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Spectral Properties of Probes Containing Benzothioxanthene Chromophore Linked with Hindered Amine in Solution and in Polymer Matrices

Pavol Hrdlovič · Štefan Chmela · Martin Danko ·
Mohamed Sarakha · Ghislain Guyot

Abstract Absorption and emission spectroscopy as well as laser flash photolysis was employed in order to characterize the spectral properties of novel probes based on benzothioxantheneimide chromophore covalently linked with different types of sterically hindered amines. These were chosen as 2-(2,2,6,6-tetramethyl-4-piperidyl)-thioxantheno[2,1,9-dej]isoquinoline-1,3-dione (BTXINH), the equivalent stable nitroxyl radical, i.e. 2-(1-oxo-2,2,6,6-tetramethyl-4-piperidyl)thioxantheno[2,1,9dej]isoquinoline 1,3-dione (BTXINO) and the alkoxy derivative 2-(1-(1′-phenylethoxy)-2,2,6,6-tetramethyl-4-piperidyl)-thioxantheno[2,1,9-dej]isoquinoline-1,3-dione (BTXINOR). Spectral properties, in solutions and in various polymer matrices such as polystyrene, polymethyl methacrylate, polyvinyl chloride and polypropylene, were compared with the compound 2-(1-dodecyl)-thioxantheno[2,1,9-dej]-isoquinoline-1,3-dione (BTXID) taken in the present study as a reference compound. By means of the fluorescence decay and in the contrary to three other probes, BTXINO probe clearly showed a biexponential decay while the three other probes led to monoexponential decay. Two different singlet excited states with lifetimes of about 0.4 and 5 ns were proposed. They correspond to two dispositions of the nitroxyl radical chain above and along the fluorescent moiety of the molecule. Such behaviour depends on the surrounding media. Moreover, an efficient intramolecular quenching of the fluorescence emission was only observed with the short lived singlet excited state. The ratio BTXID/BTXINO was found equal to about 4 and 9 in solutions and polymer matrices respectively. Laser flash photolysis indicated that the novel probes as well as the model compound yielded transient absorption with maximum at 530 nm, corresponding to the triplet states. The intermolecular quenching of such species by molecular oxygen and by free N-oxyl, such as 1-oxy-2,2,6,6-teramethylpiperidine (TEMPO) and 1-oxy-2,2,6,6-teramethyl-4-hydroxypiperidine (TEMPOL), and the intramolecular quenching was not efficient.

Introduction

Various fluorescence probes of different structure are used to monitor processes in environments as well as in solutions, micelles, solid amorphous matrices etc. [1–5]. The spectral parameters, which exhibit strong dependence on medium, are well exploited in these probes. The advantages of parameters connected with fluorescence for example, are the high sensitivity, simple detection, easy quantitative evaluation for selected chromophores and distinct medium effect. Simple chromophores as aromatic hydrocarbons or dyes are not always suitable for monitoring selected parameters. Generally, more complex molecules containing different structural units—modules—are needed.

In the 1990s specific groups of probes were developed, which are based on intramolecular quenching. In these probes the chromophores are linked with the structural unit easily oxidized on aminooxide of N-oxyl type, which exhibits paramagnetic properties [6]. Fluorescence switch

P. Hrdlovič (✉) · Š. Chmela · M. Danko
Polymer Institute, Slovak Academy of Sciences,
Dúbravská cesta 9,
842 36 Bratislava, Slovakia
e-mail: upolhrdl@savba.sk

M. Sarakha · G. Guyot
Laboratoire de Photochimie Moléculaire et Macromoléculaire,
UMR CNRS 6505, Université Blaise Pascal,
F-63177 Aubière-Cedex, France

off or on occurs as a result of chemical reaction on such reactive site. Therefore, the easy detection and monitoring of free radicals by nitroxide scavenging is possible [7].

We have tested this concept for construction of probes for chromophores as 1-naphthoic, 1-naphthylacetic [8], 4-(1-pyrene)alcanoic acids [9, 10], 1,8-naphthalene-imide [11], and anthracene [12]. These chromophores were linked with sterically hindered amine (HAS—Hindered Amine Stabilizers) of piperidine (2,2,6,6-tetramethyl-4-hydroxypiperidine, 2,2,6,6-tetramethyl-4-aminopiperidine) and piperazine type (7,15-diazabicyclo[5,1,5,3]hexadecane) [13]. HAS was in the form of parent amine or in the form of stable nitroxyl radical. The radical centre of N-oxyl type was prepared by oxidation of parent amine or by using N-oxyl as starting material. Spectral properties and other properties were evaluated in solutions and in polymer matrices.

Generally, the efficiency of intramolecular quenching in these probes depends on the size of linker as well as on the medium. All chromophores, tested in the probes, do not exhibit high stability under conditions of photo-oxidation, although the sterically hindered amines are efficient long-term light and heat stabilizers for polyolefins. The linkage of chromophore with sterically hindered amine does not seem to protect the respective chromophore from the photo-oxidative damage [14–16]. The most efficient intramolecular quenching of the singlet [11] and of the triplet state [17] was observed for adducts such as 1,8-naphthalenedicarboxylic anhydride and HAS.

Other authors tried this concept of linking suitable chromophore with N-oxyl and employed it for various applications. The Hungarian group prepared and optimized synthesis of double (fluorescent and spin) sensor molecules based on dansyl and aminophthalimide chromophores. They monitored reactive oxygen species in thylakoid membranes [18, 19]. Dual fluorophore-nitroxides probes were used for determination of vitamin C in biological liquids [20]. Nitroxide linked naphthalene was used as a fluorescent probe for hydroxyl radicals [21]. Novel naphthalene-hindered amine linked with long alkyl chain was tested as thermo- and photo-stabilizers [22]. In order to get some insight into radical processes occurring during induction period in thermal degradation of polyolefines novel pro-fluorescent probe based on phenanthrene was used [23].

