CHAPTER V

EXPLORING ENCAPSULATION OF AN ANTISPASMODIC DRUG ALIBENDOL WITH β-CYCLODEXTRIN MOLECULE BY SPECTROSCOPIC METHODOLOGIES

Abstract: The encapsulation of Alibendol (AB), the biologically potent muscle relaxant, within the cavity of β-cyclodextrin(β-CD) was studied in assistance of some spectroscopic techniques such as Uv-Vis spectroscopy, ^{1}HNMR spectroscopy, 2D ROESY, FT-IR spectroscopy. The study confirmed 1:1 stoichiometry of the host-guest inclusion complex. All the experiments showed a good correlation to establish a feasible inclusion through wider rim of the cyclodextrin molecule. The thermodynamic parameters explained the process of inclusion as exergonic and spontaneous.

Keywords: Alibendol, β–CD, 2D ROESY, 1:1

1. Introduction:

The muscle relaxant is a medication that reduces the contraction of muscles by disturbing skeletal muscle functioning. It may be used to reduce the indications which include hyperreflexia, muscle spasms, and pain. The muscle relaxants are categorized as spasmolytics and neuromuscular blockers. The latter category acts by entering at the neuromuscular end and does not affect the central nervous system. They can cause short-term paralysis during surgery. Spasmolytics are muscle relaxants, that basically focus on to reduce spasms and musculoskeletal pain. Sometimes spasmolytics and the neuromuscular blockers are conjointly spelled as muscle relaxants but mainly spasmolytics are the muscle relaxants.

The natives of South America who lived in the Amazon bed in the early century, used specially designed arrows that have poison on the tip and can cause skeletal muscle paralysis which bring about death. The research had done a lot of scientific studies thereafter and this leads to the discovery of many muscle relaxants/antispasmodic drugs that relax the muscle spasms [1, 2]. Antispasmodics are the spasmolytics that are given when there occurs smooth muscle spasms. They are immensely effective in the

gastrointestinal tract to diminish the aching in the intestine, stomach, and in urinary bladder. [3]

Alibendol, or 5-allyl-2-hydroxy-N-(2-hydroxyethyl)-3-methoxybenzamide, is an antispasmodic drug that reduces the muscle spasms. Different antispasmodic drugs including choleretic, cholekinetic contains this compound as an active ingredient.

For the treatment of dyspepsia causes from biliary insufficiency, alimentary intolerance, and constipation of hepatic origin, the use of this drug is commendable. [4]

A number of applications of cyclodextrins has been included in the literature till now . The fundamentals of interaction of cyclodextrins with other molecules, initiated by Schardinger and viliers 100 years back. CDs are the macrocyclic oligosaccharides and of natural occurrence. Three types of CDs α, β and γ having 6,7, 8 glucopyranose units (joined by α-1,4 lingkages) respectively, particularly generated from the enzymatic degradation of starch . The hydrophobic toroid shaped cavity of the CD can put specific molecule inside it, which possesses definite shape and can create some non-covalent interactions with CD to produce the inclusion complex .Outer surface is hydrophilic for the CD, which makes it water soluble. With this benefit, CDs are employed for drug carriers and new drug designs chiefly for the drugs, which have low solubility, bioavailability and have toxicity ,which causes side effects. [5]

In this work, we have designed an inclusion complex of alibendol and β –CD to overcome the above mentioned limitations of the drug (alibendol).The encapsulation of the above molecules was characterised by different techniques.

2. Experimental section

2.1. Source and purity of samples:

The drug (Alibendol) selected for experiment and β-CD were purchased from TCI Chemicals and Sigma-Aldrich, Germany respectively and used as purchased. The mass fraction purity of AB (Alibendol) and β-cyclodextrin were ≥0.98, 0.97 respectively.

2.2. Apparatus and procedure

Solubility of β-CD and the drug have been verified in triply distilled, deionized and degassed water. It was detected that the drug AB is poorly soluble in water. All the stock solutions of the drug were prepared by mass (Mettler Toledo AG-285 with uncertainty 0.0001 g) and the working solutions were prepared by mass dilution at 298.15 K. [6]. Necessary precautions were made to reduce the evaporation during mixing.

^{1}H NMR spectra were recorded in d_6- DMSO at 400 MHz using Bruker AVANCE 400 MHz instrument at 298.15K. Signals were quoted as values in ppm.

UV–visible spectra were recorded by Agilent 8453 Uv-visible spectrophotometer attached with a thermostat to control the temperature.

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

2.3. Preparation of Solid Inclusion Complex of AB with β -CD:

For the formation of inclusion complex, β-CD of 1.134 g was mixed with 30 mL triply distilled and degassed water in a round bottom flask and stirred continuously over magnetic stirrer for few hours. Then 0.258g of AB was taken into a beaker along with 10 mL ethanol-water mixture (20%) and stirred over magnetic stirrer until a homogeneous mixture (completely dissolved) is formed. Here, 1:1 M ratio of β-CD and AB has been used. Guest mixture was then added into β-CD solution and stirred for 48 h without a break. The reaction mixture was then put in refrigerator for 52 h without any disturbance. After 2 days, a white solid was observed. The residue was filtered and washed for several times with distilled water. Finally, the dry white powder was acquired after drying in oven at 50°C for about 24 h. The resultant solid was the inclusion complex between AB and β-CD [7]. It was further analysed by FT-IR, NMR methods.

