J Fluoresc (2010) 20:809–820
DOI 10.1007/s10895-010-0623-3

ORIGINAL PAPER

Intramolecular Charge Transfer Effects on Flutamide Drug

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Received: 11 July 2009 / Accepted: 8 February 2010 / Published online: 10 March 2010

Abstract Spectral characteristics of flutamide drug have been studied in various solvents and β-cyclodextrin (β-CD). The inclusion complex of flutamide with β-CD is analysed by UV-visible, fluorimetry, FT-IR, 1H NMR, SEM, DSC and AM1 methods. In all solvents, flutamide exhibits a dual fluorescence. The longer wavelength emission (A band ~380 nm) is due to intramolecular charge transfer state (ICT) and the shorter wavelength emission (B band ~285 nm) originates from a locally excited state. In β-CD, the increase in the fluorescence intensity of 'A' band indicates ICT emission enhanced in the β-CD medium. β-CD studies shows isopropyl group is present in the interior part of the β-CD cavity whereas amino and CF_3 groups are present in the outside of the β-CD cavity. A mechanism is proposed to explain the 1:1 inclusion process.

Keywords Flutamide · β-cyclodextrin · Intramolecular charge transfer · Solvent effects

Introduction

The subject of cyclodextrin chemistry has experienced an enormous amount of interest over the last three decades. In 1993, Inoue pointed out in his review [1] of NMR studies of CD, there still remained no clear arrangement of the major driving force, behind the inclusion of best species within the CD cavity. Indeed in view of the wide variety of guest types which can complex with CD these are likely to be several different types of driving forces acting in various combinations. Inoue lists the main factor influencing complexation between CD with guest as vander Walls interactions, hydrophobic interactions, H–bonding between guest and hydroxyl groups of the CD, and the effects of solvent surface tension.

The most important property of CD is their ability to admit a variety of appropriately sized guest molecules into the cavity with formation of inclusion complexes [2–4]. The recognised potential of the CD-guest interaction as a model for enzyme activity sites has attracted the attention of many investigators [5]. Further, the photophysical and photochemical properties of organic and drug molecules included in to the CD cavity are also largely different from those of the molecules in aqueous solutions. Fluorescence intensity enhancement [6], intra [7] and intermolecular excimer formation [8], intramolecular exciplex formation [9], room temperature phosphorescence emission [10], the rate of proton dissociation [11], changes in proton induced quenching [12], and intramolecular charge transfer (ICT) [13, 14] have been reported to occur in solution in the presence of CD. Due to this ability, CDs have been widely used for analytical purpose [13] and have a wide range of practical application in cosmetics, food, chemical and other industries. Furthermore, they have very important uses in pharmaceutical industries to improve solubility, enhanced stability and bioavailability [14].

Cyclodextrin of different sizes are expected to influence the ICT emission differently [15, 17]. The reduced polarity of the CD environment as compared to water has some influence on the luminescence properties. Thus, the overall effect is likely to be rather complex and is likely to depend on the cavity size of the CD. Further, compared to the locally excited (LE) state, ICT emission has been found to be enhanced tremendously on complexation with β-CD than solvents, this being ascribed to the lowering of polarity of the CD microenvironment which in turn would lead to lowering of the non radiative

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decays from the ICT state to the lowering states. If the fluorophore is tightly packed with β-CD cavity, the ICT emission is expected to be suppressed severely. In the following sections it is reported that both LE and ICT emissions of flutamide are enhanced on complexation with β-CD.

The binding of 1,4-disubstituted benzenes (flutamide drug consider as 1,4-disubstituted benzene) within CD offers a good system in which to investigate factors influencing complexation. This is due to the effect of the tightly fitting benzene ring which ensures that guest molecules can only take up a limited number of conformations within the cavity [2]. For a small guest with CDs, the number of conformations is much larger and as a consequence, the predictability of the system suffers as seen in the complex formation for 1,4-disubstituted benzenes [3], a reasonable correction obtained for binding constants for CD. For CD, the NMR studies indicate the principle factor affecting orientation in dipole-dipole interactions.

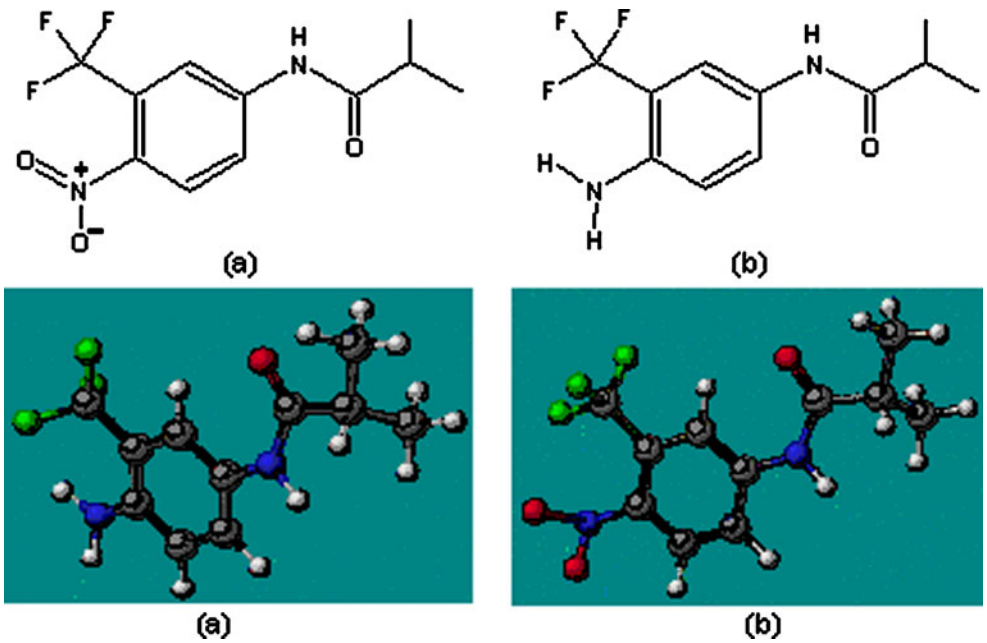
In this work, we not only studied spectral properties of flutamide–β-CD complex by UV-visible and fluorescence, but also prepared its solid inclusion complex by co-precipitation method and determined its formation by FTIR, ^{1}H NMR, SEM and DSC methods. The formation constant of the inclusion complex was obtained according to the data of UV-visible, and fluorescence using modified Benesi-Hildebrand equation. The solid studies on the inclusion complex of CD with guest molecule have been performed to obtain direct evidence for the formation of the inclusion complexes [15–17]. The obtained results indicate that the solid structure of these complexes is designable by appropriately selecting type, length and functional substitutent group in the guest.

