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Proton transfer and supramolecular complex formation between Nile Blue and tetraundecylcalix[4]resorcinarene—a fluorescence spectroscopic study

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The interactions between a calixarene host comprising dissociable protons, and an organic dye guest existing in a neutral base and in a protonated form, have been studied on model systems obtained by dissolving Nile Blue base (**1**) and tetraundecylcalix[4]resorcinarene (**2**) in two apolar solvents, dichloromethane and toluene. Steady state absorption and fluorescence spectroscopy and fluorescence lifetime measurements were applied as experimental methods. It has been established that the equilibrium composition of these systems is controlled predominantly by three reactions: (1) formation of the weakly bound complex $\mathbf{1}\cdot\mathbf{2}$; (2) simultaneous formation of the tightly bound ionic species with zero net charge $\mathbf{1}^+\cdot\mathbf{2}^-$ ($\mathbf{1}^+$ = protonated form of **1**, $\mathbf{2}^-$ = monophenolate form of **2**); (3) protonation of the latter product in a subsequent step yielding $\mathbf{1}^+\cdot\mathbf{2}$. The equilibrium constants for these reactions have been determined from the absorption spectra by an iterative procedure. The high value of the equilibrium constant for reaction (2) in toluene (9.8×10^6) is in accordance with the strongly solvatophobic nature of cation $\mathbf{1}^+$ in this solvent. The fluorescence lifetime of neutral base **1** increases whereas that of its protonated form, $\mathbf{1}^+$, markedly decreases upon complexation. These effects have been interpreted in terms of intra- and intermolecular decay channels.

Introduction

The strategic goal of calixarene chemistry is the synthesis of novel supramolecular receptors capable of selective recognition of charged and neutral species.[1] The calixarene core in these hosts can play two different roles. In the majority of these receptors the macrocyclic skeleton is merely a lipophilic matrix of appropriate conformation holding the properly designed coordination sphere where the ionic guest can be bound through non-covalent interactions.[2] (Some exceptions are known where cation–π interactions between the guest—a soft metal ion[3] or an organic ammonium ion[4]—and the calixarene core take part in the binding process.) The situation is quite different with organic guests, which can penetrate into the calixarene cavity, forming a great variety of inclusion complexes. Of these, the calixarene complexes with 'fluorescent probes'—aromatic hydrocarbons and fluorescent dyes—have received special attention, the properties of which can be very efficiently studied in dilute solutions by combining UV–visible absorption and fluorescence spectroscopic methods.

The first papers in this field were published by Shinkai and co-workers,[5–8] who demonstrated that such experiments provide invaluable information on the nature of the host–guest interaction and on the ring-size selectivity of the host in these systems. Following these studies there has been a growing interest in this research area, as an important part of the investigation of various types of nanocavities. The fluorescent guests studied in supramolecular structures with calixarene (calix[4]arene, calix[6]arene, calix[8]arene and calix[4]resorcinarene) derivatives were 2-anilinonaphthalene,[5] 8-anilinonaphthalene-1-sulfonate anion,[6,8] pyrene,[7–9] pyrene-modified *N*-alkylpyridinium cations,[10,11] 2-(2′-hydroxyphenyl)-4-methyloxazole[12] and the cationic dyes Auramine O,[13] Brilliant Cresyl Blue[14] and Acridine Red.[15]

A great number of dyes, including some of the fluorescent dyes mentioned above, have a basic and at least one (singly) protonated form. As has been pointed out by Shinkai *et al.*,[16] the interaction between these indicator dyes and calixarene based supramolecular receptors in protic solvents can be described by a complicated reaction scheme, in which the proton transfer from the solute to the free and complexed forms of the basic guest is taken into account. The scheme was applied to the analyses of systems formed by *p*-sulfonated calixarene hosts and indicator dye guests, the latter in neutral and in monocationic form (Phenol Blue, Anthrol Blue)[16] or in monocationic and in dicationic form (4-[4-(dimethylamino)styryl]-1-methylpyridinium iodide),[17] in aqueous solutions.

