J Fluoresc (2015) 25:1151–1158
DOI 10.1007/s10895-015-1610-5

ORIGINAL ARTICLE

The Fluorescence Properties of Three Rhodamine Dye Analogues: Acridine Red, Pyronin Y and Pyronin B

Xian-Fu Zhang[1,2] · **Jianlong Zhang**[1] · **Xulin Lu**[1]

Received: 15 April 2015 / Accepted: 30 June 2015 / Published online: 11 July 2015

Abstract The fluorescence spectra, fluorescence quantum yield, and fluorescence lifetime of Acridine Red (AR), Pyronin Y (PYY), and Pyronin B (PYB) in aqueous and organic solvents were measured by steady state fluorescence, time-correlated single photon counting, and electronic absorption methods. The rate constants of radiation and non radiation process (k_f and k_{ic}) were calculated to elucidate the structural effect on the fluorescence mechanism. The data for each compound are compared with that of the corresponding rhodamine dye. AR showed significant longer lifetime and higher quantum yield than PYY and PYB, because the alkyls on N atom enhance the internal conversion (IC), the longer the alkyl the faster the IC. However, the structural variation does not alter the rate constant of radiation process (k_f) but does change k_{ic} significantly. The phenyl in Rhodamine B or Rhodamine 6G shows only a slight effect on the fluorescence properties. Ethanol is the solvent in which all five compounds exhibit longest lifetime and highest fluorescence quantum yield.

Keywords Rhodamine · Pyronine · Acridine red · Fluorescence · Solvent effect

✉ Xian-Fu Zhang
zhangxianfu@tsinghua.org.cn

[1] Institute of Applied Photochemistry & Center of Analysis and Measurements, Hebei Normal University of Science and Technology, Qinhuangdao, Hebei Province, China 066004

[2] MPC Technologies, Hamilton, ON, Canada L8S 3H4

Introduction

The excellent fluorescence properties of rhodamine dyes have gained wide spread applications in chemistry and biology [1–4]. The factors that affect the fluorescence properties of rhodamine have been the subject in many reports [5–42]. Pyronin B (PYB), Pyronin Y (PYY), and Acridine Red (AR) are structural analogues of rhodamine dyes (Fig. 1). Their main difference from a corresponding rhodamine is the absence of a carboxyphenyl moiety. They are the chromophores and fluorophores of rhodamine dyes responsible for the strong light absorption and fluorescence emission in the visible region. One advantage of these dyes over rhodamines is the absence of lactone form due to the absence of the carboxyphenyl moiety. Acridine Red is used chiefly for dyeing leather and mordanted cotton. It is highly fluorescent in solution but its fluorescence properties (quantum yield and lifetime values in different solvents) are not available in literature. Pyronin Y is used for staining RNA while Pyronin B is used in the methyl green-pyronin method for coloration of nucleic acids.

The comparison of fluorescence properties of AR, PYB and PYY with each other and with that of rhodamines can provide important information on how the alkyls on N and phenyls attached on xanthene core affect the fluorescence efficiency and lifetime. It is surprising that the comparison has not been found in literature.

Based on this consideration, we measured the fluorescence spectra, fluorescence quantum yields (Φ_f), and fluorescence lifetimes of PYY, PYB, and AR and compared the data with that of corresponding rhodamines reported by us previously [43].

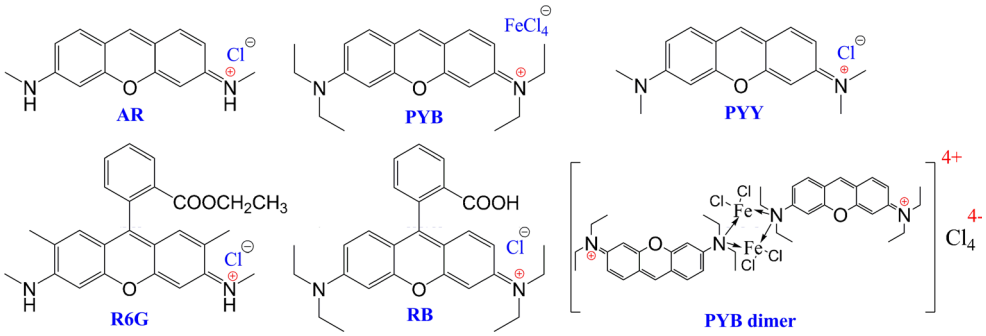

Fig. 1 The chemical structures of AR, PYB, PYY, R6G, RB and PYB dimer

Materials and Methods

Materials

The dyes in the study (structures are shown in Fig. 1) include Acridine Red (AR), Pyronine B (PYB), and Pyronine Y (PYY), Rhodamine 6G (R6G), Rhodamine B (RB). All dyes were purchased with analytical grade or better and used as received. The purity was checked by TLC and only one spot was detectable.

