CHAPTER VIII

Probing Inclusion Complexes of Pentoxifylline and Pralidoxim inside Cyclic Oligosaccharides by Physicochemical Methodology

1. Introduction

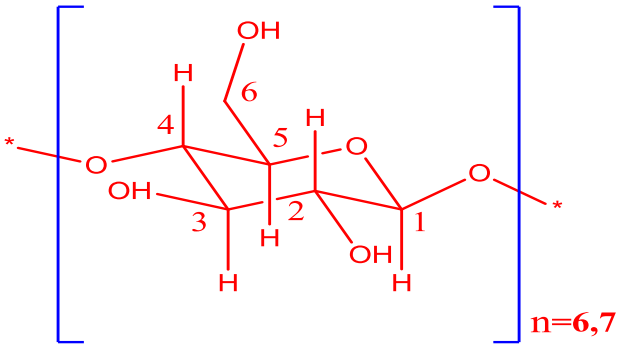
Pentoxifylline, $C_{13}H_{18}N_4O_3$, (3,7-dimethyl-1-(5-oxohexyl)purine-2,6-dione), (PTX)[1] ,is a non-selective methyl xanthine phosphodiesterase inhibitor, which improves the blood flow by decreasing viscosity[2]. At the position 1 of PTX (Fig. 1) the 'N' atom is linked with aliphatic hexyl group containing a polar electrophilic carbonyl group at the end. It has the suitable structure to form inclusion complexes with various host molecules. In 2 and 6 positions there are two ketone groups. The oxygen atoms of ketones may be able to form H-bonds with the H atoms of OH groups present in the wider rim of the CDs.

Parlidoxime is an important drug and act as a nerve agent for the treatment of organophosphorus poisoning in the nervous system[3],[4] Parlidoxime or 2-pyridine aldoxime methochloride (2-PAM) is an oxime based drug molecule. It has a suitable charged pyridine moiety and an oxime part resides at C-2 position of pyridine ring (Fig.1). The cationic pyridine moiety helps the 2-PAM for the formation of inclusion with CD through non covalent interactions.

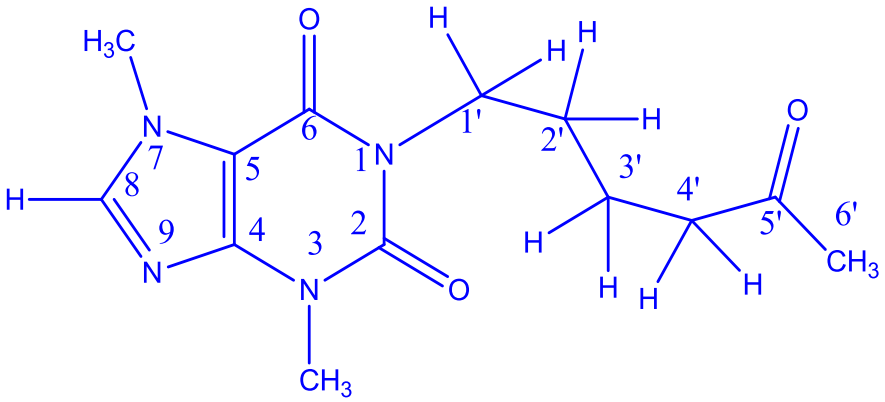
Cyclodextrins are unique host molecules containing hydrophobic inner cavity. They can form host-guest inclusion complexes with various guest molecules. Cyclodextrins (CDs) are the members of the cyclic oligosaccharides family made up of six to twelve α-D-glucopyranose units connected at 1 and 4 positions giving a rigid structure. The cyclic oligosaccharides with six to eight members are named as α-Cyclodextrin, β-Cyclodextrin and γ-Cyclodextrin respectively CDs have the lipophilic cavity and hydrophilic outer surface. Among all these, α-CD and β-CD have the suitable size for many guest molecules to form Host-Guest[5] inclusion complexes. α–CD and β –CD are easily available whereas γ-CD is comparatively expensive. In general, the special ability of cyclodextrins is the formation of host-guest inclusion complex with different organic molecules through the interaction with the inner cavity that provides hydrophobic environment to trap a hydrophobic molecule[6]. Various non-covalent interactions, such as hydrogen

bonding, Van der Waals interaction, hydrophobic interactions and also electrostatic attraction play vital role to form host-guest inclusion complex[7]**.** In pharmaceutical industries CDs are mainly used as the complexing agent to increase solubility, availability, stability, reduction of irritation, prevention of incompatibility, odour and taste masking and material handling benefits.

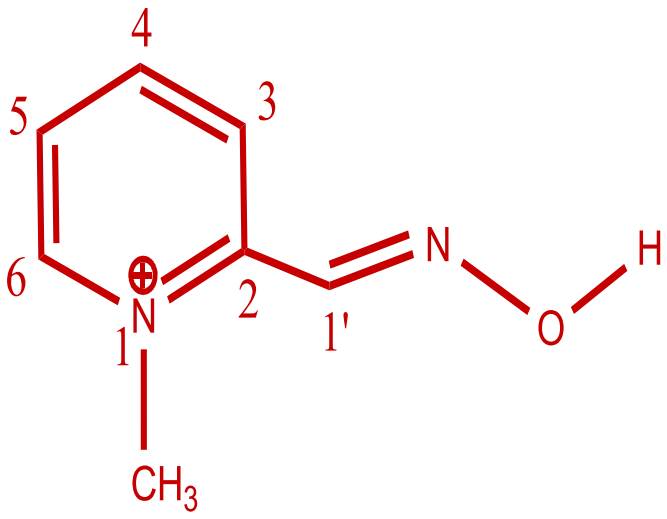
Here, in this work, a comparative study has been performed with two structurally significant drug molecules with α-CD and β-CD in aqueous environment by physicochemical and spectroscopic concern.



(a)



(b)



(c)

Fig.1. Molecular structure of (a) one unit of CD, (b) PTX and (c) 2-PAM

2. Experimental Section

2.1. Materials

α-Cyclodextrin, β-cyclodextrin, Pentoxifylline, Pralidoxime of puriss grade were purchased from Sigma Aldrich. The mass fraction purity of α-CD and β-CD used was ≥0.99 and that of PTX and 2-PAM was ≥0.99. All the above salts were dried heating at 373K for 48 hours and they were stored in a desiccator after cooling.

2.2 Apparatus and procedure

Before the start of the experimental work the solubility of the compounds under experiment were precisely checked and found the selected drug (PTX), 2-PAM, α-CD and β-CD to be freely soluble in triply distilled and degassed water (with a specific conductance of $1\times10^{-6}S\cdot cm^{-1}$). Different solutions of drug for experiment were prepared by mass (Mettler Toledo AG-285 with uncertainty ±0.0003g), and the working solutions were prepared by the procedure of mass dilution at 298.15 K.

The solid inclusion complexes namely PTX+α-CD, PTX+β-CD, 2-PAM+α-CD, and 2-PAM+β-CD have been prepared pouring ethanol solution of drug drop by drop to the aqueous CD solution. The aqueous solution of CD and ethanolic solution of drug have been prepared dissolving 1.0 mM of cyclodextrin in 20 mL of water and 1.0 mM of drug

in 20 mL of ethanol respectively and stirring separately for 3h. The mixtures have then been stirred for 48 h at 50^0C. The solid obtained in each case has been filtered at the same temperature and then allowed to cool to 5^0C and kept for 12 h. Then washed with ethanol repeatedly and dried at 100^0C.

^{1}H NMR spectra of the pure solutions and mixtures of hosts and guests were recorded on Bruker ADVANCE 300 MHz spectrometer using D_2O at 298.15K. Signals were quoted as δ values in ppm using residual protonated solvent signals as internal standard (D_2O: δ 4.79 ppm). All the Data were reported as chemical shift.

FT-IR spectra were recorded by Perkin Elmer FT-IR Spectrometer applying KBr Desk technique with scanning range 400 to 4000 cm^{-1}.

UV-visible spectra were recorded by JASCO V-530 UV-VIS Spectrophotometer, with an uncertainty of wavelength resolution of ±2 nm. The temperature was controlled by an automated digital thermostat.

With the help of platinum ring detachment method using a Tensiometer (K9, KRŰSS; Germany) at the studied temperature the surface tension experiments were carried out. The precision of the measurement was within ±0.1 $mN{\cdot}m^{-1}$. Temperature of the system was maintained throughout the experiment using circulating auto-thermostated water through a double-wall glass vessel containing the solution.

The densities (ρ) of the solvent and different solutions were measured using vibrating u-tube Anton Paar digital density meter (DMA 4500M) with accuracy of ±0.00005gcm^{-3} maintained at ±0.01K of the experimental temperature. The system was calibrated by passing doubly distilled, deionized, degassed water and dry air.

The viscosities (η) were measured using a Brookfield DV-III Ultra Programmable Rheometer with spindle size-42 fitted to a Brookfield digital bath TC-500. The viscosities were obtained by the equation below.

η = (100/RPM) X TK X torque X SMC

Here, TK (0.09373), SMC (0.327) and , RPM to be the viscometer torque constant, spindle multiplier constant and speed of the spindle respectively. The instrument was calibrated with the help of the standard viscosity samples which was supplied with the instrument, water and aqueous $CaCl_2$ solution. The temperature was maintained ±0.01^0

C using a Brookfield TC-500 thermostat digital bath. The viscosities of the different solutions were measured with an accuracy of ±1%. Every measurement reported here as an average of three readings taken successively with a precision of 0.3%.

3. Result and Discussion

3.1 Surface Tension Study

Measurement of surface tension for the formation of inclusion complexes describes the inclusion phenomena of the drug molecules. The drug 2-PAM has a cationic pyridine part and a hydrophobic side chain (oxime residue) on the other hand PTX has hydrophilic part (purine like moiety) and long hydrophobic side chain which make both the drugs considerably surface active material in aqueous medium. In our previous investigation we observed that the surface tensions of both the investigated cyclodextrins were similar in nature and their surface tension (γ) values does not show any significant changes in compared to pure water [5]. On the other way, α-CD and β-CD can be considered almost surface inactive in aqueous medium at studied conditions. Hence, any change in surface tension curve that may be due to the involvement (or insertion into CDs cavity) of drug molecule with the cyclodextrins. Here, γ - values of 10.0mM solution of drugs have been measured with the increasing concentration of α-CD and at 298.15K (Table S1 to S4). The observed increasing trend of γ with increasing concentration of CDs may be because of subtraction of the drug molecules (surface active) from the surface of the solution and insertion into the hydrophobic cavity of CDs to form stable host-guest complexes[8]. From the plot (Fig 2) it is also observed that there is a single break point in each case, the break point found at a concentration higher for β-CD than that obtained in case of the α-CD, after which the line becomes almost flattened. The trend of the values of γ clearly reveals the formation of inclusion complex and from the study of the break point of the curve of γ against concentration the stoichiometry can be interpreted. The single break point (Table S1) in each case of surface tension curve indicates 1:1stoichiometry (Fig. 2) of host-guest IC. However, some factors may be described which act as the driving forces for the formation of favourable IC i.e., (i) release of water molecules from energetically unfavourable CDs cavity (ii) enhancement of hydrophobic-hydrophobic interaction after getting incorporated of hydrophobic side part of drug molecule into the CDs. (iii) formation of

H-bonds with the –OH group present on the wider rim of CDs and (iv) hydration of the inclusion complexes by the neighbouring water molecules.