3. Result and discussion:

3.1. UV–visible spectroscopy

3.1. A. Job plot for determination of stoichiometry:

The stoichiometry of the IC has been discerned utilising continuous variation Job's method [8] in the solution medium by plotting ΔA × R against R (where ΔA is the difference in absorbance of AB without and with CD and R is [AB]/([AB]+[CD]) and the spectral data have been listed in Table 1. Sets of solution were made from AB and β-CD by changing the mole fraction of guest from 1-0 or vice versa in 20% ethanol-water mixture. The value of R at the maxima on curve provides the stoichiometry ratio of IC (IC-$_{1:2}$, R = 0.33; IC-$_{1:1}$, R = 0.5; IC-$_{2:1}$, R = 0.66) [9]. It has been found that the resultant curve shows the maximum at R = 0.5 suggesting that the complex has a stoichiometry of 1:1 (Fig. 1).

3.1.B. Association Constant: Interaction of AB With β-CD in liquid environment.

The UV absorption spectra of AB in [20%EtOH-H_2O (v/v)] β-CD medium have been analysed. The spectral data of AB in various concentration of β-CD at different temperatures have been registered in TableS1, S2 and S3. The strong absorption peaks of AB appears at 212 nm and with addition of β-CD blue shift occurs. No significant isosbestic point is spotted in the spectra. The absorbance intensities of AB gradually increase with increasing the concentration of β-CD. This fact confirms the encapsulation of guest molecule into the β-CD cavity due to the presence of hydrophobic and van der Waals interaction between the guest monomer and β-CD molecules [7, 10, 11]. Thus, such non-covalent interactions act as the main driving forces to incorporate the guest molecule into the CD cavity to form 1:1 IC throughout the complexation process by promoting the dissolution of the guest molecule (AB).

From the Job plot, it is confirmed that AB and β-CD form 1:1 IC. Hence, the IC formed between AB and CD can be represented as,

CD + AB⇄CD⋯AB

For a 1:1 complexation process the association constant (k_a) has been estimated by using the double reciprocal plots on the basis of Benesi-Hildebrand equation [12]. The absorption values are used in the following Benesi-Hildebrand Eq. (1) [13].

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon[AB]ka}\frac{1}{[CD]} + \frac{1}{\Delta\varepsilon[AB]} \quad (1)$$

Fig.2 depicts the plots of 1 / ΔA against 1 / [CD] at temperatures 293.15K, 303.15K and 313.15 K. A good linear correlation was observed at all the three temperatures, showing that the IC is of 1:1 stoichiometry. The values of k_a were evaluated by using the Eq. (1) from the intercept/slope of the plots (Fig.2). The values are presented in Table 2. It has been found that the association constants have high positive values that designate that the interaction of the guest and host are strong to form inclusion complex.

3.1.C. Thermodynamic parameters show Spontaneity of formation of Inclusion Complex

The free energy change (ΔG) is a very vital thermodynamic parameter that has been estimated by using the Eq. (3) [14, 15]. From the plot of Association constants (k_a) against 1/T, enthalpy and entropy values have been obtained with the help of van't Hoff equation Eq. (2).

$$2.303 log k_a = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (2)$$

It is found that the entropy is small negative. This fact supports the association of the host and guest. The enthalpy has also been found to be negative, which again is a strong evidence of interaction of the host and guest whereas the negative value of free energy change supports the spontaneity of the formation of the IC and support the fact that the complexation is an exergonic process.

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

3.2. ^{1}H NMR: Supports inclusion:

The formation of IC can be explained on the light of the ^{1}H NMR spectroscopy study [16]. This method is based on the changes of chemical shifts of protons due to encapsulation of guest molecule into the β-Cyclodextrin [17]. In β-CD structure the H-3

(near wider opening side) and H-5 (close to narrow rim) are located inside of the β-Cyclodextrin cavity. The H-6 of methylene group (bearing the primary OH group) remains on the narrow opening side of β-Cyclodextrin and the rest of the other H atoms H-1, H-2, H-4 are situated on the outer surface of β-CD. The ^{1}H NMR spectra of β-CD, pure AB and the solid IC are represented in Fig. 4. It is clearly observed from Table 3 and Table 4 that for H3 and H5, a large up-field shift has been occurred. The considerable changes of chemical shifts (δ) suggested that the AB monomer entered into the nano hydrophobic hole of β-CD. The upfield shift of H-3 (δ = 0.050ppm) is much greater than the H-5 shifting (δ = 0.005ppm). On the other hand, minor chemical shifts are observed for 1'H, 2'H,3'H that are the part of the guest AB molecule, which further support the complexation process. A significant downfield shift for the protons 1'H, 2'H, 3'H of AB has been detected. In the IC these 'H's of AB are situated in hydrophobic hollow space of β-Cyclodextrin. The hydrophobic environment is responsible for the downfield shift of the protons of guest [18]. These all changes clearly indicate that well encapsulation of the guest AB into interior hydrophobic cavity of β-CD has been occurred and it enters through the wider ring opening side. The detailed variations of chemical shifts of the two binding partners before and after forming IC have already been mentioned in Table3 & 4.

