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ORIGINAL PAPER

Encapsulation of Labetalol, Pseudoephedrine in β-cyclodextrin Cavity: Spectral and Molecular Modeling Studies

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Abstract The absorption and fluorescence spectra of labetalol and pseudoephedrine have been studied in different polarities of solvents and β-cyclodextrin (β-CD). The inclusion complexation with β-CD is investigated by UV-visible, steady state and time resolved fluorescence spectra and PM3 method. In protic solvents, the normal emission originates from a locally excited state and the longer wavelength emission is due to intramolecular charge transfer (TICT). Labetalol forms a 1:2 complex and pseudoephedrine forms 1:1 complex with β-CD. Nanosecond time-resolved studies indicated that both molecules show triexponential decay. Thermodynamic parameters (ΔG, ΔH, ΔS) and HOMO, LUMO orbital investigations confirm the stability of the inclusion complex. The geometry of the most stable complex shows that the aromatic ring is deeply self included inside the β-CD cavity and intermolecular hydrogen bonds were established between host and guest molecules. This suggests that hydrophobic effect and hydrogen bond play an important role in the inclusion process.

Keywords Labetalol · Pseudoephedrine · Cyclodextrin · TICT · Inclusion complex · Molecular modeling

Introduction

Cyclodextrins (CDs) have been widely used in drug delivery systems, bioencapsulation processes as well as in separation technologies [1, 2]. All the practical applications of CDs are based on their ability to form inclusion complexes with different organic compounds. To this end, complex formation of CDs is comprehensively studied and a great number of articles devoted to this subject have been published. Moreover, various modified CDs synthesized in recent years are frequently used as host molecules because they are more soluble in water and in some cases display higher binding affinity to guest molecules in comparison with native CDs.

Complex formation of parent cyclodextrins with the simplest aromatic and drug molecules has been investigated in detail by different experimental methods [3–17]. It has been shown that most of the compounds are inserted in CD cavity and 1:1 binding takes place in all cases except γ-CD, which is able to form 1:2 complexes in the presence of excess amount of aromatic compounds [6]. To the best of our knowledge, interactions of β-CD with labetalol and pseudoephedrine were not studied. Therefore, the aim of this work was to examine the binding affinity of β-CD to labetalol and pseudoephedrine.

Our recent studies on various drugs have shown that flutamide [10], bicalutamide [11], norepinephrine/epinephrine/isoprenaline [12] and sulphanilamide [13, 14] drugs can undergo TICT emission and form 1:1 inclusion complex whereas tramadol [15] dothiepin/doxepin [16], imipramine/carbamazepine [17] drugs form 1:2 inclusion complex with β-cyclodextrin. As a continuation of the previous investigations [10–17], a detailed study of the luminescence behaviour of labetalol was undertaken keeping in view of all the striking prospects of labetalol and compared with pseudoephedrine.

In this article, we have examined the spectral properties of labetatol (2-hydroxy-5-(1-hydroxy-2-((1-methyl-3-phenyl propyl) amino) ethyl) benzamide) and pseudoephedrine (α-(1-methyl aminoethyl) benzylalcohol) with the following points in mind: (i) whether labetalol and pseudoephedrine have the same or different spectral characteristics and (ii) whether the presence of alkyl chain increase or decrease the conjugation. A striking feature of the examined compound was that neither the absorption nor fluorescence excitation spectra gave significant indications of the existence of ICT (TICT) emissions. It is anticipated that the ICT (TICT) emission could be exhibited in solvents and/or cyclodextrin

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solutions. To explore the host-guest interaction, the complex is systematically characterized. Additionally, the molecular modeling by PM3 method is used to verify the geometrical configuration of the complex from experimental results. Labetalol and pseudoephedrine are used as an antihypertensive and sympathomimetic treatment respectively.

Experimental

Reagents and Materials

Labetalol, pseudoephedrine and β-CD were obtained from Sigma-Aldrich and used as such. All used solvents of the highest grade (spectrograde) were commercially available. Solutions in the pH range 2.0–12.0 were prepared by adding the appropriate amount of phosphate buffer (NaOH and H_3PO_4). 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 the order of 4×10^{-4} to 4×10^{-5} M. The concentration of β-CD was varied from 1×10^{-3} to 1×10^{-2} M. The experiments were carried out at room temperature 303 K.

Instruments

Absorption spectral measurements were carried out with a Shimadzu UV 1601 PC model UV-Visible spectrophotometer and fluorescence measurements were made by using a Shimadzu spectrofluorimeter model RF-5301. The pH values in the range 2.0–12.0 were measured on an Elico pH meter (model LI-120).

The fluorescence lifetime measurements were performed using a picoseconds laser and single photon counting setup from Jobin-Vyon IBH. A diode pumped Millena CW laser (Spectra Analysis) 532 nm was used to pump the Ti-Sapphire rod in Tsunami picosecond mode locked laser system (Spectra physics Model No. 4690 M3S). The Ti-Sapphire rod is oriented at Brewster's angle to the laser beam. The wavelength turning range is 720–850 nm, i.e., standard pico configuration. The fluorescence decay of the sample is further analysed using IBH data analysis software. The fluorescence decay profiles were fitted to the expression:

$$I(t) = A_1 \exp\left(\frac{-t}{\tau_1}\right) + \mathrm{A_2}\exp\left(\frac{-t}{\tau_2}\right) \quad (1)$$

$$I(t) = A_1 \exp\left(\frac{-t}{\tau_1}\right) + \mathrm{A_2}\exp\left(\frac{-t}{\tau_2}\right) + \mathrm{A_3}\exp\left(\frac{-t}{\tau_3}\right) \quad (2)$$

Where τ_1, τ_2 and τ_3 are lifetimes of the three components, a_1 a_2 and a_3 are the pre-exponential factors of the same and t is time. The average fluorescence lifetime is calculated by using the equation.

$$\langle\tau\rangle = \sum \tau_i a_i \quad (3)$$

Molecular Modeling Studies

The theoretical calculations were performed using the Gaussian view 5.0 software package. The initial molecular geometries of guest molecules, β-CD and inclusion complexes were fully optimized using the PM3 (Parametric method 3) method. The corresponding frequencies were calculated to ensure that the obtained stationary points were true minima. These semiempirical methods are very convenient for the modeling of large molecular systems, such as cyclodextrin inclusion complexes [18, 19].

Results and Discussion

Effect of Solvents

Absorption, fluorescence maxima, log ε and Stokes shifts of labetatol and pseudoephedrine were obtained in different solvents with various polarities and the data are complied in Table 1. Due to very low solubility of the above drugs in cyclohexane, the spectra were measured using 2 % ether solution of cyclohexane. Table 1 clearly shows that the absorption and emission maxima of labetalol is more red shifted in any one of the solvent compared to those of pseudoephedrine. The solvatochromic shifts for labetalol (cyclohexane: λ_{abs}~300, 225 nm, λ_{flu}~429 nm; methanol: λ_{abs}~305, 225 nm, λ_{flu}~340, 451 nm) are found to be more than that of pseudoephedrine (cyclohexane: λ_{abs}~268, 218 nm, λ_{flu}~283, 325 nm; methanol: λ_{abs}~267, 221 nm, λ_{flu}~283, 333 nm) indicating that in both S_0 and S_1 states, the charge transfer interaction of carbonyl group in labetalol is larger than pseudoephedrine.