New approach for detection of carbon-centered radicals in enzymatic processes was developed using pre-fluorescent probes based on quinoline as chromophore linked with TEMPO [24]. Fluorescent imaging using a pre-fluorescent radical probe was employed for mapping photo-generated radicals in thin polymer films [25]. Fluorescent sensor applications as detectors for DNA damage, free radical formation and microlithography has been recently reviewed [26].

Adducts of 1,8-naphthylimide and sterically hindered amine were used as 'one step' brighteners and stabilizers [27].

In order to extend the range of application of the probes, a chromophore with specific properties is searched for. This chromophore should exhibit a high fluorescence emission and high stability against different agents and in the same time it should be linked with HAS by performing simple reactions.

Recently adducts of benzothioxanthone 3,4-dicarboxylic anhydride with suitable sterically hindered amines has been prepared [28]. Aminoalkoxy methacrylates, containing this chromophore have been used for preparation of fluorescent polymer particles by emulsion polymerization [29]. Polycarbonate particles dyed with benzothioxanthene core have recently been prepared by miniemulsion polymerization [30].

Benzothioxanthene-3,4-dicarboxdiimine modified with methylene amine has been used as fluorescent labels for DNA hybridization [31].

This type of chromophore, which contains 1,8-naphthaleneimide unit, seems to be suitable for construction of novel probes operating with intramolecular quenching. Although this chromophore composed of thioxanthene and isoquinoline units exhibits rather complex behaviour after photo-initiation, it is worth to apply it in a molecular structure suitable as sensors. The aim of this paper is to investigate this type of molecules as probes in different environments stressing the importance of polymer matrices. In any meaningful application the embedding in solid (polymer) matrix will be the most important. The main attention is devoted in this paper to the spectral properties of this type of probes in solution as well as in polymer matrices and also on the evaluation of the extent of intramolecular quenching of singlet as well as triplet excited states.

Experimental

The structures of fluorescence probes used in this paper are given in Scheme 1. Benzothioxanthendicarboxylic anhydride, BTXA, used as a starting material for synthesis was a commercial product (HY-anh., Clariant Huningue S.A., France). It was used as received. The details of synthesis of BTXINH and BTXINO are described in [32]. The details of synthesis of BTXINOR and BTXID will be given elsewhere as well. Some characteristics are given here.

2-(2,2,6,6-Tetramethyl-4-piperidyl)thioxantheno[2,1,9-dej]isoquinoline-1,3-dione (BTXINH) was prepared by reaction of suspension of BTXA in dry DMF with solution of 4-amino-2,2,6,6-tetramethylpiperidine in DMF. The purity of the orange powdered product (m.p. 300–302 °C) was confirmed by NMR and FTIR spectroscopy.

Scheme 1 Fluorescent probes

With R :

BTXINH — 2-(2,2,6,6-tetramethyl-4-piperidyl-thioxantheno[2,1,9,dej]isoquinoline-1,3-dione

BTXINO — 2-(1-oxo-2,2,6,6-tetramethyl-4-piperidyl-thioxantheno[2,1,9,dej]isoquinoline-1,3-dione

BTXINOR — 2-(1-(1'-phenylethyl)oxy-2,2,6,6-tetramethyl-4-piperidyl-thioxantheno[2,1,9,dej]isoquinoline-1,3-dione

—CH₂[CH₂]₁₀CH₃ — BTXIID — 2-(1-dodecyl)-thioxantheno[2,1,9,dej]isoquinoline-1,3-dione
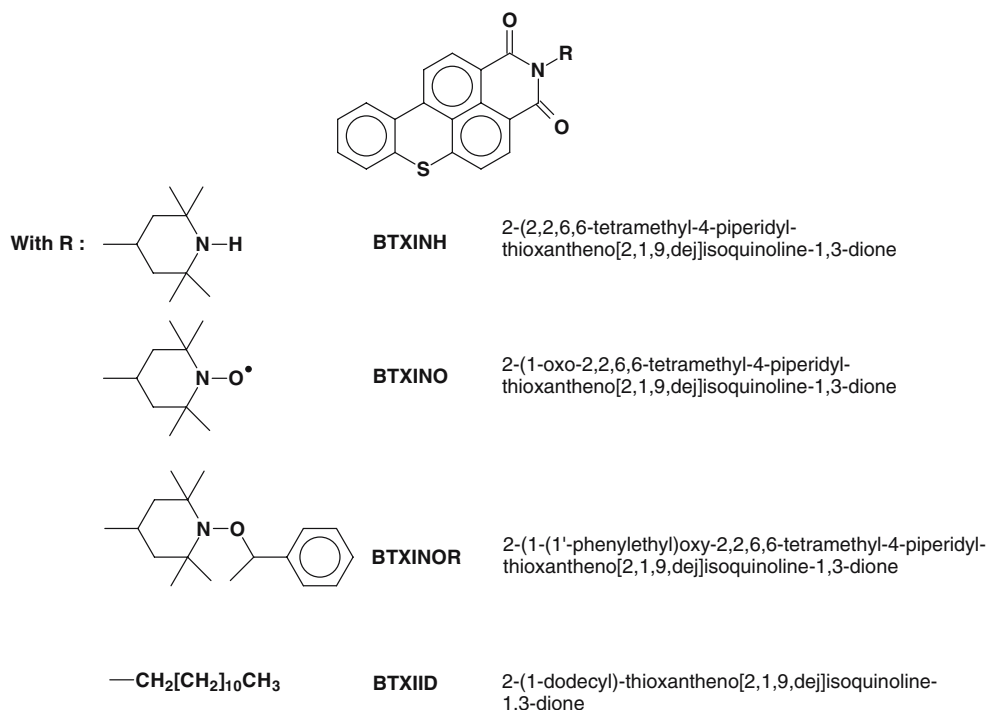
^{1}H NMR (CDCl$_3$): δ (ppm) 1.21 (s, 6H, 2×CH$_3$ 2+6 axial.), 1.37 (s, 6H, 2×CH$_3$ 2+6 equat.), 1.64–1.69 (dd, 2H, CH$_2$ 5+3 axial.), 2.44–2.53 (t, 2H, CH$_2$ 5+3 equat.), 5.64–5.78 (tt, 1H, >CH–N), 7.26–7.41 (m, 3H, arom.), 7.43–7.52 (d, 1H, arom.), 8.19–8.22 (m, 2H, arom.), 8.38–8.41 (d, 1H, arom.), 8.58–8.61 (d, 1H, arom.).

