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Synthesis and Characterization of Host Guest Inclusion Complexes of Cyclodextrin Molecules with Theophylline by Diverse Methodologies

Ashutosh Dutta [a], Niloy Roy [b], Koyeli Das [b], Debadrita Roy [b], Raja Ghosh [b], Mahendra Nath Roy [b*]

[a] *Department of Technology, Uttar Banga Krishi Viswavidyalaya, Coochbehar-736165, India*

[b] *Department of Chemistry, University of North Bengal, Darjeeling-734013, India*

Abstract

Steady host–guest inclusion complexes have been produced with medicinally important guest molecule theophylline within aqueous α-Cyclodextrin and HP-β-Cyclodextrin. α-and HP-β-Cyclodextrins have been established with favorable structural features for inclusion with Theophylline which include diversified applications in modern science such as controlled delivery in the field of pharmaceuticals, food processing, pesticides, foodstuffs etc. Theophylline is one of the most widely accepted drugs for the treatment of asthma and chronic obstructive pulmonary disease (COPD) worldwide, even if it has been used clinically for many years. With both *α* and HP-*β*-Cyclodextrins it is found that 1:1 hosts-guest inclusion complexes are formed with the guest molecule theophylline. The construction and quality of the inclusion complexes have been characterized by using conductivity measurement, surface tension study, and Job's method. The inclusion phenomenon has been confirmed by FTIR spectroscopy, proton NMR study. Association constants and thermodynamic parameters have been evaluated for the created inclusion complexes by ultraviolet spectroscopy.

Keywords:

Theophylline;
Hydroxypropyl-β-Cyclodextrin;
α-Cyclodextrin;
Host–Guest Inclusion Complex;
Non-Covalent Interaction.

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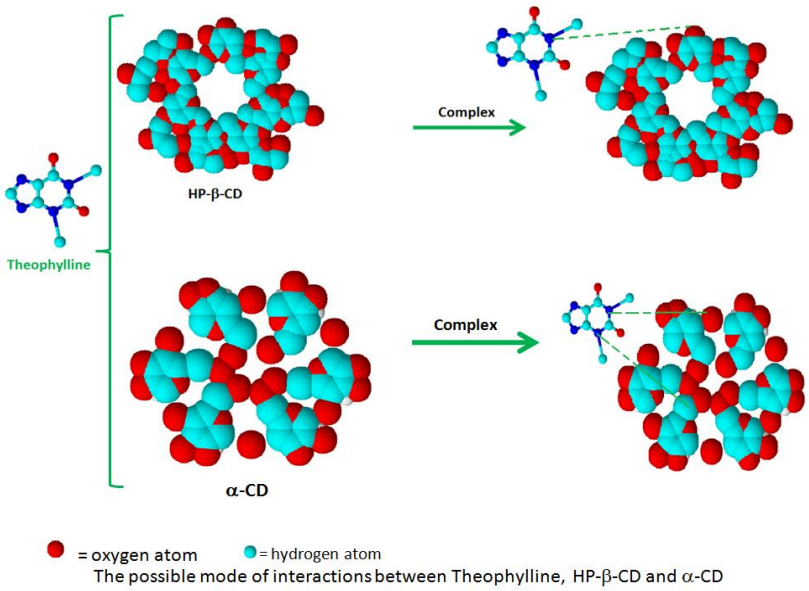
Graphical Abstract



The possible mode of interactions between Theophylline, HP-β-CD and α-CD

* **CONTACT**: Mahendraroy2002@yahoo.co.in

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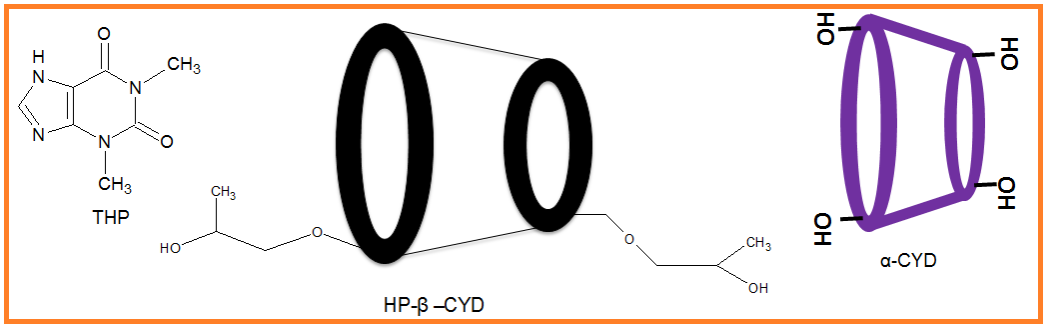
1- Introduction

Cyclodextrins (CYDs) are made up of 6, 7 and 8 glucopyranosyl units attached to α-(1, 4)-glycosidic linkages are identified as α, β, γ-Cyclodextrins respectively. The CYDs are of biomedical and pharmaceutical interest are cyclic oligosaccharides composed of six to eight dextrose units connected through one to four bonds [1, 2]. The utilization of CYDs previously has an extended history in pharmaceuticals, pesticides, foodstuffs etc. for the solubility, bioavailability, safety, stability and as a transporter of the guest molecules [3]. Beta Cyclodextrin has been extensively used due to ready availability as well as low price although it has some disadvantages like low solubility and nephrotoxicity [4]. Derivatives of β-Cyclodextrin with improved water solubility (e.g. Hydroxypropyl-β-Cyclodextrin i.e HP-β-CYD) are most commonly pharmaceutical formulation [5]. CYDs have been revealed to enhance the solubility of sparingly soluble drugs by making inclusion complexes. Among the variety of customized β-Cyclodextrins, hydroxypropyl-β-cyclodextrin (HP-β-CYD) and sulfoxybutyl ether-β-cyclodextrin are the negligible amount of toxic and may be useful in the improvement of parenteral dosage forms of these drugs [6]. It is essential to use as small amount of CYDs as likely in pharmaceutical formulations. In this respect, aqueous solubility of α-CYD is more than β-CYD, taking extra advantages for this investigation (solubility in water (w/v) at 25 °C (298K): for α-CYD is 145 mg/ mL and β-CYD is 18.5 mg/ mL) [7]. 2-hydroxylpropyl-β-cyclodextrin (HP-β-CYD) is an substitute to α, β and γ-cyclodextrin, with enhanced water solubility of approximately 500mg/mL and may be further toxicologically benign, mostly when dosed orally, and exhibits only narrow toxicity, formed extra slight hematological changes but no histopathological changes [8]. Amongst these CYDs, β-CYD and its hydrophilic derivative, such as hydroxypropyl-β-cyclodextrins (HP-β-CYD) are the first choices because of their appropriate cavity sizes and modest cost [9]. HP-β-CYD can be used in safety as a transporter for parenteral delivery of drugs. HP-β-CYD is not absorbed from the gastrointestinal tract. It is rapidly and almost entirely cleared from the systemic circulation by the kidneys after intravenous injection, and is cleared from the lung by being absorbed into the systemic circulation following administration in an aerosol [10]. Amongst the three cyclodextrin homologues *(α,* β and γ) β-cyclodextrin is the slightest expensive. Undesirably, β-cyclodextrin has only inadequate water solubility, and its complexes are consequently only a little water-soluble. Thus, β-cyclodextrin is frequently chemically customized to increase its water solubility. One of its derivatives, hydroxypropyl-β-cyclodextrin (HP-β-CYD) was found to be extremely water-soluble. Hence, HP-β-CYD is used in this study [11]. Recently, the anticancer consequence of HP-β-CYD has been revealed and proved in vivo in mouse model of leukemia [12].

Theophylline [1,3-dimethyl-1*H*-purine-2,6-(3*H*,7*H*)–dione] is one of the most extensively approved drug used in therapy for respiratory diseases such as for the treatment of asthma and chronic obstructive pulmonary disease (COPD) worldwide, although it has been used clinically for more than 82 years. However, in rising countries, Theophylline (THP) is at a halt the first-line treatment in patients with asthma and COPD, because it is low-priced and widely accessible. A growing amount of confirmation has recommended that low-dose THP has anti-inflammatory and immune modulatory effects in asthma and COPD and thus, THP has fascinated a large amount of awareness and importance [12, 13]. THP fast metabolizers, as are started especially in the middle of children and smoking adults, may necessitate a further, regular interval than once-a-day dosing, and greater fluctuations in theophylline levels should be predictable [14]. Main toxicity after THP intoxication differs by variety of overdose [15]. In this effort, we have investigated the formation of complexes of the guest molecule THP with host molecules α-CYD and HP-β-CYD in aqueous m environment. The complexes were characterized by Conductance measurement, Surface tension, ^{1}H NMR, FTIR and UV-visible spectra. The structure of the THP, α-CYD and HP-β-CYD are shown in scheme 1.



Scheme1. Molecular structure of THP, α-CYD and HP-β-CYD

2- Experimental Section

2-1- Materials

The THP (99%) and α-CYD (99%) were bought from Sigma-Aldrich, Germany and HP-β-CYD (98%) TCI used as purchased. The CAS Registry Nos., suppliers, and mass fractions are listed in Table 1. All the chemicals are used without further purification.

Table 1. Names, Suppliers and Mass Fractions of the Chemicals.

