueous solutions (0.2 M NaCl) upon variation of pH for the transition: random coil (I)-α-helix (II)-compact structure-globule (III), M=7000.

TABLE 9. Intramolecular mobility (IMM) of polymer chains of individual blocks of three-block copolymers and IMM (τ_w) of homopolymers with the same chemical structure and similar molecular weight in selective solvents, 25°C.

Polymer in toluene-octane	octane content, weight %			
	0	12	17	22
	τ_w, ns			
PMMA	4.3	6.2	10.5	51
PS-PMMA*-PS	4.3	6.2	10.5	51
PMMA*-PS-PMMA*	4.3	6.2	33	82
PS	3.6	3.9	4.1	4.2

Polymer-polymer complexes.

Polymer-polymer complexes of all types afford another example of multicomponent polymer systems the formation and properties of which are extensively studied by the PL method. They include polycomplexes (PC) consisting of polymer chains complementary to each other with respect to the structure and chemistry (19,20) complexes of the molecules of synthetic polymers with proteins (31) and stereopolycomplexes. When a PC is formed, the mobility of polymer chains changes by one order of magnitude or more. In (PMAA-PEG) PC (PEG is polyethyleneglycol) the hindered IMM of PMAA becomes even more hindered (77 ns for free PMAA and 300 ns for PMAA in the (PMAA-PEG) PC in aqueous solutions). However, the motion of PEG chains that exhibit very high mobility in the free state (τ_w < 1 ns for free PEG chains in water) becomes also hindered: τ_w=300 ns for PEG in (PMAA-PEG) PC in aqueous solutions.

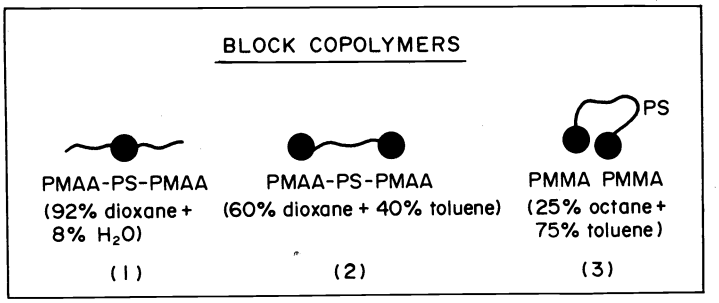


Fig.5. Structural transformations in the molecules of three-block copolymers in selective solvents.

When polymer chains of iso- and syndiotactic PMMA form stereopolycomplexes (in DMF), relaxation times characterizing the IMM of PMMA increase by one order of magnitude: from 9 to 90 ns. The PL method has been used in the study of the relationships for the formation of PC (19,20) and stereo-PC and for establishing the duration of contacts stabilizing PC (20). The study of stereo PC allowed the establishment of factors affecting the formation of regular sequences in the growing PMMA chains.

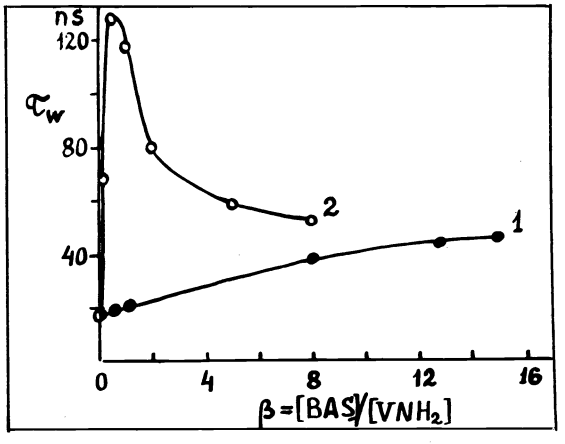


Fig.6. IMM of the polymer carrier of BAS vs BAS content in aqueous solution, 25°C.
BAS-$C_mH_{2m+1}OSO_3Na$ alkyl sulphates, 1-m=8, 2-m=15. A copolymer of vinyl pyrrolidone and vinylamine ($VP-VNH_2$) (90:10 weight %) was used as polymer carrier. Polymer concentration in the solvent was 0.5 to 1 mg/ml. $\beta=[BAS]/[VNH_2]$ is the content of BAS and vinyl amine units in moles and moles of the monomer unit (21).

