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ORIGINAL PAPER

Fluorimetric Study on Molecular Recognition of β-cyclodextrin with 2-amino-9-fluorenone

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Abstract The molecular recognition interaction of β-cyclodextrin (β-CDx) was investigated using 2-amino-9-fluorenone (2AFN) by UV, steady-state fluorescence and time-resolved fluorescence measurements in aqueous solution at various pH. The effect of acidity on the ground and excited state equilibria between the neutral and the monocationic forms of 2AFN in water and in β-CDx environments are studied. Based on the change in the fluorescence spectrum and lifetimes of 2AFN by the addition of β-CDx, it is found that the unsubstituted part of the 2AFN is encapsulated in the hydrophobic cavity of β-CDx. The unusual red shift obtained for the protonation of amino group in water and β-CDx solution is due to large solvent relaxation of the monocation. The structure of the 1:1 inclusion complex between 2AFN and β-CDx has been proposed on the basis of ground and excited state pK_a values and the bond distances obtained by MOPAC/AM 1 data.

keywords 2-Amino-9-fluorenone · β-cyclodextrin · Excited state acidity constants · Fluorimetric titration

Introduction

Molecular recognition [1–7] in chemistry and biology is of current interest in supramolecular chemistry and can be characterized quantitatively and qualitatively by fluorescence [8–10] due to alteration of the photophysical processes, induced by environmental stimuli [11]. Cyclodextrins (CDxs) are one of the most important host molecules in supramolecular chemistry. They are cyclic oligosaccharides obtained from the enzymatic degradation of starch by bacteria. The most commonly used forms of cyclodextrins are α, β, and γ-CDxs, containing six, seven, and eight glucose units, respectively, bonded via α(1,4)-linkages. CDxs are torus-shaped and when dissolved in water, the hydroxyl groups arrange on the outer surface of the ring, resulting in an internal cavity that is relatively hydrophobic, consisting of a circular configuration of hydrogen atoms and glucoside oxygen atoms. This arrangement permits the CDxs to accommodate guest molecules within the cavity so forming inclusion complexes [12–14].

Ketones possess interesting photophysical properties because of their various complex characteristics such as intramolecular charge transfer, hydrogen bond formation, etc. [15–18]. Reaction dynamics and mechanism of proton transfer have received the attention and efforts of chemists for a long time [19]. Various reports are there on the excited state dynamics of biphenyl and fluorene derivatives in detail [20–22]. The excited state acid–base properties of aminoaryls in an organized media would reveal the environmental effects on such molecules. In this paper, we report the photophysical and photoprototropic behavior of 2-amino-9-fluorenone (2AFN) in β-cyclodextrin.

Experimental

2-Amino-9-fluorenone (Aldrich) was purified by recrystallization from methanol. β-Cyclodextrin (S.D. Fine Chemicals) was used as received. The purity of 2AFN was checked

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Table 1 Absorption and fluorescence spectral data of 2AFN with different concentrations of β-CDx.

Concentration of β-CDx (M)	Absorption maximum λ_{abs} (nm)/(log ε)	Fluorescence maximum λ_{flu} (nm)[a]
0	272.5/(3.91)	316
		354
		474
2.0×10^{-4}	273.0/(3.94)	340
		470
4.0×10^{-4}	273.0/(3.95)	340
		470
8.0×10^{-4}	273.0/(3.95)	338
		470
1.2×10^{-3}	273.5/(3.96)	336
		470
1.6×10^{-3}	273.5/(3.96)	336
		470
2.0×10^{-3}	273.5/(3.96)	336
		470

[a]Excitation wavelength = 260 nm.