3.3. 2D-ROESY Study to Confirm the Inclusion Phenomena:

The principle of 2D ROESY relies on the interaction of the protons which are present in the close proximity of 0.4 nm range to each other to produce NMR cross peaks [19] [20]. In our study, we have investigated the inclusion of the drug inside the nano-cavity of β-CD. The NMR study was carried out in d_6-DMSO. It is clear that the H-3 and H-5 protons of β- CD are present inside the cavity and hence if inclusion occurs, there should be the presence of such close proximity of 0.4 nm of the AB protons with H-3 and H-5 protons of CD which can produce rotating-frame nuclear overhauser effect to give cross peaks [21, 22]. In Fig.5 there are the presence of cross peaks due to the interaction of H-3 proton of β-CD and 2'H proton of AB and H-5 protons of β-CD with 3'H protons of AB. This clarifies the root of insertion of guest inside host through the wider rim. In the dynamic process of the inclusion the cross peaks are generated due to the insertion of the side chain of the guest but it is not possible of entering the second AB molecule as it

is sterically unfavourable. Hence, this incident signifies the inclusion phenomena of the said drug molecule into the CD cavity.

3.4: FTIR study:

Formation of the inclusion complex is enlightened with the help of FTIR spectroscopic study. Here, the deviation of peak shape, position and intensity of the FT-IR spectra of solid IC is used to confirm the inclusion [23]. The characteristic IR frequencies of AB, β-CD, and their solid IC are listed in Table5 along with the chemical bonds responsible for the corresponding stretching frequencies and the spectra are shown in Fig.6. In the IR spectra, Stretching of =C-H of the pure AB was observed at 3039cm^{-1} but the peak was not seen in the IR spectra of IC. The stretching of aromatic -C=C was found at 1606 cm^{-1} for the pure AB but the considerable shift of the spectra was observed in the inclusion complex at 1631cm^{-1} . On the other hand the peak due to O-H stretching of β-CD was observed at 3408 cm^{-1} which was shifted in the spectra of IC to 3385 cm^{-1}. The stretching frequency of the C-H from CH_2 was also found to be shifted from 2941 cm^{-1} to 2922cm^{-1}. This may be due to the interaction of the O-H groups with the polar part of the guest AB. Short band due to O-H bending at 1404 cm^{-1} was found absent. Band at 1465 cm^{-1} due to bending of $–CH_2-$ of AB was also found to be absent in IC. These are due to the interaction of the host and guest after formation of the IC. From the analysis of the 2D NMR spectra it is clear that the propenyl group of AB is inserted inside the CD molecule. Moreover the absence of O-H bending frequency, shifting of alkyl C-H stretching, shifting of C-N stretching conclude the outer sphere polar interaction of the host and guest. [24]

3.5. Effects of CD: interaction of AB with β‑CD in liquid environment

Cyclodextrin has a unique structure to accommodate guest molecules of various dimensions. The cavity diameter of β-CD is 6.0-6.5 Å. Here, alibendol is a molecule of suitable size that can easily be incorporated by the host beta-cyclodextrin. The propenyl group of the alibendol at the C-5 position of the aromatic ring is hydrophobic in nature. This hydrophobic part of AB can easily enter inside the cyclodextrin cavity and participate in the hydrophobic interaction with the hydrophobic part of the CD molecule. The 2D NMR shows that 3'H interacts with the H-5 of cyclodextrin and 2'H

interacts with the H-3 of cyclodextrin. The C=O group at the C-1 position as well as the methoxy group at the C-3 position of the aromatic ring interact with the O-H Proton of cyclodextrin and thus a stable inclusion complex is formed. During the inclusion complexation, the guest enters through the wider rim of the CD molecule.

According to Shikari and his co-workers the water molecules which are polar in nature bound by the polar-apolar interaction inside the hydrophobic cavity of cyclodextrin molecule, which is in fact not so strong and as a consequence the relatively more stable inclusion complex is formed due to stronger apolar-apolar interaction removing the water molecule from the cavity [25] [26]. As a result a more stable lower energy state of the system is obtained where the ring strain of cyclodextrin moiety is reduced.

4. Conclusion: The incorporation of the antispasmodic AB within β-CD and their interactions have been studied by Uv-vis, ^{1}HNMR, 2D ROESY and FTIR spectroscopy. The 1:1 complexation was attributed by job's plot. The values of thermodynamic parameters also suggest the same. The generation of cross peaks in the 2D ROESY spectra is a confirmation that the inclusion complex has been formed between the drug and cyclodextrin. This work mainly focused on the establishment of the phenomenon of inclusion so that certain properties of the drug can be changed for betterment of its activity. The sound IC can be treated as a modified version of the drug which may lead to increase of water solubility of the drug and decrease its side effects (toxicity) or also may contribute in control drug delivery in near future with retention of its therapeutic activity.

Conflict of interest: There is no conflict of interest.

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TABLES

Table: 1: Data of Job's plot of (AB+β-CD) system obtained from Uv-visible spectroscopy.

Guest conc. [D] (µm)	β-CD (µm)	R= [D]/ ([D]+[β-CD])	A @λmax 212 nm	ΔA (2.02707-A)	ΔAx[AB]/ ([AB]+[β-CD])
100	0	1	2.02707	0	0
90	10	0.9	1.91375	0.11332	0.101988
80	20	0.8	1.69433	0.33274	0.266192
70	30	0.7	1.51287	0.5142	0.35994
60	40	0.6	1.27714	0.74993	0.449958
50	50	0.5	1.05889	0.96818	0.48409
40	60	0.4	0.8708	1.15627	0.462508
30	70	0.3	0.6174	1.40967	0.422901
20	80	0.2	0.38435	1.64272	0.328544
10	90	0.1	0.17471	1.85236	0.185236
0	100	0	0.07143	1.95564	0

Table.2: Values of Association constants (K_a) obtained by Benesi-Hildebrand method from UV-vis spectroscopy and corresponding free energy change (ΔG^0), enthalpy (ΔH^0), entropy (ΔS^0) of the AB.β-CD inclusion complex at 293.15K, 303.15K and 313.15K.