^{13}C NMR (CDCl$_3$): δ (ppm) 26.9 (2C, axCH$_3$), 33.9 (2C, eqCH$_3$), 40.2 (2C, 2x *CH$_2$*–CH–N), 46.5 (1C, CH–N), 60.7 a 60.8 (2C, 2x C–N), 118.1 (1C, =C=arom.), 119.4 (1C, =CH–arom.), 120.6 (1C, =CH–arom.), 121.3 (1C, =C=arom.), 125.4 (1C, =C=arom.), 126.3 (1C, =CH–arom.), 126.6 (1C, =CH–arom.), 127.8 (1C, =CH–arom.), 127.9 (1C, =C=arom.), 130.2 (1C, =CH–arom.), 130.4 (1C, =C=arom.), 130.9 (1C, =CH–arom.), 131.7 (1C, =C=arom.), 132.7 (1C, =CH–arom.), 136.9 (1C, =C=arom.), 141.0 (1C, =C=arom.), 164.4 (1C, C=O), 164.8 (1C, C=O).

FTIR (CHCl$_3$): ν(C=O asym.) 1,646 cm^{-1}, ν(C=O sym.) 1,690 cm^{-1}.

2-(1-Oxo-2,2,6,6-tetramethyl-4-piperidyl)thioxantheno [2,1,9-dej]isoquinoline-1,3-dione (BTXINO) was prepared by the same procedure as BTXINH using 4-amino-1-oxo-2,2,6,6-tetramethylpiperidine. The purity of red-orange crystals (about 95%) with m.p. 269–279 °C of BTXINO was determined by EPR spectrum, which revealed the typical triplet with lines of equal intensity in toluene. The hyperfine splitting is the result of interaction of the unpaired electron with the nucleus ^{14}N. The integral of EPR spectra of BTXNO was compared with the integral of the standard (4-hydroxy-1-oxo-2,2,6,6-tetramethylpiperidine, TEMPOL). The values of the integrals are proportional to the number of radicals.

The concentration of radicals in standard is assumed to be 100%. The value of relative concentration 95% proves the acceptable purity of BTXINO.

Elemental analysis for C$_{27}$H$_{25}$N$_2$O$_3$S (M_w= 457.57). Calcd. C: 70.87, H: 5.51, N: 6.12%. Found: C: 70.58, H: 5.59, N: 5.92%.

FTIR (CHCl$_3$): ν(C=O asym.) 1,646 cm^{-1}, ν(C=O sym.) 1,690 cm^{-1}.

2-(4-(1-(1′-Phenylethyl)oxy-2,2,6,6-tetramethyl)piperidine)-thioxantheno[2,1,9-dej]isoquinoline-1,3-dione (BTXINOR). Synthesis of BTXI-NOR was performed by addition of nitroxide radical BTXI-NO to styrene using Mn(OAc)$_3$ × 2H$_2$O followed by reduction of intermediate by NaBH$_4$. Crystallization of product from ethyl acetate gave bright orange crystals with m.p. 212–215 °C.

^{1}H NMR (CDCl$_3$): δ (ppm) 0.75 (s, 3H, CH$_3$ axial.), 1.25 (s, 3H, CH$_3$ axial.), 1.38–1.39 (m, 6H, 2×CH$_3$ 2+6 equat.), 1.45–1.67 (dd, 2H, CH$_2$ 5+3 axial.), 1.53 (d, *J*= 6.6 Hz, 3H, *CH$_3$*–CH–Ph), 2.82 and 2.94 (t, 2H, CH$_2$ 5+3 equat.), 4.86 (q, *J*=6.7 Hz 1H, CH$_3$–*CH*–Ph), 5.54 (m, 1H, CH–N), 7.22–7.37 [m, 9H (5H Ph+4H arom.)], 8.0–8.10 (m, 2H, arom.), 8.27 (d, 1H, arom.), 8.45 (d, 1H, arom.).

^{13}C NMR (CDCl$_3$): δ (ppm) 20.8 (1C, *CH$_3$*–CH–Ph), 23.3 (2C, axCH$_3$), 34.1 a 34.4 (2C,eqCH$_3$), 41.5 (2C, 2x *CH$_2$*–CH–N), 45.7 (1C, CH–N), 60.7 a 60.8 (2C, 2x C–N), 83.2 (1C, CH–Ph), 118.4–140.1 (21C, Ph + arom.), 145.6 [1C, C(Ph)], 164.0 (1C, C=O), 164.4 (1C, C=O).

