Role of molecular interactions and structural defects in the efficient fluorescence quenching by carbon nanotubes

Dilip K. Singh a, Parameswar K. Iyer b, P.K. Giri a,c,*

a Centre for Nanotechnology, Indian Institute of Technology Guwahati, Guwahati 781039, India
b Department of Chemistry, Indian Institute of Technology Guwahati, Guwahati 781039, India
c Department of Physics, Indian Institute of Technology Guwahati, Guwahati 781039, India

ABSTRACT

Fluorescence quenching effect of single walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) on three most common fluorophores fluorescein, rhodamine 6G and quinine sulphate has been studied and compared. Comparative studies of quenching efficiency shows that SWCNTs are more efficient fluorescence quencher than the MWCNTs. Nature of Stern–Volmer plot was found to be highly non-linear indicating combined effect of dynamic and static quenching. The contribution of dynamic quenching component was assessed through the fluorescence lifetime measurements. Studies on vacuum annealed SWCNTs with low defect contents suggest that structural defects primarily contribute to the large quenching. Fluorescence quenching was found to be dominant even in the cases where adsorption was low implying that surface adsorption play a minor role in the quenching, except for rhodamine 6G. Adsorption isotherms have been studied using Langmuir and Freundlich models. Freundlich model was found to be closer in behaviour implying a multilayer adsorption of molecules on the surface. The contributions of metal nanoparticles and carbon impurities present in different allotropic forms to the fluorescence quenching were also assessed. We speculate that defect mediated nonradiative energy transfer through dipole–dipole coupling may be the dominant mechanism of high efficiency quenching by SWCNTs.

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1. Introduction

Interaction of carbon nanotubes (CNTs) with numerous biomolecules is under extensive research such as biosensor [1], multifunctional biological transporter for selective cancer cell destruction [2] and interaction of DNA with single walled carbon nanotubes (SWCNTs) [3]. Despite its immense potential applications, a proper understanding of fluorescence quenching effect of CNTs and its various contributors like molecular interactions and structural defects are lacking in the literature and it requires systematic studies. The fluorescence quenching effect of CNTs has been reported for a variety of molecules, such as π-stacked pyrenes, porphyrins [4], chromophores [5–8], quantum dots [9,10], and conducting polymers [11]. However, the exact mechanism of quenching remains unclear in most of these studies. In a few cases, this novel property of CNTs has been used for imaging using reverse contrast in fluorescence microscopy [12], nanomanipulation [13]. Recently, Guo et al. [14] studied the single-walled carbon nanotubes based quenching of free FAM-aptamer for selective determination of ochratoxin A, one of the most abundant food-contaminating mycotoxins in the world. Zhu et al. have reviewed the role of CNTs as a fluorescence quencher [15]. More recently, our group has shown that

* Corresponding author at: Centre for Nanotechnology, Indian Institute of Technology Guwahati, Guwahati 781039, India. Fax: +91 361 2582749.
E-mail address: giri@iitg.ernet.in (P.K. Giri).
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surface defects in SWCNTs are responsible for high efficiency quenching of fluorescein [16]. Despite several reports on fluorescence quenching by CNTs, a number of questions need to be addressed such as nonlinear Stern–Volmer plots and specific mechanisms of quenching, difference in the nature of quenching in SWCNTs and MWCNTs and most importantly its quenching efficiency for various commonly used fluorophores, such as fluorescein, rhodamine 6G and quinine sulphate.

The absorption spectrum of a typical SWCNT spans over a wide range of wavelengths (approximately 500–900 nm), significantly overlapping with the fluorescence spectra of various fluorophores allowing Förster resonance energy transfer (FRET). Since CNTs contain highly delocalized π-electrons, the surface of CNTs can be easily functionalized through π–π interactions with compounds that possess a π-electron rich structure. Ahmad and Balasubramanian showed that energy transfer occurs from nile blue A to CNTs through fluorescence resonance energy transfer mechanism and dynamic quenching process was accounted to play a role [17]. In this process, neither a chemical alteration nor a complex formation takes place. The energy transfer is through long-range dipole–dipole interactions between the fluorophore and the quenchers [18]. In contrast, fluorescence quenching in rhodamine B was found to be a static quenching process [19]. Quenching by energy transfer was observed in case of squarylium dye to SWCNTs [20]. Polymeric water soluble zinc-tetraaryl porphyrin was found to form long-lived electron transfer products with MWCNTs, resulting into soluble zinc-tetraaryl porphyrin was found to form long-lived

