Influences of molecular structures on the spectral properties and photostability of rhodamine dyes

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Research Article

Keywords: Rhodamine dyes, Photostability, Absorption spectrum, Fluorescence emission spectrum, Density functional theory (DFT)

Posted Date: February 15th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2578761/v1

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Abstract

Although the syntheses and applications of rhodamine dyes have been extensively researched, the photophysical properties and photostability have rarely been studied. In this study, we systematically investigated the photophysical properties and photostability of six rhodamine dyes by the spectrum measurements, photodegradation experiments and theoretical calculations. It was found that the following structural modifications can lead to the redshift of absorption and fluorescence emission spectra: Increasing the alkyls of amino on xanthene; forming rigid ring between the N-linked alkyl and xanthene; esterification of –COOH on benzene ring. The degradation experiments under 525 nm light irradiation indicated that increasing the alkyls of amino on xanthene improves the photostability, fixing the N-linked alkyls on the xanthene ring to form rigid rings would significantly reduce the photostability of rhodamine dyes, and the esterification of –COOH on benzene ring can increase the photostability to some extent. The results of DFT calculations showed that the esterification of –COOH decreases the dihedral angle $D_1$ and increases $D_2$, the variation trend of calculated absorption and emission wavelengths is consistent with the measured results, and the rhodamine dyes with higher electrophilic index usually have higher photostability.

Introduction

Rhodamine is a type of important fluorescent dye, belonging to the xanthene derivatives [1]. Since it was first discovered in 1887 [2], rhodamine dyes have gained widespread applications owing to their excellent photophysical and photochemical properties such as large molar extinction coefficient, high fluorescence quantum efficiency, high photostability, and so on [3–6]. So far, they have been extensively used as fluorescent metal sensors [7–9], pH sensors [10, 11], laser dyes [12, 13], molecular switches [14], explosive detectors [15–17], imaging in living cells [18], and single-molecule imaging [19]. To meet the requirements of different applications, a variety of rhodamine dyes with desired properties have been developed by the method of molecular structure modification. For instance, by incorporating an additional amine group with fused rings into rhodamine molecules, Zhang et al. synthesized a new near-infrared (NIR) rhodamine dye with a long emission wavelength (648 nm) and large Stokes shift (73 nm) [20]. Liu et al. designed a series of rhodamine fluorescent dyes with maximum absorption wavelength of 581–631 nm, by extending the $\pi$ conjugation of chromophore [21].

In the actual applications of rhodamine dyes, the photostability is an important performance that directly affects their long-term reliability [22]. In this regard, the researchers have explored many strategies to improve the photostability of rhodamine dyes such as introducing the electron-withdrawing sulfonic group [23], replacing their N,N-dimethyl amino substitutions with quaternary azide ring [24], and introducing the weakly coordinated anion as an anti-anion to increase the electrostatic attraction and steric effect between anions and cations [25]. In the previous works, the syntheses and applications of rhodamine dyes have been extensively studied. However, the researches have rarely focused on the influence of rhodamine structures on their photophysical properties and photostability, especially the law and intrinsic mechanism of structure-function relationship.
In this work, we employed six rhodamine dyes with different structures as the research targets and investigated the influences of molecular structures on the photophysical properties in water and CHCl\textsubscript{3} solutions. The photostabilities of these dyes were evaluated by monitoring their residual rates in water under 532 nm LED light irradiation via a home-made photoreactor. The configurations of rhodamine dyes were optimized by the density functional theory (DFT) method at the B3LYP-D3/6-311G(d,p) level \cite{26-30}. Based on the calculated results, their HOMO and LUMO energy levels as well as the maximum absorption and emission wavelengths were estimated. Finally, the electrophilic indexes of the rhodamine dyes were calculated to account for the influence of molecular structures on photostability.

