

CHAPTER V

EXPLORING β -CD GRAFTED GO NANOCOMPOSITES WITH AN ENCAPSULATED FLUORESCENT DYE DULY OPTIMIZED BY MOLECULAR DOCKING FOR INNOVATIVE APPLICATIONS

ABSTRACT

Here, we have designed and synthesized β -cyclodextrin grafted Graphene Oxide based fluorescent probe with encapsulated fluorescent dye. The nanocomposites were characterized by several spectroscopic methods such as FTIR, DLS, zeta potential, UV-vis and fluorescence spectroscopy. Thermal gravimetric analysis (TGA) was employed to account for thermal stability of β -CD grafted graphene oxide nanocomposites. Nile blue molecules are well encapsulated into the cyclodextrin cavity and embedded on the surface of Graphene Oxide sheet. Molecular Docking study helps us to understand the feasibility of encapsulation process of fluorescent dye inside our synthesized nanocomposites. Various physicochemical properties like UV-vis and fluorescence spectra of composite in different solvent and photophysical properties like fluorescence quantum yield, molar extinction coefficient, stokes shift have been calculated.

1. INTRODUCTION:

Now-a-days, cancer has become a major issue throughout the world, although, there are numerous diagnostic methods such as magnetic resonance imaging (MRI), ultrasound, positron emission tomography (PET) imaging, and single-photon emission computed tomography (SPECT) are available [1]. Recently, fluorescent probes are widely used for cancer cell imaging in cell biology. When photosensitizing dye is being given to a cancer patient, it localizes in the cancerous tissue and when light of appropriate wavelength is placed, it is activated and evolves strong fluorescent colour [2]. Recently, Polymer nanoparticles as well nanocomposites are used extensively in biomedical applications [3].



However, one has to be very selective when choosing the photosensitizers [4]. An efficient fluorescent probe is based on dye where emission maximum is found to be in the far-red end of the visible spectrum i.e, above 600-700 nm to minimize background interference [5,6]. In our study, we have chosen Nile blue chloride (NB) as our fluorescent dye. It is a cationic dye and is readily soluble in water. It is a photostable organic dye from the benzo[a]phenoxazine family, which shows strong fluorescence. It has redshifted absorbance spectra and its emission maximum falls in the NIR region with high fluorescent quantum yields, making it a potential fluorescent probe for biological imaging and photodynamic action [7,8].

Graphene oxide (GO), the precursor of graphene, being water-soluble has attracted tremendous interest in the field of biomedicine in past few years [9]. It consists of several functional groups (hydroxyl, epoxy, carbonyl, carboxylic groups) and high surface area which makes it potential nano-carriers for drug and gene delivery [10]. It has been reported that GO having enormous oxygen rich functional group can be modified by many non-toxic functional group to extend biological application [11,12].

Cyclodextrins (CDs) are generally cyclic oligosaccharides of six to eight d-glucose monomers linked by α -1, 4-glucose bonds [13]. They have the ability to encapsulate various drug molecules forming supramolecular host-guest complex [14]. They are extensively used as drug carrier [15], enzyme mimics [16], and photochemical sensors [17]. They can be used as catalyst [18], separating agent [19] and chemosensing [20].

In a recent study, Banerjee et al., showed that fluorescent guest encapsulated β CD complexes are embedded or adsorbed on the surface of GO nanosheet to understand the electron transfer mechanism from complex to GO [21]. Whereas, Ray et al., worked with analogous dye Nile red with β -cyclodextrin to investigate various spectroscopic properties [22]. In our present study, supramolecular assembly of NB with β -cyclodextrin and Graphene oxide (GO), i.e., GO- β CD-NB nanocomposites, was designed and synthesized ([Scheme 1](#)). In this work, we have been able to prepare NB



and GO nanocomposites with β CD host molecules for enhancing applicability in biological aspects.

2. METHODS AND EXPERIMENTAL SECTION:

2.1 Chemicals and Materials:

All the materials such as graphite flake, β CD and Nile blue were obtained from Sigma Aldrich India PVT. LTD. Deionized water (DW, 18 M Ω cm) was used for preparing aqueous solutions.

2.2 Instruments:

UV-Vis spectra and Fluorescence spectra were recorded by an Agilent 8453 spectrophotometer and a PTI Quanta Master fluorescence spectrophotometer (Quantamaster-40, USA) respectively. The SEM micrographs of nanocomposites were obtained by JEOL JSM-IT 100 Microscope. The zeta potentials and size of nanocomposites were measured by a Malvern Nanosizer. Infrared spectra of the samples were recorded by a Fourier transform infrared spectrometer (Perkin Elmer FT-IR). All TGA spectra were recorded by TA Instrument Q-50 TGA.

2.3 Preparation of GO:

GO was synthesized using modified Hummers method from purified natural graphite flakes as reported earlier [23]. According to the method, a mixture of graphite flakes (3.0 g, 1 wt equiv) and KMnO₄ (18.0 g, 6 wt equiv) were added to 9:1 mixture of concentrated H₂SO₄/H₃PO₄ (360:40 mL), the mixture get warmed upto 40°C showed exothermic nature. The reaction was kept at 55°C and stirred with magnetic stirring for 12 hrs. After cooling at room temperature, the reaction mixture was poured into crust ice (~400 mL) with 30% H₂O₂ (3 mL). The solid material obtained after filtration was then wash twice successively with 200 mL of water, 200 mL of 30% HCl and 200 mL of ethanol. After that, solution was centrifuged at 4000 rpm for 10 mins and again washed with distilled water so that various chlorides,



sulphate ions got free. Finally, the solution was dried by rotary evaporator under reduced pressure and obtained 1.7g of product.

2.4 Preparation of GO- β CD:

To prepare rGO- β CD composite, a relatively greener approach was chosen rather than using highly toxic hydrazine as reducing agent [24]. Prior to the experiment, GO was ultrasonicated in distilled water for 10 mins for getting a homogenous solution. Then, 200 ml of 1 mg/mL β CD aqueous solution was mixed with 200 mL of 0.5 mg/mL GO aqueous suspension. The mixture was stirred at room temperature for 12 hrs. Then the pH of the mixture was adjusted to 12 by adding aqueous solution of NaOH (1.0 M). Finally, the solution was heated at 75^oC and stirred at 370 rpm for 6 hrs. After the reaction, the stable black dispersion of the rGO- β CD mixture was centrifuged at a relative centrifugal force 4000 rpm so that unreacted β CD got removed from the solution followed by washing with distilled water for three times. After that a solid rGO- β CD nanocomposites was obtained by using rotary evaporator under reduced pressure.

2.5 Preparation of GO- β CD-NB:

Once, CD was attached on the surface of GO via chemical reaction between hydroxyl groups of CD and epoxide groups of GO, rGO- β CD nanocomposites was prepared. Then, 1mM of NB was dissolved in 20 mL of water and it was added in 20 mL of prepared rGO- β CD nanocomposites. The solution was heated at 55^oC and stirred at 370 rpm for 12 hrs for evaporation.

