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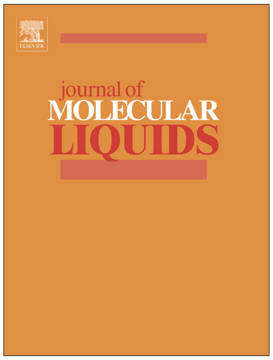

Bimolecular fluorescence quenching of benzoxazole/ benzothiazole-based functional dyes

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Bimolecular fluorescence quenching of benzoxazole/benzothiazole-based functional dyes

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ABSTRACT

Four dyes that belong to the group of hemicyanines and possess benzoxazole and benzothiazole moieties are synthesized and described. The spectroscopic properties of these compounds in 1-methyl-2-pyrrolidinone (MP) were studied. Synthesized dyes absorb in the UV-Vis region. The emission spectra are broad with a maximum located at approximately 500-520 nm. The deactivation of excited states of the synthesised molecules by various quenchers is also presented. The fluorescence quenching parameters were calculated using the Stern-Volmer equation. It was found, that the fluorescence quenching of the excited dye by quencher molecules occurs. The fluorescence quenching rates (k_q) are ~10^{10} $M^{-1} \times s^{-1}$.

Keywords: hemicyanine dyes, spectroscopic properties, fluorescence quenching, quencher, Stern-Volmer equation

1. Introduction

Mauveine was the first discovered dye. In 1856 Perkin synthesised this compound and started a new era of synthesis of dyes [1]. Since then, searching and designing new dyes using novel methods of synthesis, which might exhibit unique and useful physico-chemical properties, became very important for a wide group of scientists [2]. The dye industry is still developing, this is indicated by the number of dyes produced. It is estimated, that more than 7×10^5 tons of synthetic dyes are produced in the world with about 10^4 types of commercially available dyes [3, 4].

Cyanines (polymethines) have been of great interest for many years because of their multidimensional applications. Typical cyanine dye is composed of a methine chain $(\text{-CH=CH-})_n$ with two aza-heterocyclic moieties, such as benzothiazoles, benzoxazoles, pyridines, quinolines, indoles etc., which are attached to the conjugated double bond system. The introduction of appropriate substituents instead of heterocycles results in diversification of structure and properties of these compounds.

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Both, the type of terminal groups and the length of the polymethine chain affect the position of the maximum absorption for cyanine dyes. This is related to delocalisation of electrons in the molecule, which causes the absorption over a wide spectrum range, from visible to the near infrared region [5-7].

Cyanines have narrow absorption bands and are characterised by very high molar extinction coefficients. Moreover, these compounds display large microscopic polarisabilities, intensive fluorescence in the visible region, high affinity to cell structures and the ability to form highly ordered self-assemblies (J-aggregates) [8-12]. These unique properties aforementioned, allow their use in other different aspects such as antitumor agents in Photodynamic Therapy (PDT) [13-15], fluorescence probes in biological imaging [16, 17], sensitisers in dye-sensitised solar cells (DSSCs) [18-20], sensors for detection of various substances [21-23], optical recording and data storage media [24, 25], and many others.

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Chemical molecules exhibiting intensive fluorescence are of interest in organic synthesis [26]. The study of the fluorescence phenomenon of molecules is extremely significant and shows huge potential in the diagnosis and treatment of different types of cancer. This fact is confirmed by numerous of articles presenting the justified application of dyes in PDT, which is defined as a minimally invasive therapeutic technique.

For example, in 2020, Lima and co-workers [27] described novel indolenine-based squaraine dyes that provide a potential application as photosensitisers in therapeutic systems against carcinoma cells. These compounds display strong, sharp absorption within the so-called “phototherapeutic window” (650-850 nm), high photostability and the ability for the generation of singlet oxygen ($^1O_2$). The reactive oxygen species are the main cytotoxic agent, which induce infected cell death *via* oxidative stress mechanisms, thus the system is selective [28].

In 2019 Bokan *et al.*, presented a new method for targeted drug delivery (TDD) by means of a combination an anticancer medicine with a proper fluorogenic dye. The novel, water-soluble phenolo-cyanine dyes were bound to the chlorambucil (CLB) medicament through a biodegradable ester linker. The developed conjugates possess a single reactive carboxylic group that is able to bind with a specific target cancer carrier, *e.g.*antibodies or peptides. The hydrolytic cleavage of the ester bond in the cell medium, causes the drug to be released and this fluorescent system exhibits emission in over a wide region of the spectrum with a maximum located at 720 nm. Therefore, the proposed molecules are really promising for tumour drugs delivery to target infected structures [29].