**Experimental**

**Materials and instruments**

The investigated rhodamine dyes in this work include Rhodamine 19 (Rh19), Rhodamine 590 (Rh590), DR21 methyl ester (DR21-Me), Rhodamine B (RhB), Rhodamine 101 (Rh101), Rhodamine 101 methyl ester (R101-Me), as shown in Fig. 1. All the rhodamine dyes were analytical or spectra grade, while all the solvents were analytical grade and used without further purification. The UV-vis absorption and the fluorescence emission spectra of the rhodamine dyes were measured on a Shimadzu UV-vis 2450 spectrophotometer and FLS980 fluorescence spectrometer, respectively.

**Measurements of dye photostability**

The photostability of the rhodamine dyes were measured by a home-made photoreactor, in which four 12 W LED lamp panels emitting 525 nm light were used as the light source. The light intensity at the center of the photoreactor was measured to be \(~52\text{ mW/cm}^2\). For each measurement, 80 mL of rhodamine solution was added into the 100 mL quartz tube equipped with an oxygen balloon to provide oxygen atmosphere. For each measurement, the rhodamine solution was continuously stirred by a magnetic stirrer to make it uniform and an electric fan was used to avoid the increase of reaction temperature. At each given time interval, 1 mL of the solution was taken and its absorption spectrum was measured and the degradation rates of rhodamine dyes were calculated by the following equation:

\[
\text{Degradation rate of dye (\%)} = (A_0 - A_t)/A_0 \times 100\%
\]

Where \(A_0\) is the absorbance of rhodamine dye before light irradiation, while \(A_t\) represents the absorbance of rhodamine dye after a certain time light irradiation.

**Theoretical calculation**

The ground state geometry optimizations of the rhodamine dyes were carried out using The density functional theory (DFT) at B3LYP-D3/6-311G(d,p) level \cite{30}, while the excited state geometry
optimizations were performed at the same level using TD-DFT method. All the theoretical calculations were carried out with the Gaussian 09 program package [31]. Visualization analyses of the calculated results were performed by GaussView 5.0 program [32].

Results And Discussion

Effect of molecular structure on the spectral property

Figure 2 and Fig. 3, respectively, show the normalized absorption and fluorescence emission spectra of the six investigated dyes in CHCl₃ and water solutions. In Fig. 2, all the rhodamine dyes present a symmetry absorption band, in which the stronger peak at longer wavelength is assigned to the 0→0 band of S₀→S₁ transition, while the weaker shoulder peak at shorter wavelength is ascribed to the 0→1 vibrational band of S₀→S₁ transition [22]. From the corresponding spectral data summarized in Table 1, it can be seen that all the rhodamine dyes exhibit small stokes shifts in both CHCl₃ and water solvents (15 – 28 nm in CHCl₃; 27 – 32 nm in water), which is due to the small geometry relaxation of rhodamine dyes under light irradiation [33].

For the influence of dye structures on the absorption and fluorescence emission spectra, it can be seen that the maximum absorption wavelength (λₐₕₗₜ) and fluorescence emission wavelength (λₑₘ) increase from 524 nm to 580 nm and from 549 nm to 612 nm, respectively. Compared to the monoethylamino-substituted Rh19, the diethylamino-substituted RhB displays an obvious red-shift (31 – 34 nm) in terms of λₐₕₗₜ and λₑₘ. As illustrated in Scheme 1, the rhodamine chromophore has three resonance structures a, b and c. The N-linked alkyl group plays the role of +I inductive effect and thereby stabilizes the positive charge at the amino N atom as π-donor and stabilizes [33]. As a result, the statistical weights of the resonance configurations a and c would increase, leading to the bathochromic shift of absorption and emission wavelengths. By comparing the absorption and emission spectra of RhB and Rh101 as well as R590 and DR21-Me, it can be seen that fixing the N-linked alkyl groups to the xanthene ring leads to the further bathochromic shift of λₐₕₗₜ and λₑₘ. This bathochromic shift is ascribed to the increase of molecular rigidity and planarity, which can increase the electron delocalization and decrease the bandgap between MOMO and LUMO orbits. By comparing the absorption and emission spectra of Rh19 and Rh590, as well as Rh101 and Rh101-Me, we can know that the esterification of PhCOOH also leads to the bathochromic shift of absorption and emission spectra. It is known that the plane of the xanthene ring is perpendicular to that of benzene ring, which is beneficial to the intramolecular electrostatic interaction between the COOR group and the xanthene ring. The − COOH of benzene ring is more effective to stabilize the positive charge of central carbon on the xanthene ring, thereby increasing the statistical weight of the ‘b’ configuration (Fig. 2E). In this configuration, the C – N bonds between xanthene and amino groups have more single bond component, decreasing the conjugated degree of xanthene and amino groups [34]. In contrast, the esterification of PhCOOH group increases the statistical weight of the ‘a’ and ‘c’ configuration, which increases the conjugated degree of xanthene and amino groups to a certain extent, and consequently leads to the bathochromic shift in absorption and emission spectra.
Table 1
The spectral data of the six rhodamine dyes in CHCl$_3$ and water

