Journal of Fluorescence, Vol. 13, No. 3, May 2003 (© 2003)

Ionic Equilibria of Fluorophores in Organized Solutions: The Influence of Micellar Microenvironment on Protolytic and Photophysical Properties of Rhodamine B

N. O. Mchedlov-Petrossyan,[1,2] N. A. Vodolazkaya,[1] and A. O. Doroshenko[1]

Received October 15, 2002; revised December 9, 2002; accepted December 10, 2002

Ionic equilibria and fluorescence decay of rhodamine B were studied in micellar solutions of sodium *n*-dodecyl sulfate (SDS) with various additives (NaCl, pentanol-1, crown ether, and tetra-*n*-butyl ammonium salt), and five nonionic surfactants (Brij 35, nonyl phenol 12, Triton X-100, Triton X-305, Tween 80), as well as in β-cyclodextrin solutions. The apparent dissociation constants, K_a^a, of rhodamine B ($HR^+ \rightleftarrows R + H^+$) were obtained. The distribution of the dye species HR^+ and R in the ultramicroheterogeneous systems was studied using absorption and emission spectra, fluorescence lifetimes, τ, and the plots of pK_a^a versus surfactant concentration. The pK_a^a values under conditions of complete binding, pK_a^{ac}, were found to be markedly higher than that in water (pK_a^w). The medium effects, $\Delta pK_a^{ac} = pK_a^{ac} - pK_a^w$, in organized solutions studied are in accord with the charge type of the acid-base couple +/ ± , confirming the zwitterionic nature of rhodamine B neutral species. Both τ and ΔpK_a^{ac} values of the dye were shown to sense the changes in the micellar microenvironments, and the possibility of using rhodamine B as an interfacial acid-base indicator, for example, for monitoring of surface potentials and of bulk ionic strength, were demonstrated.

KEY WORDS: Rhodamine B; sodium dodecyl sulfate; nonionic surfactants; apparent ionization constant; absorption and emission spectra; fluorescence lifetime.

INTRODUCTION

This paper is aimed to study ionic equilibria and fluorescence emission of rhodamine B in micellar media of sodium *n*-dodecyl sulfate (SDS), as well as in some other organized solutions. The goal of our successive examination of dye partitioning between water and micelles, of its photophysical properties in micelles, of protolytic behavior of the fluorophore in various micellar systems, was to clarify the possibility of rhodamine B functioning as an interfacial acid-base indicator, for example, for surface potentials monitoring. The results obtained allow to predict the changes in the fluorescence and acid-base properties of rhodamine B in organized solutions of various types.

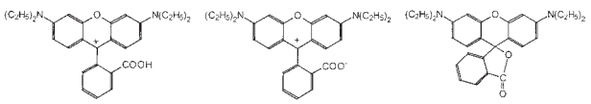
Unique fluorescent properties of rhodamine dyes cause their enormously wide application in various fields of chemistry and physics [1–26]. These dyes are also of significance in biochemistry and related sciences [1,7,11,14,15]. For example, they are utilized as probes for monitoring membrane fusion, for distance determinations in biological aggregates, for site-selective labeling of a cysteine side chain of a muscle protein [7,15], as a main component of fluorosensors for on-line monitoring of ammonia [13], as guest molecules in supramolecular chemistry [3,17,20,21,24], and as components of Langmuir-Blodgett films [16]. Rhodamine dyes are traditionally used for examination of colloid systems, for instance for the study of fluorescence depolarization and excitation transport in micelles, photoinduced intermolecular electron transfer in micelles, and the study of reversed micelles nanosecond dynamics [7,14,22], for studying sol-gel systems [9,23,26] and metallic particles as well [25]. The phenomena of fluorescence polarization of rhodamines

[1]Kharkov V. N. Karazin National University, Kharkov, 61077, Ukraine.
[2]To whom correspondence should be addressed. E-mail: mchedlov@univer.kharkov.ua

1053-0509/03/0500-0235/0 © 2003 Plenum Publishing Corporation

in surfactant solutions is of importance in analytical chemistry and biochemistry [18].

Rhodamine B is one of the mostly used fluorophore, laser dye, and fluorescent analytical reagents [1–14,17, 20,21,23–25]. It exists in solutions as a cation HR^+ and a neutral form R. The latter is represented by an equilibrium mixture of the colored and fluorescent zwitterion $R^{\pm}$ and the colorless lactone R°, which exists in some solvents, but not in water, where the fraction of R° species is too small to be registered experimentally.



HR^+ $R^{\pm}$ R^{o}

One significant feature of rhodamine B requires permanent attention: both the absorption and excitation spectra of the species $R^{\pm}$ and HR^+ are rather similar, at least in water and in water-rich systems. So, in pure water their λ_{max}^{abs} values are 553 nm and 557 nm, respectively, while their molar absorptivities practically coincide (108×10^3 mole^{-1}·cm^{-1}·dm^3). These peculiarities may cause a drawback in interpretation of spectral data in solutions with low buffer capacity or in the presence of surfactant micelles, liposomes, biomolecules, or nanoparticles of various nature, which are known to shift acid-base equilibria of dyes in solutions, and to change their absorption and emission spectra as well.

The equilibria among the above species, as well as protonation of the nitrogen atoms and dimerization of $R^{\pm}$, were subjects for much study [1,2,3,8,27–28]. All the data of such kind are of significance for correct interpretation of photochemical and photophysical phenomena connected with rhodamines [6,8,29–32], especially when they are studied in micellar solutions of colloidal surfactants [4,7,14,22]. In some cases, introduction of surfactants into aqueous solutions of rhodamines dyes facilitate the appearance or enhancement of their spontaneous emission (laser action). However, the influence of these media on protolytic properties of rhodamines is studied only fragmentarily [33].

Micellar solutions of colloidal surfactants in water belong to the most widely used type of organized solutions. At the same time they can be regarded as reduced models of biological systems, because of the existence of water–micelle interfaces and of hydrophobic internal regions of micelles. Therefore we decided to study the acid-base equilibria of rhodamine B and photophysical properties of its cationic and neutral species in micellar media systematically.

Studies of the following equilibrium in water [19,20] and in alcohols [27,34–37]

$$HR^+ \rightleftharpoons R + H^+, K_a \qquad (1)$$

allowed to conclude, that the zwitterion of rhodamine B, $R^{\pm}$, at transfer from water to organic solvents behaves, in the first approach, as a particle with two separate opposite charges [35,37].

A key characteristic of an acid-base indicator couple in micellar and other microheterogeneous media is the so-called apparent dissociation constant (K_a^a) [38–44]. The pK_a^a value is calculated by using the pH value corresponding to the bulk (aqueous) phase, and the ratio of equilibrium concentrations of protonated and deprotonated forms of the indicator, expressed in moles per dm^3 (1 mole dm^{-3} ≡ 1*M*) of the total solution volume, for example, $[HR^+]_t$ and $[R]_t$. The latter values are determined spectrophotometrically.

Dyes of such type are generally believed to be located in the hydrophilic portion of micelles, and their acid-base properties are sensitive to the nature of the microenvironment [38–44]. In the present study, Brij 35, nonyl phenol 12, Triton X-100, Triton X-305, and Tween 80 were used as nonionic surfactants and SDS, $CH_3(CH_2)_{11}OSO_3Na$ as an anionic one. The SDS micellar solutions were modified by adding NaCl, pentanol-1, a crown ether, and a tetraalkyl ammonium salt. We decided also to compare the shift of acid-base equilibrium of rhodamine B, caused by surfactants, with that caused by a cyclodextrin.

EXPERIMENTAL

The samples of SDS (purity 99%), Brij 35, Triton X-100, Triton X-305, and Tween 80, Sigma, were used without further purification. The sample of nonylphenol 12 (NP 12), n–C_9H_{19} – C_6H_4 – O – $(CH_2CH_2O)_{12}H$, was received from Soyuzneftekhimprom, Kazan, Russia. Pentanol-1 was purified through distillation at atmospheric pressure (fraction with t_{boil} 136.5–137°C was used). The samples of β-cyclodextrin (× ca. 9 *M* H_2O), Chinoin, Merk, and Fluka, were kindly put at our disposal by Professors A. I. Grizodoub and S. N. Shtykov. Dicyclohexyl-18-crown-6 ether (DCH-18-crown-6), cis-anti-cis (isomer B) (purity >99%) was purchased from Institute of General and Inorganic Chemistry, Moscow. Rhodamine B was chromatographically pure; its purity was checked also by fluorescence synchroneous scanning spectra. Hydrochloric and acetic acids, as well as tetra-*n*-butyl ammonium iodide, were analytical-grade reagents,

sodium chloride was purified by recrystallization. The standard sodium hydroxide solution was prepared using CO_2-free water and was protected from the atmosphere.

Absorption spectra of the dye solutions were measured using SF-46 apparatus, against solvent blanks. pH determinations were performed at 25.0 ± 0.1°C on a P 37-1 potentiometer and pH-121 pH-meter equipped with ESL-63-07 glass electrode and an Ag | AgCl reference electrode in a cell with liquid junction (1 *M* KCl). Standard buffers (pH 1.68, 4.01, 6.86, and 9.18) were used for cell calibration. The fluorescence spectra were determined on Hitachi F 4010 fluorimeter. The fluorescence decay was studied on a nanosecond pulse fluorometer, and then mathematically treated with the Demas and Adamson phase plane method [45]. The dye concentrations, C_{dye}, were (0.6–9) × 10^{-6} *M*.