2. Experimental

Commercially available CNTs grown by CVD were used for the present study. The SWCNTs have diameter <2 nm and length 5–15 nm. The purity of CNTs was >90% and purity of SWCNTs was >50%. The ash content in the sample was <2 weight% and amorphous carbon content was <5 weight%. Specific surface area was >400 m²/g, as measured using BET analysis. MWCNTs of two different diameters with purity >95%, surface area 40–300 m²/g and amorphous carbon <3% have been studied. MWCNT-1 has diameters in the range 10–20 nm and MWCNT-2 has diameters in the range 20–40 nm. MWCNTs-2 contains very low amount of metallic impurities, as estimated from TGA analysis [24]. For comparison, graphite powder and activated charcoal procured from Sigma–Aldrich were studied. In order to eliminate structural defects, some of the SWCNTs were vacuum annealed at 700 °C for 2 h and these are named as VA-SWCNTs [25]. FTIR studies of pristine SWCNTs show absence of chemically functional groups (Fig. S1, Supplementary information). In order to study the influence of structural defects, defects were introduced in graphite powder by a ball milling process for 140 h at 350 rpm using PM100 Retsch, Germany ball mill machine using contamination-free Zirconia balls and vial in the 1:10 power to ball weight ratio. Fluorophores fluorescein, rhodamine 6G and quinine sulphate (molecular structure shown in Supplementary information, Fig. S2) were procured from Sigma–Aldrich and used for the quenching studies.

Standard fluorophores solution of 2µM-molar concentration was prepared to avoid high optical density and turbidity that can result in decrease of fluorescence intensity. Fluorescein, rhodamine 6G and quinine sulphate were prepared in 0.1 M NaOH, water and 0.1 M H₂SO₄, respectively. Concentration of fluorophores was estimated using their extinction coefficient at absorption wavelengths (λabs) from Beer–Lambert’s law. Steady state fluorescence measurements were performed at room temperature using a commercial fluorimeter (Thermo Electron, FA-357). In each step, 40 µl of the standard fluorophore solution was taken out and 40 µl of the nanotube solution was added continuously to a 3 ml of the standard fluorophore solution taken in 10 mm quartz cuvette so that the concentration of fluorophore remains constant. Emission spectra of fluorescein, rhodamine 6G and quinine sulphate were recorded using 496, 488 and 350 nm excitations, respectively. Time resolved fluorescence measurements were performed using a commercial high resolution fluorimeter (Life Spec II, Edinburgh Instruments) with a time resolution of 50 ps. Fluorescein and rhodamine 6G were excited with 475 nm laser and emission was monitored at 513 and 550 nm, respectively. Quinine sulphate was excited with 375 nm laser and emission was monitored at 445 nm. Confocal laser fluorescence scanning microscopy (CLFSM) images were recorded using Zeiss LSM 510 Meta system.

For the adsorption analysis, the concentration of fluorophore (qₐ) was estimated after subtracting the value concentration of fluorophore before and after addition of CNTs using Beer–Lambert’s law. Adsorbed amount of fluorophore (qₐ) was estimated from the decrease in fluorescence intensity for rhodamine B and nile blue A, respectively. None of the reports have analysed the influence of other possible factors such as amorphous carbon, nature of nanotubes: SWCNTs or MWCNTs, metallic impurities, structural/surface defects, etc. that may contribute towards the fluorescence quenching effect.

