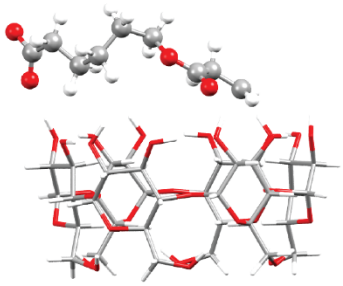
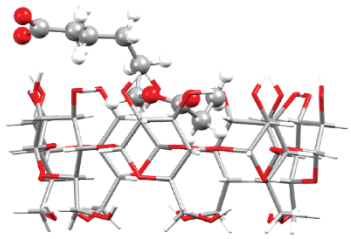
CHAPTER-VII

Subsistence and Energy Optimization of Inclusion Complexes of Azelaic Acid with α and β-CD Molecules and their Extensive Comparison by Physicochemical and Computational Methodologies



Inclusion of Azelaic acid in α-CD



Inclusion of Azelaic acid in β-CD

ABSTRACT

In this study, we have compared the ability of inclusion complex formation of Azelaic acid with α and β-CD and the phenomenon has been investigated using UV-Visible, NMR spectroscopic methods, HRMS, SEM study along with DFT study .Conductivity and Job's plot method was used to determine the stoichiometry. Benesi-Hildebrand equation was employed to determine association constant for both systems. The β-CD inclusion complex got stabilized by the intermolecular hydrogen bonding and DFT data reveals that. Enthalpy and Gibbs free energy of formation indicates that the formation of β-CD inclusion complex is spontaneous. This encapsulation is noteworthy for its stabilization against external hazards like oxidation, degraDAtion, and photolytic cleavage, persistance for the efficient release of an required amount of drug at the targeted site when applied for a number of dermatoses and acne treatment.

Keywords

Azelaic acid(AA), α-Cyclodextrin αα(α-CD), β-Cyclodextrin (β-CD), DFT Study, Inclusion complex(IC).

EXPERIMENTAL SECTION

Materials used

Azelaic acid (≥ 98%), Methyl orange(ACS reagent,dye content 85%), α-Cyclodextrin (≥ 98%) and β-Cyclodextrin (≥ 97%) were purchased from Sigma-Aldrich and used without further purification.

Distilled water (specific conductivity < 1μS·cm^{-1}) was used for the preparation of all solutions. The details of chemicals used in this work are listed in Table.S1.

Apparatus and Procedure

Samples were weighed using Mettler Toledo AG-285 (uncertainty ±0.0003 g). Solutions of different strengths were prepared by mass dilution at 298.15 K.

Conductivities of the solutions were measured by Mettler Toledo Seven Multi conductivity meter having uncertainty 1.0 μS m^{-1}. The experiment was carried out in a thermostated water bath at 298.15 K with uncertainty ±0.01 K. HPLC grade water with specific conductance 6.0 μS m^{-1} was used during measurement. The conductivity cell was calibrated using 0.01 M aqueous KCl solution.

FT-IR spectroscopic study was performed by Perkin Elmer FT-IR Spectrometer 8300 applying KBr Desk technique with scanning range 400-4000 cm^{-1}.

UV-Visible spectroscopy was performed using Agilent 8453 spectrophotometer and JULABO F-32 thermostat was used to maintain the experimental temperature.

2D ROESY and $^{1H\ NMR}$ study, spectra of the pure solutions and the ICs were recorded at 400 MHz Bruker Advance at 298.15K in D_2O. Signals were denoted as δ values in ppm using residual protonated solvent (HDO) signals as internal standard (D_2O: δ 4.79 ppm). The differences in chemical shifts were recorded to analyze the interaction between TMSI and cyclodextrin molecules.

The Mass Spectroscopic analysis was performed in Agilent Accurate-Mass Q-TOFLC/MS6520.

Scanning Electron Microscope (SEM) study was performed in JEOL JSM-IT100 instrument.

1. INTRODUCTION

In the era of globalisation, molecular encapsulation and release have become imperative in the field of pharmacology and drug delivery science[235, 236]. Macrocyclic host molecules show a tendency to encapsulate guest molecules and are served as drug carriers, drug stabilizers, and drug bioavailability enhancers [237]. Through inclusion, host molecules can protect the drug molecules from degradation by hindering the closest approach of other guest molecules. Such encapsulation can facilitate the specificity of the drugs through the enhanced permeability and retention effect for a specific time period. This strategy has been extensively probed for naturally occurring hosts such as cyclodextrins, crown ethers, calixarenes etc [238-240]. During the formation of inclusion complexes, a guest gets trapped within steric barriers created by a host lattice and finally gives rise to a stable., guest-free, framework [240, 241]. Guests and hosts are associated with weak intermolecular forces such as van der Waals, hydrogen bonding, and charge-transfer interactions [240-244]. Such interactions significantly influence the physical characteristics of inclusion complexes [245].

The study of collective properties of inclusion compounds enables the determination of the contributions that a guest's conformation, size, and performance make to the bulk properties. Inclusion complexes provide useful vehicles for isolation and study of specific guest-host interactions [246]. Azelaic acid (nonanedioic acid) is an oxidation product of oleic acid that occurs in rancid fats. It has anti-inflammatory, antibacterial, and keratolytic effects [247]. Azelaic acid is widely used to treat a number of dermatoses and is universally accepted as an acne treatment [104, 248]. The compound has broad-spectrum bactericidal activity in vitro [105, 249, 250],which can be greatly enhanced by reducing the pH of the medium [251, 252]. On the other hand, The cyclodextrins (CDs) draw the attention of the chemists because of their amphiphilic nature[253]. Cyclodextrins are cyclic oligosaccharides formed by (α-1,4)-linked α-d-glucopyranose units, having a hydrophilic outer surface and a lipophilic central cavity [17, 254, 255]. Cyclodextrins (CD) composed of six, seven, or eight glucose units, are well-known molecular hosts in supramolecular chemistry and those are referred to as α-, β-, and γ-cyclodextrin respectively [256].

In particular, α-Cyclodextrin (α-CD) has an internal cavity shaped like a bottomless bucket of about 7.8 Å in depth and 4.7-5.3 Å in diameter.β-Cyclodextrin (β-CD)has an internal cavity shaped like a bottomless bucket of about 8 Å in depth and 6.0–6.5 Å in diameter (the smaller value is for the end with the primary hydroxyl group (primary face) and the larger is for the end with the secondary hydroxyl group (secondary face) [257-259]. The primary hydroxyl moieties of cyclodextrins form hydrophobic interior and secondary hydroxyl moieties are found on the hydrophilic rims [260, 261]. Because of having their inherent structure, CDs can be served as versatile molecular hosts for various biological, pharmaceutical, organic and inorganic guest molecules by forming host-guest inclusion complexes [261, 262]. Their truncated shape and hydrophobic cavity impart them to have the ability to form inclusion complexes (ICs) through non-covalent interaction with a wide range of guest molecules which can fit either partially or fully inside their cavity, including ILs, surfactants, amino acids, drugs and polymers [263]. Nowadays cyclodextrins (α- and β-CDs) are being widely used as solubilizing agents in bio-sensing, drug and gene delivery, energy protection and cosmetics industry [264, 265].

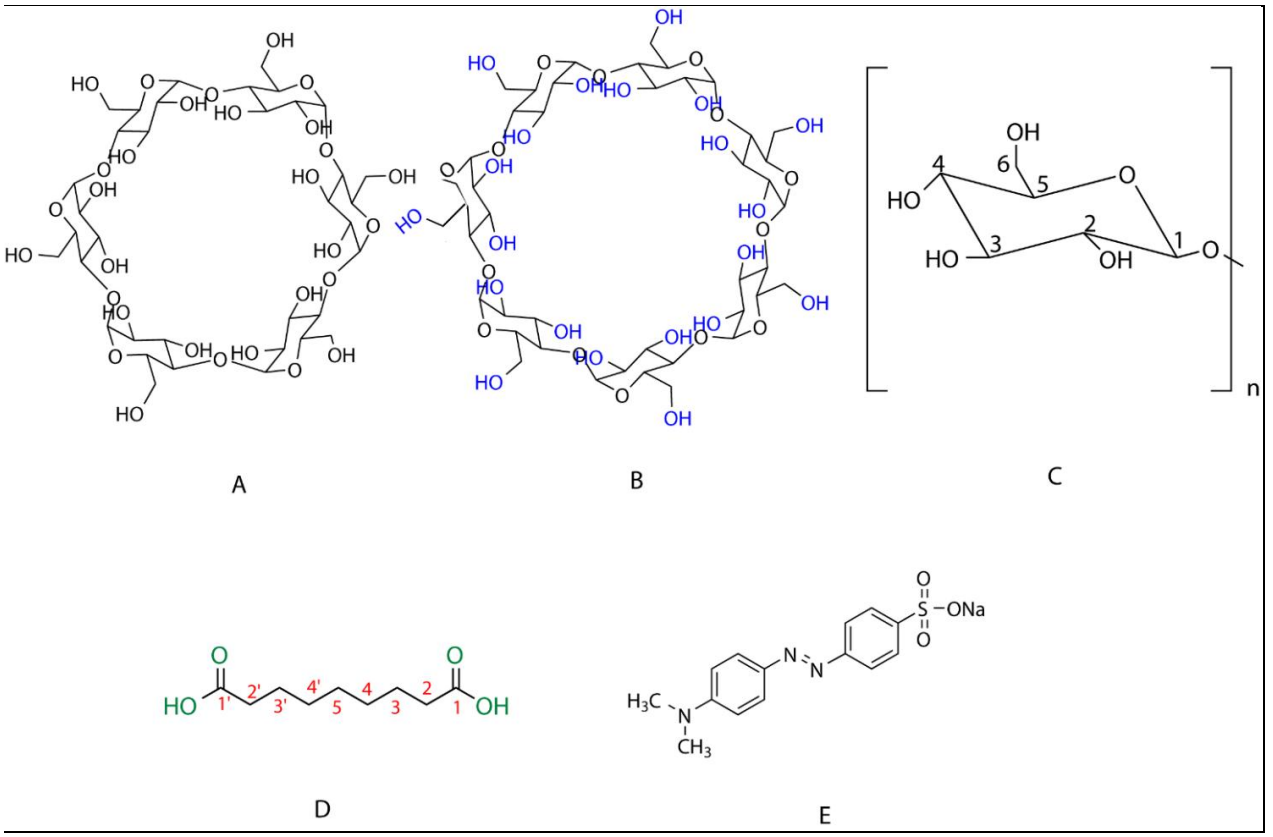
In the area of pharmacology, the encapsulation of AA molecule within the cavities of CD molecules is a matter of priority because of their stabilisation and controlled release [266]. Hence, to protect the drug molecule from external hazards and controlled delivery over a specific time period, it is necessary to investigate whether the drug molecules can be encapsulated within the cavities of CD molecules [267],[268]. In this work, we have synthesised the inclusion complexes of AA within the cavities of α- and β-CD and compared their degree of encapsulation. We have employed the Benesi-Hildebrand equation to determine the association constants. Other thermophysical parameters such as enthalpy, entropy and free energy change of the processes at three different temperatures have also been calculated. UV-

