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A Novel Porphyrin-Labeled Poly(*N*-Isopropylacrylamide): Spectral and Luminescent Properties

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A novel water-soluble tetraarylporphyrin-containing polymer has been synthesized by the reaction of bromoalkyl-containing poly(*N*-isopropylacrylamide) with 5-(4-pyridyl)-10,15,20-tri(4-methoxyphenyl)porphyrin. Some physicochemical properties of the obtained polymers are reported. It has been shown that a strong interaction between the porphyrin units takes place in liquid aqueous medium at temperatures below the lower critical solution temperature (LCST). This phenomenon results in considerable broadening of the Soret band in the absorption spectrum and in strong quenching of fluorescence. Higher than LCST fluorescence enhancement is observed.

KEY WORDS: Poly(*N*-isopropylacrylamide) copolymers; porphyrin derivatives; phase transition; fluorescence quantum yield; fluorescence lifetime.

INTRODUCTION

Polymers containing chromophore units are extensively synthesized and studied in connection with their diverse applications in science and technology. One of the important trends of the investigation of such polymers is the creation of molecular systems modeling biological objects [1].

Specifically, in biophysical processes an important and, to a large extent, not yet elucidated role is played by conformational changes in biopolymers, in the first instance, proteins, including chromoproteins and pigment–protein complexes [2]. The development of the generation of so-called "smart" polymers [3] makes it possible to model such transformations and to control them by changing external factors [4], such as temperature.

Owing to the exceptional role of tetrapyrrole pigments, chlorophyll and heme, in animate nature, polymers bearing porphyrinic units are of considerable interest as biomimetic models. For this reason, we are carrying out research in the field of synthesis and investigation of the "smart" polymers, derivatives of poly(*N*-isopropylacrylamide) (PNIPAM) containing porphyrin and metalloporphyrin units [5].

Since, in prospect, one may expect that in this way controllable photochemical systems can be elaborated, we pay primary attention to spectral-luminescence investigations of the polymers obtained. It is necessary to note that fluorescence methods are sensitive to conformational changes of macromolecules. Thus, it has been shown by Winnik that the intensities of the monomer and excimer fluorescence of pyrene fragments linked to PNIPAM change sharply on the phase transition in the copolymer [6].

Polymers containing porphyrin moieties in their side chains can be prepared by the polymerization of porphyrins containing vinyl groups [7–9] or by reactions of prepolymers with porphyrin derivatives [10].

In the framework of the second approach we have proposed to use for the preparation of porphyrin-labeled PNIPAM the reaction of quaternization of monopyridyl-

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containing porphyrin derivatives by side bromoalkyl groups of a prepolymer [11].

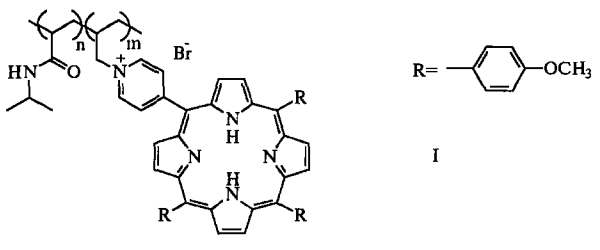
In the present article we report spectral and physicochemical properties of the polymer prepared by the reaction of 5-(4-pyridyl)-10,15,20-tri(4-methoxyphenyl) porphyrin with the copolymer of *N*-isopropylacrylamide (NIPAM) and allylbromide.

EXPERIMENTAL

The porphyrin-containing PNIPAM, **I** (Fig. 1), was prepared by the reaction of 5-(4-pyridyl)-10,15,20-tri(4-methoxyphenyl)porphyrin with the copolymer of NIPAM and allylbromide (3-bromo-1-propene) in refluxing 1,4-dioxane. The polymer was purified by precipitation from a tetrahydrofuran (THF) solution by dry diethyl ether and then air-dried. Distilled water and 1,4-dioxane freshly distilled over calcium hydride were used as solvents for spectral measurements.