The stock solutions of surfactants and indicators were prepared by dissolving weighted amounts of substances in appropriate amounts of water. The nearly transparent working solutions were prepared by the volume method, taking aliquots of stock solutions at 25.0 ± 0.1°C. All spectra were referenced against solvent blanks containing all components except dyes. Suitable pH values of solutions were created by HCl (pH ≤ 3.5) and by mixtures of sodium acetate (0.01 *M*) with acetic acid. The required ionic strength was maintained by addition of NaCl. The theoretically calculated pH values practically coincided with the measured values (±0.02 units) both in the presence and in the absence of surfactants. The sequence of adding stock solutions did not affect the results. The pK_a^a values were determined spectrophotometrically (25°C) according to the standard procedure; C_{dye} = (6–8) × 10^{-6} *M*. The following equation was used for calculations:

$$pK_a^a = p\mathrm{H} + \log\frac{A_\mathrm{R} - A}{A - A_\mathrm{HR}}, \qquad (2)$$

where *A* is the absorbance at the current pH at chosen wavelength and constant dye concentration, and A_R and A_{HR} are absorbances under conditions of complete transformation of the dye into the correspondent form (R, HR^+). Contrary to aqueous solutions and analogous to alcohols [34–36] and other organic solvents [35,37,46], the pH region of HR^+ predominating in micellar systems is very wide, because the ability of the cation to protonation strongly decreases in organic environments. An example of the absorption spectra of the cationic (acid) and the neutral (basic) forms in micellar systems is presented in Fig. 1; a similar resolution of the bands is observed in Brij 35 solutions.

The number of working solutions used for each pK_a^a determination was 5–7. Though 13 wavelengths within the range of 520–580 nm were used as analytical positions, the differences of *A* at 540 nm and 570 nm were utilized for final calculations because the dependence of such a function versus pH is more pronounced. The pK_a^a were determined with confidence interval ±(0.01–0.05). The results were only slightly temperature dependent.

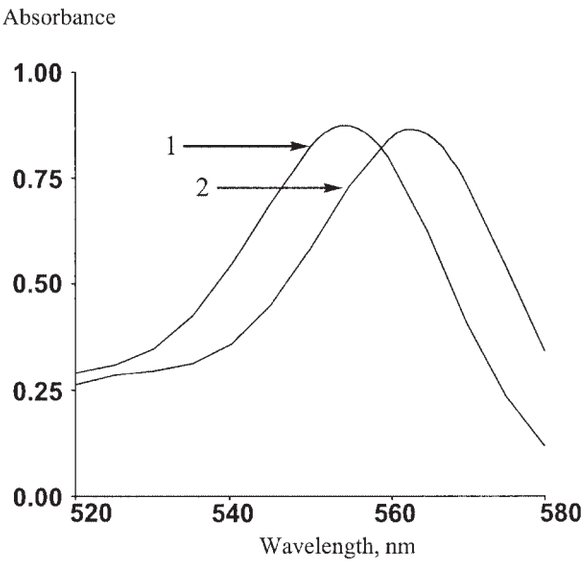


Fig. 1. Absorption spectra of neutral (1), and cationic (2) rhodamine B species in micellar solutions of SDS (0.01 *M*) at ionic strength 0.2 *M* (NaCl); C_{dye} = 8.3 × 10^{-6} *M*; pH = 12 (1) and 1.2 (2).

In micellar solutions of Triton X-100 and Brij 35, at high pH values, the intensity of $R^\pm$ absorption in 3 h after preparation begins to decrease, and in 24 h the color disappears irreversibly, probably as a result of the traces of ethylene oxide. In NP 12 solutions of the dye was stable; in the case of HR^+ solutions (pH ~ 2), the spectra were unchanged in all the systems.

RESULTS AND DISCUSSION

The Completeness of Dye Binding as Checked by Using the Apparent Dissociation Constants

Any discussions on the influence of micellar microenvironment on protolytic properties must be preceded by some necessary tests proving the complete binding of the acid-base couple by the micelles or by the extrapolation of the pK_a^a to complete binding. The so-called binding constants are suitable for description of the dye

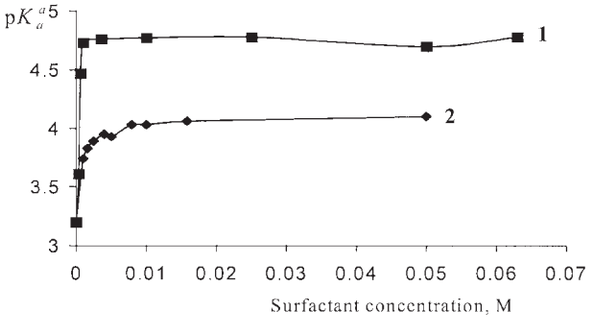


Fig. 2. The pK_a^a dependence of rhodamine B versus surfactant concentration in solutions of sodium dodecyl sulfate at ionic strength 0.2 *M* (NaCl) (1) and in solutions of Brij 35 at ionic strength 0.01 *M* (NaCl) (2).

species partition between the bulk (aqueous) phase and the micellar pseudophase [42,43]:

$$K_{b,i} = \frac{[i_m]_t}{[i_w]_t} \times \frac{1}{C_{\text{surf}} - c.m.c.} \quad (3)$$

here $[i_m]_t$ and $[i_w]_t$ are equilibrium concentrations of corresponding species, bound to the micelles or staying in water, respectively, C_{surf} is the total surfactant concentration, *c.m.c.* is the critical micelle concentration. The $K_{b,i}$ values can be derived from the analysis of the pK_a^a dependences upon C_{surf} using the well-known equation [42,43]:

$$pK_a^a = pK_a^{w\otimes} + \log \frac{1 + K_{b,\text{HR}}(C_{\text{surf}} - c.m.c.)}{1 + K_{b,\text{R}}(C_{\text{surf}} - c.m.c.)} \quad (4)$$

Here $K_a^{w\otimes}$ is the dissociation constant at the given ionic strength in the absence of the surfactant. The linearized formula is more suitable for further consideration:

$$\frac{1-(10^{pK_a^{w\otimes} - pK_a^a})}{C_{\text{surf}} - c.m.c.} = -K_{b,\text{R}} + (10^{pK_a^{w\otimes} - pK_a^a})K_{b,\text{HR}} \quad (5)$$

The dependences of pK_a^a versus C_{surf} are depicted in Figs. 2 and 3. The following values of the binding constants are calculated for rhodamine B species in Brij 35 solutions, using Eq. (5): $K_{b,\text{HR}}{}^{+} = 4 \times 10^3\ \text{M}^{-1}$ and $K_{b,\text{R}} = 5.9 \times 10^2\ \text{M}^{-1}$ (with relative error ±10% and ±14%, respectively), r = 0.987; the *c.m.c.* values (Table I) are taken from literature [44,47–49].

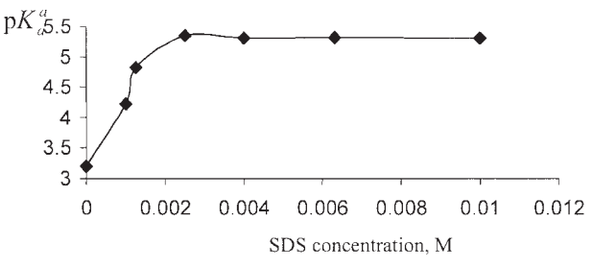


Fig. 3. The pK_a^a dependence of rhodamine B versus SDS concentration, ionic strength 0.05 *M* (NaCl).

Thus obtained K_b values can be utilized for calculation of the pK_a^a value, characterizing the acid-base properties of completely bound dye, pK_a^{ac}:

$$pK_a^{ac} = pK_a^a + \log \frac{(K_{b,\text{R}})^{-1} + C_{\text{surf}} - c.m.c.}{(K_{b,\text{HR}})^{-1} + C_{\text{surf}} - c.m.c.} \quad (6)$$

The pK_a^a value on the plateau of the pK_a^a versus C_{surf} dependence also can be regarded as pK_a^{ac}. For micellar solutions of all the nonionic surfactants the complete binding of both HR^+ and R finally occurs at high enough C_{surf}. At $C_{\text{surf}} = 0.05\ M$ only 0.5% of dye cations and 3% of neutral molecules stay in the bulk, and the deviation of pK_a^a from pK_a^{ac} is ~0.01 unit. The pK_a^{ac} values are presented in Table I; variations in the pK_a^{ac} values within the set of nonionic surfactants do not exceed 0.2 units. The most probable location site of rhodamine B dye in micelles of such a type is the oxyethylene mantle. The internal portion of micelles seems to be less suitable for rhodamine B species: the relatively low K_b values demonstrate that the dye is not hydrophobic enough for solubilization in the hydrocarbon core. As can be seen from K_b values, the cation HR^+ is less hydrated compared with the neutral form, which is in accord with its zwitterionic nature, denoted as $R^{\pm}$.

In SDS solutions, complete binding of the acid-base couple is already reached at C_{surf} values close to *c.m.c.* at the given ionic strength. As pK_a^a values are in these solutions essentially higher than $K_a^{w\otimes}$ it automatically means that $K_{b,\text{HR}}{}^{+}$ is higher than $K_{b,\text{R}}$. And indeed, the negative charge of the micellar surface makes the conditions for adsorption of the cation even more favorable than in the case of nonionic micelles. Studies of rhodamine B diffusion in agarose hydrogel with and without SDS micelles, at pH 7 and 2.5, at ionic strength 0.2 *M*, also confirm a stronger binding of HR^+ species to micelles as compared with $R^{\pm}$ [12]; such interpretation [12] is correct if the diffusivities of the cation and zwitterion are equal.

However, it is necessary to confirm the information on the dye partition in organized solutions under study by using spectral methods as well.

