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Characterization of non-ionic surfactant aggregates by fluorometric techniques

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Abstract

Steady-state and nanosecond time-resolved studies have been carried out on the fluorescence quenching of excited pyrene by *N*,*N*-dibutylaniline in an aqueous solution of non-ionic micelles of the six Tritons, (oxyethylene)$_m$-*p*-(1,1,3,3-tetramethylbutyl)phenyl ethers with *m* ranging from 8 to 70. The aggregation numbers and the rate constants of intramicellar quenching have been determined. The critical micelle concentrations of investigated Tritons were determined using the dependence of the fluorescence spectrum of pyrene on the microenvironment. The local polarity was obtained from the intensity ratio of the first to the third peak (I_1/I_3) in the fluorescence spectrum of pyrene. The microviscosity of the micellar core was estimated to be about 200 cP at ambient temperature on the basis of fluorescence spectra of 1,3-bis(1-pyrene)propane, from the excimer to the monomer emission intensity ratio using the calibration curve determined for a number of solvents of known viscosities.

Keywords: Fluorescence quenching; Tritons micelles; Pyrene; Aggregation number

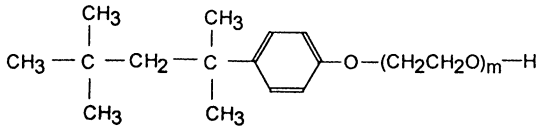
1. Introduction

Non-ionic surfactants belonging to polyethylene oxide family are widely used in industrial and domestic applications. The structure and dynamical properties of the surfactant micelles play an important role in their applications. Triton X-100 is one of the non-ionic surfactants widely used and extensively studied. Techniques such as light scattering, NMR viscometry, small-angle X-ray scattering, ultrasonic absorption, rheological measurements and spectrometry have delivered substantial data on the TX-100 micelle. The results of these investigations show that the non-polar hydrocarbon chains are packed in the interior micellar core, whereas the hydrophilic polyoxyethylene chains stay outside the core forming a larger shell with a certain number of water molecules included. However, the details of micellar properties such as a degree of packing of hydrophobic chains in micellar core or even the shape of micelle are controversial [1–4]. The literature concerning other Tritons is rather scarce. The aim of this work was to obtain the parameters for characterization of micellar properties of six polyoxyethylene-*p*-(1,1,3,3-tetramethylbutyl)phenyl ethers distinguished by the length of their polyoxyethylene chains. Steady-state and time-resolved fluorescence techniques have been used by us to determine aggregation numbers, *N*, critical micelle concentration (CMC), microviscosity (microfluidity) and micropolarity of the micellar interior.

2. Experimental

2.1. Reagents

The six studied polyoxyethylene-*p*-(1,1,3,3-tetramethylbutyl)phenyl ethers of the general formula



belong to popular family of non-ionic surfactants known as Tritons®. The specification of used materials is shown in Table 1. Each Triton is a mixture of homologues, which differ in the hydrophilic chain length, so *m* is the average value.

Experiments were carried out with pyrene (99% Fluka) and 1,3-bis(1-pyrene)propane (P3P) (Molecular Probes, USA) as a fluorescent probes. P3P was used without additional purification. Pyrene was purified by column chromatography [5]. The quencher *N*,*N*-dibutylaniline (DBA)

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Table 1
Investigated surfactants[a]

Trade name	Source	MW	*m*	Remarks
TX-114	Fluka	558	8	Liquid
TX-100	Fluka	624	9.5	Liquid
TX-165	Sigma	910	16	70% aqueous solution
TX-305	Merck	1526	30	Solid
TX-405	Fluka	1966	40	70% aqueous solution
TX-705	Sigma	3286	70	70% aqueous solution

[a] Triton® is the trademark of Röhm and Haas Deutschland GmbH.

was purchased from Aldrich and cetylpyridinium chloride was purchased from Sigma. Solutions were prepared in ethanol (96% p.a., Polmos, Poland), in distilled water treated in a Millipore Milli-Q Plus system, and in dioxane (Fluka).

2.2. Experimental procedures

The steady-state fluorescence measurements were done on a Perkin-Elmer luminescence spectrometer LS50B with the thermostated cuvette holder. One centimeter optical path cuvettes were used.

For the determination of CMC, the appropriate amount of pyrene ethanolic stock solution (2.9 mM) was introduced into a volumetric flask containing water. The solution of pyrene, concentration equal to about 2 μM, was divided into two parts. In one part, the proper amount of the detergent was dissolved. By mixing the obtained two solutions in desired ratios, one can prepare a series of solutions of various detergent concentrations and the same, constant pyrene concentration. The probe was excited at 337 nm. The measurements were done at $22 \pm 1\,^{\circ}C$.

For the determination of aggregation number, the detergent solutions were prepared by exact weighing and dissolution in water. The proper volume of pyrene stock solution was introduced into a dry flask. After evaporation of ethanol, the residue was dissolved in earlier prepared detergent solution by sonication. In this manner, a series of solutions of the detergent concentrations in the range 2.5–40 mM and of pyrene concentration in the range 0.5–8 μM were obtained. Each solution was divided into two parts and into one part, DBA was added in such quantity that its final concentration was equal to 0.1 mM. Next, by mixing two parts in appropriate ratios, a few solutions were obtained of various quencher concentrations (5–100 μM) and the same, constant detergent and pyrene concentrations. The high microviscosity of Tritons micelles requires to use very efficient quenchers of pyrene fluorescence in order to obtain reliable values of the micelle aggregation numbers. DBA clearly fulfil this requirement but not cetylpyridinium chloride. In some experiments, the samples were deoxygenated by bubbling nitrogen. Pyrene was excited at 337 nm. During measurements the temperature was kept at $22 \pm 1\,^{\circ}C$. Time-resolved fluorescence measurements were performed on flash photolysis system or by the single-photon timing technique. The flash photolysis system consists of a nitrogen laser [5] (Laser Photonics, LN120C) providing single light pulses (337.1 nm, duration 300 ps and energy 70 μJ), the detection system: monochromator Acton Research SpectraPro 275 or Baush & Lomb, photomultiplier Hamamatsu R 3896 or 1P28, and power supply Stanford Research System PS325.

