Interactions of Environmental Pollutant Aromatic Amines With Photo Excited States of Thiophene Substituted 1,3,4-Oxadiazole Derivative: Fluorescence Quenching Studies

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Abstract
In the present work, the fluorescence quenching of novel thiophene substituted 1,3,4-oxadiazole derivative 2-(4-(4-vinylphenyl) phenyl)-5-(5-(4-vinylphenyl)thiophen-2-yl)-1,3,4-oxadiazole (TSO) by five different environmental pollutant aromatic amine derivatives like 2,4-dimethylaniline, 3-chloroaniline, 4-chloroaniline, o-anisidine, and m-toluidine has been studied at room temperature through steady-state and time-resolved methods. It is observed that, the quenching efficiency is highest in the case of o-anisidine and least in the case of 3-chloroaniline. The fluorescence quenching mechanism between TSO and aromatic amines is analysed through different quenching models. The results suggest that, the fluorescence quenching is due to diffusion assisted dynamic or collisional quenching according to the sphere of action static quenching model and according to the finite sink approximation model, the bimolecular quenching reactions are due to the collective effect of dynamic and static quenching. Further, cyclic voltammetry and DFT studies suggest that the fluorescence quenching is due to electron transfer. Binding equilibria analysis confirms the 1:1 stoichiometric ratio between fluorophore and the quencher.

Keywords Fluorescence quenching · Stern–Volmer · 1,3,4-oxadizole · Aromatic amines · 2,4-dimethylaniline · m-toluidine · o-anisidine

Introduction
Fluorescence quenching is a process, in which the electronic excitation energy of an excited fluorophore is transferred to a non-fluorescent quencher molecule via several mechanisms such as energy transfer, charge transfer, molecular re-arrangement, excited state reactions, diffusion, ground state complex formation, etc. This can be explained either by dynamic (collisional) or static quenching mechanisms [1, 2]. Fluorescence quenching is affected by various factors such as temperature, viscosity, solvent polarity, etc., of the surrounding medium, as well as on the nature of the quencher also. Experimentally, the fluorescence quenching mechanisms can be studied by determining the quenching parameters using Stern–Volmer (S-V) plots. If S-V plots are linear, then quenching mechanism is mainly due to diffusion assisted dynamic or collisional quenching. But if the S-V plots are non-linear with some positive deviation, then this may be attributed to processes like intersystem crossing, charge transfer complex formation, static quenching along with the dynamic process. The fluorescence quenching of organic molecules by various quenchers like aromatic amines, bromobenzene, carbon tetrachloride, metal ions, halide ions, etc. are mainly used to understand the nature of molecular interactions. Further, from quenching studies, one can reveal the diffusion rate of quenchers, localization of fluorophores in proteins and membranes and their accessibility to quenchers. The quenching studies are also very important in biological, chemical and medical sciences [3, 4].

The detection of aromatic amines has become very important, because they have been considered as severe destroyers of environmental ecological equilibrium and
toxic to human health via breathing, skin contact, etc. The detection is also important in terms of monitoring industrial and environmental pollution, diagnosis of certain diseases, for checking the quality of food products, etc. The vapours of aromatic amines can diffuse into the atmosphere from foul water, car exhaust, incineration of garbage, construction materials, industrial emissions, cigarette smoke, etc. Aniline and its derivatives are of high toxicity to all living organisms even at very low concentrations and have been considered as a potential carcinogen and are listed as Group 3 root causes by “The International Agency for Research on Cancer” (IARC) [5–7]. It has been reported that, acute inhalation exposure to high levels of aniline and its derivatives can lead to lung cancer in human beings. Further, detection of carcinogenic organic compounds like aromatic amines, o-toluidine, nitroaromatics, etc. is important since tobacco smoke contains a large number of them and are known to cause bladder cancer. In spite of this, aromatic amines have been widely used in cosmetics, medicines, pesticides, dye intermediates, herbicides, rubber, coal conversion and various industries like chemical, pharmaceutical, military, etc. Therefore, developing highly sensitive, selective and efficient detection methods for aromatic amines has gained the large attention of researchers around the world over the last so many years [8–10].

Sriramulu et al. have developed novel fluorescent probes viz. perylenediimide and perylenemonomide for the detection of common amines in solution [11]. Bozkurta et al. have shown that, a novel pyrazoline derivative can be used for aniline detection via fluorescence quenching [2]. Liu et al. have reported that, aniline in an aqueous solution can be degraded by using non thermal plasma generated in micro bubbles [12]. Li et al. have reported on the efficient treatment of aniline containing waste water in bipolar membrane microbial electrolysis cell- Fenton system [13]. Song et al. have synthesised five isomorphic lanthanide metal organic frameworks and reported the selective fluorescence sensing of aniline [14].

In the present study, we have used novel thiophene substituted 1,3,4-oxadiazole derivative TSO as a fluorophore. The 1,3,4-oxadiazole derivatives are the most stable among the oxadiazole isomers and provide better thermal, luminescent, and electron transporting or hole blocking abilities that could play an important role and meet the requirements to construct an efficient sensor for the detection of explosive nitroaromatic compounds. In the recent years, 1,3,4-oxadiazole derivatives have also attracted researchers due to their vital role in medicinal chemistry, material chemistry, high range of pharmacological and biological activity [15], small-molecule materials [16–18], polymeric materials [19–21], construction of OLEDs, optoelectronics, organic photovoltaics, biomedical imaging, fluorescence sensors, metal ions sensing and as donor-π-acceptor molecules to work as efficient sensitizers in dye-sensitized solar cells [22–24].