Absorption and Fluorescence Spectra of Rhodamine B and Dye Partition in Micellar Systems

Absorption spectra also provide some information about the binding of rhodamine B to micelles. The bands

Table I. The Apparent pK_a^a Values of Rhodamine B Under Conditions of Complete Binding by Micellar Pseudophase (i.e., pK_a^{ac}), 25°C

Surfactant	C_{surf}, M	Ionic strength of the buffer (M)	*c.m.c. (M)*	pK_a^{ac}	ΔpK_a^{ac}
none	0	$\rightarrow 0$	–	3.22 (pK^w_a)	0
SDS	0.01	0.01	$4.1\cdot10^{-3}$ [a]	5.70 ± 0.03	2.48
SDS	0.002–0.016	0.05	$1.7\cdot10^{-3}$ [a]	5.32 ± 0.03	2.12
SDS	0.001–0.063	0.20	$6.0\cdot10^{-4}$ [a]	4.75 ± 0.05	1.53
SDS, DCH-18-crown-6 (0.005M)	0.02	0.01	–	5.45 ± 0.05	2.23
SDS, pentanol-1 (0.3M)	0.02	0.01	–	5.05 ± 0.02	1.85
SDS, $N(C_4H_9)^+_4$ (0.01M)	0.001	0.005[b]	–	4.33 ± 0.02	1.11
Brij 35	0.001–0.016	0.01	$6.0\cdot10^{-5}$	4.08 ± 0.04[c]	0.86
Brij 35	0.05	0.01	$6.0\cdot10^{-5}$	4.10 ± 0.01	0.88
Brij 35	0.05	0.20	$6.0\cdot10^{-5}$	4.18 ± 0.07	0.96
Nonyl Phenol 12	0.05	0.01	$1.0\cdot10^{-4}$	4.28 ± 0.01[d]	1.06
Triton X 100	0.05	0.01	$2.0\cdot10^{-4}$	4.15 ± 0.03	0.93
Triton X 305	0.05	0.01	$3.0\cdot10^{-4}$	4.22 ± 0.02	1.00
Tween 80	0.05	0.01	$1.2\cdot10^{-5}$	4.17 ± 0.02[e]	0.95
β-cyclodextrin (0.01M)[f]	–	0.01	–	3.69 ± 0.01	0.47

[a]Calculated by using the equation log *c.m.c.* $= -3.72 - 0.74\log[Na^+_w]$[44], which agrees satisfactorily with the equation, reported by others: log *c.m.c.* $= -3.6973 - 0.69823 \log[Na^+_w]$ [49]; the $[Na^+_w]$ values are somewhat higher than the initial ionic strength of the buffer.
[b]Ionic strength of acetate buffer, in addition to 0.01 M $N(C_4H_9)_4I$.
[c]Calculated by using Eq. (6).
[d]$pK_a^a = 4.03$ at $C_{surf} = 0.004$ *M* and ionic strength 0.05 *M* [33].
[e]$pK_a^a = 3.89$ at $C_{surf} = 0.001$ *M* and ionic strength 0.05 *M* [33].
[f]See the text.

of the dye species in micellar solutions (Fig. 1) are shifted as compared with those in H_2O (Table II).

It must be pointed out, that in aprotic solvents with low content of water, 98.3 mass. % acetone and 91.3 mass. % dimethyl sulfoxide, the HR^+ absorption band has the same λ^{abs}_{max} as in water or displays a slight red shift {$\Delta\lambda^{abs}_{max} = 0$ nm and 8 nm, respectively; the values in pure $(CH_3)_2SO$, CH_3CN, dimethyl formamide, and benzene are similar [30]}, while for the R species an expressed blue shift is reported ($\Delta\lambda^{abs}_{max} = -26$ nm and -48 nm,

Table II. Absorption and Emission Maxima and Fluorescence Lifetimes of Rhodamine B HR^+ and $R^{\pm}$ Species in Water and Micellar Media ($C_{dye} = 6 \times 10^{-6}$ *M*)

	λ^{abs}_{max} (nm)	λ^{em}_{max} (nm)	τ (ns)
HR^+			
Water	557	586[a]	1.54
0.01 *M* SDS, 0.2 *M* NaCl	564	590	2.77[b]
0.05 *M* Brij 35	564[c]	593	2.30
$R^{\pm}$			
Water	553	582[d]	1.73
0.01 M SDS, 0.2 M NaCl	555	581	3.57
0.05 M Brij 35	552[e]	582[f]	2.91

[a]At $C_{dye} = 0.6 \times 10^{-6}$ M $\lambda^{em}_{max} = 582$ nm.

[b]At $C_{dye} = 3 \times 10^{-6}$ M $\tau = 2.58$ ns.

[c]In 0.05 *M* Triton X-100 solutions: $\lambda^{abs}_{max} = 564$ nm.

[d]At $C_{dye} = (2–3) \times 10^{-6}$ M $\lambda^{em}_{max} = 580$ nm, at $C_{dye} = 0.6 \times 10^{-6}$ M $\lambda^{em}_{max} = 578$ nm; such λ^{em}_{max} dependence on C_{dye} is in agreement with data published by others [2,3,29].

[e]In 0.05 *M* Triton X-100 solutions: $\lambda^{abs}_{max} = 552$ nm.

[f]in 0.05 *M* Triton X-100 solutions: $\lambda^{em}_{max} = 578$ nm.

respectively) [46]. In methanol [36] and ethanol, *n*-propanol, and *n*-butanol [2,29], blue shifts are reported for both cation (4–5 nm) and neutral form (2–9 nm). Thus the micellar shifts of the absorption spectra are qualitatively similar to those in aprotic solvents and alcohols, respectively.

However, it must be pointed out, that the intensity of absorption bands change insignificantly. Probably, if the aforementioned equilibrium ($R^{\pm} \rightleftarrows R^{o}$) is even shifted toward the right in micellar media as compared with aqueous solution, the fraction of R molecules existing as lactone R^{o} is still negligible. It could be understood, if one takes into account that though the effective dielectric constant of the micellar pseudophase is known to be markedly lower than that of water [38], it is the ability for H-bond formation that is the decisive property of the media, which prevents the lactone formation [28,30,37].[3] As the water–micelle interfaces, where the dye molecules are situated, are sufficiently hydrated, the neutral form is represented by $R^{\pm}$ species. Hence the below pK_a^a values, like pK_a^w values, can be attributed to the dissociation of the COOH group of the cation: $HR^+ \rightleftarrows R^{\pm} + H^+$.

Fluorescence spectra give a more expressed picture: not only the λ_{max}^{em} but the emission intensity changes markedly as a result of binding to micelles (Figs. 4 and 5). At Brij 35 concentration 0.01 *M* the dependence of fluorescence intensity versus C_{surf} (not shown here), like the dependence of pK_a^a versus C_{surf}, reaches the plateau, which also proves the complete binding of rhodamine B species to micelles.

Interestingly, the absorption, excitation, and emission spectra of rhodamine B in the tetraethyl orthosilicate solutions during the sol-gel transition, as well as of the dye, encapsulated in silica gel network, were found to be similar to those of rhodaminc B in neutral and alkali solutions and therefore attributed to the $R^{\pm}$ form [9]. However, as the sol (and then the gel) was prepared by hydrolysis in the presence of HCl, and as here the pK_a^a value could be expected to be higher than pK_a^w, the species HR^+ seem more probable; their spectra within the geleous microenvironment can be close to that of $R^{\pm}$ in water.

Another approach to obtain more versatile information on the dye partitioning and properties in organized solutions is based on the fluorescence lifetimes measuring.

[3]However, the reported predomination of $R^{\pm}$ in glacial acetic acid [28] seems to be improbable, due to conversion into HR^+ [2,30,35]. This is a typical example of errors in interpretation of spectral data, caused by the similarity of the visible spectra of the cationic and zwitterionic species.

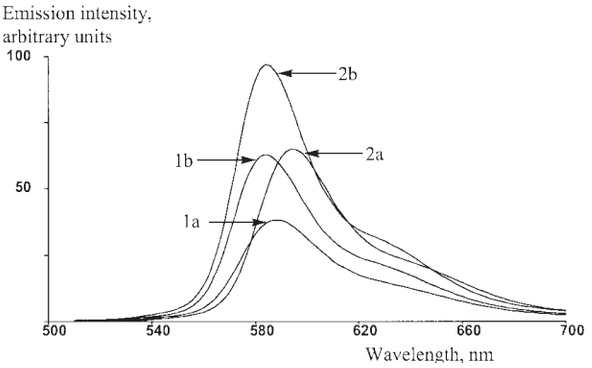


Fig. 4. Emission spectra of rhodamine B species HR^+ and $R^{\pm}$ in water (la, lb) and in micellar solution of SDS (0.01 *M*) (2a, 2b) at ionic strength 0.2 *M* (NaCl); a −pH = 1.2 (HCl); b −pH = 12 (NaOH); $C_{dye} = 6 \times 10^{-6}$ *M*.

Fluorescence Lifetimes of Dye Species Bound to Surfactant Micelles

The binding of the dye species to micelles at $C_{dye} = (5–8) \times 10^{-6}$ *M* is also confirmed by measurements of fluorescence lifetimes. The dependences of τ versus C_{surf} are presented in Fig. 6. The τ values of the species R and HR^+ in aqueous media are 1.73 and 1.54 ns, respectively. In aqueous media, the τ values increase along with decrease in C_{dye}; so, at $C_{dye} = 1 \times 10^{-6}$ *M* τ (R) = 1.88 ns. Our results in general agree with the data, available from literature. For the neutral form the τ values 1.7 [2], 1.67 [4], 1.50 (25°C), and 1.77 (19°C) [7], 1.9 [8], 1.76 [10], 1.5-1.6

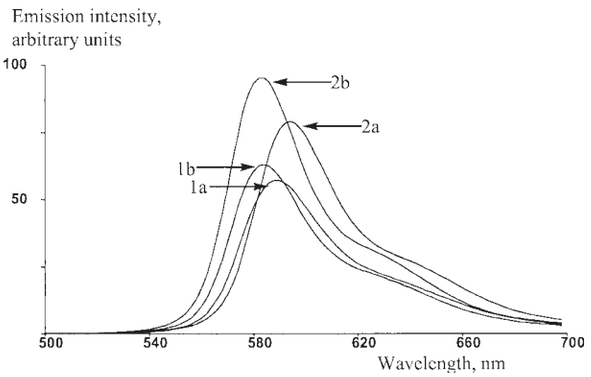


Fig. 5. Emission spectra of rhodamine B species HR^+ and $R^{\pm}$ in water (la, lb) and in micellar solution of Brij 35 (0.01 *M*) (2a, 2b); a −pH = 1.2 (HCl); b −pH = 12 (NaOH); $C_{dye} = 6 \times 10^{-6}$ *M*; ionic strength 0.01 *M* (NaCl).

