

*Chapter*

**5**

# **GO/surfactant-inspired photophysical modulation of dye in DESs with or without additives**

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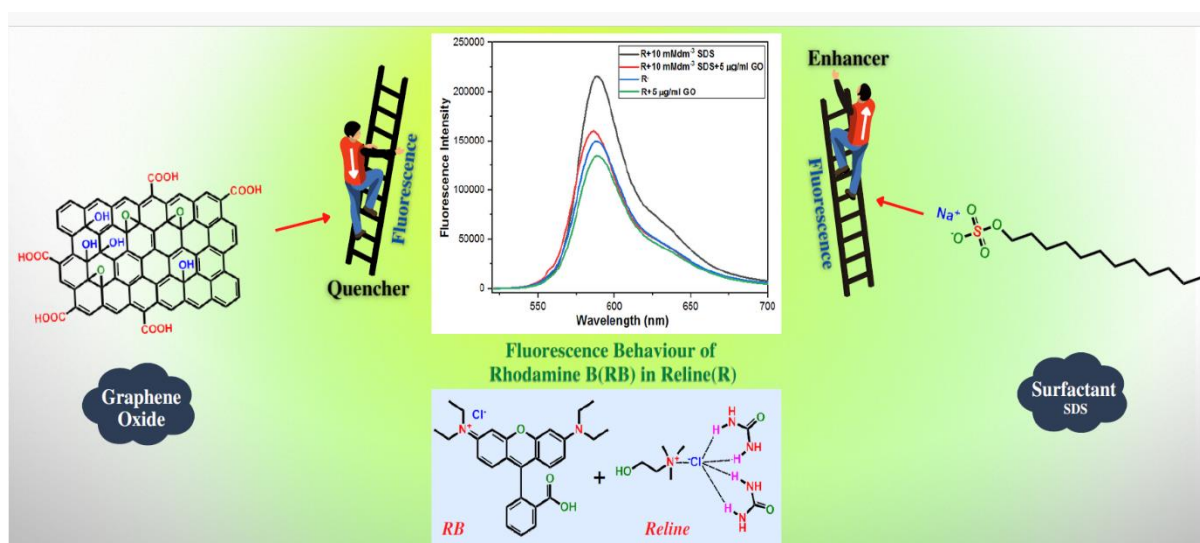
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This chapter is mainly focused on comparative study of Reline and conventional solvents (water or methanol) on the fluorescence behaviour of RB has been performed with or without GO. The study was extended by either adding water to the pure Reline or by partial replacement of urea with glycerol. These changes are applied to make Reline more appropriate to dissolve both types of ionic surfactants (anionic or cationic). The role of ionic surfactant on RB-GO interaction (photophysical behaviour) has also been studied to get insight into controlling the transport of RB towards GO surface with a concomitant modification in the photophysical process.



## 5.1 Introduction

In the last decades, fluorescent chemo-sensors have been preferred over other conventional analytical tools due to their better sensitivity and selectivity [1–3]. Rhodamine dyes (Rhodamine B (RB) and derivatives) have been widely used in single-molecule detection, fluorescence levelling, or for DNA sequencing [4,5]. The choice of solvent medium and dye concentration is a pre-requisite for the specific use. In the past, the majority of the work addressed individual solvent effects with a limited concentration range [6–8]. In a later study, it has been reported that solvent polarity affects the linear and non-linear properties of the chromophores [9]. The photophysical properties of rhodamine dyes made them important members of laser dyes [10]. The association behaviour of ionic dyes is distinctly influenced by several factors such as concentration, pH, temperature, and nature of the medium [11–14]. In a few studies, it has been shown that solvent nature governs the photophysical process shown by fluorescent molecules with or without nanomaterials of the graphene family [15–18]. Therefore, a wider

window exists to investigate the photophysics of the systems involving carbon-based nanomaterial, dye molecules, and solvents with a mechanism of fluorescence modulation.

In light of the above facts, the role of solvent is decisive in molecular-level interactions of chromophores with biomolecules [19]. As discussed in previous chapters, deep eutectic solvents (DESs) have many characteristics analogous to conventional ILs [23]. Since ILs are reported to form mixed micelles with surfactants, one can expect DES-inspired modification of the solution behaviour of a surfactant [24–26]. DES can be an alternative to conventional solvents and ionic liquids themselves as they are cheaper, non-toxic, easy to prepare, non-volatile, and non-flammable [22,27]. Among the various investigated DES, reline (ChCl: urea, 1:2 molar ratio) is the most important member from the point of view of investigations and applications in various fields [28–32]. In Reline, ChCl acts as hydrogen bond acceptor (HBA) and urea as hydrogen bond donor (HBD), with hydrogen bonding interactions at the level of the chloride ion and urea [33]. Recently, research has been performed to investigate the role of water addition in changing the characteristics of Reline (as solvent) [33–36]. To the best of our knowledge, there are only a few reports on the role of DES in the modulation of the photophysical behaviour of fluorophores [37–40].

Graphene oxide (GO) has been reported both as a quencher and enhancer of fluorescence for various materials [18,41–44]. This may be due to the presence of epoxy and hydroxyl groups in its basal planes and acidic groups in the peripheral region, which impart interactivity with solvents and fluorophores. Presently, researchers are exploring the synergistic effects of GO and solvent on the photophysical processes by changing solvent nature and GO doses [18,19,45–47]. The presence of surfactant in solution is known to change the behaviour and interaction with dyes in an aqueous medium [48–52]. In a recent report, the transport of amphiphilic molecules to GO surface using mixed surfactant aggregates was investigated [53]. However, the role of surfactant in modifying the GO-dye interactions (in DES) has not been studied a single time. Fluorescence measurements could be an ideal technique for the study of *in-situ* functionalization of GO in the presence of RB/DES or surfactant [54].