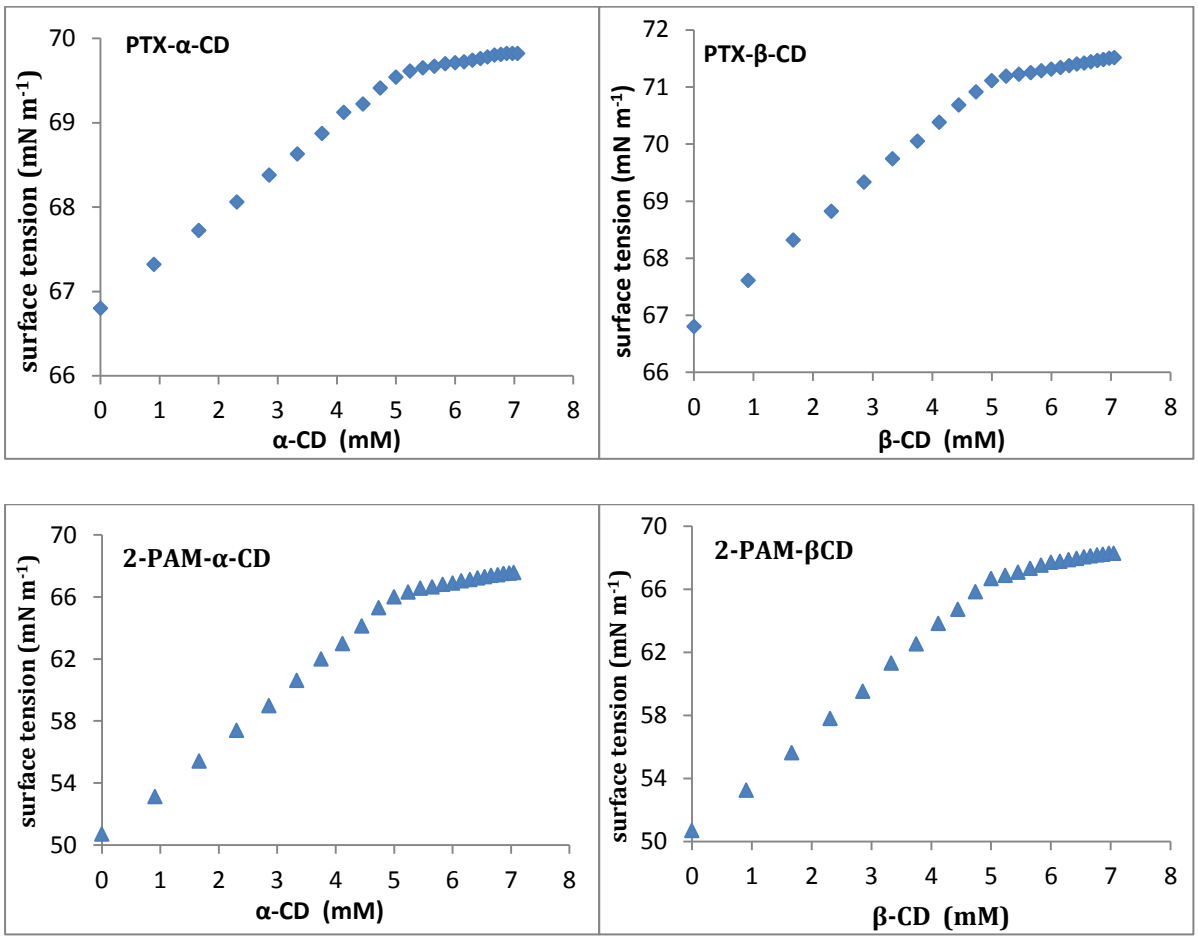


Fig.2. Surface tension of PTX with α –CD (**top left**), PTX with β–CD (**top right**) 2-PAM with α –CD (**bottom left**) 2-PAM with β – CD (**bottom right**) at 298.15 K.

The break point, in the surface tension curve for β-CD with drugs have been found at a lower concentration with drugs [Table1], indicates the higher possibility of inclusion for β-CD than α-CD [Fig.2]. In the inclusion complex the hydrophobic long chain part of the guest molecule gets inserted inside into the hydrophobic cavity of the CDs and the aromatic part remains outside the cavity. On the other hand, β-CD provides wider space and can more easily encounter the guest molecule inside into it compared to the α-CD[9]. Again the hydrophilic part of the guest outside the cavity can interact with the hydrophilic rim of CD forming H-bonds. This has also been agreed by the result obtained from the density and viscosity measurements.

Table1. Values of surface tension (γ) and at the break point with corresponding concentration of aqueous α and β-cyclodextrins at 298.15 K[a]

	Surface tension			
	PTX+α-CD	PTX+β-CD	2-PAM+α-CD	2-PAM+β-CD
Conc. Of CD/ mM	5.061	4.958	5.148	5.084
γ/mN m^{-1}	69.554	71.119	66.319	66.888

[a]Standard uncertainties in temperature u are: u(T) = 0.01 K.

3.2 UV-Vis study

3.2.1 Job's Method

The binding stoichiometry for the ICs has been confirmed by the continuous variation method for the studied systems. In this method, the mole fractions of the guest and the host were varied, while the total concentrations of the mixture of the guest and the host were kept constant (Table S2-S5)[10]. The values of absorbance were measured at respective λ_{max} (Fig.S1) for each solution at 298.15K. The Job's plots for this complex system were obtained by plotting ΔA x R against R (where, ΔA is the difference in absorbance of the drug with and without CDs and R= [drug]/([CDs] + [drug]). The value of R at the maximum deviation gives the clear indication about the stoichiometry of the inclusion complex (IC), *i.e.,* in ICs guest: host = 1:2 if R = 0.33; 1:1 if R = 0.5; 2:1 if R = 0.66 etc. It is indicative of a 1:1 binding stoichiometry between the CDs and drugs and reached a maximum at a ratio of 0.5 for [drug]/([CDs]+[drug])[11]. This behaviour thus comprises the 1:1 inclusion complexes between both the CDs and drug molecules (Fig. 3).

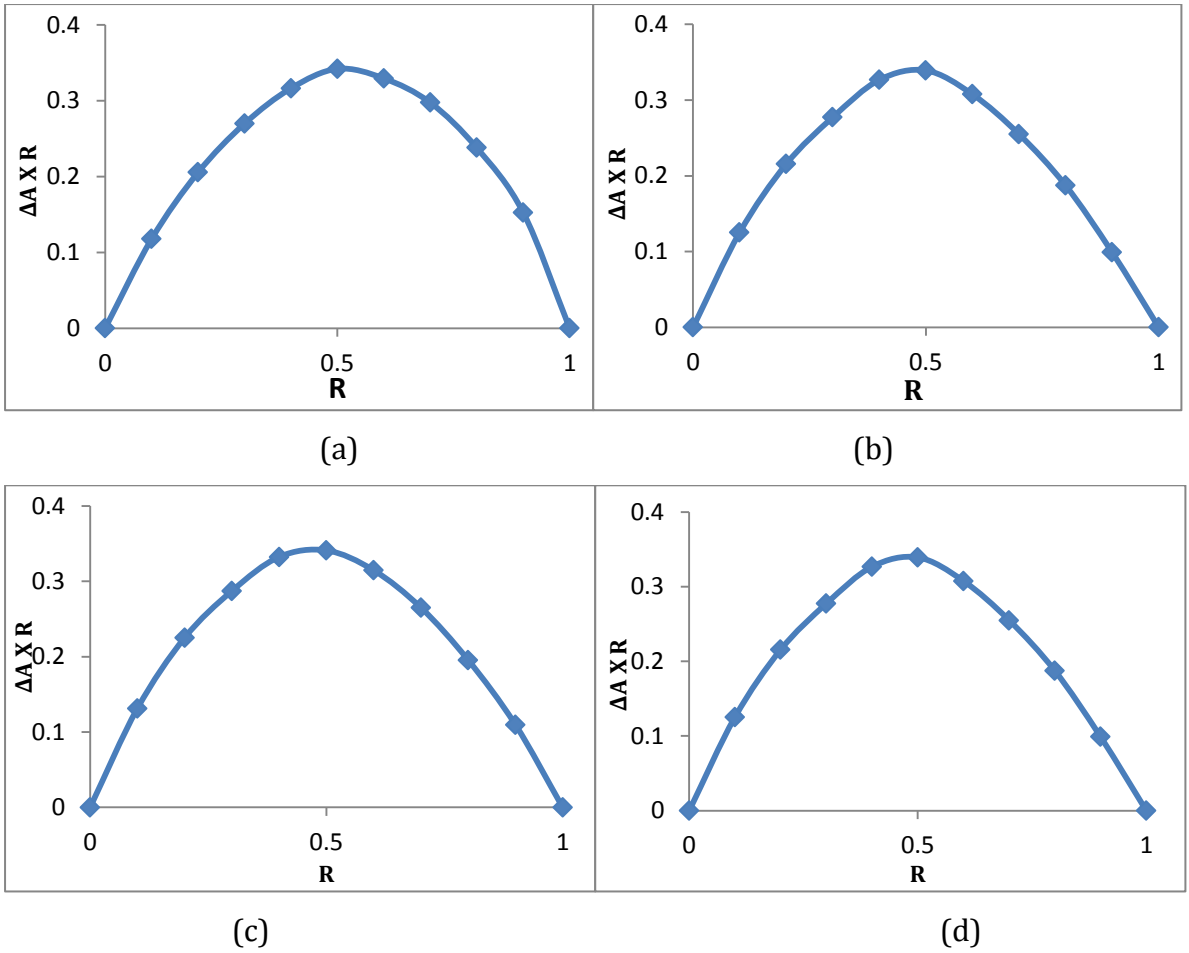


Fig.3 Job plot of (a) PTX-α-CD at λ_{max}=272 nm, (b) PTX-β-CD at λ_{max}=272 nm, (c) 2-PAM-α-CD at λ_{max}=292 nm, (d) 2-PAM-β-CD at λ_{max}=292 nm. ΔA= (A_{IC}-A_{CD}), R= [Drug]/([Drug]+[CD]) (Total concentration of the Soln. =100 µM).

