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ORIGINAL PAPER

Unusual Spectral Shifts of Imipramine and Carbamazepine Drugs

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Abstract The absorption and fluorescence spectra of imipramine and carbamazepine have been recorded in solvents of different polarity and β-cyclodextrin (β-CD). The inclusion complexes for both drugs are investigated by UV-visible, fluorimetry and DFT. Solvents study shows isotropic polarizability structure is present in imipramine while the amide group inhibits the above structure in carbamazepine. The band width half a maximum of carbamazepine decreased in polar solvents suggest that different species present in non-polar solvents and among that one of this species is affected in protic solvents. Both drugs form two different 1:2 inclusion complexes with β-CD. The structured longer wavelength emission in β-CD solution suggests viscosity plays major roles in the inclusion complex. This study also confirms van der Waals forces and hydrophobic interactions are the driving forces in imipramine and hydrogen bonding interactions play major roles in carbamazepine.

Keywords Imipramine · Carbamazepine · β-cyclodextrin · Inclusion complex · Solvent effects

Introduction

Medicinal chemistry is concerned with the understanding of chemical and biological mechanism by which the action of drug molecules can be explained [1–4]. It also tries to establish relations between chemical structure and biological activity and to link the latter to the physical properties of the drug molecules. The discovery of a new and biologically important active compound usually gives rise to an extended search for closely related compounds of similar more effective, more specific or even opposite activity [5–9]. In many cases, substitution of one atom or group of atoms in the parent compound (drug) results to surprising actions.

A drug molecule in its electronically excited state has different electronic charge distribution at various atomic centers in comparison to that of the ground state and as a result its physical and chemical properties would differ from those in the ground state. Hence, the study of photochemical and photophysical process of drug molecules has always been of spectral interest to chemist [10–18] and pharmacists [8–14]. In addition to the solvent data, studies on the inclusion complexation of drugs with cyclodextrin (CD) also provide some useful information about the geometry of guest molecules. A generally accepted reason for choosing CDs, a class of cyclic oligosaccharides with 6-8 D-glucose units linked by α-1,4-glucose bonds, as the starting materials to construct the supramolecular architectures is that the truncated cone-shaped hydrophobic cavities of CDs have a remarkable ability to include various guest molecules either in solution or in the solid state to form the functional host-guest inclusion complexes [19, 20] which can be subsequently used as the building blocks of supramolecular aggregates.

Further, β-CDs have been shown to be interesting micro vessels for several molecules and the resulting supramolecular species serve as excellent miniature models for enzyme substrate complexes [5–9]. The reduced polarity and the restricted space provided by the β-CD cavity markedly influence by a number of photophysical properties of the drug molecules. In addition, especially in pharmaceutical industries, the inclusion process of drug molecules to β-CD

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leads to important modifications of pharmaceutical properties of drug molecules. For example, the pharmaceutical interest in β-CD extends to enhance solubility, chemical stability and bioavailability of poorly soluble drugs, to reduce toxicity and to control the rate of release [6, 7]. The size of the β-CD and the drug molecule plays an important role to determine the complex type or stoichiometry. The drug can be present in the different parts of the β-CD depending on the type of inclusion complex formed. The reason for choosing β-CD is that the imipramine (10, 11-dihydro-N,N-dimethyl-5H-dibenz(b,f)azepine-5-propamine) and carbamazepine (5H-dibenz (b,f)azepine-5-carboxamide) drugs (Fig. 1) are quite large and thus it will encapsulate only partially to the β-CD cavity. Imipramine is used as antidepressant and carbamazepine is used as grandmal epilepsy and psychomotor seizures treatment.

Experimental

Absorption spectral measurements were carried out with a Shimadzu (model 1601 PC) UV-Visible spectrophotometer and fluorescence measurements were made with a Shimadzu spectrofluorimeter (model RF-5301). Imipramine, carbamazepine and β-CD were obtained from Sigma-Aldrich and used as such. All used solvents of the highest grade (spectrograde) were commercially available. Triply distilled water was used for the preparation of aqueous solutions. The solutions were prepared just before taking measurements. The concentration of the drug solutions were of the order 4×10^{-4} to 4×10^{-5}M. The concentration of β-CD was varied from 1×10^{-3} to 10×10^{-2}M. The experiments were carried out at room temperature 303 K.

Results and discussion

Effect of solvents

The absorption and fluorescence spectral data, log ε and Stokes shifts of imipramine and carbamazepine in the solvents of different polarity and hydrogen bond forming tendency are complied in Table 1. Because of the very low solubility in cyclohexane, both drugs spectral maxima were obtained using 1% diethylether solution of cyclohexane. These maxima will be very near to the maxima if obtained from pure cyclohexane, because the polarity of diethylether is close to cyclohexane. Furthermore, the trend observed in the spectral maxima of both drugs in cyclohexane is similar to the trend in other solvents. There is a red shift observed from cyclohexane to water in the absorption spectral maxima of both drug molecules (Table 1). However, in polar solvents, only small absorption spectral shifts are observed. In all the solvents, in carbamazepine (cyclohexane: λ_{abs}~280, 220 nm, λ_{flu}~398 nm, 371 nm; methanol: λ_{abs}~284, 218 nm, λ_{flu}~344, 316 nm) there is a

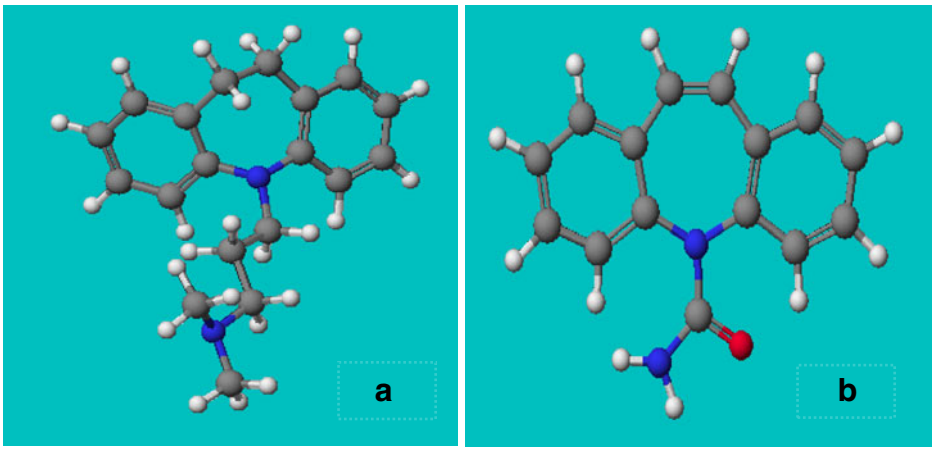


Fig. 1 CAChe structure—(**a**) imipramine and (**b**) carbamazepine

(a) Imipramine : ΔH = 40.18406 KCal = 168.13009 KJ

H_1- H_4 = 4.9832	H_9- H_{12} = 4.9538	N_1- N_2 = 4.9559	N_1- C_{19} = 6.2382
N_1- H_{24} = 7.0507	C_{15}- H_{24} = 5.8727	C_{15}- C_{19} = 4.9640	C_3- C_8 = 7.2558
H_3- H_{10} = 9.1318	C_2- C_9 = 6.6452		

