



Molecular Interaction Between Cationic Polymer Polyethyleneimine and Rose Bengal Dye: A Spectroscopic Study

Tuğba BAYRAKTUTAN^{1*}  

¹Department of Biochemistry, Faculty of Arts and Science, Iğdır University, Iğdır, Turkey

Abstract: The binding mechanism and polymer–fluorescence probe interactions between polyethyleneimine (PEI) and Rose Bengal (RB) were investigated by using UV–Vis absorption, steady-state and time-resolved fluorescence spectroscopy techniques. The spectroscopic data indicated that unusual interactions and binding constant was calculated at $3.75 \times 10^2 \text{ M}^{-1}$ with high linearity for the PEI-RB system. The photophysical parameters of the dye, such as band shifts, fluorescence quantum yields, and fluorescence lifetimes, were determined. Non-radiative (k_{nr}) and radiative (k_r) rate constants were calculated and then compared.

Keywords: Polyethyleneimine, Rose Bengal, Absorption and Fluorescence Spectroscopy, Dye-Polymer Interaction

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***Corresponding author: E-mail:** tugba.bayraktutan@igdir.edu.tr.

INTRODUCTION

Among the studies on macromolecule-small molecule interactions, comprehensive studies are available on dye compounds and binding through water-soluble synthetic or natural polymers. Many dyes were found to exhibit very different binding properties to unmodified PEI. Previous studies in this area have shown that the binding affinity of polyethyleneimine (PEI) is suitable (1, 2). Among the cationic polymers, polyethyleneimine (PEI) is an applicable transfection reactant derived from the polyamine group (3). It is a polymer with linear or branched forms. PEI acts as a good polymer chelating agent due to its water solubility and suitable molecular weights. It is a polymer where chelating properties are examined by using potentiometric and spectrophotometric methods (4, 5). In this study, a branched PEI containing primary, secondary, and tertiary amino groups at approximately 1/4, 1/2 and 1/4 rates respectively, were used (6). In this study, PEI provides a change in the spectral properties for Rose Bengal (RB) dye through π - π stacking and electrostatic interaction.

Rose Bengal (RB) is an anionic dye derived from xanthene. It is a water-soluble, xanthene - derived, an anionic dye, which has a high absorbance coefficient in the UV-visible region and high sensitivity to light. The tendency to transfer long life radicals from the triplet excited state and intersystem crossing efficiency is high (7, 8). Since it is a dye that is highly sensitive to a micro-environment, it is much preferred in photophysical studies.

This study aims to examine the polymer-dye interactions between Polyethylenimine (PEI) and Rose Bengal (RB) by using UV-Vis absorption and fluorescence spectroscopy techniques. In this context, PEI is a good candidate because polyamine polymers have a high concentration of amine groups, which provides an effective macromolecular environment in the aqueous solution. In this study, we will provide RB-PEI interaction, and the photophysical parameters will be discussed and an attempt presented for the polymer-dye interaction mechanism. The formation of complex and aggregation in the dye-polymer interactions is one of the most common

situations in the literature. The dye-polymer studies are a powerful tool for determining the binding region, the driving forces in the binding, conformational changes of the polymer and which polymer has an affinity to the dye. In this respect, this study is significant in terms of adding a dye-polymer relationship to the literature.

EXPERIMENTAL

Chemicals

Rose Bengal and Polyethyleneimine (the molecular structures are shown in the Figure 1a and 1b, respectively) were purchased from Sigma. Methanol was purchased from Fluka. RB and PEI were stored in the dark as concentrated stock solutions of 1.0 mM in methanol. The chemicals were used without further purification. All experiments were done in distilled water.