The above facts inspired us to investigate tuning of the fluorescence behaviour of RB in DESs and its potential improved sensing (as tracer) application. In this chapter, a comparative study of Reline and conventional solvents (water or methanol) on the fluorescence behaviour of RB has been performed with or without GO. The study was extended by either adding water to the pure Reline or by partial replacement of urea with glycerol (ChCl: urea: glycerol, 1: 2-x: x, where  $x=0.5/1$ ) [55,56]. These changes are applied to make Reline more appropriate to dissolve both

types of ionic surfactants (anionic or cationic). The role of ionic surfactant on RB-GO interaction (photophysical behaviour) has also been studied to get insight into controlling the transport of RB towards GO surface with a concomitant modification in the photophysical process. For this purpose, two ionic surfactants, sodium dodecyl sulfate (SDS, anionic surfactant) and cetyltrimethylammonium bromide (CTAB, cationic surfactant), are employed to modify DES media. Ionic surfactants are well-known for the modification of solution properties [57–62]. The study may help in developing the procedure for sustained transport of other amphiphilic molecules (drugs or pesticides) [63,64].

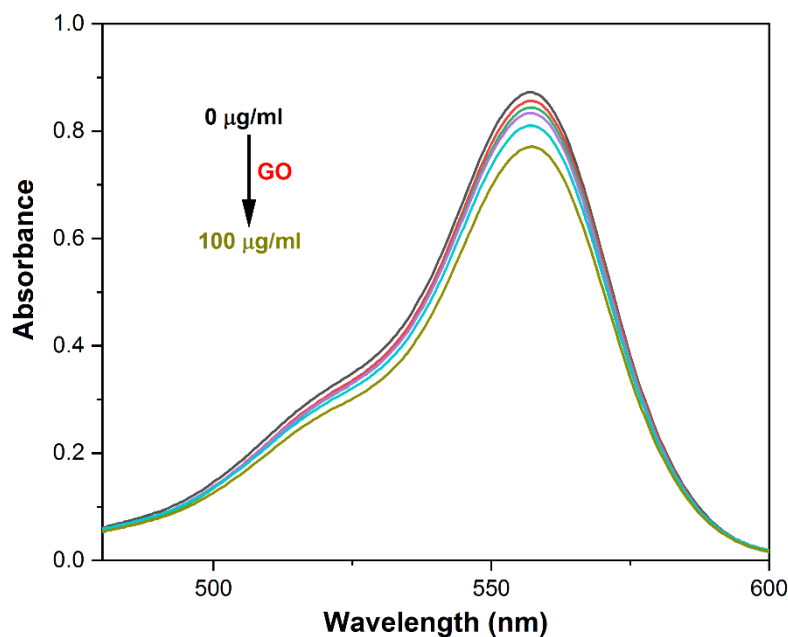
## 5.2 Experimental section

The materials and methods used are discussed in chapter 2.

## 5.3 Results and Discussion

### 5.3.1 RB absorption spectra in Reline

UV-Vis. absorption study of RB ( $3 \times 10^{-4}$  Mdm<sup>-3</sup>) has been performed in Reline with or without the addition of GO and spectra are shown in **Figure 5.1**. An intense peak has been observed at 557 nm with an insignificant hump ( $\lambda_{\text{hump}} \sim 520$  nm) in the blue region side. Such peaks for the aqueous solution of RB were observed for the monomeric state in the past [65,66]. Aggregation behaviour of RB has been reported in a recent study but [RB] of the present study rules out safely any possibility of aggregation [67]. In another study on the solvent effect, it has been reported that RB  $\lambda_{\text{max}}$  increases from 550 nm to 560 nm as one passes from water to dichloromethane (DCM) to N, N-dimethyl formamide (DMF) [68]. Though Reline has been reported as basic in nature,  $\lambda_{\text{max}}$  of RB in Reline falls between water and DMF with no significant shift from conventional solvents [69]. Even in the presence of GO (**Figure 5.1**), there was no noticeable change in  $\lambda_{\text{max}}$ . However, a fall in absorbance seems directly dependent on the amount of GO taken into the system. This behaviour can be understood in light of the fact that Reline (basic medium) may cause deprotonation of both RB and GO. The interaction between RB and GO will be governed by three interactive forces, namely: i) the strong interaction of iminium with an augmented charge on the GO surface (due to deprotonation); ii) the presence of an electron-deficient ring system in RB may provide additional  $\pi$ - $\pi$  interactions with the GO surface, and iii) the repulsive interaction between deprotonated acidic groups of both RB and GO. The fall of absorbance (in **Figure 5.1**) with the content of GO can be interpreted in terms of the dominance of the above first two factors over the last one. A similar explanation has been given for the binding of RB with the GO surface by varying the aqueous pH [70].



**Figure 5.1:** Absorption spectral profile of rhodamine B (RB,  $3 \times 10^{-4} \text{ Mdm}^{-3}$ ) in Reline with or without graphene oxide (GO, 0 to  $100 \mu\text{g/ml}$ ) at  $30^\circ\text{C}$ .