(b) Carbamazepine : ΔH = 81.97655 KCal = 342.98987 KJ

H_1- H_4 = 4.8964	C_3- C_8= 8.0328	H_3- H_8 = 10.0516	C_2- C_9 = 7.5894
H_2- H_9 = 9.2785	H_7- H_{10} = 4.8656	C_5- N_2 = 5.8998	C_5- H_{12} = 6.6776
H_5- H_{12} = 7.7403	C_6- N_2 = 5.6859	C_6- H_{12} = 6.5785	H_6- H_{12} = 7.5872

Table 1 Absorption, fluorescence spectral data (nm) and Stokes shifts (cm^{-1}) of imipramine and carbamazepine in selected solvents

No.	Solvents	Imipramine				Carbamazepine			
		λ_{abs}	log ε	λ_{flu}	$\Delta\bar{\nu}_{ss}$ cm^{-1}	λ_{abs}	log ε	λ_{flu}	$\Delta\bar{\nu}_{ss}$ cm^{-1}
1	Cyclohexane	280	3.85	336	5953	280	sat	398	10590
		250	3.64	425w		220		371	8835
		212	4.20						
2	Diethyl ether	274	3.78	340	7006	282	4.22	330	5157
		252	3.85			222	4.19		
3	Dioxane	275	3.79	340	6873	284	4.24	330	4907
		252	3.86			224	4.18		
4	Tetrahydro furan	275	3.80	341	6889	285	4.15	330	4785
		252	3.82			225	3.93		
		211	4.13						
5	Ethyl acetate	274	4.04	345	7511	285	4.17	335	4785
		252	3.80			225	3.98		
		212	4.20						
6	Dichloro methane	274	4.04	345	7511	285	4.14	335	4785
		252	3.80			225	3.96		
7	Acetonitrile	270sh	3.56	363	9490	285	4.09	335	4785
		250	3.77	402sh		226	4.18	314	
		212	4.14						
8	*t*-Butyl alcohol	270sh	3.74	355	8867	286	4.15	336	5155
		250	3.88	407sh		219	4.27		
		212	4.29						
9	2-Butanol	270sh	3.86	356	8886	285	4.24	339	5590
		250	3.95	407sh		218	4.32		
		212	4.35						
10	2-Propanol	270sh	3.48	361	9967	284	4.12	339	5712
		250	3.64	407sh		219	4.23	316	
		212	4.06						
11	Glycol	270sh	3.56	361	9967	284	4.30	338	5748
		250	3.77	407sh		219	4.40	316	
		212	4.14						
12	Ethanol	270sh	3.86	372	10157	284	4.12	344	6140
		250	3.95	408sh		218	4.23	316	
		212	4.35						
13	Methanol	270sh	3.80	372	10157	284	4.34	344	6140
		250	3.92	408sh		218	4.42	316	
		212	3.85						
14	Water	270sh	3.89	377sh	10157	283	4.03	345	6175
		250	3.98	425		218	4.21	331sh	
		212	4.44						
15	Dipole moment (μ_g)	1.12				2.98			
16	Onsager cavity radius Å	5.59				4.87			
17	Correlation Coefficient $\Delta\nu_{ss}$ *vs* $E_T(30)$				0.8652				0.8473
	$\Delta\nu_{ss}$ *vs* BK				0.6512				0.6426

red shift observed in the absorption spectra than that of imipramine (cyclohexane: λ_{abs}~280, 250, 212 nm, λ_{flu}~336, 425w nm; methanol: λ_{abs}~270, 250, 212 nm, λ_{flu}~372, 408 nm). The above results suggest that in carbamazepine the presence of CH=CH and $-CONH_2$ groups increase the conjugation between the aromatic rings.

Figure 2 shows the fluorescence spectra of the both drugs in selected solvents. The absorption spectral maximum of the both molecules are weakly solvent dependent but the emission properties are strongly solvent dependent suggesting that the character of both molecules may change in the excited state. The emission maximum of imipramine is regularly red shifted with increasing polarity of solvents (Fig. 2). However, in carbamazepine there is red shifted emission maximum observed in cyclohexane than polar solvents. Further, in water there is a large red shift observed in the emission maximum of imipramine (λ_{flu}~377sh, 425 nm) than carbamazepine (λ_{flu}~331sh, 345 nm). The above results indicating that, in carbamazepine even though both aromatic rings are linked with CH=CH group, the presence of electron with drawing amide group decreases the conjugation between the aromatic rings.

In carbamazepine, the longer wavelength (LW) fluorescence appears only in hydrocarbon solvent whereas in imipramine it appears only in water. In non-polar solvent, the LW emission for carbamazepine is not only red shifted and the half-width of the fluorescence band also increased (Fig. 2). Interestingly, the LW emission maximum of imipramine is largely red shifted and the half-width of the fluorescence band is increased in polar solvents than non-polar cyclohexane. We observed that a clear difference in the LW emission of both molecules changed dramatically with the tertiary nitrogen atom substitution. The above results indicate that the addition of different substituent in tertiary nitrogen atom changed the fluorescence spectral behaviour of both drugs.

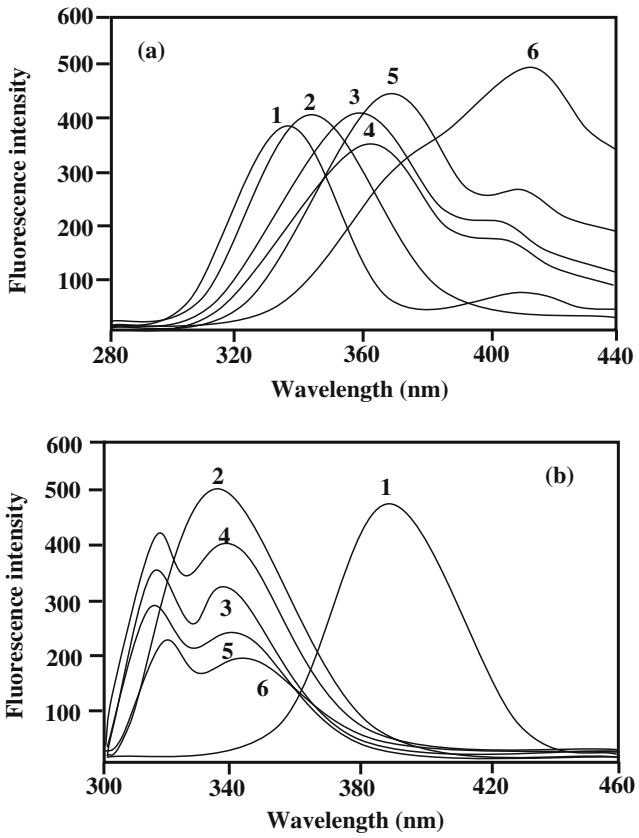


Fig. 2 Fluorescence spectra of (**a**) imipramine and (**b**) carbamazepine in selected solvents at 303 K: 1. cyclohexane, 2. ethyl acetate, 3. acetonitrile, 4. 2-propanol, 5. methanol 6. Water

