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Intramolecular Fluorescence Quenching of Crowned 7-Aminocoumarins as Potential Fluorescent Chemosensors

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The effects of the nature of solvent, temperature and complex formation with alkali and alkaline-earth metal cations, as well as protonation, on the efficiency and the kinetics of fluorescence of 3-azacrowned 7-diethylaminocoumarins have been studied. For the crown-ethers under investigation, the ratio of a dipole moment to the radius of Onsager cavity $\Delta\mu/\rho$ is a constant value, and a macrocycle does not affect $\Delta\mu$ and ρ. The fluorescence of coumarin 1 in acetonitrile is quenched by an electron donor, triethylamine, with the Stern-Volmer constant being equal to (0.474 ± 0.009) M^{-1}. The decrease in coumarin 1 fluorescence quantum yield upon the introduction of *N*-alkylazacrown moiety into position 3 is caused by an intramolecular photoinduced electron transfer from the nitrogen atom of macroheterocycle to the coumarin moiety, where the excitation is localized. The fluorescence quenching has an activation energy 2.32 ± 0.05 kcal/mol in various hydrocarbons, and does not depend on the solvent viscosity. The fluorescence kinetics of free crowned coumarins in methanol is not monoexponential because of the existence of macrocycle conformers, or because of the hydrogen bond complex formation between the solvent and the nitrogen atom of macrocycle, in which the efficiency of intramolecular electron transfer is different. Upon complex formation with alkali and alkaline-earth metal cations and upon protonation, the fluorescence quantum yield increases and fluorescence decay becomes monoexponential.

KEY WORDS: Crown-ethers; 7-aminocoumarins; fluorescence quenching; complex formation; electron transfer; fluorescent chemosensors.

INTRODUCTION

Crown-ethers with side chromophoric moieties are sensitive to the presence of metal cations and widely used in quality and quantity analyses, as well as fluorescence labels in chemistry and biology [1–3]. The introduction of a macrocyclic substituent into a fluorophore moiety can result in its fluorescence efficiency decrease [4].

The introduction of azamacrocyclic moiety into the molecule of coumarin 153 decreases the fluorescence quantum yield, moreover, the fluorescence decay becomes biexponential [5,6]. The fluorescence quantum yield increases and the fluorescence decay becomes monoexponential upon the complex formation with alkali and alkaline-earth metal cations.

The reasons of low efficiency of crowned 7-aminocoumarins fluorescence are not yet clear. The purpose of the present work is to ascertain the regularities and mechanism of radiationless deactivation of azacrowned 7-aminocoumarins.

EXPERIMENTAL

The structural formulas of the derivatives under investigation of 7-diethylamino-4-methylcoumarin (**1**) and 2,3,6,7-tetrahydro-9-methyl-1*H*,5*H*,11*H*-[1]benzopyrano[6,7,8-*ij*]quinolizine-11-one (**2**) are given in the Fig. 1.

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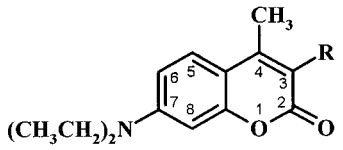


1a, R = H

1b, R = $CH_2N(CH_2CH_2O)_3CH_2CH_2$

1c, R = $CH_2N(CH_2CH_2O)_4CH_2CH_2$

1d, R = $CH_2N(CH_2CH_2O)_5CH_2CH_2$

1e, R = $CH_2NCH_2CH_2OCH_2CH_2$

1f, R = $CH_2N^+(CH_3)CH_2CH_2OCH_2CH_2$

1g, R = $CH_2NHCH_2CH{=}CH_2$

1h, R = $CH_2NH^+(CH_2CH_2O)_3CH_2CH_2$

1i, R = $CH_2NH^+(CH_2CH_2O)_4CH_2CH_2$

1j, R = $CH_2NH^+(CH_2CH_2O)_5CH_2CH_2$

1k, R = $CH_2NH^+CH_2CH_2OCH_2CH_2$

1l, R = $CH_2NH_2^+CH_2CH{=}CH_2$

1m, R = $CH_2N(CH_2CH_2O)_3CH_2CH_2\cdot Na^+$

1n, R = $CH_2N(CH_2CH_2O)_3CH_2CH_2\cdot Ca^{2+}$

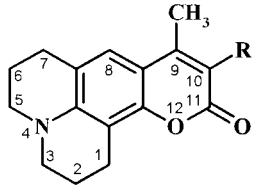
1o, R = $CH_2N(CH_2CH_2O)_4CH_2CH_2\cdot Li^+$

1p, R = $CH_2N(CH_2CH_2O)_4CH_2CH_2\cdot Mg^{2+}$

1q, R = $CH_2N(CH_2CH_2O)_4CH_2CH_2\cdot Na^+$

1r, R = $CH_2N(CH_2CH_2O)_4CH_2CH_2\cdot Ca^{2+}$

1s, R = $CH_2N(CH_2CH_2O)_4CH_2CH_2\cdot Ba^{2+}$



2a, R = $CH_2NCH_2CH_2OCH_2CH_2$

2b, R = $CH_2N(CH_3)(CH_2)_{17}CH_3$

2c, R = $CH_2NH^+CH_2CH_2OCH_2CH_2$

2d, R = $CH_2NH^+(CH_3)(CH_2)_{17}CH_3$

Fig. 1. The structural formulas of the derivatives under investigation of coumarin 1 (**1**) and coumarin 102 (**2**).

The absorption spectra were recorded on the spectrophotometer "Shimadzu UV-3100," the fluorescence spectra—on the spectrofluorimeter "Elumin-2M." In order to represent the fluorescence spectra in wave numbers, the fluorescence intensities were multiplied by squared wavelengths [7]. The fluorescence quantum yield was determined by comparing of the squares under the corrected fluorescence spectra of a substance under study and quinine bisulfate solution in 1 N H_2SO_4 ($\phi = 0.546$ [8]). The fluorescence quantum yield of cooled solutions was corrected for changing of optical density as a result of the dependence of solvent density on the temperature.

The fluorescence lifetimes were measured using single photon counting technique by nanosecond spectrometer SP-70. Coumarin 1 (**1a**) was "quantum electronic" grade and was used without further purification. Synthesis of crowned dyes **1b**, **1c**, **1d** was performed according to the procedure described in [9]. Compounds **1e**, **1f** (iodide), **1g**, **2a** and **2b** were kindly granted by Prof. M. A. Kirpichenok. The solvents used were purified according to the known procedures [10]

The protonation of compounds **1b**, **1c**, **1d**, **1e** and **1g** at the nitrogen atom of 3-substituent and **2a** and **2b** at the nitrogen atom of 10-substituent was performed with sulfuric acid (10^{-5} M) in polar solvents (THF, acetone, acetonitrile, methanol), and trifluoroacetic acid (10^{-5} M) in nonpolar solvent *n*-hexane. The protonation of the nitrogen atoms at the position 7 of coumarins of series **1** and at the position 4 of coumarins of series **2** does not take place.

