SPECTROFLUORIMETRIC STUDY ON INCLUSION COMPLEXATION OF 2-AMINO-6-FLUOROBENZOTHIAZOLE WITH β-CYCLODEXTRIN

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The interaction between 2-amino-6-fluorobenzothiazole (AFBT) and β-cyclodextrin (β-CDx) has been investigated in aqueous solution and in the solid state. The stoichiometry and binding constant of the complex between AFBT and β-CDx in solution were determined by steady-state and time-resolved fluorescence spectroscopy. The FT-IR spectral data and SEM images of the solid complex confirmed the formation of inclusion complex. The proton transfer behaviour of AFBT has been investigated in aqueous and β-CDx solutions.

Keywords: Inclusion complexes; Cyclodextrins; Benzothiazole; Acidity constant; Fluorimetry.

Cyclodextrins (CDx) are cyclic oligosaccharides which have six, seven and eight D-glucopyranose units for α-, β- and γ-cyclodextrin, respectively. They form inclusion complexes with guest molecules of suitable polarity and size[1–6], because of their special molecular structure with a hydrophobic central cavity and a hydrophilic outer surface. This microheterogeneous property has been widely used for the analytical purposes[7–11]. The difference in polarity within the molecule and the restricted space provided by CDx cavity significantly influence photophysical and photochemical processes of guest molecules.

2-Amino-6-fluorobenzothiazole (AFBT) is less fluorescent in aqueous solution but its fluorescence intensity increases on the addition of β-CDx. The supramolecular complex between amino-substituted benzothiazole and substituted phenethylamide through the donor–acceptor complex was studied[12]. The amino-substituted benzothiazoles are used in the synthesis of Schiff bases[13]. Nowadays β-CDx complexation is widely used for the se-

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lective synthetic reactions[14,15]. The formation of AFBT inclusion complex with β-CDx may give a way for its synthetic utility.

Protonation or deprotonation of a guest molecule in β-cyclodextrin cavity is influenced by inclusion complexation. Consequently, the ground- and excited-state pK_a values in the presence of β-CDx are different from those in aqueous solution. The acidity constants were also used to determine the geometry of the complex[16,17]. Earlier we reported the solvent and pH effect on 2,6-diaminoanthraquinone[18] and the effect of inclusion complexation on the acidities of amino substituted ethers[19], fluorenes[20] and benzisothiazole[21]. In the present work we report the effect of β-CDx on fluorescence and prototropic characteristics of AFBT.

EXPERIMENTAL

Materials and Methods

2-Amino-6-fluorobenzothiazole was obtained from Aldrich Co. and purified by recrystallisation from aqueous ethanol. β-Cyclodextrin was purchased from S.D. Fine Chemicals and used as received. Triply distilled water was used for the preparation of solutions. A modified Hammett acidity scale (H_0) was employed for the solutions below pH 1.5 (aqueous H_2SO_4). The concentration of AFBT in the experimental solutions was 2.7×10^{-4} mol l^{-1}. To measure the fluorescence intensities during fluorimetric titration, the isosbestic wavelength was used for λ_{exi}. The wavelength used for excitation is given in figure captions.

Absorption spectra were recorded with a Hitachi model U-2001 spectrophotometer while fluorescence measurements were made using a Jasco FP-550 spectrofluorimeter. pH values in the range 1–12 were measured using an Elico LI-10T model pH meter. Fluorescence lifetimes were determined using a time-correlated picosecond photon counting spectrofluorimeter (Tsunami, Spectraphysics, U.S.A.). FT-IR spectra were obtained with Avatar-330 FT-IR spectrophotometer using KBr pellets. The spectral range was 500–4000 cm^{-1}. Microscopic morphological structure measurements were performed with JEOI-JSM 5610 LV scanning electron microscope (SEM).

RESULTS AND DISCUSSION

Inclusion Complexation

The absorption maxima, log ε and fluorescence maxima of AFBT at different concentrations of β-CDx at pH 1.1 and 6.2 are given in Table I. 2-Amino-6-fluorobenzothiazole exists as monocation at pH 1.5 and neutral form at pH 7.0.

Upon increasing the concentrations of β-CDx, the absorption maxima were slightly blue-shifted with a gradual increase in the molar absorption coefficients for both neutral and monocationic forms. The absorbance of

each form increases with increasing β-CDx concentration up to 1.2×10^{-2} mol l^{-1}. At higher concentrations (1.4×10^{-4} mol l^{-1}) the absorption maxima and molar absorption coefficients remain unchanged.

