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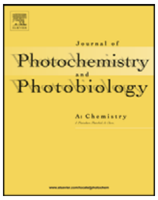

Synthesis and spectral characteristics of substituted 1,8-naphthalimides: Intramolecular quenching by mono-nitroxides

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ABSTRACT

Novel series of chromophore-nitroxide adducts were synthesized for the monitoring of photophysical and photochemical processes in solution as well as in a polymer matrix. These adducts were: (1) N-butyl-4-(1′-oxy-2′,2′,6′,6′-tetramethyl-4′-piperidylamino)-1,8-naphthalimide, BUNO – paramagnetic probe has been synthesized having the nitroxyl radical substituent acting as a quencher attached to the naphthalene in position 4 of 1,8-naphthalimide while imino nitrogen was substituted by n-butyl group. (2) N-(1′-oxy-2′,2′,6′,6′-tetramethyl-4′-piperidyl)-4-dimethylamino-1,8-naphthalimide – DMANO, where nitroxyl unit is on imino nitrogen of 1,8-naphthalimide while in position 4 of 1,8-naphthalimide there is dimethylamino substituent. Diamagnetic parent amine (BUNH, DMANH) were used as standard samples for comparison of the efficiency of intramolecular quenching. Absorption and fluorescence spectra of probes were taken in solution as well as in polymer films. In their absorption spectrum the longest wavelength band lies in the range from 400 up to 440 nm depending on the medium. In fluorescence spectrum the maximum lies in the range from 460 to 530 nm. When compared with less polar media the maxima of absorption and fluorescence spectra in polar media are red-shifted. The fluorescence is observed for all probes but there is distinctly much higher for parent amine and alkoxyamine than for respective mono-nitroxyl radical. The extent of intramolecular quenching, represented by the ratio of quantum yields of diamagnetic and paramagnetic forms, for both pairs BUNH/BUNO and DMANH/DMANO is in the range 2–15 depending on the medium. Intramolecular quenching in solvents for both pairs of mono-nitroxyl radical (BUNH/BUNO and DMANH/DMANO) exhibits an additive effect when compared with bi-nitroxyl radical (DINH/DINO) investigated previously.

1. Introduction

Low molecular probes monitoring various processes in vivo and in vitro may have complex structure. Preferably they exhibit fluorescence which is extensively studied and exploited phenomenon [1–6]. Such probes form basic part of fluorescent sensors and switches. Fluorescence of these systems is the parameter of choice for monitoring because it exhibits high sensitivity, simple detection and quantitative evaluation and pronounced medium effect. The most important unit of any system is the fluorophore which reports the change. Therefore, its selection is of high importance.

The 1,8-naphthalimide (benzo[de]isoquinoline-1,3-dione), substituted in various position, was widely employed as basic unit in different probes during the nineties and first part of this decade [7–14]. This structural unit substituted in different positions by electron donating substituents exhibits interesting spectral properties and strong fluorescence. Some time ago we have prepared and spectrally characterized probe composed from 1,8-naphthalimide (NI) and parent amine or nitroxide of 2,2,6,6-tetramethylpiperidine type in solution and in polymer matrices [8]. Later, adduct of NI-2,2,6,6-tetramethylpiperidine was functionalized with double bond and these products were tested as polymer light stabilizers and fluorescent dye (brightener) simultaneously [9]. The 1,8-naphthalimide was used as a chromophore in the probe sensing anions [10]. The dyad with dansylamide was employed for switching of proton induced fluorescence [11]. Recently, poly(amidoamine) dendrimer of second generation functionalized with 1,8-naphthalimide units was tested as potential sensor for metal cations [12]. Strong solvatochromism of the 4-methoxy-N-methyl-1,8-naphthalimide was employed for testing of ethanol–water mixtures [13]. Similar effect was observed for 4-phenoxy derivative as well [14]. Novel luminescent dyes containing 1,8-naphthalimide units were prepared and used as well [15]. The adduct formed by combination of 1,8-naphthalimide chromophore with 2-hydroxyphenylbenzotriazole and 2,2,6,6-tetramethylpiperidine as light stabilizers exhibited the best photostability due to synergism of two stabilizer fragments with different action combined in one molecule [16].

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Various 4-aminonaphthalimide compounds were investigated as probes with the strong pH dependance on the fluorescence quantum yield [17] and as sensors/switches [18]. The authors refer that the spacer between receptor and chromophore influence a photoinduced electron transfer reaction responsible for the fluorescence quenching.

Strong electron donating group as amine, alkyl amine or dialkylamine in the 4-position of the 1,8-naphthalimide causes bathochromic and bathofluoric shifts in the absorption and fluorescence spectra, respectively. Recently, we have prepared and characterized dual chromophore-nitroxide probes. Double substituted 1,8-naphthalimide with nitroxides in position N and 4 was employed as the chromophore [19]. Nitroxides represented 4-amino-1-oxy-2,2,6,6-tetramethylpiperidine. The strong bathochromic and bathofluoric shift was observed. The 4-amino-2,2,6,6-tetramethylpiperidine structural unit behaves as strong electron donor such as, e.g. dimethylamino group.

The goal of this study is to explore the spectral properties of substituted 1,8-naphthalimide probes with nitroxide only in position 4 or N as 4-(2′,2′,6′,6′-tetramethyl-4′-piperidylamino)-N-butyl-1,8-naphthalimide and N-(2′,2′,6′,6′-tetramethyl-4′-piperidyl)-4-dimethylamino-1,8-naphthalimide. Diamagnetic parent amine and alkoxyamine was synthesized as well in order to determine the extent of intramolecular quenching. The spectral characteristic and efficiency of intramolecular quenching of probes containing one nitroxide was compared with the structurally similar probe containing two nitroxides [19] to determine the contribution of the individual nitroxides.

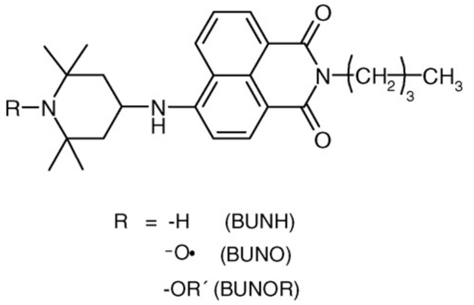
2. Experimental

2.1. Synthesis of probes

Derivatives of N-butyl-1,8-naphthalimide substituted in position 4 by hindered amine according to Scheme 1 (BUNR series) were prepared as follows.

