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Spectral properties of probes based on pyrene and piperazine: the singlet and triplet route of deactivation

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Abstract

Probes, composed of pyrene (Py) and piperazine (7,15-diazadispiro[5.1.5.3]hexadecane or 7,15-diazadispiro[5.1.5.3]hexadecane-14,16-dione) (PA) linked directly or through methylene group (Me) were prepared. Probe with direct link is 15-(1-pyrenyl)-7,15-diazadispiro[5.1.5.3]hexadecane (PyPA). In 15-(1-pyrenylmethyl)-7,15-diazadispiro[5.1.5.3]hexadecane-14,16-dione (PyMePAH) pyrene is connected with piperazine through methylene bridge. The parent amine of PyMePAH was oxidized to the form of stable nitroxyl radical (PyMePAO) in position 7. Oxidation of the amine PyPA did not yield any aminooxide.

The deactivation processes of the probes were monitored by fluorescence (singlet excited state route) as well as by nanosecond laser flash photolysis techniques (triplet excited state route). The spectral features of the multifunctional probes were compared. Spectral measurements were performed in methanol and polymer matrices as polymethyl methacrylate (PMMA) and polystyrene (PS). The absorption and the fluorescence spectra of the probe PyPA do not exhibit vibrationally resolved bands while the probes with the methylene link PyMePAH (or PyMAPAO) have well resolved vibrational structure similar to that obtained with pyrene. Absorption spectra of the probes have got similar shape in solvent as well as in polymer matrix. The quantum yield of fluorescence of the PyPA relative to anthracene is rather high as compared with PyMePAH or PyMePAO in aerated methanol. The quantum yield of the PyMePAH or PyMePAO relative to anthracene is higher in deaerated methanol and polymer matrices except polystyrene. The lifetime of fluorescence of PyPA is rather short about 5 ns and those of PyMePAH or PyMePAO lies in the range above 10–300 ns. It is strongly dependent on the experimental conditions. The extent of intramolecular quenching expressed as the ratio of relative quantum yields of PyMePAH and PyMePAO and respective lifetimes is rather low indicating that the intramolecular quenching is not very effective.

Nanosecond laser flash photolysis was also used to examine the triplet excited state route of deactivation of these novel probes. The formation of this excited state which exhibits a T–T absorption band in the range 360–550 nm, occurred at 355 nm laser excitation in these probes as well as for parent pyrene. Triplet states of pyrene chromophore of all probes were efficiently quenched by oxygen with rate constant about $2 \times 10^9\ \mathrm{dm^3\ mol^{-1}\ s^{-1}}$. Intermolecular quenching of triplet states of the all probes by *N*-oxyl radical (1-oxo-2,2,6,6-tetramethylpiperidine, TEMPO) was rather low. The rate constant was evaluated to about $5 \times 10^7\ \mathrm{dm^3\ mol^{-1}\ s^{-1}}$. The intramolecular triplet quenching expressed as the ratio of the rate constants of triplet decay of the oxidized probe PyMePAO to PyMePAH was 5 in methanol and 7.7 in cyclohexane.

Keywords: Multifunctional probes; Pyrene; Piperazine; Methylene link; Singlet; Triplet; Deactivation

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1. Introduction

Pyrene itself or its derivatives are widely employed as chromophore for construction of probes based on monitoring of fluorescence in various complex systems as micelles

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[1,2] polymers [3,4] and biological structural units as vesicles and cells [5]. It might be used itself or in combination with some functional group for sensors. The sensitivity of the spectral parameters as position of the absorption or the emission maxima, change in vibrational structure, quantum yield of emission, formation of homo or heterodimers, lifetime, external or internal quenching, etc, to the change of the environment, might be used for this purpose.

These spectral parameters are related mainly to singlet excited state. Less attention was paid to monitoring of triplet excited state probably because it is less accessible at normal conditions. Moreover, monitoring its build up and decay by transient absorption spectroscopy is less sensitive than to monitor singlet excited state by steady-state or dynamic fluorescence.

Interesting spectral features are observed in class of compounds where electron donor and acceptor group are substituted on aromatic ring. Much often, they emit dual fluorescence and relax from a roughly planar conformation (locally excited state (LE) with partial charge transfer character) to a roughly perpendicular one (twisted intramolecular charge transfer (TICT) state with full CT character) [6]. The intramolecular CT properties of molecules with large donor group as 4-(1-pyrenyl)-benzonitrile have been investigated in details [7]. Both experimental results and quantum chemical calculations indicate that such pyrene derivative relaxes after excitation by mutual twisting of the two subunits towards a more planar geometry [7]. Recently we have observed that the bulky electron donating group as 7,15-diazadispiro[5,1,5,3]hexadecane linked with pyrene strongly influences the spectral properties and consequently electronic relaxation processes of pyrene chromophore [8].

Linking of pyrene with the free radical of sterically hindered amine type such as *N*-oxyl, which is paramagnetic, strongly influences both singlet as well as triplet excited states. Intermolecular quenching of these excited states of aromatic hydrocarbons and ketones was studied in detail [9–17]. In the early nineties fluorescence probes in which simple chromophore was combined with a free radical centre of the *N*-oxyl type were prepared [18,19]. Later pyrene combined with 1-oxo-2,2,6,6-tetramethylpiperidine was employed for construction of this type of probes [20,21]. Formation or decay of the free radical in these probes is connected with switching off or on the chromophore emission as a result of the intramolecular quenching.

Mechanism of inter- or intramolecular quenching of excited states by *N*-oxyls of different type is still the matter of discussion. The following processes are considered as catalytic enhancement of intersystem crossing as a result of an increase in spin–orbital coupling due to the paramagnetic effect or catalytic enhancement of the efficiency of internal conversion. Alternatively, quenching can involve transfer of electronic energy of resonance or exchange type or transfer of electron and formation of cation or anion radicals.

The majority of mechanistic studies of the singlet excited state quenching of aromatic hydrocarbons with *N*-oxyl radicals concluded that the enhancement of intersystem crossing is the most probable route for dissipation of energy. The quenching of a triplet excited state occurs through internal conversion [9–17]. The photophysical process is a preferred route for deactivation of excited state by intramolecular quenching as well [18]. On the other hand, the photoinitiated intramolecular electron transfer from *N*-oxyl to diimide under formation of diimide monoanion has been observed recently [19]. These studies indicate that *N*-oxyl radical is able to quench the excited state by various mechanisms depending on the structure of the couple quenchee–quencher and medium.

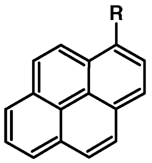
Recent study of the triplet route of deactivation of 2,2,6,6-tertamethyl-4-piperidinyl-1-pyrenealkanoate parent amine showed that quenching by oxygen is efficient ($(1–5) \times 10^9$ dm^3 mol^{-1} s^{-1}). On the other hand, intermolecular quenching by the same *N*-oxyl structural unit (TEMPO) is distinctly less effective (ca. 1×10^7 dm^3 mol^{-1} s^{-1}). For this class of probes intramolecular quenching based on comparison of parent amine and 1-oxo-derivative is rather low [22].

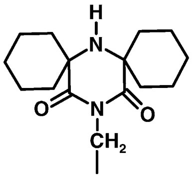
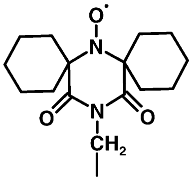
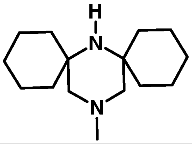
In this paper the spectral properties of pyrene directly linked with bulky functional group as 7,15-diazadispiro[5.1.5.3]hexadecane or 7,15-diazadispiro-[5.1.5.3]hexadecane-14,16-dione separated by methylene bridge are compared. We expect that the spectral properties (namely position and intensity of fluorescence) of these combined probes will reflect specific sensitivity on the environment. Beside the singlet excited state route of deactivation monitored by fluorescence spectroscopy, the triplet excited state route is also followed. Based on absorption of triplet transition the decay of triplet state is compared for pyrene and its derivatives with bulky functional group as piperazine in the various environments (polar, non-polar). The aim of the present work is to obtain more spectral data related to photophysical features of these combined probes.

