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Fluorescence Quenching Study of Nano Graphene Oxide Interaction with Naphthalimide Dye: Thermodynamic and Binding Characteristics

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ABSTRACT

quenching study was reported for interacting a fluorescence naphthalimide derivative with graphene oxide (GO) as a quencher. 1,8naphthalimide fluorophore with two amine functional side arms (NN2) was synthesized and characterized. Many different 1,8-naphthalimide fluorescence dye derivatives have been designed for fluorescence probe application. Fluorescence quenching-based platforms in nanoscale have extensively been used in sensing systems. Raman, FTIR, UV-Vis, and fluorescence spectroscopic techniques were used to study GO and NN2 characteristics and their photophysical and quenching mechanisms at different temperatures. The results indicated that graphene plays an effective quencher against the naphthalimide molecule, with quenching efficiently at 91 %. The Stern-Volmer analysis results show a mix of static and dynamic quenching mechanisms. The binding constant of the quencher and fluorophore and the number of binding sites have been reported. Thermodynamic parameters of their interaction were evaluated. The negative values of the ΔG confirm that the complexation process is spontaneous. Meanwhile, the positive entropy value confirms the favourable pathway process. Prog. Color Colorants Coat. 16 (2023), 243-253© Institute for Color Science and Technology.

1. Introduction

Nano graphene oxide with unique optical and physicochemical properties attracted much attention for sensor application. Its excellent electrical conductivity, fast electron mobility, tunable optical properties, high mechanical strength, and functionalization capability make it an efficient platform for developing sensor applications [1-8]. One of the most sensitive optical sensors is based on the quenching process between a fluorophore and a quencher. Reported quenching mechanisms of graphene oxide (GO) as a quencher are based on one of these models, the photoinduced electron transfer (PET) process or Förster resonance energy transfer (FRET). The fluorophore directly affected by the graphene quencher makes it suitable for optical sensing platforms [9-14]. When GO and the fluorophore are close, the energy or excited electron is transferred from the fluorophore to GO. In this case, the fluorescence signal decreases.

Among fluorescence dyes, 1,8-naphthalimide has been applied in different areas. They are used in the paint and textile industry, sensors, laser diodes, and biological probes [15, 16]. Their excellent stabilities. such as photo, thermal, and chemical stability, attracted much attention. Their derivatives showed high fluorescence efficiency [17, 18]. These properties make it to be a good candidate for sensitive fluorescent sensors. Easy modification of the fluorescence core achieved through imide or core position resulted in a desirable design for specific interaction and an efficient fluorescent probe [18]. The design and development of novel platforms based on graphene as the energy acceptor have been a remarkable success in recent years. Our team is developing the design and application of fluorochrome dyes for probe applications. Gharagozlou et al. reported a new reusable mercury-sensitive turn-on nano-chemosensor based on CoFe₂O₄@SiO₂ magnetic nanocomposite. It was functionalized by napthalimiderhodamine sites [19]. Seraj et al. investigated the fluorescence quenching mechanism of a naphthalimide dye derivative by graphene oxide [20]. Seraj et al. in 2019, developed a naphthalimide-based optical turn-on sensor for monosaccharide recognition using a boronic acid receptor [21]. Also, Seraj et al. in 2021 reported the synthesis and fluorescence quenching mechanism of a novel naphthalimide derivative by nanographene oxide [22].

Recently we worked on the effect of graphene oxide as a quencher on the different naphthalimide derivatives. Studies on the quenching mechanism between graphene oxide and a symmetric di-amino functionalized 1,8-naphthalimide dyes have yet to be reported. This work investigated the synthesis of a water-soluble 1,8-naphthalimide dye. The mechanism of quenching was studied by UV-Vis and fluorescence spectroscopic techniques. Also, the thermodynamic pathway of interactions was investigated. The quenching parameters were obtained, and involved mechanisms were reported.

