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Effect of Cyclodextrins on the Photophysics of Three Indoloquinoline Derivatives: An Intriguing Fluorometric Study

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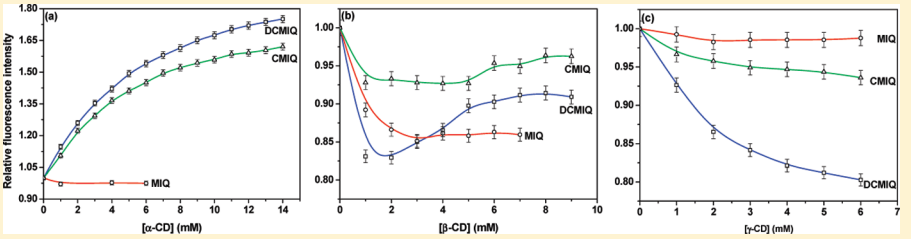
ABSTRACT:



Effect of cyclodextrin encapsulation on the photophysics of three indoloquinoline derivatives, namely, 5-methyl-5*H*-indolo[3,2-c]-quinoline (MIQ), 8-chloro-5-methyl-5*H*-indolo[3,2-c]quinoline (CMIQ), and 2,8-dichloro-5-methyl-5*H*-indolo[3,2-c]quinoline (DCMIQ), has been studied using steady state and time-resolved fluorescence spectroscopy. The three compounds, which are basically cryptosanguinolentines, exist mainly in their zwitterionic forms in the excited state. Appreciable emission from the $\pi-\pi^*$ state can be observed on excitation of the compounds at a specific wavelength. The existence of zwitterions in the excited state leads to mutual interaction to form dimers triggered by the presence of the hydrophobic nanocavities of cyclodextrins (CDs) through Coulombic interaction. This is evidenced by steady state fluorescence measurements and treating the fluorophores with CDs of different cavity space. The photophysical behavior of the compounds gets dramatically modulated when they are treated with α-, β-, and γ-CD hosts. Presence of chloro substituent/s on the parent molecule and the extent of encapsulation by CDs of different dimensions exhibit enormous alterations in the fluorescence characteristics of the compounds. Solvation of the chromophoric moiety by water molecules deviates in character due to the guest−host interaction. Trapped water molecules inside the bigger cavity of γ-CD seem to play a vital role in quenching the fluorescence of the zwitterions of the molecules.

INTRODUCTION

Indoloquinoline alkaloids represent a new class of drug leads in the field of traditional medicines that contributed greatly to the discovery and development of new therapeutic agents. To mention a few, cryptolepine (5-methyl-5*H*-indolo[3,2-b]quinoline), neocryptolepine (5-methyl-5*H*-indolo[2,3-b]quinoline), and isocryptolepine (5-methyl-5*H*-indolo[3,2-c]quinoline), extracted from the African medicinal plant *Cryptolepis sanguinolenta*, are isomeric tetracyclic compounds of particular interest.[1] They have a broad spectrum of biological activities including antiparasitic, antifungal, antibacterial, cytotoxicity, anti-inflammatory, and antihyperglycaemic.[2,3] Chemically these compounds are isomeric indoloquinolines, which inhibit DNA replication and transcription[2] and also exhibit strong antiplasmodial activity.[3] Natural products have provided important resources for the synthesis of anticancer drug candidates.[4] The heterocyclic ring structures including pyrrole, purine, pyrimidine, indole, and quinoline are often used to optimize the anticancer activity of molecules.[5] For example, *N*′-(11*H*-indolo[3,2-c]quinolin-6-yl)-*N*,*N*-dimethylethane-1,2-diamine (IQDMA), an indoloquinoline compound, is a novel antineoplastic agent with a broad spectrum of antitumor activity against many human cancer cells.[6] IQDMA causes a marked increase in apoptosis, which was accompanied by increased levels of Bax, activated caspase-3, -8, and -9, and cleaved PARP.[6] The antiprotozoal activities of two dimeric indoloquinoline alkaloids, cryptoquindoline and biscryptolepine, were compared with those of a new synthetic indoloquinoline isomer, isoneocryptolepine, and a quaternary derivative, *N*-methyl-isocryptolepinium iodide, by Van Miert and coworkers.[7] The latter compounds showed a high antiplasmodial activity against the chloroquine-resistant *Plasmodium falciparum* strain. A number of 2-substituted indoloquinolines have been synthesized and evaluated in antifungal screens, and several have been shown to increase potency and expand the antifungal spectrum of cryptolepine.[8]

Similar indoloquinoline derivatives of cryptosanguinolentine are also regarded as important indoloquinoline alkaloids.[9,10] Indoloquinoline compounds and their methyl derivatives are known to possess suitable skeleton for DNA intercalation.[11] This results into dramatic changes in DNA conformation and can inhibit DNA replication and transcription.[12,13] In our laboratory we have been involved in the photophysical characterization of the parent cryptosanguinolentine or 5-methyl-5*H*-indolo[3,2-c]quinoline (MIQ) and two of its derivatives, 8-chloro-5-methyl-5*H*-indolo[3,2-c]quinoline (CMIQ)

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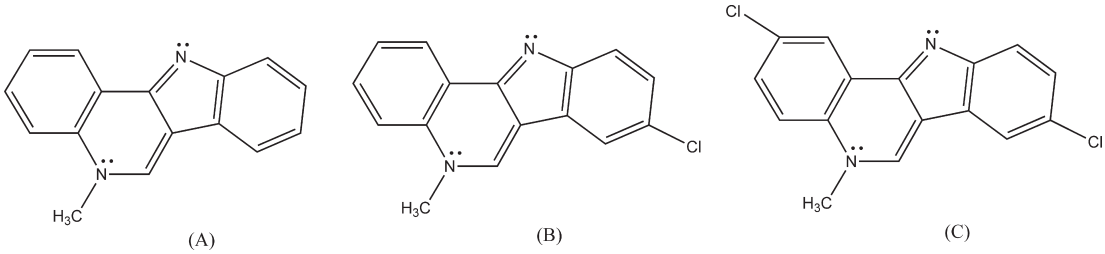
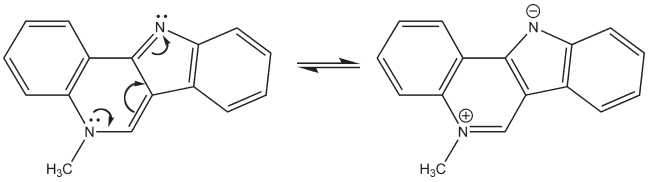
Scheme 1. Representative Structures of (A) 5-Methyl-5*H*-indolo[3,2-c]quinoline (MIQ), (B) 8-Chloro-5-methyl-5*H*-indolo[3,2-c]quinoline (CMIQ), and (C) 2,8-Dichloro-5-methyl-5*H*-indolo[3,2-c]quinoline (DCMIQ)