In this study, the Stokes shifts (Table 1 and Fig. 3) of both molecules were determined in different solvents of varying polarity and correlated with the BK [21] and $E_T(30)$ [22] parameters. Figure 3 indicates that in aprotic solvents, the unspecific interactions are the key factors in shifting the fluorescence maxima to the red for these molecules. As evident from the slope of the plots, these interactions are large in case of imipramine and may be attributed to the increasing dipole moment on excitation. Rigid molecules having only a limited degree of freedom, the solvent cages change the structure of the molecules, hence the dipole moment of the molecules changed in the excited state. This process induces a large Stokes shifts in imipramine in polar solvents. A good correlation of Stokes shift with the $E_T(30)$ scale in Fig. 3 indicates the fact that the dielectronic solute-solvent interactions are responsible for the solvatochromic shifts in both molecules.

Unusual emission in imipramine and carbamazepine

Several mechanisms have been proposed to account for this anomalous red shifted emission. The initial proposal of solvent assisted level reversal of S_2 and S_1 by Lippert et al. [23], excimer formation proposed by McGlynm et al. [24], a proton transfer in the excited state by Kosower et al. [25], exciplex formation with polar solvent molecules by Chandross et al. and Viesse [26, 27]. The results obtained in our present work can be explained as follows [28]: (i) hydrogen bond formation between the protic solvents and electron donor group facilitates the formation of the TICT state in the S_1 state [28] and (ii) hydrogen bond formation between the protic solvent and the electron withdrawing carbonyl group will lead the electron withdrawing group to become coplanar with the benzene ring [28]. In other words, this hydrogen bonding seems to make the migration of electron density from benzene ring to the electron withdrawing group more facile.

The possible origin of the dual fluorescence is discussed in the following section. The dual fluorescence resulting from impurities and photochemical products can be rejected [29] on the ground state that (i) the fluorescence excitation spectra of both drugs recorded at different λ_{max} (flu) resemble the absorption spectra, (ii) no changes were

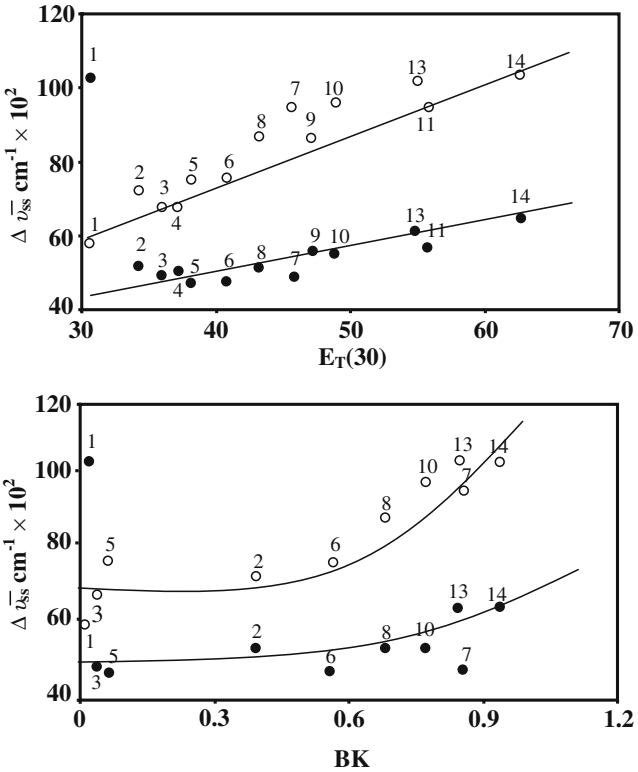


Fig. 3 Plots of Stokes shifts (cm^{-1}) of imipramine (○) and carbamazepine (●) *versus* $E_T(30)$ and BK solvent parameters: 1. cyclohexane, 2. diethyl ether, 3. dioxane, 4. tetrahydrofuran, 5. ethylacetate, 6. dichloromethane, 7. acetonitrile, 8. *t*-butyl alcohol, 9. 2-butanol, 10. 2-propanol, 11. glycol, 12. ethanol, 13. methanol 14. Water

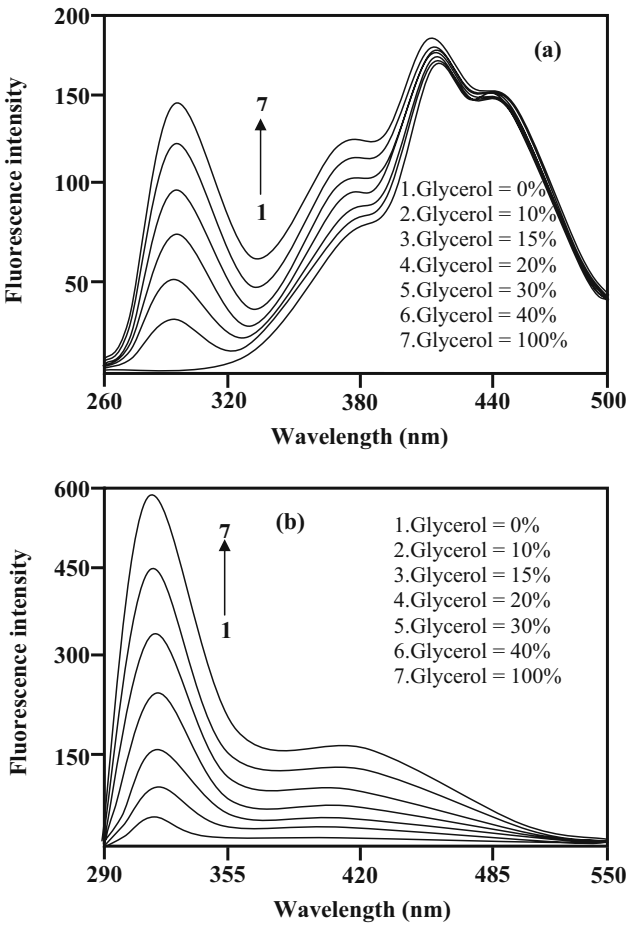


Fig. 4 Fluorescence spectra of (**a**) imipramine and (**b**) carbamazepine in water-glycerol mixture. 1. 0%, 2. 10%, 3. 15%, 4. 20%, 5. 30%, 6. 40% 7. 100% glycerol