Component	CAS reg. No.	Suppliers	Mass fraction	Analysis method
Theophylline (THP)	58-55-9	Sigma Aldrich	≥99%	Used as purchased
α-Cyclodextrin (α-CYD)	10016-20-3	Sigma Aldrich	≥98%	Used as purchased
Hydroxy Propyl-β-Cyclodextrin (HP-β-CYD)	128446-35-5	TCI Chemicals (India) PVT Ltd.	≥98%	Used as purchased

2-2- Apparatus and Procedure

Prior to the start of the experimental work solubility of the chosen THP, α-CYD and HP-β-CYD in triply distilled and degassed water (with a specific conductance of 1×10^{-6} S·cm^{-1}) have been precisely checked and it was observed that the selected drug freely soluble in all proportion α-CD and HP-β-CYD solution.

The surface tension experiments were done by platinum ring detachment method using a Tensiometer (K9, KRŰSS; Germany) at the studied temperature. The precision of the measurement was within ±0.1 mN·m^{-1}. Temperature of the system has been maintained circulating auto-thermo stated water through a double-wall glass vessel containing the solution.

The conductance measurements were carried out in a Systronics-308 conductivity meter (accuracy ±0.01%) using a dip-type immersion conductivity cell, CD-10, having a cell constant of approximately (0.1 ± 0.001) cm^{-1}. Measurements were completed in a water bath maintained within T = (298.15 ± 0.01) K.

UV-Visible spectra were obtained by a JASCO V-530 UV-VIS spectrophotometer, with an uncertainty of wavelength resolution of ±2 nm. The measuring temperature was held constant by a thermostat.

Infrared spectra were analyzed in 8300 FT-IR spectrometer (Shimadzu, Japan). The details of the instrument have formerly been described [12]. The FTIR measurements were performed in the scanning range of 4000−400 cm^{-1} having resolution of 4 cm^{-1} at room temperature.

NMR spectra were obtained in D_2O unless otherwise stated. ^{1}H NMR spectra were obtained at 300 MHz using a Bruker AVANCE and instrument at 298.15 K. Signals are stated as δ-values in ppm by using residual protonated solvent (HDO) signals as internal standard (D_2O: δ-4.79 ppm). Data are reported as chemical shifts.

2-2-1- Preparation of solid inclusion complex of THP with α-CYD & HP-β-CYD

The solid inclusion complexes of (THP+α-CYD and THP+HP-β-CYD) have been prepared by taking 1:1 molar ratio of both components. Both components are dissolved in triply distilled and degassed water separately and stirred over magnetic stirrer until it makes a clear solution. After that the drug solution i.e., THP is added into α-CYD and HP-β-CYD solution respectively and stirred for 48 h at 60 °C without a break. A precipitation is appeared after cooling. The precipitate is filtered and washed for several times with triply distilled water. Finally, we have got a dry white powder after drying the washed precipitate in oven at 40 °C for 24 h. These solids were further analyzed and characterized by means of FTIR, NMR spectroscopic methods.

3- Results and Discussions

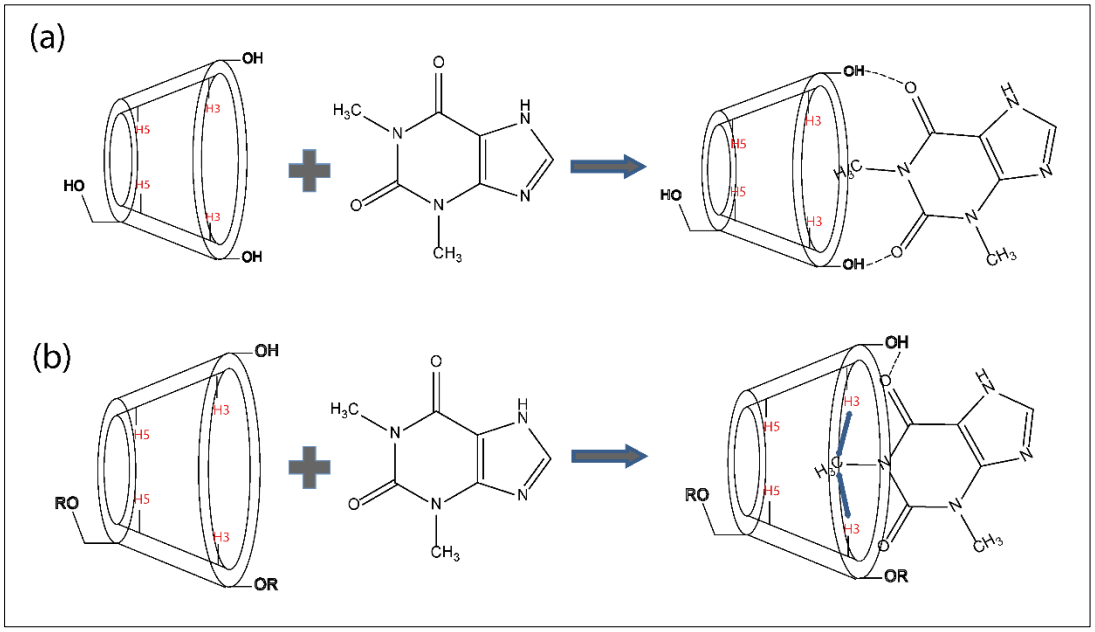
3-1- Surface Tension

Surface tension (γ) measurements clears the fact whether inclusion can occur or not but also to deduce the stoichiometry of inclusion complexes [16, 17]. It was proved that no notable alteration occurs for the surface tension of pure water while α-CYD and HP-β-CYD are added in water, demonstrating that α- and HP-β-CYD are approximately surface inactive compounds in pure water mixtures [18]. γ value raise with accumulation of CYDs are owing to the fact that surface activity decreases with rising number of CYD molecules into the THP (Schemes 2) solution. Each curve, (Figure. 1a and b), evidently exhibits a single cut-off point in surface tension at a certain concentration, i.e., the γ value enhance with the increase in concentration, achieve a sure point (cut-off point), and then become almost steady, which observably indicates the construction of selective 1:1 inclusion complex. By probing the facts of γ-values (Table 2) it is understood that HP- β-CYD is more proficient for the creation of inclusion complexes than that of α-CYD. This is markedly due to the fact that HP-β-CYD furnishes further practical trait (Scheme 1) for the construction of possible inclusion complexes than α-CYD. Also, we predict the non-polar methyl groups of the THP to be inserted via the wider rim through hydrophobic and hydrophilic interaction, so as to make highest contact with the CYD cavity, while the charged polar head side remains either in the wider rim of CYD or in the bulk solution through H-bonding or other non-covalent interactions.

Table 2. Values of Surface tension and at the break point with corresponding concentration of α-CYD and HP-β-CYD for THP at 298.15 K.

	THP & α-CYD	THP & HP-β-CYD
Conc. Of CYD/ mM	5.07	5.00
Surface tension (γ/mNm^{-1})	70.02	70.5

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



Scheme 2. The proposed inclusion mode of (a) THP+α-CYD (b) THP+HP-β-CYD and their significant ROESY correlations.

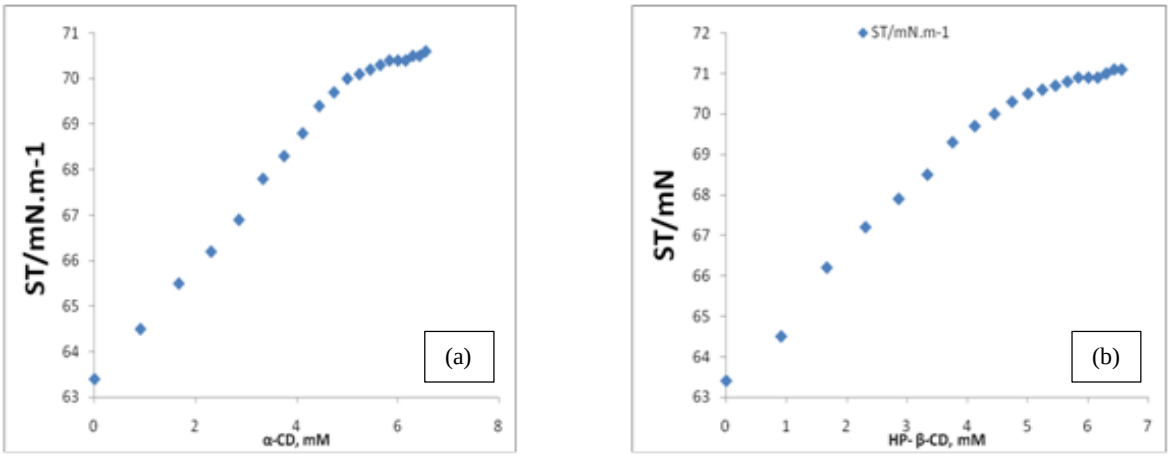


Figure 1. (a) Surface tension of THP with α-CYD and (b) Surface tension of THP with HP-β-CYD at 298.15 K.

3-2- Conductivity Study

Conductivity study demonstrates inclusion technique and their stoichiometric ratio. The selected drug THP is liberally soluble in water. The solution conductivity of THP is noticeably changed by the addition of ∝-CYD & HP-β-CYD (CYDs). Conductivity (κ) measurement is an important contrivance to illuminate the inclusion incident in solution phase [19-21].