Sample Preparation

All solvents of analytical grade were dried and redistilled immediately before use. Deionized water was purified by Milli-Q system (Millipore, USA). pH in aqueous solution was maintained at 7.4 by using 0.1 mM phosphate buffer.

Methods

Absorption spectra were recorded on a StellarNet Black Comet BLK-CXR-SR fiber optic spectrometer using 1 cm matched quartz cuvettes. Fluorescence spectra were performed by using a FLS 920 fluorospectrometer of Edinburgh Instruments with excitation at 485 nm (21 °C). The emission and excitation slits were both 1.0 nm. The fluorescence was measured at 90° to the incident excitation beam. The fluorescence intensity at all wavelengths was calibrated against the detector response and the excitation light intensity. Wavelength was calibrated against the detector response and the excitation light intensity. The fluorescence quantum yield was measured by using

$$\Phi_f = \Phi_f^0 \cdot \frac{F_s}{F_0} \cdot \frac{A_0}{A_s} \frac{n_s^2}{n_0^2},$$

in which **F** is the integrated fluorescence intensity, **A** is the absorbance at excitation wavelength, n is the refractive index of the solvent used, the subscript 0 stands for a reference compound and s represents samples. R6G in ethanol was used as the reference (Φ_f^0=0.95) [39], since it is esterified and has no deprotonation process. Φ_f values of RB, R6G thus obtained in ethanol also match the reported values [39, 44–48]. Several measurements for a Φ_f value were performed and averaged by choosing different concentration and excitation wavelength.

Measurements of the fluorescence lifetimes were performed with standard time-correlated single-photon counting method. The excitation light was a portable diode laser (EPL-515, Edinburgh Instruments), laser beam was guided into the samples, and fluorescence (the wavelength at the emission maximum of a dye) was detected with a PMT (Hamamatsu R928) cooled to −21 °C. The repetition rate is 10 MHz whilst the count rate did not exceed 20 kHz (0.2 %) in order to avoid pile-up effects. The bandwidth for excitation as well as for

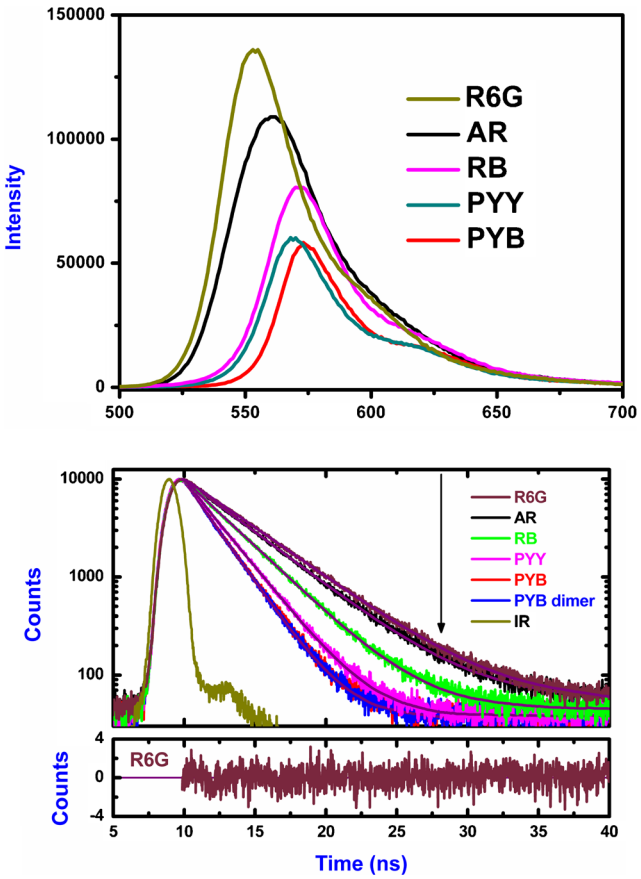

Fig. 2 *Top*: Fluorescence spectra in ethanol with excitation at 485 nm (absorbance 0.090). *Bottom*: Fluorescence decay curves in ethanol with excitation at 509 nm (70 ps) and monitoring at emission maximum