Besides the proton transfer between such dye guests and solvent molecules, an additional type of proton transfer may take place between the guest and the host, if the host comprises dissociable protons. In the present work we studied the role of the latter process in a model system. We have chosen Nile Blue as a dye guest existing in a basic and in a protonated form (**1** and $\mathbf{1}^+$ in Scheme 1, cation $\mathbf{1}^+$ is often referred to as "Nile Blue A") and tetraundecylcalix[4]resorcinarene (**2** in Scheme 2, its monophenolate form will be denoted by $\mathbf{2}^-$) as a host with

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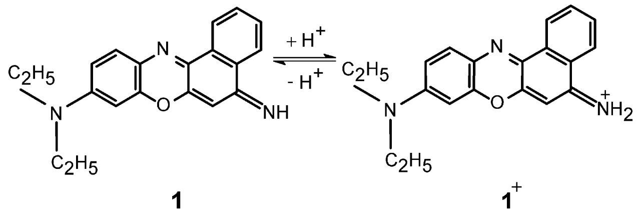
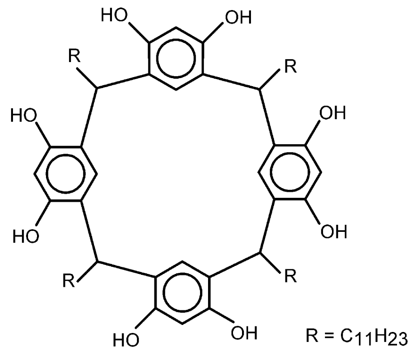
Table 1 Absorption and fluorescence spectral data of Nile Blue A (1^+) perchlorate and of Nile Blue base (**1**)–tetraundecylcalix[4]arene (**2**) mixtures

	Solute composition/M					
Solvent	[1^+]	$[1]_0$	$[2]_0$	Absorption spectrum λ_{max}/nm (ε/M^{-1} cm^{-1})	Fluorescence spectrum λ_{max}/nm (relative fluorescence intensity)	
Dichloromethane					λ_{ex} = 505 nm	λ_{ex} = 585 nm
	2×10^{-6}			647 (97 400)		660 (1160)
		2×10^{-6}		505 (45 200)	579 (886)	
		2×10^{-6}	4×10^{-5}	649 (96 500)	595 (36)	666 (103)
Toluene					λ_{ex} = 490 nm	λ_{ex} = 585 nm
	Satd. soln.[a]			627 (—)		645 (43)
		2×10^{-6}		490 (36 800)	544 (271)	
		2×10^{-6}	4×10^{-5}	647 (55 900)	567 (9)	658 (20)

[a] Estimated concn. ~ 1.2×10^{-7} M.



Scheme 1



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Scheme 2

dissociable protons. Nile Blue is a versatile oxazine dye with many applications in analytical chemistry, photoscience, materials science and molecular biology. The proton addition of neutral base **1** and the reverse process in various types of solvents were the subject of a thorough spectroscopic study by Douhal.[18] The characteristics of the $S_0 \longrightarrow S_1$ transition and of the following deactivation of cation 1^+ in polar solvents are known in detail from our former time-resolved spectroscopic measurements[19–21] and quantum chemical calculations.[22] Calixresorcinarene **2** forms stable hydrogen-bonded complexes with strongly polar guests like sugars and these complexes are soluble in apolar solvents.[23]

In this work, in addition to steady state UV–visible absorption and fluorescence spectroscopy, fluorescence lifetime measurement has also been applied as an experimental method. The recently published results of the first fluorescence decay experiments, carried out on an organic dye guest–calixarene host system, prove that these measurements provide important information on the nature of host–guest interactions.[12]

Results and discussion

Steady state spectra

Solutions of **1** and **2** prepared with various solvents were mixed and the interactions between the two solutes were monitored by comparing the UV–visible absorption spectra of the pure components to those of the mixtures. On dissolving **1** in protic solvents (water, alcohols), we obtained equilibrium mixtures of **1** and its protonated form, 1^+, before adding any **2** to the solutions. In these solvents, as well as in aprotic, polar solvents (*e.g.* acetonitrile), upon the addition of **2** no change in the spectrum was observed. In aliphatic, aromatic and chlorinated hydrocarbons the mixing resulted in substantial changes in the spectrum. Of the latter systems, toluene and dichloromethane solutions were studied in detail. The absorption and fluorescence spectra of pure **1** and of pure 1^+-perchlorate were taken, then the spectra of mixtures with the same initial concentration of **1** (2×10^{-6} M) and various initial concentrations of **2** (from 5×10^{-7} to 4×10^{-5} M) were recorded in the two solvents (1 M = 1 mol dm^{-3}).

The data of the absorption spectra and fluorescence spectra of the solutions of pure **1**, of pure 1^+-perchlorate, and of the mixtures with the highest **2** excess are summarized in Table 1. The variations of absorption and fluorescence spectra with the amount of added **2** are displayed in Figs. 1 and 2, respectively.