FTIR (CHCl$_3$): ν(C=O asym.) 1,646 cm^{-1}, ν(C=O sym.) 1,688 cm^{-1}.

2-(1-Dodecyl)-thioxantheno[2,1,9-dej]isoquinoline-1,3-dione (BTXID). It was prepared by the same procedure as BTXINH using dodecylamine and BTXA as reagents. Repeated crystallization from ethyl acetate gave red-orange crystals with m.p. 120–123 °C.

^{1}H NMR (CHCl$_3$) δ: (ppm) 0.87 (t, 3H, CH$_3$), 1.18–1.43 (m, 18H, CH$_2$–CH$_2$–CH$_2$), 1.72 (qui, 2H, N–CH$_2$–CH$_2$), 4.13 (t, 2H, CH$_2$–N), 7.32–7.39 (m, 4H, arom.), 8.02–8.05 (d, 1H, arom.), 8.06–8.10 (m, 1H, arom.), 8.30–8.33 (d, 1H, arom.), 8.47–8.50 (d, 1H, arom.).

^{13}C NMR (CHCl$_3$) δ: (ppm) 14.1 (1C, CH$_3$), 22.7 (1C, CH$_2$–CH$_3$), 27.2 (1C, CH$_2$), 28.0 (1C, CH$_2$), 29.37 (1C, CH$_2$), 29.42 (1C, CH$_2$), 29.6 (1C, CH$_2$), 29.65 (1C, CH$_2$), 29.66 (1C, CH$_2$), 29.68 (1C, CH$_2$), 31.9 (1C, –CH$_2$–CH$_2$–N), 40.5 (1C, CH$_2$–N), 118.0 (1C, =C=arom.), 119.0 (1C, =CH–arom.), 120.2 (1C, =CH–arom.), 121.2 (1C, =C=arom.), 125.3 (1C, =C=arom.), 125.9 (1C, =CH–arom.), 126.3 (1C, =CH–arom.), 127.5 (1C, =CH–arom.), 127.8 (1C, =C=arom.), 129.9 (1C, =CH–arom.), 130.1 (1C, =C=arom.), 130.6 (1C, =CH–arom.), 131.5 (1C, =C=arom.), 132.3 (1C, =CH–arom.), 136.4 (1C, =C=arom.), 140.2 (1C, =C=arom.), 163.3 (1C, C=O), 163.7 (1C, C=O).

FTIR (CHCl$_3$): ν(C=O asym.) 1,646 cm^{-1}, ν(C=O sym.) 1,688 cm^{-1}.

Anthracene was zonally refined (Lachema n.e., Brno, CR). Solvents cyclohexane (Merck, Darmstad, F.R.G.) ethanol and methanol were UV spectroscopy quality. Chloroform, tetrahydrofuran (Lachema n.e., Brno, CR) were analytical reagents. The quenchers: 1-oxo-2,2,6,6-tetramethylpiperidine (TEMPO) was received from Aldrich and 1-oxo-2,2,6,6-tetramethyl-4-hydroxypiperidine (TEMPOL) was the same as previously [9].

Polymer films doped with fluorescence probes 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 (28×35 mm). The films of polypropylene (PP; Tatren HPF, non stabilized) were prepared by penetrating of PP powder with chloroform solution of the probe for 24 h. After slow evaporation of chloroform films were prepared by hot pressing at 190 °C. Films of poly(vinylchloride) (PVC; Neralit, Spolana Neratovice s.e., CR) were prepared by casting from tetrahydrofuran solution (5 g/100 ml). The final concentration of the probe in film was 0.002 mol kg^{-1}.

Absorption spectra were recorded on a spectrometer UV 1650PC (Shimadzu, Japan).

Perkin-Elmer MFP 4 (slits 10 nm for excitation and 2–10 nm for emission, R928 multiplier) was used for recording the fluorescence spectra. Emission of polymer films was measured in front face arrangement on the solid sample holder. The quantum yield of doped polymer films was determined using anthracene as standard, assuming small effect of the medium. The relative quantum yields in solution and in film were corrected on different absorption at the wavelength of excitation according to [33].

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 [34] using program of J. Snyder based on [35]. The standard deviation $G^{1/2} = \sum \left(\left(I_{exp} - I_{calc} \right)^2 / n \right)^{1/2}$, where I_{exp} and I_{calc} are intensity of emission experimental and calculated respectively, is used to judge if the decay is mono-exponential. It is assumed that the decay curve satisfies the monoexponential when $G^{1/2}$ is lower than 5%. The more complex decay curves of BTXINO in various matrices were fitted to bi-exponential using modified program of Fluorofit Mat Lab package based on [36].

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 266 nm (fourth 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 osciloscope (HP 54522A) and analyzed on 32 bit RISC work station [17, 37].

EPR spectra were measured with X band spectrometer E-4 Varian (USA) interfaced with PC using program Symphonia Bruker.