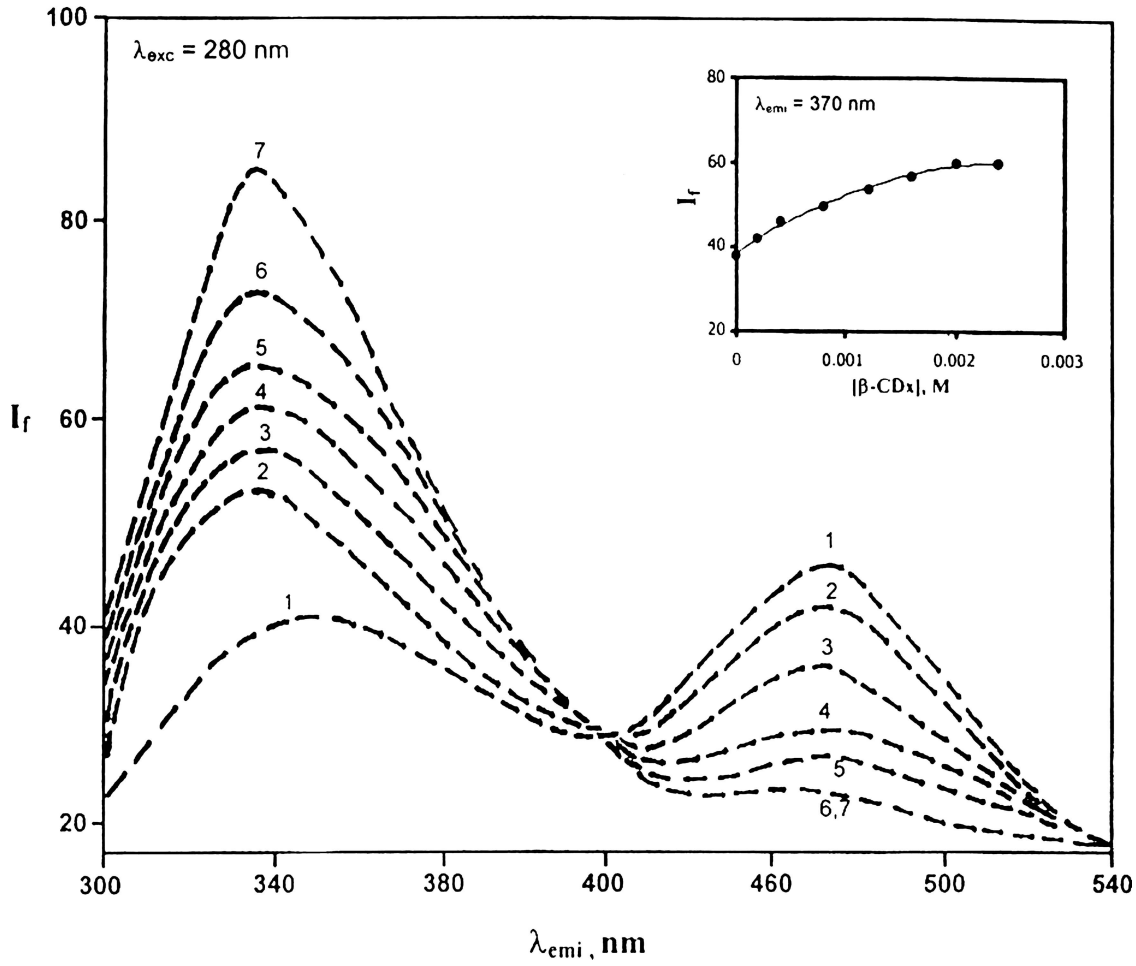


Fig. 1 Fluorescence spectra of 2AFN with various concentrations of β-CDx: 1. 0 M, 2. 2×10^{-4} M, 3. 4×10^{-4} M, 4. 8×10^{-4} M, 5. 1.2×10^{-3} M, 6. 1.6×10^{-3} M, 7. 2×10^{-3} M. Inset: Increase in the intensity of fluorescence of 2AFN with β-CDx concentration

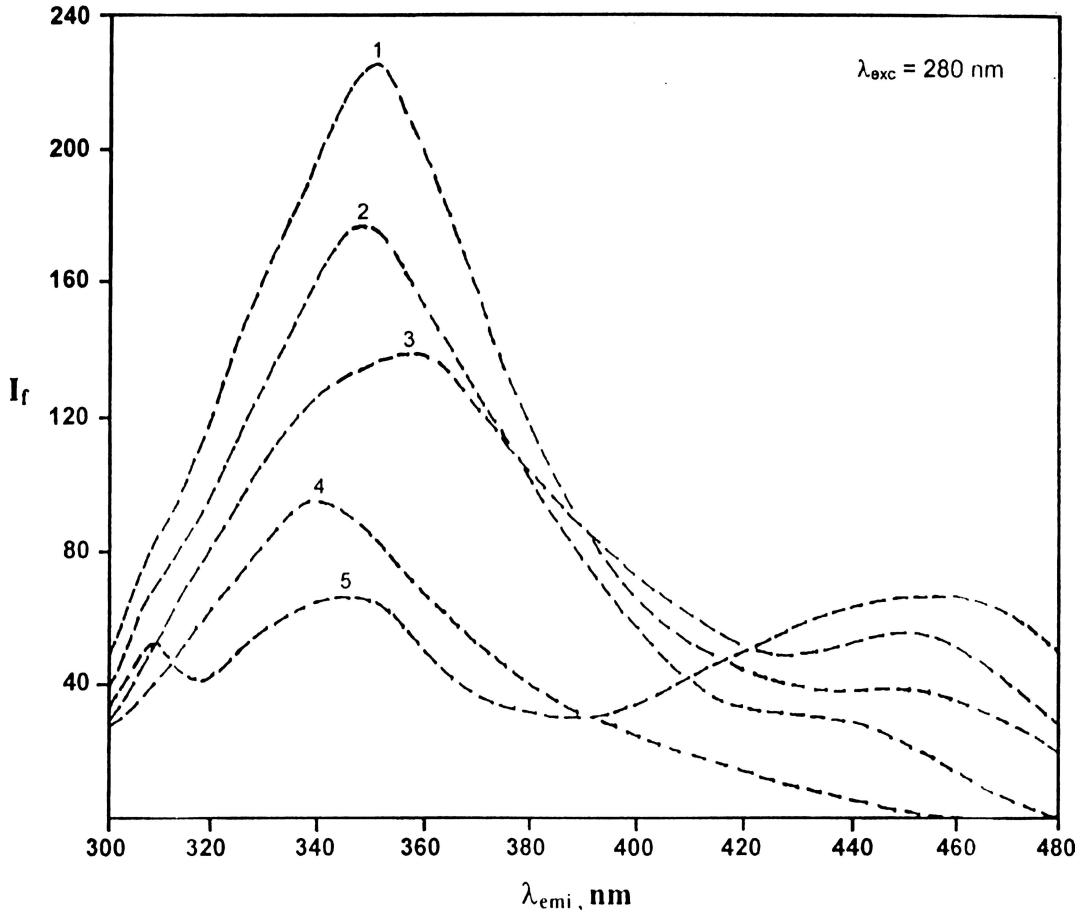


Fig. 2 Fluorescence spectra of 2AFN with various solvents: 1. dioxane, 2. acetonitrile, 3. methanol, 4. cyclohexane, 5. water