The absorption maxima and molar absorption coefficients of AFBT with different concentrations of β-CDx at pH 1.5 are significantly different when compared with the same solutions at pH 7.5. But no significant change was observed in the absorption spectra of AFBT with different concentrations of β-CDx even at pH 7.0 and above. The blue shift and change in molar absorption coefficient suggest the efficient formation of an inclusion complex between AFBT and β-CDx.

The binding constant and stoichiometry of inclusion complex of AFBT with β-CDx can be determined from the changes in absorbance of AFBT by the addition of β-CDx using the Benesi–Hildebrand (BH) equation[22]. The BH equation for 1:1 complex is given below.

TABLE I
Absorption and fluorescence maxima of 2-amino-6-fluorobenzothiazole at different concentrations of β-CDx

c(β-CDx) mol l^{-1}	pH 6.2			pH 1.1			
	λ_{abs} (log ε)	λ_{abs} (log ε)	λ_{emi} nm	λ_{abs} (log ε)	λ_{abs} (log ε)	λ_{abs} (log ε)	λ_{emi} nm
0	259.6 (3.47)	221.7 (3.95)	322.0	289.5 (3.19)	281.3 (3.23)	255.6 (3.41)	317.2
2×10^{-3}	258.8 (3.67)	221.7 (3.99)	355.2 422.0	289.4 (3.26)	280.6 (3.30)	255.6 (3.47)	350.8
4×10^{-3}	258.8 (3.78)	221.6 (4.03)	353.4 422.0	289.3 (3.32)	279.9 (3.36)	255.2 (3.52)	348.6
6×10^{-3}	258.8 (3.83)	221.5 (4.06)	350.2 421.0	289.2 (3.37)	279.4 (3.42)	255.3 (3.37)	347.6
8×10^{-3}	258.7 (3.85)	221.5 (4.07)	350.0 421.0	289.1 (3.42)	279.1 (3.48)	255.0 (3.62)	347.4
10×10^{-3}	258.6 (3.86)	221.5 (4.10)	349.8 421.0	288.9 (3.47)	278.8 (3.53)	254.8 (3.66)	346.0
12×10^{-3}	258.6 (3.91)	221.5 (4.13)	349.6 420.5	288.7 (3.52)	278.6 (3.58)	254.7 (3.70)	346.0

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon[\mathrm{AFBT}]_0} + \frac{1}{K[\mathrm{AFBT}]_0 \Delta\varepsilon[\beta\text{-CDx}]_0}, \qquad (1)$$

where ΔA is the difference between the absorbance of AFBT in the presence and absence of β-CDx, $\Delta\varepsilon$ is the difference between the molar absorption coefficients of AFBT in the presence and absence of β-CDx. $[\mathrm{AFBT}]_0$ and $[\beta\text{-CDx}]_0$ are the initial concentrations of AFBT and β-CDx, respectively, and K is the binding constant.

Figure 1 gives the plots of $1/\Delta A$ vs $1/[\beta\text{-CDx}]$ for both monocationic and neutral forms. The linearity of the plot shows the formation of 1:1 complex for both neutral and cationic forms of AFBT with β-CDx. From the slope of the straight line, the binding constant '*K*' was calculated to be 219.11 and 189.3 l mol^{-1} for the neutral and cationic forms of AFBT at 303 K, respectively.

Figures 2 and 3 show the fluorescence emission spectra of AFBT at pH 1.1 and 6.2 with different concentrations in aqueous solution of β-CDx. It was observed that the fluorescence intensity, I_f, increases with increasing concentrations of β-CDx at both the pH. The data suggest that a stable complex is formed between cationic and neutral forms of AFBT with β-CDx. An increase in the fluorescence intensity of a guest while increasing the concentrations of β-CDx during the formation of an inclusion complex has been reported earlier[23,24].