Table 1 The absorption and fluorescence properties in different solvents

	AR	PYB	PYB-dimer	PYY	RB	R6G
Solvent: DMF						
λ_{abs}/nm	535	557	555	552	559	534
λ_{em}/nm	562	581	582	579	587	562
$\Delta\nu$/nm	27	24	27	27	28	28
Φ_f	0.87	0.35	0.36	0.35	0.43	0.80
τ_f/ns	3.87	1.73	1.71	1.94	2.24	3.87
χ^2	1.08	1.07	1.04	1.07	1.12	1.05
k_{ic}, 10^9 s^{-1}	0.034	0.38	0.37	0.34	0.25	0.05
k_f, 10^9 s^{-1}	0.22	0.20	0.21	0.18	0.19	0.21
Solvent: Water (pH 6.8)						
λ_{abs}/nm	528	552	552	546	554	526
λ_{em}/nm	562	572	572	566	579	552
$\Delta\nu$/nm	34	20	20	20	25	26
Φ_f	0.51	0.18	0.20	0.22	0.23	0.69
τ_f/ns	4.18(79 %), 1.61	1.32(95 %), 4.27	1.30(98 %), 4.53	1.98	1.78	4.22
χ^2	1.05	1.06	1.06	1.14	1.32	1.09
k_{ic}, 10^9 s^{-1}	0.12	0.62	0.62	0.39	0.43	0.073
k_f, 10^9 s^{-1}	0.12	0.14	0.15	0.11	0.13	0.16
Solvent: THF						
λ_{abs}/nm	538	555	556	549	554	536
λ_{em}/nm	564	579	579	572	582	560
$\Delta\nu$/nm	26	24	23	23	28	24
Φ_f	0.88	0.32	0.35	0.42	0.43	0.67
τ_f/ns	3.66	2.48	2.49	2.00 (93 %), 1.07	3.57(89 %), 1.71	3.8
χ^2	1.11	1.1	1.09	1.03	1.04	1.11
k_{ic}, 10^9 s^{-1}	0.033	0.27	0.26	0.29	0.16	0.087
k_f, 10^9 s^{-1}	0.24	0.13	0.14	0.14	0.12	0.18
Solvent: CH_3CN						
λ_{abs}/nm	526	553	552	547	555	522
λ_{em}/nm	550	575	575	571	583	550
$\Delta\nu$/nm	24	22	23	24	28	28
Φ_f	0.74	0.29	0.28	0.50	0.40	0.91
τ_f/ns	4.10	1.44	1.40	2.06	1.86	4.17
χ^2	1.10	1.13	1.14	1.06	1.12	1.03
k_{ic}, 10^9 s^{-1}	0.063	0.49	0.51	0.24	0.32	0.022
k_f, 10^9 s^{-1}	0.18	0.20	0.20	0.24	0.22	0.22
Solvent: EtOH						
λ_{abs}/nm	534	553	553	547	546	530
λ_{em}/nm	562	574	574	569	572	553
$\Delta\nu$/nm	28	21	21	22	26	23
Φ_f	1.00	0.47	0.46	0.48	0.74	0.95
τ_f/ns	3.87	2.01	1.98	2.32	3.06	4.17
χ^2	1.10	1.08	1.06	1.07	1.12	1.01
k_{ic}, 10^9 s^{-1}	0.00	0.26	0.27	0.22	0.085	0.012
k_f, 10^9 s^{-1}	0.30	0.23	0.23	0.21	0.24	0.23
Solvent: Dioxane						
λ_{abs}/nm	534	554	554	543	557	533
λ_{em}/nm	561	582	581	570	588	562
$\Delta\nu$/nm	27	28	27	27	31	29

Table 1 (continued)

	AR	PYB	PYB-dimer	PYY	RB	R6G
Φ_f	0.71	0.01	0.03	0.14	0.34	0.77
τ_f/ns	3.88(93 %), 1.42	1.34(77 %),0.39	1.27 (92 %), 3.46	1.02(60 %), 3.16	1.56	1.95
χ^2	1.07	1.36	1.06	1.07	1.06	1.07
k_{ic}, 10^9 s^{-1}	0.075	0.74	0.76	0.84	0.42	0.12
k_f, 10^9 s^{-1}	0.18	0.030	0.020	0.14	0.22	0.20

emission was <2 nm. The prompt response function of the system had an fwhm is less than 70 ps. The convolution method was used to fit the $I(t) = A+Be^{(-t/\tau_f)}$ or $I(t) = A+B_1e^{(-t/\tau_{f1})}+B_2e^{(-t/\tau_{f2})}$ to obtain the fluorescence lifetime.

Results and Discussion

Figure 2 shows the fluorescence spectra and lifetime decay curves of the five dyes measured under the same conditions. The differences in emission intensity and lifetime are very obvious, although they all share the same fluorophore (Fig. 1). Table 1 lists their fluorescence and absorption properties. A large change occurred in their fluorescence quantum yield and lifetime values (Table 1) upon the variation of substitution on the fluorophore. To understand the reason, a more detailed study is given below.

UV–vis Absorption Spectra

Figure 3 compares the UV–vis absorption of the five dyes, PYY, PYB, AR, R6G and RB in ethanol. The spectral shapes are all similar for these dyes. The main band of the absorption spectra for the dyes is located at 542(±11) nm with a shoulder at 500(±10) nm, and a minor band sits at 350(±10) nm. The carboxyphenyls in R6G and RB only show a slight influence on their absorption spectra, since the carboxyphenyls are not π-conjugated with the main π-system but act like substituents. Solvents, either less polar THF or highly polar DMF, also do not exhibit profound effect on the spectral shape.

