CHAPTER VI

PROBING INCLUSION COMPLEXES VIA DIVERSIFIED INTERACTIONS BY PHYSICOCHEMICAL APPROACH

6.1. INTRODUCTION

Cyclodextrins (CYDs) are made up of 6, 7 and 8 glucopyranose 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 but it has some demerits 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 a 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 298.15K: for α-CYD is 14.5 mg/ mL and β-CYD is 1.85 mg/ mL). [7] 2-hydroxylpropyl-β-cyclodextrin (HP-β-CYD) is an substitute to α, β and γ-cyclodextrin, with enhanced water solubility 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 environment. The complexes were characterized by Conductance measurement, Surface tension, 1H NMR, IR and UV-visible spectra. The structure of the THP, α-CYD and HP-β –CYD are shown in scheme 1.

6.2. Experimental Section

6.2.1 Materials

The THP (99%) and α-CYD (99%) were bought from Sigma-Aldrich, Germany and HP-β –CYD (98%) TCI used as purchased.

6.2.2. 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 $S \cdot cm^{-1}$) 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 $mN \cdot m^{-1}$. 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 recorded 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} at room temperature.

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

6.2.3. 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.

6.3. Results and discussions

6.3.1. 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 and 3) solution. Each curve, (Figure. 1a and b), obtained from the data [Table 1(a), 1(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 [Table2.] it is understood that HP- β-CYD is less proficient for the creation of inclusion complexes than that of α-CYD. This is markedly due to the fact that α-CYD furnishes further practical trait (Scheme1) for the construction of possible inclusion complexes than HP-β-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.

6.3.2. 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[Table3(a) and 3(b)] at 298.15 K are depicted in Figure 2(a), (b). Through this method the stoichiometry of the inclusion complexes can be deduced from the breaks (Table 4.) 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 m mol L^{-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 favorable structures for the formation of selective inclusion complexes with the investigated THP. This is also supported by the above mentioned surface tension experiment.

6.3.3. Job's plot

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[Table5(a) and 5(b)] . 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 deviation gives the stoichiometry of the inclusion complex (IC), *i.e.,* ratio of guest and host is 1:2 if R = 0.33; 1:1 if R = 0.5; 2:1 if 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 (Figure 3a. & 3b).

6.3.4. 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 6), On the basis of the consistent Benesi–Hildebrand method for a 1: 1 host–guest complex, the double reciprocal plots[Fig4(a),4(b),4(c), 4(d),4(e), 4(f).] have been drawn using the equation[-1] as follows [33, 34]

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

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]. 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 (eqn (VI.2)) as follows:

$$\ln K_a = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad \text{(VI.2)}$$

There is a linear relationship between $\ln K_a$ and 1/T in the above mentioned equation (eqn(VI.2)) (Figure 5(a). to 5(b). Based on eqn(VI.2), the thermodynamic parameters ΔH^o, ΔS^o and ΔG^o for the formation of the inclusion complex can be obtained (Table3). 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 (Table 6). 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.

6.3.5. FTIR spectroscopy

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 [Table 7(a), (b), (c), (d), (e)] and spectroscopic change in wave number before and after inclusion are shown in Figure 4. Due to non covalent interactions the changes of bands are 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 -O-H 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 -O-H 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 3436.38cm^{-1},2952.02cm^{-1},1639.83cm^{-1},1295.85cm^{-1}and1030-236.86cm^{-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 1620cm^{-1} in α-CYD +[THP] & 1614.19cm^{-}

[1] 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 favour 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 insertion of the THP in α-CYD and THP in HP-β-CYD in the resultant complex (Figure 6). 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.

6.3.6. NMR spectroscopy

1H NMR analysis is one of the most satisfactory methods for the study of inclusion complex [37, 38]. 1H NMR spectra of the 1:1 mixture of solid inclusion complex have been recorded in D_2O at 298.15 K. (Figure 7) and the chemical shift ($\Delta\delta$) 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 towards the cyclodextrin ring through the wider rim side rather than the narrower dimension can be envisaged from the chemical shift displacements ($\Delta\delta$) of the H3 of α-CYD is more pronounce as it is located near the wider rim. 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 7, Scheme 2, 3) [39]. Insertion of a guest molecule inside into 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 (1H) 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 compared to H5. In the present study the molecular interactions of THP with α and HP-β- Cyclodextrin have been studied using the 1H 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 of the alkaloid undergone down field shift probably its proton is relatively more shielded than inside of the CYD cavity. [42-44]

In the 1H 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

Upon inclusion the upfield chemical shift values (Δδ) of the H3 and H5 protons of **α** and HP-β-Cyclodextrins have been listed in [Table8(a),(b), (c) (d)] 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 may also be mentioned that after inclusion some non aromatic proton peaks of the THP was completely disappeared in the proton NMR spectra of the THP and Cyclodextrin ICs, indicating strong evidence for the inclusion complex formation.

6.4. Conclusion

The present study reveals an exclusive behaviour 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 and guest was found to be 1:1 by Job's method. ^{1}H NMR data as well as FTIR also confirms the inclusion phenomena. The determination of association constants and various thermodynamic parameters quantitatively clarify the consequence of the study. Consequently, this distinct study has diversified applications in the broad field of biology and Chemistry i.e. in Biochemistry.

4.5. REFERENCES

References of CHAPTER VI are given in BIBLIOGRAPHY (Page No.295-297)

Tables

Table1(a). 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.

Table1(b). Data for the Surface tension study of aqueous **THP VS HP-β-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 HP- β-CYD	Total vol.	Conc. Of drug(THP)	Conc.of HP- β-CYD	ST/mN.m-1
10	0	10	10	0	63.4
10	1	11	9.091	0.909	64.5
10	1	12	8.333	1.667	66.2
10	1	13	7.693	2.307	67.2
10	1	14	7.143	2.857	67.9
10	1	15	6.667	3.333	68.5
10	1	16	6.25	3.75	69.3
10	1	17	5.883	4.117	69.7
10	1	18	5.556	4.444	70
10	1	19	5.264	4.736	70.3
10	1	20	5	5	70.5
10	1	21	4.762	5.238	70.6
10	1	22	4.546	5.454	70.7
10	1	23	4.348	5.652	70.8
10	1	24	4.167	5.833	70.9
10	1	25	4	6	70.9
10	1	26	3.847	6.153	70.9
10	1	27	3.704	6.296	71
10	1	28	3.572	6.428	71.1
10	1	29	3.449	6.551	71.1

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

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

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

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

Table3(a). 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

Table3(b). Variation of Conductivity of THP VS HP- β-CYD at 298.15K

added HP-β-CD	Total volm	conc of THP	conc of HP- β-CYD	conductance
mL	mL	mM	mM	µS m-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 4. Values of Conductivity and at the break point with corresponding concentration of α-CYD and HP- β-CYD for THP at 298.15 K

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

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

Table5(a). Data for Job plot obtained from UV-spectroscopy for aqueous THP+ α-CYD system at 298.15K[a]

THP(ml)	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

Table5(b).). Data for Job plot obtained from UV-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	0.290382718	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 6. Association constant (K_a) and thermodynamic parameters ΔH^o, ΔS^o and ΔG^o of THP-CYDs inclusion complexes at 293.15K, 298.15K , 303.15K temperatres

IC	Temp/K[a]	K_a[b] (×10^{-5})/M^{-1}	ΔH^{ob}/kJ mol^{-1}	ΔS^{ob}/J mol^{-1} K^{-1}	ΔG^{ob} (298.15 K)/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 = ∓ 0.02 ×10-5 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}.