Visible Spectroscopy, 1H NMR spectroscopy, two-dimensional (2D) NMR rotational nuclear Overhauser effect spectroscopy (ROESY) was used to find out the degree of encapsulation, whereas High-Resolution Mass Spectrometry (HRMS), Fourier transform infrared (FTIR) spectroscopy and Scanning Electron Microscopic (SEM) study were used to characterise the solid IC. Molecular encapsulation of the Azelaic acid molecule provides the extent of drug loading ability of α- and β-cyclodextrins. DFT study reveals the extent of inclusion in both cases which assists in the application of the proposed utilization of the encapsulated drug in the fields of biomedical and chemical engineering.

2. EXPERIMENTAL SECTION

2.1. Source and purity of samples

Azelaic acid, Methyl orange, α-Cyclodextrin (α-CD) and β-Cyclodextrin (β-CD) were purchased from Sigma-Aldrich and used without further purification. The details of the chemicals used in this work are listed in Table. S1. Distilled water (specific conductivity < 1μS·cm-1) was used for the preparation of all solutions.



Scheme1. Structures of compounds used: (A) α-Cyclodextrin (n=6), (B) β-Cyclodextrin (n=7), (C) Cyclodextrin monomer, (D) Azelaic acid and (E) Methyl Orange , where n= no of monomeric subunits.

3. RESULT AND DISCUSSION

3.1. Synthesis of the inclusion compound

β-CD.12H_2O (0.5 mmol) was dissolved in water (20 mL) at 60°C and Azelaic acid (0.5 mmol) was added. The inclusion complex of Azelaic acid and β-CD was prepared by coevaporation method [269]. 0.5 mmol Azelaic acid in 10 mL water was added drop wise to the 20 mL aqueous solution of 0.5 mmol β-CD, and the solution was stirred at 323.15 K for 18 h in water bath. Then it was filtered and cooled to room temperature, put the mixture into the refrigerator at 277 K overnight. The white precipitate was filtered and washed successively with distilled water & ethanol. The solid was dried in vacuum drying oven to obtain a white Azelaic acid-β-CD inclusion complex. Same procedure was followed to obtain inclusion complex of α-CD.

3.2. Conductivity Study

The stoichiometry of inclusion complex can be predicted primarily from the breakpoint in the curves of the molar conductivity versus the concentration of cyclodextrin molecules [270-273]. In this case, the conductance (Λ) was measured at 298.15 K and the variation was performed with respect to the concentration of cyclodextrin, following conventional conductance technique (**Table.S2.**). At first, the conductivity cell constant was calibrated using aqueous 0.01(M) KCl solution. After proper callibration , conductivity of 10 mL pure AA solution was measured. Then aqueous solution of cyclodextrin was progressively added to 10mL Azelaic acid solution with the help of micro-pipette and the conductance was measured after thorough mixing at temperature equilibrium. The conductance (Λ, Sm cm^{-1}) for all studied systems was evaluated at room temperature i.e. 298.15K .

The conductance curves (Λ versus c) for the above systems were found to be gradually decreasing and depicted in **Fig.S1.** On increasing the concentration of cyclodextrin in Azelaic acid solution, the decrease in Λ was observed [161]. The decreasing tendency of the molar conductance versus concentration curve clearly indicates that the AA molecule gets encapsulated within the cavities of α- and β-CDs molecules, one by one, consequently the movement of AA becomes restricted and it in turn diminishes the conductivity of the overall system [274].The fact may be attributed to the following factors-(i) formation of intermolecular Hydrogen bonding between the –OH groups of Azelaic acid and hydrophilic –OH groups of cyclodextrin molecules and (ii) on increasing the concentration of CD molecules, more guest molecules are getting encapsulated inside the hollow cavity of CD itself [275].

The inflection point in case of AA.β-CD complex appears at a concentration of 5.24 mM (approx), indicating that the stoichiometry of the Azelaic acid and the β-CD should be equimolar. On the other hand, for the AA.α-CD inclusion complex, the appearance of the sharp inflection point is not observed, but there is a decrease in conductance. Such behaviour may arise because of the dynamic equilibrium between α-CD and Azelaic acid molecule[276] as α-CD has a smaller diameter which prevents the

guest molecule to get encapsulated tightly. At the inflection point, all of the guest molecules are encapsulated in the hollow cavities of the cyclodextrin molecules, then the concentration of CD increases gradually exceeding the concentration of Azelaic acid. But From **Table.S2.** , it is observed that the rate of decrease in molar conductance of AA in α-CD is not so sharp like the β-CD one, which primarily indicates better feasibility of inclusion phenomena in case of β-CD [275] .

3.3. FTIR Spectroscopy:

The IR spectra were recorded on a Perkin–Elmer RX1 FT-IR spectrometer 8310 with samples using KBr pelleting. All the spectra were run in the range of 400–4000 cm^{-1} at room temperature. As a part of our preliminary investigations, we performed FT-IR Spectroscopy to ensure the inclusion phenomenon by considering the deviation of stretching frequency [267, 277-280]. The characteristic IR frequencies of Azelaic acid, α-CD, β-CD and their corresponding solid ICs are enlisted in **Table.S3.** The relevant spectra are shown in **Fig.S2.**

The characteristic IR absorption peaks of pure AA are found at 2938 cm^{-1} (symmetrical stretching of –C–H in $–CH_2$), 1706 cm^{-1} (stretching of –C=O in -COOH), 1453 cm^{-1} ($-CH_2$ bending), 1296 cm^{-1} (-C-O stretching), 937cm^{-1} (-O-H bending) and 720 cm^{-1} ($-CH_2$ bending obtained due to long chain). Broad bands due to –O–H stretching for α-CD and β-CD are observed at 3430 cm^{-1} and 3427 cm^{-1} respectively. In case of the β-CD IC, the –O–H frequency is found to be shifted to the lower region at 3379 cm^{-1}, which arises due to the formation of hydrogen bond between the –COOH group of AA and –OH group of cyclodextrin molecule [193, 200]. The absorption bands of AA in the AA.β-CD complex are shifted to a considerable extent. In pure Azelaic acid –C=O stretching frequency was observed at 1706 cm^{-1}, but in case of AA.β-CD the -C=O stretching was found at 1686 cm^{-1}. Such a drop in stretching frequency of the –C=O bond again indicates the possibility of the hydrogen bond formation between the guest and host molecule. The shifts in stretching frequencies in case of AA.β-CD inclusion complex (Table.S3.) indicates effective insertion of the AA molecule inside the cavity of β-CD. Whereas, no remarkable shift in stretching frequencies was observed in case of the AA.α-CD IC. This observation may be attributed to the inefficient fitting of AA inside the cavity of α-CD [235].

3.4. UV-Vis Spectroscopy

3.4.1. Association Constant and Thermodynamic Parameters

UV-Visible spectroscopy is one of the simplest methods for determining both the stoichiometry and binding constant of inclusion complexes, which can be characterized by continuous change in the optical density (OD) along with or without a spectral shift depending upon the addition of host molecule to the guest solution [281].