2. Experimental

Solvents: methanol, cyclohexane, and chloroform were for UV spectroscopy. Pyrene (Scheme 1, Py) (Lachema Brno, CR) were zonally refined. The free radicals: 1-oxo-2,2,6,6-tetramethylpiperidine (TEMPO), used as quencher, was commercial product (Aldrich, Steinheim, Germany). The pH of the solutions in methanol was adjusted by adding either concentrated perchloric acid or sodium hydroxide. It is important to note that since the pH scale in methanol is completely different from that obtained in water [23,24], the pH values are given as indication for the acid and basic character of the solution.

The structures of combined probes are given in Scheme 1. Details of synthesis of PyMePAH and PyMePAO are given below.



	R	Probe
Py	H	Pyrene
PyMePAH		15-(1-pyrenylmethyl)-7,15-diazadispiro[5.1.5.3] hexadecane -14,16-dione
PyMePAO		7-oxo-15-(1-pyrenylmethyl)-7,15-diazadispiro[5.1.5.3] hexadecane-14,16-dione
PyPA		15-(1-pyrenyl)-7,15-diaza dispiro[5.1.5.3]hexadecane

Scheme 1.

2.1. 15-(1-Pyrenylmethyl)-7,15-diazadispiro[5.1.5.3]hexadecane-14,16-dione (PyMePAH)

In the flask equipped with magnetic stirring bar, 7,15-diazadispiro[5.1.5.3]hexadecane-14,16-dione (2.5 g, 10 mmol) was partially dissolved in dry dimethylformamide (DMF, 50 mL) and the mixture was heated to 65 °C. To a stirred suspension, natrium hydride (0.5 g, 20 mmol, 60% suspension in oil) was added in small portions during 15 min and the reaction mixture was kept at this temperature for 1 h. After this time, 1-chloromethylpyrene (3.25 g, 13 mmol) in dry DMF (10 mL) was added drop-wise and the reaction mixture was stirred at room temperature for 16 h. DMF was then partially evaporated and the residue was dissolved in 50 mL of dichloromethane and extracted with water. Organic layer was separated, dried over Na_2SO_4 and the solvent was removed by the vacuum rotary evaporation. Purification by column chromatography with 2:1 chloroform:isohexane, yielded 2.4 g (52%) of yellow crystals with m.p. 122–127 °C.

FT-IR (KBr) (cm^{-1}): ν(C=O) 1715, ν(C=O) 1664, δ(CH_2-cyclohexyl) 1450 and ν(pyrenyl) 845.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20–1.85 (m, 20H, spiro-cyclohexyl CH_2), 5.70 (s, 2H, CH_2-pyrene), 7.95–8.45 (m, 9H, pyrene).

^{13}C NMR ($CDCl_3$) (20.7 (C, *C*(3), *C*(5), *C*(10), *C*(12)), 25.4 (2C, *C*(4), *C*11)), 35.0 and 35.4 (4C, *C*(2), *C*(6), *C*(9), *C*(13)), 41.5 (1C, *C*H_2-Py), 58.1 (2C, *C*(1), *C*(8)), 123.2–131.3 (16C, pyrene), 177.3 (2C, *C*(14), *C*(16)).

2.2. 7-Oxo-15-(1-pyrenylmethyl)-7,15-diazadispiro[5.1.5.3]hexadecane-14,16-dione (PyMePAO)

The parent amine PyMePAH (0.93 g, 2 mmol) was dissolved in 30 mL of dichloromethane and cooled to 0 °C. Under stirring, 3-chloroperoxobenzoic acid (0.7 g, 4 mmol) was added in small portions for 20 min and the solution became orange. It was allowed to warm to room temperature and stirred for 3 h. Reaction mixture was extracted with aqueous K_2CO_3 solution and with water. Organic layer was dried over Na_2SO_4 and the solvent was removed by the vacuum rotary evaporation. Crude product was purified by column chromatography using a mixture of dichloromethane:isohexane 2:1. After solvent evaporation the yellow crystals were washed with diethyl ether and dried to give 0.41 g (43%) of crystals with m.p. 121–124 °C.

FT-IR (KBr) (cm^{-1}): ν(C=O) 1728, ν(C=O) 1685, δ(CH_2-cyclohexyl) 1450, ν(N–O) 1350 and ν(pyrenyl) 838.

Structure and purity of the radical was proved by ESR spectroscopy as well as by TL chromatography. ESR quantitative measurements were performed in benzene solutions ($c = 1 \times 10^{-3}$ mol dm^{-3}). Integral of ESR spectra for PyMePAO was compared with the integral of standard measured under the same conditions. As the standard 4-hydroxy-2,2,6,6-tetramethyl-piperidine-*N*-oxyl was used. ESR spectrum of the PyMePAO as well as standard were triplets with equal line intensities. The values of integrals are proportional to the number of radicals so the relative concentration c_r provides information about amount of radical in the sample. We assume that the concentration of radicals in the standard is 100%. The value c_r for PyMePAO was 95%, which is the proof of the very high purity.

Preparation of 15-(1-pyrenyl)-7,15-diazadispiro[5.1.5.3]-hexadecane (PyPA) has already been described elsewhere [8].

Absorption spectra were taken on a M-40 UV–vis-spectrometer (C. Zeiss, Jena, Germany) and Shimadzu UV-160 (Shimadzu, Japan), ^{1}H NMR on 300 MHz spectrometer (Bruker, FRG), mass spectra on HP 5971A (Hewlett Packard, Palo Alto, USA) and FT-IR spectra on Impact 400 (Nicolet, USA). ESR spectra were measured with X-band spectrometer E-4 Varian (USA) interfaced on PC with program Symphonia Bruker. Emission spectra were recorded on a Perkin-Elmer model MP-3L and MPF-4 spectrofluorimeter (Perkin-Elmer, Norfolk, CT, USA), which was connected through interface and A/D converter to an ISA slot of PC using ORPHY GTI program for data collection. Origin 5.0 software from Microsoft was used for data plotting and mathematical fitting. Emission of solutions was measured at right angle in a 1 cm cell. The quantum yields were determined relative to anthracene in methanol or cyclohexane. Emission of polymer films was measured in front-face arrangement to the solid sample holder. The quantum yield in polymer films was determined using anthracene as standard and assuming low effect of the medium. The relative quantum yields in solution and films were evaluated according to the expression [25]:

$$\Phi_\mathrm{F} = \Phi_\mathrm{F}^\mathrm{S} \frac{\int_0^\infty I_\mathrm{F}(\nu)\,\mathrm{d}\nu}{\int_0^\infty I_\mathrm{F}^\mathrm{S}(\nu)\,\mathrm{d}\nu} \left(\frac{1-10^{-A^\mathrm{S}}}{1-10^{-A}} \right)$$

where $\Phi_\mathrm{F}^\mathrm{S}$ is the quantum yield of the standard (equal 1), integrals $\int_0^\infty I_\mathrm{F}(\nu)\,\mathrm{d}\nu$ and $\int_0^\infty I_\mathrm{F}^\mathrm{S}(\nu)\,\mathrm{d}\nu$ the areas under curves of the probe and the standard, while $I_\mathrm{F}(\nu)$ and $I_\mathrm{F}^\mathrm{S}(\nu)$ the intensity of fluorescence of probe and standard as function of wave-number, respectively, and A and A^S the absorptions of the probe and standard.

