

## Inclusion of a coumarin derivative inside the macrocyclic hosts: A spectroscopic, thermodynamic and theoretical investigation



Sayeed Ashique Ahmed <sup>a</sup>, Soma Seth (Duley) <sup>b</sup>, Rajesh Kumar Gautam <sup>a</sup>, Debabrata Seth <sup>a,\*</sup>

<sup>a</sup> Department of Chemistry, Indian Institute of Technology Patna, Patna 801103, Bihar, India

<sup>b</sup> Department of Chemistry, Nabadwip Vidyasagar College, West Bengal, India

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### ABSTRACT

In aqueous medium, the guest-host interaction of 7-(diethylamino)coumarin-3-carboxylic acid *N*-succinimidyl ester (7-DCCAE) with cucurbit[7]uril (CB7) and cucurbit[8]uril (CB8) are very interesting. The interaction of 7-DCCAE with CB7 and CB8 have been investigated in a systematic way by different types of spectroscopic techniques such as UV-Vis spectroscopy, steady state and time resolved fluorescence emission spectroscopy, <sup>1</sup>H-NMR along with thermodynamic approach by isothermal titration calorimetry measurement. We observed that the photophysical properties of the aqueous solution of 7-DCCAE are modulated in the presence of CB7 and CB8. We observed that 1:1 complex is formed between 7-DCCAE with CB7 and CB8. From ITC measurement, we obtained different thermodynamic parameters associated with host-guest complexation. We have drawn up the driving forces involved for the host-guest complexation from ITC measurement. By using density functional theory geometry and frequency of the 7-DCCAE-CBn complexes were optimized. We observed that both the complexation processes were exothermic in nature from ITC as well as from computational study.

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### 1. Introduction

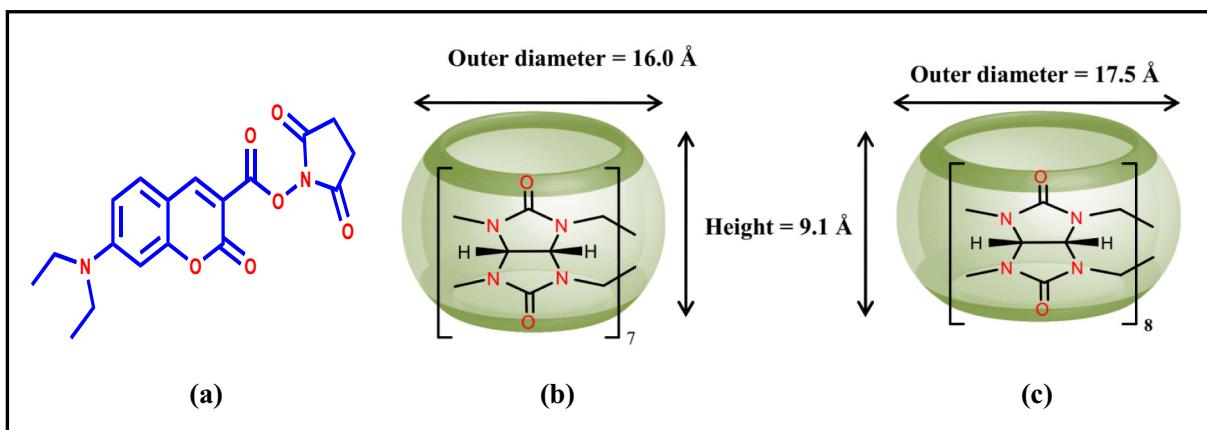
The chemistry of cucurbit[n]urils (CBn) is very expeditiously developing research area. Cucurbit[n]uril was first synthesized in 1905 [1]. CBn are highly symmetric and rigid macrocyclic host having two identical carbonyl portal ends on both side of the cavity [2,3]. CBn consists “n” number of glycoluril units affixed by a pair of methylene groups to form a cyclic structure. CBn have different cavity size and the cavity size is dependent on the number of glycoluril units ( $n = 6, 7, 8, 10, 12, 14$ ) [3]. This rigid macrocyclic hosts do not possess monopole, dipole or octapole moment but possess quadrupole moment due to the presence of high symmetry [4]. It has highly polarisable carbonyl edges with hydrophobic cavity. The incorporation of small biologically active organic molecules inside the macrocyclic hosts are flourishing area of research as it has wide ranges of applications such as drug delivery, fluorescence switching, catalysis, supramolecular dye lasers and many other applications over the past decennium [3,5–17]. CBn have possess extra ordinary affinity towards guest molecule, (the affinity of CBn depends on the size of the guest molecules) for this reason the uses of CBn are receiving more attention in different field of chemistry such as host-guest complexation, drug-delivery, molecular recognition, nanotechnology and also biologically relevant works due to its low toxicity, etc. [18–22].

The host-guest complexation process modifies the different properties of the guest molecule [23]. As a result of which physical properties of the guest molecule in bulk medium and inside the hydrophobic cavity of macrocyclic host molecule is often different. To study the modulation of the chemical properties of the guest molecule on complexation, in a systematic way, different types of spectroscopic techniques are very useful tools [24–26]. Here, we have investigated the photophysical properties of aqueous solution of 7-(diethylamino)coumarin-3-carboxylic acid *N*-succinimidyl ester (7-DCCAE) in presence of two macrocyclic hosts cucurbit[7]uril (CB7) and cucurbit[8]uril (CB8) (Scheme 1). CB7 has a portal diameter of 5.4 Å, cavity diameter of 7.3 Å and height of 9.1 Å, where seven glycoluril units present and which are connected by two methylene groups. CB8 has a portal diameter of 6.9 Å, cavity diameter of 8.8 Å and height 9.1 Å and eight glycoluril units are present in CB8 which are connected by a pair of methylene groups. The study of changes in chemical properties or photophysical properties of guest molecule in presence of CB7 and CB8 are alluring area of research.

Coumarin dyes are very attractive molecule for their photophysical properties and their wide ranges of biological applications [27–29]. These dyes have numerous applications in different fields such as pharmaceutical industry, drug delivery system, biomedical analysis, used as optical chromophores, as gain media in blue-green tuneable organic dye lasers [28–31]. Some of the derivatives also have anticancer activity, anti-inflammatory, anti oxidant, antibacterial, antifungal and HIV inhibitory activities [27–29]. Apart from these biological applications of the coumarin derivatives, they also have very interesting photophysical

\* Corresponding author.

E-mail address: [debabrata@iitp.ac.in](mailto:debabrata@iitp.ac.in) (D. Seth).



**Scheme 1.** Schematic representation of (a) 7-DCCAE, (b) CB7 and (c) CB8.

properties. Coumarin molecules are very attractive for their interesting fluorescence properties like charge transfer as well as twisting in the excited state. 7-DCCAЕ is a coumarin class of dye. It can be used to label cells and not only cells labelling but also have significant applications to generate blue fluorescent in bioconjugates [32]. 7-DCCAЕ is an important molecule for the determination of amino acids using capillary zone electrophoresis based on the laser fluorescence detection [33]. The photophysics of the 7-DCCAЕ are highly sensitive towards polarity, viscosity of the surrounding media [34–35]. Here, we have discussed about the changes of photophysical properties of 7-DCCAЕ due to formation of supramolecular host-guest complex with CB7 and CB8, respectively. Here, the modulation of photophysical properties of 7-DCCAЕ were deeply investigated through different types of spectroscopic techniques. We have performed isothermal titration calorimetry (ITC) measurements for deeper understanding about the changes in thermodynamic properties due to the supramolecular complexation process and also able to find out the driving forces involved for the host-guest complexation. We have theoretically studied the host-guest complexation to decipher the molecular orientation of 7-DCCAЕ in the presence of both CBn. In aqueous medium, the twisted intramolecular charge transfer (TICT) state of 7-DCCAЕ is non emissive in nature. Where the molecule present in excited state and the non-radiative deactivation pathway converts to intramolecular charge transfer (ICT) state which very quickly converts to TICT state and here the twisting motion inside the molecule is taking place around the N—C bond between the amino group and rest of the chromophore. Our main focus in this present study is to formulate the nature of supramolecular host-guest complexation of 7-DCCAЕ with both CBn. This work will very much helpful for understanding the photo-physics of 7-DCCAЕ molecule in presence of these two very interesting macrocyclic hosts. This study will be also helpful for understanding the driving forces involved for the complex formation in the studied systems and the change in thermodynamic parameters due to complexation between 7-DCCAЕ with the studied CBn. One of the most interesting finding is that the binding constant value of 7-DCCAЕ with CB7 and CB8 obtained from fluorescence measurement are just in reverse trend than that of the binding trend obtained from ITC measurement. This study will also helpful for understanding the reason for difference in binding strength obtained from fluorescence and ITC measurements.

## 2. Materials and methods

### *2.1. Materials*

7-(Diethylamino)coumarin-3-carboxylic acid *N*-succinimidyl ester (7-DCCAЕ), Cucurbit[7]uril (CB7) and Cucurbit[8]uril (CB8) were purchase from Sigma-Aldrich with highest available purity (**Scheme 1**). These chemicals were used as received without further purification.

Triple distilled water was used throughout the experiment for preparation of the solutions. All experiments were performed by using freshly prepared solution to avoid any photophysical changes. The concentration of the aqueous solution of 7-DCCAE was maintained at  $2.0 \times 10^{-6}$  (M) for all spectroscopic measurements.

