CHAPTER IV

Subsistence of Inclusion Complex via Assembly of a Drug into cyclic Oligosccharide: Its Formation, Mechanism, Behaviour and Importance

IV.1. HIGHLIGHTS

- β-Cyclodextrin (β-Cyd) forms Inclusion complex with DEPM with 1:1 stoichiometry.
- Association constant measuements in two different pH ranges show the effect of pH.
- ^{1}H NMR provides confirm evidence about the solid inclusion phenomenon (ICs).
- Solid complex formation between β-Cyd and DEPM is established by FTIR spectroscopy.
- Direct evidence of formation of solid ICs has got from powder XRD method and SEM.

IV.2. INTRODUCTION

Cyclodextrin (Cyd) is the cyclic oligosachharide having torus shaped cavity with wide and narrow openings rather than the perfect cylindrical shaped [1]. Based on the structural design they containing α-1,4 linked glucopyranose unit with hydrophilic primary hydroxyl groups on narrow side and secondary hydroxyl groups on wide edge and hydrophobic cavity in the core (**Scheme1**) which can hold completely or at least partially a large variety of polar and non-polar guest molecules [2] e.g. drugs [3,4], amino acids[5,6], polymers[7,8], ionic liquids [9,10] vitamins [11], dyes [12] and so on. Actually, the hydrophobic part of the guest molecule is accommodated into the hydrophobic cavity of the cyds whereas the hydrophilic part (if any) of the guest are associated via non-covalent bonds (hydrophobic interactions, van der Wall attractions, hydrogen bonding etc.) with polar side of cyds causing stabilisation of inclusion complex(s). The encapsulation capacity of Cyd with guest molecules has introduced great advantages that are widely applied in various industries. The most common industrial uses of such inclusion complexes are to enhance the solubilisation rate of poorly soluble or immiscible drugs in aqueous environment and promote to improve its

bioavailability [13-16]. Additionally, they can be used to convert liquid drugs into powders, prevent drug–drug interactions, reduce or eliminate unpleasant smell or taste [13]. Encapsulation of guest molecules into Cyd modifies some physicochemical properties such as state, solubility, stability against heat, light and oxidation, reduction of volatility of the guest molecules without affecting the framework structure of host molecules and more benefits applications are obtained after formation of inclusion complex [15-20].

Diethyl phenylmalonate (DEPM) belongs to drug family. The water insoluble mono-substituted aromatic malonic ester (DEPM) has enormous potentials in the synthesis of another kind of drugs that acts as central nervous system depressants [21]. So, DEMP is very effective drug and has huge applications in designing drug delivery system.

The purpose of the present work is to investigate and justified the interaction between DEPM and β- Cyd (**Scheme1**) with proper stoichiometry ratio; enrichment of solubility of DEPM in water and finally, ^{1}HNMR, FTIR, powder XRD and SEM methods have been carried out to confirm the formation of solid inclusion complex (IC). The most benefit of this work is that the smell of DEPM is removed and the pharmaceutical industries can be able to overcome the complications with water immiscible DEPM drug. It is hoped that the complexation with the drug receptor β-Cyd may permit for the development of analytical and remediation actions.
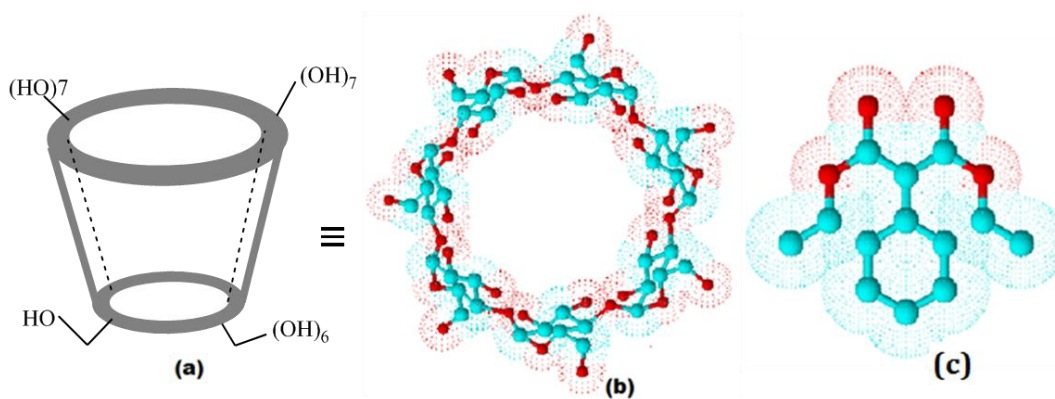
Scheme 1. Geometry of β- Cyd (a) and Ball & stick representation of (b) β- Cyd and (c) DEPM; blue for carbon and red for oxygen.