The present fluorophore TSO contain electron deficient 1,3,4-oxadiazole as an electron acceptor and electron rich thiophene group as an electron donor. The presence of electron deficient 1,3,4-oxadiazole makes charge transfer possible from the electron rich aromatic amines and subsequently leads to the fluorescence quenching of the fluorophore. The vast applications of 1,3,4-oxadiazole derivatives and the charge transfer abilities motivated us to carry out this research work on the novel fluorophore TSO for the detection of hazardous aromatic amines through fluorescence quenching mechanisms.

**Experimental**

**Synthesis and Characterisation of TSO**

The novel thiophene substituted 1,3,4-oxadiazole derivative TSO is synthesized and characterised according to the method reported by Deshapande et al. [25].

**Instruments and Methods**

The steady state absorption and fluorescence spectra were recorded in the absence and the presence of the quenchers using Cary-100 UV–Vis spectrophotometer (USA) and Hitachi F-7000 fluorescence spectrophotometer (Japan) respectively at room temperature with 10 nm excitation and emission slit widths and 1500 nm/min scan speed. The fluorescence spectrometer has a 150 W xenon lamp used as a light source. In all the spectroscopic measurements quartz cuvettes having 1 cm width and 5 cm height are used.

Fluorescence lifetime measurements were carried out using an ISS Chronos-BH (ISS-90021) time correlated single photon counting spectrometer (USA). The pulsed light emitting diode with excitation wavelength 340 nm operating at 8 MHz frequency is used as an excitation source. The fluorescence decay curves were recorded setting the emission wavelength at 330 nm and decay curves were fitted biexponentially with a χ2 value close to unity to measure the fluorescence lifetime. Further, fluorescence decay curves were analysed using a Vinci multimedia software program.

Cyclic voltammetry (CV) studies were carried out using an electrochemical analyzer/Work station (model: CHI1112C, series, USA) at 0.1 M tetrabutylammonium hexafluorophosphate (TBAPF₆) as supporting electrolyte with a scan rate 100 mVs⁻¹.

The theoretical computations were performed with the help of Gaussian 09 W software by using DFT with basis sets B3LYP/6.11 + + G(d,p).
The Stern–Volmer (S-V) plots were drawn from the knowledge of fluorescence intensity of the fluorophore in the presence (I) and absence (I<sub>0</sub>) of different concentrations of the Quenchers (Q).

**Materials**

Aromatic amines namely 2,4-dimethylaniline (2,4-DMLA), 3-chloroaniline (3-CRA), 4-chloroaniline (4-CRA), o-anisidine (o-ANS), and m-toluidine (m-TLD) and also spectroscopic grade ethanol were procured from Spectrochem Pvt. Ltd. Mumbai, India. All the amines were used as quenchers in the present study and are double distilled before use. The quencher concentration is varied from 1 to 10 mM in all the cases. The solvent ethanol is used without any further purification. Throughout our studies, the concentration of the TSO is fixed at 10<sup>-5</sup> M to avoid the self-quenching process. All the measurements were carried out at room temperature.

The molecular structure of fluorophore TSO is shown in Fig. 1. The molecular structures of all the aromatic amines are given in Fig. S1.

**Results and Discussion**

**Steady-state Absorption and Fluorescence Studies**

The absorption and fluorescence spectra of TSO were recorded in ethanol and are given in Fig. S2. The wavelength corresponding to the absorption and fluorescence maxima are respectively at 330 nm and 400 nm.

The absorption spectra of TSO with varying concentrations (1 mM-10 mM) of aromatic amines in ethanol were recorded and are given in Fig. S3. It is observed that, the absorbance increases with an increase in the quencher concentration without any shift in the absorption maxima. This suggests that, there is no formation of the ground state complex between probe and quenchers [3, 4, 26, 27].

The fluorescence spectra of TSO in the absence and presence of aromatic amines (1 mM-10 mM) were recorded in ethanol and are shown in Fig. 2. It is observed that, as the concentration of aromatic amines increases, an appreciable quenching is observed in the fluorescence intensity of TSO without any change in the shape and position of the fluorescence maxima. This suggests the presence of significant molecular interactions between probe and quenchers.

Further, the steady state Stern–Volmer (S-V) equation given in Eq. (1) [3, 4, 26–29] is used to identify the type of fluorescence quenching mechanism between the fluorophore and quencher.

\[
\frac{I_0}{I} = 1 + K_{SV}[Q]
\]

where I<sub>0</sub> and I are the fluorescence intensities of the fluorophore in the absence and presence of quencher, [Q] is quencher concentration and K<sub>SV</sub> is the S-V constant. The S-V constant [26] is described as given in Eq. (2).

\[
K_{SV} = \frac{k_q}{\tau_0}
\]

where, k<sub>q</sub> is the bimolecular quenching rate parameter and \( \tau_0 \) is fluorescence lifetime of the fluorophore in the absence of quencher.