The cuvette orientation was carefully adjusted and appropriate optical filters were used to reduce scattered light interference from the laser pulse. The signal output from photomultiplier was digitized and recorded using a Tektronix TDS 680C or Hewlett-Packard 54510A oscilloscope, and transferred via a GPIB to the computer.

Fluorescence life-time measurements were also made using a FL900CDT single-photon pulse fluorimeter from Edinburgh Analytical Instruments. For all experiments, the excitation wavelength was 337 nm and the excitation and emission bandpasses were 3.6 nm. The instrument response function was recorded by collecting scattered light from a Ludox silica suspension. Fluorescence decay from both the sample and scattering solution was acquired to 1.0×10^4 counts in the peak. The counting rate was less than 2% of the lamp repetition rate. The fluorescence decay curves recorded experimentally were fitted to Eq. (5) using a non-linear weighted least squares procedure [5,6], using the computer program written by Dr. M. Wojcik from IARC.

For the determination of the microviscosity, the stock solution of P3P was prepared in dioxane of such concentration that 20 μl aliquots diluted to 3 ml gave absorbance of a 0.25 at 345 nm. Twenty microliters of the stock solution was introduced to a cuvette and the solvent was removed by nitrogen flow. The deposited film was dissolved in 3 ml detergent solution of the concentration equal to 10 mM by stirring at elevated temperature (313 K) for 2 days. Oxygen was removed from the samples by nitrogen or argon stream. The ratio I_E/I_M (I_E is the intensity of excimer band at 480 nm and I_M the intensity of vibronic peak of monomer at 373 nm) for P3P strongly depends on the presence of oxygen, because the quenching of the excimer band is much stronger with respect to that of the monomer. Some Triton samples were deaerated using the freeze–thaw technique with a vacuum line and by bubbling with nitrogen or argon. The obtained ratio I_E/I_M using both the methods was the same, so we decided to remove the air by nitrogen bubbling, which is more convenient. We have examined the P3P fluorescence spectra in Triton solutions with the Aminco–Bowman spectrofluorimeter used by one of us earlier [7] to obtain a calibration plot (i.e. the plot of I_E/I_M of P3P* versus viscosity of solvents of known viscosities).

3. Results and discussion

3.1. CMC determination

Among the most widespread methods for investigating microheterogeneous media of all kinds there are techniques based on the spectroscopic properties of molecules acting

as probes. Pyrene is the most popular fluorescent probe to investigate polarity changes in micro-organized systems. Its monomer fluorescence spectrum in solution shows significant vibronic fine structure consisting of the five major peaks. The intensity ratio of the first and the third vibrational bands (I_1/I_3) has been used as an indicator of the microenvironment polarity [8–10]. Since pyrene is a hydrophobic compound in aqueous micellar solutions, it tends to solubilize in the micellar core and its spectrum undergoes some variations in comparison with that in detergent solutions of the concentrations below CMC or in pure water. Monitoring the ratio I_1/I_3 of pyrene spectrum in detergent solutions of various concentrations one can determine the CMC. This method is very often used for CMC determination [2,8–10]. The dependence of I_1/I_3 ratio on TX-705 concentration is shown in Fig. 1. The concentration in the vicinity of the inflection point on the sigmoidal curve indicated in the Fig. 1 was considered as the CMC. The second derivative of the fitting curve equals zero at CMC. The intensity ratio I_1/I_3 decreases as the temperature is increased. For example, in the case of TX-405 the change in the I_1/I_3 value is given by equation: $I_1/I_3 = 1.704 - 0.001T$ (T, temperature in K). This is probably caused because of "squeezing out" of associated water molecules as the polyoxyethylene chains tighten [11,12], but it can also be explained as decrease in medium polarity with increasing temperature, what was noted for pure solvents [12] (in water we have found the following equation: $I_1/I_3 = 5.021 - 0.01065T$ well describes the influence of temperature on the I_1/I_3 value). The micropolarities measured by pyrene in Tritons (I_1/I_3∼1.4–1.5) are similar to that recorded in methanol ($I_1/I_3 = 1.39$, dielectric constant $\varepsilon = 32.6$), and are slightly higher than the polarity that the probe samples in cationic micelles of hexadecyltrimethylammonium chloride (HTAC) ($I_1/I_3 = 1.36$) [5]. The environment corresponding to the value 1.4 is thought to be the external surface of the hydrophobic core of the Triton micelles.