Fluorescence lifetime measurements were performed on a LIF 200 (Lasertechnik Ltd., Berlin, F.R.G.), which operates as a stroboscope. The excitation source was a nitrogen laser ($\lambda = 337$ nm) and emission was selected by the use of cut-off filters. The box-car integrator was connected to PC through interface. The fluorescence decay curves were evaluated by simple phase plane method [26] using program of J. Snyder 1988 based on [27]. The standard deviation, $G^{1/2} = \sum((I_\mathrm{exp} - I_\mathrm{calc})^2/n)^{1/2}$, where I_exp and I_calc are the experimental and calculated intensities of emission, respectively, was used to judge the quality of fit. It was assumed that decays were monoexponential if $G^{1/2}$ is <5%. Static and time-resolved fluorescence measurements were performed on aerated and deaerated solutions (10 min purge with argon for a 1 cm cell). Measurements on polymer films were performed in the presence of air.

Measurements of transient absorption spectra within the time scale 20 ns to 500 μs were carried out on a nanosecond laser flash photolysis LKS 60 from Applied Photophysics Ltd (London, England). The laser excitation at 355 nm (third harmonic) from Quanta Ray GCR 130-1 Nd:YAG (pulse width ~ 9 ns) was used in a right angle geometry with respect to the monitoring light beam. The transient absorbance at the pre-selected wavelength was monitored by a detection system composed of a pulsed Xe-lamp (150 W), monochromator and a 1P28 photomultiplier. A unit controlled synchronising of the pulse lamp, programmable shutters and high voltage power supply with laser output. The signal from photomultiplier was displayed on digital oscilloscope (HP 54522A) and analyzed on 32 bit RISC work station [22,28].

3. Results and discussion

The probes of type chromophore-link-radical centre (Scheme 1) exhibit more complex decay routes after excitation. At the beginning the electronic excitation is mainly localized on absorbing chromophore. The presence of paramagnetic centre opens up the new routes of deactivation, which can be monitored by fluorescence or time-resolved spectroscopy. The extent of opening of any new deactivation channel on singlet level might be estimated by the ratio of quantum yields ($\Phi_\mathrm{NH}/\Phi_{\mathrm{NO}^\bullet}$) or lifetimes ($\tau_\mathrm{NH}/\tau_{\mathrm{NO}^\bullet}$) of fluorescence chromophore with and without radical centre. On triplet level the extent of opening of new channel is based on the ratio of the decay rate in presence and the absence of radical centre ($k_{\mathrm{NO}^\bullet}/k_\mathrm{NH}$).

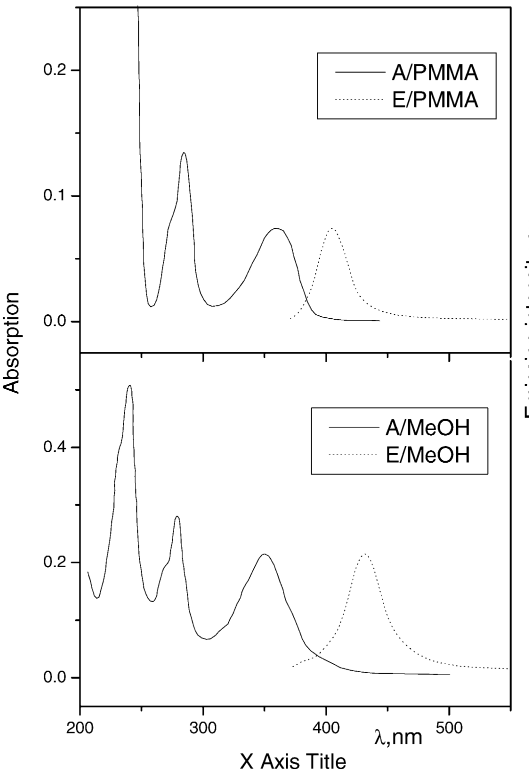
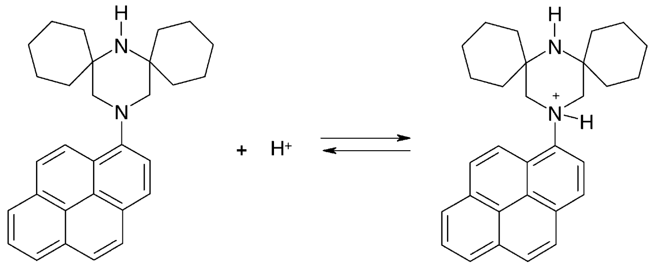


Fig. 1. Absorption (A) and fluorescence (E) spectra of 15-(1-pyrenyl)-7,15-diazadispiro[5.1.5.3]hexadecane (PyPA) in methanol (10^{-5} mol dm^{-3}) and PMMA (2×10^{-3} mol kg^{-1}).

Some spectral feature of PyPA has already been partly discussed [8]. Here they are mainly analyzed for comparison of the singlet and triplet excited states routes of deactivation of the probes with the direct linkage of pyrene with piperazine and that of bridged with methylene.

The absorption spectra of the probe based on pyrene directly linked to sterically hindered piperazine PyPA in methanol (polar medium) are shown in Fig. 1 and in cyclohexane (non-polar medium) in Fig. 2. As clearly shown, the typical well known vibrational resolution of the absorption spectra of pyrene is completely lost for PyPA in polar as well as in non-polar environments. Loss of vibrational structure is more likely due to the direct linking of pyrene on piperazine nitrogen, which brings about the loss of symmetry and also due to electron donating alkyl amino like group on the pyrene moiety. The vicinity of the amino group to pyrene part leads to a protolytic equilibrium as follows:



The emission spectra of both forms in methanol are given in Fig. 3. They were obtained in the presence of 2 mol L^{-1} perchloric acid (pH = 0.4) and in the presence of 2 mol L^{-1} sodium hydroxide to the solution (pH = 12.5) (see Section 2). The protonated one presents a fluorescence maximum at 400 nm while the neutral form emits with a maximum at 431 nm. No significant change of the spectrum is observed coming from neutral to basic solution. Such observations indicate that the pK_a lies within the region 4–5. This is in good agreement with the pK_a values of compounds such as aminoanthracene and aminonaphthalene [29].

The main features of absorption spectra of probe PyPA are preserved in methanol and cyclohexane as well as in polymer matrices. There is no distinct shoulder at longest wavelength edge of the absorption spectra indicating low energy n–π* state. If such state exists, it is overlapped by more intense π–π* band at around 350 nm.

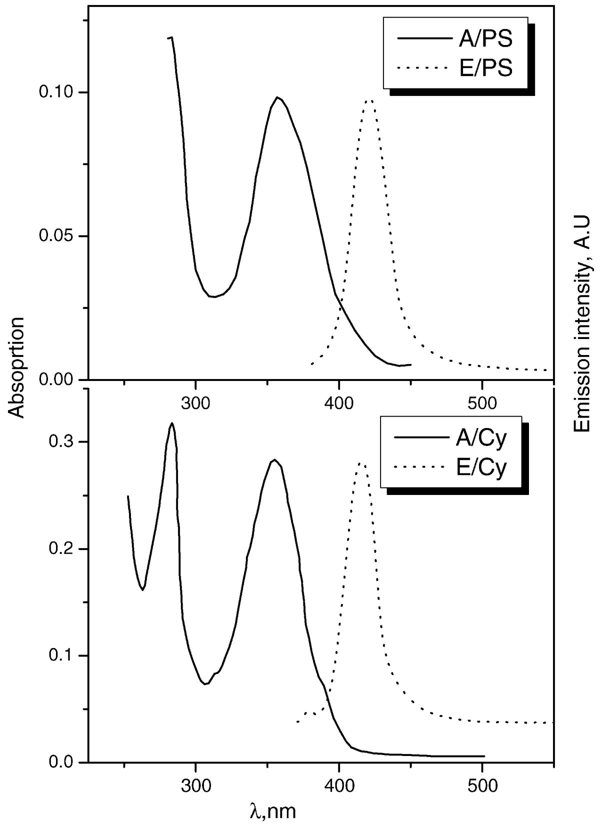


Fig. 2. Absorption (A) and fluorescence (E) spectra of PyPA in cyclohexane (10^{-5} mol dm^{-3}) and polystyrene (2×10^{-3} mol kg^{-1}).