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Dye</th>
<th>$\lambda_{ab}$ (nm)</th>
<th>$\varepsilon \times 10^{-4}$ ($M^{-1} cm^{-1}$) $^a$</th>
<th>$\lambda_{em}$ (nm)</th>
<th>Stokes shift (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl$_3$</td>
<td>Rh19</td>
<td>524</td>
<td>2.56</td>
<td>539</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Rh590</td>
<td>534</td>
<td>8.95</td>
<td>561</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>DR21-Me</td>
<td>551</td>
<td>7.05</td>
<td>576</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>RhB</td>
<td>555</td>
<td>9.21</td>
<td>571</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Rh101</td>
<td>577</td>
<td>11.62</td>
<td>605</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Rh101-Me</td>
<td>580</td>
<td>12.46</td>
<td>607</td>
<td>27</td>
</tr>
<tr>
<td>water</td>
<td>Rh19</td>
<td>522</td>
<td>2.99</td>
<td>549</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Rh590</td>
<td>525</td>
<td>8.59</td>
<td>557</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>DR21-Me</td>
<td>541</td>
<td>6.8</td>
<td>569</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>RhB</td>
<td>553</td>
<td>10.08</td>
<td>583</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Rh101</td>
<td>574</td>
<td>9.56</td>
<td>605</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Rh101-Me</td>
<td>580</td>
<td>10.74</td>
<td>612</td>
<td>32</td>
</tr>
</tbody>
</table>

$^a$ $\varepsilon$ is the molar extinction coefficient of the rhodamine dyes.

**Influence of molecular structure on photostability**

Figure 4A shows the degradation curves of the six rhodamine dyes in the oxygen-saturated water solution under 525 nm light irradiation. After light irradiation for 6 h, the degradation rates of RhB, Rh19 and Rh590 were 23%, 80% and 60%, while the degradation rates of DR21-Me, R101 and R101-Me reached nearly 100% after light irradiation for 4 h (Fig. 4A). The degradation kinetics of these dyes were further investigated by plotting the value of ln($A_0/A$) against the light irradiation time. It can be seen that the degradation reactions of the three dyes follow pseudo-first-order kinetics (Fig. 4B). As listed in Table 2, the degradation rate constants of Rh19, Rh590, DR21-Me, RhB, R101, and Rh101-Me are $0.266 \text{ h}^{-1}$, $0.158 \text{ h}^{-1}$, $0.8566 \text{ h}^{-1}$, $0.044 \text{ h}^{-1}$, $1.834 \text{ h}^{-1}$, and $1.212 \text{ h}^{-1}$, respectively. From the above results, we can know that the photostabilities of these rhodamine dyes follow the order of RhB Rh590 Rh19 DR21-Me Rh101-Me Rh101.