The relatively high polarity at the probe site supports the idea that water penetrates deeply into micellar aggregates. According to Robson and Dennis [13], the TX-100 micelle has an oblate ellipsoid shape with a semi-axes of 52 and 27 Å, and its structure consists of hydrated polyoxyethylene chains, which take up around 80% of the total micellar volume. The non-polar hydrocarbon chains, phenyls and first adjacent oxyethylene group form the micellar core [14]. The penetration of water into the micellar core is excluded [4]. Consequently, Triton micelles are molecularly non-homogenous showing biphasic nature of two microenvironments: hydrocarbon-like core and hydrophilic polyethylene mantle. Such structure of the micellar aggregates suggests that local polarity inside the micelle is lower in the core and the higher near to the micellar surface. Therefore, various indicator molecules report a wide range of effective dielectric constants ($15 \leq \varepsilon \leq 40$) inside Tritons micelles [15–17]. We have observed that detergents with long polyoxyethylene chains tend to give larger I_1/I_3 values, which indicated that those micelles provide pyrene a more polar environment than the ones with short chains. The polarities sensed by pyrene in the examined micelles do not differ much, but clearly pyrene in Triton micelles with long ethylene oxide chains is partially associated with water molecules.

Solubility of pyrene in micelles allows to observe pyrene excimer formation below concentrations at which this phenomenon occurs in homogeneous solutions [18]. The emission spectrum of dimer (∼480 nm, Fig. 2) arises if at least two pyrene molecules enter the hydrophobic micelle's core. When the detergent concentration increases this band disappears. It is due to the increasing number of micelles, in this circumstances the probability of meeting two pyrene molecules inside the same micelle falls down to zero. Measuring the intensity ratio of the excimer and the third vibrational monomer bands (I_E/I_3) in dependence of detergent concentration it is also possible to determine the CMC.

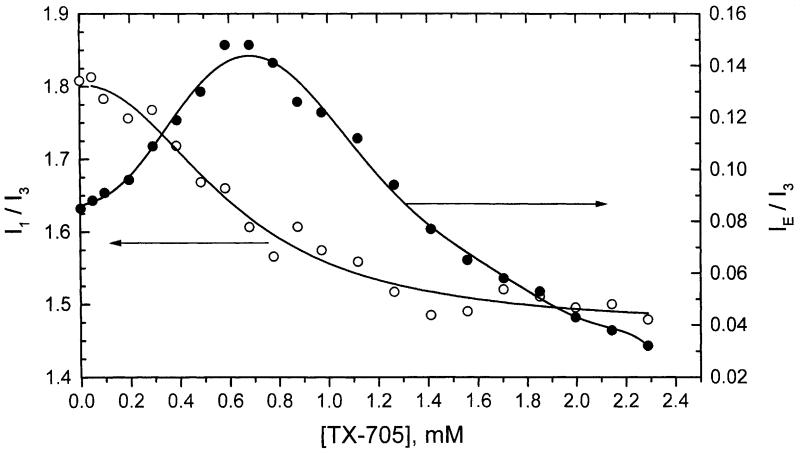

Fig. 1. Dependence of the pyrene emission intensity ratio of the first to the third vibronic peaks, I_1/I_3, and that of excimer emission to the third peak, I_E/I_3, on Triton X-705 concentration ([pyrene] = 2 μM, λ_{exc} = 337 nm and T = 298.5 K).

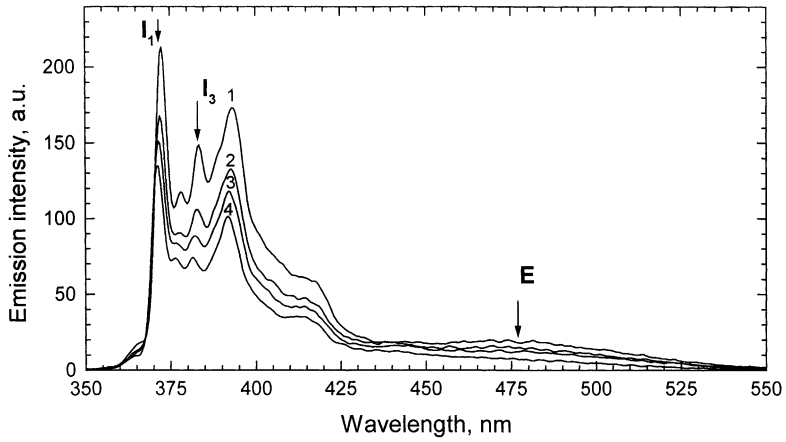

Fig. 2. Fluorescence spectra of pyrene in aqueous solutions of Triton X-165 of various concentrations: (1) 0; (2) 0.264; (3) 0.395; (4) 0.593 mM ([pyrene] = 2 μM, λ_{exc} = 337 nm and T = 292.5 K). I_1 and I_3 are the intensities of the first and the third vibronic peaks in the emission spectra of pyrene, and E the intensity of the excimer peak.

Table 2
CMC of Tritons

Triton	CMC from I_1/I_3 ratio (mM)	CMC from I_E/I_3 ratio (mM)
TX-114	0.22	0.23
TX-100	0.16	0.19
TX-165	0.37	0.36
TX-305	0.66	0.61
TX-405	1.03	1.05
TX-705	0.63	0.67

Fig. 1 presents the relationship of the I_E/I_3 ratio on the TX-705 concentration. The detergent concentration corresponding to the maximum of the fitting curve (polynomial function) was considered as the CMC (first derivative equal to zero). The determined CMC values for all the studied detergents are collected in Table 2, and can be compared with the literature values for: TX-405 0.9 mM [19], 0.18 mM [20], 0.81 mM [21]; TX-100 0.273, 0.277 mM [22], 0.28 mM [19], 0.24 mM [23], 0.15 mM [2], 0.25 mM [24], 0.25, 0.28 mM [25], 0.26 mM [26], 0.29 mM [27], 0.25, 0.27 mM [28], 0.33 mM [29], 0.272 mM [30], 0.23 mM [31], 0.33 mM [9,10]; TX-114 0.28 mM [11], 0.26 mM [32], 0.265, 0.212 mM [22], 0.22, 0.283, 0.247 mM [21], 0.27 mM [31]; TX-165 0.43 mM [21]; TX-305 0.7 mM [19].