Table 7a. Data for IR spectra of THP

Theophylline (THP)	
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

Table 7b. Data for IR spectra of α-CYD

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

Table 7c. Data for IR spectra of HP-β-CYD

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

Table 7d. Data for IR spectra of α-CYD +[THP] inclusion complex.

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	-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

Table 7e. Data for IR spectra of HP-β-CYD+THP complex

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

Table 8a. Data for NMR spectra of **HP-β-CYD &** THP+ **HP-β-CYD** complex(IC-1)

Type of proton	δ (ppm)		**Shift (Δδ)**
	HP-β-CYD	THP+ **HP-β-CYD** complex(IC-1)	
H-1	5.144-4.969	5.129-4.959	**-0.015**
H-2	3.504	3.504	**-0.001**
H-3	3.906	3.9085	**0.002**
H-4	3.394	3.436	**0.042**
H-5	3.754-3.603	3.734	**0.222**
H-6	3.754-3.603	3.753	**0.112**
$-CH_3$	1.042-1.022	1.017-1.037	**-**

Table 8b. Data for NMR spectra of α-CYD and THP+**α-CYD** complex(IC-2)

Type of proton	δ (ppm)		**Shift (Δδ)**
	α-CYD	THP+**α-CYD** complex(IC-2)	
H-1	4.910-4.900	4.941-4.930	**-**
H-2	3.49-3.500	3.535-3.492	**-**
H-3	3.840-3.830	4.019-3.989	**0.186**
H-4	3.420-3.460	3.467-3.438	**-**
H-5	3.680-3.820	3.770-3.716	**-0.140**

Table8c. Data for NMR spectra for significant peak of THP and **THP**+ HP-β-CYD complex(IC-1).

Type of proton	δ (ppm)		**Shift (Δδ)**
	THP	**THP**/ HP-β-CYD complex(IC-1)	
NCHN**H**	7.155-7.127 (Unsymmetrical doublet, , J=8.49Hz), , &	7.131-7160	**0.021**
NC**H**NH	6.890-6.862(Unsymmetrical doublet, , J= 8.61 Hz),	6.860-6.888	**0.027**
(-N-CH_3 near to aromatic ring)	2.783-2.570	Peak disappeared	**-**
[-CON(C**H**$_3$)CO-N-CH_3]	2.590-2.570	Peak disappeared	**-**

Table8d. Data for NMR spectra for significant peak of α-CYD and THP+**α-CYD** complex(IC-2)

Type of proton	δ (ppm)		**Shift (Δδ)**
	α-CYD	THP+**α-CYD** complex(IC-2)	
NCHN**H**	7.155-7.127 (Unsymmetrical doublet, J=8.49Hz), &	7.176-7.147	**0.007**
NC**H**NH	6.890-6.862(Unsymmetrical doublet, , J= 8.61 Hz),	6.917-6.889	**0.027**
(-N-CH_3 near to aromatic ring)	2.783-2.570	2.865-2.706	**0.082**
[-CON(C**H**$_3$)CO-N-CH_3]	2.590-2.570	Peak disappeared	**-**

Figures

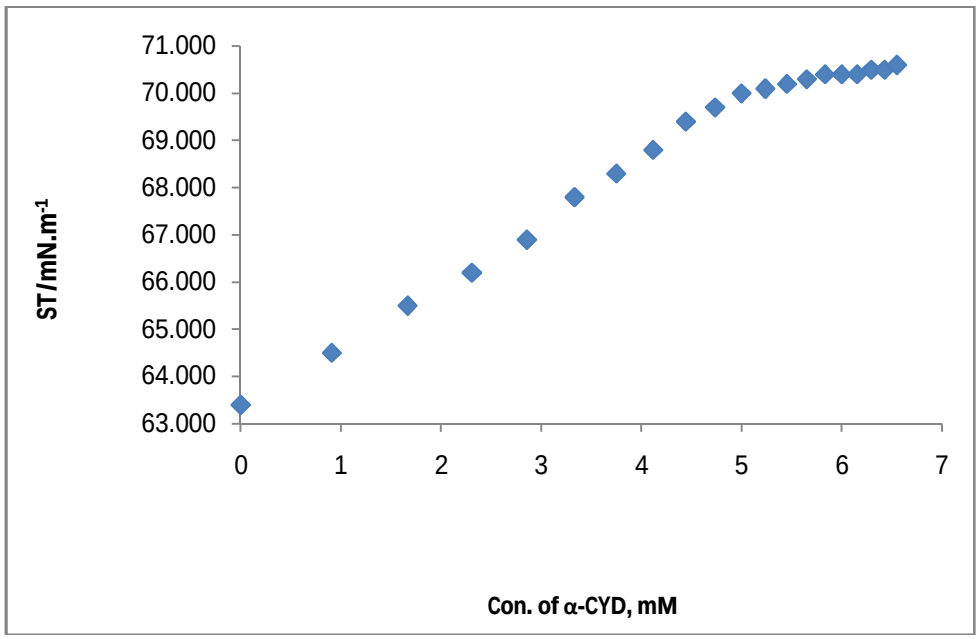
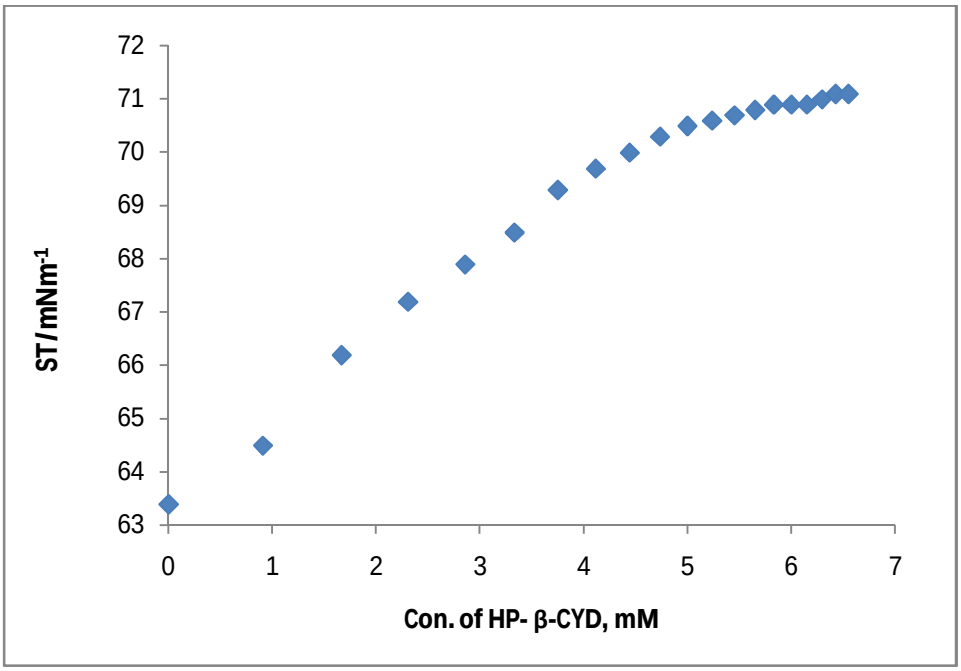


