Physicochemical approaches to inclusion complexes and different interactions

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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.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**.** "Beta Cyclodextrin has been extensively used due to ready availability but it has some demerits like low solubility and nephrotoxicity". Derivatives of β-Cyclodextrin with improved water solubility (e.g. Hydroxypropyl-β-Cyclodextrin i.e HP-β-CYD) are most commonly pharmaceutical formulation.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.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 14.5 mg/ mL and β-CYD is 1.85 mg/ mL). 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 haematological changes but no histopathological changes. 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. 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. **Amongst the three cyclodextrin homologues *(α,* β and γ) β-cyclodextrin is the slightest expensive. Undesirably, β-cyclodextrin has only a 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.** Newly, the anticancer consequence of HP- β - CYD has been revealed and proved in vivo in mouse model of leukemia.

Theophylline (THP) is one of the most extensively approved drugs 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, 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. 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. Main toxicity after THP intoxication differ by variety of overdose. 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,1H NMR, IR and visible spectra.

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-thermostated 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.

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

The solid inclusion complex 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 respectvely 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 analysed and characterised by means of FTIR, NMR spectroscopic methods.

3. Results and discussions

3.1. Surface tension

Surface tension (γ) measurements be able to use to make clear 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 3 and 4) solution. Each curve, Fig.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 1) 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 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.

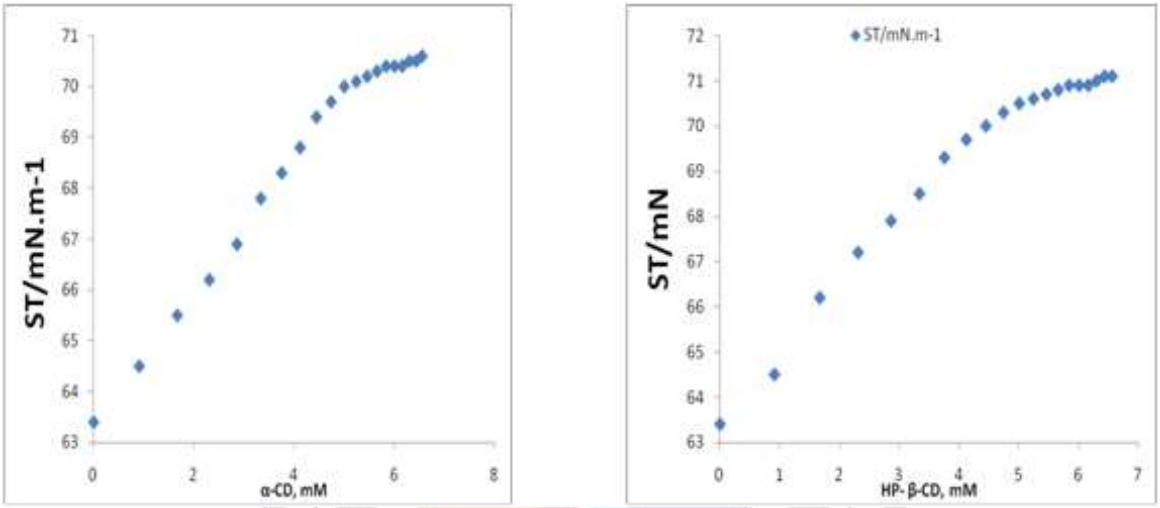

Figure 1(a) Surface tension of THP with α-CYD and (b) Surface tension of THP with HP-β-CYD at 298.15 K.

Table 1. Values of surface tension (γ) and at the break point with corresponding concentration of α-CYD and HP- β-CYD for THP at 298.15 K[a]

	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.

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.

It indicates the construction as well as the stoichiometry of the IC produced. CYD concentrations at 298.15 K are depicted in Fig.1c & d. Through this method the stoichiometry of the inclusion complexes can be deduced from the breaks (Table 2) in the conductivity curves. 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 mmol L^{-1} for CYDs, suggesting that the stoichiometry of the inclusion complex is equimolar. 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.