Adsorption of dyes on the fluorophores surface leads to decrease in their concentration in solution, sometimes leading to the incorrect interpretation of observed decrease in fluorescence intensity. The exact quantification of relative contributions of fluorescence quenching and surface adsorption are separately required for useful applications of fluorescence quenching effect in SWCNTs. Adsorption related applications of CNTs depends on their internal pore volume. A wide range of values for adsorption surface areas (15–3000 m²/g) have been reported for CNTs [22,23]. In order to understand the mechanism of quenching, we have analysed the process of surface adsorption of three different fluorophores on CNTs using Langmuir and Freundlich models. Surface adsorption studies confirm it to be a minor component contributing towards decrease in fluorescence intensity observed experimentally. We have attempted to quantify the nature of molecular interactions that leads to surface adsorption and its contribution to the quenching process.
CNTs in each step. Weight of the fluorophore adsorbed [A] was calculated by dividing the number of micro-moles to the molecular weight, 332.31 g for fluorescein, 479.02 g for rhodamine 6G and 391.47 g for quinine sulphate.

3. Results and discussion

3.1. UV–vis absorption spectroscopy

Absorption spectra of the fluorophores are shown in Fig. 1(a). Fluorescein shows a prominent absorption peak at 492 nm along with a shoulder at 472 nm, while rhodamine 6G and quinine sulphate show peaks at 525 and 350 nm, respectively. Fig. 1(b) shows the monotonic decrease in 525 nm absorption peak with increasing concentration of SWCNTs in rhodamine 6G solution. Similarly, absorption spectra were recorded after addition of VA-SWCNTs and MWCNT-1. These spectra were fitted using two Gaussian peaks. Variation in the absorbance with concentration of different types of CNTs is shown in Fig. 1(c). Fig. 1(d) shows the variation in the absorption intensity of 525 nm peak in rhodamine 6G with addition of nanotubes. Similarly, Fig. 1(e) shows the change in the intensity of 350 nm absorption peak of quinine sulphate after addition of SWCNTs and MWCNT-1. Quantitative analysis of surface adsorption of fluorophores on SWCNTs is shown in Fig. 1(f), after normalising the absorption intensity. It is clear that absorbance monotonically decreases with increasing concentration of SWCNTs. In case of fluorescein, vacuum annealed SWCNTs shows highest change in absorbance, indicating comparatively higher surface adsorption of fluorescein on less defective SWCNTs, while MWCNT-1 shows the least change in adsorption among the three, as shown in Fig. 1(c). Rhodamine 6G shows equal changes in absorbance for SWCNTs and MWCNT-1. Quinine sulphate on the other hand shows mixed behaviour. At a low concentration of CNTs, equal change in absorbance was observed both in SWCNTs and MWCNTs. With increase in concentration of CNTs, no change in absorbance was observed for MWCNT-1, whereas

Fig. 1 – (a) Absorption spectra of fluorescein, rhodamine 6G and quinine sulphate. (b) Absorption spectra of rhodamine 6G as a function of SWCNTs concentration. (c) Change in absorbance with increasing concentration of SWCNTs, VA-SWCNTs and MWCNT-1 in fluorescein; (d) absorbance of rhodamine 6G as a function of concentration of SWCNT and MWCNT; (e) decrease in absorbance of quinine sulphate with increasing CNTs concentration; (f) comparison of absorbance for fluorescein, rhodamine 6G and quinine sulphate with concentration of SWCNTs.
SWCNTs continues to show monotonic decrease in absorbance. From Fig. 1(f), it is interesting to note that rhodamine 6G shows relatively higher adsorption on SWCNT surface as compared to fluorescein and quinine sulphate. Small fluctuations observed in the data points are due to nanotube–nanotube interaction leading to bundling.