## 2.2. Methods

### **2.2.1. Instruments**

**2.2.1.1. Steady-state measurements.** Ground state absorption spectra were collected by using UV-vis spectrophotometer (Model: UV-2550, Shimadzu). The steady-state fluorescence emission measurements were performed by using Fluoromax-4P spectrophotometer (Horiba Jobin Yvon). Absorption and fluorescence measurement were performed by using quartz cuvette having path length of 1 cm. The fluorescence quantum yield ( $\phi_f$ ) of 7-DCCA in different systems were measured using the fluorescence quantum yield ( $\phi_f$ ) of Coumarin 480 in water solution ( $\phi_r = 0.66$ ) as reference [36]. The fluorescence quantum yields ( $\phi_f$ ) of the present systems were obtained by using the following Eq. (1)

$$\phi_s = \phi_r \times \left( \frac{I_s A_r n_s^2}{I_r A_s n_r^2} \right) \quad (1)$$

where,  $s$  and  $r$  represent the studied sample and reference, respectively. Here  $A$ ,  $I$  and  $n$  represent the absorbance of the sample at excitation wavelength, the integrated area under the fluorescence curve and the refractive index of the medium, respectively.

**2.2.1.2. Time-resolved fluorescence measurements.** The fluorescence time resolved decays were collected by using a picosecond time-correlated single-photon counting (TCSPC) technique at temperature 298 K. We have used a time-resolved fluorescence spectrophotometer from Edinburgh Instruments (model: Life Spec-II, U.K.). We have used picoseconds diode laser with excitation wavelength at 405 nm. The full width at half maximum (FWHM) of our system is ~80 ps. The fluorescence transients were detected at magic angle ( $54.7^\circ$ ) polarization using Hamamatsu MCP PMT (3809U) as a detector. The fluorescence emission decays are fitted by using the following expression (Eq. (2)) after deconvoluting IRF;

$$I(t) = A + \sum_{i=1}^N B_i \exp(t/\tau_i) \quad (2)$$

The parameter  $A$  and  $B_i$  represent the background and the pre-exponential factor along with the characteristic lifetime  $\tau_i$ , respectively.

The time-resolved emission decays were analyzed with the help of F900 software. The fluorescence anisotropy decays ( $r(t)$ ) were measured by using the same instrument. The following equation was used to obtain the value of  $r(t)$ :

$$r(t) = \frac{I_{\parallel}(t) - G I_{\perp}(t)}{I_{\parallel}(t) + 2G I_{\perp}(t)} \quad (3)$$

where,  $I_{\parallel}$  and  $I_{\perp}$ , represent the emission intensities at parallel and perpendicular polarizations, were collected alternatively by fixing the time for both the decays, respectively. We have used motorised polarizer's to collect the parallel and perpendicular decays. We have used F-900 software to analyze the anisotropy decays.

**2.2.1.3. Isothermal titration calorimetry.** We have used iTC200 microcalorimeter from GE healthcare for isothermal titration calorimetry measurement. Temperature was kept constant at 298 K throughout the measurement.

**2.2.1.4. Nuclear magnetic resonance.**  $^1\text{H-NMR}$  spectral data were collected by Bruker 400 MHz NMR spectrometer in MeOD and D<sub>2</sub>O at room temperature.

**2.2.1.5. pH of the solution** was 6.4. pH of the solution was measured by using a pH meter from Oakton Instrument (model: pH 700) using a microelectrode.

## 2.2. Computational study

Structures of 7-DCCAЕ, Cucurbit[7]uril, Cucurbit[8]uril and the host–guest complexes have been optimized by using B3LYP/STO-3G level of theory and the frequency calculation also has done at the same level of theory. All theoretical calculations have been performed by using Gaussian 09 and Gauss view 03 packages [37,38]. For all cases we found that the number of imaginary frequency is zero which confirms the complexes are energy minima structures.

## 3. Results & discussion

### 3.1. Steady-state absorption studies

7-DCCAЕ shows characteristic absorption maximum at 444 nm in aqueous medium as per the earlier reports [34,35,39]. Bathochromic shift was observed with gradual addition of CB7 to the aqueous solution of 7-DCCAЕ, but the change in absorbance value is very small on gradual addition of CB7. The changes in the absorption spectra of 7-DCCAЕ on addition of CB7 are shown in Fig. 1(a). On final addition of  $2.1 \times 10^{-5}$  (M) CB7 to the aqueous solution of 7-DCCAЕ, we observed 8 nm bathochromic shift with respect to the absorbance maximum of the

aqueous solution of 7-DCCAЕ. The changes in spectral feature indicate that there must be some changes in the environment around 7-DCCAЕ in the presence of CB7. The observed changes in absorbance spectra may be due to the interaction taken place between 7-DCCAЕ and CB7. Now the interesting question is about why bathochromic shift was observed in case of CB7 but not hypsochromic shift like in case of  $\beta$ -CD and  $\gamma$ -CD [39]. To explain it, we have to take under consideration about two factors, one is usual hydrophobic interaction along with charge-dipole type of interaction. So, cyclodextrins can provide hydrophobic type of interaction towards the guest molecule as well as hydrogen bonding but not able to provide the charge-dipole or dipole-dipole type of interaction towards the guest molecule, hence it is expected that the complex is formed between 7-DCCAЕ with CB7 is more stronger as compared to the complex formed between 7-DCCAЕ with  $\beta$ -CD and  $\gamma$ -CD, respectively. Here, the bathochromic shift of absorbance maximum of 7-DCCAЕ in the presence CB7 may be observed due to hydrophobic interaction in the association with the charge-dipole type interaction. Such phenomenon was explained by considering the decrease of the polarity around the coumarin molecule and it will destabilized the ground state leading to the observed bathochromic effect [27,40]. In case of CB8, very small bathochromic shift in absorbance maximum along with a change in the absorption spectral shape of the guest molecule was observed (Fig. 1(b)). CB8 can provide hydrophobic environment towards the guest molecule due to the presence of hydrophobic cavity and can also provide charge-dipole or dipole-dipole type interaction due to the presence of carbonyl portal ends on both side of its cavity. From the changes in absorbance spectral features of 7-DCCAЕ in presence of both the macrocyclic hosts, we can say that the interaction may be taken place between 7-DCCAЕ with CB7 and CB8 in aqueous medium, respectively. When 7-DCCAЕ presents in aqueous medium its ground state stabilization energy is more but when CBn were added the polarity around 7-DCCAЕ was reduced as a result of which ground state stabilization energy of 7-DCCAЕ is reduced. Since cavity size of CB8 is higher than CB7 and number of high energy water molecule is more in CB8 compare to CB7 [3]. In the case of CB8 more number of water molecules remains inside the cavity as compared to CB7 due to formation of host-guest complex, as result of which the polarity sensed by 7-DCCAЕ inside the cavity of CB8 is more as compared to CB7. It may be the possible reason for greater bathochromic shift of 7-DCCAЕ in presence of CB7 compared to CB8.

### 3.2. Steady state fluorescence emission studies

7-DCCAЕ shows an emission maximum ~480 nm in aqueous solution (Fig. 2). 7-DCCAЕ has weak fluorescence intensity ( $F$ ) in aqueous medium. The fluorescence quantum yield ( $\phi_f$ ) of the aqueous solution of 7-DCCAЕ was found to be 0.01. It was observed that the fluorescence intensity ( $F$ ) gradually increased with increase in the concentration of

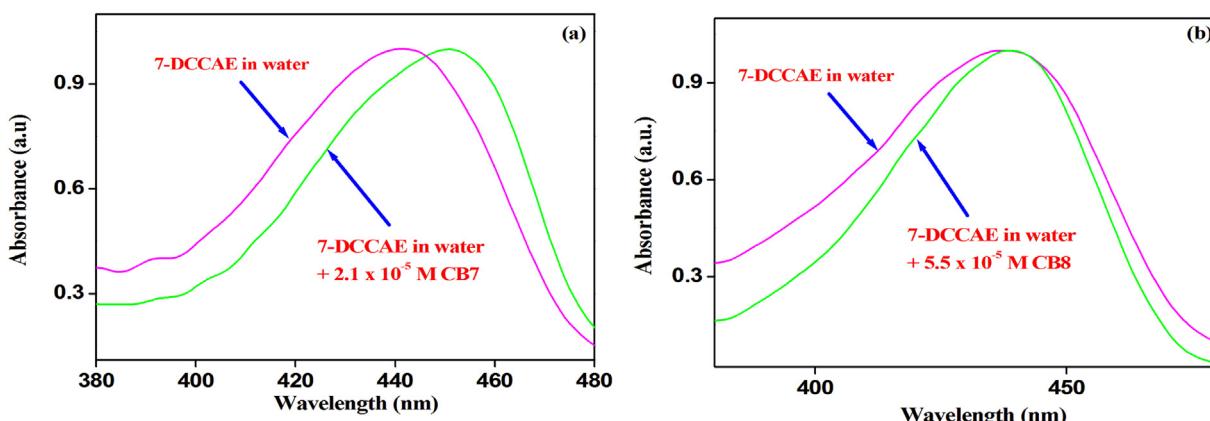
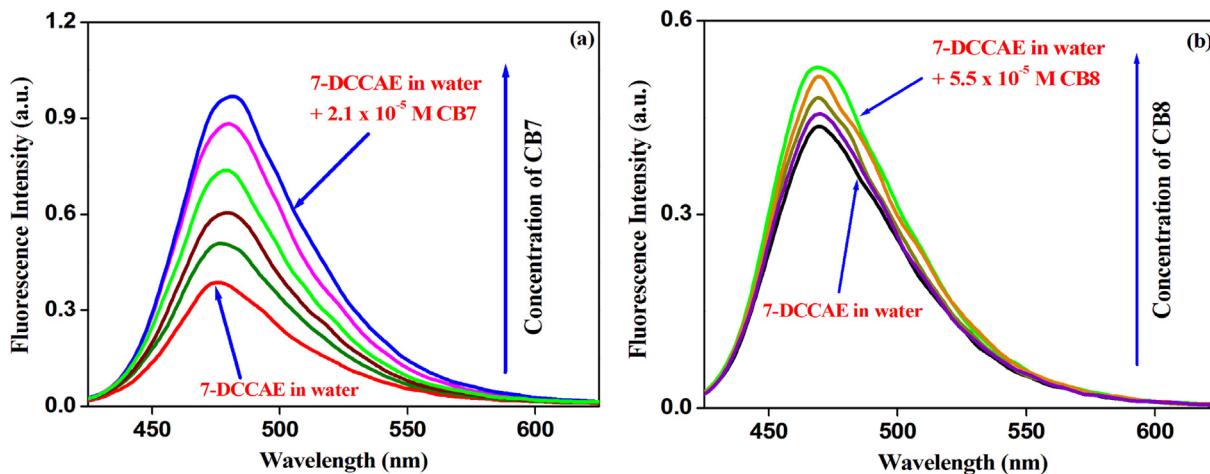


Fig. 1. The absorption spectral changes of aqueous solution of 7-DCCAЕ with addition of (a) CB7 and (b) CB8.