The methods based on excimer formation, except of TX-165 and TX-305, gave slightly higher CMCs. The process when micelles appear and are shaped takes place in a certain concentration range and it is difficult to indicate a sharp boundary of the concentration when micelles are formed. The determined values of CMC depend on the method used [31,33]. The CMC values increase as the Tritons' ethoxylate chain is longer and follow the straight line CMC (mM) = $0.0253m - 0.0482$ with the correlation factor 0.989, with the exception of TX-705. Similar dependence of the CMC on the length of ethoxylate chain was obtained for the CMC determined basing on pyrene excimer formation. The linear relationship of CMC on the length of ethoxylate chain is in good agreement with the earlier measurements [31] for another set of Tritons. The hydrophilic chain longer, the solubility in water is better and the tendency for agglomeration is weaker. So micellization takes place at higher surfactant concentration.

Tritons are the mixtures of the compounds of various length of the ethoxylated, hydrophilic chain. The hydrophobic parts are the same in all studied Tritons and one can suspect that the formed micelles, especially their cores, should have the same shape, at least when the hydrophilic chains are short. The longer ethoxylic chains are probably entangled and can wrap around the hydrophobic core which influences the size of the micelle, the aggregation number are lower.

3.2. Determination of micelle aggregation number

The method based on the fluorescence quenching to determine micelle sizes was proposed by Turro and Yekta [34]. The procedure relies on the following assumptions:

1. the micelles must be monodisperse;
2. the probe and the quencher must be hydrophobic and located in the micelles, and their residence time should be much longer than the unquenched fluorescence life-time of the probe;
3. the observed fluorescence is emitted from probes located in micelles only when there is no quencher, i.e. the static quenching occurs;
4. the random association of probe and quencher with micelles is described by Poisson distribution.

Putting x as the average number of quencher (Q) molecule per micelle (M), x = [Q]/[M] the probability P_n of a particular micelle to contain n quencher molecules is

$$P_n = \frac{x^n}{n!}\exp(-x) \quad (1)$$

The probe molecule can fluoresce if it is alone in micelle—there is no quencher, $n = 0$, and the probability equals to

$$P_0 = \exp(-x) \tag{2}$$

The intensity I of probe emission is therefore proportional to P_0. When the surfactant solution does not contain any quencher, the probe fluorescence intensity is I_0, and the probability that fluorescence occurs is 1. Thus, the following expression is valid

$$\frac{I}{I_0} = \exp(-x) = \exp\left(-\frac{[\mathrm{Q}]}{[\mathrm{M}]}\right) \tag{3}$$

The semi-logarithmic plot of the ratio I_0/I against quencher concentration gives a straight line with the slope equal to reciprocal of micelle concentration [M]. Knowing total bulk detergent concentration [S] and the concentration of detergent non-associated in micelles, which is practically equal to the CMC [35], one can calculate aggregation number

$$N_\mathrm{s} = \frac{[\mathrm{S}] - \mathrm{CMC}}{[\mathrm{M}]} \tag{4}$$

The aggregation numbers obtained from steady-state measurements for 10 mM Tritons are given in Table 3 and can be compared with literature values of N for: TX-100 104 (T = 298 K, c = 0.055 M) [36], 106 (T = 298 K, c = 0.055 M) [2], 66 (T = 303 K) [37], 121 (T = 303) [38], 125, 156 [38], 111, 140, 120, 100, 121, 134 [16], 110 [39], 111 (T = 293 K, c = 0.03 M) [40], 82–86 (T = 293 K, c = 0.01 M) [11], 143 [41], 140 (T = 294 K, c = 0.1 M) [28]; TX-114 80 (T = 295 K) [42]; TX-405 4 [43]. For the validity of the used method for N_s determination, it is important to choose a hydrophobic probe and quencher. As already mentioned, pyrene and DBA fulfil this condition satisfactorily. Also the quantitative relations between the concentrations of the quencher and micelle essentially affect the results [35]. Too low detergent concentrations [S] and simultaneously the micelle concentrations [M] give underestimated values of N_s. Tritons of the average length of hydrophilic chain m could not form ideally monodisperse micelles. This obviously influences the obtained values of aggregation numbers.

Assuming that micelles in an established range of the detergent concentration have the same shape and size, the aggregation number N_s determined for various Triton concentrations should be the same. The quenching of Py^* by DBA in TX-165 micelles occurs in the manner predicted by Eq. (3) as shown in Fig. 3. The aggregation numbers calculated from the slope of each straight line in Fig. 3 are shown in the inset of Fig. 3. The aggregation numbers for concentrations of TX-165 higher than 10 mM are within the experimental error the same (N = 54–59). The similar dependence has been obtained using TX-100 and TX-114 micellar solutions. This is a common phenomenon observed for non-ionic surfactants [11]. In contrast, the aggregation number depends significantly on the surfactant concentration in the case of TX-305, -405 and -705. These small micelles grow with increasing surfactant concentration above 30 mM threshold. A distinct change in the micellar aggregation number can be related with the transition from ellipsoidal micelles to the lamellar phase (as was proposed [44] for TX-114 above 40% surfactant (w/v)).

