*Journal of Inclusion Phenomena and Macrocyclic Chemistry* **34:** 105–114, 1999.



# Binding of Acridinedione Dyes with $\alpha$, $\beta$ and $\gamma$-Cyclodextrins: Fluorescence Quenching and Estimation of Thermodynamic Parameters

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(Received: 17 March 1998; in final form: 20 September 1998)

**Abstract.** Using absorption and fluorescence spectroscopy, acridinedione (ACD) was found to be included into the $\beta$-cyclodextrin ($\beta$-CD) cavity to form a 1 : 1 inclusion complex. As a consequence of inclusion, the fluorescence of ACD is strongly quenched. Lifetime data confirm that the quenching is purely static. The association constant for the formation of the complex was calculated using linear and non-linear equations. $\Delta$H and $\Delta$S values obtained from the temperature dependent association constants of the $\beta$-CD/ACD(B) system are reported. It was also observed that these dyes complexed very weakly with $\alpha$ and $\gamma$-CD.

**Key words:** cyclodextrin, acridinedione, fluorescence quenching, inclusion.

## 1. Introduction

Cyclodextrins (CDs) are water soluble cyclic oligosaccharides consisting of six, seven and eight glucopyranose units ($\alpha$, $\beta$, and $\gamma$-CD respectively). All the glucose units are in a chair conformation, linked by $\alpha$(1,4) glycosidic oxygen bridges. This special arrangement gives a truncated cone like structure with a central hydrophobic cavity. The inner diameters of $\alpha$, $\beta$, and $\gamma$-CD are 4.7–5.3, 6.0–6.5 and 7.5–8.3 Å respectively [1]. The presence of the central hydrophobic cavity makes these molecules capable of forming inclusion complexes with many organic and inorganic guest molecules [1–4].

A number of investigators have examined CD inclusion complexation. CD exerts an appreciable influence on fluorescence, the lasing properties of dye molecules, water solubility and photoreactivity [5–8]. The complex formation was determined from the spectral shift and enhancement in the fluorescence quantum yield [9–12]. The binding interaction involved in the complex formation and the stability of the inclusion complex have been attributed to van der Waals forces,

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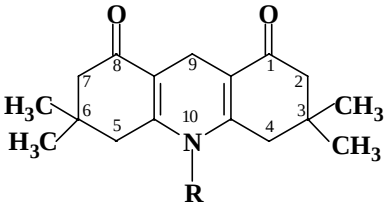
hydrogen bonding and release of high energy water molecules from the CD cavity [1]. Reports on fluorescence quenching upon binding with CD in the literature are scanty. Schuette et al. [13] discussed the fluorescence quenching of acridine upon complexation with $\beta$-CD. Fraiji et al. [14] demonstrated the fluorescence quenching of 2-acetylnaphthalene in CD. The quenching of Lumichrome [15] fluorescence by $\beta$-CD was also reported.




| Compd | -R |
|---|---|
| ACD(A) | -H |
| ACD(B) | -$CH_3$ |

In the present study, we have chosen ACD dyes having N—H and N—$CH_3$ substituent. These have been recently reported as a class of laser dyes [16]. These dyes are important because of their structural similarity to 1,4-dihydropyridines and NADH, which act as coenzymes in biological systems [17]. The ACD dyes have a bichromophoric structure enabling them to act both as electron donors and acceptors in the ground and excited states [18–21].

They have potential application as photosensitisers and as good initiators in photopolymerisation [22]. The photochemistry [23] and photophysics [24, 25] of these dyes have been studied recently. Solvent effect studies on the dyes used in this study show that they have a high fluorescence quantum yield of about 0.9 [24] and have good lasing action [16] comparable to that of coumarin 102 using methanol as solvent.

## 2. Experimental

Absorption spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer. Fluorescence spectra were obtained with a Perkin-Elmer LS-5B Luminescence Spectrometer equipped with a thermostated cell holder. The temperature was maintained by using a Christ FDC-1 thermostat, all the solutions were equilibrated for 30 minutes prior to the measurements. Acridinedione dyes were synthesised by procedures reported in the literature and characterised [26]. Methanol HPLC was obtained from Qualigens India Ltd. The water used was triply distilled over alkaline permanganate in an all glass apparatus. $\alpha$, $\beta$ and $\gamma$-CD (Fluka) were used as received.