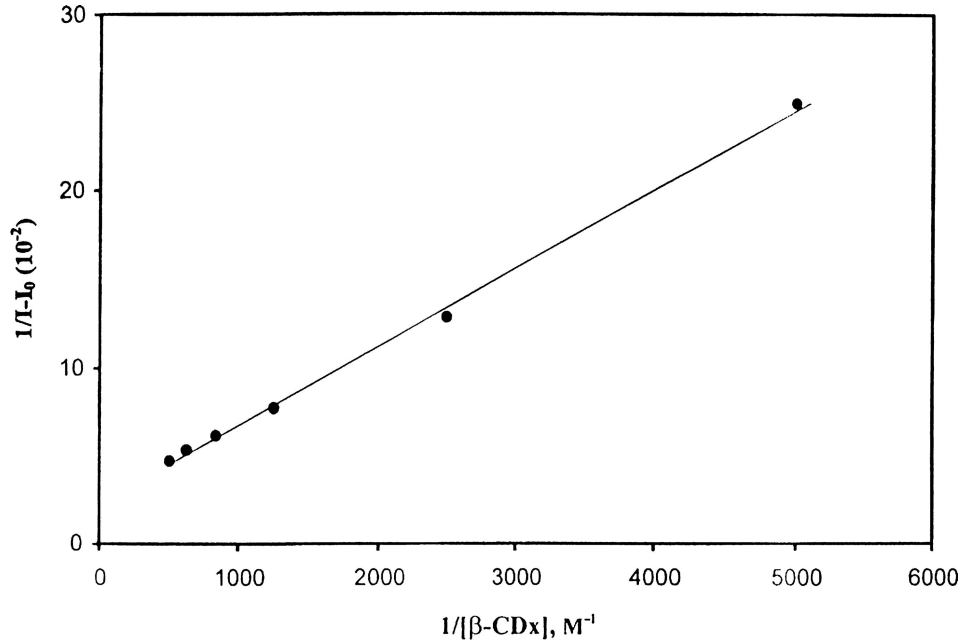


Fig. 3 Benesi–Hildebrand plot for the 1:1 complexation of 2AFN in β-CDx

Table 2 Time-resolved fluorescence spectral data of 2AFN with different concentrations of β-CDx.

Concentration of β-CDx (M)	Lifetime (s)/(standard deviation)	Amplitude (%)	χ^2
0	$2.36 \times 10^{-9}/(1.63 \times 10^{-10})$	100	0.99
8.0×10^{-4}	$2.45 \times 10^{-9}/(1.81 \times 10^{-10})$	45.60	1.01
	$8.88 \times 10^{-9}/(1.43 \times 10^{-10})$	54.40	
1.6×10^{-3}	$2.19 \times 10^{-9}/(1.78 \times 10^{-10})$	33.14	1.01
	$9.29 \times 10^{-9}/(1.68 \times 10^{-10})$	66.86	
2.4×10^{-3}	$2.10 \times 10^{-9}/(1.87 \times 10^{-10})$	17.52	1.01
	$9.41 \times 10^{-9}/(1.48 \times 10^{-10})$	82.48	

Note. Excitation wavelength = 279 nm, detection wavelength = 360 nm.

by identical fluorescence spectra when excited at different wavelengths. Triple distilled water was used to prepare the aqueous solutions. Absorption spectra were recorded using a JASCO-650 spectrophotometer and fluorescence measurements were done on a JASCO FP-550 spectrofluorimeter. Time-resolved fluorescence measurements were made using a time-correlated single photon counting spectrofluorimeter (TSUNAMI). pH of solutions in the range of 2–8 was measured using a ELICO LI-1OT pH meter. A modified Hammett's acidity scale [23] was used to prepare solutions below pH 2 (using a H_2SO_4-H_2O mixture). Due to the poor solubility of 2AFN in water, a stock solution was prepared in methanol. The concentrations of the experimental solutions were of the order of 10^{-5}–10^{-4} M.

Results and discussion

The absorption spectral data of 2AFN with different concentrations of β-CDx keeping the concentrations of the fluorophore constant is given in Table 1.

Addition of β-CDx shifts the absorption maximum slightly to the red (272.5–273.5 nm) with a small and regular increase in absorbance. This may be due to the enhanced dissolution of the guest molecule through the detergent action of β-CDx and the formation of 2AFN-β-CDx inclusion complex. The fluorescence emission spectra of 2AFN ($\lambda_{ex} = 280$ nm) with varying concentrations of β-CDx are shown in Fig. 1. A dual emission is observed in water with two emission maxima at 354 nm (SW band) and 474 nm (LW band). With the addition of β-CDx, the intensity of SW band increases with a simultaneous blue shift in all the bands. The increase in the intensity of fluorescence at SW band (354 nm) with concentration of β-CDx is shown in the inset of Fig. 1. The blue shift at SW band (18 nm) is more than the blue shift at LW band (4 nm). At the highest concentration of β-CDx (2.4×10^{-3} M) only the SW band is observed. To analyze the longer wavelength emission in water, the fluorescence spectra in different solvents were recorded and analyzed. The fluorescence spectra of 2AFN in cyclohexane, dioxane, acetonitrile, methanol and water are shown in Fig. 2. In cyclohexane, only the SW band with the maximum at 344 nm is observed. But LW band starts appearing in other solvents and its intensity increases with the increasing polarity and the hydrogen bonding ability of solvents. 2AFN has C=O and NH_2 groups which can form hydrogen bonds with protic and aprotic solvents. So the longer wavelength maximum in water and hydrogen bonding solvents is due to the solute–solvent exciplex. In β-CDx, the 2AFN molecule is encapsulated in the hydrophobic cavity and so this inclusion complexation results in the decrease of LW band and increase of SW band. The blue shift in SW band confirms the inclusion of 2AFN in the hydrophobic part of β-CDx. This is also revealed by the similarity of the fluorescence spectra of 2AFN in β-CDx and cyclohexane.