observed during the experiment in the spectral features or in the emission spectra for carbamazepine is measured freshly prepared samples and (iii) the appearance of the dual luminescence in non-polar solvents implies that the spectral behaviour is not due to solute-solvent specific interaction i. e. complex formation [26]. The possibility of excimer formation can also be rejected on the basis of the following reasons [29], (i) the ratio of the intensities at the band maxima does not change with an increase of concentration of the fluorophore in the range 2×10^{-6} to 2×10^{-4} M and (ii) the dual fluorescence band is also observed in high viscous solvent (glycol) at room temperature. The appearance of the LW fluorescence in a non-polar solvent clearly indicates that solvent-assisted excited state reversal [23] is not the mechanism responsible for dual fluorescence. In order to further see the effect of ICT, the fluorescence spectrum at 303 K was recorded in solution consisting of different compositions of glycerol-H_2O mixtures (Fig. 4). In carbamazepine, the decrease in the intensity of fluorescence and absence of regular red shift with increase of water content suggest ICT emission is not present in this molecule [27]. We have also compared the FWHM of the fluorescence bands in solvents; FWHM is slightly decreases in polar solvents than cyclohexane indicating that intramolecular charge transfer (ICT) is not the process responsible for dual fluorescence.

The fluorescence excitation spectra of both drugs in different solvents are recorded at different emission wavelength (340, 430 and 450 nm). The excitation spectra differ from each other suggest that different species are present in both drugs [24]. The FWHM of the fluorescence band is nearly invariant in the polar solvents [24]. The FWHM of the longer wavelength absorption, as well as longer wavelength fluorescence excitation band is nearly equal to each other in polar solvent. This suggests that not much change is occurring in the geometry of the molecule [30] on excitation.

It is already reported [30] if the LW maximum is due to TICT, this should be more red shifted in protic solvents because TICT more pronounced in protic solvents than other solvents. For these reasons, we can be interpreted for carbamazepine, the LW in non-polar solvent is due to the hydrogen bond formation of an amide group. The LW emission is moved to 400 nm for carbamazepine than imipramine (336 nm), this is because the electron with-

drawing substituent is present in the tertiary nitrogen position. The sudden change of the band half-width in this region suggests that different species are present in non-polar solvents and in protic solvents one of this species affected due to proton donating nature of these solvents.

When the amount of water is increased in imipramine, a red shifted fluorescence band is observed in the aprotic/non-polar solvents; i.e., the fluorescence maximum in the cyclohexane solution is changed significantly on addition of water/methanol showing a dual emission. It is noted that, both SW and LW emission in water is further red shifted than that of other polar solvents. These observations suggest that the dual emission for imipramine in water seems to be influenced by the enhanced intermolecular hydrogen bonding of the =N- atom in the excited state. Supporting this, the fluorescence spectrum in the aqueous solution is changed significantly on addition of glycerol showing a dual emission and an isoemissive point in glycerol-water mixture (Fig. 4).

The large red shifted fluorescence band maximum reflects the greater delocalization of the π–cloud of the =N- group with the aromatic ring. The difference between both drugs is the presence of lone pair of electrons in the '*p*' orbital on =N- group. The lone pair of electrons takes part in the intramolecular charge transfer towards the π-cloud of the homocyclic rings and thus polarizing the imipramine molecule as shown below [31, 32] (Scheme 1):

The above isotropic polarizability structure is present in imipramine drug only. For carbamazepine, the presence of electron withdrawing carbonyl group at =N- atom inhibit the isotropic polarizability structure. This is because, the electron withdrawing carbonyl group withdraw the lone pair of electrons from =N- atom. Further, in carbamazepine, solvents can easily interact with more polar amide group than =N- atom. This reflected in carbamazepine, hence no large red shift is observed in protic solvents. Due to this, in polar solvents a large red shift is observed in imipramine than that of carbamazepine.

Effects of β-CD

Absorption spectral studies

Table 2 depicts the absorption and emission maxima for imipramine and carbamazepine in different β-CD concentrations and the spectra are shown in Figs. 5 and 6. Upon increasing the concentration of β-CD the absorbance of imipramine and carbamazepine are increased at the same wavelength. The increase in the absorbance is due to the encapsulation of both molecules in the β-CD cavity [33, 34]. The above behaviour may be attributed to the enhanced dissolution of the guest molecule through the hydrophobic interaction between the guest and non-polar cavity of the β-CD. These results indicate that both drugs are encapsulated in the β-CD cavity.

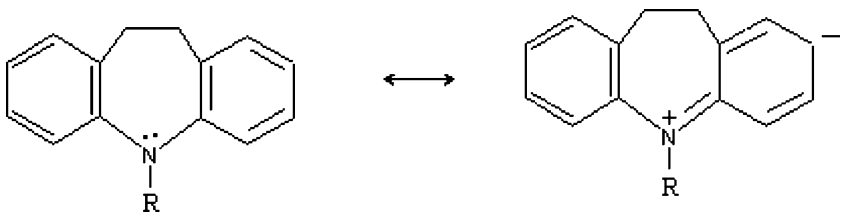
No clear isosbestic point is observed in the absorption spectra, and the changes that are observed in the absorbance are very small. In general, the existence of an isosbestic point in the absorption spectra is an indication to the formation of well defined 1:1 complex [33, 34]. The possibilities proposed for this deviation are: (i) more than one guest molecule got accommodated within a single β-CD cavity, (ii) due to the space restriction of β-CD cavity, more than one type of complex each having 1:1 stoichiometry might have been formed (iii) solution containing methanol (1%) could have made some interaction between these components [35–39] and (iv) the strong detergent action of β-CD could have prevented the formation of isosbestic point.

Binding constant

The binding constant for the inclusion complex formation has been determined by analysing the changes in the intensity of absorption and fluorescence maxima with the β-CD concentration. In order to determine the stoichiometry of the inclusion complex, the dependence on β-CD of the both drugs absorbance and fluorescence intensity were analysed by using the Benesi-Hildebrand equation [40] 1:1 complex (Eq. 1) and the 1:2 complex (Eq. 2) between both drugs with β-CD as shown below:

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

$$\frac{1}{I-I_0} = \frac{1}{I'-I_0} + \frac{1}{K(I-I_0)[\beta-CD]^2} \quad (2)$$



Scheme 1 Isotropic polarizability structure of imiparamine R= $CH_2CH_2CH_2N(CH_3)_2$

Table 2 Absorption and fluorescence maxima (nm) of imipramine and carbamazepine at different concentrations of β-CD