2.6 Preparation of modified for GO- β CD Molecular Docking:

The crystal structure of β -cyclodextrin was taken from Chembridge Crystal data centre (CCDC) as CIF file (ID: 762697). The two-dimensional structure of graphene oxide (PubChem CID: 124202900) was used in the canonical smiles format from <https://pubchem.ncbi.nlm.nih.gov>. The flat 2D structure then converted into a three dimensional arrangement using an online server at https://www.mn-am.com/online_demos/corina_demo. The structures of graphene oxide and



cyclodextrin reduced graphene oxide have been modified according to our need and hydrogen bonds and formal charges were added to the ligand before optimizing the whole structures. Finally, we performed molecular docking studies in MOE 2015 software to better understand the interaction of NB with the different surface modified GO- β CD conjugates.

3. RESULTS AND DISCUSSION:

UV-vis spectroscopic study was used to investigate the interaction between pure NB with GO- β CD nanocomposites. Although GO exhibits a peak around at 231 nm which may be due to the π - π^* transition of C=C double bond [25] but in our case, according to the spectra shown in [Figure S1](#), GO dispersion showed the maximum absorption at the wavelength of 264 nm which is somewhat about 30 nm longer. After the formation of GO- β CD nanocomposites, the peak got shifted to 258 nm, which indicate the grafting of β CD on the Graphene oxide nanosheets. When UV-vis spectrum of pure NB was studied, two distinct peaks at 279 nm in far-ultraviolet region due to long wavelength π - π^* transitions of aromatic ring and 634 nm in visible light region due to n - π^* transitions of C=N were observed. But when NB was encapsulated inside the GO- β CD nanocomposites, the intensity got reduced which indicate that encapsulation was taking place during the synthesis [26]. The [Figure S2](#) shows the digital photograph of GO, GO- β CD, GO- β CD-NB and NB. Typically, GO exhibits pale yellow colour whereas GO- β CD dispersion appears dark black colour. However, GO- β CD-NB composite showed faded blue colour with aggregated small particle possibly due to encapsulation in hydrophobic cavity of β CD obtained from DLS study.

Cushing et al., showed that GO was able to show excellent fluorescence and the fluorescence emission spectra of GO is very broad around 400-700 nm [27]. The GO sheets exhibited three different fluorescence emission peaks at 455 nm, 567 nm and 635 nm as shown in [Figure S3](#) and all the three peaks have different relative intensities [28]. Upon 260 nm excitation, graphene oxide showed three emission peaks at 455 nm, 567 nm and 635 nm due to $\sigma^* \rightarrow n$ electronic transition of C-OH, $\pi \rightarrow \pi^*$ transition of the graphitic C=C double bond and $\pi^* \rightarrow n$ transition of the C=O



associated groups respectively, thereby, confirming that GO has been formed which is also quite similar as in the previous literatures [29,30]. After the formation of GO- β CD nanocomposites, the emission peak have been shifted towards the lower region i.e., at 450 nm. During the reaction course, β CD molecules were grafted on the surface of the graphene oxide.

GO spectrum shows a peak at around 1723 cm^{-1} which is basically due to the presence of several C=O functional group like aldehyde(-CHO), ketone(-C=O) etc. linked on the surface of GO structure. [Figure S4](#) showed all the different components. The peaks at 1616 cm^{-1} and 1118 cm^{-1} of the GO spectrum were ascribed to the functional groups of aromatic C=C and C-O, respectively, whereas peak at 1376 cm^{-1} might be due to the C-OH stretching vibrations [31,32]. After the functionalisation by β CD on GO nanosheets, the disappearance of a peak at 1723 cm^{-1} and the appearance of C-O/C-C stretching vibrations at 1018 cm^{-1} and 1125 cm^{-1} , O-H/C-H bending vibrations at 1410 cm^{-1} and aromatic C=C stretching around 1573 cm^{-1} ensures that graphene oxide has been reduced resulting in the formation of GO- β CD composite [33,34].

In the spectrum of NB, the C-H stretching frequency of CH/CH₂ groups and N-H bending vibration were observed at 2915 cm^{-1} and 1587 cm^{-1} respectively. But after the formation of GO- β CD-NB composites, there was disappearance of a peak at 2915 cm^{-1} and the characteristic peak at 1587 cm^{-1} was found to be shifted to lower region around 1576 cm^{-1} which suggest that Nile blue was encapsulated and stabilized onto the GO- β CD composite.

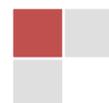
The surface morphology of GO, GO- β CD, GO- β CD-NB and NB are shown in [Figure S5](#). It is clearly observed that the GO surface appeared wrinkled, sharp edged and irregularly shaped. In GO- β CD nanocomposites, the particles were viewed as an aggregated form [35,36]. Free NB appeared to have small ball like shape, while after GO- β CD-NB formation, there was an alteration in its size and shape. All these observations provide a qualitative idea about the surface morphology of NB encapsulated GO- β CD.



The size distribution of aqueous dispersion of GO, GO- β CD and GO- β CD-NB were studied by DLS technique. The hydrodynamic diameter of GO, GO- β CD and GO- β CD-NB were found to be 0.576 μm , 0.739 μm and 1.532 μm respectively as shown in supporting information (Figure S6). According to Liu et al., [37] the effective size of particles in GO and rGO are 0.56 μm and 2.93 μm respectively, which very well matches the values of our experimental data [38].

Zeta (ξ) potential is also an important parameter to characterize the different graphene based materials [39]. According to literature, only those composites are considered to be stable whose zeta potential values are either more positive than +30 mV zeta values or more negative than -30 mV [40,41]. In our study, we found that GO suspension had $\xi = -49.1$ mV. This is due to the presence of hydroxyl and other oxygenated functionalisation on the surface of GO (Figure S7). In case of GO- β CD, the particle showed a negative zeta potential of -32.7 mV which indicates its fair stability in solution. However, in case of GO- β CD-NB, the surface potential significantly decreased to -25.4 mV and the decrease of negative zeta potential indicates the reduction of graphene oxide via grafting of cyclodextrin on the surface of GO and the nanocomposites becomes quite aggregated as can be seen from figure S2. However, having low dispersion stability of the nanocomposites, thermal stability obtained by TGA analysis (Figure S8) makes the nanocomposites more efficient as a guest surface coated nanocomposites.

Functionalisation of β CD molecules on the surface of graphene oxide was determined by thermal gravimetric analysis. The TGA curves for GO, β CD and GO- β CD are shown in Figure S8. As per the literature, GO has low thermal stability around 100 $^{\circ}\text{C}$ with a moderate weight loss caused due to the removal of adsorbed water. The oxygen-containing functional groups such as epoxides, carboxyl and hydroxyl groups were decomposed between 100-200 $^{\circ}\text{C}$, and more than 99% of GO had been reduced when the temperature was raised upto 600 $^{\circ}\text{C}$ [42]. β CD showed a rapid weight loss at 100 $^{\circ}\text{C}$ and tends to decompose at about 320 $^{\circ}\text{C}$, but with the rise of temperature to 350 $^{\circ}\text{C}$ it almost decomposes to about 68%.