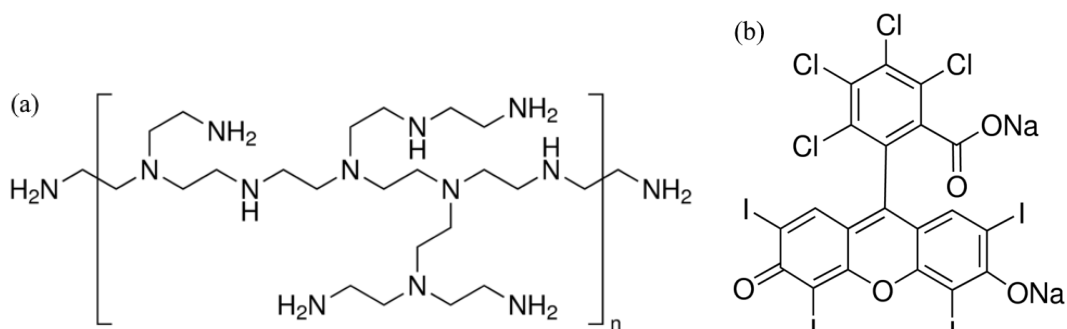


Figure 1. Molecular structures of (a) Polyethyleneimine and (b) Rose Bengal (Rose Bengal b sodium salt).

Equipments

Absorption and fluorescence spectra of samples were taken with Lambda 35 UV / Vis Spectrophotometer and Shimadzu RF-5301PC, respectively. Time-resolved fluorescence measurements were recorded in the Photon Technology Instrument (PTI) Time Master TM3. The excitation and emission slits were adjusted at 1.0 nm. The absorption spectra were recorded in the region of 455 nm to 620 nm at room temperature. For the fluorescence spectra, the system was excited at 520 nm and the fluorescence spectrum was recorded between 525 nm and 620 nm. A more detailed description of the method time depending fluorescence measurements is given other where (9).

Fluorescence Quantum Yield Measurements

Fluorescence quantum yields (Φ_f) were calculated using a reference solution and using equation 1.

$$\phi_S = \phi_r \left(\frac{D_S}{D_r} \right) \left(\frac{n_S}{n_r} \right)^2 \left(\frac{1-10^{OD_r}}{1-10^{OD_S}} \right) \quad (\text{Eq.1})$$

In equation 1, D_S and D_r refer to the integrated area under the corrected fluorescence spectra for

sample and reference, n_S and n_r are refractive indexes of sample and reference solutions, respectively. The ODs are the sample and the ODr is the reference optical density read at the excitation wavelength (10, 11). The fluorescence quantum yield of 1uM Rh101 is 1.00 in methanol (12).

RESULT AND DISCUSSION

Effects of PEI on the absorption spectra of RB

PEI does not show any absorbance in the visible region. The UV-Vis absorption spectra of the RB (2.0 uM) at various PEI concentrations changing from 0.02 to 0.90 mg/mL are shown in Figure 2. Figures 2a and 2b show the absorption spectra and normalized absorption spectra of the dye at room temperature and in a distilled water medium, respectively. RB shows maximum fluorescence absorption at 549 nm. When the various concentration polymer solutions are titrated to the dye solution, spectral shifts occur as a function of the PEI concentration. The biggest shift of about 15 nm was observed at the highest PEI concentration.