Flutamide (Scheme 1) is an oral antiandrogen drug [18, 19] primarily used to treat prostate cancer. It is also be used to treat excess androgen levels in women [18]. It competes with testosterone and its powerful metabolite, dihydrotestosterone (DHT) for binding to androgen receptors in the prostate gland. Flutamide has been largely replaced by a newer member of this class due to a better side-effect profile. Flutamide is not a steroid and it is excreted in various forms in the urine, the primary form being 2-amino-5-nitro-4-(trifluoromethyl) phenol.

Experimental

Instruments

Absorption spectral measurements were carried out with a Shimadzu Model-1601 UV-visible spectrophotometer and fluorescence measurements were made using a Perkin spectrofluorimeter Model LS-55. FT-IR spectra were obtained with Avatar-330 FT-IR spectroscopy using KBr pelleting. The range of the spectra is from 500 to 4,000 cm^{-1}. Microscopic morphological structure measurements were performed with JEOL JSM 5610 LV scanning electron microscope (SEM) and Shimadzu-60 Differential Scanning Colorimeter (DSC) was used to measure the thermal curves. Bruker Advance DRX 400 MHz super conducting NMR spectrophotometer was used to study ^{1}H NMR spectra (IISc, Bangalore).



Scheme 1 CAche structure of flutamide: **a** nitro form, **b** amino form

Reagents and materials

Flutamide and β-CD were obtained from E-merck and recrystallized from aqueous ethanol. The purity of the compound was checked by similar fluorescence spectra when excited with different wavelengths. All used solvents used were of the highest grade (spectrograde) commercially available. Triply distilled water was used for the preparation of aqueous solutions. The concentration of the flutamide solutions was the order of 4×10^{-4} to 2×10^{-5} mol dm^{-3}. The concentration of β-CD solution was varied from 0.001 to 1×10^{-2} mol dm^{-3}. The solid inclusion complex is prepared by co-precipitation method.

Results and discussion

Effect of solvents

The absorption and fluorescence maxima and molar extinction coefficient of flutamide drug (chemical name~ 2-methyl-N-[4-nitro-3-(trifluoro methyl)phenyl]-propan amide) is studied in various solvents (Table 1). In flutamide, the absorption band in all solvents consists of two peaks. The absorbance in longer wavelength band (LW ~315 nm) is very weaker than its middle wavelength band (MW ~260 nm). Both the LW and MW bands are slightly red shifted from non polar to aprotic solvents (318 nm), whereas it is slightly blue shifted in protic solvents (310 nm). The spectral shifts observed in the absorption spectrum of this drug in protic and aprotic solvents are consistent with the characteristic behaviour of amino groups [15, 16]. The location of the LW band related to the former one can be assigned to π–π* transition involving the whole electronic system of the compounds with a considerable charge transfer (CT) character. Such a CT originates mainly from the aromatic ring or amino group to the CF_3 or C=O (carbonyl) group which is characterized by a high electron accepting character i.e. this band is due to ICT character. Because of the greater charge transfer effect of the carbonyl group with the NH_2 group, a large red shift is observed in flutamide. The red shift observed in the

Table 1 Absorption, fluorescence spectral data (nm) and Stokes shift (cm^{-1}) of flutamide in different solvents

No.	Solvents	λ_{abs}	log ε	λ_{flu}	Stokes shift	$E_T(30)$	BK	*f*(D,n)
1	Cyclohexane	314 261	2.30 3.45	360 283	4670 2978	30.9	−0.001	−0.0004
2	Diethyl ether	317 263	2.22 3.37	366 284	4223 2811	34.6	0.397	0.167
3	1,4-Dioxane	317 262	2.14 3.33	366 284	4223 2956	36.0	0.043	0.021
4	Dichloro methane	315 262	2.25 3.29	365 282	4348 2706	41.1	0.586	0.218
5	Ethyl acetate	316 262	2.14 3.42	370 285	4618 3080	38.1	0.488	0.201
6	Acetonitrile	318 263	2.20 3.30	373 286	4636 3057	46.0	0.864	0.305
7	*t*-Butyl alcohol	313 262	2.21 3.32	375 286	5282 3202	43.9	0.673	0.245
8	2-Butanol	313 262	2.18 3.41	375 285	5282 3080	47.1	0.734	
9	2-Propanol	313 262	2.24 3.33	377 286	5423 3202	48.6	0.766	0.274
10	1-Butanol	313 262	2.10 3.34	375 285	5282 3080	50.2	0.754	0.263
11	Ethanol	313 262	2.28 3.42	376 286	5353 3202	53.7	0.812	0.289
12	Methanol	312 256	2.21 3.28	376 287	5435 4219	55.5	0.858	0.309
13	Water	310 256	2.13 3.15	382 287	5942 4219	63.1	0.913	0.320
14	Correlation co-efficient							
a)	$E_T(30)$ Vs $\Delta\bar{\nu}_{ss}$			0.9124				
b)	BK Vs $\Delta\bar{\nu}_{ss}$			0.7658				
c)	*f*(D,n) Vs $\Delta\bar{\nu}_{ss}$			0.7864				

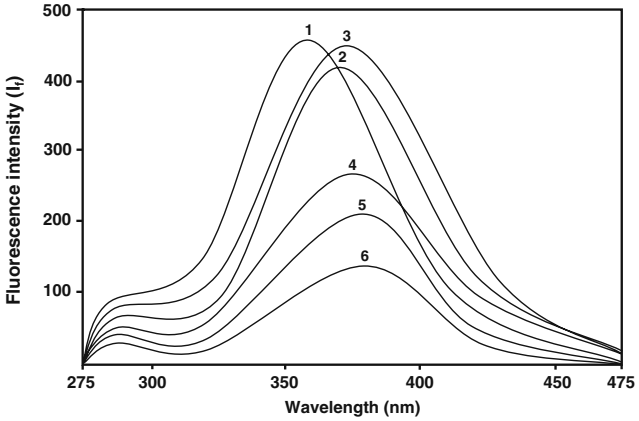


Fig. 1 Fluorescence spectra of flutamide in different solvents: 1. cyclohexane, 2. ethyl acetate, 3. acetonitrile, 4. 2-propanol, 5. methanol, 6. water

absorption spectra from cyclohexane to aprotic solvents is due to the dispersive interactions and the most proton accepting nature of the solvents. Since, methanol and water can act as proton donor solvents and thus flutamide produce blue shifted maxima in the absorption spectra. This shows the interaction of the amino group is larger when compared to the carbonyl group.

Dual emission

Figure 1 depicts the fluorescence spectra of flutamide in various solvents. In the S1 state, flutamide exhibits dual fluorescence in all solvents. Among the two bands, one occurs in the shorter wavelength region (SW, around 285 nm) and the other in longer wavelength region (LW, around 380 nm). In all solvents, the LW emission is more red shifted than SW intensity and the intensity of LW band is higher than SW. Further, the LW fluorescence intensity increases with an increase in the $\lambda_{excitation}$ from 260 nm to 280 nm. This may be due to the extended π-conjugation that would induce an excited state resonance, contribute from carbonyl group to amino group resulting in the increased polarity to facilitate the interaction with polar solvents [16].

