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ABSTRACT: Supramolecular interaction between an intramolecular charge transfer (ICT) probe, N,N-dimethylaminonaphthyl-(acrylo)-nitrile (DMANAN), and two well-recognized macrocyclic hosts, cucurbit[7]uril (CB7) and β-cyclodextrin (β-CD), has been studied in aqueous medium by absorption, emission, time-resolved measurements, and 1H NMR spectroscopic methods. The changes in the profiles of the fluorescence spectra illustrate significant modifications in fluorescence intensity, decay time, and quantum yield upon confinement of probe within the hydrophobic cavity of the hosts. Using the Benesi–Hildebrand relationship, the stoichiometric ratio as well as the binding constant of the host–guest complexation has been estimated. The stable inclusion complexes of the probe with different hosts have been supported by DFT and ONIOM based quantum chemical calculations. These methods of measurement establish that the acceptor group of the probe resides inside the hydrophobic cavity of the macrocycle. The competitive binding of metal ions and cationic surfactants to CB7 has been excellently mapped with this guest fluorosensor.

Supporting Information

INTRODUCTION

Rigid molecular containers with different cavity size, capable of encapsulating small molecules, are of much interest because of their widespread applications in nanotechnology, separations, catalysis, nanoreactors, sensors, and drug delivery.1−3 To this end, supramolecular chemists have judiciously designed and synthesized a wide variety of non-natural receptors, including cyclodextrins, crown ethers, calixarenes, and cyclophanes, and evaluated their recognition properties as drug carriers. Their primary aim is to enhance the solubility, stability, and bioavailability of drug molecules.4−7 A new class of similar supramolecular hosts is the pumpkin-shaped cucurbit[n]urils (CBn), which are composed of n (5−10) glycoluril units linked by a pair of methylene groups (Scheme 1). The supramolecular hosts CBn have fairly rigid hydrophobic cores of different sizes with low polarity and polarizability.8−10 Although Behrend11 first prepared CB molecules in 1905, its structure was determined in 1981 by Mock and co-workers.12 CBs have often educated comparisons with well-studied cyclodextrins (CDs) molecules.13−15 The CB6, CB7, and CB8 hosts have comparable cavity volumes and sizes to those of α-CD, β-CD, and γ-CD, respectively.16,17 However, the composition and binding properties of CBs are quite different from CDs. CDs are natural products generated by the action of the enzyme cyclodextrinase on starch, and these are composed of chiral glycopyranose units joined by acetal linkages.13−15 On the other hand, CBs, as synthetic products, are prepared by the acid-catalyzed condensation of symmetric glycoluril repeating units with formaldehyde and consist of glycoluril monomers joined by pairs of methylene bridges. Both the cavity openings

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are identical in CBs. On the contrary, the two cavity openings of CDs differ by size and chemical composition, where the smaller opening is lined by primary hydroxyl groups and the larger opening is lined by secondary hydroxyl groups (see Scheme 1). Similar to CDs, the extremely nonpolarizable rigid cavity of CBs can encapsulate small organic molecules such as fluorophore/drug/drug-template through hydrophobic interaction. On the contrary, ions and molecules can bind through charge–dipole as well as hydrogen bonding interactions at the carbonyl oxygen lining of both the CB cavity openings. The CB molecules are attractive as a synthetic receptor due to their highly rigid framework and ability of forming complexes with ions and molecules.18–20 Confinement of any guest molecules inside the CBn macrocycle cavity can change markedly the chemical and physical properties,21–23 photostability,24,25 and acidity26–28 of the guest. Only CB7 is a water-soluble host, thereby making it the most potentially useful host among all cucurbit[n]uril.s.

So far, studies on host–guest complexation reaction involving CDs and CBs with a variety of small organic molecules such as dyes have been reported quite extensively involving CDs and CBs with a variety of small organic molecules.35 The synthetic protocol for compound N,N-dimethylaminonaphthyl-(acrylo)-nitrile (DMANAN),38 an intramolecular charge transfer (ICT) probe, is used to investigate its photophysics upon complexation with CB7 and β-CD. The possibility of an ICT process in naphthalene isomers of the medium. In the present work, the complexation reaction of any fluorophore with these two hosts, including their mode of binding, binding constant, and visually good residual plot. Mean (average) goodness of fit: Watson (DW) parameter,

\[ R = \frac{I_{UV} - GI_{UV}}{I_{UV} + 2GI_{UV}} \]  

\[ G = \frac{I_{UV}}{I_{HII}} \]

in which \( I_{UV} \) and \( I_{HII} \) are the emission intensities when the excitation polarizer is vertically oriented and the emission polarizer is oriented vertically and horizontally, respectively. \( G \) is the correction factor.