The high values of the molecular extinction coefficient and a small change in the absorption spectra in polar and hydrogen bonding solvents (Table 1) suggests that the n–π* transition is not present in both molecules [10–17]. Distinction between π–π* and charge transfer bands can be made from a correlation of the magnitude of the Stokes shift with the nature of the substituent and solvent. In general, large Stokes shift and greater solvent dependence are observed for the charge transfer band. The data in Table 1 clearly shows that the lowest energy transition in the drug molecules are π–π* character. In water the blue shifted absorption maxima suggests the formation of hydrogen bond with the lone pair of the imino group (solute) inhibiting its interaction with the

Table 1 Absorption and Fluorescence spectral data (nm) observed for labetalol and pseudoephedrine in different solvents

No	Solvents	Labetalol				Pseudoephedrine			
		λ_{abs}	log ε	λ_{flu}	Stokes shift	λ_{abs}	log ε	λ_{flu}	Stokes shift
1	Cyclohexane	300 225	3.72 3.83	429	10023	268 218	2.38 3.68	283 325	1977 6543
2	1,4-Dioxane	303 228	3.69 3.78	453–435	10014	268 220	2.34 3.55	285 328	2245 6825
3	Ethyl acetate	303 228	3.50 3.93	453–430	9747	269 221	2.34 3.62	285 329	2087 6780
4	Acetonitrile	306 228	3.48 3.87	452–434	10556	269 221	2.37 3.67	285 329	2087 6780
5	*t*-Butyl alcohol	306 228	3.54 3.88	340 450	3440 10458	268 221	2.29 3.55	286 330	2348 7010
6	2-Butanol	306 229	3.56 3.93	340 449	3440 10408	268 221	2.34 3.55	286 333	2348 7283
7	2-Propanol	305 225	3.40 3.89	340 448	3440 10466	268 220	2.32 3.61	285 333	2245 7283
8	Methanol	305 225	3.63 4.02	340 451	3440 10660	267 221	2.28 3.58	286 333	2488 7283
9	Water (pH=6.5)	305 225	3.48 4.00	340 453	3440 10772	267 222	2.49 3.75	286 333	2488 7283
10	Onsager cavity radius (Å)			8.29				4.55	
11	Correlation coefficient $E_T(30)$ *Vs* $\Delta\bar{\nu}_{ss}$				0.9124				0.9156
	BK *Vs* $\Delta\bar{\nu}_{ss}$				0.7132				0.7042

π-cloud. The red shift in aprotic solvents is due to the usual dipole-dipole effect on the π–π* transition or hydrogen donating character of the hydroxy group [10–17].

The fluorescence spectrum is regularly red shifted as the polarity and proton donor capacity of the solvent increase. Generally, the fluorescence spectra results can be explained by charge migration from the electron donating group and phenyl ring to the electron withdrawing carbonyl group. That is, the charge density of the oxygen atom decreases resulting in an increase in the proton donor ability of the electron donating (-OH) group. Usually, the H-bonding interactions of the solvent molecules with the -OH group of aromatic compounds enhances molecular fluorescence as a result of destabilization of the CT state, thus reducing the CT character of the emitting state (π–π*). The fluorescence enhancement of labetalol in H-bonding solvents can also be explained by using the same principles. The fluorescence spectrum in each solvent is broader, that is having broader full width at half maximum height (FWHM). The FWHM of the fluorescence spectrum is not increased in polar solvents.

Dual Emission

In contrast to the weak solvent dependent of absorption maximum, the emission properties of labetalol is strongly solvent dependent indicating a possibility of a change in the character of the electronic state. In all solvents pseudoephedrine gives dual emission maximum. In contrast, labetalol gives a single emission maximum in the non-polar and aprotic solvents whereas a dual luminescence in polar solvents. Among the two bands one occurs in shorter wavelength region (SW) around 345 nm and the other in longer wavelength region (LW) around 450 nm. The LW band emission intensity is larger than that of the SW. The fluorescence intensity of the LW band increases with increase in the λ_{exc} 290 nm to 310 nm. This may be the extended π-conjugation would induce an excited state resonance contribution of the carbonyl group to the benzene ring resulting in the increased polarity to facilitate the interaction with polar solvents.

The LW emission is strongly solvent dependent with the Stokes shift reaching its maximum value in the water. The broad Stokes shifted emission in water is common in molecules having an electron withdrawing group such as=N- or carbonyl group attached to an aromatic nucleus. However, the nature of such emission is not always easy to ascertain, since it can be the result of a variety of causes, including dimer formation in the ground state (or other kinds of aggregates) and excimer formation or charge transfer processes. In labetalol, the donor is –OH group and the acceptor is the carbonyl group. In water, the carbonyl group becomes more conjugated with the aromatic π-system, in this situation there is a marked charge separation occur within the molecule.

The present results explained by Rettig [20] suggestion as follows: (i) hydrogen bond formation between the protic solvents and electron donor group facilitates the formation of the TICT state in the S_1 state [21] and (ii) hydrogen bond formation between the protic solvent and the electron withdrawing carbonyl group will lead the electron donating group to become coplanar with the benzene ring [22]. In other words, this hydrogen bonding seems to make the migration of electron density from the benzene ring to the electron withdrawing group more facile. The fluorescence spectrum in the cyclohexane solvent is changed significantly on the addition of water/methanol showing a dual emission. It is noted that, both LW and SW emission is nearly independent of the hydrogen bonding ability of the solvents.

These observations suggest that the dual emission of labetalol in polar solvents seems to be influenced by the enhanced intermolecular hydrogen bonding of the carbonyl group in the excited state. The fluorescence spectrum in the aqueous solution is changed significantly on addition of dioxane, showing a dual emission and an isoemissive point in dioxane-water mixture (Fig not shown) is supporting for the above prediction. The fluorescence intensity increases along with red shift with increase of water content suggest TICT is present in the labetalol [23]. The FWHM of the fluorescence bands increases in polar solvents than that of the cyclohexane indicating that TICT is the process which is responsible for the dual fluorescence. Further, the greater Stokes shift in polar and non-polar solvents would also suggest that the TICT is present in the labetalol.

Further the fluorescence spectrum of labetalol in water shows a different feature depending on the excitation wavelength (280 nm and 310 nm). In 280 nm excitation, the emission spectrum exhibits the dual emission (335 nm and 450 nm). However, with an excitation at 310 nm the dual emission intensity at 450 nm is increased. This excitation wavelength dependence of the dual emission is similar to the typical red edge effect [22] observed in the TICT fluorescence which is usually observed under the restrictive molecular mobility environment like the polymer system [24, 25]. Further, Modiano et al. reported whenever two phenyl rings are separated by the groups like SO_2, CH_2, CO, NH etc., they form a TICT state [25]. Thus, it can be speculated that the enhanced 450 nm emission should originate from the TICT state. The TICT emission is observed in polar solvents suggesting that the hydrogen bonding plays the major role in the TICT emission [26]. The excitation spectrum for the 450 nm emission is different from that for the 340 nm emission. These results suggest that, in polar solvents TICT present in labetalol.

Effect of β-CD

Figures 1 and 2 depicts the absorption and fluorescence spectral maxima of labetalol and pseudoephedrine (4×10^{-5} M) at different β-CD concentrations (pH~7). The inset Figs. 1 and 2 depict the changes for the absorbance and fluorescence intensities were observed as a function of the concentration of β-CD added. Upon increasing the concentration of β-CD, the absorption maximum of the labetalol is blue shifted (λ_{abs}~305 to 300 nm) from water to β-CD whereas no significant spectral shift is observed in pseudoephedrine (λ_{abs}~267, 222 nm). With an increasing the β-CD concentration, the absorbance is increased in pseudoephedrine but it decreased in labetalol. The above results suggest both drugs are transferred from more protic environment (bulk aqueous phases) to less protic β-CD cavity environment [27–29]. The blue shifted absorption spectrum reveals that the β-CD cavity providing non-polar environment to the labetalol.