The typical S-V plots with all aromatic amines are shown in Fig. 3. In many cases, the Stern–Volmer plots ((I<sub>0</sub>/I) versus [Q]) were found to be linear, in which the quenching mechanism is primarily due to the dynamic process, where the diffusion process is dominant [27]. But in our studies, we observed that, the S-V plots for all the aromatic amines are found to be non-linear and shows an upward positive deviation at higher concentrations. The positive deviation in the steady-state S-V plots suggests that, the quenching mechanism is not purely dynamic (collisional quenching), and may be due to the formation of either the ground state complex or the static quenching process [3, 4]. Similar results are also reported by the other researchers [26, 27, 29].

**Ground-state Complex Formation Model**

The possibility of ground-state complex formation is studied using the modified S–V equation [28] given below.

\[
\frac{[I_0/I] - 1}{[Q]} = (K_{SV} + K_g) + K_{SV}K_g[Q]
\]

where, K<sub>SV</sub> and K<sub>G</sub> are the Stern–Volmer constant and the ground-state association constant respectively.

The plots of [(I<sub>0</sub>/I)-1]/[Q] vs.[Q] are shown in Fig. 4 and are found to be linear. From the knowledge of intercept and slope, the values of K<sub>SV</sub> and K<sub>G</sub> are determined and are found to be imaginary for all the quenchers studied and the values are given in Table S2. These experimental findings ruled out the possibility of ground-state complex formation between fluorophore and quenchers [26, 27].
Fig. 2  Steady state spectra of fluorophore TSO ($\lambda_{ex} = 330$ nm) with increasing concentration (1 mM-10 mM) of aromatic amines
To establish the static quenching phenomenon in the excited state, the sphere of action static quenching model is invoked as suggested by Frank and Wawilow [3, 4]. According to this model, instantaneous or static quenching occurs if the quencher molecule is very close or in contact with the fluorescent molecule at the moment of its excitation. It can be explained by the fact that only a certain fraction \( W \) of the excited state is quenched by the collisional mechanism. The fraction of the fluorophore molecules in the excited state which is \( (1 - W) \), are de-activated almost instantaneously after being formed because a quencher molecule happens to be randomly positioned in the closeness at the time the molecules are excited and interacts very strongly with them. In addition to this model, several other methods were also used (Smoluchowski model, etc.) to describe the static quenching process, all leading to the modified form of the S-V equation as given in Eq. (4) [26–29].

\[
\frac{I_0}{I} = 1 + \frac{K_{SV} [Q]}{W}
\]  

(4)

Here, \( W \) is the fraction of fluorophore quenched due to the collision in the excited state, \( Q \) is the quencher concentration. All other terms have their usual meaning.

In order to study the non-linearity in the S-V plot, Eq. (4) is modified as follows [26–29].

\[
\frac{1 - \frac{I}{I_0}}{Q} = K_{SV} \left[ \frac{I}{I_0} \right] + \frac{(1 - W) [Q]}{W}
\]  

(5)

According to Eq. (5), the plots of \( 1 - (I/I_0)/[Q] \) against \( [Q] \) were made and are shown in Fig. 5. All the least square fit plots are found to be linear.

Further, the S-V constant \( (K_{SV}) \) is determined from the knowledge of the slope. The bimolecular quenching rate parameter \( k_q (= K_{SV}/\tau_0) \) is determined using the S-V constant and the
values are given in Table 1. It is observed that, the values of S-V constants are in the same range as reported by others [26, 29]. The larger magnitude of the S-V constant represents the strong quenching interaction between the fluorophore and aromatic amines [28]. From Table 1, the S-V constant ($K_{SV}$) value for o-ANS is large as compared to other aromatic amines and this may be due to the strong electron-donating group present in the o-ANS.

To support static and dynamic (collisional) quenching in the excited state, the magnitudes of the static quenching constant ($V$) and radii of the sphere of action or kinetic distance ($r$) were determined according to the Eqs. (6) and (7) [3, 4, 26–29].

$$W = e^{-V[Q]} \text{ or } ln(1/W) = V[Q]$$  

(6)

$$\frac{V}{N^3} = \frac{4}{3} \pi r^3$$  

(7)

where $N$ is Avogadro's number per millimole.

The determined values of $V$ and $r$ are given in Table 1. The values of $V$ and $r$ are found to be in the same range as reported by others [26–29]. From Table 1, it is observed that the $K_{SV}$ values are larger relative to the values of $V$ for 4-CRA, o-ANS, and m-TLD, indicating the higher contributions of dynamic processes in the overall quenching mechanism [26].