It indicates the construction as well as the stoichiometry of the IC produced [22]. CYD concentrations at 298.15 K are depicted in Figures 2(a) and 2(b). Through this method the stoichiometry of the inclusion complexes can be deduced from the breaks (Table 3) in the conductivity curves [23, 24]. The amazingly falling specific conductivity with increasing CYDs concentrations indicates the inclusion complex formation between CYDs and the THP individually and hence movement of the THP is controlled and the free ions per unit volume is decreased; as a result the conductivity decreases. At a certain concentration of CYDs, this linear decrease of specific conductance with THP concentration halted rather rapidly to show no or little further reduce with further CYDs additions and which represents the saturation point of inclusion. A distinctive break in the conductivity curve occurred at a concentration of about 5.0 mmolL^{-1} for CYDs,

suggesting that the stoichiometry of the inclusion complex is equimolar [25-29]. This indicates that the principal inclusion complexes of CYDs with THP in this range are of 1:1 ratio which indicates that the THP are almost wholly in complexed form. This certainly illustrates that both the CYDs have the favourable structures for the formation of selective inclusion complexes with the investigated THP. This is also supported by the above mentioned surface tension experiment.

Table 3. Values of Conductivity and at the break point with corresponding concentration of α-CYD and HP- β-CYD for THP at 298.15 K.

	THP & α-CYD	THP & HP-β-CYD
Conc. Of CYD/ mM	5.23	5.17
Conductivity (k/mSm^{-1})	76.70	82.50

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

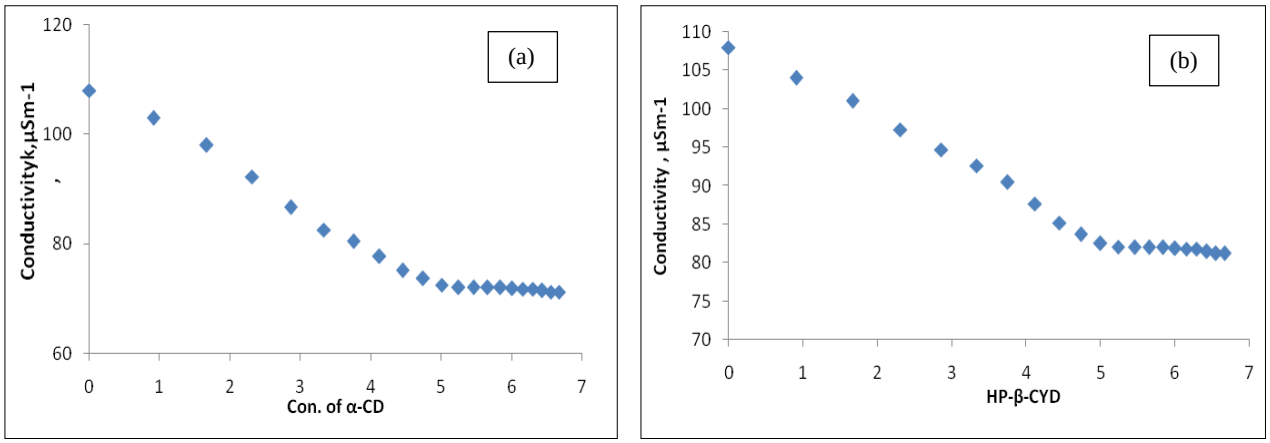


Figure 2. Variation of conductivity of aqueous THP with (a) α-CYD solution and (b) HP-β-CYD solution respectively with increasing concentration of α-CYD & HP- *β*-CYD at 298.15 K.

3-3- Job's Plot

3-3-1- Job's Plot Reveals the Stoichiometry of the Host-guest Inclusion Complex

One of the best method used to identify the stoichiometry of the host-guest inclusion complexes is the Job's method, well-known as the continuous variation method, which has been applied here by using UV-visible spectroscopy [30]. A set of solutions for THP & α-CYD as well as THP & HP-β -CYD was prepared varying the mole fraction of the guest in the range 0–1. Job's plots were generated by plotting Δ A × R against R, where Δ A is the difference in absorbance of the THP without and with α-CYD & HP-*β*-CYD where R = [THP]/([THP]+[CYD]) .Absorbance values were measured at respective λ_{max} for each solution at 298.15 K. The value of R at the maximum peak gives the stoichiometry of the inclusion complex (IC), *i.e.*, ratio between guest and host is 1:2 if R = 0.33; 1:1 for R = 0.5; 2:1 for R = 0.66 etc. In the present work maxima for each plot was found at R = 0.5, which suggest 1:1 stoichiometry of the host-guest inclusion complexes (Figures 3a and 3b).

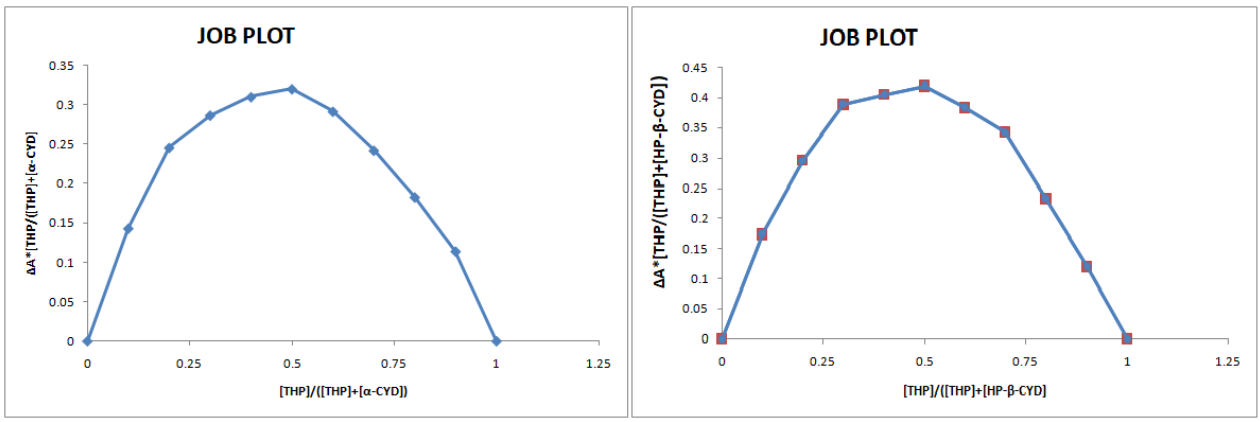


Figure 3. (a) Job plot of THP+α-CYD and (b) THP+HP-β-CYD.

3-4- Ultraviolet Spectroscopy: Association Constants and Thermodynamic Parameters

The association constants K_a for THP-Cyclodextrin systems have been evaluated by spectroscopic methods on the basis of changes of molar absorptivity of the THP when complexed with the cyclodextrin molecules.

This is most probably caused by the insertion of guest molecule inside into the apolar cavity of cyclodextrin from the aqueous environment [31, 32]. Changes in absorption intensity was studied as a function of concentration of cyclodextrin to establish the value of K_a **(**Tables 4**)**, (Tables S1 and S2). On the basis of the consistent Benesi–Hildebrand method for a 1:1 host–guest complex, the double reciprocal plots have been drawn using the Equation 1 as follows (Figures S1 to S6): **[**33, 34].

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon[guest]Ka}.\frac{1}{[Host]} + \frac{1}{\Delta\varepsilon[guest]} \quad (1)$$

Table 4. Association constant (K_a) and thermodynamic parameters ΔH^o, ΔS^o and ΔG^o of THP-CYDs inclusion complexes.

IC	Temp/K[a]	K_a/M^{-1}	ΔH^o/kJ mol^{-1}	ΔS^o/J mol^{-1} K^{-1}	ΔG^o/kJ mol^{-1}
THP+α-CYD	293.15	10.4569	-27.86	-75.37	-5.766
	298.15	9.1948			-5.390
	303.15	7.1664			-5.013
THP+HP-β-CYD	293.15	11866	-180.15	-534.96	-23.41
	298.15	6779			-20.74
	303.15	1027			-18.07

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

[b] Mean errors in $K_a = \mp$ 0.02 M^{-1}; ΔH^o= ±0.01 kJ mol^{-1}; ΔS^o= ±0.01 J mol^{-1} K^{-1}; ΔG^o = ±0.01 kJ mol^{-1}.

The values of the association constants for the systems were evaluated by dividing the intercept by the slope of the straight line of the double reciprocal plot [35]. In case of THP/HP-β-CYD system, the association constant value was found to be 11865 M^{-1}, 6779 M^{-1}, 1027M^{-1} at 293.15K, 298.15K and 303.15K respectively. However, in case of THP/α-CYD, Ka was 10M^{-1}, 9M^{-1}, 7M^{-1} at 293.15K, 298.15K and 303.15K respectively. So, in all the three different temperature, association constant value was found to be higher for THP/HP-β-CYD system than that of THP/α-CYD. It may be due to the bigger cavity size of HP-β-CYD than that of α-CYD and due to the presence of hydroxypropyl group at the wider rim, binding between guest and host becomes prominent.

Thermodynamic parameters can easily be derived from the association constants found by the above mentioned technique with the help of the Van't Hoff equation {Equation 2} as follows:

$$\ln K_a = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (1)$$

There is a linear relationship between $\ln K_a$ and 1/T in the above mentioned equation {Equation 2} (Figures S7 to S8). Based on Equation 2, the thermodynamic parameters ΔH^o, ΔS^o and ΔG^o for the formation of the inclusion complex can be obtained (Table 4). The value of ΔG^o was established to be negative, which suggests that the inclusion method proceeds impulsively. ΔH^o and ΔS^o were also set up to be negative, signifying that the inclusion process is exothermic and entropy controlled, not entropy determined. This is estimated, as while the inclusion complex is produced between cyclodextrin and any guest molecule a molecular association occurs, resulting in a fall of entropy, which is adverse for the spontaneity of the inclusion complex creation. Conversely, this effect is occupied by the higher negative value of ΔH^o, making the overall inclusion process thermodynamically favourable.