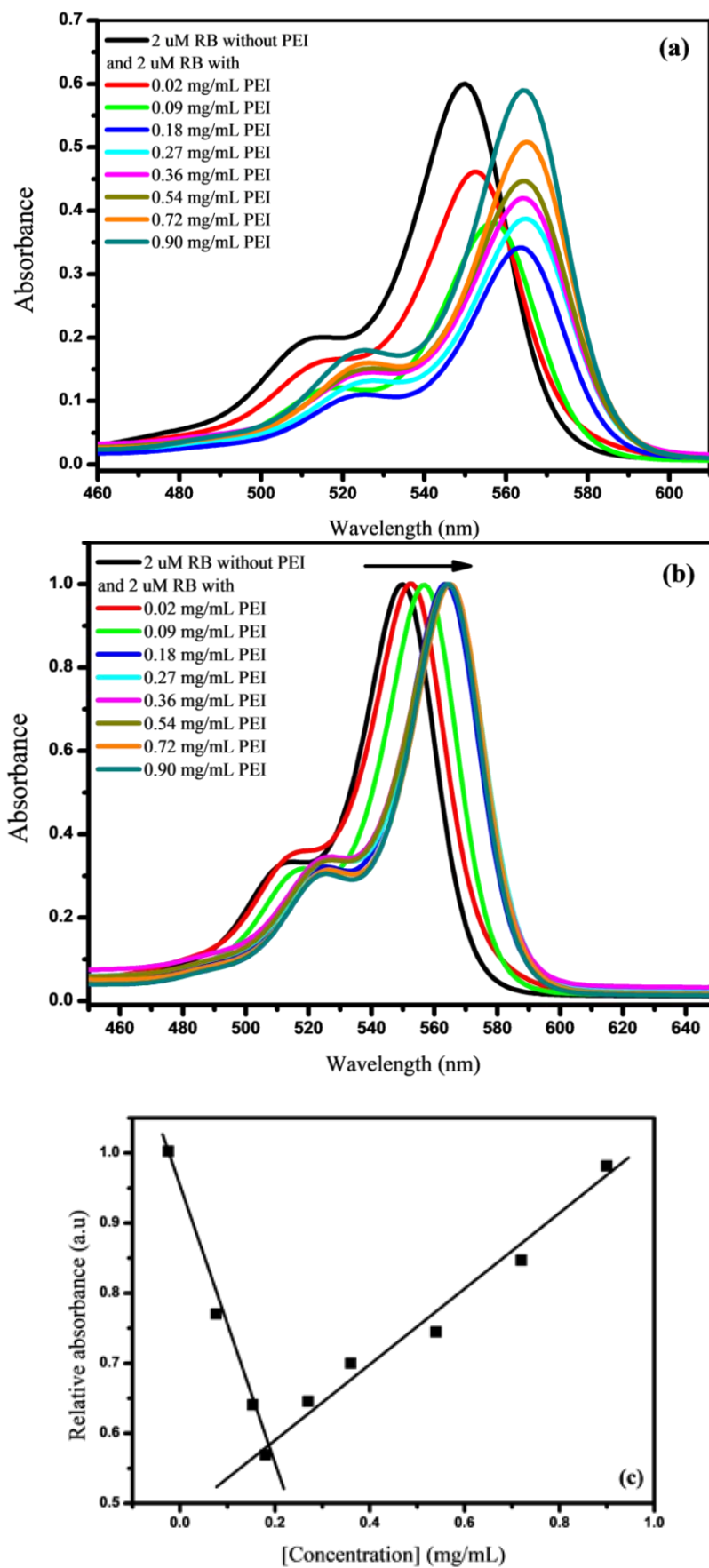


Figure 2. (a) Observed and (b) normalized UV-Vis absorption spectra of RB with various concentrations of PEI in distilled water. (c) Relation between the relative absorbance and the ratio of as a function of concentration of PEI.

According to the theory of molecular exciton, the transition moments are final for the electric dipole changeover from the ground state to the excited state or from the ground state to the higher energized excited state. It is known that a linear transition dipole will cause dimer, aggregation, or higher complexity. Such interactions always result in strong red shifts for the dimer, aggregate, and complex transition (13, 14). The absorption band of RB-PEI expands about 5 nm, and a strong red shift is observed in the comparative absorption bands of the RB alone. These spectral changes confirm the RB attaches to the polymer chain of PEI (15). These interactions lead to a decrease in absorbance at lower PEI concentrations and an increase in absorbance in higher PEI concentrations. This is clearly illustrated in the graph in Figure 1c showing the relative absorbance values based on the PEI concentrations. The change of the interaction of the resulting complex is clearly shown.

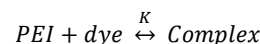
Determination of binding constant and evaluation of the quantities of binding.

Absorbance measurements were made for pure dye and PEI-dye mixture. For dye, A_{dye} refers to absorbance of RB dye alone; A refers to absorbance for the PEI- RB and the difference occurred in absorbance is calculated with the following formula:

$$A - A_{dye} = \Delta A = C_B (\epsilon_{complex} - \epsilon_{dye}) = C_B \Delta \epsilon \text{ (Eq.2)}$$

where C_B is the concentration of bound dye and $\Delta \epsilon$ is the change of the molar absorption coefficient of the bound dye or complex and the

free dye. The balance of complex formation between polymer and dye is shown as follows



where K is the binding constant. If the complex is considered to be composed of 1: 1

$$K = \frac{[complex]}{C_F [PEI]_{free}} = \frac{C_B}{C_F [PEI]_{free}} \text{ (Eq.3)}$$

where C_F refer to the free dye concentration. When the FEI is overdone, $[PEI]_{free} \sim [PEI]_{total}$ and represents $[PEI]_{total}$ as $[PEI]$ be simplified;

$$K = \frac{C_B}{C_F [PEI]} \text{ or } \frac{C_B}{C_F} = K [PEI] \text{ (Eq.4)}$$