The sum of the molecular radii $R (= R_Y + R_Q)$ is referred to as encounter distance or contact distance or reactive distance. This value of $R$ is compared with the value of the radius of the sphere of action ‘$r$’ to verify whether the reaction is due to the “static effect” or not. The radii of the fluorophore ($R_Y$) and the quencher ($R_Q$) molecules were determined by adding the atomic volumes of all the atoms constituting the molecule as suggested by Edward [30]. From Table 1, it is observed that the values

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**Table 1** The values of steady-state S-V constant ($K_{SV}$), the quenching rate parameter ($k_q$), intercept [(1-W)/Q], range of W, static quenching constant ($V$), and radius of the sphere of action ($r$) for quencher

| Quencher | $K_{SV}$ (M$^{-1}$) | $k_q \times 10^{10}$ (M$^{-1}$ s$^{-1}$) | Intercept | Range of W | $V$ mol$^{-1}$ dm$^3$ | $r$ in A$^0$ | R in A$^0$ |
|----------|-------------------|---------------------------------|-----------|-----------|-----------------|-----------|-----------|
| 2,4-DMLA | 36.592            | 4.194                           | 56.303    | 0.437–0.944 | 68.581          | 30.066    | 7.659     |
| 3-CRA    | 26.945            | 3.065                           | 60.129    | 0.399–0.940 | 74.594          | 30.121    | 7.491     |
| 4-CRA    | 78.618            | 8.944                           | 41.463    | 0.583–0.959 | 41.427          | 26.588    | 7.491     |
| o-ANS    | 106.78            | 12.147                          | 63.761    | 0.362–0.936 | 78.240          | 31.415    | 7.615     |
| m-TLD    | 65.046            | 7.400                           | 43.066    | 0.569–0.967 | 49.571          | 27.983    | 7.475     |
of kinetic distance ‘r’ are greater than the encounter distance R for all the aromatic amines, suggesting the static effect in the excited state [3, 4, 26, 29]. Therefore, according to Andre et al. [31] and Zeng and Durocher [32] the static effect takes place in the case of the steady state method irrespective of ground-state complex formation, provided that the reactions are diffusion limited. These results reveal that the sphere of action static quenching model is valid in our case.

**Finite Sink Approximation Model**

To find whether the reactions are diffusion-limited, we invoked the finite sink approximation model [33]. According to this model, the modified S-V relation can be given as below.

\[
K_{SV}^{-1} = (K_{SV}^0)^{-1} - \left( \frac{2\pi N'}{4\pi N'Dr} \right)^{1/3}[Q]^{1/3}
\]  

(8)

Here, \(K_{SV}^0\) is S-V constant at \([Q]=0\) and D is the mutual diffusion coefficient.

In order to determine \(K_{SV}^{-1}\), the S-V constant \((K_{SV})\) is determined for different concentrations of quencher from 1 to 10 mM using Eq. (9) [33].

\[
K_{SV} = \left[ \left( \frac{I_0}{I} \right) - 1 \right]/[Q]
\]  

(9)

**Table 2** The values of \(K_{SV}^0\) (steady-state quenching constant at \([Q]=0\)), mutual diffusion coefficient D (finite sink model), distance parameter R', 4\(\pi\)N' R' D, R the quenching rate parameter \(k_q\)

| Quencher | \(K_{SV}^0\) M\(^{-1}\) | \(D\times10^{-5}\) cm\(^2\) s\(^{-1}\) | R' in A\(^0\) | \(k_q\times10^{10}\) (M\(^{-1}\) s\(^{-1}\)) | \(k_d\times10^{10}\) (M\(^{-1}\) s\(^{-1}\)) |
|----------|-----------------|------------------|------------|------------------|------------------|
| 2,4-DMLA | 61.350          | 4.478            | 20.590     | 4.194            | 6.979            |
| 3-CRA    | 62.112          | 4.453            | 20.963     | 3.065            | 7.066            |
| 4-CRA    | 80.000          | 6.694            | 17.961     | 8.944            | 9.101            |
| o-ANS    | 78.740          | 4.770            | 24.809     | 12.147           | 8.957            |
| m-TLD    | 81.766          | 7.574            | 16.225     | 7.400            | 9.302            |

According to Eq. (8), the plots of \(K_{SV}^{-1}\) against \([Q]^{1/3}\) were made and are shown in Fig. 6. The plots are found to be linear with a negative slope.

From Fig. 6, the mutual diffusion coefficient D is determined from the slope and from the known values of N’ and \(\tau_0\). Similarly, from the intercept, \(K_{SV}^0\) is determined. Further, using the values of mutual diffusion coefficient D and \(K_{SV}^0\) the distance parameter R’ is calculated for all the quenchers using Eq. (10) [26, 29] and the values are given in Table 2.

\[
K_{SV}^0 = 4\pi N'R'D\tau_0
\]  

(10)

In order to confirm that, the bimolecular reactions are diffusion-limited, we have compared the values of distance parameter R’ and the encounter distance R. The bimolecular fluorescence quenching reactions are said to be diffusion limited, if R’> R [26, 27, 29]. From the Table 2 it is observed
that \( R' > R \) in all the cases. Hence, it may be concluded that the bimolecular reactions are diffusion limited.

According to Joshi et al. \[34\], the bimolecular reactions are said to be diffusion-limited, if the value of the bimolecular quenching rate parameter \( (k_q) \) is greater than the \( k_d \). The values of the diffusion-controlled rate parameter \( (k_d) \) is determined using Eq. \( (11) \) \[26, 27, 29\] and are presented in Table 2.

\[
k_d = 4\pi N'R'D
\]

From Table 2, it is noticed that, the value of \( k_q \) is greater than \( k_d \) only for o-ANS suggesting that diffusion-limited reactions take place in o-ANS with TSO. But for the remaining quenchers, the values of \( k_q \) are smaller than \( k_d \). This may be due to the reduced values of the diffusion coefficient \[26, 27\]. Hence, we may infer that diffusion assisted static quenching is responsible for fluorescence quenching in the case of 2,4-DMLA, 3-CRA, 4-CRA, and m-TLD.