A Time Correlated Single Photon Counting (TCSPC) spectrophotometer (Horiba Jobin Yvon) was used for the recording of the fluorescence lifetime of the probe in different media. A nanosecond diode laser of \( \lambda_e = 340 \) nm (IBH, nanoLED-07) was used as the exciting light source with TBX-04 as the detector. The fluorescence signals from the samples at different emission maxima were collected with an emission polarizer kept at the magic angle (\( \sim 54.70^\circ \)), and Data Station v-2.5 decay analysis software was used for the evaluation of decay. The instrument response function for this experimental setup was \( \sim 70 \) ps. The parameters which are considered here for goodness of fit are \( \chi^2 \) values, Durbin–Watson (DW) parameter, and visually good residual plot. Mean (average) fluorescence lifetime \( \langle \tau \rangle \) (from the multiexponential decay fitting was determined from the decay time constants \( \tau \) and the preexponential factors \( a \) using the following equation:

\[ \langle \tau \rangle = \sum_i a_i \tau_i \]  

The following equations were used for calculating radiative and nonradiative decay rate constants:

\[ k_i = \frac{\Phi_i}{\langle \tau \rangle} \]  

\[ k_{nr} = \frac{1 - \Phi_i}{\langle \tau \rangle} \]

We have measured time-resolved fluorescence anisotropy decay using the same TCSPC spectrophotometer. The
polarized fluorescence decays for the parallel $[I_p(t)]$ and perpendicular $[I_v(t)]$ emission polarizations with respect to the vertical excitation polarization were first collected at the emission maxima of the host–guest complex. Then the following equation was used for the evaluation of the anisotropy decay function $r(t)$ using these measured $I_p(t)$ and $I_v(t)$ decays:

$$r(t) = \frac{I_p(t) - G I_v(t)}{I_p(t) + 2G I_v(t)}$$

(7)

$G$ is termed as the correction factor for the detector sensitivity to the polarization detection of the emission.

The functional form of the biexponential anisotropy decay, $r(t)$, of the guest in the host can be represented as

$$r(t) = r_0 \times [\alpha_1 \exp(-t/\tau_1) + \alpha_2 \exp(-t/\tau_2)]$$

(8)

Here, $r_0$ is the limiting anisotropy and $\alpha_i$ is the pre-exponent that provides the fraction of the $i^{th}$ rotational relaxation time ($\tau_i$).

The average rotational correlation time has been calculated using the following equation:

$$\langle \tau_i \rangle = \sum_i \alpha_i \tau_i$$

(9)

The geometry of DMANAN has been optimized via the ab initio Density Functional Theory (B3LYP/6-31G**) method by using the Gaussian 09 programs.\(^3^9\) The DFT method (B3LYP/6-31G**) as well as ONIOM (B3LYP/6-31G**:PM3)\(^4^1\) hybrid calculations were performed on the CB7-DMANAN inclusion complex. On the contrary, ONIOM (B3LYP/6-31G**:PM3) hybrid calculations were performed on a $\beta$-CD–DMANAN inclusion complex. Computationally ONIOM is a highly efficient method for large system, and the precision is comparable to that of DFT with medium sized basis sets. Here in the ONIOM procedure, Density Functional Theory with the B3LYP functional and 6-31G** basis set was performed on the guest compounds, DMANAN, and the semiempirical PM3 method was used for CB7 and $\beta$-CD. It is impossible to study all the possible conformers due to size and conformational flexibility of the inclusion complexes. We have tried several starting points for searching the lowest energy structures of the inclusion complexes in the DFT and ONIOM hybrid optimizations. The stabilization energies ($\Delta E$) of host–guest complexations were calculated as follows:

$$\Delta E_{\text{DFT}} = E[H + G]_{\text{DFT}} - E[H]_{\text{DFT}} - E[G]_{\text{DFT}}$$

(10)

$$\Delta E_{\text{ONIOM}} = E[H + G]_{\text{ONIOM}} - E[H]_{\text{PM3}} - E[G]_{\text{DFT}}$$

(11)

where $H$ and $G$ stand for host and guest, respectively. $\Delta E_{\text{DFT}}$ and $\Delta E_{\text{ONIOM}}$ are the difference in energies between the host–guest complex ($E[H + G]$) and the total energy of the free host ($E[H]$) and the guest ($E[G]$) by the two methods of calculations: DFT and ONIOM, respectively.

\section*{RESULTS AND DISCUSSION}

\subsection*{Absorption spectral behavior of DMANAN in the presence of CB7.}

The absorption spectra of DMANAN with gradual addition of CB7 in aqueous solution are presented in Figure 1a. It is already reported that DMANAN shows an absorption band at $\sim 355$ nm for the $S_0 \rightarrow S_1$ transition ($\pi \pi^*$ transition).\(^3^8\) With the incremental addition of CB7 in an aqueous solution of DMANAN, the lower energy absorption band is blue-shifted from $\sim 355$ to $340$ nm with a concomitant increase in the absorbance value. These modifications of the absorption profiles of the probe with the introduction of CB7 clearly demonstrate some interaction of the probe with the host and are due to the formation of an inclusion complex between the probe DMANAN and CB7. Probably the combination of a hydrogen bonding interaction and a hydrophobic effect contributes to the binding force. On the contrary, as displayed in Figure 1b, addition of $\beta$-CD to the aqueous solution of DMANAN results in a gradual increase in the DMANAN absorbance with an unnoticeable change of the spectral band position. The change of the absorption spectrum of the probe in the presence of $\beta$-CD also dictates some interaction between the two partners. In both the cases (Figure 1), enhancement in absorbance indicates that macrocyclic hosts improve the water solubility of the probe.