Absorption spectra were recorded on a Varian Cary 500 Scan spectrophotometer. Fluorescence spectra were measured using a laboratory handmade setup described in Ref. 12. It is based on two diffraction grating monochromators MDR-23. A 3-kW high-pressure xenon lamp with a water cooling system was used for excitation. Fluorescence from the sample was modulated by a disk modulator and then directed to the recording monochromator. A photomultiplier with the S1 type of photocathode with an additional cooling system was used for the emission detection. The procedure for the fluorescence quantum yield measurements is described in Ref. 13. A time-correlated single-photon counting spectrofluorometer (PRA 3000) was used for fluorescence lifetime measurements.

Aqueous solutions viscosities were measured at 20°C with an Ubbelohde-type viscometer. The viscosity-average molecular mass of the polymer was calculated using the equation given in Ref. 14. The phase transition



I

n/m=1300

Fig. 1. Chemical structure of polymer **I**. The bromine atoms of the residual bromoalkyl groups are not shown.

of aqueous solutions was investigated by monitoring the transmittance of a monochromatic light beam (λ = 490 nm) at different temperatures. Commercial thermostat MLW UH8 and the cell described earlier [5] were used for these measurements. The rates of heating up and cooling down of the sample cells were adjusted at 0.7°C/min.

RESULTS AND DISCUSSION

The polymer obtained is soluble in acetone, chloroform, dioxane, ethanol, methanol, tetrahydrofuran, cold water, and some other solvents which are capable of forming reasonably strong hydrogen bonds with the polymer.

Aqueous solutions of PNIPAM and its derivatives are known to undergo phase separation at about 30–40°C on heating. The phase transition diagram of aqueous solution of polymer **I** is shown in Fig 2b. It is typical for a system exhibiting the lower critical solution temperature [15]. Comparison with the porphyrin-free prepolymer (Fig. 2a) shows that the presence of voluminous porphyrin groups makes the phase transition somewhat smoothed.

The spectral and luminescence properties of polymer **I** have been studied in dioxane and in water. The characteristics of polymer **I** and the prepolymer are presented in Table I. Dioxane has been chosen as a typical organic solvent of moderate polarity. Water is the most important solvent since it is in water that the phase transition takes place. As mentioned above, the long-range goal of the investigations is the modeling of biological photoprocesses, and water is always present in biological systems.

The absorption spectra given in Fig. 3 are very similar to the spectra of *meso*-tetraphenylporphyrin (TPP) in organic solvents. This is not surprising since the absorption spectra of porphyrins are weakly sensitive to the influence of environment and the spectra of *p*-substituted derivatives of TPP do not differ essentially from that of TPP. The absorption bands of the *meso*-tetraarylporphyrin chromophore are observed on a continuous background whose nature is unclear.

At the same time, in aqueous solution the absorption spectrum has characteristic features. The $Q_2(0,0)$ and $Q_2(0,1)$ bands are shifted bathochromically, so that the $Q_2(0,0)$ band partially overlaps with the $Q_1(0,1)$ band. There is an analogy of this feature with, e.g., the spectrum of the water-soluble sulfo derivative of TPP in water [16]. Presumably, the hydrophobic porphyrin units in aqueous solution are not completely sheltered in the polymer globule and interact with water. Moreover, visible bands and, especially, the Soret band are broadened in aqueous