**Time Resolved Fluorescence Studies**

The steady-state method results reveal that fluorescence quenching is due to the static effect and diffusion limited. In order to verify these results, we have carried out fluorescence quenching experiments through time-resolved fluorescence with varying concentrations of aromatic amines. As this method is considered to be more accurate than the steady-state method, the fluorescence lifetime of TSO with varying concentrations of aromatic amines is measured and the decay profiles are shown in Fig. 7. In all the cases, the fluorescence decay profile was fitted bi-exponentially with a residual \( \chi^2 \) value which is nearly equal to one. Fluorescence lifetime values are presented in Table-S1. The dynamic and static fluorescence quenching mechanisms can be distinguished with time-resolved measurements. In the static quenching process, the fluorescence decay lifetime of the fluorophore will remain unchanged as the concentration of the quencher is increased. However, in collisional quenching, a decrease in the average fluorescence lifetime of the excited molecule will be observed. From Fig. 7, it is observed that, the fluorescence lifetime decreases with an increase in the quencher concentration. This suggests that, the fluorophore is quenched through collisional interactions with the quencher \[3, 4\].

The Stern–Volmer (S-V) equation \[3, 4, 26\] for the time-resolved method is given by.

\[
\frac{\tau_0}{\tau} = 1 + K_{sv}'[Q]
\]

where, \( \tau_0 \) and \( \tau \) are the fluorescence lifetime of fluorophore in the absence and presence of the quencher respectively. [\( Q \)] is quencher concentration and \( K_{sv}' \) is the time resolved S-V constant. The time resolved S-V constant \( (K_{sv}') \) can be expressed as

\[
K_{sv}' = k_q'\tau_0
\]

where \( k_q' \) is the bimolecular quenching rate parameter. Using Eq. \( (12) \), the time resolved S-V plots were made and is shown in Fig. 8. It is observed that, the time resolved S-V plot shows linearity with increasing concentration of quencher. This result reveals that, there is an existence of dynamic quenching between the fluorophore and aromatic amines \[3, 4\]. Further, from the knowledge of the slope of the S-V plot, the time resolved S-V constant \( (K_{sv}') \) and bimolecular quenching constant \( (k_q') \) were calculated and are given in Table 3.

From the Table 1 and Table 3 it is observed that, the time-resolved S-V constant values are lower than the steady-state S-V constants. This result implies the presence of static fluorescence quenching interactions between the TSO and aromatic amines \[3, 4\]. Hence, the steady state and time resolved fluorescence methods suggest that, fluorescence quenching is due to static as well as dynamic quenching processes.

**Cyclic Voltammetry (CV) Studies**

To understand the reason for the differences in the fluorescence quenching abilities of all the quenchers, we have carried out an electrochemical analysis. Further, a quencher is always within the encounter distance with an excited fluorophore and thus electron transfer can occur effectively as a unimolecular process, without involving any diffusion of the reactants. The larger value of quenching efficiency suggests the possibility of electron transfer from aromatic amines to the fluorophore.

It is known that, the aromatic amines are electron donors and thiophene substituted 1,3,4-oxadiazole derivatives are electron acceptors. Thus, the oxidation potentials of aromatic amines and the reduction potential of TSO are essential to understand the electron transfer in the fluorescence quenching mechanisms. With the knowledge of oxidation and reduction potential, the possibility of electron transfer from aromatic amines to the fluorophore can be determined by electrochemical analysis \[35\].

The cyclic voltammogram for the probe TSO in the presence of supporting electrolyte (TBA\textsubscript{2}PF\textsubscript{6}) in ethanol is shown in Fig. 9 and the reduction potential value of TSO is found using the CV graph. The oxidation potential values of aromatic amines used in the present work were taken from the literature \[36\]. The oxidation and reduction potential values of all the amines and TSO are given in Table 4.
Fig. 7  Fluorescence decay profile of fluorophore TSO with increasing concentration of aromatic amines
The possibility of the electron transfer from the excited-state of TSO to the ground-state of the quencher is dependent on the standard free energy change for electron transfer reaction $\Delta G_{et}$. The $\Delta G_{et}$ value can be calculated using the Rehm-Weller equation given below [37].

$$\Delta G_{et} = E^{(\text{ox})}_{1/2} - E^{(\text{red})}_{1/2} - E^* - \frac{e^2}{\varepsilon r}$$

where $E^{(\text{ox})}_{1/2}$ is the ground-state of the oxidation potential of aromatic amines (donor), $E^{(\text{red})}_{1/2}$ is the reduction potential of TSO (acceptor). $E^*$ is the singlet excited-state energy of TSO in ethanol, $(e^2/\varepsilon r)$ is the coulombic energy term, ‘$e$’ is the charge of an electron, ‘$\varepsilon$’ is the dielectric constant of the solvent used and $r$ is the separation between donor and acceptor. The value of $E^*$ is determined by the relation $E^* = 1240/\lambda$, where ‘$\lambda$’ is the absorption maxima (330 nm) and is found to be 3.757 eV.