3-5- FTIR Spectroscopic Study

FT-IR study of the solid inclusion complexes produced was performed for the investigation of the creation of the solid ICs. There are changes in frequencies of bands of the inserted guest molecules over and above some bands are not present in the spectra of complex. This may be owing to the construction of the ICs [35]. Data for pure compounds and inclusion complexes are recorded in Tables 5(a) to 5(e) and spectroscopic changes in wave number before and after inclusion are shown in Figure 4. Due to non-covalent interactions, the shifting of bands is observed. In the spectra of α-CYD and HP-β-CYD the broad band's obtained at 3415.85 cm^{-1} and 3415.82 cm^{-1} are due to the valence vibrations of -OH groups linked by H-bond. The O-H stretching for α-CYD and HP-β-CYD obtained at 3415.85 cm^{-1} and 3415.82 cm^{-1} were obtained in the complexes 3412.17 cm^{-1} and 3412.04 cm^{-1} respectively, may be due to the interaction of the oxygen atom of the carbonyl -C=O group of the six membered ring from THP and the oxygen atom of -OH group of α-CYD and HP-β-CYD respectively.

The -C-H stretching and bending are obtained at 2915.12, 2907.74 cm^{-1} and 1376.95 cm^{-1} for pure ∝-CYD and HP-β-CYD, but only stretching of CYDs are shifted in the both ICs to 2915.12 cm^{-1} and C-H bending are absent. The –N-H, -C-H, carbonyl -C=O, -C-N of imidazole ring(strong peak), C-N(medium peak) stretching bands for pure THP are observed at 3436.38 cm^{-1},2952.02 cm^{-1},1639.83 cm^{-1},1295.85 cm^{-1} and 1030-1236.86 cm^{-1}. Stretching band due to N-H of imidazole ring from THP is absent or shifted at 2915.12cm^{-1} in both the ICs [36]. The C-H stretching due to methyl group are shifted to 2915.12cm^{-1} in both ICs and carbonyl C=O stretching shifted to 1620 cm^{-1} in α-CYD+THP & 1614.19cm^{-1} in HP-β-CYD+THP due to the interaction within the hollow space of cyclodextrin. In the ICs no additional significant signal was obtained which denies the chance of chemical reaction. Thus, the study provides significant proof in favor of the development of the ICs in the solid state. The important intensities changes and the shifting in distinguishing bands of the two binding partners in each case certainly confirm the interaction of the THP in α-CYD and insertion THP in HP-β-CYD in the resultant complex (Figure 4). The non-covalent interactions like hydrogen bond (H-bond), hydrophobic interaction and Vander Waals interaction that appear in complex are held responsible for the changes.

Table 5. (a) Theophylline (THP); (b) α-CYD; (c) HP-β-CYD; (d) α-CYD+THP; (e) HP-β-CYD+THP

(a)

wave number (cm^{-1})	Group
3436.38	Stretching for -N-H of THP
2952.02	Symmetrical Stretching vibration of –C-H of -CH_3
1639.83	Stretching of -C=O from THP
1236.86	C-N stretching of imidazole ring strong peak
1174.19 – 1030.41	C-N stretching medium peak

(b)

Wave number (cm^{-1})	Group
3415.33	stretching of -O-H
2915.12	stretching of –C-H from –CH_2
1376.95	bending of –C-H from –CH_2 and bending of O-H
1148.38	bending of -C-O-C
1028.35	stretching of -C-C-O
949.30	skeletal vibration involving α-1,4 linkage

(c)

Wave number (cm^{-1})	Group
3415.82	stretch of O−H
2907.74	stretch of −C−H from − CH2
1623.96	bend of − C−H from − CH2 and bending of O−H
1376.95	bend of –C- H of -CH_3
1152.07	bend of C−O−C
1023.04	stretch of C−C−O
938.64	skeletal vibration involving α-1,4 linkage

(d)

Wave number (cm^{-1})	Group
3412.17	Stretching of –O-H of α-CYD & stretching of –N-H of THP
2915.12	Symmetrical stretching of –C-H from –CH_3 of THP
1620.00	-C=O stretching from THP
1029.92	Bending of -C-C-O Of α-CYD
984.46	stretching of C-C-O of α-CYD

(e)

Wave number (cm^{-1})	Group
3412.04	Stretching of –O-H of HP- β-CYD & stretching of –N-H of THP
2915.12	Symmetrical stretching of –C-H from –CH_3 of THP
1614.19	-C=O stretching of THP
1030.41	stretch of C−C−O of HP-β-CYD

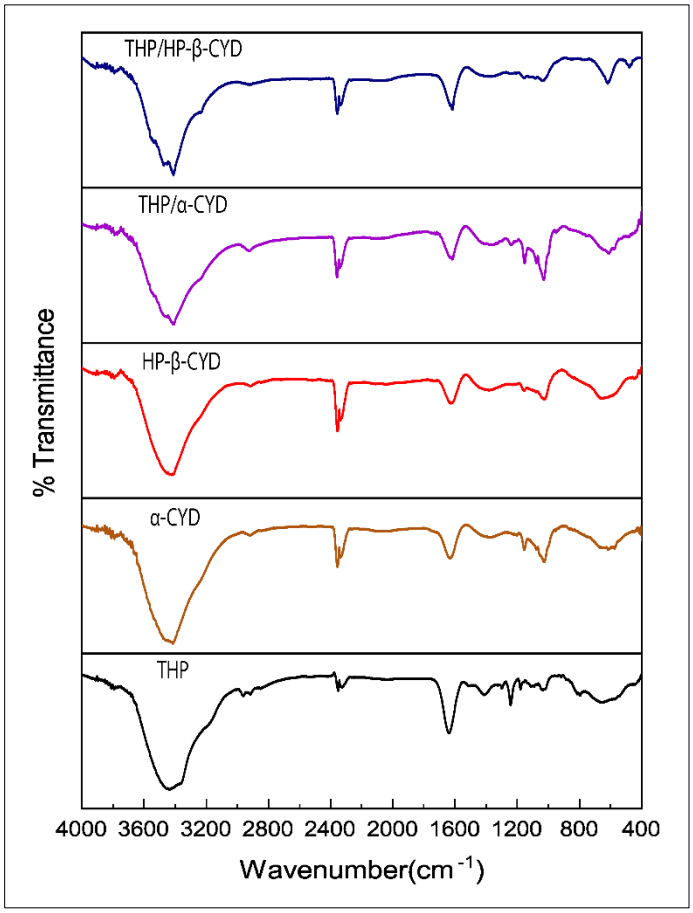


Figure 4. FTIR spectroscopy of THP with respect to HP-β-CD and α-CD and its inclusion complexes.

3-6- NMR Spectroscopic Study

^{1}H NMR analysis is one of the most satisfactory methods for the study of inclusion complex [37, 38]. ^{1}H NMR spectra of the 1:1 mixture of solid inclusion complex have been recorded in D_2O at 298.15 K (Figure 5) and the chemical shift (Δδ) for protons of both α-CYD, HP-β-CYD and THP are studied. Since, under this condition, only shift changes of the signals occur, it follows that the inclusion phenomenon is a dynamic process in which a fast exchange exists between the free and the bound states. The upfield shift of α-CYD, HP-β-CYD protons and downfield shift in guest protons made known the presence of THP molecules into α-CYD and HP-β-CYD cavity. Incorporation of the THP guest molecule into the cyclodextrin ring through the wider rim side rather than the narrower dimension can be envisaged from the chemical shift displacements (Δδ) of the H3 proton. Probably the guest molecule doesn't fit in the cavity firmly for HP-β-CYD. Therefore our work confirms the inclusion complexation has taken in α-CYD more appropriately as depicted in the mentioned NMR {Figure 5, Schemes 2(a) and 2(b)} [39]. Insertion of a guest molecule inside the cavity of a cyclodextrin results in the modification of the NMR frequencies of the signals of the guest as well as of the host. FT-NMR (^{1}H) spectra are used to verify the host-guest interaction of ICs in the CYD systems [40].

In the CYD the H3 and H5 protons are situated inside the conical cavity, mainly, the H3 is oriented towards the wider rim while H5 is placed near the narrower rim, the others are positioned at the outside of the CYD molecule [41].

As most of the guest molecules are inserted through the wider rim, the H3 proton is more shifted to the upfield region compared to H5. In the present study the molecular interactions of THP with α and HP-β-Cyclodextrin have been studied using the ^{1}H NMR spectra by taking a 1:1 molar ratio of the THP and α or HP-β-CYD in D_2O at 298.15 K. Here the alkaloid's hydrophobic part was inserted into the both α & HP-β-CYD's cavity. Hence, chemical shift value of the CYD protons and protons of the alkaloid are support the formation of ICs. One of the interesting observation here is that the non-aromatic protons of the alkaloid undergone down field shift probably its proton is relatively more shielded than inside of the CYD cavity [42-44].

In the ^{1}H NMR experiment of the Inclusion Complexes, it can be observed that the signals of the interior H3 and H5 atoms of the CYDs show upfield shift and that of the approaching non-aromatic protons of THP showed downfield shifts, confirming the formation of ICs. The characteristic non-aromatic peaks THP after inclusion showing downfield shift of N-C**H**$_3$ proton proves the inclusion of -CON(C**H**$_3$)CO-N-C**H**$_3$ (far away from aromatic ring inside the CYD rim). Thus, NMR study is in tune with the results of the previous investigations.