In order to know the binding stoichiometry, we used the continuous variation Job's method, in which the total concentration of the guest and [CD] was kept constant, while the mole fraction of the solution

with host and guest is varied in deionized water [282]. The maximum absorbance reached at a mole ratio of 0.5 for the AA.β-CD inclusion complex, but no such maximum absorbance has been found for the AA.α-CD. This demonstrates the formation of 1:1 inclusion complex, in the measured concentration range between CD and Azelaic acid [190, 283]. UV-Vis absorption spectra were recorded at three different temperatures ranging from 293.15K to 313.15 K. We have used the optical probe methyl orange to characterize the interaction nature of Azelaic acid with cyclodextrin molecules in an aqueous medium. Methyl orange has two phenyls and one azo group as chromophoric groups. The two benzene rings are connected to each other via azo-bridge (Scheme1). The concentration of M.O. was 0.00001(M) or 1×10^{-5} mol dm^{-5} and cyclodextrins (α-CD and β-CD) concentration were ~1×10^{-2} mol dm^{-5} for spectral measurement. The absorption spectra of 0.00001 (M) M.O. was measured in distilled water at concentrations using a quartz cell of path length 1 cm. **Fig.S3.** shows that the absorption spectrum of 10^{-5} (M) M.O, recorded only in the spectral region 220-650 nm.

At first, we recorded the absorption spectra of pure Methyl orange and The absorption band due to MO was observed at λ_{max} = 467 nm [284]. Then, the absorption spectra of methyl orange (MO) in Azelaic acid was recorded in the presence of cyclodextrins to check the formation of inclusion complexes in an aqueous medium. Since α- and β-CDs hardly show absorption through the wavelength range (300-500 nm), hence participation of the CD molecules was considered to be negligible [274]. The methyl orange (MO) interacts electrostatically with the –OH groups of guest molecule to reveal their extent of interaction through variations in the intensity in the probe's absorption pattern [282, 285] .

It was observed that with an increased β-CD concentration, the intensity of the absorption peak in the vicinity of 267 nm (due to Azelaic acid) increases considerably while the concentration of AA remains the same and three isosbestic points at 310nm, 385nm and 565 nm appeared. Although the small change in the intensity of the absorption patterns has been observed in case of (AA+ α-CD) system no such isosbestic points have been found here (**Fig.S4**.), which in turn ensures the successful inclusion phenomena between Azelaic acid and β-CD molecule [274]. Such observation is ascribed to the fact that inclusion complex formation entirely takes place between AA and the cyclodextrin molecule [282].

Binding constant is one of the key guidelines to Fig. out non-covalent binding behaviour of host-guest interactions. The binding constant of Azelaic acid (guest molecule) with Cyclodextrins (host molecule) has been calculated using the following equation [274].

$$[AA]+[CD]\underset{\longleftarrow}{\overset{K_a}{\longrightarrow}}[IC] \qquad (1)$$

Where, [IC], $[AA]_f$ and $[CD]_f$ signifies the equilibrium concentration of inclusion complex, free Azelaic acid and free cyclodextrin respectively. The plot of 1/[CD] vs 1/ ΔA was found to be linear

(Supporting information **Fig.S5.**).The experimental observation indicates the formation of 1:1 inclusion complex. From the higher value of K_a depicted from the Benesi-Hildebrand equation, it is quite clear that the incorporation of AA into β -CD results in a tight fit (**Table.1**.).

The enthalpy and entropy parameters are dependent upon the extent of interactions between the host and the guest molecules during the inclusion phenomena [200]. The thermodynamic parameters like enthalpy ΔH^0 and free energy ΔG^0 can also be calculated using Van't Hoff equation.

$$\ln k_a = -\frac{\Delta H_0}{RT} + \frac{\Delta S_0}{R} \tag{2}$$

Table.1. shows that the values of ΔH^0 and ΔS^0 are negative. The decrease in the energy of the system favours the formation of the ICs. It has been observed that as the value of free energy of AA.β-CD complex is almost twice of the α-CD one, hence the AA.β-CD inclusion complex is much more stable than the AA.α-CD.

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon[V]K_a} X \frac{1}{[CD]} + \frac{1}{\Delta\varepsilon[V]} \tag{3}$$

Where, ΔA is the difference between the absorbance of [Azelaic acid in Methyl Orange] in the presence and absence of α- and β-CDs. Δϵ is the difference between the molar absorption coefficient of [Azelaic acid] in the presence and absence of CDs and K_a is the absorption constant. A good linear correlation is obtained from the 1/ΔA versus 1/CDs plot **(Fig.S5.)** [126, 187, 286]. The association constants (K_a) for the inclusion complexation can be calculated from the slope of the Benesi-Hildebrand plot according to the following equation

$$Ka = \frac{1}{\text{Slope}(A\text{-}A_0)} \tag{4}$$

The values of ΔH_0 and ΔS_0 for the formation of ICs were found negative **(Table.1.)** suggesting that the inclusion process is exothermic and entropy controlled [187].

Table.1. Association constants (K_a), Gibb's free energy (ΔG^0), enthalpy (ΔH^0) and entropy(ΔS^0) of (AA+CD) systems from UV-Visible spectroscopy

IC	k_a (M^{-1})			ΔG^0	ΔH^0	ΔS^0
	293.15K	303.15K	313.15K	(kJ mol^{-1})	(kJ mol^{-1})	(J mol^{-1}) K^{-1}
AA.β-CD	4640	3220	2230	-20.40	-28.00	-25.10
AA.α-CD	73	61	54	-10.40	-11.10	-2.30

3.4.2. Job Plot

Job's method of continuous variations has been often applied for the study of inclusion complexes in solution. [287],[189, 190, 288]. (See supporting information (**Table.S4. and Table.S5.).** ΔA x R values have been plotted against R, where ΔA signifies the difference in absorbance of the pure AA and its inclusion complexes and R is [AA]/([AA] + [CD]). λ_{max} was found at 267 nm at 298.15,303.15 and 313.15 K. The stoichiometric ratio of guest and host molecule can be obtained from the R-value at the maxima of the Job Plot such as R = 0.33, for 1:2 IC, R= 0.5 for 1:1 IC, R= 0.66 for 2:1 IC etc. In the case of AA.β-CD complex, the R-value was found to be approximately 0.5, whereas no such specific R-value has been obtained in case of the AA.α-CD **(Fig.1.).**This result indicates the extent of inclusion phenomena is greater in the case of AA.β-CD complex.

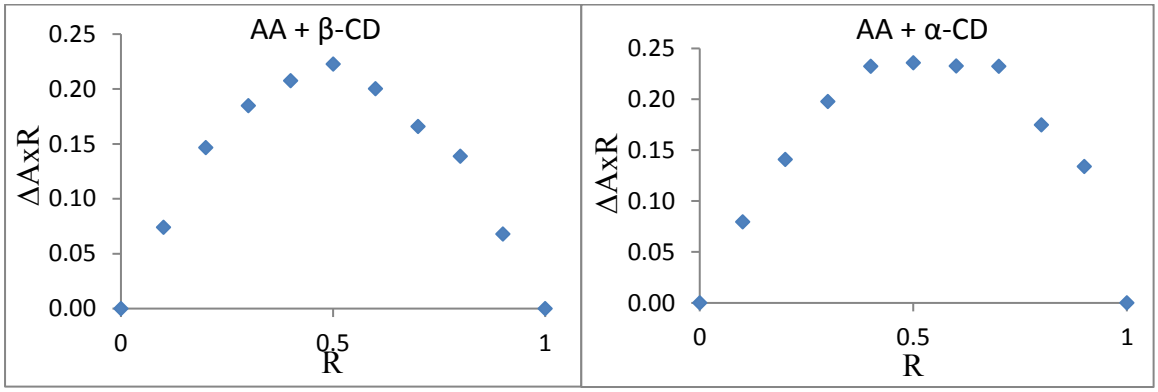


Fig.1. Job Plot for (AA+β-CD) and (AA+α-CD) system.

3.5. ^{1}H NMR analysis

The insertion of a guest molecule into the hydrophobic cavity of α and β-CD reflects in changes in the values of chemical shift of the protons in the $^{1H\ NMR}$ spectra and it is a direct evidence for the complexation of Azelaic acid with CDs [289],[290]. The most plausible mode of interaction of inclusion complexes can be also established with the help of $^{1H\ NMR}$ spectroscopy. It is also an effective tool to study the changes of the electronic environment around the different protons of Azelaic acid molecule in presence of α and β-Cyclodextrin [291]. NMR samples were prepared by adding Azelaic acid to cyclodextrin in NMR tubes by mass.

D_2O solvent was added with a syringe while the samples were kept under N_2 flow. NMR spectra of the solvent were also taken to ensure that they were free from contaminants. All measurements were performed at 298.15 K using a 400 MHz spectrometer. At first, $^{1H\ NMR}$ spectra of the pure compounds in D_2O solvent were taken (Fig.S6., Fig.S7. & Fig.S8.).

The structure of cyclodextrin molecules show that the H3 and H5 hydrogens are placed inside the hollow conical cavity, more specifically, the H3 and the H5 are located near the wider rim and

narrower rim of the cyclodextrin molecule respectively. The other protons named as H1, H2 and H4 hydrogens are located at the exterior of the cyclodextrin molecule **(Fig.2.)** [189]. On entering the hollow cavity of β-CD moecule, AA preferably interacts with H3 and H5 protons, which results in upfield shift, on the other hand, H1, H2 and H4 too experience such upfield chemical shift although this extent is very small compared to other interior protons [292] **(Fig.S10).** In AA.β-CD inclusion complex, the resonance of H2 (or H2′) was shifted to 0.04 ppm to high field The H3(or H3′) proton was also shifted up to significant extent and the peaks of quintet have been diminished and a singlet appeared instead of it and the chemical shift of H4, H5 and H4′ were only slightly affected (0.02 ppm) **(Fig.S10.).**But in case of the AA.α-CD inclusion complex no considerable chemical shifts in protons have been observed **(Fig.S9.).**Consequently, taking account of the respective chemical shifts in the spectrum of α and β inclusion complexes, it can be concluded that the extent of interaction is greater in case of β-CD complex than that of the α one. This finding is consistent with the DFT explanation.