The decomposition curve of GO- β CD was found to exhibit the combination of the curves of GO and raw β CD. The weight loss of 10% at around 100-150^oC may be attributed to the decomposition of β CD while at around 150-250^oC a weight loss of 15% emerged. When the temperature was raised upto 600^oC, only 50% weight percentage was reduced indicating that β CD molecules could be functionalized at the edges of the reduced GO sheets. These observations showed that functionalized graphene oxide showed much higher thermal stability [43].

To investigate the encapsulating power of GO- β CD composite with NB, fluorescence spectroscopic technique has been used. In this study, for three different sets were prepared where, 10 μ M of NB was used as pure in each case, in the second set, 10 μ M β CD was added and finally in the third set, 0.05 mg/mL GO- β CD composites were added and shaken for an hour. It can be seen from [Figure 1](#) that the fluorescence intensity of NB was quenched by β CD to approximately 38.4%. While the quenching of NB by GO- β CD composite was enhanced to about 77.5% which was possibly due to the energy transfer from dye to graphene oxide and adsorption of some dye on the surface of GO [44].

A knowledge regarding the binding constant is also significant in terms of drug delivery issues. In order to get the value of binding constant, Benesi-Hildebrand method was employed and titration curve were shown in [Figure 1](#) [45]. The double reciprocal plot of $1/(A_0-A)$ versus $1/[\beta\text{CD}]$ was obtained on titration of NB with different concentrations of β CD ([Table S1](#)). A good linear relationship between $1/(A_0-A)$ and $1/[\beta\text{CD}]$ suggest the 1:1 stoichiometry of inclusion complexes ([Figure S9](#)) [43]. The binding constant (K_a) of the 1:1 NB- β CD complexes were calculated to be $2.1 \times 10^4 \text{ M}^{-1}$ [46].

To understand the encapsulation mechanism, binding of NB- β CD and GO- β CD-NB inclusion complex were studied by molecular docking study [47]. Generally, if the binding energy is more negative, the interaction between the host and guest will be stronger. Before docking all the structures were optimized. From all the optimized structures shown in [Figure 2](#), it can be seen that the phenoxazine moiety of Nile blue molecule is localized into the cavity with its $-\text{N}(\text{C}_2\text{H}_5)_2$ group near the



narrow rim, oxazine ring near the wider rim and benzene ring inside the hydrophobic hollow space of β CD. The Gibbs free energies of the docked optimized structures of NB- β CD, two NB with GO grafted one β CD, NB with GO grafted two β CD and two NB with GO grafted two β CD were found to be -5.36 kcal/mol, -5.53 kcal/mol, -5.82 kcal/mol and -4.88 kcal/mol respectively. The negative Gibbs free energy values indicate the encapsulation process to be thermodynamically favourable. This gives us a strong idea that there is usually strong binding of the molecule with GO- β CD nanocomposites rather than pure β CD [48].

Figure 3 shows the variation of absorption maximum and fluorescence of GO- β CD-NB nanocomposites with pH. In strong acidic medium, $\text{pH} \leq 2$, the absorption maximum got diminished. However, absorption peak was observed at 638 nm with $\epsilon \sim 1.58 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ and $\sim 1.63 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ respectively at $\text{pH} = 3$ and 4. Then again at $\text{pH} = 5$ and 6, such absorption peak got diminished. At neutral region $\text{pH} = 7$, a peak at 638 nm dramatically enhanced with $\epsilon \sim 1.32 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$.

At moderate alkaline medium, $\text{pH} = 8$ & 9, further no absorption peak was found to appear, but in highly alkaline medium, new absorption peaks at 237 nm appeared with $\epsilon \sim 2.02 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ and $\sim 1.20 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ at $\text{pH} = 10$ and 11 respectively [49,50].

When 0.5 mg/mL of GO- β CD-NB composites were taken in different pH solutions, fluorescence intensity reached maximum in case of pH at 4. Then, a gradual decrease in fluorescence intensity for different pH medium was observed. This is possibly due to aggregation of Nile blue loaded inside GO- β CD cavity in neutral medium. These kinds of aggregations are most probably due to hydrophobic interactions. Generally, Pure NB can act as pH probe due to its high pH sensitivity. According to Martinez et al., in aqueous solution the acidic form of Nile blue molecules results in H-aggregation which causes disappearance of its fluorescence and thereby, effecting photophysical behaviour of Nile blue [51]. In our study, it was observed that the GO- β CD-NB nanocomposites showed considerable fluorescence in highly acidic medium, $\text{pH} = 2, 3, 4$ and in highly basic medium, $\text{pH} = 10$. Hence, GO- β CD-NB composites can be used as pH sensitive probe.



In order to characterize the spectral properties of the nanocomposites, we first studied its solvent dependency [52]. Out of six different solvents, two polar protic and three polar aprotic solvents have been used. As shown in [Figure 4](#), the probe exhibits single absorption and emission bands mainly contributed by NB moieties and the peaks due to reduced GO have been totally diminished. The peaks centred at around 630 nm correspond to the maximum absorption and emission wavelengths of NB [53]. The maximum absorption and emission wavelengths of nanocomposites have been shown in [Table 1](#) (λ_{abs} , 625–634 nm and λ_{em} , 652–688 nm for MeOH to CHCl_3). The absorption maxima of our GO- β CD-NB nanocomposites have been found to increase linearly. While, the fluorescence intensity gradually decreases upto acetonitrile with increasing solvent polarity (owing to the ICT process that enables this dye to respond to environmental polarity) and then again tends to increase upto chloroform [54].

The additional red shift of NB in protic solvents, such as alcohols and probably chloroform, is associated with the hydrogen-bond formation with the carbonyl group of the dye. According to the previous literature [55], NB in MeOH showed its absorption maximum at 625 nm, and emission maximum at 655 nm with molar extinction coefficient $80,800 \text{ M}^{-1} \text{ cm}^{-1}$. However, for our NB encapsulated nanocomposites, the emission maximum, λ_{em} , was shifted to 669 nm with highest stokes shift of 44 nm followed by reduction of molar extinction coefficient to $35,282 \text{ M}^{-1} \text{ cm}^{-1}$.

In case of polar aprotic solvents like dichloromethane, irradiation with 624 nm wavelength showed strong fluorescence with least stokes shift of 28 nm. According to previous literature [56], in DMSO, Nile blue showed stokes shift of about 33 nm, however, absorption peak of NB in GO- β CD nanocomposites was observed at 634 nm and emission maximum at around 688 nm but stokes shift increases to 54 nm. This study helps us to know that DMSO is more effective polar aprotic solvent for our nanocomposites. Therefore, solvent-dependent spectral properties of nanocomposites make them ideal scaffolds for the development of improved biological indicators.