The complexes of crowned dyes with alkali and alkaline-earth metal cations were obtained by adding of anhydrous perchlorates to a solution (the salt concentration was 10^{-2} M). The completeness of protonation and complex-formation was controlled spectrophotometrically. Because of the high sensitivity of neutral aza-crowned compounds to medium acidity, the registration of spectra and fluorescence lifetimes was performed in the presence of tetrabutylammonium hydroxide (10^{-5} M in acetonitrile and 10^{-4} M in methanol). It was shown that tetrabutylammonium hydroxide, in concentrations up to 10^{-2} M, does not influence on the fluorescence spectra of **1c** in methanol.

RESULTS AND DISCUSSION

The wavelengths of absorption and fluorescence spectra maxima of the compounds **1a**, **1b**, **1c**, **1d**, **1e**, **1f**, **1g**, **2a** and **2b** , the protonated forms **1h**, **1i**, **1j**, **1k**, **1l**,

Table I. Spectral Properties of the Compounds of the Series **1** and **2** in Various Solvents (λ_{abs} and λ_{fl} Are the Wavelengths of Absorption and Fluorescence Spectra maxima, φ is the Fluorescence Quantum Yield)

	Solvent	λ_{abs} (nm)	λ_{fl} (nm)	φ		Solvent	λ_{abs} (nm)	λ_{fl} (nm)	φ
1a	Hexane	349	392	0.53	**1h**	Acetonitrile	392	458	0.44
	THF	362	418	0.85		Methanol	392	465	0.22
	Acetonitrile	367	437	0.65	**1i**	Hexane	389	427	0.86
	Methanol	375	455	0.38		THF	387	441	0.90
1b	Hexane	356	404	0.07		Acetonitrile	393	461	0.34
	THF	366	429	0.05		Methanol	394	468	0.20
	Acetonitrile	371	443	0.06	**1j**	Hexane	388	425	0.76
	Methanol	380	459	0.07		THF	385	441	0.85
1c	Pentane	355	403	0.02		Acetonitrile	390	458	0.38
	Hexane	356	403	0.02		Methanol	393	467	0.23
	Decalin	359	408	0.02	**1k**	Hexane	392	425	0.93
	Toluene	365	419	0.02		THF	388	441	0.88
	THF	365	430	0.03		Acetonitrile	393	462	0.25
	Acetonitrile	374	446	0.03		Methanol	394	467	0.16
	Methanol	379	461	0.25	**1l**	Hexane	389	425	1.0
1d	Hexane	356	406	0.08		THF	383	440	0.91
	THF	366	431	0.08		Acetone	386	451	0.44
	Acetonitrile	372	447	0.10		Acetonitrile	386	461	0.34
	Methanol	380	459	0.55		Methanol	391	466	0.21
1e	Hexane	356	401	0.37	**1m**	Methanol	381	462	0.67
	THF	370	429	0.38	**1n**	Methanol	398	473	0.49
	Acetonitrile	374	445	0.37	**1o**	Methanol	383	464	0.43
	Methanol	379	459	0.45	**1p**	Methanol	386	466	0.64
1f	Methanol	400	469	0.12	**1q**	Methanol	381	463	0.64
1g	Hexane	353	402	0.07	**1r**	Methanol	401	474	1.0
	THF	364	429	0.53	**1s**	Methanol	395	471	1.0
	Acetone	367	438	0.58	**2a**	Methanol	396	480	0.87
	Acetonitrile	371	445	0.61	**2b**	Methanol	393	479	0.33
	Methanol	378	462	0.74	**2c**	Methanol	411	487	0.96
1h	Hexane	389	422	0.82	**2d**	Methanol	411	485	0.64
	THF	384	440	0.86					

2c and **2d**, and the metal-complexes **1m**, **1n**, **1o**, **1p**, **1q**. **1r** and **1s** are given in the Table I. When passing from the nonpolar solvents to the polar ones, one can observe the low-frequency shift of absorption (800–1800 cm^{-1}) and fluorescence (2000–3000 cm^{-1}) spectra, caused by the universal and specific (a hydrogen bond) interaction between the fluorophore and the solvent. A greater red shift of the fluorescence spectra in comparison with the absorption spectra, when solvent polarity raises, is caused by an increase in the dipole moment of 7-aminocoumarin molecule upon the excitation ($\Delta\mu = 7.2$ D for **1a**) [11].

The influence of the medium polarity on the position of absorption and fluorescence spectra can be described by Lippert equation [12].

$$\Delta\tilde{\nu} = \frac{2(\Delta\mu)^2}{hc\rho^3}\left(\frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{2n^2+1}\right) + \text{const}$$
$$= \frac{2(\Delta\mu)^2}{hc\rho^3} f(\varepsilon, n) + \text{const} \qquad (1)$$

Here, $\Delta\tilde{\nu}$ is Stokes' shift of the fluorescence spectrum, $\Delta\mu$ is a change of the dipole moment upon the excitation, ρ is a radius of Onsager sphere, h is Planck's constant, c is the speed of light in vacuum, ε and n are dielectric constant and refractive index of the solvent, respectively, const is a certain constant value for a given substance. The dependence of Stokes' shifts of the compounds **1a**, **1b**, **1c**, **1d** and **1g**, having 7-aminocoumarin as the same chromophore moiety, on the solvent function $f(\varepsilon, n)$ according to the Eq. (1) is shown in the Fig. 2. It can be seen that the slopes of these straight lines (3100, 2800, 2700, 2700, 3000 ± 200 cm^{-1}, respectively) for all the compounds are nearly identical, in spite of the fact that they have different sizes of 3-substituent and therefore must differ in the radius of the Onsager sphere. The data obtained allow us to conclude that for the compounds under investigation the ratio $\Delta\mu/\rho$ is a constant value, and a macrocycle in crowned coumarins does not affect the change of dipole moment upon the excitation and the radius of Onsager sphere.

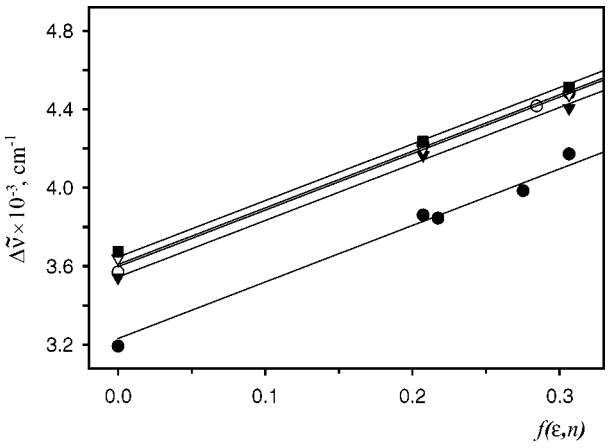


Fig. 2. The dependence of Stokes' shifts of the compounds **1a** (●), **1b** (▼), **1c** (∇), **1d** (■) and **1g** (○) on the solvent function $f(\varepsilon, n)$.