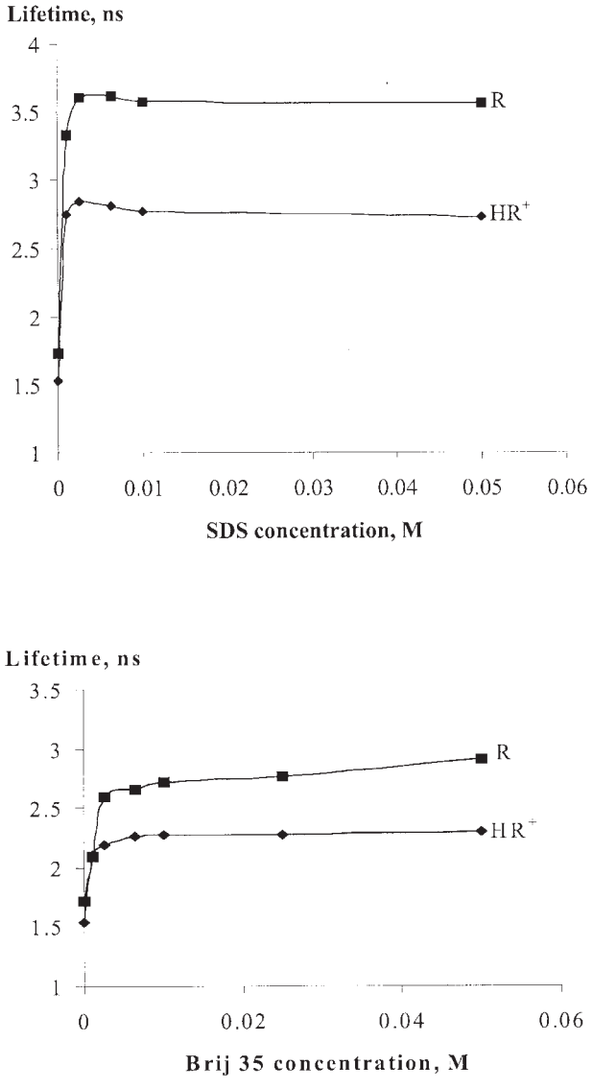


Fig. 6. The fluorescence lifetimes dependence of rhodamine B neutral and cationic species on SDS and Brij 35 concentrations; ionic strength: 0.2 *M* (NaCl) and 0.01 M, respectively; $C_{dye} = 6 \times 10^{-6}$ *M*.

[14], 1.68 [19], and 1.5 ns [29] have been published; for the cationic species the reported lifetimes are: 1.6 [2], 1.7 [8], 1.52 [19], and 1.3 ns [29]. Note that in all cases if measurements are made for both cation and neutral (zwitterionic) forms, the latter show a higher τ value. This regularity is also observed in alcoholic media [2,6,8,19,29], where the τ values are ~1.4-2.0 times higher than in water.

In the given micellar solution, the τ values and fluorescence intensities, registered by us, were always higher for the neutral species than for the cation. On the other hand, under conditions of complete binding to micelles, the τ values of R and HR^+ of rhodamine B are on the average 1.5–2.1 times higher than in water (Table II). Contrary to aqueous solutions, in micellar media the τ and C_{dye} values change symbatically.

The data for $R^{\pm}$ in Triton X-100 micellar solutions, reported in literature, are: $\tau = 3.25$ ns ($C_{dye} = 1 \times 10^{-5}$ *M*) [4] and 2.89 ns ($C_{dye} \leq 1.4 \times 10^{-6}$ *M*) [14]. We obtained the value $\tau = 2.88$ ns in 0.05 *M* Triton X-100 solution at $C_{dye} = 3 \times 10^{-6}$ *M*. In nonionic micelles of Brij 35, under conditions of practically complete binding ($C_{surf} = 0.05$ *M*, $C_{dye} = 8 \times 10^{-6}$ *M*), the following lifetimes were registered: 2.30 ns (HR^+) and 2.91 ns ($R^{\pm}$). Beginning from *C*(Brij 35) 6.3×10^{-3} *M*, the value $\tau(HR^+)$ stays constant, while the τ(R) values continue to increase (Fig. 6), which is in accord with the above $K_{b,\ HR^+}$ and $K_{b,R}$ values. The τ values of $R^{\pm}$ registered by us at equal C_{dye} values in micelles of NP 12, Triton X-100, and Brij 35, coincide within ±1%.

In studies of octadecyl rhodamine B fluorescence anisotropy decay in Triton X-100 micelles [7] along with a short correlation time 2 ns, an extremely long lifetime of 9 ns was registered, reflecting the rotation time of the whole micelle [7].

In general, in dispersed systems the fluorescence decay is frequently fitted by the biexponential function [4,14,26]. However, certain deviations from such behavior could be expected. The nonexponential character might be the evidence of dye aggregation, as in the case of octadecyl rhodamine B in hydrocarbon media [7]. A multi–exponent decay might be caused by the conformational rebuilding, for example, by "butterfly-like" motions of the xanthene moiety in the excited state, resulting in appearance of additional components with $\tau < 0.25$ ns, as for rhodamine B adsorbed on various solid surfaces [5]; according to Kemnitz and coauthors [5], charge transfer effects are less probable for adsorbed dye. Finally, multiplicity of binding sites of dyes in microheterogeneous systems could also manifest itself in the character of their fluorescence [50].

Most of our fluorescence decay curves could be satisfactorily approximated by a single exponent; the "admixture" of an additional exponent with "aqueous" τ value was negligible or not detectable at all. Note that the dye concentration in fluorescence studies was essentially lower than that at absorption spectra measurements. At small surfactant concentrations, close to *c.m.c.*, the reported τ values must be regarded as *averaged lifetimes*.

The dye species in nonionic micelles are probably located in the oxyethylene mantle (see above). However, the τ values for $R^{\pm}$ are somewhat higher in media with oxyethylene and hydroxy groups, than in nonionic micelles; in ethylene glycol $\tau = 3.39$ ns (20°C) [11] and

2.7 ns (28°C) [29], in glycerol: 3.40 ns (25°C), 3.50 ns (19°C) [7], 3.6 (20°C) [2], and 3.61 (5°C) [11]. The reason for such discrepancy is rather evident: the hydrophilic micellar layer contains water molecules as well. In PVA film $\tau(R^{\pm}) = 5.1$ ns [2]; the fluorescence lifetime of 5-iodoacetamidotetramethylrhodamine in PVA films is 3.3 ns, whereas in water it is 2.3 ns [15].

In SDS micelles the τ values are higher than in nonionic micelles or cationic ones ($\tau = 2.10$ ns [14]). The surface of such micelles is known to be rougher [44] and more hydrophilic [39–41, 44] than that of nonionic and cationic micelles. The high hydrophilicity of SDS demonstrates the E_T^N value 0.841, whereas for NP 12 micelles it equals 0.685 [41] (for water $E_T^N \equiv 1.00$). The E_T^N values for micelles formed by cationic surfactants are close to those for nonionic ones. For ethanol and *n*-butanol E_T^N, values equal 0.654 and 0.602, whereas the τ values for rhodamine B $R^{\pm}$ species are 2.7–3.0 and 2.9–3.1, respectively [2,6,8,29].

Note that data on fluorescence decay in anionic microenvironments, reported for $R^{\pm}$ by others, are also rather high. In polystyrene latex, with particles having both hydrophobic domains and anionic sulfate groups, $\tau = 3.56$ ns [10]. In reversed sodium *bis*(2-ethylhexyl) sulfosuccinate micelles in hydrocarbon bulk solvents, the τ value varies from 3.5–3.7 to 2.1 ns, along with the growth of "water pools" and hence with increase in the fraction of "normal" water molecules [7], in accordance with the IR spectroscopy data [51]. In SDS micellar solutions, with and without NaCl, at $C_{dye} = (6.0–8.8) \times 10^{-6}$ *M*, we obtained the value τ ($R^{\pm}$) = 3.55 ns. At $C_{dye} = 1 \times 10^{-6}$ *M* the value τ ($R^{\pm}$) was found to be 3.00 ns; all the results are numerously reproduced. The relatively low value of 2.88 ns in SDS micelles, reported in literature even for somewhat higher concentrations [14], can be explained by interconversion of $R^{\pm}$ into HR^+, because in SDS micelles without salt background the pK_a^{ac} value is 5.7 or even higher. By using the Förster-Weller cycle, the excited state pK_a^{a*} value can be estimated as ~6.3, while the pH value of CO_2-saturated water can be even lower,[4] hence the measured τ value must be attributed to $\tau(HR^+)$, which is usually smaller than $\tau(R^{\pm})$.