A clear isosbestic point observed in pseudoephedrine suggest it forms 1:1 inclusion complex with β-CD, whereas absence of isosbestic point in labetalol which may rule out the possibility of a single equilibrium involving 1:1 complexation between the drug molecules and β-CD [27–29]. The possibilities are proposed for this deviation: (i) more than one guest molecule can be accommodated with in 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 may be formed and (iii) the changes detected in the absorption spectra when β-CD is added to the both solutions containing methanol (1 %) can also made the interaction between both compounds. Since in this experiment the concentration of methanol is practically constant with respect to β-CD concentration it may affect the isosbestic point.

The emission spectra of both drug molecules in β-CD solutions are shown in Fig. 2. An addition of labetalol in the β-CD, the shorter wavelength (SW) fluorescence intensity is increased with slight red shift and the longer wavelength

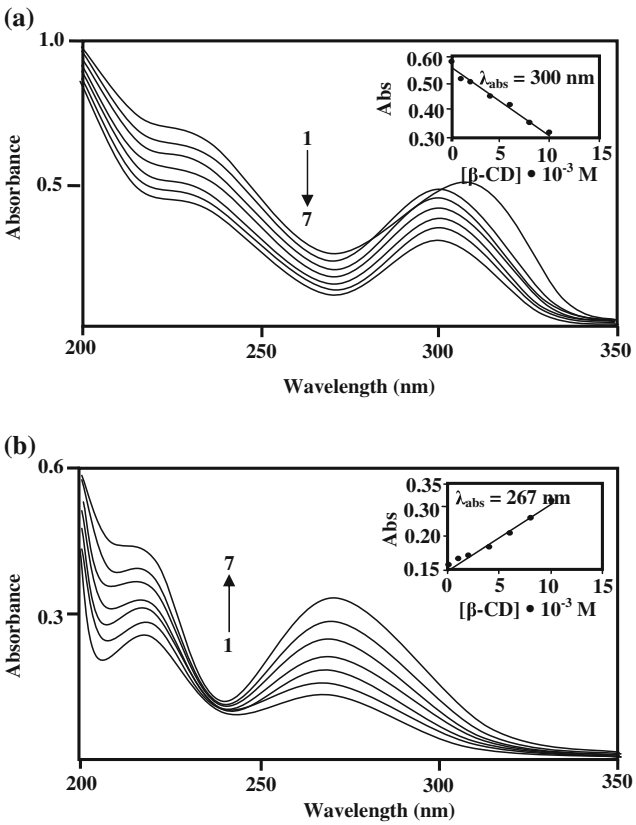

Fig. 1 Absorbance spectra of labetalol (**a**) and pseudoephedrine (**b**) in different β-CD concentrations (M): (1) 0, (2) 0.001, (3) 0.002, (4) 0.004, (5) 0.006, (6) 0.008 and (7) 0.01. Inset figure: absorbance vs. β-CD concentrations

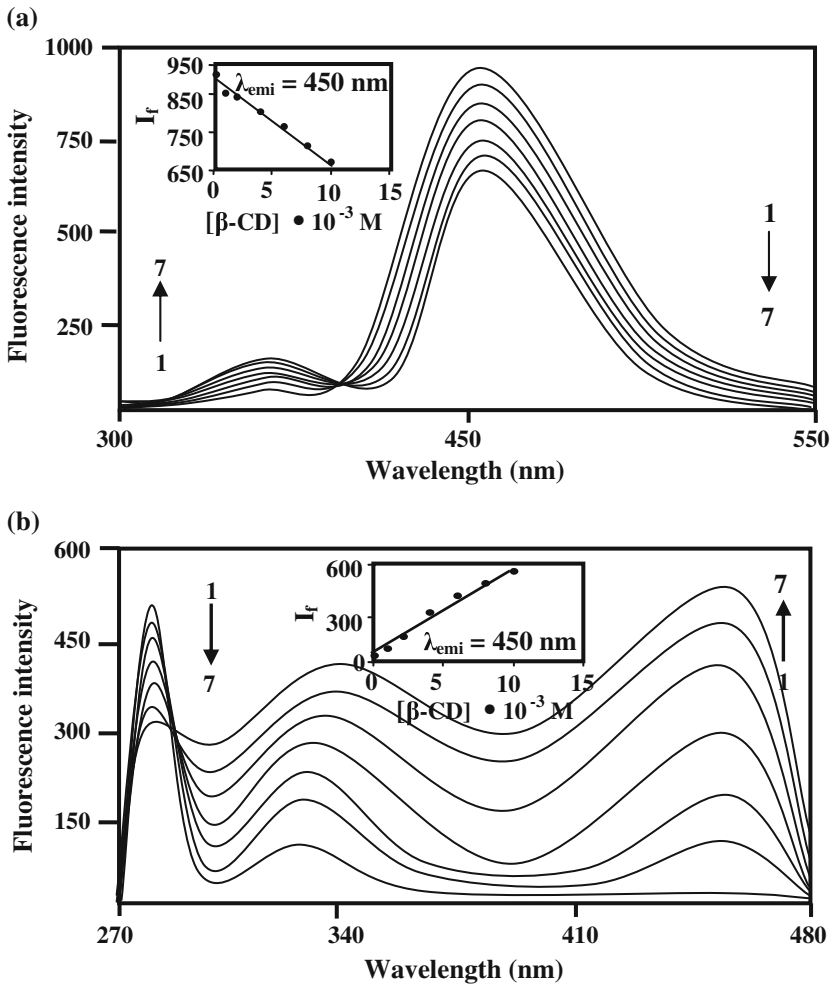

Fig. 2 Fluorescence spectra of labetalol (**a**) and pseudoephedrine (**b**) in different β-CD concentrations (M): (1) 0, (2) 0.001, (3) 0.002, (4) 0.004, (5) 0.006, (6) 0.008 and (7) 0.01. Inset figure: fluorescence intensity vs. β-CD concentrations

(LW) fluorescence intensity is decreased at the same wavelength (λ_{flu}~339, 450 nm). In contrast in pseudoephedrine, the SW fluorescence intensity (λ_{flu}~285, 330 nm) is decreased whereas the LW fluorescence intensity (λ_{flu}~450 nm) is increased in β-CD. In aqueous β-CD free solutions, single emission maximum is observed in pseudoephedrine (λ_{flu}~285 nm, 330 nm) but multiple emission is noticed in the β-CD solutions (pseudoephedrine: λ_{flu}~ 285 nm, 330 nm, 450 nm). The above results indicate that, the above molecules form different type of inclusion complexes with β-CD.

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 drugs absorbance and fluorescence were analysed by using the Benesi-Hildebrand equation [30]. A plot of $1/I\text{-}I_0$ versus 1/[β-CD] (both absorption and fluorescence), labetalol gives an upward or downward curves while plot of $1/I\text{-}I_0$ versus $1/[\beta\text{-CD}]^2$ gives straight line (Figs. 3 and 4). However, plot of $1/I\text{-}I_0$ versus $1/[\beta\text{-CD}]^2$ in pseudoephedrine gives an upward or downward curves while $1/I\text{-}I_0$ versus 1/[β-CD] gives linear line. This analysis reflects labetalol forms 1:2 inclusion complex (binding constant (M^{-1})=abs~ 10,245, flu~10,542) whereas pseudoephedrine forms 1:1 inclusion complex (abs~540 M^{-1}, flu~674 M^{-1}) with β-CD [27–29]. The values of binding constant are calculated from the slope and the intercept of the plot. 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 labetalol and two molecules of β-CD. The binding constant values for both molecules are significantly changed which reveals that different type of inclusion associated with β-CD.