Results and discussion

Singlet deactivation route

The absorption spectra of probes in organic solutions, where the benzothioxantheneimide (BTXI-) chromophore is directly linked with sterically hindered amine (–NH, –NO, –NOR and D), are characterized in the visible region by an intense absorption band within 400–525 nm region (Figs. 1 and 2). The fluorescence spectra show an intense band within 450–700 nm. All the probes have got basically features similar to that of the reference compound, namely
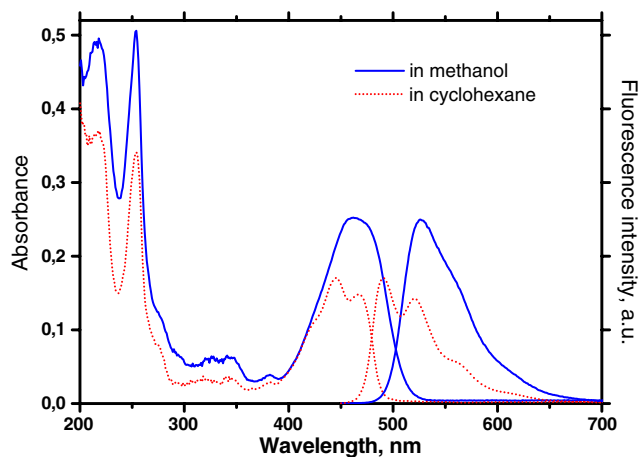
Fig. 1 Absorption and fluorescence spectra of BTXID in methanol and cyclohexane solution at 10^{-5} moldm^{-3}

BTXID, which contains a long alkyl group instead of sterically hindered amine units. Three solvents with different polarity were used to study the effect of the medium on the absorption as well as on the fluorescence spectra, methanol, chloroform and cyclohexane. As clearly shown in Figs. 1 and 2, in the less polar solvent, a vibrational structure of the absorption and the fluorescence bands were observed. All the spectroscopic data are gathered in Table 1. The Stoke's shift, which could yield the information concerning the structure and the geometry of the excited state varied with the polarity of the solvent. In all the cases, it was found to increase by increasing the polarity. This indicates some changes do occur in the excited state, namely extending the molecular structure. The probes BTXID, BTXINH and BTXINOR exhibited very intense yellow fluorescence. However, for BTXINO the fluorescence is much less intense and this difference can simply be seen by naked eye. The quantum yields
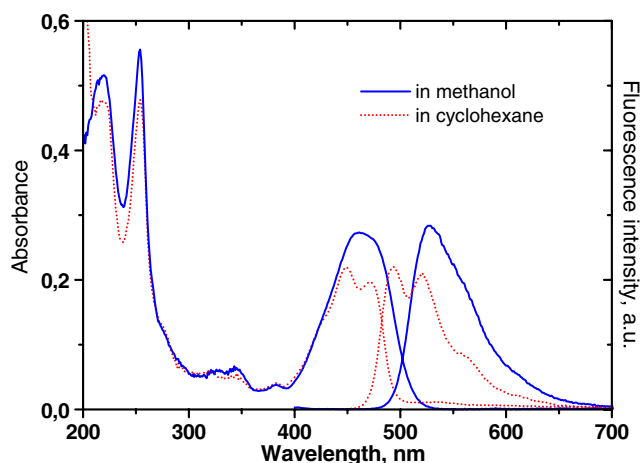

Fig. 2 Absorption and fluorescence spectra of the stable nitroxyl radical, BTXINO, in methanol and cyclohexane solution at 10^{-5} moldm^{-3}

Table 1 Spectral properties of BTXI-derivatives in different solvents

Probe	Spectral features	MeOH	Chloroform	Cyclohexane
BTXINH	$\lambda_{absorption}$, nm (logε)	460 (4.30)	461 (4.34), 478sh	446, 468
	$\lambda_{fluorescence}$, nm	528	517, 546sh	491, 521
	Stoke's shift, cm^{-1}	2,800	1,600	1,000
BTXINO	$\lambda_{absorption}$, nm (logε)	460 (4.26)	463 (4.45), 480sh	448, 472
	$\lambda_{fluorescence}$, nm	527	518, 544sh	498, 523
	Stoke's shift, cm^{-1}	2,800	1,500	1,100
BTXINOR	$\lambda_{absorption}$, nm (logε)	527	515, 544sh	493, 522
	$\lambda_{fluorescence}$, nm	2,800	1,500	1,100
	Stoke's shift, cm^{-1}	460 (4.26)	461(4.42), 479sh	446, 468
BTXID	$\lambda_{absorption}$, nm (logε)	460 (4.28)	460(4.41), 478sh	446, 467
	$\lambda_{fluorescence}$, nm	526	515, 544sh	491, 521
	Stoke's shift, cm^{-1}	2,700	1,500	1,000

determined in chloroform by using anthracene as a reference is given in Table 2. They show that except for the nitroxyl radical derivative, all the other probes have roughly similar fluorescence quantum yield. This leads to the conclusion that an efficient emission quenching by the radical moiety is occurring. Moreover, the measurements of the fluorescence decay were undertaken in chloroform solutions and showed a monoexponential decay with a lifetime of 6.9 ns except for BTXINO which clearly present a biexponential decay with τ_1=0.43 ns and τ_2=5.7 ns. Such result led to the conclusion that two different excited state species were present: a short and a long lived one. In chloroform solution the short lived one appeared to be dominant species—roughly 96%. The position of the nitroxyl radical chain (NO) in BTXINO above and along the fluorescent part of the molecule (BTXI) could be the reason for such behaviour. The former situation is more likely that of the short lived species.
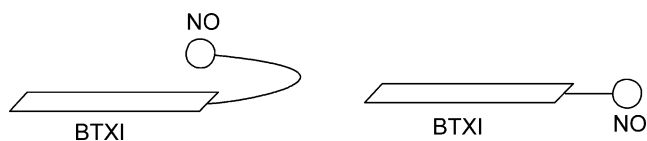
In polymer matrices, PMMA, PS, PVC and PP, the longest wavelength absorption band is broader and vibrational resolution is less distinct (Figs. 3 and 4; Tables 3 and 4). The absorption spectra of all probes and model

Table 2 Quantum yield relative to anthracene of the different probes and their fluorescence lifetime in chloroform solutions

Probe	BTXINH	BTXINO	BTXINOR	BTXID
Φ_i	4.2	1.0	3.3	3.8
Φ_{BTXID}/Φ_i	0.91	3.8	1.15	1.0
τ, ns	6.9	0.43 (96%)	6.7	6.9
		5.7 (4%)		

The standard error in lifetime is evaluated to 3%

compound at shorter wavelengths show weaker absorption in the region from 400 to 250 nm with maxima at 387, 347 and 326 nm. At the short wavelength edge there is strong absorption at 256 nm. This applies for all the polymer matrices except PS where the short wavelength edge cannot be observed.