2. Experimental

2.1. Materials

4-Bromo-1,8-naphtalic anhydride and ethylenediamine were purchased from Merck Chemical Co. and used as received. All solvents were of analytical grade. The used graphene oxide was purchased from Nano SANY Co. (product number; US 7906, CAS 7732-18-5). A stock solution of dye (10^{-3} M) was used for spectroscopic investigations.



Scheme 1: Schematic of NDN2 quenching by graphene oxide.

2.2. Synthesis of fluorescence dye (NN2)

NN2 was synthesized from 4-bromo-1,8-naphtalic anhydride, according to our previous work [21, 22]. 0.3 g 4-Bromo-1,8-Naphtalic anhydride was dissolved in 12 mL Ethanol. 40 mL ethylenediamine was added in 2 steps intervals. The resulting solution was heated under reflux for 24 h. After cooling, the ethylenediamine was distilled under a vacuum to afford a crude product. After recrystallization from ethanol, yellow needles were obtained (90 %). m.p. 235 °C, FTIR (KBR) cm⁻¹: 3439, 3080, 2934, and 1729 cm⁻¹. ¹H NMR (400 MHz, DMSO-d6): d (ppm) = 8.55 (d, 1H, Ar-H), 8.32 (d, 1H, Ar-H), 8. (d, 1H, Ar-H), 7.62 (t, 1H, Ar-), 6.73 (d, 1H, Ar-), 4.83 (s, 4H, -NH₂), 4.41 (s, 1H, -NH-), 4.04 (t, 2H, -CH₂-), 3.92 (t, 2H, -CH₂-), 3.22 (t, 2H, -CH₂-) ,3.00 (t, 2H, -CH₂-). Anal. Calcd. for C₁₆H₁₈N₄O₂ (%): C, 64.41; H, 6.08; N, 18.78. Found: C, 64.51; H, 6.01; N, 18.68.

2.3. Apparatus

¹HNMR spectra were recorded with Brucker spectrophotometer at 500 MHz (solvent: DMSO). FTIR spectra were recorded on a SPECTRUM ONE spectrometer. A Perkin-Elmer LS55 fluorescence spectrophotometer was used for all fluorescence measurements. UV-VIS absorption spectra were measured on a CECIL-CE9200 spectrophotometer.

2.4. Preparation of NN2/GO

A stock dispersed GO solution was prepared by 20 min sonication of appropriate GO powder in distilled water. To a 0.1 mL of the stock solution of NN2 (10^{-3} M), an appropriate amount (0, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 µg/mL) of 30 µg/mL GO was added, sonicated for 10 min, and, diluted to 5 mL was transferred into a 5 mL tube.

3. Results and Discussion

3.1. Synthesis of fluorescence dye (NN2)

The synthetic route for the preparation of NN2 is shown in Scheme 2. A simultaneous nucleophilic substitution on two positions of naphthalic anhydride molecule by ethylenediamine has been performed. Position, 4 of naphthalic anhydride ring is prone to nucleophilic attack. Also, the anhydride ring and the carbonyl groups have a suitable position for the nucleophilic attack, subsequently forming a stable amide ring. NN2 was obtained with a good yield of 90 %. Results of FTIR and HNMR, and CHN analysis firmly identified the structure of compound NN2.

3.2. Photophysical properties

NN2 is related to the 1,8- naphthalimide category of dyes, which have photophysical properties, and strongly depend on the dicarboximide and amino-substituted groups. The amino-substituted groups with

the ethylene space show a photoinduced charge transfer process (PET) to carbonyl groups. The electron charge transfer between the donor-acceptor (the imide moiety or C-4 position of the chromophoric core and the carbonyl groups) and resulting polarization is responsible for the spectral characteristics of these derivatives [15, 16].