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The possibility of the electron transfer from the excited-state of TSO to the ground-state of the quencher is dependent on the standard free energy change for electron transfer reaction $\Delta G_{et}$. The $\Delta G_{et}$ value can be calculated using the Rehm-Weller equation given below [37].

$$\Delta G_{et} = E^{(\text{ox})}_{1/2} - E^{(\text{red})}_{1/2} - E^* - \frac{e^2}{\varepsilon r}$$

where $E^{(\text{ox})}_{1/2}$ is the ground-state of the oxidation potential of aromatic amines (donor), $E^{(\text{red})}_{1/2}$ is the reduction potential of TSO (acceptor). $E^*$ is the singlet excited-state energy of TSO in ethanol, $(e^2/\varepsilon r)$ is the coulombic energy term, ‘$e$’ is the charge of an electron, ‘$\varepsilon$’ is the dielectric constant of the solvent used and $r$ is the separation between donor and acceptor. The value of $E^*$ is determined by the relation $E^* = 1240/\lambda$, where ‘$\lambda$’ is the absorption maxima (330 nm) and is found to be 3.757 eV.

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In polar solvents such as ethanol, the coulombic term is very small compared to the other contributions and can be neglected. The calculated values of $\Delta G_{et}$ for fluorophore and quenchers are presented in Table 4. The larger negative values of $\Delta G_{et}$ suggests the pronounced thermodynamically favourable electron transfer from aromatic amines to the excited TSO. From the Table 4 it is observed that, as the oxidation potential decreases, the $\Delta G_{et}$ value also decreases, whereas the quenching rate parameter ($k_q$) increases. From these observations, it is inferred that, as $\Delta G_{et}$ value decreases, the quenching efficiency increases.

In order to confirm the electron transfer from the quencher to the fluorophore, the HOMO–LUMO values of TSO and all the quenchers were obtained theoretically from ab initio computations using DFT level of theory with basis sets B3LYP/6.11 + G(d,p) and are presented in the Table 5. A schematic energy level diagram for the possible photo induced electron transfer from aromatic amines to fluorophore is shown in the Fig. 10. It is observed that the LUMO level of all the aromatic amines lies well above the LUMO of the fluorophore and ensures the required driving force for electron transfer from aromatic amines to the fluorophore.

### Analysis of Binding Equilibria

In order to understand the stoichiometry between the fluorophore and quencher and to estimate the binding constant, the binding equilibria analysis [38] is carried out using Eq. (15).

$$\log \left[ \frac{(I_0 - I)}{I} \right] = \log K + n \log [Q] \tag{15}$$

where K, n, are the binding constant and number of binding sites respectively and the remaining terms have their usual meaning. The plots of log [(I₀ − I)/I] vs. log [Q] for all the quenchers in ethanol were made and are shown in Fig. 11. The plots exhibited the best linear fit for all the quenchers.

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### Table 4

| Quencher  | $E_{1/2}^{red}$ (eV) | $E_{1/2}^{ox}$ (eV) | $k_q \times 10^{10}$ (M⁻¹ s⁻¹) | $\Delta G_{et}$ (eV) |
|-----------|----------------------|---------------------|-------------------------------|---------------------|
| 2,4-DMLA  | -0.873               | 0.734               | 4.194                         | -2.150              |
| 3-CRA     | 0.952                | 3.065               | -1.932                        |                     |
| 4-CRA     | 0.880                | 8.944               | -2.004                        |                     |
| o-ANS     | 0.712                | 12.147              | -2.172                        |                     |
| m-TLD     | 0.843                | 7.400               | -2.041                        |                     |

### Table 5

| Molecules | LUMO(eV) | HOMO(eV) | $\Delta E$ (eV) |
|-----------|----------|----------|-----------------|
| TSO       | -2.462   | -5.906   | 3.444           |
| 2,4-DMLA  | -0.194   | -5.605   | 4.945           |
| 3-CRA     | -0.103   | -5.139   | 5.059           |
| 4-CRA     | -0.161   | -5.480   | 5.319           |
| o-ANS     | -0.301   | -5.074   | 4.773           |
| m-TLD     | -0.083   | -5.321   | 5.238           |

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Fig. 10 The schematic energy level diagram of photoinduced electron transfer from amines to TSO.
This indicates that there is a 1:1 stoichiometric ratio between the fluorophore and aromatic amines.

The values of binding sites (n) and binding constant (K) were determined using the least-square fit method with the help of slope and intercept and are given in Table 6.

It is observed from Table 6 that, the binding constant (K) value is higher for o-ANS compared to the other aromatic amines and this may be due to the strong electron-donating group present in the o-ANS. The higher values of the binding constant (K) confirms the strong binding of the fluorophore with aromatic amines [39]. The ‘K’ values are found to be in the same range as reported by others [40].