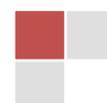
Fluorescence quantum yield for fluorescent dye as well as fluorescent nanocomposites is very vital [57,58]. It measures the efficiency of the conversion of absorbed photons into emission photon. A typical fluorescence spectrometer is used for the measurement of relative fluorescence quantum yield. For pure NB molecules, ϕ was found to be 0.27 in ethanol as reported in literature [59]. However, in case of our nanocomposites, relative fluorescence quantum yield of NB decreased to 0.19 and when the same experiment were carried out after three days with the same solution, it got reduced to 0.03. So, there is an obvious decrease in relative fluorescence quantum yield (Table 2).

4. CONCLUSION:

In this work, a unique process of encapsulation of NB molecules by β CD grafted reduced graphene oxide (GO) in the aqueous medium has been synthesized and established emphatically through vivid spectroscopic investigations. The experimental results are rationalized by molecular docking studies and showed the encapsulation of NB molecules inside free β CD as well as reduced GO- β CD sheets. Exploiting the host-guest chemistry of NB with GO- β CD, it was demonstrated that encapsulation power of GO- β CD nanocomposites revealed that NB molecules are entering inside the cavity of GO- β CD nanocomposites twice than that of pure β CD. This study also suggested that how specific cyclodextrin functionalities can help to increase biodegradability of GO leading to an efficient biodegradable carriers based on graphene nanomaterials, and the thermal stability of nanocomposites have been increased. The result demonstrated that the pH responsive characteristic of graphene-based nanocomposites is vital in case of fluorescence imaging, designing a nanocomposites with improved photophysical performance and better delivery system to release the loaded NB molecules to acquire highly efficient biological activity.

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CHAPTER V

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Authors contributions:

N.R., P.B., D.R., B.G. and M.N.R contributed to the design and analysis of experiments. N.R., P.B., D.R., and B.G. performed the experiments in this manuscript. N.R., P.B., wrote the manuscript. N.R., P.B., and M.N.R critically reviewed the manuscript.

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Competing Interests: The authors declare no competing financial interest.

Additional Information

Supporting Information: UV-vis spectra, Photograph of different composites, DLS, Zeta potential measurements, SEM microphotograph, Table for Benesi-Hildebrand plot for association constant, Linear plot of $1/\Delta A$ vs $1/[\beta CD]$ (M^{-1}).



TABLES

No	Solvent	Absorbance		Fluorescence		Stokes Shift (nm)	ϵ Values ($M^{-1} cm^{-1}$)
		Wavelength (nm)	A.U	Wavelength (nm)	A.U		
1.	MeOH	625	1.76410	669	720345	44	35282
2.	EtOH	630	2.17974	671	752811	41	43184
3.	DMSO	634	2.297323	688	355092	54	45946
4.	MeCN	633	1.044811	669	274006	36	20896
5.	DCM	633	0.203392	656	696870	23	4000
6.	$CHCl_3$	624	1.096071	652	1826493	28	21921

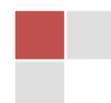
Table 1: Photophysical properties of the composites where 50 μM of NB solution were taken in 0.5 mg/mL of GO- β CD was taken in different solvent

Sample	Integrated emission intensity (I)	Absorbance at ~ 636 nm (A)	Refractive Index of solvent (η)	PLQY (ϕ)
GO- β CD-NB	187918	1.60213565	1.3605	0.19683
GO- β CD-NB After Three days	32724	1.57810974	1.3606	0.03480

Table 2: Relative fluorescence quantum yield calculation of GO- β CD-NB and after three days

[NB] / μM	[β CD] / μM	A_o	A	ΔA	$1/[\beta CD]$ / M^{-1}	$1/\Delta A$	Intercept	Slope	K_a/M^{-1}
50	5	0.849308	0.884011	0.034703	200000	28.81576			
50	10	0.849308	0.917808	0.0685	100000	14.59854			
50	15	0.849308	0.941678	0.09237	66666	10.826			
50	20	0.849308	0.954901	0.105593	50000	9.470306			
50	25	0.849308	0.965287	0.11598	40000	8.622201	2.75252	0.0001285	21,420
50	30	0.849308	0.975467	0.126159	33333	7.926493			
50	35	0.849308	0.986621	0.137313	28571	7.282612			
50	40	0.849308	1.034042	0.184735	25000	5.413165			
50	45	0.849308	1.03831	0.189003	22222	5.290935			
50	50	0.849308	1.068188	0.21888	20000	4.56871			

Table S1: Calculation for association constant at 293.15K by applying Benesi-Hildebrand equation



FIGURES

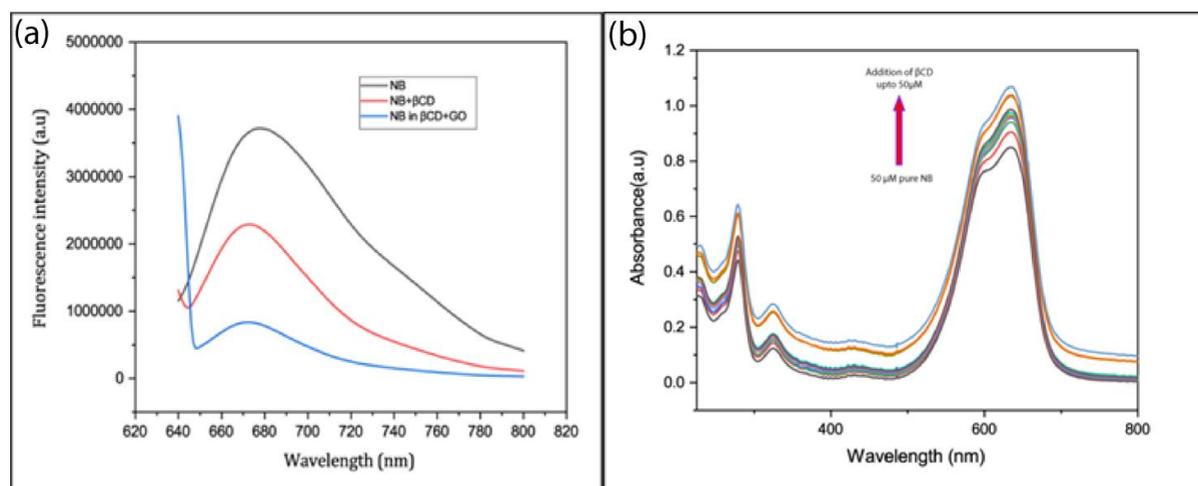


Figure 1: (a) Fluorescence intensity of 10 μM NB (black), 10 μM NB + 10 μM $\beta\text{-CD}$ (red) and 10 μM NB + 0.05 mg/mL GO- βCD nanocomposites (blue) (b) Spectral titration of NB with βCD solution to calculate association constant