Because of the significant interest in looking for alternative renewable sources of energy, in the last years, the development of dye-sensitised solar cells has been rapidly increased. In DSSCs, the sensitiser plays a key role in absorbing the light and initiating the electrochemical reactions for electricity production [30].

Confirmation of the usability of cyanines as sensitisers in solar cells was explored by Ghann and co-workers [18]. The group determined the photovoltaic efficiency of conversion to be 0.04%-0.24%. In 2019, new systems containing organic dyes with quantum dots (QDs) were proposed. The surface deposition of cyanine using QDs increased the efficiency of DSSCs to 0.89% in comparison to typical solar cells only based on the dye [31].

The examples mentioned, confirm the important role of searching for new dyes and studying their properties. These works show an application of novel designed dyes in various fields, such as science, engineering, technology, pharmacy, and medicine. Due to a wide range of applications for cyanine-based dyes, it is necessary to examine their features. In our study, we present spectral properties and fluorescence quenching of four synthesised hemicyanines with different molecules used as quenchers.

2. Experimental

2.1. Materials and general methods

All reagents for the synthesis of the hemicyanines and spectroscopic grade solvent: 1-methyl-2-pyrrolidinone (MP) were purchased from Sigma-Aldrich (Poland) and used without further purification. The commercially available compounds used as quenchers were purchased from Sigma-Aldrich (Poland) or Alfa Aesar and the others were synthesised in our laboratory by methods described in the literature [32, 33].

Absorption and emission spectra were recorded using an Agilent Technology UV-Vis Cary 60 Spectrophotometer and a Hitachi F-7000 Spectrofluorimeter, respectively. The measurements were carried out at room temperature with 1-methyl-2-pyrrolidinone as the solvent.

The fluorescence quantum yield for each dye (Φ_{dye}) was determined relative to Coumarine I, as a reference standard, and calculated based on the following equation [34]:

$$\Phi_{\mathrm{dye}} = \Phi_{\mathrm{ref}} \times \frac{\mathrm{I_{dye}A_{ref}}}{\mathrm{I_{ref}A_{dye}}} \times \frac{\mathrm{n_{dye}^2}}{\mathrm{n_{ref}^2}} \tag{1}$$

where: Φ_{ref} is the fluorescence quantum yield of the reference, I_{dye} and I_{ref} are the integration of the fluorescence intensity for the dye and reference sample, A_{dye}, A_{ref} are the absorbances of dye and

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reference at the excitation wavelength, n_{dye}, n_{ref} refer to the refractive indexes of solvents used for dye and reference, respectively.

The fluorescence lifetimes, as well as fluorescence quenching, were measured by the use of a single-photon counting system UV-VIS-NIR Fluorolog 3 Spectrofluorimeter (Horiba Jobin Yvon). This apparatus utilises molecule excitation with a picosecond diode laser (370 nm), which generates pulses of *ca*. 55 ps. A successful analysis of fluorescence decay signals with a resolution of few picoseconds is possible as a result of the short laser pulses in conjunction with fast microchannel plate photodetector and ultrafast electronics. The concentration of the dye was fitted to provide equivalent absorbance at excitation wavelength equal to 370 nm (0.2 in the 10 nm cell). The rate constant for the quenching process of the hemicyanines by all quenchers were studied in 1-methyl-2-pyrrolidinone. The concentration of quenchers varied from 1.0×10^{-4} M to 9.0×10^{-4} M (for R1/I1 system the concentration of quencher was 10-times higher).

3. Results and discussion

3.1. Synthesis

Four hemicyanines were synthesised according to the well-known procedure based on Knoevenagel condensation of a *N*-alkyl heterocyclic base containing active methyl group in the 2-position with an aromatic aldehyde [33, 35]. The synthetic routes of compounds under study are shown in Scheme 1. In general, the neutral dyes R1 and R2 were obtained by heating equimolar amounts of 2-methylbenzoxazole or 2-methylbenzothiazole (respectively for R1 and R2) and *p*-(*N*,*N*-dimethylamino)benzaldehyde in presence of boric acid (0.05 g per 0.01 M of aldehyde) at 195-200 ^{0}C for 5h. The ionic dyes were synthesised by the heating of appropriate amounts of compounds R1 or R2 with 1,3-butanesultone at 120 ^{0}C for 1h. The products of the reactions were crystallised from ethanol (neutral dyes) or mixture ethanol:hexane (10:1) and dried at ambient temperature.