No.	Concentration of β-CD M	Imipramine			Carbamazepine		
		λ_{abs}	log ε	λ_{flu}	λ_{abs}	log ε	λ_{flu}
1	Water	270sh	3.48	422	285	3.81	319
		250	3.64		219	4.22	
		212	4.06				
2	0.001	270sh	3.54	417	285	3.86	419
		250	3.70	377	220	4.27	320
		212	4.15				
3	0.002	270sh	3.62	417	285	3.94	427
		250	3.78	375	222	4.30	320
		212	4.22				
4	0.004	270sh	3.69	416	285	4.00	426
		250	3.84	373	222	4.35	322
		212	4.28				
5	0.006	270sh	3.76	416	285	4.04	426
		250	3.90	373	222	4.40	324
		212	4.35				
6	0.008	270sh	3.80	416	285	4.14	428
		250	3.92	373	222	4.45	328
		212	4.44				
7	0.010	270sh	3.89	416	285	4.18	428
		250	3.98	373	222	4.48	328
		212	4.48				
8	Excitation wavelength	250			285		
9	Binding constant(M^{-1})	568		763	586		780
10	ΔG KJ/mol	−15.97		−16.72	−16.05		−16.77

where K is the formation constant, I_0 is the initial absorption/fluorescence intensity of free drug, I′ the absorption/fluorescence intensity of β-CD inclusion complex and I is the observed absorption, fluorescence intensity. According to Eq. 1 a plot of $1/I\text{-}I_0$ *versus* $1/[\beta\text{-CD}]$ and Eq. 2 plot of $1/I\text{-}I_0$ *versus* $1/[\beta\text{-CD}]^2$ (both absorption and fluorescence) gives an upward or downward curves (Figs. 7 and 8). The values of binding constant are calculated from the slope and the intercept of the plot. This analysis reflects both drugs forms mixture of different 1:2 inclusion complex. The plot of $1/I\text{-}I_0$ *versus* $1/[\beta\text{-CD}]^2$ with intercept unity using absorption and fluorescence data suggests that the inclusion complex is formed between one molecule of drug and two molecules of β-CD. Both molecules binding constants values are significantly changed which reveals that different types of inclusion complexes are formed. These values for the relevant free energy changes for these systems at 303 K are given in Table 2. The binding constant values are higher than with compared to other guest molecule with β-CD complexes such as benzene derivatives [39, 40]. This is probably these molecules are tightly included into the cavity.

The free energy change was calculated from the formation constant (K):

$$\Delta G = -RT \ln K \tag{3}$$

The values of thermodynamic parameter ΔG for the formation of the guest molecule to β-CD are given in Table 2. As can be seen from the Table, ΔG is negative which suggests that the inclusion proceeded simultaneously at 303 K. The experimental results are indicating that the inclusion reactions of the β-CD with both drugs are an exothermic process.

Fluorescence spectral studies

Figure 6 shows the fluorescence spectra of both drugs in aqueous solution as a function of β-CD concentration. Since no clear isosbestic point is observed in the absorption spectrum, the excitation wavelength is selected in such a manner that the absorbance changes are very small.

(i) **Carbamazepine**: In aqueous solution, single fluorescence maximum was observed at 319 nm. Upon

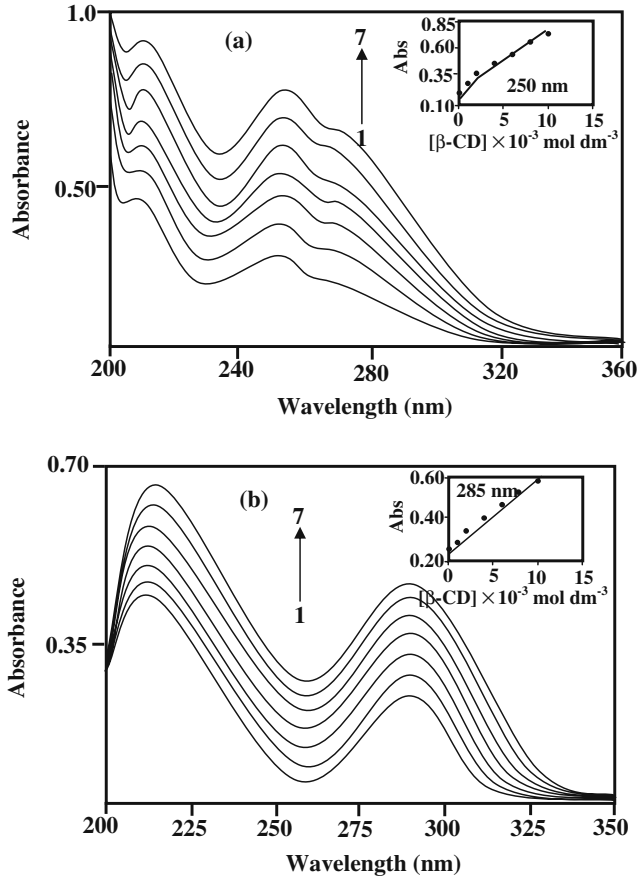


Fig. 5 Absorption spectra of (**a**) imipramine and (**b**) carbamazepine in β-CD solutions with different concentrations (M): (1) 0 (2) 0.001 (3) 0.002 (4) 0.004 (5) 0.006 (6) 0.008 (7) 0.01

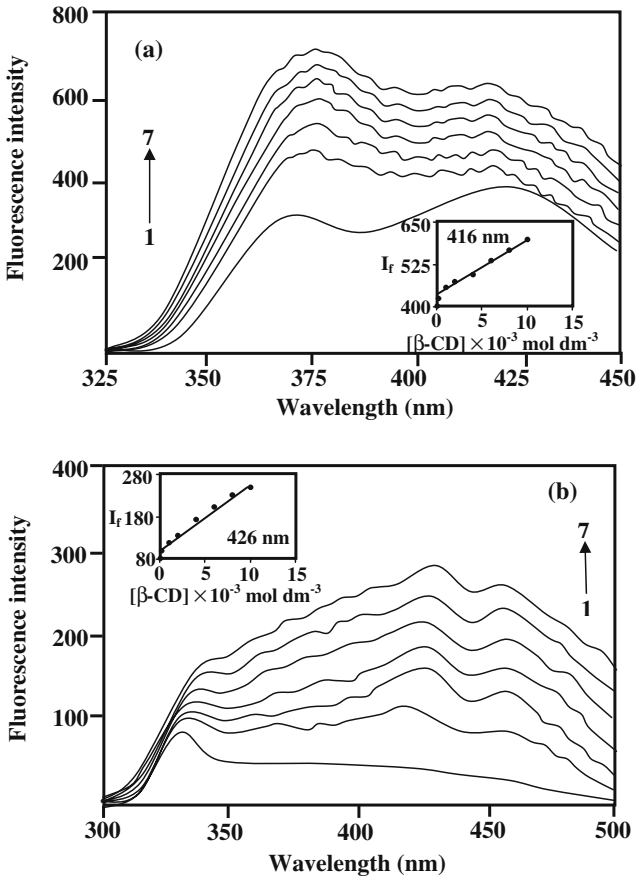


Fig. 6 Fluorescence spectra of (**a**) imipramine and (**b**) carbamazepine in β-CD solutions with different concentrations (M): (1) 0 (2) 0.001 (3) 0.002 (4) 0.004 (5) 0.006 (6) 0.008 (7) 0.01

addition of β-CD, the shorter wavelength (SW) emission band is gradually increased with appearance of structured longer wavelength (LW) emission around 425 nm and 450 nm. With the addition of β-CD both LW and SW intensities increases, however, the rate of enhancement of the LW emission is greater than that for the SW band. Further, with an increasing the β-CD concentration, a regular red shift is observed in the SW band (320–330 nm) whereas no significant emission spectral shift is observed in the LW band (425 nm and 450 nm). This can be seen more clearly from the Fig. 6, the LW intensity in 0.01 M β-CD solution is increased to 6 times than that of aqueous medium and the SW emission is enhanced to approximately 2 times.