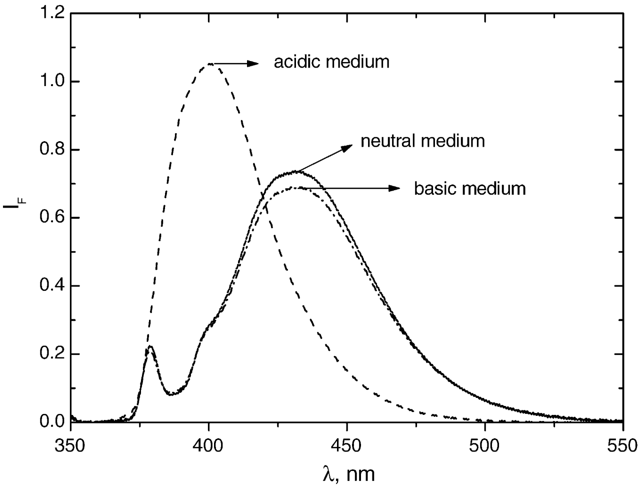


Fig. 3. Fluorescence spectra of PyPA in neutral, acidic (2 M $HClO_4$) and basic (2 M NaOH) methanolic solutions.

The fluorescence of PyPA probe is a rather broad band (Figs. 1 and 2). Some spectral data of probe PyPA are given in Table 1 for different media. It has to be pointed out that the important bathochromic shift in the fluorescence spectra observed for deaerated solution is due to the decrease of the pH of the solution. This effect is owing to the removal of dissolved carbon dioxide by the bubbling process. Such fluorescence of PyPA is efficiently inhibited by oxygen in acidic as well as in basic solution. Nice Stern–Volmer plots were obtained and they lead to the values $k_q\tau = 273$ and 139 mol^{-1} dm^{-3} where k_q is the rate constant for the quenching process and τ the lifetime for the emissive excited state.

The comparison of some spectral parameters (Table 1) shows that the longest wavelength absorption band ($\log \varepsilon \sim 4.0$) is not sensitive to medium. The relative quantum yield of PyPA indicates that this compound exhibits intense fluorescence from aerated as well as deaerated methanol. The lifetime of PyPA is shorter in aerated (about 3–4 ns) as compared to deaerated (about 5 ns) solutions. The main spectral features of PyPA are similar when this probe is doped in polymer matrices. The longest wavelength band in the absorption spectrum is not influenced by the medium. The fluorescence is more intense as compared with anthracene in aerated and deaerated solutions and polymer matrices. The lifetime of PyPA is slightly longer in polymer matrices than in solution. The fluorescence spectrum yields only limited structural information as compared to pyrene since it exhibits a broad band without any vibrational structure. There is, however, distinct difference in the shape of fluorescence of PyPA in methanol and cyclohexane (Figs. 1 and 2). The emission band of PyPA in cyclohexane is narrower and more symmetrical while in methanol the emission band of PyPA is broader. Such difference may be related to the polarity of the solvent.

The spectral characteristics of probes pyrene–methylene–piperazine in solution and in polymer matrices are summarized in Table 2. The absorption and fluorescence spectra

Table 1
Spectral characteristics of probe PyPA in various media

Medium	Conditions	$\lambda_{abs}{}^{a}$ (nm)	$\log \varepsilon^{b}$ (mol^{-1} cm^{-1})	$\lambda_{em}{}^{c}$ (nm)	$\Phi_r{}^{d}$	τ^{e} (ns)	$G^{1/2,f}$ (%)	$\Delta\nu^{g}$ (cm^{-1})
MeOH	Neutral solution	348	4.32	420, 431^{h}	7.1, 8.6^{h}	4.7	5.0	5059, 5838^{h}
	Acidic solution	341	4.30	400				
	Basic solution	349	4.33	431				
Cy		355		415, 415^{h}	1.2, 1.9^{h}	3.8, 4.8^{h}	2.3, 1.4	4060
PS		353	3.97	421	9.3	7.6	4.7	4570
PMMA		349	3.84	405	1.2	10.8	5.2	3390

Medium: MeOH: methanol, 10^{-5} mol dm^{-3}; PS: polystyrene, 0.002 mol kg^{-1}; PMMA: poly(methyl methacrylate, 0.002 mol kg^{-1}. The standard error of spectral measurements is around 10%.

[a] Maximum of the absorption bands.

[b] Molar decadic extinction coefficient.

[c] Maximum of the emission band.

[d] Quantum yield relative to anthracene in the respective medium.

[e] Lifetime of fluorescence.

[f] Standard error of lifetime (τ).

[g] Stoke's shift.

[h] Deaerated solution (5 min purge with argon).

of probes of the type chromophore–methylene–piperazine both parent amine PyMePAH and aminooxide PyMePAO exhibit vibrational structure in methanol as well as in polymer matrix PMMA (Figs. 4 and 5). Similar features of PyMePAH and PyMePAO are observed for non-polar PS (Fig. 6) and polar PVC (Fig. 7). The longest wavelength band exhibits nearly the same vibrational structure as an unsubstitute pyrene but it is slightly red shifted. The longest wavelength absorption band is not sensitive on polarity of the medium and the maxima of the vibrational bands are found at 310, 320, 335 and 355 nm.

The fluorescence of both parent amine PyMePAH and aminooxide PyMePAO is in the same region as monomer fluorescence of pyrene namely around 378, 393 (shoulder) and 400 nm. In polar methanol the most intense band is at 378 nm. In other matrices it is around 400 nm. The quantum yield relative to anthracene of both derivatives is low in aerated methanol but it is high in deaerated methanol (Table 2). The fluorescence intensity lies between these values in polymer matrices. The lowest one is in polystyrene. Lifetime of PyMePAH in deaerated methanol is long (331.6 ns) and rather short in aerated methanol (19.7 ns). The quenching rate of oxygen is 2.4×10^{10} dm^{3} mol^{-1} s^{-1} taking into account the oxygen concentration in methanol 2.2×10^{-3} mol dm^{-3} [30]. This is quite reasonable value of quenching rate constant for pyrene fluorescence. The data on ratio of quantum yields ($\Phi_{NH}/\Phi_{NO^\bullet}$) or lifetimes ($\tau_{NH}/\tau_{NO^\bullet}$) indicating the extent of intramolecular quenching through the new deactivation singlet channel are rather low about 1.5. These values for PMMA are about 1 indicating that no intramolecular quenching oc-

Table 2
Spectral characteristics of probes pyrene–methylene link–piperazine in the different environments

Probe^{a}	M^{b}	$\lambda_{abs}{}^{c}$ (nm)	$\log \varepsilon^{d}$ (M^{-1} cm^{-1})	$\lambda_{em}{}^{e}$ (nm)	Φ^{f}	$\Phi_{NH}/\Phi_{NO^\cdot}{}^{g}$	τ^{h} (ns)	$\tau_{NH}/\tau_{NO^\cdot}{}^{i}$
PyMePAH	MeOH	354	4.41	397	0.40^{j}	1.5	19.7^{j}	1.3
				378^{k}	8.60^{k}	2.3	331.6^{k}	1.2
PyMePAO	MeOH	351	4.51	397	0.26^{j}		15.4^{j}	
				378^{k}	3.81^{k}		273.7^{k}	
PyMePAH	PMMA	349	4.17	398	3.30	0.9	155.7	1.05
PyMePAO	PMMA	351	4.34	398	3.72		148.1	
PyMePAH	PS	355	4.16	400	0.81	1.7	176.3	1.3
PyMePAO	PS	354	4.20	400	0.46		140.8	
PyMePAH	PVC	361	4.31	399	3.07	1.4	83.6	4.2
PyMePAO	PVC	366	4.45	399	2.17		20.1	

The standard error of spectral measurements is around 10%.