The Benesi–Hildebrand plot following Eq. (1) [24, 25] drawn with the fluorescence measurements at 370 nm for the 1:1 complexation of 2AFN in β-CDx shows a linearity (Fig. 3) and the binding constant is calculated to be 1215.80 M^{-1}.

$$\frac{1}{I - I_0} = \frac{1}{I' - I_0} + \frac{1}{(I' - I_0)K[\beta - \mathrm{CDx}]} \tag{1}$$

The fluorescence lifetime data of 2AFN at 355 nm with various concentrations of β-CDx are given in Table 2. The decay curves in the presence of β-CDx gave a best fit for biexponential decay with good χ^2 values (~ 1.00). The amplitude and lifetime of the β-CDx complexed form of 2AFN also increased up to a concentration of 2.4×10^{-3} M β-CDx and no further change was observed above this concentration of β-CDx. This lifetime data confirms the formation of inclusion complex.

The free energy change ΔG of this inclusion complex formation was determined at 30 °C using the following equation.

$$\Delta G = -RT \ln K \tag{2}$$

The negative value of -17.9 kJ/mole for ΔG indicates that the formation of inclusion complex between 2AFN and β-CDx is spontaneous.

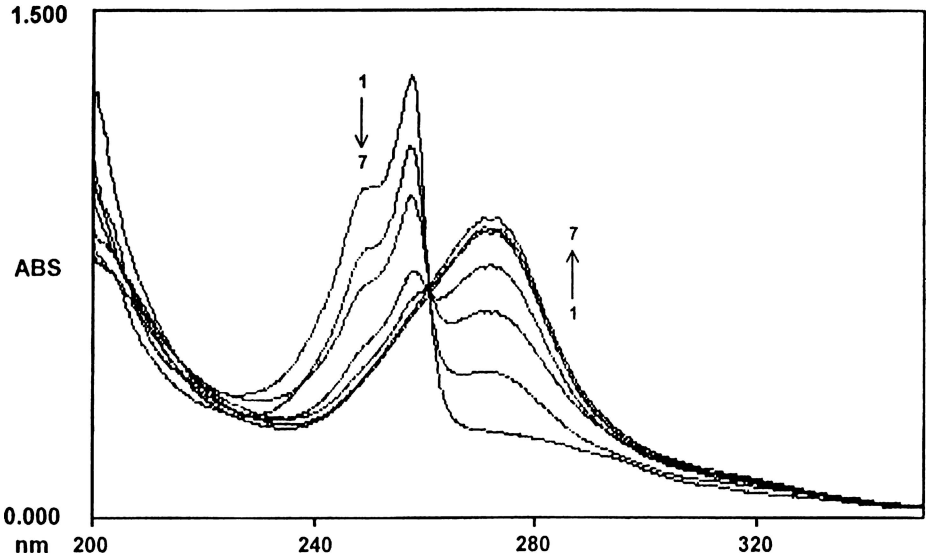


Fig. 4 Absorption spectra of 2AFN without β-CDx at various pH: 1. pH 3.2, 2. pH 3.6, 3. pH 4.0, 4. pH 4.4, 5. pH 4.8, 6. pH 5.2, 7. pH 5.6

Effect of acidity

The effect of acidity on the absorption spectra of 2AFN has been studied in the range of H_0 -3 to pH 7 in aqueous and in β-CDx solutions (Figs. 4 and 5). The absorption maxima of the neutral form of 2AFN at pH 7 in water and β-CDx solutions are at 272 and 273.5 nm, respectively. When pH is decreased a blue-shifted spectrum is obtained around pH 3.0 for both the solutions. These spectra correspond to the monocation of 2AFN obtained by the protonation of the amino group. The absorption maxima of monocation in aqueous and β-CDx solution are 241.0 and 241.5 nm, respectively. Further increase in acid concentration does not change the absorption spectrum significantly. On increase of pH from 7.0, there is no significant change in the absorption spectra even up to pH 12.0. For the monocation–neutral equilibrium

Table 3 Ground and excited state pK_a values of the monocation–neutral equilibrium of 2AFN.

	pK_a	
Equilibrium	Ground state	Excited state
Without CDx	4.50	-1.9
With β-CDx	4.33	-1.55

of 2AFN clear isosbestic points at 257 and 258 nm are observed in aqueous and β-CDx solutions, respectively. The ground state pK_a value for the monocation–neutral equilibrium of 2AFN in aqueous and β-CDx solutions were determined spectrophotometrically to be 4.5 and 4.33, respectively.