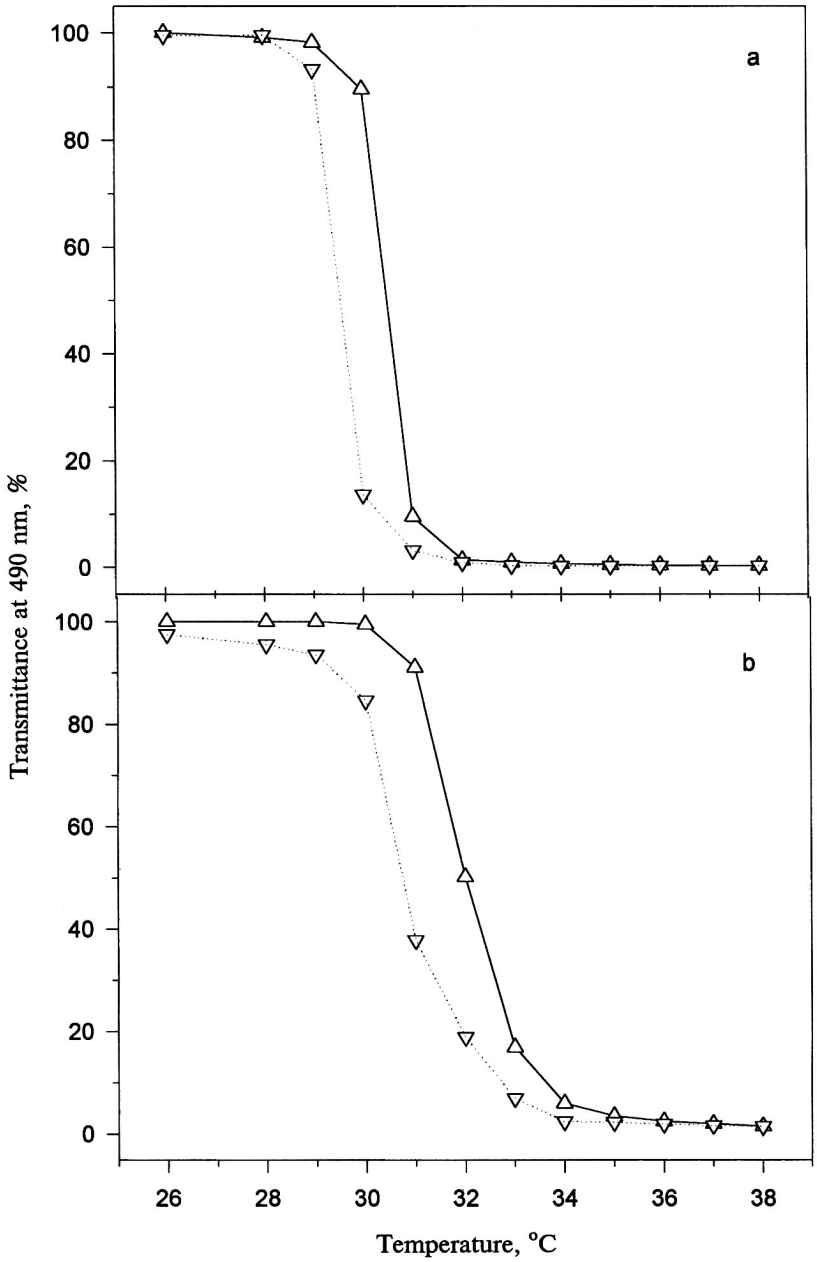


Fig. 2. Phase transition diagrams of the polymers studied: (a) starting polymer; (b) polymer **I**. Solid line, heating-up; dotted line, cooling-down.

media, which is indicative of interchromophore interactions. The latter phenomenon is similar to that in homopolymers of the vinyl group-containing porphyrins [17].

The fluorescence spectra of the investigated polymer in dioxane and water (Fig. 3) are similar (the spectrum in dioxane corresponds closely to the fluorescence spectrum of TPP in the same solvent). However, in water an ≈10-nm red shift is observed. Since the position of the $Q_1(0,0)$ absorption bands in dioxane and water is almost the same, the Stokes shift in water obtained is larger by ca. 100 cm^{-1}. We believe that this is due to the rearrangement of the solvation shell of the porphyrin unit in the excited electronic state S_1.