Scheme 2. Equilibrium between the Neutral and the Zwitterionic Forms of MIQ



and 2,8-dichloro-5-methyl-5*H*-indolo[3,2-c]quinoline (DCMIQ). The structures of these compounds are shown in Scheme 1. All three compounds exist in their neutral and zwitterionic forms in aqueous solution (Scheme 2). This is in contrast to similar compounds of this sort, norharmane and 2-methylharmine, where the species that exist in equilibrium are neutral, zwitterionic, and cationic under similar conditions.[14−16] Reluctance to produce the cationic form by our compounds may be attributed to the existence of two phenyl rings on the two flanks, which make the possible prototropic centers to be feebly active. In the excited state, the zwitterionic species get dominance over the neutral ones. During the solvatochromic studies we observed that the molecules have peculiar solvation pattern that demonstrates the coexistence of solvation of the central chromophoric part and the two flanks. We inferred about this observation from the obtained time-resolved fluorescence data where we witnessed the existence of two contributing species to the fluorescence decay.[17]

Scheme 2 suggests a possibility of intermolecular stacking between the zwitterions when two such species come close enough to each other considering their existence in solution in the excited state. Coulombic interaction can bring such interaction to keep the neutral excimers together with the zwitterionic monomers. Such "stacking" is not new as the possibility of their formation considering other systems was shown through theoretical calculations by Oda and Sato.[18] We have shown here that the equilibrium of the dimer formation in the excited state can be modulated through application of cyclodextrins (CDs) of different dimensions; namely, α, *β*, and *γ*-CDs, with lipophilic inner cavities and hydrophilic outer surfaces, can interact with a large variety of guest molecules to form noncovalent inclusion complexes. Basically, CDs are cyclic oligosaccharides containing at least six D-(+) glucopyranose units attached by α-(1, 4) glucosidic bonds generating the variety called α-CD. Seven and eight glucopyranose rings constitute the *β*- and *γ*-CD, respectively. CDs are both large (MW ranging from almost 1000 to over 2000 Da) and hydrophilic with a significant number of H-donors and acceptors. No covalent bonds are formed or broken during the complex formation, and drug molecules in the complex are in rapid equilibrium with free molecules in the solution.[19] The cavity diameters of α-, *β*-, and *γ*-CDs are 4.7−5.3 Å, 6.0−6.5 Å, and 7.5−8.3 Å, respectively, and the depth is about 7.9 Å.[20] For complexation, the cavity size of CD and the size of the guest molecule are the essentially most important factors.

While working with norharmane, Mallick et al. observed the formation of two types of inclusion complexes between the fluorophore and *β*-cyclodextrin (*β*-CD) depending on the relative population of the two.[21] However, they did not observe any appreciable interaction between the guest and the α- and *γ*-CDs. In another report, while studying the effect of CD nanocavity confinement on the photophysics of a *β*-carboline analogue, it was found that, upon encapsulation, the charge transfer fluorescence of the fluorophore exhibits hypsochromic shift along with enhancement in the fluorescence yield through an 1:1 guest/CD inclusion complex formation in α- and *β*-CDs.[22] Depending on the concentration of *γ*-CD, formation of both 1:1 and 1:2 guest−host complexes were observed.[22] As has already been indicated that the molecular structures of MIQ, CMIQ, and DCMIQ are similar to norharmane, they have some fundamental peculiarities in chemical properties. These get reflected during the study on the encapsulation of the compounds in the CDs.

EXPERIMENTAL SECTION

Materials. MIQ, CMIQ, and DCMIQ were synthesized, purified, and analyzed in the organic chemistry laboratories of BITS, Pilani following the procedure mentioned elsewhere.[23] Stock solutions of the compounds (1.001×10^{-3} M) were prepared in pure methanol, 0.1 mL of which was poured in a 10 mL volumetric flask and left for a few hours for complete evaporation of methanol before dissolving in different solvents to acquire the desired concentrations. The final concentration of the compounds was 10×10^{-6} M. All of the solvents were procured either from Spectrochem, India or Merck, India. High-performance liquid chromatography (HPLC) grade water purchased from Fisher Scientific, PA, USA has been used throughout the experiment.

Methods. The absorption spectra were recorded using a Varian Cary 300 Bio UV−vis spectrophotometer. Fluorescence measurements were performed using a PerkinElmer LS 55 scanning spectrofluorimeter. The fluorescence lifetimes were measured by the method of time-correlated single-photon counting using a picosecond spectrofluorimeter from Horiba Jobin

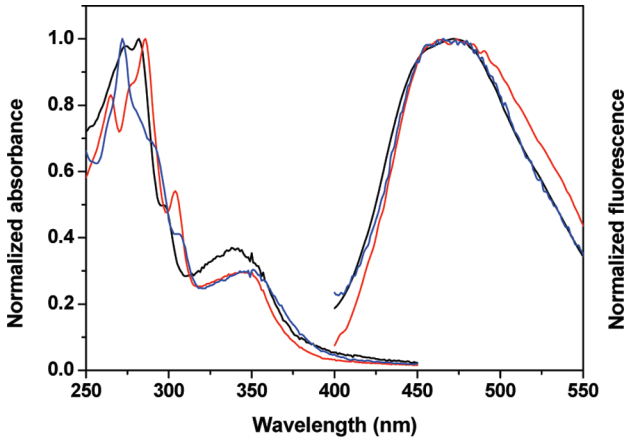


Figure 1. Normalized absorption and fluorescence spectra of MIQ (solid black line), CMIQ (solid red line), and DCMIQ (solid blue line). The excitation wavelength for obtaining the fluorescence spectra was 290 nm (this wavelength was chosen after examining the excitation spectra of the compounds in water).[17].

Yvon IBH. The instrument was equipped with FluoroHub single photon counting controller, Fluoro3PS precision photomultiplier power supply, and FC-MCP-50SC MCP-PMT detection unit. A laser head or a nano-LED pulsed diode powered by a pulsed diode controller (IBH) was used as the excitation light source. The excitation wavelength was 281 nm. The typical response time of this laser head was <1 ns. To calculate the lifetime, the fluorescence decay curves were analyzed by an iterative fitting program provided by IBH.