Considering 4-N,N-dimethylamino benzonitrile (DMABN) [20] as a reference molecule, the SW or locally excited (LE) band and the LW or Intermolecular charge transfer (ICT) band are defined as the 'B' band and the 'A' (anomalous) band respectively. The ratio of the fluorescence intensity of 'A' band to that of 'B' in flutamide at their respective maxima in cyclohexane is decreased with an increasing in the polarity of the solvents. The fluorescence maximum of LW band is more red shifted than SW band in going from non polar to polar solvents.

Several mechanisms have been proposed to explain the 'A' band emission. The mechanism proposed by Grabowski et al. [20, 21] still is the best. The present results can also be explained on the same lines by rejecting the other mechanisms as follows. For example, Lippert et al. [22] have suggested the reversal of S_1 and S_2 states because the solvent interaction of the excimer formation proposed by Khalil et al. [23], a proton transfer in the excited state [24], complex formation with the solvent [25] and the formation of exciplex with free electron pairs of the solvents [26]. As suggested by Rettig [27], the results obtained in the present work can be explained as follows:

(1) The appearance of the 'A' fluorescence in cyclohexane implies that the spectral behaviour of the flutamide molecule is not due to solute-solvent specific interactions, (2) the fluorescence band correspond to the same ground state forms, because the excitation spectra do not differ, (3) hydrogen bond formation between the protic solvents and electron donor group facilitates the formation of the ICT state in the S_1 state [16, 27, 28] and (4) hydrogen bond formation between the protic solvents and the electron withdrawing group will lead the electron withdrawing group to become coplanar with benzene ring [16]. In other words, this hydrogen bonding seems to make the migration of electron density from amino group/benzene ring to the electron withdrawing group more facile. Like Cazeau-Dybroca et al. [29], a red shifted fluorescence band at room temperature is also observed when the amount of water is increased in the aprotic/non-polar solvents; (i.e.) the fluorescence spectrum in the cyclohexane solution is changed significantly an addition of water showing a dual emission (Fig. 2). In contrast to other polar solvents, dual emission in water is further red shifted to ~390 nm (compared to methanol 375 nm), even though the maximum of the normal emission around ~285 nm is nearly independent of the hydrogen bonding ability of solvents. These observations suggest that, the dual emission in water seems to be influenced by the enhanced intermolecular hydrogen bonding of the carbonyl group in the excited state. Supporting this fluorescence spectrum in the aqueous

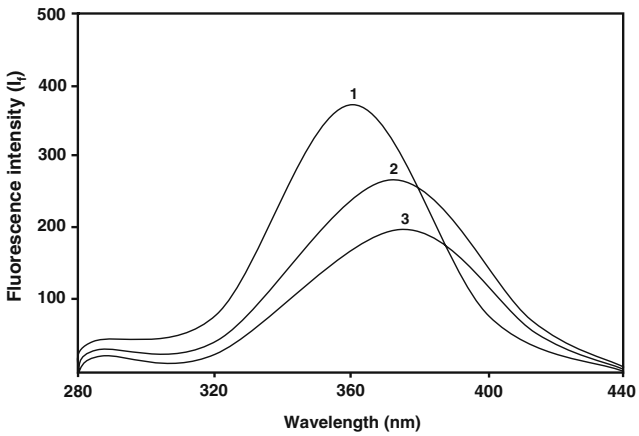


Fig. 2 Fluorescence spectra of flutamide: 1. cyclohexane, 2. cyclohexane with 1% methanol, 3. cyclohexane with 1% water

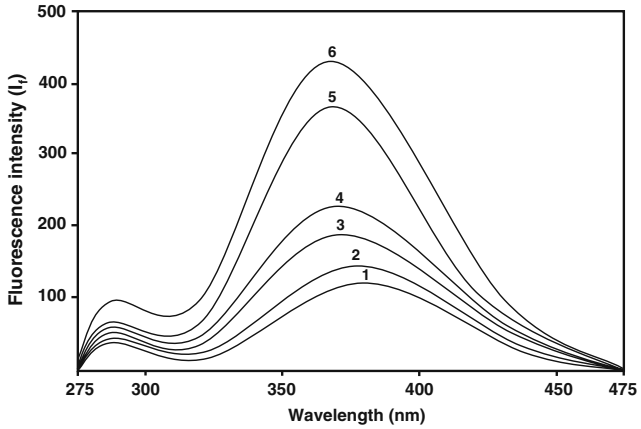


Fig. 3 Fluorescence spectra of flutamide in water 1,4-dioxane mixture: 1. 100% water, 2. 1% 1,4-dioxane, 3. 5% 1,4-dioxane, 4. 10% 1,4-dioxane, 5. 20% 1,4-dioxane, 6. 30% 1,4-dioxane

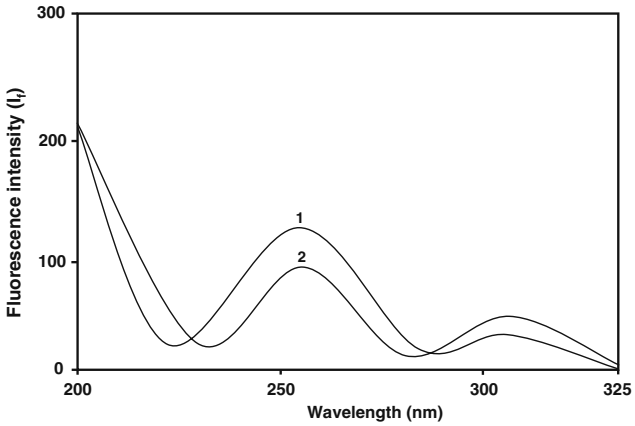


Fig. 5 Excitation spectra of flutamide in methanol: 1. 285 nm, 2. 380 nm

solution is changed significantly on addition of dioxane showing a dual emission and an isoemissive point in dioxane-water mixture (Fig. 3).

It is also noted that the fluorescence spectrum of flutamide in water shows a different feature depending on the excitation wavelength (260, 270 and 280 nm) (Fig. 4). In the case of 260 nm excitation, the emission spectrum exhibits the dual emission (285 nm and 380 nm). However, with an excitation at 275 nm the intensity of LW emission at 380 nm is increased than 260 nm excitation. This excitation wavelength dependence of the dual emission is similar to the typical red edge effect [29] observed in the ICT fluorescence which is usually observed under the restrictive molecular mobility environment like the polymer system [30].