3.2. Steady state fluorescence spectroscopy

A systematic decrease in the fluorescence intensity of fluorophores was observed after addition of CNTs. CLFSM images show dark regions near the CNTs due to the decrease in fluorescence intensity by SWCNTs (Fig. S3, Supplementary information). Fig. 2(a) shows a systematic decrease in the fluorescence intensity of fluorescein with increasing concentration of SWCNTs. Similarly, Fig. 2(b) and (c) show decrease in intensity of 550 and 445 nm emission bands of rhodamine 6G and quinine sulphate, respectively. Fig. 2(d) shows a comparison of the Stern–Volmer plots for all the three fluorophores. Here, $F_0$ and $F$ are the fluorescence intensity without and with SWCNTs, respectively. $[Q]$ indicates the concentration of SWCNTs added. Here $[Q]$ is expressed in terms of volume of the nanotube in microliter added from the dispersed solution of CNTs having concentration 1 mg/ml to 3 ml of fluorophore solution. In other words, $[Q]$ represents the mass of nanotubes in microgram added assuming uniform dispersion of nanotubes in the solution. The non-linear nature of Stern–Volmer plot indicates the possible involvement of combined dynamic and static quenching [26]. From Fig. 2(d), it is clear that among all the fluorophores, fluorescence of rhodamine 6G is quenched most by the SWCNTs. In contrast, fluorescence of fluorescein and quinine sulphate is relatively less quenched. Upon addition of SWCNTs, although the change in the absorption is same for both fluorescein and quinine sulphate, their quenching efficiencies are different. Quenching of fluorescence intensity usually happens through two mechanisms: (a) static quenching, and (b) dynamic quenching. Static and dynamic quenching processes are distinguished by lifetime measurements. Since collisional quenching (dynamic quenching) is a rate process which depopulates the excited state, it results into equivalent decrease in fluorescence intensity, while fluorescence lifetime remains unchanged in the case of static quenching [26].

When a fluorophore is quenched by collisions and by complex formation both, it is represented by:

$$
\frac{F_0}{F} = (1 + K_s [Q] + K_d [Q]) = 1 + K_{app} [Q]
$$

where $K_s$, $K_d$ represents static and dynamic Stern–Volmer constants, $F_0/F$ represents the fractional fluorescence remaining given by the product of fraction not complexed and fraction not quenched by collisional encounters. Deviations from the standard Stern–Volmer equation represented by Eq. (1) could be due to the limited accessibility of quenchers to the nanotubes due to bundling effect. But such effects would lead to negative deviation, contrary to the observed positive deviation. Contrary to the behaviour expected from Eq. (1), the plot of $(F/F_0 - 1) \frac{1}{[Q]}$ versus $[Q]$ is not linear, as shown in Fig. 3(a) and it is quite similar to that reported by Ahmad et al. [19]. Interestingly, the present data follows a

![Fig. 2](image-url)

Fig. 2 – Fluorescence spectra showing monotonic decrease in FL intensity of (a) fluorescein (b) rhodamine 6G (c) quinine sulphate with varying concentration of SWCNTs. (d) Stern–Volmer plot for fluorescein, rhodamine 6G and quinine sulphate as function of concentration of SWCNTs.
the quenching efficiency is unusually high that follows a faster-than-exponential growth behaviour with $z = 1.307$, as compared to the modified Stern–Volmer equation with $z = 1$. It is known that in addition to defects in SWCNTs, strain, curvature, helicity of the low diameter SWCNTs have profound effect on reactivity and oxidative stability of the nanotubes [27] and this may partly contribute to the observed high efficiency quenching. In case of VA-SWCNTs, it is found to follow $z = 1.303$, close to 1.0, while MWCNT follows the modified Stern–Volmer equation perfectly with $z = 1.0$. It has been reported that compressed-exponential growth can occur from a local rearrangement of the molecules or micro-collapse of particles and is rarely observed in physical systems [28]. Such rearrangement of molecules is quite plausible in case of adsorption of fluorophores on the CNTs with highly reactive sites due to the structural defects and high strain. Rhodamine 6G and quinine sulphate also show a similar behaviour. In case of quinine sulphate, exponential fit and modified Stern–Volmer equation (Eq. 3) gives equally good fit. Exponential dependence of Eq. (2) arises from the Poisson distribution of the probability of finding a volume $V$ with $n$ quenchers given by $P(n) = \frac{\lambda^n}{n!} e^{-\lambda}$, where $\lambda$ is the mean number of quenchers per volume $V$. We approximately estimated the radius of sphere of action using the parameter obtained from fitting Eq. (2). The number of SWCNTs/cm$^3$ added in each step has also been calculated. Typically, for a (8,0) SWCNT of length $15 \mu$m, there are $114 \times 10^4$ carbon atoms, which weighs $227.33 \times 10^{-13}$ g. Now, addition of $40 \mu$l of SWCNTs solution in each step leads to increase in concentration by $\sim$1.955 $\times$ 10$^8$ SWCNTs/cm$^3$. This leads to $\lambda = 0.0149$ and the equivalent radius of sphere of action comes out to be $1.27 \mu$m. Such a large radius of sphere of action represents a highly efficient quenching. The large sphere of action for SWCNTs can arise from the extended cylindrical geometry, facilitating a number of adsorption sites owing to defects and strain, and the possibility of quenching of large number of fluorophore molecules by a single SWCNT molecule.