For the same solvents we have measured the fluorescence quantum yields φ_F and investigated the kinetics of fluorescence (see Table I). The measurements have shown that in a dioxane solution the fluorescence of porphyrin chromophores linked to the polymer chain is practically

Table I. Physicochemical and Spectral Properties of the Polymers

Polymer	Solvent	Molecular weight	LCST (°C)	Porphyrin content (mol/g)	Polymer concentration (g/L)	Fluorescence quantum yield	Fluorescence lifetime, ns (contribution of monoexponential component)
Starting	Water	$4.6*10^4$	31	—	3	—	—
I	Dioxane	—	—	$6.7*10^{-6}$	1.25	0.06	8.8 (100%) 0.49 (83%)
	Water	—	32	—	3.5	0.009	6.9 (17%)

not quenched compared with TPP. Contrary to this, fluorescence in water is strongly quenched and its kinetics is nonexponential. The kinetic results show that there are two types of porphyrin units, having $\tau_F = 0.49$ and 6.9 ns.

The acquired data on the kinetics of fluorescence and the quantum yields enable us to draw some conclusions.

1. In dioxane solutions porphyrin units exist as a single species. Of course, certain inhomogeneity is possible, but the spread of the parameters of different centers is not large and is not seen in the experiments. The interaction of the chromophore with the polymer chain practically does not lead to shortening of τ_F, i.e., to quenching, compared to TPP in *n*-propanol [18].
2. In aqueous solutions two forms exist, with the same reservation concerning the homogeneity. One form is close to the form in the dioxane solution regarding the τ_F value, but the τ_F in water is somewhat shorter. The other form has a considerably shorter τ_F (14-fold compared to the first form), i.e., its fluorescence is strongly quenched. The pattern of the absorption spectra shows that the S_0–S_1 transition moment does not change essentially on passing from dioxane to water; hence the concomitant rate constant of the radiative transition, k_F, should not change significantly. Therefore, when estimating the photophysical parameters qualitatively and semi-

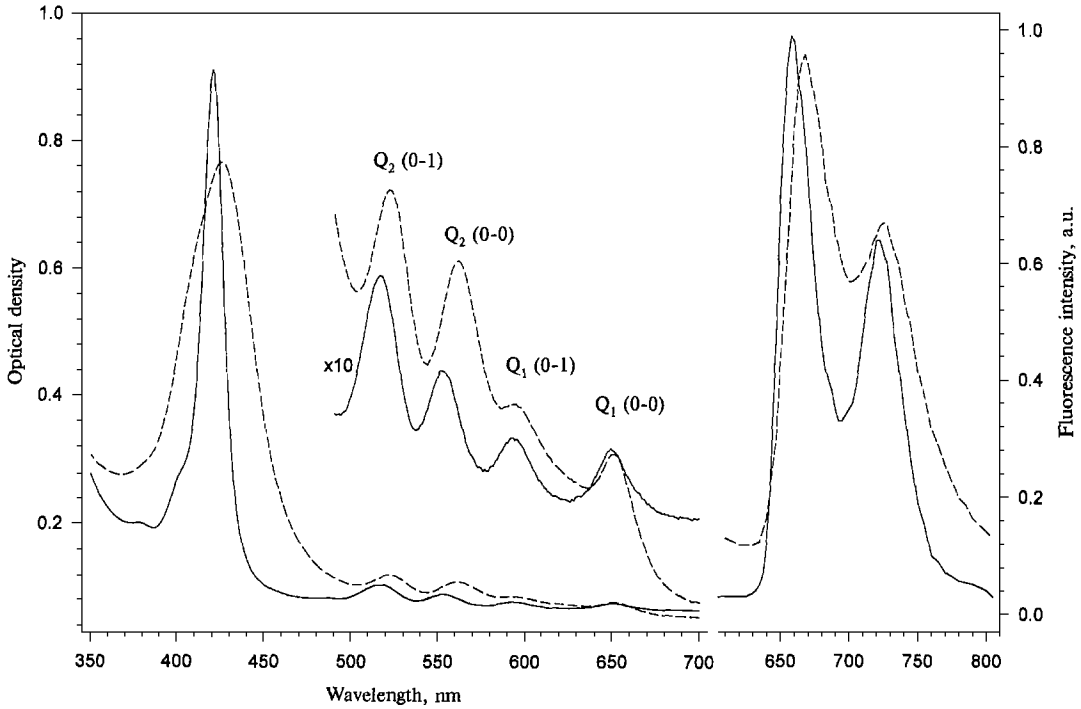


Fig. 3. Absorption and fluorescence emission spectra (λ_{exc} = 424 nm) of polymer **I** at 293 K in dioxane (solid line) and in water (dashed line).