The effect of excitation wavelength in the range of 260–280 nm has been studied in cyclohexane and methanol (Fig. 5). It is observed that fluorescence band maxima of both bands are independent of excitation. This shows that the emission from both states occur from their most relaxed

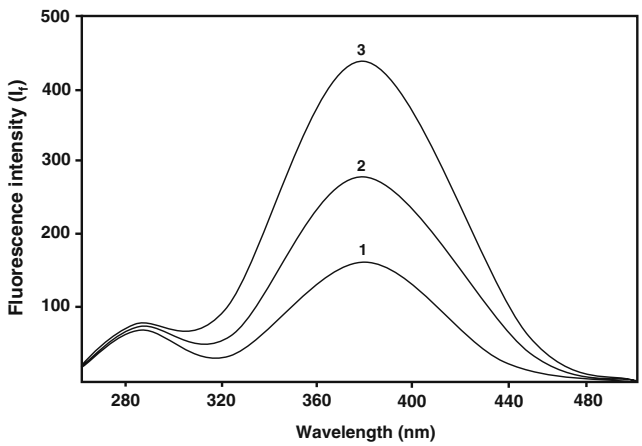


Fig. 4 Fluorescence spectra of flutamide in water measured at different excitation wavelength: 1. 260 nm, 2. 270 nm, 3. 280 nm

states and the solvent relaxation times of these solvents are smaller than the radiative decay rate of the fluorophore. The fluorescence excitation spectra recorded in cyclohexane/methanol resembles one another as well as with the absorption spectra. This indicates the absorbing species in the ground state for both emissions are same. Large Stokes shifts have been observed in molecules showing ICT behaviour [31]. The small Stokes shifted band can be assigned to the locally excited state 'B' band and the large Stokes shifted band 'A' to the ICT state. It is well known that, the energy barrier for the formation of A* from B* decreases as the polarity of the medium increases [32]; i.e. the emission intensity of A* should increase from non polar to polar solvents can be explained in the following manner: the dipole moment of the ICT state is very large and the dipolar interactions of the ICT will increase with the increase in polarity of the solvents. Thus the decrease in the fluorescence intensity of the 'A' band in polar solvents could be due to large stabilization of the highly polar ICT state by strong dipole-dipole and hydrogen bonding interaction and consequent rapid non-reductive transition to the ground and/or low lying triplet states [33]. Further, the fluorescence spectrum of water changed significantly to the addition of dioxane showing a dual emission suggests that ICT emission is present in this molecule (Fig. 3). Thus, it can be speculated that the enhanced 380 nm emission should originate from the ICT state. Supporting this implication, the excitation spectra exhibit the monitoring wavelength dependence as shown in Fig. 5. The excitation spectrum for the 380 nm emission is similar to 285 nm emission suggests that the ICT state is present in this molecule.

In order to confirm this, Reichardt's solvent parameter $E_T(30)$ [34], BK [35] and $f(D,n)$ [36] solvent parameter are used and compared with Stokes shifts. The Stokes shifts $(\Delta\bar{\nu}_{ss}cm^{-1})$ of the fluorescence spectra of flutamide in different solvents are correlated with $E_T(30)$, BK and $f(D,n)$ are displayed in Fig. 6. The difference between the

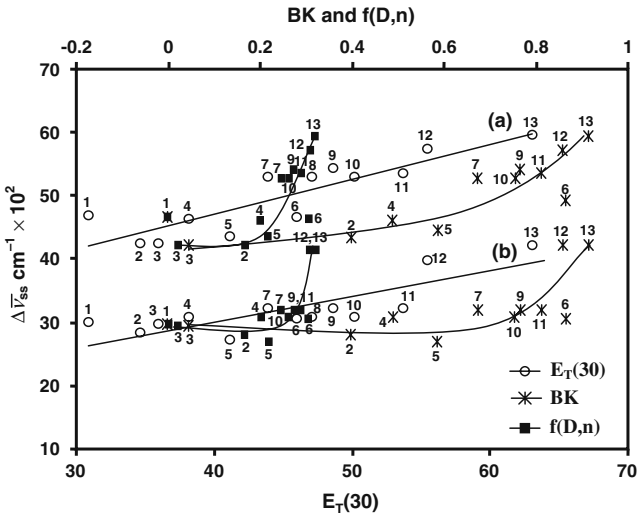


Fig. 6 Plot of Stokes shifts (cm^{-1}) of flutamide versus $E_T(30)$, BK and *f*(D,n) solvent parameters: 1. cyclohexane, 2. diethyl ether, 3. 1,4-dioxane, 4. ethyl acetate, 5. dichloro methane, 6. acetonitrile, 7. *t*-butyl alcohol, 8. 2-butanol, 9. 2-propanol, 10. 1-butanol, 11. ethanol, 12. methanol, 13. water

solvatochromic slopes for the 'A' band is larger than 'B' band. The solvatochromic slopes in Fig. 6 which is indicative of the fact that the dipole moment difference between the excited state and the ground state is large for the ICT state.

Effect of β-CD

Figures 7 and 8 show the absorption and fluorescence spectra of flutamide in aqueous solutions (pH~7) containing different concentrations of β-CD. In absorption, no significant spectral shift (λ_{abs}~305 nm and 255 nm) is observed upon increasing the concentration of β-CD from

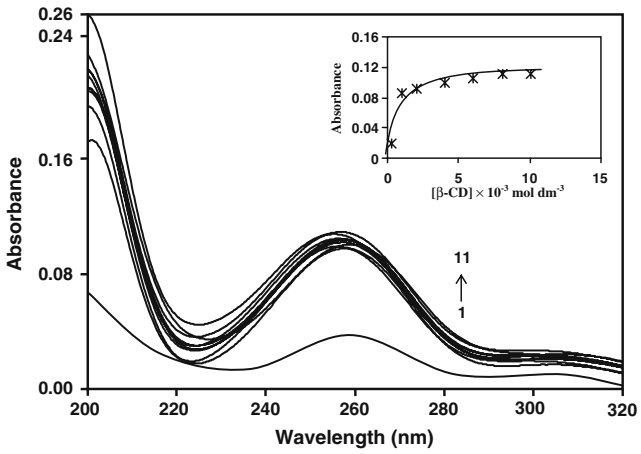


Fig. 7 Absorption spectra of flutamide in different β-CD concentrations (× 10^{-3} M): 1) 0, 2) 1, 3) 2, 4) 3, 5) 4, 6) 5, 7) 6, 8) 7, 9) 8, 10) 9, 11) 10. Insert Fig. Plot of absorbance versus [β-CD]

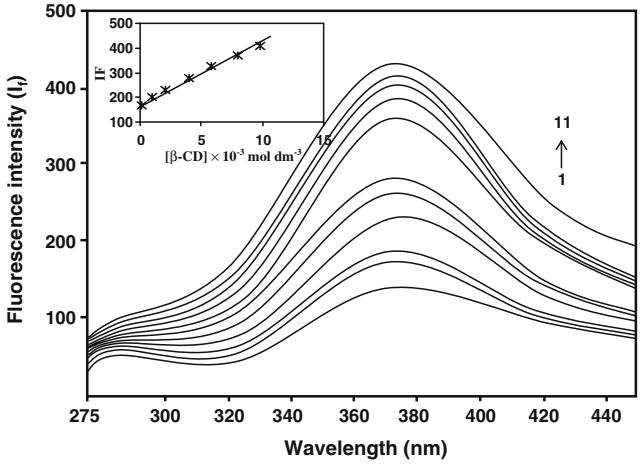


Fig. 8 Fluorescence spectra of flutamide in different β-CD concentrations (×10^{-3} M): 1) 0, 2) 1, 3) 2, 4) 3, 5) 4, 6) 5, 7) 6, 8) 7, 9) 8, 10) 9, 11) 10

0.001 to 0.01 M. However, the molar extinction coefficient is increased when the concentration of β-CD increases. The absorbance and fluorescence intensities measured at 255 nm and 380 nm wavelength respectively as a function of [β-CD] is plotted in insert Figs. 7 and 8. The slight increase in absorbance is due to the detergent action of β-CD and it is attributed to the additional dissolution of flutamide adsorbed on the surface of the walls of the container [37–40]. Increase in the absorbance is noticed in β-CD solutions indicates the nonpolar part of this molecule which may be encapsulated in the non polar β-CD cavity and the regular blue shift shows the amino group encapsulated in the β-CD cavity [37–40].