Fig. 4 shows the quenching efficiency of MWCNTs with different fluorophores and comparison of quenching efficiency with that of the SWCNTs. Fig. 4(a) shows a systematic decrease in fluorescence intensity of rhodamine 6G with increasing concentration of MWCNTs. Fig. 4(b) shows the Stern–Volmer plot for fluorescein with MWCNTs and its comparison with pristine SWCNTs and VA-SWCNTs. Pristine SWCNTs clearly show a large $F_0/F$ as compared to VA-SWCNTs and MWCNTs. Although VA-SWCNTs exhibit higher adsorption of fluorescein [Fig. 1(c)], it shows much smaller quenching efficiency. Thus, surface adsorption cannot account for the observed large quenching for SWCNTs. Note that our earlier studies have shown that VA-SWCNTs contain fewer defects as compared to the pristine SWCNTs [25]. Thus, the low quenching efficiency of VA-SWCNTs clearly suggests that structural defects in SWCNTs play a crucial role in the quenching process. Similarly, Fig. 4(c) and (d) show comparison of quenching efficiency of SWCNTs and MWCNTs for rhodamine 6G and quinine sulphate, respectively. It is evident that the quenching efficiency of MWCNT is much lower than that of SWCNTs, except for rhodamine 6G where the quenching efficiency of MWCNTs was significant. This is consistent with the absorbance data shown in Fig. 1(d), where almost
identical absorption behaviour was observed for SWCNTs and MWCNT-1 in rhodamine 6G. It is important to note that even at a low concentration of SWCNTs, $F_0/F$ value is quite high for rhodamine 6G as compared to that of fluorescein and quinine sulphate. In case of rhodamine 6G, the high quenching efficiency is partly due to the higher absorption. Interestingly, the absorption range of SWCNTs closely overlaps with the emission range of studied fluorophores (Supplementary information, Fig. S4), indicating a strong possibility of energy transfer, such as FRET, being responsible for the observed quenching.

In order to ascertain the role of structural defects/dangling bonds and to assess the possible contributions of other factors such as different forms of carbon and residual metallic impurities in SWCNTs (as observed under HRTEM shown in Fig. S5, Supplementary information) to the observed quenching, systematic studies have been carried out on graphite powder, carboxylic acid functionalized SWCNTs, activated charcoal and MWCNTs-2 (that had extremely low metal content). Fig. 5(a) shows the fluorescence quenching by graphite powder in fluorescein and Fig. 5(b) shows the corresponding Stern–Volmer plot. It’s clear that the $F_0/F$ varies linearly with $[Q]$, i.e., concentration of dispersed graphite.