IV.3. EXPERIMENTAL SECTION

IV. 3.1. Reagents

The selected drug diethyl phenylmalonate (DEPM) (mass fraction purity ≥ 0.98) and β- Cyd of purris grade are purchased from sigma-Aldrich, Germany and have been used without further purification. Ethanol (purity ≥ 0.99) is purchased from Merck. Buffer capsules having pH 7.0 ± 0.05 and pH 9.2 ± 0.05 are brought from Merck. Triply distilled and degassed water is used to ready all solutions. Each buffer capsule is twisted and pulled apart and then dissolved in 100 ml triply distilled and degassed water to prepared desired pH solution.

IV. 3.2. Apparatus

UV-visible spectra were recorded by JASCO V-530 UV/VIS Spectrophotometer. All the absorption spectra were recorded at 25°C ±1°C.

NMR spectra were recorded at 300 MHz Bruker ADVANCE at 298.15K in D_2O. Signals are quoted as δ values in ppm using residual protonated solvent signals as internal standered (D_2O: δ 4.71 ppm). Data are reported as chemical shift.

Infrared spectra were measured in Perkin Elmer, FTIR spectrometer.

The powder XRD patterns of the compounds were recorded by using Cu-Kα radiation (Bruker D8 Discover; 40kV, 30 mA).

The scanning electron microscopy (SEM) images were obtained using Nova Nanosem 600, FEI Field emission scanning electron microscope (FESEM).

IV.3.3. Preparation of solid inclusion complex of DEPM with β- Cyd

First 1.134 gm of β- Cyd with 30 ml triply distilled and degassed water is mixed in a round bottom flask and is stirred enough over magnetic stirrer. Then 0.236 gm of DEPM is placed into a beaker and 10 ml ethanol is poured on it and stirred over magnetic stirrer until it makes a homogeneous mixture (completely dissolved). 1:1 molar ratio of β- Cyd and DEPM has been used. After that, the guest mixture is added into β- Cyd solution and stirred for 48hrs without a break. The reaction mixture has been put in refrigerator for 52hrs without any disturbance. After 2 days later a white precipitation was observed. 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 50°C for 24 hrs. The resultant solid is inclusion complex between DEPM and β- Cyd [22]. It is further analysed by FTIR, NMR, powder XRD and SEM method.

IV. 4. RESULTS AND DISCUSSION

IV.4.1. Job plot: Elucidation of Stoichiometry behaviour of β- Cyd: DEPM inclusion complex

The stoichiometry ratio of β- Cyd: DEPM (host: guest) IC has been provided by using the continuous variation Job's method [23]. The Job plot has been done in neutral (7.0±0.5) and alkaline (9.2±0.5) medium. In this method, the total molar concentration of the two binding partners ([β- Cyd] + [DEPM]) is kept constant at 100µM but their mole fraction are varied so that the mole fractions of DEPM complete the range of 0-1(R= [DEPM]/ [β- Cyd] + [DEPM]) (**Table 1, Table 2**) [24, 25]. The absorbance values are measured at $\lambda_{max\ at}$ 298.15K for a series of solutions. The plot of ΔA ×R against R represents the job plot (where ΔA = A_o – A, is the shifts of absorbance at λ_{max} of β- Cyd -DEPM complex [A] from absorbance at λ_{max} of pure DEPM [A_o]) as shown in **Figure. 1**. The resultant plot shows the maximum deviation of R at 0.05 in both cases with well symmetrical shapes which signify the occurrence of 1:1 stoichiometry between β- Cyd and DEPM [26].
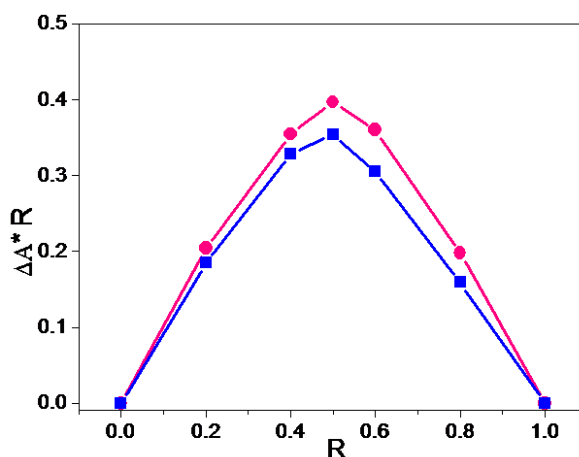
Figure 1. Job plot of DEPM-β- Cyd systems at 298.15K in neutral (pink, ●) and alkaline (blue, ■) medium.