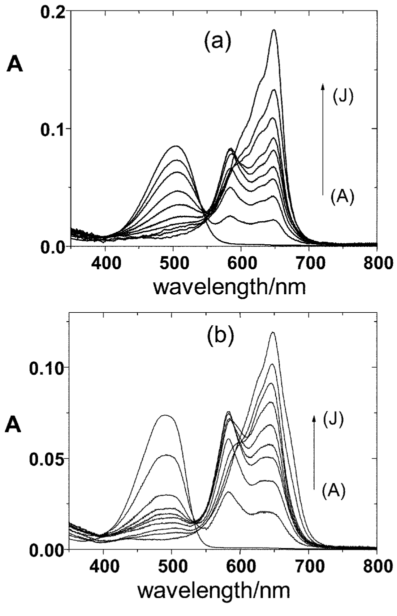

Fig. 1 Absorption spectra of Nile Blue base (**1**)–tetraundecylcalix[4]resorcinarene (**2**) mixtures (a) in dichloromethane and (b) in toluene. Initial concentrations: $[1]_0 = 2 \times 10^{-6}$ M in each solution, $[2]_0$ = (A) 0, (B) 1×10^{-6}, (C) 2×10^{-6}, (D) 3×10^{-6}, (E) 4×10^{-6}, (F) 6×10^{-6}, (G) 8×10^{-6}, (H) 1.2×10^{-5}, (I) 1.6×10^{-5}, (J) 4×10^{-5} M.

These spectra indicate that Nile Blue exists in these systems in at least five different forms: (i) unreacted base **1**, (ii) a supramolecular complex consisting of a neutral host and a neutral guest, **1·2**, (iii) a supramolecular complex of ionic nature, $1^+\cdot2^-$, (iv) complexed cation $1^+\cdot2$ and (v) uncomplexed cation 1^+. The 1 : 1 stoichiometry of $1^+\cdot2^-$ has been directly proved with the help of the 'continuous variations' method. The compositions of the other two complexes could not be determined

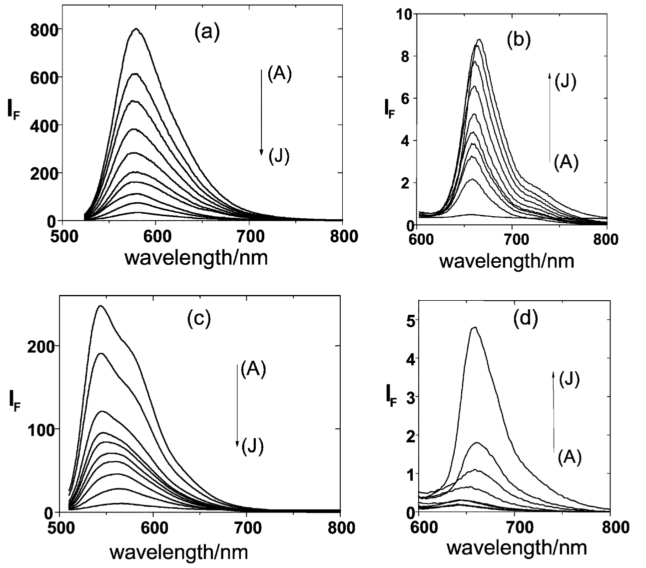

Fig. 2 Fluorescence spectra of **1**–**2** mixtures (a) in dichloromethane, excitation wavelength (λ_{ex}) = 505 nm; (b) in dichloromethane, λ_{ex} = 585 nm; (c) in toluene, λ_{ex} = 490 nm; (d) in toluene, λ_{ex} = 585 nm. Initial concentrations as in Fig. 1.

with simple standard methods, because their formation can be observed only in the presence of the host in high excess. The presence of each complex in the solutions, and their stoichiometries, however, have been established by calculating the equilibrium constants of the occurring reactions from the absorption spectra, by the steady-state fluorescence spectra and by the fluorescence lifetime measurements, the details of which will be given later in this paper.

The position of the absorption band belonging to the Nile Blue base is not sensitive to the addition of **2** to the solutions. The formation of **1·2** is indicated primarily by the fluorescence measurements. The fluorescence band of the base loses intensity and shifts gradually to higher wavelengths with increasing **2** content. In the case of CH_2Cl_2 solutions the maximum of this band, λ_{max}, is located at 579 ± 2 nm in the spectra of all systems with initial calixresorcinarene concentrations $[\mathbf{2}]_0 = 0\text{–}8 \times 10^{-6}$ M, then it is shifted gradually to 595 nm observed at $[\mathbf{2}]_0 = 4 \times 10^{-5}$ M, the highest applied excess of the host. In toluene the corresponding band, appearing at 544 nm in the absence of **2**, shows a clear shift to higher wavelengths even at low **2** contents, *e.g.* it appears at 560 nm in the spectrum of the solution with $[\mathbf{2}]_0 = 8 \times 10^{-6}$ M. As can be seen in Fig. 2c, the shape of the band also changes in the same interval (the shoulder gradually disappears). This is followed by a further moderate shift to 567 nm, as $[\mathbf{2}]_0$ is increased to 4×10^{-5} M. These results suggest that the adduct **1·2** is more stable in a strongly apolar medium, like toluene.

