

# Heterogeneous Wide Range *pH*-sensing Materials Allowing Ratiometric Fluorescence Detection Based on Structurally Rigid Analogs of 2,6-distyrylpyridine

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**Abstract** The new sensing materials based on the micro-sized silica gel powder with non-covalently immobilized structurally rigid analogs of 2,6-distyrylpyridine ((3E,5E)-3,5-dibenzylidene-8-phenyl-1,2,3,5,6,7-hexahydrodicyclopenta[*b,e*]pyridines) were developed and tested. Most of the investigated compositions demonstrate linear ratiometric fluorescence response on *pH* in the physiologically important interval (*pH* 6–9). The compound with the greatest number of protolytic centers within the studied series demonstrated the widest *pH* sensitivity range, however in this case the analytical signal was the lowest. The prospects for the practical application of the investigated materials in the fiber optics sensing devices were outlined.

**Keywords** *pH* sensing · Ratiometric fluorescence detection · Microsized silica · Fluorescence probes · 2,6-distyrylpyridine analogs · Dicyclopentano[*b,e*]pyridine

## Introduction

During the last decades design and application of various sensing materials became one of the leading trends in the environmentally, biologically and medically oriented chemical analysis [1].

Fluorescence spectroscopy is the most favorable among the methods of optical detection owing to its high sensitivity and selectivity. Thus, the principle of fluores-

cence detection was often used in developing of sensing materials and devices. Wide classes of fluorescent compounds could be involved in design of chemical sensors, such as cyanine dyes [2, 3], azlactones [4], Schiff bases [5], 3-hydroxyflavons [6], etc.

Sensors for the physiological *pH* range are the most demanded in various biophysical applications, their  $pK_a$ 's should fall into the 6–9 *pH* units interval, typical to the most of biological systems. Wolfbeis and co-workers proposed for this purpose the series of coumarin derivatives [7, 8] and 8-hydroxy-1,3,6-pyrenetrisulfonate [9]. The possibility of application for this purpose of fluorescein [10] and eosin-phenol red [11] or other mixed indicator dye systems [12] were shown as well. Widening of *pH* sensing interval were generally achieved by using mixtures of indicators [13, 14] or by applying indicator compounds with multiple reactive centers of the different nature [15]. Very promising results were obtained for the indicators utilizing the photoinduced electron transfer (PET) reaction [16, 17] influenced by the protolytic interactions by the “on-off” principle.

Generally, heterogeneous fluorescent *pH* sensors, suitable to express analysis basing on the application in fiber optics systems have several advantages over the traditional fluorescent probes working in the homogeneous regime [17, 18]. The first one allows exact positioning in the studied object, longer time period of functioning, reproducibility and so on. Most of the discussed sensing materials are composed of the corresponding dyes chemically or non-covalently immobilized in the thin easily penetrated near-surface layers of polymeric materials [19, 20], macroporous glass [21], zeolites [22] or silica gel [23–25].

Most commonly, fluorescent sensors are based on the intensometric principle, according to which, changes in the fluorescence intensity act as the analytical signal. They

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require preliminary calibration by the standard samples, which could not be convenient and easy in cases of the complicated biological and/or environmental systems analysis. To our understanding, the newly-redeveloped ratiometric principle of fluorescence detection could provide more adequate results for the latter analysis, because no special calibration is needed for ratiometric sensing [26]. Owing to the obtaining of the fast and “absolute” analytical signal, ratiometric fluorescence sensing could be applied in developing of the expressed test-systems [27].

Structurally rigid analogs of 2,6-distyrylpyridine, the main objects of this paper, have all prospects to their application as sensing materials, because their  $pK_a$ 's lies in and near the physiological  $pH$  range, most of these compounds are rather effective fluorophores with pronounced changes in their optical parameters at protonation [28]. Excellent spectral resolution of the emission bands belonging to their various protolytic forms makes possible the ratiometric  $pH$  sensing. The insolubility of the title compounds in water allows their easy non-covalent immobilization on surface of the suitable inorganic materials for the further application to analysis of aqueous media. In the case of choice of optically transparent inorganic sorbents for the sensor dyes immobilization, the resulting materials became prospective to their future application in the fiber optics detecting systems.

## Experimental

### Chemicals and materials

Structurally rigid analogs of 2,6-distyrylpyridine, derivatives of (3*E*,5*E*)-3,5-dibenzylidene-8-phenyl-1,2,3,5,6,7-hexahydrodicyclopenta[*b,e*]pyridine, which molecular structure is shown on the Fig. 1, were used for manufacturing the heterogeneous  $pH$  sensing compositions on the base of microsized silica gel powder. Synthesis and investigation of the spectral and protolytic parameters of the title compound were reported in [29].