The absorption maximum value is increased in the same order in all solvents: R6G < AR < PYY < PYB ≈ PYB dimer < RB (Fig. 3). A relatively large jump in absorption and emission maxima occurs at the transition from AR to PYY (Fig. 4); this corresponds to the replacement of the H on N atom by a CH_3 group (Fig. 1). This red shift is intensified from PYY to PYB, when the CH_3 on the N atoms is substituted by a CH_2CH_3 group. This change indicates that the stronger the electron donating capability of a substituent on N ($H < CH_3 < CH_2CH_3$) leads to a larger red-shift. This is mainly due to that the energy gap between HOMO-LUMO becomes smaller.

However, the carboxyphenyl in R6G and RB shows different effects on the absorption maximum, the former causes a blue shift compared to AR, while the latter leads to a red shift compared to PYB. This is because $PhCOOCH_2CH_3$ in R6G is a neutral electron withdrawing group, while PhCOOH in RB is easily deprotonated to negatively charged $PhCOO^-$ which makes it electron donating.

Fluorescence Spectra

The normalized fluorescence spectra are shown in Fig. 3. Compared to absorption spectra, each dye shows only the emission band which is mirror symmetrical to its maximum absorption band, and the emission maximum of each fluorescence spectrum is slightly red shifted from its absorption with a Stokes shift (Δν) of 25±4 nm. The small Stokes shift suggests that the molecular structure of a dye in the excited state is very similar to that in its ground state. The shape of emission

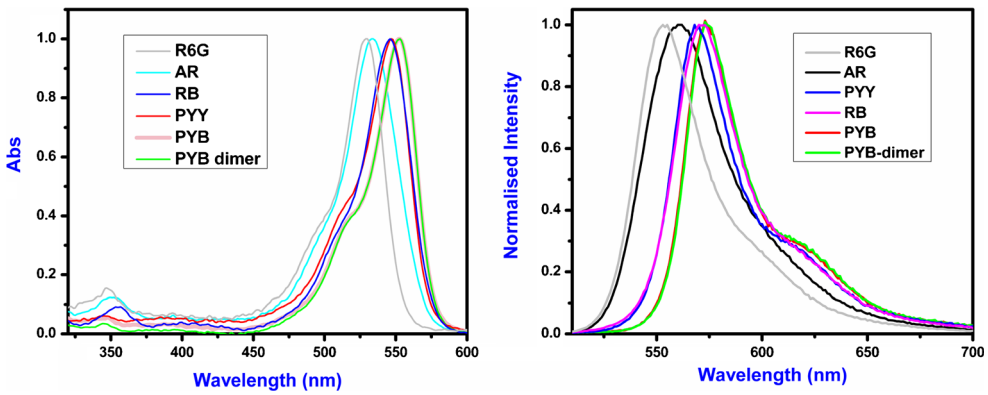

Fig. 3 Normalized absorption (*Left*) and fluorescence (*Right*) spectra of different dyes in ethanol. The excitation wavelength is 485 nm for all emission spectra

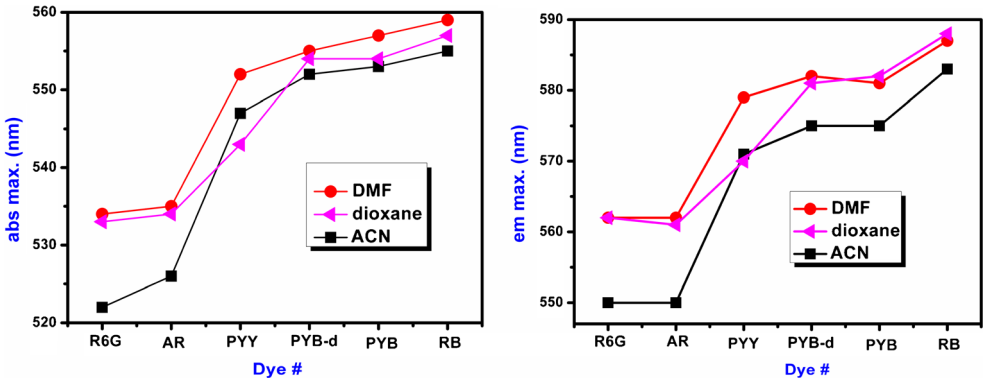

Fig. 4 Change of absorption and emission maxima upon dye structure. The compounds are listed by the substituent's electron donating ability which is increased from left to right (ranked by the number and chain length of the alkyls on N atoms)

bands is all similar for these dyes, which means the excited state fluorophore structure for all the dyes are very similar. The main band of emission spectrum for a dye is located at 562(±11) nm together with a shoulder at 620(±20) nm. Similar to the absorption cases, the emission maximum is increased in the same order in all solvents: R6G < AR < PYY < PYB ≈ PYB dimer < RB (Fig. 4). The reasons for this change have been discussed in the previous section.

