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Spectral study and global analysis of fluorescence decays of the inclusion complexes of 2-amino-4,6-dimethyl pyrimidine with α- and γ-cyclodextrins

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Abstract

The steady state and time-resolved fluorescence study of 2-amino-4,6-dimethyl pyrimidine (ADMP) have been studied in aqueous solution of α- and γ-cyclodextrins (CDs). In the presence of CDs, the fluorescence spectra of ADMP point out an enhancement of the emission without significant shift. The experimental data show the presence of only one kind of complex for ADMP in α- and γ-CDs. Although, ADMP partially included within the interior CD cavities, it encapsulated inside the γ-CD cavity more deeply, compared with the α-CD. The results also revealed that the polar amino group along with the endocyclic nitrogen remains located near the aqueous environment.

Keywords: Inclusion complexes; Pyrimidine; α- and γ-cyclodextrins; Association constants; Hydrogen bonding; Fluorescence decays

1. Introduction

Cyclodextrins (CDs) are naturally occurring cyclic polysaccharides. The number of glucose units (6,7 or 8 for α-, β- and γ-CD) determines the diameter (ca. 5–8 Å) and internal volume (ca. 170–430 $Å^3$) of the cavity. CDs are versatile host molecules that can include a variety of organic and inorganic compounds [1–3]. These oligosaccharides have also been employed as biomimetic cavities [4,5] for analytical applications [6,7] and for industrial applications, such as solubilization agents and drug carriers [8,9] as well as for biological receptor–substrate interactions [10]. A wealth of thermodynamic parameters is available for the complexation of guests to CDs [1–3]. The binding efficiency of guests to CDs is to a great extent determined by the size complementarity of the guest to the CD cavity and the hydrophobicity of the guest molecule. The interaction of ADMP with β-CD has been studied by means of UV absorption, steady state and time-resolved fluorescence techniques [11]. The results imply the formation of 1:1 complex.

The dynamics of the excited-state double-proton transfer of pyrimidines mediated by hydrogen-bonded complexes has been reported [12]. Dual hydrogen bonding formation with 1:1 stoichiometry has been observed between ADMP and acetic acid in non-polar solvents [12].

In this paper we present the effect of cyclodextrin cavity size on the complexation with ADMP using steady state and time-resolved fluorescence spectroscopy.

2. Experimental

ADMP (Aldrich) was purified by recrystallization twice from acetonitrile. Its purity was checked by the fluorescence excitation spectra in cyclohexane. α- and γ-CDs (Aldrich) were recrystallized twice using deionized triply distilled water and dried under vacuum. Cyclohexane (Aldrich), ethanol (Merck) and methanol (Merck) were used without further purification.

The absorption and fluorescence spectra were recorded on a Shimadzu 2450 UV-Vis specrtophotometer and a Shimadzu RF-540 spectrofluorimeter, respectively. The fluorescence lifetimes were measured with time-correlated single photon counting fluorimeter (Edinburgh Instruments, Model OB900), the details of which are described elsewhere [13,14]. To analyze the lifetime data at different concentrations, a global iterative program based on Marquardt algorithm [15] was used. The decay profiles were analyzed at different concentrations of cyclodextrin solutions. Lifetime data were both individually and globally analyzed by

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using single, double and triple-exponentials. The fluorescence decay data was fitted by the iterative convolution to the sum of exponents:

$$I(t) = \sum_{i=1}^{n} \beta_i \exp\left(-\frac{t}{\tau_i}\right), \quad (1)$$

where $I(t)$ is the intensity of the fluorescence at time t, β_i the pre-exponential factor for the fraction of the fluorescence intensity, τ_i the fluorescence lifetime of the emitting species and n is the total number of emitting species. The results were judged by the statistical fitting parameter χ^2 and by shape of the autocorrelation function of weighed residuals.

Fluorescence quantum yields (Φ_f) were measured using quinine sulphate as standard in 0.1N H_2SO_4 ($\Phi_f = 0.546$) [16].

The geometry of the molecule was optimized using AM1 of the MOPAC 97 program.

3. Results and discussion

3.1. Steady state fluorescence and absorption measurements

Fig. 1A shows the absorption spectra of ADMP (1×10^{-5} M) in various media. The absorption intensity of ADMP in water increases upon addition of α- or γ-CD (2×10^{-3} M). This behavior has been attributed to the enhanced dissolution of the guest molecule through the hydrophobic interaction between ADMP and non-polar cavity of CDs.