**Fig. 2.** The fluorescence emission spectral changes of the aqueous solution of 7-DCCAЕ with varying concentration of (a) CB7 and (b) CB8 ( $\lambda_{\text{ex}} = 445 \text{ nm}$ ).

CB7 to the aqueous solution of 7-DCCAЕ (Fig. 2(a)). The  $\phi_f$  value of 7-DCCAЕ was found to be 0.04 on addition of  $2.1 \times 10^{-5}$  (M) CB7 to the aqueous solution of 7-DCCAЕ. The significant changes in photophysical properties of 7-DCCAЕ indicates that it present in different microenvironment as compared to aqueous medium. Such changes in the microenvironment are only possible when interaction taking place between 7-DCCAЕ and CB7, where the guest molecule facing more restricted environment as compared to the aqueous medium. The increase in  $\phi_f$  may be due to the reduction of different non-radiative processes. The increase in  $\phi_f$  value may be due to retardation of twisting rotation around the single bond (a) (Scheme 2). Here, we have considered that the complexation of 7-DCCAЕ with macrocyclic host takes place through —N(Et)<sub>2</sub> group. Again when the complex is formed between 7-DCCAЕ and CB7, the nitrogen atom of —N(Et)<sub>2</sub> preferred to have more positive charge density due to the close approximation of carbonyl portal of CB7 having negative charge density and for which the stronger interaction is taken place between them. Hence, the guest molecule was strongly associated with macrocyclic host, for this reason twisting rotation around the bond (a) is highly restricted in the presence of CB7.

The considerable changes in fluorescence emission spectra of 7-DCCAЕ were observed on addition of CB8 to the aqueous solution of 7-DCCAЕ. The fluorescence intensity ( $F$ ) gradually increases with increase in the concentration of CB8 to the aqueous solution of 7-DCCAЕ (Fig. 2(b)). The quantum yield value ( $\phi_f$ ) was increased to 0.013 on addition of  $5.5 \times 10^{-5}$  (M) of CB8. The increase in fluorescence intensity ( $F$ ) as well as  $\phi_f$  value were observed due to the interaction between 7-DCCAЕ and CB8. The increase in  $\phi_f$  indicates that 7-DCCAЕ faces more

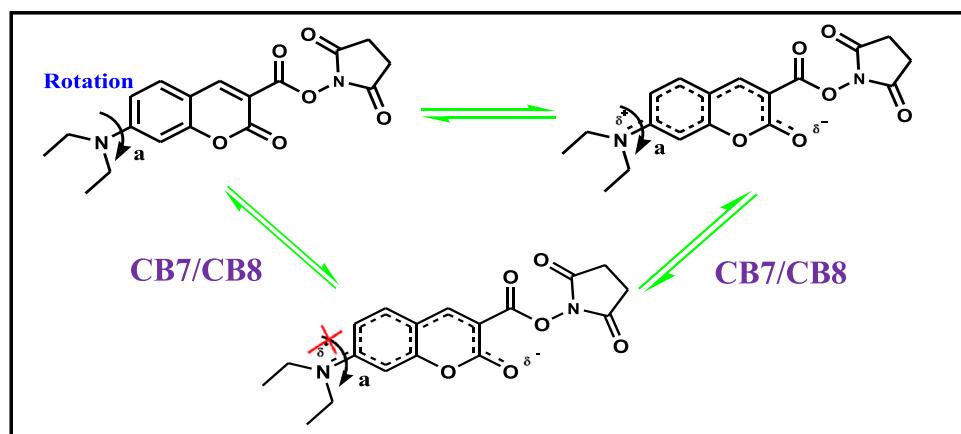
restricted environment as compared to free 7-DCCAЕ in aqueous medium. The increase in fluorescence intensity ( $F$ ) may also be possible due to decrease in non-radiative decays. If we compare the  $\phi_f$  value of 7-DCCAЕ in presence of CB7 and CB8, it was found that the  $\phi_f$  was higher in presence of CB7 as compared to CB8. To explain it, we have to take under consideration about the hydrophobic cavity size and terminal inner cavity diameter. The hydrophobic cavity size as well as terminal inner cavity diameter is smaller for CB7 as compared to CB8. Hence, it can expect that 7-DCCAЕ faces more restricted microenvironment on complexation with CB7 as compared to CB8. One most interesting observation is about the changes in fluorescence properties of 7-DCCAЕ was very remarkable on addition of CB7 and CB8 in the order of  $10^{-5}$  (M) whereas, in case of cyclodextrins,  $10^{-3}$  (M) concentration were needed to observe the considerable changes [39].

We have carried out Job's method of continuous variation to find out the stoichiometry of the host-guest complex from fluorescence emission measurements. Total concentration of 7-DCCAЕ and CBn was maintained at  $1 \times 10^{-5}$  M. The Jobs plots were shown in Fig. 3. From these plots it was observed that 7-DCCAЕ for 1:1 complex with CB7 and CB8.

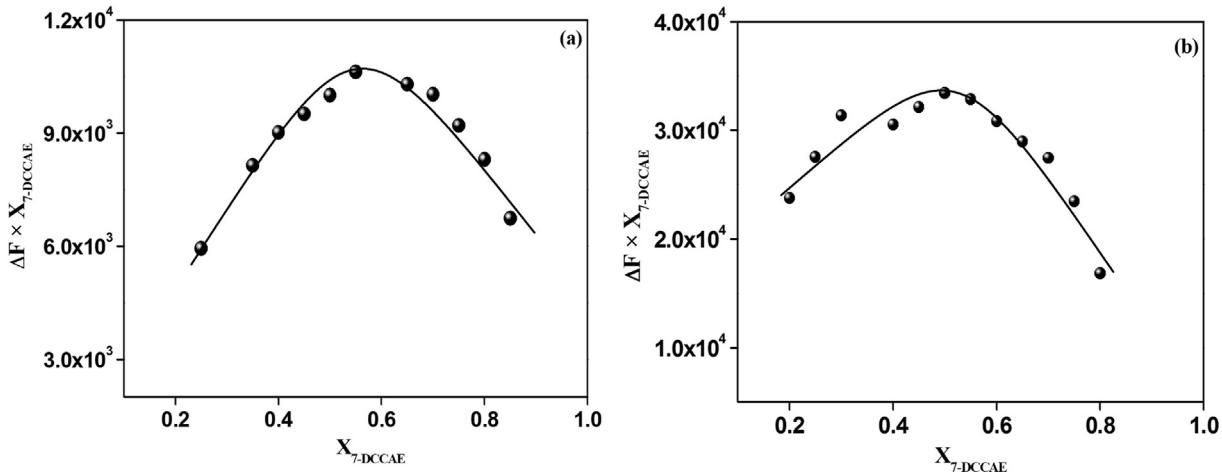
To describe the host-guest interaction between 7-DCCAЕ with CB7 and CB8, the following equilibrium has been considered;



where,  $K$  represents the binding constant between the host and the guest molecule. The binding strength between the host and guest



**Scheme 2.** Charge redistribution in the excited state of 7-DCCAЕ in aqueous medium and in presence of macrocyclic hosts.



**Fig. 3.** Job's plot to find the stoichiometry of the complex formed between 7-DCCAЕ with (a) CB7 and (b) CB8 using fluorescence measurements.

molecule is one of the important parameter from which we can draw an idea about how strongly they are associated with each other. The binding constant for the formation of host-guest complexes were estimated from the changes observed in fluorescence intensity. The binding constant value ( $K$ ) of host-guest complexes was estimated by using the nonlinear least-squares regression analysis by using fluorescence data, where the experimental data are directly fitted by using the relevant equation. The Eq. (4) was used to estimate the binding constant of the 1:1 (7-DCCAЕ-CBn) complexes [41]:

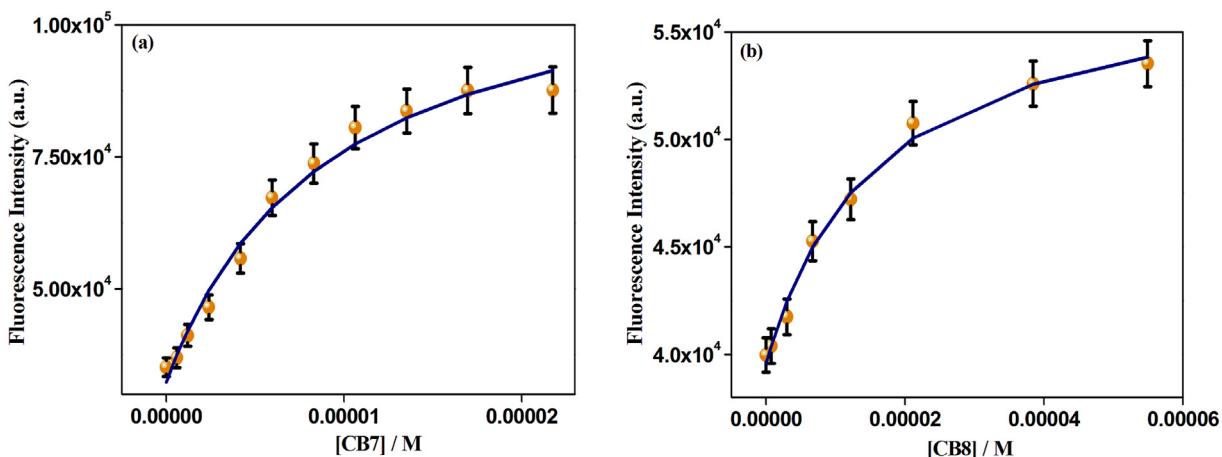
$$F = \frac{F_0 + F_{\max}K[CBn]}{1 + K[CBn]} \quad (4)$$

where,  $F_0$  represents the fluorescence intensities of the aqueous solution of 7-DCCAЕ in absence of CB7.  $F_{\max}$  is the fluorescence intensity in presence of maximum concentrations of macrocyclic host (CB7 or CB8), where 1:1 binding has been completed and  $F$  is the fluorescence intensities at different concentrations of CB7 or CB8. The term  $[CBn]$  represents concentration of CB7 or CB8 in the medium. By using the fluorescence data, it was observed that 7-DCCAЕ forms 1:1 complex with CB7 and CB8, respectively. We have obtained well fitted curve with good correlation coefficient ( $R^2$ ) value of 0.99 and 0.99 for 7-DCCAЕ with CB7 and CB8, respectively (Fig. 4). The binding constant values of 7-DCCAЕ with CB7 and CB8 were found to be  $3.69 (\pm 0.40) \times 10^5 \text{ M}^{-1}$  and  $6.26 (\pm 1.14) \times 10^4 \text{ M}^{-1}$ , respectively.