Figure 1(a). Surface tension of THP with α-CYD at 298.15 K and



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

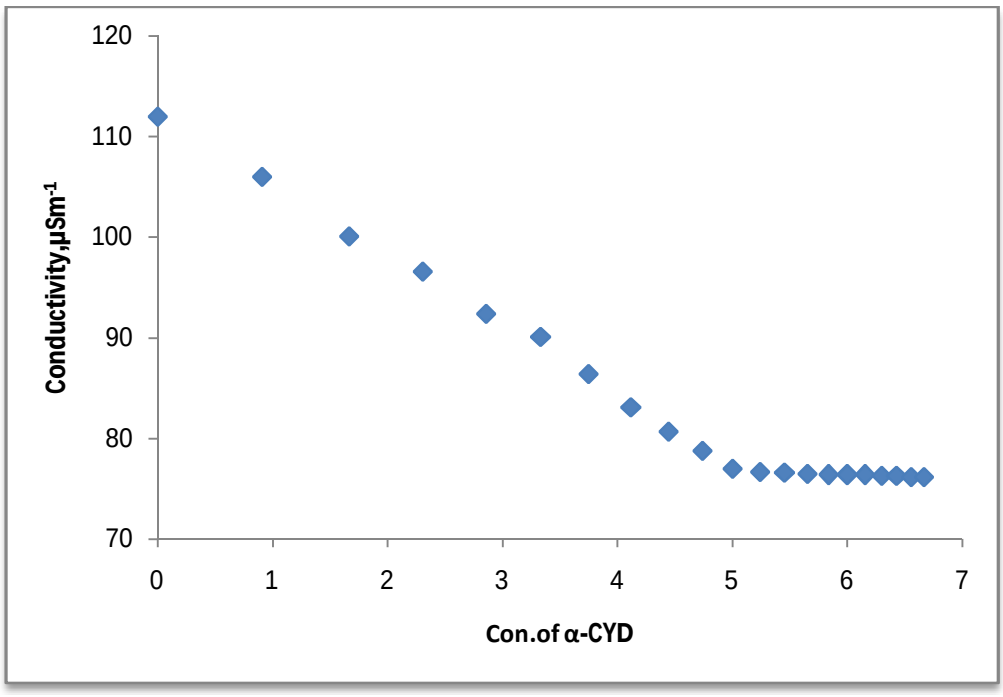
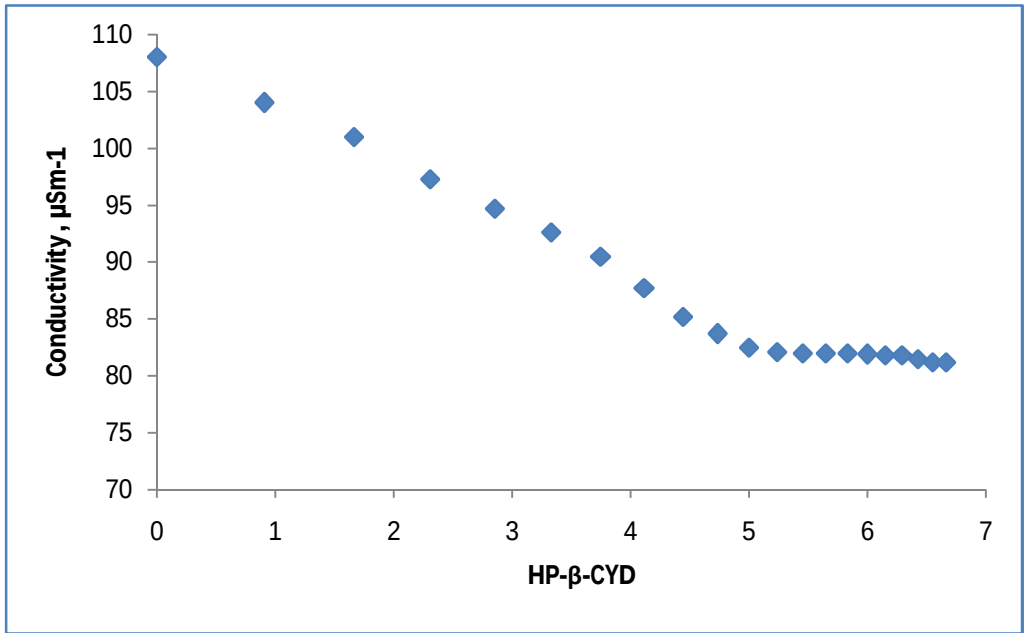


Figure 2: Variation of conductivity of aqueous THP with (**a**) α-CYD solution at 298.15 K. and



(**b**) HP-β-CYD solution respectively with increasing concentration of **α**-CYD & HP- *β*-CYD at 298.15 K.