Complex	k_a ($10^3 M^{-1}$)			ΔG^0 (kJ mol^{-1})	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1}) K^{-1}
	293.15K	303.15K	313.15K			
AB - β-CD	6.78	4.42	1.26	-21.14	-63.86	-143.31

Table3. ^{1}H-NMR spectral data of Alibendol (AB), β-CD, AB.β-CD(IC).

Alibendol (400 MHz, Solvated DMSO) δ /ppm	2.507-2.516 (1H, S); 3.289-3.371 (2H, td, J=6.0, 6.8 Hz); 3.513-3.542 (2H, t, J= 6.0 Hz); 3.718-3.818 (5H, t, J=20.0Hz); 5.036-5.113 (2H m); 5.926-6.027 (1H, tt, J= 6.8Hz, 3.6Hz); 6.924-6.928 (1H, d, J= 1.6Hz); 7.27 (1H, d; δ8.75 J=2.0Hz).
β-CD (400 MHz, Solvated DMSO) δ /ppm	2.33–2.56 (6H, t, J = 9.2 Hz), h5=3.27–3.37 (6H, dd, J = 9.6, 3.2 Hz), h3=3.55–3.66 (18H, m), 4.44-4.47 (6H, t, J = 9.2 Hz), 5.67–5.74 (6H, d, J = 3.6 Hz).
Alibendol- β-CD IC (400 MHz, Solvated DMSO)δ /ppm	2.33–2.56 (6H, t, J = 9.2 Hz), 2.507-2.516 (1H, S);**h5**=3.26–3.37(6H, dd, J = 9.6, 3.2 Hz), 3.323-3.412(2H, td, J=6.0, 6.8 Hz); 3.513-3.542 (2H, t, J= 6.0 Hz); **h3**=3.51–3.60(18H, m),3.718-3.818 (5H, t, J=20.0Hz); 4.44-4.47 (6H, t, J = 9.2 Hz), 5.046-5.124(2H m);5.67–5.74 (6H, d, J = 3.6 Hz).5.947-6.044 (1H, tt, J= 6.8Hz, 3.6Hz); 6.924-6.928 (1H, d, J= 1.6Hz); 7.27 (1H, d; δ8.75 J=2.0Hz).

[a] mixed in 1:1 molar ratio, 400 MHz, Solvent: DMSO-d_6;

Table.4: The chemical shift values of the protons of β-CD, Alibendol in pure state and in complexed state and their deviations from pure to complex.

	σ(ppm)			
Protons	β -CD	IC	Alibendol	$\Delta\sigma$ ($\sigma_{complex}$-σ_{Pure})
H-3	3.605	3.555		-0.050
H-5	3.320	3.315		-0.005
1'H		3.36	3.33	0.03
2'H		5.99	5.97	0.02
3'H		5.08	5.07	0.01

Table.5: Data obtained from FT-IR spectroscopic study of β-CD, AB& β-CD+AB IC

Group	Wave number (Cm^{-1})		
	β-CD	AB	β-CD+ AB
stretching of O-H	3408		3385
stretching of –C-H from $–CH_2$	2941		2922
bending of –C-H from $–CH_2$ and bending of O-H	1404		…….
bending of C-O-C	1160		1155
vibration involving α-1,4linkage	954		949
Alkyl -C-H Stretching		3306	…..
Stretching =C-H		3039	…..
Stretching aromatic -C=C		1606	1631
$-CH_2-$ bending (m)		1465	…..
C-N stretching		1264	1266

Table S1. Data of Benesi-Hildebrand double reciprocal plot of the system (Alibendol+β-CD) obtained from Uv-Vis spectroscopy at 293.15 K.

A0	A1	ΔA	1/ΔA	1/CD
0.61996	0.75569	0.13573	7.367568	50000
0.61996	0.81599	0.19603	5.10126	33333
0.61996	0.86508	0.24512	4.079634	25000
0.61996	0.91828	0.29832	3.352105	20000
0.61996	0.97096	0.351	2.849003	16667
0.61996	1.00208	0.38212	2.616979	14286

Table S2. Data of Benesi-Hildebrand double reciprocal plot of the system (Alibendol+β-CD) obtained from Uv-Vis spectroscopy at 303.15 K.

A0	A1	ΔA	1/ΔA	1/CD
0.69196	0.81161	0.11965	8.35771	50000
0.69196	0.85686	0.1649	6.064281	33333
0.69196	0.91998	0.22802	4.38558	25000
0.69196	0.94354	0.25158	3.974879	20000
0.69196	0.97856	0.2866	3.489184	16667
0.69196	1.02998	0.33802	2.958405	14286

Table S3. Data of Benesi-Hildebrand double reciprocal plot of the system (Alibendol+β-CD) obtained from Uv-Vis spectroscopy at 313.15 K.