The binding constant value of 7-DCCAЕ-CB7 or 7-DCCAЕ-CB8 is much more higher than that of the binding constant value of 7-DCCAЕ-CDs system [39]. In our present studies, the binding strength were observed in the order of  $\sim 10^5 \text{ M}^{-1}$ , whereas the binding order for 7-DCCAЕ-CD is in the order of  $\sim 10^2 \text{ M}^{-1}$  i.e. approximately thousand times higher for 7-DCCAЕ-CBn. Hence, we can conclude from our present study that both CBn can use as stronger macrocyclic hosts for encapsulation of 7-DCCAЕ as compared to  $\beta$ -CD and  $\gamma$ -CD. The binding constant value of the 7-DCCAЕ-CB7 complex was found to be greater as compared to 7-DCCAЕ-CB8 complex. To explain it, we assumed that  $-\text{N}(\text{Et})_2$  group is present inside the hydrophobic cavity and for this reason the TICT process hindered. It reinforces the concept that  $-\text{N}(\text{Et})_2$  terminal of the guest are residing inside the hydrophobic cavities of CBn forming 1:1 complex. The terminal inner cavity diameters are  $5.4 \text{ \AA}$  and  $6.9 \text{ \AA}$  for CB7 and CB8, respectively [3]. The terminal inner cavity diameter of CB7 is smaller as compared to the terminal inner diameter of CB8. Hence, it is expected that the binding strength is higher in case of 7-DCCAЕ-CB7 complex as compared to 7-DCCAЕ-CB8.

### 3.3. $^1\text{H}$ NMR studies of 7-DCCAЕ-CBn host-guest complexes

To understand the host-guest complex formation between 7-DCCAЕ with CBn, we have used  $^1\text{H}$  NMR spectroscopy. We have analyzed the  $^1\text{H}$  NMR spectra of 7-DCCAЕ in  $\text{CD}_3\text{OD}$  solution whereas, the host-guest interaction between 7-DCCAЕ with CB7 was analyzed in  $\text{D}_2\text{O}$ . 7-DCCAЕ is



**Fig. 4.** (a) The binding interaction between 7-DCCAЕ with CB7 forming 1:1 complex and (b) the binding interaction between 7-DCCAЕ with CB8 forming 1:1 complex. These plots were obtained from experimental fluorescence data and the fitted curves were obtained by using Eq. (4) with very good  $R^2$  value.

very weakly soluble in D<sub>2</sub>O, hence we are not able to get good spectra in D<sub>2</sub>O. We observed that the protons present at position 'a' show upfield shift around  $\delta = 1.27$  to 0.50 ppm and the protons present at 'b' show upfield shift,  $\delta = 3.58$  to 2.78 ppm (Fig. 5). We also observed upfield shift for the protons present at 'd' and 'g' from  $\delta = 6.58$  to 6.38 ppm and 6.86 to 6.26 ppm respectively (Fig. 5). The proton present at 'e' ( $\delta = 7.62$  ppm) does not show any noticeable chemical shift in presence of CB7. The proton present at 'f' ( $\delta = 8.62$ ) shows slightly downfield shift to 8.78 ppm (Fig. 5). We are not observed any shift for protons present at 'c' in presence of CB7 ( $\delta = \sim 2.91$ ) (Fig. 5). Therefore, the complexation is taken place from the side of —N(Et)<sub>2</sub> moiety and there is no complexation through the side of ester moiety. Therefore 1:1 complex is formed between 7-DCCAЕ with CB7. In 7-DCCAЕ-CB7 complex, —N(Et)<sub>2</sub> moiety and some part of benzene ring (at which —N(Et)<sub>2</sub> moiety is attached) are present inside the hydrophobic cavity of CB7. The host-guest interaction of 7-DCCAЕ with CB8 was analyzed in D<sub>2</sub>O. In the presence of CB8 we have observed that the protons present at position 'a' and 'b' show upfield shift from  $\delta = 1.27$  to 0.55 ppm and  $\delta = 3.58$  to 2.88 ppm, respectively (Fig. 5). The protons present at 'd', 'g' and 'e' from  $\delta = 6.58$  to  $\sim 5.90$  ppm,  $\delta = 6.86$  to  $\sim 5.94$  ppm and  $\delta = 7.62$  to 7.22, respectively (Fig. 5). The proton present at 'f' ( $\delta = 8.74$ ) shows the downfield shift to 8.88 ppm (Fig. 5). No shift observed for the protons present at 'c' ( $\delta = \sim 2.91$ ) (Fig. 5). Therefore, the complexation is taking place between 7-DCCAЕ and CB8 through the side of —N(Et)<sub>2</sub> moiety not through ester moiety. Hence, this study confirmed that 1:1 host-guest complex is formed between 7-DCCAЕ with macrocyclic host CB8. The probable structure of the complexes on the basis of <sup>1</sup>H NMR is shown in Scheme 3.

### 3.4. Time resolved fluorescence measurements

Time resolved fluorescence measurements serve as a very sensitive indicator for the deeper understanding about the changes in environment around the guest molecule. For this reason it is useful tool for understanding the host-guest interaction. Fluorescence decay pattern of 7-DCCAЕ in aqueous medium and in presence of CBn with residuals of fitting are shown in Figs. 6 and S1. All the emission decays profile of 7-DCCAЕ in presence of CB7 and CB8 are fitted by tri-exponential function (Table 1). The average fluorescence lifetime value of 7-DCCAЕ in aqueous medium is found to be  $\sim 45$  ps [34]. The average fluorescence lifetime value of 7-DCCAЕ in presence of  $2.1 \times 10^{-5}$  (M) CB7 was found to be 350 ps (Table 1). The change in fluorescence lifetime value of each components along with change in their relative amplitude in presence of CB7 indicates that there must be some change in environment around the guest molecule and this change in environment around the guest molecule is due to the host-guest complex formation between 7-DCCAЕ with CB7 (Table 1). The average fluorescence lifetime value of 7-DCCAЕ was found to be 175 ps in presence of  $5.5 \times 10^{-5}$  (M) CB8 (Table 1). Here also, we observed that the fluorescence lifetime value as well as their relative amplitude of each component was found to be significantly modified on addition of  $5.5 \times 10^{-5}$  (M) CB8 (Table 1). The average lifetime value of 7-DCCAЕ molecule was found to be 88 ps and 270 ps in the presence of  $\beta$ -CD and  $\gamma$ -CD, respectively [39]. Here, the changes were observed due to the complex formation between 7-DCCAЕ with CBn. The changes in fluorescence lifetime value are found to be more in presence of CB7 as compared to CB8. To explain this, we have to take the cavity size as well as the terminal inner