Determination of the guest−host stoichiometry and the binding constant with α-CD has been done using the double reciprocal method of Benesi−Hildebrand.[24,25] The equations used for the 1:1 (eq 1) and 1:2 (eq 2) guest−host complexation are as mentioned below:

$$\frac{1}{F - F_0} = \frac{1}{F_m - F_0} + \frac{1}{K[CD]_0(F_m - F_0)} \quad (1)$$

$$\frac{1}{F - F_0} = \frac{1}{F_m - F_0} + \frac{1}{K'[CD]_0^{\,2}(F_m - F_0)} \quad (2)$$

Here, F_0 and F_m are the fluorescence intensities at zero and the maximum concentrations of CD, F denotes the fluorescence intensities at different concentrations of CD, $[CD]_0$ is the total CD concentration, and K and K' are the respective binding constants.

RESULTS

Steady State Spectral Analysis in Presence and Absence of CDs. Normalized absorption and fluorescence spectra of MIQ, CMIQ, and DCMIQ in aqueous solution are shown in Figure 1. The absorption spectra for the three compounds show vibronic structures due to the $\pi-\pi^*$ electronic transitions in the molecules. $n-\pi^*$ transitions are also probable, but the bands due to those could get masked by the $\pi-\pi^*$ transition spectra.[17] Addition of CDs to the aqueous solutions of the compounds hardly changes the absorption spectra. This indicates that the photophysical changes (if any) might take place in the excited states of the compounds. This is also confirmed by the excitation spectra of the probes. It has been mentioned earlier that in the excited state the zwitterionic species exists in higher proportion than the neutral ones in aqueous solution.[17]

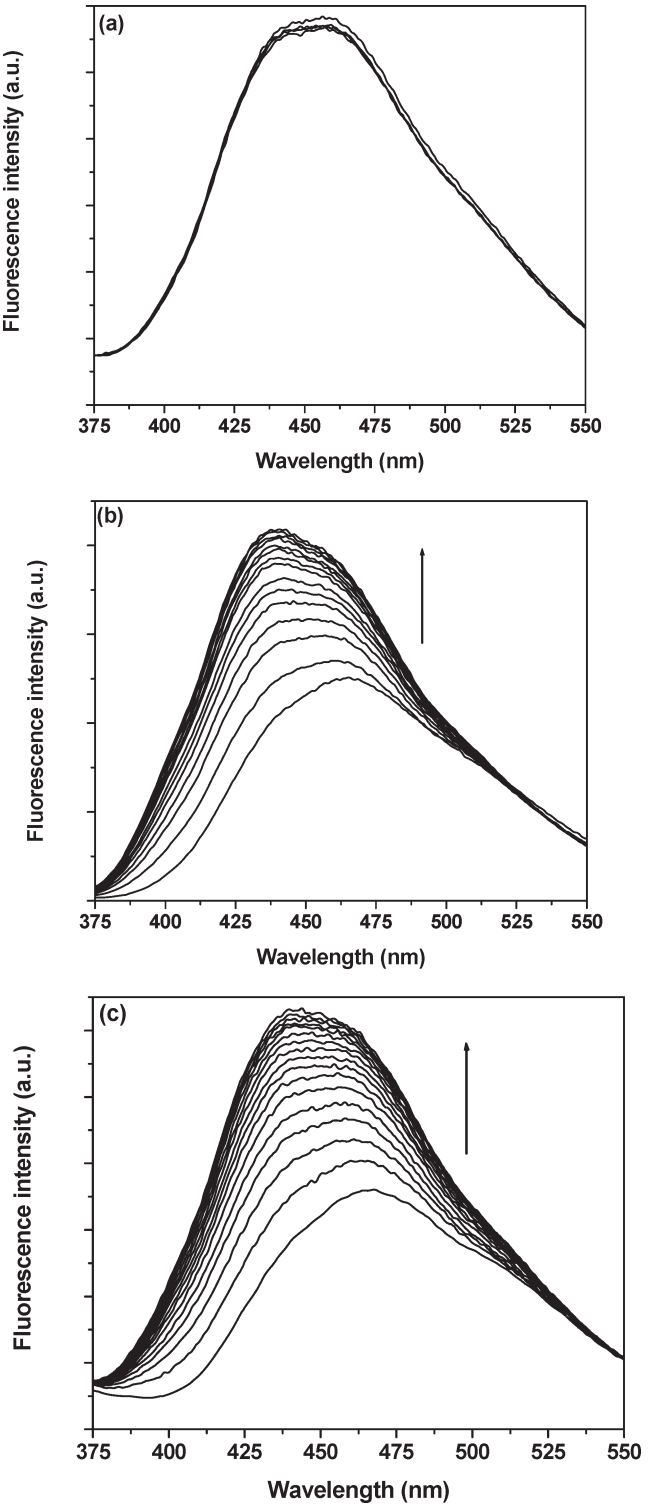


Figure 2. Emission spectra of (a) MIQ, (b) CMIQ, and (c) DCMIQ in aqueous and aqueous α-CD solutions. For MIQ the concentrations of α-CD was varied from 0 to 6 mM (no change observed), and for CMIQ and DCMIQ the concentration of α-CD was varied from 0 to 14 mM (λ_{ex} = 290 nm).

Emission spectra of the compounds in aqueous solution at room temperature show a broad stature at the maximum due to