The joint linear regression with the same slope for all the straight lines gives the value 3070 ± 90 cm^{-1}, which corresponds to $\Delta\mu = 7.2$ D [11] on the assumption that the radius of the Onsager sphere is equal to 5.6 Å. This assumption is in a good agreement with the value of 5.5 Å, obtained as half of the largest interatomic distance of the **1a**, calculated using semi-empirical AM1 method. For comparison, the value of $\Delta\mu$, obtained at the investigation of solvatochromism of **1a**, is 8.02 D [13].

Upon protonation of the compounds **1b**, **1c**, **1d**, **1e**, **1g** at the nitrogen atom of 3-substituent and **2a** and **2b** at the nitrogen atom of 10-substituent, a low frequency shift of absorption and fluorescence spectra (300–1300 and 800–2500 cm^{-1}, respectively) is observed, caused by the influence of the intramolecular electric field of trialkylammonium cation on the chromophoric part of the molecule. The magnitude and the direction of displacement are in agreement with the model of intramolecular electrochromism and electrofluorochromism [14]. In polar solvents (acetonitrile, THF), the shifts of absorption and fluorescence spectra (800–1500 and 300–700 cm^{-1}) are less than in nonpolar solvents (hexane), 2500 and 1300 cm^{-1}, respectively.

The complex formation of the compounds **1b** and **1c** with alkali and alkaline-earth metal cations, as well as protonation, leads to the low frequency shift of absorption and fluorescence spectra. The value of this shift depends on the charge of a cation and its radius. As one can see in the Table I, in the case of cations of small radius (0.70 Å and 0.75 Å for Li^+ and Mg^{2+} [15]), when the cation is essentially less than the macrocycle cavity of **1c** (1.0 ± 0.1 Å) [5], the shift caused by the complex formation increases nearly in proportion to the charge of cation (198 and 411 cm^{-1} in the case of Li^+ and Mg^{2+} for absorption spectrum maximum of **1c**, 116 and 243 cm^{-1} for fluorescence spectrum maximum). At the same time, the complex formation with the cations Ca^{2+} and Ba^{2+}, which have significantly larger radii (1.06 and 1.38 Å, respectively [15]), produces the greater shifts of absorption (1450 and 1070 cm^{-1}) and fluorescence (560 and 460 cm^{-1}) spectra, although, due to the smaller magnitudes of the ratio of the charge to the radius, one might expect lesser shifts for these cations in comparison with Mg^{2+}. This fact enables us to suppose that the cations Ca^{2+} and Ba^{2+} form a coordination bond with the oxygen atom of the carbonyl group of **1b** and **1c**, which is directly conjugated with the chromophore part of the molecule. The similar assumption, that the coordination of the carbonyl group with a complexing agent is also possible, was made for the crowned coumarin 153 [5] and was proved later by the observation of a low frequency shift of the vibrational band of the pyrone carbonyl group by 37 cm^{-1} for Mg and Ca-complexes of the crowned coumarin 343 [16]. The absorption and fluorescence spectra of the crowned coumarin **1b**, its protonated form **1h** and complexes **1m** and **1n** in methanol are given in Fig. 3, as typical ones.

The fluorescence quantum yield of **1a** is within 0.2–0.9 depending on the solvent, passing through the maximum in a series "non-polar–polar–protic" solvent [17]. The introduction of azacrown macrocycle in the molecule of **1a** results in a decrease of the fluorescence quantum yield in aprotic solvents more than ten times (Table I).

One of the possible mechanisms of increasing the efficiency of radiationless deactivation upon the

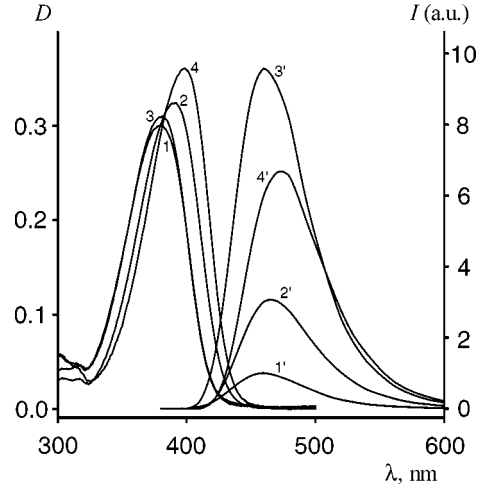


Fig. 3. Absorption (1, 2, 3, 4) and fluorescence (1′, 2′, 3′, 4′) spectra of the compounds **1b**, **1h**, **1m** and **1n** in methanol, respectively. Squares under the fluorescence spectra of the compounds are proportional to its fluorescence quantum yields.

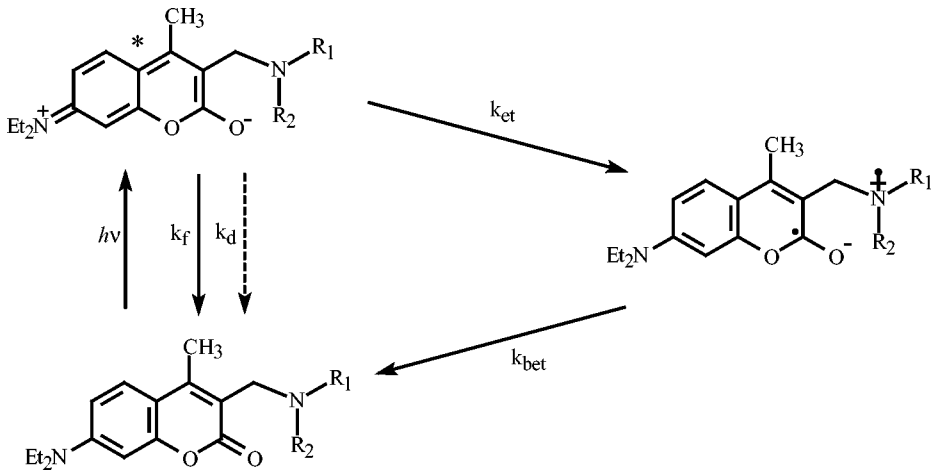


Fig. 4. The scheme of photophysical and photochemical processes in the molecule of 3-alkylamino-7-aminocoumarin. k_d is the rate constant of radiationless deactivation, k_f is the rate constant of radiative deactivation, k_{et} is the rate constant of photoinduced electron transfer, k_{bet} is the rate constant of dark back electron transfer.

introduction of azamacrocycle moiety into the coumarin molecule may be an intramolecular reaction of electron transfer in the excited state from the donor fragment of trialkylamine of 3-substituent to the acceptor fragment of aminocoumarin (Fig. 4). In this case, one can also expect that the fluorescence efficiency of aminocoumarin upon the introduction of any aminoalkyl substituent in the position 3 of aminocoumarin decreases, which was confirmed experimentally (**1g** and **1a**, Table. I). The efficiency of electron transfer must decrease, when the lone pair of nitrogen atom participates in the formation of a coordination bond. According to this, the fluorescence quantum yield of the crowned coumarins **1b**, **1c**, **1d** increases upon protonation, upon complex formation with alkali and alkaline earth metal cations, as well as when passing from aprotic solvents to protic ones.