\subsection*{Emission spectral changes of DMANAN on complexation with CB7.}

As reported earlier,\(^3^8\) the molecule DMANAN shows a solvent polarity dependent red-shifted ICT emission due to excited state charge transfer from the donor to the acceptor group. Interestingly, it is found that fluorescence spectral profiles such as the fluorescence intensity, quantum yield, and fluorescence lifetime of the CT band of DMANAN are affected by the nature of the solvent.\(^3^8\) This behavior is due to the significantly higher dipole moment of the excited CT state ($S_1$) compared to the ground state ($S_0$) of the molecule ($\Delta \mu = 7.7$ D).\(^3^8\) This in turn induces a large Stokes

![Figure 1](https://example.com/f1.png)
shift of the guest upon electronic excitation. This solvent dependent CT emission of the probe has already prompted us to study the photophysics of DMANAN in a micellar nanocage, since micelles, CDs, and CBs are capable of providing micropockets of desired polarity which are very similar to the biological environments. As in the absorption spectral studies, confinement of DMANAN in the CB7 cavity has a pronounced effect on the fluorescence behavior. The molecule DMANAN shows weak emission with a maximum at ~500 nm in water. As shown in Figure 2a, addition of CB7 would modify significantly upon interaction of the probe with CB7. Such an increase in fluorescence intensities as well as blue shift, as revealed in Figure 2a, is also observed during the inclusion complex formation of the probe with β-CD. With the addition of 12 mM β-CD in the aqueous solution of DMANAN, enhancement of LE as well as the CT emission band intensity is ascertained with the consecutive 17 and 10 nm blue shifts, respectively (Figure 2b). Dissimilarity is observed only in the case of the CT emission intensity. As pictured in Figure 3a, while, in the case of CB7-DMANAN inclusion phenomenon, major changes occur in the local emission intensity, CT emission along with LE experience enhancement along with blue shift during confinement of DMANAN in β-CD (Figure 3b). Enhancement in LE of the probe in the presence of CB7 is more pronounced than that for the interaction between β-CD and DMANAN (Figure 3a and 3b). This may be attributed to much more fitter and tighter binding of probe within the CB7 cavity, which makes it difficult for the probe to twist for CT emission, so that enhancement of the LE state is more pronounced. The decrease in polarity of the surrounding environment of the encapsulated probe inside the hosts is reflected by the corresponding blue shift of the emission maxima of the probe. Besides this, water acts as a quencher of CT emission. Hence, the possibility of non-radiative decay reduces to a greater extent as the guest molecules are less exposed to water due to encapsulation inside the hydrophobic micropockets of CB7 and β-CD. Decrease of polarity near the vicinity of the probe inside the cavity results in an increase in energy gap between the CT state and the triplet/ground states, thereby leading to a reduction in the non-radiative decay, and hence, CT emission is enhanced in the presence of these macrocyclic hosts. This decrease in polarity also results in an increase in the energy barrier for transition.

Figure 2. Modifications of the fluorescence spectrum of a 50 μM aqueous solution of DMANAN (λ_{ext} = 350 nm) on addition of (a) (1 → 14) 0, 0.04, 0.06, 0.1, 0.12, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45, and 0.5 mM CB7 and (b) (1 → 10) 0, 1, 2, 3, 4, 6, 8, 9, 10, and 12 mM β-CD.

Figure 3. (a) CB7 and (b) β-CD concentration dependence of the local emission intensity (λ_{max} = 414 nm) and charge transfer fluorescence intensity (λ_{max} = 490 nm) of DMANAN (λ_{ext} = 350 nm). (c) Variation of quantum yield values of DMANAN with the concentration of CB7 and β-CD.
between the LE state and the ICT state, thereby enhancing the LE intensity. The main nonradiative channel of the local emission, via the ICT pathway, is restricted inside the nanocavity, which also causes an enhancement of the intensity of the LE band. The fluorescence quantum yield ($\Phi_f$) of the probe increases by about 9-fold and 5.4-fold for the formation of inclusion complexes with CB7 and $\beta$-CD, respectively (Figure 3c). This is caused by the reduction of the nonradiative decay rate because of the geometrical confinement as well as lower polarity in the corresponding cavities.

The modulation of the excitation spectra of the probe with and without the supramolecular assemblies at the emission maxima (~500 nm) is presented in Figure S1. The modification of the spectra with the increasing host concentration is observed to be reasonably similar to the case of the absorption spectra (Figure 1). This observation concludes that the origin of the emitting state is the same and a single type of equilibrium (1:1) exists in the host–guest complex system.