quantitatively one may consider that τ_F changes proportionally to φ_F.

3. The quenching of the porphyrin units in the polymer investigated seems to be due to interchromophore interactions. In the case of a loose polymer chain (in dioxane) the porphyrin chromophores interact only slightly. In aqueous solutions more compact globules are formed, and hydrophobic porphyrin units completely or partially move inside into the globule. Thereby the porphyrin units come closer to one another, which may lead to fluorescence quenching. It has been shown for a similar copolymer containing pyrene labels that in aqueous solutions the excimer fluorescence appears while the monomer fluorescence is quenched [6]. In our case only fluorescence quenching is observed (at present there is no evidence concerning the excimer fluorescence of porphyrins). However, it cannot be excluded that a certain contribution to the quenching is brought by the external heavy-atom effect. As is known, alkyl halides (bromides and iodides) are effective fluorescence quenchers by this mechanism. The presence of bromoalkyl groups in the polymer will lead to fluorescence quenching whose efficiency is determined by the proximity of the porphyrin units to the bromoalkyl groups. Possibly, for weakly quenched centers (τ_F = 4.7 ns) the quenching occurs due to this mechanism.

The data obtained enable the estimation of the contribution of the centers of separate types to the fluorescence of the polymer under study.

The fluorescence decay law for the mixture of two forms

$$I_F = L_1 e^{-t/\tau_1} + L_2 e^{-t/\tau_2} \quad (1)$$

is given by PRA fluorometer as values of τ_i and L_i. It allows one to determine the concentration ratio of these forms if their extinction coefficients, ε_i, for λ_{exc} and the fluorescence rate constants, k_{Fi}, are known

$$\frac{C_1}{C_2} = \frac{L_1}{L_2} \cdot \frac{\varepsilon_2 k_{F2}}{\varepsilon_1 k_{F1}} \quad (2)$$

Strictly speaking, in our case the values of ε_i and k_{Fi} are not known, but taking into account the similarity of the absorption spectra to the spectrum of TPP, one may believe, as already mentioned, that $\varepsilon_1 \approx \varepsilon_2$ and $k_{F1} \approx k_{F2}$. Then $C_1/C_2 \approx L_1/L_2$, and components' contributions of luminescence given in Table I directly reflect the concentration of the forms, i.e., the concentration of the