[a] Probe according to Scheme 1.

[b] Medium: MeOH—methanol, PMMA—poly(methyl methacrylate), PS—polystyrene, PVC—polyvinyl chloride.

[c] Maximum of the longest wavelength absorption band.

[d] log of decadic extinction coefficient in dm^{3} mol^{-1} cm^{-1}.

[e] Maximum of the emission.

[f] Quantum yield relative to anthracene.

[g] Ratio of the relative quantum yield of the parent amine (NH) and oxidized form (NO) in the respective medium.

[h] Lifetime.

[i] Ration of the lifetimes of the parent amine (NH) and oxidized form (NO) in the respective medium.

[j] Aerated solution.

[k] Deaerated solution by stream of argon for 5 min.

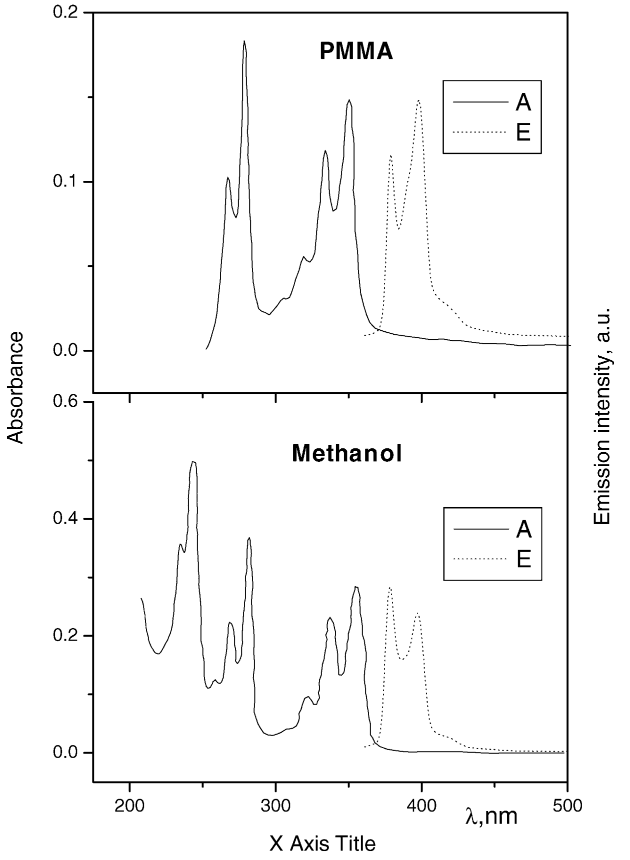


Fig. 4. Absorption (A) and fluorescence (E) spectrum of 15-(1-pyrenylmethyl)-7,15-diazadispiro[5.1.5.3]hexadecane-14,16-dione (PyMePAH, parent amine) in methanol (10^{-5} mol dm^{-3}) and PMMA (2×10^{-3} mol kg^{-1}).

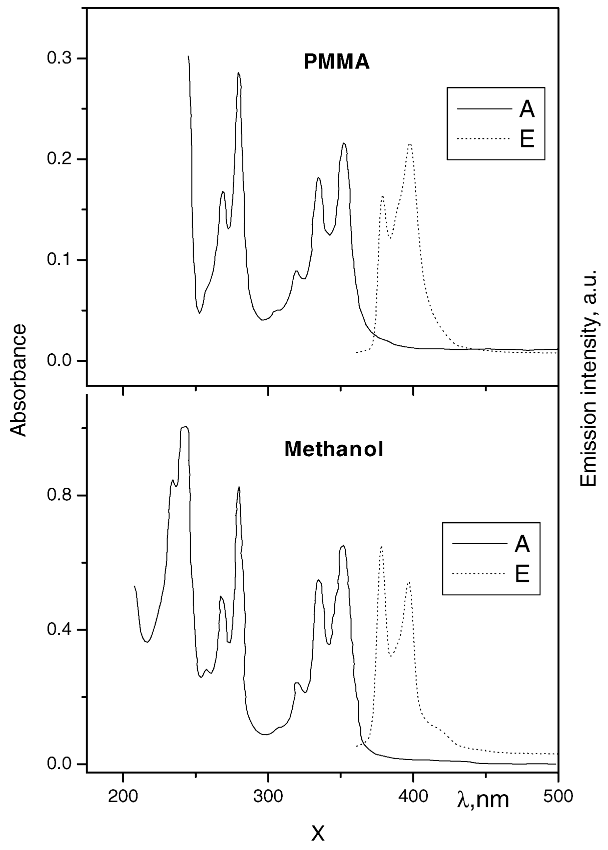


Fig. 5. Absorption (A) and fluorescence (E) spectrum of 7-oxo-15-(1-pyrenylmethyl)-7,15-diazadispiro[5.1.5.3]hexadecane-14,16-dione (PyMePAO, aminooxide) in methanol (10^{-5} mol dm^{-3}) and PMMA (2×10^{-3} mol kg^{-1}).

curs. The rather high value of ($\tau_{NH}/\tau_{NO\bullet}$) is observed for PVC around 4.2 which might be caused by influence of this matrix on lifetime of the probes.

The comparison of the absorption and fluorescence spectra of probes, where pyrene is directly linked with piperazine and linked with the methylene bridge, clearly shows that the direct linkage has got strong influence on the spectral properties. On the other hand, the methylene as link between pyrene and piperazine causes that pyrene behaves like unsubstituted derivative and the extent of singlet intramolecular quenching is rather low.

The triplet deactivation route was examined by laser flash photolysis using 355 nm excitation. The absorption spectra of pyrene (Py) and other probes containing pyrene (Figs. 1–7) secure that the 355 nm excitation is directed on long-wavelength edge of these probes and reasonable absorption about 0.4 can be reached at 10^{-4} mol dm^{-3} concentration. Using 355 nm laser excitation, the triplet state of all probes under study is formed causing transient absorption. The relevant kinetic and spectroscopic data concerning the transient absorption of these probes are given in Tables 3–5. For Py, PyMePAH and PyMePAO the maximum of this transient absorption is around 410–420 nm with band or shoulder at 380–390 nm and less intense absorption band in the range of 450–550 nm (Figs. 8–10). The band at 410 nm is more or less solvent independent in position but it has got higher intensity in non-polar cyclohexane. On the other hand, the band around 500 nm is red shifted in cyclohexane as compared with methanol but the intensity is comparable. These features have got transient spectra in both aerated and deaerated solvents.

For probe PyPA the transient spectrum is resolved with maximum at 410 nm and less intense at 520 nm for cyclohexane, which is rather similar to pyrene and its alkyl derivatives (Fig. 11). In polar methanol there is strong transient absorption in the range from 410 up to 520 nm, which is opposite behaviour as compared to probes Py and PyMePAH, PyMePAO.

Kinetic data obtained for Py and PyMePAH in aerated and deaerated methanol differ by more than one order of magnitude. The lifetime of the triplet state in aerated solution, given as $\tau = 1/k$ (Table 3) is about 0.25 μs and in deaerated methanol up to 20 μs. The oxygen concentration in aerated organic solvents is 2.2×10^{-3} mol dm^{-3} [30]. The bimolecular quenching rate of triplet state by oxygen can be estimated. This rate constant is about 2.0×10^{9} dm^{3} mol^{-1} s^{-1}, which is quite reasonable value for quenching of triplet state.