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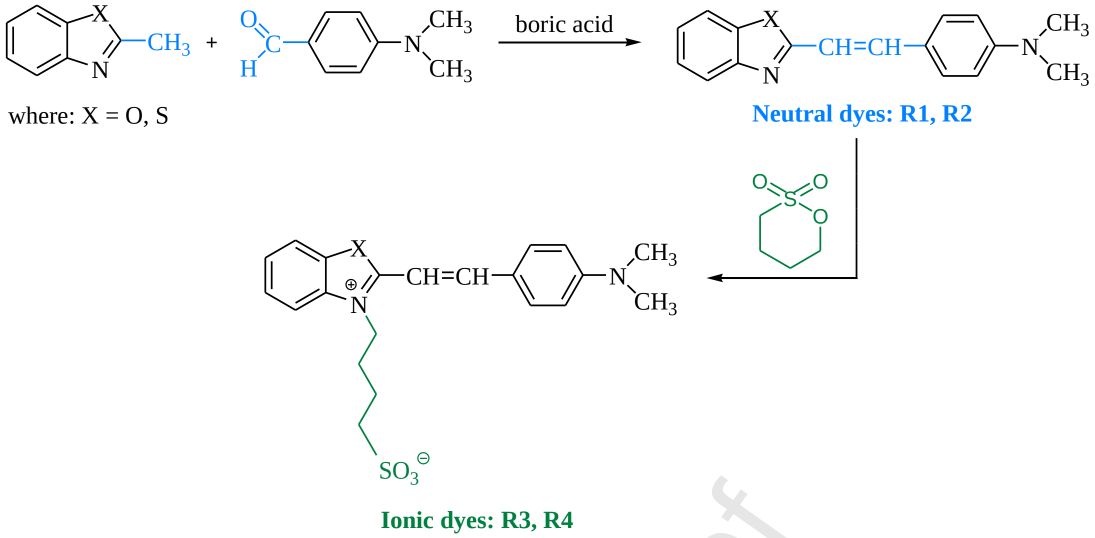


Scheme 1. General synthesis pathway of tested compounds R1-R4.

The structures of synthesised dyes were confirmed using ^{1}H and ^{13}C NMR spectroscopy (data presented in [33]). These compounds belong to the group of hemicyanines (styryl dyes) possessing two nitrogen atoms linked with a chain of conjugated double bonds, wherein one of nitrogen atom is not a part of the heterocyclic ring [7]. In Fig. 1, basic structural information about the systems is presented.

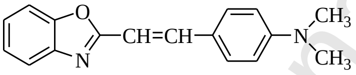
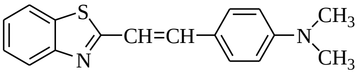
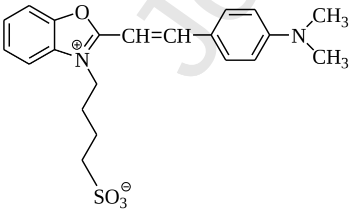
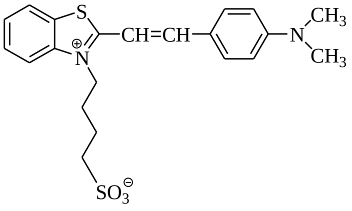
Hemicyanines	
 2-[*p*-(*N*,*N*-dimethylaminostyryl)]benzoxazole **R1**	 2-[*p*-(*N*,*N*-dimethylaminostyryl)]benzothiazol **R2**
 2-[*p*-(*N*,*N*-dimethylamino)styryl]benzoxazole butanesulfonate **R3**	 2-[*p*-(*N*,*N*-dimethylamino)styryl]benzothiazole butanesulfonate **R4**

Fig. 1. Chemical structures, names and abbreviations of hemicyanine dyes.

