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Synthesis and characterization of NADH model compound modified β-cyclodextrin and its role as an energy donor in FRET

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ABSTRACT

β-Cyclodextrin is chemically modified to selectively introduce an acridinedione moiety on the primary face. The synthesis involves the substitution of one of the primary hydroxyl groups of β-cyclodextrin by a tosyl group which facilitates the introduction of an ethylene diamine modification which is finally condensed to a tetraketone. The acridinedione modified β-cyclodextrin thus obtained was fully characterized by IR, NMR and Mass spectrometry. The photophysical properties of the compound were also analyzed. The potential of the modified β-cyclodextrin to act as an energy donor in FRET was investigated with a suitable acceptor.

1. Introduction

Cyclodextrins (CDs) are a family of cyclic oligosaccharides that are composed of α-1,4-linked glucopyranose subunits existing in a chair conformation [1]. CDs are synthetically obtained from starch by enzymatic degradation. The three most common types of CDs well known are α-CD, β-CD and γ-CD having 6, 7 and 8 glycosyl units, respectively. These are referred to as parent, native or first generation CDs among which β-CD is the most accessible, the lowest priced and generally the most useful.

CDs possess a doughnut-like structure with wide and narrow hydrophilic tops delineated by O(2)H and O(3)H secondary and O(6)H primary hydroxyl groups respectively and by a hydrophobic annular core lined with H(3), H(5) and H(6) hydrogen atoms and O(4) ether oxygen atoms [2]. It is the central hydrophobic cavity of β-CD with an inner diameter of 6–6.5 Å units, which makes these molecules capable of forming inclusion complexes with many organic and inorganic guest molecules [3]. Interesting strategies are emerging in this field and a wide range of organic molecules has been found to form complexes with CDs and hence they find apt application ranging from solubilization of pharmaceutical compounds to chiral chromatography [4].

CDs and their chemically modified derivatives have been the subject of numerous investigations for a variety of reasons ranging from achieving solubility in a desired solvent to analyzing the mechanisms of enzyme-catalyzed reactions [5,6]. For synthetic chemists, CDs are of interest because they are chemically stable and can be modified in a regioselective manner [7]. Such modifications produce enormous opportunities and at the same time more challenges to the chemists. Opportunities are provided by the fact that, through modifications, CDs can produce exquisite molecules that can be invaluable in investigations at the frontiers of chemistry like enzyme like catalytic activity and antibody like binding activity to aesthetically pleasing molecules. But the presence of hydrophobic cavity and a large number of hydroxyl groups provide a great challenge to the selective modification of CD [8]. There always seems to occur a competition between the hydroxyl groups at positions 2, 3 and 6 for the reagent and makes the selective modification extremely difficult. In recent days, suitably modified CD derivatives acting as fluorescent sensors for detecting a variety of organic molecules including biologically important substances have been indicated, e.g., pyrene, naphthalene and dansyl modified CDs [9]. Reports are also available on the increased stability of inclusion complexes using bridged bis (β-CD)s. It has been observed that the cooperative binding of one guest by two CD moieties in a single host molecule is the reason for such a multifold enhancement in the stability of inclusion compounds [10,11].

CDs are essentially inert to photochemical excitation but their chemical modifications with chromophoric moieties may convert them into a spectroscopically active molecule. This property of modified CDs could be extensively used to construct host–guest sensory systems [12,13] with which a number of molecular species

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can be detected and hence-forth they can be considered as biological markers or sensors for the detection volatile organic compounds [14]. Detailed studies on pyrollinone modified CDs have been carried out in this context wherein the pyrollinone appended to the β-CD acting as a hydrophobic cap to increase the binding ability of the host have been already reported [15]. Such a chemical modification paves a convenient method for constructing a fluorescence sensory system wherein even spectroscopically inert guest molecules can be examined directly [16].

Other interesting fields in which the modified CDs acquire importance are the molecular modeling [17] and mechanism of excitation energy transport [18] and Fluorescence Resonance Energy Transfer (FRET). Extensive investigations have been already carried out in the case of multichromophoric CDs being used in the interpretation of photophysics involved in energy hopping [19]. FRET is a fast emerging field owing to its selectivity and sensitivity in providing information about the structure and dynamics of macromolecules as well as molecular assemblies [20].

In this paper, our attention is focused on the synthesis and a preliminary investigation on the photophysical properties of a chromophoric CD *viz*., β-CD linked to an acridinedione, which has been reported as a class of laser dyes [21]. Apart from having a high fluorescence quantum yield and lasing action comparable to that of coumarin-103, the acridinedione chromophores also acquire importance in biological systems due to their structural similarity to 1,4-dihydropyridines and NADH which are active biological co-enzymes [22].

Hence, with the synthesis of such an acridinedione modified β-CD, we further aim at the study of potential application of these dyes as photosensitizers [23], fluorescent sensors [24,25] and probes [26], initiators in photopolymerization reactions and also suitable substitutions on the acridinedione molecule which would further develop the fluorescent behaviors of such compounds. Also, such modified β-CD provides an opening into an area of further research, wherein, another chromophore of suitable size can be included into the cavity of β-CD and hence a bifluorophoric study can be initiated. i.e., they can be considered as a variety of chemosensors and hence a study of its signal transduction properties can be carried out.

2. Materials and methods

Resorcinol and *p*-Toluenesulfonyl chloride used for the synthesis were obtained from S.D. Fine Chemicals Pvt. Ltd. Ethylenediamine also obtained from S.D. Fine chemicals was doubly distilled before use. *N*,*N*-Dimethyl formamide and methanol used were of HPLC grade and were obtained from Qualigens India Ltd. β-CD received from Cyclolab was recrystallized from water before use. Cyclohexane-1,3-dione for synthesis was obtained from Sigma–Aldrich Chemicals Pvt. Ltd. and used as received. Toluene, P_2O_5 used were also obtained from Qualigens India Ltd. Molecular sieves with pore sizes of 4 Å were used to store DMF.

Absorption spectra were recorded in an Agilent 8453 diode array spectrophotometer. Fluorescence spectra were recorded using a Horiba Jobin Yvon Fluoromax 4P Spectrofluorimeter. Fluorescence quantum yields were obtained from the corrected fluorescence spectrum using quinine sulphate in 0.1 N H_2SO_4 [27]. Time resolved fluorescence decays were obtained by the time-correlated-single-photon-counting (TCSPC) method. The excitation source used was a 375 nm LED (model N-375 LH) with a pulse width of 600 ps purchased from Horiba Jobin-Yvon. The emission intensity were counted by a MCP PMT (Hamamatsu R 3809) and processed through CFD, TAC and MCA. The fluorescence decay was analyzed by using the software provided by IBH (DAS-6).