Quantitatively different data were obtained for PyMePAO. In this case stable nitroxyl radical has got some effect on

Table 3
Kinetic data of decay of transient absorption of the pyrene and pyrene based probes at 355 nm excitation

Probe[a]	Solvent[b]	Cond.[c]	λ[d] (nm)	F[e]	k[f] (s^{-1})	A[g] (%)	τ[h] (μs)
Py	MeOH	Air	410	M	4.13×10^6		0.24
		N_2	410	B	3.11×10^5	55	
					6.52×10^4	45	
				M	1.28×10^5		7.80
	Cy	Air	410	M	4.43×10^6		0.23
		N_2		B	2.04×10^5	52	
					4.19×10^4	48	
					9.69×10^4		10.30
PyMePAH	MeOH	Air	410	M	4.01×10^6		0.25
		N_2	410	B	3.12×10^5	44	
					5.10×10^4	56	
				M	8.69×10^4		11.50
	Cy	Air	410	M	4.16×10^6		0.24
		N_2	410	B	3.35×10^5	53	
					6.58×10^4	47	
				M	1.10×10^5		9.10
PyMePAO	MeOH	Air	410	M	4.76×10^6		0.21
		N_2	410	B	6.33×10^5	88	
					1.48×10^5	12	
				M	4.4×10^5		2.27
	Cy	Air	410	M	5.72×10^6		0.17
		N_2	410	B	1.25×10^6	85	
					2.32×10^5	12	
				M	8.46×10^5		1.18
PyPA	MeOH	Air	490	B	1.3×10^5		7.70
					7.19×10^5	56	
					6.17×10^4	35	
		N_2	490	M	2.23×10^5		4.50
				B	4.91×10^5	74	
					4.96×10^4	26	
	Cy	Air	410	M	1.17×10^6		0.85
				B	2.87×10^6	60	
					3.23×10^5	40	
		N_2	410	M	1.00×10^5		10
				B	4.89×10^5	56	
					3.38×10^5	44	

[a] Structure of the probe according to Scheme 1.
[b] Solvents: MeOH—methanol, Cy—cyclohexane.
[c] Experimental conditions: air-aerated solutions, N_2-bubling with stream of nitrogen for 10 min.
[d] Monitoring wavelength.
[e] Fitting to monoexponential M, or biexponential, B.
[f] Rate constant of the decay.
[g] Fraction at the bimolecular decay.
[h] Lifetime $\tau = 1/k$.

the triplet. In the aerated solution, stable nitroxyl radical cannot effectively compete with quenching by oxygen and the lifetime is similar to that of Py and PyMePAH. On the other hand, in the deaerated solutions, the lifetime of PyMePAO is much shorter due to intramolecular quenching by nitroxyl radical. The rate constants of PyMePAO of triplet decay are 5 times higher in methanol and 7.7 times faster in cyclohexane.

Triplet state of the PyPA, where pyrene is directly linked with piperazine, is not quenched so effectively with oxygen as Py and PyMePAH. The triplet lifetime of PyPA is 20 times longer in aerated methanol and 4 times in aerated cyclohexane. It should be noted that the spectrum in methanol is different as compared with cyclohexane and moreover it is not influenced by oxygen.

Substantially lower rate of decay of the triplet state in the range of 10–50 s^{-1} (the lifetime is in the range 20–100 ms) was observed in micellar solutions of sodium dodecyl sulphate and tetradecyltrimethylammonium bromide at pyrene concentration 2×10^{-4} mol dm^{-3} and detergent concentration

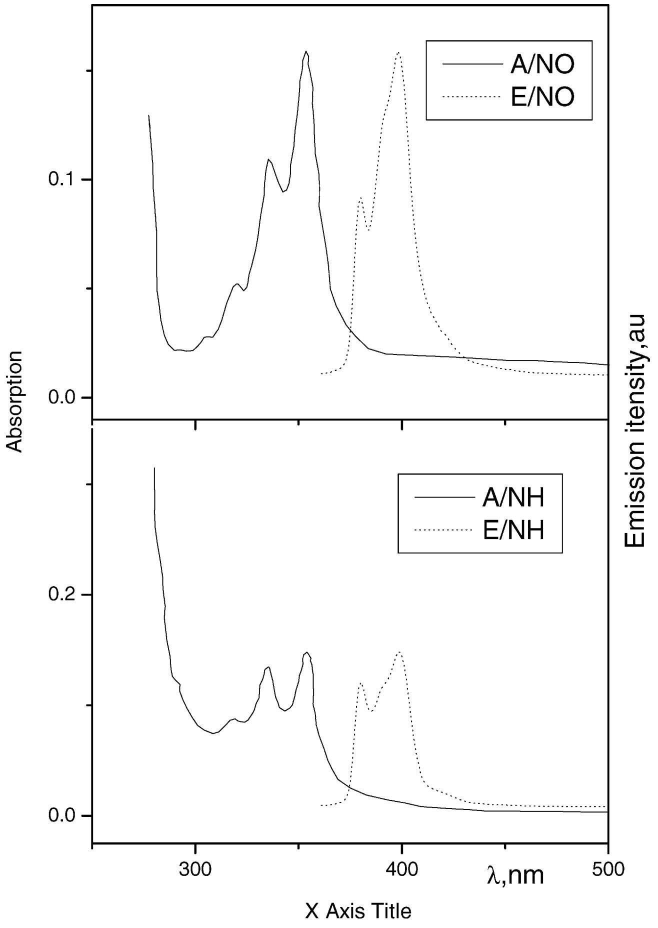


Fig. 6. Absorption and emission spectrum of parent amine PyMePAH and aminooxide PyMePAO in PS at 2 × 10^{-3} mol kg^{-1}.

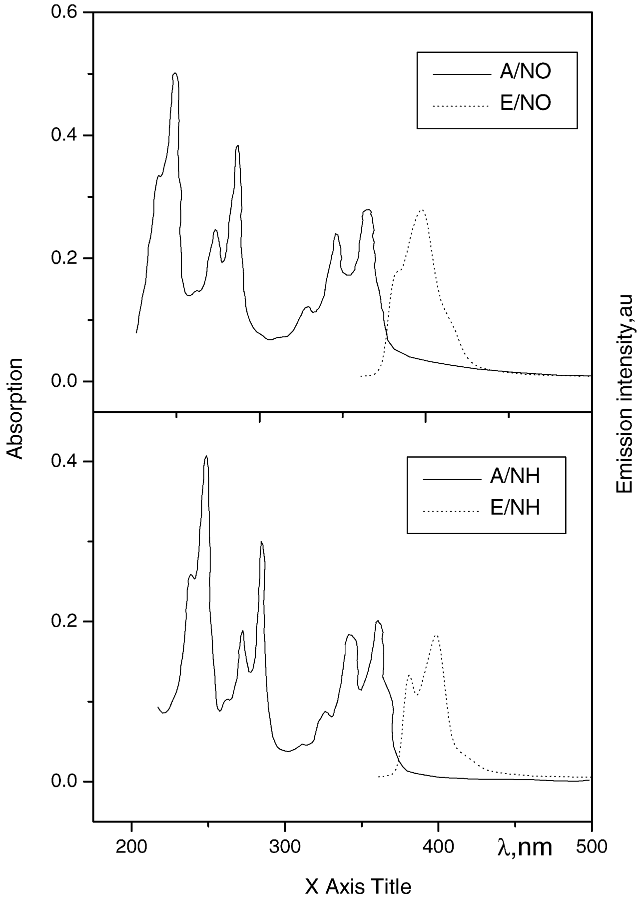


Fig. 7. Absorption and emission spectrum of parent amine PyMePAH and aminooxide PyMePAO in PVC at 2 × 10^{-3} mol kg^{-1}.