3.2. Spectroscopic properties of hemicyanines

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The photophysical data of the hemicyanine dyes were studied and are summarised in Table 1. As can be seen in Fig. 2, the compounds absorb in the UV-Vis region and emit between 450 nm to 600 nm range. The visible band of absorption arise from electronic transitions involving π electrons along the polymethine chain. Generally, the dyes exhibit a maximum absorption (λ_{ab}) band at about 400 nm. For R4, it was observed that the second maximum of absorption is located at 530 nm. The emission spectra are single and broad with a maximum of fluorescence (λ_{fl}) located at about 500 nm for benzoxazole-based dyes and at *ca*. 520 nm for the benzothiazole derivatives. Moreover, the Stokes shifts achieved relatively high values. These characteristics suggest that for fluorescence lifetimes, the crucial structural changes take place in the S_1 state of the solvated molecule relative to the primary state S_0.

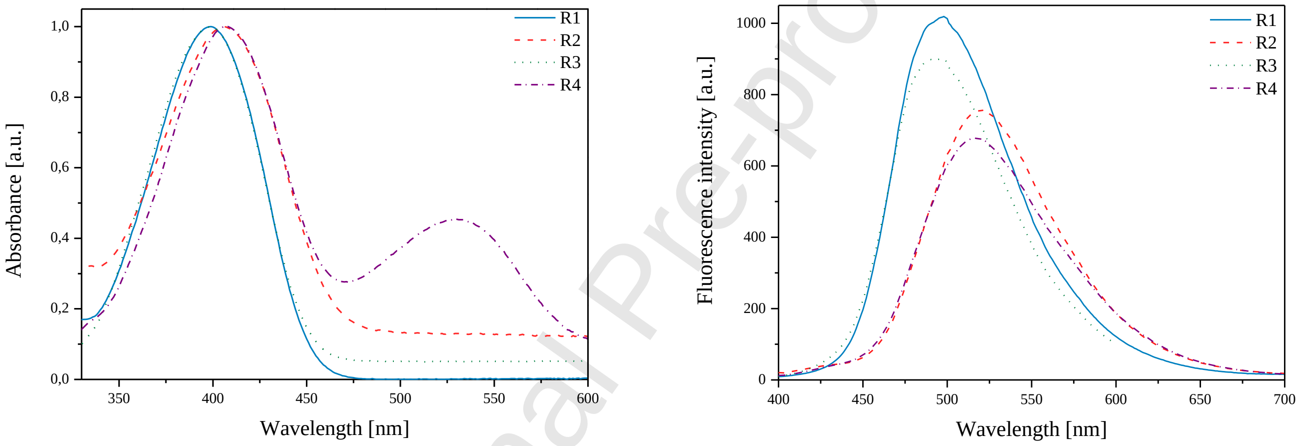

Fig. 2. Normalised absorption and fluorescence spectra of the tested dyes recorded on 1-methyl-2-pyrrolidinone (MP) at room temperature.

High molar extinction coefficients for the synthesised benzoxazole and benzothiazole derivatives were determined. The fluorescence quantum efficiency (Φ_{fl}) was calculated from the ratio of the number of photons emitted by the molecule to the number of absorbed [36]. The quantum yields achieved quite low values and the excited state energy levels (E_{00}) are similar for all tested dyes. Low values of Φ_{fl} may be indicative of the radiative or non-radiative deactivation processes that transform the molecule from the excited to ground state.

Table 1
Spectroscopic properties of studied dyes in 1-methyl-2-pyrrolidinone (MP).

Dye	**Parameter**							
	λ_{ab} [nm]	FWHM$_{ab}$ [cm^{-1}]	ε [10^4 M^{-1} × cm^{-1}]	λ_{fl} [nm]	FWHM$_{fl}$ [cm^{-1}]	Stokes shift [cm^{-1}]	Φ_{fl} [10^{-2}]	E_{00} [eV]