The absorption spectral maxima are blue shifted in labetalol suggest the lone pair of amide and hydroxyl groups may interact with the secondary hydroxyl group of the β-CD. 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

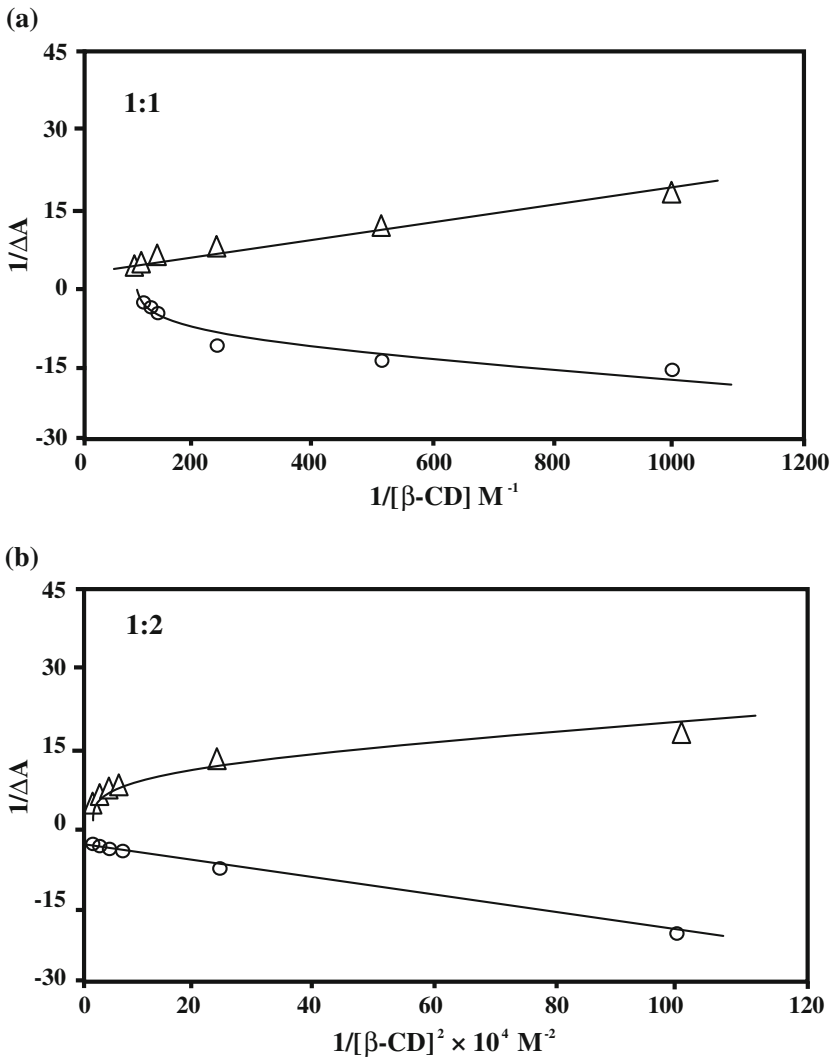

Fig. 3 Absorption spectra of Benesi-Hildebrand plot for the complexation of labetalol (*white circle*) and pseudoephedrine (*white triangle*) with β-CD. (**a**) Plot of $1/\Delta A$ vs. $1/[\beta\text{-CD}]$ and (**b**) Plot of $1/\Delta A$ vs. $1/[\beta\text{-CD}]^2$

bond interaction responsible for the higher/lower binding constants found, when compared to those of the substituted/unsubstituted molecules. The higher formation constant of the drugs implies that they are easily embedded in the β-CD cavity.

With the addition of β-CD the TICT emission at 450 nm exhibit a marked quenched in labetalol, but the SW emission maximum at 340 nm is slightly increased. Kim et al. reported similar results in biphenyl molecules [28], when biphenyl formed complexation with β-CD, the excited state geometry may change toward coplanar conformation of the biphenyl becomes accessible. But, the coplanar geometry is not possible in labetalol because it is well known in the excited state, coplanar geometry possible only in biphenyl molecules. In labetalol, the presence of alkyl chain between the aromatic rings prevents the coplanar geometry in the excited state. Further, the LW maximum (450 nm) decreased in labetalol suggest both the aromatic rings are completely entrapped in the β-CD cavity, because the cavity provide a non-polar environment like cyclohexane. As the carbonyl group encapsulated in the β-CD cavity, the TICT is significantly reduced because the interaction between the carbonyl and water is decreased. Moreover, the LW fluorescence intensity decreased in labetalol, suggest that both aromatic rings are encapsulated in the non polar β-CD cavity.

Further, if the carbonyl group is present in the hydrophilic part of the β-CD cavity, it should increases the TICT emission in the β-CD environment because it is well known that out side of the β-CD cavity provide polar environment. Therefore, we conclude, labetalol forms 1:2 inclusion complex with β-CD and the interaction of carbonyl group with the protic polar solvent (water) is greatly suppressed in the 1:2 complex, hence TICT emission decreased in the β-CD. Pseudoephedrine forms 1:1 inclusion complex because the size of this

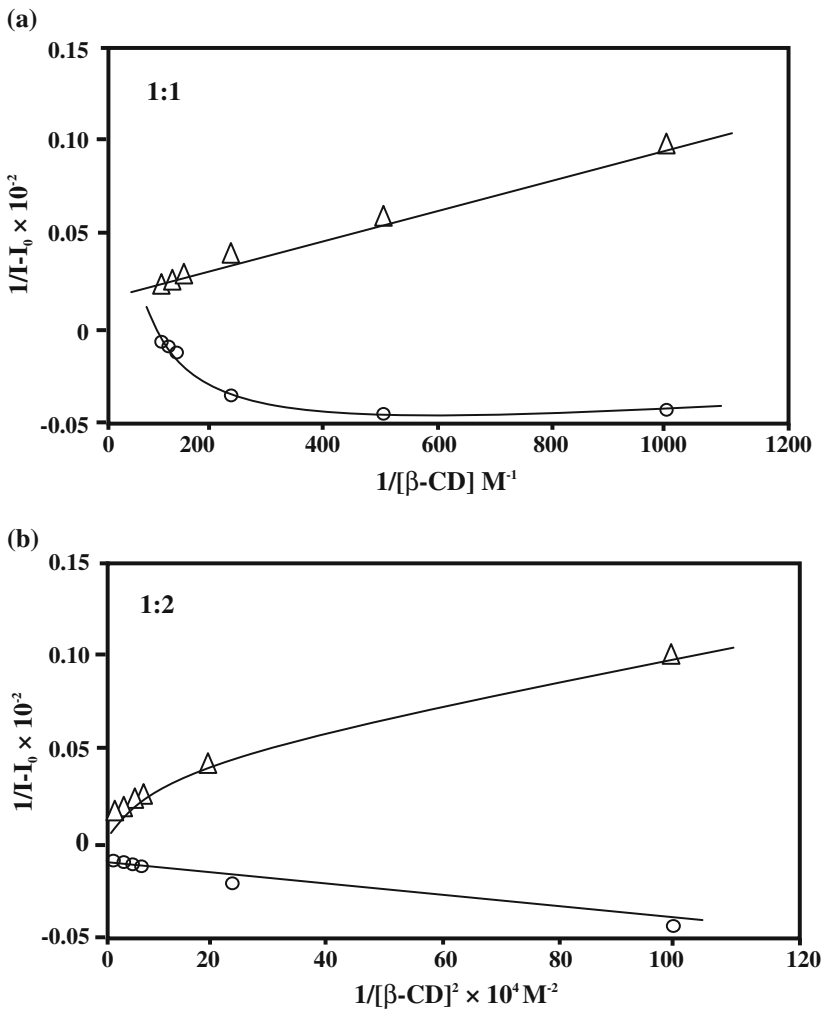

Fig. 4 Fluorescence spectra of Benesi-Hildebrand plot for the complexation of labetalol (*white circle*) and pseudoephedrine (*white triangle*) with β-CD. (**a**) Plot of $1/I\text{-}I_o$ vs. $1/[\beta\text{-CD}]$ and (**b**) Plot of $1/I\text{-}I_o$ vs. $1/[\beta\text{-CD}]^2$

drug is small and is not having polar substituent, therefore, it completely entrapped in the interior part of the β-CD cavity.