2.1. Synthesis of Mono-6-deoxy-6-aminoethylaminoacridinedione – β-CD:(β-CDen-ADR)

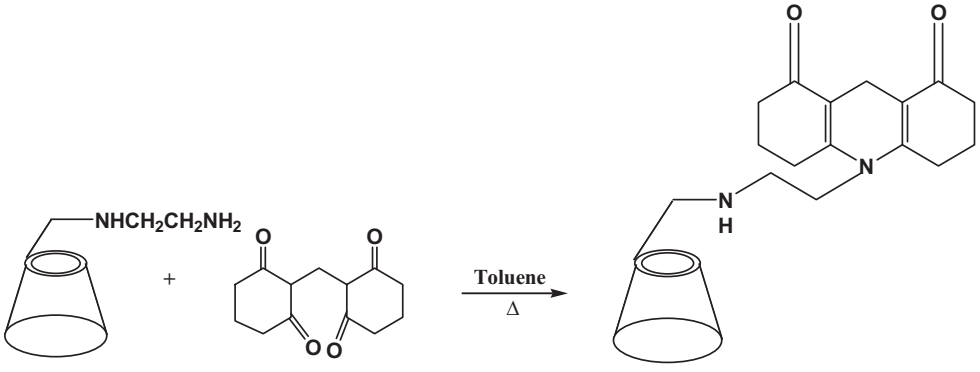
Starting from β-CD, one of the primary hydroxyl groups was modified into a tosyl group by following reported procedures [28]. The mono-6-deoxy-6-tosyl modified β-CD was further modified to obtain mono-6-deoxy-6-aminoethylamino modified β-CD (β-CDen) by treating it with freshly distilled ethylene diamine at high temperature [29] In parallel, the required tetraketone was also prepared by treating cylohexane1,3-dione with formaldehyde in a medium of methanol [30,31].

The modified compound β-CDen-ADR was obtained in the final step as shown in Scheme 1. 2.3 g (2 mmol) of β-CDen was treated with 0.40 g (2 mmol) of tetraketone in 60 mL of toluene and refluxed in a Dean-Stark apparatus for 48 h. The completion of reaction was checked with TLC; the solvent was removed by distillation. The obtained crude product was purified through multiple crystallization from 1:1 MeOH:$CHCl_3$ mixture. The resultant dark brown colored crystals were collected and dried. Yield: 0.8 g, 40%, M.p: 202–04 °C.

3. Results and discussion

The attachment of fluorophores to natural or synthetic receptors has received increased interest over last few years in endeavors to furnish new fluorescent sensors. In particular, fluorescent CDs have generated considerable interest among the synthetic chemists as it is witnessed in recent articles dealing on sensing properties.

The literature data offer several methods for the modification of CDs. Among the hydroxyl groups in β-CD, the primary ones are more nucleophilic and hence they can be easily modified into other



Scheme 1. Synthesis of β-CDen–ADR.

functional groups than their secondary counterparts. Tosylation at the 6th position is the most popular method for mono-modification of CDs, and is found to offer to be a very good precursor for a variety of modifications. This is due to the nature of the tosyl substitution which behaves as a good leaving group, especially in presence of nucleophiles [32]. As illustrated in the scheme, native β-CD was mono modified as aminoethylamino-acridinedione-β-CD (β-CDen-ADR). The purity of the intermediate products were verified and the results were found to be in good agreement with the reported values [24,33]. The final compound was well characterized at using IR, NMR and Mass analysis.

3.1. Characterization

3.1.1. Mono-6-deoxy-6-aminoethylaminoacridinedione – β-CD

IR (KBr) spectrum showed absorption peaks corresponding to acridinedione-like moiety, one at 1716 cm^{-1}, characteristic of the carbonyl groups and the other one at 3275 cm^{-1} indicating the interaction between the carbonyl and hydroxyl groups.

^{1}H NMR (500 MHz, DMSO-d_6) showed the signals of both the entities of the new compound namely, acridinedione and the native β-CD. The acridinedione couplings were found to appear between 2.3 and 1.9 in the spectrum and the signals were assigned to four sets of protons viz., δ 2.65(CH_2), δ 2.37–2.27 (t, 4H, $C^{2,7}$), δ 2.25–2.15 (t, 4H, $C^{4,5}$), δ 1.3–0.9 (q, 4H, $C^{3,6}$). The signals of cyclodextrin moiety were found to appear at δ 8.0 (s, 1H (N–H), δ 5.85–5.60 (m, 14H $OH^{2,3}$), δ 4.95–4.8 (m, 7H, H^1), δ (4.6–4.4 (m, 6H, OH^6), δ 3.8–3.6 (m, 35H, H-^{3,5,6}), δ 3.45–3.2 (m, H, ^{2,4}) overlap with HOD) (Fig. S1).

^{13}C NMR (125 MHz, DMSO-d_6) showed the signals corresponding to the carbonyl and olefinic groups of acridinedione at 195 ppm, 145 ppm respectively. The signals corresponding to aliphatic carbons appeared at 46, 39, 36, 21 ppm and 9 ppm respectively (Fig. S2). The signals of β-CD ring were assigned as follows: C^1 at 101 ppm, C^4 at 82 ppm, $C^{2,3,5}$ at 73,72.8 and 72.4, C^6 at 60 ppm, respectively (Fig. S2).

An electron impact mass spectrum was recorded for the compound which showed the molecular weight as 1376, which was in good agreement with the calculated value (Fig. S3).

3.2. Photophysical studies

For all spectroscopic measurements of β-CDen-ADR, 6% methanol was used as the solvent.

The absorption and emission spectra of all acridinedione dyes show a maximum around and 395 nm and 460 nm, respectively in 6% methanol solution. This band has been assigned to an intramolecular charge transfer from the ring nitrogen to the ring carbonyl oxygen rich centre within the ADR fluorophore [34].

In order to establish the photophysical behavior of the modified molecule, we made a comparison between the absorption and emission characteristics of free ADR and β-CDen-ADR. The absorption spectrum of the free ADR molecule is found to show maxima at 250, 273 and 395 nm and when excited at 395 nm shows a characteristic emission with maximum intensity at 465 nm. The absorption spectrum of the β-CDen-ADR recorded in the UV–vis region showed a similar observation; the absorption maxima were found to occur at 247, 263 and a shoulder around 390 nm (Fig. S4).

Fig. 1 depicts the excitation and emission spectra of the acridinedione modified β-CD. The maximum absorbance of the excitation spectrum was observed at 367 nm and when excited, the fluorescence spectrum showed a characteristic emission with the maximum intensity centered at 462 nm. The inset shows the life time profile of the molecule when excited at 375 nm.