4-Bromo-N-butyl-1,8-naphthalimide. 4-Bromo-1,8-naphthalic anhydride (1.8 g, 6 mmol) and n-butylamine (0.6 g, 8 mmol) were dissolved in 100 mL of absolute ethanol and solution was heated at 60 °C under stirring overnight. Excess of n-butylamine and ethanol was then partially evaporated and the residue was dissolved in 100 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 chloroform:isohexane (2:1), yielded 1.5 g (75%) of yellow crystals with m.p. = 103–105 °C.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.95 (t, *J* = 4.8 Hz, 3H, C*H*$_3$), 1.45 (m, 2H, C*H*$_2$), 1.70 (m, 2H, C*H*$_2$), 4.15 (m, 2H, N–C*H*$_2$) 7.85 (t, *J* = 5.4 Hz, 1H, C*H* (napht.-6)), 8.00 (d, *J* = 3.9 Hz, 1H, C*H* (napht.)), 8.35 (d, *J* = 3.9 Hz, 1H, C*H* (napht.)), 8.50 (d, *J* = 4.2 Hz, 1H, C*H* (napht.)), 8.60 (d, *J* = 4.2 Hz, 1H, C*H* (napht.)).



Scheme 1.

FTIR ($CHCl_3$), cm^{-1}: ν(CO$^{sym.}$) 1685, ν(CO$^{asym.}$) 1649, ν(C=C) 1590, ν(C–N) 1361.

4-(2′,2′,6′,6′-Tetramethyl-4′-piperidylamino)-N-butyl-1,8-naphthalimide (BUNH). 4-Bromo-N-butyl-1,8-naphthalimide (0.67 g, 2 mmol) and 4-amino-2,2,6,6-tetramethylpiperidine (0.47 g, 3 mmol) was dissolved in 50 mL of 1-methyl-2-pyrrolidone. The mixture was heated for 24 h at 70 °C and excess of 4-amino-2,2,6,6-tetramethylpiperidine and 1-methyl-2-pyrrolidone were recovered by vacuum distillation. Residual mixture was dissolved in chloroform and extracted with water. Organic layer was separated and dried over Na_2SO_4. After solvent evaporation the crude product was purified by column chromatography on silica gel using a mixture of chloroform and isohexane (3:1) yielding 0.55 g (67%) of yellow wax.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.00 (t, *J* = 4.8 Hz, 3H, C*H*$_3$/n-butyl/), 1.20 (s, 6H, 2× C*H*$_3$ax), 1.40 (s, 6H, 2× C*H*$_3$eq), 1.45 (m, 2H, C*H*$_2$/n-butyl/), 1.65 (m, 2H, 2× C*H*H), 1.70 (m, 2H, C*H*$_2$/n-butyl/), 2.30 (m, 2H, 2× CH*H*), 4.10 (m, 1H, C*H*), 4.20 (m, 2H, N–C*H*$_2$), 5.00 (wide s, 1H, N*H*), 7.00 (d, *J* = 5.1 Hz, 1H, C*H* ((napht.-3)), 7.65 (t, *J* = 5.2 Hz, 1H, C*H* (napht.-6)), 8.00 (d, *J* = 5.4 Hz, 1H, C*H* (napht.)), 8.35 (d, *J* = 6.0 Hz, 1H, C*H* (napht.)), 8.55 (d, *J* = 6.3 Hz, 1H, C*H* (napht.)).

GC–MS *m*/*z*: 407 [M$^+$]. Elemental analysis. Calcd. for $C_{25}H_{33}N_3O_2$: C, 73.68%; H, 8.16%; N, 10.31%. Found: C, 74.11%, H, 8.27%, N, 10.09%.

FTIR ($CHCl_3$), cm^{-1}: ν(CO$^{sym.}$) 1686, ν(CO$^{asym.}$) 1649, ν(C=C) 1589, ν(C–N) 1363.

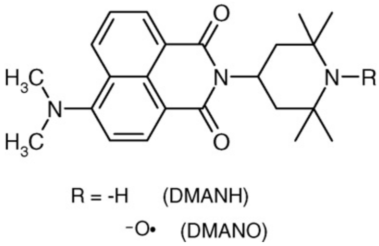
4-(1′-Oxy-2′,2′,6′,6′-tetramethyl-4′-piperidylamino)-N-butyl-1,8-naphthalimide (BUNO). 4-Bromo-N-butyl-1,8-naphthalimide (0.67 g, 2 mmol) and 4-amino-1-oxy-2,2,6,6-tetramethylpiperidine (0.5 g, 3 mmol) was dissolved in 50 mL of 1-methyl-2-pyrrolidone. The mixture was heated for 24 h at 70 °C and excess of 4-amino-2,2,6,6-tetramethylpiperidine and 1-methyl-2-pyrrolidone were recovered by vacuum distillation. Residual mixture was dissolved in chloroform and extracted with water. Organic layer was separated and dried over Na_2SO_4. After solvent evaporation the crude product was purified by column chromatography on silica gel using a mixture of chloroform and isohexane (1: 1) yielding 0.5 g (59%) of orange crystals with m.p. = 84–87 °C. Purity of the radical was proved by EPR spectroscopy. The value of radical concentration for BUNO was 93%. This value was calculated as the relative concentration to the amount of radicals from 4-hydroxy-1-oxy-2,2,6,6-tetramethylpiperidine used as a standard. We assume that the concentration of radicals in standard is 100%.

GC–MS *m*/*z*: 422 [M$^+$]. Elemental analysis. Calcd. for $C_{25}H_{32}N_3O_3$: C, 71.06%; H, 7.63%; N, 9.94%. Found: C, 71.18%; H, 7.83%; N, 9.41%.

FTIR ($CHCl_3$), cm^{-1}: ν(CO$^{sym.}$) 1686, ν(CO$^{asym.}$) 1649, ν(C=C) 1590, ν(C–N) 1363.

4-(1′-Phenylethoxy-2′,2′,6′,6′-tetramethyl-4′-piperidylamino)-N-butyl-1,8-naphthalimide (BUNOR). 4-(1′-oxy-2′,2′,6′,6′-tetramethyl-4′-piperidylamino)-N-butyl-1,8-naphthalimide, BUNO (0.3 g, 0.7 mmol), $Mn(OAc)_3$ (0.75 g, 2.8 mmol) and styrene (0.29 g, 2.8 mmol) were suspended in 15 mL solvents mixture (ethanol/toluene/acetic acid 2:2:1) and stirred vigorously at room temperature. $NaBH_4$ (0.16 g, 4.2 mmol) was added slowly in very small portions and reaction was monitoring by TLC. After 5 h the mixture was filtered and washed two times with dichloromethane. The organic layers were combined, solvents were evaporated and the residue was dissolved in 30 mL of dichloromethane. The solution was washed with aqueous $NaHCO_3$ solution and with water. Organic layer was separated, dried over Na_2SO_4 and the solvent was evaporated. Purification by column chromatography, eluting with dichloromethane/isohexane (2:1), gave 0.28 g (76%) of yellow wax.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.75 (s, 6H, 2× C*H*$_3$ax), 1.00 (t, *J* = 4.5 Hz, 3H, C*H*$_3$/n-butyl/), 1.30 (s, 6H, 2× C*H*$_3$eq), 1.45 (s, 3H,



Scheme 2.