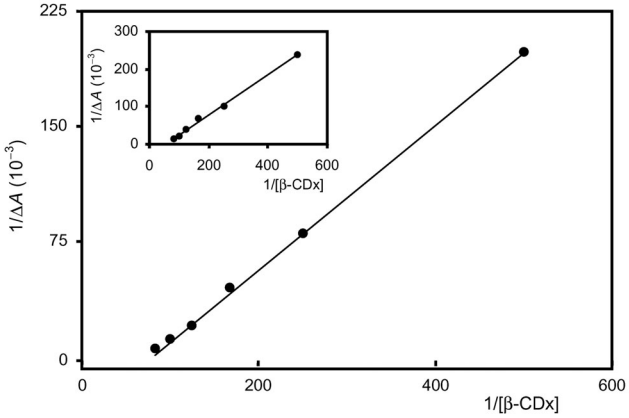

FIG. 1
Benesi–Hildebrand absorption plot for the 1:1 complex of neutral form of AFBT in β-CDx at pH 6.2 (Inset: Benesi–Hildebrand absorption plot for the 1:1 complex of cationic form of AFBT in β-CDx at pH 1.1)

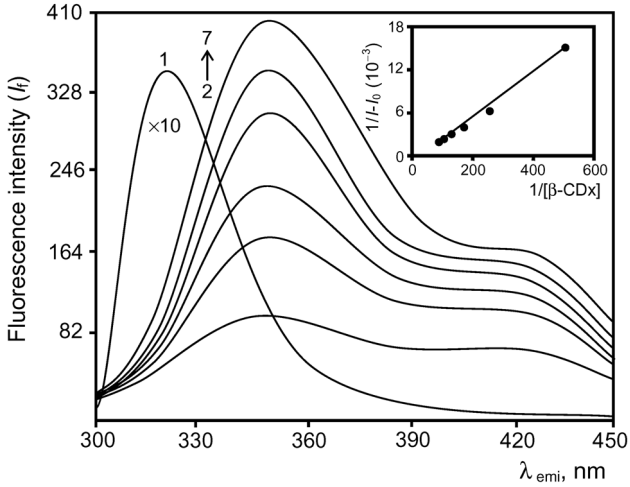

FIG. 2

Fluorescence spectra of AFBT with increasing concentrations of β-CDx (in mol l^{-1}) at pH 6.2: 1 0, 2 2×10^{-3}, 3 4×10^{-3}, 4 6×10^{-3}, 5 8×10^{-3}, 6 10×10^{-3}, 7 12×10^{-3} (λ_{ex} = 280 nm) (Inset: Benesi–Hildebrand fluorescence plot for the 1:1 complex of neutral form of AFBT in β-CDx at pH 6.2)

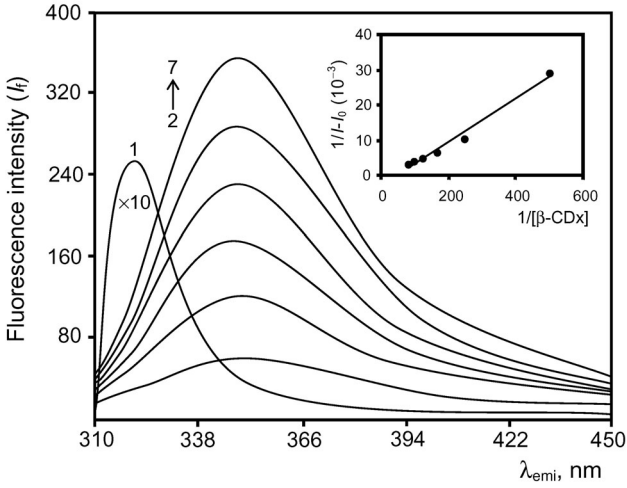

FIG. 3

Fluorescence spectra of AFBT with increasing concentrations of β-CDx (in mol l^{-1}) at pH 1.1: 1 0, 2 2×10^{-3}, 3 4×10^{-3}, 4 6×10^{-3}, 5 8×10^{-3}, 6 10×10^{-3}, 7 12×10^{-3} (λ_{ex} = 286 nm) (Inset: Benesi–Hildebrand fluorescence plot for the 1:1 complex of cationic form of AFBT in β-CDx at pH 1.1)

The fluorescence intensities of both forms increase with the addition of β-CDx up to 1.2×10^{-2} mol l^{-1}. There is no increase in the fluorescence intensity on further addition of β-CDx. This shows that the complexation is complete at 1.2×10^{-2} mol l^{-1} β-CDx for both the forms of AFBT. The formation of the inclusion complex is also confirmed by the fluorescence decay curves for neutral form of AFBT at different concentrations of β-CDx. The lifetimes and the amplitudes of AFBT in the presence or in the absence of β-CDx at pH 6.2 are given in Table II. The time-resolved fluorescence spectra of AFBT with β-CDx show a biexponential decay indicating the equilibrium between free and complexed forms. The lifetime and amplitude of the complexed form increased with increasing concentration of β-CDx up to 0.012 mol l^{-1}. Above that value, no change in lifetimes and amplitudes of complex and free forms of AFBT was observed. The χ^2 values for the single and biexponential fittings are less than 1.23. The increase in lifetime and amplitude values of complexed AFBT with β-CDx and decrease in the lifetime and amplitude values of free form of AFBT during complexation had been reported earlier[25–27].