Reliable absorption and fluorescence spectral data for the pure protonated dye, $\mathbf{1}^+$, (in the form of its perchlorate salt) could be obtained only in dichloromethane. The solubility of this salt is very low in toluene and the absorption spectra indicate that a part of the dissolved cations is instantly deprotonated.

The absorption bands arising from free $\mathbf{1}^+$ cation, and from its complexed forms, $\mathbf{1}^+\cdot\mathbf{2}$ and $\mathbf{1}^+\cdot\mathbf{2}^-$, strongly overlap, thus, they cannot be easily separated in the spectra.

The absorption feature with a single maximum around 650 nm, which becomes predominant at high **2** concentrations, has been assigned to $\mathbf{1}^+\cdot\mathbf{2}$. As can be seen in Table 1, the λ_{max} and ε_{max} values for complex $\mathbf{1}^+\cdot\mathbf{2}$ and for uncomplexed cation $\mathbf{1}^+$ in dichloromethane are very close. The corresponding data for the toluene solutions cannot be directly compared, because of the aforementioned poor solubility of $\mathbf{1}^+$-perchlorate in this solvent.

The presence of $\mathbf{1}^+\cdot\mathbf{2}^-$, a supramolecular structure of ionic nature, is indicated by the appearance of two overlapping bands with maxima around 585 and 645 nm in the absorption spectra in Fig. 1. The intensities show that in both solvents the concentration of this species rapidly increases with the amount of the added host up to ~ $[\mathbf{2}]_0 = 4 \times 10^{-6}$ M, the addition of further **2** to the systems leads to the conversion of this product into $\mathbf{1}^+\cdot\mathbf{2}$. The range of the visible absorption of $\mathbf{1}^+\cdot\mathbf{2}^-$ clearly shows the cationic character of its Nile Blue moiety, and through this the ionic nature of the whole supramolecular structure. $\mathbf{1}^+$-perchlorate dissolved in polar or amphiphilic solvents has a single absorption band in the visible range (λ_{max} = 635 nm in water, 628 nm in ethanol, 627 nm in butan-1-ol, 624 nm in chloroform).[24] In contrast, in 1-chloronaphthalene the absorption band (λ_{max} = 646 nm) has a shoulder on the lower wavelength side,[25] the relative intensity of which does not change with the concentration,[18] thus, it does not seem to be related to aggregation. Instead, the splitting may be a consequence of the imperfect solvation of the oppositely charged ions. Nile Blue may be present in the form of tight ion pairs in this rather apolar solvent, in other words, the electrostatic interaction between cation $\mathbf{1}^+$ and the perchlorate anion is expected to be stronger in 1-chloronaphthalene than in more polar solvents. The electrostatic forces may be even stronger between the $\mathbf{1}^+$ and $\mathbf{2}^-$ moieties of the ionic complex in the systems studied, where the solvent molecules are unable to weaken the electrostatic part of the host–guest interactions, which may be the reason for the splitting observed in the spectra in Fig. 1.

As can be seen in Fig. 2d, the fluorescence of the supramolecular systems in toluene is very weak when they are irradiated at 585 nm, where the free and the two complexed forms of cation $\mathbf{1}^+$ absorb strongly. This confirms that the cations are present predominantly in the complexed forms, as $\mathbf{1}^+\cdot\mathbf{2}$ and $\mathbf{1}^+\cdot\mathbf{2}^-$. Probably, neither of the complexes exhibits detectable emission in toluene, the weak fluorescence of the solutions with high excess of **2** may arise from a small amount of free cations.

On the other hand, dichloromethane solutions show fluorescence, when irradiated at the above wavelength. The fluorescence intensity increases with the amount of **2** in the systems, but even in cases of high excess of **2**, where the absorption spectra indicate that almost the whole amount of base **1** is converted to protonated species, it remains weaker by one order of magnitude than that of pure $\mathbf{1}^+$-salts. This suggests that there is only a small amount of strongly fluorescing free $\mathbf{1}^+$ cations in these systems, and—in contrast to the toluene solutions—the complexation of the cations in the forms of $\mathbf{1}^+\cdot\mathbf{2}^-$ and $\mathbf{1}^+\cdot\mathbf{2}$ results in a dramatic reduction of fluorescence, but not in complete quenching.