(ii) **Imipramine:** Contrary to carbamazepine, the SW band of imipramine is more sensitive in β-CD than LW band (Fig. 6). In β-CD solutions, the LW emission intensity (422 nm) gradually increased and the dual emission appears at the SW (375 nm). The LW emission band shows a small enhancement with a regular hypsochromophoric shift (422 nm to 415 nm). Even though, both LW and SW emission intensities increased with β-CD concentrations, the rate of enhancement of the SW emission is greater than that for the LW band.

The above result indicates that, both molecules form different type of inclusion complex with β-CD [41–45]. The enhancement of both bands may be explained as follows [43, 44]. The enhancement of the LW band in β-CD may be due to lowering of solvent polarity at higher β-CD concentration [43, 44]. Inside the β-CD cavity both drugs feels much less polar environment and the main non-radiative path of the SW band (through intramolecular charge transfer (ICT) is restricted which also causes an enhancement of the LW band. Further, the geometrical limitations of the cavity would restrict the free rotation of the amide group or alkyl chain in the β-CD cavity and thus hinders the free rotation also causing an enhancement of LW band.

In general, several driving forces have been postulated for the inclusion complexation of CD with guest compounds [45]: (i) van der Waals forces; (ii) hydrophobic interactions; (iii) hydrogen bonding; (iv) release of distor-

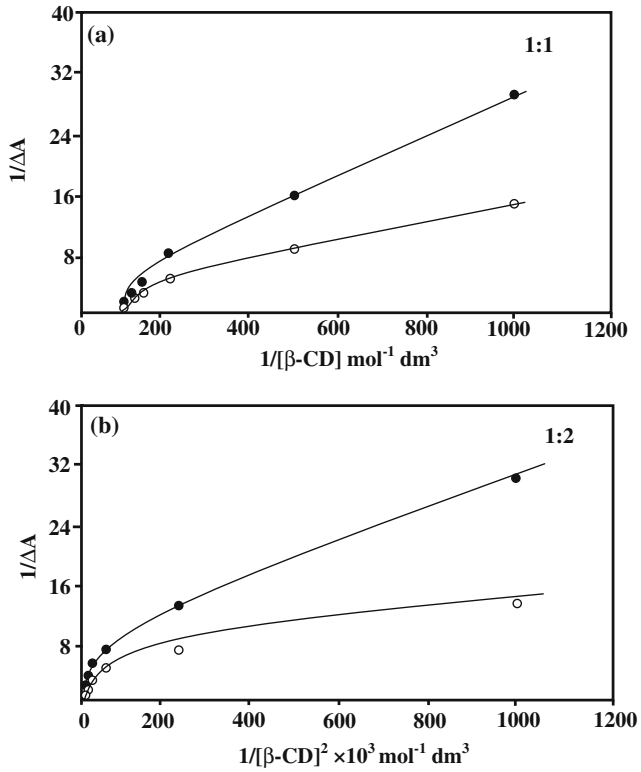


Fig. 7 Absorption spectra of Benesi-Hildebrand plot for the complexation of imipramine (○) and carbamazepine (●) with β-CD. (**a**) Plot of $1/\Delta A$ *Vs* $1/[\beta\text{-CD}]$; (**b**) Plot of $1/\Delta A$ *Vs* $1/[\beta\text{-CD}]^2$

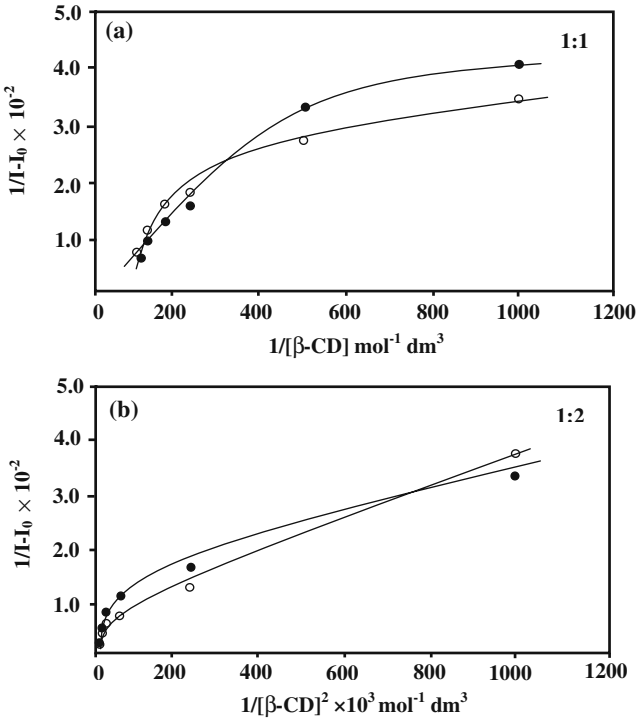


Fig. 8 Fluorescence spectra of Benesi-Hildebrand plot for the complexation of imipramine (○) and carbamazepine (●) with β-CD (**a**) Plot of $1/I\text{-}I_0$ *Vs* $1/[\ \beta\text{-CD}]$; (**b**) Plot of $1/I\text{-}I_0$ *Vs* $1/[\ \beta\text{-CD}]^2$

tional energy of CD by binding guest; and (v) extrusion of 'high energy water' from the cavity of CD upon inclusion complex formation. Tabushi [46] proposed a thermodynamic model for the process of CD inclusion complex formation. Based on the thermodynamic parameter (ΔG) calculated for the inclusion of these drugs, we conclude that the hydrogen bonding interaction, van der Waals interaction, and breaking of the water cluster around this polar guest compound (drugs) mainly dominate the driving force for inclusion complex formation.

It is well known that the strength of interaction is also dependent on the size of the CD cavity and size of the substituent in the complex [47]. This means that the interaction is more sensitive to the size of substituents and the CD in the complexation. The CDs are truncated, right-cylindrical, cone-shaped molecules, 7.8 Å heights with a central cavity. The diameters of the narrower and wider rim of the cavity for β-CD are 6.5 Å and 5.8 Å, respectively [48]. It is well known that the van der Waals force including the dipole–induced dipole interactions are proportional to the distance between the drugs and the wall of the CD cavity and to the polarizabilities of the two components. It is thus a short range interaction; therefore, the drug may embedded by β-CD cavity. In general, β-CD cavity is in non-polar form which favours the non polar part of the guest. The aromatic ring moiety may achieve a maximum contact area [49] with the internal surface of the cavity of the β-CD; hence, the interaction of the phenyl ring with β-CD would play an important role.