Fluorescence Quantum Yield and Lifetime

Figure 2 shows the typical fluorescence decay curves. The fluorescence lifetime values could be obtained by mono exponential fitting with chi squared values between 1.00 and 1.10, except that in dioxane. A mono exponential decay indicates that only one emitting species exists. In dioxane, biexponential fitting are needed to fit the decay curves, suggesting the existence of two emitting exponents. Dioxane is a base and proton acceptor, it could cause the following reaction (Fig. 5).

Figure 6 shows how the structure and solvent affect the fluorescence quantum yield and lifetime. PYB and its dimer PYB-d have the same Φ_f and τ_f in all solvents, i.e., the aggregation due to Fe^{3+} coordinating shows no influence on the fluorophores. In other words, the 3d electrons in Fe^{3+} do not have spin-orbit coupling with the π-systems of the connected fluorophores.

Both Φ_f and τ_f decrease in the same order in all solvents: R6G ≥ AR >> RB > PYY > PYB ≈ PYB dimer (Fig. 6). A relatively large decrease occurs from AR to RB (Fig. 6); this corresponds to the replacement of the H on N atom by a CH_3 group (Fig. 1). This decrease is intensified from PYY to PYB, when the CH_3 on N atom is substituted by a CH_2CH_3 group. This result indicates that the larger decrease in Φ_f and τ_f is caused when the substituent on N is changed in the order: H < CH_3 < CH_2CH_3. Note that the N atoms are sp^2 and conjugated with the π-system, the two alkyl groups are on the π-planar ring.

The fluorescence properties of an organic compound is determined by the photophysical processes associated with S_1, the lowest lying excited state. In the absence of photochemical reactions, S_1 can return to the ground state (S_0) through three competing processes: fluorescence (FL), internal conversion (IC, i.e., heat releasing via vibration and rotation of chemical bonds), and intersystem crossing (ISC). The corresponding rate constant for each process is k_f, k_{ic}, and k_{isc}. $\tau_f = (k_f+k_{ic}+k_{isc})^{-1}$, while $\Phi_f = k_f/(k_f+k_{ic}+k_{isc}) = k_f \bullet \tau_f$.

To understand which process (ISC, IC or FL) contributes to the structural effect on the fluorescence, the radiation constant (k_f) is calculated and listed in Table 1. One can see that k_f in each solvent does not show significant changes for all the dyes, indicating that the presence of phenyl or alkyls on N do not change the radiation process for the fluorophore. Therefore either IC or ISC must be responsible for the difference in photophysical properties of these dyes.

We then tried to detect the triplet state and measure its properties with the laser flash photolysis technique for these

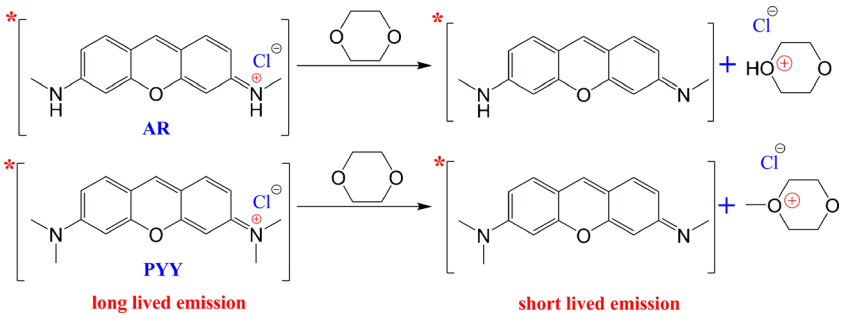

Fig. 5 The excited state reaction of AR or PYY with dioxane

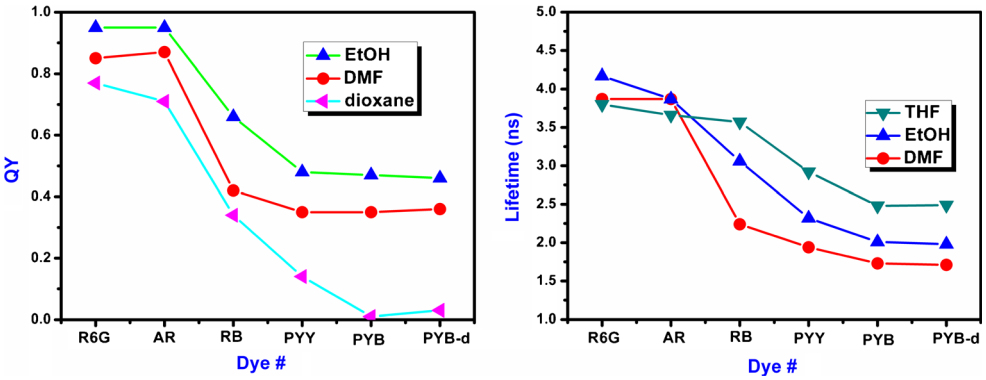

Fig. 6 Change of fluorescence quantum yield (QY) and lifetime with dye structure (ranked by the number and chain length of the alkyls on N atoms)