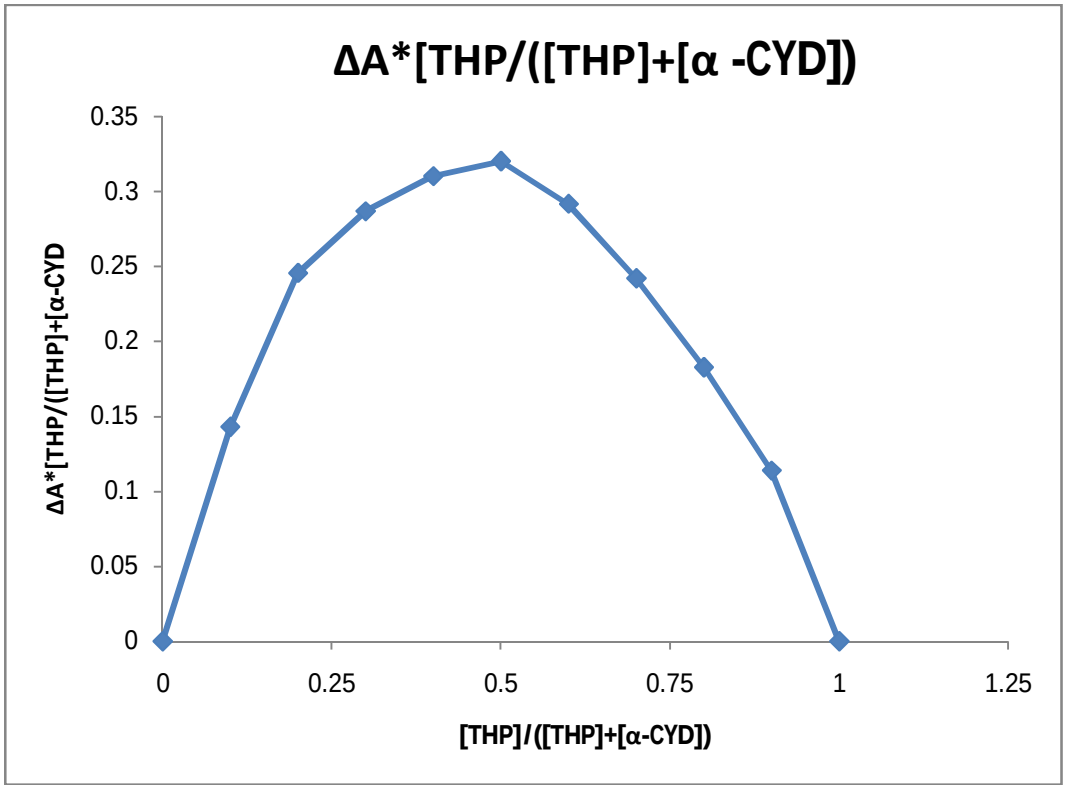
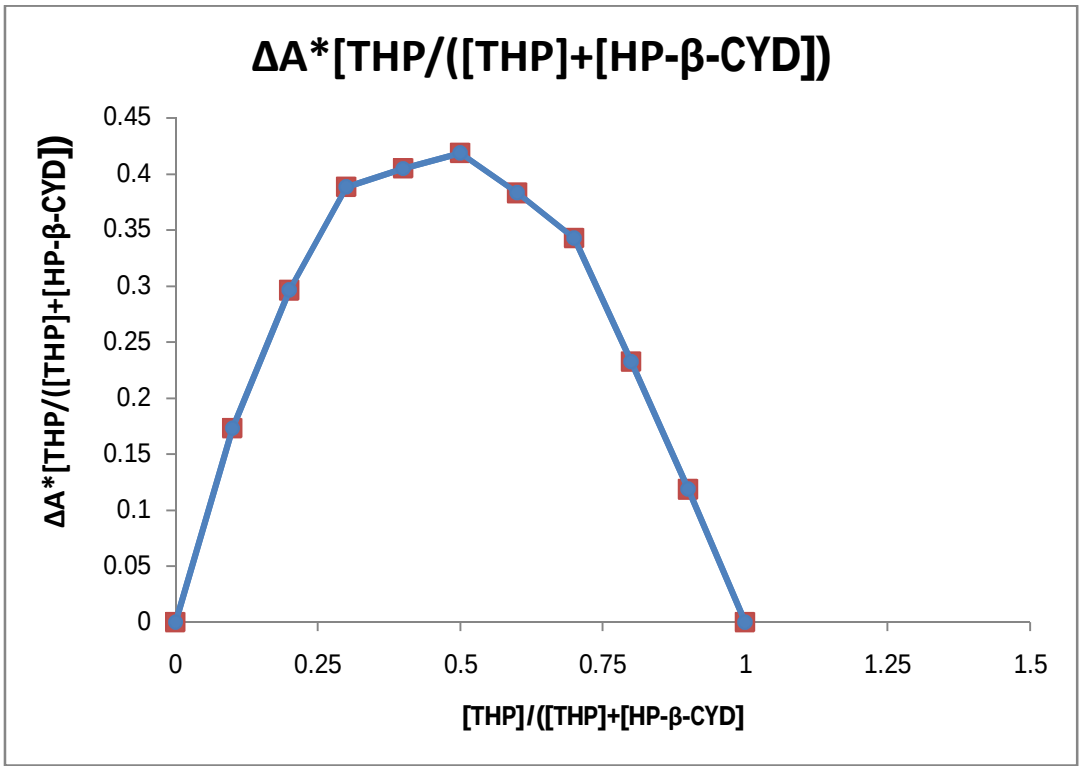


Figure 3(a). Job plot of THP+ α-CYD system at 298.15K and



3(b) Job plot of THP+ β-CYD system at 298.15K

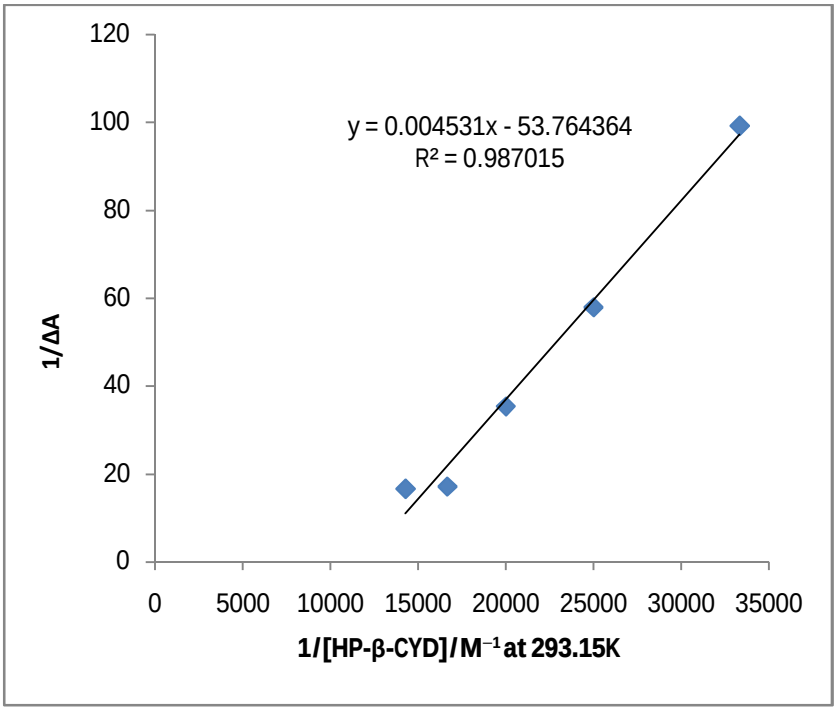


Figure 4(a). .Benesi-Hildebrand double reciprocal plots for the effect of HP- β-CYD on the absorbance of THP at 293.15K

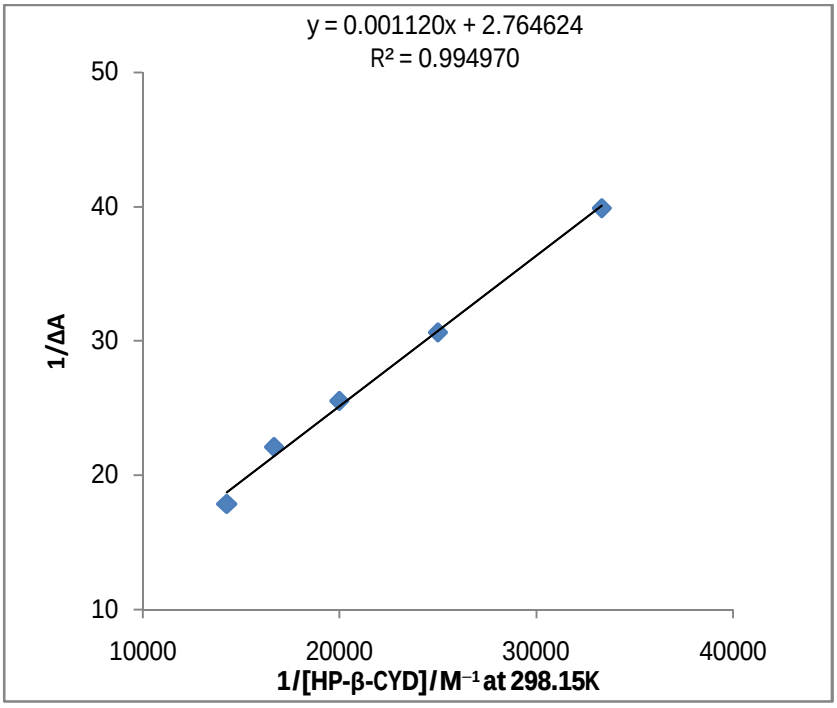


Figure4(b). .Benesi-Hildebrand double reciprocal plots for the effect of HP-β-CYD on the absorbance of THP] at 298.15K