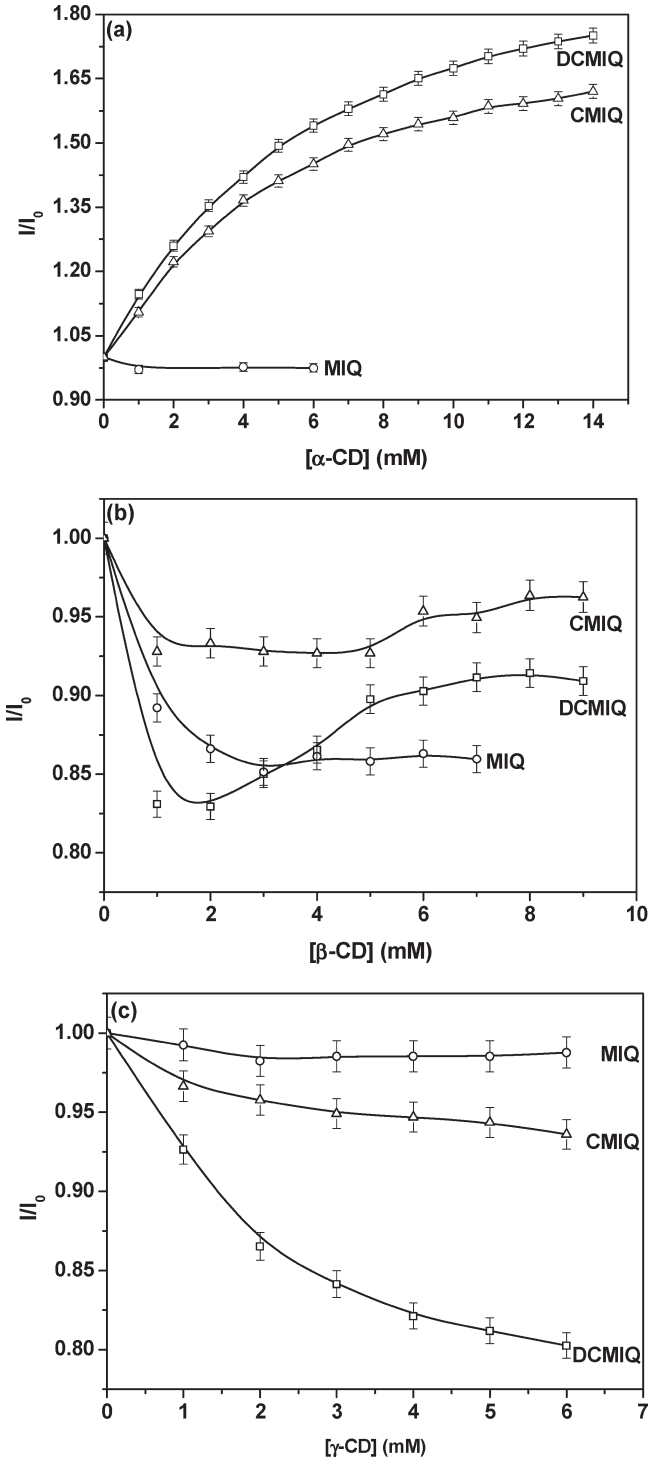


Figure 3. Relative change in the fluorescence intensity of MIQ, CMIQ, and DCMIQ in absence (I_0) and on addition (I) of (a) α-, (b) β-, and (c) γ-CD. The fluorescence intensities at 456 nm (arbitrarily chosen) have been monitored and plotted.

the existence of the two solvated species as explained in a previous study on the molecules.[17] The band ~470 nm is expected to be due to the flank solvation of the molecules and that ~440 nm is due to the centrally solvated chromophoric species. On addition of α-CD to MIQ in water, we could not see any appreciable change to the fluorescence spectrum, whereas

Table 1. Fluorescence Decay Times of MIQ, CMIQ, and DCMIQ in Aqueous and Aqueous CD Media[a]

[α-CD] (mM)	τ_1 (ns)	τ_2 (ns)	χ^2	[β-CD] (mM)	τ_1 (ns)	τ_2 (ns)	χ^2	[γ-CD] (mM)	τ_1 (ns)	τ_2 (ns)	χ^2
MIQ											
0	4.45	-	1.06	0	4.45	-	1.06	0	4.45	-	1.06
1	4.49	-	1.04	1	4.48	-	1.04	1	4.45	-	1.04
3	4.52	-	1.04	3	4.57	-	1.05	2	4.48	-	1.04
5	4.54	-	1.03	5	4.71	-	1.03	3	4.50	-	1.07
9	4.56	-	0.99	7	4.85	-	1.04	5	4.54	-	1.06
CMIQ											
0	1.9	3.21	0.97	0	1.9	3.21	0.97	0	1.9	3.21	0.97
1	2.01	3.04	0.99	1	1.83	2.94	1.01	1	1.86	3.8	0.99
3	2.04	2.69	0.99	3	1.88	2.90	1.01	2	1.89	3.93	1.01
5	2.08	2.56	0.96	5	2.01	3.00	1.03	3	1.86	4.24	0.95
9	2.13	2.59	0.98	7	2.02	3.15	0.95	5	1.85	4.27	1.02
DCMIQ											
0	1.71	3.62	1.09	0	1.71	3.62	1.09	0	1.71	3.62	1.09
1	1.79	2.98	1.03	1	1.73	2.78	1.06	1	1.89	5.06	1.12
3	1.75	2.92	1.02	2	1.74	2.55	1.07	2	1.91	5.55	1.09
5	1.74	2.76	1.01	5	1.74	2.92	1.05	3	1.88	5.60	1.09
9	1.75	2.73	1.03	7	1.75	2.94	1.09	5	1.87	5.65	1.12

[a] The χ^2 values indicate the goodness of the fits.

CMIQ and DCMIQ displayed a remarkable modulation of their respective emission bands (Figure 2). While simple norharmane does not show any appreciable effect of α-CD on the molecular fluorescence[21] as also the MIQ molecule, we see dramatic enhancement of the 470 nm band of CMIQ and DCMIQ. The latter compounds are the chloro derivatives of MIQ, and it is thus easily understandable that the presence of the hydrophobic chloro groups on the flank/s of MIQ promotes interaction of the molecules with α-CD.

A closer look at the relative change in the fluorescence intensities of MIQ, CMIQ, and DCMIQ with an increase in concentration of α-CD shows that the host−guest interaction not only increases in case of the latter two compounds but also the interaction in case of DCMIQ is superior to CMIQ (Figure 3a). If we hold the presence of the chloro group/s to be responsible for such difference in behavior, then it is quite feasible to state that in case of DCMIQ, which contains two chloro groups on the two flanking phenyl rings leads to better encapsulation by α-CD resulting into higher enhancement of fluorescence. Besides, we observed a complete change in the photophysical manifestation of the three fluorophores on addition of β-CD (Figure 3b). An initial quenching of the fluorescence of the zwitterions followed by a small recovery is observed. The extent of quenching of fluorescence is highest in case of DCMIQ, whereas it is lowest in case of CMIQ. Quenching of MIQ fluorescence is intermediate among the three and plateaus at higher concentrations of β-CD. The recovery of fluorescence starts after addition of a certain amount of β-CD into the solutions. On application of γ-CD to the aqueous solutions of the fluorophores, we witnessed a progressive quenching of the fluorescence of the zwitterions of CMIQ and DCMIQ. MIQ does not show a remarkable effect of added γ-CD keeping the

fluorescence more or less the same as was in bulk water. All of these observations steer our views toward the pronouncing effect of the three CDs on MIQ, CMIQ, and DCMIQ depending on the structure of the formed zwitterions in aqueous solution.