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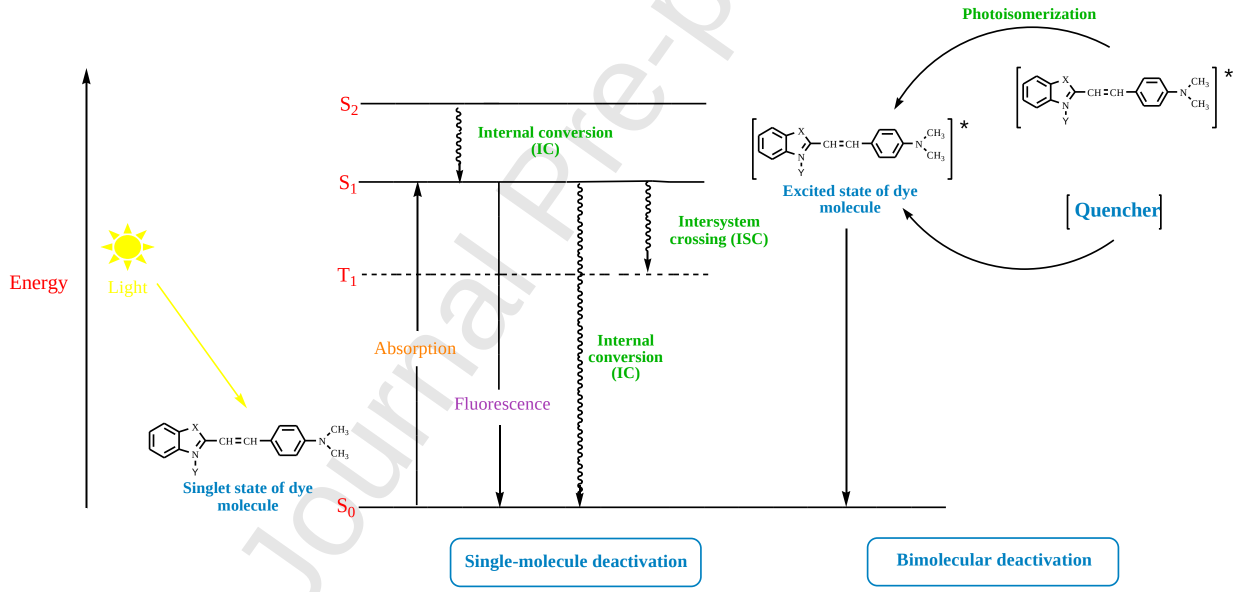
R1	399	4642	3.61	498	3116	4982	1.59	2.77
R2	406	4257	2.30	521	3106	5437	2.03	2.65
R3	399	4303	4.99	496	3517	4901	1.92	2.78
R4	408 (530)	4006	3.08	517	3290	5167	1.54	2.64

3.3. Deactivation of excited states of cyanine dyes

Absorption of light results in excitation of molecule and transition from ground to excited singlet state. Methods for deactivation of excited states depend on the type of absorbing molecule. As presented in Scheme 2, there are major quenching routes from the excited singlet to ground singlet state for typical polymethines:

- single–molecule deactivation: fluorescence, internal conversion, and intersystem crossing.
- bimolecular deactivation: photoisomerisation and reaction between the excited state of dye and quencher molecule [37].



Scheme 2. Simplified energy level diagram of dyes under study showing routes for deactivation of an excited state of the molecule.

Fluorescence quenching (FQ) is defined as the physicochemical process of relaxation, that occurs without photon emission. This process results in the reduction of the fluorescence lifetime and fluorophore intensity. Deactivation of the excited state of the molecule can be directly monitored by measurement of fluorescence lifetime as a function of intensity *vs* time the after light excitation [38-40].

FQ of fluorophores, using different molecules acting as the quenchers, has been widely studied to understand the mechanism of reaction that occurs in transient and steady-state conditions

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[41]. The excited state quenching is usually classified into three major groups: static, dynamic, and resonance energy transfer. Generally, the quenching of the fluorescence process can be studied experimentally by estimating the characterizing rate parameters on the basis of the Stern-Volmer equations, which are presented below [42, 43]:

$$\frac{I_0}{I} = 1 + K_{SV} \times [Q] \tag{2}$$

where: I_0 and I are the fluorescence intensities of solute molecules in the absence and in the presence of a quencher, respectively, K_{SV} is the Stern-Volmer (S-V) constant, and [Q] is the concentration of quencher.

The K_{SV} constant is expressed by Eq. (3):

$$K_{SV} = k_q \times \tau_0 \tag{3}$$

where: k_q is the quenching rate parameter, and τ_0 is the lifetime of the fluorophore in the absence of quencher.

Equation (2) is appropriate for linear variation of the obtained experimental results.

Fluorescence quenching of excited state of dye – fluorescence lifetime

As summarised in Table 2, the synthesised hemicyanines show similar fluorescence lifetimes. The emission lifetime of the molecule is short and occurs within *ca.* 6 ns. This is an impermanent phenomenon, which is typical for organic molecules.

Table 2
Fluorescence lifetime values determined for R1-R4 dyes.

	Dye			
	R1	R2	R3	R4
Fluorescence lifetime [10^{-9} s]	6.75	5.66	5.82	6.58

The FQ in the presence of the quencher is more effective than that observed for dye without a quencher due to the possibility of a strong interaction between the singlet fluorescent state of fluorophore and quencher.