The choice of the title fluorophores was determined by the nature of their main protolytic center—pyridine nitrogen atom and by the donor/acceptor character of substituents in the side phenyl moieties of 1–6. Compound 1 with the only one reactive group—pyridine cycle, acts as the

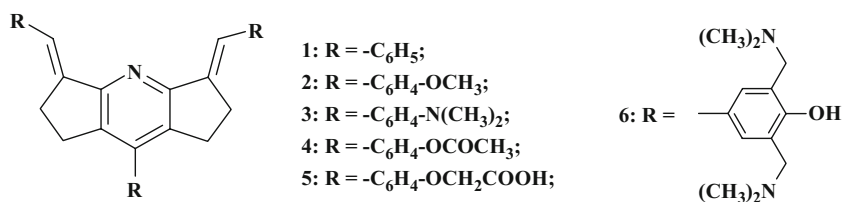
model compound. Compounds 2,4 have the same number of protonation sites, while as 3 has three additional ones. Compound 5 could demonstrate additional equilibria connected with its COOH groups dissociation in the basic surrounding. The most complicated protolytic interactions were expected for the compound 6, which allow both protonation of its amine and pyridine nitrogen atoms and dissociation of the phenolic hydroxy groups. Proton binding to the tertiary amine groups of compound 6 has no effect on its absorption spectra, making quantitative estimation of the corresponding protonation constants hardly possible. From the other side, one could expect influence of this process onto the fluorescence spectra owing to the PET effect, however this could not make estimation of the discussed constants less complicated. Thus, in this paper we have to consider the protolytic equilibria of compound 6 only on the qualitative level.

Heterogeneous  $pH$ -sensitive samples of compounds 1–6 were prepared on the base of their solutions in chloroform and microdispersed silica gel powder (for the thin-layer chromatography, 5/40  $\mu$ , CHEMAPOL). Commercially available chloroform were additionally purified and dried according to [30].

Buffer solutions necessary for the  $pH$  titration of the manufactured solid samples were prepared on the base of the distilled water, acids, bases and salts solutions according to [31], such as: oxalic acid and potassium oxalate (for  $pH$  range 1.0–2.5), tartaric acid and potassium hydroxide ( $pH$  range 3.0–4.5), phthalic acid and potassium hydroxide ( $pH$  range 5.0–6.0), potassium dihydrophosphate and phosphate ( $pH$  range 6.5–8.0), boric acid and potassium hydroxide ( $pH$  range 8.5–11.0), aminoacetic acid and potassium hydroxide ( $pH$  range 11.5–12.5). The above mentioned solutions were mixed one with another in corresponding proportions to prepare buffer solutions with the  $pH$  in the range from 1 to 12 with the step of 0.5  $pH$  units according to the recommendations of [32]. The  $pH$  of the buffer solutions was controlled by the universal ionometer EV-74 with the glass electrode ESL-11g-05, which was calibrated by the standard aqueous buffer solutions (Merck).

The fluorescence spectra of the dye 1 immobilized on silica at different  $pH$  are shown as an example on the Fig. 2 together with the corresponding intensometric fluorescence titration plot.

**Fig. 1** Molecular structure of the investigated derivatives of (3*E*,5*E*)-3,5-dibenzylidene-8-phenyl-1,2,3,5,6,7-hexahydrodicyclopenta[*b,e*]pyridine (1–6)



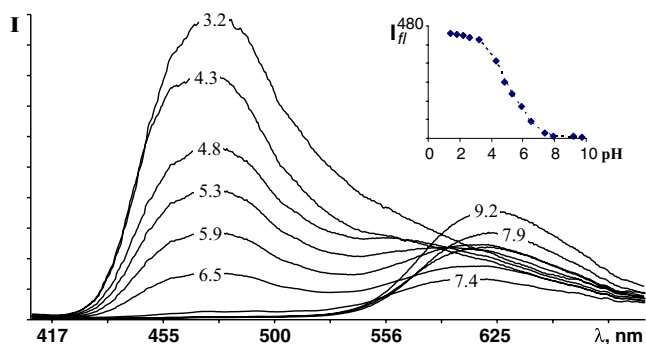
The photostability of the dyes 1–6 immobilized on silica was tested at UV/Vis irradiation by the high-pressure Mercury lamp DRSh-250 (LOMO, St.-Petersburg, Russia) combined with the mirror monochromator MDR-12 (LOMO, St.-Petersburg, Russia).

### Procedure

Immobilization of the dyes 1–6 on silica were conducted from their chloroform solutions according to the following common procedure: 2 g of silica gel powder was added to 2 ml of  $\text{CHCl}_3$  solution containing approximately  $10^{-6}$  g of the dye, and then the mixture was heated to boil at continuous stirring. Discoloration of organic phase during 10–15 min normally took place, indicating the complete sorption of the dyes on silica surface. Then the mixture was cooled, the solid phase was filtered off and dried on air at 20–25 °C in the dark place up to the disappearance of chloroform odor.