3.2.2 Binding Constant:

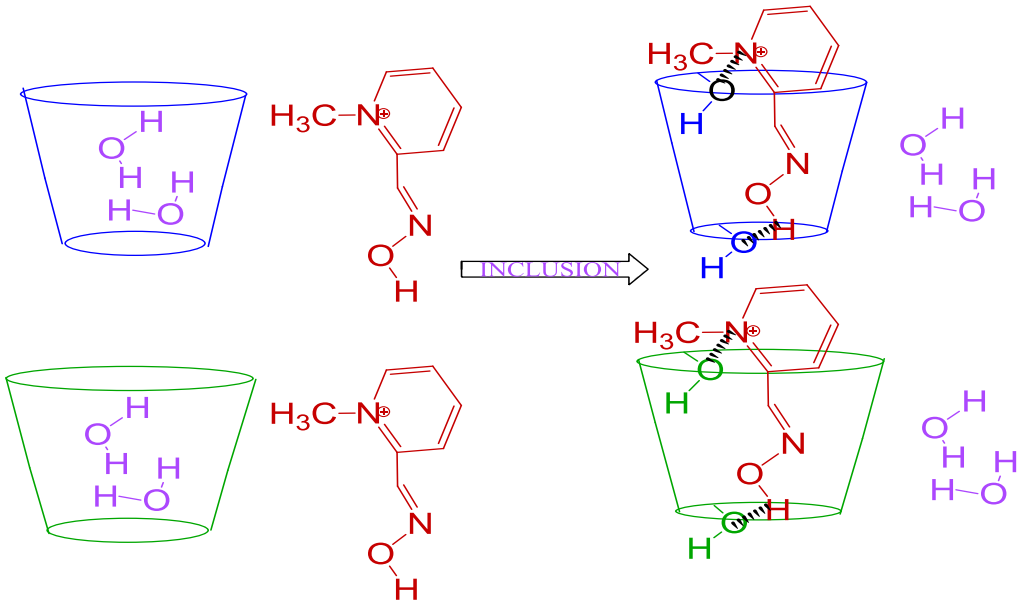
The binding constant of a host–guest complex is often considered as an essential parameter to evaluate the non-covalent binding strength between the host and the guest molecules. The binding constant (K_a) were calculated for the ICs by UV-Visible spectroscopy as a result of changes in molar extinction coefficient (Δε) of the drug when complexed with CDs molecule, which is due to the encapsulation-induced environment change from the polar bulk surroundings to apolar microenvironments of CDs (Table S6)[12],[13]. The double reciprocal Benesi–Hildebrand method was employed for calculating the binding constant (K_a) for 1:1 host-guest IC eqn [14].

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon[V]K_a} X \frac{1}{[CD]} + \frac{1}{\Delta\varepsilon[V]}$$

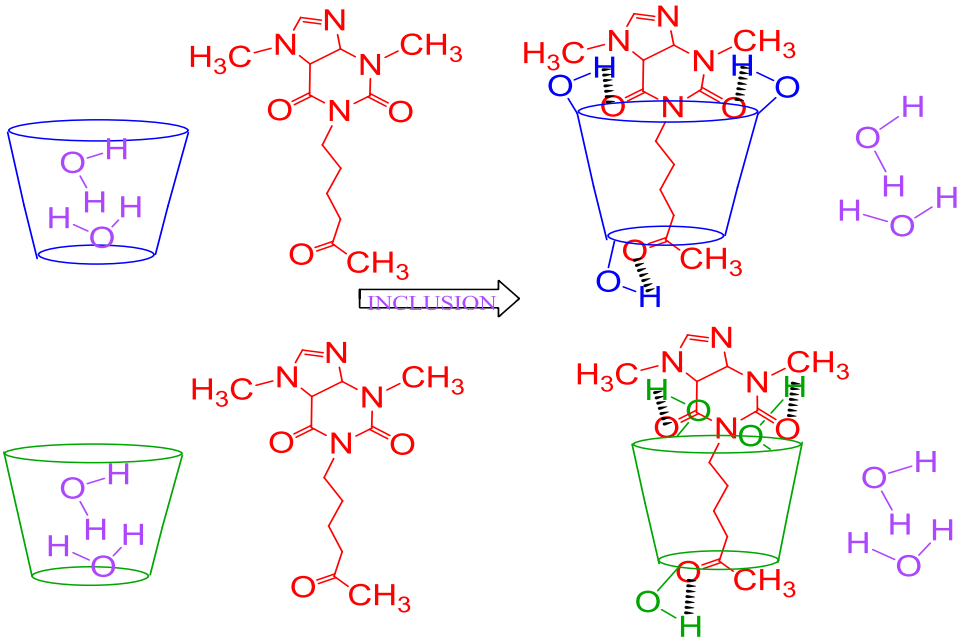
Fig.3 shows reciprocal plots that determine the stoichiometry ratio of the ICs. A very good linear relationship was obtained for $1/\Delta A$ *vs.* 1/ [CDs]. This reciprocal plot clearly indicates the stoichiometry ratio of 1:1 for the ICs formed between drug and CD[15].

The apparent formation constant was determined based on the reciprocal plot from the data obtained. The binding constant (Table S6) obtained $5.05\pm0.03\times10^{2}$ L.mol^{-1} and $1.39\pm0.01\times10^{3}$ L.mol^{-1} for α-CD and β-CD respectively with 2-PAM and $1.54\pm0.04\times10^{3}$ and $1.98\pm0.03\times10^{3}$ for α-CD and β-CD respectively with PTX. Thus higher magnitude of formation constant in β-CD suggests that the inclusion in β-CD is more efficient than α-CD for both the cases.

It is also clear that inclusion of PTX with the CDs are more efficient than that of 2-PAM. Here, the structural difference is the key factor for the binding variation to occur. 2-PAM is the oxime based compound having a positively charged nitrogen atom which interacts with the H atoms of the –OH groups present on the wider rim of the CDs. Also, the methyl group, which gets inserted into the hydrophobic cavity, is bound by the hydrophobic interaction. On the other hand, PTX is bound with the CDs as shown in the (Fig. 4) where the two carbonyl oxygen atoms form hydrogen bonds with the H atoms of the CDs of the wider rim and the hydrophobic chain gets inserted into the hydrophobic cavity of the CD molecules. Here, the hydrophobic interaction predominates with respect to that of 2-PAM molecule which has shown the variation of the binding constants.



(a)

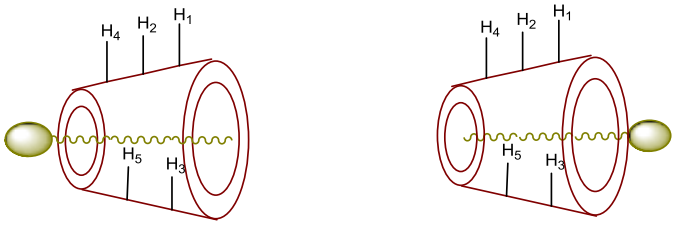


(b)

Fig. 4: Schematic diagrams of inclusion (a) 2-PAM with α-CD and β-CD, (b) PTX with 2-PAM with α-CD and β-CD.

3.3. NMR Spectroscopic Study:

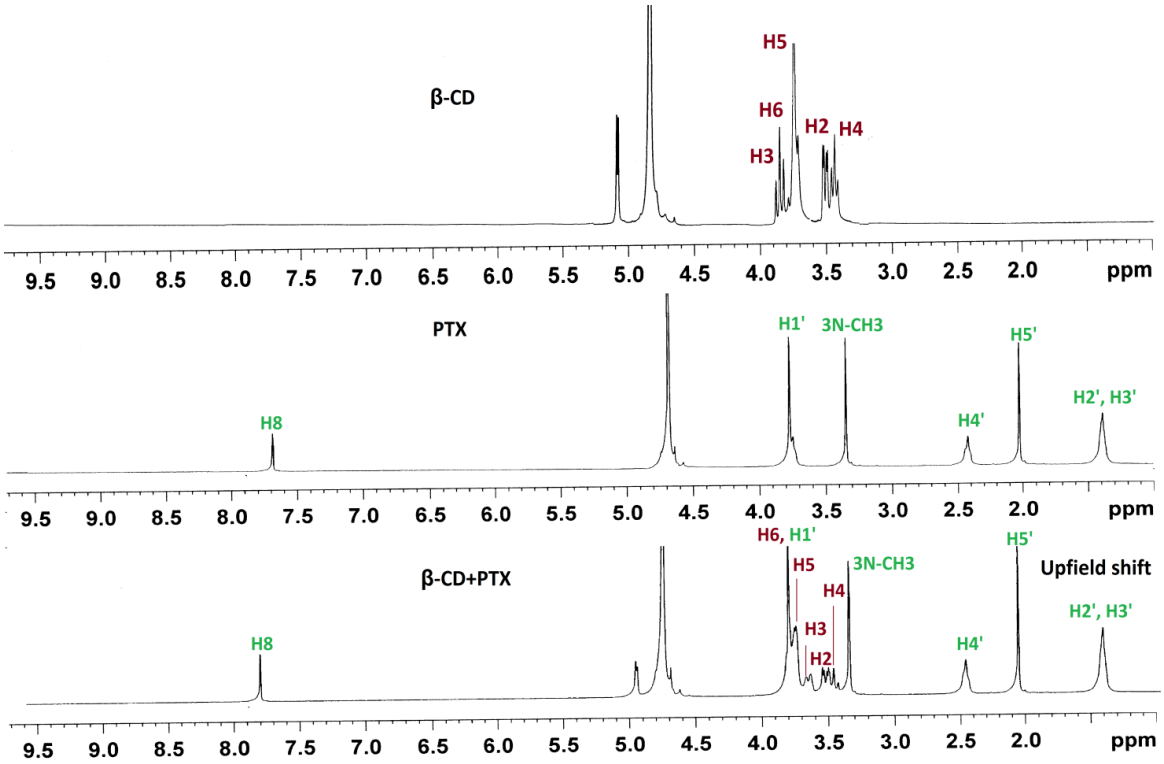
The ^{1}H-NMR spectroscopic study provides adequate evidence about the host-guest inclusion [16]. Here, the inclusion of PTX with α-CD and β-CD respectively are shown by the change in the chemical shift of protons of host and guest molecules in the complex, in comparison with the chemical shifts of the same protons in the free host and guest molecules. Proton NMR spectra of pure host and guest molecules and the complex in a 1:1 molar ratio are presented in Fig 5 and Fig. S1. The C-3 protons and C-5 protons are located inside the cavity of the CDs. Near the wider rim of the CD cavity the C-3 protons are located while the C-5 protons form a ring near the narrower rim formed by the methylene protons C-6. The protons with C-1, C-2 and C-4 atoms are situated on the outer side of the molecule. It has been found that the proton C-3 shows a significant up field shift (Table S7&S8, Fig.6 & S2) while proton C-5 shows up field shifts but less than proton C-3. The protons in the hydrophobic chain of the drug show shift also. As in both the cases Guest molecule is same, it is better to consider the shift of the host molecules[17].