CH_3–CH–Ph), 1.50 (m, 2H, CH_2/n-butyl/), 1.70 (m, 2H, CH_2/n-butyl/), 1.85 (m, 2H, 2× CHH), 2.60 (m, 2H, 2× CH*H*), 4.00 (m, 1H, C*H*), 4.20 (m, 2H, N–CH_2), 4.80 (m, 1H, CH_3–C*H*–Ph), 5.00 (wide s, 1H, N*H*), 7.00 (d, *J* = 5.1 Hz, 1H, C*H* ((napht.-3)), 7.25–7.40 (m, 5H, Ph), 7.65 (t, *J* = 5.1 Hz, 1H, C*H* (napht.-6)), 8.00 (d, *J* = 5.1 Hz, 1H, C*H* (napht.)), 8.35 (d, *J* = 5.4 Hz, 1H, C*H* (napht.)), 8.55 (d, *J* = 5.4 Hz, 1H, C*H* (napht.)).

GC–MS *m*/*z*: 527 [M$^+$]. Elemental analysis. Calcd. for $C_{33}H_{41}N_3O_3$: C, 75.11%; H, 7.83%; N, 7.96%. Found: C, 75.42%; H, 7.98%; N, 8.33%.

FTIR ($CHCl_3$), cm^{-1}: ν(CO$^{sym.}$) 1686, ν(CO$^{asym.}$) 1648, ν(C=C) 1590, ν(C–N) 1362.

The preparation of DMANH from the DMANR series (Scheme 2) has already been partially described [20]. The preparation of DMANO was performed as follows.

To the solution of 4-dimethylamino-1,8-naphthalic anhydride (0.24 g, 1 mmol) in DMF (3 mL) was slowly added 1-oxy-4-amino-2,2,6,6-tetramethylpiperidine (0.24 g, 1.4 mmol) in DMF (3 mL) and reaction mixture was stirred for a 45 min. After this time AcOH (0.2 mL) was added. Mixture was stirred at room temperature for 40 min and 12 h at 100 °C. Reaction mixture was then cooled, concentrated and washed with diethylether (20 mL). Orange solid was filtered off and purified by column chromatography using Al_2O_3 and dichloromethane/isohexane 3:2 as eluent. The product was crystallized from ethanol to yield orange crystals (62 mg, 17%), m.p. = 199–203 °C. Structure and purity of the radical was proved by FTIR and EPR spectroscopy as well as by TL chromatography. The value of radical concentration for DMANO was 92%.

^{1}H NMR (300 MHz, $CDCl_3$, in the presence of pentafluorophenylhydrazine): δ = 1.20 (s, 6H, 2× CH_3ax), 1.35 (s, 6H, 2× CH_3eq), 1.65 (m, 2H, 2× C*H*H), 2.50 (m, 2H, 2× CH*H*), 3.11 (s, 6H, 2× CH_3N), 5.20 (s, 1H, N–O*H*), 5.70 (m, 1H, C*H*N), 7.10 (d, *J* = 8.1 Hz, 1H, C*H* (napht.-3)), 7.70 (t, *J* = 6.6 Hz, 1H, C*H* (napht.-6)), 8.40–8.60 (m, 3H, C*H* (napht.)). GC–MS *m*/*z*: 394 [M$^+$]. Elemental analysis. Calcd. for $C_{23}H_{28}N_3O_3$: C, 70.03%; H, 7.15%; N, 10.65%. Found: C, 70.21%; H, 7.45%; N, 10.52%.

2.2. Spectral measurements

^{1}H NMR spectra were recorded in solution on a Bruker AC-300P (300.1 MHz) spectrometer, with the TMS proton signal as an internal standard. FTIR spectra were measured on Impact 400 (Nicolet, USA) and ESR spectra on X-band spectrometer E-4 Varian (USA) interfaced with PC using program Symphonia Bruker. GC–MS analysis was performed with Agilent 6890N (Agilent Technologies, Santa Clara, CA). Total C, H and N contents were determined using elemental analyser (Fisons EA-1108 Fisons Instruments, Milano, Italy).

Anthracene was zonally refined (Lachema n.e., Brno, CR). Methanol was for UV spectroscopy. Chloroform, tetrahydrofuran (Slavus, SR) was analytical reagents. Quencher: 1-oxo-2,2,6,6-tetramethyl-4-hydroxypiperidine (TEMPOL) was received from Aldrich.

Polymer films doped with fluorescence probes of both types were prepared by casting from solution. Films of polystyrene (PS) (Chemische Werke Huels, F.R.G.), poly(methyl methacrylate) (PMMA) (Diacon, ICI, England) were prepared by casting of 1 mL chloroform solution of polymer (5 g/100 mL) containing the respective amount of probe on a glass plate (28 mm × 35 mm). The solvent was evaporated slowly. Films of poly(vinylchloride) (PVC) (Neralit, Spolana Neratovice s.e., CR) were prepared by similar way by casting from tetrahydrofuran solution.

UV–Vis absorption spectra were taken on a spectrometer UV 1650PC (Shimadzu, Japan).

Emission spectra were recorded on spectrofluorophotometer RF-5301PC (Shimadzu, Japan) and on spectrofluorimeter Perkin-Elmer MPF-4 (Perkin-Elmer, Norfolk, Conn. USA), which was connected through interface and A/D convertor to ISA slot of PC using home made program for data collection. Fluorescence of solution was measured in 1 cm cuvette in the right angle arrangement. The quantum yield was determined relative to anthracene in cyclohexane, chloroform or methanol. Fluorescence of polymer films was taken in front face arrangement on the solid sample holder. The relative quantum yields in solution were corrected on different absorption at the wavelength of excitation [21]. The fluorescence spectra were taken at excitation into the maximum of the longest wavelength absorption band.

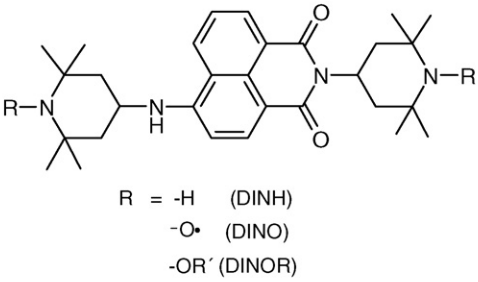
The fluorescence lifetime measurements were performed on a LIF 200 (Lasertechnik Ltd., Berlin, F.R.G.), which operates as a stroboscope. The excitation source is a nitrogen laser emitting at 337 nm and the emission is selected by cut-off filter. The output signal of Box Car Integrator was digitized and transferred to the PC using home made program. The fluorescence decay curves were evaluated by simple phase plane method [22] using program of J. Snyder based on [23]. The standard deviation $G^{1/2} = \Sigma((I_{exp} - I_{calc})^2/n)^{1/2}$, where I_{exp} and I_{calc} are intensity of emission experimental and calculated respectively, is used to judge if the decay is monoexponential. It is assumed that the decay curve satisfies the monoexponential when $G^{1/2}$ is lower than 5%. The fitting of fluorescence decay curves for a model of biexponential decay was preformed using adapted FluoFit MatLab package [24].