The absorption and emission spectra of dispersed NN2 in H₂O are shown in Figure 1. The dye shows a yellow-green color (λ_{abs} = 420 nm). The wavelength band around 420 nm (ϵ = 13900 L mol⁻¹.cm⁻¹) confirms a charge transfer (CT) band due to ($\pi \rightarrow \pi^*$) transition of the S0 \rightarrow S1 transition [23]. There is a band in ABS spectra at about 550 nm attributed to intermolecular charge transfer (ICT) [24]. The dye emission was shifted to the visible region with a maximum (λ_{em} = 540 nm) with an intense yellow-green emission and a significant 120 nm stock shift.



Scheme 2: Synthesis of NN2.



Figure 1: A) Absorption and B) emission spectra of NN2 (10^{-5} M) in H₂O.

3.3. Characterization of GO

SEM and UV-Vis techniques were applied to characterize the commercial GO used, as shown in Figure 2a and b. SEM image shows the planar morphology of GO. These results showed that the used GO was indeed a single-layer sheet. As shown in Figure 2b, maximum absorption at 229 nm confirms the π - π * transition of aromatic C=C bonds. Also, a shoulder around 300 nm is related to the n- π * transition of C=O bonds [25].

Raman analysis was also conducted for the absorption of NN2 and GO, shown in Figure 3. The Raman spectra of GO show GO's characterized D and G bands at (1348 cm^{-1}) and (1599 cm^{-1}) , respectively, approving the presence of GO. The GO spectrum, after interaction with NN2 remarkable intensity reduction at the D and G bands without any significant shift. These results can be further evidence of the interaction between GO and NN2.



Figure 2: a) SEM, b) UV-Vis spectra of used GO.



Figure 3: Raman spectra of GO before (blue) and after adsorption of NN2 (red).

Figure 4 shows FTIR spectra of individual GO, NN2, and a mixture of GO⁺NN2. As obvious in the figures, characteristic peaks of C=O vibration for GO are at 1723, 1572, and 3000-3200 cm⁻¹. Also NN2 shows C=O vibration at 1732, N-C stretching at 1600, 1532, and NH₂ vibration at 3331 cm⁻¹. Remarkable changes occur on these characteristic peaks for the mixture of GO and NN2. The peak at 3445 is going to be wider, with different shapes for GO in this region. The C=O vibration peak is shifted to the lower vibration energy at 1627 cm⁻¹, respecting the individual GO and NN2 C=O peaks. Our previous work obtained the same results [21, 22].

3.4. Quenching mechanism

A wide variety of processes can decrease the intensity of fluorescence. Such decreases in intensity are called quenching. Quenching can occur via different pathways. Energy transition takes place by nonradiative process, where two species come close together to transfer the energy. Collisional quenching occurs when the excited-state fluorophore is deactivated upon contact with some other molecule in the solution, which is called the quencher. Then the fluorophore is returned to the ground state. A wide variety of molecules can act as collisional quenchers. Examples include oxygen, halogens, amines, and electron-deficient molecules [26].

The fluorescence emission spectra of NN2 are illustrated in Figure 5. It shows a maximum intensity at 540 nm, gradually decreasing with increasing GO concentration. Changes indicate that GO is a quencher of the naphthalimide fluorophore (NN2). Also, GO has no fluorescence at the NN2 excitation wavelength. The quenching was due to the interaction between NN2 and GO, not an inner filter effect or reabsorption. As previously reported in the literature, GO was the oxidized form of graphene with the carboxylic acid, hydroxyl, and epoxy groups [27-29]. Therefore, GO could have π - π stacking interaction with aromatic rings of aromatic molecules, herein, NN2 dye. The quenching efficiency is defined as $\eta = (F0-F)/F0 \times 100$, for which a high value of (91.33 %) was obtained for the NN2.



Figure 4: FTIR spectra of GO, NN2, and GO+NN2.



Figure 5: left) Fluorescence spectra of (10⁻⁵ M) NN2 in the presence of various concentrations of GO, right) Photographs of NN2 during GO addition under UV light(up) and Vis light (down).