Polymer carriers of biologically active substances (BAS)

The application of the PL method to the study of the formation of internal structure in the molecules of polymer carriers of BAS due to the action of BAS allows the determination of the conditions for the formation of a polymer-BAS complex in aqueous solutions depending on the chemical structure of the polymer and BAS and the pH and ionic strength of solution (21,22). The change in the IMM of the polymer carrier under the influence of BAS upon variation of the polymer: BAS ratio is described by curves in Fig.6 for two BAS with different structures.

Competing interaction of components in multicomponent polymer systems.

The PL method permits to obtain information on the competing interaction of components in multicomponent polymer systems when labelled and nonlabelled interacting components are used. The PL method was employed to study the competing interaction in reactions of the formation of polymer-polymer complexes (PC) (20) or in the polymer-BAS reactions (22), i.e., in reactions affecting the IMM of the polymer (Fig.7).

In Fig.7 the change in 1/P describes the course of the substitution reaction in aqueous solution in the system

$$PC(PAA^{*}\ PEG) + PMAA \longrightarrow PAA^{*} + PC\ (PMAA\ PEG)$$

where PAA^{*} is the polyacrylic acid containing a luminescent marker. When the PMAA concentration in the (PAA^{*} PEG) PC solution increases, the fraction of PAA^{*} molecules displaced from PC increases and 1/P also increases since the IMM of free PAA molecules (τ_w = 23 ns) is much higher than that of PAA molecules in PC (τ_w = 50 ns).

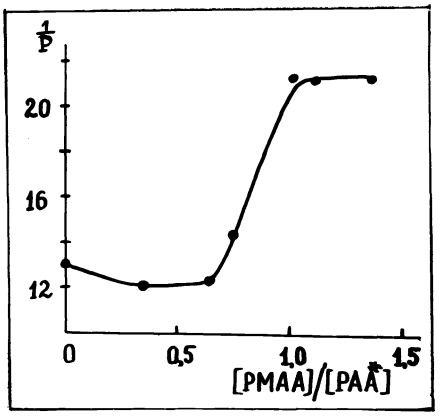


Fig.7. Dependence of 1/P for an aqueous solution of PC (PAA^{*}-PEG) on the concentration of PMAA in solution (in moles of the monomer unit) [PAA]/[PEG] = 1.

Branched polymers with covalent junctions.

The use of the PL method in the study of the formation of branched and cross-linked polymers in the copolymerization of methyl methacrylate (MMA) with a bifunctional anthracene-containing monomer permitted the some new relationships to be established (9). The main conclusion is that it is the number of interchain bridge bonds per linear cross-linked chain that determines the formation of the gel fraction and the position of the gel point rather than the relative content of the bridging reagent and the monomer in the reaction mixture. On the basis of the PL method a procedure was developed for the determination of the content of luminescent bridge bonds in the system and that of the bridge reagent reacting at one end. This determination is based on different mobilities of the anthracene group in the bridge and in a side radical bonded at one end. The dependence of 1/P for a toluene solution of copolymer of MMA and luminescent bifunctional reagent on the time of copolymerization describes the insertion of the bifunctional monomer into the bridge bond (Fig.8).

Insoluble polymer networks.

The PL method is widely used to study insoluble cross-linked polymers in a solvent for linear chains comprising the network (10). For this investigation

the insoluble cross-linked polymer is dispersed into particles of the size of 0.5 μ. The effect of cross-linking on the IMM of linear fragments was investigated taking as an example cross-linked PMAA and PAA in water containing a marker in the linear fragment. The value of τ_w for a cross-linked system increases several times as compared to that for linear chains (at 2% content of the cross-linking agent in the reaction mixture) (Table 10).

TABLE 10. Intramolecular mobility (τ_w ns) for the molecules of linear PMAA and PAA homopolymers and linear fragments of cross-linked polymers; PMAA-C and PAA-C in water at 25°C. α is the degree of ionization.

Polymer	τ_w, ns	
	$\alpha = 0$	$\alpha = 1$
PMAA	90	15
PMAA-C	410	45
PAA	23	10
PAA-C	36	20

When linear PMAA chains are crosslinked, their internal structure is retained. When the carboxy groups of PMAA are ionized, the degradation of those parts of linear PMAA fragments in the network that exhibit an internal structure occurs cooperatively, just as in linear chains, and cross-linking displaces the transition towards higher α values (degree of ionization).