Binding constant (K) for the formation of ground state inclusion complex is obtained from the concentration dependence of absorption spectra by using Benesi-Hildebrand equation [41] indicates 1:1 complex formed between flutamide and β-CD. For 1:1 complex between β-CD and guest molecule flutamide the following equilibrium can be written:

$$\text{Flutamide} + \beta\text{−CD} \rightleftharpoons \text{Flutamide} \ldots \beta\text{−CD} \quad (1)$$

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon} + \frac{1}{K[\text{Flutamide}]_0 \Delta\varepsilon[\beta\text{−CD}]_0} \quad (2)$$

where ΔA is the difference between the absorbance of flutamide in the presence and absence of β-CD, Δε being the difference between the molar absorption coefficient of flutamide and the inclusion complex $[\text{flutamide}]_0$ and $[\beta\text{-CD}]_0$ are the initial concentration of flutamide and β-CD, respectively. Figure 9 depicts a plot of 1/ΔA as a function of 1/[β-CD] for flutamide. A good linear correlation is obtained, confirming the formation of a 1:1 inclusion complex. From the intercept and slope values of this plot K is evaluated [K=96 M^{-1}] at 303 K.

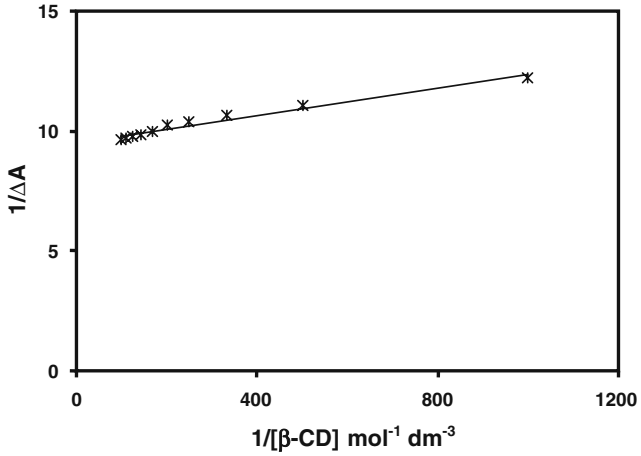


Fig. 9 Plot of 1/ΔA versus 1/[β-CD] for flutamide

The β-CD dependence of flutamide fluorescence can be analysed by the Benesi-Hildebrand [41] plot as given by Eq. 3:

$$\frac{1}{I-I_0}=\frac{1}{I'-I_0}+\frac{1}{K(I'-I_0)[\beta-CD]_0}\cdots \quad (3)$$

where $[\beta\text{-CD}]_0$ represents the initial concentration of β-CD, I_0 and I are the fluorescence intensities in the absence and presence of β-CD respectively and I′ is the limiting intensity of fluorescence. The ($K=315\ M^{-1}$) values are obtained from the slope and the intercept of the plots. The Benesi-Hildebrand plots (Fig. 10) shows an excellent linear regression ($r=0.9772$) supporting the formation of the 1:1 inclusion complex. The K value in the S_1 state is higher than S_0 state indicates in the excited states, the drug is more deeply included in the β-CD cavity. The greater increase in the fluorescence intensity confirms this implication.

The fluorescence spectra of flutamide molecule at various β-CD concentration reflects a remarkable change in the emission properties of the locally excited (LE) and ICT states upon complexation (Fig. 8). The ICT intensity in pure aqueous medium is slightly greater than LE intensity. With an addition of β-CD both the LE and ICT intensity increase, however, the rate of enhancement of the ICT emission is greater than that of the LE band. This can be seen clearly from looking at Fig. 8, while the LE intensity in 0.010 M β-CD solution is increased to 2 times than that of aqueous medium and the ICT emission is enhanced to approximately 8 times. Such changes caused by the formation of an inclusion complex of this drug with β-CD. Considerable increase in the fluorescence intensity compared with absorbance means that the quantum yields of this molecule are increased in the presence of β-CD. The common characteristic observed is β-CD enhanced ICT fluorescence intensity. The enhancement by β-CD of the ICT fluorescence is much stronger than the LE state which should be attributed to the hindrance of the twisting of this molecule.

In the present case, both SW and LW maxima increase in CD solutions suggest that, this drug present in partial polar environment. Further, if the CF_3 and $-NH_2$ groups present in the inside of the CD cavity, the ICT emission should decrease in the CD environment, because the inside CD cavity provides nonpolar environment. However, in this drug, CF_3 and $-NH_2$ groups are exposed to water, so that TICT emission is increased. This observation implies a partial implication of the flutamide inclusion complex formation in the reduced polar environment. Therefore, it is assumed that, flutamide form 1:1 inclusion complex with CD, and the interaction of CF_3 and $-NH_2$ groups with the protic polar solvent (water) is greatly increased in the 1:1 complex. Hence, TICT emission increases in β-CD. Further, it is well known that, the interior part of the CD cavity is hydrophobic and isopropyl group is also more hydrophobic than CF_3 and $-NH_2$ groups, hence isopropyl group more easily encapsulated in to the β-CD cavity than CF_3 and $-NH_2$ groups. This should be the reason why the longer wavelength fluorescence is more enhanced than SW. Also, it is clear that the TICT can be increased in the inclusion complex because this drug partially encapsulated in the CD cavity.

This is further supported by using semiempirical quantum mechanical calculations. The internal diameter of the β-CD is approximately 6.65 Å and its height is 7.8 Å. To determine the dimensions of flutamide, the geometry of flutamide in the ground state has been optimized by using the AM1/CAche program (Schemes 1 and 2). This calculation reveals that the length of flutamide molecule is greater than β-CD cavity. Since the length between amino and isopropyl groups in flutamide is larger than that of the upper rim of β-CD and hence one of the above groups should be projected outside the cavity and will be available in the bulk solution due to the size restriction in the β-CD cavity [37, 38].