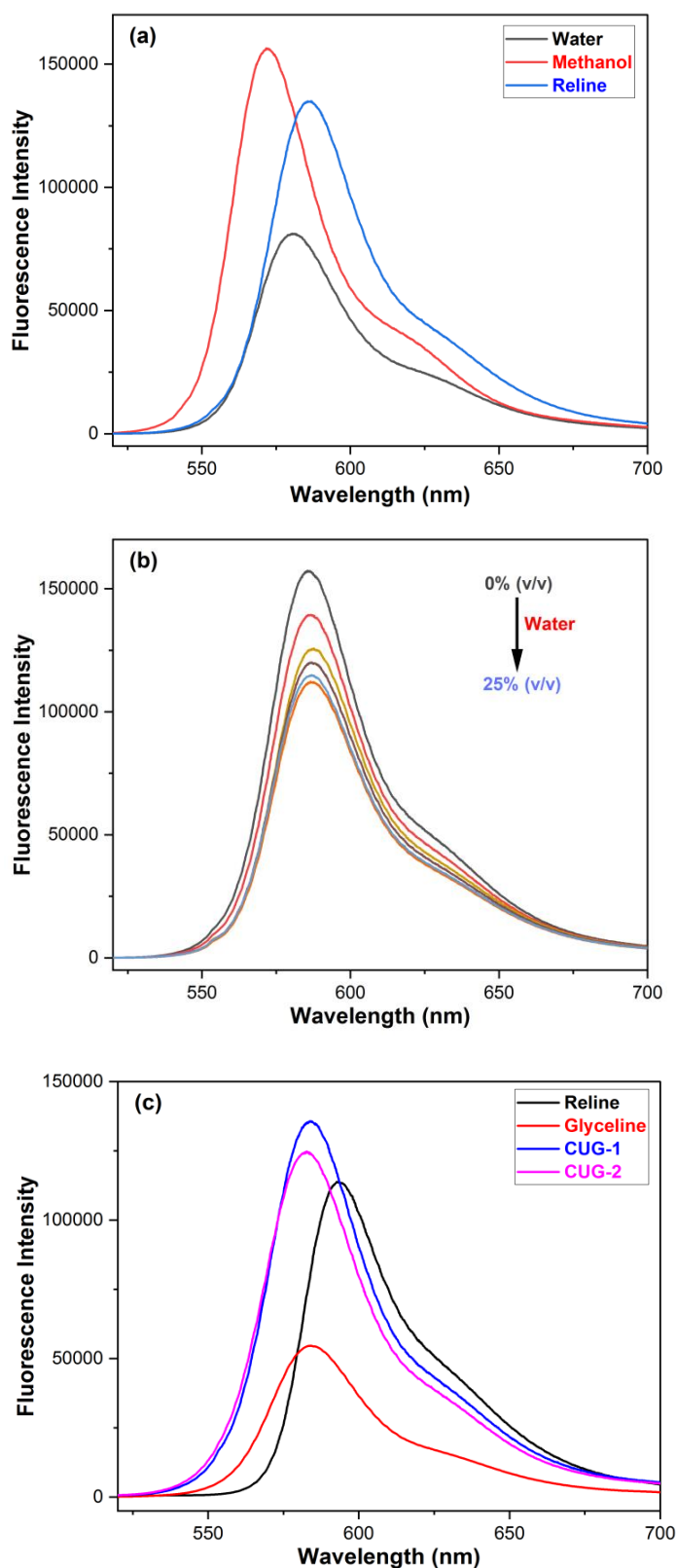
### 5.3.2 Fluorescence spectra of RB

#### 5.3.2.1 RB in Reline with and without second HBD (water or glycerol)

To exploit DES's solvent properties, researchers are varying the nature of both HBA and HBD [38,71–73]. The photophysical process in such designer solvents is an entirely new development, though such behaviour has been studied both with water and polar/non-polar non-aqueous solvents [68]. **Figure 5.2 (a-c)** shows the comparative behaviour of the variation of fluorescence intensity of RB ( $3 \times 10^{-4} \text{ Mdm}^{-3}$ ) in terms of pure conventional solvents (water and methanol), Reline, and Reline + second HBD (water or glycerol). The [RB] has been decided on the basis of the concentration effect on fluorescence in three different solvents (water, methanol, and Reline, **Figure 5.3 (a-c)**, supplementary information). Peak wavelengths ( $\lambda_{\text{em}}$ ) for water and methanol are similar as observed earlier [74]. It has been observed that the appearance of the above peak is dependent on the nature and concentration of solvent and fluorophore, respectively [17,67]. In this context,  $\lambda_{\text{em}}$  of RB in Reline has been found red-shifted. This may be due to the presence of a polar hydroxyl group and a non-polar alkyl chain in one of the components of Reline (ChCl). This structural feature may play a role in interaction with RB. This interpretation finds support from an earlier report where the chain length effect of alcohol and the consequent