A0	A1	ΔA	1/ΔA	1/CD
0.78975	0.93989	0.15014	6.66045	50000
0.78975	0.99162	0.20187	4.953683	33333
0.78975	1.09739	0.30764	3.250553	25000
0.78975	1.1664	0.37665	2.654985	20000
0.78975	1.1883	0.39855	2.509095	16667
0.78975	1.24439	0.45464	2.199542	14286

Table S4. The van't Hoff equation data for the calculation of thermodynamic parameters (ΔH^0, ΔS^0, ΔG^0) of the inclusion complex (AB+β-CD).

k_a of the complex * 10^3	ka of beta complex	T	1/T	log(k_a)
6.78	6780	293.15	0.003411	3.83123
4.42	4420	303.15	0.003299	3.64542
1.26	1260	313.15	0.003193	3.10037

FIGURES

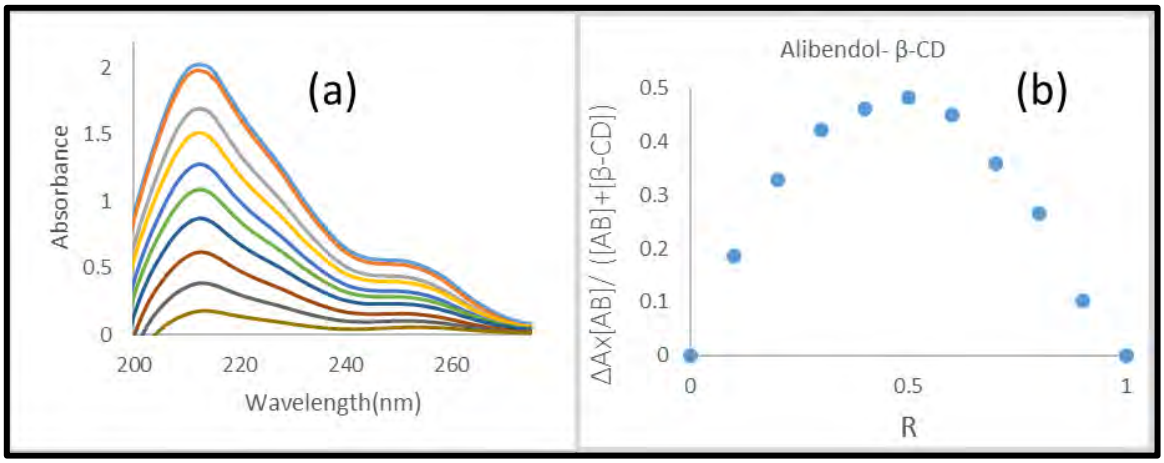


Fig.1. (a) Spectra of Job's plot of AB.β-CD at λ_{max}=212 nm, (b) Job plot of 1:1 stoichiometry where R=0.5; R= [Drug]/([Drug]+[CD])

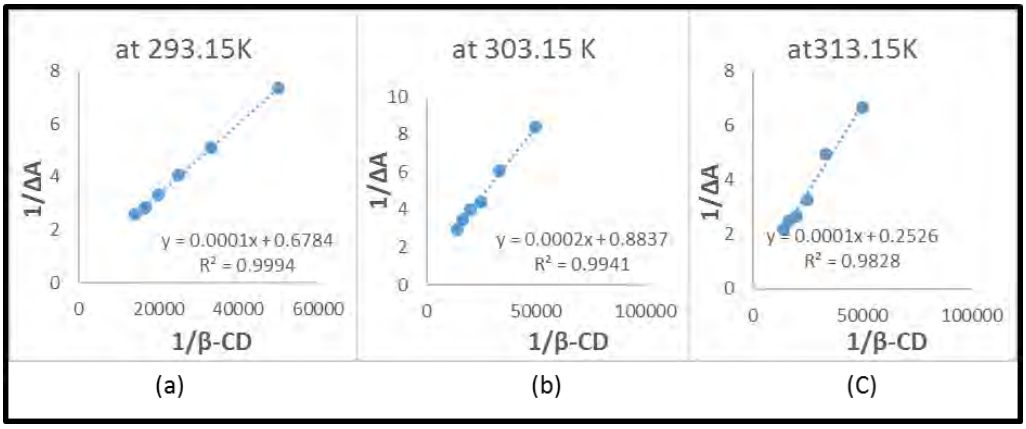


Fig. 2: Benesi-Hieldebrand double reciprocal plots of 1/ΔA against 1/β- [CD] at (a)293.15K, (b)303.15K and (c) 313.15 K.

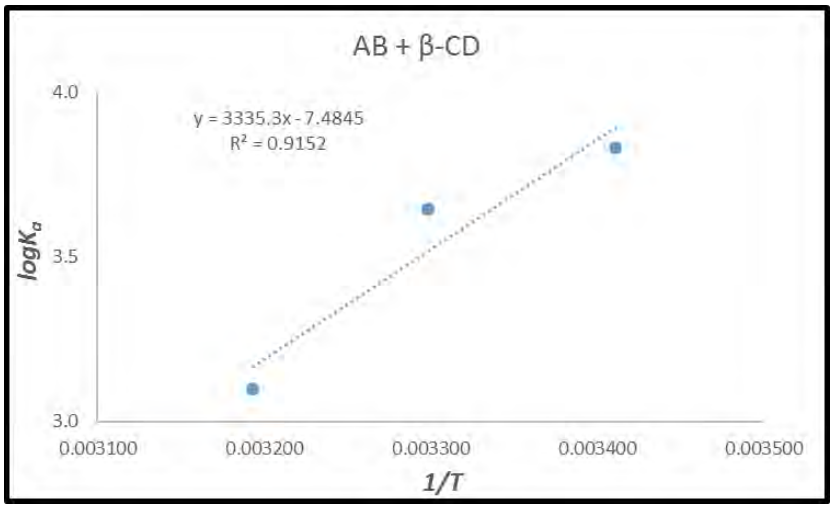


Fig. 3: Plot of $\log k_a$ Vs 1/T for the determination of thermodynamic parameters.