Table 1. Data for Job plot obtained from UV-spectroscopy for aqueous (neutral)-ethanol β- Cyd -DEPM system at 298.15K[a].

DEPM (mL)	β-Cyd (mL)	[DEPM] (μM)	[β-Cyd] (μM)	$R=\dfrac{[DEPM]}{[DEPM]+[\beta\text{-Cyd}]}$	Absorbance (A)	ΔA	ΔA*R
0	3	0	100	0.0	0.0	1.12039	0.0
0.6	2.4	20	80	0.2	0.09776	1.02263	0.2045
1.2	1.8	40	60	0.4	0.23275	0.88764	0.3551
1.5	1.5	50	50	0.5	0.32572	0.79467	0.3973
1.8	1.2	60	40	0.6	0.52000	0.60039	0.3602
2.4	0.6	80	20	0.8	0.87265	0.24774	0.1982
3	0	100	0	1	1.1204(A_o)	0.0	0.0

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

Table 2. Data for Job plot obtained from UV-spectroscopy for aqueous (alkaline)-ethanol β- Cyd -DEPM system at 298.15K[a].

DEPM (mL)	β-Cyd (mL)	[DEPM] (μM)	[β-Cyd] (μM)	$R=\dfrac{[DEPM]}{[DEPM]+[\beta\text{-Cyd}]}$	Absorbance (A)	ΔA	ΔA*R
0	3	0	100	0.0	0.0	1.12039	0.0
0.6	2.4	20	80	0.2	0.19338	0.92701	0.1854
1.2	1.8	40	60	0.4	0.30038	0.82001	0.3280
1.5	1.5	50	50	0.5	0.41272	0.70767	0.3538
1.8	1.2	60	40	0.6	0.61186	0.50853	0.3051
2.4	0.6	80	20	0.8	0.92128	0.19911	0.1593
3	0	100	0	1	1.12039(A_o)	0.0	0.0

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

IV.4.2. Association Constant: Interaction of DEPM with β- Cyd in liquid environment

The UV absorption spectra of DEPM in aqueous β- Cyd medium are carried out in pH = 7.0±0.5 and pH =9.2± 0.5 respectively. The spectral data of DEPM in various concentration of β- Cyd in different pH has been listed in **Table 3**. Both the **Figure.2a** and **Figure.2b** depicts the absorption spectra of DEPM (2×10^{-4} M) in absence and presence of β- Cyd solutions. The strong absorption peaks of DEPM (2×10^{-4}M) appears at 208 nm and with addition of β- Cyd blue shift is occurred in both case. No considerable isosbestic point is noticed in the spectra. In each case the absorbance intensities of DEPM gradually increase with increasing the concentration of β- Cyd. This fact confirms the encapsulation of guest molecule into the β- Cyd cavity due to the presence of hydrogen bonding, hydrophobic and van der Waals interaction between the guest monomer and β-Cyd molecules [26, 27]. So, such non- covalent interactions act as the main driving forces to be integrated the guest molecule into the β-Cyd cavity to form 1:1 IC throughout the complexation process by promoting the dissolution of the guest molecule (DEPM).
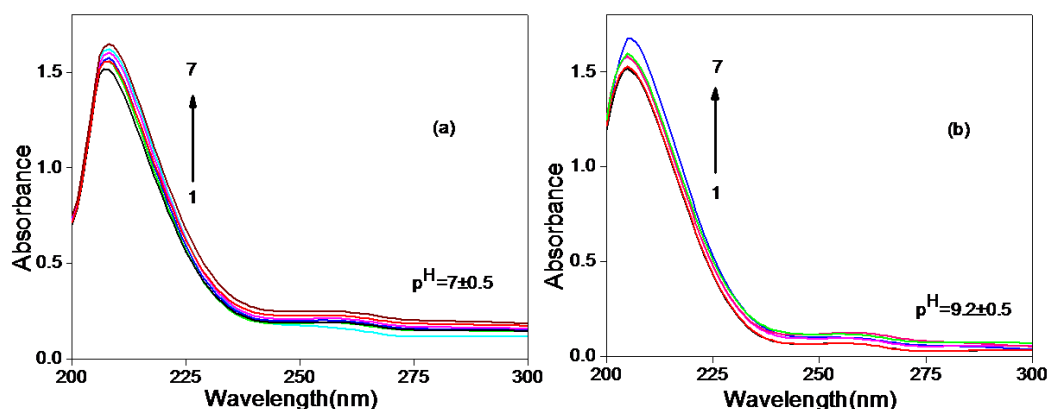
Figure 2. Adsorption spectra of DEPM (2 x 10-4 M) in (a) pH=7.0±0.5 and (b) pH=9.2±0.5 at different concentration of β-Cyd (1) absence of β- Cyd, (2) 0.002M, (3) 0.004M, (4) 0.006M, (5) 0.008M, (6) 0.010M, (7)0.012M.