A substantial reduction of the fluorescence intensity has been reported for the majority of supramolecular systems studied so far, with cationic dye guests and calixarene hosts having dissociable protons,[10,11,14,15] but there are also examples where such systems exhibit fluorescence enhancement.[12,13] (All these systems were studied in aqueous solutions.)

The applied absorption and fluorescence spectroscopic methods do not provide much information about the inclusion type nature of the complexes formed by **1** and **2**. The inclusion of dye molecules by calixarene receptors can be studied efficiently by measuring the shifts in the ^{1}H NMR spectra of the dye moieties upon the addition of calixarenes, as was shown in two papers by Shinkai *et al.*[16,17] The solubility of their hosts, guests and complexes in D_2O was high enough for such measurements. We made an effort to carry out similar experiments by measuring the ^{1}H NMR spectra of **1**, of $\mathbf{1}^+$ (perchlorate salt), of **2** and of **1**–**2** mixtures in two solvents—$CDCl_3$ and CD_2Cl_2—in which the UV–VIS spectra indicate complex formation. These experiments clearly indicated the proton transfer from **2** to **1**, but did not give reliable information concerning the inclusion type structure of the complex. In $CDCl_3$ the solubility of $\mathbf{1}^+$-perchlorate is too low for NMR measurements, in CD_2Cl_2

the solubility of **2** is too low to prepare mixtures which contain at least the 'ionic' complex $\mathbf{1^+{\cdot}2^-}$ in higher concentration.

Reactions and equilibrium constants

In the majority of studies on supramolecular systems the formation of a single supramolecular product is observed, when the stoichiometry of the complex can be justified, and the association constant can be determined with the help of a simple Benesi–Hildebrand plot of the absorbance values or of the fluorescence intensities. Since the spectra revealed the existence of three different supramolecular complexes in our systems, this route could not be followed. Instead, various schemes with parallel and consecutive reactions have been set up and tested by checking if the values obtained for the equilibrium constants for the individual reactions are independent of the composition. The equilibrium concentrations of the reactants and of the products have been determined from the absorption spectra. The spectral data of twelve solutions in each solvent have been taken into account, with the same total Nile Blue concentration of 2×10^{-6} M: solution of pure base **1**, ten mixtures with various amounts of added calixresorcinarene from $[\mathbf{2}]_0 = 5 \times 10^{-7}$ to 8×10^{-6} M, and a mixture with $[\mathbf{2}]_0 = 4 \times 10^{-5}$ M. The spectrum of the latter system has been taken as the spectrum of $\mathbf{1^+{\cdot}2}$. The concentration of the free $\mathbf{1}^+$ cations was taken as zero and the absorption of the free host (in the visible range) was neglected. By application of Beer's law for such a system, the spectrum—the absorbance of a sample in a cell with light-pass λ—can be expressed as a sum of the contributions from the components, which are present in their equilibrium concentrations:

$$A(\lambda) = \{\varepsilon_1(\lambda){\cdot}[\mathbf{1}] + \varepsilon_{1\cdot2}(\lambda){\cdot}[\mathbf{1{\cdot}2}] + \varepsilon_{1^+\cdot2^-}(\lambda){\cdot}[\mathbf{1^+{\cdot}2^-}] + \varepsilon_{1^+\cdot2}(\lambda){\cdot}[\mathbf{1^+{\cdot}2}]\}{\cdot}l$$

The visible absorption spectrum of $\mathbf{1{\cdot}2}$ was taken to be identical with that of **1** for the ten systems involved in the analysis, which seems to be a reasonable assumption at least for dichloromethane solutions, where the effect of the added **2** is insignificant even on the fluorescence behavior. The spectrum of $\mathbf{1^+{\cdot}2^-}$, a component available only in equilibrium mixtures, was obtained at the end of an iterative subtraction of the bands representing **1** (together with $\mathbf{1{\cdot}2}$) and $\mathbf{1^+{\cdot}2}$. The result of this procedure is illustrated in Fig. 3, showing the contributions of the components to the spectrum of two selected systems: one in dichloromethane and one in toluene.