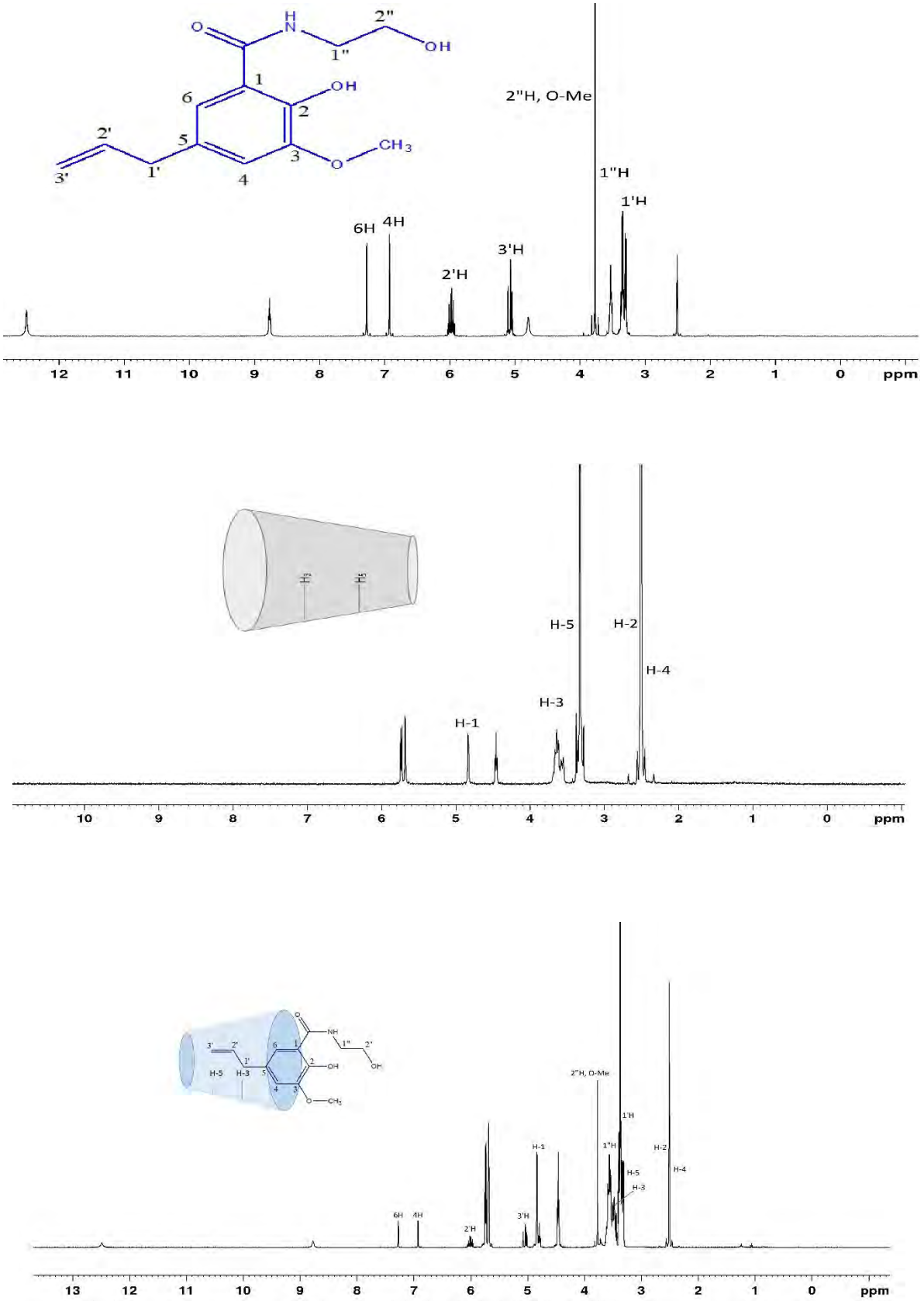


Fig.4.[1]H NMR spectra of pure Alibendol (top), pure β–CD (middle), AB - β–CD IC (bottom). (In d_6-DMSO, 400 MHz)