1H NMR data

Azelaic acid (AA) : 2.26 (2H,t,J=8 Hz), 1.48 (4H,quint,J=8 Hz), 1.21(2H,m) **(Fig.S6.)**

α- Cyclodextrin (α-CD): $^{1H\ NMR}$ (D_2O, 298.15 K): δ/ppm 3.50 (6H, t, *J* =9.00 Hz), 3.55(6H, dd, *J*= 8 Hz), 3.73-3.86 (18H, m), 3.89 (6H, t, *J* =9 Hz), 4.96 (6H, d, *J*=3 Hz) **(Fig.S7.).**

β- Cyclodextrin (β-CD): $^{1H\ NMR}$ (D_2O, 298.15 K): δ/ppm 3.50 (7H, t, *J* = 9.2 Hz), 3.55-3.57 (7H, dd, *J* = 9.6, 3.2 Hz), 3.77-3.81 (21H, m), 3.88(7H, t, *J* = 9 Hz), 4.98 (7H, d, *J* =3.6 Hz) **(Fig.S8.).**

AA.α-CD Inclusion Complex: 3.50 (6H, t, *J* =9.00 Hz), 3.56 (6H, dd, *J*= 8 Hz),),3.70-3.78 (18H, m), 3.86 (6H, t, *J* =9 Hz), 4.96 (6H, d, *J*=3 Hz), 2.27 (2H,t,*J*=8 Hz), 1.48 (4H,quint,*J*=8 Hz), 1.23 (2H,m) **(Fig.S9.).**

AA.β-CD Inclusion Complex: 3.50 (7H, t, *J* = 9.2 Hz), 3.55-3.57 (7H, dd, *J* = 9.6, 3.2 Hz), 3.66-3.76 (18H, m), 3.80 (7H, t, *J* = 9.2 Hz), 2.29 (2H,t, *J*=8 Hz), 1.57 (4H,quint, *J*=8 Hz), 1.31(2H,m) **(Fig.S10.)**

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3.6. 2D-ROESY

2D ROESY experiment was done to obtain information about the spatial proximity between protons of the host and guest molecules having an intermediate molecular weight between 1000 and 1500 by observing the intermolecular dipolar cross-correlations. The principle of 2D ROESY is based upon the extent of interaction of the protons which are present in the close proximity of 0.4 nm range to each other to produce cross peaks [293].

The structure of CD reveals that the H3 and H5 protons of CDs are present inside the hollow cavity; the H3 hydrogen is located near the wider rim, whereas the H5 hydrogen is placed near the narrower rim and the other H1, H2, and H4 hydrogens are situated at the exterior of the CD molecule **(Fig.2.)**

[294]. If inclusion takes place, the spatial proximity of the atoms of the guest molecule with H3 and H5 protons of CD should be observed and it is reflected in the intermolecular dipolar cross-correlations [235, 295].

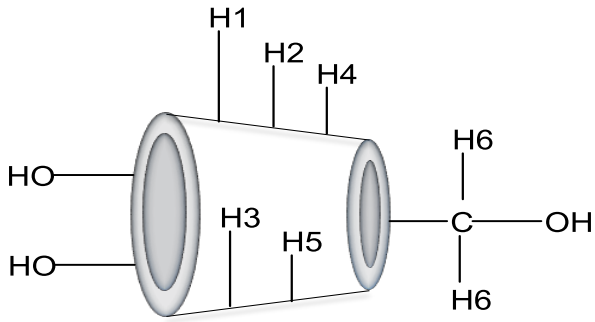
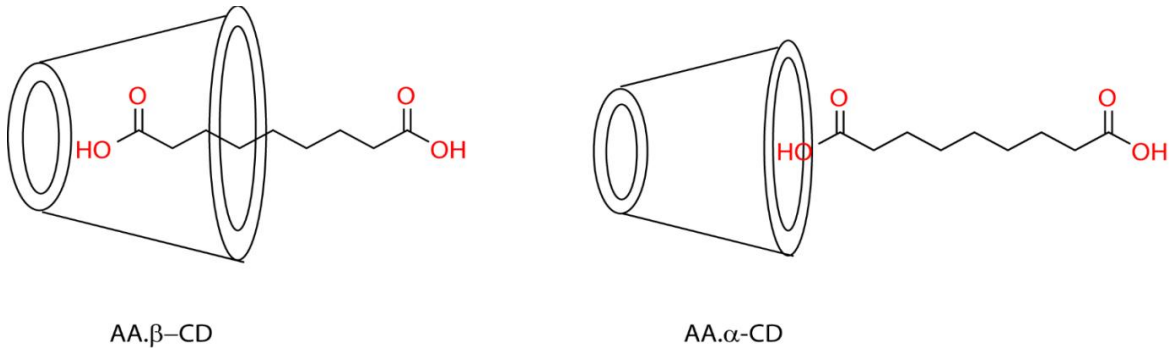


Fig.2. Location of different types of protons in Cyclodextrin molecule.

The shape of the signals has been very characteristic for the H3 (triplet) and H5 protons of β-CD. Thus, there was no doubt that the NOE responses were due to spatial proximity of Azelaic acid protons with the H3 and H5 protons of β-CD and not with the overlapping external H6 and H2 protons of β-CD, respectively. All of these results strongly support the tentative structure of the AA.β-CD complex as shown in Scheme.2., the presence of cross peaks of H3 and H5 protons of β-CD with H2, H2′, H3, H3′ and H4, H5, H4′ protons of the guest molecule was obtained.In the process of inclusion, the cross peaks arises due to the insertion of the alkyl chain part of the AA inside the cavity of β-CD **(Fig.S12.)** but in case of α-CD no such cross-peaks have been obtained **(Fig.S11.)**,which clearly dictates that the inclusion is not so much fruitful in the later case. The fact may be attributed to the smaller cavity size of α-CD which resist the AA molecule to get encapsulated.

This observation definitely indicates that the aliphatic carbon chain of AA deeply enters the cavity of β-CD from its secondary wider rim and the rest part remains close to the cavity on its secondary opening but the inclusion phenomena have not been proved efficient in case of α-CD perhaps due to smaller cavity size compared to the β-CD molecule.



Scheme.2. Proposed tentative structures of Azelaic acid and cyclodextrin inclusion complexes.

3.7. HRMS Study

Electrospray ionization (ESI) mass spectrometry (MS) experiment was performed on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Agilent Technologies).AA.αCD and AA.βCD inclusion complexes were prepared in methanol and were introduced into the ion source of the mass spectrometer with a flow rate of 20ml/min using a syringe pump. The temperature of the inlet capillary was 200^0C and the detection was performed in the positive ion mode. The peaks were given in m/z (% of basis peak).

The peak at 1162.0697 and 1324.2087 in $[M+H]^+$ mode indicates the formation of AA.α-CD and AA.β-CD ICs respectively and for $[M+Na]^+$ mode we got m/z values at 1184.0517 and 1346.1877.The m/z values have been given in **Table.2.** and the spectra have been revealed in **Fig.S13.** and **Fig.S14.** The spectrum of each complex shows peak at 1338.98 and 1176.84 respectively which are almost equal to the sum of host and guest molecule. It indicates the formation of the IC of 1:1 stoichiometric ratio [187].

Table.2. The calculated values and obtained m/z values of the solid inclusion complexes

Ions	Exact Mass (calculated) $[M+H]^+$	m/z values Obtained	Exact Mass (calculated) $[M+Na]^+$	m/z values Obtained
[α-CD+ Azelaic acid]	1162.0711	1162.0697	1184.0529	1184.0517
[β-CD+ Azelaic acid]	1324.2147	1324.2087	1346.1965	1346.1877

3.8. DFT calculation on the inclusion complexation

In order to interpret the nature of the interaction between the cyclodextrin and azelaic acid, density functional calculation was carried out in gas phases, by considering all the possible binding modes [281]

The geometries were optimised at the UB3LYP (6-31G)(d) level. For the AA.α-CD and AA.β-CD complexes, possible geometries were optimised. These structures are represented in the and **Fig.4(c & d)** respectively and will be denoted as complex α1, α2, β1 and β2 respectively. The first observation that strikes to the sight is that the cavity of α-CD is not large enough to allow a deep encapsulation of then guest molecule. This was further supported by the calculated NMR chemical shifts, which showed negligible changes matching the experimental observation for H3 and H5. In terms of formation energies of the theoretical β-CD·Azelaic acid complexes, the β-CD complex is more stable. than the α-CD one, while the α-CD complex is less stable. by $1.61x10^6$ kJ/mol. After energy optimization, it has also been observed that the H-bond length in case of the AA.β-CD complex was $1.67A^0$, which is

smaller than that of AA.α-CD complex (H bond length=1.82A^0), being that a reason for the lower energy of the former structure.