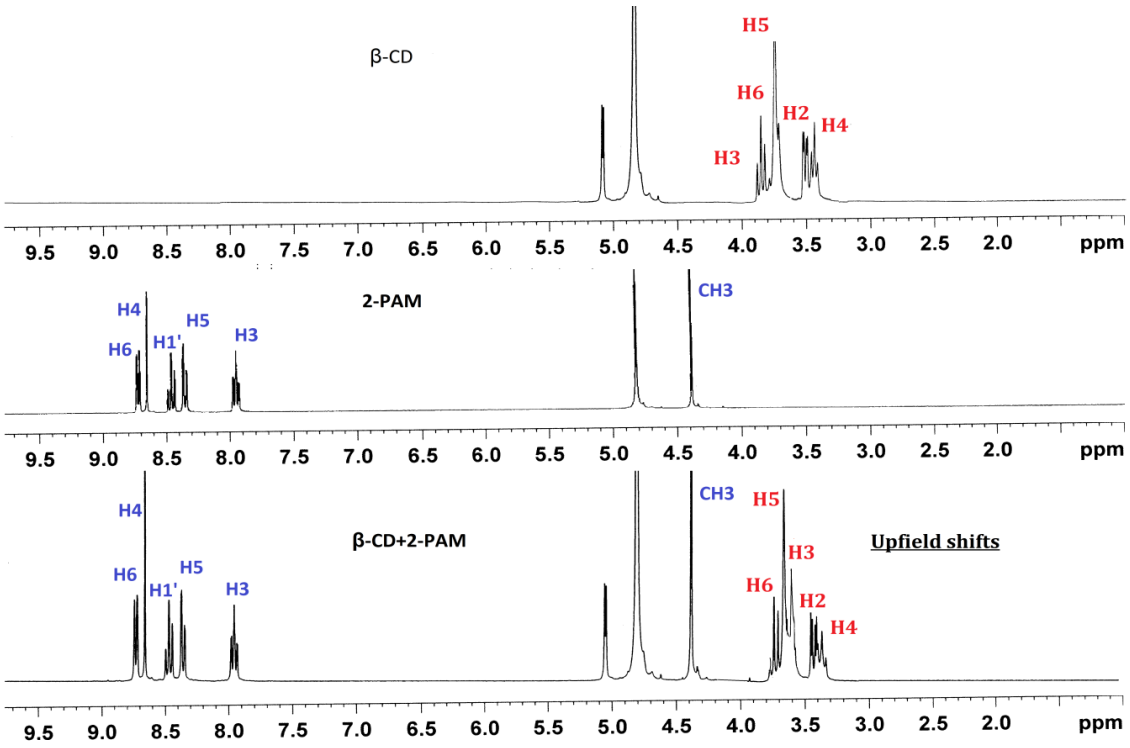
Restricted inclusion from narrower rim Feasible inclusion from wider rim

Fig. 5: Schematic diagrams of inclusion through the narrower and wider rim of the cyclodextrin molecule.

The shift of the peak due to H3 and H5 protons were found to be in more up field position for β-CD than α-CD in both the cases of inclusion (TableS8). There are two possibilities of the guest molecule to enter into the CD molecules through the wider rim and narrower rim (Fig.5) of the CD. However, the NMR spectroscopic result show more up field shift of the H3 proton of CDs which is the consequence of the inclusion of the guest through the wider rim, which in turn again support the above phenomena of inclusion. Moreover, the higher shift in case of β-CD shows more favoured inclusion[5].



(a)



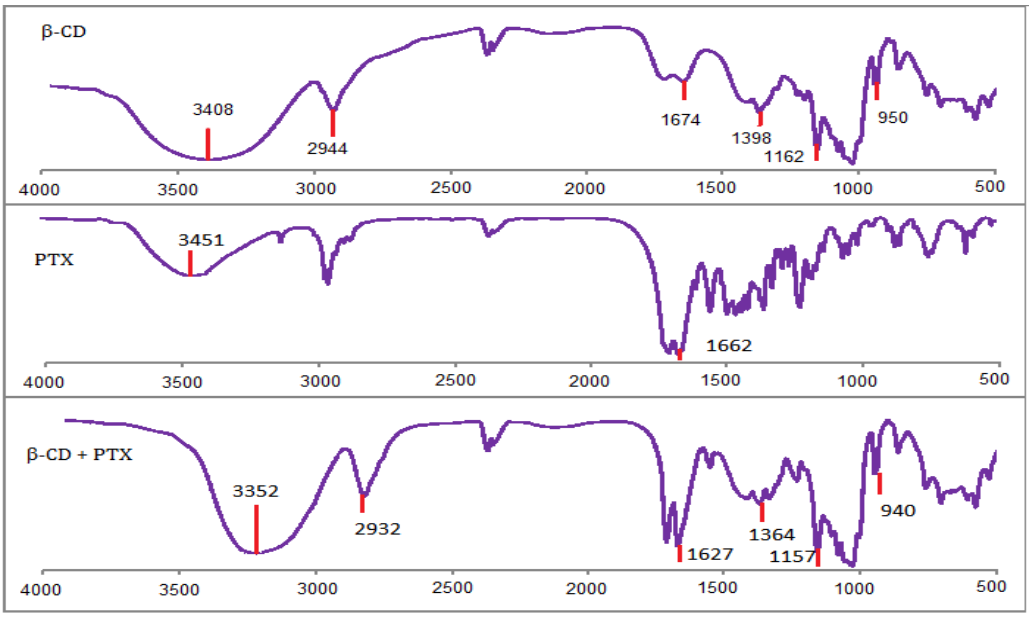
(b)

Fig.6 ^{1}H NMR spectra of (a) pure β –CD, PTX and β-CD + PTX (b) 2-PAM, pure β –CD and β-CD + PTX. (Green and blue coloured protons are of PTX and 2-PAM respectively while red coloured protons are of CD).

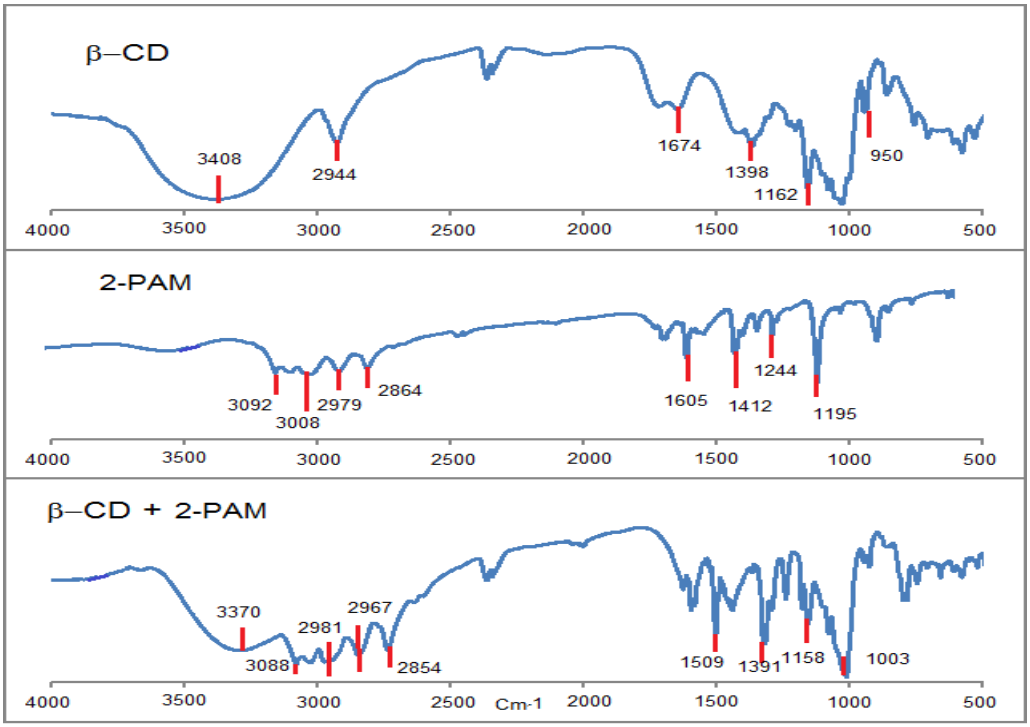
3.4. FT-IR Spectroscopy

The inclusion phenomena can also be explained by FT-IR spectroscopic study[18] [19] [20]. Characteristic IR frequencies of PTX, 2-PAM, α-CD, β-CD listed in the Table S9 explains the inclusion phenomena to occur. Here, it is seen that some peaks are shifted from their original positions in the spectra of complexes**.** The –O-H stretching frequencies of both α-CD and β-CD are seen to be shifted to a lower frequency region for all the four ICs which is due to the involvement of the –O-H groups of the CD molecules in hydrogen bonding with the guest molecules which enter into the host molecules to form inclusion complexes. The –O-H stretching frequency from the guest 2-PAM is found to be absent in the spectra of IC, clearly indicates the insertion of the guest into the cavity of CDs. It is also recorded that the C=O stretching frequency of the PTX is shifted to a lower frequency region in the spectrum and which also proves the inclusion of the PTX inside into the CDs. The inner part of the CD is hydrophobic in nature and as a consequence the drug molecules after inclusion experience the hydrophobic environment which leads to the change of the absorption peaks. The shift of the –O-H

frequency is higher in case of the PTX compared to that of the 2-PAM. The shift is again higher for β-CD than α-CD (Fig. 7 and Fig. S3). However, this observation can reveal the more feasible inclusion with β-CD compared to α-CD as well as the more effective inclusion complexation with PTX than 2-PAM. Structurally α-CD and β-CD are different only in dimension, where β-CD provides more hydrophobic space (the radius of the wider rim of α-CD is 4.7-5.3Å and of β-CD is 6.0-6.5Å)[21]. Now fact is that both the drug molecules enter into the cavity but PTX molecule fits inside into the cavity more potentially which leads to the more feasible inclusion.



(a)



(b)

Fig.7 FT-IR Spectra of (a) pure β –CD, PTX, and β-CD + PTX and (b) 2-PAM, pure β –CD, β-CD + PTX.

3.5. Density Measurement:

The calculation of apparent molar volume (Φ_v) and limiting apparent molar volume (Φ_v^0) from the study of density of the mixtures of drug and the CDs help to understand the interactions taking place in the solution Table2. The sum of the geometric volume of the solute molecules and the change in the solvent volume due to its interaction with the solute is called the apparent molar volume. Using equation (1) the values of apparent molar volume (Φ_v) were measured (Table S10 & S11).

$$(\Phi_v) = M/\rho - 1000.\ (\rho - \rho^0) / m.\rho.\rho^0 \qquad (1)$$

Where, M is the molar mass of the solute molecule, m is the molarity of the solution, ρ and ρ^0 are the densities of the solution and aqueous solvent of CD respectively.