Thus, the graphite shows a linear Stern–Volmer plot indicating the involvement of only static quenching. Note that the $F_0/F \sim 2$ observed experimentally for graphite is much lower than that of the SWCNTs and MWCNTs for the same concentration. Ball milled graphite powder, where more defects and strain may be expected, also shows linear Stern–Volmer plot. Ball milled graphite powder shows slight decrease in quenching efficiency perhaps due to a decrease in $sp^2$ content required for the $\pi-\pi$ interaction. This eliminates any possible role of graphitic phase in the observed quenching in SWCNTs. Due to absence of any absorption peak in graphite (zero band gap conductor), there is no significant change in quenching with graphite concentration. Fig. 5(c) shows the effect of functionalized SWCNTs on fluorescence intensity of fluorescein. In this case, the dangling bonds/defects of carbon are passivated after functionalization with COOH group. Fig. 5(d) shows the Stern–Volmer plot for the SWCNT–COOH quenched fluorescein, rhodamine 6G and quinine sulphate. Quenching efficiency of SWCNT–COOH was found to be much lower than the pristine SWCNTs, but higher than the VA-SWCNTs (less defective). Thus, the studies on functionalized SWCNTs further suggest that the dangling bonds/defects indeed contribute strongly to the quenching. Due to functionalization, decrease in dangling bonds leads to a decrease in the value of $F_0/F$. It is interesting to note that due to higher adsorption, SWCNT–COOH shows higher quenching efficiency for rhodamine 6G as compared to fluorescein and quinine sulphate. Fig. 5(e) shows the Stern–Volmer plot for activated charcoal. Here the $F_0/F$ value is quite low (only $\sim 2.8$) as compared to the high value in SWCNTs. We find that the fluorescence quenching by the SWCNTs does not recover even after several hours, while the activated charcoal and the graphite powder give a turbid solution in a short time. Another possible mechanism of quenching could be surface energy transfer (SET) from fluorophores to metal nanoparticles. SET originates from the interaction of the electro-magnetic field of the donor dipole with
the free conduction electrons of the accepting metals. Metals, e.g., Fe, Ni, Co nanoparticles that were used in the synthesis of SWCNT as catalyst also possess quenching properties [29]. In the SWCNTs, the metal residue was estimated to be 2% by weight using thermogravimetric (TGA), and energy dispersive X-ray (EDX) analyses. EDX studies show the presence of Co and Mo in the SWCNTs. We studied the extent of quenching due to these metal nanoparticles as well as their salt solution and found that the quenching efficiency was extremely low by the metal nanoparticles alone. We also studied quenching effect of two different MWCNTs having different amount of metallic impurity contents. Fig. 5(f) shows a comparison of quenching efficiencies of MWCNT-1 and MWCNT-2. It is interesting to note that MWCNT-2 containing much lower amount of metallic impurity show relatively higher value of $F_0/F$. Thus, no correlation is found between the metal nanoparticles and the quenching efficiency and our study confirms that metallic impurities do not play any significant role in the quenching of fluorescence. In fact, ESR studies on MWCNT-2 (see Supplementary information, Fig. S6) reveals that MWCNT-2 contains more dangling bonds than the MWCNT-1, providing strong support to the hypothesis that dangling bonds contribute to the quenching process.

Static quenching may arise due to the formation of non-fluorescent complex between defect sites generated during acid purification of CNTs and the fluorophores. Non-covalent attachment of dye occurs through hydrophobic interaction [12]. Since we did not observe any additional peak in UV–vis spectra after addition of nanotube, the binding occurs preferably through non-covalent π-staking interaction. Although some of the earlier reports denied the quenching through energy transfer to the CNTs [30], recent studies show that a
small number of extra holes or electrons in CNT can quench excited states through a non-radiative Auger recombination mechanism [31].

### 3.3. Time resolved fluorescence spectroscopy

Fig. 6(a) shows a typical time resolved spectra for fluorescein and quinine sulphate. Prompt spectrum was recorded separately and response time of instrument was subtracted from the life time of fluorophores obtained by exponential fitting. Similar process was followed for rhodamine 6G as well. Fluorescein and rhodamine 6G showed single life time component of 4.05 and 4.12 ns, respectively, before the addition of the quencher, while the quinine sulphate required two exponentials to fit the data, representing two lifetimes 20.54 ns (channel-1) and 0.89 ns (channel-2), channel-1 accounting for 98.9% of the process. Fig. 6(b) shows variation of $s_0/s_\infty$ with concentration of SWCNTs, and MWCNT-1. It is interesting to observe that $s_0/s_\infty$ changes marginally with increasing concentration [Q], rather than remaining constant at $s_0/s_\infty = 1.0$ (a condition for perfect static quenching). However, it does not follow $F_0/F = s_0/s_\infty$ condition either (necessary for a perfect dynamic quenching). Here $s_0/s_\infty$ shows an intermediate behaviour. This clearly indicates the involvement of combined static and dynamic quenching, with relatively small contribution from dynamic quenching. The lifetime measurement with graphite powder shows $s_0/s_\infty \sim 1.0$ indicating only static quenching. Dynamic quenching component is more prominent in SWCNTs than the VA-SWCNTs and MWCNT-1. This is because the number of dangling bonds in pristine SWCNTs is much higher than that in VA-SWCNTs and MWCNTs. Fig. 6(c) and (d) shows a small decrease in the lifetime for quinine sulphate and rhodamine 6G, respectively. $s_0/s_\infty$ was found to follow nearly an exponential behaviour except in case of Rhodamine 6G quenched by SWCNTs.