The fluorescence spectra of 2AFN in aqueous solution at different H_0/pH values are shown in Fig. 6. The neutral

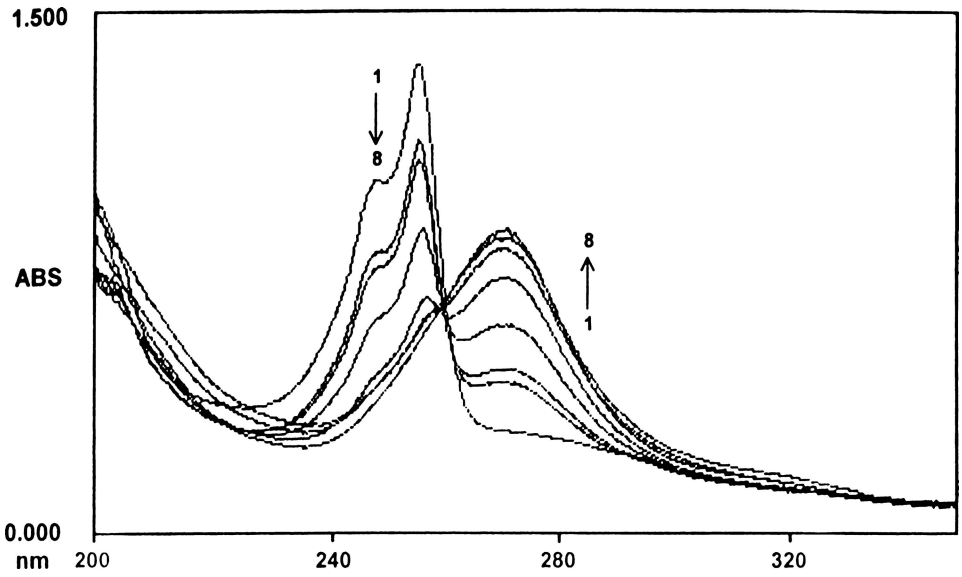


Fig. 5 Absorption spectra of 2AFN with β-CDx at various pH:1 pH 2.8, 2. pH 3.2, 3. pH 3.6, 4. pH 4.0, 5. pH 4.4, 6. pH 4.8, 7. pH 5.2, 8. pH 5.6

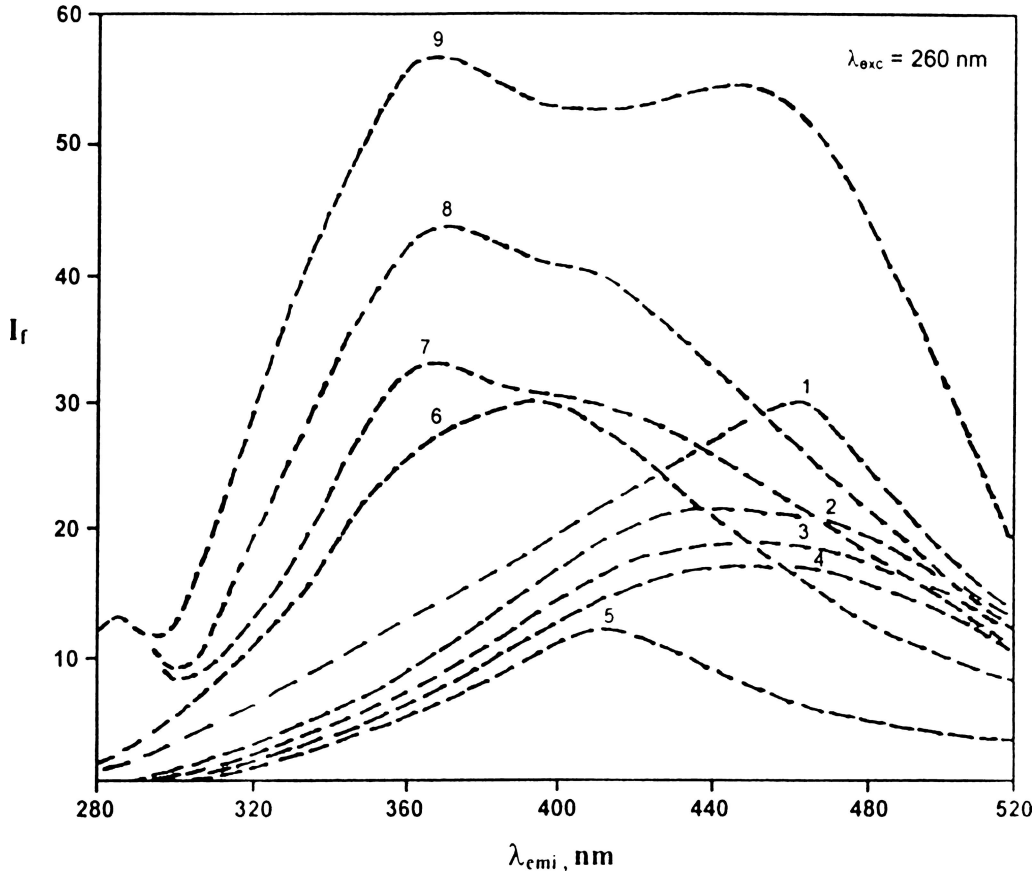


Fig. 6 Fluorescence spectra of 2AFN without β-CDx at various H_0/pH: 1. H_0 −2.76, 2. H_0 −1.85, 3. H_0 −1.38, 4. H_0 −0.26, 5. pH 0.44, 6. pH 0.83, 7. pH 1.5, 8. pH 2.0, 9. pH 3.5

species at pH 7 exhibits two fluorescence maxima at 354 and 470 nm. When pH is decreased the band at 470 nm begins to disappear. The fluorescence is quenched with the increase in acid concentration. At pH 0.83, a new spectrum begins to appear with the maximum around 414 nm. Further increase in acidity increases the red shift and the intensity of fluorescence up to H_0 −2.76. Above H_0 −2.76 no significant change in the spectrum is observed. This spectrum may be due to the formation of the monocation. Protonation of amino group usually results in blue shift both in absorption and fluorescence. But in this case an unusual red shift is observed in fluorescence on protonation. This effect is also opposite to the effect observed in other amino compounds. To explain this unusual shift, the fluorescence spectrum of the monocation in a nonpolar solvent, cyclohexane has been recorded. In cyclohexane, the monocation of 2AFN is formed by the addition of trifluoroacetic acid (TFA). The fluorescence spectra of monocation is blue shifted to the fluorescence spectrum of the neutral form as obtained in amino compounds (Fig. 7). This reveals that the red shift observed in aqueous solution is due to the large solvent relaxation in polar water medium in the excited singlet state. This kind of behavior has also been reported in 2,7-diaminofluorene [26] and 4,4′-diaminodiphenyl [27].