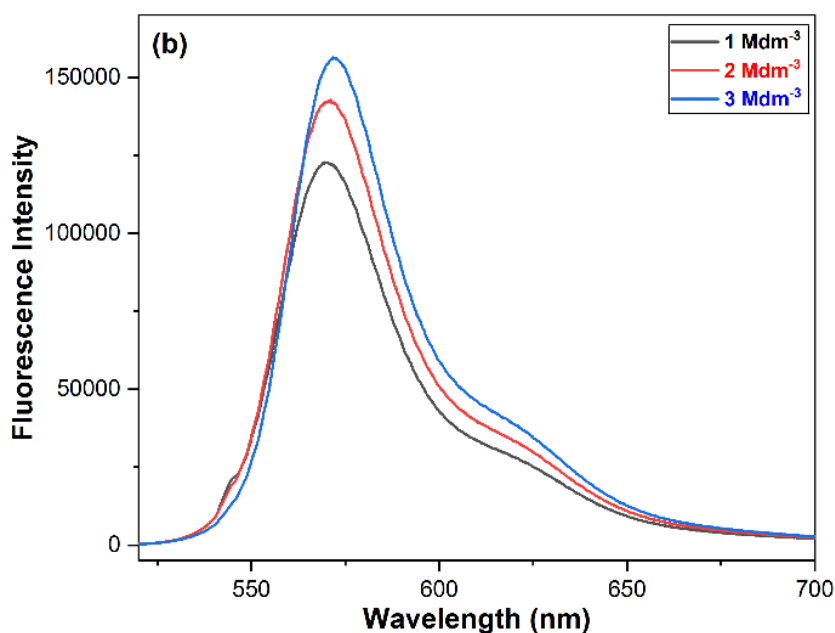
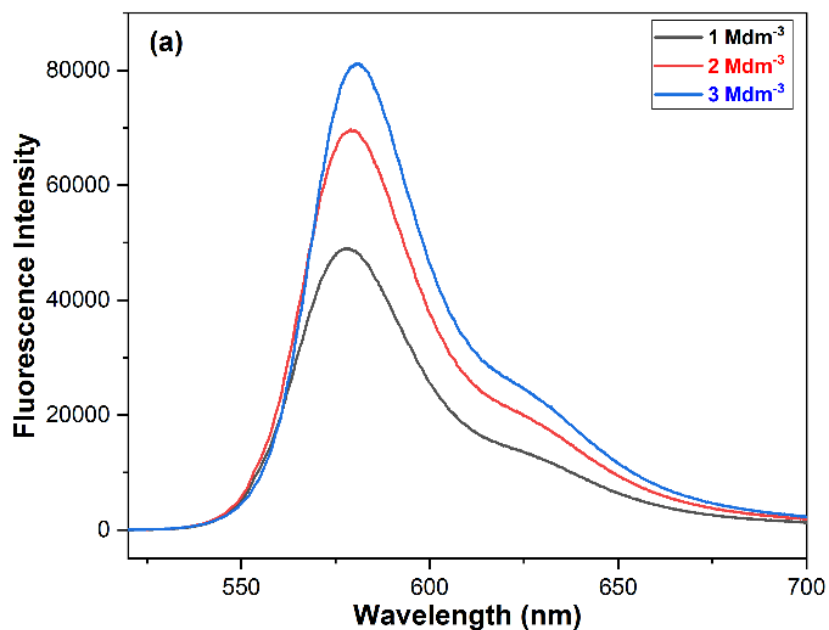
red shifting in  $\lambda_{em}$  were observed [7,75–77]. To get further insight into the designer solvent effect, Reline has been modified by adding a third component (water or glycerol), and fluorescence data are also presented in **Figures 5.2 (b & c)**. **Figure 5.2 (b)** shows a decrease in fluorescence intensity with a minor red shift in  $\lambda_{em}$ . By perusal of **Figures 5.2 (a & b)**, one can observe fluorescence intensity differences for pure water and pure Reline, and obviously, a water-Reline mixture will give intensities in between the above two pure solvents. This is indeed observed in **Figure 5.2 (b)**. Interactions between Reline components diminished gradually as the water was added [33,34,78]. It may be mentioned here that the pH of Reline moves towards neutrality (from basic, *vide supra*) on water addition (**Table 5.1**, supplementary information). Probably, these two factors are responsible for the solvation of RB and bathochromic shift in  $\lambda_{em}$  with water addition [79]. **Figure 5.2 (c)** shows partial replacement of the urea component of Reline with glycerol (another HBD). To our surprise, a hypsochromic shift was observed in  $\lambda_{em}$  together with enhanced fluorescence intensity of RB by partially replacing the urea component of Reline (1:1.5:0.5, CUG-1, and 1:1:1, CUG-2). However, CUG-1 shows a relatively greater enhancement than CUG-2. The trend continues when urea is completely replaced by glycerol (Glyceline) [80]. Since the chloride ion (of ChCl) is involved in forming hydrogen bonds with HBDs (urea, glycerol, or water), the RB interaction (through an iminium group) with Cl<sup>-</sup> would depend upon the effective negative charge left on Cl<sup>-</sup> after the formation of H-bonds. If we compare pure Reline and Glyceline, four hydrogens of NH<sub>2</sub> (due to two moles) groups are available with urea in comparison to two hydrogens (due to two moles) of glycerol [81]. Therefore, the magnitude of H-bonding interaction with Cl<sup>-</sup> will be more leaving less negative charge in comparison to Glyceline. If this is correct, then a higher magnitude of negative charge will be available in case of Reline to interact with RB. Hence, more [RB] will be available in the background solution of Reline in comparison to Glyceline. Therefore, higher fluorescence intensity is expected with Reline in comparison to Glyceline. This indeed was observed in **Figure 5.2 (c)**. Moreover, in the case of CUG-1 and CUG-2, effectively four hydrogen atoms and three hydrogen atoms are available respectively to interact with Cl<sup>-</sup> of ChCl. With CUG-1, steric hindrance will also work against RB interactions with Cl<sup>-</sup>. Hence, more RB will be available in the background solution in the case of CUG-1 than CUG-2. These reasonings will be responsible for more enhanced fluorescence intensity with CUG-1 as observed in **Figure 5.2 (c)**.