According to the rough estimation based on the average density and particles dimensions of the silica gel powder (data of the manufacturer), the ratio of the masses of the dyes and the sorbent corresponded to the sorption of approximately 1 dye molecule on 500 Å<sup>2</sup> of silica surface. Model quantum-chemical calculations (semiempirical PM6 method [33], MOPAC 2007 package [34]) were applied to evaluate the area of the 1–6 dyes molecules of the order of 450–900 Å<sup>2</sup>. This means we obtained nearly monomolecular dyes layers on silica gel powder during the immobilization procedure.

For elucidation of *pH* influence onto the spectral properties of the immobilized dyes 1–6, the obtained solid samples were wetted by the buffer solutions, after that their reflectance and fluorescence spectra were measured. Color changes of the dye-immobilized silica were being observed immediately at the contact with the buffer solution, while as colorization of buffer solution never took place even at



**Fig. 2** Fluorescence spectra of compound 1 non-covalently immobilized on silica at different *pH* and the intensometric fluorescence titration plot built on their background

continuous contact of the solid with the aqueous phase indicating the absence of the dyes desorption from silica. This is the consequence of the low solubility of the protolytic forms of the investigated dyes 1–6 in water and their strong binding with silica surface.

### Data treatment and analysis

Electronic UV/Vis spectra of the investigated samples were measured on fluorescence spectrometer HITACHI F-4010 and spectrophotometers Specord M-40 and HITACHI U-3210.

Reflectance spectra were presented in the Kubelka-Munk function [35] by Eq. 1, which is proportional to the molar extinction and is the analog of absorptivity in the electronic absorption spectroscopy:

$$F = \frac{(1 - R_r)^2}{2R_r} = \frac{K_r}{S} \sim \varepsilon \cdot c \quad (1)$$

here  $F$ —Kubelka-Munk function,  $R_r$ —relative diffuse reflection ( $R_r = I_\lambda/I_\lambda^0$ ),  $K_r$ —absorption at reflectance,  $S$ —scattering,  $\varepsilon$ —molar extinction,  $c$ —molar concentration of the dye.

Ionization constants for the investigated dyes were estimated on the base of their fluorescence spectra with the common Eq. 2:

$$\log K_b = pH + \log \frac{[HL^+]}{[L]} \quad (2)$$

Ratio of the equilibrium concentrations of the dyes protolytic forms were estimated with the help of their related contributions into the experimental fluorescence spectrum by Eq. 3:

$$\frac{[HL^+]}{[L]} = \frac{g(HL^+)}{g(L)} = \frac{J_j(HL^+)/J_0(HL^+)}{J_j(L)/J_0(L)} \quad (3)$$

here  $J_j$ —integral intensity of the corresponding emission band in the experimental spectrum,  $J_0$ —the same integral intensity at boundary conditions (*pH*, at which no other protolytic forms exist).

Mathematical deconvolution of the fluorescence spectra onto individual emission bands was conducted by the specialized software based on the Fletcher-Powell algorithm [36] and approximation of the individual emission band shape by the asymmetric log-normal function [37].

## Results and discussion

### Spectral properties of the immobilized dyes

Usually, immobilization of organic dyes into heterogeneous systems leads to the strong changes of their optical

parameters [3, 4, 38]. However, all the *pH*-induced spectral changes in heterogeneous media observed for the investigated dyes 1–6 correlate well with those for homogeneous solutions. Acidity increasing leads to the protonation of the pyridinic nitrogen atom, as it was shown earlier for the 80%<sub>v/v</sub> water ethanol [28].

Data of the Fig. 3 demonstrate definite bathochromic shift of the absorption bands for both neutral and protonated forms of the dye 2 on silica compared to those in homogeneous media. This circumstance reflects the additional polarizing effect of silica surface onto the investigated compounds in comparison to such a rather polar media as 80%<sub>v/v</sub> water ethanol. The acidic origin of silica surface manifests itself in the existence of the long-wavelength absorption band belonging to the protonated form of the silica immobilized dye 2, which did not disappeared even at *pH*~10. The same features were typical to the fluorescence spectra of this dye as well (Fig. 4).