Prototropic Reactions in β-CD Medium

To know the effect of β-CD on the prototropic equilibrium between monocation, neutral and monoanion on the pH dependent changes in the absorption and emission spectra for both drugs in aqueous and β-CD medium have been recorded. The absorption and emission maxima have been studied in 8×10^{-3} M β-CD solutions in the pH range from 0.1 to 11. On comparison with aqueous and β-CD medium, no significant spectral shift noticed in the labetalol monocation (λ_{abs}~310, 247 nm; λ_{flu}~455 nm) and monoanion (λ_{abs}~332, 245 nm; λ_{flu}~non fluorescent). However, in the ground state, a blue shift is noticed in the labetalol neutral species (λ_{abs}~305, 238 nm to 300, 235 nm; λ_{flu}~450, 338 nm to 450, 340 nm) and the appearance of LW emission in pseudoephedrine (λ_{flu}~288, 325 nm to 288,325,455 nm) indicates, amide group is present in the hydrophobic part of the β-CD. The tendencies of these shifts in λ_{abs} and λ_{flu} of both molecules are attributable to the inclusion in to the β-CD cavity.

Fluorescence Lifetime

We have recorded the fluorescence lifetime of labetalol and pseudoephedrine in different environments. The typical time resolved fluorescence decay profile is presented in Table 2. The fluorescence decay behaviour of both molecules in acetonitrile, water and β-CD environments are triexponential fit could yield acceptable results such behaviour may be attributed to the possible existence of different species i.e., locally excited state molecule (LE), hydrogen bonded and twisted intra molecular charge transfer (TICT) species are formed. In both molecules, triexponential species observed in the SW and LW emission. In these environments TICT species have more lifetimes in the S_1 state compared to other two species. The average fluorescence lifetime at LW emission is increased than that of SW emission in all the environments.

Table 2 Fluorescence decay curves of labetalol and pseudoephedrine in acetonitrile, water and 0.01 M β-CD

Drug	Medium	Lifetime (ns)			Pre exponential factor			<τ>
		τ_1	τ_2	τ_3	a_1	a_2	a_3	
Labetalol	Acetonitrile (λ_{emi}: 430 nm)	1.39	2.50		0.08	0.04		0.23
	Water (λ_{emi}: 320 nm)	0.57	3.31	10.05	0.14	0.03	0.01	0.29
	(λ_{emi}: 430 nm)	0.15	2.03	5.59	0.002	0.09	0.01	0.30
	β-CD (λ_{emi} :320 nm)	0.008	1.75	7.02	0.86	0.09	0.02	0.31
	(λ_{emi}: 450 nm)	1.11	4.28			0.05	0.10	0.51
Pseudoephedrine	Acetonitrile (λ_{emi}: 340 nm)	0.23	2.18	7.01	0.22	0.02	0.02	0.25
	Water (λ_{emi}:340 nm)	0.07	1.26	5.49	0.65	0.08	0.01	0.27
	β-CD (λ_{emi}: 340 nm)	0.33	1.66	6.60	0.25	0.05	0.01	0.24
	(λ_{emi}: 450 nm)	0.41	2.02	6.36	0.14	0.07	0.02	0.35

The increase in the fluorescence lifetime of three components of both molecules upon inclusion in the β-CD is explained from a reduction in the polarity in the vicinity of the fluorophore. In β-CD the excited singlet state lifetime of labetalol is higher than pseudoephedrine. The above results indicates the tendency of complexation of β-CD, in other words labetalol have more interactions with β-CD.

Molecular Modeling

Quantum mechanical calculations were performed in order to obtain further information related to the structural characteristics of the inclusion complexes between the guest and the CD. The ground state geometry of guest molecules, β-CD and inclusion complexes were optimized by using PM3 (Parametric method 3) level of theory. Due to large dimensions of the molecular structures, vibration frequency analysis was performed to characterize the local stationary points as the minima. Calculations were carried out in the gas phase, the effects of the solvent being not taken in the account. β-CD was built up with α-D-glucopyranose residues, that were connected with other residues by α-(1-4)-glycoside oxygen bridges. For the inclusion process, the glycosidic oxygens of β-CD were placed onto the x, y plane and their centre was defined as the center of the coordination system, the β-CD was kept in this position, while the guest molecule approached this β-CD cavity along the z-axis. Initially, the guest molecules included manually in the β-CD cavity then, the geometry of the inclusion complex was fully optimized by the PM3 method. The optimized structure, bond distances, bond angles and most interesting dihedral angles in labetalol and pseudoephedrine molecule before and after complexation calculated by PM3 method are presented in Tables 3 and 4. The optimized structure of the isolated drugs and the inclusion complexes are presented in Figs. 5 and 6. The corresponding frequencies were calculated to ensure that the obtained stationary points were the minima. All the semiempirical calculations were performed using Gaussian 03 W software.

As shown in Fig. 6, taking the labetalol, pseudoephedrine, β-CD system as an example, the energy decreases sharply from the starting point A (Z_A=8 Å; ZA being the Z coordinate of point A) until it reaches point B (Z_B=1 Å), which is a local minimum. Then, the energy increases rapidly and achieves a local maximum at point C (Z_C=−1 Å). After that, the energy decreases again until it reaches point D (Z_D=−2 Å), the global minimum for the whole curve. This indicates that labetalol, pseudoephedrine and β-CD could form the most stable inclusion complex in the Head-up pattern. Lastly, the energy increases until point E (Z_E=−8 Å). Similarly, we give a simple presentation of the energy changes obtained from labetalol, pseudoephedrine passing through the cavity of β-CD from the wide side (Head-down). In Fig. 6, the energy decreases sharply from the starting point a until reaching point B (Z_B=2 Å), which is the global minimum of the whole curve and indicates that labetalol, pseudoephedrine and β-CD could form another stable inclusion complex in the Head-down pattern. Subsequently, the energy increases rapidly until reaching point C (Z_C=−8 Å). It can be seen that labetalol and pseudoephedrine with β-CD can also form stable inclusion complexes both for Head-up and Head-down interactions.