dyes. Take PYB as an example, with laser excitation at 532 nm only transient species absorbing at 420 and 450 nm were found, but their lifetimes are only a few nanoseconds and not affected by the presence of oxygen. This means that the quantum yield of triplet formation for the dye is less than 0.01, i.e., $k_{isc}/(k_f+k_{ic}+k_{isc}) = k_{isc}\bullet\tau_f<0.01$, or $k_{isc}<0.25\times 10^7\ M^{-1}\ s^{-1}$. Compared to $k_f\sim0.20\times10^8\ M^{-1}\ s^{-1}$, ISC makes negligible contribution to the difference in the dyes fluorescence parameters.

k_{ic} values are calculated by $k_{ic} = \Phi_{ic}/\tau_f$, with $\Phi_{ic}=1-\Phi_f-\Phi_T\approx1-\Phi_f$, the values are listed in Table 1. k_{ic} for the dyes are obviously changed significantly. We then conclude IC process plays the mail role of changing the fluorescence properties when substitution occurs on the fluorophore.

From H, CH_3 to CH_2CH_3, the rigidity of the fluorophore is lowered which usually cause the decrease in Φ_f and τ_f values. Also the increase of the chain length causes more vibrational and rotational movements from S_1 state, i.e., the rate of internal conversion (k_{ic}) is enhanced and leads to the decrease in Φ_f and τ_f values.

On the other hand, the electron donating capability becomes stronger from H, CH_3 to CH_2CH_3. This favors the excited state charge transfer from NR_2 to the π-ring and may cause the decrease in Φ_f and τ_f values.

The presence of carboxyphenyls, however, causes slight increase in Φ_f and τ_f values. This is due to the large steric hindrance caused by the presence of COOR which presents the phenyl from rotating.

Solvent effect is remarkable (Fig. 6 and Table 1). For any particular dye, Φ_f and τ_f values are generally highest in ethanol but lowest in dioxane. OH groups in ethanol molecules can form hydrogen bonding (O—H—N) with N atoms of the fluorophore. The presence of H-bonding strengthens the rigidity and reduces vibration or rotation. It has been known that this type of hydrogen bonding favors the enhancement of fluorescence emission.

When dioxane or THF is used as a solvent, the photochemical process in Fig. 5 competes with the radiation process and therefore lowers Φ_f and τ_f values.

Conclusions

We have measured the fluorescence properties of AR, PYY, and PYB in different solvents and compared them with that of RB and R6G. Based on the comparison, we could examine the effect of phenyl and alkyls on the fluorescence properties. AR showed significant longer lifetime and higher quantum yield than PYY and PYB. The shorter lifetime and lower quantum yield of PYB and PYY is mainly due to the enhanced non radiation process by alkyl groups, the longer the alkyls the faster the non radiation is. However, the structural change does not lead to the variation of the rate constant of radiation process (k_f) or intersystem crossing (k_{isc}) for all compounds. The presence of the phenyl in PYY and PYB shows only slight effect on fluorescence properties. The fluorescence properties of PYB dimer show no difference from that of PYB itself. Due to hydrogen bonding effect, ethanol is the best solvent in which each compound exhibits longest lifetime and highest fluorescence quantum yield than that in other solvents. Dioxane, on the other hand, is the least favored solvent for the fluorescence efficiency of the rhodamine dyes. These observations advance the understanding on rhodamine fluorescence mechanism, and help in design and synthesize new rhodamine fluorescent material which can be applied in biological, chemical and environmental science.

Acknowledgments We thank the financial support from Hebei Provincial Hundred Talents Plan (Contract E2013100005), Hebei Provincial Science Foundation (Contract B2014407080) and HBUST (Contract CXTD2012-05).

References

1. Chen X, Pradhan T, Wang F, Kim JS, Yoon J (2012) 1 fluorescent chemosensors based on spiroring-opening of xanthenes and related derivatives. Chem Rev 112:1910–1956
2. Dsouza RN, Pischel U, Nau WM (2011) 2 fluorescent dyes and their supramolecular host/guest complexes with macrocycles in aqueous solution. Chem Rev 111:7941–7980