The time-resolved fluorescence quenching (TRFQ) data for surfactant solutions were analyzed in the frames of the model developed by Infelta et al. [45] and Tachiya [46]. The model assumes that a micelle contains a maximum one probe, which does not exit the micelle during the life-time of excited state, and the occupation of micelles by the solutes (probe and quencher) follows Poisson statistics. Using a non-linear least-squares procedure the fluorescence decay curves $I(t)$ have been fitted by Eq. (5):

$$I(t) = A_1 \exp\{-A_2 t - A_3[1 - \exp(-A_4 t)]\} \tag{5}$$

where $A_1 = I(0)$ is the emission intensity at zero time (the end of laser pulse). In the case of the so-called immobile quencher, not undergoing intermicellar exchange during the life-time of the excited state of the luminophore, the parameters A_2–A_4 are given by Eqs. (6)–(8), respectively:

$$A_2 = k_0 \tag{6}$$

$$A_3 = \frac{[\mathrm{Q}]}{[\mathrm{M}]} = \langle n \rangle \tag{7}$$

$$A_4 = k_\mathrm{qm} \tag{8}$$

where $\langle n \rangle$ denotes the mean occupancy of micelles by the quencher, k_0 is the rate constant of fluorescence decay in the absence of the quenchers (s^{-1}) and k_qm the

Table 3
Aggregation numbers, microviscosity, and rate constants of pyrene probe in Triton micelles

Triton	Aggregation number		$k_\mathrm{qm} \times 10^{-7}$ (s^{-1})	$k_0 \times 10^{-6}$ (s^{-1})[a]	$k_0' \times 10^{-6}$ (s^{-1})[b]	Microviscosity (cP)
	Steady state	Time resolved				
TX-114	68	156	0.4	2.70	4.12	256
TX-100	64	111	0.15	2.86	4.12	280
TX-165	57	63	1.0	2.85	4.14	338
TX-305	16	17	0.2	2.79	4.02	245
TX-405	10	20	1.0	2.85	4.01	198
TX-705	16	17	1.0	2.88	4.02	187

[a] Rate constant for deaerated sample.
[b] Rate constant for non-deaerated sample.

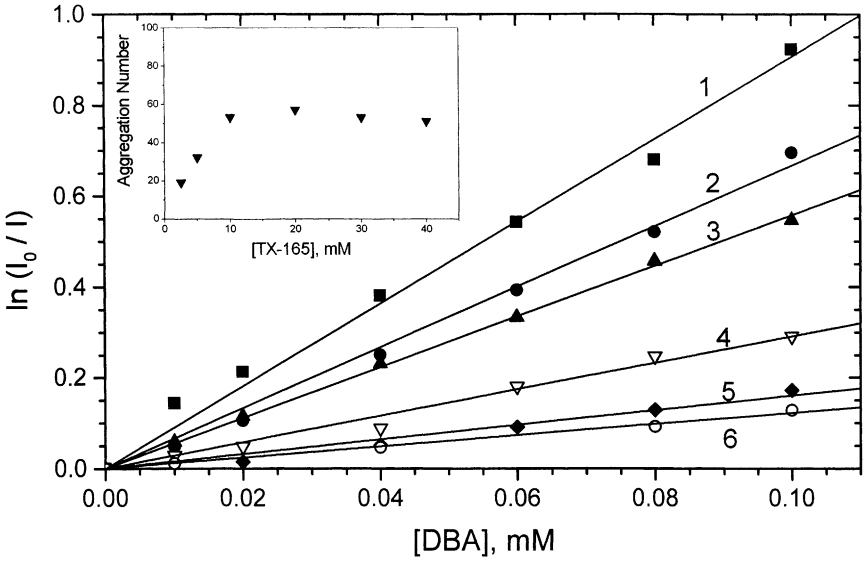

Fig. 3. The influence of quencher (DBA) concentration on the intensity of pyrene fluorescence in the micellar solution of TX-165 of various concentrations: (1) 2.5; (2) 5; (3) 10; (4) 20; (5) 30; (6) 40 mM ([pyrene] = 2 μM, λ_{exc} = 337 nm and T = 292.5 K).

pseudo-first-order rate constant of intramicellar fluorescence quenching (s^{-1}). Fig. 4 shows an example of the kinetic data fitting. The A_2 parameter was independent of the quencher concentration and equal to k_0 (within the experimental uncertainty), while the A_3 parameter increased linearly versus DBA concentration as shown in Fig. 5. From the slopes of the straight lines, the concentrations of micelles were determined according to Eq. (7), and the aggregation numbers N_D were calculated from the relation (9):

$$N_D = A_3 \frac{[S] - CMC}{[Q]} \quad (9)$$

The aggregation numbers N_D, determined from TRFQ data, were found to be Triton concentration independent in the range 10–30 mM. This observation is in agreement with steady-state measurements of the aggregation numbers.