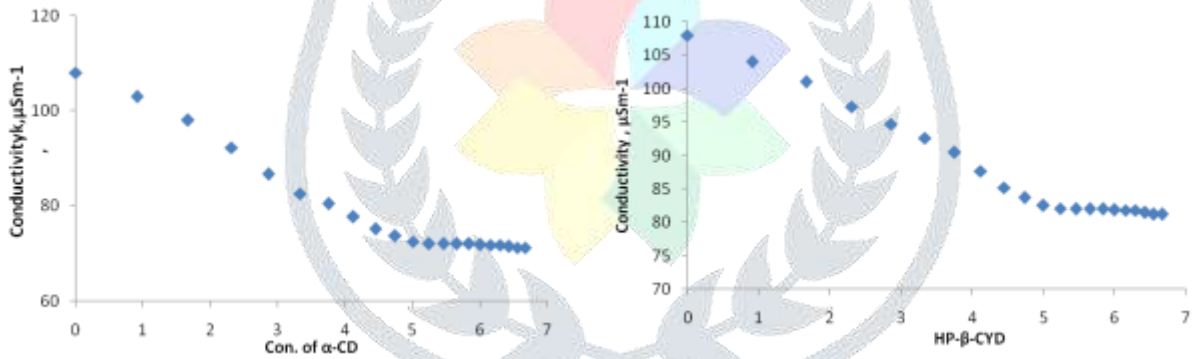

Figure 2: Variation of conductivity of aqueous THP with (**a**) α-CYD solution and (**b**) HP-β-CYD solution respectively with increasing concentration of **α**-CYD & HP- *β*-CYD at 298.15 K.

Table 2. Values of Conductivity and at the break point with

corresponding concentration of α-CYD and HP- β-CYD for THP at 298.15 K[a]

Conductivity

	THP & α-CYD	THP & HP-β-CYD
Conc. of CYD/ mM	5.23	5.17
k/mS m^{-1}	76.70	82.50

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

3.3. 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. Changes in absorption intensity was studied as a function of concentration of cyclodextrin to establish the value of K_a (Tables Sup). 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.

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

The values of the association constants for the systems was evaluated by dividing the intercept by the slope of the straight line of the double reciprocal plot **(Table 3).** 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 (2)) as follows:

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

There is a linear relationship between $\ln K_a$ and 1/T in the above mentioned equation (eqn(2)). Based on eqn (2), the thermodynamic parameters ΔH^o, ΔS^o and ΔG^o for the formation of the inclusion complex can be obtained (**Table 3**).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 3).** 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 favorable.

Table 3: 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 [b] ($\times 10^{-5}$)/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}.

^{1}H NMR study confirms the inclusion phenomenon

The inclusion of a guest molecule into the hydrophobic cavity of α and HP- β-CYD results in the chemical shift of the protons of the cyclodextrin molecules in the ^{1}H NMR spectra, which is because of the interaction of the host cyclodextrin with the guest molecule.

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In the structure of cyclodextrin the H3 and H5 hydrogens are located within the conical cavity, predominantly; H3 is placed near the wider rim while H5 is placed near the narrower rim of the cyclodextrin molecule. The other H1, H2 and H4 hydrogens are placed at the exterior of the cyclodextrin molecule.

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Thus when a guest molecule enters into the cavity of cyclodextrin it interacts with the H3 and H5 protons, resulting in the upfield chemical shift of these protons. As H3 is located near the wider rim of cyclodextrin, through which usually the guest enters, the shift is higher for it than that for the H^5 proton which is located near the narrower rim at the interior of cyclodextrin. The other H1, H2 and H4 hydrogens also show an upfield chemical shift, but it is lower compared to that of the interior proton

In the present work 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-Cyclodextrin in D_2O at 298.15 K. It has been established that there are significant upfield shifts (Δδ) of the interior H3 and H5

protons, but little shifts of the exterior H1, H2 and H4 protons of cyclodextrins, as well as those of the interacting protons of the THP.

This establishes that the inclusion phenomenon has occurred between the chosen host and guest molecules. Upon inclusion the upfield chemical shift values (Δδ) of the H3 and H5 protons of **α** and HP-β-Cyclodextrins have been listed in Table 1, 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.

Conclusion

The present study reveals a 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 additionto 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 the consequence of the study. Consequently, this limited study has diversified applications in the broad field of biology and Chemistry i.e. in Biochemistry.

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