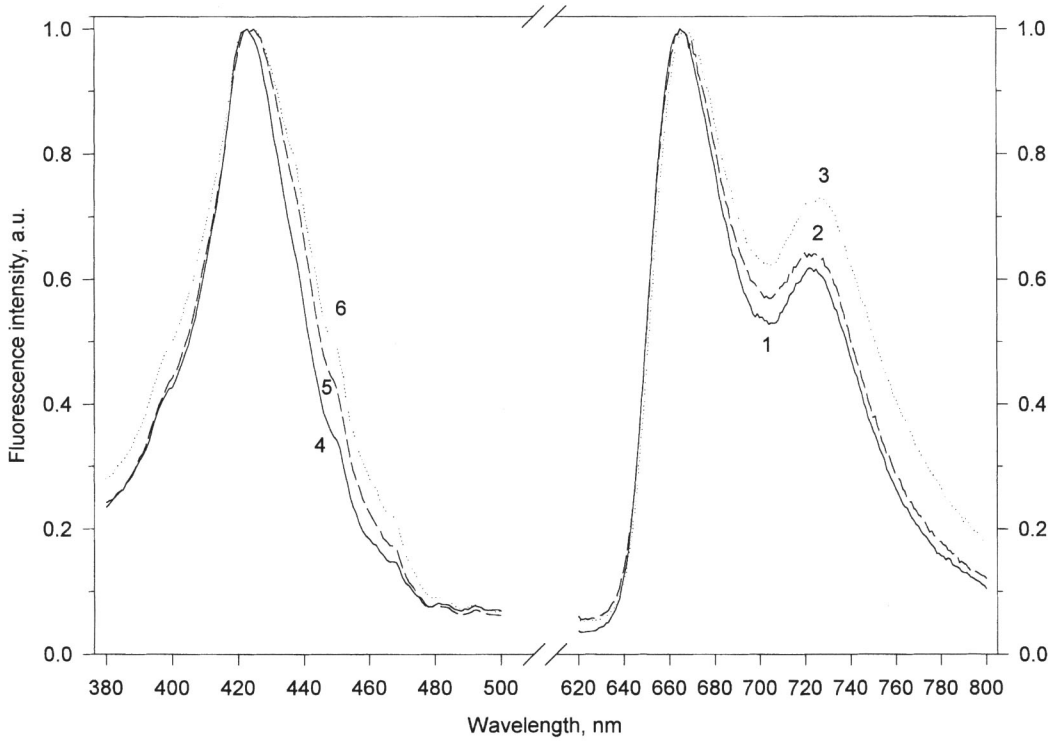


Fig. 4. Fluorescence emission (1–3) and excitation (4–6) spectra of an aqueous solution of **I** at 293 K: (1) λ_{exc} = 424 nm; (2) λ_{exc} = 405 nm; (3) λ_{exc} = 445 nm; (4) λ_{mon} = 650 nm; (5) λ_{mon} = 665 nm; (6) λ_{mon} = 680 nm.