In addition, we also investigated the influence of alcohol on the photostability of rhodamine dyes by comparing the degradation rates of RhB, Rh101 and Rh101-Me in O$_2$-saturated 20%EtOH-H$_2$O solution with those in water. As shown in Fig. 5, the degradation rates of Rh101 and Rh101-Me in 20%EtOH-H$_2$O
after light irradiation for 6 h were 97.8% and 86.2%, respectively, while that of RhB after light irradiation for 72 h was 50%. Their degradation reaction kinetics were fitted and the corresponding degradation rate constants were 0.0096 h\(^{-1}\), 0.6719 h\(^{-1}\), and 0.3375 h\(^{-1}\) for RhB, Rh101, and Rh101-Me, respectively. On one hand, the photostability order of the three dyes in 20%EtOH-H\(_2\)O is the same as that in water, i.e., RhB Rh101-Me Rh101; on the other hand, the photostabilities of RhB, Rh101 and Rh101-Me in 20%EtOH-H\(_2\)O are much higher than those in water, respectively. By carefully comparing the relationship between the structures of rhodamine dyes and their photostabilities, it can be concluded that the dialkyl-substituted rhodamine dyes display higher photostability compared than the mono-alkylated rhodamine dyes, fixing the N-linked alkyls on the xanthene ring to form rigid rings would significantly reduce the photostability of rhodamine dyes, and the esterification of –COOH on benzene ring can increase the photostability to some extent.

<table>
<thead>
<tr>
<th>Dye</th>
<th>Rh19</th>
<th>Rh590</th>
<th>DR21-Me</th>
<th>RhB</th>
<th>Rh101</th>
<th>Rh101-Me</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_1) (h(^{-1})) in water</td>
<td>0.2657</td>
<td>0.1577</td>
<td>0.8566</td>
<td>0.0436</td>
<td>1.8306</td>
<td>1.2124</td>
</tr>
<tr>
<td>(k_2) (h(^{-1})) in 20% EtOH/H(_2)O</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0096</td>
<td>0.6719</td>
<td>0.3375</td>
</tr>
<tr>
<td>(k_1/k_2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.5416</td>
<td>2.7245</td>
<td>3.5922</td>
</tr>
</tbody>
</table>

The phenomenon that forming rigid ring between the N-linked alkyl and xanthene can significantly decrease the photostability of rhodamine dyes can be explained by the twisted intramolecular charge transfer (TICT) mechanism: It is known that the tetraalkylrhodamine dyes such as RB can easily form a TICT state in the excited state owing to their lower ionization potential [35]. As shown in Fig. 6A, after absorbing a photon, the rhodamine molecule goes to the excited state and an electron is transferred from the amino group to the xanthene part, along with the distortion of C – N bond and the formation of TICT state. The molecule in TICT state go back to the ground state mainly by the way of non-radiative inactivation. The rhodamine dye on TICT state loses energy mainly in the form of nonradiative inactivation, which decreases the probability of intersystem crossing and the formation of triplet state (\(T_1\)) dye. Since the \(T_1\) dye can react with dissolved O\(_2\) to form singlet oxygen (\(^1\)O\(_2\)) [36, 37], which is the main reactive species leading to the degradation of dyes under light irradiation. Due to the low triplet content, tetraalkylrhodamines possess relatively higher photostability. On the contrary, the rhodamine dyes with rigid rings not only can return to the ground state through the radiative decay pathway, but also can form the triplet state molecules by intersystem crossing, which further react with O\(_2\) to produce \(^1\)O\(_2\) and other reactive oxygen species. These reactive oxygen species further react with the surrounding excited state and ground state dye molecules, leading to the degradation of dye molecules [Fig. 6B]. As a result, the rhodamine dyes with rigid rings usually show lower photostability than tetraalkylrhodamines.