The fluorescence lifetimes were recorded on a IBH-5000, UK, Single photon Counting Spectrofluorimeter. A nanosecond flash lamp with a pulse width of 1.4 ns filled with hydrogen was used for excitation and a Hamamatsu (3235) photomultiplier tube was used for the detection of the fluorescence. The fluorescence

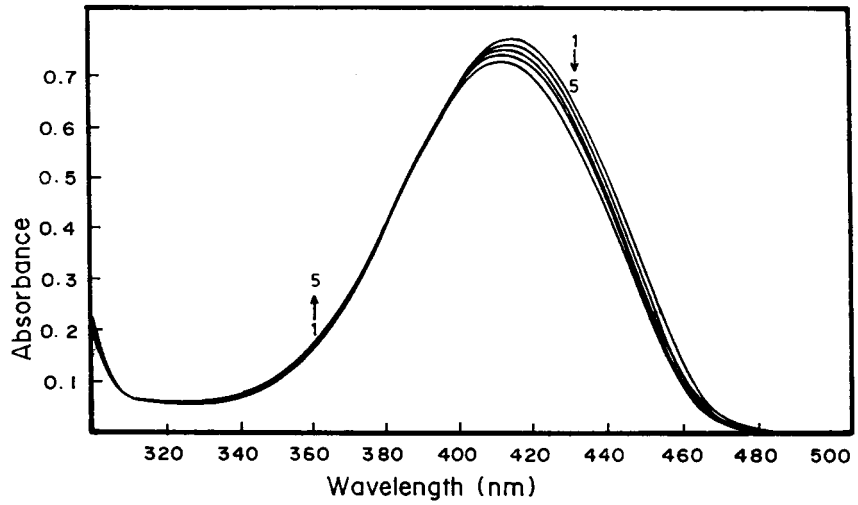



*Figure 1.* Absorption spectra of ACD(B) in the presence of 0–0.008 M of $\beta$-CD at 300 K. [$\beta$-CD] = 0 (1); 0.001 M (2); 0.002 M (3); 0.004 M (4); and 0.008 M (5).

decay curves were analysed by an iterative fitting program provided by IBH. The goodness of the fit was estimated from the $\chi^2$ value.

Due to the poor solubility of ACD dyes in aqueous medium, methanol (0.6%) was chosen as co-solvent and the inclusion studies carried out with $\alpha$, $\beta$ and $\gamma$-CDs. The inclusion complex was prepared by the following procedure. Approximately 0.2 mM dye solution in 6% methanol was prepared. 0.5 mL was constantly added to different volumes of CDs and the contents were made upto 5 mL. The corresponding concentration of CDs was used as reference for absorption measurements.

## 3. Results and Discussion

### 3.1. EFFECT OF $\beta$-CYCLODEXTRIN

Figure 1 shows the absorption spectra of ACD(B) in the absence and presence of $\beta$-CD in 0.6% methanol. The dye shows an absorption maximum at 415 nm in the absence of CD. As the $\beta$-CD concentration is increased, the absorbance at 415 nm decreases along with a blue shift of about 5 nm in the maximum. It is important to note that the change in the absorption spectrum indicates the formation of a host-guest complex consisting of the dye and $\beta$-CD. In addition to this a clear single isosbestic point around 390 nm was observed. This indicates formation of a 1 : 1 association complex between $\beta$-CD and ACD(B), and the blue shift indicates the dye molecule experiences a less polar environment.