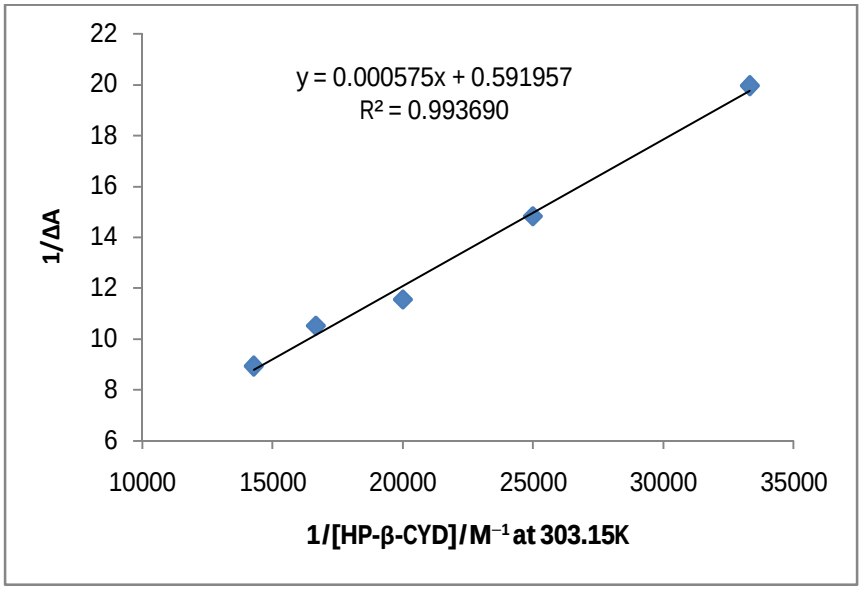


Figure 4(c). **Benesi-Hildebrand double reciprocal plots for the effect of HP-β-CYD on the absorbance of THP at 303.15K**

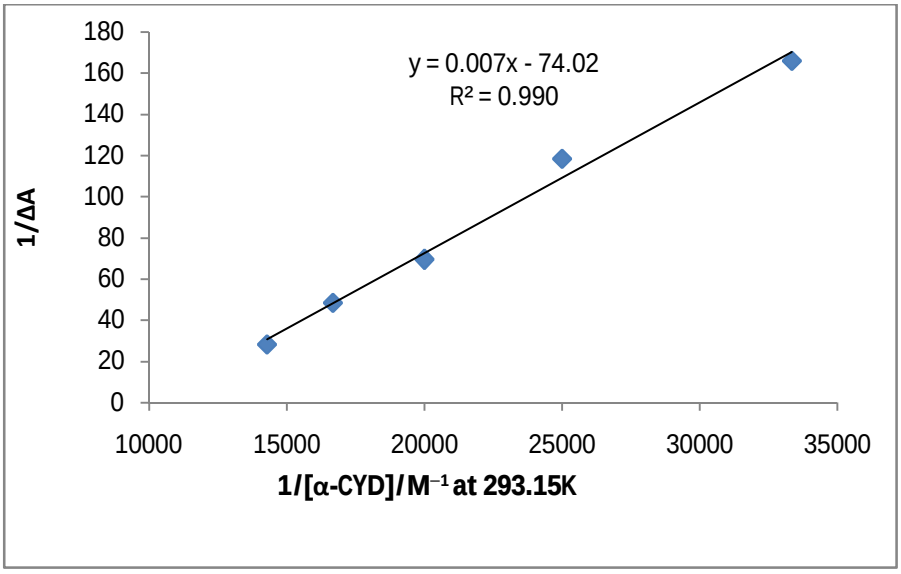


Figure4(d). **Benesi-Hildebrand double reciprocal plots for the effect of α-CYD on the absorbance of THP at 293.15K**

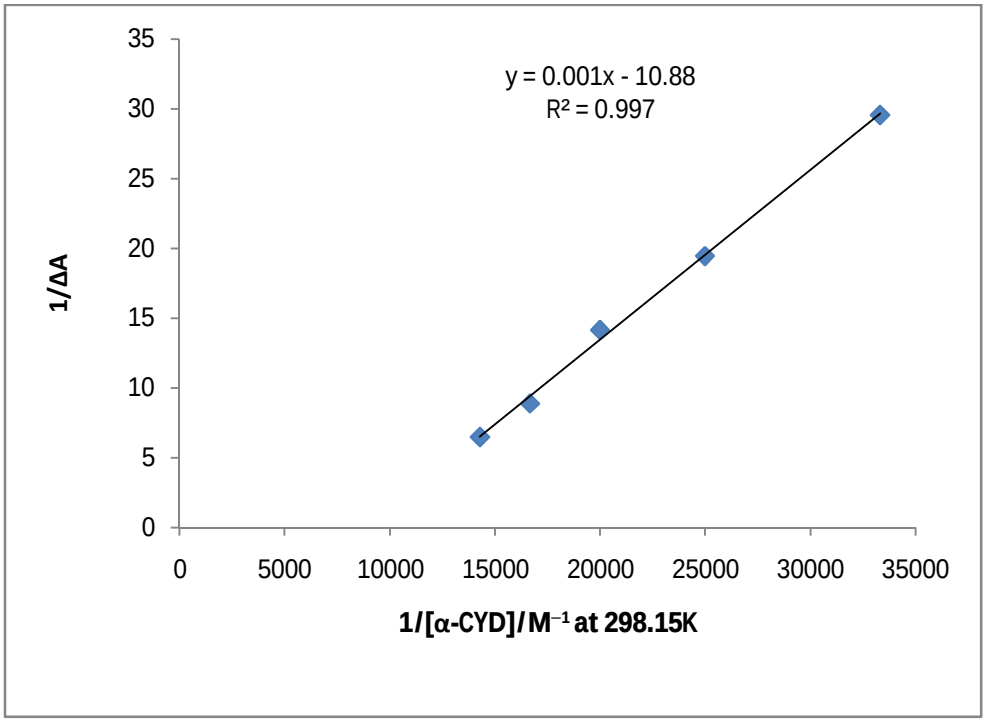


Figure 4(e). Benesi-Hildebrand double reciprocal plots for the effect of α-CYD on the absorbance of THP at 298.15K