Time-Resolved Emission Studies in Presence and Absence of CDs. To obtain a better view on the observed changes in fluorescence of MIQ, CMIQ, and DCMIQ, we performed time-resolved fluorescence experiments with the fluorophores in aqueous and aqueous-CD environments. The results are presented in Table 1. The raw data for the fluorescence decay of the MIQ molecules in the three CDs show the presence of a single species that matches more or less with that in the bulk aqueous phase. There was nearly no change in the steady state fluorescence pattern of MIQ in α-CD, and the same trend is also reflected in the time-resolved fluorescence data. On the other hand, CMIQ and DCMIQ show a two-component fit to the raw data obtained from the time-resolved fluorescence experiments. In 9 mM α-CD solution, the fluorescence lifetime of the slower component of CMIQ decreases by 940 ps compared to that in bulk water. The faster decaying component does not show much change (a slight increase in the lifetime was recorded). A similar trend was observed for DCMIQ in α-CD. The lifetime of the slower component got lowered by 1.22 ns at 9 mM α-CD concentration.

Recall that in the case of encapsulation by β- and γ-CD appreciable variations of the steady state fluorescence of the probes were observed. An increase in β-CD concentration showed an initial quenching of the MIQ fluorescence followed by saturation. In the time-resolved fluorescence decay results we observed an increase in the lifetime of MIQ by 400 ps at 7 mM β-CD concentration. CMIQ showed a fluorescence quenching up to ~3 mM of β-CD, and after that there was a small recovery of the fluorescence. This is corroborated by the time-resolved fluorescence decay times when we noticed that there is a decrease in the decay time of the slower component by 1.10 ns up to 3 mM β-CD concentration followed by an increase. The faster decaying component shows a slight increase in the lifetime with the increase in β-CD concentration. DCMIQ showed highest initial quenching of the steady state fluorescence in the presence of β-CD, which is consistent with the initial decrease of the value of the slower decaying component, where we see a lowering by 1.37 ns followed by an increase in the fluorescence lifetime value.

Encapsulation of MIQ in γ-CD does not affect either the steady state or time-resolved fluorescence too much, whereas CMIQ and DCMIQ showed a progressive quenching of the steady state fluorescence with increase in γ-CD concentration. The magnitudes of the slower decaying components in these two cases got enhanced by 1.06 and 2.03 ns, respectively. The faster component does not show any remarkable change in the lifetime. Figure 4 demonstrates the effect of γ-CD on DCMIQ.

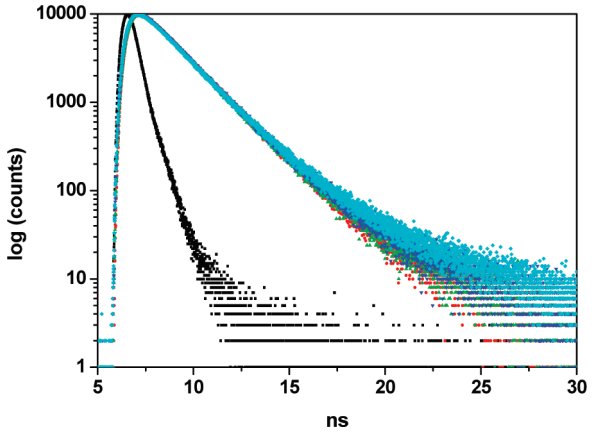
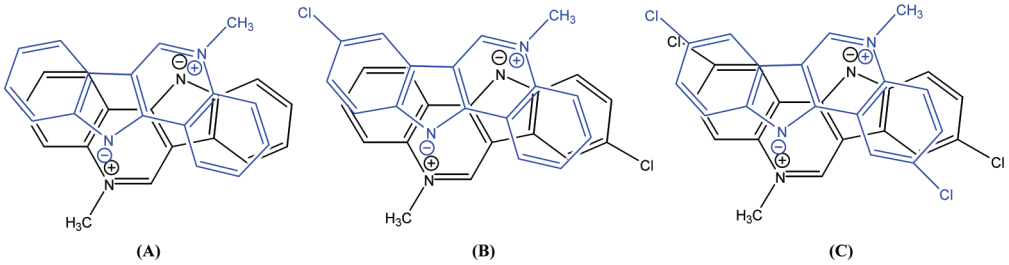


Figure 4. Fluorescence decay profiles of DCMIQ in different concentrations of γ-CD (red circle, 0 μM; green triangle, 1 μM; blue inverted triangle, 2 μM; cyan diamonds, 6 μM; and the black squares signify the probe lamp profile).

DISCUSSION

The results indicate remarkable and intriguing interactions between the three indoloquinoline derivatives and the CDs. The photophysics of the compounds get dramatically affected presumably due to the dual effect of the size of the nanocavities of the CDs and the structural features of the fluorophores under investigation. No change in the absorption spectra of the compounds in the presence of CDs indicates that the modulations in the photophysics of MIQ, CMIQ, and DCMIQ are excited state phenomena. In our previous work with these compounds we noticed that the zwitterionic form of the molecules dominate in the excited state.[17] In studying the effect of encapsulation of the compounds in the CD cavities, we naturally presume that the zwitterionic species is playing the most important role. The structural features of the molecules show that the chromophoric part is located at the central part of the compound where we observe the presence of the delocalizing π-cloud. The zwitterions may have the propensity to form dimers in presence of some sort of driving force for their formation through Coulombic interaction, thus reducing the presence of free zwitterionic monomers.[18] In such a condition the fluorescence yield of the monomeric zwitterions would get reduced. This is known as inherent quenching of fluorescence of the system under study. Scheme 3 demonstrates the stacking motifs of MIQ,

Scheme 3. Stacking Motifs of (A) MIQ, (B) CMIQ, and (C) DCMIQ[a]



[a] One of the molecules has been shown in blue and the other in black for clarity. They form parallel stacks with opposite charges one above the other. The planar molecular structure may facilitate the possibility of the formation of such dimers in the excited state.