^{1}H NMR (300 MHz, Solvated in D_2O, Theophylline, δ/ppm): 7.155-7.127 (Unsymmetrical doublet, NCHN**H**, *J*=8.49Hz), 6.890-6.862(Unsymmetrical doublet, NC**H**NH, *J*= 8.61 Hz), 4.69-4.61 (Residual solvent peak, HOD), 3.995-3.844(M, *J*=7.8 Hz), 2.783-2.570(N-CH_3 near to aromatic ring) & 2.59-2.57[-CON(C**H**$_3$)CO-N-CH_3 far away from aromatic ring].

^{1}H NMR (300 MHz, Solvated in D_2O, HP-β-CYD, δ/ppm): 5.144-4.969 (Unsymmetrical doublet, H1), 3.908 (H3), 3.734 (H5, H6), 3.504 (H2), 3.394 (H4), 1.042-1.022 (CH_3).

^{1}H NMR (300 MHz, Solvated in D_2O, Theophylline+HP-β-CYD, δ/ppm): 7.131-7160(Unsymmetrical Doublet, NCHNH), slight deshielding 6.860-6.888 (d), slight shielding H1 (5.129-4.959), H3 (3.908) slight deshielding, H5 (3.734), H6 (3.753), H2 (3.504) H4 (3.436), CH_3 (1.017-1.037).

^{1}H NMR (400 MHz, Solvated in D_2O, α-CYD, δ/ppm): 3.42-3.46 (H4, 6H, t, *J*= 9.2 Hz), 3.49-3.50 (H2, 6H, dd, J_1=10 Hz, J_2=3.2 Hz), 3.68-3.82 (H6, H5, 18H, m), 3.84-3.83 (H3, 6H, dd , J_1 = 9.6 Hz, J_2=8.8 Hz), 4.909-4.91 (H1, 6H, d, *J* = 3.6 Hz).

^{1}H NMR (300 MHz, D_2O, Theophylline+α-CYD): 7.176-7.147(Unsymmetrical doublet, NCHN**H**, *J*=8.7 Hz) deshielding, 6.917-6.889(Unsymmetrical doublet, NC**H**NH, *J*= 8.61 Hz) deshielding, 4.941-4.930 (H1, 6H, d, *J* = 3.6 Hz) strong deshielding, 4.019-3.989(H3), 3.927-3.827(H5), 3.770-3.716 (H5, H6), 3.535-3.492(H2), 3.467-3.438(H4), 2.865-2.706, deshielding, 1.013-0.994 (Unsymmetrical doublet).

Upon inclusion the upfield chemical shift values (Δδ) of the H3 and H5 protons of **α** and HP-β-Cyclodextrins have been listed in Tables 6a to 6d, which confirm that the interaction of the guest THP with H3 is greater than that with H5, signifying that the inclusion has taken place through the wider rim of the α and HP-β-Cyclodextrins.

It also may be mentioned that upon inclusion some non-aromatic peak of the THP was completely disappeared in the proton NMR spectra of the THP, leave strong evidence of inclusion complex formation.

Table 6. Chemical shift values (Δδ) of the H3 and H5 protons of α and HP-β-Cyclodextrins

(a)

Type of proton	Spin multiplicity	δ (ppm)		Shift (Δδ)
		HP-β-CYD	THP/ HP-β-CYD complex	
H-1	d	5.144-4.969	5.129-4.959	-0.015
H-2	dd	3.504	3.504	-0.001
H-3	dd	3.906	3.9085	**0.0025**
H-4	dd	3.394	3.436	0.0420
H-5	m	3.754-3.603	3.734	**0.2220**
H-6	dd	3.754-3.603	3.753	0.1122
-CH_3	-	1.042-1.022	1.017-1.037	-

(b)

Type of proton	Spin multiplicity	δ (ppm)		Shift (Δδ)
		α-CYD	THP/ α-CYD complex	
H-1	d	4.91-4.90	4.941-4.930	-
H-2	dd	3.49-3.50	3.535-3.492	-
H-3	dd	3.84-3.83	4.019-3.989	**0.186**
H-4	dd	3.42-3.46	3.467-3.438	-
H-5	m	3.68-3.82	3.770-3.716	**-0.14**
H-6	-	-	-	-

(c)

Type of proton	Spin multiplicity	δ (ppm)		Shift (Δδ)
		HP-β-CYD	THP/ HP-β-CYD complex	
NCHN**H**	d	7.155-7.127 (Unsymmetrical doublet, *J*=8.49Hz)	7.131-7160	**0.021**
NC**H**NH	dd	6.890-6.862 (Unsymmetrical doublet, *J*= 8.61 Hz)	6.860-6.888	**0.027**
(-N-CH_3 near to aromatic ring)	dd	2.783-2.570	Peak disappeared	-
[-CON(C**H**$_3$)CO-N-CH_3]	dd	2.59-2.57	Peak disappeared	-

(d)

Type of proton	Spin multiplicity	δ (ppm)		Shift (Δδ)
		α-CYD	THP/ α-CYD complex	
NCHN**H**	d	7.155-7.127 (Unsymmetrical doublet, *J*=8.49Hz)	7.176-7.147	**0.0065**
NC**H**NH	dd	6.890-6.862 (Unsymmetrical doublet, *J*= 8.61 Hz)	6.917-6.889	**0.027**
(-N-CH_3 near to aromatic ring)	dd	2.783-2.570	2.865-2.706	**0.082**
[-CON(C**H**$_3$)CO-N-CH_3]	dd	2.59-2.57	Peak disappeared	-

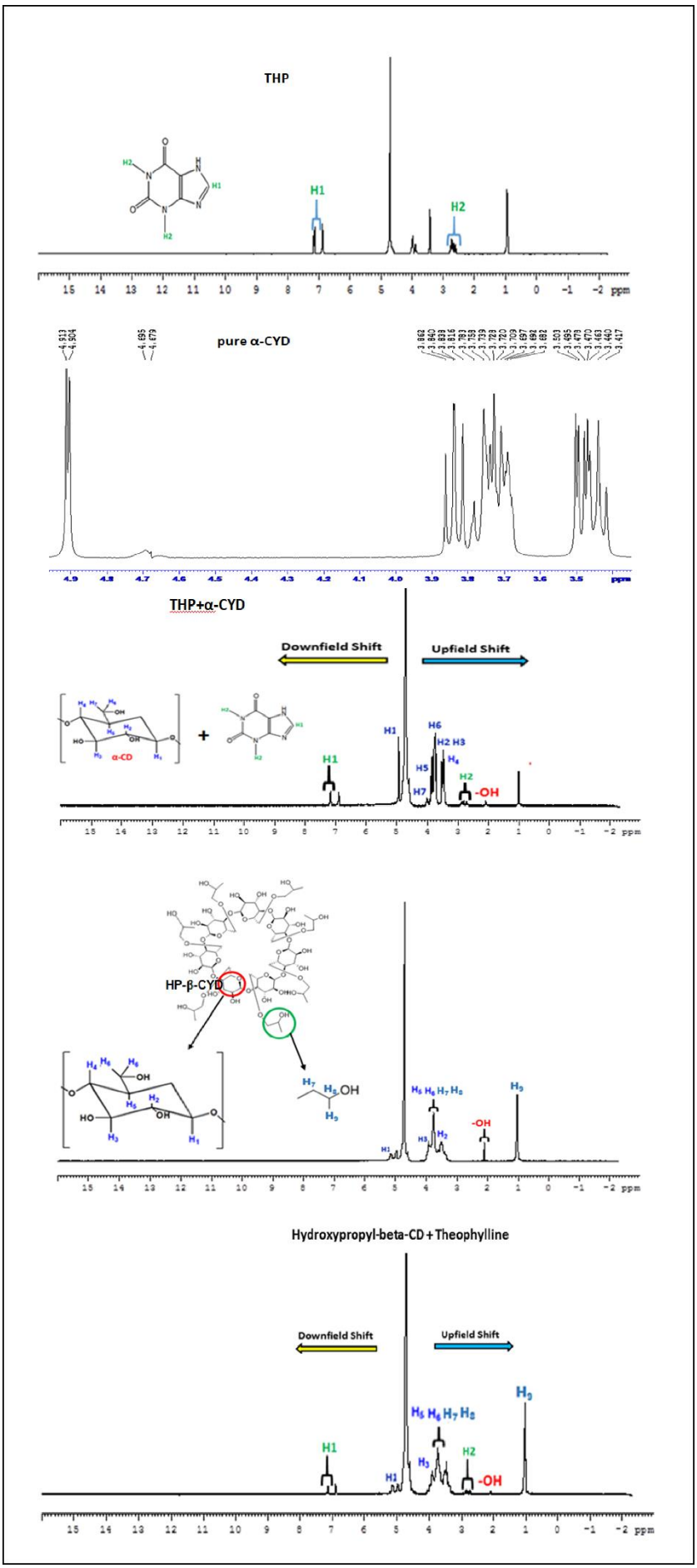


Figure 5. NMR spectra of the pure compounds (THP), α-CYD, HP-β-CYD and their inclusion complexes.