The fluorescence spectra of ACD(B) obtained by exciting at the isosbestic point (390 nm) is remarkably quenched along with a blue shift of around 5 nm as shown

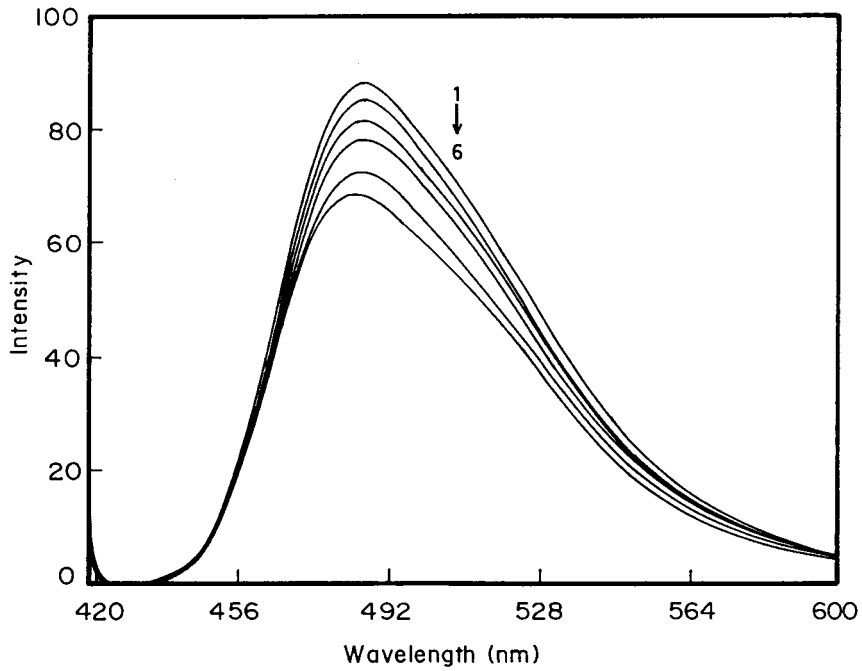



*Figure 2.* Emission spectra of ACD(B) in the presence of 0–0.008 M $\beta$-CD at 300 K. [$\beta$-CD] = 0 (1); 0.0005 M (2); 0.001 M (3); 0.002 M (4); 0.004 M (5); and 0.008 M (6).

in Figure 2. The blue shift in the emission maximum is due to the reduced polarity experienced by the dye molecule. The decrease in the fluorescence quantum yield is attributed to the specific complex formation between $\beta$-CD and the dye ACD(B).

The fluorescence lifetime of ACD(B) in 0.6% methanol was measured and it should be noted that the fluorescence decay curve is single exponential with a lifetime of 3.7 $\pm$ 0.1 ns with a $\chi^2$ value of 1.1 This value is unchanged while increasing the concentration of $\beta$-CD, which rules out the possibility of dynamic quenching (Figure 3). The lifetime and spectral data confirm that the quenching is purely static. The fluorescence quenching of ACD(B) by $\beta$-CD can be analysed by the following linear equation[15] assuming 1 : 1 association.

$$\frac{(1 - I/I_0)}{[\text{CD}]} = (1 - I_0'/I)K - K(1 - I/I_0) \tag{1}$$

$I_0$ is the initial fluorescence intensity of the dye alone at the maximum, $I_0'$ is the fluorescence of the totally complexed ACD(B) with $\beta$-CD, and I is the observed fluorescence intensity. A plot of $(1 - I/I_0)/[\beta\text{-CD}]$ *vs* $(1 - I/I_0)$ yields a straight line (Figure 4) indicating 1 : 1 association between $\beta$-CD and ACD(B); from the slope the association constant was found to be 226 $\pm$ 5 $M^{-1}$ at 300 K. The slope of a straight line is more sensitive to the ordinate value of the point having the smallest concentration. In order to determine the exact association constant the following non-linear equation [13a] is used.