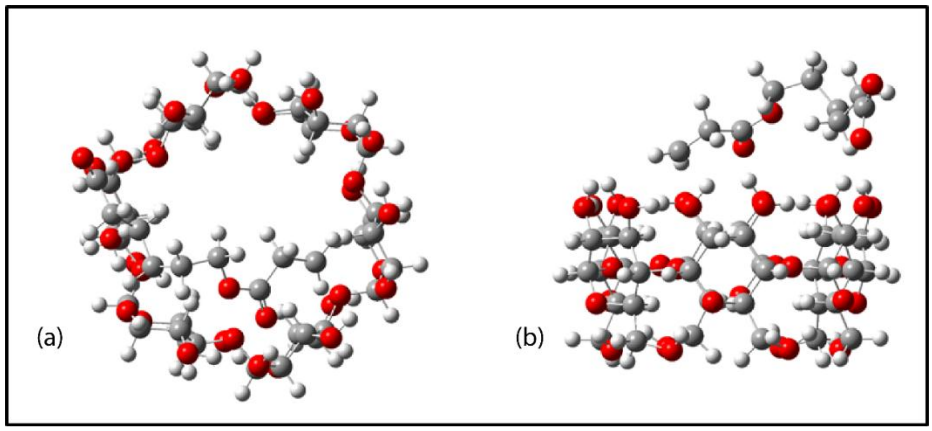


Fig.3. Energy-Optimized Structure of Azelaic acid.α-CD by DFT calculations (a) α1 and (b) α2.

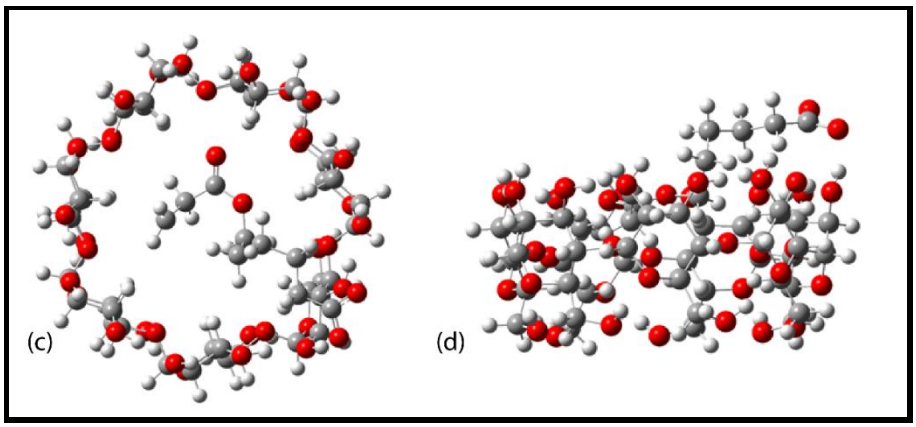


Fig.4. Energy-Optimized Structure of Azelaic acid.β-CD by DFT calculations (c) β1 and (d) β2.

3.9. Scanning Electron Microscope (SEM) Study

The surface morphologies of the pure compounds along with their inclusion complexes were analyzed by Scanning Electron Microscope study. These images are used to Fig. out the effect of the coprecipitation process on the morphology of the solids used for the formation of solid systems. Scanning Electron Microscope(SEM) Study reveals that pure α-CD particles have prismatic shape with well-developed faces and β-CD was observed as plate-shaped crystals [296]. It has been revealed in SEM images that the AA crystals are in the form of bar-shaped structures and smooth surfaces. Pure α-CD particles exist in prismatic shape having well-developed faces and β-CD shows plate-shaped crystals. In contrast, AA.α-CD the samples appear in bar-shaped structures of variable sizes whereas irregular shape was observed for the AA.β-CD complex.

Furthermore, if we compare the morphological changes of the pure components with the inclusion complexes, we find that the morphology of AA.β-CD inclusion complex is totally different from both of the pure components whereas there are some similarities between the morphologies of AA.α-CD and azelaic acid itself [**Fig.5.**(a,b & c) and **Fig.6.**(d & e)].

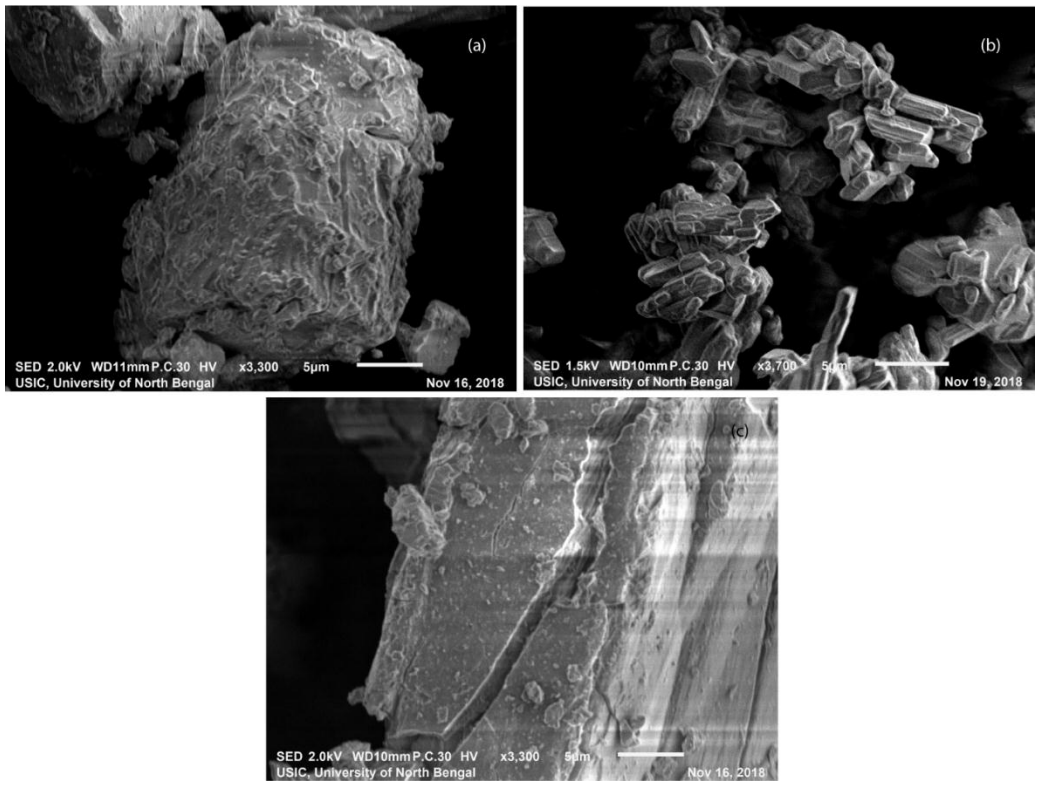


Fig.5. SEM images of pure compounds (a) α-Cyclodextrin (b) Azelaic acid and (c) β-Cyclodextrin.

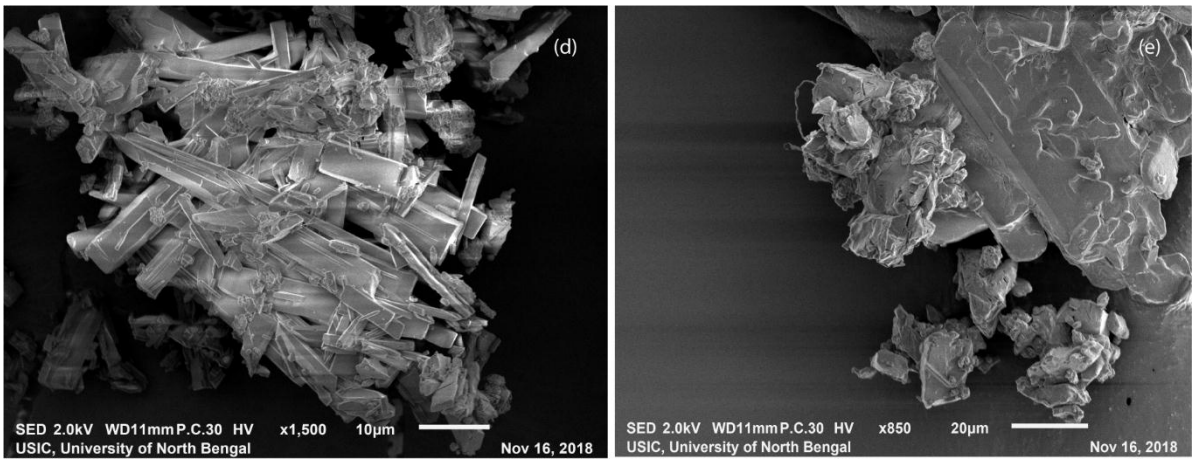


Fig.6. SEM images of inclusion complexes (d) AA.α-CD (e) AA.β-CD.

4. CONCLUSION

The present work dictates about the inclusion phenomena of Azelaic acid and cyclodextrin molecules. The data obtained from conductivity primarily indicates that the AA molecule successfully enters in the hollow cavity of the β-CD molecule. The Job's plot method ensures the 1:1 stoichiometry for the inclusion complex formed. From the Absorption pattern obtained in UV-Vis spectroscopic measurement and the values of association constants, it has been observed that the extent of host-guest interaction is more extensive in case of the AA.β-CD complex than the other one, which is in good agreement with DFT study. The morphological analysis also provides successful inclusion of Azelaic acid into the cavity of the β-CD molecule.