0.05 mol dm^{-3} and carefully removing of oxygen [31]. For all micelles containing two or more solubilizate molecules, when one of these molecules is excited to the triplet state, its lifetime is going to be short (about 10^3 times). In this case the triplet state is deactivated by ground state of pyrene in a triplet excimer mechanism. In this paper no special precautions were made to extent the triplet lifetime measurements. Therefore, the lifetime of the triplet state of the parent amine might be shortened by bimolecular quenching due to ground state of pyrene derivatives or other quenchers as rest of oxygen, etc. On the other hand, the more rapid decay in aminooxide is due to paramagnetic centre and will not be influenced by any micellar formation.

Another process, which might influence the rate of decay, is triplet–triplet annihilation leading to delayed fluorescence [32]. In this study at low laser intensity no delayed fluorescence was observed. Therefore, this process does not influence the observed rate of triplet decay in parent amines PyPA and PyMePAH and aminooxide PyMePAO.

The intermolecular quenching rate constant of these probes with 1-oxo-2,2,6,6-tetramethyl-piperidine (TEMPO) is around 5×10^7 dm^3 mol^{-1} s^{-1} in methanol but slightly higher around 10^8 dm^3 mol^{-1} s^{-1} in cyclohexane which is less efficient as compared to oxygen. More efficient quenching with *N*-oxyl radical was observed for probes based on naphthalene and 1,8-naphthaleneimide [22]. The intramolecular quenching for probes with oxidized amine PyMePAO based on comparison of rate constant for decay (fitted to monoexponential) $k_{\mathrm{NO}^\bullet}/k_{\mathrm{NH}}$ is about 5. As discussed above this value might be higher due to slower decay of parent amine. On the other hand, the intermolecular quenching with TEMPO occurs in PyMePAO at the same rate as intramolecular one, which indicates that the intramolecular quenching has got some limitations most probably due to the steric factors. Methylene linkage and bulky structure of piperazine unit probably prevents more effective intramolecular quenching. To determine the efficiency of the intramolecular quenching at direct linkage of piperazine unit on pyrene was not possible because preparation of respective radical by oxidation of PyPA was not successful.

The triplet route of deactivation of these probes, where pyrene is chromophore linked to sterically hindered amine (piperazine), seems to be a feasible way of electronic energy dissipation. It was clearly demonstrated that triplet state is formed and quenched by oxygen and *N*-oxyl type of quencher. However, quenching with *N*-oxyl pyrene and its derivatives under study is less efficient as quenching

Table 4
Spectral and kinetic data of triplet pyrene and PyPA at 355 nm excitation

Probe[a]	Solvent[b]	λ_{max}[c] (nm)	$k_q(O_2)$[d] ($dm^3\,mol^{-1}\,s^{-1}$)	k_q(TEMPO)[e] ($dm^3\,mol^{-1}\,s^{-1}$)
Py	MeOH	410	2.0×10^9	4.6×10^7
		450		
		490		
	Cy	390	2.2×10^9	1.8×10^8
		410		
		520		
PyPA	MeOH	480	1.0×10^8	3.4×10^7
	Cy	420	5.4×10^8	4.9×10^7
		520		

[a] Structure of the probe according to Scheme 1.
[b] Solvents: MeOH—methanol, Cy—cyclohexane.
[c] Maximum of the transient spectra around 1 μs after excitation.
[d] Bimolecular rate constant of quenching by oxygen $k_q(O_2)$ determined from single point measurement in aerated solution.
[e] Bimolecular rate constant of quenching by 1-oxo-2,2,6,6-tetramethylpiperidine (TEMPO) determined at three concentration of quencher (0.001, 0.005 and 0.01 mol dm^{-3}) using rate constant obtained by fitting to monoexponential.

Table 5
Spectral and kinetic data of triplet of parent amine PyMePAH and amineoxide PyMePAO at 355 nm excitation

Probe[a]	Solvent[b]	λ_{max}[c] (nm)	$k_q(O_2)$[d] ($dm^3\,mol^{-1}\,s^{-1}$)	k_q(TEMPO)[e] ($dm^3\,mol^{-1}\,s^{-1}$)	$k_{NO\bullet}/k_{NH}$[f]
PyMePAH	MeOH	410	2.0×10^9	1.8×10^7	
		500			
	Cy	420	2.0×10^9	1.1×10^8	
		520			
PyMePAO	MeOH	410	2.1×10^9	1.8×10^7	5.1
	Cy	420	2.6×10^9	1.2×10^8	7.7
		520			

[a] Structure of the probe according to Scheme 1.
[b] Solvents: MeOH—methanol, Cy—cyclohexane.
[c] Maximum of the transient spectra around 1 μs after excitation.
[d] Bimolecular rate constant of quenching by oxygen $k_q(O_2)$ determined from single point measurement in aerated solution.
[e] Bimolecular rate constant of quenching by 1-oxo-2,2,6,6-tetramethylpiperidine (TEMPO) determined at three concentration of quencher (0.001, 0.005 and 0.01 mol dm^{-3}) using rate constant obtained by fitting to monoexponential.
[f] Ratio of the rate constants of the decay of parent amine and aminooxide based on monoexponential fit of the decay curve.

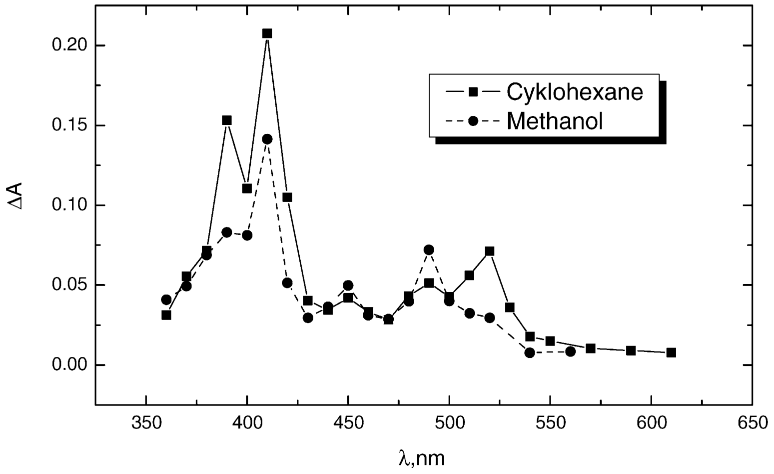


Fig. 8. Transient absorption spectra of pyrene (Py) in cyclohexane at 1.5 μs and methanol at 1.5 μs at 355 nm excitation.

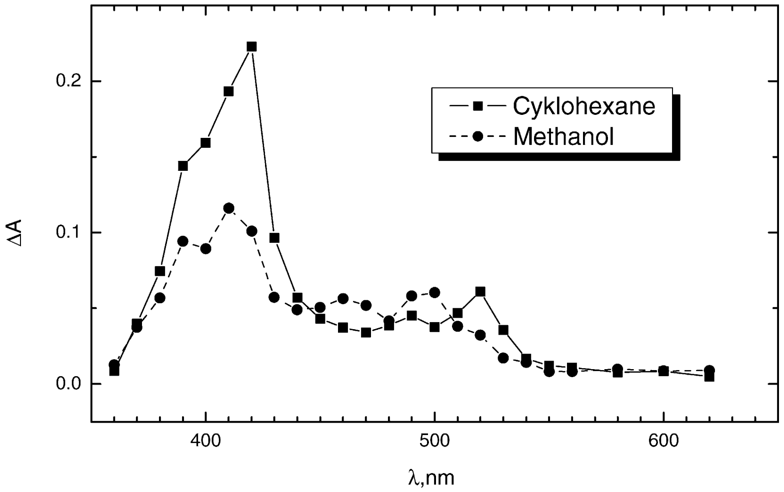


Fig. 9. Transient absorption spectra of PyMePAH in methanol (2 μs) and cyclohexane (2 μs) at 355 nm excitation.