Table2: Limiting apparent molar volume (φ_v^0), Experimental slope (Sv^*), Viscosity B and A coefficient of PTX and 2-PAM in different concentration of aqueous α-CD and β-CD solvent mixtures.

mole fraction (w_1)	φ_v^0 X 10^6	Sv^*	B	A
PTX				
α-CD				
0.0010	195.5	-48.37	0.425	0.032
0.0030	198.4	-57.87	0.512	0.010
0.0050	201.1	-76.43	0.616	0.002
β- CD				
0.0010	198.3	-61.23	0.707	0.005
0.0030	201.2	-70.26	0.785	0.004
0.0050	204.1	-89.11	0.856	0.004
2-PAM				
α-CD				
0.0010	169.85	-109.5	0.420	0.034
0.0030	172.16	-116.54	0.498	0.012
0.0050	175.17	-136.37	0.577	0.009
β- CD				
0.0010	171.49	-115.77	0.692	0.003
0.0030	174.37	-130.09	0.779	0.003
0.0050	178.12	-147.99	0.842	0.001

The experimental values of density of pure CD solutions and the drug-CD mixtures are given in the (Table S10 and S11). It has been found that the values of Φ_v i.e., apparent molar volume are positive, which is the clear indication of presence of solute-solvent interaction[22]. Also the values of Φ_v are found to be increased with the increase in the mole fraction of the CDs which again proves that with the increase of the mole fraction there is the increase of the solute-solvent i.e., drug-CD interaction.

Limiting apparent molar volume (Φ_v^0) calculated from the Masson Equation (2)

$$\Phi_v = \Phi_v^0 + S_V^*.\sqrt{C} \qquad (2)$$

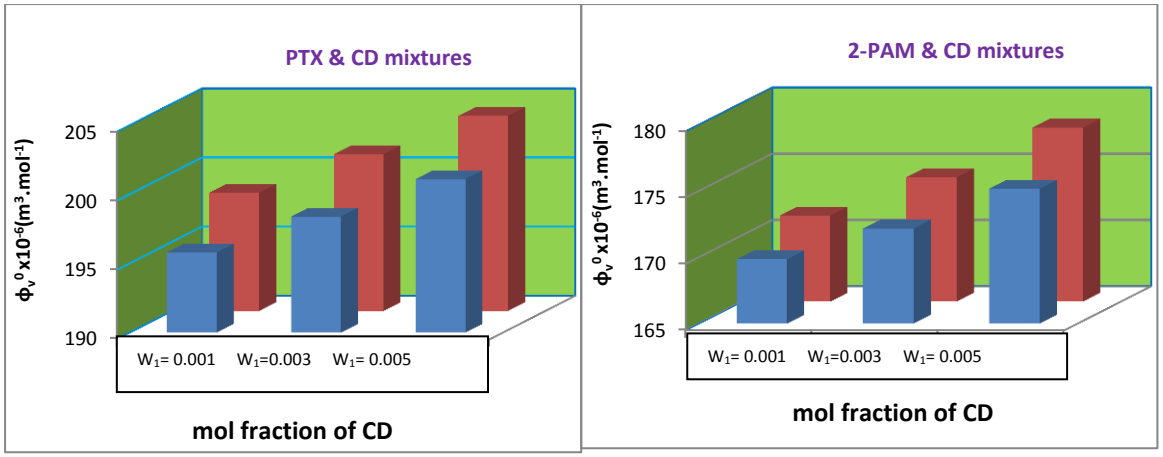
Where, Φ_v^0 is the partial molar volume at infinite dilution and S_V^* is the experimental slope. The plots of Φ_v with the molar concentration in all the cases were found to be linear and with negative slopes (Fig. 8). The values of Φ_v^0 and S_V^* are reported in the Table1. It is found that the values of Φ_v^0 are positive and increase with the mass fraction of the co-solute, the values of S_V^* are found negative. This phenomenon reveals the presence of higher solute-solvent interaction over the solute-solute interaction[23]. Thus, the density study reveals the higher drug-CD interaction than CD-CD or drug-drug interaction, which is the strong support of the inclusion.

3.5. Viscosity calculation:

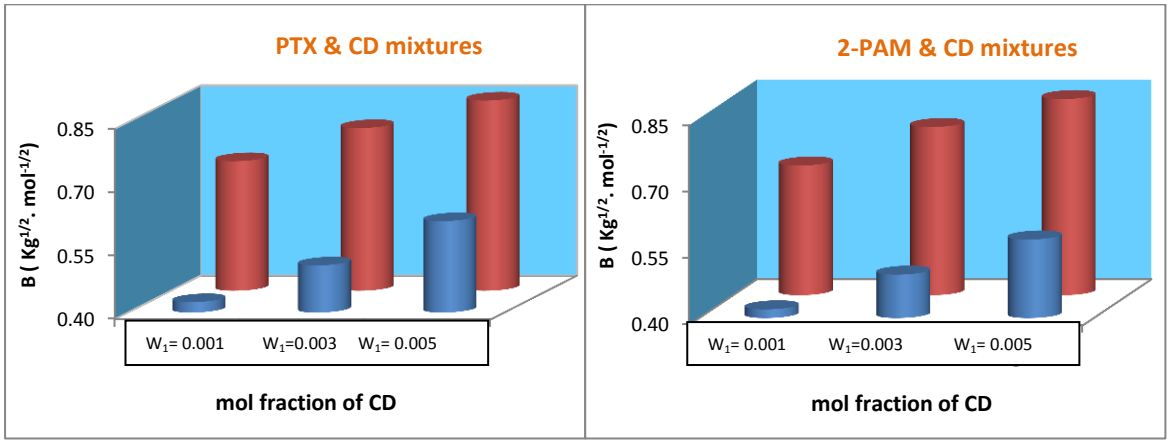
The experimentally obtained data are given in Table S10 & S11. Using Jones-Dole equation [3] the data were calculated.

$$(\eta/\eta_0\text{-}1)\ m^{1/2} = A+Bm^{1/2} \qquad (3)$$

Where, η and η_0 are the viscosity coefficients of the solution and the pure solvent respectively, M is the molarity of the ternary solution. A and B are the empirical constants, known as viscosity A and B co-efficient respectively[24] [21]. A signifies the solute – solute interaction and B signifies the Solute- Solvent interaction in the solute solvent mixture. It is seen that with the increase in the mole fraction of CDs in the solutions the values of A decreases slightly. This also supports the values obtained for Sv*. The B values (Table2) increase with the increase in the mass fraction (w_1) of the CDs in the solvent mixture. From the significantly increasing values of B with mass fraction, it can be explained that the solute-solvent interaction is predominating over the solute-solute interaction[25]. The B co-efficient values found higher for the PTX-CD system than 2-PAM-CD system and also more preciously the values are higher for the complexes with β-CD than that of with α-CD (Fig. 8). The higher B values express more compactness of the host-guest system and which is the result of the higher interaction taking place between them. This clears the fact that the feasibility of formation of IC is more with PTX and which is also higher with β-CD.



(a)



(b)

Fig. 8 (a) Limiting apparent molar volume (φ_v^0) and (b) viscosity B-coefficient of PTX and 2-PAM in different mole fractions (w1) of aqueous (■)α-CD and (■)β-CD solution at 298.15K.

4. Structural effect

The size of the guest molecule plays vital role for the formation of the host-guest inclusion complex. The host-guest complexation occurs only when there is a structural compatibility. The dimensions of the guest molecule should be such that it can fit inside the cavity of the CD molecule. Another structural suitability of the CD molecule for the hydrophobic guest molecule has been explained by Shekaari and his co-worker [26] where, polar water molecules inside the hydrophobic CD molecule are bound by polar-apolar interaction which is however not so strong and as a consequence the relatively more hydrophobic drug molecules (PTX and 2-PAM) form inclusion complex with relatively stronger apolar-apolar interaction removing the water molecules from the

cavity (Fig. 4). Again, among all the possibilities of inclusion only 1:1 inclusion occurs as discussed above. One fact is that dimensions and structures of the drug molecules are such that only one molecule can enter inside the cavity and after that possibility of inclusion of another molecule becomes restricted. The 1H-NMR study clears the fact of inclusion to occur through the wider rim, which is due to the lesser possibility of inclusion through narrower rim. As shown in the Fig.4 the C=O groups in PTX and the N atom of the pyridinium ring and the O-H group in 2-PAM stabilize the complexes by interacting with the O-H groups of the CD molecules and thus block the side to enter another molecule. Thus it can be concluded that due to the structural effects 1:1 inclusion occurs through the wider rim of the CD molecule. Now the structural compatibility of the drugs and the β-CD leads to form more feasible inclusion complexation than α-CD. Moreover, the drug molecule PTX can stabilize the complex by forming hydrogen bonds with its C=O group that again leads to a further better inclusion with high association constant of $1.98\pm0.03x10^3$ with β-CD.

5. Conclusion

The entire study reveals the formation of host-guest inclusion complex of 1:1 stoichiometry. The ^{1}H-NMR and FT-IR studies confirm the formation of inclusion complexes between the drug molecule and CD molecule. The same conclusion is also supported by the experimental data derived from density and viscosity measurements. The single break point at the concentration near to 5mM of CD in each case obtained from the surface tension study indicates the formation of the inclusion complexes with 1:1 stoichiometry. From the UV-VIS spectroscopic data, binding constant calculated using Job's plot, clearly explains the formation of more effective inclusion complex with PTX than 2-PAM and the formation of IC with β-CD is more feasible than α-CD. Taking all the parameters and results in account the plausible mechanism of the inclusion has been depicted. The qualitative and quantitative idea of the formation of host-guest ICs of α-CD and *β*-CD with PTX and 2-PAM reveal substantial applications in drug industries and medicinal sciences [16].