The fluorimetric titration curves (FT curves) for the monocation–neutral equilibrium of 2AFN (Fig. 8) in aqueous solution at two different wavelengths 360 and 460 nm show that there is no correspondence between the fluorescence quenching of the neutral form and the formation of the monocation. This suggests that there is proton-induced fluorescence quenching prior to the formation of monocation in 2AFN in aqueous solution. The pK_a^* value for the monocation–neutral equilibrium determined from the mid point of the formation curve of the monocation is −1.9.

The fluorescence emission of 2AFN with β-CDx at various H_0/pH values are shown in Fig. 9. The results are more or less similar to those observed in aqueous solution. The fluorescence is quenched on increase of acidity from pH 6.0. At pH 0.13 there is formation of monocation and the red-shifted fluorescence spectrum at 458 nm corresponding to the monocation appears. In β-CDx also the monocation fluorescence is red shifted to the neutral form as observed in aqueous solution. This reveals that the NH_2 group is in the

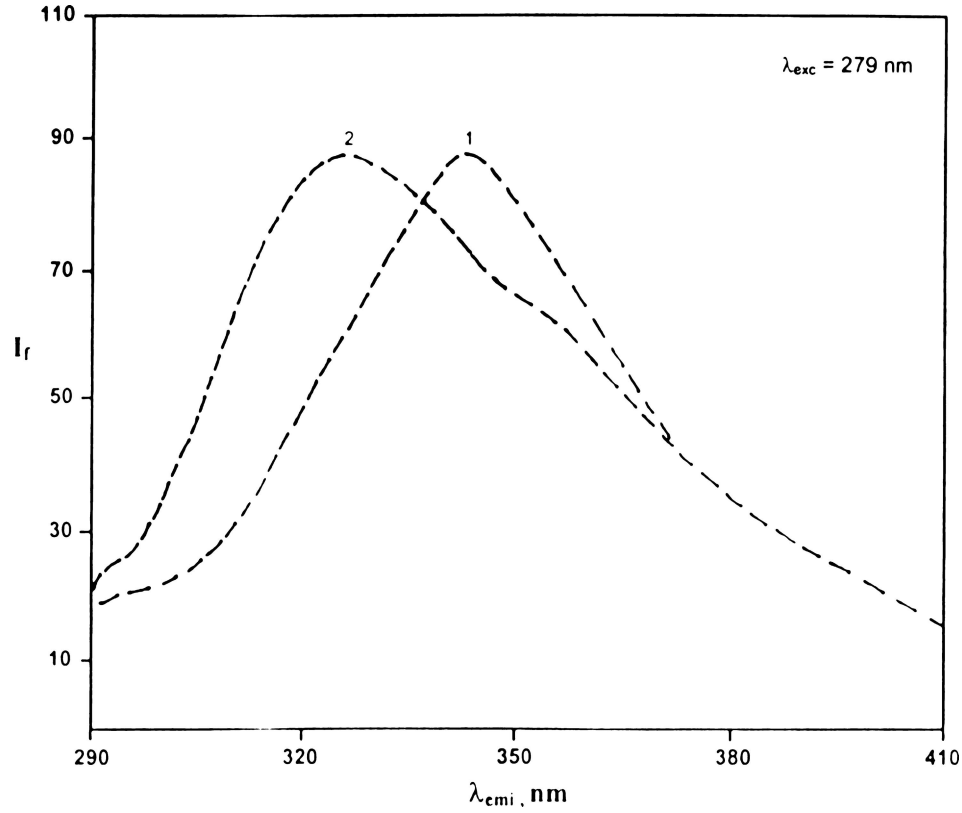


Fig. 7 Fluorescence spectra of 2AFN with cyclohexane and trifluoroacetic acid: 1. 2AFN in water, 2. 2AFN in cyclohexane + 10% TFA