3-7- 2D-NMR Study

2D-NMR spectroscopy is a very important tool to identify whether inclusion complex have been formed or not. According to the literature, if the guest molecules have included inside the cavity of the cyclodextrin molecules and the proton of the guest and H-3, H-5 proton of the cyclodextrin molecules come closer than 4Å, then there will be a cross peak present in the 2D-NMR spectra [45]. In our case, cross peak have been found in case of HP-β-CYD, where H-3 proton of cyclodextrin molecules have interacted with N-CH_3 moiety of the guest molecules but no cross peak has been observed in case of α-CYD. These observations conclude that in case of THP/HP-β-CYD inclusion complex, it forms a stable complex both in solid and as well as solution state. However, in case of THP/α-CYD, may be in solution state, inclusion phenomena occur in such a dynamic way that interaction between guest and host is not that prominent and consequently, we did not observed any cross peak in 2D-NMR. The observation is well matched with the low association constant data obtained from UV-vis spectroscopic study (Figures 6a and 6b).

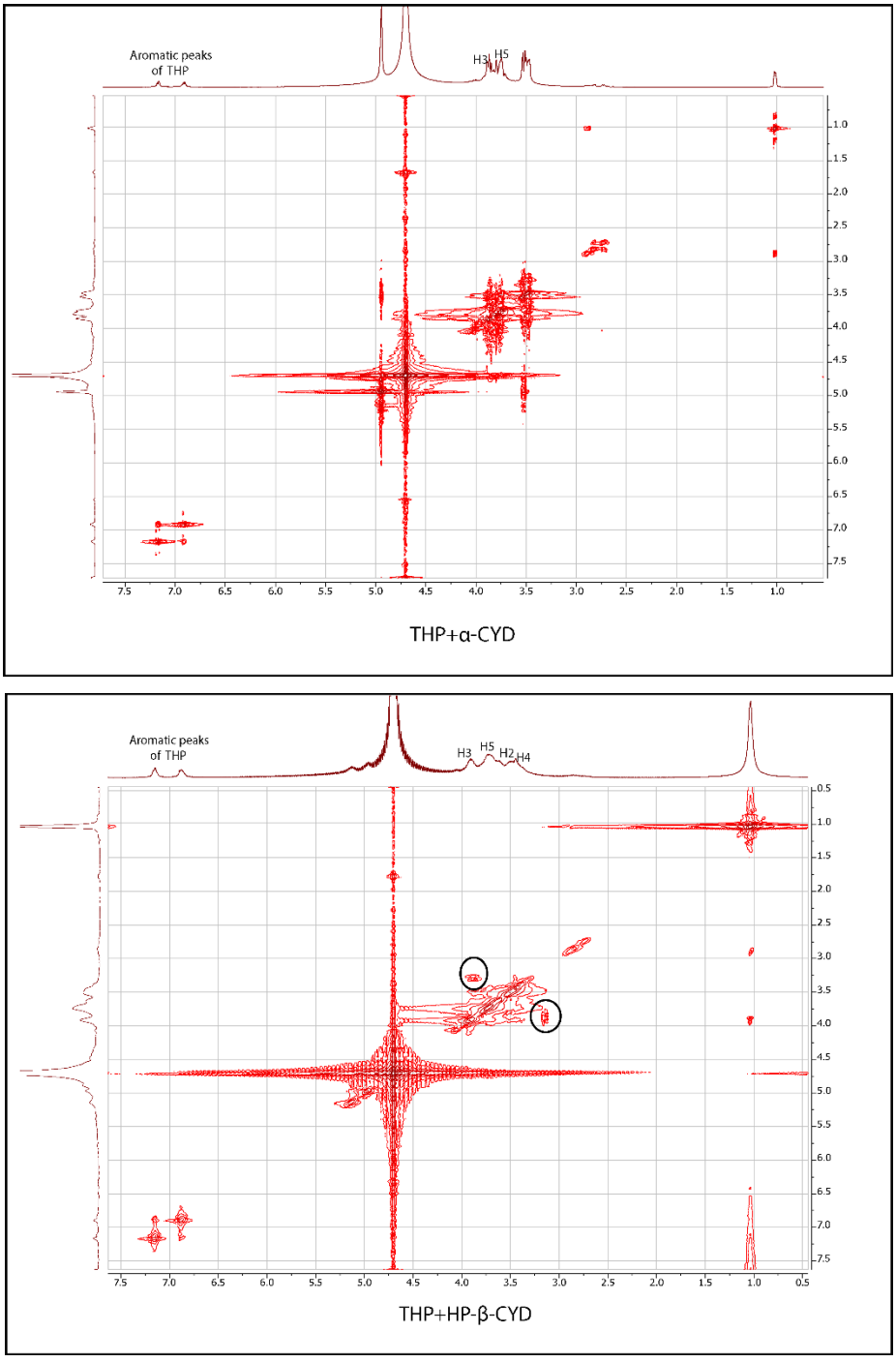


Figure 6. (a) 2D-NMR spectra of inclusion complexes of THP+α-CYD; (b) 2D-NMR spectra of inclusion complexes of THP+HP-β-CYD.

4- Conclusion

The present study reveals an exclusive behavior of the aqueous cyclodextrin-theophylline system. It establishes the possibility of formation of host–guest inclusion complexes between cyclodextrins and THP by physicochemical as well as spectroscopic methods. Surface tension and Conductivity measurement support that α-cyclodextrin and HP-β-cyclodextrin form inclusion complex with THP. In addition to that the ratio of host: guest was found to be 1:1 by Job's method. ^{1}H-NMR data as well as FTIR also confirms the inclusion phenomenon. The determination of association constants and various thermodynamic parameters quantitatively clarify that interaction between HP-β-CYD with THP was quite higher than that with α-CYD and. 2D-NMR data confirms that when HP-β-CYD is taking as host molecules, some sort of interaction is occurring with the guest THP molecules which is not possible if α-CYD is taken as host. Another observation is that HP-β-CYD is taking as preferable host; solubility of the THP+HP-β-CYD system is much more than that of THP+α-CYD. Theophylline is a drug which involves various applications in muscular relaxation, increment of heart rate, anti-inflammations, and neurotransmitter. Due to these potential applications of the drug it could be used in the host guest complexation with Cyclodextrins. As cyclodextrin act as a drug delivery vehicle thereby showing the controlled release of the drug in the target zone, moreover it manifolds the solubility of the drug and its bioavailability. Consequently, this limited study has diversified applications in the broad field of biology and Chemistry i.e. in Biochemistry.

5- Abbreviations

Cyclodextrins = CYDs

Alpha cyclodextrin = α-CYD

Hydroxypropyl beta Cyclodextrin = HP-β-CYD

Theophylline = THP

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7- Conflict of Interest

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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Appendix I

Table S1. Data for the Surface tension study of aqueous THP VS α-CYD (concentration of stock solution of THP = 10mM, concentration of stock solution of CYD = 10mM) at 298.15K[a].

Vol. of drug(THP)	Vol. of α-CYD	Total vol.	Conc. Of drug(THP)	Conc. of α-CYD	ST/mN.m-1
10	0	10	10	0	63.400
10	1	11	9.091	0.909	64.500
10	1	12	8.333	1.667	65.500
10	1	13	7.693	2.307	66.200
10	1	14	7.143	2.857	66.900
10	1	15	6.667	3.333	67.800
10	1	16	6.25	3.75	68.300
10	1	17	5.883	4.117	68.800
10	1	18	5.556	4.444	69.400
10	1	19	5.264	4.736	69.700
10	1	20	5	5	70
10	1	21	4.762	5.238	70.1
10	1	22	4.546	5.454	70.2
10	1	23	4.348	5.652	70.3
10	1	24	4.167	5.833	70.4
10	1	25	4	6	70.4
10	1	26	3.847	6.153	70.4
10	1	27	3.704	6.296	70.5
10	1	28	3.572	6.428	70.5
10	1	29	3.449	6.551	70.6

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

Table S2. Variation of Conductivity of THP VS α-CYD at 298.15K.

added α-CYD	Total volm	conc of THP	conc of α-CYD	conductance
mL	mL	mM	mM	μS m^{-1}
0	10	10.000	0	112
1	11	9.091	0.909090909	106
2	12	8.333	1.666666667	100.1
3	13	7.692	2.307692308	96.6
4	14	7.143	2.857142857	92.4
5	15	6.667	3.333333333	90.1
6	16	6.250	3.75	86.4
7	17	5.882	4.117647059	83.1
8	18	5.556	4.444444444	80.7
9	19	5.263	4.736842105	78.8
10	20	5.000	5	77
11	21	4.762	5.238095238	76.7
12	22	4.545	5.454545455	76.6
13	23	4.348	5.652173913	76.5
14	24	4.167	5.833333333	76.4
15	25	4.000	6	76.4
16	26	3.846	6.153846154	76.4
17	27	3.704	6.296296296	76.3
18	28	3.571	6.428571429	76.3
19	29	3.448	6.551724138	76.2
20	30	3.333	6.666666667	76.2

Table S3. Variation of Conductivity of THP VS HP- β-CYD at 298.15K.

added HP-β-CD (mL)	Total volm (mL)	conc of THP (mM)	conc of HP-β-CYD (mM)	conductance (μSm^{-1})
0	10	10.000	0	108
1	11	9.091	0.909090909	104
2	12	8.333	1.666666667	101
3	13	7.692	2.307692308	97.3
4	14	7.143	2.857142857	94.7
5	15	6.667	3.333333333	92.6
6	16	6.250	3.75	90.5
7	17	5.882	4.117647059	87.7
8	18	5.556	4.444444444	85.2
9	19	5.263	4.736842105	83.7
10	20	5.000	5	82.5
11	21	4.762	5.238095238	82.1
12	22	4.545	5.454545455	82
13	23	4.348	5.652173913	82
14	24	4.167	5.833333333	82
15	25	4.000	6	81.9
16	26	3.846	6.153846154	81.8
17	27	3.704	6.296296296	81.8
18	28	3.571	6.428571429	81.5
19	29	3.448	6.551724138	81.2
20	30	3.333	6.666666667	81.2

Table S4. Data for Job plot obtained from UV-vis spectroscopy for aqueous THP+ α-CYD system at 298.15K[a].