Tables:

Table S1: Surface Tension (γ) values of aqueous PTX and 2-PAM with α-CD and β-CD at 298.15 K

conc.of α-CD	Surface Tension of PTX+α-CD (mN.m-1)	Surface Tension of 2-PAM+α-CD (mN.m-1)	conc.of β-CD	Surface Tension of PTX+β-CD (mN.m-1)	Surface Tension of 2-PAM+β-CD (mN.m-1)
0.000	66.80	50.71	0.000	66.80	50.71
0.909	67.32	53.13	0.909	67.61	53.27
1.667	67.72	55.41	1.667	68.315	55.63
2.307	68.06	57.41	2.308	68.82	57.82
2.857	68.38	59.00	2.857	69.33	59.54
3.333	68.63	60.62	3.333	69.74	61.32
3.75	68.87	62.00	3.750	70.05	62.55
4.117	69.12	63.00	4.118	70.38	63.83
4.444	69.22	64.12	4.444	70.68	64.74
4.736	69.41	65.31	4.737	70.91	65.84
5.000	69.54	66.00	5.000	71.11	66.69
5.238	69.61	66.32	5.238	71.19	66.89
5.454	69.64	66.56	5.455	71.22	67.08
5.652	69.66	66.65	5.652	71.25	67.33
5.833	69.67	66.81	5.833	71.28	67.53
6.000	69.70	66.89	6.000	71.31	67.71
6.153	69.72	67.03	6.154	71.34	67.78
6.296	69.74	67.13	6.296	71.37	67.87
6.428	69.76	67.23	6.429	71.40	67.97
6.551	69.78	67.31	6.552	71.42	68.07
6.666	69.80	67.39	6.667	71.44	68.12
6.774	69.81	67.44	6.774	71.46	68.18
6.875	69.82	67.52	6.875	71.48	68.23
6.969	69.82	67.54	6.970	71.50	68.28
7.058	69.82	67.57	7.059	71.51	68.29

[a] Standard uncertainties in temperature u are: u(T) = ± 0.01K

Table S2: Data for Job's Plot performed by UV-Vis spectroscopy for aqueous Pentoxifylline-α-CD system at 298.15 K [a]

PTX (μM)	α-CD (μM)	[PTX]/ ([PTX]+[α-CD])	Absorbance (A)	ΔA	ΔAX [PTX]/ ([PTX]+[α-CD])
0	100	0.0	0.0000	1.0583	0.0000
10	90	0.1	0.0023	1.0560	0.1056
20	80	0.2	0.1521	0.9062	0.1812
30	70	0.3	0.2801	0.7782	0.2335

40	60	0.4	0.3883	0.6700	0.2680
50	50	0.5	0.4953	0.5631	0.2815
60	40	0.6	0.6173	0.4411	0.2646
70	30	0.7	0.7244	0.3339	0.2337
80	20	0.8	0.8221	0.2363	0.1890
90	10	0.9	0.9200	0.1383	0.1245
100	0	1.0	1.0583	0.0000	0.0000

[a] Standard uncertainties in temperature u are: u(T) = ± 0.01K

Table S3: Data for Job's Plot performed by UV-Vis spectroscopy for aqueous Pentoxifylline-β-CD system at 298.15 K

PTX (μM)	β-CD (μM)	[PTX]/ ([PTX]+[β-CD])	Absorbance (A)	ΔA	ΔAX [PTX]/ ([PTX]+[β-CD])
0	100	0.0	0.0000	1.1794	0.0000
10	90	0.1	0.0023	1.1772	0.1177
20	80	0.2	0.1521	1.0273	0.2055
30	70	0.3	0.2801	0.8993	0.2698
40	60	0.4	0.3883	0.7911	0.3165
50	50	0.5	0.4953	0.6842	0.3421
60	40	0.6	0.6303	0.5492	0.3295
70	30	0.7	0.7544	0.4250	0.2975
80	20	0.8	0.8820	0.2975	0.2380
90	10	0.9	1.0099	0.1696	0.1526
100	0	1.0	1.1794	0.0000	0.0000

[a] Standard uncertainties in temperature u are: u(T) = ± 0.01K

Table S4: Data for Job's Plot performed by UV-Vis spectroscopy for aqueous Pentoxifylline-β-CD system at 298.15 K

PTX (μM)	α-CD (μM)	[2-PAM]/ ([2-PAM]+[α-CD])	Absorbance (A)	ΔA	ΔAX [2-PAM]/ ([2-PAM]+[α-CD])
0	100	0.0	0.0000	1.0583	0.0000
10	90	0.1	0.2075	1.3132	0.1313
20	80	0.2	0.3947	1.1260	0.2252
30	70	0.3	0.5629	0.9578	0.2873

40	60	0.4	0.6895	0.8312	0.3325
50	50	0.5	0.8384	0.6823	0.3412
60	40	0.6	0.9961	0.5246	0.3148
70	30	0.7	1.1419	0.3788	0.2652
80	20	0.8	1.2762	0.2445	0.1956
90	10	0.9	1.3988	0.1219	0.1097
100	0	1.0	1.5207	0.0000	0.0000

[a] Standard uncertainties in temperature u are: u(T) = ± 0.01K

Table S5: Data for Job's Plot performed by UV-Vis spectroscopy for aqueous Pralidoxim-β-CD system at 298.15 K

PTX (μM)	β-CD (μM)	[2-PAM]/ ([2-PAM]+[β-CD])	Absorbance (A)	ΔA	ΔAX [2-PAM]/ ([2-PAM]+[β-CD])
0	100	0.0	0.000	1.5207	0.0000
10	90	0.1	0.2687	1.2520	0.1252
20	80	0.2	0.4432	1.0775	0.2155
30	70	0.3	0.5964	0.9243	0.2773
40	60	0.4	0.7041	0.8166	0.3267
50	50	0.5	0.8425	0.6782	0.3391
60	40	0.6	1.0081	0.5126	0.3076
70	30	0.7	1.0431	0.4776	0.2549
80	20	0.8	1.2866	0.2341	0.1873
90	10	0.9	1.4109	0.1098	0.0989
100	0	1.0	1.5207	0.0000	0.0000

[a] Standard uncertainties in temperature u are: u(T) = ± 0.01K

Table S6: Data for the Benesi-Hildebrand double reciprocal plot performed by UV-VIS spectroscopic study for aqueous Drug-CD systems at 298.15 K

	[Drug] (μM)	[CD] (μM)	A_0	A_1	ΔA	1/ΔA	1/[CD] (M^{-1})	Intercept	Slope	Ka (M^{-1})
	50	30	0.5801	0.5951	0.0150	66.9	33333			
	50	40	0.5801	0.5992	0.0191	52.5	25000			
PTX+α-CD	50	50	0.5801	0.6038	0.0237	42.2	20000	2.932852	0.001909	$1.54x10^3$
	50	60	0.5801	0.6082	0.0281	35.6	16667			
	50	70	0.5801	0.6138	0.0337	29.7	14286			

	50	30	0.5801	0.5990	0.0189	52.9	33333			
	50	40	0.5801	0.6054	0.0253	39.6	25000			
PTX+β-CD	50	50	0.5801	0.6105	0.0304	32.9	20000	2.940803	0.001489	$1.98x10^3$
	50	60	0.5801	0.6166	0.0365	27.4	16667			
	50	70	0.5801	0.6207	0.0406	24.6	14286			
	50	30	0.8381	0.8455	0.0074	135.0	33333			
	50	40	0.8381	0.8475	0.0094	107.0	25000			
2-PAM+ α-CD	50	50	0.8381	0.8497	0.0116	86.1	20000	2.055701	0.004065	$5.05x10^2$
	50	60	0.8381	0.8525	0.0144	69.3	16667			
	50	70	0.8381	0.8556	0.0175	57.2	14286			
	50	30	0.8421	0.8501	0.0080	124.7	33333			
	50	40	0.8421	0.8526	0.0105	95.6	25000			
2-PAM+ β-CD	50	50	0.8421	0.8549	0.0128	78.4	20000	5.007744	0.003605	$1.39x10^3$
	50	60	0.8421	0.8578	0.0157	63.7	16667			
	50	70	0.8421	0.8598	0.0177	56.7	14286			

Table S7. 1H-NMR spectra of PTX, 2-PAM, α-CD, β-CD, and PTX+α-CD,PTX+β-CD, 2-PAM+α-CD, 2-PAM+β-CD complexes

α-Cyclodextrin (500 MHz, Solvated in D2O) δ /ppm	PTX[b] δ /ppm
3.48-3.51 (6H, t, J= 9.00 Hz), 3.53-3.56 (6H, dd, J= 10.00, 3.00 Hz), 3.74-3.83 (18H, m), 3.87-3.91(6H,t, J = 9 Hz), 4.96-4.97 (6H, d, J = 3 Hz)	1.39 (4H,s), 2.03 (3H,s), 2.45 (2H,s), 3.29 (3H,s), 3.72 (2H,s), 3.74 (3 H,s), 7.71 (1H,s)
β-Cyclodextrin (400 MHz, Solvated in D2O) δ /ppm	2-PAM [b] δ /ppm
3.49-3.54 (6H, t, J = 9.2 Hz), 3.57-3.60 (6H, dd, J =9.6, 3.2 Hz), 3.79-3.84 (18H, m), 3.87-3.92 (6H,t, J = 9.2 Hz), 5.00-5.01 (6H, d, J = 3.6 Hz)	4.35 (3H,s), 7.91-7.97 (1H,m), 8.35 (1H, d, J=6.9Hz), 8.43-8.48(1H,m), 8.65 (1H, s), 8.71-8.73(1 H, d, J=6.0 Hz).
PTX+α-CD[a]	PTX+β-CD[a]

1.42(4H,s), 2.03 (3H, s), 2.45(2H,s), 3.32 (3H,s), 3.45-3.49(6H, t, J= 9.00 Hz), 3.51-3.54(6H, dd), 3.66-3.74(6H, t, J = 9 Hz), 3.68-3.74 (18H, m), 4.96-4.97 (6H, d, J = 3 Hz)	1.42(4H,s), 2.05 (3H, s), 2.45(2H,s), 3.32 (3H,s), 3.49-3.453(6H, t, J= 9.00 Hz), 3.51-3.54(6H, dd),3.61-3.71 (6H, t, J = 9 Hz), 3.77-3.83 (18H, m), 4.96-4.97 (6H, d, J = 3 Hz)
2-PAM+α-CD[a]	2-PAM+β-CD[a]
3.48-3.51 (6H, t, J= 9.00 Hz), 3.53-3.56 (6H, dd, J= 10.00, 3.00 Hz), 3.69-3.75 (6H, t, J = 9 Hz) 3.74-3.83 (18H, m), 4.96-4.97 (6H, d, J = 3 Hz), 4.35 (3H,s), 7.91-7.97 (1H,m), 8.35 (1H, d, J=6.9Hz), 8.43-8.48(1H,m), 8.65 (1H, s), 8.71-8.73(1 H, d, J=6.0 Hz).	3.49-3.54 (6H, t, J = 9.2 Hz), 3.57-3.60 (6H, dd, J =9.6, 3.2 Hz), 3.78-3.83 (18H, m), 3.68-3.72 (6H,t, J = 9.2 Hz), 5.00-5.01 (6H, d, J = 3.6 Hz), 4.35 (3H,s), 7.91-7.97 (1H,m), 8.35 (1H, d, J=6.9Hz), 8.43-8.48(1H,m), 8.65 (1H, s), 8.71-8.73(1 H, d, J=6.0 Hz).