The total dye concentration C_T is shown by

$$C_T = C_B + C_F \text{ (Eq.5)}$$

By dividing Eq.5 by Eq.2, we get the following:

$$\frac{C_T}{\Delta A} = \frac{C_B + C_F}{C_B \Delta \epsilon} \text{ (Eq.6)}$$

By simplifying Eq.6 with the replacement of the value of C_B / C_F from Eq.4 we have

$$\frac{C_T}{\Delta A} = \frac{1}{\Delta \epsilon} \left(1 + \frac{1}{K [PEI]} \right) \text{ (Eq.7)}$$

Eq. 7 is the linking of experimentally known or quantifiable amounts of ΔA to K and $\Delta \epsilon$ to the binding study (16). Figure 3 shown the plot of Eq.7 ($C_T/\Delta A$ vs $1 + 1/ K [PEI]$) for system. The experimental data were given in Table 1.

Table 1. Binding Constant and Extinction Coefficients for the PEI-Rose Bengal System

SYSTEM	$\epsilon_{dye} \times 10^5$ ($M^{-1} cm^{-1}$)	$\Delta \epsilon \times 10^5$ ($M^{-1} cm^{-1}$)	$\epsilon_{complex} \times 10^5$ ($M^{-1} cm^{-1}$)	$K \times 10^2$ (M^{-1})
PEI-RB	3.00	1.81	1.19	3.75

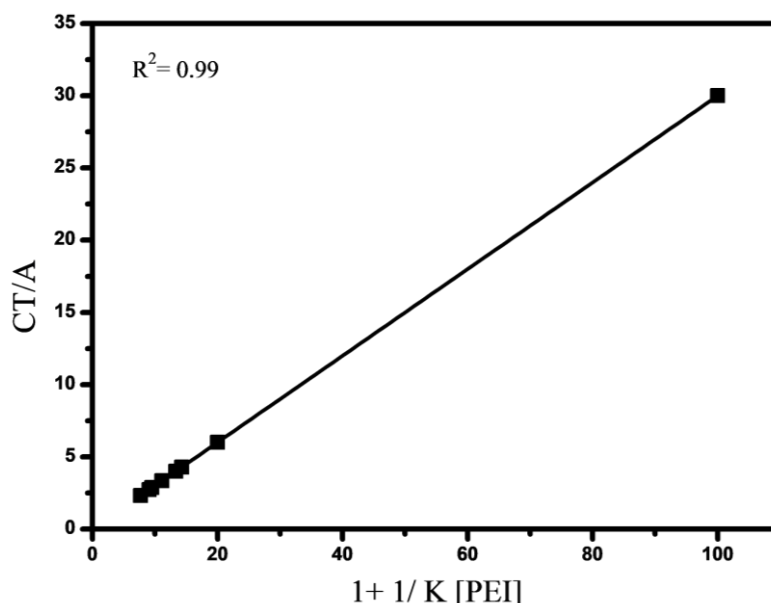
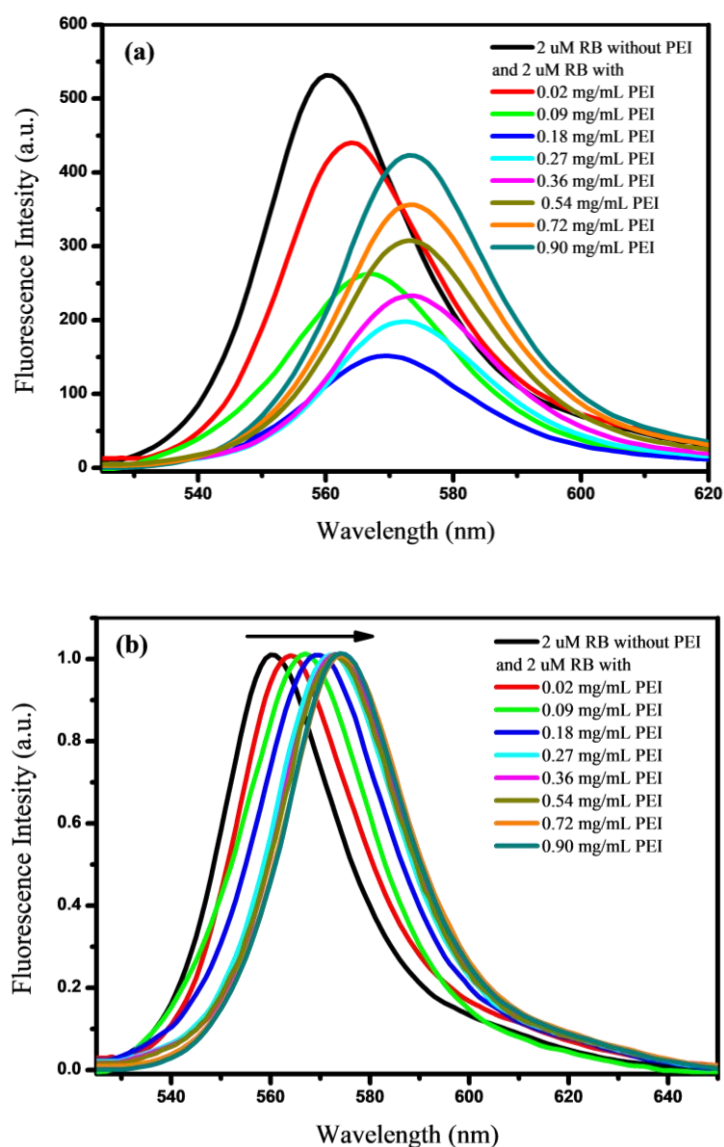