Since the complex formation of crowned coumarins with metal cations may sometimes lead to a considerable enhancement of their fluorescence, some crowned coumarins can be used as fluorescent chemosensors [5,6,18]. In order to ascertain the possibility of using the crowned 7-coumarins under study as fluorescent chemosensors, the fluorescence spectra of **1c** were recorded at various concentrations of sodium perchlorate in acetonitrile–water mixture (1:1 vol.). As it is shown in Fig. 5, the fluorescence intensity gradually increases up to several times as the concentration of sodium perchlorate grows (while the absorption spectra remain practically unchangeable).

The dependence of the fluorescence intensity of **1c** on the concentration of Na^+ upon the formation of complex with the 1:1 stoichiometry can be expressed by the following equation:

$$\frac{1}{I - I_0} = \frac{1}{K(I_C - I_0)}\frac{1}{C_M} + \frac{1}{I_C - I_0} \qquad (2)$$

where C_M is the concentration of metal cations, K is the stability constant, I is the current value of fluorescent intensity at a certain wavelength, I_0 is the fluorescence intensity of the pure ligand in the absence of complex formation, I_C is the fluorescence intensity under complete complex formation. The best fit parameters for linear regression (2) at the wavelength 460 nm are as follows: $K = 200 \pm 40 M^{-1}$, $I_C/I_0 = 4.2$.

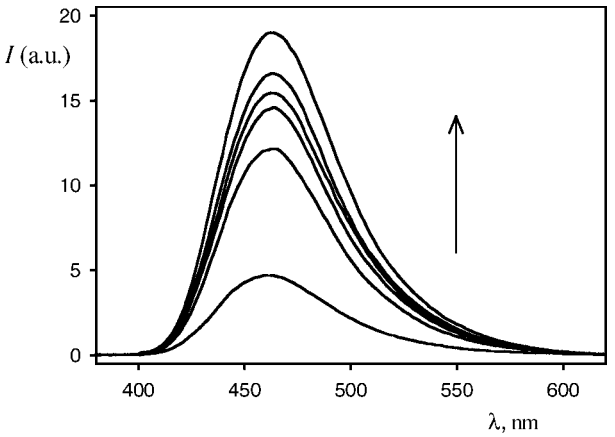


Fig. 5. Uncorrected fluorescence spectra of **1c** in the acetonitrile–water mixture (1:1 vol.) in the presence of $NaClO_4$ at the concentration 0, 0.004, 0.007, 0.014, 0.028 and 0.077 mol/L.

It is known that the fluorescence of 7-aminocoumarins is quenched by a series of organic and inorganic electron donors and electron acceptors [19]. For the donors with the potential of half-wave $E_{1/2}^{ox} < 1.0$ V and the acceptors with $E_{1/2}^{red} > -1.5$ V (relative to the saturated calomel electrode), the fluorescence quenching is limited by the diffusion. Later, the products of photoinduced electron transfer, ion-radicals, having lifetimes 100–200 ps, were found in the transient absorption spectra of 4-(trifluoromethyl)-coumarins in aromatic amines [20,21].

In order to test the assumption that the radiationless deactivation in the crowned 7-aminocoumarins takes place by electron transfer mechanism, the fluorescence quenching in the model system, coumarin 1 (**1a**) and triethylamine in acetonitrile, was studied. The reciprocal value of fluorescence quantum yield increases linearly with the concentration of triethylamine, as shown in the Stern-Volmer plot (Fig. 6). The quenching constant $K = 0.474 \pm 0.009$ M^{-1}. If we assume that the fluorescence lifetime of **1a** in acetonitrile is equal to 3.4 ns [17] and the dynamic mechanism of this quenching, the quenching rate constant is equal to 1.4×10^8 $M^{-1} \cdot s^{-1}$. Upon the fluorescence quenching of **1a** the formation of the encounter complex of the reagents (**1a** $\cdot$ NEt_3), in which electron transfer happens, takes place. We consider this reaction intermediate as a model compound for the electron transfer in the crowned 7-aminocoumarin. The replacement of an azacrown by triethylamine is quite appropriate in this model. In such a way, it has been shown that Stern-Volmer constants for triethylamine and *N*-methylmorpholine in a series of fluorescence quenching of naphthalene and anthracene derivatives are the same within a 25% accuracy [22]. The value of the electron transfer rate

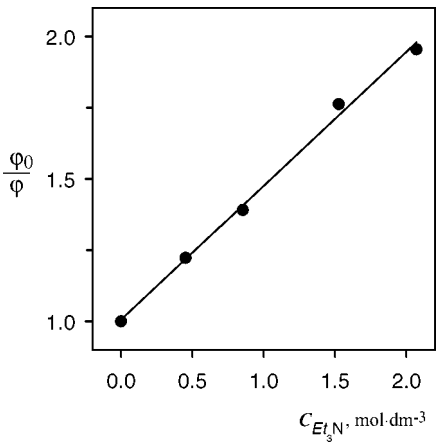


Fig. 6. The dependence of the fluorescence quantum yield (φ) of **1a** in acetonitrile on the concentration of triethylamine (C_{Et_3N}).

constant (k_{et}) in **1a** $\cdot$ NEt_3 is expressed by the following equation:

$$k_{et} = \frac{k_{-d} k_q}{k_d - k_q} \tag{3}$$

Here, k_q is a fluorescence quenching rate constant, k_d and k_{-d} are the rate constants of diffusion formation and decomposition of encounter complex of the reagents, which were calculated according to the Smoluchowski Eqs. (4) and (5), respectively [23],

$$k_d = 4\pi\, r_c (D_D + D_A) N_A \tag{4}$$

$$k_{-d} = \frac{k_d}{V_c} \tag{5}$$

where r_c and V_c are a radius and a molar volume of the encounter complex, N_A is Avogadro constant. The diffusion coefficients of donor and acceptor D_D and D_A were calculated using the Othmer–Thakar relationship [24] between the magnitudes D, molar volume V and solvent viscosity η.