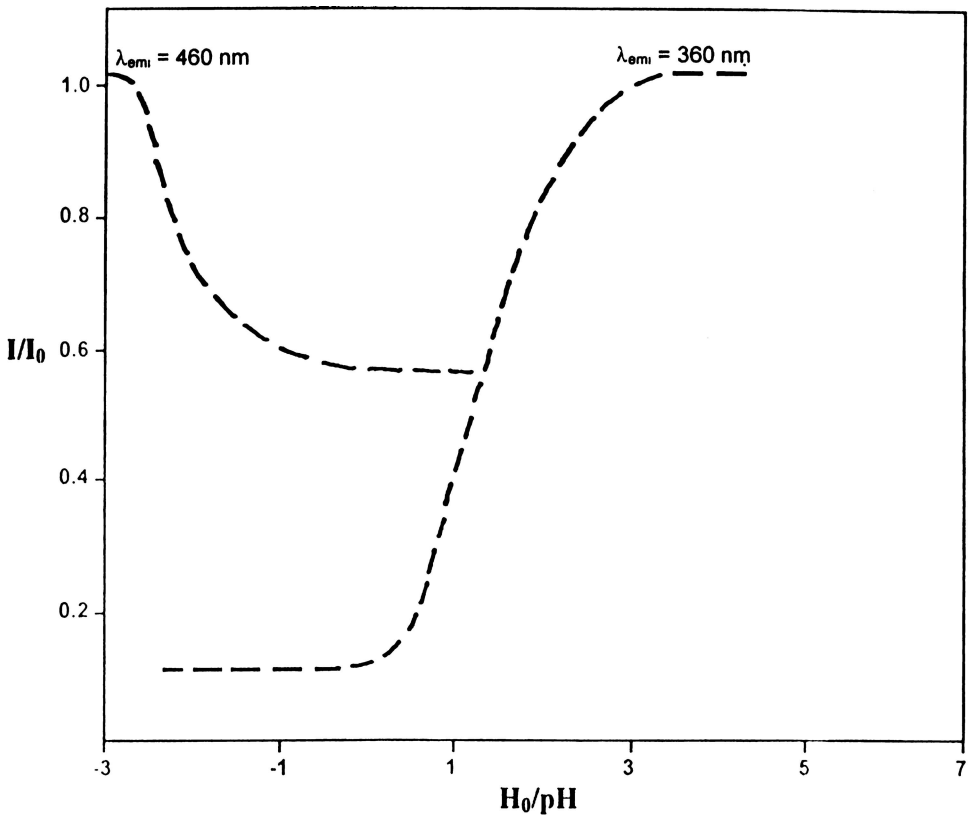


Fig. 8 Fluorimetric titration curves for the monocation–neutral equilibrium of 2AFN without β-CDx

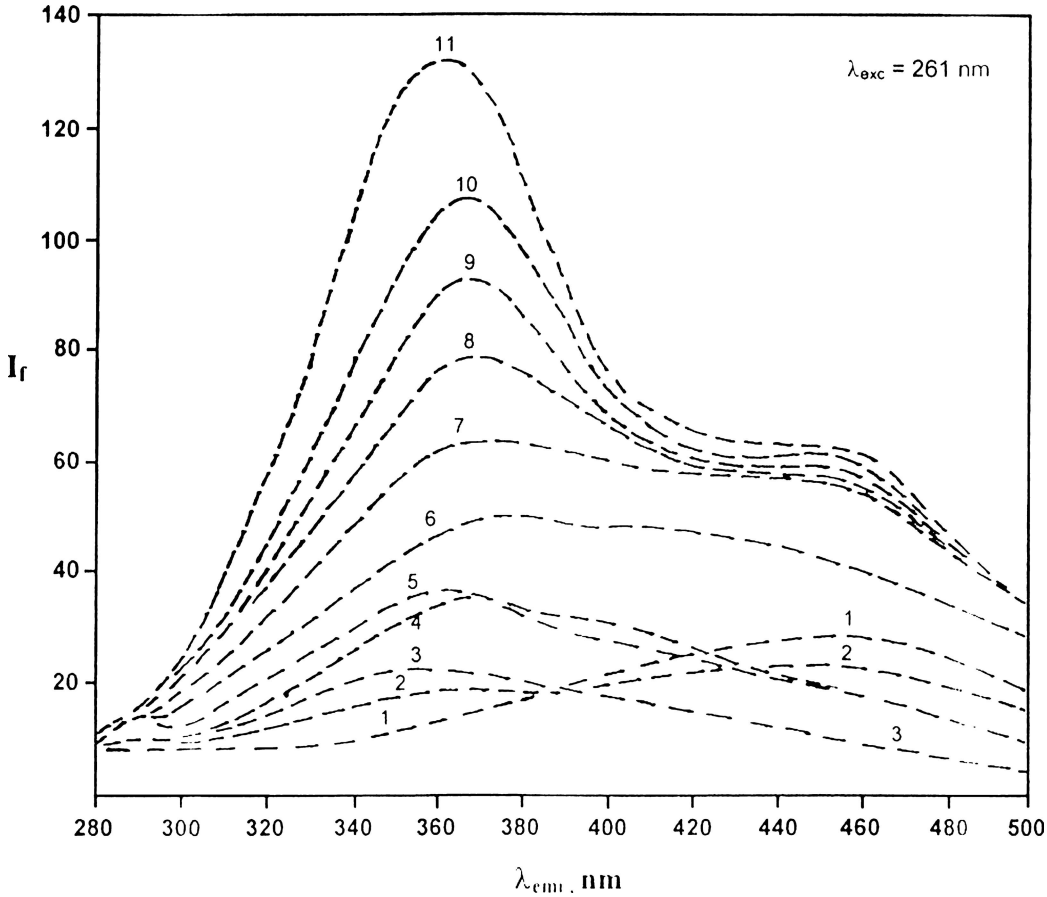


Fig. 9 Fluorescence spectra of 2AFN with β-CDx at various H_0/pH: 1. H_0 −2.76, 2. H_0 −1.85, 3. H_0 −0.26, 4. pH 0.44, 5. pH 1.0, 6. pH 1.5, 7. pH 2.0, 8. pH 2.5, 9. pH 3.0, 10. pH 3.5, 11. pH 4.0

aqueous environment and so the monocation experiences the same type of solvent relaxation as in water in the excited singlet state.

The FT curves show that there is no correspondence between the quenching of fluorescence of the neutral form of 2AFN in β-CDx and the formation of its monocation (Fig. 10). Thus, in the presence of β-CDx the pK_{a*} value for the monocation–neutral equilibrium determined from the mid point of the formation curve is -1.55. The pK_a values for the monocation–neutral equilibrium of 2AFN in aqueous and β-CDx media are listed in Table 3.