The abnormally low values of the micellar aggregation numbers for TX-305, -405 and -705 are not easy to explain. If one assumes that used TRFQ technique is appropriate for all six micellar solutions it can be concluded that there are two kinds of microstructures of Tritons. The first group—TX-100, -114 and -165, with the aggregation number higher than 100 is expected to form ellipsoidal micelles with an axial ratio of about 1.9 [13]. The aggregation number of Triton X series of the first group decreases with an increase in the oxyethylene number m. The shape and size of non-ionic surfactant micelles of the second group (TX-305, -405 and -705) are unknown. The aggregation number of these Tritons has been found independent of the number of oxyethylene groups (see Table 3). The literature concerning the aggregation number of Tritons with long hydrophilic chain is scarce. It should be stressed that for m = 40

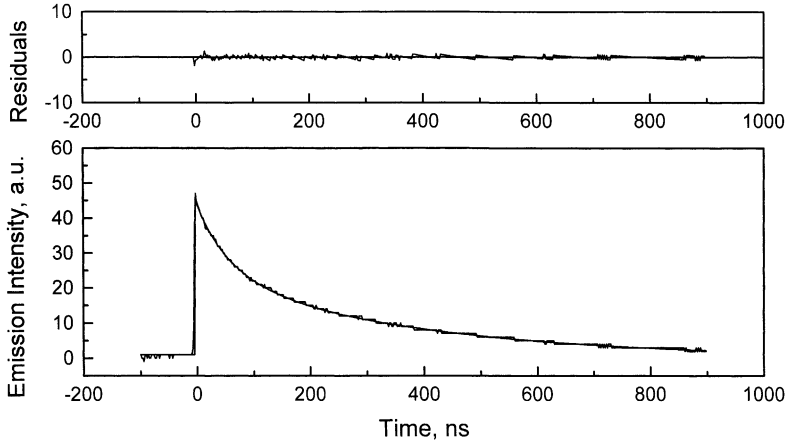

Fig. 4. An example of kinetic data fitting for pyrene/DBA in Triton X-165 solution ([pyrene] = 2 μM; [DBA] = 0.1 mM; [TX-165] = 10 mM); fitting parameters obtained by using the Infelta–Tachiya equation (5): $A_3 = 0.69$; $A_2 = 2.85 \times 10^6\ s^{-1}$; $A_4 = k_{qm} = 1.07 \times 10^7\ s^{-1}$.

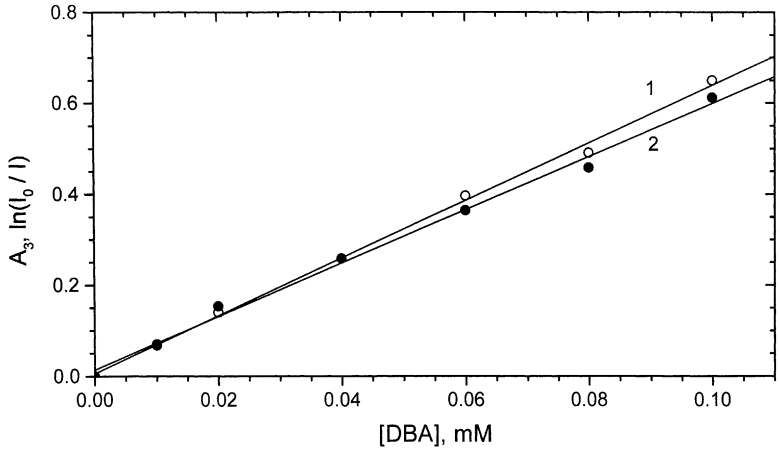

Fig. 5. The comparison of the dependence of parameter A_3 (1) from time-resolved measurements and $\ln(I_0/I)$ (2) from steady-state measurements versus quencher concentration ([TX-165] = 10 mM and T = 293 K).

(Triton X-405) the aggregation number as low as 4 has been published [43]. It is tempting to assume that the structure of Triton micelles of this group is different because of such big difference in values of an aggregation number—20 versus 100. On the other hand, such big similarity in physicochemical properties (similarity in k_0, k_{qm} and I_1/I_3, I_E/I_M ratios) of fluorescence reporters trapped in the micellar interior indicates rather similar microstructure of all the Tritons micelles. If one assumes that the shape of all micellar aggregates is the same and the sizes do not differ much, it can be concluded that Py* quenching by DBA in TX-305, -405 and -705 is not adequately described by Eq. (5) (for instance, due to loss of accessibility of Py by DBA). Using light, neutron or X-ray scattering techniques to measure the size of TX-305, -405 and -705 micelles, one can elucidate the structure of micelles in the future. The aggregation numbers of Tritons in aqueous solutions measured using TRFQ are larger than analogues numbers determined in the steady-state fluorescence quenching. The discrepancy between the two methods are due to the fact that one of the main assumptions used in deriving Eq. (3), namely $k_{qm}/k_0 \gg 1$, is not fulfilled in the case of Tritons (see Table 3). If k_{qm} is not much larger than k_0, some of the micelles containing one fluorophore probe and quencher molecule (or molecules) do emit light. This "kinetic effect" on the underestimation of the micelle aggregation number using the steady-state fluorescence quenching method is well recognized [5,35]. When k_{qm} is lower than k_0 (TX-100, -305), the steady-state method leads to dramatic underestimation of the aggregation number. The origin of the discrepancy in the aggregation numbers determined using the steady-state and time-resolved methods are due to extremely high microviscosity of the micellar core (k_{qm} is inversely proportional to viscosity [35]).

The life-time of excited state of pyrene is strongly influenced by the presence of air (Table 3) because oxygen is a very effective quencher of pyrene fluorescence [36]. The effect of molecular oxygen on the pyrene fluorescence life-time for all six Tritons solutions is the same. The life-time of Py* drops from about 350 ns in argon saturated micellar solution to less than 250 ns in the presence of air. This can be explained if one assumes that oxygen very easily penetrates the very viscous micellar core and that oxygen residence time in all micelles is similar. Note that in the case of DBA the quenching process depends on the micellar interior (k_{qm} values are changing in a wide range: 0.15×10^7–1×10^7 s^{-1}; see Table 3).