The lifetime profiles of the free ADR and β-CDen-ADR molecules were also analyzed. The free ADR is found to possess a lifetime of 5.2 ns [35] and is well fit by a single exponential function. On

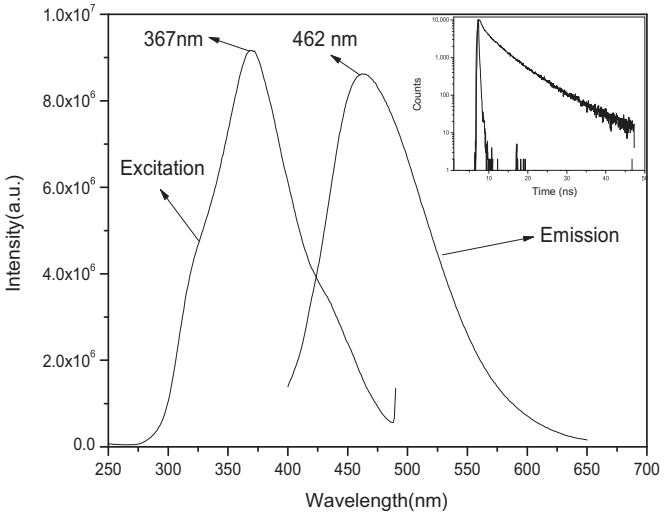


Fig. 1. Excitation spectrum (monitored at 450 nm) and Emission spectrum (excited at 375 nm) of β-CDen-ADR. Inset shows the decay curve of β-CDen-ADR when excited at 375 nm.

the other hand, the fluorescence decay of the modified β-CD is non-exponential and requires three exponential decay terms for adequate fitting with the dominant component exhibiting a decay time of 7.4 ns. The observation of triple exponential fluorescence decay for the modified dye gave us an indication for the possible existence of the molecule in more than one conformation. This may be due to the rotation of the acridinedione moiety about the $–CH_2–CH_2–$ axis. This has been ascertained by studying the intensity and lifetime of the molecule by changing the viscosity of the medium. It was observed that with an increase in viscosity, both the emission intensity (Fig. S5) and the average life time (Fig. S6) of the molecule increased, which is attributed to the arresting of the free rotation of the acridinedione moiety along the axis.

We have also carried out the TRES and TRANES analysis for the modified compound and the spectra are as shown in Figs. 2 and 3. The spectral shift of 5 ns and the observation of two isoemissive points in these figures reveal the presence of three different emitting species in the system. This was further supported by our theoretical calculations. As per the quantum chemical computations using GAUSSIAN 03 (G03W) software calculations carried out

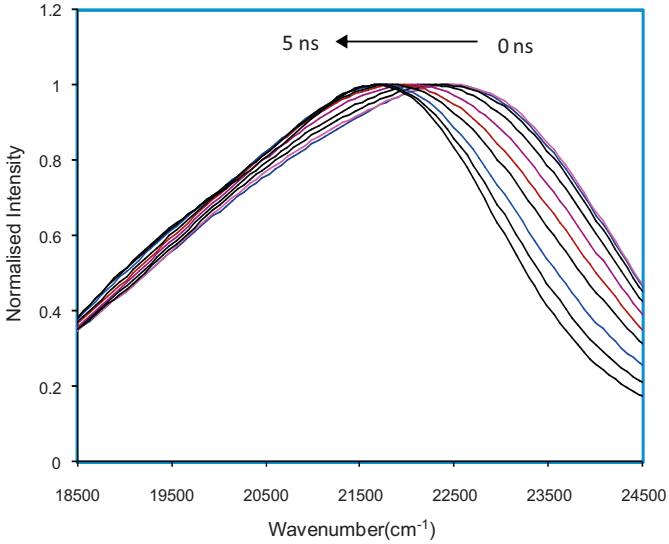


Fig. 2. TRES spectrum of β-CDen-ADR.

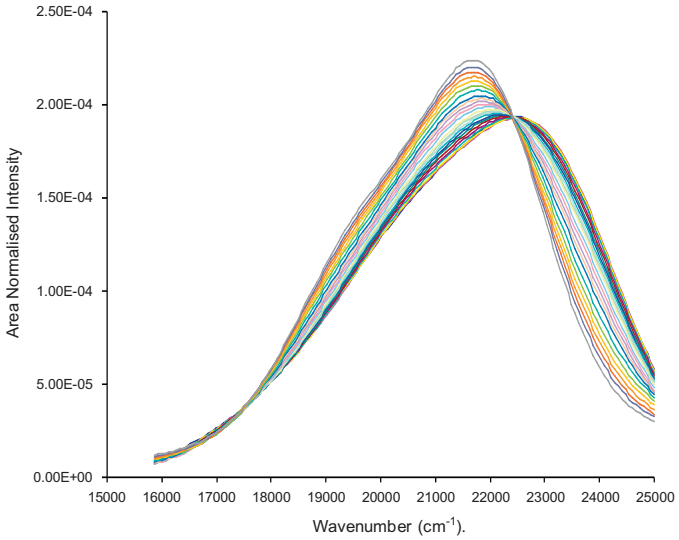


Fig. 3. TRANES spectrum of β-CDen-ADR.

the three structural configurations of the modified molecule is as shown in Fig. 4. Among the conformers, 'III' is the one with highest energy which corresponds to the partial inclusion of the acridinedione moiety into the cavity of β-CD, 'II' is the one with lowest energy and 'I' with intermediate energy, where in the acridinedione moiety is outside the cavity and shows a slight change in orientation along the $-CH_2-CH_2$ axis. Accordingly, the conformer associated with the lowest energy is attributed to the component with the highest population and the longest life time (7.4 ns, ~84%) which is close to that of a free acridinedione dye and the one with highest energy is attributed to the lowest populated component obtained from the lifetime decay analysis (0.53 ns, ~3%). The quenching observed in the lifetime of this component is justified by the hydrogen bond formation between the carboxyl group of the acridine dione moiety and the hydroxyl group of the cyclodextrin moiety as evidenced from the Fig. 4III.

From the corrected fluorescence spectrum, we also have calculated the quantum yield of the β-CDen-ADR molecule as 0.13. This can be compared with those of many free ADR dyes which are reported to have a good quantum yield i.e. in the range of 0.5–0.9. A series of ADR dyes, both free and with substitutions have been already synthesized, photophysical processes were studied, quantum yields were determined and reported by Srividya et al. [35]. Lasing actions of these dyes also have been studied in various solvents and have been reported [36–38].