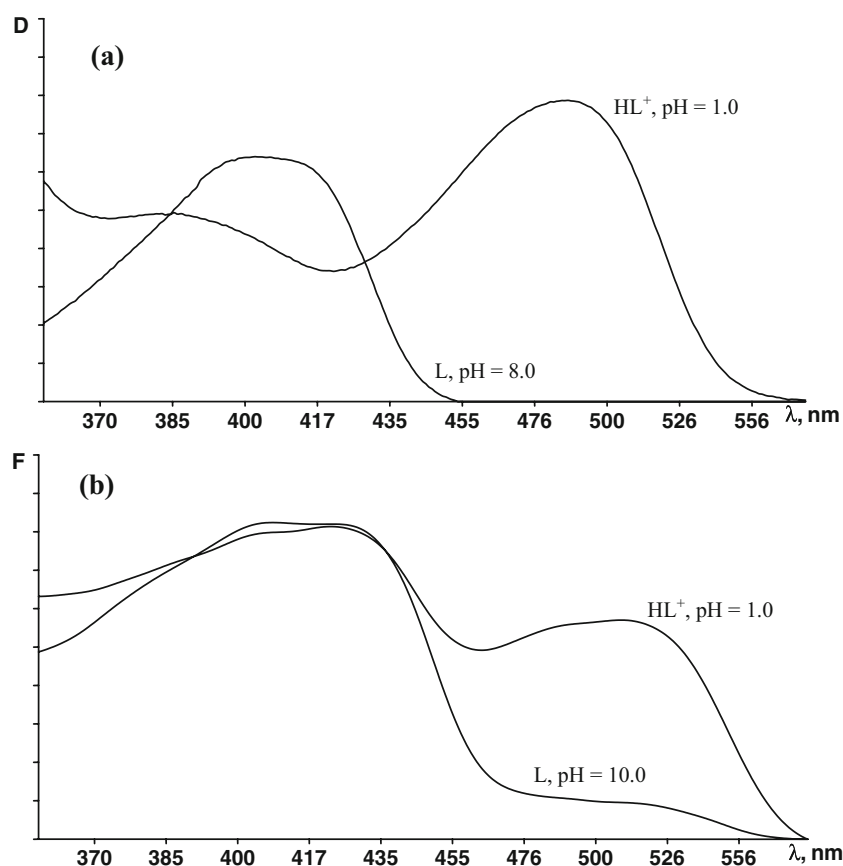
The spectral parameters of the rest of the investigated compounds are summarized in the Table 1. The protonation of the immobilized dyes with the single basic center results in the bathochromic shift of the long-wavelength absorption band onto 4,200–4,500  $\text{cm}^{-1}$ , while as proton binding in the homogeneous media leads to the lesser shifts of 3,500–

4,200  $\text{cm}^{-1}$ . Emission spectra demonstrate nearly the same values for both cases, ~3,500  $\text{cm}^{-1}$ . Thus, decreasing of the fluorescence Stokes shifts for protonated forms of the studied dyes in the immobilized state reflects rigid fixation of their molecules on silica surface in comparison to the case of fluid solutions.

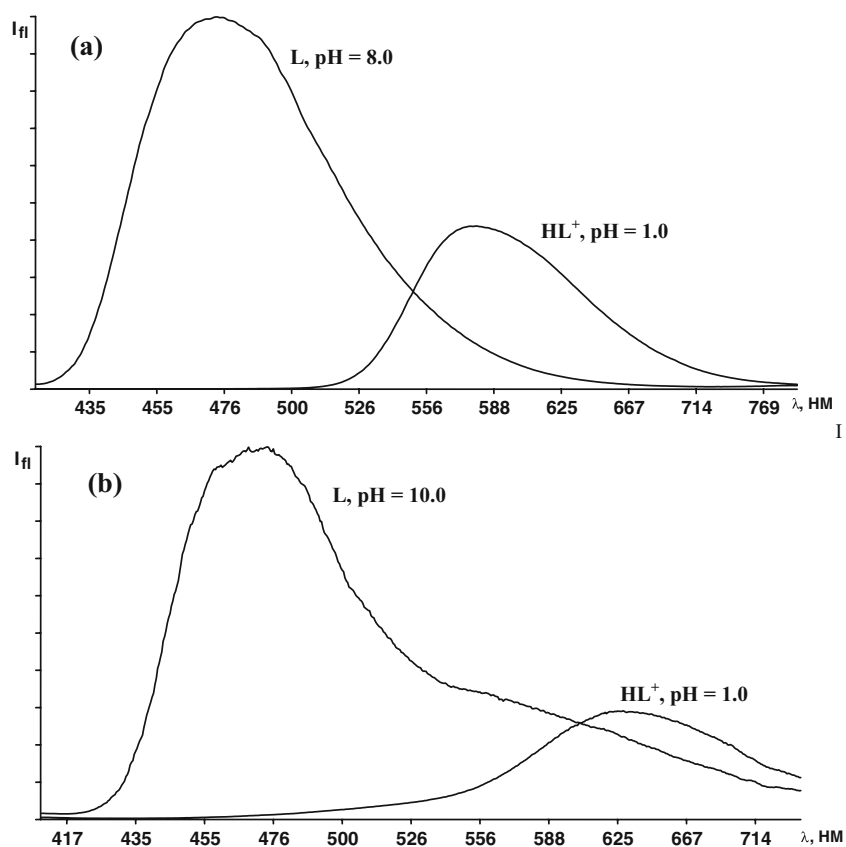
The absorption/reflectance spectral shifts on transfer of the neutral dyes from the homogeneous solution onto silica surface vary from 310 to 760  $\text{cm}^{-1}$  depending on the nature of the substituents in the side phenyl rings. The analogous values for their protonated forms are nearly twice larger. This could be the evidence of the stronger interaction of the protonated pyridinium dyes with silica surface. It should be noted, that no correlation between the magnitude of the discussed shifts and the electronic effects of substituents was observed in this study. The analogous shifts in fluorescence spectra are of the same order for the neutral and protolytic forms. Surprisingly, the most prominent bathochromic shifts on going from homogeneous media to silica surface were detected to the compound 4 possessing weak electron acceptor  $-\text{O}-\text{COCH}_3$  group in the side phenyl moieties.