$$D = \frac{1.4 \times 10^{-4}}{V^{0.6} \eta} \tag{6}$$

The rate constant k_{et}, calculated according to the Eqs. (3)–(6) and on the assumption that $V_c = \frac{4}{3}\pi\rho^3 + V_{Et_3} N$, is equal to 1.8×10^8 s^{-1}. This shows that a noticeable decrease, $(1 + k_{et}\tau)^{-1} \approx 1.6$ times, of fluorescence quantum yield of **1a** occurs in a contact pair. The similar picture is observed for the morpholine-substituted compound **1e**, the fluorescence quantum yield of which in acetonitrile is 1.7 times less than for the unsubstituted compound **1a**. A relatively small value of k_{et} in comparison with the typical values of preexponential factor for the intramolecular electron transfer (4×10^{12} s^{-1}, [25]) allows us to conclude that this reaction in **1a** $\cdot$ NEt_3 has an activation barrier. One can expect that when the solvent polarity decreases, the barrier of electron transfer reaction increases, and a temperature change will affect the rate of this process more markedly. Therefore, the fluorescence quenching of **1c** by the temperature change in non-polar solvents (*n*-pentane, decalin (racemate), toluene) was studied. The fluorescence efficiency of **1c** in these solvents increases dramatically as the temperature decreases (Fig. 7). It can be seen that the quenching efficiency does not depend on the solvent nature. The dependence of the fluorescence quantum yield of **1c** on the temperature is described by one common Eq. (7) [26] simultaneously for all three solvents.

$$\frac{1}{\varphi} = \frac{1}{\varphi_0} + \frac{k_d^0}{k_f} \exp\left(-\frac{E_a}{RT}\right) \tag{7}$$

In this expression, k_d^0 and E_a are a preexponential factor and an empirical activation energy of radiationless

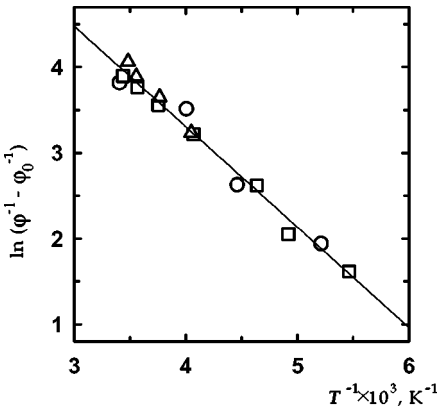


Fig. 7. The dependence of the fluorescence quantum yield (φ) of **1c** on the temperature (T) in various hydrocarbons (*n*-pentane (○), toluene (□), decalin (△)).

deactivation, R is the universal gas constant, T is the temperature, φ_0 is a limiting value of fluorescence quantum yield when $T \to 0$, k_f is a rate constant of fluorescence emission, which is suggested to be temperature independent.

Experimental data fit satisfactorily to the straight line, obtained by the linearization of the expression (7), assuming that $\varphi_0 = 0.82 \pm 0.08$, $k_d^0/k_f = (3.0 \pm 0.3) \times 10^3$, $E_a = 2.32 \pm 0.05$ kcal/mol (Fig. 7). If we estimate the value of k_f on the basis of the measurements of the fluorescence lifetimes in methanol (see below), we shall obtain $k_d^0 = (6.7 \pm 1.0) \times 10^{11}$ s^{-1}. The value obtained for φ_0 within the confidence interval is in agreement with the fluorescence quantum yield of the protonated forms **1h**, **1i**, **1j**, **1k** and **1l** in non-polar solvents in which the reaction of intramolecular electron transfer is suppressed. Accurate within the confidence interval, E_a is significantly different from the activation energies of viscous flow of pentane, toluene and decalin which are equal to 1.44, 2.14 and 3.21 kcal/mol, respectively [27].

It is known that the temperature fluorescence quenching of some donor-acceptor systems such as aminobenzonitrile [28,29], is not caused by the intramolecular charge transfer and occurs according to the mechanism of activated internal conversion and intersystem crossing. If one compares the values obtained for the preexponential factor and the activation energy with the similar values, say, for aminonaphthalenes [26,30] and aminobenzonitriles [28], one might notice that the intersystem crossing is characterized by a very small magnitude of the preexponential factor (k_{isc}^0/k_f is equal to 10 for aminobenzonitriles and to 0.5–5 for aminonaphthalenes) and has a relatively low activation energy ≈1 kcal/mol. The internal conversion is characterized by great values of the preexponent (k_{ic}^0/k_f is equal to 10^5–10^6 for aminonaphthalenes and to 10^4–10^6 for aminobenzonitriles) and a considerable activation energy ($E_a^{ic} = 3$–9 kcal/mol).

The temperature dependence of radiationless deactivation, found for **1c**, might be connected with the inhibition of mobility of macrocycle as a result of the solvent viscosity growth as the temperature decreases. The data obtained show that the fluorescence quantum yield is predominantly determined by the temperature of the system and does not depend on the solvent viscosity. In particular, at the room temperature the viscosities of pentane, toluene and decalin differ within one order (0.24, 0.60 and 2.4 cP, respectively), but the efficiency of radiationless deactivation does not change (the values of fluorescence quantum yield are significantly lower than φ_0 and are equal to 0.02). The independence of fluorescence quantum yield of viscosity indicates that the temperature-activated radiationless deactivation is not connected with the transposition of the molecular fragments relative to each other.

When considering the reaction of electron transfer as a cause of the radiationless deactivation, it is necessary to be sure that the reaction is thermodynamically possible for a series of compounds under study in the solvents used. For this purpose, a change of free Gibbs energy in the reaction of **1a** with triethylamine in polar solvent , i.e. acetonitrile, was estimated. According to the Rehm-Weller equation [31], the change of free Gibbs energy for electron transfer in an excited state is

$$\Delta G_{et} = E_{D/D}^{+} - E_{A/A}^{-} - E_{00} - C \qquad (8)$$

where $E_{D/D}^{+}$ and $E_{A/A}^{-}$ are the electrochemical potentials of donor and acceptor, respectively, E_{00} is an energy of the excited state, C is a Coulomb term, which takes into account the stabilization of reaction products due to their coming together to a finite distance. According to the work [19], $E_{D/D}^{+}$ and $E_{A/A}^{-}$ are equal to 1.1 V and −2.2 V (relative to the saturated calomel electrode) for triethylamine and for coumarin 1 in acetonitrile. The energy of 0-0 transition was estimated as a half-sum of energies of the absorption and fluorescence spectra maxima, and is equal to 3.1 eV. Without taking the Coulumb term into consideration, ΔG_{et} amounts to 0.2 eV. For high-polar solvents, the value C is usually assumed to be equal to ≈0.1 eV [19,32]. Even taking into account this term, the change of free Gibbs energy is slightly positive. This estimation points out that intermolecular fluorescence quenching of coumarin 1 derivatives by aliphatic amines must not occur very efficiently and one may expect that the constant of bimolecular quenching will be considerably less than the diffusion barrier (1.5×10^{10} M^{-1} s^{-1}). Such a result is in agreement with our investigation of the fluorescence quenching of coumarin 1 by triethylamine in acetonitrile. In some cases, however, fluorescence of coumarin 1 can be

quenched very effectively by cyclic tertiary amines, for instance, by 1,4-diazabicyclo-[2,2,2]-octane [33]. This fact matches the low oxidative potential of diazabicyclooctane (0.52 V in acetonitrile [34]).