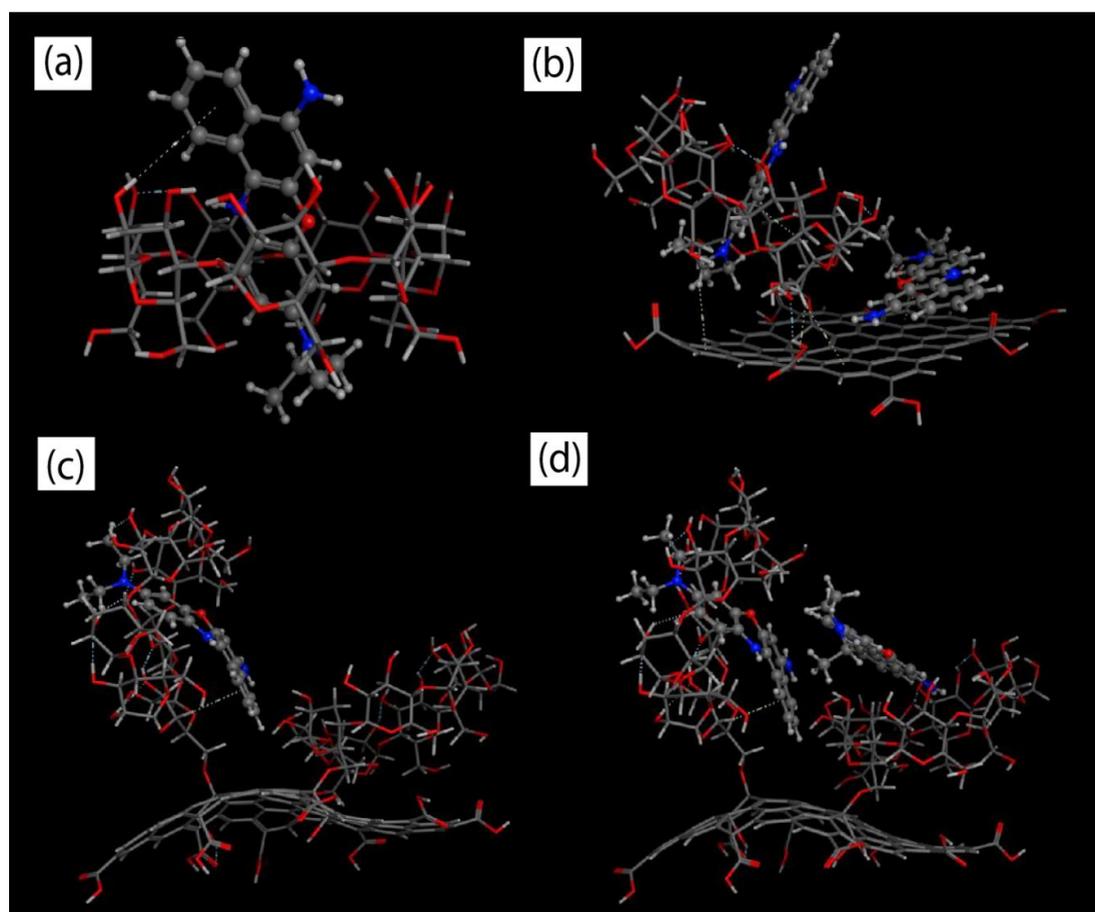


Figure 2: Molecular docking images of (a) NB- βCD (b) two NB with GO grafted one βCD (c) NB with GO grafted two βCD (d) two NB with GO grafted two βCD



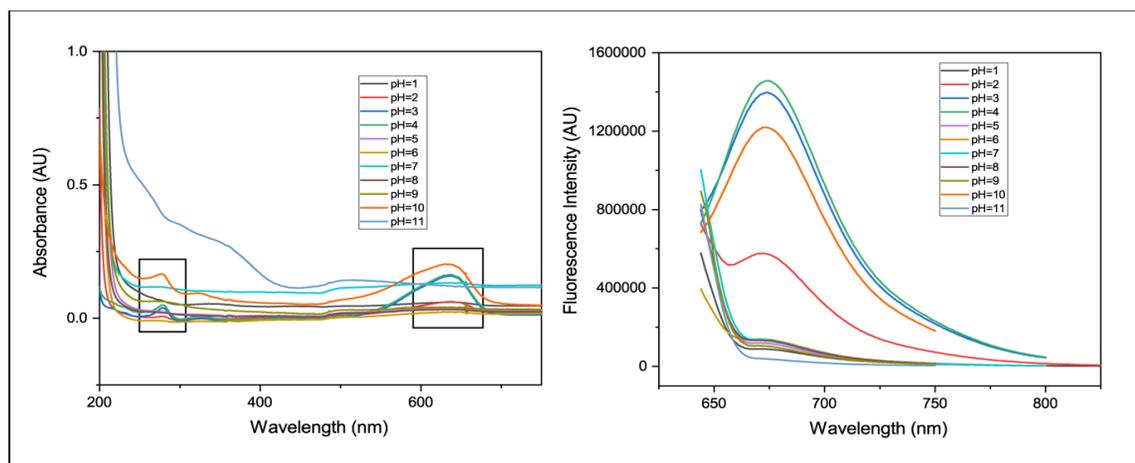


Figure 3: UV-vis and fluorescence spectra of 0.5 mg/mL GO-βCD-NB nanocomposites at different pH from 1 to 11

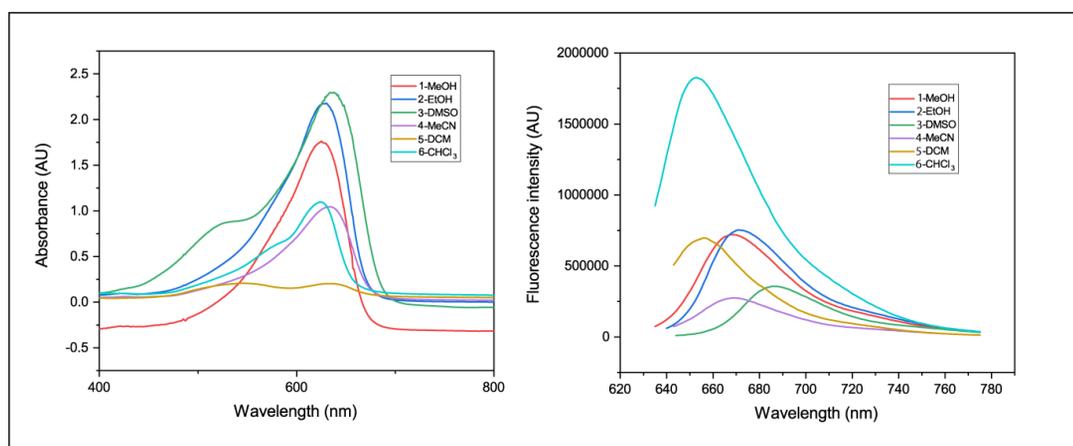


Figure 4: UV-Vis spectra (left) and fluorescence emission spectra (right) in different solvents