Bimolecular fluorescence quenching

In order to explain the effect of other molecules on the photochemical behaviour of the light-absorbing dyes, experiments based on fluorescence quenching of dyes as a function of quencher concentration were carried out. The compounds used as fluorescence quenchers are presented in

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Fig. 3. All quenchers used in the experiments are not fluorescent compounds compared to the R1-R4 dyes that undergo a radiation deactivation after excitation. These compounds belong to diphenyliodonium salts, *N*-alkoxypyridinium salts, borate salt, amines and 1,3,5-triazine and were also usually used as co-initiators in photoinitiating systems for radical polymerisation of various acrylate monomers [44, 45].

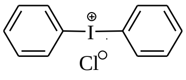
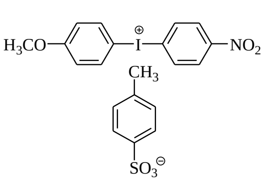
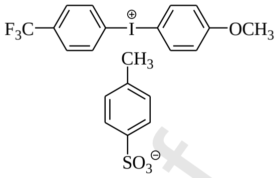
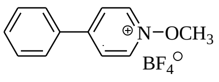
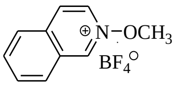
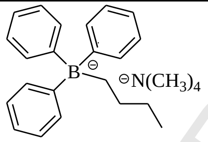
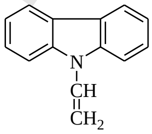
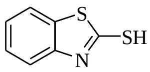
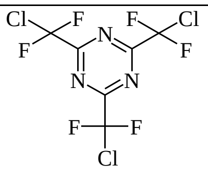
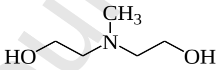
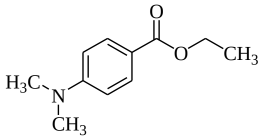
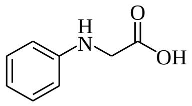
Quenchers			
			
diphenyliodonium chloride	(4-methoxyphenyl)-(4-nitrophenyl)iodonium *p*-toluenesulfonate	(4-trifluoromethylphenyl)-(4-methoxyphenyl)iodonium *p*-toluenesulfonate	*N*-methoxy-4-phenylpyridinium tetrafluoroborate
I1	**I81**	**I85**	**NO**
			
N-methoxyisoquinoline tetrafluoroborate	tetramethylammonium *n*-butyltriphenylborate	*N*-vinylcarbazole	2-mercaptobenzothiazole
NO2	**B2**	**NVK**	**MS**
			
2,4,6-tris(chlorodifluoromethyl)-1,3,5-triazine	*N*-methyl-*N*,*N*-diethanolamine	ethyl (*N*,*N*-dimethylamino)benzoate	*N*-phenylglycine
T1	**MDEA**	**EDMAB**	**NPG**

Fig. 3. Chemical structures, names, and abbreviations of the quenchers used in the study.

Figure 4 presents the fluorescence decay profiles of 2-[*p*-(*N*,*N*-dimethylaminostyryl)]benzoxazole (R1) in the presence of different concentrations of *N*-methoxyisoquinoline tetrafluoroborate (NO2), used as a quencher. The quencher has a significant effect on the fluorescence lifetime. As a result of the interaction between the dye and quencher, a

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shortening of the fluorescence lifetime was observed. An increase in the concentration of the quencher decreases the fluorescence lifetime of the dye.

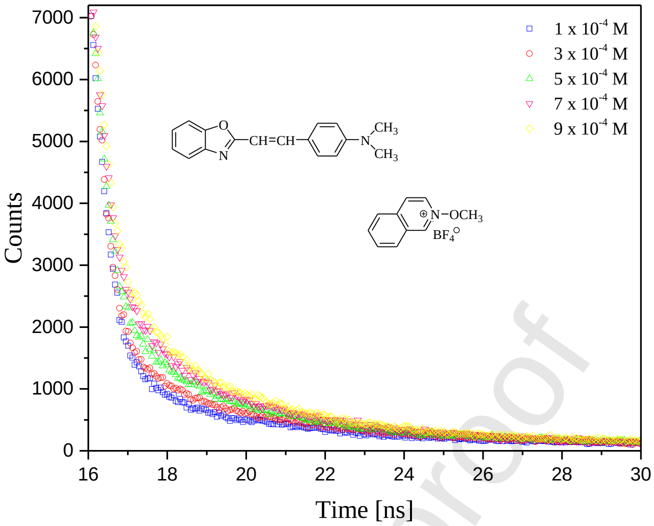

Fig. 4. Fluorescence decay profile of R1 in the presence of various concentrations of NO2.