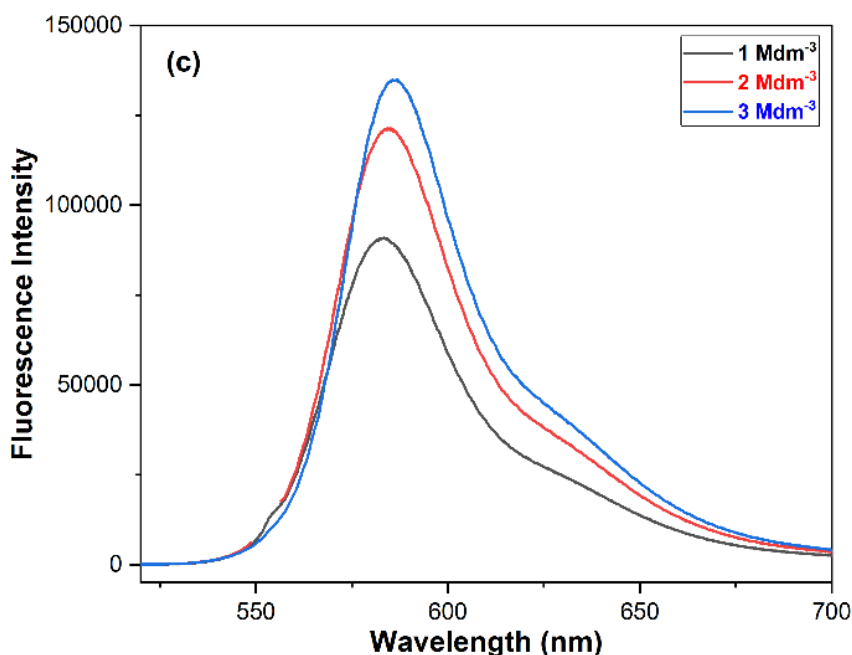
**Figure 5.2:** Emission spectra of RB ( $3 \times 10^{-4} \text{ Mdm}^{-3}$ ) in (a) Pure solvents (water, methanol or Reline), (b) Reline + water (0 to 25% (v/v)), and (c) Reline with and without glycerol (ChCl: U: Glycerol, 1:2-x:x, x=0 (Reline), x=0.5 (CUG-1), x=1 (CUG-2), x=2 (Glyceline)).

Table 5.1: Different DESs, components, molar ratio, and their respective pH.

DESs	Components	Molar ratio	pH
Reline	ChCl: Urea	1:2	10.38
CUG-1	ChCl: Urea: Glycerol	1:1.5:0.5	9.15
CUG-2	ChCl: Urea: Glycerol	1:1:1	9.80
Reline+ water	Reline: Water	75%:25% (v/v)	10.33
Glyceline	ChCl: Glycerol	1:2	4.70