3.3. Microviscosity at the probe site

We have examined the P3P fluorescence spectra in aqueous Triton micelles, and in a number of non-aqueous solvents of known viscosities, and measured the intensity ratio of the excimer to the monomer emission maxima, I_E/I_M [7].

A calibration curve for the microviscosity (I_E/I_M versus viscosity (η) at 298 K) is presented in Fig. 6. The variation of I_E/I_M with η can be reproduced [7] by Eq. (10):

$$\frac{I_E}{I_M} = 2.764\eta^{-0.57} \quad (10)$$

As seen in the inset of Fig. 6, emission maxima are observed in the P3P/TX system at λ_M = 373 nm and λ_E = 480 nm. For this TX-165 micellar solution, the emission from the excimer is weak compared to the intensity of monomer fluorescence ($I_E/I_M = 0.125 \pm 0.015$), and the microviscosity in the P3P environment estimated from the calibration curve is 228 cP ±25%. Although this value has a relatively large margin of error, the viscosity is clearly very large and indicative of a hard core. The term microviscosity is hardly appropriate for the micellar phase but it is used nevertheless in most literature. To emphasize the difference between the macroscopic viscosity and the factors governing the motion of molecules in microenvironments such as micelles the terms microfluidity [47] or space dependent viscosity [37] have been proposed. It should be pointed out that the I_E/I_M

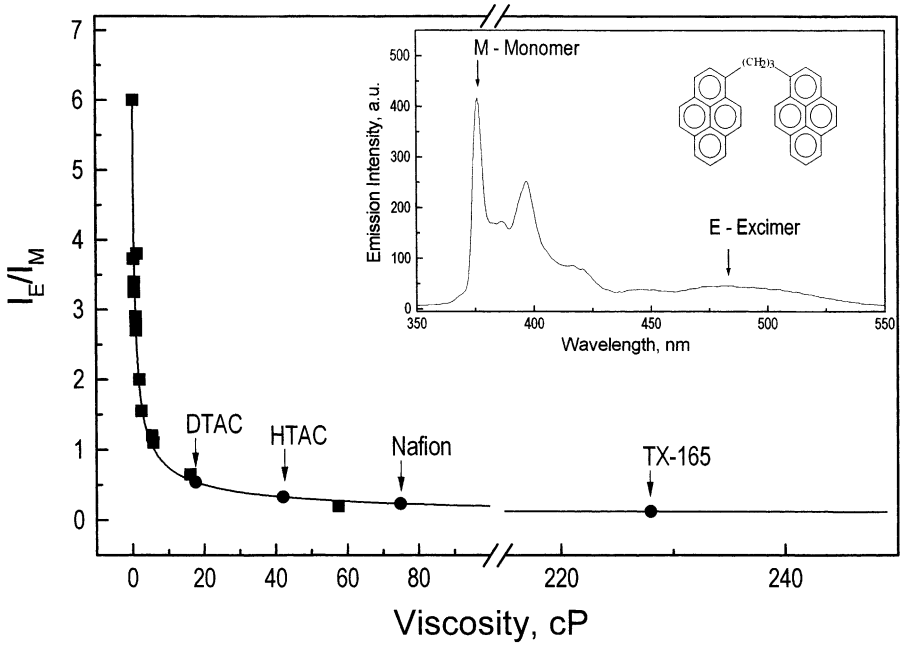

Fig. 6. Dependence of the intensity ratio of excimer to monomer fluorescence for P3P on the local viscosity (the solid line is the calibration curve for the viscosity taken from [12]). Inset: fluorescence spectrum of P3P in the aqueous solution of Triton X-165.

values recorded for a few solvents do not fulfil Eq. (10). For example, in the case of 2,4-dioxane the I_E/I_M value recorded experimentally is 1.6, while that calculated from Eq. (10) is 2.49. This means that some specific interaction P3P–microenvironment can influence the value of measured microfluidity. The microviscosity of silicone oil determined from analysis of the ratio I_E/I_M and P3P excimer life-time is around 100 cP, however bulk viscosity is equal to 2000 cP. This observation is a good illustration of the difference in the meaning of the terms viscosity and microviscosity.

Pyrene excimer emission kinetics in the micellar solution on flash excitation is well understood [37], and can be analyzed by using the equation:

$$I_E(t) = I_0 A(\exp[-\lambda_1 t] - \exp[-\lambda_2 t]) \quad (11)$$

The constants A, λ_1 and λ_2, depend on the rate constants of excimer formation and dissociation, and on the rate constants for excimer and monomer emission decays, I_0 is the initial number of excited monomers [37]. Formation of intramolecular excimer in the case of P3P is a more complex