There is a significant change in the ADMP absorption maxima on going from a non-hydrogen bonding (cyclohexane) to hydrogen bonding (water) solvents. On complexation the absorption maxima do not change their positions. Fig. 1B shows that the excitation spectra of the aqueous solutions of ADMP with and without CD remain the same. This behavior indicating that the probe in the complexes is still hydrogen-bonded to the water molecules or to the secondary hydroxyl groups of CDs which constitute the top of the doughnut shaped molecules. There are three hydrogen bonding centers: amino group and two pyrimidinal nitrogen atoms. Experimentally, we have observed hydrogen bonding in this system with water and acetic acid [11,12].

Fig. 2A and B illustrates emission spectra of ADMP in aqueous solution containing various concentrations of α- and γ-CDs, there is an enhancement of the emission, but an insignificant shift in the position of the fluorescence band. Upon inclusion or partial inclusion of molecules within their hydrophobic interior, CDs can effectively shield the excited singlet state of molecules from nonradiative process and enhance their fluorescence intensity [17]. A similar increase of the fluorescence in the presence of cyclodextrin, without a spectral shift, was found by Agbaria et al. [18] for the

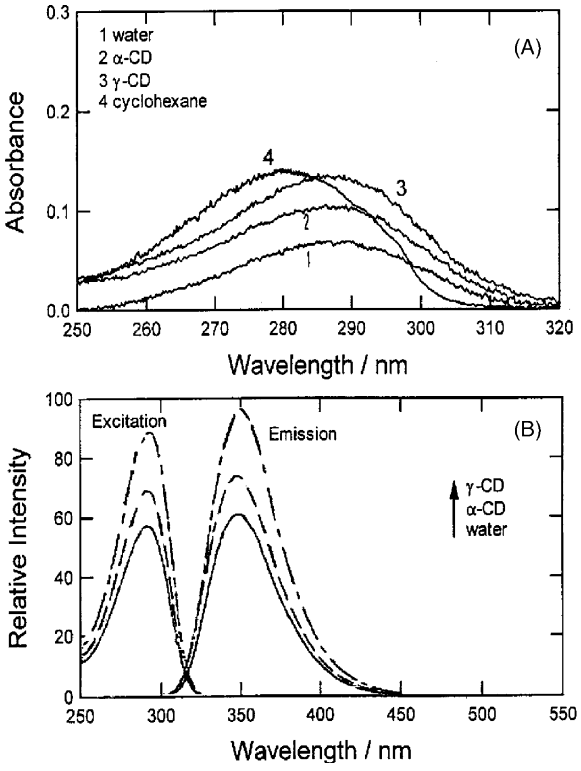

Fig. 1. (A) Absorption spectra of ADMP (1×10^{-5} M) in various media and (B) fluorescence excitation and emission spectra of ADMP (1×10^{-5} M) in various media at 298 °K.

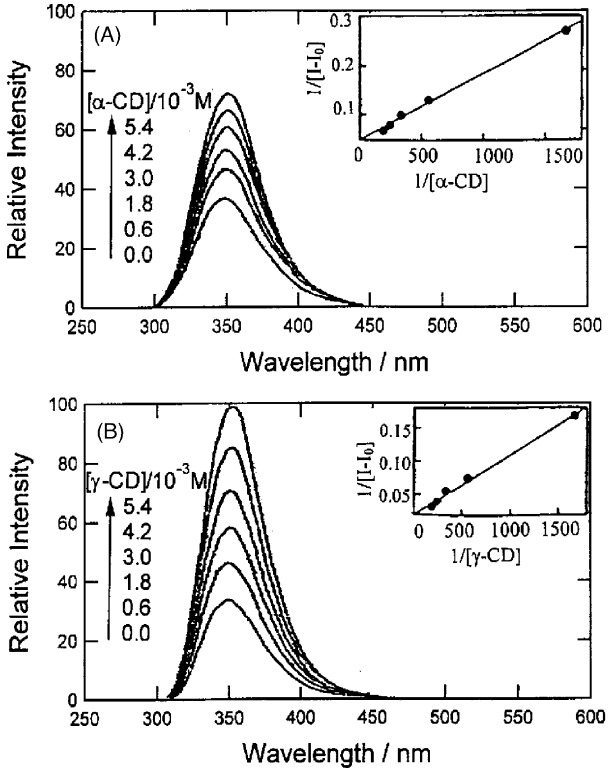

Fig. 2. Fluorescence spectra of ADMP (1×10^{-5} M) in water containing various concentrations of (A) α-CD and (B) γ-CD. The insets show Benesi-Hildebrand plots for 1:1 inclusion complex at 298 °K.