The β-CDx dependence of AFBT fluorescence can be analysed by the Benesi–Hildebrand equation for 1:1 complex[28].

TABLE II

Time-resolved fluorescence spectral data of 2-amino-6-fluorobenzothiazole at pH 6.2 of different concentrations of β-CDx (excitation wavelength 280 nm; detection wavelength 360 nm)

c(β-CDx) mol l^{-1}	Lifetime s	Relative amplitude	χ^2	S.D.
0	2.82×10^{-9}	100	1.12	3.53×10^{-10}
2×10^{-3}	2.39×10^{-9} 6.52×10^{-9}	63.14 36.86	1.23	3.98×10^{-10} 7.31×10^{-10}
4×10^{-3}	2.18×10^{-9} 6.58×10^{-9}	52.44 47.56	1.07	4.71×10^{-10} 6.12×10^{-10}
6×10^{-3}	2.01×10^{-9} 6.73×10^{-9}	41.07 58.93	1.19	5.13×10^{-10} 6.75×10^{-10}
8×10^{-3}	1.98×10^{-9} 6.73×10^{-9}	36.33 63.67	1.21	5.58×10^{-10} 5.78×10^{-10}
10×10^{-3}	1.91×10^{-9} 6.66×10^{-9}	21.12 78.88	1.03	5.82×10^{-10} 5.31×10^{-10}
12×10^{-3}	1.83×10^{-9} 6.88×10^{-9}	14.31 85.69	1.09	6.75×10^{-10} 3.21×10^{-10}

$$\frac{1}{I - I_0} = \frac{1}{I' - I_0} + \frac{1}{K[I' - I_0][\beta\text{-CDx}]}, \qquad (2)$$

where K is the binding constant, I_0 is the intensity of fluorescence of AFBT without β-CDx, I is that with a certain concentration of β-CDx, and I' is the fluorescence intensity of AFBT with the highest concentration of β-CDx. The plot of $1/I - I_0$ vs 1/[β-CDx] is given in inset Figs 2 and 3. The linearity of the plot reveals the formation of 1:1 complex between AFBT and β-CDx. From the slope of the straight line, the binding constant K was calculated to be 222.2 and 186.32 l mol^{-1} at pH 1.1 and 6.2, respectively. The binding constants obtained from absorption and fluorescence data are close to each other. The binding constant of AFBT monocation-β-CDx complex is greater than the binding constant of AFBT neutral-β-CDx complex. This reveals that the β-CDx complexation is stronger with monocation.

The Gibbs energy change ΔG of the complex formation was determined from the binding constant at 303 K using the following equation.

$$\Delta G = -RT \ln K \qquad (3)$$

The negative values of ΔG (Table III) indicate that the formation of the inclusion complex between AFBT and β-CDx is spontaneous.

A solid inclusion complex was prepared by co-precipitation method[29] and analysed by FT-IR and SEM [30,31]. The FT-IR spectra of pure AFBT, physical mixture of AFBT and β-CDx, and solid complex of AFBT with β-CDx are

TABLE III
Binding constant and Gibbs energy change values of 2-amino-6-fluorobenzothiazole in β-CDx medium