**Determination of binding constants.** Though the interaction scenario between the probe and macrocycles is reflected in the changes of spectral characteristics with addition of different hosts, a quantitative estimation of binding and the stoichiometric ratios of probe DMANAN to the hosts can be estimated using the well-known Benesi–Hildebrand (BH) relation. The cavity size of CB7 is similar to that of $\beta$-CD and the ethyl ester of $N,N$-dimethylaminonaphthyl-(acrylic)-acid (EDMANA), structurally quite similar to DMANAN, forms a 1:1 host–guest complex in an aqueous solution of $\beta$-CD. The interaction of the hosts with the probe DMANAN used in this study is also expected to be of 1:1 stoichiometric fashion. The equilibrium for a 1:1 inclusion complex can be expressed by the following equation

$$H + G \rightleftharpoons H:G$$

$$K = \frac{[H:G]}{[H][G]}$$

where $K$ is the binding constant for the formation of a host–guest inclusion complex. The derivation of the BH relationship has been discussed elsewhere, and therefore, the BH equation in terms of emission intensity for this type of complexation phenomenon is expressed here as

$$\frac{1}{(I - I_0)} = \frac{1}{(I_1 - I_0)} + \frac{1}{(I_1 - I_0)K[H]}$$  \hspace{1cm} (12)$$

where $I_0$, $I_1$, and $I$ are the emission intensities in the absence of, at intermediate, and at infinite concentration of host, respectively. [H] represents the concentration of the host. The linearity of the double reciprocal plot (Figure 4) endorses 1:1 stoichiometric inclusion complexes between the probe and both the hosts. The $K$ values are thus obtained from the slope and intercept of the respective BH plots. For CB7–DMANAN interaction, the values are $(10.51 \pm 1.47) \times 10^2$ M$^{-1}$ for the locally excited state and $(0.17 \pm 0.03) \times 10^2$ M$^{-1}$ for the ICT state (Figure 4a). Similarly, the $K$ values for the inclusion complexes of DMANAN with $\beta$-CD are found to be $(2.28 \pm 0.47) \times 10^2$ M$^{-1}$ and $(2.32 \pm 0.48) \times 10^2$ M$^{-1}$ for the local and CT states of DMANAN, respectively (Figure 4b). The magnitudes of the binding constants (LE state) for the complex of the guest with CB7 are much higher (5-fold) than those measured for the same guest with $\beta$-CD. This type of huge binding enhancement is most probably due to hydrogen bonding interactions, which are not taken into account in the case of $\beta$-CD complexes. Therefore, CB7 can be the better host compound for the quantitative estimation of DMANAN by a fluorimetric method.

**Steady state anisotropy measurements.** Steady state fluorescence anisotropy measurements have also been performed to further support our claim of inclusion complexes formation between the used hosts and guest. Since encapsulation of DMANAN in the micropockets of CB7 and $\beta$-CD imposes motional restriction of the probe, this study can be a useful tool to assess the probable location of the probe in these supramolecular assemblies. Figure 5 shows the variation of steady state anisotropy with increasing concentration of CB7 and $\beta$-CD. In both the cases, enhancement of fluorescence anisotropy is observed which reflects restriction on rotational relaxation of the excited DMANAN in both the macrocycles. Time-resolved anisotropy measurements may provide a better picture about this restricted motion of the probe inside the hosts and are discussed later.

**Time-resolved fluorescence measurements.** Low-frequency large-amplitude motion (say twisting motion) by flexible bonds in DMANAN may be the reason behind its very fast nonradiative decay, thereby showing very short excited state decay time (beyond the time resolution of the present TCSPC setup). Such flexible motion of the guest is expected to reduce largely on forming inclusion complexes (i.e., confinement of probe) with the hosts, and hence, fluorescence decay time is expected to be increased. Figure 6 represents the
fluorescence decay profiles recorded for DMANAN at 500 nm in the presence of CB7 and β-CD. Due to the rotational flexibility of the probe, it can form different conformational structures with the hosts. So the fluorescence decays may be multiexponential in nature. As shown in Table 1, fluorescence decay curves are best fitted by a triexponential function with respect to the goodness of the fit (χ^2 and DW parameters). Among the three components, one component (τ_1) has a very short lifetime, which is within the time resolution (~70 ps) of our TCSPC setup. The other two components (τ_2 and τ_3) are relatively larger in magnitude. Though the decay parameters are within the instrument response function and complex in nature, the increase in decay time suggests an increase of rigidity of the guest molecule on interaction with the supramolecular host molecule. With increasing the host concentration, the contributions of the slower components increase along with a steady decrease in the amplitude of the faster component (Table 1). Since the longer lifetime components correspond to a complex component, this enhancement in amplitude indicates confinement of the guest in the nanocages of the macrocyclic host. In order to avoid complexity, an average fluorescence lifetime (⟨τ⟩) has been considered, rather than emphasizing too much on the individual components. As listed in Table 1, similar to the individual decay time constants, ⟨τ⟩ values also increase with increasing concentration of the host, which can be interpreted by considering inclusion of the probe in the CB7 and β-CD cavities. A guest molecule encapsulated inside different host molecules experiences a hydrophobic microenvironment with a reduced polarity and polarizability, and this generally deactivates the nonradiative channels. The nonradiative decay constants (k_nr), as calculated using eq 6, have been listed in Table 1. As expected, the decreasing trend of k_nr values with an increase in CB7/β-CD concentration reflects confinement of the probe inside the hydrophobic cavity of the host and restriction on its twisting motions. Strong host–guest binding reduces the probability of deactivation via nonradiative channels, and hence, fluorescence lifetime as well as Φ_f value increases.