Table 6 The values of binding sites (n) and binding constants (K) in ethanol solvent

| Quencher  | Number of Binding sites(n) | Binding constant (K) M⁻¹ | Co-relation coefficient |
|-----------|----------------------------|--------------------------|------------------------|
| 2,4-DMLA  | 1.281                      | 16.609                   | 0.982                  |
| 3-CRA     | 1.351                      | 19.846                   | 0.994                  |
| 4-CRA     | 1.209                      | 14.717                   | 0.990                  |
| o-ANS     | 1.415                      | 29.964                   | 0.954                  |
| m-TLD     | 1.204                      | 14.083                   | 0.990                  |

Conclusions

From this study, the following conclusions are made;

- The fluorescence quenching has occurred without involving the ground state complex formation.
- The positive deviations in the steady state Stern–Volmer plots with higher values of bimolecular quenching rate parameter (kq) indicate efficient quenching.
- The positive deviation in the steady state S-V plots may be due to the combined effect of both dynamic and static quenching processes.
- The quenching reactions are due to static quenching and diffusion-limited.
- The lifetime measurement studies confirm the presence of dynamic quenching also.
- The negative free energy change (ΔGet) values indicate that, the interactions of TSO with aromatic amines are thermodynamically favourable.
- Analysis of the experimental data using Rehm-Weller relation and DFT studies confirm that, the fluorescence...
quenching may be due to electron transfer from aromatic amines to the fluorophore.

- The binding equilibria analysis studies reveal that, the stoichiometric ratio between fluorophore and quencher is of the order of 1:1.

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Availability of Data and Material Data generated or analysed during this study are included in this article and additional information are given in supplementary file.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