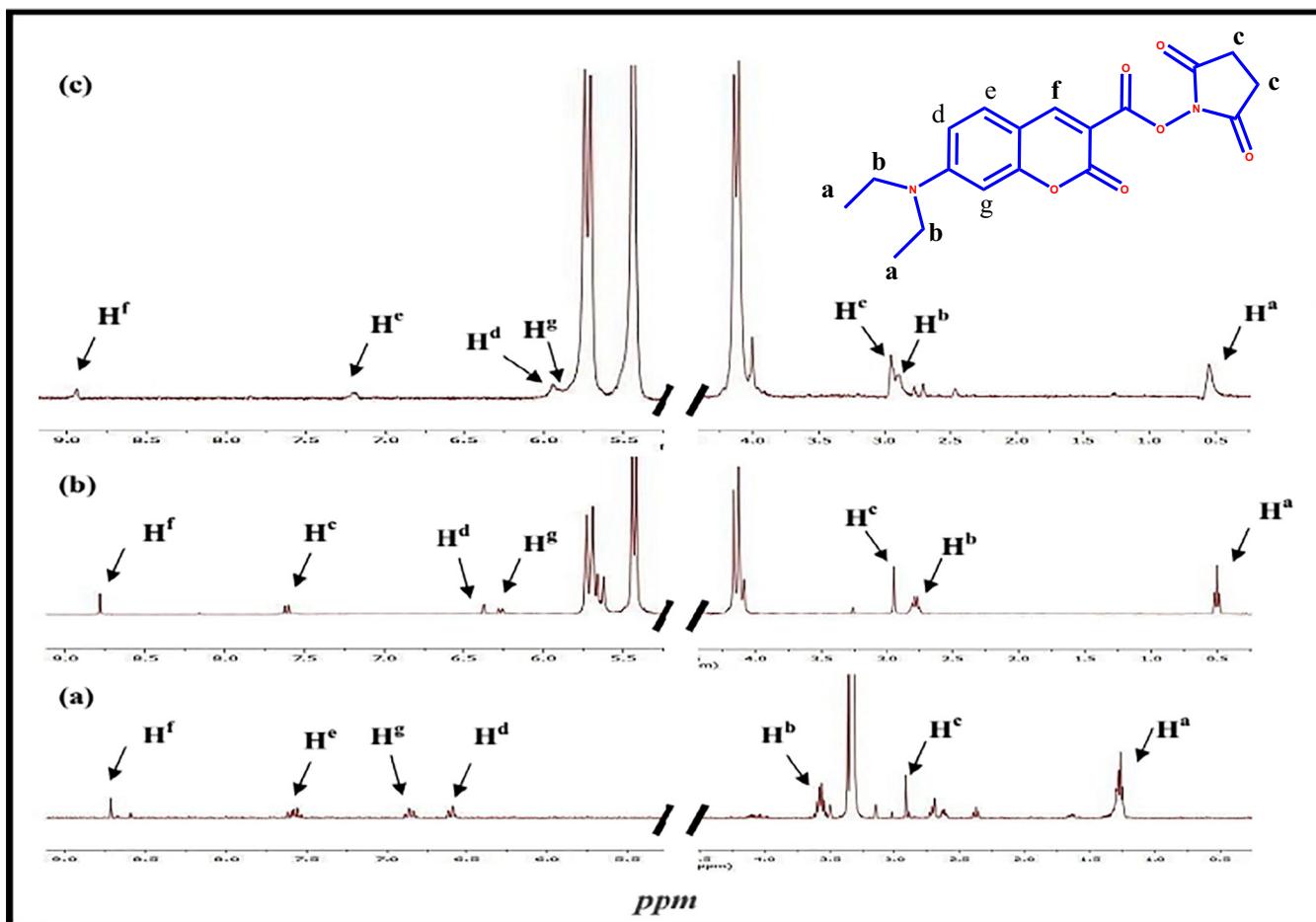
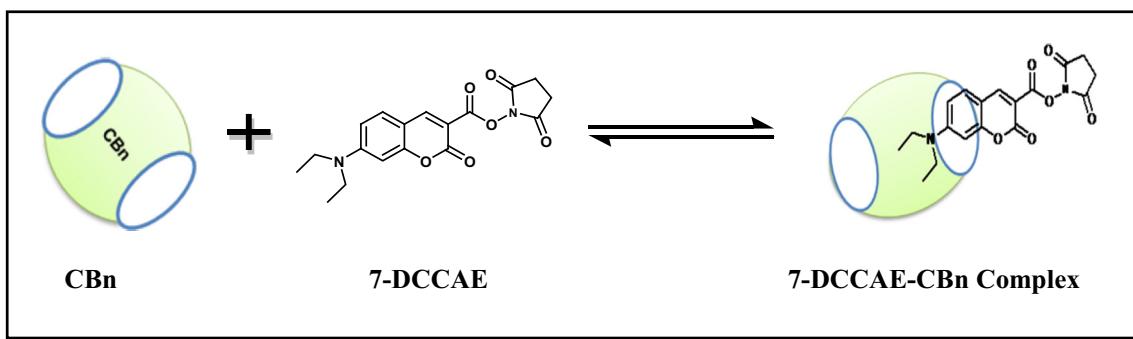


Fig. 5. <sup>1</sup>H NMR spectra (400 MHz) of (a) 7-DCCAЕ (solvent: MeOD) and different complexes of (b) CB7 and (c) CB8 in D<sub>2</sub>O.



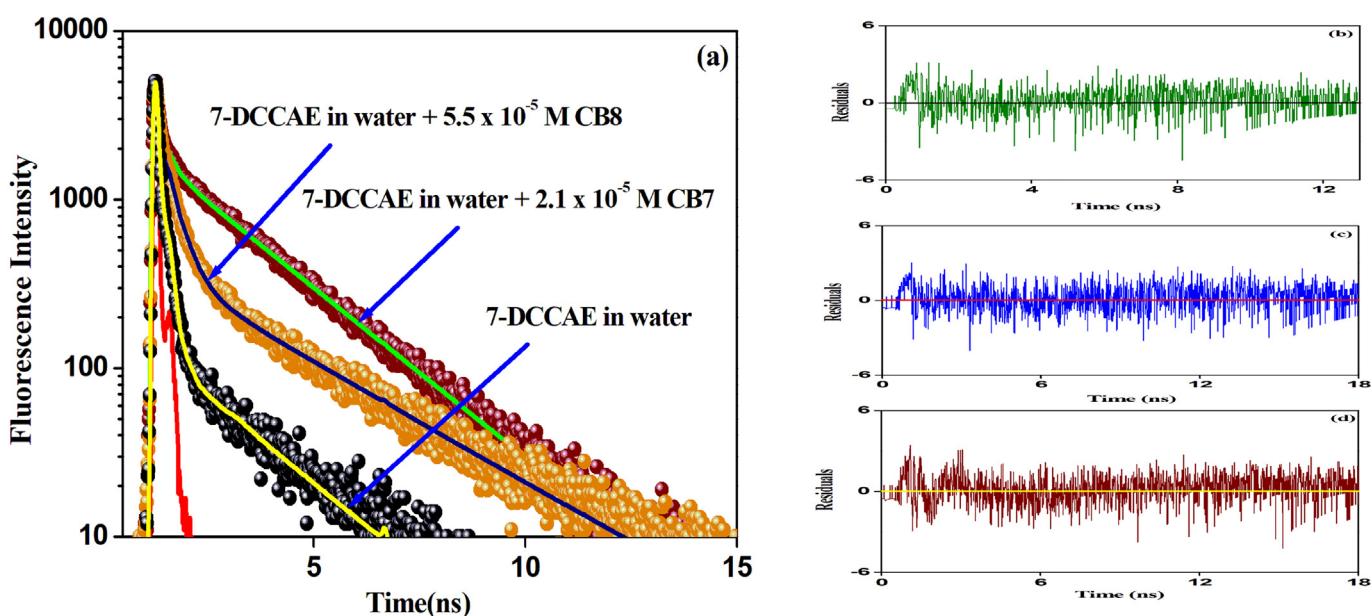
**Scheme 3.** Schematic representation of probable structure of the complex between 7-DCCAЕ with CB7 and CB8, respectively.

diameter of host molecules under consideration. CB7 has more suitable terminal inner diameter for the guest molecule as compared to the CB8, again the binding constant value (obtained from fluorescence measurement) of 7-DCCAЕ-CB7 is greater as compared to the binding constant value of 7-DCCAЕ-CB8. It indicates that the guest molecule is strongly associated in case of 7-DCCAЕ-CB7 complex as compared to 7-DCCAЕ-CB8 complex. From the fluorescence lifetime measurements, the significant increase of lifetime values of 7-DCCAЕ in presence of both the macrocyclic host is clearly depict that the host-guest complex was formed between 7-DCCAЕ with CB7 and CB8.

### 3.5. Time resolved fluorescence anisotropy measurements

In order to get the deeper understanding about the microenvironment around the guest molecule, we have performed fluorescence anisotropy measurement in the presence of CB7 and CB8 (Fig. 7). The initial anisotropy ( $r_0$ ) value depends upon the angle between absorption and emission transition dipoles. The initial value of anisotropy ( $r_0$ ) in all cases was found smaller than 0.4, it clearly indicates that the ultrafast change in emission transition moment direction or may be the occurrence of very fast depolarisation which is outside of the time resolution of our TCSPC system. The rotational relaxation time of 7-DCCAЕ in aqueous medium was fitted by single exponential function and the value of rotational relaxation time was found

to be 200 ps [39]. When we added  $2.1 \times 10^{-5}$  (M) CB7 to the aqueous solution of 7-DCCAЕ, the rotational relaxation time was found to be increased significantly to 360 ps. The anisotropy decay of 7-DCCAЕ in the presence of CB7 was fitted by single exponential function. Such significant modulation in rotational relaxation time of 7-DCCAЕ on addition of CB7, clearly depicts that the changes in environment around 7-DCCAЕ molecule. Such changes in microenvironment may due to the interaction between 7-DCCAЕ and CB7. The amplification of rotational relaxation time of 7-DCCAЕ in presence of CB7 implies that the guest molecule faces more restricted environment as compared to the aqueous medium. In case of CB8, the rotational relaxation time of 7-DCCAЕ is also significantly modified. The anisotropy decay of 7-DCCAЕ in presence of CB8 was fitted by bi-exponential function and the component were found to be 80 ps (33%) and 490 ps (67%). Such modification in rotational relaxation time of 7-DCCAЕ in presence of CB8 is observed due to the complex formation between 7-DCCAЕ and CB8. As 7-DCCAЕ faces restricted environment in presence of macrocyclic host for which the twisting process is hindered. The trend of change of rotational relaxation time of 7-DCCAЕ in presence of studied CB's are similar to that of 7-DCCAЕ in presence of CD's [39]. The changes found in rotational relaxation time of 7-DCCAЕ molecule in presence of both the macrocyclic hosts clearly indicates that the interaction is taking place between 7-DCCAЕ with CB7 and CB8, respectively.



**Fig. 6.** (a) The fluorescence emission decays of 7-DCCAЕ in aqueous solution, in presence of CB7 and CB8, respectively. Residual of fitting for (b) 7-DCCAЕ in neat water, (c) 7-DCCAЕ with CB7, and (d) 7-DCCAЕ with CB8.

**Table 1**

The fluorescence lifetime components of 7-DCCAЕ in presence of CB7 and CB8.