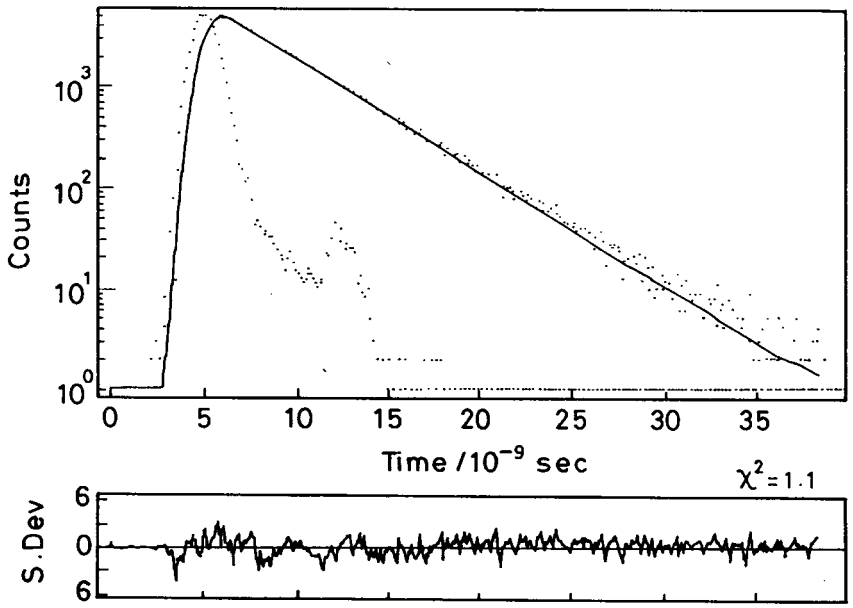



*Figure 3.* Fluorescence decay curve of ACD(B) in 0.6% methanol in the presence of 0.008 M of $\beta$-CD on excitation at 390 nm.

$$I_0 - I = \frac{I_0 K[\beta - \mathrm{CD}]}{1 + K[\beta - \mathrm{CD}]} \tag{2}$$

Thus, plotting $I_0 - I$ *vs* [$\beta$-CD] results in a curvilinear plot (Figure 5) which can be analysed by a Sigma plot non-linear regression analysis to obtain the association constant. The $K$ values obtained from the linear fit can be used as an estimate for parameters in the non-linear regression. The association constant obtained from the non-linear fit is $232 \pm 4$ M$^{-1}$ which is in close agreement with the value obtained from the linear regression.

The association constant between $\beta$-CD/ACD(B) at five different temperatures was determined. As the temperature increases the association constant decreases, this further indicates that the quenching is static in nature. Using the temperature dependent association constants the thermodynamic parameters $\Delta H$ and $\Delta S$ were calculated to be $-26.1 \pm 0.9$ kJ mol$^{-1}$ and $-41.9 \pm 0.2$ JK$^{-1}$ mol$^{-1}$ respectively from the classical plot of ln $K$ *vs* $1/T$ shown in Figure 6. The negative enthalpy changes results from the hydrophobic interaction of the dye molecule with the CD cavity [27–29]. The entropy change is the sum of several entropy changes. A positive entropy change results because of expulsion of ordered water molecules by the guest from the CD cavity [27–29]. When a larger guest molecule fills the CD cavity the movement about the glycosidic linkage of CD is restricted and results in a negative entropy change [29]. The negative enthalpy change in our case reveals the presence of hydrophobic interaction in the $\beta$-CD/ACD(B) system and the