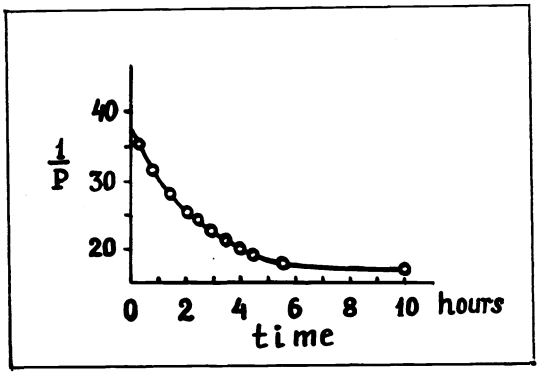


Fig.8. Dependence of 1/P for a copolymer of methyl methacrylate and dimethacrylic ester of bisoxymethyl anthracene in toluene on copolymerization time.

The PL method allows the study of the main features of protein association on a polyelectrolyte network and the mobility and density of packing of protein globules interacting with the network. These data were obtained for the lysozyme-cross-linked PMAA system.

STRUCTURAL-KINETIC HETEROGENEITY AND REACTIVITY OF FUNCTIONAL GROUPS OF COPOLYMERS.

Of the problems of polymer chemistry for the solution of which the PL method was used one can cite such an important problem as the effect of the structural-kinetic heterogeneity of copolymer molecules on the reactivity of the functional groups of the copolymer.

It has been shown for the copolymers of styrene and α-methylstyrene that the reactivity of functional groups is higher in those copolymers which contain parts exhibiting high mobility (4). Fridel-Crafts reaction of the phenyl

groups of polystyrene with chloromethylanthracene as the luminescent agent has been carried out (4). As a result of the reaction a marker appears on the polymer with the aid of which the mobility of the reacted part is studied. The data show that the functional groups of the polymer are more reactive in the parts exhibiting high mobility then in those with hindered mobility. Hence, to establish the structural-kinetic heterogeneity of copolymer molecules, the reaction of functional groups with a luminescent agent should be carried out in two stages. In one experiment reaction with the luminescent agent should be carried out and the mobility of the chain part containing the reacted groups should be measured. In another experiment first the reaction with a dark reagent with a chemical structure close to that of the luminescent agent should be carried out, and then one should carry out the reaction with the luminescent agent and measure the mobility of the parts containing those functional groups that for some reason exhibit lower reactivity than that of the groups reacting first. The mobility of these parts is found to be hindered. These data have been obtained for copolymers of methacrylate and methyl methacrylate (23). Structural-kinetic heterogeneity is exhibited most distinctly in protein molecules as has been shown taking chymotrypsinogen as an example (15).

CONCLUSION

The examples reported in the present paper briefly illustrate the possibilities of the PL method that can be widely use to study different problems. This is mainly due both to the establishment of the behaviour of LG themselves under various experimental conditions and to the successful choice of LG. The major factor in the development of the PL method was the establishment of the procedures for covalent bonding LG to the polymer and the preparation of a system of model labelled anthracene-containing polymers (more than a hundred) with the aid of which the relationships between PL and the structure and location of the markers and the structure and conformational behaviour of polymer chains were determined. It was possible to solve these problems by using a system of anthracene-containing monomers, and reagents and methods for obtaining labelled polymers developed by Krakovyak and co-workers. On this basis all anthracene-containing polymers mentioned in the present paper were obtained. It should also be noted that the amount of LG in labelled polymers is usually 1 LG per 1000 monomer units of the main polymer. Special experiments showed that this small amount of LG bonded to the polymer does not affect its properties. When new polymers and polymer systems are investigated by the PL method or when its data are interpreted, one should bear in mind the factors that affect the small-scale relaxation processes and, hence, the polarization of luminescence and take into account the changes in the viscosity and the thermodynamic strength of the solvent, the marker structure, the location of LG in the polymer chain (in the main chain, at the end of the main or side chains) and the interaction between LG and the macromolecular environment. The effect of these factors can easily be taken into account on the basis of the PL relationships for solutions of anthracene-containing polymers established with the aid of model labelled polymers. If all these relationships are taken into account, the interpretation of data obtained by the PL method is fairly simple and clear conclusions can be drawn on the main features of the structure and dynamic behaviour of polymers and complex multicomponent polymer systems.

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