[a] mixed in 1:1 molar ratio, 300 MHz, Solvent: D2O; [b]300MHz, Solvent: D2O

TableS8. Change in chemical shifts (ppm) of the H3 and H5 protons of cyclodextrin molecules in four different host-guest complexes in D2O at 298.15 K[a].

Protones of CD	PTX + α-CD	PTX + β-CD	2-PAM + α-CD	2-PAM+ β-CD
H3	0.19	0.37	0.16	0.18
H5	0.08	0.15	0.05	0.03

a Standard uncertainties in temperature u are: u(T) = 0.01 K.

Table S9: Data obtained from FT-IR spectroscopic study of α-CD, β-CD, PTX, 2-PAM, α-CD+PTX, β-CD+PTX, α-CD+2-PAM, β-CD+2-PAM

Group	Wave number (Cm^{-1})					
	α-CD	β-CD	α-CD+PTX	β-CD+PTX	α-CD+ 2-PAM	β-CD+2-PAM
stretching of O-H	3410	3408	3360	3352	3376	3370
stretching of –C-H from –CH2	2934	2944	2931	2932	…..	…..
bending of –C-H from –CH2 and bending of O-H	1424	1398	1411	1364	1328	1391
bending of C-O-C	1160	1162	1150	1157	1158	1158

stretching of C-C-O skeletal	1058	1031	1027	1001	1014	1003
vibration involving α-1,4linkage	956	950	.....		.....	.....
	PTX	2-PAM				
Stretching =C-H	3002	3008	.....	.....	2985	2981
Stretching -C=N	1557	1605	.....	.....	1516	1509
Stretching -N-O	.....	1412	.....	.....	.....	.....
Stretching -C-H from CH_3	2940	2979	.....	.....	.....	.....
Stretching of C=O from CH3C=O	1706	.....	1684	1679	.....	.....
Stretching of C=O from ring	1662	.....	1630	1627	.....	.....

Table S10. Experimental values of density (*ρ*) and viscosity (*η*) in deferent mass fractions of aq. *α*-CD and β-CD

Aqueous solvent	Molfraction	ρ×10-3 /Kg.m3	η/mP.S
α-CD	0.001	0.99733	1.30
	0.003	0.99796	1.31
	0.005	0.99864	1.32
β-CD	0.001	0.99753	1.31
	0.003	0.99817	1.32
	0.005	0.99894	1.33

a Standard uncertainties u are: u(ρ) = 5×10-5 g·cm-3, u(η) = 0.003 mP·s,

Table S11: Experimental values of densities (*ρ*) and viscosities (*η*) with varying concentration in different mass fractions of aq. *α*-CD with drug molecules.

Conc. (m)	ρ·10-3 (kg·m-3)	φ_v	η (mP·s)	ρ·10-3 (kg·m-3)	φ_v	η (mP·s)
PTX						
	α-CD			β-CD		

w_1=0.001 [b]						
0.010	0.99758	190.63089	1.31	0.997770	192.5957	1.32
0.025	0.99804	187.82337	1.32	0.998220	188.9868	1.33
0.040	0.99855	185.86813	1.33	0.998760	185.829	1.35
0.055	0.99910	184.25015	1.34	0.999330	183.8468	1.36
0.070	0.99970	182.60939	1.35	0.999930	182.2845	1.38
0.085	1.00033	181.19383	1.36	1.000565	180.8608	1.39
w_1=0.003 [b]						
0.010	0.99820	192.51273	1.32	0.99839	194.4759	1.33
0.025	0.99864	189.30618	1.33	0.99884	189.6671	1.35
0.040	0.99915	186.75097	1.34	0.99935	186.9621	1.36
0.055	0.99970	184.86075	1.35	0.99992	184.6397	1.38
0.070	1.00031	182.92173	1.36	1.00056	182.3108	1.39
0.085	1.00095	181.31341	1.37	1.00120	180.8038	1.41
w_1=0.005 [b]						
0.010	0.99889	193.37913	1.33	0.99915	195.327	1.34
0.025	0.99935	188.77296	1.34	0.99960	189.9213	1.36
0.040	0.99987	186.11940	1.35	1.00014	186.3175	1.37
0.055	1.00048	183.27468	1.36	1.00078	182.8593	1.39
0.070	1.00115	180.79083	1.38	1.00146	180.3111	1.41
0.085	1.00189	178.35900	1.39	1.00218	178.1912	1.43

[b] w_1 is the mass fractions of aq. α-CD and β-CD

Conc. (m)	$\rho \cdot 10^{-3}$ (kg·m^{-3})	φ_v	η (mP·s)	$\rho \cdot 10^{-3}$ (kg·m^{-3})	φ_v	η (mP·s)
2-PAM						
	α-CD			β-CD		
w_1=0.001 [b]						
0.010	0.99746	159.03622	1.31	0.99766	160.00521	1.32
0.025	0.99783	152.61902	1.32	0.99802	153.38887	1.33
0.040	0.99835	147.25464	1.33	0.99854	147.72488	1.35
0.055	0.99890	144.26937	1.34	0.99912	144.05673	1.36
0.070	0.99960	140.41488	1.35	0.99980	140.52853	1.38
0.085	1.00029	138.03877	1.36	1.00051	137.89177	1.39
w_1=0.003 [b]						
0.010	0.99808	160.93831	1.32	0.99828	161.90629	1.33

0.025	0.99845	153.32278	1.33	0.99866	153.29052	1.34
0.040	0.99894	148.41276	1.34	0.99917	147.88062	1.36
0.055	0.99952	144.54123	1.35	0.99979	143.41791	1.37
0.070	1.00018	141.18373	1.36	1.00048	139.86595	1.39
0.085	1.00090	138.30390	1.37	1.00125	136.62473	1.41
w_1=0.005 [b]						
0.010	0.99877	161.82685	1.33	0.99903	163.78361	1.34
0.025	0.99915	153.21531	1.34	0.99940	154.37364	1.36
0.040	0.99966	147.80806	1.35	0.99993	148.01690	1.37
0.055	1.00031	142.80135	1.37	1.00057	143.12535	1.39
0.070	1.00104	138.79599	1.38	1.00134	138.47107	1.41
0.085	1.00183	135.49745	1.39	1.00212	135.34170	1.42