emission assigned to the 9-anthroic acid at pH = 2.4 and was also reported by Oana et al. [19] in the case of the inclusion complex of the 3-carboxyphenoxathiin with β-cyclodextrin in water. On the basis of the fluorescence decay time in the presence of cyclodextrin, the authors found that the inclusion process reduces the rate of nonradiative deactivation process. In this study, the possible explanation for the fluorescence change can be found in the role of a restriction on intramolecular degrees of freedom imposed by the geometric constraints created when the guest is complexed with the CD.

The lack of spectral shift upon inclusion supporting our conclusion that the change in the local environment is not the only factor determining the enhancement of fluorescence. This behavior indicates that the NH_2 group and may be the endocyclic nitrogen of the guest molecule in the CD cavity remains located near the aqueous environment. The fluorescence spectrum of ADMP in γ-CD is similar to that observed in α-CD, although the relative emission intensity for the former is higher. This is supported by the fluorescence quantum yield values.

The fluorescence quantum yield of ADMP bound to 0.01 M of α-CD (0.18) is lower than that of methanol (0.25), whereas fluorescence quantum yield of ADMP bound to 0.01 M γ-CD (0.31) is higher than that of methanol. This reflects a significant contribution of water molecules to the probe molecule in the α-CD cavity and a lower one for γ-CD. A further reason that could be account for the increased quantum yield is better shielding of ADMP from collisions with solvent. The comparison of the respective quantum yields may also reflect a more significant binding efficiency of ADMP with γ-CD as compared with α-CD.

It should also be noted that the comparison of the fluorescence quantum yields of ADMP complexes with that in methanol is used to compare the effective polarity experienced by the molecule in the complex. Thus, one must conclude that the effective polarity experienced by the molecule in the α-CD complex is more than methanol and ADMP in γ-CD shows an environment which is less polar than methanol. With α-CD, because of very little inclusion of the molecule, one expect that probe will find a more polar environment.

The binding constants of the inclusion complexes of ADMP were estimated from the double-reciprocal plot [20] for 1:1 CD:ADMP association using fluorescence data:

$$\frac{1}{I - I_0} = \frac{1}{I_1 - I_0}\frac{1}{K[\mathrm{CD}]_0} + \frac{1}{I_1 - I_0}, \quad (2)$$

where $[CD]_0$ represents the initial concentration of CD, I_0 and I are the fluorescence intensities in the absence and presence of CD, respectively and I_1 is the limiting intensity of fluorescence. K is the association constants for 1:1 complex. The insets of Fig. 2 display a double-reciprocal plots for ADMP in α- and γ-CDs. As can be seen, the plots are well described as single straight line, from which the values of association constants have been determined. The calculated values at 298 °K are listed in Table 1. The Benesi-Hildebrand plots assuming 1:2 stoichiometry are curved, supporting the assumed 1:1 inclusion of ADMP in α- and γ-CDs. Similar behavior was obtained for ADMP in β-CD [11].

Table 1
Association constant K (M^{-1}) of ADMP with α- and γ-CDs at four different temperatures

CD	20 °C	25 °C		30 °C	35 °C
		Absorption	Fluorescence		
α-CD	178 ± 4	197 ± 4.0	209 ± 8	211 ± 4	229 ± 8
γ-CD	221 ± 6	257 ± 4.0	273 ± 11	299 ± 4	329 ± 11

Fig. 3 shows the absorption spectra of ADMP in aqueous solutions containing varying concentrations of γ-CDs. With increasing the CD concentration, the absorption peaks at 224 and 288 nm gradually increase, indicating the formation of inclusion complex between ADMP and CD.

The binding constant K and stoichiometric ratios of the inclusion complex of ADMP can be determined according to the double-reciprocal relations assuming the formation of a 1:1 host–guest complex [20]:

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon} + \frac{1}{K[\mathrm{ADMP}]_0\Delta\varepsilon[\mathrm{CD}]_0}, \quad (3)$$

where ΔA is the difference between the absorbance of ADMP in the presence and absence of CD, $\Delta\varepsilon$ the difference between the molar absorption coefficients of ADMP and the inclusion complex. $[ADMP]_0$ and $[CD]_0$ are the initial concentration of ADMP and CD, respectively.