The analysis has led to the conclusion that our systems are governed by three reactions. The weakly bound and the ionic complexes are formed in simultaneous processes

$$\mathbf{1} + \mathbf{2} \rightleftharpoons \mathbf{1{\cdot}2} \tag{1}$$

and

$$\mathbf{1} + \mathbf{2} \rightleftharpoons \mathbf{1^+{\cdot}2^-} \tag{2}$$

The ionic complex is then protonated in a subsequent step:

$$\mathbf{1^+{\cdot}2^-} + \mathbf{2} \rightleftharpoons \mathbf{1^+{\cdot}2} + \mathbf{2^-} \tag{3}$$

The experimental spectra do not provide direct information on reaction (1). As can be seen in Fig. 1, at lower **2** concentrations the formation of the ionic complex—reaction (2)—predominates in dichloromethane and this is the only observable reaction in toluene. As the concentration of **2** is increased, the ionic complex is gradually protonated and in systems with large **2** excess **1** is converted almost completely into $\mathbf{1^+{\cdot}2}$.

Thermodynamically the systems can be characterized by the apparent equilibrium constants K_1, K_2 and K_3 belonging to reactions (1), (2) and (3), respectively

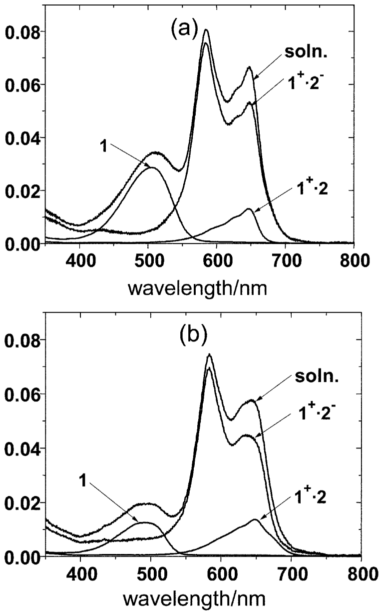

Fig. 3 Resolution of the absorption spectra of a **1**–**2** mixture in (a) dichloromethane and (b) toluene. Initial concentrations: $[\mathbf{1}]_0 = 2 \times 10^{-6}$ M, $[\mathbf{2}]_0 = 4 \times 10^{-6}$ M.

$$K_1 = \frac{[\mathbf{1{\cdot}2}]}{[\mathbf{1}][\mathbf{2}]}, \tag{4}$$

$$K_2 = \frac{[\mathbf{1^+{\cdot}2^-}]}{[\mathbf{1}][\mathbf{2}]}, \tag{5}$$

$$K_3 = \frac{[\mathbf{1^+{\cdot}2}][\mathbf{2^-}]}{[\mathbf{1^+{\cdot}2^-}][\mathbf{2}]}. \tag{6}$$

Denoting the fractions of **1** converted to $\mathbf{1{\cdot}2}$ and to $\mathbf{1^+{\cdot}2^-}$ by α and β, respectively, and the fraction of the latter product converted to $\mathbf{1^+{\cdot}2}$ by γ, the equilibrium concentrations of the products and the reactants are

$$[\mathbf{1{\cdot}2}] = [\mathbf{1}]_0{\cdot}\alpha \tag{7}$$

$$[\mathbf{1^+{\cdot}2^-}] = [\mathbf{1}]_0{\cdot}\beta{\cdot}(1 - \gamma) \tag{8}$$

$$[\mathbf{1^+{\cdot}2}] = [\mathbf{2^-}] = [\mathbf{1}]_0{\cdot}\beta{\cdot}\gamma \tag{9}$$

$$[\mathbf{1}] = [\mathbf{1}]_0{\cdot}(1 - \alpha - \beta) \tag{10}$$

$$[\mathbf{2}] = [\mathbf{2}]_0 - [\mathbf{1}]_0{\cdot}(\alpha + \beta + \beta{\cdot}\gamma) \tag{11}$$

As a first estimation, the value of α (and of K_1) was taken as zero, and the values for K_2 and K_3 were calculated from the concentrations available from the resolved spectra. In the case of dichloromethane solutions, the values obtained for K_2 and K_3 were independent of the composition, which seems to confirm that K_1 is of low value in this solvent. In contrast, in toluene the figures obtained for K_2 and K_3 showed a slight, but systematic variation with the amount of the added **2**. The equilibrium constants for the latter systems were computed in an iteration process, in which K_2 and K_3 were calculated using values for α for the ten systems corresponding to various assumed values for K_1, as long as K_2 and K_3 became independent of the composition of the mixtures.