[b] w_1 is the mass fractions of aq. α-CD and β-CD

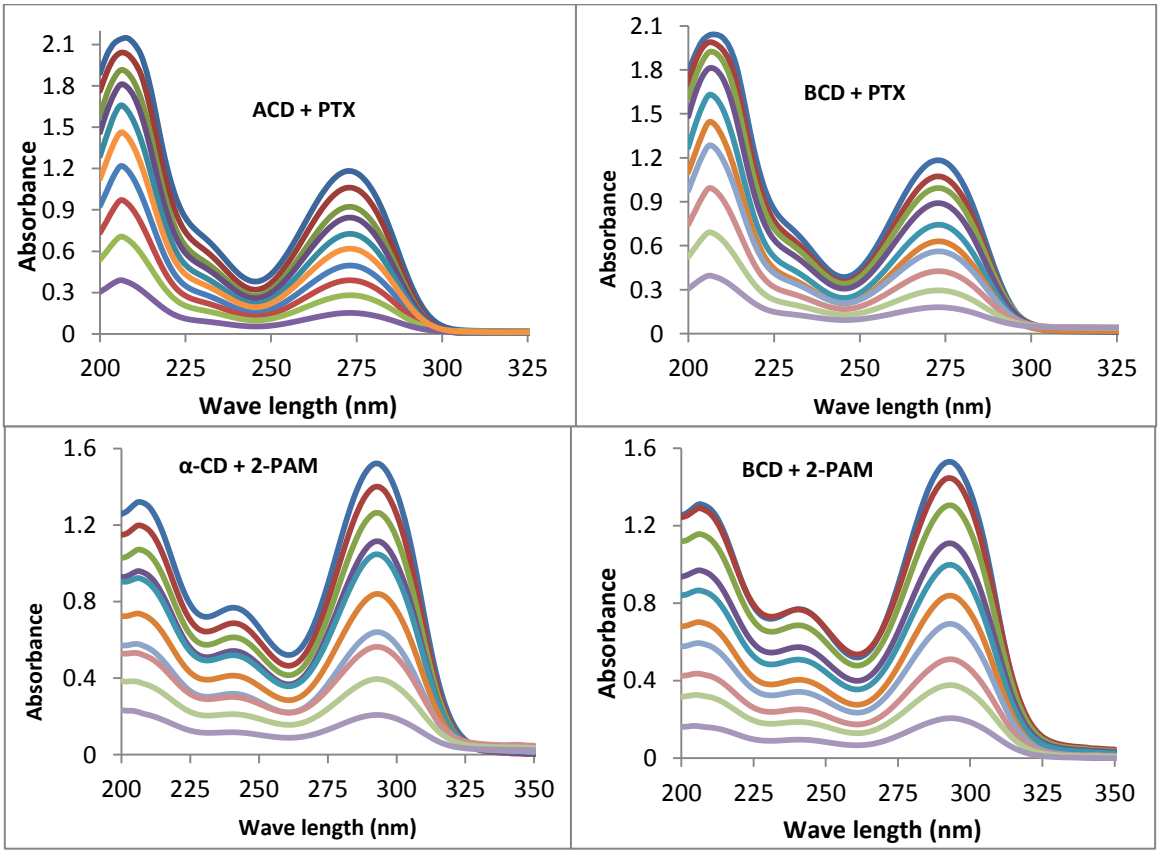
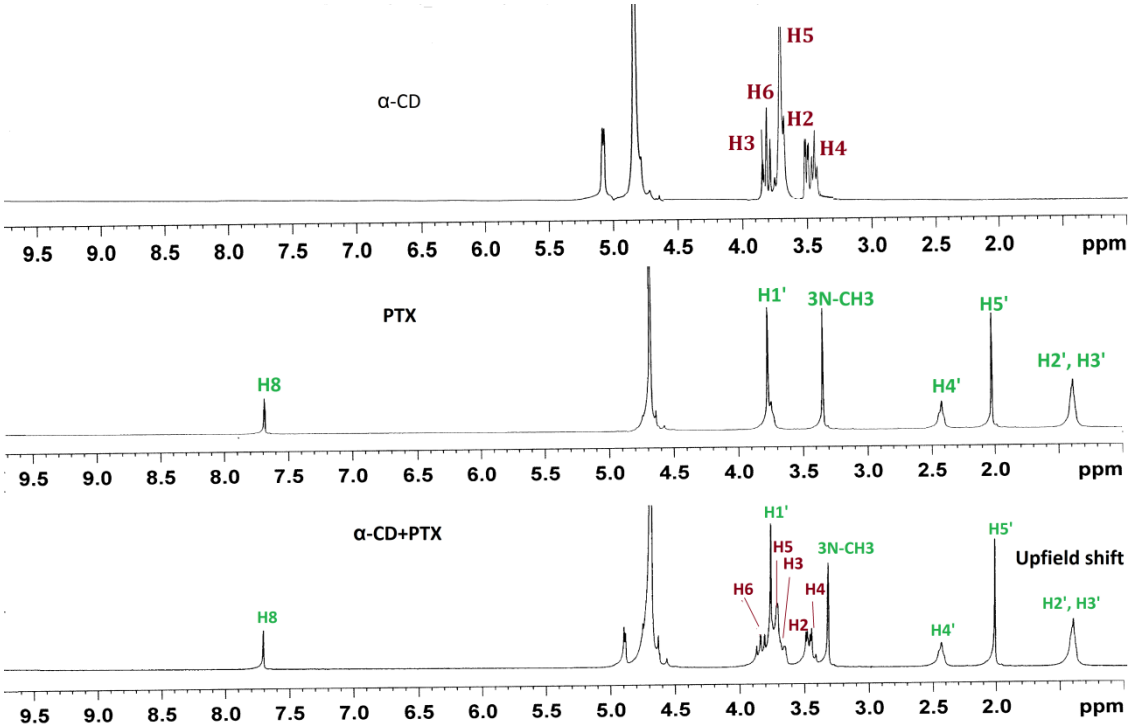
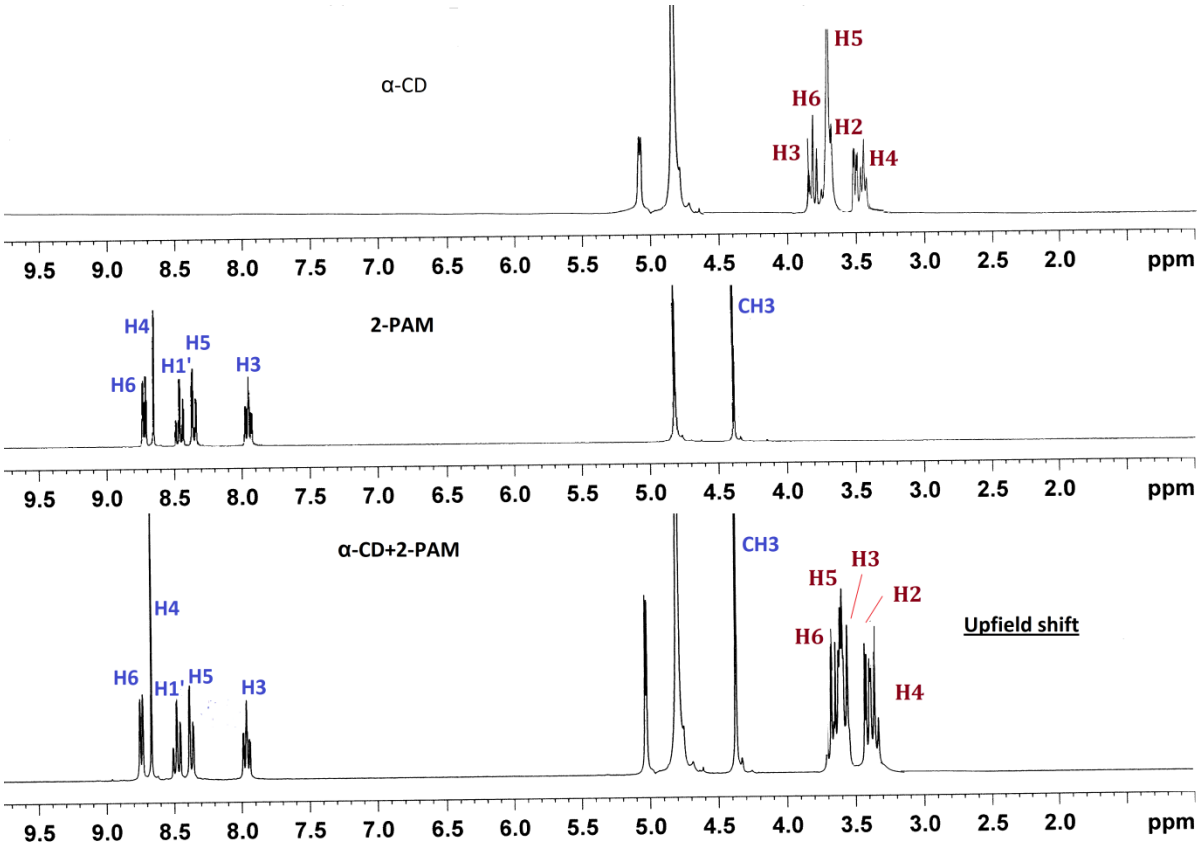


Fig.S1. Absorbance vs wave length curve of α-CD and β-CD with PTX and 2-PAM.

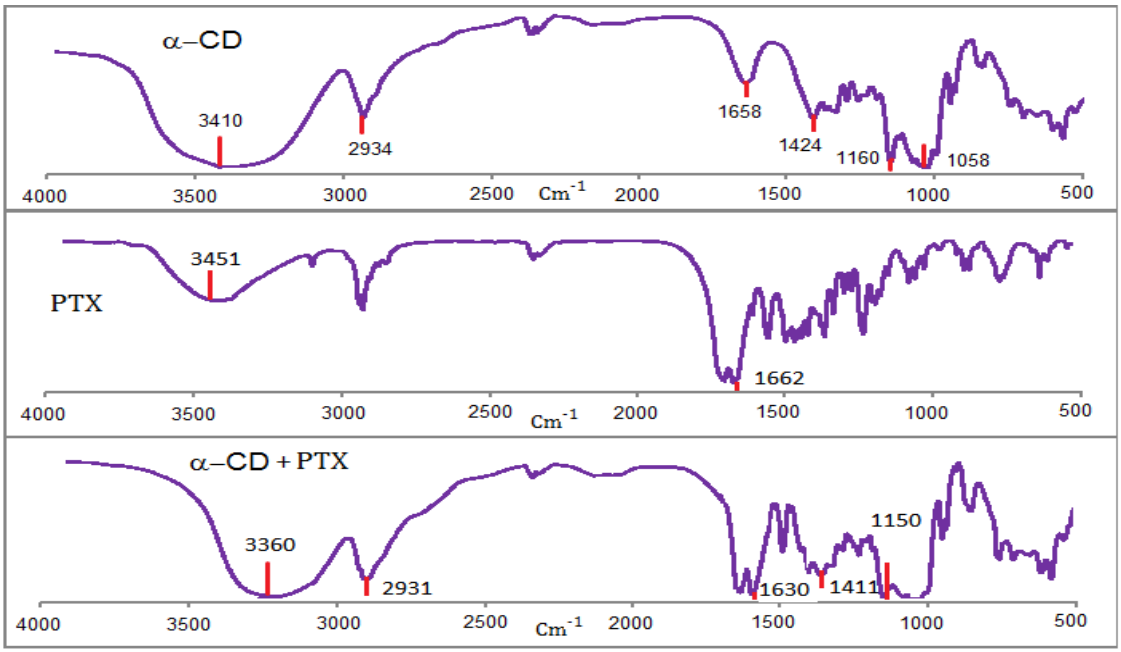

α-CD
H5
H6
H2
H3
H4
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm
PTX
H1'
3N-CH3
H5'
H2', H3'
H8
H4'
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm
α-CD+PTX
H1'
H5
H3
3N-CH3
H5'
Upfield shift
H6
H4
H2
H2', H3'
H8
H4'
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm

(a)

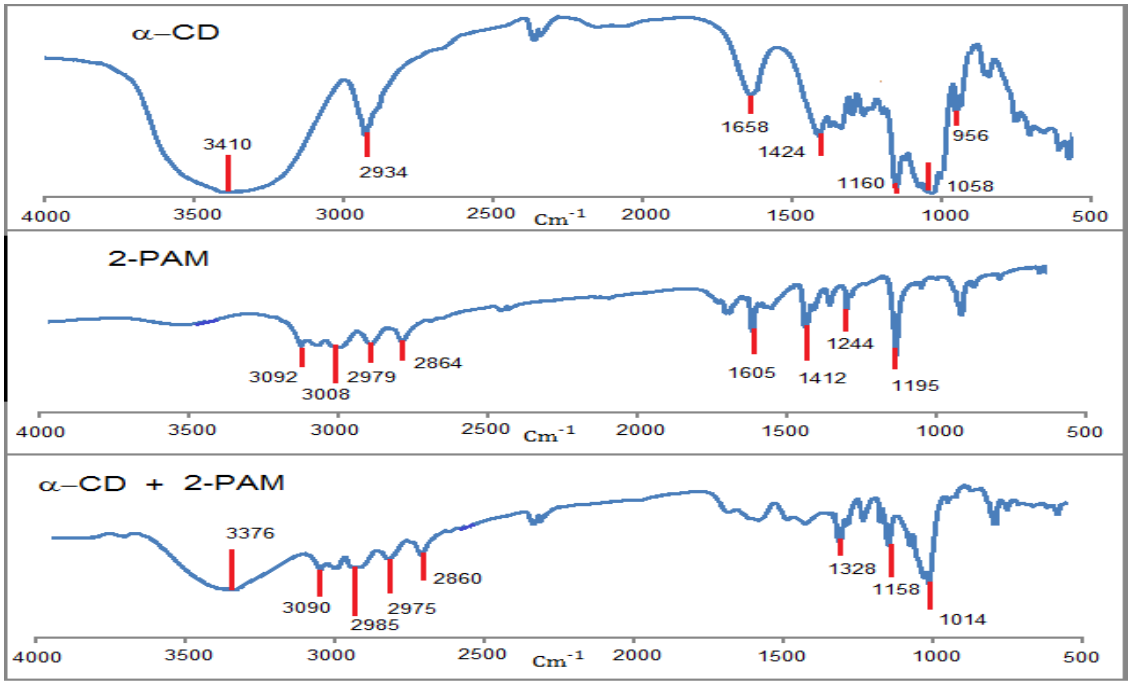

α-CD
H5
H6
H2
H3
H4
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm
2-PAM
H4
H5
H1'
H3
H6
CH3
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm
α-CD+2-PAM
CH3
H3
H5
H2
H4
H6
Upfield shift
H6
H1'
H5
H3
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm

(b)

Fig.S2. [1]H NMR spectra of (a) pureα–CD, PTX andα-CD + PTX; (b) 2-PAM, pure α –CD and α-CD + PTX; (Green and blue colored protons are of PTX and 2-PAM respectively while red colored protons are of CD).



(a)



(b)

Fig.S3 FT-IR Spectra of (a) pure β –CD, PTX, and β-CD + PTX and (b) 2-PAM, pure β –CD, β-CD + PTX