Parameters	pH 6.2		pH 1.1	
	λ_{abs}	λ_{flu}	λ_{abs}	λ_{flu}
K, l mol^{-1}	189.3	186.32	219.11	222.22
ΔG, kJ mol^{-1}	–10.87	–10.70	–12.59	–12.76
r	0.996	0.997	0.995	0.992
S.D.	0.639	0.421	1.31	1.39

shown in Fig. 4. The N–H stretching frequency of pure AFBT appears at 3386 cm^{-1}, whereas it is significantly changed to a broad band centered at 3396 cm^{-1} in the case of solid complex. In the case of physical mixture the N–H stretching appeared at 3386.91 cm^{-1} as in pure compound. The C–H aromatic stretching frequency of pure AFBT appears at 3078 cm^{-1}, whereas it is not observed in the case of solid complex. But it is present in the physical mixture. The C–F and C–S stretching frequencies of pure AFBT appear at 1112 and 707 cm^{-1}, respectively, whereas they are shifted to 1156 and 758 cm^{-1} respectively, in the solid complex. Further, the absorption intensities of most of the stretching frequencies in the solid complex are significantly weaker (10–30%) than for pure AFBT. There is no appreciable change observed in the absorption intensities between pure AFBT and physical mixture of AFBT and β-CDx. These results clearly indicate the formation of inclusion complex between AFBT and β-CDx.

The SEM images of powdered form of AFBT, physical mixture of AFBT–β-CDx and the solid inclusion complex are shown in Fig. 5. The morphologies clearly elucidated the difference of the solid states. This difference may be taken as a proof for the formation of inclusion complex between AFBT and β-CDx.

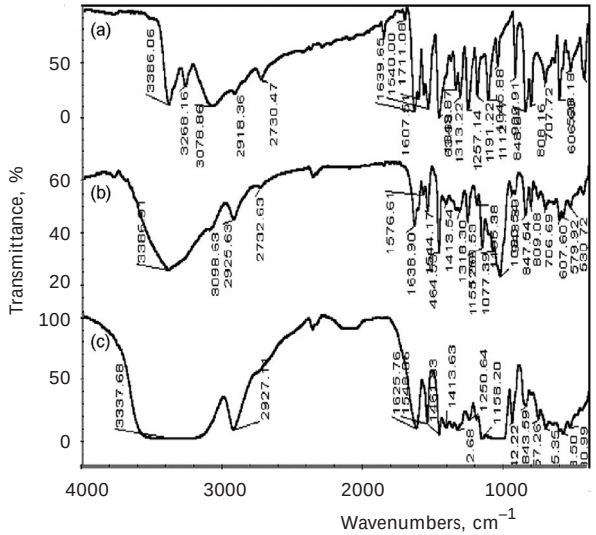

FIG. 4
FT-IR spectra of pure AFBT (a), physical mixture of AFBT with β-CDx (b) and solid complex of AFBT with β-CDx (c)

Effect of Acid-Base Concentration

The effect of acidity on the absorption spectra of AFBT has been studied in the range from H_0 = –3 to pH 7.0 in aqueous medium (Fig. 6). The absorption maxima of the neutral form of AFBT are at 259 and 221 nm; when pH is lowered, a blue-shifted maximum at 255 nm is obtained around pH 3.6. This blue-shifted (4.4 nm) maximum corresponds to the AFBT monocation obtained by protonation of the amino group. A further increase in acid

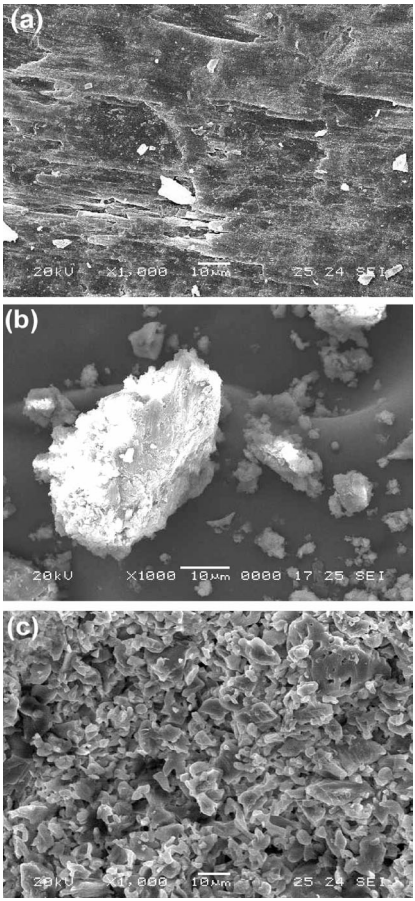

FIG. 5
SEM images of a pure AFBT (× 1000), b physical mixture of AFBT with β-CDx (× 1000) and c solid complex of AFBT with β-CDx (× 1000)