The ground and the excited state acidity constants for the monocation–neutral equilibrium of 2AFN and β-CDx are not significantly different from those in aqueous solution. From the Benesi–Hildebrand plot the stoichiometry of the inclusion complex of 2AFN in β-CDx is found to be 1:1. As the pK_a values in both the media are not much different from each other, it may be suggested that the amino group of the 2AFN molecule may be outside the cavity of β-CDx.The solute–solvent exciplex formed in aqueous solution is absent in β-CDx.This shows that the exciplex is formed by the interaction of –C=O group of 2AFN with the solvent. Since the –C=O group is inside β-CDx cavity the exciplex formation is hindered. On the basis of these results, the following structure (Fig. 11) is proposed for the 1:1 inclusion complex of 2AFN with β-CDx.

Calculation using the software MOPAC-AM1 shows the above bond lengths which are consistent with the observation that the unsubstituted part of the fluorenone could be inside the β-CDx molecule as shown in Fig. 11.

Conclusions

β-Cyclodextrin forms a 1:1 inclusion complex with 2-amino-9-fluorenone with a binding constant value of 1215.80 M^{-1} and ΔG value of -17.9 kJ/mole. Fluorescence enhancement occurs as a result of this inclusion complex formation and the longer wavelength fluorescence maximum is due to the exciplex formed by the interaction of –C=O with water. The dual emission diminishes on complexation as the –C=O group is inside the β-CDx cavity. The unusual blue shift on protonation of amino group is due to large solvent relaxation. The amino group is exposed to the aqueous environment in

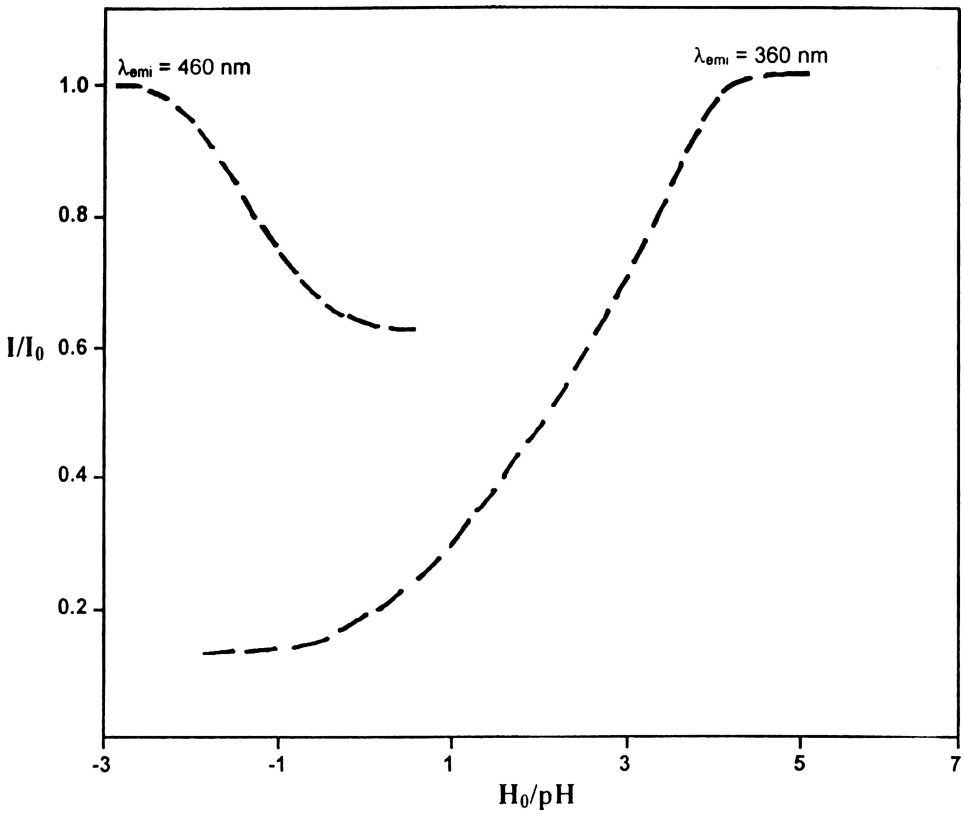
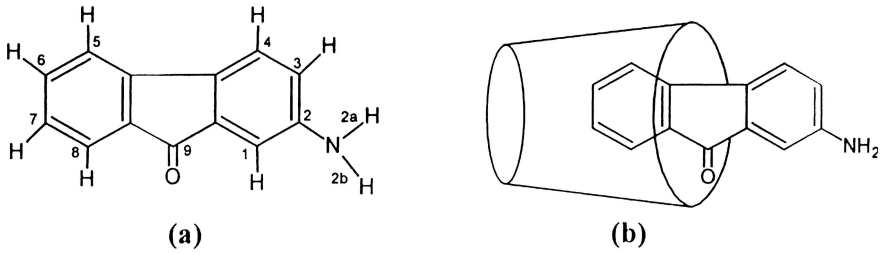


Fig. 10 Fluorimetric titration curves for the monocation–neutral equilibrium of 2AFN with β-CDx



Distances between atoms

$H^7 - H^{2b}$	=	9.034 Å
$H^5 - H^8$	=	2.833 Å
H^4 – O (carbonyl)	=	5.733 Å

Fig. 11 Schematic diagram of the 1:1 inclusion complex of 2AFN with β-CDx

the inclusion complex. The fluorimetric behavior of 2AFN in water is different from that in β-CDx solution. As β-CDx mimics enzymes, the results can be utilized for making use of 2AFN as a fluorescent probe for the investigation of biological environment.

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