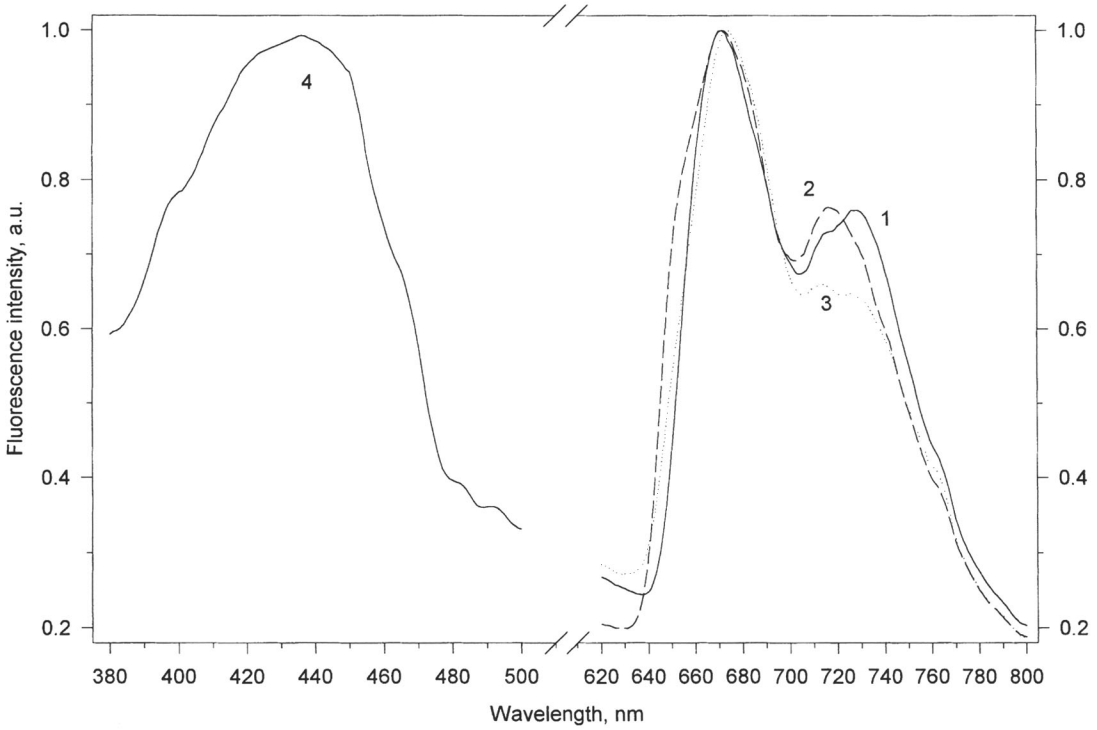


Fig. 5. Fluorescence emission (1–3) and excitation (4) spectra of a frozen aqueous solution of **I** at 77 K: (1) λ_{exc} = 445 nm; (2) λ_{exc} = 424 nm; (3) λ_{exc} = 405 nm; (4) λ_{mon} = 675 nm.

strongly quenched form is greater by a factor of 4.9 than that of the weakly quenched form.

The quantum yield of fluorescence of the two-form mixture, $\varphi_{F\Sigma}$ (which is measured in experiments under steady-state excitation) is related to the fluorescence quantum yield of individual components, φ_{Fi}, as follows:

$$\varphi_{FS} = \frac{\varphi_{F1}L_1k_{F2} + \varphi_{F2}L_2k_{F1}}{L_1k_{F2} + L_2k_{F1}} \quad (3)$$

If we assume that $k_{F1} \approx k_{F2}$,

$$\varphi_{F\Sigma} \approx \varphi_{F1}L_1 + \varphi_{F2}L_2 \quad (4)$$

To estimate φ_{F1} and φ_{F2}, we use the data for the dioxane solution considering, as stated above, τ_F and φ_F as proportional. Thus we obtain φ_{F1} = 0.0033 and φ_{F2} = 0.047. For L_1 = 0.83 and L_2 = 0.17, formula (4) gives $\varphi_{F\Sigma}$ = 0.0107.

The experimental value $\varphi_{F\Sigma}$ = 0.009 is in satisfactory agreement with the calculated value. This confirms the correctness of the implemented analysis and, at the same time, means that our polymer does not contain nonfluorescent (more strongly quenched) forms in aqueous solution. The existence of such forms has been revealed by us in an analogous way for a previously investigated polymer [19].

Additional corroboration was obtained in the investigation of the fluorescence excitation spectra. Figure 4 presents the fluorescence spectra of **I** in water at different λ_{exc} (in the Soret band region) and the fluorescence excitation spectra measured at monitoring in different intervals of the fluorescence spectrum. The analysis of the data obtained shows that the fluorescence spectrum is determined by the contributions of two components of the mixture of chromophores. The dominant component has an excitation spectrum corresponding to the absorption spectrum of the mixture shown in Fig. 3 (i.e., it is characterized by a broadened Soret band) and a somewhat lowered intensity of the fluorescence 0–0 band. For the minor component the Soret band is narrower (see curve 4 in Fig. 4) and the intensity of the fluorescence 0–0 band is somewhat higher (see curve 2). Since the differences in the fluorescence spectra are not large, one may state that the integral values of k_F for the components mentioned differ insignificantly, which corroborates the correctness of the above analysis of the kinetic data.