Rhodamine B is well known to show a higher lifetime in an adsorbed state than in aqueous solution because the internal rotation of the diethylamino group, which is believed to play the main role in the radiationless decay of this molecule, is suppressed under adsorption [10]. According to Kemnitz *et al.*, the long fluorescence lifetimes of 3–4 ns of adsorbed $R^{\pm}$ at ideal sites seem plausible [5]. However, it is of importance to understand why the τ values are so high on SDS micellar surface though the last-named is more hydrophilic than those of nonionic micelles. We suppose that electrostatic interactions between the positively charged xanthene moiety and the anionic sulfate groups ensure more tight fixation of both HR^+ and $R^{\pm}$ on the negatively charged surface. Both flat orientation of the fluorophore on the surface and its orthogonal insertion into the Stern layer as a "giant" counterion can be assumed. In all the cases the carboxyphenyl residue is with high probability exposed to the bulk aqueous phase. Such character of location is favorable to slower fluorescence decay, compared with that in a more disordered mixture of oxyethylene chains with water molecules. Nevertheless, in nonionic micelles the orientation of the fluorophore toward the micellar interior and the carboxyphenyl substituent toward the water-rich palisade seems reliable. Following the conceptions of Kemnitz [5], deduced for solid surfaces, the surfaces of SDS and nonionic micelles might be qualified as ideal and distorted adsorption sites. However, these pseudophases are rather liquid-crystalline than solid ones. Small (~4%) but systematically reproduced decrease in the τ values in SDS micelles along with C_{surf} growth (Fig. 6) can be caused by the well-known micellar "sphere → rod" transitions, which can in some way influence the dye location.

The above studies have clarified the character of dye partition in the micellar systems. Now it is possible to analyze the protolytic properties of the dye in a completely bound state.

RHODAMINE B AS AN INTERFACIAL ACID–BASE INDICATOR

Thermodynamic pK_a value in water (pK_a^w) equals 3.22 at 25°C [35–37]; further protonation of the cation HR^+, which leads to dication H_2R^{2+} formation, is impossible at pH > 2 and low ionic strength of aqueous solutions. The dimerization does not manifest itself in water, at low ionic strength and rhodamine B concentrations $<10^{-5}$ *M* [27]. In alcohols and other organic solvents, the ability of HR^+ and $R^{\pm}$ species of rhodamine B to dimer formation decreases [35,37]; further protonation of HR^+ is hindered as well [35,37].

Studies of protolytic equilibria in mixtures of water with acetone [35,37] and with dimethyl sulfoxide

[4]And indeed, when an appropriate amount of the indicator hexamethoxy red in its colorless base form was dissolved by us in SDS micellar solution, containing no other additives, the liquid became intensively red colored. The pK_a^{ac} value of this indicator in SDS micelles is close to that of rhodamine B [41].

[37,46,52] demonstrated that the pK_a values are strongly controlled by the shift of the tautomeric equilibrium ($R^{\pm} \rightleftarrows R^{o}$) toward the right. In recent decades a number of papers have been devoted to tautomerism of rhodamines [2,13,15,28,30,32,35–37,46,52]. Basing on our Vis-, IR-, and ^{13}C NMR measurements, as well as on literature data, we estimated that the fraction of rhodamine B molecules R, existing in water as lactone R^{o}, equals ~0.5–1% [35,37,46,52].

For the acid-base indicator completely bound to the hydrophilic palisade of the ionic micelle the following expression for the medium effects ΔpK_a^{ac} is valid [38–44]:

$$\Delta pK_a^{ac} = pK_a^{ac} - pK_a^{w} = \log\frac{\gamma_R}{\gamma_{HR}} + \log\frac{f_R^m}{f_{HR}^m} - \frac{\Psi F}{2.3RT} \quad (7)$$

Here γ are activity coefficients of transfer from infinitely diluted state in water to micellar phase, f^m are concentration activity coefficients of the species, bound in the Stern region, and depending on the bulk ionic strength (as a rule, the ratio f_R^m/f_{HR}^m is supposed to be equal to unity), ψ is the electric potential of the charged surface (Stern region), R is the gas constant, and T is the absolute temperature. In the case of nonionic surfactants the term containing ψ is negligible.

As a rule, the signs of the medium effects for cationic acids in nonionic micelles ($\Delta pK_a^{ac} < 0$) coincide with those registered while going from aqueous solutions to water–organic mixtures [39–42, 44]. For example, in 0.02-M solutions of NP 12 for dyes, possessing pK_a^{w} values, which are close to that of rhodamine B, hexamethoxy red ($pK_a^{w} = 3.1$) and methyl yellow ($pK_a^{w} = 3.25$), medium effects, ΔpK_a^{ac}, are equal to -0.88 and -1.85, respectively [41]. However, the essentially positive ΔpK_a^{ac} value (on average, 0.95; Table I) for rhodamine B is not surprising; the neutral form R both in micelles and in water is represented by the zwitterion $R^{\pm}$ (see above). Hence, the "charge type" of the acid-base couple [53] is $+/\pm$, not $+/0$. Therefore semiquantitative estimations [53] allow prediction of the similarity of ΔpK_a values to those for acids with charge type $0/-$. Thus the positive ΔpK_a^{ac} can be expected. For instance, the difference between pK_a^{ac} of decyl eosin in Tween 80 micellar solutions (2.61) and pK_a^{w} of ethyl eosin (1.9) equals 0.7 [44]. Thus the experimental results for rhodamine B are in agreement with the charge type $+/\pm$.

In SDS micelles, the medium effects of all the above dyes are much close; at ionic strength 0.05 M the ΔpK_a^{ac} values are equal to 2.12 for rhodamine B, and 2.63, 2.37, and 1.56 for decyl eosin, hexamethoxy red, and methyl yellow, respectively. The reason may be the aforementioned strong hydration of the SDS micellar surface, the logarithmic terms with γ values Eq. (7), reflecting the individual features of the dyes in the given media, are closer to zero than in nonionic micelles, and the total increase in the pK_a^{ac} values is governed mainly by the electrostatic contribution. The latter is probably rather similar for dyes of various type.

The widely used method of estimation of the micellar or vesicular surface potentials is based on the assumption of the equality of the combination of the activity coefficients of the given dye in nonionic and ionic micelles [38–41, 44, 54]. At 25°C Eq. (8) can be used:

$$\Psi, \text{ mV} = 59.16 \times \{pK_a^{ac} \text{ (in nonionic micelles)} - pK_a^{ac} \text{ (in ionic micelles)}\} \quad (8)$$

However, in such case the ψ values thus obtained may vary along with the nature (namely, charge type) of the chosen indicator [38–41,44,54]. For example, in the SDS micellar solution at ionic strength 0.05 M (NaCl + buffer) the calculated Ψ values are -201 mV, -192 mV, and -114 mV for methyl yellow, hexamethoxy red, and decyl eosin, respectively [41,44]. Such discrepancies are known to be typical [38–41,44,54], and are explained in terms of differences in solvation of the acid-base couples in different micelles [38,41,44] or ion pair formation in ionic micelles [38–40,54]. As in the case of SDS micelles the ion association can be expected for the cationic indicator species, some authors [39,40,54] predict systematic errors if cationic indicators (+/0) are applied.

In this context, rhodamine B may serve as a unique probe. If stable ion associates between cationic xanthene moiety and dodecyl sulfate really exist, they are probably similar for both HR^{+} and $R^{\pm}$. On the other hand, the fluorescence lifetimes (compare τ values in Table II) allow supposition that the changes in the location and solvation while going from nonionic to SDS micelles are similar for these two species. Using the pK_a^{ac} values of 5.32 in SDS micelles and 4.17 (average value for nonionic surfactants), one can obtain from Eq. (8) a value $\Psi = -68$ mV for ionic strength 0.05 M. Theoretical Ψ calculations for SDS micelles by using the approximate solution of nonlinearized Poisson-Boltzmann equation, obtained for spherical particles by Oshima, Healy, and White [54,55], are in line with our result. If 0.60 nm^2 is assumed as the most probable value of the area of the surfactant head group (the mean value between 0.663 nm^2 [49] and 0.518 nm^2 [56]), 2.0 nm as the micellar radius [54] and 0.25 as the degree of surfactant dissociation in micelles [44,54,57–59], the calculated Ψ value equals -67 mV.

Thus, rhodamine B can be proposed for monitoring electric potentials of negatively charged surfaces of

colloidal particles, which are not so well defined as SDS micelles and therefore cannot be theoretically calculated. Further studies have to show to what degree the fluorescent properties of rhodamine B can be exploited for the purpose of Ψ estimation.

In addition, taking into account a lot of new publications concerning the behavior of rhodamine B as a guest molecule in supramolecular chemistry [17,20,21,24], the acid-base properties of the dye in organized solutions of another type were examined by introduction of a receptor molecule into the aqueous solution.

Interaction of Rhodamine B with β-Cyclodextrin in Water

It is known [3,17,20,21,60] that rhodamine B interacts strongly with β-cyclodextrin (β-CD); the polarity of the microenvironment of the CD cavity is known to be commensurable with those of ethanol [61,62]. Therefore we decided to reveal the influence of this type of the nonionic microenvironment on the acid-base properties of rhodamine B, using the maximal possible concentration of this macrocyclic compound. From the result obtained (Table I) it is evident that the HR^+ form is stronger associated with β-CD than is R. Hence, in this sense, the binding character is similar to that in micellar solutions of surfactants. Assuming that the reported value of the association constant $2.9 \times 10^3\ M^{-1}$ [3] refers to the R species, one can estimate the corresponding value of $8.8 \times 10^3\ M^{-1}$ for HR^+. The values of the association constant of rhodamine B neutral form, determined recently at pH 7.2 for β-CD ($4.2 \times 10^3\ M^{-1}$) [20] as well as for its derivatives (1.2×10^3 to $2.0 \times 10^3\ M^{-1}$) [17] are in agreement with the earlier data [3]. Hence, both dye species are completely associated with β-CD, and $pK_a^a = pK_a^{ac}$. Modest alterations of the absorption spectra (1-nm red shift for HR^+, 2-nm blue shift for R), compared with the spectra in water, are in agreement with the reported absorption and fluorescent spectra [3,17,20], giving evidence for weak changes in the character of solvation. The ΔpK_a^{ac} value with 0.5 units is also rather small compared with nonionic micelles. However, such conclusion is valid only if the dye binding (association with β-CD) is really complete, whereas the details of the procedure for association constant determination are not given in the cited paper [3]. The cavity of β-CD is large enough (0.62–0.65 nm) for the interaction with groups $-C_6H_4COOH$ and $-C_6H_4COO^-$. However, taking into account the results reported by Flamigni [61,62] for interaction of hydroxyxanthenes with various cyclodextrins, no less expectable is the inclusion complex formation of β-CD with diethylamino group containing moieties of rhodamine B.