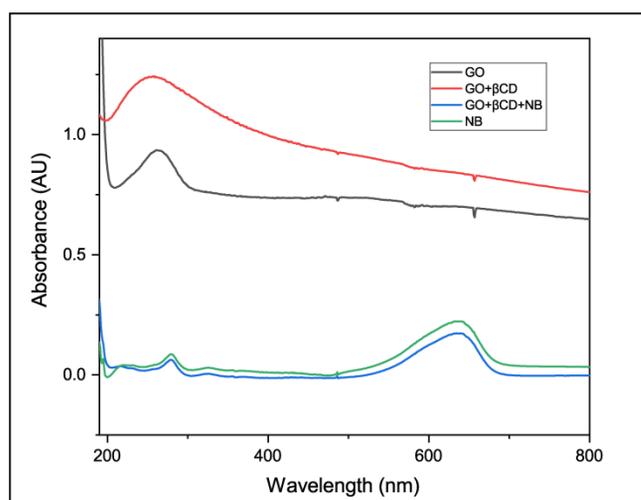
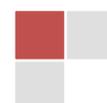


Figure S1: UV-vis spectra of aqueous solution of 0.05 mg/mL GO, GO-βCD, GO-βCD-NB and NB



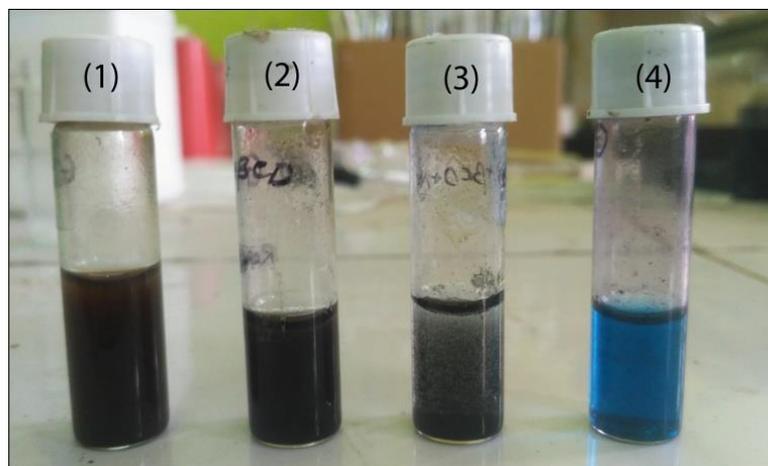


Figure S2: Photograph of different composites (1) GO, (2) GO- β CD (3) GO- β CD-NB (4) NB