Similar to the ICT state, fluorescence decay profiles are also recorded for the LE state of DMANAN in the presence of the investigated hosts, and the corresponding figures and data have been presented in Figure S2 and Table T1, respectively. A difference arises only when we consider the time constants, as they are comparatively much slower (within the time resolution of the present TCSPC setup) than that of the CT state. Similar to the CT state, individual decay time as well as ⟨τ⟩ values increase with rising concentration of the host. Furthermore, the contribution of the slower components, which are assigned to be due to the host–guest complexation, increases, thereby reducing the amplitude of the faster component (Table T1).

**Time-resolved anisotropy measurements.** Time-resolved anisotropy measurement is much more sensitive than the steady state one for obtaining further insight into the nature of the rotational dynamics of the probe in the presence of the host. It is a sensitive tool for getting information about the rotational relaxation of the fluorophore in the host–guest complex. In order to see how the rotational relaxation dynamics of the probe is influenced on confinement in the CB7 cavity, time-resolved fluorescence anisotropy measurements have been carried out and are presented in Figure 7. The rotational correlation time (τ_r) of free DMANAN in aqueous solution cannot be recorded, as it is expected to be too short to be detected with the present TCSPC setup. Figure 7a shows the typical fluorescence anisotropy decay of DMANAN in the presence of CB7, and the values of the rotational relaxation times, as obtained from biexponential (with a slow and a fast reorientation time) fitting of the anisotropy decay profile, have been listed in Table 2. The fast component is due to the independent internal motion of the probe inside the CB7 cavity, and the slow component corresponds to the overall rotational motion of the host–guest complex.

The average rotational correlation time (⟨τ_r⟩), calculated using eq 9, for DMANAN in the presence of CB7 is found to be significantly slower (0.693 ns), clearly supporting the
Table 1. Fluorescence Lifetime (\(\tau_i\)), Their Relative Contribution (in parentheses), Average Lifetime (\(\langle \tau_r \rangle\)), and Nonradiative Decay Rate Constants (\(k_{nr}\)) for the Aqueous Solution of DMANAN in the Presence of CB7 and \(\beta\)-CD (\(\lambda_{ex} = 340 \text{ nm}, \lambda_{monitored} = 500 \text{ nm}\))\(^{49}\)

<table>
<thead>
<tr>
<th>[Host] mM</th>
<th>(\tau_1) (ns)</th>
<th>(\tau_2) (ns)</th>
<th>(\tau_r) (ns)</th>
<th>(\langle \tau_r \rangle) (ns)</th>
<th>(\chi^2)</th>
<th>DW</th>
<th>(k_{nr} \times 10^9 \text{ s}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB7</td>
<td>0.1</td>
<td>0.435 (0.002)</td>
<td>4.131 (2 \times 10^{-3})</td>
<td>0.021 (0.997)</td>
<td>0.023</td>
<td>1.17</td>
<td>1.83</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>0.491 (0.015)</td>
<td>4.84 (6 \times 10^{-4})</td>
<td>0.024 (0.984)</td>
<td>0.034</td>
<td>1.13</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.637 (0.017)</td>
<td>5.521 (4 \times 10^{-4})</td>
<td>0.039 (0.983)</td>
<td>0.051</td>
<td>1.13</td>
<td>1.76</td>
</tr>
<tr>
<td>(\beta)-CD</td>
<td>3</td>
<td>0.497 (0.004)</td>
<td>3.912 (3 \times 10^{-4})</td>
<td>0.023 (0.996)</td>
<td>0.026</td>
<td>1.07</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.649 (0.005)</td>
<td>4.773 (5 \times 10^{-4})</td>
<td>0.042 (0.995)</td>
<td>0.047</td>
<td>1.07</td>
<td>1.91</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1.091 (0.006)</td>
<td>7.507 (5 \times 10^{-4})</td>
<td>0.077 (0.994)</td>
<td>0.087</td>
<td>1.14</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>1.257 (0.007)</td>
<td>8.09 (8 \times 10^{-4})</td>
<td>0.082 (0.993)</td>
<td>0.097</td>
<td>1.18</td>
<td>1.78</td>
</tr>
</tbody>
</table>

\(^{49}\)\(\chi^2\) and the Durbin–Watson (DW) parameter represent the goodness of the fitting. Standard deviation for the fitting analysis is ±5%.