The data obtained from FQ experiments are discussed on the basis of the Stern-Volmer relationship. The characterizing parameters from the S-V equation are summarised in Table 3.

Table 3

The values of the Stern-Volmer constant (K_{SV}) and rate constant of fluorescence quenching (k_q) for the systems in this study.

Dye	**Parameter**	**Quencher**											
		I1	I81	I85	NO	NO2	B2	NVK	MS	T1	MDE	NPG	EDM
R1	K_{SV} [10^2 M^{-1}]	0.47	3.12	6.18	4.73	11.21	9.14	3.69	5.69	4.90	7.39	7.32	6.85
	k_q [10^{10} M^{-1} × s	0.70	4.62	9.15	7.00	16.60	13.54	5.46	8.42	7.25	10.94	10.84	10.15
R2	K_{SV} [10^2 M^{-1}]	2.73	2.88	3.09	3.67	6.43	5.61	2.06	3.81	3.27	1.57	3.42	3.50
	k_q [10^{10} M^{-1} × s	4.82	5.09	5.45	6.48	11.35	9.90	3.63	6.72	5.77	2.78	6.05	6.17
R3	K_{SV} [10^2 M^{-1}]	4.46	4.46	5.09	4.56	3.14	7.54	3.76	5.03	4.25	5.47	5.72	5.65

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	k_q [10^{10} M^{-1} × s	7.66	7.65	8.74	7.84	5.39	12.95	6.47	8.63	7.31	9.40	9.83	9.71
R4	K_{SV} [10^2 M^{-1}]	4.03	5.46	5.95	3.91	6.34	6.82	3.54	6.66	4.77	6.25	8.77	5.92
	k_q [10^{10} M^{-1} × s	6.12	8.29	9.04	5.94	9.63	10.37	5.37	10.12	7.25	9.50	13.33	9.00

From the data presented in Table 3 and Figure 5, it is seen, that the rate of fluorescence quenching depends on the type of quencher used. It can be concluded, that there are strong interactions with the dye-quencher. A linear relationship between the change in the fluorescence lifetime and concentration of the quencher molecule is observed. The gradients of the Stern-Volmer relationship achieve relatively high values. This parameter is over a range from 0.47 × 10^2 M^{-1} to 11.21 ×10^2 M^{-1}. The most efficient quenchers for the neutral dyes are tetramethylammonium *n*-butyltriphenylborate (B2), *N*-methoxyisoquinoline tetrafluoroborate (NO2), and borate salt (B2) or *N*-phenylglycine (NPG) for ionic dyes R3 and R4, respectively. The highest values of the Stern-Volmer constant were observed when NO2 and B2 were used as quenchers for the excited state of neutral dyes R1 and R2. In the case of dyes R3 and R4, the highest values of K_{SV} were recorded for the borate salt (B2) and *N*-phenylglycine.

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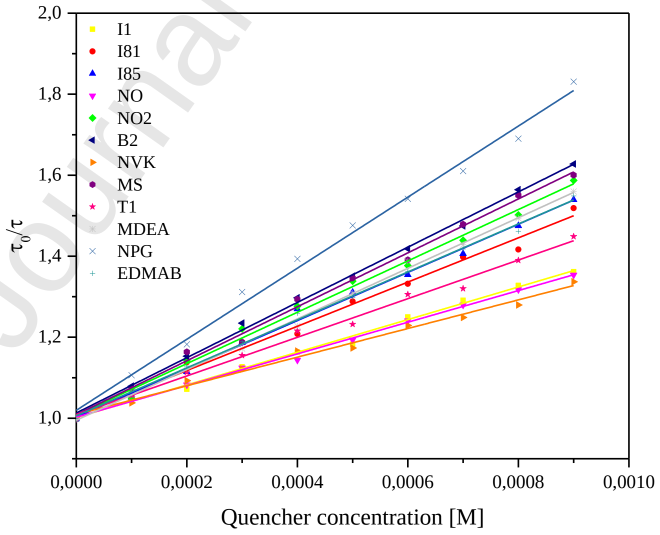

Fig. 5. The Stern-Volmer plots for the quenching of the fluorescence for 2-[*p*-(*N*,*N*-dimethylamino)styryl]benzothiazole butanesulfonate (R4) by all the quenchers outlined.