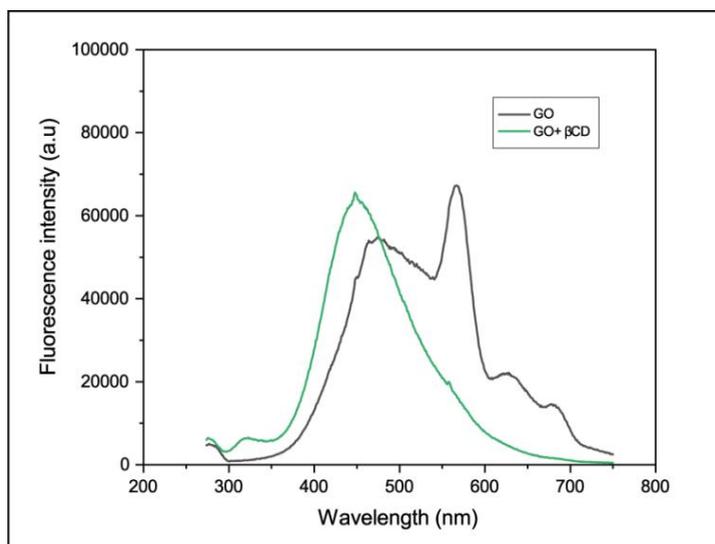


Figure S3: Fluorescence emission spectra of GO, GO- β CD composites

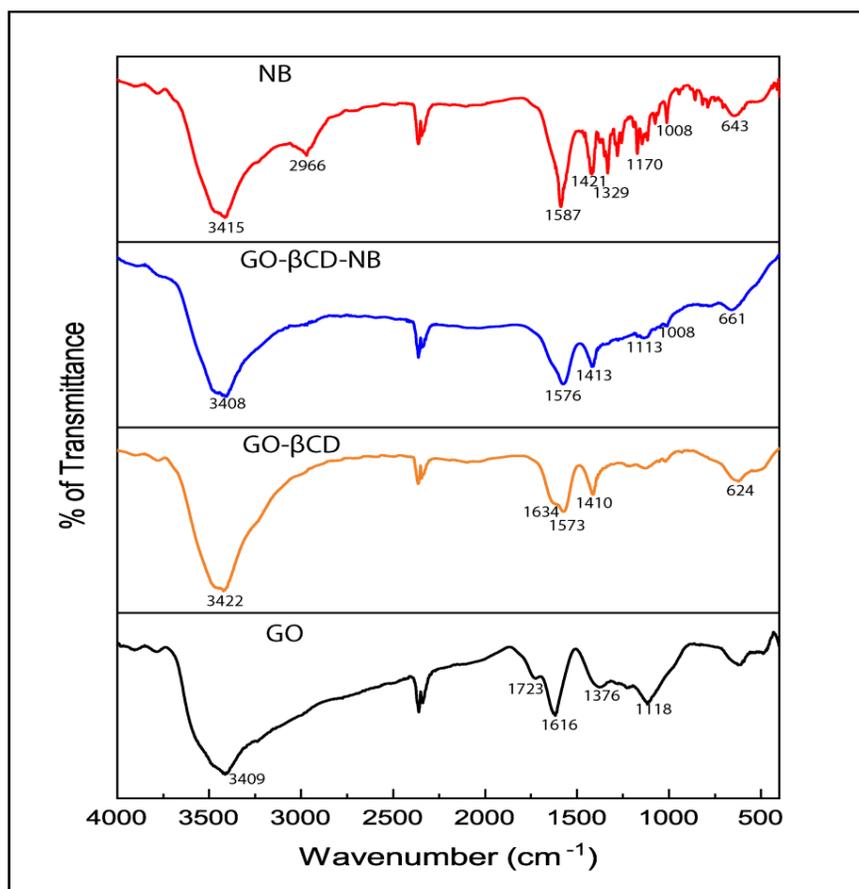


Figure S4: Infrared spectra of GO, GO-βCD, GO-βCD-NB, NB

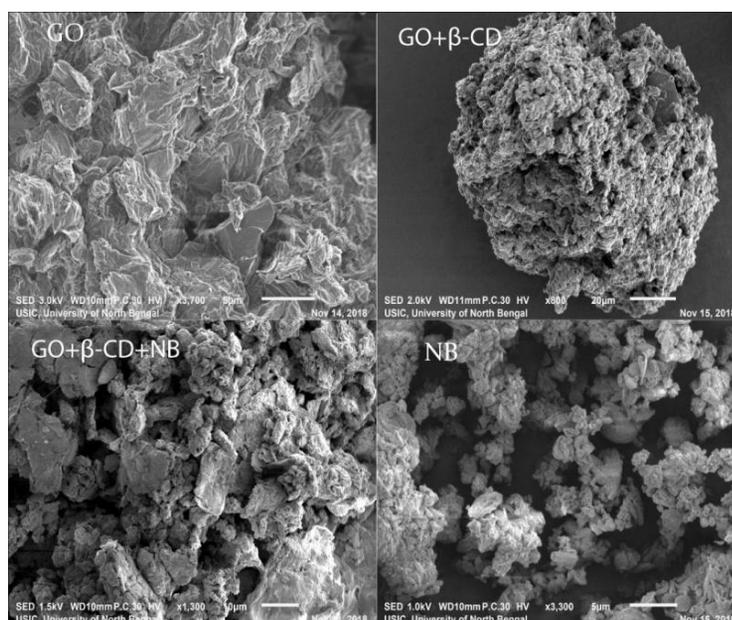
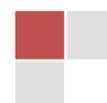


Figure S5: Scanning electron microphotograph of GO, GO-βCD, GO-βCD-NB and NB



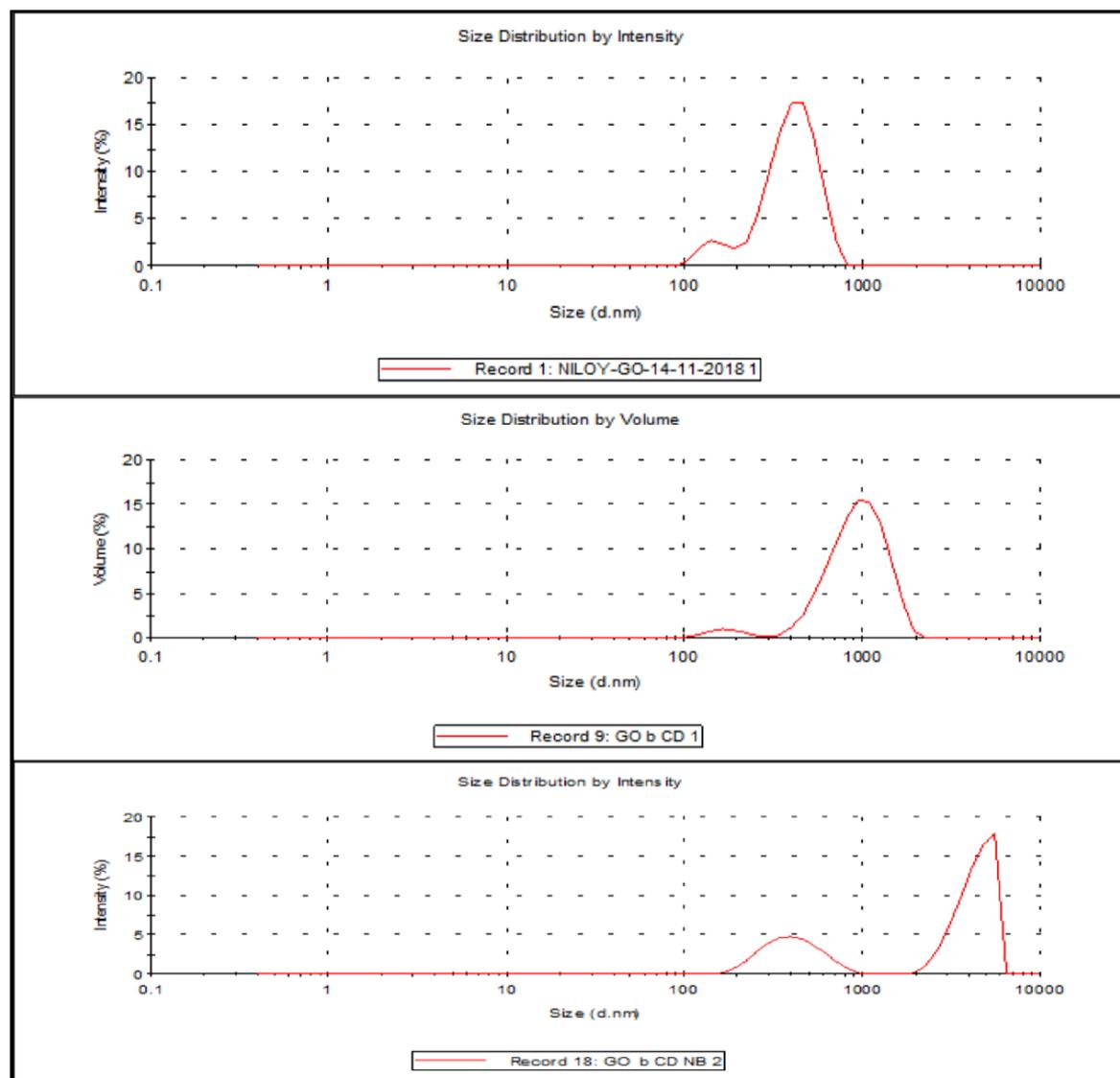


Figure S6: Dynamic Light Scattering spectra of GO, GO- β CD, GO- β CD-NB in aqueous dispersions (Samples were taken at $50\mu\text{g.mL}^{-1}$)