The inclusion of carbamazepine and imipramine with β-CD are affected by hydrophobic and electronic interactions [50]. Since CDs have a permanent dipole [51], the primary hydroxyl end is positive and the secondary hydroxyl end is negative in the glucose units of CDs. The stability of binding by hydrophobic interaction is partly the result of van der Waals force but it mainly due to the effects of entropy produced by the water molecules [52]. In aqueous solution, a hydrophobic guest compound is restricted by the water shell formed by the hydrogen bonding network [51]. It has a strong tendency to break down the water cluster and penetrate the non-polar cavity of CD. This process is exothermic due to entropic gain [51]. The association constants for the inclusion of β-CD with guest compounds were observed to be proportional to the substituent hydrophobic constant of the guest.

Since polar amide group is present in carbamazepine, the hydrogen bonding interactions play major roles in the inclusion complexation of β-CD; (i.e) in both drugs the polar groups determined by the inclusion process. The 'K' value is a reasonable measure of hydrogen bonding and the change in hydrogen bonding of these drugs is caused only by the hydrogen ion concentrations. Since the polar

substituent locates near the wider rim of the CD cavity and aromatic ring locates narrower range of the CD cavity, the 'K' values are proportional to the hydrogen bonding interactions. The difference in slope in Figs. 7 and 8 for both drugs and β-CD complexes indicates that the interactions of hydrogen atoms of carbamazepine with β-CD are much stronger than imipramine because the interactions in farmer is approximate to the hydrogen bonding contact.

The red shifted spectral maximum in carbamazepine indicates the polar amide group interact with the secondary hydroxyl group of the β-CD. This implication is based on the following reasons: The large rim of β-CD contains 14 secondary hydroxyl groups, and thus provides on environment qualitatively similar to polyhydroxy alcohols [40]. It is well known that substituents of aromatic rings capable of hydrogen bonding can bind the hydroxyl groups of the β-CD edges. The energy involved in such hydrogen bond interaction responsible for the higher/lower binding constants found, when compared to those of the substituted/unsubstituted molecules. The higher formation constant in both drugs implies that they easily embedded in the β-CD cavity.

The different type of emission spectra for both drugs in β-CD solutions are explained as follows: In carbamazepine, the presence of polar amide group at =N- increases the H-bonding interactions, hence a large red shift is noticed. This inclusion process of imipramine is different from carbamazepine, because imipramine is not having polar substituent at =N- atom. This is because, the surrounding polarities of both drugs are different in β-CD solutions. That is why bathochromophoric or hypsochromophoric shift is observed for carbamazepine and imipramine respectively. This may be possible only if the orientations of both drugs are differing from each other. From the above discussions it is clear that, hydrophobicity is the driving force for the

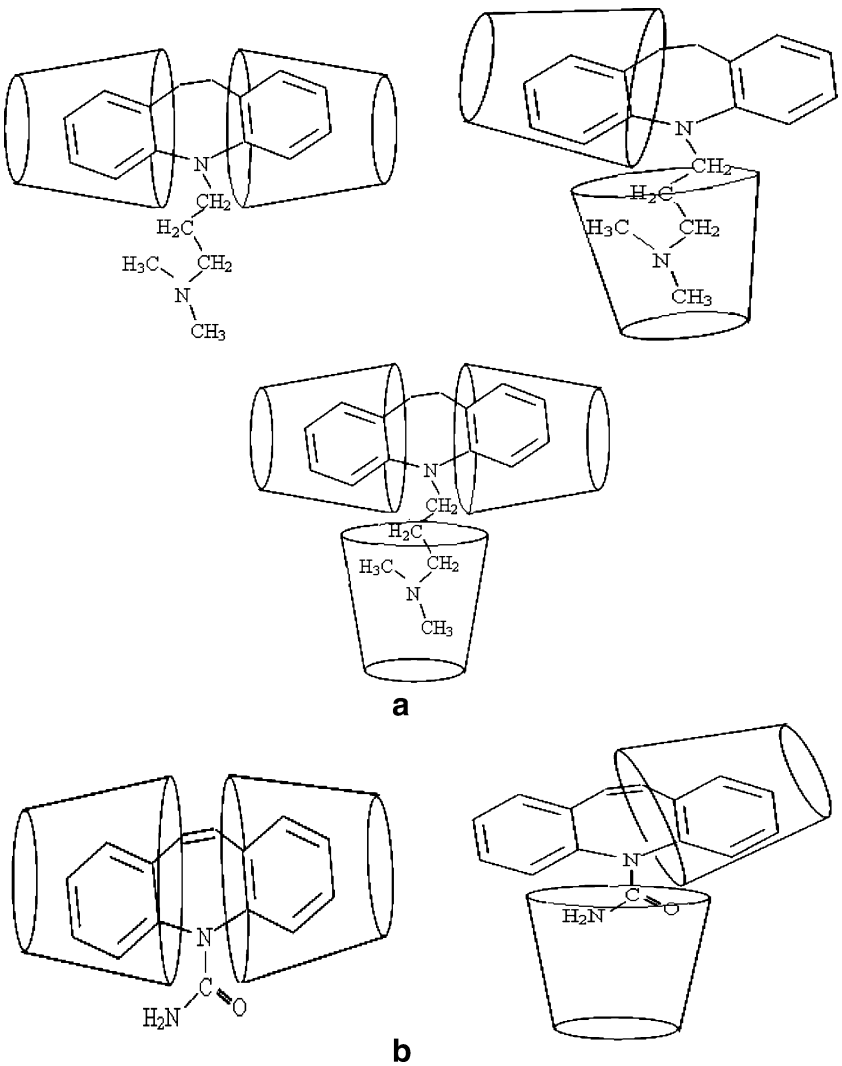


Fig. 9 Proposed inclusion complex structure of (**a**) imipramine and (**b**) carbamazepine with β-CD

inclusion process in imipramine and hydrogen bonding interactions play major roles in carbamazepine.

Further, the structured LW emission in β-CD aqueous solution suggests viscosity also plays major role. With an increasing β-CD concentration, increasing FWHM and emission intensity confirms viscosity roles in the β-CD. The aromatic rings of both drugs are partially encapsulated in the β-CD cavity because the drug sizes are greater than the β-CD cavity. For this reason, the structured LW emission is appeared in both drugs. The large size and presence of different substituent at =N- are responsible for the formation different inclusion complexes (Fig. 9).