The steady state and time resolved fluorescence measurements were performed in aerated solutions. All measurements on polymer films were performed on air.

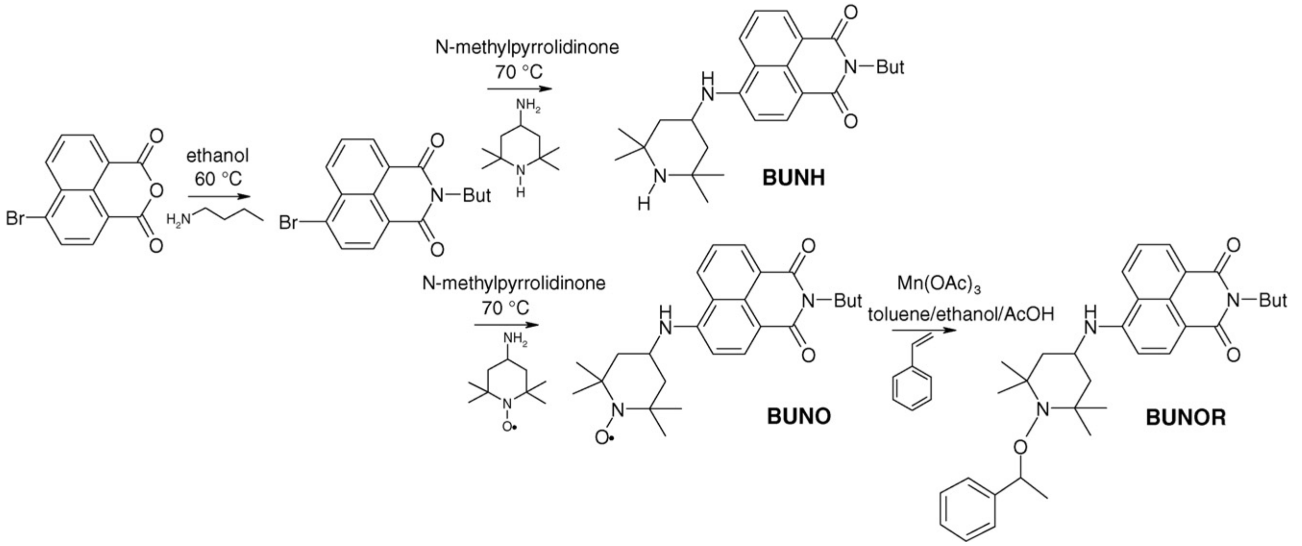
3. Results and discussion

3.1. Synthesis

The number of substituted 1,8-naphthalimides have been prepared by the selective synthetic methods. Interaction of 4-bromo-1,8-naphthalic anhydride with primary amines in 1-methyl-2-pyrrolidone at 70 °C gave double substituted N-alkyl-4-alkylamino-1,8-naphthalimide (Scheme 3) [19]. For a introduction of 2,2,6,6-tetramethylpiperidine unit exclusively on a naphthalene ring, reaction with butylamine has to be accomplished first. Resulting N-butyl-4-bromo-1,8-naphthalimide have been then converted to BUNH by interaction with 4-amino-2,2,6,6-tetramethylpiperidine (Scheme 4). BUNO has



Scheme 3.



Scheme 4.

been prepared in a similar way by reaction with 4-amino-1-oxy-2,2,6,6-tetramethylpiperidine. Synthesis of the corresponding alkoxyamine BUNOR was then carried out using $Mn(OAc)_3$ as electron transfer agent.

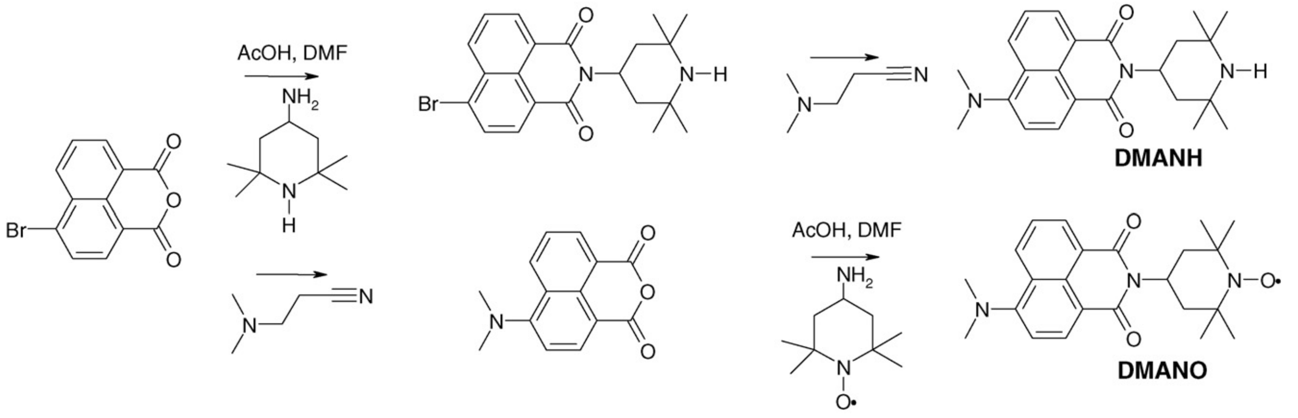
Regiospecific substitution of 4-bromo-1,8-naphthalic anhydride to 4-bromo-1,8-naphthalimide can be achieved in hot DMF at the presence of small amount of AcOH. Substitution of bromine in position 4 of naphthalene ring did not proceed under these conditions. Subsequent conversion of 4-bromo-N-(2,2,6,6-tetramethyl-4-piperidinyl)-1,8-naphthalimide to DMANH was performed by its reaction with 3-(dimethylamino)-propionitrile (Scheme 5). DMANO has been prepared by substitution of 4-bromo-1,8-naphthalic anhydride with 3-(dimethylamino)-propionitrile and by the following reaction with 4-amino-1-oxy-2,2,6,6-tetramethylpiperidine.

3.2. Spectral measurement

The spectral data of the probes under study were obtained in solution as well as in polymer matrices. Generally, it is assumed that the solvation power of solvent and polymer matrix is the same or similar if the same structural units are involved. The main difference is due to difference in mobility of the solvation envelope of low molecular solvent and polymer matrix. It may be expected that the effect of mobility is not so important for absorption spectra but it plays some role in fluorescence spectra of probes doped in polymer matrices.