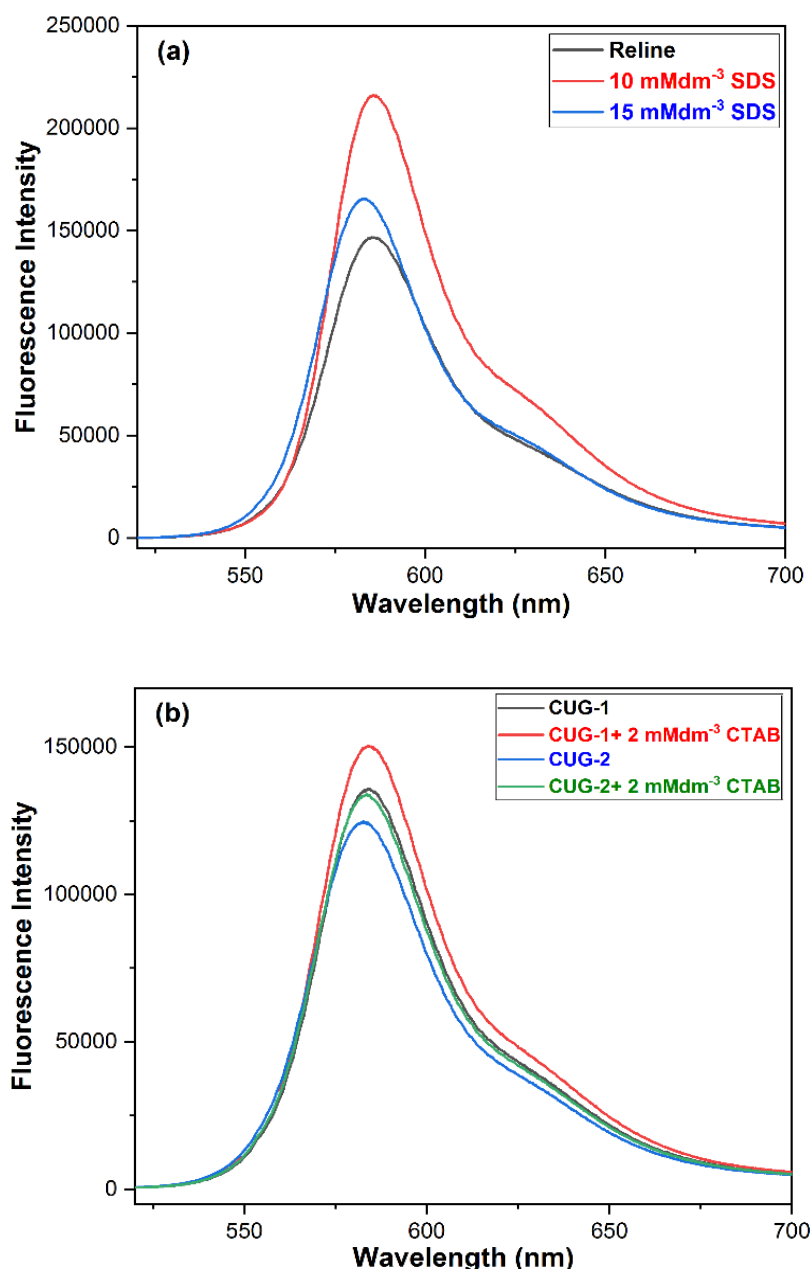


**Figure 5.3:** Emission spectra of different concentration of RB ( $1 \times 10^{-4}$  to  $3 \times 10^{-4}$   $\text{Mdm}^{-3}$ ) in (a) Water, (b) Methanol, and (c) Reline at  $30^\circ\text{C}$ .

### 5.3.2.2 RB in Reline with and without ionic surfactants

Spectroscopic investigation in an aqueous solution of rhodamine dyes with ionic surfactants has been reported in the past [82]. It has been found that various factors (charge, type, chain length, and molecular structure of surfactant/dye) influence the interaction of fluorophores with surfactant assemblies. Recently, micellization has been reported in DES media without throwing any light on changes in the photophysical behaviour of fluorophores [83–85]. Critical micelle concentration (CMC) in Reline-water (5 wt.%) mixture has been found  $\sim 13 \text{ mMdm}^{-3}$  [86]. Effects of two concentrations of SDS ( $10 \text{ mMdm}^{-3}$  and  $15 \text{ mMdm}^{-3}$ , below and above CMC, respectively) have been seen and spectra are shown in **Figure 5.4 (a & b)**. It can be seen that fluorescence intensity has increased along with a hypsochromic shift. However, concentrations above CMC ( $15 \text{ mMdm}^{-3}$ ) show no  $\lambda_{\text{em}}$  change with lower intensity in comparison to  $10 \text{ mMdm}^{-3}$  SDS. There is a probability of binding  $\text{DS}^-$  monomer with RB from the iminium ion side with a simultaneous withdrawal from the Reline. Moreover, when micelles are present (at  $15 \text{ mMdm}^{-3}$ ), one can expect RB solubilization in the interior of anionic SDS micelles. This may be due to both electrostatic and hydrophobic interactions of RB with micelles. In the latter case, the distribution of RB between micelles (by micellar solubilization) and background Reline solution will take place and be responsible for the fluorescence quenching as shown in **Figure 5.4 (a)** ( $15 \text{ mMdm}^{-3}$  SDS) [87]. It may be mentioned here that CTAB has been found sparingly soluble in Reline, and therefore the effect of CTAB micelles on the fluorescence behaviour of RB could not be

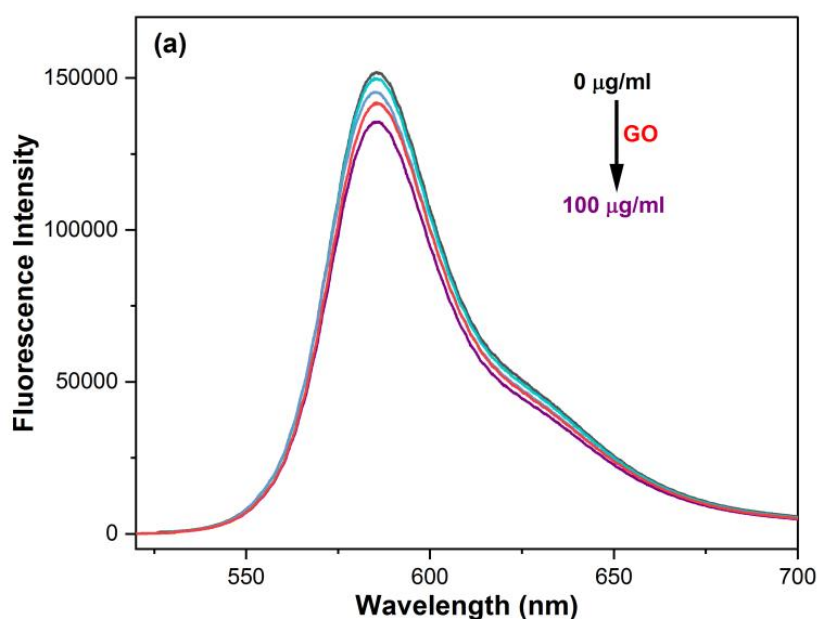
studied [86]. To make a parallel experiment with CTAB, the urea proportion of Reline was gradually replaced with glycerol and the fluorescence behaviour of RB is depicted in **Figure 5.4 (b)**. The presence of CTAB micelles causes fluorescence enhancement, which can be interpreted in terms of repulsive interactions between the positive charge of the micelle and the positive iminium group of the RB. This repulsive effect hinders micellar solubilization of RB and is responsible for the above effect.