Considering the above discussions, the possible inclusion mechanism is proposed as follows. The size of carbamazepine or imipramine are larger than β-CD cavity, both drugs formed different types of inclusion complex with β-CD (Fig. 9). For 1:1 inclusion complex, one of the aromatic ring is captured whereas in 1:2 inclusion complex both aromatic rings or one aromatic ring and substituent at = N- atom may captured in the β-CD cavity. However, the Benesi-Hildebrand analysis reflects more than the above type complex is formed. To substantiate the above discussion, the size of carbamazepine, imipramine and β-CD has been calculated by using DFT method (semiempirical quantum mechanical calculations) (Fig. 1). The internal diameter of the β-CD is approximately 6.5 Å and its height is 7.8 Å. The vertical distance between the aromatic rings ($H_5 - H_{12}$) is 7.50 Å. Since the lengths between the drugs aromatic rings are greater than that of β-CD cavity, they cannot encapsulate completely within the β-CD cavity. Therefore, more than one β-CD needs for this inclusion process.

Conclusions

The following conclusions can be drawn from the above studies: Solvents study shows isotropic polarizability structure is present in imipramine while the amide group inhibits this structure in carbamazepine. In carbamazepine, the sudden change in half-width band suggests that different species present in non-polar solvents and one of this species is affected in protic solvents. β-CD study shows (i) both drugs form two different 1:2 inclusion complexes; (ii) the structured longer wavelength emission suggests viscosity plays major roles in the inclusion complex; (iii) this study also confirms van der Waals forces and hydrophobic interactions are the driving forces for imipramine and hydrogen bonding interactions play major roles for carbamazepine. In future, (i) the inclusion complexation process of imipramine and carbamazepine may analysed with α-CD and γ-CD and (ii) the solid inclusion complex of both drugs may prepared with the above CDs and analysed by FTIR, 2D NMR, and X-RD methods.

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References

1. Dey JK, Warner IM (1998) J Photochem Photobiol A Chem 116:27
2. Zhang G, Shuang S (2005) J Photochem Photobiol A Chem 169:153
3. Borissevitch IE, Gandini SCM (1998) J Photochem Photobiol B Biol 43:112
4. Chirvony VS, Turpin PY (1997) J Photochem Photobiol B Biol 40:154
5. Li JF, Wei YX, Ding LH, Dong C (2003) Spectrochim Acta 59A:2759
6. Brahmankar DM, Sunil B (2006) Jaiswal, Biopharmaceutics and pharmacokinetics, A-Treiastise, Vallabh Prakasham Pubs., 302
7. Chao J, Meng D, Li J, Xi H, Huang S (2004) Spectrochim Acta 60A:729
8. William DA, Lenke TC (2005) Foye's principle of medicinal chemistry, 5th edition, I. Pubs. 711
9. Carswell CI, Figgitt P (2002) Drugs 62:2471
10. Wei YL, Ding LH, Dong C, Niu WP, Min Shuang S (2003) Spectrochim Acta 59A:2697
11. Fugen G, Cuijing L (1998) Chin Pharm J 33:153
12. Xiying L, Yunginf Y (1997) Chim Pharm J 32:218
13. Manzoori JL, Amjadi M (2003) Spectrochim Acta 59A:909
14. Canaparo R, Mumtoni E, Zara GP, Dellapena C, Berno E, Costa M, Endi M (2000) Biomed Chromatogr 14:219
15. Galian RE, Veglia AV, de Rossi RH (2000) Analyst 125:1465
16. Escandar GM (1999) Analyst 124:587
17. Galian RE, Veglia AV, de Rossi RH (1998) Analyst 123:1587
18. Duran-Meras I, Munoz de la pena A, Salinas F, Rodriguez Caceres I (1994) Analyst 119:125
19. Szejtli J, Osa T (1996) In: Atwood JL, Davies JE, Mac Nicol DD, Vogtle F (eds) Comprehensive supramolecular chemistry, vol. 3. Pergamon/Elsevier, Oxford
20. Uekama K, Hirayama F, Irie T (1998) Chem Rev 98:2045
21. Bilot L, Kawski A (1962) Z. Naturforsch 17A:621
22. Dimroth K, Reichardt C (1983) *Liebigs, Ann. Chem.* 661 and *Anqew.Chem.* 91 (1979) 119
23. Lippert E, Londer W, Boos H (1962) In: Mangini A (ed) Advances in molecular spectroscopy. Pergmon, Oxford, p 443
24. McGlynm SP, Khalil OS, Meeks JS (1976) Chem Phys Lett 39:457
25. Kosower EM, Dodiuk H (1976) J Am Chem Soc 98:924
26. Chandross A (1975) In Gorden M, Ware M (Eds.), Exciplex, Academic Press, New York 187
27. Visser R, Wiesenborn PCM, Varma CAGO (1985) Chem Phys Lett 113:330
28. Rettig W (1986) Angew Chem Int Ed Engl 25:599
29. Day JK, Dogra SK (1994) J Phys Chem 98:3638
30. Nayak MK, Dogra SK (2004) J Photochem Photobiol A Chem 161:169
31. Gaplousky A, Hrdlovic P, Donovalova J, Hrnciar P (1991) J Photochem Photobiol A Chem 59:221
32. Zhang X, Sun X-Y, Wang C-J, Jiang Y-B (2002) J Phys Chem 106:5577
33. Kim Y, Yoon M, Kim D (2001) J Photochem Photobiol A Chem 138:167
34. Jiang YB (1995) J Photochem Photobiol A Chem 88:109

35. Stalin T, Rajendiran N (2006) J Photochem Photobiol A Chem 177:144
36. Stalin T, Rajendiran N (2006) J Mol Stru 794:35
37. Antony Muthu Prabhu A, Rajendiran N (2009) Spectrochimica Acta 74:484
38. Antony Muthu Prabhu A, Rajendiran N (2010) J Fluoresc 20:43
39. Siva S, Rajendiran N (2009) Indian J Chem 48A:1515
40. Benesi A, Hildebrand JH (1949) J Am Chem Soc 71:2703
41. Park HR, Mayer B, Wolschann P, Kohlen G (1994) J Phys Chem 98:6158
42. Sandra S, Dogra SK (1996) J Photochem Photobiol A Chem 101:221
43. Bhattacharya K, Chowdhury M (1993) Chem Rev 93:507
44. Panja S, Bangal PR, Chakravorty S (2000) Chem Phys Lett 329:377
45. Kano K, Tawiya Y, Hashimoto S (1992) J Incl Phenom 3:287
46. Tabushi I (1984) Tetrahedron 40:269
47. Guo Q-X, Luo S-H, Liu Y-C (1998) J Incl Phenom Mol Recognit Chem 30:173
48. Li S, Purdy WC (1992) Chem Rev 92:1457
49. Connors KA, Lin SF, Wong AB (1982) J Pharm Sci 71:217
50. Davies DM, Deary ME (1995) J Chem Soc Perkin Trans 2:1287
51. Delaage M (1991) In: Delaage M (ed) Molecular recognition mechanisms, Ch. 1. VCH, New York
52. Sakurai M, Kitagawa M, Inoue Y, Chujo R (1990) Carbohydr Res 198:181