### 3.4. Surface adsorption of fluorophores on nanotube

Fig. S7 (Supplementary information) shows the adsorption isotherms of fluorescein, rhodamine 6G and quinine sulphate adsorbed by SWCNTs, VA-SWCNTs and MWCNT-1. It is interesting to note that amount of fluorescein adsorbed by VA-SWCNTs is higher than the pristine SWCNTs, and MWCNT-1 shows the least adsorption for the same weight. Although pristine SWCNTs show lesser adsorption, it shows the strongest quenching effect. This strongly suggests that structural defects and dangling bonds play a major role in the fluorescence quenching, while surface adsorption contributes negligibly. In contrast to the earlier reports, the present work enabled us to distinguish various factors that contribute to the quenching by SWCNTs. Contrary to the case of fluorescein and quinine sulphate, rhodamine 6G shows similar adsorption for both SWCNTs and MWCNT-1. Interestingly, we find that the adsorption of rhodamine 6G does not increase linearly with concentration of CNTs, but it reaches a saturation indicating that very little amount of rhodamine 6G is left in the solution. In case of quinine sulphate, the adsorption behaviour is not linear with concentration of CNTs. Similar to the fluorescein, quinine sulphate is less adsorbed by MWCNT-1 than SWCNTs.
In order to understand further the nature of interaction between fluorophores and nanotubes, the adsorption isotherms have been analysed using both Langmuir (upper panel) and Freundlich models (lower panel), as shown in Fig. 7. Langmuir isotherm model represents monolayer adsorption of molecules on the surface, while the Freundlich isotherm represents multilayer adsorption of molecules on the surface. Langmuir isotherm is expressed as

$$[A] = \frac{Q_m b}{1 + b \frac{Q}{C_{138}}} \quad \text{or} \quad \frac{Q}{A} = \frac{1}{Q_m b} \left(1 + b \frac{Q}{C_{138}}\right) \quad \text{(Linear form)} \quad (4)$$

and Freundlich isotherm is expressed as,

$$[A] = K_f \left(\frac{Q}{C_{138}}\right)^n \quad \text{or} \quad \log [A] = \log K_f + \frac{n}{R} \log \left(\frac{Q}{C_{138}}\right) \quad \text{(Linear form)}, \quad (5)$$

where [A] is the amount of fluorophore adsorbed by [Q] weight of CNTs. $Q_m$ represents maximum adsorption capacity, $K_f$ is the Freundlich affinity coefficient and $n$ represents the Freundlich exponent factor. From Fig. 7, a linear profile is expected if it follows a particular isotherm either Langmuir or Freundlich. Figs. 7(a–c) show the Langmuir isotherms while Figs. 7(d–f) show the Freundlich isotherms for adsorbed fluorescein, rhodamine 6G and quinine sulphate, respectively, by SWCNTs and MWCNTs-A. The details of the parameters extracted from the fitting are presented in Table 1.

In all the fluorophores, the Langmuir isotherm behaviour shown in Figs. 7(a–c) is far from being linear, while Freundlich isotherms of Fig. 7(d–f) show an excellent linear fit to the data points. Least square fitting parameter, $R^2$ (value closer to 1.0 represents a better fit), decides the quality of the fitting. For SWCNTs, Freundlich exponent factor ($n$) of 1.174 indicates that adsorption does occur, since it is within a range of 1–10 [32]. In MWCNTs, Freundlich isotherm shows an inferior fit to the data as compared to that of SWCNTs. However, the $n$-values indicate that adsorption does occur in this case as well.

Thus, from the isotherm analysis it can be inferred that adsorption of fluorophores on the CNTs occurs in the form of multilayer adsorption of molecules. Slight deviation observed from Freundlich model represents fluctuations arising from the probabilistic nature of adsorption sites leading to a slight non-linearity. Although there have been attempts to model this with non-linear models like Brunauer–Emmett–Teller model and Polanyi–Manes model [33], within the experimental error and taking consideration of various factors related to CNTs such as structure, purity, and dispensability,
Freundlich model is found to best represent the physical process of adsorption of fluorophores on the nanotube surface.