The fluorescence spectrum of probes is influenced by polymer matrix (Tables 3 and 4). In non-polar PP as well as PS two maxima are observed. In more polar PMMA or PVC one broad fluorescence band is observed which is red-shifted as compared to chloroform. The lowest Stoke's shift about 1,000 cm^{-1} is observed for PP and the largest shift above 2,100 cm^{-1} is observed in PMMA. In any case the Stoke's shift is rather large (around 1,500 cm^{-1}) indicating some change in the excited state structure. It is of great importance to note that that the Stoke's shift varied with the nature of the polymer matrix. In almost all the cases, it was found to decrease in the order PMMA, PVC, PS and PP. The fact that PP gives the lowest Stoke's shift value which is roughly similar to that obtained in cyclohexane, is in good agreement with the fact that PP is the less polar polymer. The main spectral features of BTXI based probes
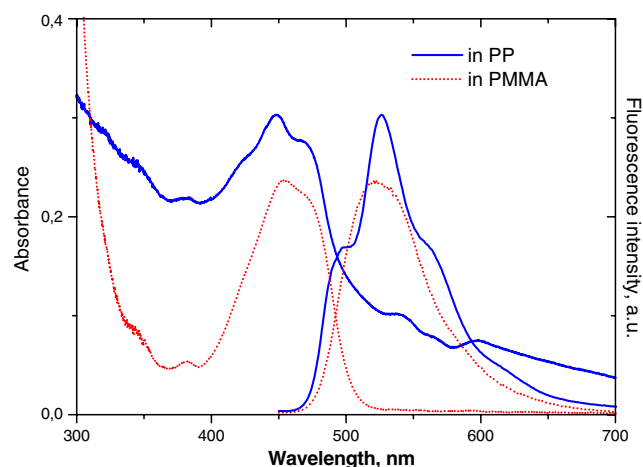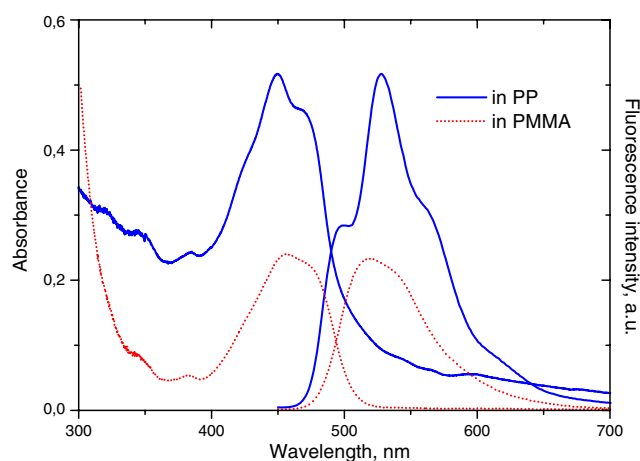
Fig. 4 Absorption and fluorescence spectra of the stable nitroxyl radical, BTXINO, in polypropylene (PP) and polymethylmethacrylate (PMMA) matrices 0.002 mol kg^{-1}

in PS and PVC lie between those in PP and PMMA and are summarized in Table 3.

The parent amine BTXINH, N-alkoxy derivative BTXINOR and model compound BTXID exhibit very intense fluorescence in solution as well as in polymer matrices. Much less intense fluorescence is observed for BTXINO as a result of intramolecular quenching due to the presence of radical centre of N-oxyl type. When the probes are doped in polymer films, the difference in fluorescence intensity for BTXINO and BTXINOR can be clearly seen by naked eye.

Similar absorption and intense fluorescence was observed also when the BTXI chromophore was bound to polymer particles during miniemulsion polymerization [29, 30].

The benzothioxanthene-3,4-carboxydiimide chromophore might be looked upon as a disubstituted less complex 1,8-naphthalimide (benzo[de]isoquinoline-1,3-dione). The absorption and fluorescence spectra show clearly that there is large bathochromic shift in absorption as well as fluorescence spectra by electron donating substituents. In fact the parent 1,8-naphtalic anhydride and 4-bromo-1,8-naphtalic anhydride in methanol exhibit the broad longest wavelength band with maximum at 300 nm and 310 nm respectively [38]. The absorption spectra of monosubstituted N′-(2′,2′,6′,6′-tetramethyl-4′-piperidyl)-1,8-naphthaleneimide and N′-(2′,2′,6′,6′-tetramethyl-4′-piperidyl)-4-bromo-1,8-naphthaleneimide exhibits the longest wavelength band as a broad bands with maximum shifted to around 335 nm and 345 nm respectively in polar methanol as a consequence of donor substituent 2,2,6,6-tetramethyl-piperidylimino. Substitution on naphthalene ring in position 4 by another electron donating substituent for example 2,2,6,6-tetramethylpiperidylimino [27, 39, 40] or other fragment results in shift of the maximum of the longest wavelength band batochromically to the range 400–450 depending on the polarity of solvent. In non polar cyclohexane there is seen some