In aqueous media at $C_{dye} = 3 \times 10^{-6}$ M, where $\tau(R^{\pm})$ equals 1.88 ns, the introduction of 0.01 *M* of β-CD leads to decrease in fluorescence lifetime down to 1.36 ns; practically the same value of 1.38 ns is registered under such conditions for $\tau(HR^+)$. This result is in accord with the well-known phenomenon of rhodamine B fluorescence quenching in dilute dye solutions in the presence of β-CD and its derivatives [17,20,60], including the system $C_{60}-\beta-CD$ + rhodamine B in 80% aqueous CH_3CN.

Some authors [20] explain the fluorescence quenching in terms of the shift of the tautomeric equilibria $R^{\pm} \rightleftarrows R^{\circ}$ toward the right, as a result of stabilization of the hydrophobic, nonfluorescent lactone by inclusion complexation. However, the alterations of the absorption spectra are rather small (decrease in intensity by ~2%) even under conditions of complete binding of the dye with the receptor, and thus the fraction of neutral species, converted into the colorless lactone, is to first approximation negligible. Then our measurements of lifetimes demonstrate that the state of fluorescing species differ from that in pure water. Thus the decrease in the τ value of the $R^{\pm}$ tautomer resulting from complexation seems to be more reliable than the decrease in the fraction of the molecules existing in zwittertionic state. On the other hand, the registered decrease in $\tau(HR^+)$ values cannot be explained in terms of lactonization. Finally, even the rough estimations show the energy of the excited lactone of rhodamine B to exceed that of zwitterion to such an extent that the lactonization in the excited state seems to be completely improbable.

Salt Effects on the Dissociation of the Adsorbed Dye

The variation of the ionic strength in the solutions of nonionic surfactants displays very small changes in the pK_a^{ac}, whereas in the case of SDS the salt effects are pronounced (Table I) because of the screening of the micellar surface charge. The combination of the electrostatic and pseudophase ion-exchange models allows prediction of the linearity of the plots of pK_a^{ac} versus logarithm of the equilibrium bulk concentration of the counterion [44]:

$$pK_a^{ac} = B - b\log[Na_w^+]. \qquad (9)$$

Generally, the slope *b* is close to the degree of neutralization of DS^- with sodium cations in the Stern region. The $[Na_w^+]$ values were calculated by using the well-known relation [44]:

$$[Na_w^+] = C_{Na} + (C_{surf} - c.m.c.)(1 - b) + c.m.c., \qquad (10)$$

Here C_{Na} values are equal to the sum of sodium chloride and alkali analytical concentrations in the buffer

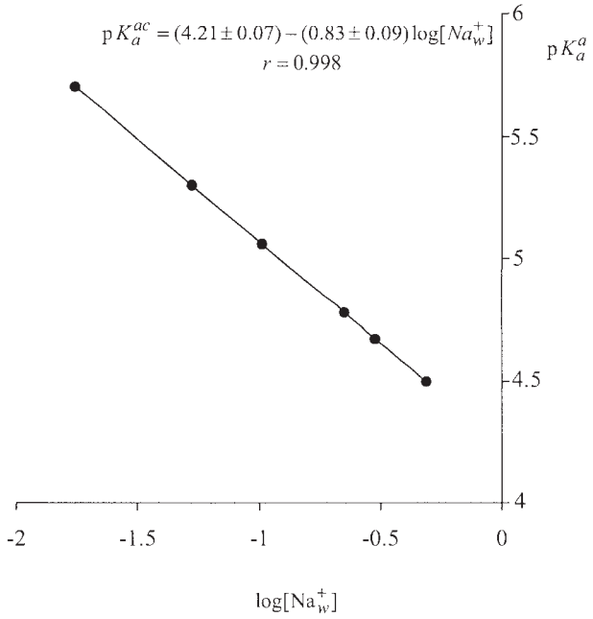


Fig. 7. The pK_a^{ac} dependence of rhodamine B in micellar solution of SDS (0.01 *M*) versus logarithm of Na^+ concentration in the bulk phase.

solutions, *c.m.c.* refer to the given C_{Na} value. For first $[Na_w^+]$ calculations *b* was equated to 0.8, and then refined by using Eqs. (9,10). The results of our experiments are depicted in Fig. 7. The *b* value for rhodamine B (0.83 ± 0.09) is an average between those for indicators with charge type 0/−(decyl eosin: 0.73 ± 0.04 [44]; 4-heptadecyl-7-hydroxycoumarin: 0.77 ± 0.05, calculated by using the data of Hartland *et al.* [54]) and for common cationic indicators (methyl yellow: 0.91 ±0.03; hexamethoxy red: 0.91 ± 0.03; quinaldin red: 0.92 ± 0.07 [41,44]). As for now, the most probable dissociation degree of SDS in micelles is 0.22–0.28 [57–59]. It corresponds to the value $b = 0.72$–0.78; the analysis of *c.m.c.* values at various $[Na_w^+]$ have recently allowed us to obtain the value 0.74 ± 0.04 [44].

Besides NaCl additives, the introduction of some organic substances was examined by us in order to reveal the character of rhodamine B response to such modifications of SDS micelles.

Modification of the Properties of SDS Micelles by Various Additives as Detected with Rhodamine B as an Interfacial Acid–Base Indicator

Some organic additives are known to strongly modify the surface of SDS micelles [41,43,44,54,63–66]. They are (i) alcohols with limited solubility in water (e.g., pentanol-1), (ii) crown ethers (e.g., DCH-18-crown-6), which form complexes with Na^+ ions, and (iii) tetraalkyl ammonium ions [e.g., $N(n-C_4H_9)_4^+$]. Our study revealed that the above additives influence the pK_a^{ac} value of rhodamine B (Table I) to essentially a lower degree, compared with the pK_a^{ac} of other indicators. So, introduction of pentanol-1 (0.3 *M*) decreases the pK_a^{ac} value of rhodamine B by 0.65 units, whereas those of decyl eosin and methyl yellow, by 1.0 and 1.7 units, respectively [41,44,46]. 0.005 *M* of DCH-18-crown-6 displays an even smaller decrease (0.25 pK_a^{ac}units), whereas for the above-mentioned two indicators the decrease in pK_a^{ac} is 1.17 and 0.88, respectively [44]. Even the effect of the hydrophobic counterion $N(n-C_4H_9)_4^+$ is, in the case of rhodamine B, less expressed (pK_a^{ac} decreases by 1.37 units) than for methyl yellow (2.25 units) [63].

All these additions act through two main mechanisms: (i) lowering of $|\Psi|$ as a result of decrease in the surface charge density, automatically leading to pK_a^{ac} decrease, in accord with Eq. (7); (ii) and dehydration of the SDS micellar surface [41,44,63–66].

The electrostatic effect can be regarded, at the first approach, as identical for all the indicators situated within the Stern region. Contrary to it, the second effect—drying action—must display essentially varying impacts, depending strongly on the chemical nature and charge type of the acid-base couple. In other words, the variations in the γ_R/γ_{HR} quantity [Eq. (7)] are to be different. Such an explanation seems reasonable, if we try to mimic qualitatively the water–micelle interface by water-organic mixed solvents. And really, the ΔpK_a values ($=pK_a - pK_a^w$), registered in water-organic mixtures, are higher for rhodamine B, than for methyl yellow and decyl (ethyl) eosin [44].

Modification of the Properties of SDS Micelles by Various Additives as Detected with Rhodamine B as a Fluorescent Probe

Finally, we examined the alterations of fluorescent properties of rhodamine B, accompanying the above pK_a^{ac} changes. In Table III, the emission maxima, fluorescent intensities, and lifetimes of cationic and zwitterionic dye species in various micellar solutions are compiled. The photophysical properties of both HR^+ and $R^\pm$ appear to be sensitive to the aforementioned alterations in the SDS micellar structure. The strong impact of the hydrophobic cation is in accord with numerous evidences of its disturbing action upon SDS micelles [67,68]; hence the strong lowering of $|\Psi|$ is here also not surprising. At the concentrations chosen the additives are to be arranged in accordance with their influence upon fluorescence lifetimes as follows: crown ether < pentanol-1 < $N(n\text{-}C_4H_9)_4^+$.