Table 3. Absorption values of DEPM (Conc. 2× 10^{-4} M) at different concentrations of β-Cyd in pH=7.0±0.5 and pH=9.2±0.5 buffer solutions at 298.15K[a].

Sl. No.	Conc. of β- Cyd (M)	pH= 7.0±0.5		pH= 9.2±0.5	
		λ $_{max}$ (nm)	Absorbance	λ $_{max}$ (nm)	Absorbance
1	0.000	208	1. 51612	208	1. 51612
2	0.002		1.54186		1.53117
3	0.004		1.56257		1.54546
4	0.006		1.58195		1.55601
5	0.08		1.60012		1.56909
6	0.010		1.62005		1.57526
7	0.120	206	1.63011	205	1.58966
K (M^{-1})			121.84		108.78
ΔG KJ mol^{-1}			-11.90		-11.62

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

In both cases, from the Job plot it is confirmed that DEPM-β-Cyd form 1:1 IC. Hence, the IC formed between DEPM and β-Cyd can be expressed as

$$\text{β-Cyd} + \text{DEPM} \rightleftarrows \text{β-Cyd} \cdots \text{DEPM}$$
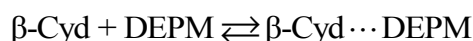
For a 1:1 complexation process the association constant (K) has been estimated by using the double reciprocal plots on the basis of Benesi-Hildebrand equation[28].The absorption values are used in the following Benesi-Hildebrand equation 1[23].

$$\frac{1}{A - A_o} = \frac{1}{Ka(A'' - A_o)} \cdot \frac{1}{[\text{β-Cyd}]} + \frac{1}{(A'' - A_o)} \tag{1}$$

Figure.3 depicts the plot of 1/A-A$_o$ against 1/ [β-Cyd] for DEPM in both pH. In both cases a good linear correlation (R^2$_{(7.0±0.5)}$ =0.999; R^2$_{(9.2±0.5)}$ =0.998) are occurred and showing that both IC is 1:1. The values of K are evaluated by using the equation 2 from the slop values of straight lines. The resultant association constant of DEPM

in neutral medium (K= 121.84) is significantly high than its alkaline medium (K= 108.78) (**Table 3**).

$$K = \frac{1}{\text{Slope}(A^{"} - A_o)} \qquad (2)$$
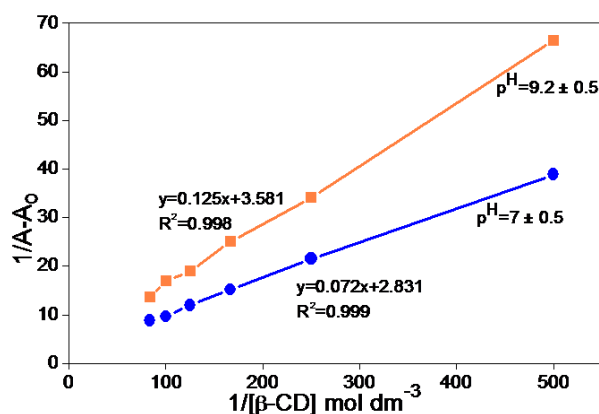
Figure 3. Benesi-Hildebrand plot of $1/A\text{-}A_o$ vs. $1/$ [β-CD] for DEPM in pH=7.0±0.5 and pH=7.0±0.5 at 298.15K.

IV.4.3. Effect of pH

At pH~7 DEPM exists as a neutral form. We have also recorded the UV spectral data in alkaline medium at pH~ 9.2 to see how much pH influences on UV spectra as well as on association constant. The spectral data shows that there is marginal shift in absorption at these pH ranges and the spectral shape of DEPM in both pH are very close to each other. Therefore, it has been concluded that as the association constant (K) is very sensitive to change in pH range [29], hence we have got the significant change of association constant (K) in neutral and alkaline medium respectively.