System	$\lambda_{\text{ex}}$ (nm)	$\lambda_{\text{emi}}$ (nm)	$\tau_1$ (ps)	$a_1$	$\tau_2$ (ps)	$a_2$	$\tau_3$ (ps)	$a_3$	$\langle \tau \rangle^{\text{a}}$ (ps)	$\chi^2$
7-DCCAЕ + 21 ( $\mu\text{M}$ ) CB7	405	480	55 ( $\pm 4\%$ )	0.793	280 ( $\pm 5\%$ )	0.074	2140 ( $\pm 1.0\%$ )	0.133	350	1.10
7-DCCAЕ + 55 ( $\mu\text{M}$ ) CB8	405	476	45 ( $\pm 3\%$ )	0.799	335 ( $\pm 2\%$ )	0.174	3000 ( $\pm 1.0\%$ )	0.027	175	1.13

$$\langle \tau \rangle^{\text{a}} = a_1 \tau_1 + a_2 \tau_2 + a_3 \tau_3.$$

Additional benefit of fluorescence anisotropy measurements is the estimation about the hydrodynamic radius of the host-guest complex. The hydrodynamic radius can be obtained by using the following Stokes-Einstein-Debye (SED) equation (Eq. (5));

$$\tau = \frac{4\pi\eta r_h^3}{3KT} \quad (5)$$

where,  $\tau$  and  $r_h$  represent time constant of the slower component of the anisotropy decays and hydrodynamic radius, respectively.  $\eta$  and  $K$  represent viscosity of the medium and Boltzmann constant, respectively.  $T$  is temperature (298 K). By using this SED equation, the hydrodynamic radius of the complex was found to be 7.51 Å for 7-DCCAЕ-CB7 complex whereas 8.40 Å for 7-DCCAЕ-CB8 complex. Therefore, the diameter of the complexes are 15.16 Å and 16.80 Å for 7-DCCAЕ-CB7 and 7-DCCAЕ-CB8 complexes, respectively. From the diameter of the complex, it is confirmed that 1:1 complex is formed in both the cases because the diameter of the complex is higher than that of the diameter of the dye molecule as well as the height of both the macrocyclic host. The height of both macrocyclic hosts is 9.1 Å [21]. The estimated diameter of the 7-DCCAЕ molecule is 8.34 Å. Not only the idea about the stoichiometry of the complexes but it also gives an idea that the some portion of 7-DCCAЕ molecule is present inside the hydrophobic cavity of the macrocyclic host and rest of the part is present outside the cavity of the host molecule. The fluorescence anisotropy measurements help us for understanding about the host-guest complex formation between 7-DCCAЕ with the macrocyclic hosts, where 7-DCCAЕ molecule is partially encapsulated inside the cavity of both CB7 and CB8.

### 3.6. Study of binding thermodynamics of 7-DCCAЕ with CB7 and CB8 using isothermal titration calorimetric measurements

ITC technique is widely used by chemists and biochemists to understand the binding thermodynamics of interacting systems [42]. We can observe the change in different thermodynamic parameters due to the

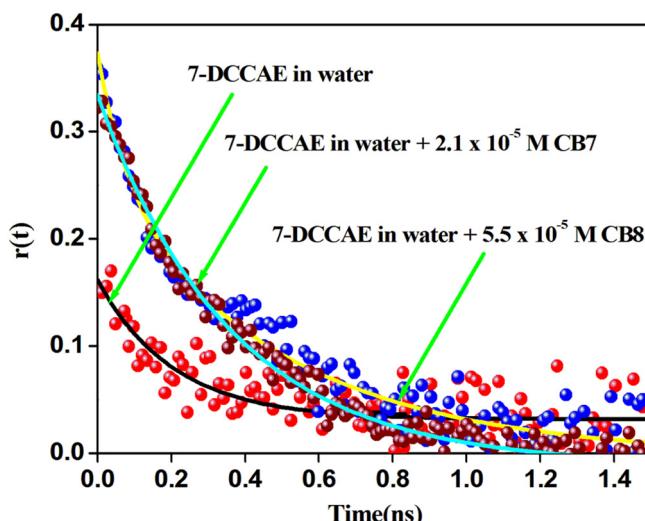
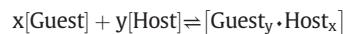


Fig. 7. The time resolved anisotropy decays of 7-DCCAЕ in aqueous solution and in the presence of CB7 and CB8, respectively.

complexation as well as the binding constant and from which we can draw up the idea about the driving forces involve for the complexation with the help of ITC measurement. This titration method also provides the change in thermodynamic parameters along with the nature of binding for the complexation of the guest molecule with macrocyclic host. The supramolecular host-guest interaction can proceed through equilibrium and the equilibrium can be represented by the following expression:



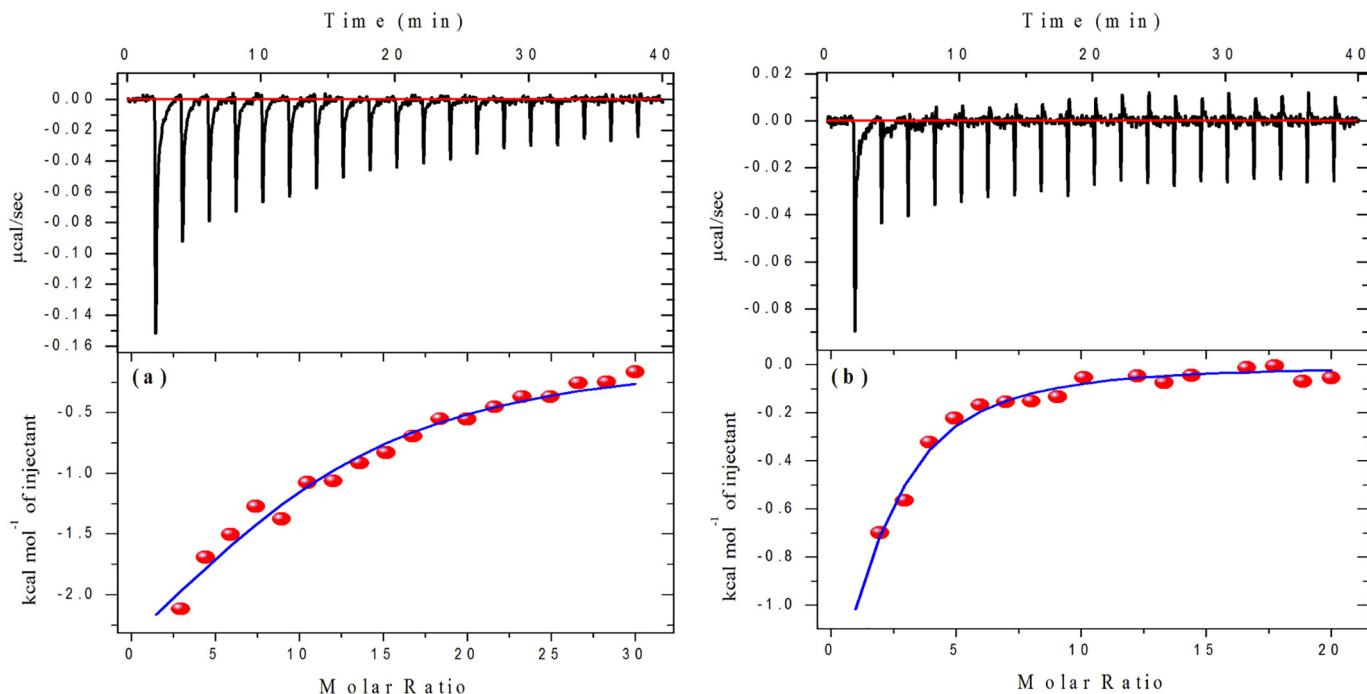
where,  $x$  and  $y$  are the stoichiometry coefficients ( $x, y = 1, 2, 3$ , etc.).

From ITC measurement, it was observed that the interaction is taking place between 7-DCCAЕ with both the macrocyclic hosts (CB7 and CB8) at 298 K (Fig. 8, Table 2). From ITC measurement, we observed that the complexation process of 7-DCCAЕ with CB7 is exothermic in nature (Fig. 8(a)). The complexation process is both enthalpy as well as entropy driven process. It was observed that 7-DCCAЕ forms 1:1 complex with CB7 at the studied temperature,  $T = 298$  K. The change in enthalpy and entropy at this temperature are given in Table 2. The binding strength of 7-DCCAЕ with CB7 is found to be  $7.31 \times 10^4 \text{ M}^{-1}$  from ITC measurement. The change in Gibb's free energy ( $\Delta G^0$ ) due to the host-guest complex formation is estimated by using the following thermodynamic expression:

$$\Delta G^0 = -RT \ln(K) \quad (6)$$

The  $\Delta G^0$  value was found to be  $-6.64 \text{ kcal} \cdot \text{mol}^{-1}$ . The negative value of  $\Delta G^0$  clearly indicates that the complexation process between 7-DCCAЕ with CB7 occurs spontaneously. 7-DCCAЕ forms 1:1 complex with CB8. The complexation process was found to be exothermic in nature (Fig. 8(b)). The complexation process between 7-DCCAЕ with CB8 is enthalpically as well as entropically favourable. The binding strength of 7-DCCAЕ with CB7 and CB8 were found to be  $7.31(\pm 2.21) \times 10^4 \text{ M}^{-1}$  and  $4.88(\pm 0.62) \times 10^5$ , respectively. The  $\Delta G^0$  value of complex formation between 7-DCCAЕ with CB7 and CB8 were found to be  $-6.64 \text{ kcal} \cdot \text{mol}^{-1}$  and  $-7.76 \text{ kcal} \cdot \text{mol}^{-1}$ , respectively. The negative value of  $\Delta G^0$  indicates that the complexation process is spontaneous. From previous study, we found that the binding strength of 7-DCCAЕ with  $\beta$ -CD and  $\gamma$ -CD are  $4410 \text{ M}^{-1}$  and  $8350 \text{ M}^{-1}$ , respectively [39]. Therefore, the binding strength of 7-DCCAЕ with CB7 and CB8 is found to be much more higher as compared to the binding strength of 7-DCCAЕ with  $\beta$ CD and  $\gamma$ CD.