The occurence of the reaction of electron transfer in the encounter complex of coumarin 1 with aliphatic amines allows us to suggest that a similar intramolecular reaction is also possible in 3-azacrowned coumarins, which leads to fluorescence quenching. It is quite unexpected that the fluorescence quantum yield of the compounds **1b**, **1c**, **1d** and **1e** does not depend practically on solvent polarity in a series of aprotic solvents, and is determined by the size of macrocycle (non-monotonically). In methanol, in spite of the suppression of electron transfer in **1g**, the fluorescence of compounds **1b** and **1c** is quenched noticeably. This fact points out to the circumstance that electron transfer in crowned coumarin occurs inner-spherically, and the solvent is already properly ordered due to macrocycle, and displaced out of the reaction zone to a considerable degree. Such a course of the process is typical for tight encounter complexes, but not for solvent-separated ones, which are observed in the excess of a quencher, when diffusion of reagents to each other is not necessary [35–37].

The independence of quenching efficiency of solvent polarity may be observed also in case when weak intermediate exciplexes are formed.

Formation of intramolecular exciplexes is possible even when the donor and the acceptor are separated by only one methylene bridge [38] (instead of a longer and more flexible chain).

Comparing fluorescence quantum yields in the series **1h**, **1i**, **1j** and **1k**, **1l** in hexane and in THF, as well as in the series **2a**, **2c** and **2b**, **2d** in methanol, one can notice that oligomer substituents themselves cause only weak fluorescence quenching. For instance, the fluorescence quantum yield of azacrowned coumarins with the amidated nitrogen atom of macrocycle is high (0.56 in acetonitrile) and practically remains unchanged upon complex formation [16].

The fluorescence kinetics of crown-ethers **1b**, **1c** and **1d** in methanol is not monoexponential and can be described as the sum of two exponents with the fluorescence lifetimes τ_i and amplitudes c_i. The magnitudes of contribution of the components into the integral fluorescence emission α_i were calculated according to the formula:

$$\alpha_i = \frac{c_i \tau_i}{\sum c_i \tau_i} \tag{9}$$

The values of $\tau_{1,2}$ and $\alpha_1(\alpha_2 = 1 - \alpha_1)$ for the neutral forms **1a**, **1b**, **1c**, **1d**, **1e**, **1g**, for the protonated forms **1h**, **1i**, **1j**, **1k**, the sodium complex **1q** and for the quaternary ammonium salt **1f** in methanol are given in the Table II.

Table II. The Lifetimes τ_1 and τ_2 of the Compounds of the Series **1** in Methanol and the First Emitter Contribution a_1 into Integral Fluorescence Emission

	a_1	τ_1 (ns)	τ_2 (ns)
1a	1.00	1.8	
1b	0.80	0.8	2.9
1c	0.66	2.9	0.4
1d	0.91	3.5	0.8
1e	1.00	2.0	
1f	1.00	0.7	
1g	1.00	3.3	
1h	0.93	1.0	3.6
1i	0.95	0.9	2.2
1j	0.75	0.8	3.0
1k	0.96	0.6	2.5
1q	1.00	2.9	

It can be seen that fluorescence decay becomes monoexponential in case of the compound **1c** upon protonation and complex formation, i.e. upon binding a lone electron pair of the 3-substituent nitrogen atom. The typical kinetic curves of fluorescence decay are given in Fig. 8 and Fig. 9. Essentially non-exponential kinetics of the crown-ether **1c** (Fig. 8) becomes monoexponential after the formation of the sodium complex **1q** (Fig. 9).

The non-exponential character of fluorescence decay of the compounds **1b**, **1c** and **1d** can be accounted for by the existence of the molecules with different conformation of macrocycle in the solution, and, as a consequence, with different rate constant of the intramolecular electron transfer and the lifetime of the excited state. Upon binding of a lone electron pair of the 3-substituent nitrogen atom, photoreaction does not occur and the lifetime of the excited molecule must depend insignificantly on the conformation of aliphatic macrocycle. Another possible explanation of the biexponentiality of fluorescence decay may be the existence of the equilibrium among the molecules with the hydrogen bond between the solvent and the nitrogen atom of macrocycle, and without hydrogen bond in the solution. In this case the long living component can be referred to the complex with hydrogen bond.

If, by analogy with the independence of dipole moment and Onsager radius of the coumarins of the series **1** of the substitution in the position 3, we assume that in this series the rate constant of fluorescence emission k_f is also determined only by the coumarin core, then fluorescence quantum yield φ will be proportional to the square under the fluorescence decay curve, and upon normalizing to the unity of contributions c_i, we can write down:

$$\varphi = k_f \bar{\tau} = k_f \sum_i c_i \tau_i \tag{10}$$

where $\bar{\tau}$ is an averaged fluorescence lifetime.

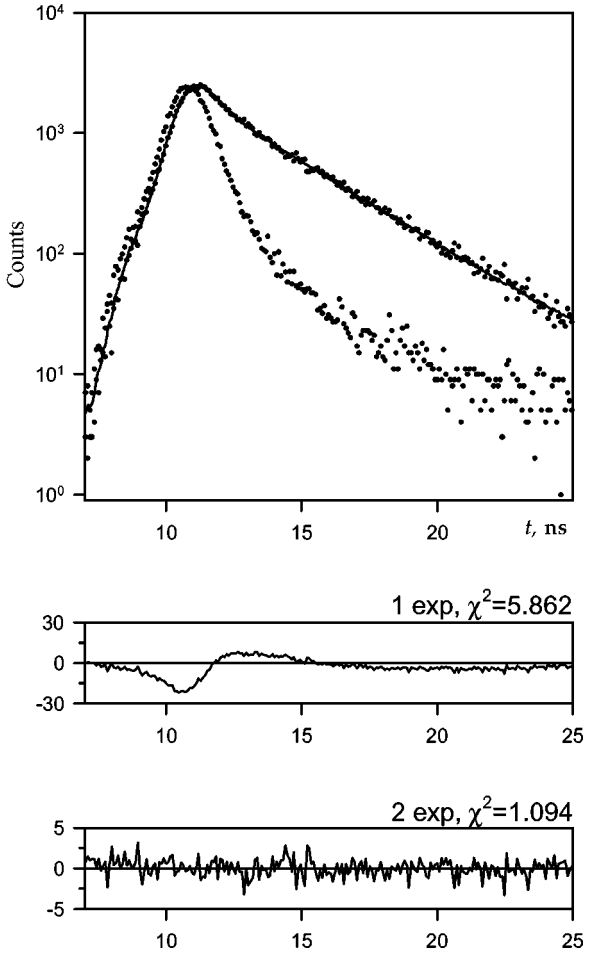


Fig. 8. The fluorescence kinetics of **1c** in methanol.