The absorption spectrum of N-substituted derivative N′-(2′,2′,6′,6′-tetramethyl-4′-piperidyl)-1,8-naphthalimide not having a substituent in the position 4 of naphthalene ring exhibits the broad band with the maximum around 340 nm in polar methanol and showing some features of the vibrational structure in non polar cyclohexane [8]. Both types of probes under this study exhibit absorption spectra as broad band bathochromically shifted when compared with only N-substituted derivative. For series BUNH, BUNO and BUNOR, where the imide nitrogen is substituted with n-butyl and position 4 with 4-amino-2,2,6,6-tetramethylpiperidine (Scheme 1), there is a shift from 340 to about 410 nm in absorption spectrum in cyclohexane and up to 435 nm in methanol. This represents a shift of more than 5 000 cm^{-1} (Fig. 1). The relevant spectral data for BUNR series are summarized in Tables 1–3. In polymer matrices the basic features of absorption spectra of BUNR probes are the same as in solvents. The longest wavelength band is rather broad and is shifted from 415 nm in PS, where it is seen some indication of vibrational structure, to 430 nm in PVC (Fig. 2).

The similar shift was observed in absorption spectra of DMANR series. The maxima of absorption spectra lie in the spectral region from 390 nm in cyclohexane to 425 nm in methanol (Fig. 3). The relevant spectral data are summarized in Tables 4 and 5. In polymer



Scheme 5.

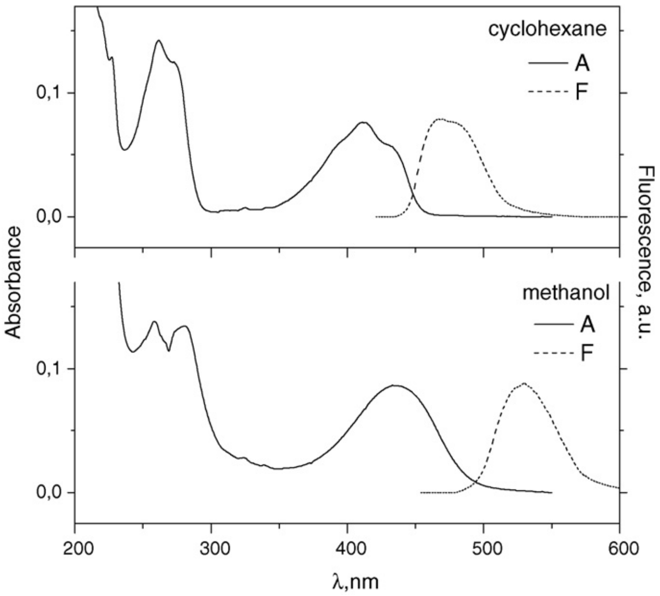


Fig. 1. Absorption (A) and fluorescence (F) spectra of BUNOR in cyclohexane and methanol at 10^{-5} mol dm^{-3}.

Table 1
Spectral characteristics of BUNH in different media.

Medium[a]	$\lambda_{max}{}^{b}$ (nm)	$\lambda_{max}{}^{c}$ (nm)	$\Delta\nu^{d}$ (cm^{-1})	Φ^{e}	$\Phi_{NH}/\Phi_{NO}{}^{g}$	τ^{h} (ns)
MeOH	437	520	3653	0.50 0.48[f]	15.6 12.9[f]	6.6
Cy	413	457, 480	2868	0.66 0.84[f]	4.6 5.0[f]	5.2
PS	414	487	3621		10.9	6.2
PMMA	428	499	3324		7.8	8.0
PVC	430	494	3013		8.7	7.7

[a] Medium: MeOH – methanol, Cy – cyclohexane, PS – polystyrene, PMMA – poly(methyl methacrylate), and PVC – poly(vinyl chloride).
[b] Wavelength of maximum absorption.
[c] Wavelength of maximum fluorescence excited at the maximum of absorption.
[d] Stoke's shift.
[e] Quantum yield calculated from Φ of anthracene; $\Phi_{Anthracene} = 0.2$.
[f] Deaerated solution.
[g] The extent of intramolecular quenching using relative quantum yield of Φ_{NO} of BUNO from Table 2.
[h] Lifetime; monoexponetial fitting.

matrices the position of absorption maxima is nearly in the same region as in solvents namely 415 nm in PMMA and 430 nm in PVC (Fig. 4). The shifts in absorption spectrum of probes of both series indicate that 4-amino-2,2,6,6-tetramethylpiperidine in position 4 of 1,8-naphthalimide ring has the same electron donating effect as dimethyl amino group.

Table 2
Spectral characteristics of BUNO in different media.

Medium[a]	$\lambda_{max}{}^{b}$ (nm)	$\lambda_{max}{}^{c}$ (nm)	$\Delta\nu^{d}$ (cm^{-1})	Φ^{e}	τ^{h} (ns)
MeOH	445	525	3424	0.032 0.038[f]	0.011 (0.97) 6.23 (0.03)[i]
Cy	406	455, 477	3171	0.140 0.168[f]	0.005 (0.96)[f] 4.9 (0.04)[i]
PS	415	482	3349		5.0
PMMA	425	494	3286		6.5
PVC	429	494	3067		6.6

[a–f, h] As for Table 1.
[i] Lifetime of fluorescence; biexponential fitting.

Table 3
Spectral characteristics of BUNOR in different media.

Medium[a]	$\lambda_{max}{}^{b}$ (nm)	$\lambda_{max}{}^{c}$ (nm)	$\Delta\nu^{d}$ (cm^{-1})	Φ^{e}	$\Phi_{NH}/\Phi_{NO}{}^{g}$	τ^{h} (ns)
MeOH	448	525	3274	0.48 0.50[f]	15.0 13.1[f]	6.6
Cy	409	458, 481	3151	0.54 0.60[f]	3.9 3.6[f]	5.1
PS	419	485	3248		11.9	7.3
PMMA	428	497	3244		10.7	8.5
PVC	433	501	3135		8.8	8.1

[a–h] As for Table 1.

The probes of both types exhibit intense fluorescence when they are in the form of diamagnetic parent amines or alkoxyamines. Distinctly less intense fluorescence was observed for paramagnetic N-oxyl derivatives but it is still observable. For BUNR series the maximum of fluorescence lies in the range from 465 nm in cyclohexane to 535 nm in methanol (Tables 1–3). The maxima of fluorescence of probes doped in polymer matrices lie in a little narrow range as for solvents. The Stoke's shift for this series is in the range 2800 up to 3600 cm^{-1} being the largest for methanol. The absolute quantum yield of diamagnetic parent amine BUNH and alkoxyamine BUNOR in solution is about 0.5–0.8 while for paramagnetic nitroxide BUNO is 0.03–0.15. The decay of fluorescence of parent amine BUNH and BUNOR is clearly monoexponential and the lifetime is in the range 5–8.5 ns. The decay of fluorescence of BUNO is more complex and the fitting to biexponential brings about the first lifetime in the subnano-second region. The extent of intramolecular quenching due to presence of radical centre of N-oxyl type expressed as the ratio of quantum yields of parent amine or alkoxyamine to quantum yield of fluorescence of probe with radical centre NO$^{\bullet}$ is in the range 5–15 in solution and 8–11 in the polymer matrix.