As far as the quantum yields the ADR dyes are concerned, the substitution on the N atom at the 10th position is found to play a great role. Such a reduction in quantum yields of the acridinedione dye series due to the substitution of bulky groups like phenyl, chlorophenyl, benzyl, phenyl naphthalimides etc., on the nitrogen atom in the 10th position have been already reported [35]. On a similar note, the substitution of a β-CDen moiety on the N is found to have decreased the quantum yield of the molecule almost to 15%. This decrease in quantum yield of the β-CDen-ADR is once again attributed to the free rotation of the β-CD moiety linked to the ADR which in turn would lead to an increase in rate of decay of the molecule in the non-radiative modes. Whereas the free ADR molecule with a rigid structure is reported to have a higher rate constant for radiative decay than that of a non-radiative one.

3.3. β-CDen-ADR in FRET

FRET occurs when the electronic excitation energy of a donor chromophore is transferred to an acceptor molecule nearby (10–90 Å) via a dipole–dipole interaction between the donor–acceptor pair [39]. The process of FRET seems to be more efficient when there is an appreciable overlap between the emission spectrum of the donor and the absorption spectrum of the acceptor [40].

Owing to the reasonable quantum yield, narrow absorption and emission spectra obtained for the chromophore modified β-CD derivative synthesized, we set to investigate the potential of this dye to act as a donor in order to initiate a photoinduced energy transfer to an acceptor molecule.

The acceptor that we have chosen for the present investigation is safranine. The choice of the acceptor was made for the following reasons: (i) it is hydrophobic, (ii) a good spectral overlap of the absorption spectrum with that of emission spectrum the donor β-CDen-ADR was found to occur, (iii) the approximate size of the acceptor matched with that of the nano-cavity of the donor. The final reason leads to an interesting discussion on whether the

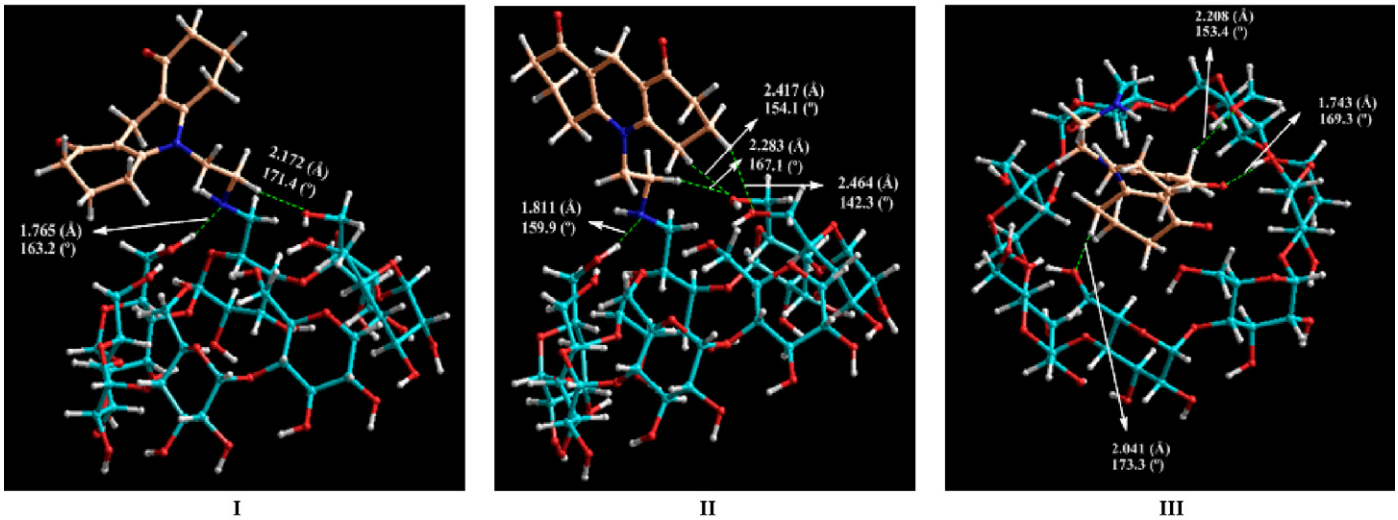


Fig. 4. Energy minimized conformers of β-CDen-ADR. Hydrogen bonding interactions (green dashed lines) between the aciridinedione fragment and CD unit in the HF/3-21G optimized conformers of β-CDen-ADR. Color code: O – red; N – blue; C – cyan; acridinedione fragment is shown in orange. The hydrogen bond lengths are in Å and the hydrogen bond angles are in°. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

acceptor is in simple interaction with the donor or it gets into the nano-cavity of the donor.

The efficiency of energy transfer by FRET mechanism is defined as "the fraction of photons absorbed by the donor that is transferred to the acceptor." This parameter can be experimentally determined by comparing the fluorescence intensities of the donor (D) in the presence and absence of acceptors (A) as given by Eq. (1).

$$E = 1 - \frac{I_{DA}}{I_D} \tag{1}$$

Since we encounter many interfering parameters in fluorescence emission measurements like inner-filter effects, background noise etc., we rely more on lifetime measurements to calculate the energy transfer efficiency as given by Eq. (2).

$$E = 1 - \frac{\tau_{DA}}{\tau_D} \tag{2}$$

where τ_{DA}, τ_D and I_{DA}, I_D are the lifetimes and intensities of the donor in the presence and absence of acceptors, respectively. Alternatively, energy transfer efficiency can also be calculated as:

$$E = \frac{1}{1 + (r/R^{\circ})^6} \tag{3}$$

where 'r' is the distance between D and A chromophores, 'R°' is the so-called Förster distance, at which the energy transfer efficiency is 50%. Thus R° is the critical distance, at which energy transfer and spontaneous decay of the excited donor are equally probable, and it may be experimentally determined from the spectroscopic data and is given by Eq. (4) as

$$R_{\circ}^6 = \frac{9000\,(\ln 10) k^2 \phi_D^{\circ}}{128\pi^5 N_A \eta_4} J(\lambda) \tag{4}$$

where κ^2 is an orientation factor and has a value of 2/3 for randomly oriented donor–acceptor dipoles, $\phi^{\circ}{}_D$ is the quantum yield of the donor in the absence of acceptor, N_A is the Avogadro number, η is the refractive index of the medium and $J(\lambda)$ is a quantitative measure of the donor–acceptor overlap [41]. Among the factors appearing in the equation, it is the spectral overlap integral $J(\lambda)$ which makes a difference with regard to efficiency of the process since all the others remain the same for a typical system.