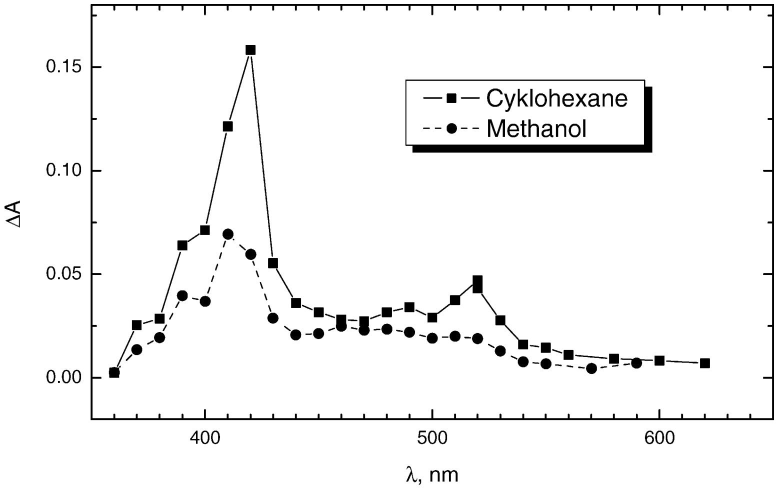


Fig. 10. Transient absorption spectra of PyMePAO in methanol (1 μs) and cyclohexane (1 μs) at 355 nm.

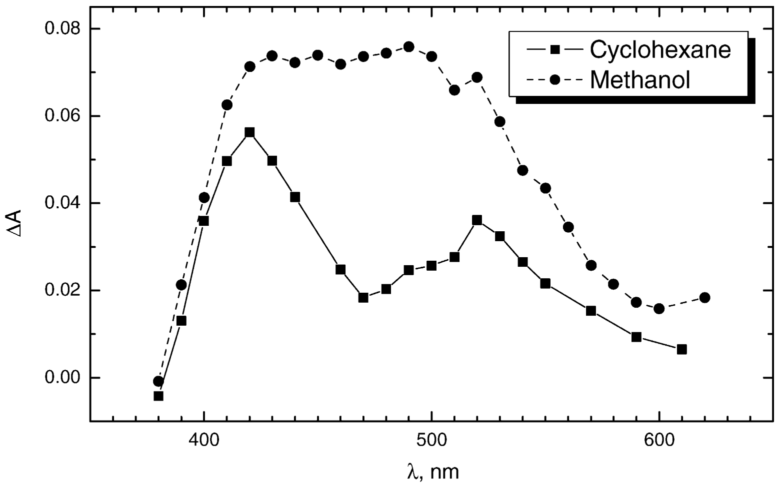


Fig. 11. Transient absorption spectra of PyPA in methanol (0.58 μs) and cyclohexane (1 μs) at 355 nm excitation.

with O_2. Similarly, the intramolecular quenching is not very efficient for probes under study with pyrene as chromophore. Similar conclusions were obtained previously [22].

The mechanism involved in intermolecular as well as intramolecular quenching is not yet well understood. The energy of the states taking part in the system is well defined on the part of donor. The singlet and triplet levels for pyrene are 323 and 210 kJ mol^{-1}, respectively [28]. The energy of the singlet and triplet levels of sterically hindered *N*-oxyls of piperidine or piperazine type has not yet been determined. The inefficient intermolecular and intramolecular quenching of pyrene triplet by *N*-oxyls indicates that triplet levels of donor and acceptor are quite similar when electronic energy transfer is involved.

In conclusion the photophysical processes in the pair pyrene–*N*-oxyl do not seem to be very effective on the singlet as well as on the triplet level. Although pyrene or its derivatives are sensitive fluorescence probes, they are not suitable for construction of molecular probes operating on intramolecular quenching on singlet or triplet level.

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References

[1] F.M. Winnik, S.T.A. Regismond, Coll. Surf. A: Physicochem. Eng. Asp. 118 (1996) 1.
[2] I. Capek, Adv. Coll. Interf. Sci. 97 (2002) 91.
[3] F.M. Winnik, Chem. Rev. 93 (1993) 587.
[4] H. Morawetz, J. Polym. Sci. A: Polym. Chem. 37 (1999) 1725.
[5] G. Duprotail, P. Lianos, in: M. Rosoff (Ed.), Vesicles, Marcel Dekker, 1996, p. 296. Chapter 8.
[6] W. Rettig, Angew. Chem. Int. Ed. Engl. 25 (1986) 971.
[7] J. Dobkowski, W. Rettig, J. Waluk, Phys. Chem. Chem. Phys. 4 (2002) 4334.
[8] P. Hrdlovič, J. Kollár, Š. Chmela, J. Photochem. Photobiol. A: Chem. 163 (2004) 289.
[9] A.R. Watkins, Chem. Phys. Lett. 29 (1974) 526.
[10] A.R. Watkins, Chem. Phys. Lett. 70 (1980) 230.
[11] A.R. Watkins, Chem. Phys. Lett. 70 (1980) 262.
[12] J.C. Scaiano, Chem. Phys. Lett. 79 (1981) 41.
[13] V.A. Kuzmin, A.S. Tatikolov, Chem. Phys. Lett. 51 (1977) 45.
[14] J.A. Green II., L.A. Singer, J. Am. Chem. Soc. 96 (1974) 2730.
[15] S.K. Chattopadhyay, P.K. Das, G.L. Hug, J. Am. Chem. Soc. 105 (1983) 6205.
[16] J. Karpiuk, Z.R. Grabowski, Chem. Phys. Lett. 160 (1989) 451.
[17] P. Hrdlovič, J.C. Scaiano, I. Lukáč, J.E. Guillet, Macromolecules 19 (1986) 1637.
[18] S.A. Green, D.J. Simpson, G. Zhou, P.S. Ho, N.V. Blough, J. Am. Chem. Soc. 112 (1990) 7337.
[19] S. Green, M.A. Fox, J. Phys. Chem. 99 (1995) 14752.
[20] P. Hrdlovič, Š. Chmela, J. Photochem. Photobiol. A: Chem. 105 (1997) 83.
[21] L. Bucsiová, P. Hrdlovič, Š. Chmela, J. Photochem. Photobiol. A: Chem. 143 (2001) 59.
[22] P. Hrdlovič, Š. Chmela, M. Sarakha, J. Lacoste, J. Photochem. Photobiol. A: Chem. 138 (2001) 95.
[23] E. Bosch, P. Bou, H. Alleman, M. Marti Roses, Anal. Chem. 68 (1996) 3651.
[24] S. Rondinini, P.R. Mussini, T. Mussini, A. Vertova, Pure Appl. Chem. 70 (1998) 1419.
[25] A. Kawski, A. Kubicki, B. Kulinski, J. Photochem. Photobiol. A: Chem. 71 (1993) 161.
[26] J.N. Demas, A.W. Adamson, J. Phys. Chem. 57 (1971) 2463.
[27] J.N. Demas, Excited State Lifetime Measurements, Appendix E, Academic Press, New York, 1983, p. 245.
[28] P. Mazellier, M. Sarakha, A. Rossi, M. Bolte, J. Photochem. Photobiol. A: Chem. 115 (1998) 117.
[29] Z. Rappoport (Ed.), Handbook of Tables for Organic Compounds Identifications, 3rd ed., CRC Press, 1967, p. 437.
[30] S.L. Murov, I. Carmicheal, L.G. Hug, Handbook of Photochemistry, 2nd ed., Marcel Dekker, New York, 1993.
[31] T.F. Hunter, A.J. Szczepinski, J. Phys. Chem. 88 (1984) 1231.
[32] C. Bohne, E.B. Abuin, J.C. Scaiano, J. Am. Chem. Soc. 112 (1990) 4226.