Fig. 3 Absorption and fluorescence spectra of BTXID in polypropylene (PP) and polymethylmethacrylate (PMMA) matrices at 0.002 mol kg^{-1}

Table 3 Spectral properties of BTXI-derivatives in various polymer matrices

Probe	Spectral features	PMMA	PVC	PS	PP
BTXINH	$\lambda_{absorption}$, nm	459, 474sh	464, 478sh	457, 473	450, 470
	$\lambda_{fluorescence}$, nm	521	523, 540sh	509, 537	494, 527, 564sh
	Stoke's shift, cm^{-1}	1,900	1,800	1,500	1,000
BTXINO	$\lambda_{absorption}$, nm	459, 475sh	450, 475sh	457, 476	450, 470
	$\lambda_{fluorescence}$, nm	519	513, 531sh	506, 531	500sh, 528, 562sh
	Stoke's shift, cm^{-1}	1,800	1,600	1,300	1,300
BTXINOR	$\lambda_{absorption}$, nm	455, 474sh	461, 475sh	455, 473	450, 470
	$\lambda_{fluorescence}$, nm	526	516sh, 531	511, 529	500sh, 527, 562sh
	Stoke's shift, cm^{-1}	2,100	1,700	1,600	1,300
BTXID	$\lambda_{absorption}$, nm	455, 473sh	461,475sh	455,475	450, 470
	$\lambda_{fluorescence}$, nm	512, 528sh	521	507, 530	500sh, 526, 562sh
	Stoke's shift, cm−1	1,600	1,900	1,300	1,300

indication of the fine vibrational structure of these derivatives as shoulders around 405 and 430 nm and a maximum around 415 nm. In polar protic methanol the longest wavelength band is red shifted up to 440 nm and it is rather broad without vibrational structure. The fluorescence of these derivatives lies in the range 440 up to 550 nm depending on the polarity of environment. Consequently, suitable substitution in the naphthalene ring of 1,8-naphthalimide shifts the absorption and fluorescence in the range of BTXI chromophore.

Although the data of relative quantum yield of the probes to anthracene in polymer matrices are charged with high error, the estimated values of relative quantum yield (Table 4) are comparable to those obtained in solutions. It is worth noting that the lifetime of model compounds BTXID and probes with reactive amino centre BTXINH and BTXINOR is in the range 6–9 ns and does not depend much on the medium. The fluorescence decay is clearly monoexponential in all media and the standard error is low (1–3%). However for probe BTXINO, as it was observed in chloroform solution, the decay was better fitted with a biexponential form. Once again two different species were present. The short lived species, where the nitroxyl group is along the fluorescent part, was predominant in PVC and PS matrices and no quenching was observed. For PMMA

matrix, the situation is completely different since the long lived species, where the nitroxyl group is above the fluorescent part, was the dominant species (roughly 97%). Concerning the intramolecular quenching process, it is clearly shown that it is highly efficient when the short lived species is the major species due to the proximity through space, of the two groups of the molecule, namely the nitroxyl and the benzothioxantheneimide parts.

The fluorescence of the model compound and all probes with benzothioxantheneimide chromophore is quenched more or less efficiently by added free nitroxyl radical as intermolecular process or in the case where nitroxyl radical is the part of complex probe as intramolecular process. The free radicals 1-oxo-2,2,6,6-tetramethylpiperidine (TEMPO) and 1-oxo-2,2,6,6-tetramethyl-4-hydroxypiperidine (TEMPOL) quench the fluorescence of all derivatives under the study quite efficiently according to Stern-Volmer mechanism (Table 5). The TEMPOL is under the same conditions more efficient probably due to hydroxyl group, which might interact slightly with some parts of quenchee. The calculated bimolecular quenching rate constant lies within the range 2×10^{10} to 5×10^{10} dm^3 mol^{-1} s^{-1}, which is in the range of diffusion controlled bimolecular rate constant for low viscosity solvent as chloroform. The values of k_q calculated based on TEMPOL quenching are higher.

Table 4 Quantum yield relative to anthracene of the different probes and their fluorescence lifetime in various polymer matrices

	BTXINH			BTXINO			BTXINOR			BTXID		
	PMMA	PVC	PS	PMMA	PVC	PS	PMMA	PVC	PS	PMMA	PVC	PS
Φi	3.1	12.0	2.0	1.0	1.4	0.5	1.6	13.0	3.7	1.9	13.2	4.4
Φ_{BTXID}/Φ_i	0.61	1.1	2.2	1.9	9.4	8.8	1.2	1.0	1.2	1	1	1
τ, ns	8.9	8.4	7.7	0.87 (3%)	0.27 (77%)	0.20 (82%)	8.3	9.2	8.7	9.1	9.3	8.9
				6.6(97%)	4.14(23%)	4.71(18%)						

The standard error in lifetime is evaluated to 3%

Table 5 Intermolecular quenching of fluorescence of derivatives of BTXI by TEMPO and TEMPOL in chloroform

| Probe[a]	$K_{SV}(TO)$[b] dm^3 (mol^{-1})	$K_{SV}(TOL)$[c] (dm^3 mol^{-1})	τ[d] (ns)	$k_q(TO)\times$ $10^{10,e}$ (dm^3 mol^{-1} s^{-1})	$k_q(TOL)$ $\times 10^{10,f}$ (dm^3 mol^{-1}s^{-1})
BTXINH	167	336	6.9	2.4	4.7
BTXINO	158	157	3.2	4.9	4.9
BTXINOR	188	219	6.7	2.8	3.2
BTXID	164	234	6.9	2.4	3.4

[a] According to Scheme 1
[b] Stern-Volmer constant of quenching by TEMPO
[c] Stern-Volmer constant of quenching by TEMPOL
[d] Lifetime of fluorescence in chloroform
[e] Bimolecular quenching rate constant for TEMPO
[f] Bimolecular quenching rate constant for TEMPOL

Triplet deactivation route

The triplet deactivation route was examined by laser flash photolysis using 266 nm excitation. This wavelength was chosen as compromise because limited choice of excitation wavelength of laser and solubility problem. Although the quantum yield of fluorescence is high especially in solid matrices, all probes under study exhibit transient absorption spectrum, which decays in the time-range typical for triplet.