Supplementary data

Subsistence and Energy Optimization of Inclusion Complexes of Azelaic Acid with α and β-CD Molecules and their Extensive Comparison by Physicochemical and Computational Methodologies

Table

Table.S1. Details of chemicals used

Name of chemicals	Source	CAS no	Purification method	Mass purity
Azelaic acid	Sigma-Aldrich, Germany	123-99-9	Used as purchased	>98.0%
Methyl orange	TCI Chemicals(India) Pvt. Limited	547-58-0	Used as purchased	>98.0%
α-Cyclodextrin	Sigma-Aldrich, Germany	10016-20-3	Used as purchased	*w*≥98%
β-Cyclodextrin	Sigma-Aldrich, Germany	7585-39-9	Used as purchased	*w*≥97%
Distilled Water	Sigma-Aldrich, Germany	7732-18-5	Used as purchased	*w*≥99%

Table.S2. Data for the conductivity study of aqueous [AA].α-CD and [AA].β-CD system (concentration of the stock solution of [α-CD] and [β-CD] = 10Mm at 298.15K[a]

Conc. of α-CD (mM)	Conc. of AA (mM)	Molar Conductivity (S cm^2 mol^{-1})	Conc. of β-CD (mM)	Conc. of AA (mM)	Molar Conductivity (S cm^2 mol^{-1})
10	0	0.170	10	0	0.170
9.091	0.909	0.167	9.091	0.909	0.157
8.333	1.667	0.164	8.333	1.667	0.149
7.692	2.308	0.157	7.692	2.308	0.141
7.143	2.857	0.153	7.143	2.857	0.132
6.667	3.333	0.149	6.667	3.333	0.127
6.250	3.750	0.145	6.250	3.750	0.121
5.882	4.118	0.141	5.882	4.118	0.113

5.556	4.444	0.138	5.556	4.444	0.109
5.263	4.737	0.134	5.263	4.737	0.106
5.000	5.000	0.132	5.000	5.000	0.103
4.762	5.238	0.129	4.762	5.238	0.102
4.545	5.455	0.126	4.545	5.455	0.102
4.348	5.652	0.123	4.348	5.652	0.101
4.167	5.833	0.122	4.167	5.833	0. 101
4.000	6.000	0.121	4.000	6.000	0. 101
3.846	6.154	0.121	3.846	6.154	0.100
3.704	6.296	0.120	3.704	6.296	0.100
3.571	6.429	0.120	3.571	6.429	0.100
3.448	6.552	0.120	3.448	6.552	0.100
3.333	6.667	0.120	3.333	6.667	0.101

[a] Standard uncertainties in temperature u are: u(T) = ± 0.01K, conductivity: ± 0.01 mS m^{-1}.

Table.S3. Data obtained from FT-IR spectroscopic study of α-CD, β-CD, AA, AA.α-CD and AA.β-CD complex.

Functional Group	Wave number (Cm^{-1})				
	α-CD	β-CD	AA	AA.α-CD	AA.β-CD
stretching of O-H	3430	3427		3320	3379
stretching of –C-H in –CH_2	2938	2940		2935	2924
bending of –C-H in–CH_2 and bending of O-H	1420	1424		1422	1370
bending of C-O-C	1162	1160		1156	1152
vibration involving α-1,4linkage	954	950		950	984
stretching of -C-H in–CH_2	…..	…..	2938	…..	…..
Stretching -C=O	…..	…..	1706	1709	1686
-CH_2- bending (m)	…..	…..	1453	…..	…..
-C-O Streching	…..	…..	1296	1301	1278
-O-H bending	…..	…..	937	…..	…..
-CH_2- bending	…..	…..	720	728	742

Table.S4. Data obtained from Job Plot of (AA+β-CD) system at 298.15 *K*[a]

Conc. of AA (μm)	α-CD (μm)	R= [AA]/ ([AA]+[CD])	A @λmax 267 nm	ΔA (0.82916-A)	ΔAx[AA]/ ([AA]+[α-CD])
0	100	0	0	0.82916	0
10	90	0.1	0.08783	0.74133	0.074133
20	80	0.2	0.09553	0.73363	0.146726
30	70	0.3	0.21293	0.61623	0.184869
40	60	0.4	0.22203	0.5193	0.20772
50	50	0.5	0.38335	0.44581	0.222905
60	40	0.6	0.49514	0.33402	0.200412
70	30	0.7	0.59209	0.23707	0.165949
80	20	0.8	0.65569	0.17347	0.138776
90	10	0.9	0.75367	0.07549	0.067941
100	0	1	0.82916	0	0

[a]Standard uncertainties in temperature =±0.01K

Table.S5. Data obtained from Job Plot of (AA+α-CD) system at 298.15 *K*[a]

Conc. of AA (μm)	α-CD (μm)	R= [AA]/ ([AA]+[CD])	A @λmax 267 nm	ΔA (0.82916-A)	ΔAx[AA]/ ([AA]+[α-CD])
0	100	0	0	0.82916	0
10	90	0.1	0.03176	0.7974	0.07974
20	80	0.2	0.12341	0.70575	0.14115
30	70	0.3	0.16932	0.65984	0.197952
40	60	0.4	0.24783	0.58133	0.232532
50	50	0.5	0.35741	0.47175	0.235875
60	40	0.6	0.44098	0.38818	0.232908
70	30	0.7	0.49706	0.3321	0.23247
80	20	0.8	0.61054	0.21862	0.174896
90	10	0.9	0.68022	0.14894	0.134046
100	0	1	0.82916	0	0

[a]Standard uncertainties in temperature =±0.01K

Table.S6. Data obtained from Benesi-Hildebrand double reciprocal plot of (AA+β-CD) and (AA+α-CD) systems at 293.15 K

	[Drug] (μM)	[CD] (μM)	A_0	A_1	ΔA	1/ΔA	1/[CD]
(AA+β-CD)	50	10	0.4531	0.47952	0.02642	37.85011	100000
	50	20	0.4531	0.50317	0.05007	19.97204	50000
	50	30	0.4531	0.52531	0.07221	13.8485	33333
	50	40	0.4531	0.54402	0.09092	10.99868	25000
	50	50	0.4531	0.56834	0.11524	8.677543	20000
	50	60	0.4531	0.58441	0.13131	7.615566	16667
	50	70	0.4531	0.59856	0.14546	6.874742	14286
	50	80	0.4531	0.60846	0.15536	6.436663	12500
	50	90	0.4531	0.61826	0.16516	6.054735	11111
	50	100	0.4531	0.628606	0.175506	5.697811	10000
(AA+α-CD)	50	10	0.4531	0.45501	0.00191	523.5602	100000
	50	20	0.4531	0.45691	0.00381	262.4672	50000
	50	30	0.4531	0.46042	0.00732	136.612	33333
	50	40	0.4531	0.46109	0.00799	125.1564	25000
	50	50	0.4531	0.46202	0.00892	112.1076	20000
	50	60	0.4531	0.46473	0.01163	85.98452	16667
	50	70	0.4531	0.46505	0.01195	83.68201	14286
	50	80	0.4531	0.46642	0.01332	75.07508	12500
	50	90	0.4531	0.46868	0.01558	64.18485	11111
	50	100	0.4531	0.47398	0.02088	47.89272	10000

Table.S7. Data obtained from Benesi-Hildebrand double reciprocal plot of (AA+β-CD) and (AA+α-CD) systems at 303.15 K

	[Drug] (μM)	[CD] (μM)	A_0	A_1	ΔA	1/ΔA	1/[CD]
AA+β-CD	50	10	0.44739	0.47051	0.02312	43.2526	100000
	50	20	0.44739	0.492619	0.045229	22.10971	50000
	50	30	0.44739	0.51342	0.06603	15.14463	33333
	50	40	0.44739	0.53173	0.08434	11.85677	25000
	50	50	0.44739	0.55402	0.10663	9.378224	20000
	50	60	0.44739	0.569273	0.121883	8.20459	16667
	50	70	0.44739	0.58485	0.13746	7.274844	14286
	50	80	0.44739	0.596142	0.148752	6.722599	12500
	50	90	0.44739	0.61588	0.16849	5.93507	11111
	50	100	0.44739	0.62448	0.17709	5.646846	10000
AA+α-CD	50	10	0.44739	0.44941	0.00202	495.0495	100000

50	20	0.44739	0.4512	0.00381	262.4672	50000
50	30	0.44739	0.45481	0.00742	134.7709	33333
50	40	0.44739	0.45576	0.00837	119.4743	25000
50	50	0.44739	0.45651	0.00912	109.6491	20000
50	60	0.44739	0.45922	0.01183	84.53085	16667
50	70	0.44739	0.45945	0.01206	82.91874	14286
50	80	0.44739	0.46082	0.01343	74.46016	12500
50	90	0.44739	0.46587	0.01848	54.11255	11111
50	100	0.44739	0.47608	0.02869	34.85535	10000