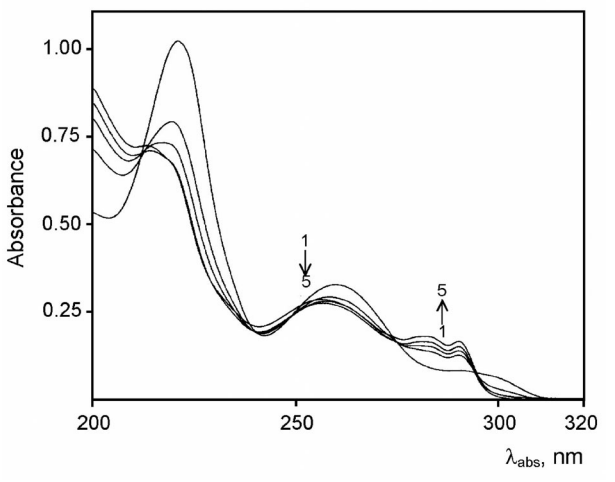

FIG. 6
Absorption spectra of AFBT without β-CDx at different pH values: 1 4.0, 2 3.6, 3 3.2, 4 2.8, 5 2.4

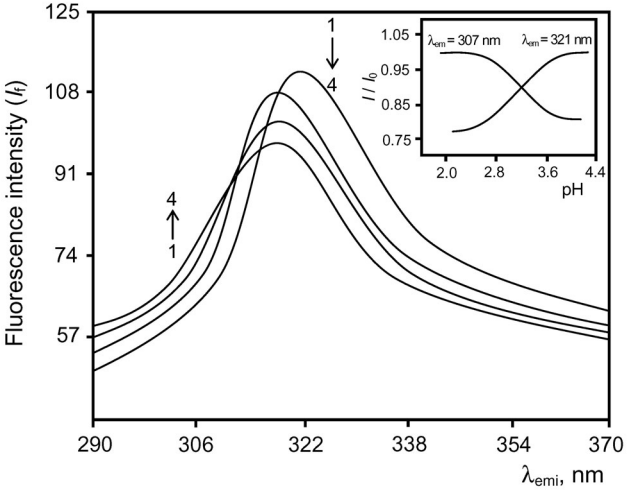

FIG. 7
Fluorescence spectra of AFBT without β-CDx at different pH values: 1 4.0, 2 3.6, 3 3.2, 4 2.8 (λ_{exi} = 271 nm) (Inset: Fluorimetric titration curves for AFBT without β-CDx)

concentration does not change the absorption spectrum significantly. Increasing pH from 7.0 to 12.0 there is no significant change in the absorption spectra. For the monocation–neutral compound equilibrium of AFBT clear isosbestic points were obtained at 295.0 and 275.0 nm. The ground-state pK_a value for the monocation–neutral compound equilibrium of AFBT in aqueous medium is determined spectrophotometrically to be 3.42.

The fluorescence spectra of AFBT in aqueous solution at different pH values are shown in Fig. 7. The neutral species at pH 7.0 shows a fluorescence maximum at 322.0 nm. When pH is decreased, the blue-shifted maximum at 317.2 nm is observed. This maximum is due to monocation of AFBT obtained by the protonation of amino group. The fluorimetric titration curve (FT-curve) for the monocation–neutral compound equilibrium of AFBT in aqueous solution obtained from fluorescence intensities is shown in inset Fig. 7. The excited-state pK_a value (pK_a*) obtained from the inflections of these curves is found to be 3.2.

The effect of acid-base concentration on the absorption spectra of AFBT has been studied in the range from H_0 = –3 to pH 12.0 in β-CDx medium (Fig. 8). The absorption maximum of the neutral molecule of AFBT in the β-CDx medium lies at 259.4 nm. When pH decreases from pH 7.0, a blue-shifted maximum at 254.5 nm is obtained. The blue-shifted maximum is

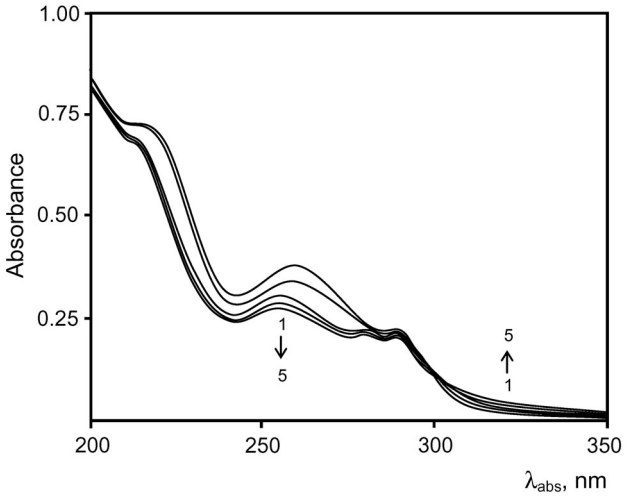

FIG. 8
Absorption spectra of AFBT with β-CDx at different pH/H_0 values: 1 4.2, 2 3.4, 3 0.83, 4 –0.26, 5 –0.84, 6 –1.38