The internal diameters of the CDs are approximately 6.5 Å for β-CD and the height of all the CDs are 7.8 Å. Considering the shape and dimensions of CD (Figs. 5 and 6) the guest molecules can not completely embed in the CD cavity. The vertical distance and the length of labetalol and pseudoephedrine is greater than the inside CD cavity and upper/lower rim of CD. Hence, the polar groups attached to benzene ring can not be fully present inside of the CD cavity. Further, the optimized structure of labetalol–CD and pseudoephedrine – CD inclusion complex by Gaussian 03 W method also confirmed the polar groups attached to phenyl ring is present in the hydrophilic part of the CD and

Table 3 Geometrical parameters of labetalol and pseudoephedrine before and after inclusion in β-CD for the most stable inclusion complexes

Properties	Labetalol		Labetalol :β-CD	Pseudoephedrine		Pseudoephedrine :β-CD
Bond length (Å)	H_1-H_{22}	16.40	15.05	H_3-H_{14}	8.16	7.68
	H_5-H_{22}	16.87	14.84	H_3-H_{10}	7.76	7.90
	N_1-N_2	8.24	7.94	C_1-H_{10}	5.96	4.30
	N_2-H_{22}	8.30	8.23	C_4-C_9	6.45	6.19
Bond angle (°)	C_{10}-N_2-C_9	113.13	114.12	C_1-C_7-C_8	109.95	109.40
	C_5-C_8-C_9	109.50	109.95	C_{10}-N-C_8	114.09	114.30
	N_2-C_{10}-C_{13}	111.47	111.66	N-C_8-C_9	113.00	113.37
Dihedral angle (°)	C_5-C_8-C_9-N_2	−162.07	−165.51	C_{10}-N-C_8-C_7	155.13	144.41
	N_2-C_{10}-C_{13}-C_{14}	61.77	56.67	C_1-C_7-C_8-C_9	158.44	132.54
	O_2-C_2-C_1-C_7	−0.74	0.13	C_1-C_7-C_8-N	−76.25	−101.73

alkyl group is present in the hydrophobic part of the CD cavity. These finding confirmed labetalol and pseudoephedrine are partially embedded in the CD cavity (Fig. 6). From the results, it is cleared that the geometrical structures of guest, after complexation is completely altered. This alteration is very significant through the great variation of dihedral angels of guest.

Considering the height and diameter of β-CD, the guest molecule prefers to insert through the axial direction (long axis) (Fig. 6). In case of labetalol, if one phenyl ring is encapsulated into the cavity, another ring should be projected outside the cavity. For this orientation, the other phenyl moiety with alkyl tail is outside the hydrophobic cavity and interacts with the hydrophilic environment and this is most favorable for the stability of the complex. In this case, the possibility of 1:2 guest: host complexes will be excluded, due to the size restriction in the CD cavity. PM3 calculations indicate the guest partially/completely included into the β-CD cavity. The difference between the complex and the isolated guest and the CD energies values justify the formation of inclusion complex.

Driving Force of Complex Formation

An important feature in complex stabilization is the ability of CDs to act as acid or weak Lewis base. This phenomenon can induce complex stabilization and is in close relationship with energies of HOMO and LUMO orbital of two guest

Table 4 HOMO - LUMO calculations for labetalol, pseudoephedrine, β-CD and inclusion complexes calculated by the PM3 method

Properties	Labetalol	Pseudoephedrine	β-CD	Labetalol : β-CD	Pseudoephedrine : β-CD
E_{HOMO} (eV)	−9.17	−9.30	−10.35	−9.25	−8.99
E_{LUMO} (eV)	−0.49	0.29	1.23	−0.32	0.27
E_{HOMO} - E_{LUMO} (eV)	−8.67	−9.59	−11.58	−8.92	−9.27
μ (eV)	−4.83	−4.50	−4.56	−4.78	−4.36
η (eV)	4.34	4.79	5.79	4.46	4.63
S (eV)	0.23	0.20	0.17	0.22	0.21
ω (eV)	2.68	2.11	1.79	2.56	2.05
Dipole (D)	4.76	1.57	12.29	9.81	10.05
E° (kJ mol^{-1})	−402.72	−132.61	−6098.86	−6568.35	−6288.38
ΔE° (kJ mol^{-1})				−66.77	−56.91
H° (kJ mol^{-1})	1116.77	643.34	3303.43	4428.31	3954.11
ΔH° (kJ mol^{-1})				8.11	7.34
G° (kJ mol^{-1})	895.05	504.64	2793.09	3774.02	3382.75
ΔG° (kJ mol^{-1})				85.88	85.02
S° (cal/mol−Kelvin)	177.74	111.19	409.13	524.49	458.01
ΔS° (cal/mol-Kelvin)				−62.38	−62.31
Zero-point energy (kJ mol^{-1})	1051.15	608.33	3098.56	4158.74	3715.09
Mullikan charges	0.00	0.00	0.00	0.00	0.00

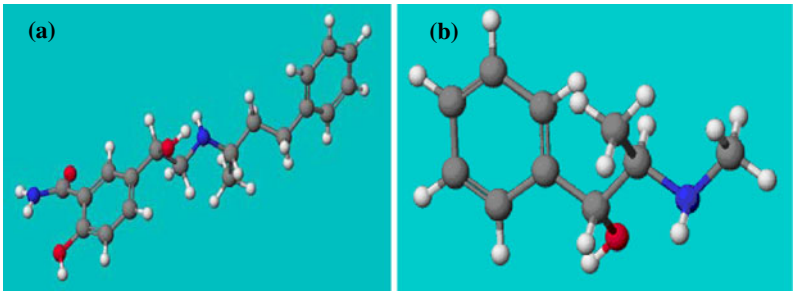

Fig. 5 The optimized structures of Labetalol (**a**) and Pseudoephedrine (**b**) at PM3 level using Gaussian 03 W software

(a) Labetalol: ΔH = -95.49 kcal/mol = -399.57 kJ/mol

N_1- N_2 = 7.60	H_3- H_6 = 7.11	C_4- C_{16} = 13.89	C_4- C_{13}= 11.27
C_1- C_{16} = 11.28	C_1- C_{13} = 8.74	C_4- N_2 = 6.46	C_4- C_{10} = 7.45
C_4- C_{11} = 8.85	H_4- H_{19} = 16.58	H_3- H_{19} = 15.94	H_{19}- N_2 = 8.51
C_{16}- N_2 = 7.48	C_{16}- C_9 = 8.87		

(b) Pseudoephedrine: ΔH = -32.02 kcal/mol = -134.01kJ/mol

H_3- C_8 = 6.09	H_3- C_9 = 8.01	C_4- C_8 = 5.03	C_4- C_9 = 6.96
C_4- N = 6.47	H_3- H_{12} = 9.10	C_1- C_9 = 4.40	C_1- H_{12} = 5.45

molecules and the host. Different types of forces have been enclosed for their role in driving complex formation including electrostatic interactions, van der Walls contributions hydrogen bonding, release of conformational strain, exclusion of high energy water in the CD cavity and charge transfer interactions. In Table 4, zero Mulliken population on the CD molecules can be observed suggesting charge transfer is not present between the host and guest molecules.

Tables 3 and 4 shows most of the labetalol values are different from pseudoephedrine. This is because the values are dependent on the size of the CD cavity and size of the substituent in the complex [31]. That is, the interaction is more sensitive to the size of substituents and the CD in the complexation. 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 polaraizabilities of the two components. The interaction of the phenyl ring with β-CD would play an important role, because phenyl moiety may achieve a maximum contact area with the internal surface of the β-CD cavity.

The values in Tables 3 and 4 shows inclusions of CDs with both drugs are also affected by hydrophobic and electronic interactions [32]. Since CDs have a permanent dipole [33] 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 is mainly due to the effects of entropy produced on the water molecules [34]. In aqueous solution, hydrophobic drugs are restricted by the water shell formed by the hydrogen bonding network [35]. It has a strong tendency to break down the water cluster and penetrate the apolar cavity of CD. This process is exothermic due to entropic gain [36]. The association constants for the inclusion of β-CD with guests were observed to be proportional to the substituent hydrophobic constant of the guest.