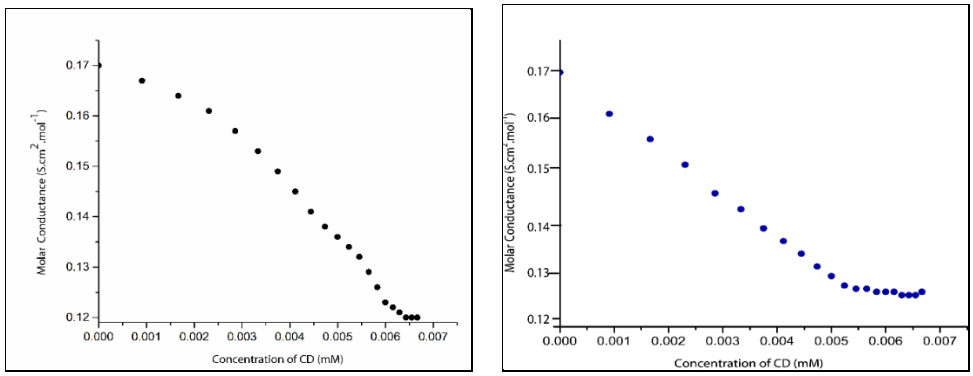
Table.S8. Data for the Benesi-Hildebrand double reciprocal plot obtained from UV-VIS spectroscopic study for (AA+β-CD) and (AA+α-CD) systems at 313.15 K

	[Drug] (μM)	[CD] (μM)	A_0	A_1	ΔA	1/ΔA	1/[CD]
(AA+β-CD)	50	10	0.43182	0.454847	0.023027	43.42728	100000
	50	20	0.43182	0.476417	0.044597	22.42303	50000
	50	30	0.43182	0.49342	0.0616	16.23377	33333
	50	40	0.43182	0.51573	0.08391	11.91753	25000
	50	50	0.43182	0.54002	0.1082	9.242144	20000
	50	60	0.43182	0.558273	0.126453	7.908077	16667
	50	70	0.43182	0.58025	0.14843	6.737183	14286
	50	80	0.43182	0.601142	0.169322	5.905907	12500
	50	90	0.43182	0.62488	0.19306	5.179737	11111
	50	100	0.43182	0.61548	0.18366	5.444844	10000
(AA+α-CD)	50	10	0.43182	0.43453	0.00271	369.0037	100000
	50	20	0.43182	0.43863	0.00681	146.8429	50000
	50	30	0.43182	0.43964	0.00782	127.8772	33333
	50	40	0.43182	0.44299	0.01117	89.52551	25000
	50	50	0.43182	0.44376	0.01194	83.75209	20000
	50	60	0.43182	0.44645	0.01463	68.3527	16667
	50	70	0.43182	0.44867	0.01685	59.34718	14286
	50	80	0.43182	0.45514	0.02332	42.88165	12500
	50	90	0.43182	0.45972	0.0279	35.84229	11111
	50	100	0.43182	0.46658	0.03476	28.7687	10000

Table.S9. ^{1}H-NMR spectra of AA, α-CD, β-CD, and (AA+α-CD), (AA+β-CD) inclusion complexes

α-Cyclodextrin (400 MHz, D_2O solvent) δ /ppm	β-Cyclodextrin (400 MHz, D_2O solvent) δ /ppm
3.50 (6H, t, J =9.00 Hz), 3.54 (6H, dd, J= 10.00, 3.00 Hz), 3.73-3.86 (18H, m), 3.89 (6H, t, J =9 Hz), 4.96-4.97 (6H, d, J=3 Hz)	3.50 (7H, t, J = 9.2 Hz), 3.56 (7H, dd, J = 9.6, 3.2 Hz), 3.77-3.81 (21H, m), 3.88(7H, t, J = 9.2 Hz), 4.98-4.99 (7H, d, J =3.6 Hz)
[AA] δ /ppm	
2.26 (2H,t,J=8 Hz), 1.48 (4H,quint,J=8 Hz), 1.21(2H,m)	
[AA].α-CD	[AA].β-CD
3.48-3.51 (6H, t, J= 9.00 Hz), 3.53-3.56 (6H, dd, J= 10.00, 3.00 Hz), 3.68(3H, s), 3.69-3.74 (18H, m), 3.62-3.67 (6H, t, J = 9 Hz), 4.96-4.97 (6H, d, J = 3 Hz), 5.30 (2H, s), 7.41-7.47 (5H,m), 8.59-8.65 (2H, dd).	3.49-3.54 (6H, t, J = 9.2 Hz), 3.57-3.60 (6H, dd, J =9.6, 3.2 Hz), 3.68(3H, s), 3.66-3.70 (18H, m), 3.60-3.64 (6H,t, J = 9.2 Hz), 5.00-5.01 (6H, d, J = 3.6 Hz), 5.25 (2Hs), 7.35-7.42 (5H,m), 8.59-8.64 (2H, dd).

Figures



(i) **(ii)**

Fig.S1. Molar conductivity of Azelaic Acid against CD at T = 298.15 K : (i) Molar conductivity of Azelaic Acid against α-CD (●) and (ii) Molar conductivity of Azelaic Acid against β-CD (●) respectively.

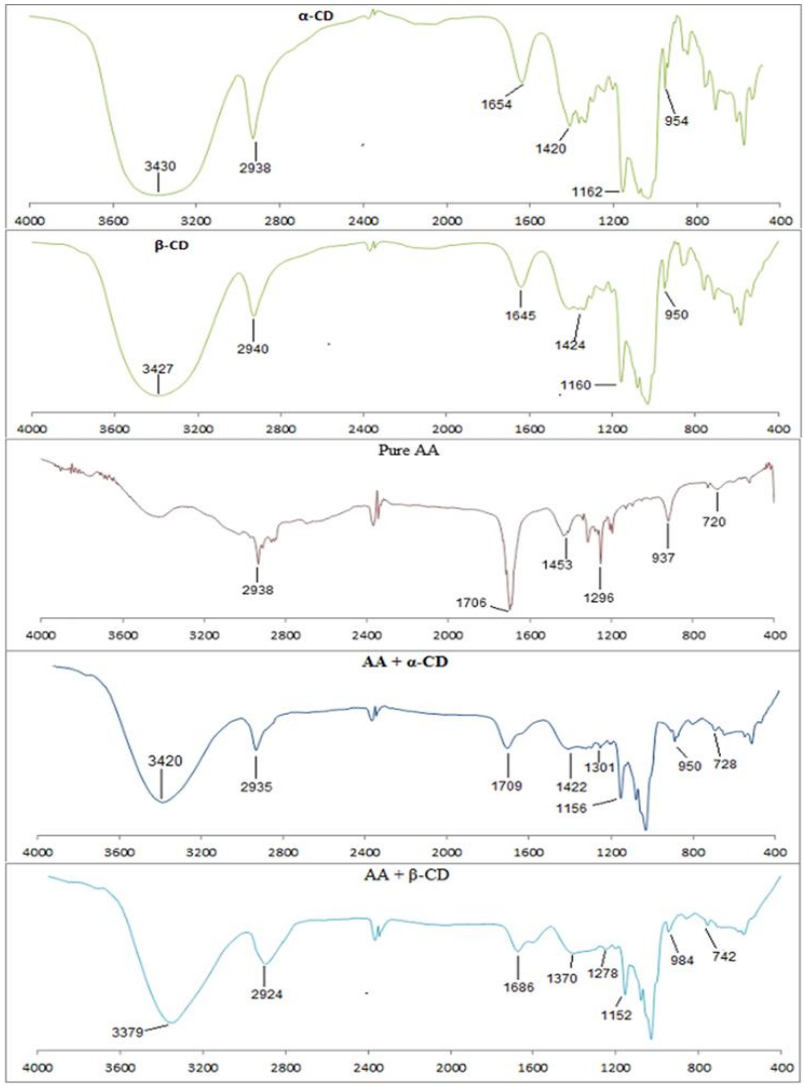


Fig.S2.FTIR Spectra of α-CD, β-CD, Pure AA, AA.α-CD and AA.β –CD inclusion complex