Based on Eq. 3, fluorescence lifetimes, and the Stern-Volmer constants the fluorescence quenching rate constants (k_q) were calculated (see Table 3 and Table 2). It was found, that this parameter is in the range from 0.70 × 10^{10} M^{-1} × s^{-1} for iodonium salt (I1) to 16.60 × 10^{10} M^{-1} × s^{-1}

for tetrafluoroborate (NO2). The correlation coefficients (R^2) in all cases for the dye/quencher systems range from 0.98 to 0.99. Generally, with increasing concentration of quencher, the k_q parameter increases. It can be concluded that all used molecules are fast and effective quenchers for the excited state of the dye. These promised values of S-V parameters may be due to the formation of an intramolecular pair which results in quenching of the fluorescence process. The quenching mechanism is based on an electron transfer process between the system's components.

4. Conclusions

In this paper, the fluorescence quenching process of hemicyanines by various quenchers is presented. All used molecules possess the ability for quenching of excited states in R1-R4 dyes. The Stern-Volmer plots confirm strong interactions between the dye and quencher that leads to high values of the fluorescence quenching rate constant parameter. The kinetics of bimolecular fluorescence quenching depends on the type of quencher. The linear S-V correlation shows that the efficiency of quenching is associated with the concentration of quencher.

Conflict of interest

The authors have no conflicts of interest to declare.

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Declaration of Interest: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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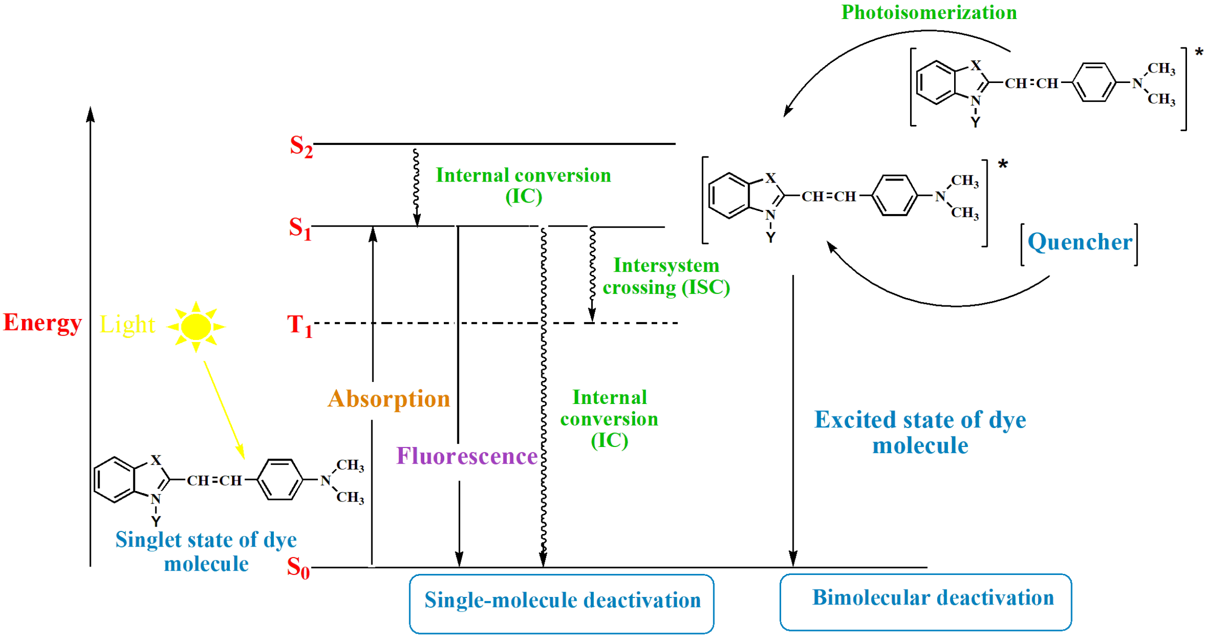
Graphical abstract



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Highlights

- Spectroscopic characteristic of benzoxazole and benzothiazole-based dyes .
- Bimolecular fluorescence quenching of hemicyanines.
- Kinetic parameters of radiation deactivation depend on the type of quencher.
- The Stern-Volmer relationship between fluorescence lifetime and concentration of quencher.

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