Analogous measurements were performed at liquid nitrogen temperature. The results are displayed in Fig. 5. It is shown in the figure that in frozen aqueous solutions the Soret band is considerably broadened and shifted to the red. The dependence of fluorescence spectra on λ_{exc}

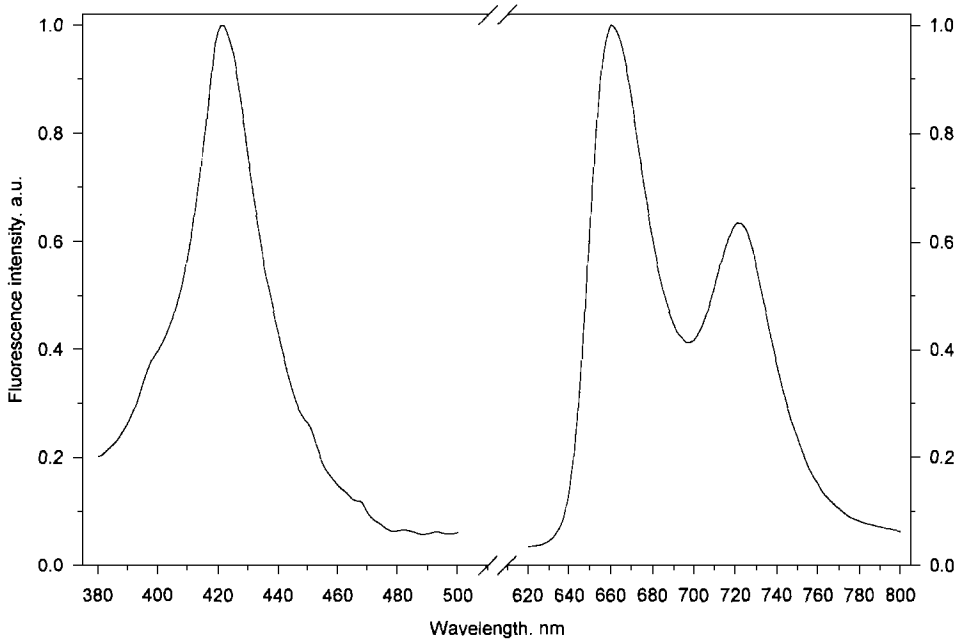


Fig. 6. Fluorescence emission (λ_{exc} = 424 nm) and excitation (λ_{mon} = 661 nm) spectra of an aqueous solution of **I** at 323 K.

becomes more complicated. One may conclude that, on freezing, the interchromophore interactions are enhanced; possibly, aggregation of chromophores takes place.

Fluorescence emission spectra at temperatures higher than the LCST (323 K) are considerably narrowed (Fig. 6) and shifted to the blue (from 665 to 661 nm for the 0–0 fluorescence band). The phase transition is accompanied by a strong enhancement of the fluorescence intensity. The fluorescence quantum yield at this temperature exceeds $\varphi_{F\Sigma}$ at 293 K by six times and practically reaches $\varphi_{F\Sigma}$ for the dioxane solution. As shown in Fig. 6, the Soret band in the excitation spectrum is also narrowed and shifted hypsochromically. It is necessary to note that the spectral-luminescent measurements reveal a certain inhomogeneity of this system. To some extent the fluorescence spectra depend on λ_{exc}, and the excitation spectra depend on the monitoring wavelength, λ_{mon}, analogously to the data in Fig. 4 (specifically, the Soret band is broadened). However, the spectra displayed in Fig. 6 belong to the predominant component of the mixture.

These results are in close agreement with the data of Winnik [6] on the fluorescence of pyrene-containing PNIPAM, for which, on the phase transition, i.e., at a temperature higher than the LCST, the fluorescence enhancement of the monomers of pyrene units was observed at the expense of the excimer fluorescence. Therefore we interpret our data in an analogous way. At temperatures lower than LCST in liquid aqueous media the interchromophore interaction is of a dynamic nature (probably, of the excimer type). Higher than cloud point the increased rigidity of the globule hinders the contact between chromophores, which leads to the luminescence of isolated porphyrinic units.

The results of our experiments show that the photophysical properties of porphyrin-labeled PNIPAMs are different for the polymers obtained by various methods [5,11,19]. There are several factors which may determine these differences, e.g., the molecular mass of a polymer, the length of spacers connecting porphyrin units with the polymer chain, and the presence of residual functional groups of a polymer. The significance of these factors will be elucidated in future investigations.

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