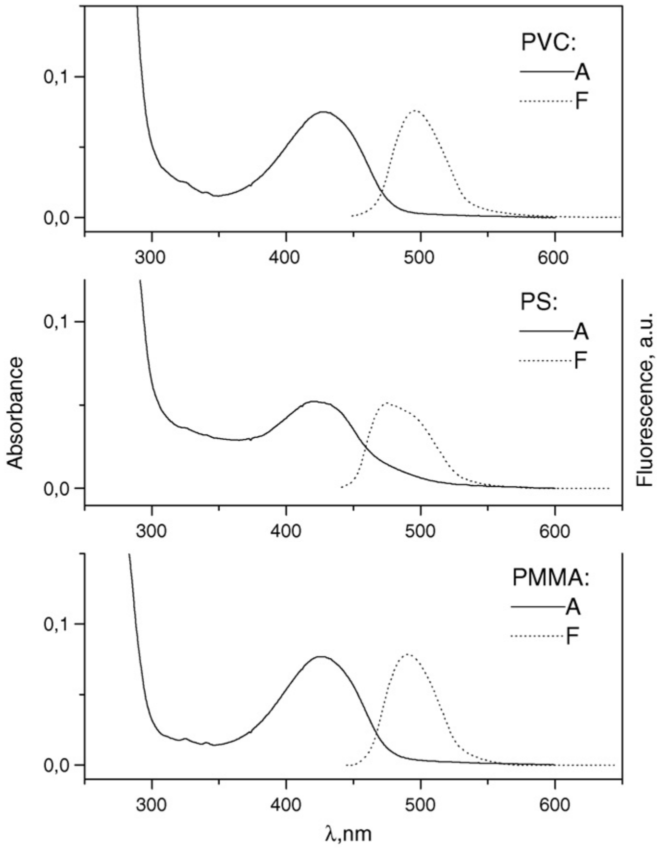


Fig. 2. Absorption (A) and fluorescence (F) spectra of BUNH in polymer matrices at concentration 0.002 mol kg^{-1}.

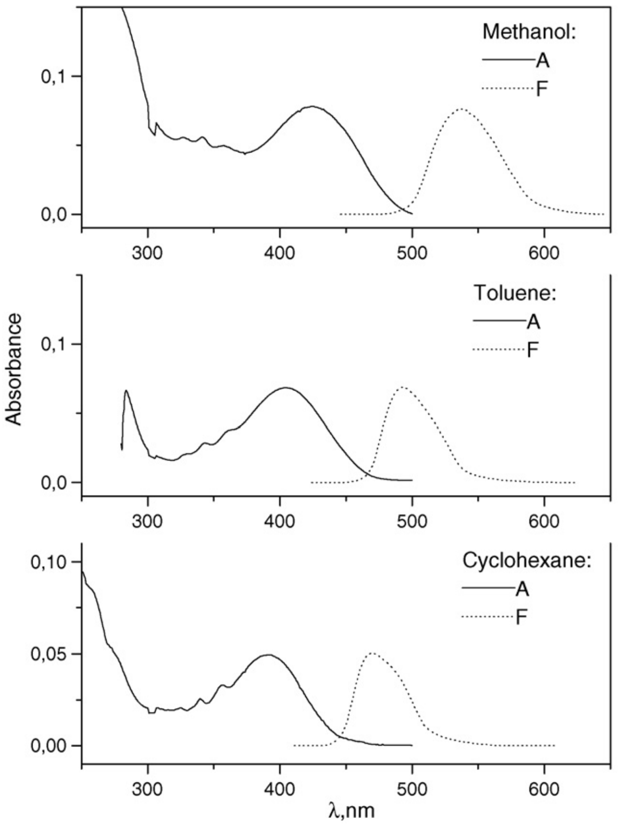


Fig. 3. Absorption (A) and fluorescence (F) spectra of DMANH in different solvents at 10^{-5} mol dm^{-3}.

As seen from Fig. 3 for DMANR series the maximum of fluorescence lies in the range from 460 nm in cyclohexane to 535 nm in methanol (Tables 4 and 5). Again the maxima of fluorescence of probes doped in polymer matrices lie in a little narrow range as for solvents 497 nm for PS and 513 nm for PVC. The Stoke's shift for this DMANR series is even in the larger range 3500–5000 cm^{-1} being the largest for methanol. The absolute quantum yield of parent amine DMANH is 0.008 in methanol and considerably higher in cyclohexane where reaches the value about 0.5.

The decay of fluorescence of parent amine DMANH is clearly monoexponential and the lifetime is in the range 6–8 ns in the same range as the lifetime of BUNH or BUNOR. The decay of fluorescence of DMANO is distorted and the fitting to biexponential brings about the first lifetime in the subnano-second region while the second lifetime is in the range of parent probe. The number of points taken for fluorescence decay data is limited in the used set up (256 points), therefore the margin of error is large and the lifetimes of BUNO and DMANO have a considerable error. The extent of intramolecular quenching due to presence of N-oxyl radical centre in DMANO expressed as the ratio of quantum yields of fluorescence of parent amine to that of probe with radical centre is above 2 in methanol and increased to 8 in nonpolar cyclohexane. Similarly, with increasing polarity of polymer matrix the value of intramolecular quenching increases from 1.2 for PVC to 3.2 for nonpolar PS.

Comparison of the intramolecular quenching due to presence of N-oxyl in solution for the pairs BUNH(OR)/BUNO and DMANH/DMANO clearly shows that the extent of quenching is strongly influenced by the medium, by the type of chemical bond

Table 4
Spectral characteristics of DMANH in different media.

Medium[a]	λ_{max}[b] (nm)	λ_{max}[c] (nm)	$\Delta\nu$[d] (cm^{-1})	Φ[e]	Φ_{NH}/Φ_{NO}[g]	τ[h] (ns)
MeOH	422	530	4829	0.008 0.024[f]	2.2 4.0[f]	
Cy	394	477	4416	0.50 0.86[f]	8.3 10.2[f]	
Toluene	404	494	4510	0.86	4.3	6.0 (±2.8%)
Chloroform	419	508	4181	1.0	2.0	7.2 (±3.2%)
PS	418	497	3803		3.2	6.7 (±3.2%)
PMMA	415	503	4216		2.1	8.0 (±3.0%)
PVC	428	513	3871		1.2	7.5 (±4.2%)

[a–f,h] As for Table 1.
[g] The extent of intramolecular quenching using relative quantum yield of Φ_{NO} of DMANO from Table 5.

Table 5
Spectral characteristics of DMANO in different media.

Medium[a]	λ_{max}[b] (nm)	λ_{max}[c] (nm)	$\Delta\nu$[d] (cm^{-1})	Φ[e]	τ[h] (ns)
Me	417	530	5113	0.004 0.006[f]	
Cy	398	461, 481	3894	0.06 0.084[f]	
Toluene	408	494	4267	0.2	0.007 (0.96) 4.9 (0.04)[i]
Chloroform	419	508	4181	0.6	0.4 (0.89) 6.1 (0.11)[i]
PS	415	490	3688		0.017 (0.92) 5.2 (0.08)[i]
PMMA	415	503	4216		5.3 (±2.8%)
PVC	425	512	3998		6.8 (±2.0%)

[a–f,h] As for Table 1.
[i] Lifetime; fluorescence decay was fitted to biexponential.