In flutamide, two different types of inclusion complex formation are possible, i.e., (1) isopropyl and amide group

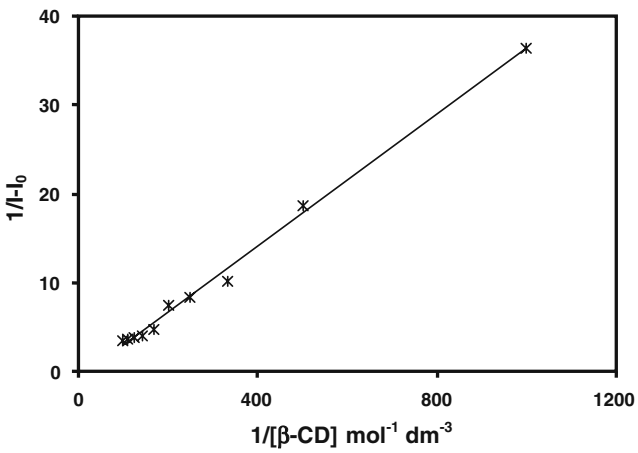
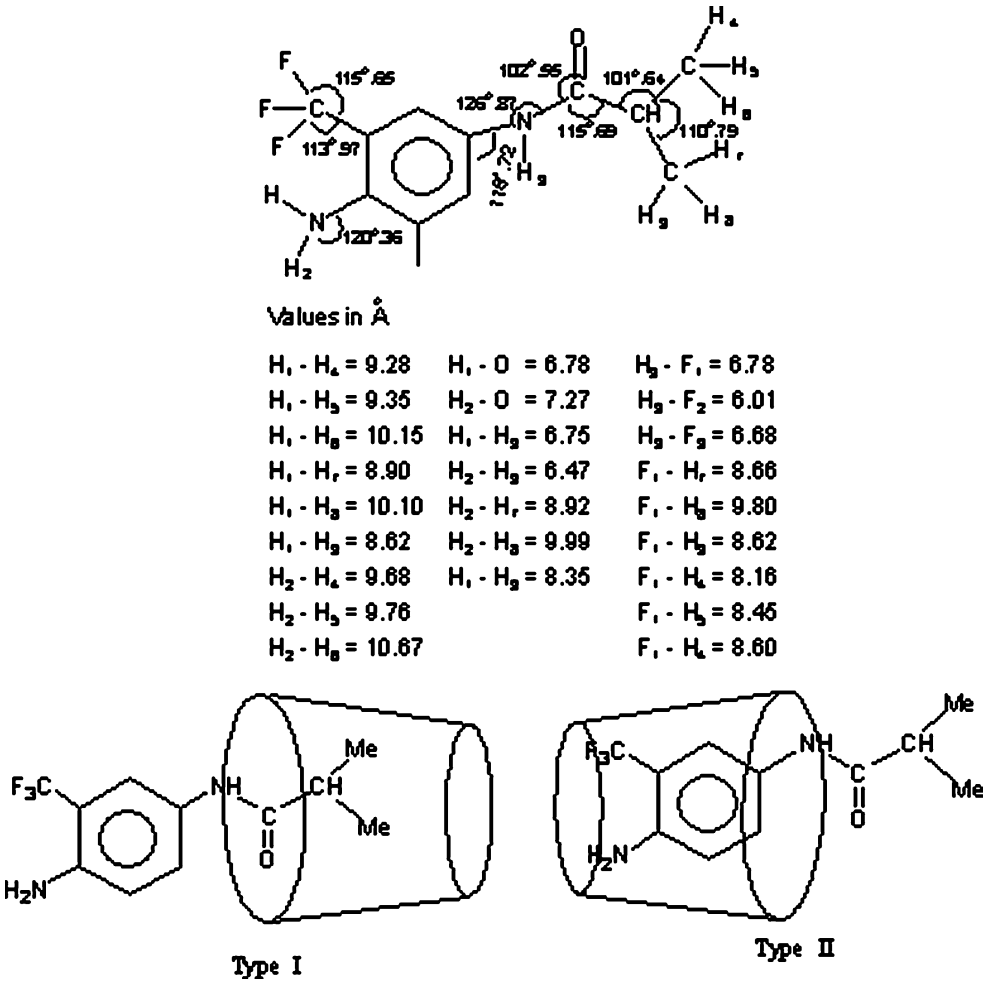


Fig. 10 Plot of $1/I\text{-}I_0$ versus 1/[β-CD] for flutamide



Scheme 2 Proposed structure of inclusion complex of flutamide with β-CD

captured or (2) amino and phenyl ring deeply present in the inner β-CD cavity. The first position (Type I) could arise when the isopropyl and amide group of the flutamide molecule is heeded towards the smaller rim of the β-CD cavity (Scheme 2) and the second position (Type II) could arise when the amino and phenyl group of the flutamide molecule is heeded towards the smaller rim of the β-CD cavity. In the case of Type I complex, the isopropyl and amide group experience the least polar and most rigid environment and therefore, give rise to a ICT fluorescence, whereas the amino and phenyl group experience an environment of higher polarity and a larger free volume because they are directed towards the water.

Further more, Nag et al. [33, 42] observed a similar observation, that the ICT fluorescence band is enhanced upon addition of β-CD to an aqueous solution of guest. Moreover, it is observed that the intensities of both ICT and LE bands are enhanced where as the ICT band is more enhanced than the LE band, which suggests that the formation of Type I complex are expected to be easy than Type II complex [38]. Further, the red shift supports the amino group is present in more polar environment than water, so that the ICT state is sufficiently solvated in high β-CD concentration solutions [37]. It is a well known fact that, hydrophobicity is the driving force to the formation of inclusion complexes [43]. Since isopropyl group is more hydrophobic than amino group, the isopropyl group may include in to the β-CD cavity. Further, the small binding constant (315 M^{-1}) implies that the isopropyl group is not more tightly embedded in the β-CD cavity and the rotation of the amino group for ICT would not be inhibited; i.e., the ICT emission is greatly enhanced in β-CD solution suggests that the rotation of the amino group is feasibly in the β-CD solutions suggests, the amino group present in the outside of the cavity.

The free energy change can be calculated from the formation constant 'K' by equation

$$\Delta G = -RT \ln K$$

As can be seen from Table 2, ΔG is negative which suggests that the inclusion process is exothermic and spontaneous at 303 K.