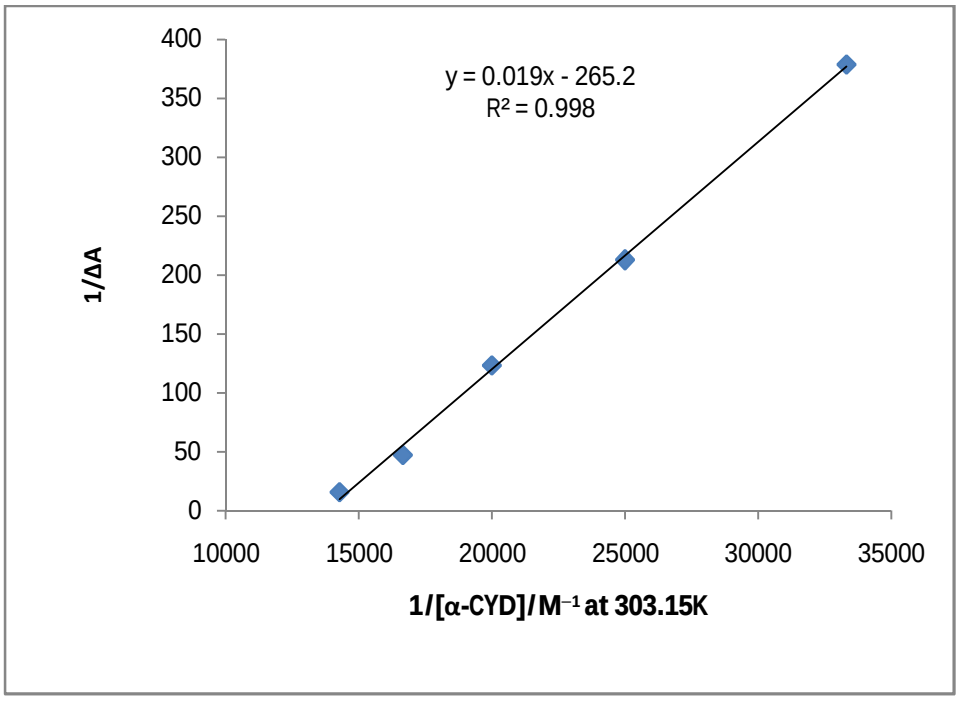


Figure4(f). Benesi-Hildebrand double reciprocal plots for the effect of α-CYD on the absorbance of THP at 303.15K

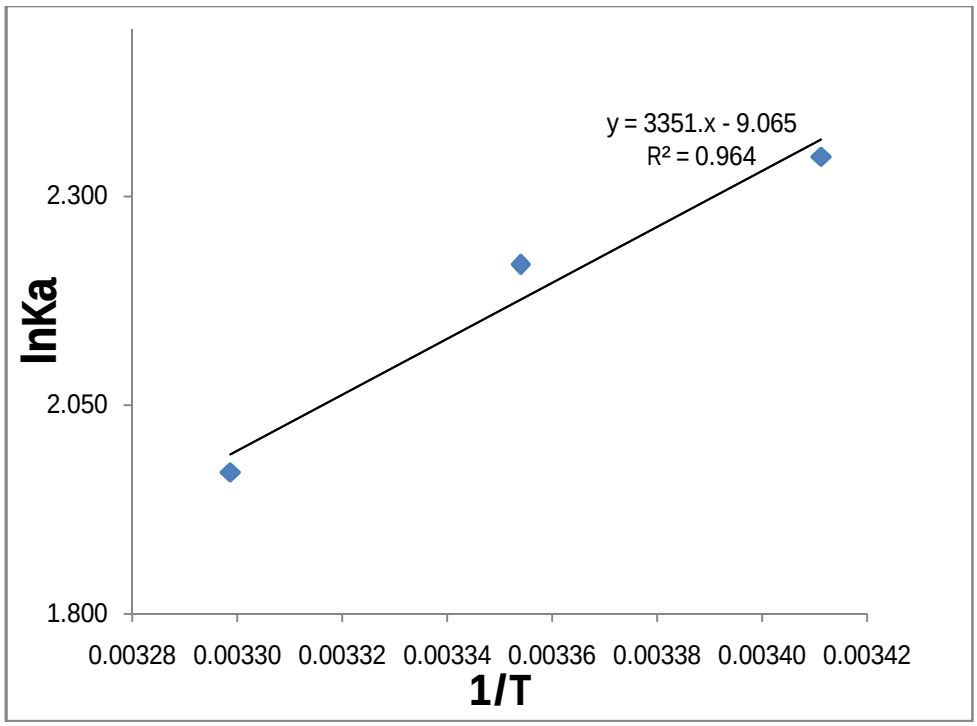


Figure5(a). lnK_a vs 1/T plot using Van't Hoff equation for determination of thermodynamic parameter of THP+ α-CYD inclusion complex.

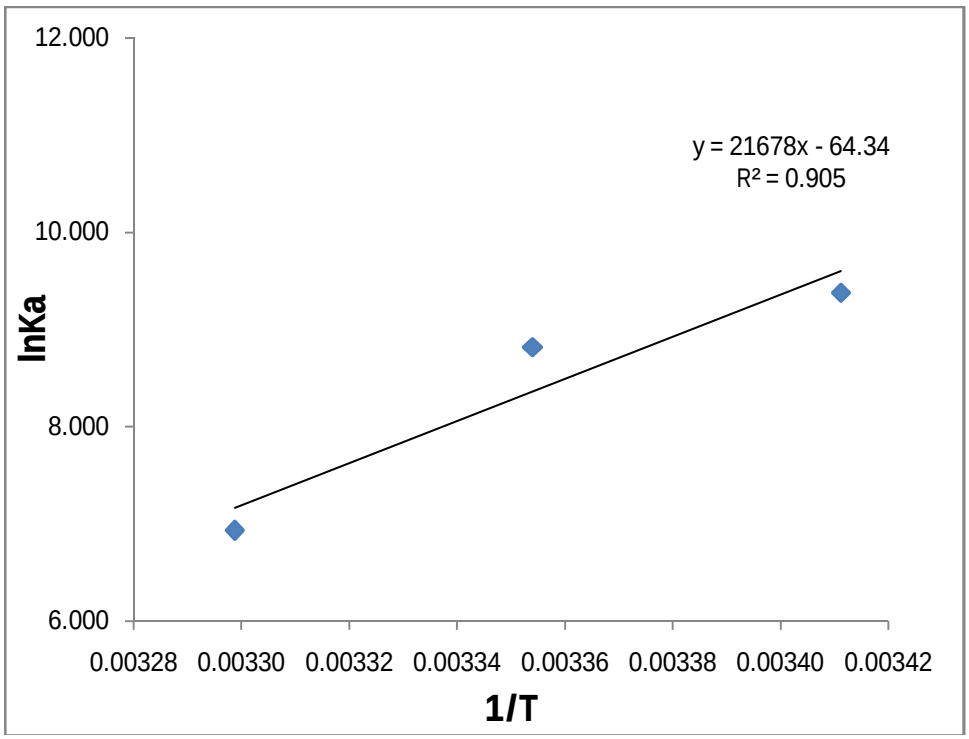


Figure 5(b). **lnK_a vs 1/T plot using Van't Hoff equation for determination of thermodynamic parameter of THP+HP-β-CYD inclusion complex.**