A relationship between fluorescence quantum yield (φ) of the compounds of the series **1** and the averaged fluorescence lifetime ($\bar{\tau}$) in methanol is shown in Fig. 10. The value of k_f is equal to $(2.2 \pm 0.1) \times 10^8$ s^{-1}.

Thus, for the crown-ethers under study, the ratio of a change of dipole moment upon excitation $\Delta\mu$ to the radius of the Onsager cavity ρ is a constant value. The introduction of macrocycle into the molecule of coumarin 1 does not affect the values $\Delta\mu$ and ρ, the fluorescence quantum yield being significantly diminished, probably, as a result of the photoinduced intramolecular electron transfer from the nitrogen atom of a macroheterocycle to the coumarin fragment, where the excitation is localized. The fluorescence quenching has an activation energy 2.32 ± 0.05 kcal/mol in various hydrocarbons, and does not depend on the solvent viscosity. The fluorescence of coumarin 1 in acetonitrile is quenched by electron donor, triethylamine, with the Stern-Volmer constant (0.474 ± 0.009) M^{-1}. The kinetics of fluorescence

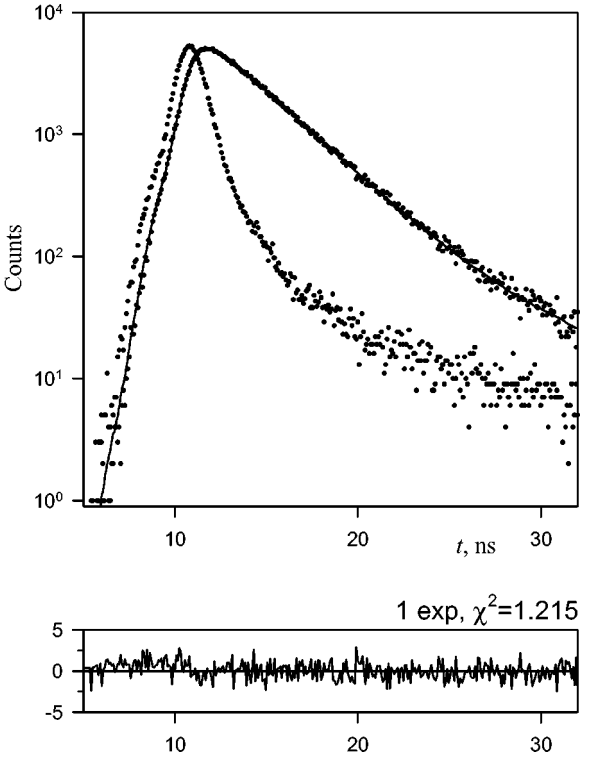


Fig. 9. The fluorescence kinetics of **1q** in methanol.

decay of free crowned coumarins in methanol is non-monoexponential due to the existence of macrocyclic conformers, or due to the formation of complex with a hydrogen bond between the solvent and the nitrogen atom of macrocycle. The efficiency of intramolecular electron transfer in these species is different. The binding of alone electron pair of nitrogen atom of macrocycle upon complex formation with alkali and alkaline-earth metal cations

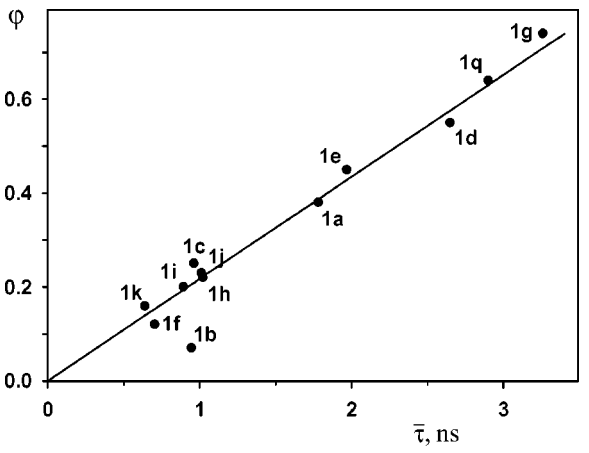


Fig. 10. The correlation between fluorescence quantum yield (φ) of the compounds of the series **1** and the averaged fluorescence lifetime ($\bar{\tau}$) in methanol.

and upon protonation, due to the inhibition of electron transfer, leads to an increase in fluorescence quantum yield. In this case the fluorescence decay becomes monoexponential.