Figure 3. Plot of Eq. (6) for RB-PEI system.

Effects of PEI on the fluorescence spectra of RB

The fluorescence spectra of the RB (2.0 μM) in different PEI concentrations ranging from 0.02 to 0.90 mg/mL are shown in Figure 4. Figures 4a and b show the fluorescence spectra and normalized fluorescence spectra of the dye in a distilled water medium and at room temperature, respectively. PEI does not release any emissions during the absorbance. The fluorescence band of the RB in distilled water was monitored at 560 nm. This band was initially quenched by forming its complex structures depending on the PEI concentration. As the concentration of the PEI continued to increase, fluorescence began to increase. This is clearly shown in Figure 4c in the

graph of relative fluorescence values to the PEI concentration. The increased fluorescence of complex is an indication that the existing interaction converts the RB molecules to aggregates (17, 18). It is known that the formation of aggregate blocks non-radiative decay and increases the fluorescence of dye by intramolecular rotation in the phenyl rings (19, 20). In view of the emission spectra of the RB, the enhanced fluorescence resulting from the increase in the presence of PEI was proven to interact with the tendency of dye. The bands at longer wavelengths arise from the excimer emission of the interplayed aromatic units and aggregate (21, 22).



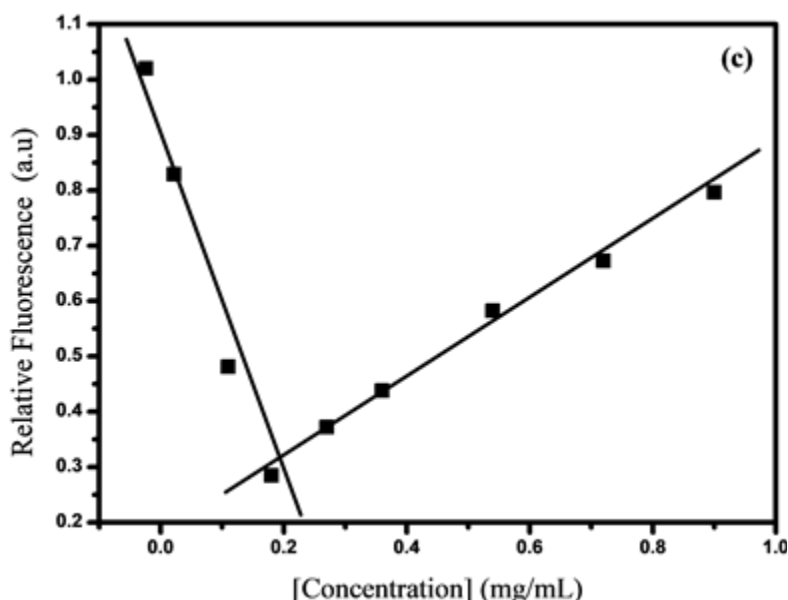


Figure 4. (a) Observed and (b) normalized fluorescence spectra of the RB with various concentrations of PEI in distilled water. (c) Relation between the relative fluorescence and the ratio of as a function of concentration of PEI.

Determination of photophysical parameters for PEI-RB system

The dye-polymer interaction resulted in the band shifting absorption. Similarly, the fluorescence spectra altered the fluorescence intensity of the RB and changed the fluorescence quantum yields (Φ_f) of the dye (Table 2). The data from Table 2 indicates that the quantum yield of RB decreases or increases depending on the fluorescence. Meanwhile, the fluorescence quantum efficiency varies greatly by the nature of the adhesion (23). The fluorescence lifetimes of the dye increased with the concentration of the polymer. The dye