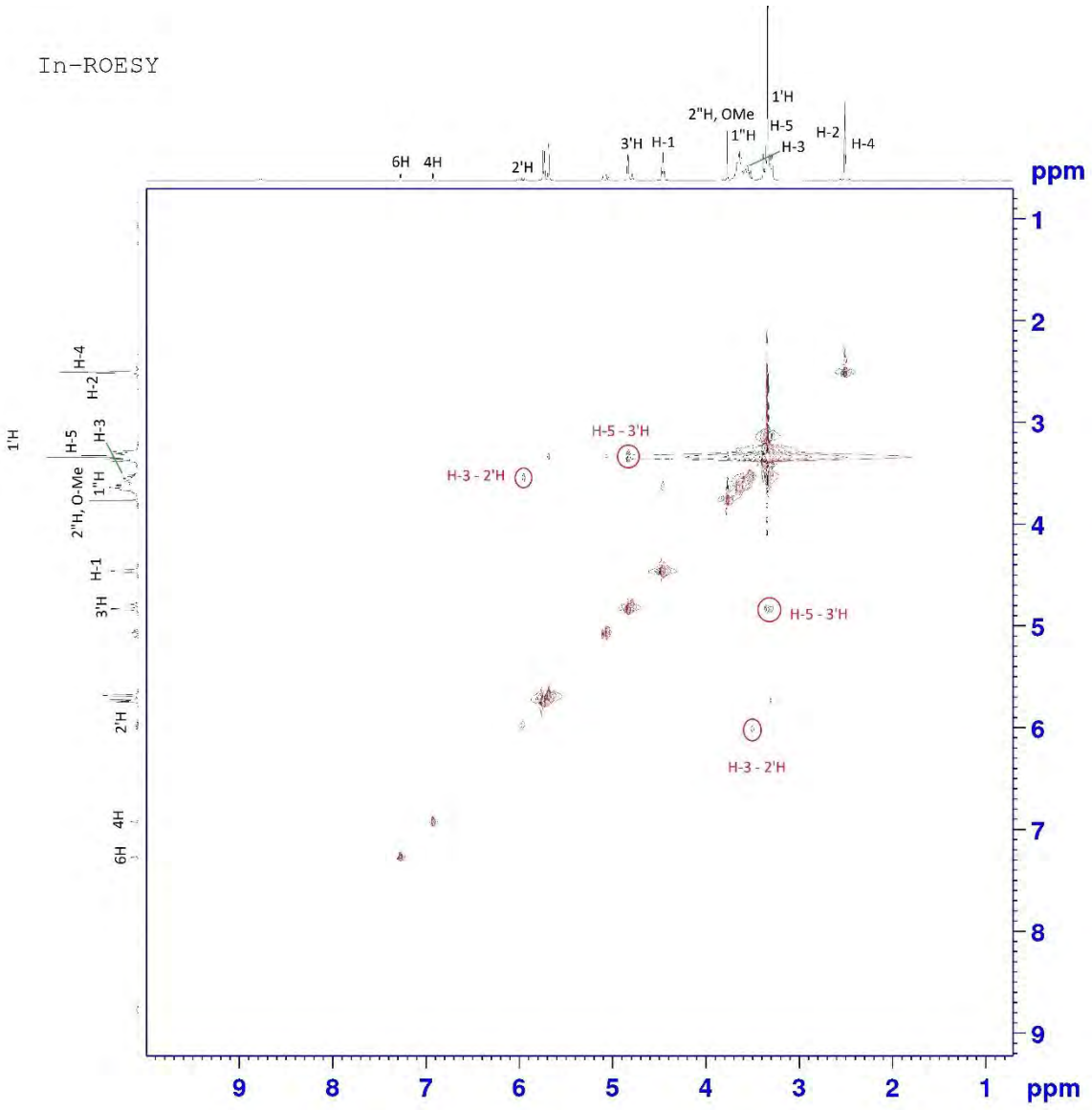


Fig 5: 2D ROESY spectra of the solid IC of AB -β-CD in d_6-DMSO. (Cross correlations are indicated by red circles)