The absorption spectrum for β-CDen-ADR for increasing concentrations of safranine acceptor is as shown in Fig. 5. Fig. 6 shows the steady-state emission of β-CDen-ADR for increasing concentrations of the safranine acceptor. The excitation wavelength of 375 nm was absorbed dominantly by the donor. Upon increasing the concentration of the acceptor, the donor fluorescence intensity decreased with concomitant increase in the fluorescence intensity of the acceptor. This gives the evidence for energy transfer from β-CDen-ADR as there is no significant contribution of safranine fluorescence by direct excitation at 375 nm. A red shift of ≈11 nm and a blue shift of ≈5 nm in the acceptor fluorescence maximum and donor fluorescence maximum, respectively, were observed with increase in the acceptor concentration. The red shift is attributed to radiative migration due to the effect of self-absorption among acceptors and the blue shift is due to the radiative transfer from donor to acceptor [41]. A decrease in intensity of the donor emission peak at 448 nm was observed with increase in concentration of safranine solution. Also a new acceptor emission peak appeared at 567 nm and its emission intensity was found to increase with continuous additions of the safranine solution.

We were able to calculate the spectral overlap integral from the overlay spectrum of the absorption spectrum of the safranine and emission spectrum of β-CDen-ADR. Based on the intensity measurements of the donor in the presence and absence of safranine, the Förster radius and efficiency of energy transfer were calculated (Table 1). The rate constant for the process was obtained from

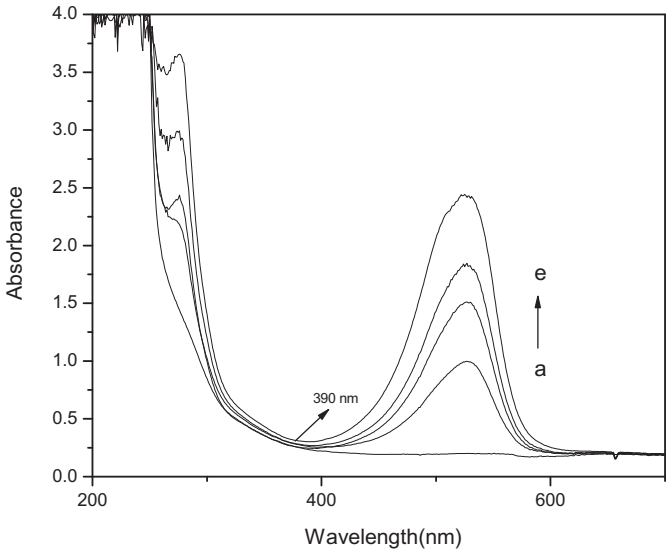


Fig. 5. Absorption spectrum of β-CDen-ADR in the presence of increasing concentrations of safranine [β-CDen-ADR] – 2×10^{-5} M, [safranine]: (a) 0 M, (b) 2×10^{-5} M, (c) 4×10^{-5} M, (d) 6×10^{-5} M, and (e) 8×10^{-5} M.

Stern–Volmer plot and was found to be 1.5×10^{12} M^{-1} s^{-1}. Such values of rate constants in the order of 10^{11}–10^{12} M^{-1} s^{-1} were very much greater than those expected for a radiative or a collisional energy transfer. Hence, a non-radiative phenomenon is proposed as the possible mechanism of energy transfer for the chosen pair of donor and acceptor.

The fluorescence decay curves and lifetime profiles of the β-CDen-ADR with the acceptor provided more information regarding the interaction between the D and A in the excited state. The lifetime of the safranine molecules in their free state was found to be 3.1 ns and fits into a single exponential decay function when excited at 375 nm (Fig. S7).

When the β-CDen-ADR/safranine was excited at 375 nm, the decay curves were recorded at the emission wavelengths of both the donor and the acceptor. Fig. 7 reveals that, a quenching was observed in the lifetime of the donor molecule to the extent of 1.87 ns at the donor decay wavelength (Table 2). Such a decrease in

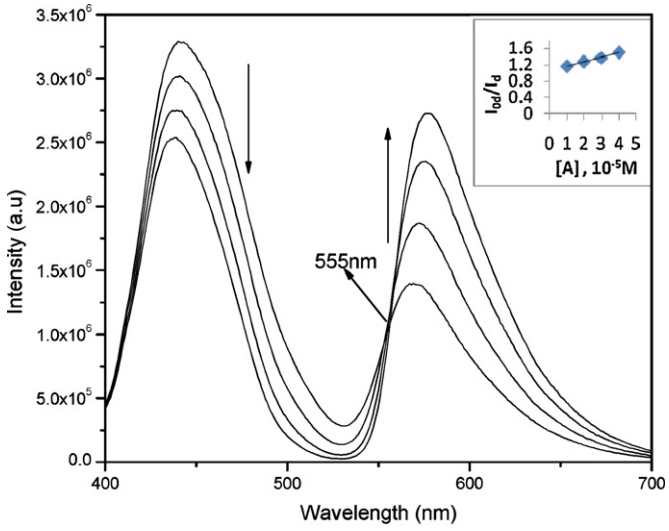


Fig. 6. Steady-state emission of β-CDen-ADR/safranine system [β-CDen-ADR]: 2×10^{-5} M, [safranine]: (a) 1×10^{-5} M, (b) 2×10^{-5} M, (c) 3×10^{-5} M, (d) 4×10^{-5} M. Inset: Stern–Volmer plot of β-CDen-ADR/safranine system. λ_{exc} = 375 nm.

Table 1
Spectral parameters calculated from steady-state emission studies.

Donor	$J(\lambda)$ in $M^{-1}\,cm^{-1}\,nm^4$	R_{DA} (Å)	R°_{DA} (Å)	Transfer efficiency (%)	Rate constant ($M^{-1}\,s^{-1}$)
β-CDen-ADR	5.63×10^{-15}	25.42	23.49	39	1.5×10^{12}
ADR	8.54×10^{-15}	37.68	34.83	38	4.2×10^{11}

Table 2
Lifetime measurements of β-CDen-ADR/safranine system at 448 nm.

Safranine (10^{-5} M)	Donor life time (ns)			Relative amplitude			χ^2
	τ_1	τ_2	τ_3	B_1	B_2	B_3	
0	2.42	7.45	0.528	12.52	84.24	3.24	1.24
2	2.25	7.08	0.574	12.51	83.34	2.74	1.09
4	2.37	7.04	0.83	12.93	82.15	2.92	1.12
6	3.18	6.82	1.19	13.43	79.41	7.16	1.06
8	2.26	5.36	0.602	17.54	77.84	4.62	1.23

fluorescence lifetime in presence of acceptor reveals it clearly that the mechanism operating is a non-radiative one.