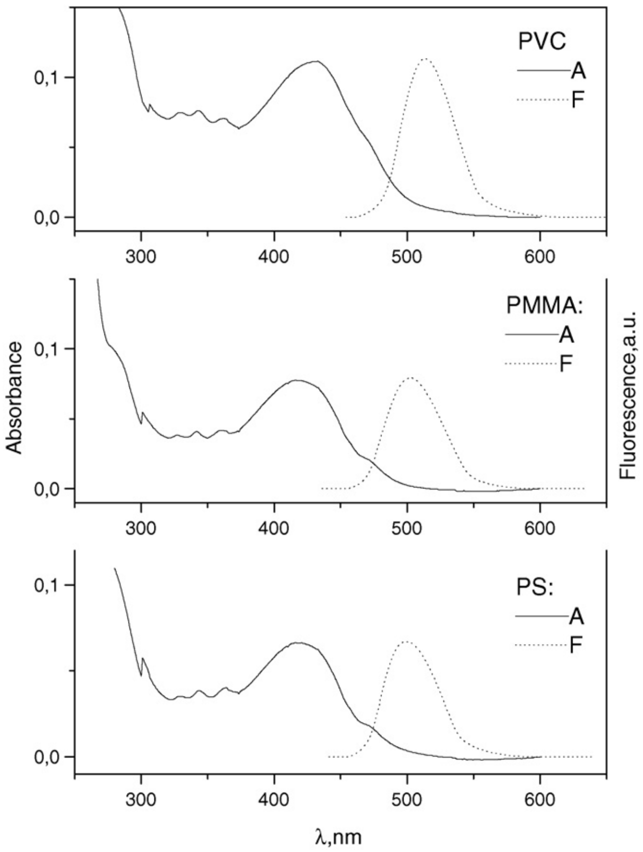


Fig. 4. Absorption (A) and fluorescence (F) spectra of DMANH in polymer matrices at 0.002 mol kg^{-1}.

Table 6
Comparison of the extent of intramolecular quenching for the probes BUNH, BUNO, DMANH, DMANO, DINH and DINO.

Medium[a]	Φ_{BUNH}/Φ_{BUNO}	$\Phi_{DMANH}/\Phi_{DMANO}$	$\Phi_{BUNH}/\Phi_{BUNO}+\Phi_{DMANH}/\Phi_{DMANO}$[b]	Φ_{DINH}/Φ_{DINO}
MeOH	15.6	2.2	17.8	19.3
	12.9[c]	4.0[c]	16.9[c]	19.2[c]
Cy	4.6	8.3	12.9	12.9
	5.0[c]	10.2[c]	15.2[c]	13.6[c]

[a] Medium: MeOH – methanol and Cy – cyclohexane.

[b] Sum of the extent of intramolecular quenching.

[c] Deaerated solution.

between chromophore and nitroxide and possibly by the distance of nitroxide and chromophore. In the case the series of BUNR where nitroxide is directly attached to chromophore the extent of intramolecular quenching in methanol (about 15) is higher as in cyclohexane (about 5). For the DMANR series where the nitroxide is attached to chromophore through diimide bond the opposite effect is observed. The intramolecular quenching lies in the range from 2 in methanol to 8 in cyclohexane. The sum of the extent of intramolecular quenching for particular mono-nitroxides BUNO and DMANO in particular solvents gives the values of extent of intramolecular quenching for the series of di-nitroxide probe containing two quenchers – DINO (Table 6 and Scheme 3). High population of excited state and relatively low efficiency of quenching by 2,2,6,6-tetramethylpiperidyl-N-oxide are the most probable reason that the quenching processes occur simultaneously. Similar results were obtained in the paper by Blough and co-workers [25]. They used naphthyl derivatives according to Scheme 6. Based on the quantum yields calculated ratio Φ_{1b}/Φ_{1a} for monosubstituted derivatives 1a and 1b was 44 in hexane. For bi-substituted derivatives 2a and 2b the ratio of quantum yields Φ_{2b}/Φ_{2a} was 92 in the same solvent. In acetonitrile the same effect was obtained. The ration of $\Phi_{1b}/\Phi_{1a}=40$ for mono-nitroxide and $\Phi_{2b}/\Phi_{2a}=77$ for bi-radical. The probes based on 2,6-dicarboxy-naphthalic acid are symmetrical and therefore the ratio of bisubstituted derivatives Φ_{2b}/Φ_{2a} is two times higher as the ratio of monosubstituted derivatives Φ_{1b}/Φ_{1a}. It should be mentioned that this additive effect was not valid for the quantum yields obtained in methanol.

The fluorescence of the probes BUNH and DMANH as well as DMANO was quenched intermolecularly by TEMPOL which is structurally related to the reaction centre of probes. The quenching process obeys the Stern–Volmer kinetics with K_{SV} constant around 250 dm^3 mol^{-1} (Fig. 5). The calculated quenching rate constant taking into account the lifetime of fluorescence is about 5×10^{10} dm^3 mol^{-1} s^{-1}. The intermolecular quenching of chromophores of the given probes by N-oxyl seems to be more efficient as in the case when the same structural units are covalently bonded