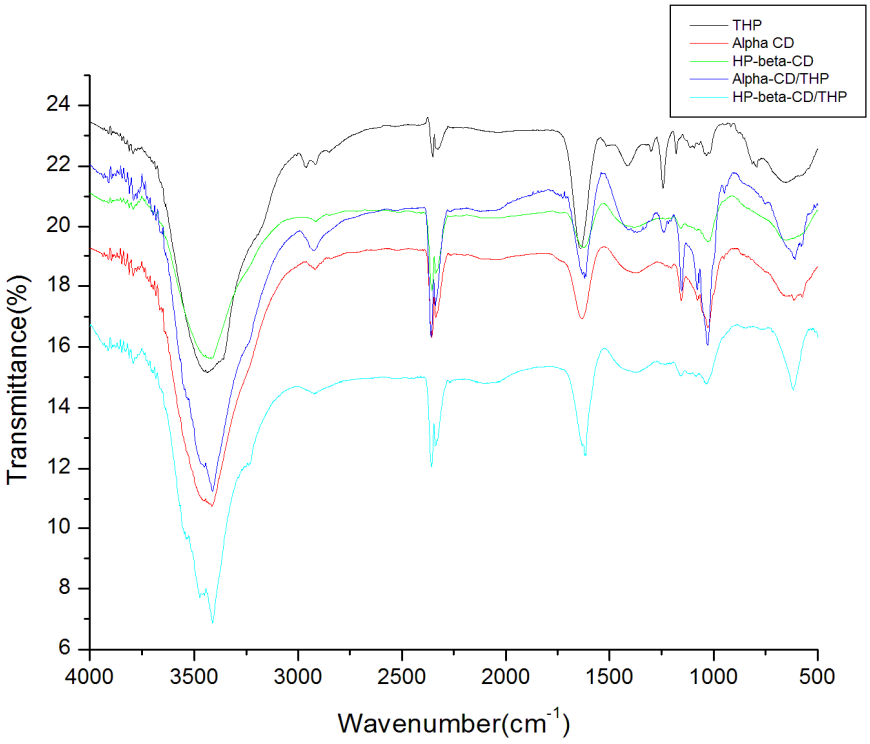


Figure6. FTIR spectroscopy of THP with respect to HP-β-CYD and α-CYD

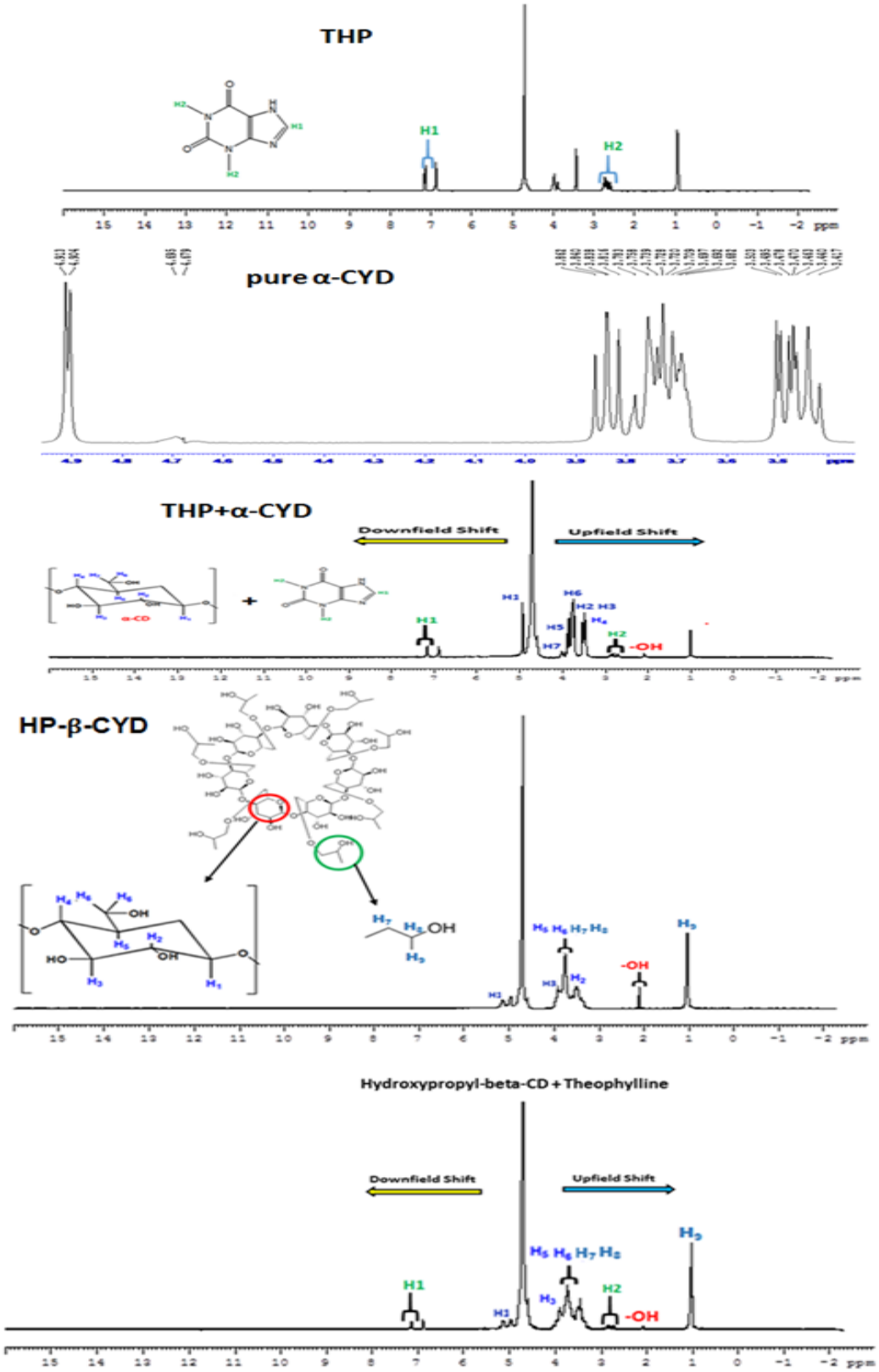
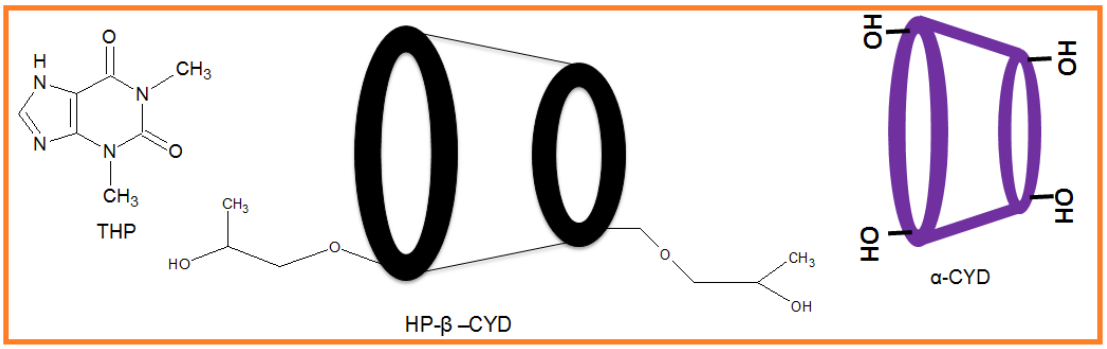
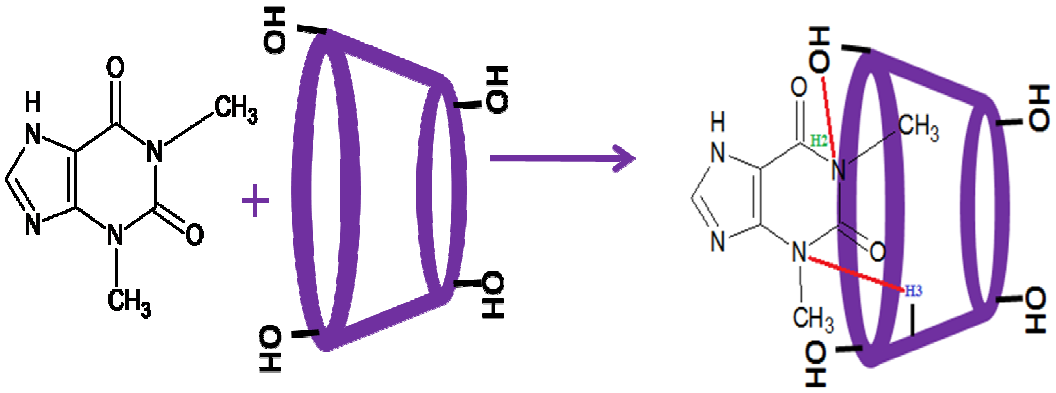