Fig. 4 depicts a plot of $1/\Delta A$ as a function of 1/[CD] for ADMP at various temperatures. Good linear correlations were obtained, confirming the formation of a 1:1 inclusion complex. From the intercept and slope values of this plot, K is evaluated at various temperatures. The values of the association constants determined at four different temperatures for each complex are given in Table 1. The obtained K values are in reasonable agreement, within about 6%, with those

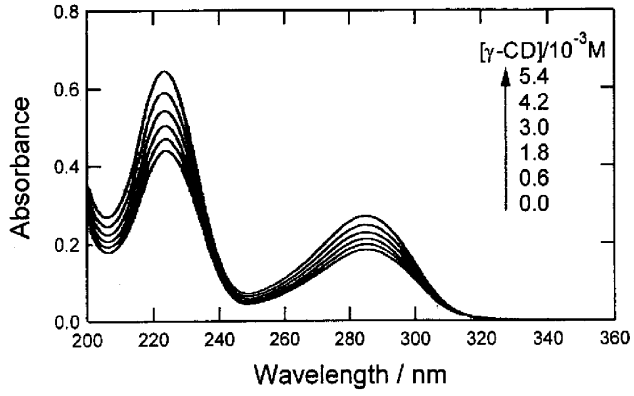

Fig. 3. Absorption spectra of ADMP (1×10^{-5} M) in water containing various concentrations of γ-CD at 298 °K.

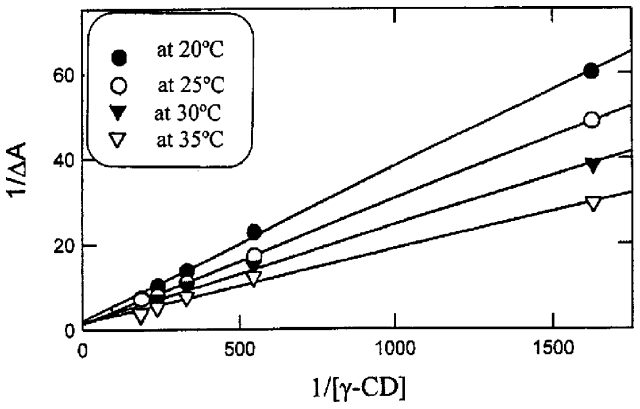

Fig. 4. Benesi-Hildebrand plots for 1:1 γ-CD:ADMP inclusion complex at various temperatures.

obtained from fluorescence spectral data. From Table 1, it is apparent that the calculated association constant values are higher for γ-CD than those of α-CD complexes. This is due to the higher a mount of ADMP complexed to γ-CD.

It is evident from the K values that γ-CD forms a stronger complex as compared to α-CD. This has been related to the stronger hydrophobic interactions experienced by the ADMP molecules with γ-CD as compared to α-CD due to the larger volume of γ-CD.

The enthalpies, entropies and free energy changes were calculated from classical van't Hoff method of plotting $\ln K$ against $1/T$. In this case, the corresponding enthalpy ΔH° and entropy ΔS° changes are obtained from the slope and intercept of the plot, respectively. The free energy change ΔG° was calculated as: $\Delta G^\circ = -RT \ln K$. The thermodynamic parameters for 1:1 complexation of ADMP with α- and γ-CDs are collected in Table 2.

The thermodynamics of cyclodextrin binding is complex due to a number of driving forces, which may be operative concurrently. In general cyclodextrin binding processes are exothermic and exhibit a fairly negative enthalpy change and entropy change which can be either positive or negative. The negative sign for the obtained ΔH° values may be accounted for the hydrophobic interactions between the fluorophore and the walls of the CD cavity [21]. The overall ΔS° association with complex formation is the sum of several entropy changes with different signs. Entropy loss accompanies binding of the fluorophore, while entropy gain results because of expulsion of water from the CD cavity and disruption of the solvent shell around the fluorophore when it binds to the CD [22,23].