THP (m)	α-CYD (ml)	THP (μM)	α-CYD (μM)	[THP]/([THP]+[α-CYD])	Absorbance (A)	ΔA	ΔA*[THP/([THP]+[α-CYD])
4	0	100	0	1	2.963566303	0	0
3.6	0.4	90	10	0.9	2.83707037	0.126496	0.11384634
3.2	0.8	80	20	0.8	2.735185719	0.228381	0.182704468
2.8	1.2	70	30	0.7	2.61772213	0.345844	0.242090921
2.4	1.6	60	40	0.6	2.477341154	0.486225	0.291735089
2	2	50	50	0.5	2.323162695	0.640404	0.320201804
1.6	2.4	40	60	0.4	2.188122196	0.775444	0.310177643
1.2	2.8	30	70	0.3	2.007714748	0.955852	0.286755466
0.8	3.2	20	80	0.2	1.735303497	1.228263	0.245652561
0.4	3.6	10	90	0.1	1.532948971	1.430617	0.143061733
0	4	0	100	0	1.452276707	1.51129	0

[a] Standard uncertainties in temperature(T)=0.01K

Table S5. Data for Job plot obtained from UV-vis spectroscopy for THP+HP- β-CYD system at 298.15K[a].

THP (ml)	HP-β –CYD (ml)	THP (μM)	HP-β –CYD (μM)	[THP]/([THP]+[HP-β-CYD])	Absorbance (A)	ΔA	ΔA*[THP/([THP]+[HP-β-CYD])
4	0	100	0	1	2.043766022	0	0
3.6	0.4	90	10	0.9	1.911896706	0.131869316	0.118682384
3.2	0.8	80	20	0.8	1.753383312	0.29038271	0.232306168
2.8	1.2	70	30	0.7	1.554103374	0.489662647	0.342763853
2.4	1.6	60	40	0.6	1.405567169	0.638198853	0.382919312
2	2	50	50	0.5	1.206616879	0.837149143	0.418574572
1.6	2.4	40	60	0.4	1.031124115	1.012641907	0.405056763
1.2	2.8	30	70	0.3	0.749193668	1.294572353	0.388371706
0.8	3.2	20	80	0.2	0.563092709	1.480673313	0.296134663
0.4	3.6	10	90	0.1	0.313292027	1.730473995	0.1730474
0	4	0	100	0	0.093270779	1.950495243	0

[a] Standard uncertainties in temperature(T)=0.01K

Table S6. Establishment of the association constant for THP and HP-β-CYD at diverse temperatures

temp/k	[THP]/μM	[HP-β-CYD]/μM	$A_。$	A	ΔA	1/[HP-β-CYD]/M−1	1/ΔA	Intercept	Slope	Ka/M^{-1}
	50	30	0.3854122	0.395494	0.010082	33333.33333	99.19	53.764364	0.004531	11865.893622
	50	40	0.3854122	0.368151	-0.01726	25000	57.93236			
293	50	50	0.3854122	0.357162	-0.02825	20000	35.39814			
	50	60	0.3854122	0.327236	-0.05818	16666.66667	17.18905			
	50	70	0.3854122	0.325506	-0.05991	14285.71429	16.69262			
	50	30	0.3108215	0.394655	-0.08383	33333.33333	39.9284	2.764624	0.00112	
	50	40	0.3108215	0.353084	-0.04226	25000	30.6619			6779.414200
298	50	50	0.3108215	0.333861	-0.02304	20000	25.5774			
	50	60	0.3108215	0.356011	-0.04519	16666.66667	22.1289			
	50	70	0.3108215	0.366705	-0.05588	14285.71429	17.8944			
	50	30	0.2880063	0.350712	-0.06271	33333.33333	19.9476	0.000575	0.591957	
	50	40	0.2880063	0.351214	-0.06321	25000	14.8208		0.000576	1027.345
303	50	50	0.2880063	0.352295	-0.06429	20000	11.5549		0.000576	
	50	60	0.2880063	0.356871	-0.06886	16666.66667	10.5213		0.000576	
	50	70	0.2880063	0.399956	-0.11195	14285.71429	8.9326		0.000576	

Table S7. Establishment of the association constant for THP and α-CYD at diverse temperatures

temp/k	[THP]/μM	[α-CYD]/μM	$A_。$	A	ΔA	1/[α-CYD]/M^{-1}	1/ΔA	Intercept	Slope	Ka/M^{-1}
	50	30	0.304157	0.31018	-0.00602	33333.33333	166.018999	0.007	74.02	10.4569
	50	40	0.304157	0.311946	-0.00779	25000	118.376102			
293	50	50	0.304157	0.320101	-0.01594	20000	70			
	50	60	0.304157	0.324763	-0.02061	16666.66667	48.5294581			
	50	70	0.304157	0.339285	-0.03513	14285.71429	28.4611345			
	50	30	0.310148	0.312788	-0.00264	33333.33333	378.766863	0.001	10.88	9.19118
	50	40	0.310148	0.325216	-0.01507	25000	212.756368			
298	50	50	0.310148	0.353561	-0.04341	20000	123.722267			
	50	60	0.310148	0.367096	-0.05695	16666.66667	47.56			
	50	70	0.310148	0.372068	-0.06192	14285.71429	16.1499			
	50	30	0.338962	0.443143	-0.10418	33333.33333	29.5986086	0.019	265.2	7.1644
	50	40	0.338962	0.407982	-0.06902	25000	19.488397			
303	50	50	0.338962	0.39111	-0.05215	20000	14.176065			
	50	60	0.338962	0.345335	-0.00637	16666.66667	8.8902521			
	50	70	0.338962	0.374052	-0.03509	14285.71429	6.49778503			

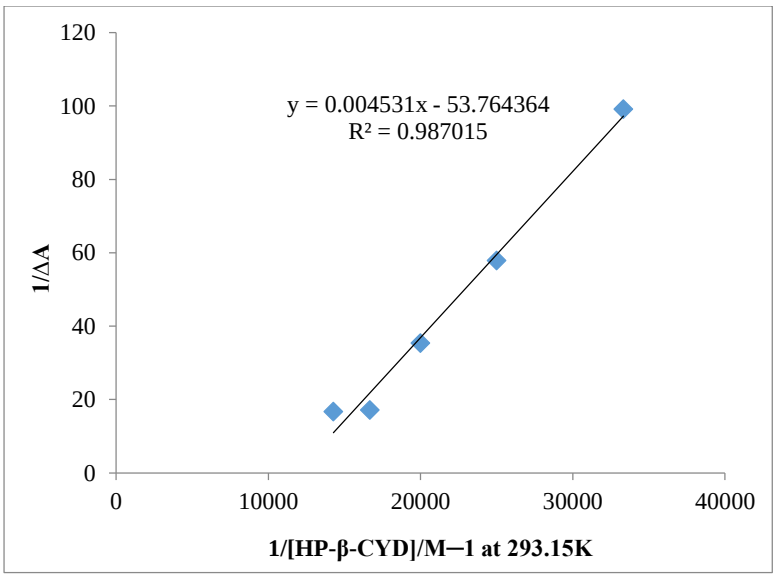

Figure S1. Benesi-Hildebrand double reciprocal plots for the effect of HP- β-CYD on the absorbance of THP at 293.15K.

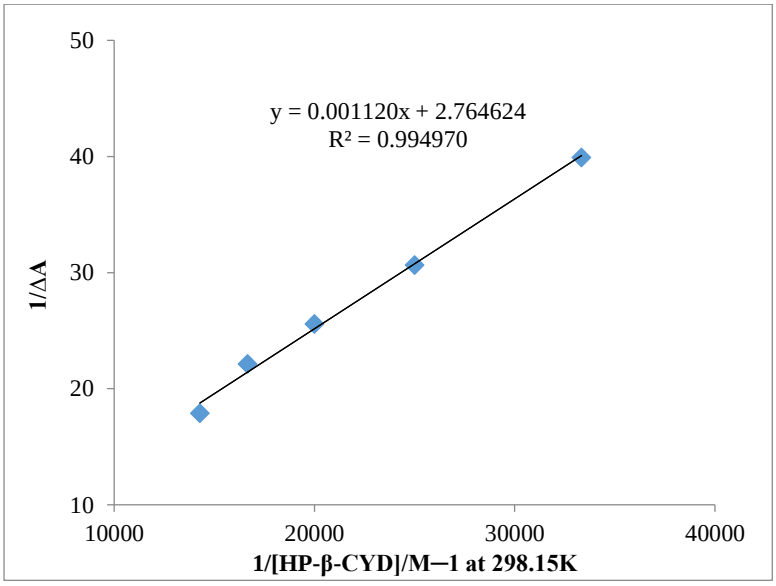

Figure S2. Benesi-Hildebrand double reciprocal plots for the effect of HP-β-CYD on the absorbance of THP] at 298.15K.