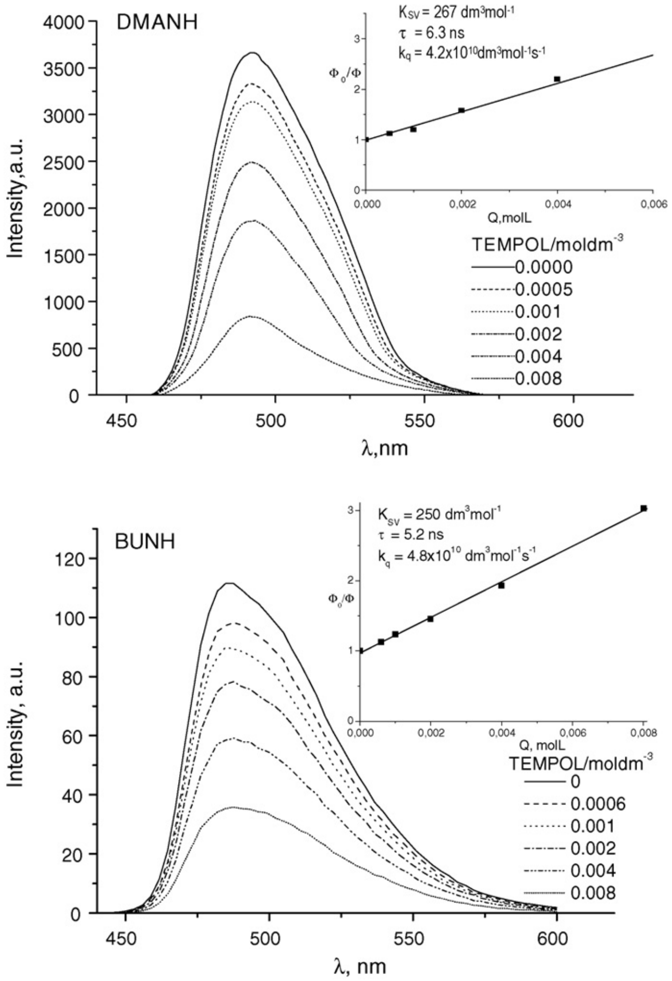
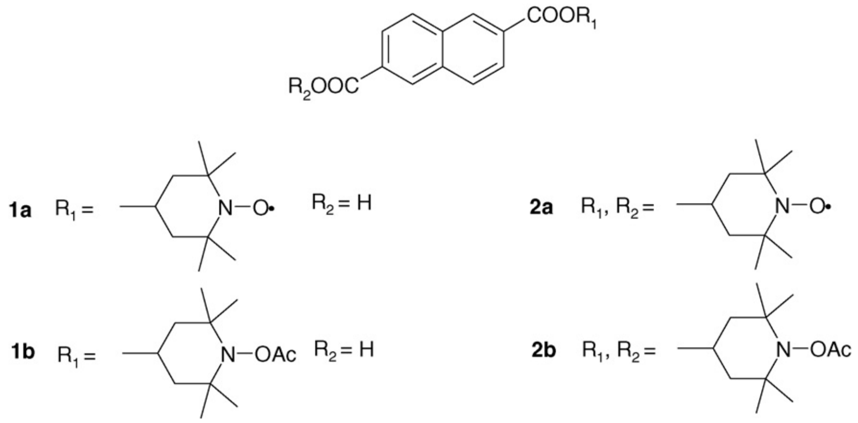


Fig. 5. Spectra of DMANH and BUNH ($c=10^{-5}$ mol dm^{-3}) in toluene quenched by TEMPOL and Stern–Volmer plot of quenching.



Scheme 6.

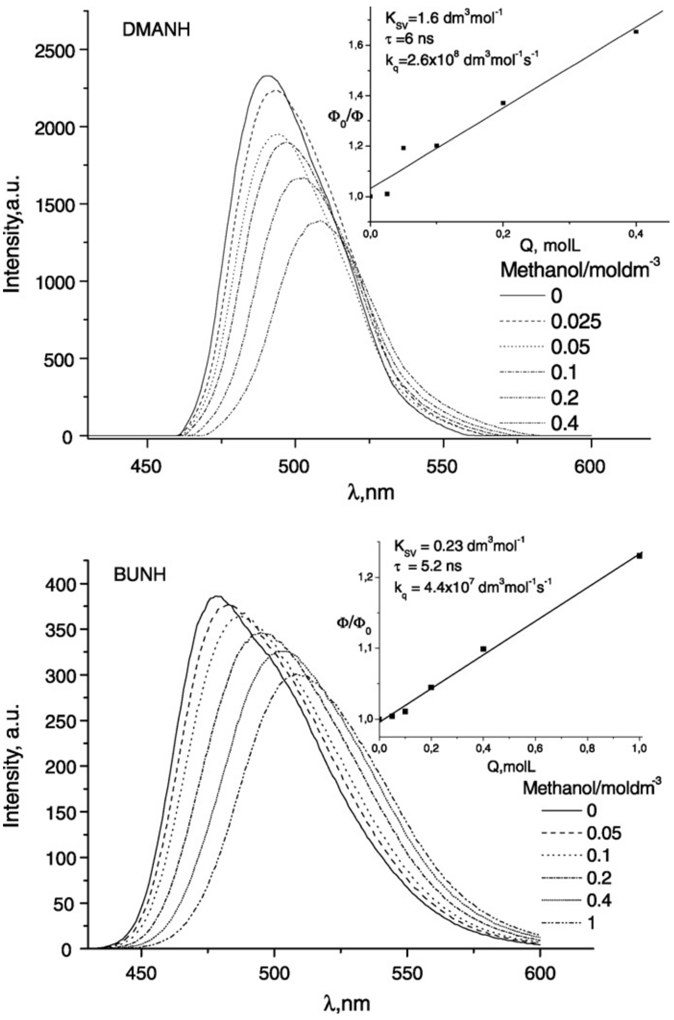


Fig. 6. Spectra of DMANH and BUNH (c = 10^{-5} mol dm^{-3}) in toluene quenched by methanol and Stern–Volmer plot of quenching.

to the given chromophore. Probably the orientation of N-oxyl plays an important role in the process of quenching. At intermolecular quenching the closer approach of N-oxyl with the excited fluorophore is possible. Consequently more efficient quenching occurs as compared with the case when fluorophore and N-oxyl are fixed. The absorption spectra of probes such as BUNR and DMANR (as R = H or O) are very similar. But concerning the fluorescence, the parent amine of the probe DMANH is more solvent sensitive as compared with BUNH. Polar hydrogen donating solvent as methanol moderately quenches fluorescence of DMANH with K_{SV} about 2 dm^3 mol^{-1} and at the same time red shift of the fluorescence maximum is observed (Fig. 6). The quenching of BUNH with methanol was above one order of magnitude slower with more significant red shift (about 1300 cm^{-1}, c_{MeOH} = 1 mol dm^{-3}). According to classification developed by Inoue and co-workers [26], the excited state of 4-aminophthalimide exhibits intramolecular charge transfer (ICT) with hard anionic character and with the highest electron density on carbonyl group. The radiationless deactivation of this state proceeds through intermolecular hydrogen bonding in plane and out of plane. The excited state of substituted 1,8-naphthalimide probably exhibits the same characteristics, since the course of quenching by polar methanol is similar as for 4-aminophthalimide.

4. Conclusions

Two types of novel probes were prepared where 1,8-naphthalimide chromophore is combined with one 2,2,6,6-tetramethylpiperidine unit in different positions. 2,2,6,6-Tetramethylpiperidine unit was in the form of the diamagnetic parent amine and alkoxyamine or paramagnetic stable nitroxyl radical form. The parent amine of both series BUNR, DMANR as well as alkoxyamine exhibit quite intense fluorescence in the range 460–530 nm depending on the medium. The fluorescence is partially quenched if the probe is in the form of free radical BUNO and DMANO.

Comparison of the intramolecular quenching in solution of the pairs BUNH(OR)/BUNO and DMANH/DMANO shows that extent of quenching is strongly influenced on the medium. While the extent of intramolecular quenching for the series of BUNR in methanol (about 15) is higher than in cyclohexane (about 5), for the series DMANR it is opposite – in the range 2 in methanol to 8 in cyclohexane. Moreover, the sum of the extent of intramolecular quenching for the series of BUNR and DMANR containing one 2,2,6,6-tetramethylpiperidine unit in particular solvents gives approximately the value of extent of intramolecular quenching for the series of DINR containing two 2,2,6,6-tetramethylpiperidine units.

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