Whereas the decay observed at the acceptor wavelength also fits into a tri-exponential decay function, but showed a rise time which indicated a formation in the excited state which is also a characteristic occurrence for a FRET process (Fig. 8). Two lifetimes were recorded at the acceptor wavelength, one longer (τ_l) and one shorter (τ_s), which indicated that the *D* and *A* exist in the excited state in two different configurations; may be one bound and the other one unbound. Hence, the longer lifetime component (τ_l) is attributed to the free β-CDen-ADR molecules and the shorter one (τ_s) with a negative amplitude to the β-CDen-ADR bound to the acceptor molecules (Table 3). It was also observed that as the acceptor concentration was increased, the rise time decreased. This indicated that the transfer rate became faster on increasing the acceptor concentration which was due to the closer proximity of the *D*–*A* molecules. Such a decrease in rise time with increase in acceptor concentrations have been already reported by Auerbach et al. theoretically [42].

With the lifetime values obtained for the donor molecule in the presence and absence of safranine at the donor decay wavelength, the efficiency of energy transfer was calculated using Eq. (2) and found to be as 29% which is less than that observed based on intensity measurements. This is in accordance with the earlier observed results that invariably in all cases, steady state FRET efficiencies are larger than those extracted from the time-resolved measurements [43].

Also the rate constant for the energy transfer processes were obtained from Stern–Volmer plot on the basis of lifetime measurements of donor–acceptor combination. Similar to the observation with the free ADR as donor, with the β-CDen-ADR donor, the rate constant for the energy transfer was found to be in the order of $10^{11}\,M^{-1}\,s^{-1}$, which supported the proposed mode of non-radiative energy transfer occurring in these systems.

In an attempt to check the efficiency of the acridinedione modified β-CDen-ADR as an energy donor in FRET analysis, same set of experiments were carried out with free ADR. A good spectral overlap was observed between the absorption and emission spectra of the acceptor and donor respectively, from which the spectral overlap integral and Förster radii were calculated and are tabulated in Table 1 The absorption spectrum of free ADR in the presence of increasing concentrations of safranine is shown in Fig. 9. Fig. 10 shows the steady state spectrum of free ADR in the presence of safranine. A blue shift of ≈8 nm and a red shift of ≈13 nm were observed at donor and acceptor fluorescence wavelengths respectively. From the intensity values of the free ADR in the presence and absence of donor, the efficiency of energy transfer process and the rate constant of the same were also calculated (Table 1). The

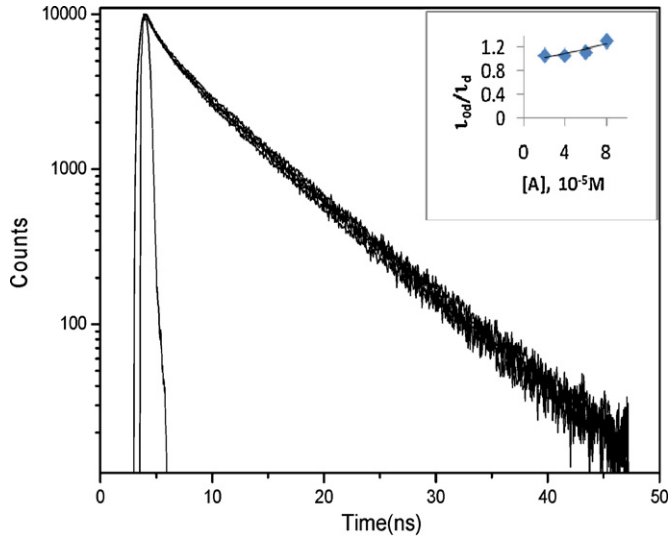


Fig. 7. Lifetime decay profile of β-CDen-ADR/safranine system at 448 nm [β-CDen-ADR]: 2×10^{-5} M, [safranine]: (a) 2×10^{-5} M, (b) 4×10^{-5} M, (c) 6×10^{-5} M, (d) 8×10^{-5} M. Inset: Stern–Volmer plot of β-CDen-ADR/safranine system. λ_{exc} = 375 nm.

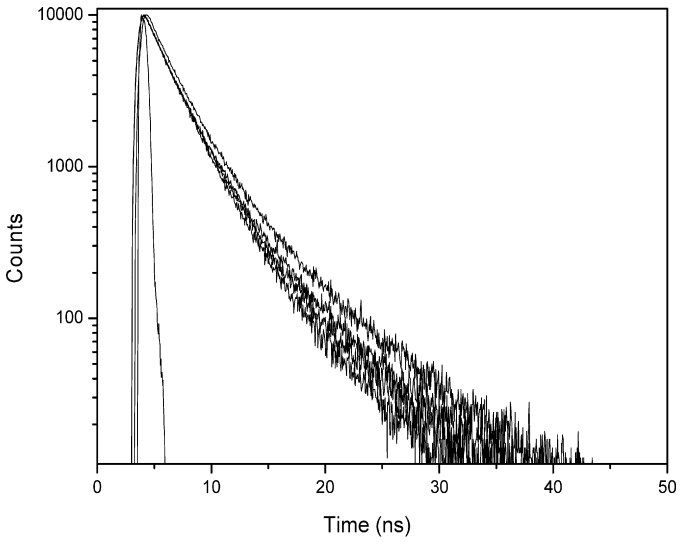


Fig. 8. Lifetime decay profile of β-CDen-ADR/safranine system at 567 nm [β-CDen-ADR]: 2×10^{-5} M, [safranine]: (a) 2×10^{-5} M, (b) 4×10^{-5} M, (c) 6×10^{-5} M, and (d) 8×10^{-5} M.

Table 3
Life-time measurements of β-CDen-ADR/safranine system at 567 nm.

Safranine (10^{-5} M)	Acceptor life time (ns)			Relative amplitude			χ^2
	τ_1	τ_2	τ_3	B_1	B_2	B_3	
2	2.27	0.27	5.36	83.67	−10.81	27.64	1.23
4	2.34	0.193	6.16	71.45	−5.35	33.90	1.01
6	2.39	0.155	5.69	83.73	−2.18	18.45	1.07
8	2.41	0.146	6.15	76.33	−1.67	25.34	1.07

Table 4
Life-time measurements of free ADR/safranine system at 467 nm and 567 nm.

Safranine (10^{-5} M)	Donor decay	Acceptor decay					
		τ_A(ns)			Relative amplitude		
	τ_D (ns)	χ^2	τ_1	τ_2	B_1	B_2	χ^2
0	4.95	1.18					
2	4.48	1.11	2.34	4.58	−78.28	178.28	1.19
4	4.13	1.17	2.39	4.45	−86.70	186.70	1.18
6	3.87	1.13	2.64	3.93	−118.96	218.96	1.20
8	3.63	1.19	2.74	3.66	−140.95	240.95	1.29

lifetime decay profiles (Figs. 11 and 12) at donor emission wavelength (467 nm) and acceptor emission wavelength (567 nm) are illustrated in Table 4. From the lifetime values of the donor in the presence and absence of acceptor, the rate constant and efficiency of the FRET process were calculated as 1.04×10^{12} M^{-1} s^{-1} and 24%.