Figure7. NMR spectra of the pure compounds and inclusion complexes

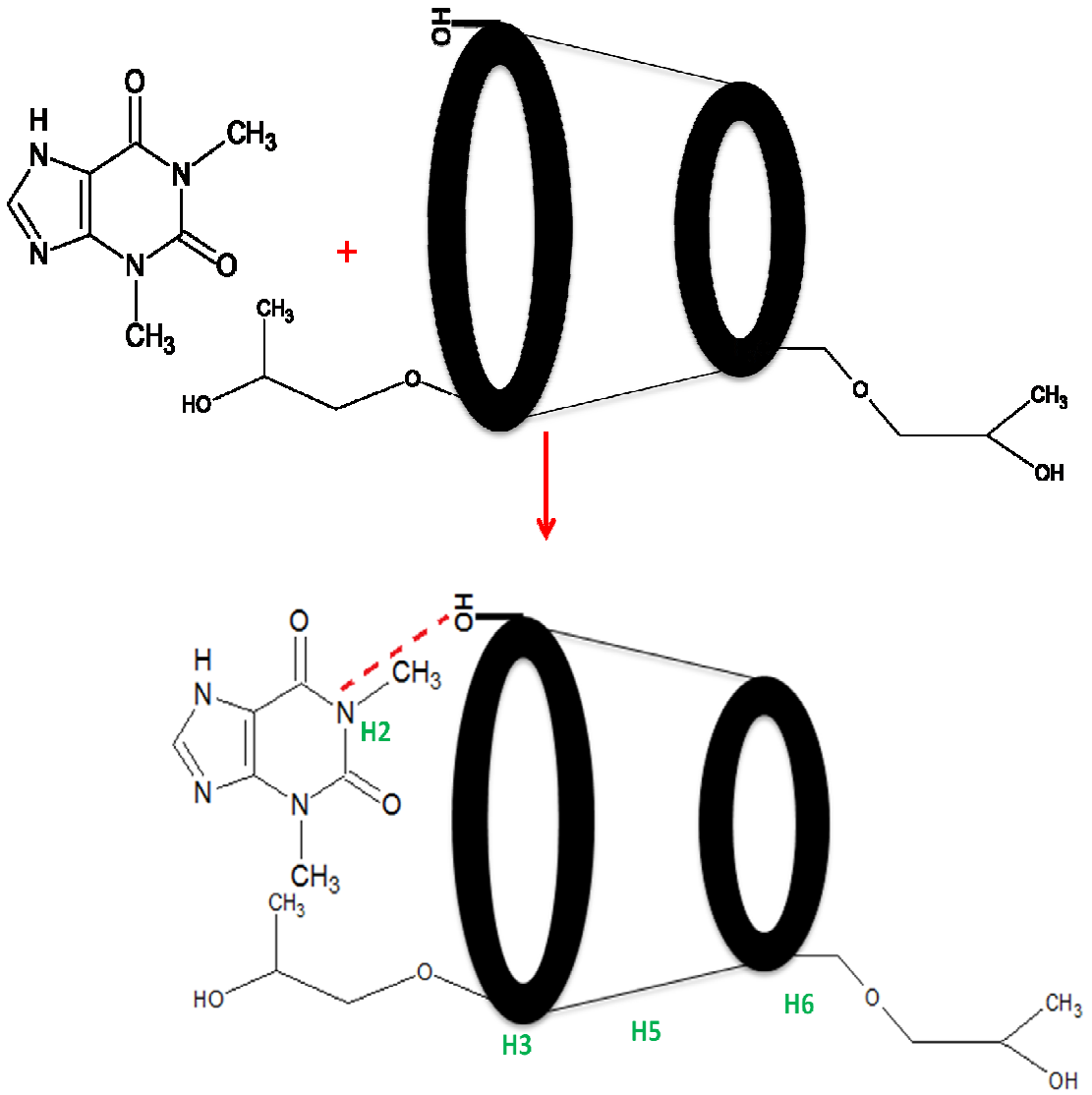
Schemes



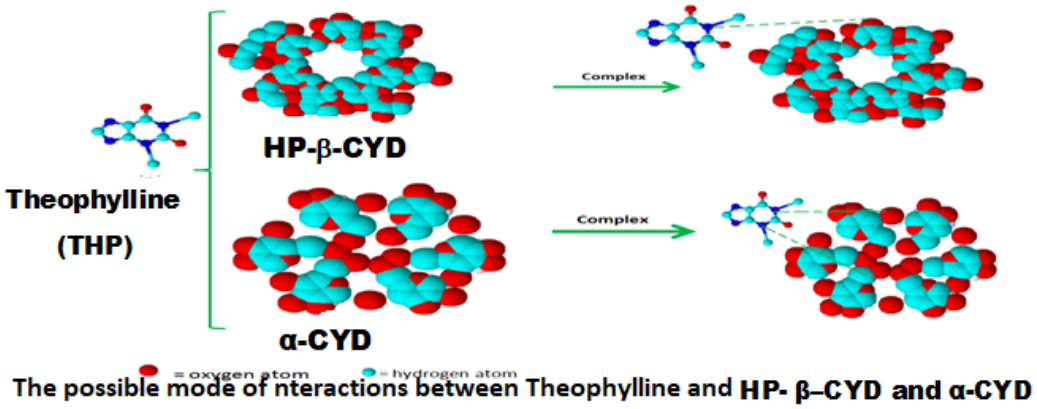
Scheme1. Molecular structure of THP, α-CYD and HP-β –CYD



Scheme2. The proposed inclusion complex geometry of THP+α-CYD



Scheme 3. The proposed inclusion complex geometry of THP+HP-β-CYD



Scheme4. The plausible mode of intection between THP+HP-β-CYD & THP+α-CYD