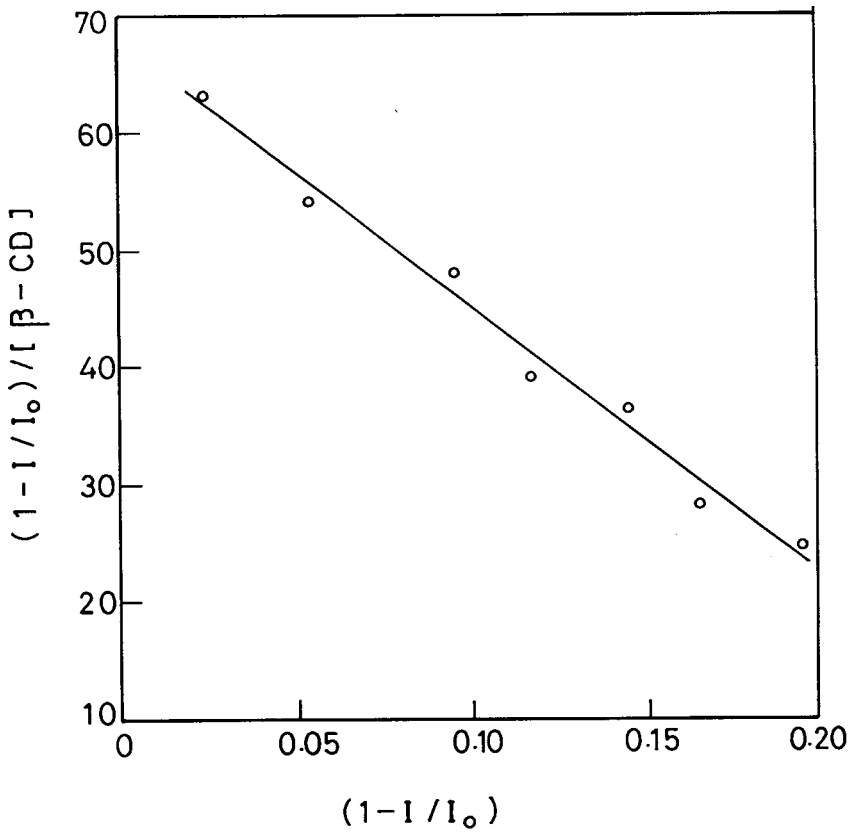



*Figure 4.* Plot of $(1 - I/I_0)/[\beta\text{-CD}]$ *vs* $(1 - I/I_0)$ for the complexation of ACD(B) with $\beta$-CD.

negative entropy change is attributed to the restricted motion of the host molecule because of inclusion of the guest molecule.

In the case of dye ACD(A) with $\beta$-CD, the absorption spectra ($\lambda_{max} - 400$ nm) show a similar trend to those of ACD(B) with an isosbestic point at 380 nm. The fluorescence spectra for ACD(A)/$\beta$-CD obtained by exciting at 380 nm shows only a very small decrease in the emission intensity. This is due to the difference in the substituent on the N atom. The presence of the methyl group on the N atom exerts a more hydrophobic character than the H atom in ACD(A). The lifetime which was found to be $7.2 \pm 0.1$ ns with a $\chi^2$ value of 1.025 for ACD(A) is also unaffected by increasing concentration of $\beta$-CD.

Interaction of the dye molecule with a saccharide unit in CD may also alter the spectral properties [30]. In order to test this, the same set of experiments were also carried out with varying concentration of (0.001–0.01 M) D(+)-Glucose (a non-cyclic saccharide) instead of CD and no change in the absorption or the fluorescence spectra of the dye molecule is observed. This shows the presence of inclusion rather than interaction with a saccharide unit.