Table 2. Rotational Relaxation Dynamical Parameters, such as Rotational Correlation Time (\(\tau_i\)), Their Relative Contribution (in parentheses), and Average Correlation Time (\(\langle \tau_r \rangle\)), from Time-Resolved Anisotropy Decay of the Aqueous Solution of DMANAN in the Presence of CB7 and \(\beta\)-CD\(^{49}\)

<table>
<thead>
<tr>
<th>[Host]</th>
<th>(\tau_1) (ns)</th>
<th>(\tau_2) (ns)</th>
<th>(\tau_r) (ns)</th>
<th>(\langle \tau_r \rangle) (ns)</th>
<th>(\chi^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB7 (0.5 mM)</td>
<td>1.546 (0.389)</td>
<td>0.150 (0.611)</td>
<td>0.693</td>
<td>1.01</td>
<td></td>
</tr>
<tr>
<td>(\beta)-CD (12 mM)</td>
<td>0.659 (0.792)</td>
<td>0.048 (0.208)</td>
<td>0.532</td>
<td>1.1</td>
<td></td>
</tr>
</tbody>
</table>

\(^{49}\)Standard deviation for the fitting analysis is ±5%.

formation of strong inclusion complex, for which the relaxation of the probe is slowed as well as restricted by CB7. The \(\langle \tau_r \rangle\) value for DMANAN in a particular CB7 concentration is observed to be higher than the \(\langle \tau_r \rangle\) value in the same, thereby suggesting incomplete depolarization within the excited state lifetime of the probe in the specified environment. Considering the dimension of DMANAN and the diameter of the CB7 cavity (~7.3 Å), it seems that the probe molecule fits tightly inside the host cavity, and hence, independent internal rotation of the probe inside the CB7 cavity may be hindered in the DMANAN–CB7 complex. The rotational depolarization of the complex is unusually slower than the excited state decay time, due to compactness and rigidity of the host environment. Thus, \(\langle \tau_r \rangle\) of the host–guest complex has a high value which corresponds to the overall rotation of the complex. This overall rotation of the complex is also reflected in the residual anisotropy (decay tails) (Figure 7). The origin of such an offset is due to the overall rotation of the DMANAN–CB7 complex, which does not decay within the present experimental time scale.47 The initial anisotropy \(\langle \tau_0 \rangle\) value was estimated to be quite high (0.2854) for the dye–CB7 complex.

According to Stokes–Einstein hydrodynamic theory,48 the \(\langle \tau_r \rangle\) values for the host–guest complex (considered as an effective sphere) can be written as a function of hydrodynamic radius \(\langle \tau_r \rangle\) as follows:

\[
\langle \tau_r \rangle = \frac{4\pi\eta r_h^3}{3KT}
\]

Here, \(T\) is the absolute temperature, \(K\) is the Boltzmann constant and \(\eta\) is the viscosity of the medium. The \(\eta\) value can be taken to be that of water at 298 K. In this case, stick-type hydrodynamic conditions are applicable, as the size of the probe is much larger than the size of the solvent molecules.49

By using the above relation, the effective hydrodynamic radius for the 1:1 DMANAN–CB7 complex is evaluated to be ~9.13 Å. This corresponds to a diameter (=2\(r_h\)) of ~18.26 Å. This value is much larger than the reported height of the CB7 cavity (~9.1 Å).50 The calculated height of the probe at the DFT/B3LYP/6-31G** level is ~10.94 Å. So it can be easily concluded that a part of the probe in the 1:1 complex is projected out of the cavity so that the height of the inclusion complex is larger than that of the CB7 cavity (Scheme S1).

Similar to CB7, time-resolved fluorescence anisotropy decay for DMANAN confined in the \(\beta\)-CD cavity has been carried out and presented in Figure 7b. As shown in Table 2, \(\langle \tau_r \rangle\) has been calculated to be longer, thereby supporting the formation of a tight association complex of the probe with the host molecule. By using eq 13, the effective hydrodynamic diameter of the complex (DMANAN–\(\beta\)-CD) is estimated to be ~16.72 Å. Since the height of \(\beta\)-CD is ~7.9 Å, it is assessed that
~8.82 Å of DMANAN is projected outside the CD cavity (Scheme S1).