Quite unexpected behavior demonstrated our dye 3, carrying the pronounced electron donor *N,N*-dimethyla-

**Fig. 3** Electronic spectra of compound 2 at different *pH*: **a** absorption spectra in the homogeneous water–alcohol media; **b** reflectance spectra of the dye non-covalently immobilized on silica



**Fig. 4** Fluorescence spectra of compound **2** at different *pH*: **a** in the homogeneous water–alcohol media; **b** non-covalently immobilized on silica



mino groups. First of all, spectral shifts on going from homogeneous media to silica surface are the least within the studied series. In water alcohol multi-step protolytic equilibria are typical to this dye resulting in the alteration of bathochromic and hypsochromic shifts of its absorption spectra with *pH* (Fig. 5a). The most long-wavelength

absorption should belong to the protolytic form of **3** with the protonated pyridinic nitrogen and unbound N,N-dimethylamino groups [28].

At the same time, only one equilibrium seems to be realized on silica for the dye **3**, thus we could assume the existence for it the only one protonated form in the

**Table 1** Spectral parameters of the dyes **1–5** in 80%<sub>vol</sub> water ethanol and immobilized on silica surface

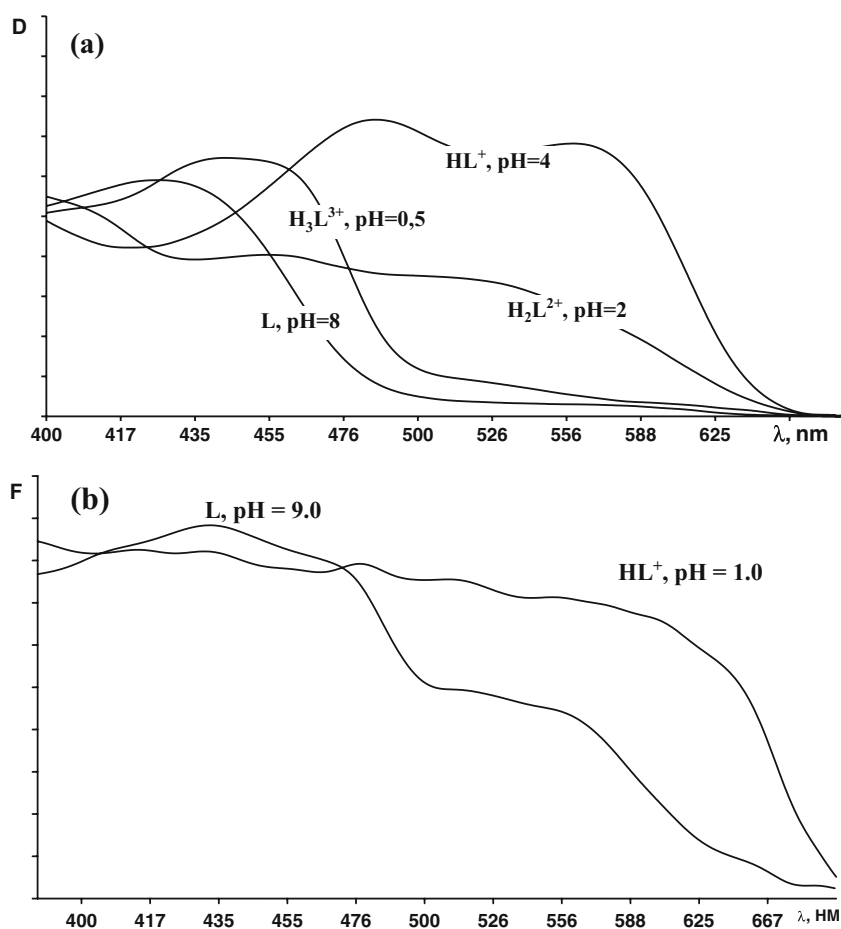
Compound	Protolytic form	$\lambda_{abs}^{max}$ , nm		$\Delta\lambda_{abs}^{max}$ nm	$\Delta\tilde{\nu}_{abs}^{max}$ cm <sup>-1</sup>	$\lambda_{fl}^{max}$ , nm		$\Delta\lambda_{fl}^{max}$ nm	$\Delta\tilde{\nu}_{fl}^{max}$ cm <sup>-1</sup>
		Silica gel	Water alcohol			Silica gel	Water alcohol		
<b>1</b>	L	402	390	12	740	453	441	12	600
	HL <sup>+</sup>	483	451	32	1,470	535	519	16	570
<b>2</b>	L	408	403	5	310	479	474	5	220
	HL <sup>+</sup>	502	487	15	630	586	579	7	210
<b>3</b>	L	426	423	3	160	448 <sup>a</sup>	573	-125	-4,870
	HL <sup>+</sup>	565	560	5	160	504 <sup>b</sup>	693	-189	-5,410
<b>4</b>	L	404	392	12	760	469	445	24	1,150
	HL <sup>+</sup>	490	457	33	1,480	581	529	52	1,690
<b>5</b>	L	406	400	6	370	491	477	14	590
	HL <sup>+</sup>	498	472	26	1,110	591	575	16	470