The binding strength of 7-DCCAЕ-CB7 in ground state was found to lower as compared to 7-DCCAЕ-CB8. In order to explain this we have to take consideration about the thermodynamic parameters obtained from ITC measurement (Table 2). In general the positive value of  $\Delta S$  is due to the hydrophobic association, charge neutralization or protonation and the negative value of  $\Delta H$  is due to the columbic force of attraction through which charge neutralization occurs, van der Waals or H-bonding interaction [43]. From Table 2, we observed that the magnitude of  $\Delta S$  was smaller for 7-DCCAЕ-CB7 as compared to 7-DCCAЕ-CB8. It was reported that high energy water molecules are present inside the hydrophobic cavity of CBn [3]. CB8 have 10 or 12 numbers of water molecules inside its inner cavity [3]. Whereas, CB7 have 7 or 8 numbers of water molecules inside its inner cavity [3]. Therefore, more number of high energy water molecules present inside the inner cavity of CB8 as compared to CB7. Therefore, on encapsulation of the guest molecule



**Fig. 8.** (a) ITC isotherm for the injection of (a) CB7 solution and (b) CB8 solution in 7-DCCA solution at 298 K. Where, in upper part data points represent integrated heats of interaction as a function of molar ratio and the solid line represents the line of best fit and in upper part data points represent integrated heats of interaction as a function of molar ratio and the solid line represents the line of best fit.

inside the hydrophobic cavity of CBn, it is expected that water molecules are released from the cavity. From the  $\Delta S$  value it is expected that the guest molecule is penetrated inside the hydrophobic cavity of the CB8 and expels more number of water molecules as compared to CB7. The release of water molecules from the cavity of the CBn contribute to increase in entropy during complexation. Again the motion of the guest molecule is less restricted inside the hydrophobic cavity of CB8 as compared to CB7, as the cavity size as well as terminal diameter of CB8 is higher as compared to CB7. Now, consider the  $\Delta H$  value, we observed that magnitude of  $\Delta H$  value is higher for 7-DCCA-CB7 as compared to 7-DCCA-CB8 as the cavity size as well as the terminal diameter of CB7 is smaller as compared to CB8. Again the negative value of  $\Delta H$  is due to columbic force of attraction. Hence, the force of attraction is more in case of 7-DCCA-CB7 as compared to 7-DCCA-CB8 (Table 2). The obtained binding constant value from ITC measurement is due to the overall contribution from  $\Delta S$  and  $\Delta H$  and the overall contribution is more for 7-DCCA-CB8 as compared to 7-DCCA-CB7 in ground state.

### 3.7. The supramolecular complexation in ground state versus excited state

The binding constant value obtained from fluorescence measurements depicts the binding interaction in the excited state whereas; the binding constant value obtained from ITC measurement represents the binding interaction in ground state. Therefore, the binding interaction observed from fluorescence measurement represents the excited state interaction and fluorescence measurement associated with the local changes surrounding the guest molecule. The binding interaction observed from ITC measurement represents the interaction in ground state and ITC measurement is associated a global change in the property. The measured binding constant value from excited state and ground

state may be same if the hydration state of the interface was unchanged. Additionally, both the measurements represent the similar strength constant when the association involves two-state transition between free and bound molecules by following a lock and key or the rigid body mechanism. The spectroscopic signal change flashes the total population of free and bound molecules. In order to get deeper knowledge about the difference between ground state and excited state binding the following points we have to take under consideration [44].

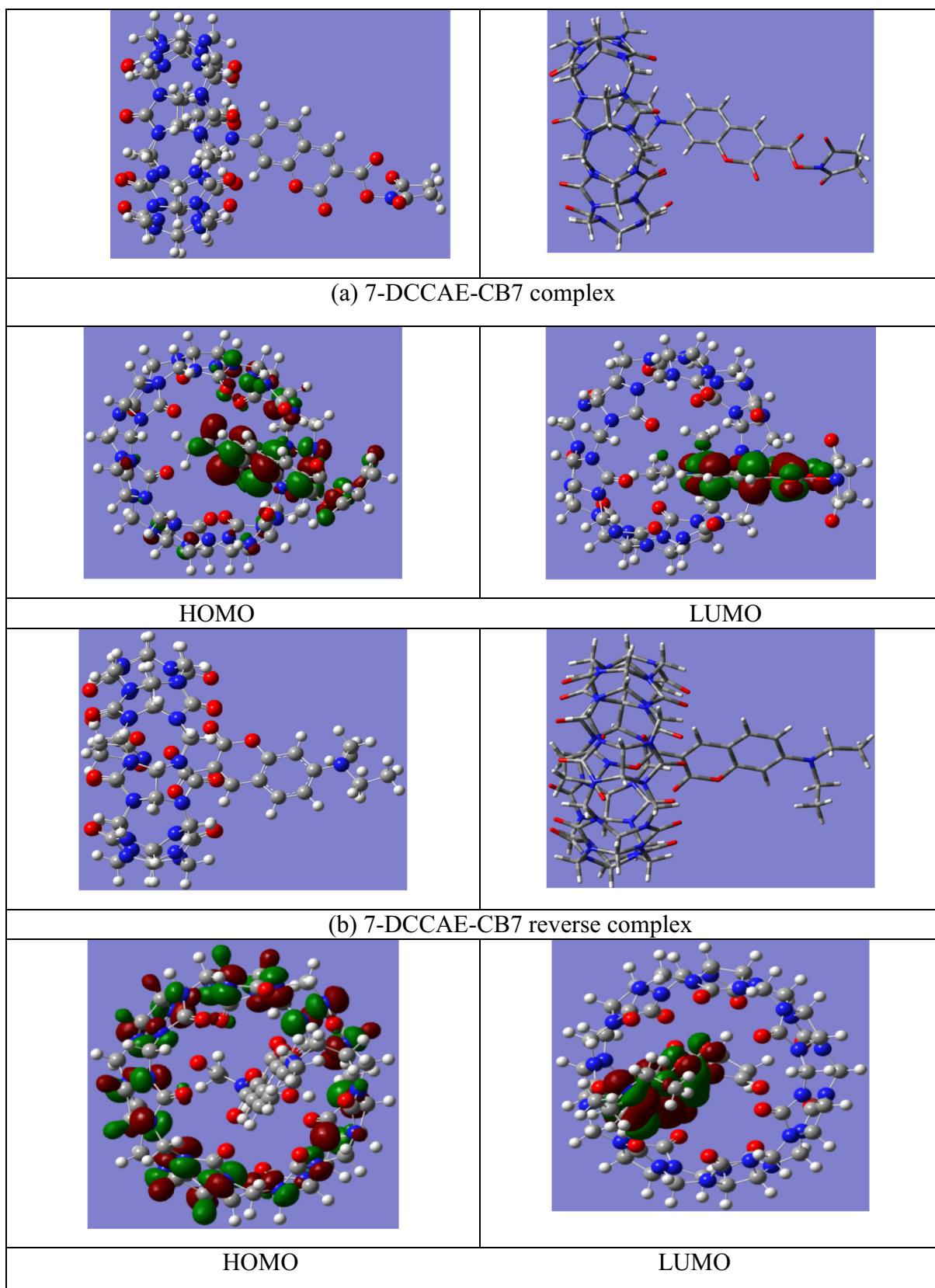
- i. The rate of inclusion of the guest inside the cavity of macrocycle.
- ii. The rate of exclusion of the guest from the cavity of the macrocycle.
- iii. The rate of decay of the excited state of the guest molecule.

Now, considering short lived excited state, the decay rate of excited state may be faster than the inclusion rate inside the host molecule or the exclusion rate of the guest molecule from the hydrophobic cavity of the host molecule. Hence, only those molecules will absorb light to remain in excited state which remains inside the hydrophobic cavity. Under such condition the exclusion rate, the inclusion rate and the binding interaction of the guest molecule with macrocyclic host will depend merely on the ground state interaction, although the electronic excitation may lead to destabilization of the supramolecular host-guest complex on absorption of light by the guest molecules. It will happen when the ground state binding strength is greater as compared to the excited state binding strength. For the excited state having long decay time, the guest molecule present outside of the hydrophobic cavity of the host molecule can enter inside the cavity of the macrocyclic host. Under this situation the inclusion rate and the exclusion rate of the guest molecule in excited state can be significantly different from ground state. It