due to the formation of monocation. Hence, the amino group of AFBT is protonated to form monocation in the β-CDx medium at H_0 = 0.26. A further increase in acid concentration does not change the absorption spectrum. For the monocation–neutral compound equilibrium, a clear isosbestic point is observed at 293.2 nm and the ground-state pK_a value of AFBT in the β-CDx medium is calculated to be –0.33.

The fluorescence emission spectra of AFBT in β-CDx medium at various H_0/pH values are shown in Fig. 9. The neutral species exhibits fluorescence maximum at 349.6 nm around pH 7.0. The fluorescence is quenched with a further increase in acid concentration. At H_0 = –0.26, a new spectrum begins to appear with the maximum around 346.0 nm. This may be due to the formation of monocation of AFBT in β-CDx. The fluorimetric titration curves for the monocation–neutral compound equilibrium of AFBT in β-CDx is shown in inset Fig. 9. Since the change in fluorescence maximum and intensity is less, the fluorimetric titration curves do not meet. Initially the decrease in fluorescence intensity is due to proton-induced quenching. In this case the middle of the monocation formation curve can be taken as pK_a* value. Hence the pK_a* is –0.2.

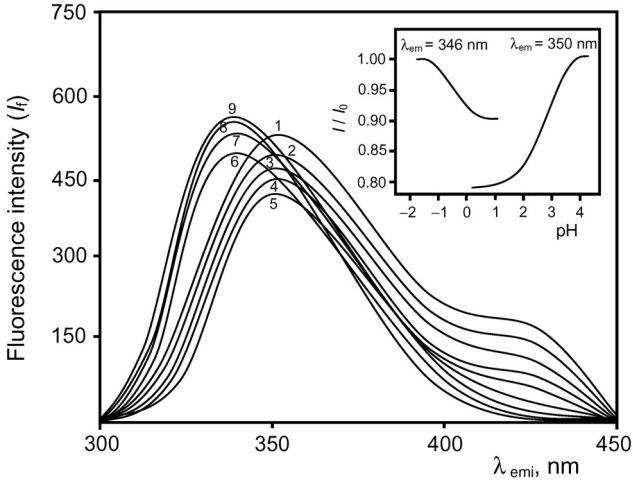

FIG. 9

Fluorescence spectra of AFBT with β-CDx at different pH/H_0 values: 1 4.0, 2 3.6, 3 2.8, 4 2.4, 5 2.0, 6 0.83, 7 –0.26, 8 –0.84, 9 –1.38 (λ_{exi} = 284 nm) (Inset: Fluorimetric titration curves for AFBT with β-CDx)

From the Benesi–Hildebrand plot it has been found that the stoichiometry of the complex is 1:1. The pK_a and pK_a* values of AFBT decrease by more than 3 units in the β-CDx inclusion complex (pK_a 3.42 to –0.33 and pK_a* 3.2 to –0.2). This indicates that the NH_2 group of AFBT lies inside the cavity of β-CDx requiring highly acidic condition for protonation. Further, the formation of an inclusion complex between AFBT and β-CDx is confirmed by time-resolved fluorescence spectroscopy in the liquid state and by FT-IR spectral and SEM image analysis in the solid state. Therefore, the complex formed is an axial inclusion complex with the NH_2 group of AFBT oriented inside the β-CDx cavity as shown in Fig. 10.

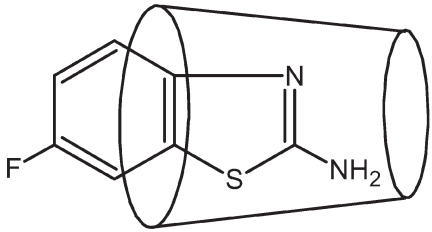

FIG. 10
Schematic diagram of the inclusion complex (1:1) of AFBT with β-CDx

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