It was found that quenching goes through either static or dynamic interaction mechanisms [30, 31]. When a complex formation between an emissive molecule and a quencher occurs, it is called static quenching. The resulting complex has no emission. Therefore, its intensity gradually decreases by adding a quencher. Dynamic quenching is called collisional quenching because a collision with a quencher causes a loss of emission. Herein there is not any complex formation [32]. The Stern-Volmer equation (Eq. 1) was used to study the dynamic quenching mechanism [26].

$$F_0/F=1+K_{sv}[Q] \tag{1}$$

 F_0 and F are the molecule's fluorescence intensity in the quencher's absence and presence, respectively. K_{sv} is the binding constant of the quencher-fluorophore, and [Q] is the concentration of the quencher. Temperature directly affects dynamic quenching because this process goes through a diffusioncontrolled pathway. The diffusion of the quencher to the fluorophore during the lifetime of the excited state is essential. Increasing the temperature resulted in a faster diffusion rate, and the bimolecular quenching constants are expected to increase. In static quenching, a non-fluorescent complex forms at the ground state; therefore, K_{sv} decreases with increasing temperature. In some processes, there is not involved only one quenching mechanism.

Generally, dynamic quenching involves the collision followed by the formation of a transient complex between an excited-state fluorophore and quencher. Recording the effect of temperature on the quenching efficiency helps identify which mechanisms are involved. It is found that the higher temperature increases the possibility of collision. It resulted in a higher quenching efficiency, known as dynamic quenching. At the same time, less quenching was observed at a lower temperature [29, 33]. The fluorescence emission results of NN2 in the presence of GO were obtained at different temperatures (288, 298, and 308 °k). Figure 6 illustrates the Stern-Volmer plot, in which a higher quenching efficiency was observed at lower temperatures. Results showed that dynamic quenching was not the primary quenching mechanism of NN2. As can be seen, the dependence of F_0/F on [Q] strayed from linearity.

The expansion is due to the strong and π - π stacking between NN2 and graphene oxide. These results showed that the responsible quenching mechanism for NN2/GO follows both dynamic and static quenching.



Figure 6: Fluorescence quenching of NDN2/ GO at different temperatures.

The resulting curves in Figure 6 show a nonlinear behavior with an upward curvature. It cannot be accurately fitted in the linear Stern-Volmer equation (Eq. 1). The modified equation (Eq. 2) has been introduced for a single species undergoing both dynamic quenching and ground-state complex static quenching [32]. K_s and K_D need to be determined in an analysis of the nonlinear steady-state fluorescence quenching model [34]. The measured K_s , K_D , and R^2 are evaluated from Figure 6 and given in Table 1.

$$F_0/F = (1 + K_S[Q])(1 + K_D[Q])$$
(2)

The number of binding sites between the fluorophore and GO can be evaluated by the site binding model (Eq. 3).

$$\log\left[\left(\frac{F_0}{F}\right) - 1\right] = \log Kb + n\log[Q] \tag{3}$$

Where K_b is the binding constant and n is the number of the binding site. Figure 7 shows the plots of log $[(F_0-F)/F]$ versus log [Q] at different temperatures, and the corresponding values of k_b and n are given in Table 1. The correlation coefficient of the curves is larger than 0.95, indicating that the interaction between NN2 and GO agrees well with the site binding model underlying (Eq.3)

The result manifested that the binding constant kb decreased as the temperature increased, resulting in the destabilization of the GO-NOP complex. The unstable complex would be partly decomposed when the temperature is increased, and hence K_b value decreased. At the experimental temperature, the values of n at the experimental temperature were approximately equal to 1, which hinted that there was a single binding site in GO and NOP.

Table 1: Quenching parameters of graphene oxide and NN2 at different temperatures, Temperature (°K).