Further, Fig. 6 shows the hydrogen bonding interactions also play major roles in the inclusion complexation of β-CD. The polar groups of the drugs are determined by the inclusion process. The 'K' value is a reasonable measure of hydrogen bonding and the change in hydrogen bonding is caused only by the hydrogen ion concentrations. Since the polar substituent locates near the wider rim of the CD cavity and phenyl ring locates narrower range of the CD cavity, the 'K' values are proportional to the hydrogen bonding interactions. The difference in slope in Figs. 3 and 4 for the complexes indicates that the interactions of hydrogen atoms of labetalol with β-CD are much stronger than pseudoephedrine because in labetalol, the interactions are approximate to the hydrogen bonding contact. Pseudoephedrine is deeply entrapped in the internal surface of the β-CD cavity because the aromatic ring of the drug is not having polar substituent. In general, β-CD cavity is in non-polar form which favours the non polar part of the guest. Therefore we concluded, the hydrogen bonding interaction, van der Waals interaction and breaking of the water cluster around this polar guest compound (drugs) mainly dominate driving force for the inclusion complex formation.

The H-bond is defined as C=O···H (or) O–H···O (or) N–H…O with the distance between O and H being less than 3.0 Å. The H-bond lengths range from 1.79 to 3.00 Å in the structures, which fall just within the reported length range for H-bonds. The hydrogen bonding interactions are found to be the most important energetic factors that facilitate formation of the inclusion complex. There are two possible hydrogen bonds between the β-CD and labetalol as shown in Fig. 6. The first

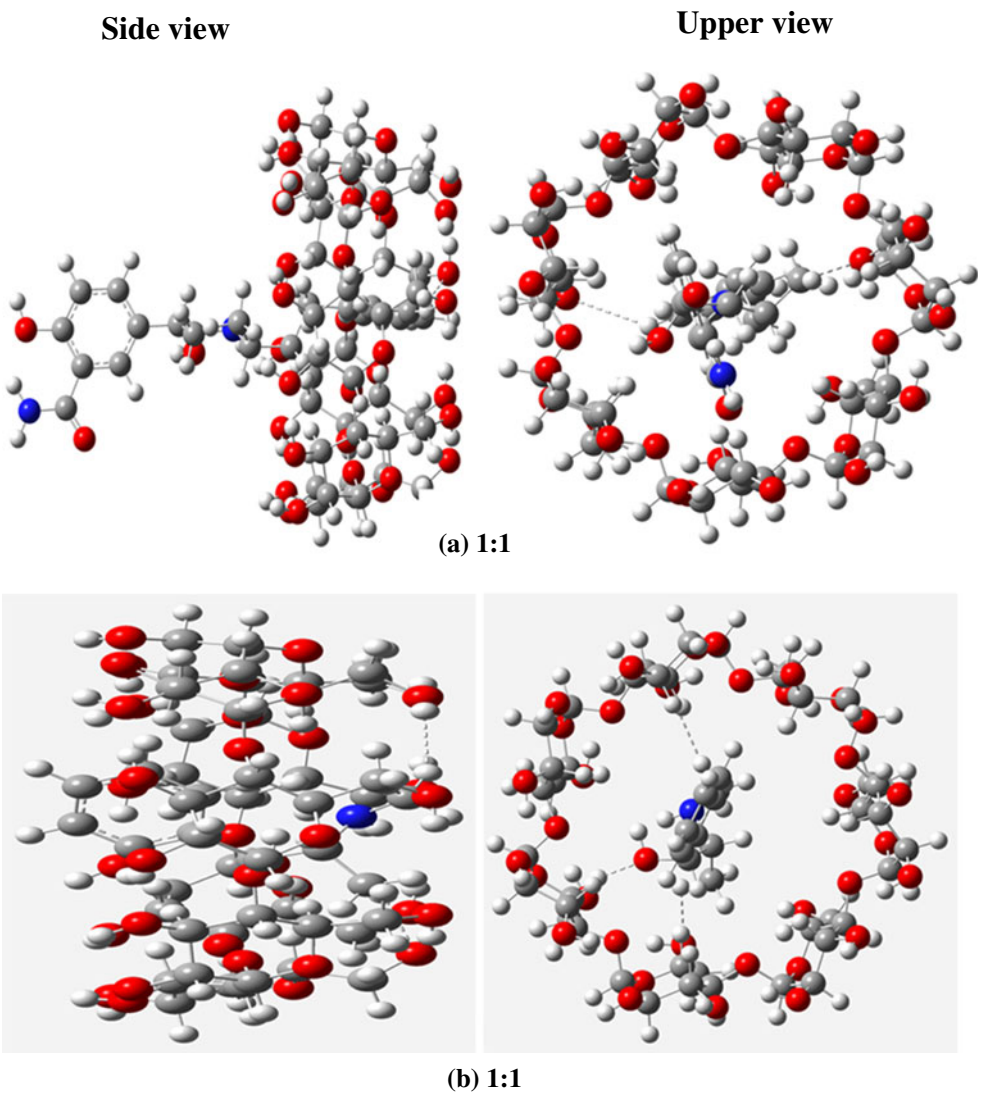

Fig. 6 Geometrical structures for the most stable inclusion complexes of labetalol/β-CD and pseudoephedrine/β-CD obtained by PM3 calculation

H-bond 1.925 Å in length, is established between the H-atom of primary group at the β-CD and the oxygen atom from the guest structure. The other H-bond 2.044 Å in length is formed between the O-atom of primary group at the β-CD and the H-atom of amino group. These hydrogen bonds separate attractive forces, causing complex stabilization and can be considered as the driving force of the complexation process. These data indicate that intermolecular H-bonds play an important role in the stability of the inclusion complexes.

Thermodynamics Parameters

To investigate the thermodynamics of the inclusion process, binding energies (ΔE), Gibbs energy changes (ΔG), enthalpy changes (ΔH) and entropy changes (ΔS) for labetalol, pseudoephedrine, β-CD and most stable complexes are summarized in Table 4.

Both theoretical and experimental free energy change values of the inclusion complex were calculated from the formation constant (K). The theoretical ΔG values for the formation of the inclusion complexes are given in Table 4. The experimental ΔG values (labetalol: abs~−23.26, flu~−23.33, pseudoephedrine: abs~−12.44, flu~−14.14 KJ/mole) negative which suggests that the inclusion proceeded simultaneously at 303 K. The experimental results are indicating that the inclusion reaction of the β-CD with labetalol, pseudoephedrine are an exothermic process.

The minimal energy of the molecular geometries was characterized by means of the stabilization energy (ΔE) between guest and the β-CD, according to eqn:

$$\Delta E = E_{complex} - \left(E_{CD} + E_{guest}\right) \quad (4)$$

where $E_{complex}$, E_{guest} and E_{CD} represent the heat of formation of the complex, free guest and free β-CD

respectively. The magnitude of the energy change variation indicates the nature of the driving force involved in the complexation process. More negative is the stabilization energy, more thermodynamically favorable is the formation of the inclusion complex. The stabilization energy for each angle rotation at the starting point Z=0 leads to exclusively negative values, which reveals that the energy of the complex is consistently lower than the sum of the isolated host and guest molecule energies. This indicates a high probability of complex formation, with guest completely included in the β-CD cavity. The binding energy for the labetalol – β-CD complex is also lower than pseudoephedrine - β-CD complex.