IV. 4.4 Spontaneity of inclusion complex

The free energy change (ΔG) is a very vital thermodynamic parameter that has been easily estimated from association constant (K) by using the following equation 3 [24, 29]

$$\Delta G = -RT \ln K \qquad (3)$$

The ΔG values for the two binding partners (DEPM and β- Cyd) are negative (-ΔG) (**Table 3**) which indicates that the host-guest IC proceeded spontaneously at 298.15K and the complexation is an exergonic process.

IV.4.5. ^{1}HNMR

The formation of IC can be explained on the light of the ^{1}HNMR spectroscopy study [30, 31] This method based on the changes of chemical shifts of protons due to encapsulation of guest molecule into the β- Cyd cavity [3,25, 30]. In β- Cyd structure the H3 (near to wider opening side) and H5 (close to narrow rim side) are located inside part of the β- Cyd cavity. The H6 of methylene group (bearing the primary –OH group) remains on the narrow opening side of β- Cyd and the rest of the other H- atoms H1, H2, H4 are situated on the outer surface of β- Cyd as shown in **Scheme2** [26,31]. The ^{1}HNMR spectra of β- Cyd, pure DEPM and the solid IC of DEPM with β- Cyd (1:1) are represented in **Figure 4.** It is clearly observed from table 1 that for H3 and H5, a large upfield shift has been occurred. The considerable changes of chemical shifts (Δδ) suggested that the DEPM monomer entered into the nano hydrophobic hole of β- Cyd. The upfield shift of H3 (Δδ= 0.142 ppm) is much greater than the H5 shifting (Δδ= 0.067 ppm) and the complexation of DEPM with β- Cyd splits the signal of H3 into doublets **Figure 4b**. On the other hand, minor chemical shifts are observed for H1, H2, H4 and H6 that are not part of the interior hydrophobic hole of β- Cyd.

Similarly, further support for complexation, 1HNMR of DEPM has also been performed in DMSO-D$_6$ and significant downfield shifting for the aromatic ring protons of DEPM that are situated in hydrophobic hollow space of β- Cyd have been found because of the interactions of DEPM with β- Cyd [32-34]. These all changes clearly indicate that well encapsulation of the aromatic ring of the guest DEPM into interior hydrophobic cavity of β-Cyd has been occurred and enters through the wider ring opening side as shown in **Scheme 2**. The detailed variations of chemical shifts of the two binding partners before and after forming IC have been mentioned in **Table 4.**
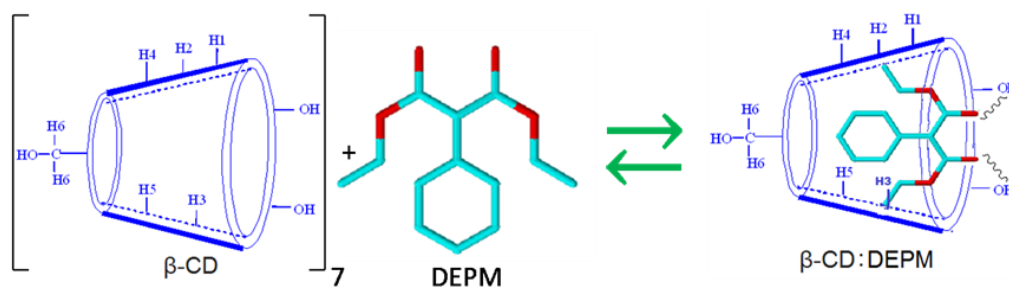
Scheme 2. The probable mechanism of the reaction of the inclusion complex of DEPM with β- Cyd.
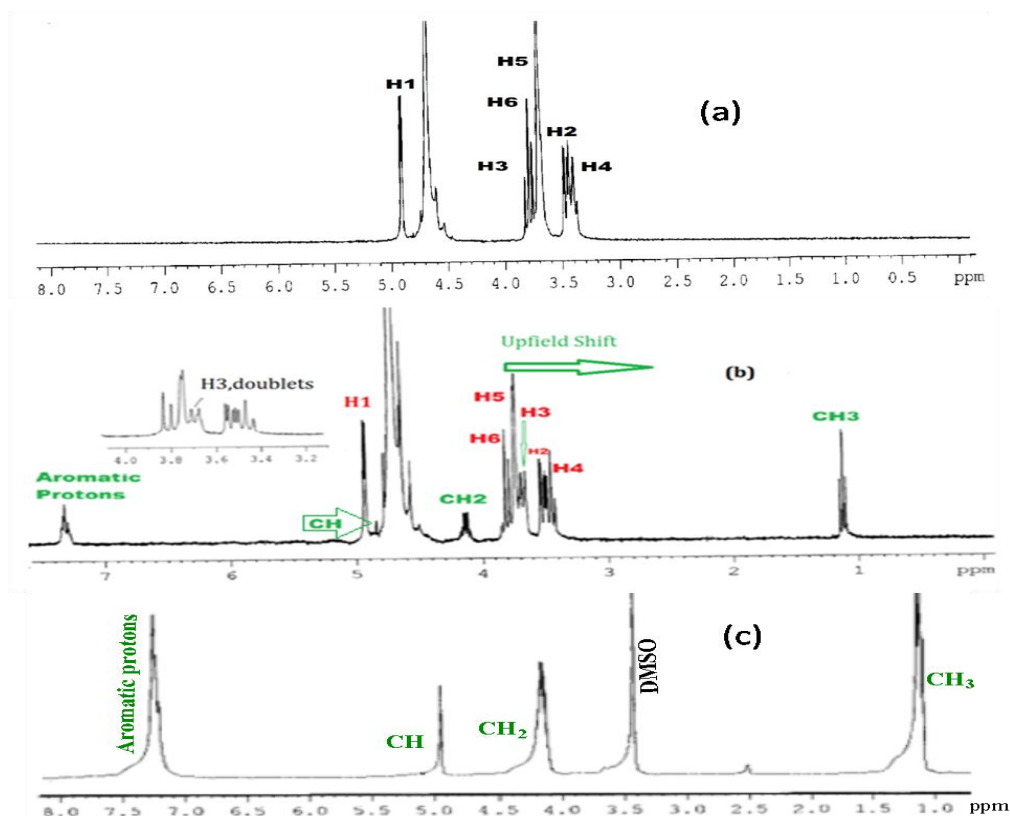
Figure4. 1HNMR spectra of (a) β- Cyd, (b) 1:1 molar ratio of β-CD:DEPM IC in D_2O and 1HNMR spectra of (c) DEPM in DMSO-D_6 at 298.15 K.