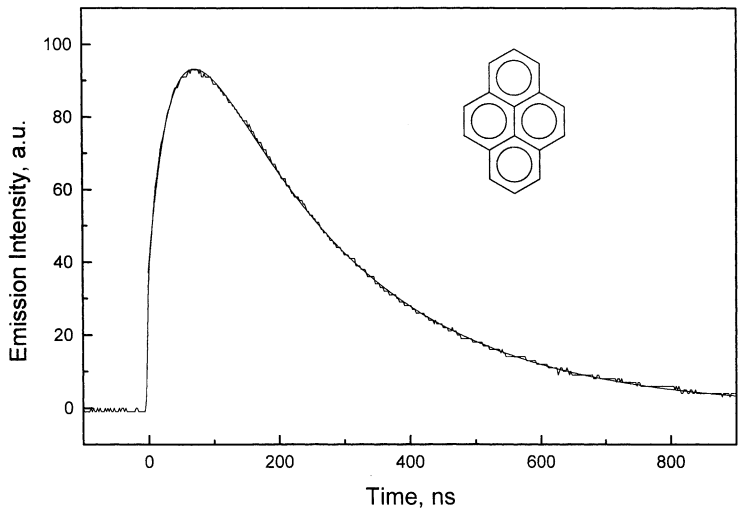

Fig. 7. Biexponential time dependence of pyrene excimer emission in the TX-100 micellar solution ([TX-100] = 10 mM, T = 295 K and [pyrene] = 0.35 mM). Fitting curve (smooth line) superimposed on the experimental run (noisy line); $\lambda_1 = 4.12 \times 10^6\,s^{-1}$ and $\lambda_2 = 2.45 \times 10^7\,s^{-1}$.

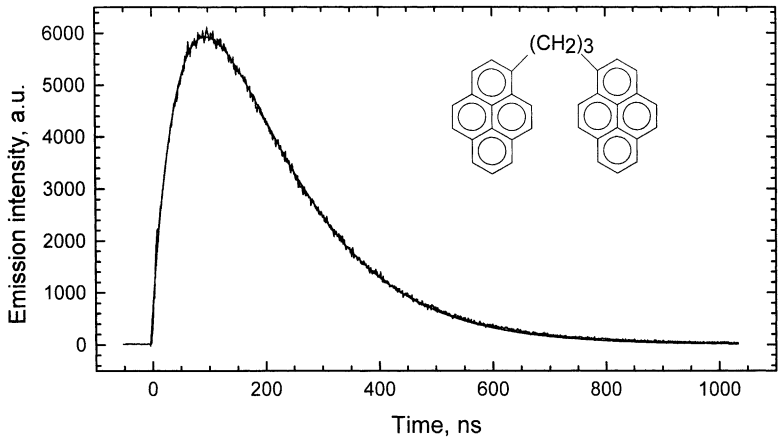

Fig. 8. Kinetics of formation and decay of P3P excimer in 10 mM Triton X-165 solution ([P3P] = 5 μM, $\varepsilon_{345\,nm} = 80000\,M^{-1}cm^{-1}$, vacuum). Fitting curve (smooth line) superimposed on the experimental run (noisy line). See Table 4 for details.

process involving formation of two conformationally different excimers with different life-times [48]. However, for the purpose of this study, we do not introduce such detailed discrimination between various kinds of excimers because in the Tritons solutions the reasonable two-exponential fit was obtained for excimer emission:

$$I_E(t) = B_1 \exp(-k_1 t) + B_2 \exp(-k_2 t) \quad (12)$$

k_1 and k_2 are the reciprocals of the fluorescence life-times of the excimer formation and decay, respectively, and B_1 and B_2 the excimer emission amplitudes.

The kinetics of pyrene excimer formation (Fig. 7) and intramolecular pyrene excimer formation in P3P (Fig. 8, Table 4) in all micellar solutions are similar. The excimer formation depends upon the rate of conformational change of propylene chain in the case of P3P and diffusion of pyrene molecules inside micellar core in the case of pyrene. Both the processes being determined by the local fluidity provide measure of effective dynamics of the microenvironment sensed by the probes. The maximum of the excimer emission intensity is reached at 100 ns in the case of TX-165 and at around 60 ns for HTAC.

Table 4
Parameters retrieved from the two-exponential fit of the P3P excimer fluorescence decays in the Triton solutions

Surfactant	B_1	$k_1 \times 10^{-7}(s^{-1})$	B_2	$k_2 \times 10^{-6}(s^{-1})$
TX-100	−6.459	1.199	6.480	7.921
TX-114	−4.641	1.291	4.675	7.280
TX-165	−4.185	1.295	4.268	6.942
TX-305	−3.029	1.249	3.143	8.983
TX-405	−3.767	1.345	3.861	8.764
TX-705	−3.740	1.410	3.782	9.133

4. Conclusions

The steady-state fluorescence measurements indicate that Tritons micelles have a polar core, comparable to the environment in methanol. Two spectrophotometric methods (i.e. determination of polarity of micellar solution using pyrene as a fluorescence probe and formation of pyrene excimer) were applied to determine critical micellar concentration of six different (oxyethylene)*m*-*p*-(1,1,3,3-tetramethylbutyl)phenyl ethers, *m* ranging from 8 to 70. In agreement with the earlier work [33], it was found that there is a good linear correlation between CMC values and the number ethylene oxide units (with the exception of TX-705, $m = 70$). The determined aggregation numbers allowed us to divide studied Tritons in two groups: with small (∼20) and high (∼100) *N* values. The steady-state fluorescence methods lead to underestimated aggregation numbers. The excited pyrene in micelles is well protected from water phase as showed by long life-time (above 350 ns) of its fluorescence. The comparison of the rate constants of intramicellar quenching of pyrene by oxygen and DBA indicates that small oxygen molecule easily penetrate deeply into micellar core.

The microviscosity at the probe site-micelle core is very high (around 200 cP) as was obtained from the intensity ratio of the excimer to the monomer fluorescence in the spectrum of 1,3-bis(1-pyrene)propane as the probe, based on the previous calibration curve obtained for solvents of known viscosities.

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