molecules are more stable when they interact with the polymer and this increases the life time values due to the molecule spending more time on excited energy levels (24). The emission band maximum of the dye, which shifted from 560 to 574 nm, proves the formation of the π -stacked binding complex (25). The fluorescence lifetime of the dye is calculated and shown in Table 2. Fluorescence lifetime and fluorescence quantum yield are associated with the non-radiative (k_{nr}) and radiative (k_r) rate constants given in Equations 8 and 9 (26, 27).

Table 2. Photophysical parameters of RB in distilled water and PEI aqueous solutions.

[PEI] (mg/mL)	λ_{Abs} (nm)	λ_F (nm)	Φ_f ($\times 10^{-3}$)	τ_f (ns)	χ^2	k_r (s^{-1}) $\times 10^9$	k_{nr} (s^{-1}) $\times 10^9$	k_{nr} / k_r
Distilled water	550	560	18.0	0.095	1.2	18.95	10.34	54.5
0.02	552	563	19.0	0.123	1.1	15.45	7.975	51.6
0.09	557	566	17.0	0.185	1.0	9.19	5.313	57.8
0.18	563	569	12.0	0.222	1.0	5.40	4.450	82.3
0.27	564	571	16.0	0.246	1.0	6.50	4.000	61.5
0.36	564	573	18.0	0.338	1.1	5.32	2.905	54.6
0.54	565	573	20.0	0.371	1.2	5.39	2.641	49.0
0.72	565	573	22.0	0.413	1.2	5.33	2.368	44.4
0.90	566	574	23.0	0.455	1.1	5.05	2.147	42.5

$$K_r = \Phi_f / \tau_f \quad (\text{Eq.8})$$

$$K_{nr} = 1/\tau_f - k_r \quad (\text{Eq.9})$$

Using these equations, k_{nr}/k_r values were calculated for dye in the PEI solutions and distilled water. Table 2 shows that the k_{nr}/k_r values for dye were higher at PEI concentrations of 0.18 mg/mL. On the other hand, k_{nr}/k_r values are often above the average in similar systems. In addition, high k_{nr}/k_r values of RB show that the

main excited-state deactivation pathway is internal conversion (28).

CONCLUSION

In the present study, the effects of PEI on the photophysical properties of RB dye were examined using spectroscopic techniques. The interactions of the RB dye and PEI in distilled water caused differences in the photophysical and spectroscopic properties of the dyes. RB (2 μM) in

the presence of PEI displays strong red shifts and changes of absorption and emission band intensities when compared to that in distilled water, which are assigned to the dye-polymer interaction. The values of k_{nr}/k_r for the dye are calculated in the PEI media. The changing photophysical parameter values of the RB are due to the local micro viscosity and polarity variations as well as the probe surrounding interactions in this medium and dye interactions. Changing and limiting the photophysical properties of the dye with the concentration of the polymer will be beneficial tools used in optical studies.

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