Table 4. ^{1}H NMR chemical shift displacements of β-Cyd, DEPM and β-Cyd/ DEPM at 1:1 molar ratio at 298.15K[a].

β- Cyd protons	free β-Cyd δ (ppm)	β- Cyd: DEPM δ (ppm)	Δ δ (ppm)
H1	4.928	4.923	0.005
H2	3.484	3.479	0.005
H3	3.860	3.718	**0.142**
H4	3.412	3.405	0.007
H5	3.793	3.726	**0.067**
H6	3.820	3.809	0.011
DEPM protons	free DEPM δ (ppm)	β- Cyd: DEPM δ (ppm)	Δ δ (ppm)

| Aromatic ring | 7.252-7.298 | 7.285-7.321 | n.c |
CH	4.938	4.798	0.14
CH_2	4.093-4.204	4.108-4.153	n.c.
CH_3	1.093-1.140	1.149-1.176	n.c.

[a] Standard uncertainties in temperature (T)=0.01K.; n.c. = not calculated.

IV.4.6. FTIR

Successful solid formation between host and guest molecules is established by FTIR spectroscopy since the frequencies of bands of the integrated guest molecule become shifted or nowhere to be found in the resulting form or their intensities altered [25,35-36]. The non- covalent interactions like hydrogen bond (H-bond), hydrophobic and Van der Waals interaction that appear in complex are responsible for the changes. From the **Figure 5** it is clear that there are prominent differences in β-Cyd (**Figure 5a**) and DEPM (**Figure 5b**) spectra with the resulting solid complex (**Figure 5c**). The broad band at 3368 Cm^{-1} that appears in pure β-Cyd spectrum indicates the valence vibrations of O−H (v_{br}(O−H)) groups connected by H-bond. The v_{sp} ^{3}(C−H) bond stretching of β-Cyd is assigned at 2923 cm^{-1}. The v_{bend}(O−H) stretching of COH groups and water molecules is recorded at 1638 cm^{-1}. The v(C−O−C) and v(C−C−O) bond stretching are found at 1157 cm^{-1} and 1030 cm^{-1} respectively [37-39]. The characteristic absorption peak due to v(>C=O) and v(C−O−) of ester groups is assigned at 1735 cm^{-1} and 1031 cm^{-1}. The former peak (1735 cm^{-1}) is appeared in complex with a new peak at 1725cm^{-1}. This suggests that the Fig. 5 shows a molecular association is arising from hydrogen bond between the oxygen atom of ester groups (>C=O) of included drug and the rim hydroxyl groups of β-Cyd via O-H···O=C< which weakens the >C=O double bond character and causing the v(>C=O) frequency mode to vibrate at a lower frequency [7,40-41]. In complex