Table 2 Absorption and fluorescence maxima (nm) of Flutamide at different Concentrations of β-CD

No.	Concentration of β-CD M	λ_{abs}	logε	λ_{flu}	I_f
1	Water	305s	2.33	375	145
		258.6	3.25	285	80
2	0.001	305s	2.35	375	178
		56.4	3.28	285	82
3	0.002	305s	2.39	375	186
		256.4	3.30	285	84
4	0.004	305s	2.44	375	264
		257.3	3.32	285	86
5	0.006	305s	2.47	375	388
		257.3	3.36	285	90
6	0.008	305s	2.49	375	407
		257.4	3.41	285	93
7	0.010	305s	2.51	375	430
		257.2	3.44	285	101
8	Binding constant M^{-1}	96		315	
9	ΔG	−11.50		−14.50	

Solid inclusion complex studies

FT-IR spectral studies

The FT-IR spectra of flutamide, β-CD and the solid inclusion complex are also studied (Fig. 11). Flutamide examined in KBr pellet, displays one absorption band at 3,360 cm^{-1}. This band represents the Ar-NH_2 stretching frequencies and it moves in the inclusion complex to 3,403, 3,361, 3,274 cm^{-1}. The Ar-NH_2 bending vibrations 1,611 cm^{-1} is moves in the inclusion complex to 1,633 cm^{-1}. The Ar-NH_2 deformations appear at 1,597 cm^{-1} is moves in the inclusion complex to 1,590 cm−1. The amide NH bending vibration 1,541 cm^{-1} is moves in the inclusion complex to 1,528 cm^{-1}. The amide C-N stretching frequency of the pure drug appears around 1,319 cm^{-1} and this frequency is shifted in the inclusion complex to 1,310 cm^{-1}. The C=O stretching frequency of the pure drug appears around 1,716 cm^{-1} is shifted to 1,654 cm^{-1} in the inclusion complex.

The aromatic C-H stretching frequency of the pure drug appears around 3,055, 3,127, 3,207 cm^{-1} whereas these frequencies are shifted to 3,057 cm^{-1} (very weak) in the inclusion complex. The C-CF_3 stretching mode 1,345, 1,319 cm^{-1} in the pure drug is moved to 1,342, 1,310 cm^{-1} in the inclusion complex. Ar-CF_3 frequencies at 522, 554, 597, 655 cm^{-1} of the flutamide are shifted in the inclusion complex to 532, 578, 609 cm^{-1}. The isopropyl stretching and bending frequencies at 1,319, 1,345, 1,391 cm^{-1} of the flutamide is moves in the inclusion complex to 1,310, 1,342, 1,297 cm^{-1} respectively. The C-H in CH_3 stretching at 2,984 cm^{-1} is moves in the inclusion complex to 2,971 cm^{-1}. The CH_3 symmetric deformation at 1,391 cm^{-1} and antisymmetric deformation (1,469 cm^{-1}) are lost in the inclusion complex. Moreover, the absorption intensity of most of the frequencies in the inclusion complex is significantly weaker (10–60%) than the flutamide molecule. These results indicates that the amino group is present in hydrophilic part and the isopropyl group is encapsulated in the β-CD cavity.

^{1}H NMR spectral shifts

^{1}H NMR spectroscopy provides an effective means of assessing the dynamic interaction site of β-CD with that of the guest molecules. Even though, only limited information can be obtained from 1H NMR data, the observation of slight up field shift of the guest protons in the presence of β-CD is consistent with the inclusion of each guest into the β-CD cavity. The resonance assignments of the protons of β-CD are well established [44–46] and consist of six types of protons. The chemical shift of β-CD protons reported by different authors [44–46] are very close to those reported in this work. The H-3 and H-5 protons are located in the interior of the β-CDs cavity, and it is, therefore likely that the interaction of the host with the β-CD inside the cavity will affect the chemical shifts of the H-3 and H-5 protons. A minor shift is observed for the resonance of H-1, H-2 and H-4 located on the exterior of β-CD [44–46].

As can be seen from the chemical shifts data for the inclusion complex was different from the free compound. The addition of flutamide (Scheme 2) into the β-CD results in a downfield chemical shift for the flutamide protons in DMSO are given below: *flutamide (inclusion complex)* ppm: NH_2 ~5.350 (5.338), NH ~9.661 (9.646), 1H ~7.728 (7.722), 2H ~7.4189 (7.426), 3H ~6.755 (6.758). A small downfield shift

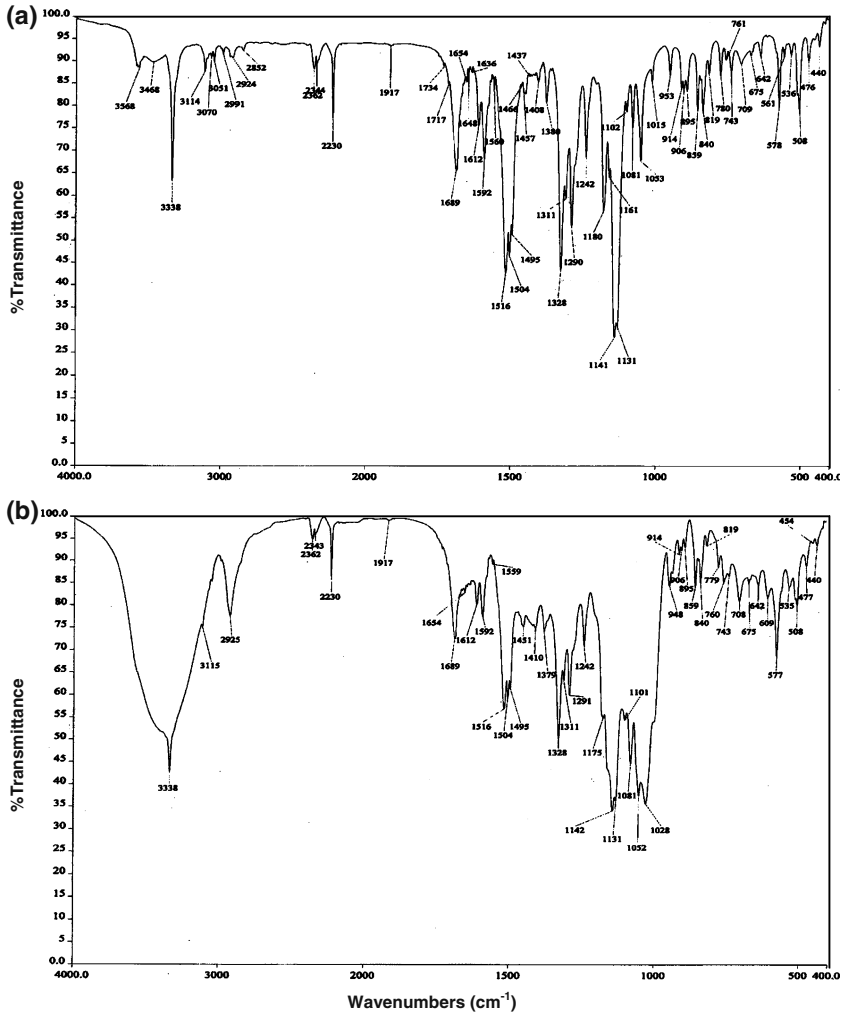


Fig. 11 FTIR spectrum of FLA in KBr **a** FLA and **b** FLA-β-CD complex

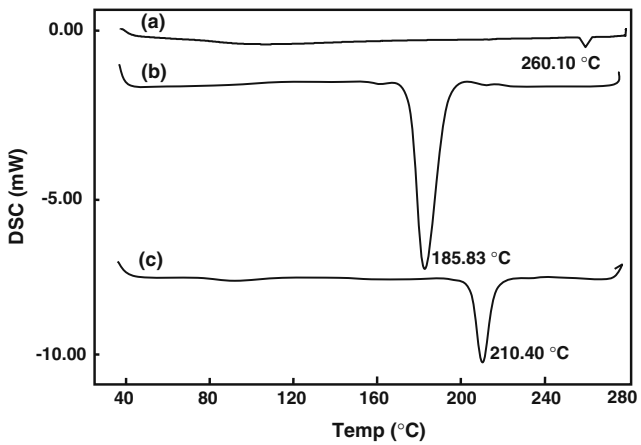


Fig. 12 Thermal spectra of a) β-CD, b) flutamide, c) flutamide-β-CD inclusion complex

on flutamide is observed in isopropyl group suggest this part encapsulated in the β-CD cavity and the other phenyl ring is present in the inner part of the β-CD cavity.