Data for multi-substituted compound **6** are not included

<sup>a</sup> corresponds to H<sub>2</sub>L<sup>2+</sup>

<sup>b</sup> corresponds to H<sub>3</sub>L<sup>3+</sup>

**Fig. 5** Electronic spectra of compound **3** at different *pH*: **a** absorption spectra in the homogeneous water–alcohol media; **b** reflectance spectra of the dye non-covalently immobilized on silica



adsorbed state, which is characterized by the most pronounced bathochromic shift in comparison with the neutral molecule,  $\sim 140$  nm ( $5,770$   $\text{cm}^{-1}$ ).

On the contrary to the absorption spectra, the fluorescence bands of the dye **3** immobilized on silica were observed in the significantly shorter wavelength region in comparison to water alcohol. There could be several versions explaining this behavior. Seemingly, the discussed emission belongs to the forms with two or three protonated N,N-dialkylamino groups of compound **3**, they were observed in homogeneous water alcohol solutions at 439 and 505 nm correspondingly. First possible explanation of this fact requires increasing probability of the side N ( $\text{CH}_3$ )<sub>2</sub>-groups protonation in the excited state of the molecule **3** absorbed on silica. Though, the most probable alternative explanation might be connected with the significantly increased surface-induced fluorescence quenching of the most long-wavelength monocationic form of the dye **3**, which makes its emission too weak to be observed on the background of the other intensively fluorescent protolytic species.

Much more complicated equilibria were realized in the case of the dye **6**, having ten potential protolytic centers. It was

too difficult to identify adequately the absorption and emission spectra of all these forms of acid-base interactions. That is why, here we make no assumptions concerning the nature of this dye both in homogeneous and in heterogeneous surroundings. Thus we have to exclude uninterpreted spectral data of **6** from the Table 1. However, absence of the detailed understanding of the protolytic interactions of the dye **6** could not affect its further practical application in the *pH* sensing (see “[Ratiometric pH Measurements](#)”).

#### Photostability of the immobilized dyes

Photostability is one of the critical parameters of the organic compounds tested for their sensing ability. Destruction of the organic molecules at continuous ultraviolet irradiation usually results in the decreasing of fluorescence signal, at the same time several other important parameters such as fluorescence lifetime and emission band shape usually remain unchanged. From another point of view, changes in the absolute fluorescence intensity at UV irradiation are important mainly to the intensometric sensing, while as sensors working by the ratiometric detection principle could keep on their workability at such

**Table 2** Logarithms of the dyes 1–5 protolytic equilibria constants in 80%<sub>vol</sub> water ethanol and non-covalently immobilized on silica

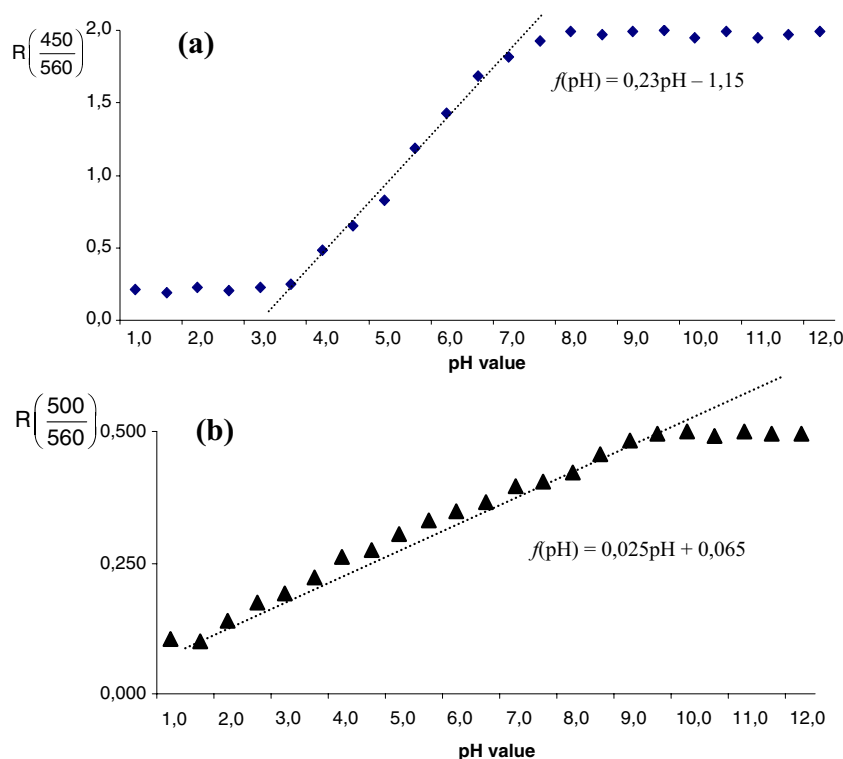
Compound	log $K_b$		$\Delta \log K_b$
	Silica gel	Water alcohol	
1	5.2±0.2	2.51±0.02	2.7
2	7.5±0.2	3.58±0.02	3.9
3	2.7±0.2	4.95±0.01 (log $K_{b1}$ )	-2.3
		3.02±0.05 (log $K_{b2}$ )	-0.3
		2.15±0.05 (log $K_{b3}$ )	0.5
4	5.8±0.2	4.98±0.01	0.8
5	7.5±0.2	2.05±0.03	5.4