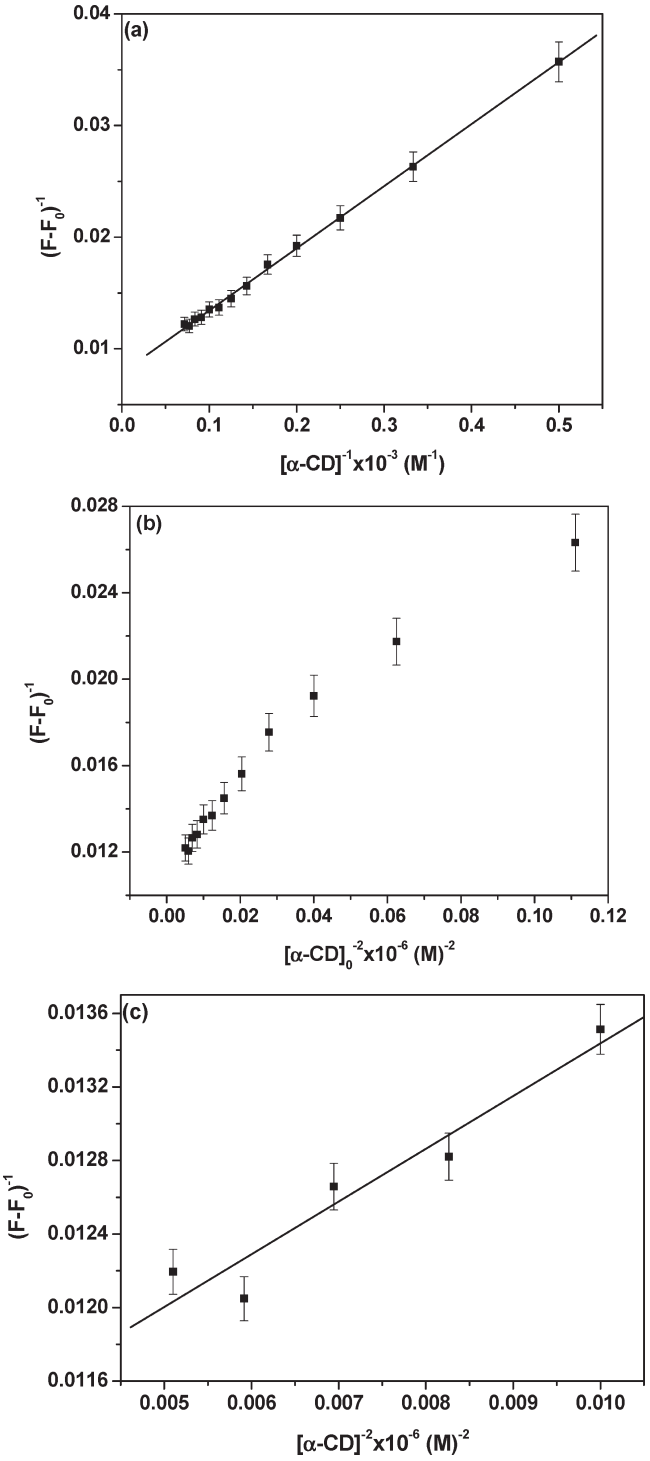


Figure 5. Double reciprocal plots according to eqs 1 and 2 to determine the binding constants and stoichiometry of the guest−host complexes between CMIQ and α-CD.

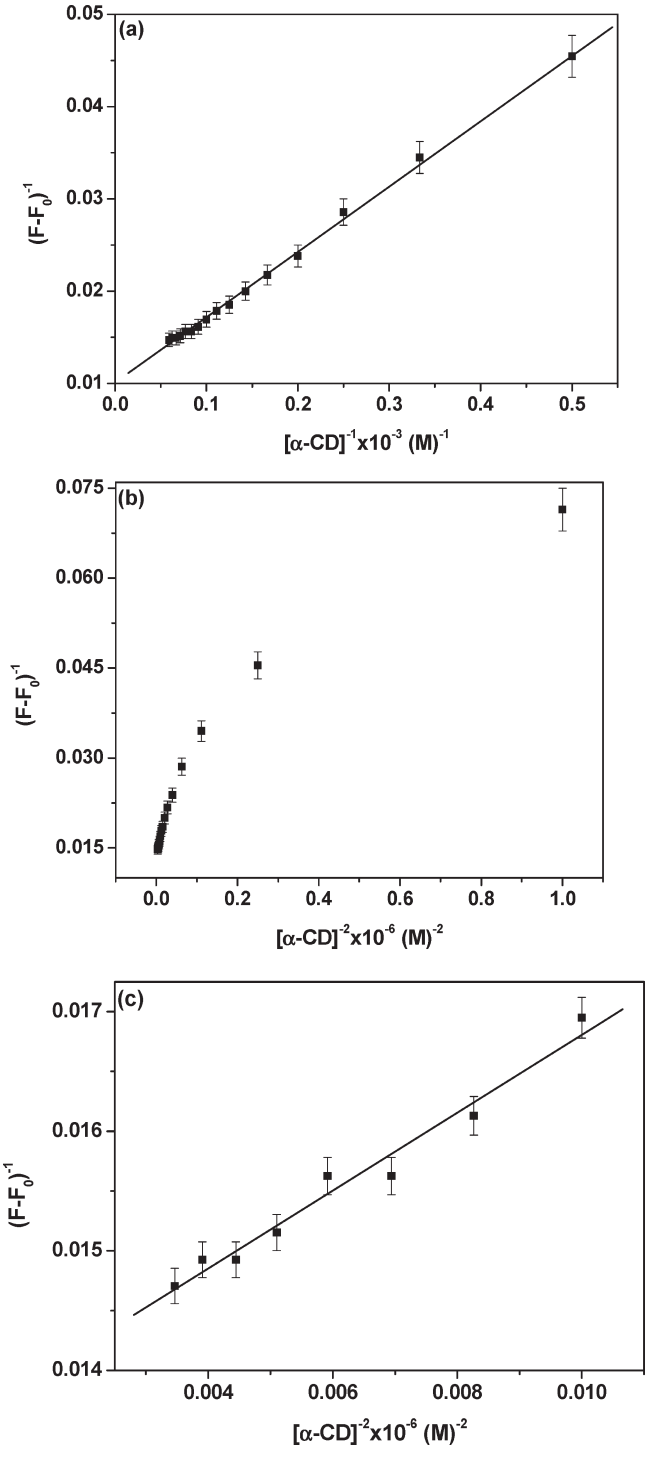


Figure 6. Double reciprocal plots according to eqs 1 and 2 to determine the binding constants and stoichiometry of the guest−host complexes between DCMIQ and α-CD.

CMIQ, and DCMIQ zwitterions. The central parts of the molecules are hydrophilic due to the charged nature and as a consequence will not entertain the CDs. Thus, the nature of encapsulation of the molecules depends on the hydrophobicity imposed by the presence of the chloro substituents. This makes clear that MIQ may show reluctance toward encapsulation by the CDs, which in turn may vary depending on the cavity size.