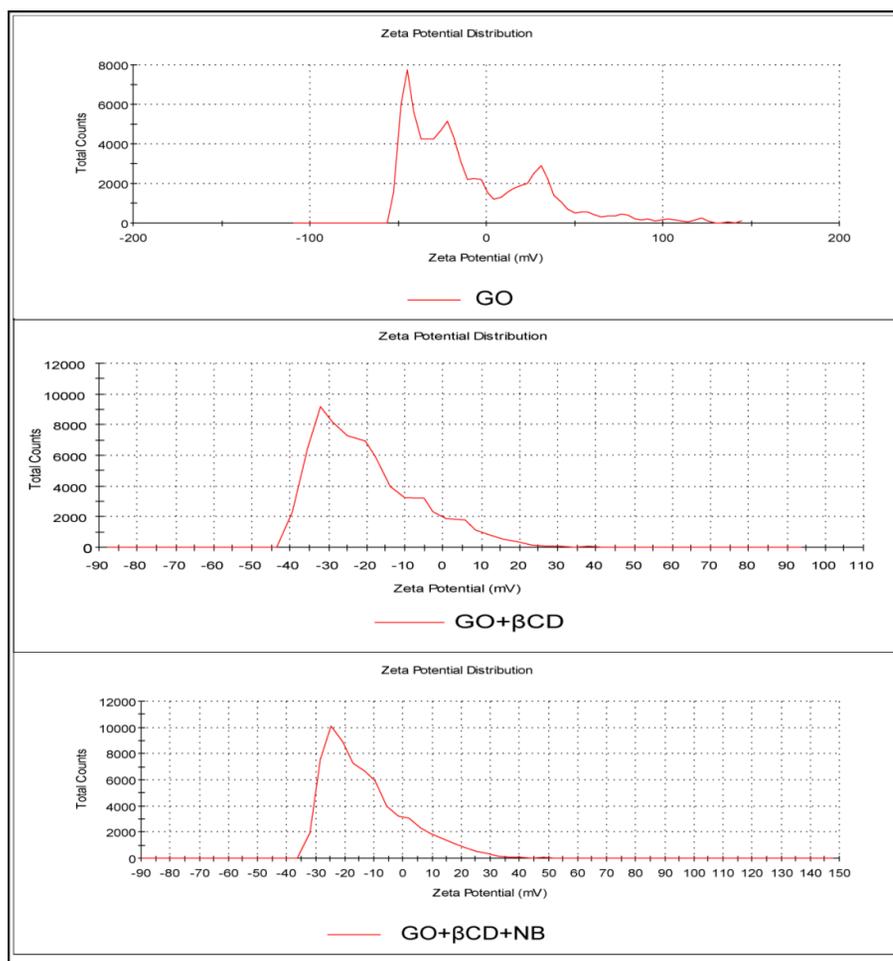


Figure S7: Zeta potential value obtained by dynamic light scattering method for GO and GO+βCD

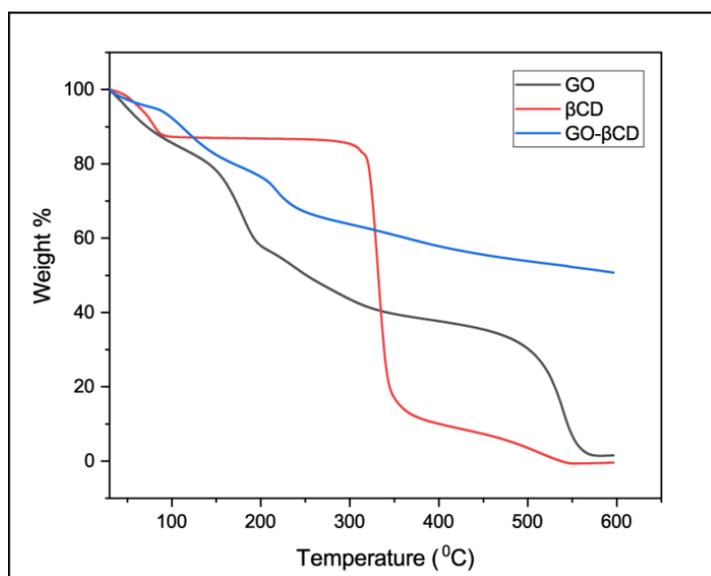
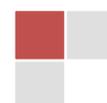


Figure S8: Thermo Gravimetric analysis of GO, βCD and GO-βCD



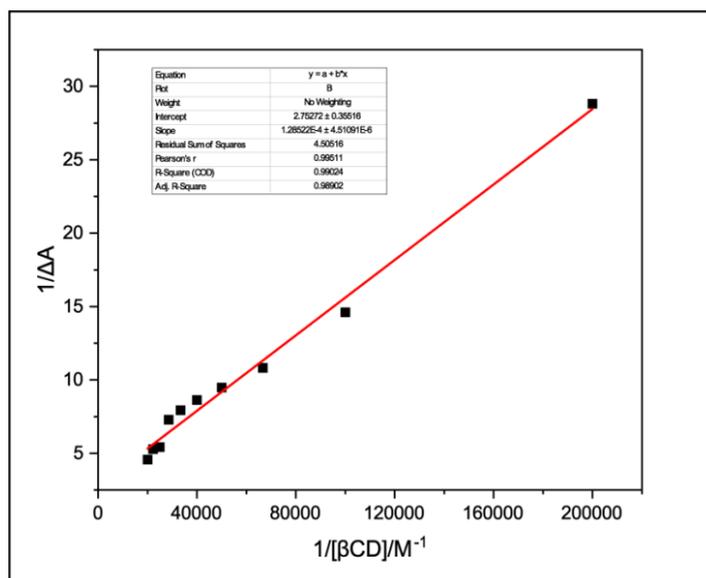
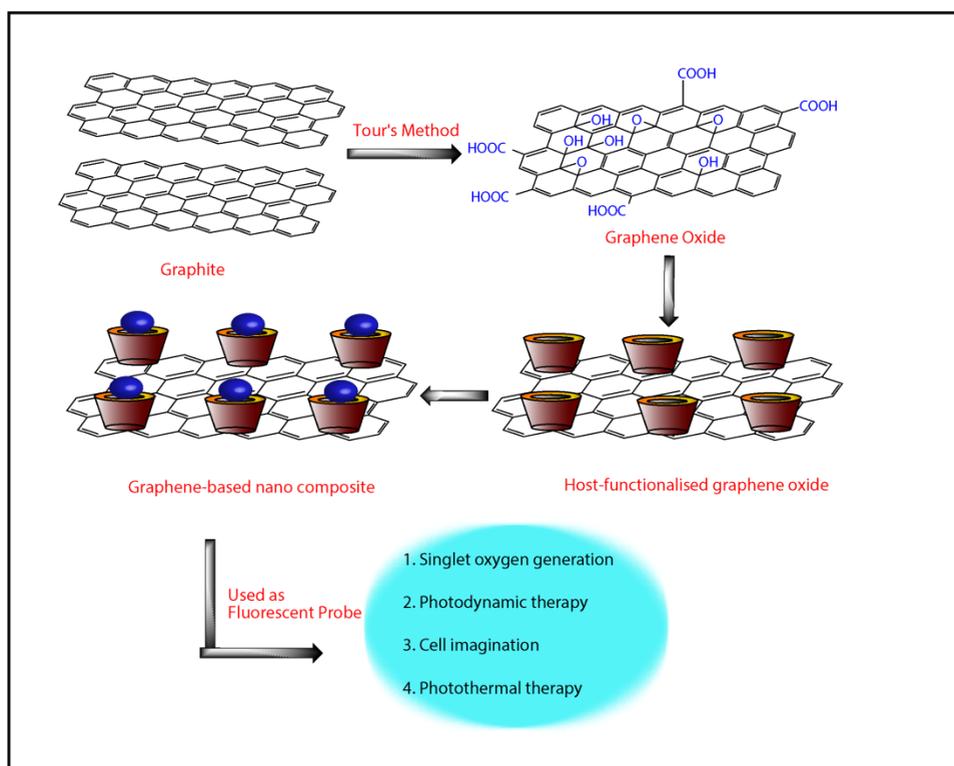


Figure S9: Double reciprocal Linear plot of $1/\Delta A$ vs $1/[\beta CD]/M^{-1}$

SCHEMES



Scheme 1: Schematic diagram for the synthesis of rGO-βCD composites and the interaction between the guest (Nile blue) and the host (βCD) moiety linked up to rGO nanosheet