The observation of an isoemissive point at 555 nm in the emission spectrum of β-CDen-ADR in the presence of safranine is an evidence for the existence of an equilibrium between the donor and acceptor [44]. In this donor–acceptor pair, a possible inclusion of safranine into the cavity of β-CD is expected due to the matching sizes of the cavity of β-CD and that of the acceptor and also due to the hydrophobicity of the CH_3 substituents. Reports are already available on such inclusion complexation of β-CD and safranine [45].

We have also carried out the experiments to find out the complexation constant of the acceptor with the β-CDen-ADR. The absorption and emission spectrum of safranine with continuous additions of β-CDen-ADR are as shown in Figs. 13 and 14 respectively.

Fixing up the concentration of the acceptor, the addition of β-CDen-ADR, showed no appreciable change in the absorbance of safranine at its λ_{max} (Fig. 13). This may be attributed to the weak binding of the acceptor into the cavity of β-CDen-ADR. An isosbestic point was observed at 476 nm which indicated the formation of 1:1 inclusion complex between the β-CDen-ADR and the safranine. The emission behavior was studied by exciting the system at 476 nm and here it was observed that there was an increase in the emission intensity of the acceptor without any change in the emission maximum (Fig. 14). And from the intensity values, the Benesi–Hildebrand plot was constructed and the complexation constant was found as 3.5×10^3 M^{-1} (inset in Fig. 14). This comes as an evidence for our proposed model of binding of safranine acceptor with the β-CD cavity.

Hence, such a FRET system with the donor and acceptor located outside and inside the cyclodextrin cavity, respectively, can keep the donor and acceptor within the effective distance of the Förster

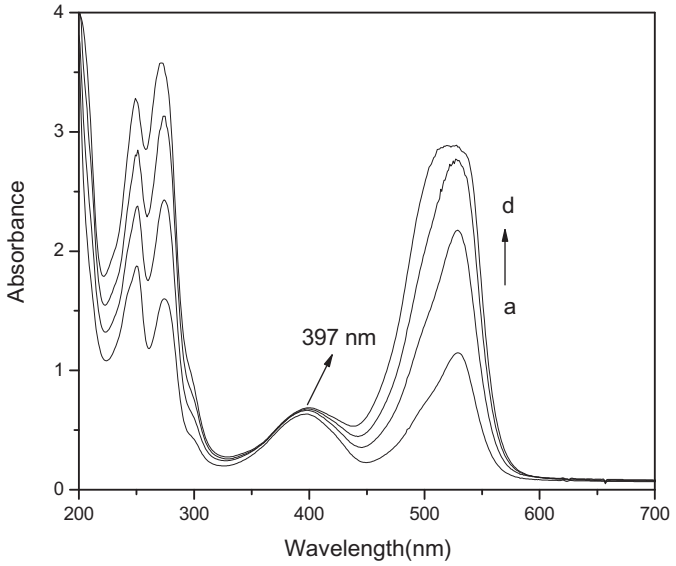


Fig. 9. Absorption spectrum of free ADR in the presence of increasing concentrations of safranine [ADR] – 2×10^{-5}M, [safranine]: (a) 2×10^{-5} M, (b) 4×10^{-5} M, (c) 6×10^{-5} M, (d) 8×10^{-5} M.

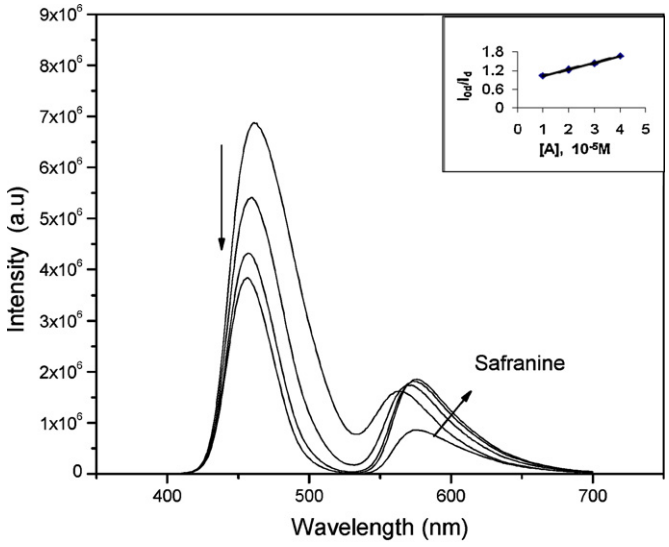


Fig. 10. Steady-state emission of free ADR/safranine system [ADR]: 2×10^{-5} M, [safranine]: (a) 1×10^{-5} M, (b) 2×10^{-5} M, (c) 3×10^{-5} M, (d) 4×10^{-5} M. Inset: Stern–Volmer plot of free ADR/safranine system. λ_{exc} = 375 nm.

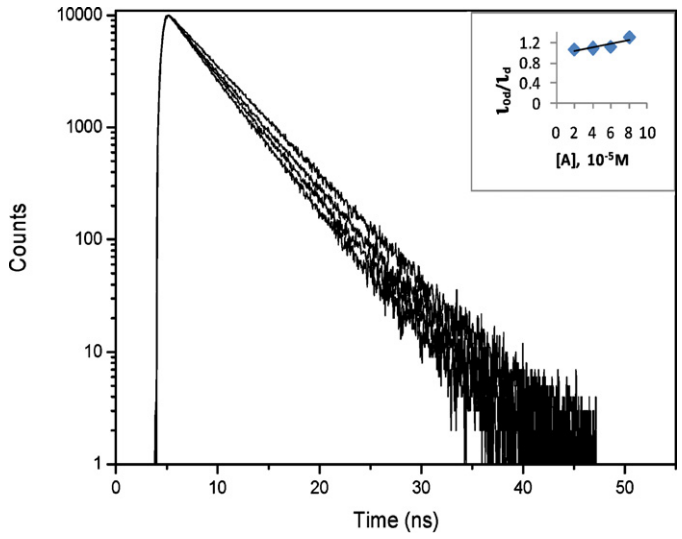


Fig. 11. Lifetime decay profile of free ADR/safranine system at 467 nm [ADR]: 2×10^{-5} M, [safranine]: (a) 2×10^{-5} M, (b) 4×10^{-5} M, (c) 6×10^{-5} M, (d) 8×10^{-5} M. Inset: Stern–Volmer plot of free ADR/safranine system. λ_{exc} = 375 nm.

energy transfer and hence can be expected to provide higher efficiency of the process. This was established from the efficiencies of the energy transfer processes of the two donors as indicated in Table 1. Though at the first sight it appears that both the donors have the same efficiency, it is worth to mention here that the free ADR provides an efficiency of 38% with a quantum yield of 0.92 [39] whereas the acridinedione modified β-CD produces an efficiency of 39% with a quantum yield of 0.13. As shown in Table 4, in case of ADR/safranine system, it was observed that the rise time increases with increase in acceptor concentration which is the reverse of what was observed with β-CDen-ADR as donor. This further supports the predicted inclusion of the acceptor into the cavity of cyclodextrin since in case of free ADR where such a cavity is not available the donor and acceptor molecules are more at random movements and hence the FRET process is rather slower and hence longer is the rise time. Also from the lifetime profiles, it was once again established that the FRET efficiency was greater with β-CDen-ADR (29%) than with free ADR (24%).