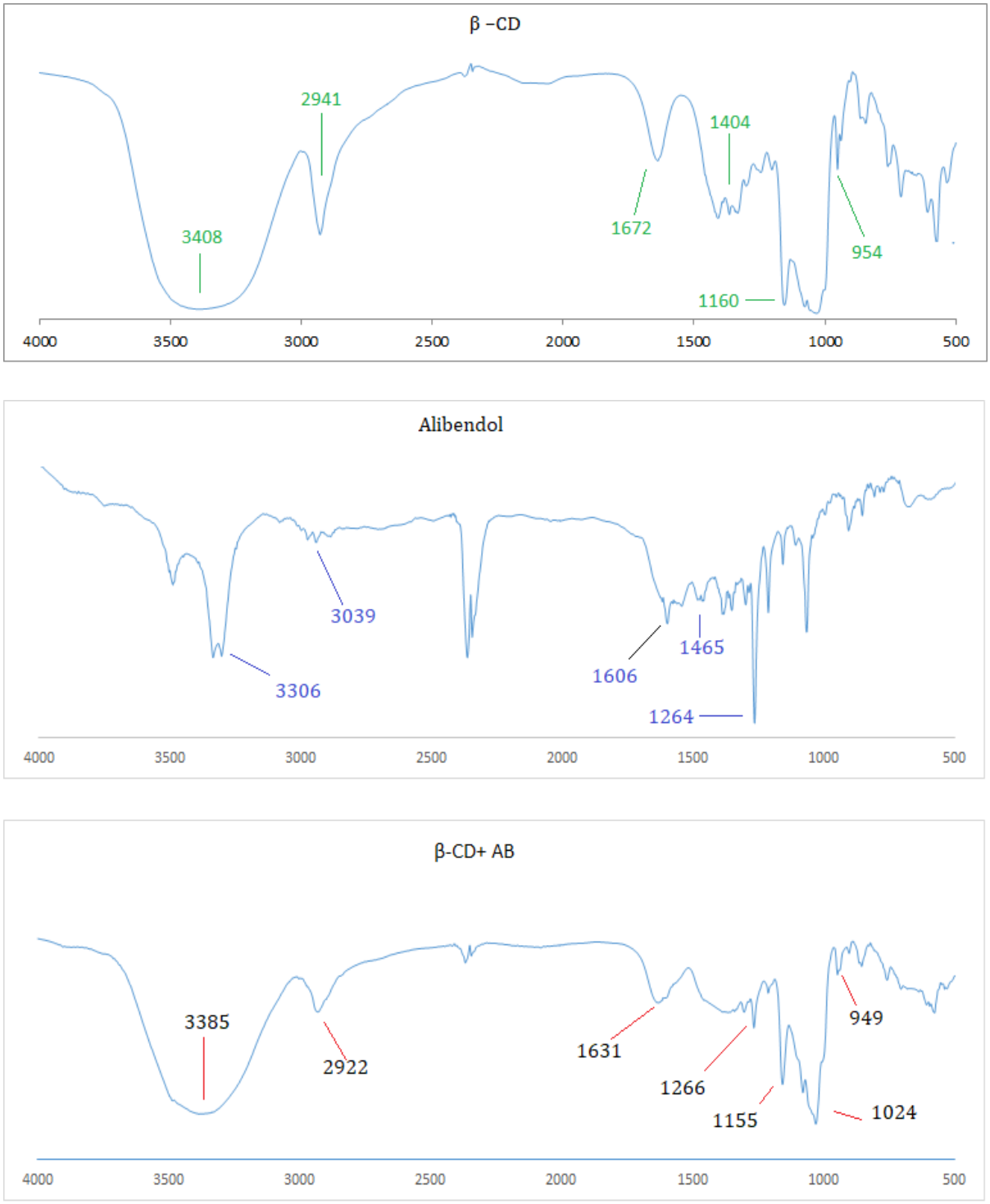
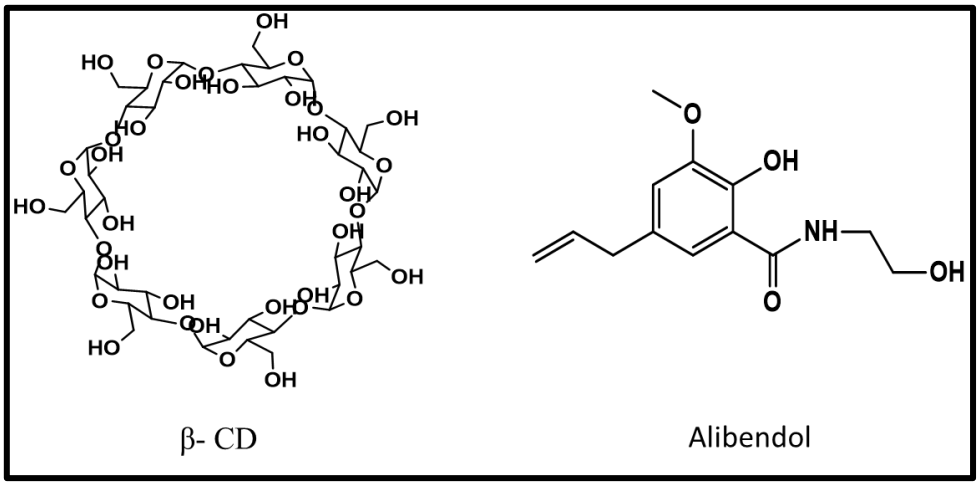
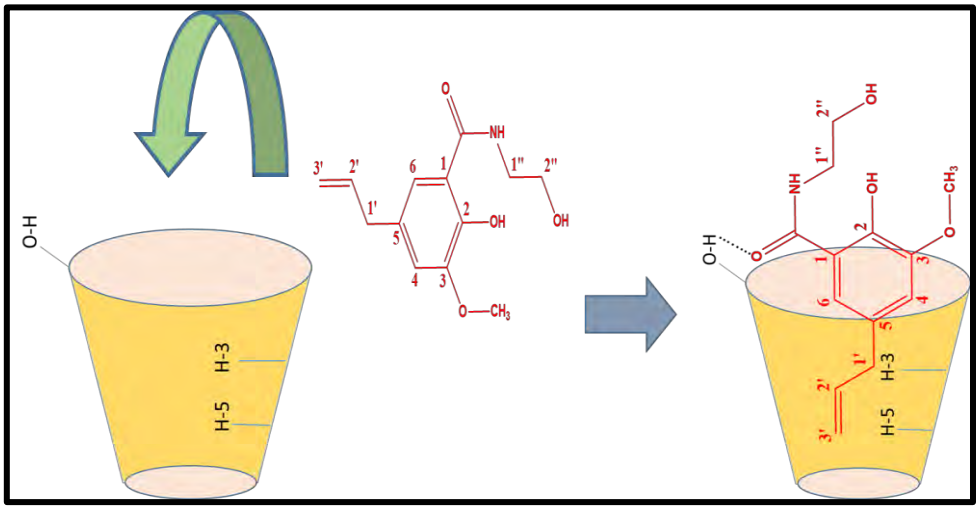


Fig.6.FT-IR Spectra of (top) pure β –CD (middle) pure Alibendol and (bottom) AB . β–CD inclusion complex.

SCHEMES



Scheme 1. Structures of the molecules



Scheme 2. Probable mechanism of inclusion.