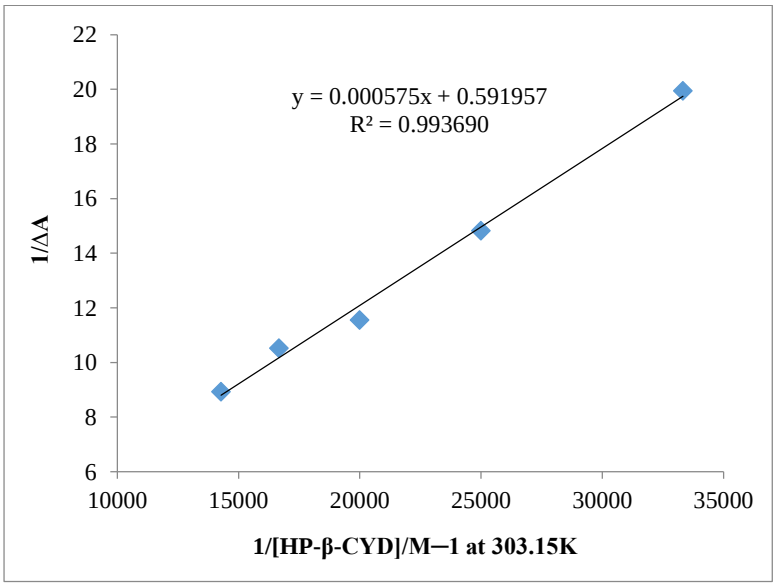

Figure S3. Benesi-Hildebrand double reciprocal plots for the effect of HP-β-CYD on the absorbance of THP at 303.15K.

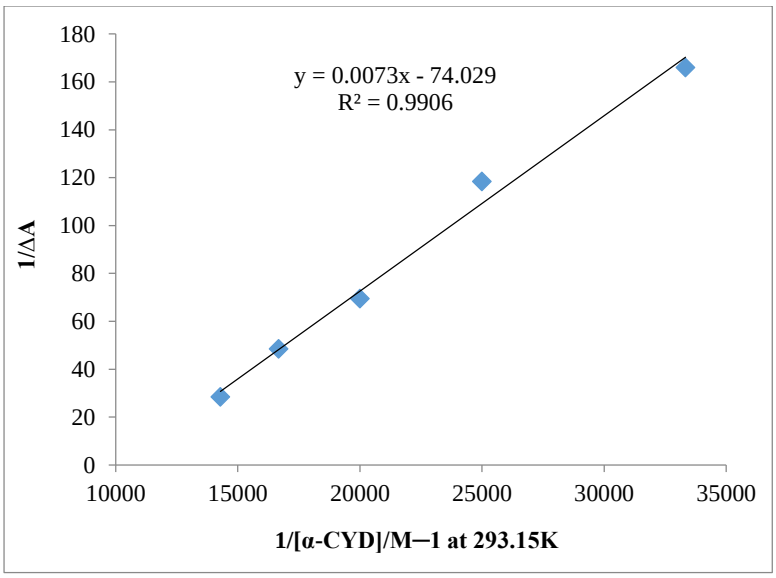

Figure S4. **Benesi-Hildebrand double reciprocal plots for the effect of α-CYD on the absorbance of THP at 293.15K.**

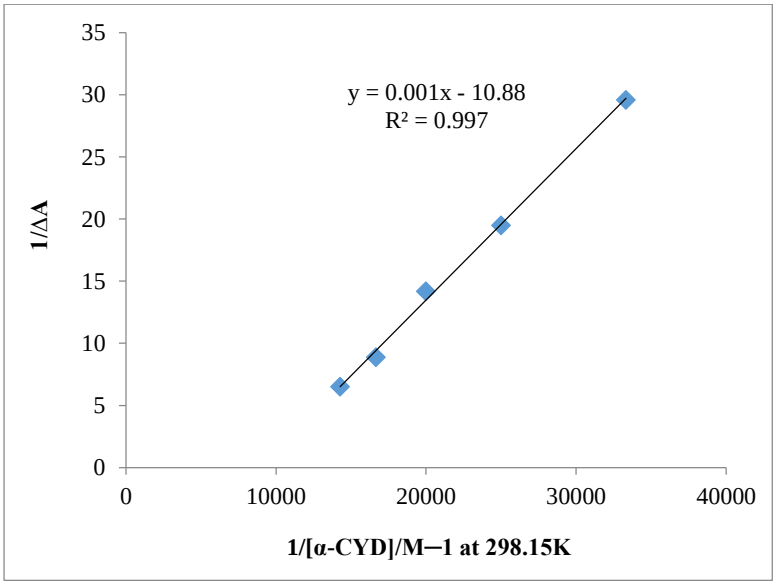

Figure S5. **Benesi-Hildebrand double reciprocal plots for the effect of α-CYD on the absorbance of THP at 298.15K.**

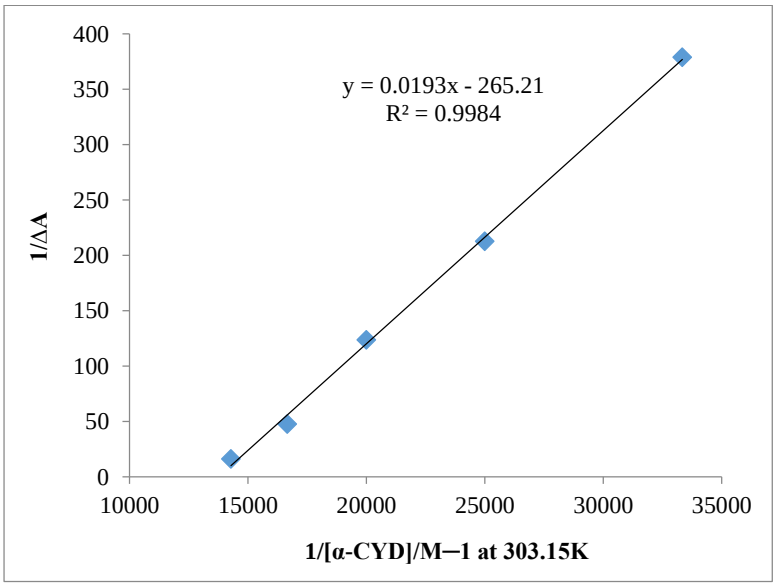

Figure S6. **Benesi-Hildebrand double reciprocal plots for the effect of α-CYD on the absorbance of THP at 303.15K.**

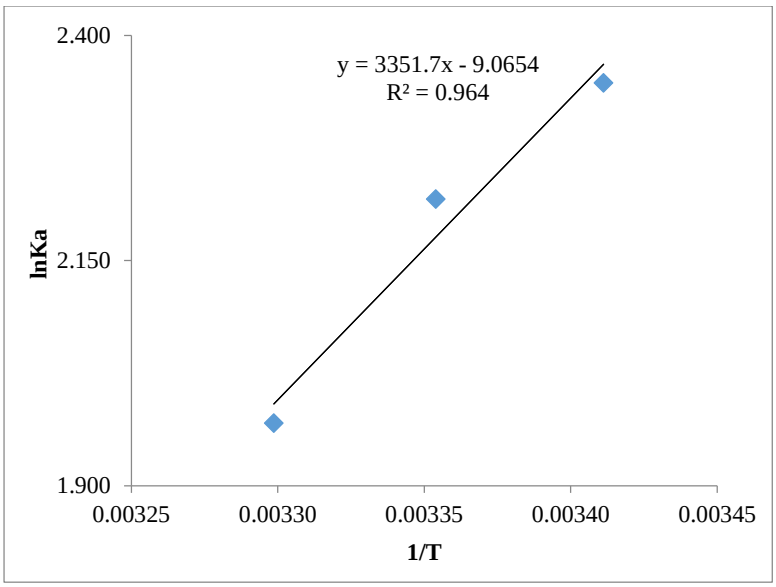

Figure S7. **$\ln K_a$ vs 1/T plot using Van't Hoff equation for determination of thermodynamic parameter of THP+ α-CYD inclusion complex.**

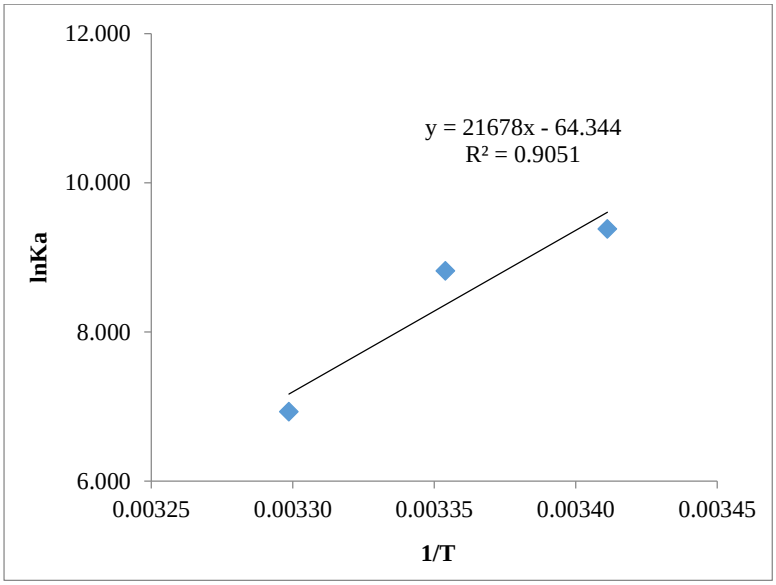

Figure S8. **$\ln K_a$ vs 1/T plot using Van't Hoff equation for determination of thermodynamic parameter of THP+HP-β-CYD inclusion complex.**