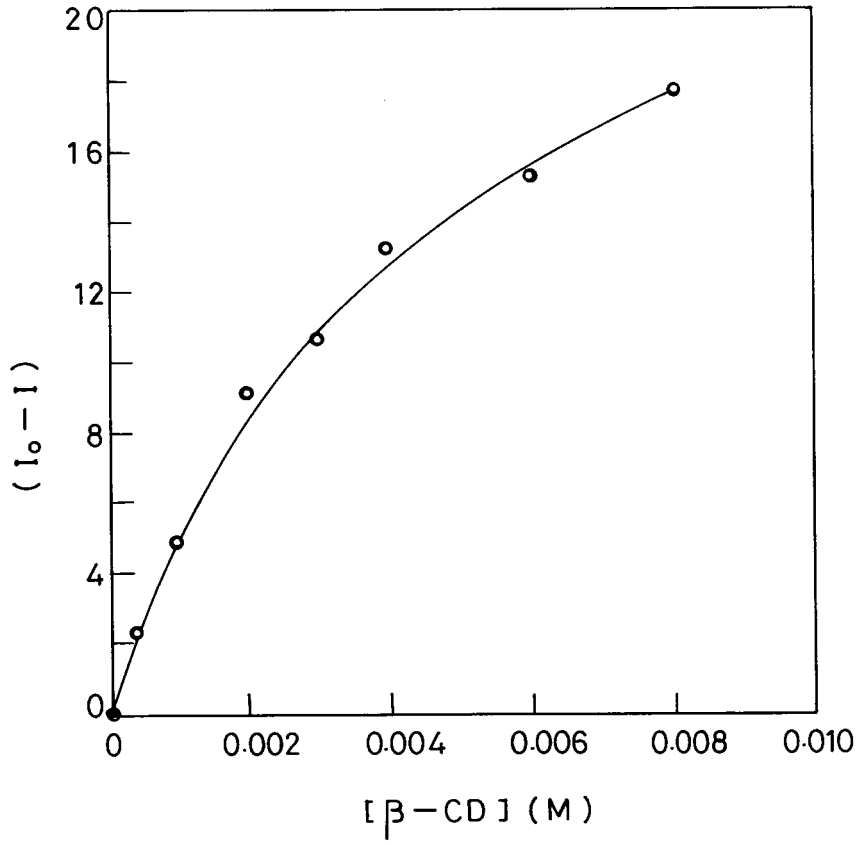



*Figure 5.* Plot of $(I_0 - I)$ *vs* [$\beta$-CD] for ACD(B) with $\beta$-CD.

### 3.2. EFFECT OF $\alpha$-CYCLODEXTRIN

The absorption spectra for the dyes ACD(A) and (B) remains unaffected with increasing concentration of $\alpha$-CD. But the fluorescence spectra obtained by exciting at the $\lambda_{max}$ values of 400 nm and 415 nm respectively show a very small decrease in intensity of around 3–5 units. This is due to the smaller cavity diameter of $\alpha$-CD. The lifetime of both these dyes were not affected with increasing concentration of $\alpha$-CD. This indicates the very weak inclusion between $\alpha$-CD and ACD(A) and (B).

### 3.3. EFFECT OF $\gamma$-CYCLODEXTRIN

The absorbance of ACD(A) and (B) progressively decreases with increasing concentration of $\gamma$-CD similar to that of $\beta$-CD with a clear single isosbestic point at 385 nm and 390 nm respectively. But the changes are very small when compared to $\beta$-CD. The fluorescence spectra obtained by exciting at the corresponding isosbestic point shows no change in the fluorescence intensity. This is also due to the weak inclusion of dye molecules with $\gamma$-CD because of its larger cavity diameter.