**1H NMR studies on the host-guest complex.** 1H NMR spectroscopy has been used to ascertain the formation of host-guest complexes between CB7 and DMANAN in aqueous solution. Figure 8 shows the NMR spectra of DMANAN in the absence and in the presence of CB7. The 1H NMR spectra of CB7 appears between 4.23 and 5.74 ppm without disturbing the proton signals of DMANAN. The 1H NMR spectra of the host-guest complex, however, clearly reveal upfield shifts of all the protons of the probe. The selection of this probe is based on two advantages: first of all, the probe is neutral and hydrophobic in nature, and second, it is expected to remain solubilized in the hydrophobic cavity of the host. It is well-known that hydrophobic interaction of the probe with the host renders an upfield shift of the proton resonances. So the upfield shifts in the presence of CB7 clearly demonstrate that the hydrophobic nature of the probe favors binding with the hydrophobic nanocage of CB7. After addition of CB7, the signals of H1, H2, and H8 of DMANAN (Figure 8b) are upfield shifted significantly (0.084, 0.095, and 0.081 ppm, respectively) with smaller shift of the other (H3, H4, H5, and H6) protons, corroborating that CB7 prefers to bind to the acceptor end of the molecule. The inclusion phenomenon of DMANAN with β-CD has also been studied by proton NMR spectroscopy and has been presented in Figure S3. The guest protons are upfield shifted on addition of β-CD. Similar to CB7, H2, H3, and H8 of the probe are shifted significantly. Gathering all these observations, we can say that hydrophobic interaction occurs in between β-CD and DMANAN and the acceptor side of the probe enters into the cavity of the host.

**Effect of cationic substances on host-guest complexation.** Recently, CBs have acquired the position of a current topic of research because of their ability to engulf cationic substances through ion-dipole interaction. The main difference arises between CBs and CDs when the encapsulated guest is cationic.16 As CBs have excellent complexation properties toward cationic guest molecules compare to those of neutral ones, a host expels the neutral molecule to welcome the cationic guest. So the binding of a metal ion to the carbonyl portal of CB7 modulates the binding constant of the inclusion complex. Having demonstrated an efficient complex formation of neutral DMANAN with the hydrophobic cavity of CB7, our next target is to find a release protocol for the trapped probe in the presence of some foreign substances. Depending on the situation, it is possible to displace fluorescent dye by adding an external analyte. Such a type of displacement mechanism is attracted due to its application in many fields, such as drug delivery,1–3 logic gates,52 etc.

In the present case, we have selected and used different chemical additives such as metal ion (NaCl, CaCl2), and cationic surfactants (CTAB) as foreign materials (stimuli) based on their varying binding affinities toward CB7. Figure S4a demonstrates the emission spectral changes observed on the 1:1 DMANAN–CB7 complex by introducing Na+ ions. Incremental addition of Na+ ions decreases the emission intensity of the inclusion complex, representing the dissociation of the host-guest complex. Figure 9a shows the changes in emission intensity with increasing metal ion concentration. The emission intensity of the host–guest complex decreases by more than 38% with 0.26 M concentration of Na+ ion. A sharper decrease in the fluorescence intensity (79% with 0.23 M concentration of Ca2+) is observed in the presence of divalent Ca2+ metal ion (Figure S4b). As pictured in Figure S4, an increasing amount of salt leads to the reappearance of the signals corresponding to the free guest. Apparently, Ca2+ ion has higher binding affinity toward CB7 portals due to purely electrostatic ground, and hence, the binding constant of the DMANAN:CB7 complex decreases more rapidly with added Ca2+ ion. The size of the metal ions has also a small effect on this competitive experiment. This competitive experiment is in accord with the previous discussions that alkali cations readily bind to the carbonyl-fringed portals of CB7, resisting the insertion of neutral DMANAN into the CB7 cavity and, thus, lowering the apparent stability of the resulting inclusion complex. Besides this, stronger ion–dipole interactions are expected in the case of divalent Ca2+ ions than in the case of monovalent Na+ ions, which is in accord with the observed findings that Ca2+ ion causes a more pronounced influence on the DMANAN:CB7 inclusion complex than that of Na+ ion. No noticeable changes have been observed with the addition of NaCl, CaCl2 to the inclusion complex of β-CD–DMANAN.

A conspicuous feature is observed with the addition of a cationic surfactant, CTAB, to the DMANAN–CB7 inclusion complex. As depicted in Figure S5a, remarkable enhancement in the fluorescence intensity of the CT state is identified after an initial dip. The changes in the fluorescence intensity ratio between the LE and CT states of the DMANAN–CB7 inclusion complex with addition of CTAB are presented in Figure 9b. In the case of the LE state, emission intensity decreases with gradual addition of CTAB, which is in agreement with the previous results. But a conflicting outcome
is perceived in the CT fluorescence intensity. Similar to the displacement mechanism, the fluorescence intensity decreases up to a certain concentration of CTAB (0.9 mM), thereby removing the probe from the cavity of CB7. After that, the cationic surfactants aggregate itself to micellization. It has been already reported that DMANAN enters into the micelle core by exhibiting huge enhancement in fluorescence intensity (Figure S5b). So the enhancement in fluorescence intensity with further addition of surfactants authenticates the inclusion of the expelled probe from the aqueous solution to the micelle core of CTAB. The observed hypsochromic shift (12 nm) also demonstrates the binding of this probe to the less polar zone of the micelle (Figure S5a).22