3. Berezin MY, Achilefu S (2010) 3 fluorescence lifetime measurements and biological imaging. Chem Rev 110:2641–2684
4. Kim HN, Lee MH, Kim HJ, Kim JS, Yoon J (2008) A new trend in rhodamine-based chemosensors: application of spirolactam ring-opening to sensing ions. Chem Soc Rev 37:1465–1472
5. Greisch J-F, Harding ME, Klopper W, Kappes MM, Schooss D (2014) Effect of proton substitution by alkali ions on the fluorescence emission of Rhodamine B cations in the gas phase. J Phys Chem A 118:3787–3794
6. Wurth C, Gonzalez MG, Niessner R, Panne U, Haisch C, Genger UR (2012) Determination of the absolute fluorescence quantum yield of Rhodamine 6G with optical and photoacoustic methods providing the basis for fluorescence quantum yield standards. Talanta 90:30–37
7. Sun YQ, Liu J, Lv X, Liu Y, Zhao Y, Guo W (2012) 4 rhodamine inspired far-red to near-infrared dyes and their application as fluorescence probes. Angew Chem Int Ed 51:7634–7636
8. Serra-Gomez R, Tardajos G, Gonzalez-Benito J, Gonzalez-Gaitano G (2012) Rhodamine solid complexes as fluorescence probes to monitor the dispersion of cyclodextrins in polymeric nanocomposites. Dyes Pigments 94:427–436
9. Savarese M, Aliberti A, De Santo I, Battista E, Causa F, Netti PA, Rega N (2012) Fluorescence lifetimes and quantum yields of rhodamine derivatives: new insights from theory and experiment. J Phys Chem A 116:7491–7497
10. Nagy AM, Talbot FO, Czar MF, Jockusch RA (2012) Fluorescence lifetimes of rhodamine dyes in Vacuo. J Photochem Photobiol A Chem 244:47–53
11. Lewkowicz A, Bojarski P, Synak A, Grobelna B, Akopova I, Gryczynski I, Kulak L (2012) Concentration-dependent fluorescence properties of Rhodamine 6G in titanium dioxide and silicon dioxide nanolayers. J Phys Chem C 116:12304–12311
12. Alessi A, Salvalaggio M, Ruzzon G (2012) Rhodamine 800 as reference substance for fluorescence quantum yield measurements in deep red emission range. J Lumin. doi:10.1016/j.bbr.2011.1003.1031
13. Sagoo SK, Jockusch RA (2011) The fluorescence properties of cationic Rhodamine B in the gas phase. J Photochem Photobiol A Chem 220:173–178
14. Sagoo SK (2011) Intrinsic properties of Rhodamine B and fluorescein gas-phase ions studied using laser-induced fluorescence and photodissociation in a quadrupole ion trap mass spectrometer. In: Chemistry. Toronto: The University of Toronto
15. Ganbold EO, Park JH, Dembereldorj U, Ock KS, Joo SW (2011) Charge-dependent adsorption of Rhodamine 6G on gold nanoparticle surfaces: fluorescence and Raman study. J Raman Spectrosc 42:1614–1619
16. Forbes MW, Jockusch RA (2011) Gas-phase fluorescence excitation and emission spectroscopy of three xanthene dyes (Rhodamine 575, Rhodamine 590 and Rhodamine 6G) in a quadrupole ion trap mass spectrometer. J Am Chem Soc Mass Spectrom 22:93–109
17. Fikry M, Omar MM, Ismail LZ (2011) Effect of host medium on the fluorescence emission intensity of Rhodamine B in liquid and solid phase. In: Modern trends in physics research: third international conference on modern trends in physics research, MTPR-08, Cairo, Egypt, 6–10 April 2008. Cairo: World Scientific Publishing Company Incorporated 210
18. Bakkialakshmi S, Menaka T (2011) Fluorescence enhancement of Rhodamine 6G by forming inclusion complexes with r-cyclodextrin. J Mol Liq 158:117–123
19. Bakkialakshmi S (2011) A study of the interaction between Rhodamine 6G and hydroxy propyl r-cyclodextrin by steady state fluorescence. Spectrochim Acta A Mol Biomol Spectrosc 81:8–13
20. Zhang XF, Zhang I, Liu L (2010) Photophysics of halogenated fluoresceins: involvement of both intramolecular electron transfer and heavy atom effect in the deactivation of excited states. Photochem Photobiol 86:492–498
21. Zhang X-F (2010) The effect of phenyl substitution on the fluorescence characteristics of fluorescein derivatives via intramolecular photoinduced electron transfer. Photochem Photobiol Sci 9:1261–1268
22. Kordel M, Schooss D, Neiss C, Walter L, Kappes MM (2010) Laser-induced fluorescence of Rhodamine 6G cations in the gas phase: a lower bound to the lifetime of the first triplet state. J Phys Chem A 114:5509–5514
23. Baumgartel T, von Borczyskowski C, Graaf H (2010) Fluorescence studies of Rhodamine 6G functionalized silicon oxide nanostructures. Nanotechnology 21:475205
24. Al-Hamdani AH, Al-Ethawi AS, Al-Hamdani R (2010) Fluorescence efficiency of Rhodamine 6G doped PMMA. J Mater Sci Eng 4:57–61
25. Nag A, Goswami D (2009) Solvent effect on two-photon absorption and fluorescence of rhodamine dyes. Phys Chem Chem Phys 206:188–197
26. Kolmakov K, Belov VN, Bierwagen J, Ringemann C, Muller V, Eggeling C, Hell SW (2009) 5 red-emitting rhodamine dyes for fluorescence microscopy and nanoscopy. Chem Eur J 16:158–166
27. Kim K, Lee YM, Lee JW, Shin KS (2009) Metal-enhanced fluorescence of Rhodamine B isothiocyanate from micrometer-sized silver powders. Langmuir 25:2641–2645
28. Fikry M, Omar MM, Ismail LZ (2009) Effect of host medium on the fluorescence emission intensity of Rhodamine B in liquid and solid phase. J Fluoresc 19:741–746
29. Chen Z, Tang YJ, Xie TT, Chen Y, Li YQ (2008) Fluorescence spectral properties of Rhodamine 6G at the silica/water interface. J Fluoresc 18:93–100
30. Wu S, Luo Y, Zeng F, Chen J, Chen Y, Tong Z (2007) Photoreversible fluorescence modulation of a rhodamine dye by supramolecular complexation with photosensitive cyclodextrin. Angew Chem Int Ed 46:7015–7018
31. Zhao Z, Ding Z, Wang Z, Tang X, Zhang Z (2006) Fluorescence of Rhodamine 101 under high pressures. Chin J High Press Phys 20:6
32. Urano Y, Kamiya M, Kanda K, Ueno T, Hirose K, Nagano T (2005) Evolution of fluorescein as a platform for finely tunable fluorescence probes. J Am Chem Soc 127:4888–4897
33. Ray K, Nakahara H, Sakamoto A (2005) Time-resolved fluorescence spectroscopic and scanning near-field optical microscopic studies of rhodamine dye adsorbed in cationic Langmuir鈥揵lodgett films. Spectrochim Acta A Mol Biomol Spectrosc 61:103–107
34. El-Rayyes AA, Al-Betar A, Htun T, Klein UKA (2005) Fluorescence emission from Rhodamine-B lactone adsorbed at solid catalysts. Chem Phys Lett 414:287–291
35. Zheng XY, Wachi M, Harata A, Hatano Y (2004) Acidity effects on the fluorescence properties and adsorptive behavior of Rhodamine 6G molecules at the air/water interface studied with confocal fluorescence microscopy. Spectrochim Acta A Mol Biomol Spectrosc 60:1085–1090
36. Vogel R, Meredith P, Harvey MD, Rubinsztein-Dunlop H (2004) Absorption and fluorescence spectroscopy of Rhodamine 6G in titanium dioxide nanocomposites. Spectrochim Acta A Mol Biomol Spectrosc 60:245–249
37. Ueno T, Urano Y, Setsukinai K, Takakusa H, Kojima H, Kikuchi K, Ohkubo K, Fukuzumi S, Nagano T (2004) Rational principles for modulating fluorescence properties of fluorescein. J Am Chem Soc 126:14079–14086
38. Schneckenburger H, Stock K, Lyttek M, Strauss WSL, Sailer R (2004) Fluorescence lifetime imaging (FLIM) of Rhodamine 123 in living cells. Photochem Photobiol Sci 3:127–131
39. Magde D, Wong R, Seybold PG (2002) Fluorescence quantum yields and their relation to lifetimes of Rhodamine 6G and