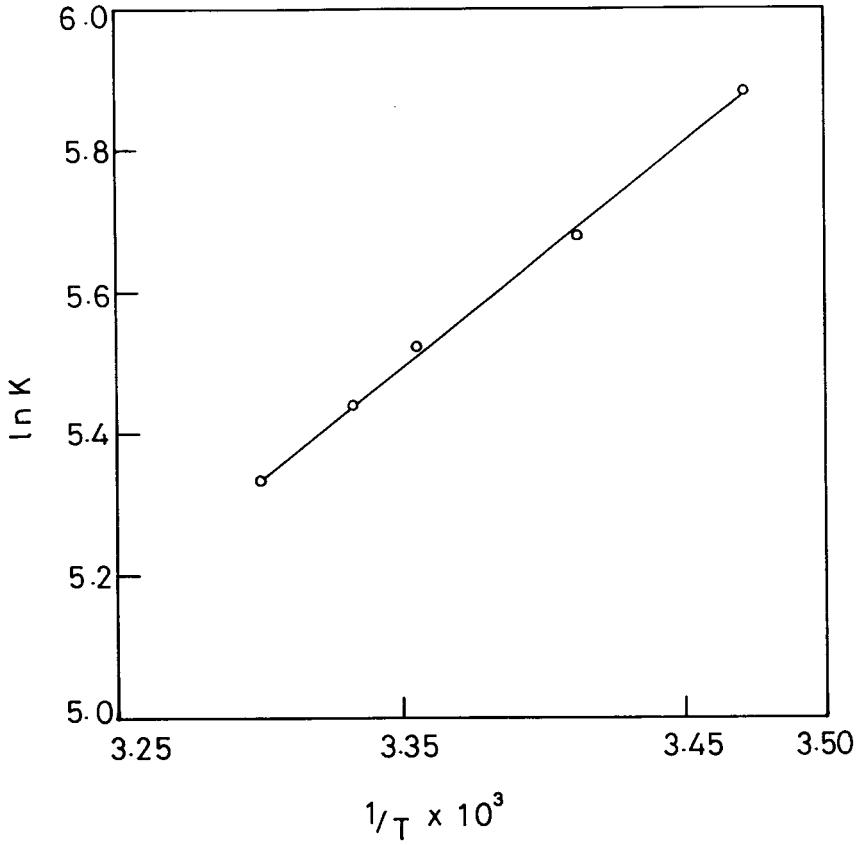



*Figure 6.* Plot of ln $K$ *vs* $1/T$ for the complexation of ACD(B) with $\beta$-CD.

The lifetime is also not affected by $\gamma$-CD indicating the formation of a ground state complex.

### 3.4. STRUCTURE OF THE COMPLEX

By using the software PC MODEL the energy minimized structure of the dye ACD(B) was elucidated. From this, the distance between the oxygens of the carbonyl group in positions 1 and 8, and the H atom present in the N methyl substituent was calculated, and was found to be 6.6 Å on one side and 6.7 Å on the other side. The complex stoichiometry was found to be 1 : 1 from the observation of the single isosbestic point in absorption experiments. This indicates that any one of these sides is included into the $\beta$-cyclodextrin cavity. The longest wavelength absorption band in the case of the dye ACD(B) around 415 nm has been assigned as due to the intramolecular charge transfer from the nitrogen to the oxygen centre. This causes an increase in the polarity of the molecule in the excited state reflected in the higher values of the excited state dipole moment compared to ground state dipole moment

values [25]. So there is a possibility of formation of a hydrogen bond between the carbonyl group and the secondary hydroxyl groups of CD in the excited state. The fluorescence quenching of these dyes by acid in the excited state has also been reported recently from our laboratory [31]. Because of this type of inclusion, the fluorescence of the dye molecule is quenched in the $\beta$-CD/ACD(B) system.

In the case of $\alpha$-CD with ACD(B) the cavity diameter of $\alpha$-CD is small and it is not able to interact effectively with the dye molecule, resulting in very small spectral changes. Similarly with $\gamma$-CD the cavity diameter is large and the complex formed is very loosely bound as reflected in the very small spectral changes.

In the case of dye ACD(A) the distance between the oxygen of the carbonyl group in positions 1 and 8, and the lone hydrogen atom on the N was calculated to be 5.4 Å and 5.5 Å respectively. Even though these distances match very well with the $\alpha$-CD cavity diameter it is not able to form a strong inclusion complex, and at same time the hydrophobicity of the H atom is much less than that of the methyl substituent in ACD(B), as indicated by the small change in the spectral properties.

## 4. Conclusion

The results reported here indicate the formation of a 1 : 1 inclusion complex between $\beta$-CD and ACD(B). The lifetime as well as the variable temperature experiments precludes the possibility of dynamic quenching in the $\beta$-CD/ACD(B) system. The association constant for $\beta$-CD and ACD(B) was determined from the static nature of the quenching by using linear and non-linear equations. We have also studied the binding of ACD(B) with $\alpha$ and $\gamma$-CD. The results indicate that the complexes formed are very weak when compared to $\beta$-CD/ACD(B). In the case of ACD(A) with $\alpha$, $\beta$ and $\gamma$-CD the results confirm that the binding is very weak. The above observations confirm that the substituent on the N atom as well as the cavity diameter of the host molecules play a major role in the inclusion complex formation.

## Acknowledgements

The authors acknowledge the University Grants Commission for the support of the infrastructure through the COSIST programme. One of the authors (PK) acknowledges DAE-BRNS for providing the SRF. The authors also acknowledge the help rendered by Dr. A. Samanta, Reader, University of Hyderabad, Hyderabad, towards the lifetime measurements.

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