Theoretical calculations on host–guest geometry. The Benesi–Hildebrand plot (Figure 4) has demonstrated the formation of a host–guest inclusion complex with 1:1 stoichiometry. Theoretical calculation has been performed to confirm the existence of a 1:1 host–guest complex. Ab initio calculations may be reliable and may predict detailed geometrical parameters and can estimate the relative stabilities of the inclusion complexes. The gas phase structure of the host–guest complex between CB7 and DMANAN has been determined by the DFT method with the B3LYP functional and 6-31G** basis set as well as by using the ONIOM (B3LYP/6-31G**:PM3) method along with the ground state geometry optimizations of free DMANAN and CB7. All the optimizations have been carried out using the Gaussian 09 suite package, and no geometric constraint was imposed on the optimization process. Different starting geometries, by placing DMANAN into the host, have been taken as input and allowed to be optimized. Two possible complexation orientations have been considered: one by entering the donor end and another by entering the acceptor end of the probe in the cavity. As illustrated in Figure 10, the most probable inclusion complex is the one in which the acceptor group is incorporated in the CB7 cavity, and this position of the probe inside the cavity is consistent with the picture obtained from the experimental observations. The stabilization energy ($\Delta E$), calculated using eq...
10, has been evaluated to be \(-8.37\) kcal/mol, which authenticates the feasibility of 1:1 complex formation. As seen in Figure 10, the stabilizing energy in the optimized structure of the complex may arise from the four hydrogen bonds generated between the hydrogen atoms of probe DMANAN and the oxygen atoms of CB7. These hydrogen bond distances range from 2.45 to 2.99 Å. On the converse, the nitrogen atom of the acceptor group is found to be at a distance of 3.7–4.3 Å from the oxygen atom of both ends of CB7.

Similarly, geometry optimization was also attempted for \(\beta\)-CD-DMANAN complexes using the ONIOM method (B3LYP/6-31G**.PM3). Among different host–guest complex orientations, the most stable one is pictured in Figure S6. As evident from this picture, the acceptor end of DMANAN prefers to insert the nanocavity of the host \(\beta\)-CD. The lowest calculated \(\Delta\beta\) value for the 1:1 complexation reaction (eq 11) is \(-5.33\) kcal/mol. This computational result can easily be correlated with the experimental findings.

### SUMMARY AND CONCLUSIONS

Supramolecular interactions of macrocyclic hosts, CB7 and \(\beta\)-CD, with naphthalene derivative, \(\beta\)N,N-dimethylaminophenyl-(acrylo)-nitrite (DMANAN), have been investigated in aqueous solution using steady state and time-resolved spectroscopy. There are distinct differences in the photophysical properties of DMANAN when it forms host–guest complexes with CB7 and \(\beta\)-CD. The observed fluorescence intensity from the LE state of the probe is enhanced largely when it is incorporated in the CB7 cavity. On the contrary, both the LE and CT state exhibit fluorescence intensity enhancement with the incremental addition of \(\beta\)-CD to the aqueous solution of DMANAN. The Benesi–Hildebrand plot dictates the formation of a 1:1 stoichiometric host–guest complex and stronger binding ability of the investigated probe in the hydrophobic interior of CB7 than that of \(\beta\)-CD. The fluorescence quantum yield and lifetime of the guest increase substantially upon complexation with the studied host. The \(\text{H}^1\) NMR experiment as well as quantum chemical calculations on the host–guest complex point toward the fact that the inclusion complexes of 1:1 stoichiometry are formed via confinement of the acceptor group of the probe into the hydrophobic nanocages of CB7 and \(\beta\)-CD, respectively. The prominent change in the fluorescence characteristics by the extrusion of DMANAN from the CB7 cavity into the aqueous phase can be investigated by competitive experiments using cationic additives. A striking feature is observed in the case of a cationic surfactant, CTAB, where the probe expelled by CTAB from the CB7 cavity further penetrates into the micelle hydrophobic core, exhibiting enhancement in CT fluorescence intensity. Since \(\beta\)-CD remains silent in this competitive phenomenon, CB7 is a much better host for this probe. This type of association and displacement mechanism can be explored for the binding and release of drug molecules in drug/dye delivery systems.

### ASSOCIATED CONTENT

* Supporting Information

Excitation spectra, time resolved fluorescence decay profiles, \(\text{H}^1\) NMR spectra, and optimized ground-state structure of the host–guest complex of DMANAN; changes in the fluorescence spectra of the DMANAN–CB7 complex with the addition of different cationic additives; table of fluorescence lifetimes, their relative contributions, and average lifetimes for the aqueous solution of DMANAN in the presence of CB7 and \(\beta\)-CD; estimation of hydrodynamic diameter for the host–guest inclusion complex of DMANAN with CB7 and \(\beta\)-CD. This material is available free of charge via the Internet at http://pubs.acs.org.

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