Table III. Emission Maxima, Relative Fluorescence Intensities and Lifetimes of Rhodamine B HR^+ and $R^\pm$ Species[a] in SDS Micellar Media with Various Additives

	HR^+			$R^\pm$		
Media	λ_{max}^{em} (nm)	I_{rel}[b]	τ (ns)	λ_{max}^{em} (nm)	I_{rel}[b]	τ (ns)
0.02 *M* SDS	588	85	2.45	578	155	3.00[c]
0.01 *M* SDS + 0.2 *M* NaCl	590	—	2.58[d]	584	—	3.27[e]
0.02 *M* SDS + 0.005 *M* DCH-18-crown-6	590	71	2.28	580	126	2.81
0.02 *M* SDS + 0.3 *M* pentanol-1	588	67	1.83	579	95	2.27
0.001 *M* SDS + 0.01 *M* $N(C_4H_9)_4^+$	591	57	1.80	579	97	1.73

[a]At pH 2 (HCl) and 12 (NaOH), respectively; $C_{dye} = 1 \times 10^{-6}$ *M*.
[b]Arbitrary units.
[c]At $C_{dye} = 3 \times 10^{-6}$ *M* $\tau = 3.16$ ns.
[d]$C_{dye} = 3 \times 10^{-6}$ *M*; at $C_{dye} = 6 \times 10^{-6}$ *M* $\tau = 2.77$ ns.
[e]$C_{dye} = 3 \times 10^{-6}$ *M*; at $C_{dye} = (6.0–8.8) \times 10^{-6}$ *M* $\tau = 3.55$ ns.

Interestingly, the sequence is the same as for the influence upon the pK_a^{ac} values of rhodamine B (see above). The penetration of relatively hydrophobic additives into the micellar interior causes the above mentioned surface charge shielding and drying action on the pK_a^{ac} values. It is reasonable to suppose that the decrease in the τ values reflects first of all the increase of the flexibility of the micellar microenvironment of the fluorophore. This confirms the model of tight fixation of the positively charged xanthene moiety in the "pure" (i.e., without organic additives) SDS micelles.

CONCLUSIONS

In this paper, protolytic equilibria of rhodamine B in organized solutions are systematically studied; a set of pK_a^a values are obtained. These new data allow distinction between the micellar effects upon the photophysical properties of HR^+ and R species of the dye, to evaluate the binding constants of these species and to reveal the response of rhodamine B photophysical properties to nonpolar additives to SDS micelles.

Both the photophysical properties and the state of the protolytic equilibrium $HR^+ \rightleftarrows R + H^+$ of rhodamine B in aqueous surfactant solutions are governed (i) by (possible) incomplete binding of dye species, (ii) through specificity of pK_a^a shifts direction in micellar media, and (iii) by effects of additives to micellar systems.

The increase in fluorescence lifetimes of cationic and zwittcrionic species in micellar media is maximal in SDS micellar solutions without hydrophobic additives, the τ values become 2 times higher than in water. The colorless lactone of rhodamine B does not appear in substantial amounts in micellar media.

The values $\Delta pK_a^{ac} = pK_a^{ac} - pK_a^w$ in surfactants micellar solutions are in accord with the charge type of the acid-base couple $+/\pm$, confirming the zwitterionic structure of rhodamine B neutral species. Rhodamine B adsorbed on the SDS micellar surface is shown to be suitable for monitoring the ionic strength of (NaCl concentration in) the aqueous phase within a wide range. The well-known effect of SDS micelles modification on adding crown ether, pentanol-1, and a tetra-*n*-butyl ammonium salt is sensed both by pK_a^{ac} values and photophysical properties of the dye species.

Further examination of rhodamine B as an interfacial fluorescent acid-base indicator seems to be promising.

REFERENCES

1. B. M. Krasovitskii and B. M. Bolotin. (1988) *Organic Luminescent Materials*. VCH Verlagsgesellschaft GmbH, Weinheim.
2. M. J. Snare, F. E. Treloar, K. P. Ghiggino, and P. J. Thistlethwaite (1982) The photophysics of rhodamine B. *J. Photochem.* **18,** 335–346.
3. Y. Degani, I. Willner, and Y. Haas (1984) Lasing of rhodamine B in aqueous solutions containing β-cyclodextrin. *Chem. Phys. Lett.* **104**(5), 496–499.
4. M.D. Ediger, R.P. Dominigue, and M.D. Fayer (1984). Picosecond studies of excitation transport in a finite volume: The clustered transport system octadecyl rhodamine B in Triton X-100 micelles. *J. Chem. Phys.* **80**(3), 1246–1253.
5. K. Kemnitz, N. Tamai, I. Yamazaki, N. Nakashima, and K. Yoshihara (1987) Site-dependent fluorescence lifetimes of isolated dye molecules absorbed on organic single crystals and other substrates. *J. Phys. Chem.* **91**(6), 1423–1430.
6. P. J. Sadkowski and G. R. Fleming (1978) Photophysics of the acid and base forms of rhodamine B. *Chem. Phys. Lett.* **57**(4), 526–529.

7. A. J. W. G. Visser, K. Vos, A. Van Hoek, and J. S. Santema (1988) Time-resolved fluorescence depolarization of rhodamine B and octadecylrhodamine B in Triton X-100 micelles and aerosol OT reversed micelles. *J. Phys. Chem.* **92**(3), 759–765.
8. F. Lopez, P. Ruiz Ojeda, and I. Lopez Arbeloa (1989) Fluorescence self-quenching of the molecular forms of rhodamine B in aqueous and ethanolic solutions. *J. Luminesc.* **44,** 105–112.
9. T. Fujii, A. Ishii, and M. Anpo (1990) Absorption and fluorescence spectra of rhodamine B molecules encapsulated in silica gel networks and their thermal stability. *J. Photochem. Photobiol. A. Chem.* **54**(2), 231–237.
10. K. Nakashima, J. Duhamel, and M. A. Winnik (1993) Photophysical processes on a latex surface: Electronic energy transfer from rhodamine dyes to malachite green *J. Phys. Chem.* **97**(41), 10702–10707.
11. J.P. Lakowicz, I. Gryczyński, V. Bogdanov, and J. Kuśba (1994) Light quenching and fluorescence depolarization of rhodamine B and applications of this phenomenon to biophysics. *J. Phys. Chem.* **98**(1), 334–342.
12. B. O. Haglund, D. E. Wurster, L. -O. Sunderlöf, and S. M. Upadrashta (1996) Effect of SDS micelles on rhodamine-B diffusion in hydrogels. *J. Chem. Educ.* **73**(9), 889–893.
13. C. Preininger, G. J. Mohr, I. Klimant, and O. S. Wolfbeis (1996) Ammonia fluorosensors based on reversible lactonization of polymer-entrapped rhodamine dyes, and the effect of plasticizers. *Analyt. Chim. Acta* **334,** 113–123.
14. N. C. Maiti, M. M. G. Krishna, P. J. Britto, and N. Periasamy (1997) Fluorescence dynamics of dye probes in micelles *J. Phys. Chem. B* **101**(51), 11051–11060.
15. M. A. M. J. van Zandvoort, D. L. J. Vossen, G. Van Ginkel, R. Torre, P. Bartolini, M. Ricci, J. Thomas-Oates, and H. Zuilhot (1999) Spectral characterization of fluorescent 5-iodoacetamidotetramethylrhodamine and its *N*-acctylcysteine derivative. *Phys. Chem. Chem. Phys.* **1**, 4571–4582.
16. K. Ishibashi, O. Sato, R. Baba, K. Hashimoto, and A. Fujishima (1999) Second-harmonic generation investigation of rhodamine B derivatives in Langmuir-Blodgett films. *J. Electroanal. Chem.* **465,** 195–199.
17. L. Jin and Y. Liu (2000) Synthesis of novel modified beta-cyclodextrins and their fluorescence studies of inclusion complexation with rhodamine B. *Chin. J. Chem.* **18,** 432–434.
18. Y. Yan and M. L. Myrick (2001) Quantitative measurement and discrimination of isochromatic fluorophores based on micelle-enhanced steady-state fluorescence polarization in fluid solution. *Analyt. Chim. Acta,* **441,** 87–93.
19. D. Magde, G. E. Rogas, and P.G. Scybold (1999) Solvent dependence of the fluorescence lifetime of xanthene dyes. *Photochem. Photobiol.* **70,** 737–744.
20. Y. Liu, Y. Chen, S. X. Liu, X. D. Guan, T. Wada, and Y. Inoue (2001) Unique fluorescence behavior of rhodamine B upon inclusion complexation with novel *bis*(beta-cyclodextrin-6-yl)-2,2′-bipyridine-4,4′-dicarboxylate. *Org. Lett.* **3,** 1657–1660.
21. D. O. Yuan, K. Koga, Y. Kourogi, and K. Fujita (2001) Synthesis of fullerene-cyclodextrin conjugates. *Tetrahedron Lett.* **42,** 6727–6729.
22. H. L. Tavernier, F. Liane, and M. D. Fayer (2001) Photoinduced intermolecular electron transfer in micelles: Dielectric and structural properties of micelle headgroup regions *J. Phys. Chem. A.* **105,** 8944–8957.
23. X. P. Hao, X. P. Fan, Z. Y. Wang, and M. Q. Wang (2001) Fluorescence properties of rhodamine B-doped SiO_2-TiO_2 films prepared by sol-gel processes. *Mater. Lett.* **51,** 245–249.
24. Y. J. Zhang, W. X. Cao, and J. Xu (2002) Interaction of sulfonated calix[n]arenes with rhodamine B and its application to determine acetylcholine in a real neutral aqueous medium. *Chin. J. Chem.* **20,** 322–326.
25. I. Gryczynski, J. Malicka, Z. Gryczynski, C. D. Geddes, and J. R. Lakowicz (2002) The CFS engineers the intrinsic radiative decay rate of low quantum yield fluorophores. *J. Fluorescence* **12** (1), 11–13.
26. C. D. Geddes, J. Karolin, and D. J. S. Birch (2002) Sol-gel nanometrology: Gated sampling can reveal initial sol formation kinetics. *J. Fluorescence* **12**(1), 113–117.
27. N. O. Mchedlov-Petrossyan (1982) The influence of inorganic salts on the spectra of rhodamine B in aqueous solutions. *Zhurn. Fiz. Khim.* **56**(1), 94–98 [in Russian].
28. I. Rosenthal, P. Peretz, and K. A. Muszkat (1979) Thermochromic and hyperchromic effects in rhodamine B solutions. *J. Phys. Chem.* **83**(3), 350–353.
29. I. Lopez Arbeloa and K. K. Rohatgi-Mukerjee (1986) Solvent effects on the photophysics of the molecular forms of rhodamine B internal conversion mechanism. *Chem. Phys. Lett.* **129**(6), 607–614.
30. D. A. Hinckley, P. G. Seybold, and D. P. Borris (1986) Solvatochromism and thermochromism of rhodamine solutions. *Spectrochim. Acta* **42A**(6), 747–754.
31. J. Ferguson and A. W. H. Mau (1972) Absorption studies of acid-base equilibria of dye solutions. *Chem. Phys. Lett.* **17**(4), 543–546.
32. E. Kanasaki (1987) Thermochromism and photochromism of rhodamine B. *Kagaku to Korjo. Chem. a. Chem. Ind.* **40**(10), 870–871 [in Japanese].
33. N. O. Mchedlov-Petrossyan, M. I. Rubtsov, L. L. Lukatskaya, T. A. Chernaya and A. Yu. Pereversev (1988) Ionic equilibria of indicators in micellar media. *Doklady Akad. Nauk SSSR* **299**(4), 921–925 [in Russian].
34. M. Faraggi, P. Peretz, I. Rosenthal, and D. Weinraub (1984) Solution properties of dye lasers. Rhodamine B in alcohols. *Chem. Phys. Lett.* **103**(4), 310–314.
35. N. O. Mchedlov-Petrossyan (1985) Ionization and tautomeric interconversions of rhodamine dyes. *Zhurn. Fiz. Khim.* **59**(12), 3000–3004 [in Russian].
36. N. O. Mchedlov-Petrossyan and L. V. Vasetskaya (1989) Ionization and tautomerism of xanthene dyes in methanol. *Zhurn. Obshch. Khim.* **59**(3), 691–703 [in Russian].
37. N. O. Mchedlov-Petrossyan, V. I. Kukhtik, and V. I. Alekseeva (1994) Ionization and tautomerism of fluorescein, rhodamine B, *N,N*-diethylrhodol and related dyes in mixed and nonaqueous solvents. *Dyes Pigments* **24,** 11–35; and references therein.
38. F. Grieser and C. J. Drummond (1988) The physicochemical properties of self-assembled surfactant aggregates as determined by some molecular spectroscopic probe techniques. *J. Phys. Chem.* **92**(20), 5580–5593; and references therein.
39. C. J. Drummond, F. Grieser, and T. W. Healy (1989) Acid-base equilibria in aqueous micellar solutions. Part 3: Azine derivatives. *J. Chem. Soc. Faraday Trans.* **85** 551–560.
40. C. J. Drummond, F. Grieser, and T. W. Healy 1989. Acid-base equilibria in aqueous Micellar Solutions. Part 4: Azo indicators. *J. Chem. Soc. Faraday Trans.* **85,** 561–578.
41. N. O. Mchedlov-Petrossyan, A. V. Plichko, and A. S. Shumakher (1996) Acidity of microheterogeneous systems: Effect of nonionic admixtures on acid-base equilibria of dyes bound to micelles of ionogenic surfactants. *Chem. Phys. Rep.* **15**(11), 1661–1678; and references therein.
42. G. P. Gorbenko, N. O. Mchedlov-Petrossyan, and T. A. Chernaya (1998) Ionic equilibria in microheterogeneous systems: Protolytic behaviour of indicator dyes in mixed phosphatidylcholine-diphosphatidylglycerol liposomes. *J. Chem. Soc. Faraday Trans.* **94**(15), 2117–2125.
43. N. O. Mchedlov-Petrossyan, A. V. Timiy, and N. A. Vodolazkaya (2000) Binding of sulfonephthalein anions to the micelles of an anionic surfactant. *J. of Mol. Liq.* **87**(1), 75–84.
44. L. P. Loginova, L. V. Samokhina, N. O. Mchedlov-Petrossyan, V. I. Alekseeva, and L. P. Savvina (2001) Modification of the properties of NaDS micellar solutions by adding electrolytes and non-electrolytes: investigations with decyl eosin as a pK_a-probe. *Colloids Surfaces A* **193,** 207–219; and references therein.
45. A. O. Doroshenko, A. V. Kirichenko, V. G. Mitina, and O. A. Ponomaryov (1996) Spectral properties and dynamics of the excited state structural relaxation of the ortho analogues of POPOP-effective