Differential scanning colorimetry (DSC) study

The DSC curves of flutamide, β-CD and inclusion complex are shown in Fig. 12. It can be seen from Fig. 12, DSC curves of inclusion complex with the DSC curves of flutamide, and flutamide –β-CD are different from one another i.e., β-CD ~260 °C, time 19.15 min; flutamide ~185.83°C, time ~15.81 min; flutamide –β-CD ~210.40 °C, time ~17.6 min. These values proved that a new inclusion complex is formed.

Microscopic morphological observation

Firstly, it is observed that powder form of flutamide and β-CD by scanning electron microscope and then we saw a

powder form of the inclusion complex (Fig. 13). Pictures clearly elucidate the difference in each case. β-CD shows sheeted/plated structure, flutamide shows stone rock structure and the complex structure is subrounded which is different from β-CD and flutamide. Modification of crystals and powder can be assumed as a proof of the formation of new inclusion complex.

Conclusions

The following conclusions can be drawn from the above studies: (1) in all solvents, flutamide exhibits a dual fluorescence. The longer wavelength emission (A band ~380 nm) is due to ICT and the shorter wavelength emission (B band ~285 nm) originates from a LE state, (2) β-CD

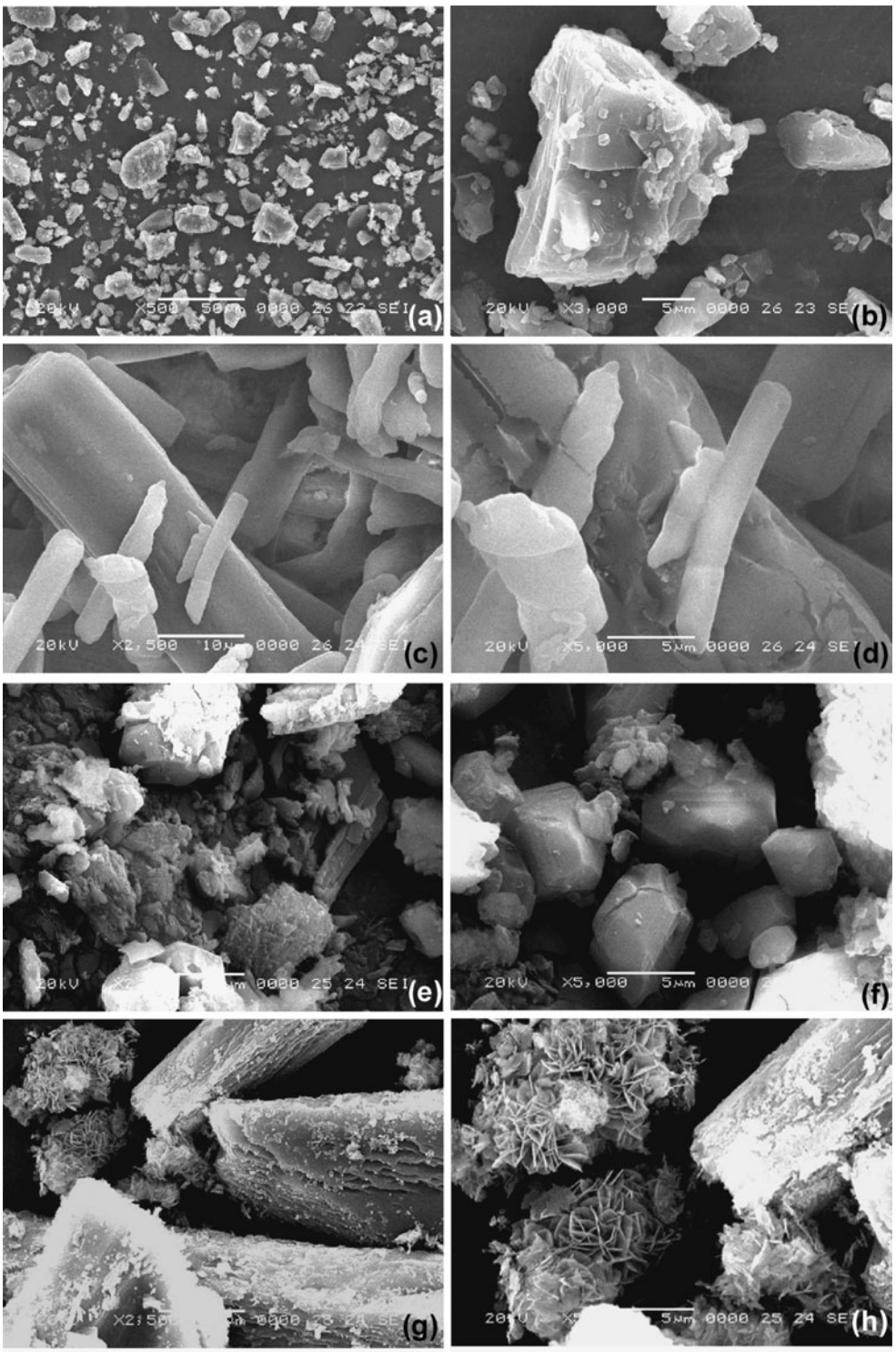


Fig. 13 Scanning electron microscope photographs (Pt. coated) of **a** β-CD (×500), **b** β-CD (×3,000), **c** flutamide-pure (×2,500), **d** flutamide-pure (×5,000), **e** flutamide-reduced (×2,500), **f** flutamide-reduced (×5,000), **g** and **h** flutamide-reduced-β-CD inclusion complex (×2,500 and ×5,000)

studies suggest that the increase in the fluorescence intensity of 'A' band indicates ICT emission enhanced in the β-CD medium, (3) isopropyl group is present in the interior part of the β-CD cavity whereas the amino and CF_3 groups are present in the outside of the β-CD cavity.

Acknowledgement This work is supported by the Department of Science and Technology, New Delhi, (Fast Track Proposal Young Scientist Scheme No. SR/FTP/CS-14/2005) and University Grants Commission, New Delhi (Project No. F-31-98/2005 (SR). We also thank Professor K. Lawrence, Department of English, Annamalai University, to carry out the grammatical and spelling corrections in this article.

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