HOMO as Ionization energy (IE) and LUMO as electron affinity (EA) are used for calculating the electronic chemical potential (μ) which is half of the energy of the HOMO and LUMO:

$$\mu = (E_{HOMO} + E_{LUMO})/2 \quad (5)$$

The hardness (η) as half of the gap energy between HOMO and LUMO was calculated using the following expression:

$$Gap = E_{HOMO} - E_{LUMO} \quad (6)$$

$$\eta = E_{LUMO} - E_{HOMO}/2 \quad (7)$$

The electrophilicity of the components has been calculated in HF method using the following equation:

$$\omega = \mu^2/2\eta \quad (8)$$

Quantum mechanical calculations show that the E_{HOMO}-E_{LUMO} energy levels (Fig. 7) and heat of formation calculations for the complexes are lower than those for isolated molecules. These calculations express that the energies of the complexation are lower than those for isolated host and guest. The HF calculation indicates that the stability of labetalol inclusion complex is higher than pseudoephedrine. The polarity of the CD cavity decreases after the guest enters into the CD cavity. As listed in Table 4, the dipole moment of the β-CD is 12.29 D, labetalol complex is 9.81 D, and pseudoephedrine complex is 10.05 D, lower than the dipole moment for β-CD. The above quantum

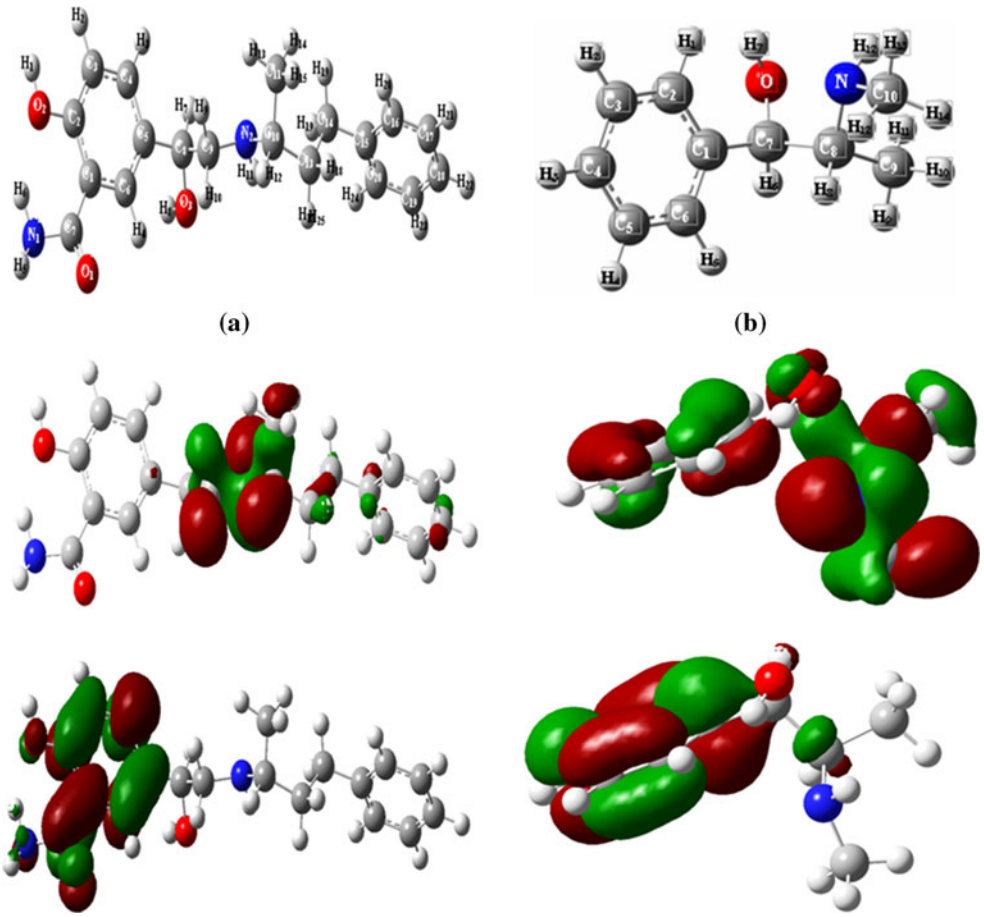

Fig. 7 The optimized structures and HOMO-LUMO energy structures of (**a**) labetalol and (**b**) pseudoephedrine

mechanical calculation values show a strong correlation with the complexation behaviour.

The data in Table 4 indicate that the inclusion complex is more stable, which can be explained as follows: β-CD having the optimal size for its internal cavity (7.8 Å) to encase the labetalol, pseudoephedrine molecules. The standard formation enthalpies (ΔH°) of the complexes labetalol–β-CD and pseudoephedrine–β-CD are 8.11 and 7.34 kJ/mol respectively, which indicate that the formation reactions for the two complexes are weak exothermic processes. Negative values for the standard Gibbs energy changes (ΔG°) indicate that the formations of all of the complexes are spontaneous processes. The entropy effects ($T\Delta S^{\circ}$) for formation of the inclusion complexes are positive and has very little contribution to the negative value of the standard Gibbs energy than the heat effect. The positive entropy effect may be due to the combined results of the host-guest reaction (negative contribution to entropy) and releasing of water molecules from the cavity (positive contribution to entropy). Finally, formation of the inclusion complexes labetalol:β-CD, pseudoephedrine:β-CD are enthalpy-entropy synergistically driven processes.

Further the binding energies (ΔE) of the complexes formed by labetalol, pseudoephedrine passing through the cavity of β-CD from its wide side are about 10.0 $kJmol^{-1}$ lower than those approaching from the narrow side. Thus, it is predicted that the labetalol, pseudoephedrine is favored to enter the cavity of CD from its wide side. The positive ΔH and ΔS values suggest that formation of the inclusion complexes is an entropy-driven process. However, the experimental data indicate that formation of both the inclusion complexes (labetalol–β-CD, pseudoephedrine–β-CD) are enthalpy-entropy synergistically driving processes. The theoretical free energy values are different from experimental value is due to solvent effect. Unfortunately, because of limitations in the calculation ability of our computer and the large molecular size of CD calculations for these systems could not be performed for aqueous solutions. However, it is observed that the solvent effect on the host-guest interactions easily changes the inclusion reaction from a non-spontaneous process in the gas phase to a spontaneous one in the aqueous phase. The host-guest interaction causes an enthalpy-entropy compensating process in the gas phase whereas the same interaction causes an enthalpy-entropy co-driven process in aqueous solution, because inclusion complexation releases a number of water molecules from the cavity of CDs.

Generally, the inclusion process is associated with a relatively large negative value of ΔH while the ΔS can be either positive or negative. The hydrophobic interactions are related to a slightly positive ΔH and large positive ΔS indicating that the process is entropy driven. The positive ΔS values are due to the breaking of highly ordered aqueous rich environments surrounding the hydrophobic part of the guest molecules upon binding the CD. In the present case, the obtained results indicate that the inclusion of guest in the β-CD in aqueous solution is favoured by both the enthalpy (ΔH >0) and entropy (ΔS >0) terms.

Conclusion

The following conclusions can be drawn from the above studies: (i) solvent study shows, dual fluorescence is observed for pseudoephedrine, (ii) labetalol shows single fluorescence in non-polar and aprotic solvents while dual luminescence observed in polar solvents, (iii) the normal emission originates from a locally excited state and the longer wavelength emission is due to TICT, (iv) β-CD studies reveal that, labetalol forms a 1:2 inclusion complex while pseudoephedrine forms 1:1 inclusion complex, (v) the increase in fluorescence lifetime in the presence of β-CD indicates that both molecules undergo effective microencapsulation. Nanosecond time-resolved studies indicated that both molecules show triexponential decay. Thermodynamic parameters (ΔG, ΔH, ΔS) and HOMO, LUMO orbital investigations confirm the stability of the inclusion complex. The geometry of the most stable complex shows that the aromatic ring is deeply self included inside the β-CD cavity and intermolecular hydrogen bonds were established between host and guest molecules. This suggests that hydrophobic effect and hydrogen bond play an important role in the inclusion process.

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