Table 2
The thermodynamic parameters of inclusion complexes between ADMP and α- and γ-CDs in water at 298 K

CD	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})	ΔG° (kJ mol^{-1})
α-CD	−14.21 ± 1.5	−4.93 ± 2.0	−13.09 ± 2.0
γ-CD	−18.49 ± 0.9	−5.67 ± 1.5	−13.75 ± 3.0

Table 3
Fluorescence lifetimes, amplitudes and χ^2 values obtained by global analysis of the fluorescence decays of ADMP (1×10^{-5} M) in the presence of different concentrations of α and γ- CDs in water at 298 K

[CD] (mM)	τ_1 (ns)	β_1	τ_2 (ns)	β_2	χ^2
ADMP + α-CD					
0	4.37	1.00			1.13
0.80	4.56	0.97	0.19	0.03	1.02
8.0	4.67	0.95	0.20	0.05	1.11
11	4.66	0.93	0.28	0.07	1.12
20	4.64	0.91	0.34	0.09	1.01
28	4.60	0.90	1.03	0.10	0.99
ADMP + γ-CD					
0	4.37	1.00		–	1.13
0.80	4.39	0.95	1.05	0.05	1.01
1.20	4.31	0.92	0.90	0.08	1.09
8.0	4.33	0.90	0.95	0.10	1.03
11	4.26	0.86	1.12	0.14	1.09

3.2. Fluorescence lifetime measurements

The fluorescence decays were measured for solutions of a fixed ADMP concentration and different CD concentrations ([CD] ranging from 0 up to 0.028 M for α-CD and up to 0.011 M for γ-CD). To prevent self-association, a relatively low concentration (1×10^{-5} M) of ADMP was used in the time-resolved fluorescence measurements. Table 3 shows the results of the global analysis of each fluorescence decay of ADMP, calculated according to Eq. (1), measured at 347 nm for various CD concentrations.

In water, the fluorescence decay of ADMP obtained from monitoring the emission at 347 nm is a single-exponential with lifetime value 4.37 ns (τ_1). Fluorescence lifetimes were also measured for solutions of fixed ADMP in the presence of different concentrations of both α- and γ-CDs. The fluorescence decay is biexponential with lifetimes τ_1 and τ_2 (see Fig. 5 and Table 3). It is evident from Table 2 that the long-lived species (τ_1) is close to the measured lifetime of ADMP in pure water, and that of the short-lived species τ_2 are correspond to the 1:1 complex. The curves were also fitted using triple-exponential analysis but χ^2 did not improve. It is clear that, even at the highest concentrations of the CD, there is a significant contribution from the τ_1 component, i.e. the molecule in water. This shows that some probe molecules are always present in the CD solution in the free and uncomplexed form. This analysis supports the assumption about the presence in solution of two emitting individuals possessing different fluorescence lifetimes.

It is also seen from Table 3 that the τ_1 values are approximately the same for all the analyzed data sets, while their contribution (pre-exponential factor) decrease with increasing the concentration of both α- and γ-CDs. The shorter fluorescence lifetime component (τ_2) and its pre-exponential factor (β_2) increase with increasing the CDs concentration. However, the lifetime of ADMP inside of α- and γ-CDs is short compared to the lifetime in solution.

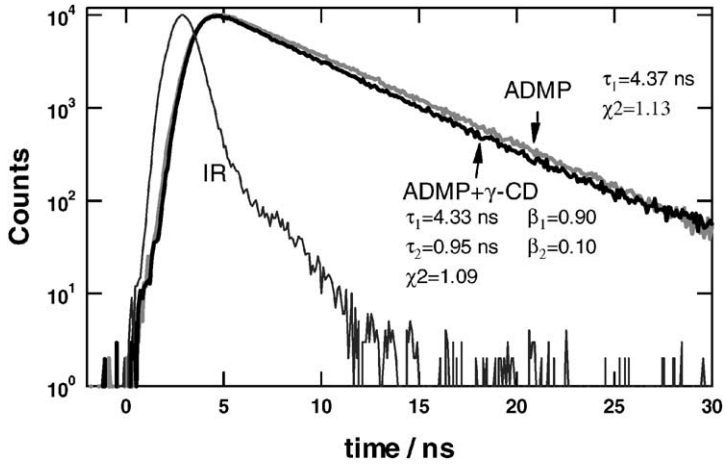

Fig. 5. Fluorescence decays for analysis of 1×10^{-5} M of ADMP in the absence and presence of 8×10^{-3} M γ-CD ($\lambda_{ex} = 288$ nm and $\lambda_{em} = 347$ nm).

Similar short-live decay was also reported in the case of inclusion complex of the 2-(2′-hydroxyphenyl)benzimidazole with CDs [24]. The short lifetime of the 1:1 inclusion complex is probably attributable to intermolecular hydrogen bond formation between one of the endocyclic nitrogen of the guest compound and the alcoholic hydrogen of the CDs.