**Theoretical calculations**
Rhodamine molecule is usually composed of diamino-substituted oxanthracene ring and a single bond-connected benzene ring. According to the previous literature [38], in a neutral water solution, R19, R590, DR21-Me, and R101-Me are presented in the form of cations, while RB and R101 exist in the form of zwitterions. Herein, the ground and excited state configurations of the six rhodamine dyes in water were optimized by using B3LYP-D3/6-311G(d,p) level of DFT and TD-DFT method, respectively. The six dyes have no virtual vibration frequency, revealing that the optimized geometric configurations have the minimum points on the potential energy surface. As an example, the geometries of Rh590 in the ground and excited states were displayed in Fig. 7A. As the main chromophore, the xanthene ring displays excellent planarity and rigidity and has a larger conjugated $\pi$ system. In Fig. 7B, the dihedral angle $D_1$ is the angle between the xanthene ring and the benzene ring, while $D_2$ represents the angle between the benzene ring and its substituted group (carboxyl or ester group). Table 3 presents the dihedral angles $D_1$ and $D_2$ of the six rhodamine dyes in $S_0$ and $S_1$ states. In the ground state ($S_0$), the $D_1$ for Rh19, Rh590, and Rh101-Me are 90.4°, 90.9°, and 90.1°, respectively, indicating that the two planes of xanthene and benzene ring are almost mutually perpendicular, while those of DR21-Me, RB, and R101 are slightly twisted to 93.4°, 96.7°, 95.3°, respectively. In contrast, all the dihedral angles of $D_2$ are near to 0°, indicating that the two planes of benzene ring and its substituent are parallel to each other. As listed in Table 3, from the ground state to the excited state ($S_1$), the rhodamine molecule undergo an evident torsion, corresponding to the dramatic changes of $D_1$ and $D_2$ (except for R19). In fact, the torsion of $D_1$ and $D_2$ could be attributed to the Coulombic and nonclassical interactions between the two moieties and solvent molecules. By comparing the excited state $D_1$ and $D_2$ of Rh19 and Rh590 as well as those of Rh101 and Rh101-Me, it can be seen that the esterification of $-\text{COOH}$ on benzene ring can lead to the decrease of $D_1$ and increase of $D_2$.

<table>
<thead>
<tr>
<th>Dye</th>
<th>$D_1$ (°)</th>
<th>$D_2$ (°)</th>
<th>$D_1$ (°)</th>
<th>$D_2$ (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S_0$</td>
<td>$S_1$</td>
<td>$S_0$</td>
<td>$S_1$</td>
</tr>
<tr>
<td>Rh19</td>
<td>90.4</td>
<td>62.4</td>
<td>1.5</td>
<td>4.6</td>
</tr>
<tr>
<td>Rh590</td>
<td>90.9</td>
<td>60.0</td>
<td>1.4</td>
<td>22.6</td>
</tr>
<tr>
<td>DR21-Me</td>
<td>93.4</td>
<td>74.5</td>
<td>1.4</td>
<td>32.0</td>
</tr>
<tr>
<td>RhB</td>
<td>96.7</td>
<td>59.8</td>
<td>0.0</td>
<td>19.4</td>
</tr>
<tr>
<td>Rh101</td>
<td>95.3</td>
<td>69.5</td>
<td>0.0</td>
<td>25.2</td>
</tr>
<tr>
<td>Rh101-Me</td>
<td>90.1</td>
<td>55.8</td>
<td>1.8</td>
<td>35.7</td>
</tr>
</tbody>
</table>
The theoretical absorption and emission data of the six rhodamine dyes were calculated by using TD-DFT method and listed in Table 4, along with the tested results. The results of the linear regression analysis indicates that the theoretical and experimental data have excellent correlation coefficients of 0.94 for $\lambda_{ab}$ and 0.88 for $\lambda_{em}$, indicating that the B3LYP-D3 basis set can give good theoretical results to calculating the absorption and emission wavelengths of rhodamine dyes. The deviation between theoretical and experimental values is mainly caused by the systematic underestimation of wavelengths, which can be eliminated by appropriate scaling factor.