abnormally large Stokes shift luminophores. *J. Photochem. Photobiol. A. Chem*. **94,** 15–26.
46. N. O. Mchedlov-Petrossyan, R. Salinas Mayorga, and Yu. N. Surov (1991) Ionization and tautomerism of xanthene dyes in mixtures of water with dimethyl sulfoxide. *Zhurn. Obshch. Khim.* **61**(1), 225–233 [in Russian].
47. K. L. Mittal (Ed.) *Micellization, Solubilization and Microemulsions*, New York, Plenum Press. (1977).
48. N. Schönfeldt (1982) *Grenzflächenaktive Äthylenoxid-Addukte*. [Russian translation] Moscow, Khimiya.
49. J. F. Rathman and J. F. Scamehorn (1987) Counterion binding on mixed micelles: Effect of surfactant structure. *Langmuir* **3,** 372–377.
50. K. K. Karukstis, A. A. Frazler, C. T. Loftus, and A. S. Tuan (1998) Fluorescence investigation of multiple partitioning sites in aqueous and reverse micelles. *J. Phys. Chem.* **102**(42), 8163–8169.
51. G. Onori and A. Santucci (1993) IR investigation of water structure in aerosol OT reverse micellar aggregates *J. Phys. Chem*. **97**(20), 5430–5434.
52. N. O. Mchedlov-Petrossyan, L. A. Fedorov, S. A. Sokolovskiy, Yu. N. Surov, and R. Salinas Mayorga (1992) Structural transformations of rhodamines in solutions. *Izv. Akad. Nauk.* **3,** 512–521 [in Russian].
53. R. Bates (1972). *Determination of pH* [Russian translation]. Leningrad, Khimiya.
54. G. V. Hartland, F. Grieser, and L. R. White (1987) Surface potential measurements in pentanol-sodium dodecylsulphate micelles. *J. Chem. Soc. Faraday Trans*. **83,** 591–613.
55. H. Oshima, T. W. Hcaly, and L. R. White (1982) Accurate analytic expressions for the surface charge density/surface potential distribution for a spherical colloidal particle. *J. Coll. Interface Sci*. **90**(1), 17–26.
56. S. G. Oh and D. O. Shah (1993) Effect of counterions on the interfacial tension and emulsion droplets size in the oil/water/dodecyl sulfate system. *J. Phys. Chem.* **97**(2), 284–286.
57. S. S. Shah, A. Saeed, and Q. M. Sharif (1999) A study of micellization parameters and electrostatic interactions in micellar solution of sodium dodecyl sulfate. *Colloids Surfaces A*. **155,** 405–412.
58. R. Ranganathan, L. Tran, and B. L. Bales (2000) Surfactant- and salt-induced growth of normal sodium alkyl sulfate micelles well above their critical micelle concentrations. *J. Phys. Chem. B* **104**(10), 2260–2264.
59. B. L. Bales (2001) A definition of the degree of ionization of a micelle based on its aggregation number. *J. Phys. Chem. B* **105**(29), 6798–6804.
60. I. R. Politzer, K. T. Crago, S. Garner, J. Joseph, J. H. Boyer, and M. Shah (1990) Effects of β-cyclodextrin on selected xanthene dyes, coumarins and pyrromethene-BF_2 complexes in aqueous solutions. *Proc. Int. Conf. Lasers*, 434–440.
61. L. Flamigni (1993) Inclusion of fluorescein and halogenated derivatives in α-, β-, and γ-cyclodextrins: A steady-state and picosecond time-resolved study. *J. Phys. Chem*. **97**(38), 9566–9572.
62. L. Flamigni (1994) Effects of complexation by cyclodextrins on the photoreactivity of rose bengal and erythrosin B. *J. Chem. Soc. Faraday Trans*. **90**(16), 2331–2336.
63. N. O. Mchedlov-Petrossyan and A. S. Pulyaeva (1995) The sodium/alkyl-ammonium ion exchange at the micellar surface of dodecyl sulfate micelles as studied with the help of pK_a-probes. *Funct. Mater*. **2,** 530–531.
64. D. D. Miller, D. F. Evans, G. G. Warr, J. R. Bellare, and B. W. Ninham (1987) Vesicle and micelle formation in a double-chained anionic surfactant: Counterion complexation by a macrocyclic ligand. *J. Colloid Interface Sci*. **116,** 598–601.
65. T. Myassoedova, D. Grand, and S. Hautecloque (1992) Effects of crown ethers on the interfacial structure of micelles and vesicles. *J. Photochem. Photobiol. A. Chem*. **64,** 159–169.
66. P. Baglioni, A. Bencini, J. Teixeira, and L. Kevan (1994) Structure and aggregation of lithium dodecyl sulphate micellar solutions in the presence of a macrocyclic cage. *J. Phys. Condens. Matter* **6,** 369–373.
67. J. B. S. Bonilha, R. M. Zumstein Georgetto, E. Abuin, E. Lissi, and F. Quina (1990) Exchange between alkylammonium and sodium ions at the surface of dodecylsulfate micelles. *J. Colloid Interface Sci*. **135**(1), 238–245.
68. E. Szajdzinska-Pietek and J. L. Gebicki (1995) Location of hydrophobic solutes in sodium dodecyl sulfate micelles and the effect of added tetraalkylammonium counterions on the structure of the head-group region: Pulse radiolytic study on scavenging of hydrated electrons. *J. Phys. Chem*. **99**(36) 13500–13504.