conditions: despite of decreasing the absolute intensity at photodestruction, ratiometric analytical response would still reflect the monitored parameters of the investigated object.

Recently we have found that all the investigated 2,6-distyrylpyridine analogs underwent effective E,Z-photoisomerization in fluid solutions [39], which significantly influenced their spectral and protolytic properties.

Our experiments for ultraviolet irradiation (250 W Hg lamp, 365 nm) of the dyes 1–6 immobilized on silica during 2 h demonstrate the total decrease of their fluorescence intensity on nearly 40–50%. At the same time, no changes in the emission spectra shape were observed. This means that irradiation does not have any effect onto the ratiometric signal produced by the investigated sensing compositions.

**Fig. 6** Plots of the fluorescence intensity ratio at the optimal analytical wavelengths  $R(\frac{\lambda_1}{\lambda_2})$  versus pH for 1 (a) and 6 (b) non-covalently immobilized on silica



For comparison reasons, let us mention the photostability experiments for one of the popular fluorescent pH indicators—carboxyfluorescein. Its 65% emission decreasing at irradiation of the Hg 200 W lamp during 1 h was reported [8], which seem to be significantly greater, than in our case. Thus, we could consider our dyes immobilized on silica are enough photostable for their application as ratiometric sensing materials.

Protolytic properties of the silica immobilized dyes

Immobilization of organic sensing compounds on the solid surface significantly changes not only their spectral parameters, but also their protolytic interactions. Binding of a dye with the negatively charged surface usually results in mean decreasing of its acidity and strengthening of basicity. The positively charged surface has the opposite effect. pH indicators immobilized into xerogel demonstrated enhancement of their acidic properties owing to the formation of hydrogen bonds between them and the deprotonated silanolic groups of the matrix [40]. At the same time, weakening basicity is typical to aliphatic amines covalently bound to silica in comparison to their unbound analogs [41].

Non-covalent immobilization of the investigated 2,6-distyrylpyridine analogs on silica has significant influence onto their protolytic equilibria (Table 2). In case of compounds 1, 2, 4 and 5 sorption makes them more pronounced bases. Probably, surface hydroxy groups take part in transferring proton from the bulk of the water

**Table 3** Analytical parameters of the heterogeneous ratiometric fluorescent sensing materials based on structurally rigid 2,6-distyrylpyridine analogs non-covalently immobilized on silica

Compound	$\lambda_1$ , nm	$\lambda_2$ , nm	pH sensing interval	$R = \frac{I_{\lambda_1}}{I_{\lambda_2}} = \alpha \cdot pH + \beta$	
				$\alpha$	$\beta$
1	450	560	3.5–7.5	0.23	−1.15
2	465	610	6.0–9.0	0.66	−7.18
3	500	715	2.0–4.5	0.33	−0.96
4	475	640	4.0–8.0	0.65	−3.98
5	490	590	5.0–9.0	0.21	−1.79
6	500	560	1.5–9.5	0.025	0.071

surrounding to the protonation center of the absorbed dyes. Definite fraction of the dye molecules on silica are in their protonated form, and never return back to the neutral basic form even at contact of the samples with the strongly basic buffer solutions (*pH* 10, Figs. 3 and 4).

Donor substituents (compounds 2, 5) have much stronger effect on the protolytic characteristics of the studied dyes on silica: their  $\Delta \lg K_b$  demonstrate approximately twice increase in comparison with the unsubstituted compound 1. Weak electron acceptor substituents in the molecule of compound 4 results in significantly lower changes of this value.

The anomalously low basicity of compound 3 could be explained by the fact, that only one protolytic equilibrium is realized for it on silica (Fig. 5) in comparison to the homogeneous media, in which all the possible equilibria could be clearly characterized. Quite probably several N,N-dimethylamino groups of this molecule became protonated at the moment of sorption, changing their electronic properties from pronounced donor to weak acceptor.