**Table 4**
Experimental and B3LYP-D3 calculated spectral data of the investigated rhodamine dyes in water as solvent.

<table>
<thead>
<tr>
<th>Dye</th>
<th>Absorption</th>
<th>Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cal. $\lambda_{abs}$ (nm)</td>
<td>$f$</td>
</tr>
<tr>
<td>R19</td>
<td>443.1</td>
<td>0.9373</td>
</tr>
<tr>
<td>R590</td>
<td>444.4</td>
<td>0.9318</td>
</tr>
<tr>
<td>DR21-Me</td>
<td>452.2</td>
<td>1.0214</td>
</tr>
<tr>
<td>RB</td>
<td>456.1</td>
<td>0.8849</td>
</tr>
<tr>
<td>R101</td>
<td>471.0</td>
<td>0.9032</td>
</tr>
<tr>
<td>R101-Me</td>
<td>481.9</td>
<td>0.9873</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.94</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ H: HOMO; L: LUMO

The effect of dye molecular structure on $\lambda_{ab}$ and $\lambda_{em}$ in the theoretical results follows the trend in the experimental results. The introduction of alkyl groups on N atoms leads to a bathochromic shift of the spectral bands, while fixing the N-linked alkyls on the xanthene ring can increase the conjugated degree of xanthene ring and amino groups, leading to the bathochromic shift of absorption and emission spectra. Moreover, the esterification of carboxyl acid on the benzene ring also leads to the red-shift of $\lambda_{ab}$ and $\lambda_{em}$. According to the main transition configuration, the absorption bands with high oscillator strength (0.8) are assigned to the electronic transitions from $S_0$ to $S_1$, which almost completely originates from HOMO→LUMO excitations (99.0%). The strong emission bands for normal rhodamines mainly originate from LUMO→HOMO de-excitation, while for RB and R101, the strong emission bands are from the de-excitation of L→H-2 and L→H-1, respectively.
Table 5
Energy of HOMO and LUMO (eV), chemical potential (µ, eV), chemical hardness (η, eV), electrophilicity index (ω, eV) of all investigated dyes in water at B3LYP/6-311G(d,p) level

<table>
<thead>
<tr>
<th>Dye</th>
<th>(E_{\text{LUMO}}) (eV)</th>
<th>(E_{\text{HOMO}}) (eV)</th>
<th>µ (eV)</th>
<th>η(eV)</th>
<th>ω (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh19</td>
<td>-2.918</td>
<td>-5.908</td>
<td>1.568</td>
<td>1.495</td>
<td>0.822</td>
</tr>
<tr>
<td>Rh590</td>
<td>-2.933</td>
<td>-5.916</td>
<td>1.575</td>
<td>1.491</td>
<td>0.832</td>
</tr>
<tr>
<td>DR21-Me</td>
<td>-2.615</td>
<td>-5.646</td>
<td>1.411</td>
<td>1.515</td>
<td>0.657</td>
</tr>
<tr>
<td>RhB</td>
<td>-2.896</td>
<td>-5.794</td>
<td>1.555</td>
<td>1.449</td>
<td>0.834</td>
</tr>
<tr>
<td>Rh101</td>
<td>-2.480</td>
<td>-5.354</td>
<td>1.339</td>
<td>1.437</td>
<td>0.624</td>
</tr>
<tr>
<td>Rh101-Me</td>
<td>-2.775</td>
<td>-5.506</td>
<td>1.489</td>
<td>1.366</td>
<td>0.811</td>
</tr>
</tbody>
</table>

According to the literature [39], the electrophilic index \(ω\) is a kind of global molecular reactivity index, which is usually used to describe the stabilization energy of an ideal electron transferred from the environment to the molecular system. There is a linear relationship between the photostability of dyes and the electrophilic index, that is, the higher the \(ω\) value is, the more stable the molecule is when electron transfer occurs [40, 41]. Herein, we described the chemical reactivity of rhodamine dyes in water by electrophilic index, so as to further explain the photostability of dyes. The electrophilic index \(ω\) is calculated as follows:

\[
M = -\frac{1}{2} (E_{\text{HOMO}} + E_{\text{LUMO}}) \quad \text{(Eq. 3)}
\]

\[
η = \frac{1}{2} (E_{\text{LUMO}} - E_{\text{HOMO}}) \quad \text{(Eq. 4)}
\]

\[
ω = \frac{\mu^2}{2\eta} \quad \text{(Eq. 5)}
\]

Where \(µ\) represents chemical potential, \(η\) represents chemical hardness, while \(E_{\text{HOMO}}\) and \(E_{\text{LUMO}}\) represent the energies of frontier molecular orbitals, respectively. The electrophilic indexes of Rh19, Rh590, DR21-Me, RhB, Rh101, and Rh101-Me in water were calculated to be 0.822 eV, 0.832 eV, 0.657 eV, 0.834 eV, 0.624 eV, 0.811 eV, which are in good concordance with the experiment results of photostability measurements.