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References

1. Naik L, Khazi IA, Malimath GH (2018) Turn-off fluorescence studies of novel thiopeptide substituted 1,3,4-oxadiazoles for aniline sensing. Sens Actuator A: Phys 284:145–157
2. Bozkurt E, Gul HI (2019) Fluorescence quenching of novel pyrazoline derivative with aniline in different solvents. J Photochem Photobiol A: Chem 383:111996–1111002
3. Evale BG, Hanagodimath SM (2010) Static and dynamic quenching of biologically active coumarin derivative by aniline in benzene-acetonitrile mixtures. J Lumin 130:1330–1337
4. Geethanjali HS, Nagaraja D, Melavanki RM (2015) Exploring the mechanism of fluorescence quenching in two biologically active boronic acid derivatives using Stern-Volmer kinetics. J Lumin 209:669–675
5. Feng HJ, Xu L, Liu B, Jiao H (2012) Europium metal-organic frameworks as recyclable and selective turn-off fluorescence sensors for aniline detection. Dalton Trans 45:17392–17400
6. Gong T, Li P, Sui Q, Chen J, Xu J, Gao EQ (2018) A stable electron-deficient metal-organic framework for colorimetric and luminescent sensing of phenols and anilines. J Mater Chem A 6:9236–9244
7. Huang XL, Liu L, Gao ML, Han ZB (2016) A luminescent metal-organic framework for highly selective sensing of nitrobenzene and aniline. RSC Adv 6:87945–87949
8. Karthikeyan S, Gupta VK, Boopathy R, Titus A, Sekaran G (2012) A new approach for the degradation of high concentration of aromatic amine by heterocatalytic Fenton oxidation: Kinetic and spectroscopic studies. J Mol Liq 173:153–163
9. Peng H, Ding L, Liu T, Chen X, Li L, Yin S, Fang Y (2012) An ultrasensitive fluorescent sensing nanofilm for organic amines based on cholesterol-modified perylene bisimide. Chem Asian J 7:1576–1582
10. Sandeep A, Praveen VK, Kartha KK, Karunakaran V, Ajayaghosh A (2016) Supercoiled fibres of self-sorted donor–acceptor platforms: a turn-off/turn-on platform for sensing volatile aromatic compounds. Chem Sci J 7:4460–4467
11. Sriramulu D, Vaiyaveettil S (2016) Perylene derivatives as a fluorescent probe for sensing of amines in solution. Dyes Pigm 134:306–314
12. Liu Y, Zhang H, Sun J, Liu J, Shen X, Zhan J, Zhang A, Ogner S, Cavadias S, Li P (2018) Degradation of aniline in aqueous solution using non-thermal plasma generated in microparticles. Chem Eng J 345:679–687
13. Li X, Jin X, Zhao N, Angeliddaki I, Zhang Y (2017) Efficient treatment of aniline containing wastewater in bipolar membrane microbial electrolysis cell-Fenton system. Water Res 119:67–72
14. Song JF, Wen HF, Luo JJ, Jia YY, Zhang XY, Su LJ, Zhou R (2018) Five isomorphous lanthanide metal-organic frameworks constructed from 5-(3-carboxyphenyl)-pyridine-2-carboxylic acid and oxalate: synthesis, crystal structures and selective fluorescence sensing for aniline. J Solid State Chem 269:43–50
15. Tong H, Zhou G, Wang L, Jing X, Wang F, Zhang J (2003) Novel highly selective anion chemosensors based on 2,5-bis(2-hydroxyphenyl)-1,3,4-oxadiazole. Tetrahedron Lett 44:131–134
16. Kim TH, Lee CH, Kwak CG, Choi MS, Park WH, Lee TS (2007) Bis(2-hydroxyphenyl)-1,3,4-oxadiazole derivative for anion sensing and fluorescent patterning. Mol Cryst Liq Cryst 463:255–261
17. Mashaquai SH, Sundaram S, Bhasikuttan AC, Kapoor S, Sapre AV (2007) Novel fluoroionophores incorporating diaryl-1,3,4-oxadiazole and aza-crown ring. Potentially sensitive Mg2+ ion sensor. Sens Actuators B: Chem 122:347–350
18. Ding J, Day M (2006) Novel Highly fluorinated poly(arylene ether-1,3,4-oxadiazole)s, their preparation, and sensing properties to fluoride anion. Macromolecules 39(18):6054–6062
19. Zhou G, Cheng Y, Wang L, Jing X, Wang F (2005) Novel Polyphenylenes containing phenol-substituted oxadiazole moieties as fluorescent chemosensors for a fluoride ion. Macromolecules 38:2148–2153
20. Kim TH, Kim HJ, Kwak CG, Park WH, Lee TS (2006) Aromatic oxadiazole-based conjugated polymers with excited-state intramolecular proton transfer: Their synthesis and sensing ability for explosive nitroaromatic compounds. J Polym Sci A Polym Chem 44:2059–2068
21. Maslat A, Abussaud M, Tashtouh H, Talib MA (2002) Synthesis, antibacterial, antifungal and genotoxic activity of bis-1,3,4-oxadiazole derivatives. Pol J Pharmacol 54(1):55–59
22. Ambrosi G, Rossi P (2010) Efficient fluorescent sensors based on 2,5-diaryl-[1,3,4]oxadiazole: a case of a specific response to zn(ii) at physiological pH. Inorg Chem 49(21):9940–9948
23. Kwak CK, Lee CH, Lee TS (2007) A new series of 2,5-bis(4-methylphenyl)-1,3,4-oxadiazole derivatives: their synthesis and fluorescence properties for anion sensors. Tetrahedron Lett 48:7788–7792
24. Shang XY, Wang SJ, Ge XC, Xiao M, Meng YZ (2007) Synthesis and photoluminescent properties of poly(arylene ether)s containing oxadiazole and fluorene moieties. Mater Chem Phys 104(2):215–219
25. Deshpande N, Pujar GH, Sunagar MG, Gaonkar S, Belavagi NS, Inamdar SR, Khazi IAM (2016) Synthesis, characterization and optoelectronic investigations of thiophene substituted 1,3,4-oxadiazole derivatives as fluorescent materials. Int J Curr Res 8:38580–38586
26. Suresh Kumar HM, Kunabenchii RS, Biradar JS, Math NN, Kadadevarmath JS, Inamdar SR (2006) Analysis of fluorescence quenching of new indole derivative by aniline using Stern-Volmer plots. J Lumin 116:35–42
27. Deepa HR, Thipperudrappa J, Suresh Kumar HM (2015) A study on fluorescence quenching of a laser dye by aromatic amines in alcohols. Can J Phys 93:1–6
28. Lakowicz JR (2006) Principles of Fluorescence Spectroscopy, third ed., Springer, New York
29. Kadadevarmath JS, Malimath GH, Melavanki RM, Patil NR (2014) Static and dynamic model fluorescence quenching of laser dye by carbon tetrachloride in binary mixtures. Spectrochim Acta, Part A 117:630–634
30. Edward JT (1970) Molecular volumes and the Stokes-Einstein equation. J Chem Educ 47:261–270
31. Andre JC, Niclause M, Ware WR (1978) Kinetics of partly diffusion-controlled reactions. I. Transient and apparent transient effect in fluorescence quenching. Chem Phys 28:371–377
32. Zeng H, Durocher G (1995) Analysis of fluorescence quenching in some antioxidants from non-linear Stern-Volmer plots. J Lumin 63:75–84
33. Keizer J (1987) Diffusion effects on rapid bimolecular chemical reactions. J Phys Chem 87:167–180
34. Joshi GC, Bhatnagar R, Doraiswamy S, Periasamy N (1990) Diffusion-controlled reactions: transient effects in the fluorescence quenching of indole and n-acetyl tryptophanamide in water. J Phys Chem 94:2908–2914
35. Tablet C, Hillebrand M (2007) Quenching of the fluorescence of 3-carboxy-5,6-benzocoumarin by aromatic amines. J Photochem Photobiol A: Chem 189:73–79
36. Pavitt AS, Bylaska EJ, Paul G, Tratnyek PG (2017) Oxidation potentials of phenols and amines: correlation analysis of electrochemical and theoretical values. Environ Sci: Processes Impacts 19:339–349
37. Weller RA (1970) Kinetics of fluorescence quenching by electron and h-atom transfer. Isr J Chem 8(2):259–271
38. Hu YJ, Liu Y, Wang JB, Xiao XH, Qu SS (2004) Study of the interaction between mono ammonium glycyrrhizinate and bovine serum albumin. J Pharm Biomed Anal 36(4):915–919
39. Valeur B (2001) Molecular Fluorescence: Principles and Applications
40. Nirupama JM, Khanapurmath NI, Chougala LS, Shastrli LA, Bhanjtri RF, Kulkarni MV, Kadadevarmath JS (2019) Effect of amino amines on the fluorescence of coumarin derivative. J Lumin 208:164–173

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