fluorescein in nine solvents: improved absolute standards for quantum yields. Photochem Photobiol 75:327–334
40. Acemioglu B, Arık M, Onganer Y (2002) Solvent effect on nonradiative process of pyronin Bin protic and aprotic solvent systems. J Lumin 97:153–160
41. Marcano OA, Urdaneta I (2001) Fluorescence quantum yield of Rhodamine 101 in the presence of absorption saturation. Appl Phys B Lasers Opt 72:207–213
42. Abugo OO, Nair R, Lakowicz JR (2000) Fluorescence properties of Rhodamine 800 in whole blood and plasma. Anal Biochem 279: 142–150
43. Zhang X-F, Zhang Y, Liu L (2014) Fluorescence lifetimes and quantum yields of ten rhodamine derivatives: structural effect on emission mechanism in different solvents. J Lumin 145:448–453
44. Kubin RF, Fletcher AN (1983) Fluorescence quantum yields of some rhodamine dyes. J Lumin 27:455–462
45. Snare MJ, Treloar FE, Ghiggino KP, Thistlethwaite PJ (1982) The photophysics of Rhodamine B. J Photochem 18:335–346
46. Arden J, Deltau G, Huth V, Kringel U, Peros D, Drexhage KH (1991) Fluorescence and lasing properties of rhodamine dyes. J Lumin 48:352–358
47. Drexhage KH (1990) Structure and properties of laser dyes. In: Schafer FP (ed) Topics in applied physics. Springer, Berlin, pp 147–172
48. Karstens T, Kobs K (1980) Rhodamine B and Rhodamine 101 as reference substances for fluorescence quantum yield measurements. J Phys Chem 84:1871–1872