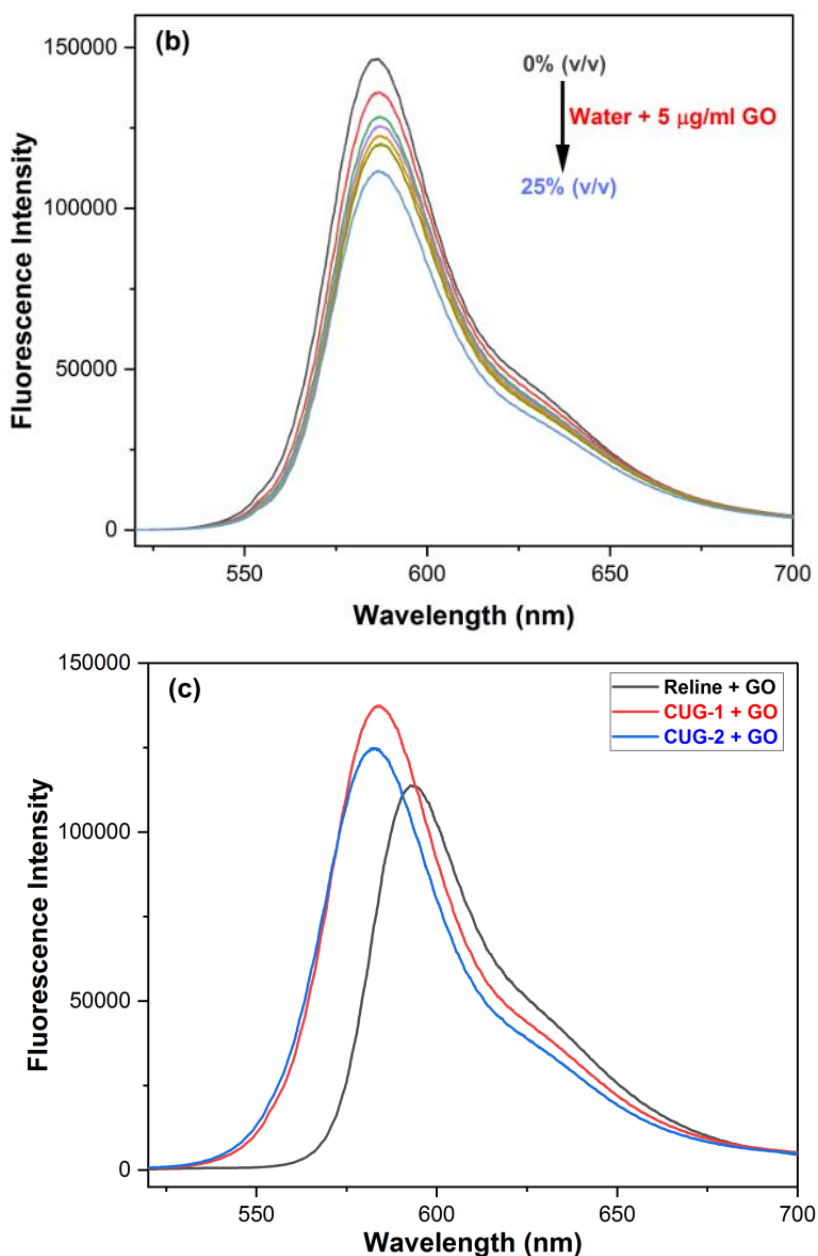


**Figure 5.4:** Emission spectra of RB ( $3 \times 10^{-4}$  Mdm<sup>-3</sup>) in (a) Reline with (below (10 mMdm<sup>-3</sup>) or above CMC (15 mMdm<sup>-3</sup>)) and without SDS, and (b) CUG-1/CUG-2 with and without CTAB (2 mMdm<sup>-3</sup>).

### 5.3.2.3 RB in Reline with and without GO

GO has been known for quenching the fluorescence intensity of various classes of compounds such as organic dyes, quantum dots, or fluorescent sensors [88–90]. GO-driven fluorescence quenching occurs due to several interactions, such as H-bonding, Coulombic interactions, and/or  $\pi$ - $\pi$  interactions with fluorophores. The basic medium provided by Reline can deprotonate both RB and GO surfaces. In addition, RB contains an electron-deficient ring and a positive iminium group. Therefore, the deprotonated acidic group of GO will interact with RB dye and be responsible for the gradual quenching effect of GO. This indeed is observed in **Figure 5.5 (a)**. Fluorescence data with 5  $\mu\text{g}/\text{ml}$  GO with varying amounts of water in a Reline-water mixture (within the eutectic range) are shown in **Figure 5.5 (b)**. Even the presence of water shows a similar quenching effect as was observed in the case of GO addition (**Figure 5.5 (a)**). However, the case of gradual urea replacement with glycerol will create a situation in which extra two OH groups (which are not H-bonded with  $\text{Cl}^-$  of  $\text{ChCl}$ ) will try to form H-bonded structure with the GO network and hinders the movement of RB towards the GO surface due to already coverage by CUG-1 or CUG-2. Within CUG-1 and CUG-2, both steric hindrance and coverage of the GO group by OH of glycerol in the case of CUG-1 will restrict the movement of RB towards the CUG-1 or GO surface. Therefore, fluorescence intensity would be higher with CUG-1 in comparison to CUG-2. In fact, the data in **Figure 5.5 (c)** are in conformity with the above interpretation.