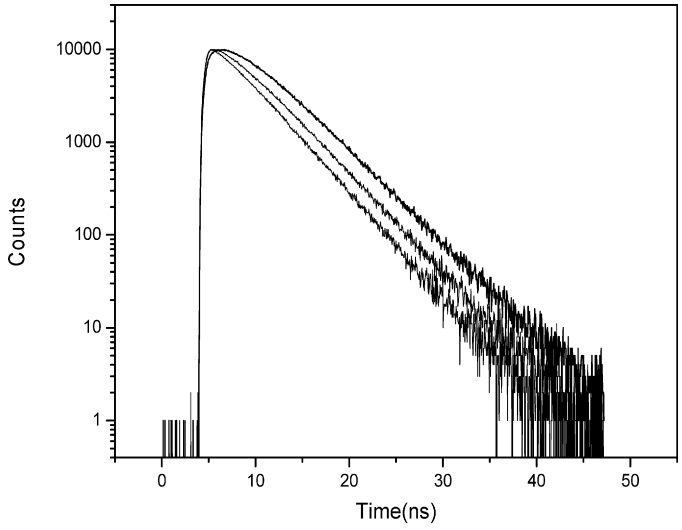


Fig. 12. Lifetime decay profile of free ADR/safranine system at 567 nm [ADR]: 2×10^{-5} M, [safranine]: (a) 2×10^{-5} M, (b) 4×10^{-5} M, (c) 6×10^{-5} M, (d) 8×10^{-5} M. λ_{exc} = 375 nm.

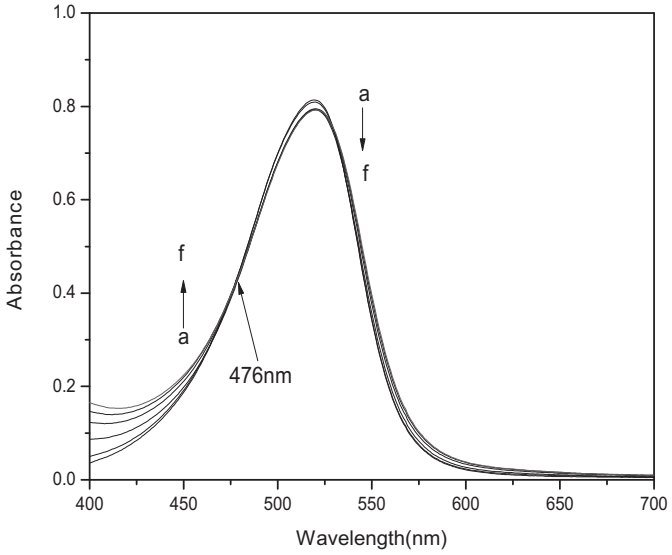


Fig. 13. Absorption spectrum of 2×10^{-4} M Safranine with sequential additions β-CDen-ADR of concentrations (a) 0 M, (b) 5×10^{-5} M, (c) 1×10^{-4} M, (d) 2×10^{-4} M, (e) 4×10^{-4} M, and (f) 8×10^{-4} M.

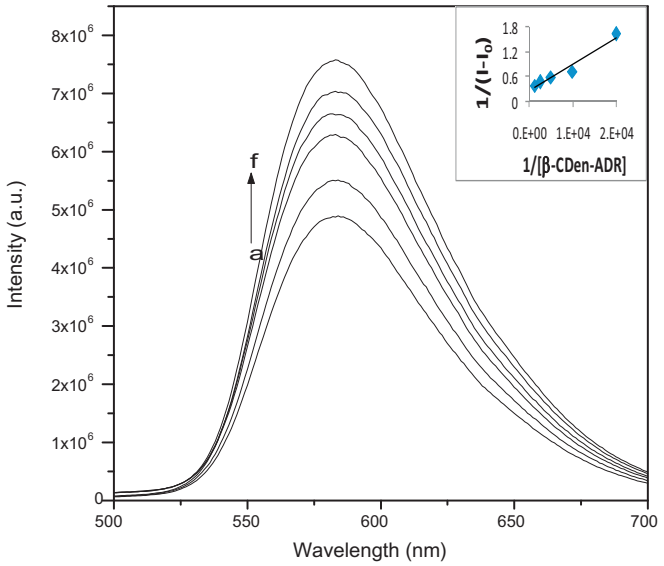


Fig. 14. Emission spectrum of 2×10^{-4} M Safranine with sequential additions β-CDen-ADR of concentrations (a) 0 M, (b) 5×10^{-5} M, (c) 1×10^{-4} M, (d) 2×10^{-4} M, (e) 4×10^{-4} M, and (f) 8×10^{-4} M. Inset: Benesi–Hildebrand plot of β-CDen-ADR/safranine system. λ_{exc} = 476 nm.

4. Conclusion

We have synthesized a modified cyclodextrin namely, mono-6-deoxy-6-aminoethylaminoacridinedione-β-CD. The above said compound was prepared via the synthesis of mono-6-deoxy-6-tosyl-β-CD and Mono-6-deoxy-6-aminoethylamino-β-CD in the preceding steps by following reported procedures. The intermediates and the compound were well characterized by IR, NMR and mass techniques. The chromophore attached β-CD thus obtained was found to possess a good quantum yield. The absorption, excitation and emission spectra recorded for the compound were found to be in close resemblance to those characteristic of the acridinedione family. A decrease in donor fluorescence intensity with a concomitant increase in acceptor intensity; shortening of decay time of the donor with a parallel species formation in the excited state as inferred from the rise time at the acceptor wavelength,

with increasing concentrations of the acceptors indicate that FRET is happening between the β-CDen-ADR and the acceptors. The FRET efficiency of acridinedione modified β-CDen-ADR was compared with that of a free ADR and found it was more in case of the modified compound, the reason being the inclusion of acceptor into the cavity of cyclodextrin. Thus, the β-CD modified dye was found to behave as a persuasive energy donor in the FRET experiments carried out.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jphotochem.2011.12.007.

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