3.5. Discussion

In the absorption spectra of fluorophores with and without CNT, absence of any red shift in the overall spectrum suggests that there is minimal complexation of the dye with the nanotubes [21,34]. It has been reported that the planar molecules easily approach CNTs through a face-to-face conformation, which is favourable for π-π interactions between the conjugated aromatic chromophore skeleton and the nanotubes. On the contrary, the non-planar molecules are kept apart from CNTs due to spatial resolution, resulting in low π-π interactions with the CNTs [35]. Through absorption studies we observed a stronger adsorption for rhodamine 6G. We believe that the higher quenching efficiency observed in rhodamine 6G is due to the combined effect of high surface adsorption and defect induced FRET process. Quantitative analysis of adsorption isotherms in support of multilayer adsorption of fluorophores on the nanotube surface, as represented by Freundlich model.

CNTs act as efficient quenchers in various pH ranges, since the three fluorophores studied were prepared in different pH medium, fluorescein in alkaline medium, rhodamine 6G in water and quinine sulphate in acidic medium. The amount of fluorescein adsorbed by VA-SWCNTs is much higher than that in pristine SWCNTs and MWCNTs (Fig. S7, Supplementary information). MWCNTs show lowest adsorption of fluorescein. Due to reduced defect density, the annealed samples exhibit higher thermal stability (Fig. S8, Supplementary information) and lower intensity of D-band in the Raman spectra (Fig. S9, Supplementary information). Pristine SWCNTs show lesser adsorption but have stronger quenching efficiency. Thus, the surface adsorption alone cannot fully account for the extent of quenching observed. Besides the surface adsorption, role of other species like metallic nanoparticles and other forms of carbons particularly amorphous carbon and graphite present as impurities in CNTs samples were systematically eliminated through controlled experiments and careful analysis. These results make us believe that the structural defects primarily control the fluorescence quenching of SWCNTs. Through time resolved fluorescence studies we showed that static quenching plays the dominant role in the observed high quenching. Weak association between CNTs and fluorophores through π-π interaction do not form a ground state complex, instead it appears that static component is due to the quencher being adjacent to the fluorophore at the moment of excitation. These closely spaced fluorophore–quencher pairs immediately quench the fluorescence and thus appear to be dark complexes in the CLFSM images. This type of apparent static quenching is usually interpreted in terms of a “sphere of action” model. However, the experimental data point follows a compressed-exponential behaviour, rather than the “sphere of action” model. This is due to a very small contribution of dynamic quenching. We believe that a local rearrangement of molecules arising from the adsorption in CNTs with the highly reactive sites of SWCNTs owing to presence of defects and strain is one possible pathway giving rise to the compressed-exponential behaviour. Further, the structural defects in SWCNTs may increase the dipole moment and interact more efficiently with the fluorophore in the close vicinity. Thus, tuning the nature of defects in CNTs would enable better control of quenching in fluorophores and would find advanced applications in biosensing and bioimaging.

4. Conclusions

We have presented a thorough analysis of fluorescence quenching efficiency of pristine SWCNTs, VA-SWCNTs, MWCNTs and various other forms of carbon in three common fluorophores. It is shown that structural defects in SWCNTs are primarily responsible for the high efficiency quenching of fluorescence. It is likely that structural defects in the SWCNT are reactive sites that may increase the dipole moment and interact more efficiently with the fluorophores. Contribution of surface adsorption to quenching was found to be small, except the case of rhodamine 6G. The observed quenching may be explained on the basis of energy transfer, such as FRET, between defect sites of SWCNTs and fluorophores. Stern–Volmer plot was found to follow a faster-than-exponential behaviour and the compressed-exponential behaviour is believed to result from local rearrangement of molecules. Rhodamine 6G shows highest efficiency of quenching due to the combined effects of surface adsorption and defect mediated energy transfer. Pristine SWCNTs show a quenching efficiency of ~98.1% for fluorescein, while nearly defect-free VA-SWCNTs show a quenching efficiency of only ~65.6%. Adsorption isotherms reveal that surface adsorption on CNTs follows the Freundlich isotherm, indicating a multilayer adsorption of fluorophores on the nanotube surface.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbon.2012.05.030.

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