Effect of α-CD. MIQ does not show any change in the emission band when treated with α-CD (1−6 mM) (Figure 3a). The small cavity of α-CD may not be able to encapsulate any part of MIQ. It also shows a single component fit to the time-resolved fluorescence data and behaves like that in bulk water. There is a small gradual enhancement in the fluorescence lifetime with increase in concentration of α-CD probably due to lowering of the flank solvation induced by the

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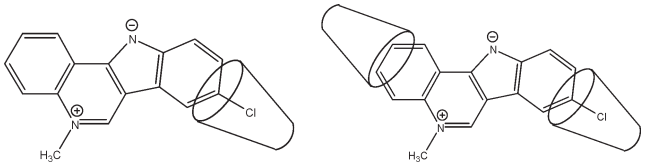
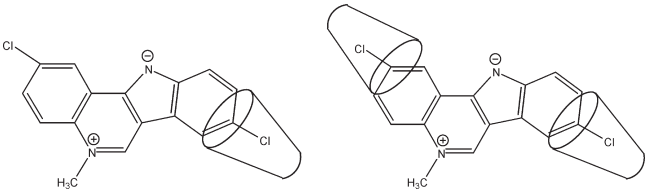
presence of the hydrophobic cavities of the CD in the vicinity. On the other hand, CMIQ fluorescence undergoes a remarkable progressive enhancement with increase in the α-CD concentration. As has been mentioned earlier that the possibility of internal stacking of the fluorophores will reduce the fluorescence yield, the present condition does not point toward any facilitation of such stacking due to the presence of the α-CD nanocavities because of space restriction. The CMIQ excimer shows the presence of two chloro groups projecting from opposite sides (Scheme 3). These hydrophobic substituents may lead to better encapsulation by the hydrophobic α-CD cavities as shown in Scheme 4. We observed enhancement of the 440 nm band that is reported to be due to the centrally solvated zwitterionic species.[17] The encapsulation of the −Cl group of CMIQ makes it more electron withdrawing due to retaining of the individuality of the group. This reduces the electron density on the N^- center of CMIQ, which in turn triggers the dynamics of more zwitterions formation as per the laws of chemical equilibrium. This phenomenon will increase the fluorescence intensity of the zwitterions and at the same time, due to the dynamism, reduce the lifetime of the species. Thus, we observe a reduction in the excited state lifetime of the slow component by ∼940 ps at 14 mM α-CD concentration. Using eq 1 we could able to justify the 1:1 complex formation between CMIQ and α-CD as shown in Figure 5a. At higher concentration of α-CD some 1:2 complexes may also develop as shown in Figure 5b and c. The calculated binding constants for the two types of complexes have been calculated to be 141.45 M^{-1} and 36 849 M^{-2}, respectively.

Figure 6 shows the respective plots for DCMIQ to calculate the binding constants and stoichiometry. DCMIQ has two chloro substituents on opposite sides of the molecule, and we observe an enhancement in fluorescence also in this case. The difference from CMIQ is that here the emission increases by about 10% more than that of the former on addition of the same amount of α-CD to the aqueous solution. Similar to CMIQ we can get a schematic for the encapsulation of DCMIQ in α-CD (Scheme 5). The compound shows 1:1 complexation with a binding constant of 139.58 M^{-1}, which is close to that for CMIQ. This indicates that the mechanism is similar in both the cases. Contrary to CMIQ, DCMIQ shows 1:2 complex at a moderate concentration of α-CD (∼7 mM) with a binding constant of 42 713 M^{-2}, which is higher than that for CMIQ. Thus, it is evident that DCMIQ has higher potency to form 1:2 complexes compared to CMIQ.

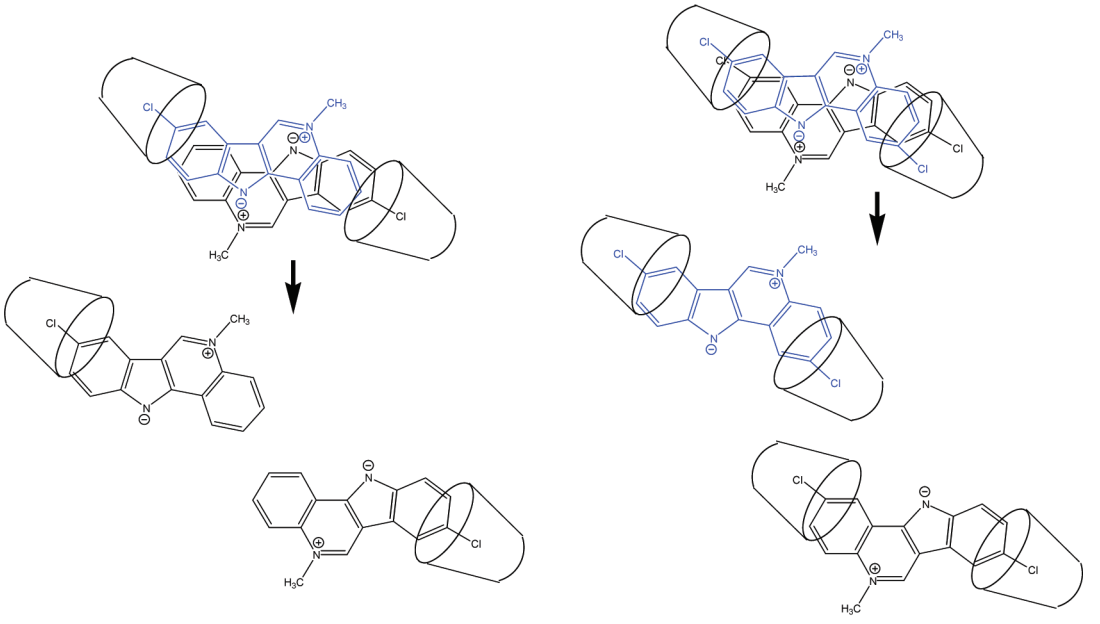
Scheme 4. Schematic of Encapsulation of CMIQ by α-CD Showing 1:1 and Probable 1:2 Guest−Host Complexes



Scheme 5. Schematic of Encapsulation of DCMIQ by α-CD Demonstrating the Formation of 1:1 and 1:2 Guest−Host Complexes



Scheme 6. Schematic of Encapsulation of CMIQ by β-CD Showing (A) 1:1 and (B) 1:2 Guest−Host Complex Formations at Higher Concentrations of β-CD



(A) (B)

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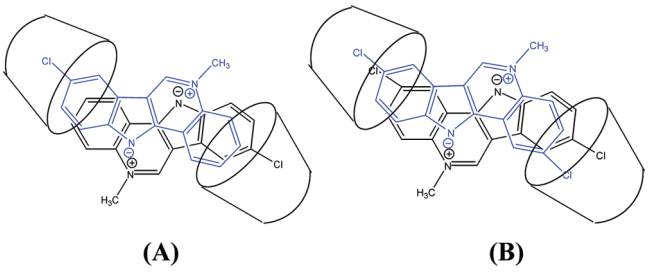
Scheme 7. Encapsulation of (A) CMIQ and (B) DCMIQ by γ-CD



(A) (B)

Similar explanations can be stated in case of DCMIQ as was discussed during describing the CMIQ-α-CD complexation. Presence of two chloro groups of either sides of DCMIQ makes it more vulnerable toward forming 1:2 complexes with α-CD at higher concentrations of the host. The enhancement of fluorescence in this case is greater compared to CMIQ since the two −Cl groups on the flanks will play their parts in withdrawing electrons from the rings providing forward dynamism toward formation of more zwitterions. Thus, the reduction in lifetime of the zwitterioninc species is greater than that of CMIQ.