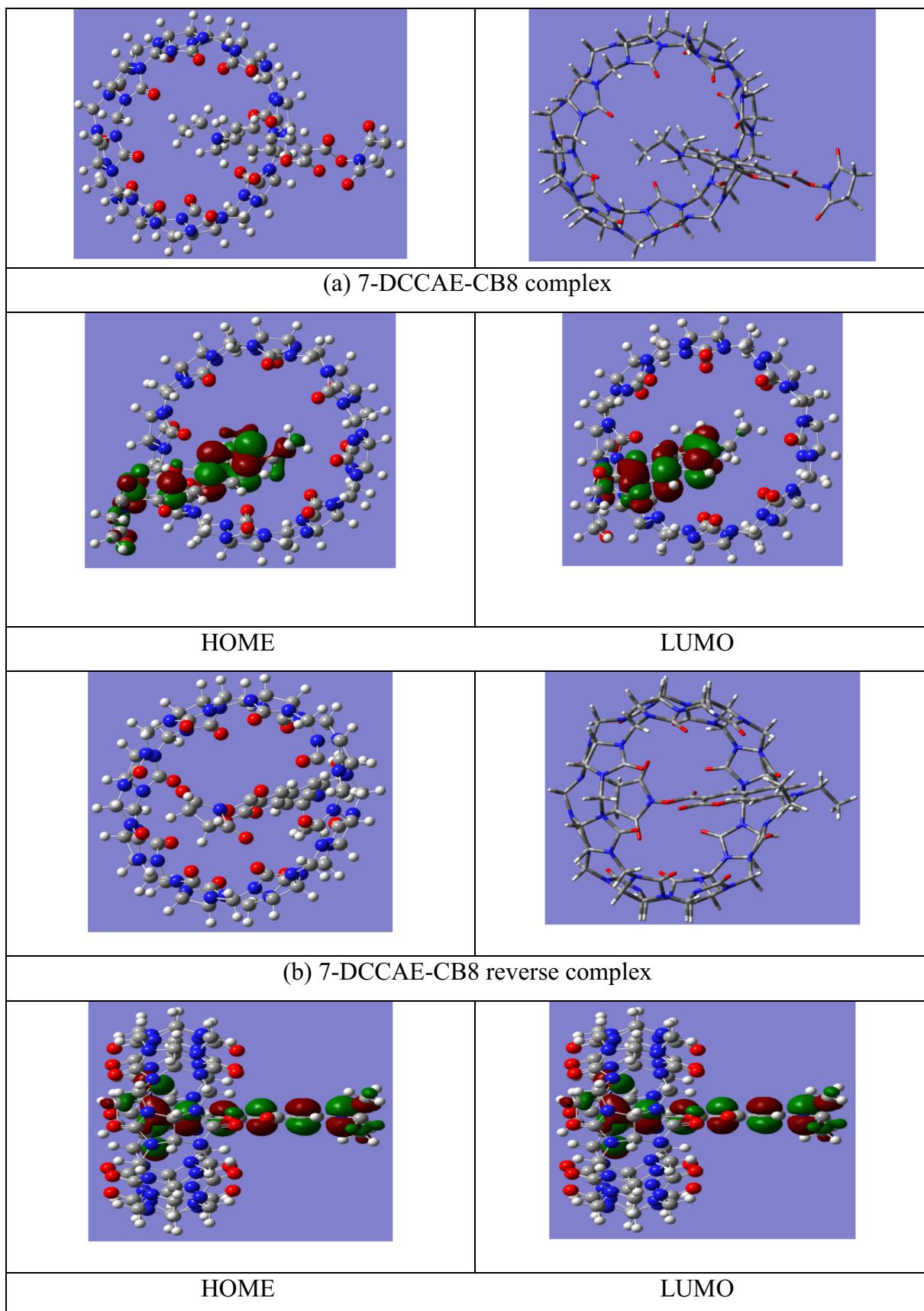
**Table 2**

The binding constant values for the binding interaction of 7-DCCA with CB7 and CB8, respectively ( $T = 298$  K) obtain from ITC measurement.

System	$K_{1:1} (\text{M}^{-1})$	$\Delta H (\text{kcal}\cdot\text{mol}^{-1})$	$\Delta S (\text{kcal}\cdot\text{mol}^{-1})$	$\Delta G^0 (\text{kcal}\cdot\text{mol}^{-1})$
7-DCCA + CB7	$7.31 (\pm 2.21) \times 10^4$	-3.86 ( $\pm 0.94$ )	2.78	-6.64
7-DCCA + CB8	$4.88 (\pm 0.62) \times 10^5$	-2.75 ( $\pm 2.2$ )	4.35	-7.76



**Fig. 9.** Optimized geometries and the frontier molecular orbital (FMO) pictures of the 7-DCCAЕ-CB7 complex and 7-DCCAЕ-CB7 reverse complex.



**Fig. 10.** Optimized geometries and the frontier molecular orbital (FMO) pictures of the 7-DCCAЕ-CB8 complex and 7-DCCAЕ-CB8 reverse complex.

may also lead to the difference of the binding interaction in ground state form excited state. A supramolecular complex is found to be stable when rate of inclusion ( $k_{in}$ ) is greater as compared to rate of exclusion ( $k_{out}$ ) and the binding constant value can be well represented as  $K = \frac{k_{in}}{k_{out}}$  [45]. The binding constant value of host-guest complex can be represented by the following equation;

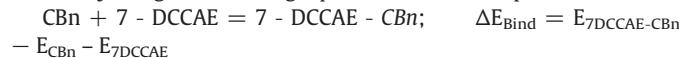
$$K = \frac{\tau_{out}}{\tau_{in}} \quad (7)$$

where,  $\tau_{out}$  and  $\tau_{in}$  are the time constant for the exclusion of the guest from the host cavity and the time constant of the guest inclusion inside the cavity of the host, respectively. The difference between ground state binding and excited state binding may be due to the difference in the inclusion rate at excited state and ground state, respectively.

### 3.8. Computational study

The formation of inclusion complex in between CB7 and CB8 with 7-DCCAЕ molecule was studied theoretically in gas phase. To find out the stability of the complex we optimized the 7-DCCAЕ-CBn ( $n = 7, 8$ ) complexes by the doping the dye molecule inside the hydrophobic cavity of the CBn molecule.

To find out the exact orientation of the interaction of the guest to the CBn ( $n = 7, 8$ ) molecules we optimized the complexes by doping both the diethylamine moiety as well as ester moiety of the guest molecule to hydrophobic cavity of CB7 and CB8 and forms 7-DCCAЕ: CBn ( $n = 7, 8$ ) complex and reverse complex. Figs. 9 and 10 represent the optimized geometries and the frontier molecular orbital pictures of the 7-DCCAЕ-CB7 and 7-DCCAЕ-CB8 complexes in both orientations. The frontier molecular orbital pictures are generated through the Gaussview 03 package [38]. The calculated values of internal energies ( $E$ ), binding energies ( $\Delta E_{Bind}$ ), enthalpy change ( $\Delta H$ ) and HOMO/LUMO energy of the complexes are tabulated in Tables 3 and 4. The number of the imaginary frequency (NIMAG) values of all the optimized geometries are zero which confirms their existence at the minima on the potential energy surface (PES). From Tables 3 and 4 we found that complex in both 7-DCCAЕ-CB7 and 7-DCCAЕ-CB8 has higher negative binding energy than of the reverse one. Higher negative binding energy and enthalpy values imply that the complex is more stable energetically and thermodynamically than the reverse complex. Negative values of  $\Delta H$  (change of enthalpy during the reaction) proven the reactions are found to be an exothermic in nature during the formation of complexes in between CBn and 7-DCCAЕ molecule. The binding energy ( $\Delta E_{Bind}$ ) has been calculated by using the following equation for both complexes:



From the frontier molecular orbital pictures of 7-DCCAЕ-CB7 complexes we found that in LUMO electronic delocalization is observed on the guest molecule whereas, HOMO of reverse complex implies the sigma delocalization is attributed overall the CB7 molecule. In case 7-DCCAЕ-CB8 complexes we found that electronic delocalization is attributed overall the guest molecule.

**Table 3**

Calculated values of internal energies, binding energies, reaction enthalpies and the corresponding HOMO and LUMO energies of the complex between CB7 and 7-DCCAЕ.

Molecule/complex	Energy (au)	$\Delta E_{Bind}$ (au)	$\Delta H$ (au)	$E_{\text{HOMO}}$ (eV)	$E_{\text{LUMO}}$ (eV)
CB7	-4157.60660	-	-	-3.56605	3.47163
7-DCCAЕ	-1241.40441	-	-	-3.45585	0.37171
CB7:7DCCAЕ complex	-5399.01680	-0.00579	-0.00258	-2.30508	1.23703
CB7:7DCCAЕ reverse complex	-5399.01436	-0.00335	-0.00025	-2.31596	1.69092

**Table 4**

Calculated values of molecular energies, binding energies, reaction enthalpies and the corresponding HOMO and LUMO energies of the complex between CB8 and 7-DCCAЕ.

Molecule/complex	Energy (au)	$\Delta E_{Bind}$ (au)	$\Delta H$ (au)	$E_{\text{HOMO}}$ (eV)	$E_{\text{LUMO}}$ (eV)
CB8	-4751.54771	-	-	-3.54646	3.45966
7-DCCAЕ	-1241.40441	-	-	-3.45585	0.37171
CB8:7DCCAЕ complex	-5992.96687	-0.01475	-0.01053	-2.40875	1.14016
CB8:7DCCAЕ reverse complex	-5992.95651	-0.00439	-0.00036	-2.31596	1.69092

### 4. Conclusion

In this study, we have investigated the supramolecular interaction of 7-(diethylamino)coumarin-3-carboxylic acid *N*-succinimidyl ester (7-DCCAЕ) with two macrocyclic hosts CB7 and CB8 in aqueous medium. We observed very interesting phenomenon on addition of CBn to the aqueous solution of 7-DCCAЕ. Fluorescence intensity, fluorescence quantum yield value of 7-DCCAЕ was increased on addition of CBn to the aqueous solution of 7-DCCAЕ. Fluorescence lifetime value of 7-DCCAЕ was found to be increased on addition of both CBn. The rotational relaxation time of 7-DCCAЕ increased on addition of both CBn. By using the SED equation we have estimated the diameter of the complex and observed that 1:1 complex is formed. From fluorescence measurement and isothermal calorimetric titration, we observed that 1:1 stoichiometric complex was formed between 7-DCCAЕ with CB7 and CB8, respectively. From <sup>1</sup>H NMR, it was confirmed that 1:1 complex was formed between 7-DCCAЕ with CBn and the binding site of 7-DCCAЕ with CBn is through —N(Et)<sub>2</sub> moiety not through ester moiety. From Job's plot we have confirmed that 1:1 complexes are formed between 7-DCCAЕ with CB7 and CB8. We observed that the binding strength of 7-DCCAЕ with both the CBn is much higher as compared to the binding strength of 7-DCCAЕ with CD's in ground state as well as in excited state. Therefore, CBn can be used as macrocyclic host more preferably than CD's for encapsulation of 7-DCCAЕ. From ITC measurement we observed that both the complexation processes are exothermic in nature. Both the complexation processes are enthalpically as well as entropically favourable process. By using density functional theory geometry and frequency of the 7-DCCAЕ-CBn complex was optimized. Both the complexation processes are enthalpically as well as entropically favourable process. From computational study, we found that the complexation process is exothermic in nature for both the complexation process, which we also observed from ITC measurement. In the present study we found that good correlation was established between the theoretical results and experimental observations.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molliq.2018.05.081>.

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