REFERENCES

1. F. Vögtle and E. Weber (Ed.) (1985). *Host Guest Complex Chemistry Macrocycles: Synthesis, Structures, Applications*, Springer-Verlag, Berlin.
2. A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher, and T. E. Rice (1997). Signaling recognition events with fluorescent sensors and switches. *Chem. Rev.* **97**(5), 1515–1566.
3. H.-G. Löhr and F. Vögtle (1985). Chromo- and fluoroionophores. A new class of dye reagents. *Acc. Chem. Res.* **18**, 65–72.
4. A. P. de Silva and S. A. de Silva (1986). Fluorescent signalling crown ethers. 'Switching On' of fluorescence by alkali metal ion recognition and binding in situ. *J. Chem. Soc., Chem. Commun.* 1709–1710.
5. J. Bourson, J. Pouget, and B. Valeur (1993). Ion-responsive fluorescent compounds. 4. Effect of cation binding on the photophysical properties of a coumarin linked to monoaza- and diaza-crown ethers. *J. Phys. Chem.* **97**(17), 4552–4557.
6. J. Bourson, M.-N. Borrel, and B. Valeur (1992). Ion-responsive fluorescent compounds. Part 3. Cation complexation with coumarin 153 linked to monoaza-15-crown-5. *Anal. Chim. Acta* **257**, 189–193.
7. J. R. Lakowicz (1983). *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.
8. W. H. Melhuish (1961). Quantum efficiencies of fluorescence of organic substances—effect of solvent and concentration of fluorescent solute. *J. Phys. Chem.* **65**(2), 229–235.
9. V. L. Lapteva, M. V. Rusalov, V. V. Samoshin, M. A. Kirpichenok, S. I. Druzhinin, B. M. Uzhinov, and N. S. Zefirov (1995). New coumarin-containing fluoroionophores. *Doklady Chem.* **344**(1–3), 202–204.
10. A. Weissberger, E. S. Proskauer, J. A. Riddick, and E. E. Toops (1955). *Organic Solvents. Physical Properties and Methods of Purification*. Interscience Publishers, New York.
11. N. A. Nemkovich, H. Reis, and W. Baumann (1997). Ground and excited state dipole moments of coumarin laser dyes: Investigation by electro-optical absorption and emission methods. *J. Lumin.* **71**, 255–263.
12. E. Lippert (1955). Dipolmoment und Elektronenstruktur von Angeregten Molekülen, *Z. Naturforsch. A* **10**(7), 541–545.
13. W. Rettig and A. Klock (1985). Intramolecular fluorescence quenching in aminocoumarines—identification of an excited-state with full charge separation. *Can. J. Chem.* **63**(7), 1649–1653.
14. S. I. Druzhinin, B. D. Bursulaya, and B. M. Uzhinov (1991). Charged substituents and the effect of their electric-field on the electronic-spectra of some aminocoumarins. *Chem. Phys.* **158**(1), 137–142.
15. A. F. Clifford *et al.* (Eds.) (1964). *International Encyclopedia of Chemical Science*, D. Van Nostrand Company, Toronto.
16. J.-L. Habib-Jiwan, C. Branger, J.-Ph. Soumillion, and B. Valeur (1998). Ion-responsive fluorescent compounds V. Photophysical and complexing properties of coumarin 343 linked to monoaza-15-crown-5. *J. Photochem. Photobiol. A: Chem.* **116**, 127–133.
17. G. Jones II, W. R. Jackson, and A. M. Halpern (1980). Medium effects on fluorescence quantum yields and lifetimes for coumarin laser dyes. *Chem. Phys. Lett.* **72**(2), 391–395.
18. Chao-Tsen Chen and Wan-Pei Huang (2002). A highly selective fluorescent chemosensor for lead ions. *J. Am. Chem. Soc.* **124**, 6246–6247.
19. G. Jones II, S. F. Griffin, Ch. Choi, and W. R. Bergmark (1984). Electron donor–acceptor quenching and photoinduced electron transfer for coumarin dyes. *J. Org. Chem.* **49**, 2705–2708.
20. S. Nad and H. Pal (2000). Electron transfer from aromatic amines to excited coumarin dyes: Fluorescence quenching and picosecond transient absorption studies. *J. Phys. Chem. A* **104**(3), 673–680.
21. S. Nad and H. Pal (2000). Electron transfer from diphenyl and triphenyl amines to excited coumarin dyes. *J. Photochem. Photobiolog. A: Chem.* **134**, 9–15.
22. R. A. Beecroft, R. S. Davidson, D. Goodwin, J. E. Pratt, and X. J. Luo (1986). Quenching of the triplet states of aromatic hydrocarbons by tertiary amines. *J. Chem. Soc., Faraday Trans. 2* **82**, 2393–2397.
23. C. A. Parker (1968). *Photoluminescence of Solutions*, Elsevier, Amsterdam.
24. D. F. Othmer and M. S. Thakar (1953). Correlating diffusion coefficients in liquids. *Ind. Eng. Chem.* **45**(3), 589–593.
25. A. Demeter, S. Druzhinin, M. George, E. Haselbach, J.-L. Roulin, and K. A. Zachariasse (2000). Dual fluorescence and fast intramolecular charge transfer with 4-(diisopropylamino) benzonitrile in alkane solvents. *Chem. Phys. Lett.* **323**(3–4), 351–360.
26. S. I. Druzhinin and B. M. Uzhinov (1982). Proton transfer and nonradiative deactivation in excited ion pairs of *N*,*N*-dimethyl-1-naphthylamine with carboxylic acids. *Theor. Exp. Chem.* **18**(5), 515–522.
27. R. C. Reid, J. M. Prausnitz, and Th. K. Sherwood (1977). *The Properties of Gases and Liquids*, McGraw-Hill, New York.
28. S. I. Druzhinin, Y.-B. Jiang, A. Demeter, and K. A. Zachariasse (2001). Internal conversion with 4-(azetidinyl)benzonitriles in alkane solvents. Influence of fluoro substitution. *Phys. Chem. Chem. Phys.* **3**(23), 5213–5221.
29. S. I. Druzhinin, A. Demeter, V. A. Galievsky, T. Yoshihara, and K. A. Zachariasse (2003). Thermally activated internal conversion with 4-(dimethylamino)benzonitrile, 4-(methylamino) benzonitrile, and 4-aminobenzonitrile in alkane solvents. No correlation with intramolecular charge transfer. *J. Phys. Chem. A* **107**(40), 8075–8085.
30. I. Rückert, A. Demeter, O. Morawski, W. Kuhnle, E. Tauer, and K. A. Zachariasse (1999). Internal conversion in 1-aminonaphthalenes. Influence of amino twist angle. *J. Phys. Chem. A* **103**(13), 1958–1966.
31. D. Rehm and A. Weller (1970). Kinetics of fluorescence quenching by electron and H-atom transfer. *Isr. J. Chem.* **8**(2), 259–272.
32. C. A. M. Seidel, A. Schulz, and M. H. M. Sauer (1996). Nucleobase-specific quenching of fluorescent dyes. 1. Nucleobase one-electron redox potentials and their correlation with static and dynamic quenching efficiencies. *J. Phys. Chem.* **100**(13), 5541–5553.
33. K. I. Priyadarsini and J. P. Mittal (1991). Effect of 1,4-diazabicyclo-[2,2,2]-octane on the laser properties of 7-amino coumarin dyes. *J. Photochem. Photobiolog. A: Chem.* **61**(3), 381–388.
34. S. S. Jayanthi and P. Ramamurthy (1998). Excited singlet state reaction of phenosafranine with electron donors. Role of the heavy-atom effect in triplet induction. *J. Chem. Soc., Faraday Trans.* **94**(12), 1675–1679.
35. Yu. Nagasawa, A. P. Yartsev, K. Tominaga, A. E. Johnson, and K. Yoshihara (1993). Substituent effects on intermolecular electron transfer: Coumarins in electron-donating solvents. *J. Am. Chem. Soc.* **115**(17), 7922–7923.
36. Ch. Wang, B. Akhremitchev, and G. C. Walker (1997). Femtosecond infrared and visible spectroscopy of photoinduced intermolecular electron transfer dynamics and solvent-solute reaction geometries: Coumarin 337 in dimethylaniline. *J. Phys. Chem. A* **101**(15), 2735–2738.
37. H. Shirota, H. Pal, K. Tominaga, and K. Yoshihara (1998). Ultrafast intermolecular electron transfer in coumarin-hydrazine system. *Chem. Phys.* **236**, 355–364.
38. T. Okada, T. Fujita, M. Kubota, S. Masaki, N. Mataga, R. Ide, Y. Sakata, and S. Misumi (1972). Intramolecular electron donor–acceptor interactions in the excited state of (anthracene)–$(CH_2)_n$–(*N*,*N*-dimethylaniline) systems. *Chem. Phys. Lett.* **14**(5), 563–568.