For all compounds containing benzothioxantheneimide chromophore - BTXI, the weak transient absorption is observed in the range under 440 nm and then stronger transient absorption above 500 nm after 266 nm excitation (Fig. 5). The transient absorption exhibited maximum at about 530 nm in methanol.

The relevant kinetic data are summarized in Table 6. The transient absorption of probes as well as model compound decays monoexponentially under nitrogen. The ratio of rate constants in probe with radical centre BTXINO and those of non radical BTXINH and BTXINOR is rather low 1.7 and 1.1 respectively. The more rapid decay of transient absorption occurs in the presence of oxygen (aerated
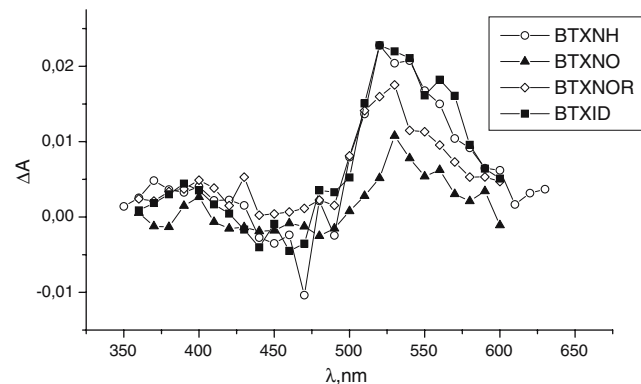
Fig. 5 Transient absorption spectrum of derivatives of benzothioxanthene in methanol (4×10^{-5}mol dm^{-3}) after 1 μs at 266 nm excitation

Table 6 Rate constants of decay of absorption transients of BTXI-derivatives

Probe	k (N$_2$)[a] (s^{-1})	K_{BTXID}/k_i[b]	k (Air)[c] (s^{-1})
BTXINH	2.5×10^5	1.12	4.2×10^5
BTXINO	4.2×10^5	0.67	6.9×10^5
BTXINOR	4.0×10^5	0.70	5.8×10^5
BTXID	2.8×10^5	1	4.3×10^5

[a] Rate constant of decay of transient absorption under nitrogen
[b] Ratio of rate constants of transient decay under nitrogen of BTXINO and BTXINH or BTXINOR
[c] Rate constant of decay of transient absorption in aerated solution

solutions). From this data the quenching rate constant for oxygen around 1×10^8 dm^3 mol^{-1} s^{-1} can be calculated assuming the concentration of oxygen in aerated methanol 2.2×10^{-3} mol dm^{-3} (Table 7).

The transient absorption of probes and model compound is quenched by TEMPO as well as TEMPOL in the concentration range up to 0.01 mol dm^{-3}. The relevant kinetic data are summarized in Table 7 and they show that for both quencher k_q is around 1.0×10^8 dm^3 mol^{-1} s^{-1} similarly as for oxygen.

We assume that the transient absorption is due to triplet state. The intermolecular quenching of this state by oxygen and TEMPO or TEMPOL is rather low or non-operating indicating that triplet level of benzothioxantheneimide chromophore is low. It could be comparable to oxygen TEMPO or TEMPOL. The intramolercular quenching in the probe BTXINO due to radical centre linked with this more complex chromophore is less efficient as for probe derived from structurally related 1,8-naphthyldicarboxylic anhydride [10, 16].

Conclusions

The absorption and fluorescence spectra of novel probes BTXINH, BTXINO and BTXINOR have got the same

Table 7 Rate constants of quenching of transient absorption of BTXI-derivatives in methanol

Probe	k_q (TO)[a] (dm^3 mol^{-1} s^{-1})	k_q (TOL)[b] (dm^3 mol^{-1} s^{-1})	k_q (Air)[c] (dm^3 mol^{-1} s^{-1})
BTXINH	1.2×10^8		9.0×10^7
BTXINO	1.7×10^8		1.2×10^8
BTXINOR	8.6×10^7	1.3×10^8	8.0×10^7
BTXID	9.5×10^7	1.1×10^8	8.0×10^7

[a] Rate constant of quenching of transient absorption under nitrogen by TEMPO
[b] Rate constants of quenching of transient decay under nitrogen of by TEMPOL
[c] Rate constant of quenching of transient absorption in aerated solution by oxygen

features as model compound BTXID. The linkage of BTXI type chromophore with reaction centre on sterically hindered amine type does not influence these spectral properties. All probes exhibit the absorption and fluorescence in the visible range of spectrum. The fluorescence of all probes is rather intense in solution and very intense when doped in polymer matrix. Two different excited states were obtained with BTXINO: with the nitroxyl group above and along the benzothioxantheneimide. The former situation lead to a short lived species and permits an efficient intramolecular quenching. Moreover, switching on and off the fluorescence might be observable by naked eye since it occurs in the region where eye is rather sensitive. This feature could be of some interest at sensor construction.

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