Effect of β-CD. Until now we saw that employing α-CD we could not induce the stack formation among the zwitterions. The small size of the α-CD cavity cannot encapsulate the stack; would that have been the case, then we should have observed further quenching of the zwitterions fluorescence due to the stabilization of the excimers. With this view in mind we opted for comparatively bigger cavities of β-CDs. We have seen that there is an initial quenching of the entire fluorescence spectrum for all three compounds to different degrees followed by some recovery of the quenched emission (Figure 3b). For MIQ there was saturation after the initial quenching of the fluorescence emission. The bigger hydrophobic cavity of β-CD may trigger the zwitterionic monomers to form the dimer. Since β-CD has more room (probably just enough) to fit the stacked system hence at lower host concentration the stacked guest may try to find space inside the hydrophobic cavity. This reduces the number of monomers in solution and thus lowers the fluorescence. On increasing the concentration of β-CD we provide more rooms for the monomers to fit in. This process appears to be thermodynamically favorable compared to that at the lower concentration of β-CD. This process revives the zwitterion monomers and their fluorescence. The two phenomena would compete with each other and depending on the nature of encapsulation would exhibit their spectra. However, for MIQ the degree of encapsulation is less, and thus we observe a single component fit for the time-resolved fluorescence data as in bulk water. The lifetime of the species was found to increase more compared to the α-CD case at higher concentrations of β-CD. This, we suppose, is due to more available space in β-CD. Attainment of higher concentration of β-CD stopped the quenching of fluorescence probably due to the equilibrium between the monomers and the dimers.

For CMIQ we see a smaller quenching of the bands followed by a plateau and a small enhancement in the intensity of the 440 nm band at higher β-CD concentrations (above 5 mM). We believe that the asymmetry in the structure of CMIQ is responsible for the small decrease in fluorescence intensity since there will be an immediate competition between double and single occupancy of the host by the guest. Thus, we observe the recovery of fluorescence at a fairly higher β-CD concentration (∼5 mM). This is justified further by the time-resolved fluorescence data where it can noted that initially there is a decrease in the lifetime of the zwitterions followed by an enhancement in higher β-CD concentrations. Scheme 6A demonstrates the process clearly.

DCMIQ demonstrates an interesting behavior of the fluorescence from the zwitterions (Figure 3c). There is a sharp initial quenching of fluorescence at lower concentrations of β-CD (up to ∼3 mM) followed by a rapid partial recovery. The degree of fluorescence quenching is greatest among the three compounds. Similar to the discussions above, the excimer may try to get encapsulated inside the CD cavity initially followed by the generation of the entropically favorable 1:2 complex at higher host concentrations (Scheme 6B). The steep quenching is due to better encapsulation reinforced by the presence of more chloro groups in the stack. At low β-CD concentrations, this phenomenon stabilizes the excimer and quenches the fluorescence from the zwitterions. At higher concentrations of the CD, most likely, due to the tight fitting of the stack, the 1:2 guest−host complexes find their formation entropically favorable, and the stacks break. Thus the monomeric fluorescence returns back. The steady state fluorescence spectral modification is well-corroborated by the time-resolved data where we observe an initial lowering of the lifetime of the zwitterions followed by an enhancement.

Effect of γ-CD. Looking at the stability of the stacks in the previous set of experiments, we moved toward exploring the concept using even bigger cavities of γ-CD. The interior of γ-CD is reported to contain water molecules along with guests inside.[26] For MIQ, we did not see much change in the steady state as well as the time-resolved fluorescence since the encapsulation is not effective enough. However, CMIQ and DCMIQ show remarkable modulations of their fluorescence inside γ-CD. More cavity space permits better encapsulation of the excimer along with reluctance for the stack to break due to their thermodynamic stability. The encapsulation phenomenon is demonstrated by Scheme 7.

CMIQ forms excimer and goes inside the γ-CD cavity. Enhancement in the encapsulation of the excimers inside the CD cavities provides more stability to the stack. On the other hand, better stacking is promoted by better formation of the zwitterions and their period of existence in the excited state. The molecules inside the cavity dwell with some water molecules that can solvate the chloro groups at the flanks. This will lower the electron withdrawing capability of the −Cl groups, thus lowering the dynamism discussed previously. This provides greater excited state lifetime to the zwitter ions and better formation of the excimers. This hypothesis is also supported by the time-resolved fluorescence data in this CD system. DCMIQ has two chloro groups on its opposite flanks that provides better stability to the zwitterions, which in turn may lead to the formation of excimers that get encapsulated by γ-CD from both sides. This should lead toward higher quenching of the zwitterionic fluorescence.

CONCLUSIONS

Interaction of three indoloquinoline derivatives, namely, MIQ, CMIQ, and DCMIQ, with three CDs of different

dimensions (α-, β-, and γ-) has been studied. The results indicate that the zwitterions predominating in the excited state of the molecules have pronounced behavioral differences in the said CD environments. The limited confinement by α-CD cannot change the fluorescence behavior of MIQ remarkably, but on addition of chloro group/s to the indolyl and the quinolinoyl phenyl rings, we experience a profuse enhancement in fluorescence. Tight confinement of generated excimers of the compounds inside the β-CD cavities demonstrate initial quenching of the zwitterionic fluorescence followed by a recovery. γ-CD provides more space to the excimers and thus loses the fluorescence from the monomeric zwitterions. Zwitterions have been extremely important in biological applications but limited studies on their behavior in confined biomimicking environments did not help the possibilities of their applications to flourish. We have demonstrated simple applications of CD cavities of different sizes on indoloquinoline derivatives that are potentially used as anticancer drugs. The formation and stability of the formed excimers from the molecular zwitterions could effectively be controlled by using different concentrations of CDs of different dimensions. The dimension of the hydrophobic interior of the CDs appears to act as the driving force toward the excimer formation.

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