Conclusions

In this study, we systematically investigated the photophysical properties and photostability of six rhodamine dyes by the spectrum measurements, photodegradation experiments and theoretical calculations. By comparing their absorption and fluorescence spectra in CHCl\(_3\) and water, it can be seen that the following structural modification can increase the conjugated degree of xanthene and amino groups and consequently lead to the redshift of absorption and fluorescence emission spectra: (1) Increasing the substituted alkyls of amino on xanthene; (2) forming rigid ring between the N-linked alkyl
and xanthene; (3) esterification of $-\text{COOH}$ on benzene ring. From the degradation experiments under 525 nm light irradiation, it can be concluded that, the dialkyl-substituted rhodamine dyes display higher photostability compared than the mono-alkylated rhodamine dyes, fixing the N-linked alkyls on the xanthene ring to form rigid rings would significantly reduce the photostability of rhodamine dyes, and the esterification of $-\text{COOH}$ on benzene ring can increase the photostability to some extent. On the basis of DFT calculations, the following conclusions can be deduced: (1) Esterification of $-\text{COOH}$ on benzene ring can lead to the decrease of dihedral angle $D_1$ and the increase of dihedral angle $D_2$, which is relative to the photostability of rhodamine dyes; (2) The variation trend of calculated absorption and emission wavelengths of the rhodamine dyes is consistent with the measured results; (3) The photostability of the rhodamine dyes are basically is positively related to the calculated electrophilic indexes, i.e., the higher electrophilic index usually means higher photostability. We think that this work can provides a useful reference to investigate the structure-function relationship of rhodamine and other fluorescence dyes.

**Declarations**

**Ethical Approval**

Not applicable.

**Competing interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work in this paper.

**Authors' contributions**

F.C and Y.D.L did the experiments; L.S., S.Z.C. and Z.Z.Z analysed the data; Y.D.L. and B.Z.T. wrote the manuscript; J.L.Z. revised the manuscript. All authors reviewed the manuscript.

**Funding**

This research was funded by the Shanghai Industrial Collaborative Innovation Project (XTCX-KJ-2022-2-01), National Natural Science Foundation of China (U1862112), and Fundamental Research Funds for the Central Universities (JKD01211701, 222201717003, 50321041917001, 50321042017001).

**Availability of data and materials**

Not applicable.

**Acknowledgments**

All authors gratefully acknowledge the support of Science and Technology on Particle Transport and Separation Laboratory.
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**Scheme 1**

Scheme 1 is available in Supplementary Files section.

**Figures**
Figure 1

Molecular structures of the six rhodamine dyes.
Figure 2

Normalized UV-vis absorption spectra of rhodamine dyes in (A) CHCl₃ and (B) water solutions.
Figure 3

Normalized fluorescence emission spectra of rhodamine dyes in (C) CHCl$_3$ and (D) water solution.
Figure 4

(A) Degradation curves and (B) Fitted reaction kinetic curves of the six rhodamine dyes under 525 nm light irradiation in O\textsubscript{2}\,-saturated water solution.
Figure 5

Comparison of dye residual rate of three rhodamine dyes in O2-saturated water and 20% ethanol aqueous solution.
Figure 6

Excitation-deexcitation process of (A) tetraalkylrhodamine dyes and (B) N-linked alkyl fixed rhodamine dye.
Figure 7

(A) Optimized geometric configurations of Rh590 in the $S_0$ and $S_1$ states. (B) Dihedral angles D1 and D2 of Rh590.

Supplementary Files
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- Scheme1.png