#### Ratiometric *pH* measurements

First problem for any kind of fluorescence analysis is the proper choice of the excitation wavelength. In this investigation we used 410 nm excitation for all the studied compounds, because all of them and their protolytic forms immobilized on silica absorb light at such wavelength in comparable extent outputting analytical fluorescent signal of the satisfactory intensity.

The principle of ratiometric fluorescence detection was applied to the resolving task of *pH* sensing with the obtained heterogeneous materials. On the contrary to peak or even integral fluorescence intensities, detection of which could be influenced by the various unaccounted factors for the case of the dyes immobilized on silica, the fluorescence intensity ratio would be determined mainly by the *mass action law* demonstrating linear correlation with *pH* at conditions of the protolytic forms coexistence.

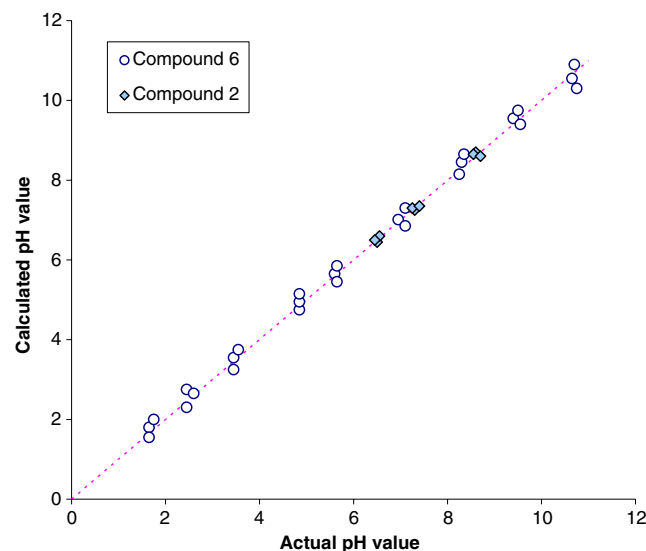
The plots of the fluorescence emission ratios were built for all the investigated dyes and the wavelength pairs giving the most pronounced variation of this parameter

were selected for analysis (Fig. 6, parameter  $R \left( \frac{\lambda_1}{\lambda_2} \right)$  denotes fluorescence intensity at  $\lambda_1$  divided to that of  $\lambda_2$ ).

Data of the Fig. 6 demonstrate good linearity in the *pH* interval between 3.5 and 7.5 for 1, which correspond to its titration range. Analogous procedure was applied to another five investigated dyes (Table 3). The estimated linear functions could be used for resolving the task of *pH* sensing. Their slope values (parameter  $\alpha$ , Table 3) characterize the sensitivity of ratiometric fluorescence detection method and correspondingly—the sensitivity of the investigated heterogeneous sensing materials to *pH*.

All the examined composites demonstrate linear ratiometric analytical signal in the physiological *pH* range with the exception of compound 3, which working *pH* interval is shifted to more acidic media. The most prominent *pH* sensitivity was detected to dyes 2 and 4 owing to the better resolution of their protolytic forms emission bands.

Compound 6 with its greatest number of protolytic centers among the studied series of dyes demonstrated the widest *pH* detection interval, however the relative *pH*



**Fig. 7** Reproducibility of the buffer solutions *pH* values estimated with the ratiometric fluorescence response functions (Table 3) at the optimal analytical wavelengths for the dyes 2 and 6 non-covalently immobilized on silica



sensitivity ( $\alpha$  value) is the lowest in comparison to the other investigated dyes 1–5. It should be noted also, that optimization of the experimental details in case of 6 was more difficult, because we had to find appropriate balance between the sensing parameters of this compound and its extremely complicated protolytic interactions scheme.

The *pH* detection reproducibility with the proposed sensing materials was tested at comparison of the experimental results obtained with the ratiometric response functions (Table 3) and the *pH* values for the same buffer solutions estimated by the potentiometric method with the use of glass electrode (Fig. 7). As it follows from the results presented on this figure, no systematic deviations were found for any of the tested *pH* sensors, however the scattering of the estimated *pH* values were expectedly higher in the case of compound 6.

Owing to the clear and linear ratiometric fluorescence analytical signal, low solubility in water, satisfactory photostability the structurally rigid 2,6-distyrylpyridinic dyes non-covalently immobilized on the micro-sized silica gel powder could be recommended for the express *pH* sensing with their possible application in the fiber optics sensing devices.

## Conclusion

The principle ability for the fluorescence ratiometric *pH* sensing in water media with the use of the structurally rigid analogs of 2,6-distyrylpyridine non-covalently immobilized on silica was shown. On the contrary to the 80%<sub>vol</sub> water ethanol the base-acid titration intervals of the studied indicator dyes on silica were shifted on several *pH* units towards the physiological *pH* range. The highest *pH* sensitivity was detected to the dyes substituted with CH<sub>3</sub>CO–O– groups, while as the widest *pH* range was covered by the dye with the most quantity of protolytic centers within the investigated series.

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