spectra (**Figure 5c**) there is a sharp peak at 1032 cm^{-1} which indicates that either the C–O– bond encapsulates into the β-Cyd cavity and shifts to 1032 cm^{-1} or it is being masked with the v(C–C–O) bond frequency of β-Cyd but the former seems more reliable (**Scheme 2**). In IR spectra of DEPM, the stretching frequency of $v_{benzene}$ (C–H$_{sp}^2$) at 3066 cm^{-1}, 3032 Cm^{-1} and $v_{benzene}$ (C=C) at 1603 cm^{-1}, 1498 cm^{-1} are recorded. The vibration spectra v_{sp}^3(C–H) of –CH$_2$–CH$_3$ moiety is found at 2983 cm^{-1}. For mono-substituted benzene ring the peak is appeared at 728 cm^{-1}.

In the spectra of β-Cyd -DEPM complex, it is already mentioned above that the peak of >C=O group is altered. Additionally, it is observed that the frequency peak of $v_{benzene}$ (C–H$_{sp}^2$), $v_{benzene}$ (C=C) and v_{sp}^3(C–H) does not appear in complex. The mono-substituted sharp and prominent peak that appeared at 728cm^{-1} is absent in **Figure 5c**. This indicates that the aromatic ring part is in good agreement with the β-Cyd hydrophobic cavity core. From the above discussion it could be concluded that the DEPM guest molecule fits properly and entrapped into β-Cyd hollow space and resulting complex is "Host-Guest" type.
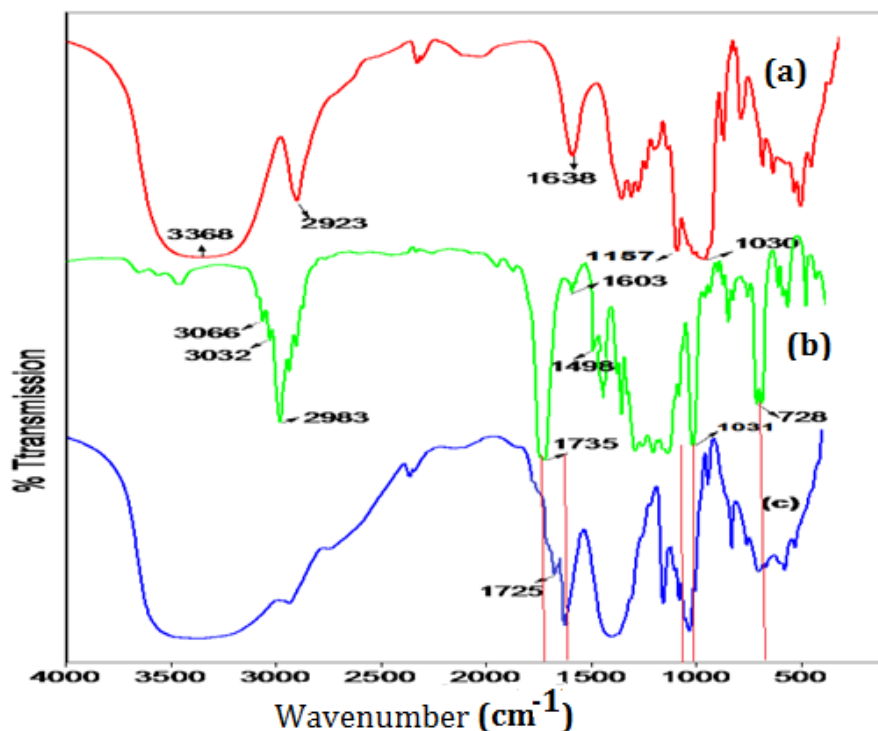
Figure 5. FT-IR spectra of (a) β- Cyd (in KBr), (b) DEPM and (c) DEPM: β- Cyd (1:1 molar ratio) solid complex (in KBr).