The calculated equilibrium constants are presented in Table 2. As can be seen, the association constants, particularly that for the formation of $\mathbf{1^+{\cdot}2^-}$ in toluene, are significantly higher

Table 2 Equilbrium constants for reactions in Nile Blue base–tetraundecylcalix[4]resorcinarene mixtures

Solvent	$K_1/10^5\ M^{-1}$	$K_2/10^5\ M^{-1}$	K_3
Dichloromethane	—	7.5 ± 1.3	0.012 ± 0.003
Toluene	2.0 ± 0.5	98 ± 8	0.023 ± 0.005
Chlorobenzene	—	2.8 ± 0.5	0.34 ± 0.06

than the values (with 10^2–10^4 order of magnitude) typical of organic guest–calixarene systems.[6,8,11,13–15,17,26] Equilibrium constants in the range 10^5–10^7 were obtained for the complexation of pyrene by water soluble calixarenes in aqueous solutions.[6,8] It seems plausible that cation $\mathbf{1}^+$, solvatophobic in apolar solvents (like pyrene is solvatophobic in water), forms a very stable complex with anion $\mathbf{2}^-$. The solubility of this complex in apolar media is due partly to the zero net charge, partly to the isolation of the oppositely charged functional groups by the aromatic rings and the long aliphatic chains.

In order to study the behavior of the system in a saturated and in a chlorinated aromatic hydrocarbon, analogous experiments have been carried out in *n*-heptane and in chlorobenzene. In the former solvent, in which the solubility of **2** is poor, the simultaneous formation of the "neutral" (**1·2**) and the "ionic" ($\mathbf{1}^+\cdot\mathbf{2}^-$) complexes could be observed in slow heterogeneous reactions by measuring the absorption and fluorescence spectra repeatedly. In chlorobenzene the system behaved in a similar manner as in dichloromethane. The equilibrium constants for reactions (2) and (3) have been evaluated, their values are also presented in Table 2.

Fluorescence lifetimes

In order to separate the fluorescence emissions of the pure and/or complexed forms of base **1** from those of pure and/or complexed forms of cation $\mathbf{1}^+$, the exciting pulses were produced by two semiconductor lasers, operating at 404 nm (in the absorption range of the former species) and at 632 nm (in the absorption region of the latter species); the emission monochromator was set to wavelengths 545 and 660 nm, close to the fluorescence maxima of neutral and cationic forms of Nile Blue, respectively. Most of the decay curves show a single exponential character. The exceptions are those obtained by exciting the cationic species in dichloromethane, which have been found biexponential, with two strongly different amplitudes. The (dominant) fluorescence lifetimes (τ_F) obtained with the two different excitation and observation wavelengths are presented in Table 3.

Values for τ_F for **1** have not been found in the literature. The observation that the addition of **2** does not influence the rate of fluorescence decay in dichloromethane—τ_F has a constant value of 1260 ± 20 ps—is in accord with the result of the steady-state experiments: reaction (1) does not play an important role in this solvent. In contrast, the lifetimes measured at 545 nm in toluene provide further evidence for the formation of **1·2**. The value for τ_F is 390 ps for pure **1** and 550 ps for complex **1·2**, the latter measured at the highest applied excess of **2**. The decay curves of the solutions with lower **2** concentrations have seemingly also a single-exponential character with lifetimes between the two above values, in other words, the lifetimes of the two co-existing species cannot be obtained separately in the fitting procedure. (Model calculations by Gardini *et al.*[27] demonstrated that the correct values for two relatively close fluorescence lifetimes may not be obtained from the analysis of the decay curves arising from the two coexisting species, instead a single exponential function may give a satisfactory fit.)

Fluorescence lifetimes for cation $\mathbf{1}^+$ in various sets of solvents were reported in Refs. 19,20,28–30. Its lifetime decreases with increasing solvent polarity, from ~3100 ps measured in chloroform to ~400 ps measured in water. Our result for the dichloromethane solution practically coincides with the τ_F value of 2900 ps reported in Refs. 29,30. Lifetimes for $\mathbf{1}^+$-salts dissolved in an apolar solvent such as toluene have not been found in the literature, the value in Table 3 fits the trend observed for decreasing solvent polarity.

The fluorescence of Nile Blue in the presence of **2** in toluene was very weak when the 632 nm laser was applied for excitation, which did not allow reliable measurement of the fluorescence lifetimes of the cationic species in this solvent. In accordance with the steady-state spectra in Fig. 2, much stronger fluorescence was observed when the dichloromethane solutions were excited with this laser, which allowed recording the above mentioned decay curves with double exponential character. As can be seen in Table 3, the value of τ_F belonging to the dominant component decreases with increasing concentrations of the calixresorcinarene up to $[\mathbf{2}]_0 = 8 \times 10^{-6}$ M, and it remains unchanged at higher **2** concentrations. Its lowest value of ~2100 ps can be assigned to $\mathbf{1}^+\cdot\mathbf{2}$. The longer decays observed at lower **2** contents may be associated with the presence of free $\mathbf{1}^+$ cations in low concentrations. The relative amplitude of the minor component increases with the concentration of **2** up to $[\mathbf{2}]_0 = 4 \times 10^{-6}$ M, and shows an opposite trend above this value, suggesting that it originates from the ionic complex $\mathbf{1}^+\cdot\mathbf{2}^-$. The amplitude of this component is too small for