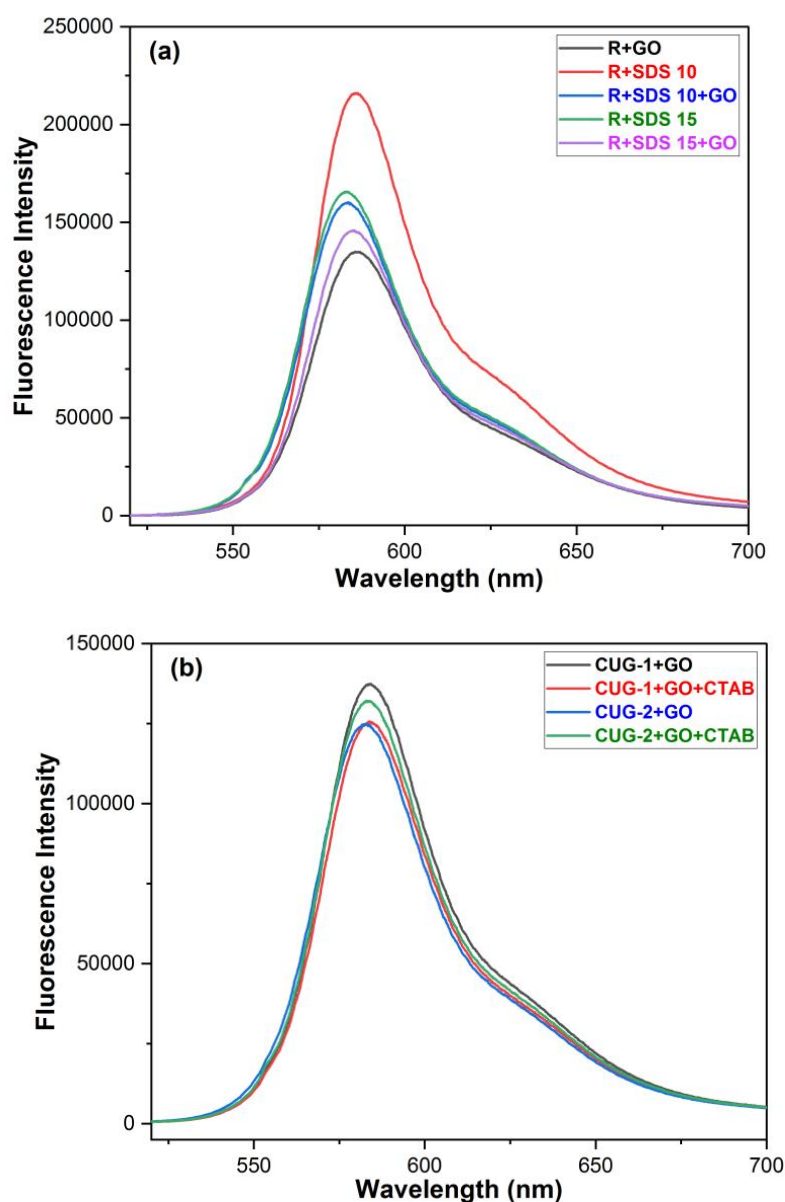


**Figure 5.5:** Emission spectra of RB ( $3 \times 10^{-4}$  Mdm<sup>-3</sup>) with GO in (a) Reline (GO varies from 0 to 100 µg/ml), (b) Reline with and without water (0 to 25% (v/v)) having 5 µg/ml GO, and (c) Reline, CUG-1 or CUG-2 with 5 µg/ml GO.

#### 5.3.2.4 RB in Reline with both GO and an ionic surfactant

Fluorescence data in the combined presence of both GO and SDS (10 mMdm<sup>-3</sup> and 15 mMdm<sup>-3</sup>) show lower intensity than without GO. In the presence of GO, one can expect RB distribution both in the micelle and at the GO surfaces. Further, in the presence of monomeric SDS, ion-pair formation between SDS monomer and RB (with a negative acidic group) will restrict RB interactions with GO due to Coulombic repulsion. The above ion pairs will impart a higher fluorescence intensity to the system than without GO (**Figure 5.6 (a)**). A similar interpretation

was put forth in an aqueous medium for an ionic surfactant system [91]. When CTAB and GO are present in CUG-1 and CUG-2, higher fluorescence intensities result in comparison to those without CTAB (**Figure 5.6 (b)**). Cationic micelles will compete with RB for the GO surface and force the RB molecule to remain in the background solution, which is responsible for higher fluorescence intensity. However, the CTAB effect was less remarkable in comparison to anionic SDS. Probably, ion pair formation of  $DS^-$  with iminium group of RB will restrict their motion towards GO surface or Reline surface. A good comparison could result when both SDS and CTAB are dissolved in a common DES.



**Figure 5.6:** Emission spectra of RB ( $3 \times 10^{-4} \text{ Mdm}^{-3}$ ) with and without  $5 \mu\text{g/ml}$  GO in (a) Reline +SDS (below ( $10 \text{ mMdm}^{-3}$ ) or above CMC ( $15 \text{ mMdm}^{-3}$ )), and (b) CUG-1/CUG-2 with and without CTAB ( $2 \text{ mMdm}^{-3}$ ).

## 5.4 Conclusion

In conclusion of this chapter, RB fluorescence can be significantly modulated (enhanced/quenched) both in the presence of a surfactant or GO. Data show that SDS ( $< \text{CMC}$ ) enhances RB fluorescence  $\sim 1.6$  times more than in pure Reline + GO system. Probably, the basic nature of Reline produces RB zwitterion which can form ion pair with  $\text{DS}^-$  monomer ( $\text{RB}^+ - \text{DS}^-$ ) and restrict its interaction to GO surface (due to similar negatively charged deprotonated acidic group in RB and GO). The fluorescence intensity depends on the [RB] at the GO surface, at the Reline surface, in the background solution, or in negatively charged ion-pair form. The  $\text{RB}^+ - \text{DS}^-$  form can be used as a model for a sustained movement of similar materials (drugs, dyes, proteins, etc.) towards their delivery sites. Strategies used here may find potential applications in chemical sensors, delivery vehicles or in biotechnology.



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