Temperature (°K)	K _S	K _D	\mathbf{R}^2	Log K _b	n	\mathbf{R}^2
288	209.06	0.00030	0.98	1.59	2.11	0.96
298	128.83	0.00035	0.98	1.52	1.98	0.95
308	88.83	0.00033	0.96	1.37	1.76	0.95



Figure 7: Plots of log $[(F_0 - F)/F]$ versus log [Q] at different temperatures.

The quenching of NN2 could occur through the following possible processes, an energy transfer or electron transfer. For an energy transfer via the FRET mechanism, an overlap between the absorption spectra of a quencher (acceptor) and the emission spectra of emissive species (donor) is needed. However, there is no overlap between the absorption spectra of GO (acceptor) and the emission spectra of NN2 (donor). Therefore, the FRET mechanism made energy transfer between NN2 and GO impossible. The fluorescence quenching of NN2 and GO seems to follow an electron transfer process. Further studies were performed to investigate the ground state and excited state energy levels of NN2. Cyclic voltammetry and UV-Vis spectra were used to calculate the band gap energy of GO and NN2. The energies obtained are -3.28 eV and -5.22eV for LUMO and HOMO energy levels of NN2, respectively. The calculated band gap, ΔE , is 1.94 eV for NN2. The reported valence bound of GO is to be -4.7 eV. Therefore, as shown in Figure 8, electron transfer is possible from the excited state of the NN2 to the GO plates. NN2 acts as a donor in the exited state to the graphene surface as an acceptor. The same behavior for GO and coumarin dye was reported previously [33].

3.5. Thermodynamic parameters

Generally, the binding constants are affected by temperature. Therefore, we follow the quenching process in different temperatures (288, 298, and 308 °k). From the value of the stability constant at different temperatures, the enthalpy can be calculated from the Van't Hoff equation (Eq. 4) based on the relationship between the binding constant (k) and temperature (T).

$$\Delta G = -2.303 \text{ RT} \log K \tag{4}$$



Figure 8: Energy levels of NN2 and GO.



Figure 9: Plot of log K_{vs} vs. 1/T.

Table 2: Thermodynamic parameters of NN2/GO quenching at (288,298 and 308 °k)

Temperature (°K)	ΔG (KJ.mol ⁻¹)	$\Delta H (J.mol^{-1})$	ΔS (J.mol ⁻¹ .K ⁻¹)
288	-8.768	0.019	30.44
298	-8.673	0.019	29.10
308	-8.01	0.019	26.23

R is the gas constant, T is the temperature, and K is the binding constant [33]. The enthalpy (ΔH) of the complexation process was obtained from the slope of the plot of log K_b vs. 1/T using the graphical representation of Van't Hoff's equation (Figure 9), and the entropy (ΔS) could then be calculated as follows using Eq. 5.

$$\Delta G = \Delta H - T \Delta S \tag{5}$$

The thermodynamic parameters of complex formation between NN2 and GO are included in Table 2. It seems that the enthalpy (Δ H) did not vary significantly over the studied temperature range, and cab assume it is constant. As can be seen, all the thermodynamic parameters are negative. The negative ΔG values confirm a spontaneous complexation process. The positive ΔS is evidence of a favorable entropy change during the complexation process.

4. Conclusions

GO efficiently interacts with NN2 via fluorescence quenching. Raman and FTIR investigations approved the adsorption of NN2 on the nano GO sheets. Fluorescence spectroscopy results also confirmed the efficient interaction between a donor and acceptor species combined with energy transfer followed by quenching of the fluorescence molecule. The results of the Stern-Volmer plot show that the primary quenching mechanism is a combination of static and dynamic quenching. The obtained binding constant of the quencher and fluorophore and the number of binding sites confirmed the presence of two sites for interaction. The binding study is of great importance in pharmacy and biochemistry. These experimental and theoretical results are potentially important in understanding the interaction mechanism between graphene and naphthalimide dye. They would be helpful guides for designing and developing efficient

graphene-based sensors. Also, the negative values of the ΔG confirm that the complexation process is spontaneous, and the positive entropy value confirms the favorable pathway process.

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