An enhancement of fluorescence quantum yield and decrease in the lifetime of the 1:1 inclusion complex suggest that the inclusion process increases the radiative rate constant $k_f = \Phi_f/\tau_2$ and probably reduces the deactivation rate constant. This conclusion is consistent with CD constrained intramolecular rotational degrees of freedom of the guest upon inclusion. Similar conclusion was presented by Agbaria et al. [18] for 9-Anthroic acid.

It is interesting to compare the pre-exponential factors β_1 of ADMP complexes with α- and γ-CDs. The relatively higher β_1 values for γ-CD compared with α-CD is probably due to steric effect or size incomplementarity. This is consistent with the estimated association constants.

3.3. Semiempirical calculations

In order to obtain the molecular dimensions of ADMP and compare them with the cavity size of α- and γ-CDs, we have performed a theoretical AM1 calculation on the probe molecule. The distance between atoms 15 and 12 which, represents the long axis of the molecule is 6.68 Å, Fig. 6. This is less than the reported depth of the CD cavities (7.99 Å; the cavity depth is believed to be the same in all the cyclodextrins) [21]. Thus, the entire molecule can lie within the cavity of cyclodextrins provided it gets inside. Since the cavity diameter of α-CD is 4.9 Å [21], which is less than the atoms 10–18 distance, a portion of the molecule has to lie outside the cavity. The situation is however, different for γ-CD, which has a cavity diameter of 8 Å. Thus, we see that the molecular dimensions of ADMP and the cavity size of γ-CD are such that ADMP can completely reside inside the cavity. It is however to be seen whether ADMP is completely encapsulated or not. In the case of α-CD complex, the question to be answered is which side of the probe molecule is enclosed (amino group or methyl group).

As hydrogen bonding is evident in CD complexes of both α- and γ-CDs, NH_2 group and may be at least one of the pyrimidinal nitrogen atoms must be exposed to the aqueous environment. Therefore, the ADMP should enter the CD cavity through the side of one methyl group of the molecule. As the distance between atoms 12 and 15 is larger than the wider rim of α-CD, then a small portion of the ADMP must be inside the cavity upon inclusion, which explains the small variation in the fluorescence lifetimes in going from water to the complex, Table 3.

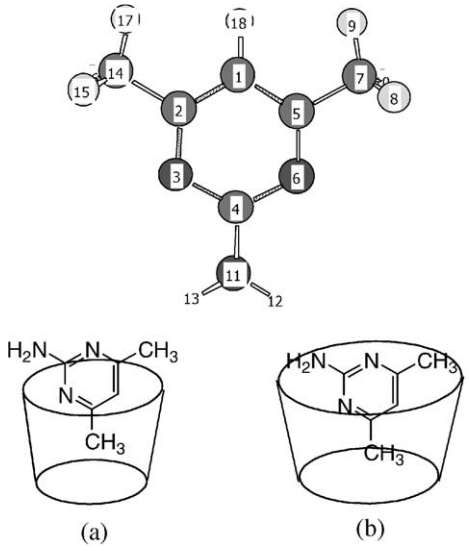

Fig. 6. The optimized structure of ADMP and the proposed structures of the inclusion complexes of ADMP with α- and β-CD.

The same line of arguments can be used to determine the possible geometry of the γ-CD:ADMP inclusion complex. Since hydrogen bonding is also evident in this case, the inclusion of the ADMP should also take place from one of the methyl group side of the molecule. Since the distance between atoms 12 and 15 is less than the wider rim of γ-CD, then the ADMP may be encapsulated inside the γ-CD cavity more deeply, compared with the α-CD. This again consistent with the relatively higher fluorescence quantum yield of γ-CD:ADMP than α-CD:ADMP complexes. The proposed structure of the complexes of α- and γ-CD complexes are shown in Fig. 6.

4. Conclusions

The experimental data point out the formation of 1:1 complexes of ADMP with α- and γ-CDs. The inclusion process determines an enhancement of the fluorescence, but an insignificant shift in the position of the fluorescence band. The results indicate that both the hydrophobic cavity and the hydrogen bonding between ADMP and water are the dominant factors in the controlling the phtochemistry of ADMP in α- and γ-CD complexes. It has been established that the inclusion of the ADMP should take place from the side of one methyl group of the molecule.

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