Table 3 Fluorescence lifetimes of Nile Blue base (**1**) and of Nile Blue A cation ($\mathbf{1}^+$) in pure solvents and in Nile Blue base (**1**)–tetraundecylcalix[4]arene (**2**) mixtures

	Solute composition/M			Fluorescence spectrum/ps[a]	
Solvent	$[\mathbf{1}^+]$	$[\mathbf{1}]_0$	$[\mathbf{2}]_0$	**1** $\lambda_{ex} = 404$ nm $\lambda_{ob} = 545$ nm	$\mathbf{1}^+$ $\lambda_{ex} = 632$ nm $\lambda_{ob} = 660$ nm
Dichloromethane	2×10^{-6}				2910
		2×10^{-6}		1260	
		2×10^{-6}	2×10^{-6}	1260	2690
		2×10^{-6}	4×10^{-6}	1240	2430
		2×10^{-6}	8×10^{-6}	1270	2110
		2×10^{-6}	1.6×10^{-5}	1280	2110
		2×10^{-6}	3×10^{-5}	1270	2090
Toluene	$\sim 6 \times 10^{-8}$				3210
		2×10^{-6}		390	
		2×10^{-6}	2×10^{-6}	380	
		2×10^{-6}	4×10^{-6}	390	
		2×10^{-6}	8×10^{-6}	430	
		2×10^{-6}	1.6×10^{-5}	470	
		2×10^{-6}	3×10^{-5}	550	

[a] Estimated errors within ±5%.

accurate determination of the respective lifetime. Its estimated value is ~ 800 ps.

As pointed out in our former study, the fluorescence decay of cation **1**$^+$ has an intramolecular and an intermolecular channel.[21] The predominant intramolecular process is probably the conversion of the electronic excitation energy into the excitation of the closely leveled internal rotation of the diethylamino group. The intermolecular channel is of significance in protic solvents, as it is associated with a fast excited state proton transfer. In the case of **1·2**, the longer decay of the complex, compared to that of pure **1**, may be due to the rigidifying of the diethylamino group by the hydrogen bonds between the host and the guest. In contrast, in **1**$^+$·**2** and particularly in **1**$^+$·**2**$^-$, the decay of the excited **1**$^+$ moiety becomes faster than that of the uncomplexed **1**$^+$ cation, possibly *via* proton transfer from the guest.

Conclusion

The combined application of steady-state absorption and fluorescence spectroscopy and of fluorescence lifetime measurement, to identify the supramolecular products and to determine the equilibrium constants for the reactions occurring in this model system may be helpful in the analysis of many related systems, where proton transfer between the host and the guest may take place. In addition, knowledge of the properties of the Nile Blue–calixresorcinarene complexes in apolar solvents may be interesting in relation to the applications of this dye in more complex apolar environments. This is the case when Nile Blue salts (and in particular, the salts of its lipophilised derivatives) are employed as indicator dyes in optical sensors with hydrophobic polymer matrices;[31] or when they are applied in photodynamic therapy studies, as special photosensitizers which localize in the lysosome of tumor cells.[32]

Experimental

Materials

Nile Blue A perchlorate was purchased from Fluka and used as received. Its basic form, **1**, was prepared by precipitating it from the aqueous solution of **1**$^+$-perchlorate with NaOH, extracting with CH_2Cl_2 and evaporating the solvent. Calixresorcinarene **2** was prepared through the cyclooligomerisation of resorcinol and dodecanal as described in the literature.[33]

Instrumentation

All spectroscopic measurements were carried out at 25 °C. The UV–VIS absorption spectra were recorded using a GBC Cintra 10E spectrometer. The fluorescence spectra were taken by using a Perkin-Elmer LB50 spectrofluorimeter.

Fluorescence lifetimes were measured with the time-correlated single-photon counting technique. The 404 and 632 nm exciting pulses were provided by two Picoquant diode lasers, with pulse duration of 60 and 90 ps, respectively. The emission was collected at a 90° angle through an Oriel 0.25 m monochromator, located in front of a Hamamatsu H5783 photosensor module. The output signal was connected to a Picoquant Timeharp 100 computer board module (channel width 36 ps). Data were analyzed by a non-linear least-squares deconvolution method.

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