IV.4.7. Powder X-ray diffraction pattern

The direct evidence for the complexation between host and guest molecule can be obtained from the analysis of powder X-ray diffraction spectrum [42-44]. **Figure 6** shows that the diffraction pattern and the characteristic peak intensity of the resultant IC are quite different from the diffractgram of β-Cyd monomer which suggests the formation of a new phase of solid inclusion complex. The characteristic peaks of β-Cyd are assigned at 2θ (degree) values of 9.04, 10.65, 12.43, 14.71, 15.30, 17.06, 19.61, 21.12, 22.64, 24.33, 27.12, 31.94 and 34.72 (**Figure 6a**) and these peaks were not overlay with the peaks of the solid inclusion complex (**Figure 6b**). In β-Cyd: DEPM solid, the new peaks appeared at 2θ (degree) values of 3.11, 5.54, 6.56,

11.18, 14.49, 15.04, 17.55, 23.51. This study clearly established that DEPM forms IC successfully with β-Cyd.
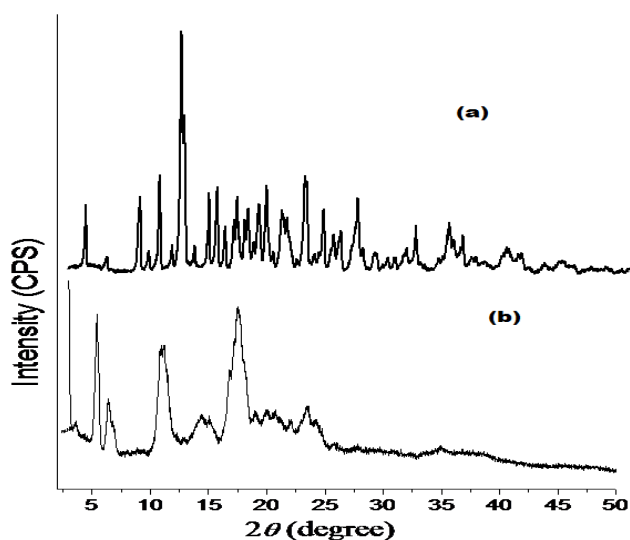
Figure 6. Powder X-ray diffraction pattern of (a) β-Cyd and (b) DEPM: β-Cyd (1:1 molar ratio) inclusion complex.

IV.4.8 Scanning Electron Microscopy (SEM)

Electron Microscopy (SEM) is a very well known technique for analyzing the surface texture and particle size of solid materials [26,42,45-46] The surface morphological structures of β-Cyd and the solid IC (DEPM: β-Cyd) are shown in **Figure 7a** and **Figure 7b** respectively. From **Figure 7** it is clear that the two morphological structures are totally different from each other. This gives clear evidence that the DEPM fits enough into the hydrophobic cavity of β-Cyd to form of solid IC.
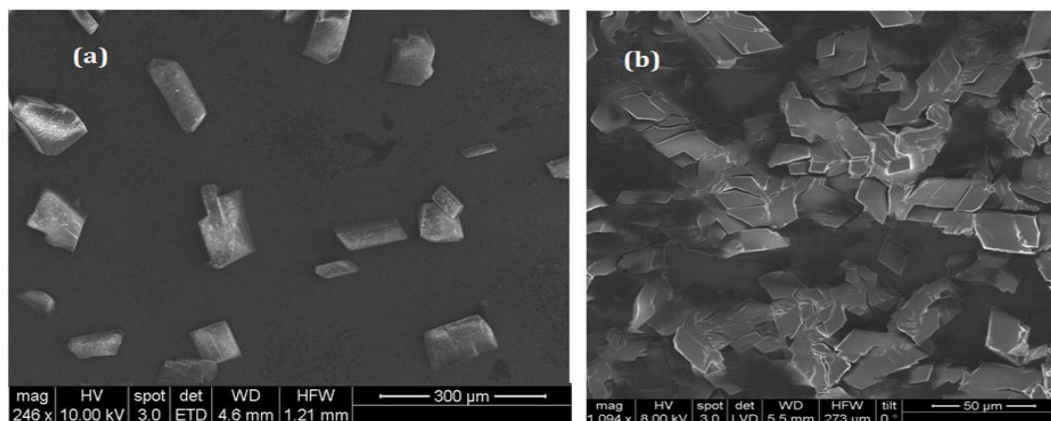
Figure7. Scanning electron microscope (SEM) morphology of (a) β-Cyd and (b) DEPM: β- Cyd (1:1 molar ratio) inclusion complex.

IV.5. CONCLUSION

The interactions of DEPM with β-Cyd are investigated in liquid medium by UV-spectra in terms of Job plot and association constant (K). Both satisfy the formation of IC with 1:1 stoichiometry. Different pH environment shows that it has great influence on association constant (K). Determination of ∆G value indicates that the process is thermodynamically controlled. The results come from ^{1}HNMR, FT-IR, powder XRD and SEM studies suggest that the selected guest molecule DEPM monomer formed IC with nano hydrophobic core of β-Cyd effectively which will make possible DEPM to be solubilised in water successfully.

IV.6. REFERENCES

References of CHAPTER IV are given in BIBLIOGAPHY (Page: 228-231).