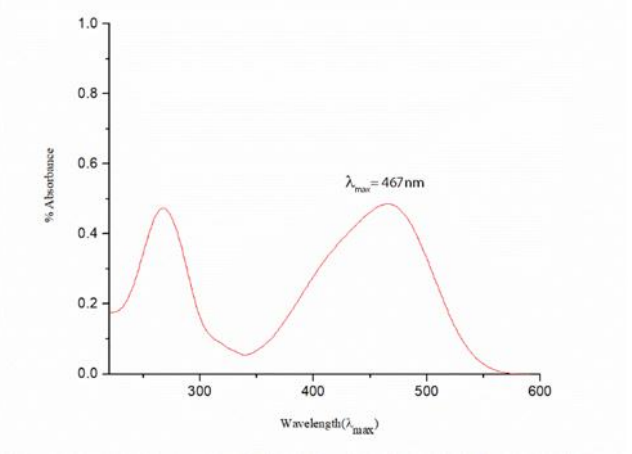
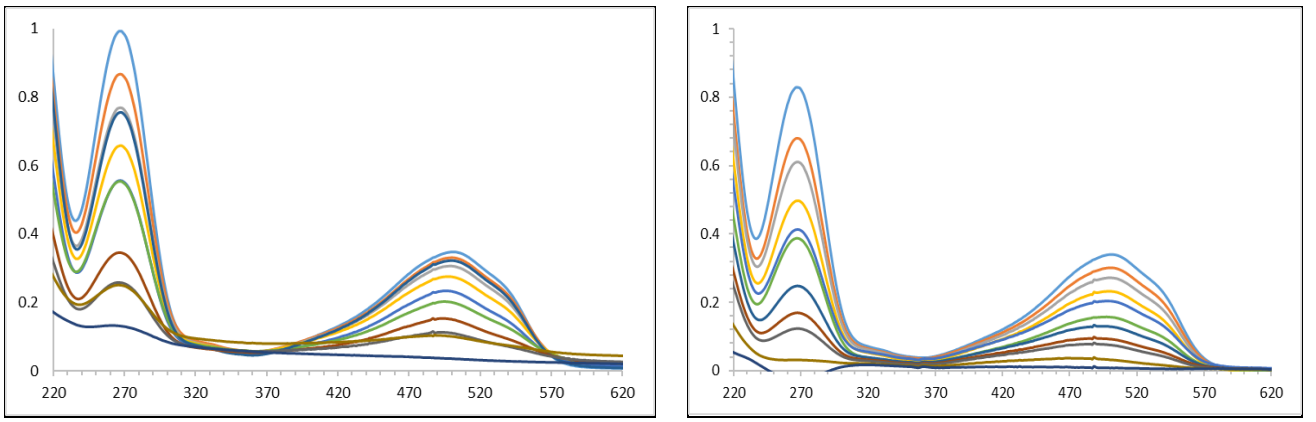
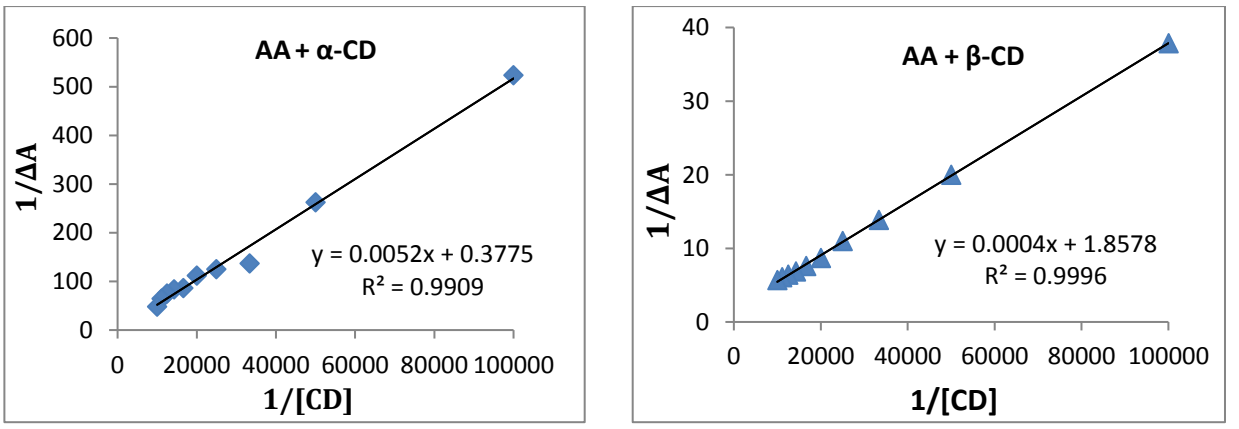


Fig.S3. Absorption spectra of 1×10^{-5} mol dm^{-5} Methyl Orange in aqueous medium, λ_{max}= 467 nm

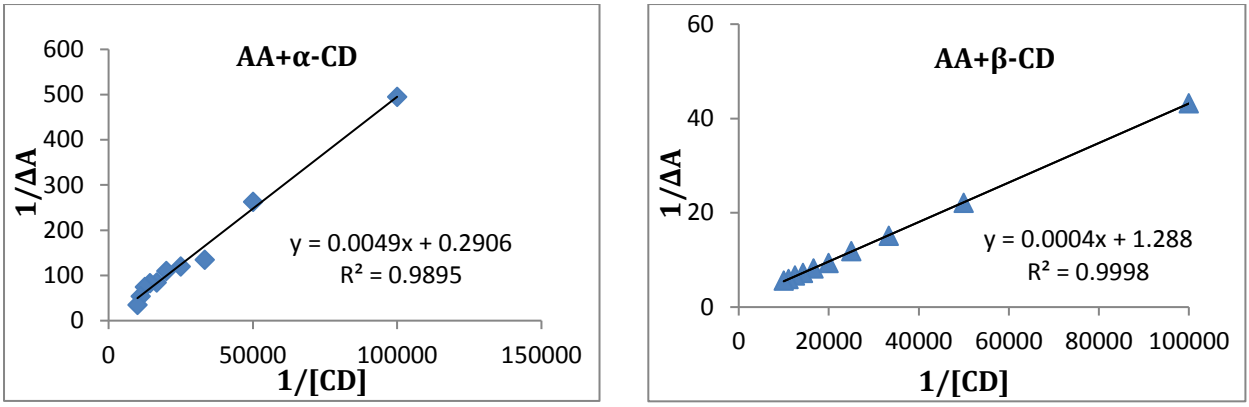


A **B**

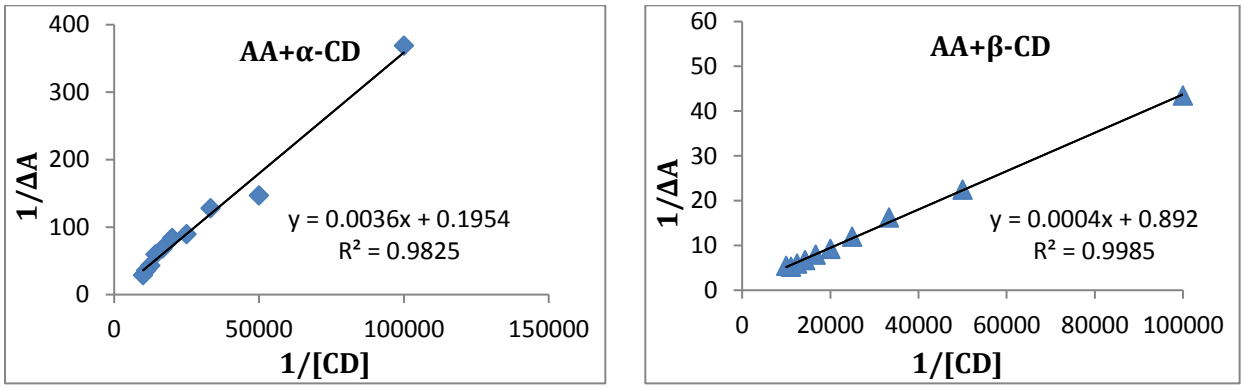
Fig.S4. Absorption pattern for (A) (AA+β-CD) system and (B) (AA+α-CD) system.



a



b



c

Fig.S5.Benesi-Hildebrand double reciprocal plot of AA.CD inclusion complexes at (a) 293.15K,(b) 303.15K ,(c) 313.15K

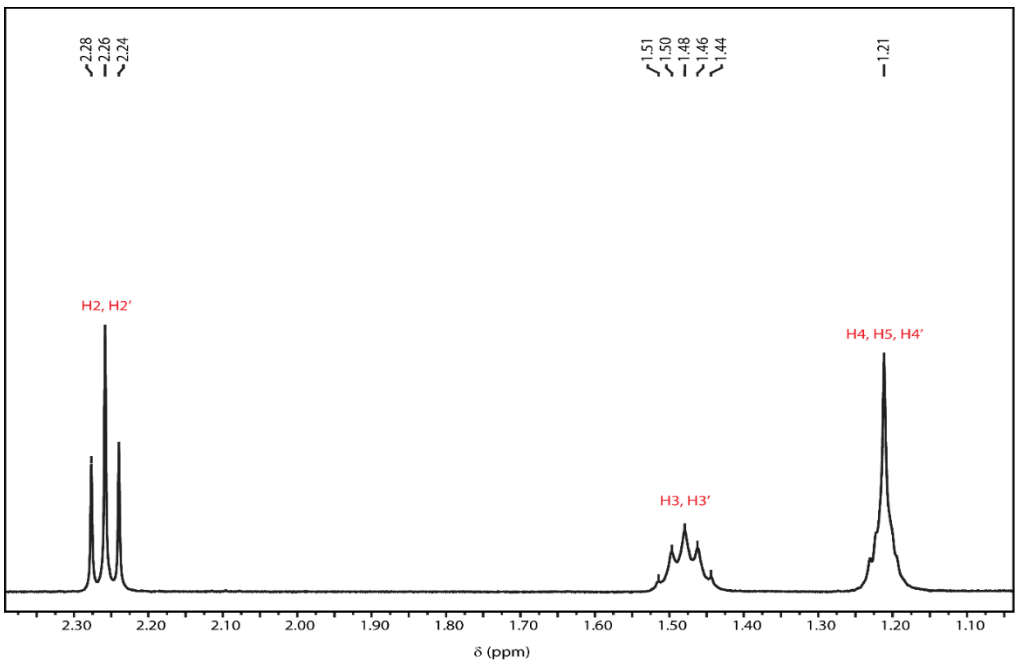


Fig.S6. : ^{1}H-NMR spectra of Azelaic acid in D_2O at 298.15K

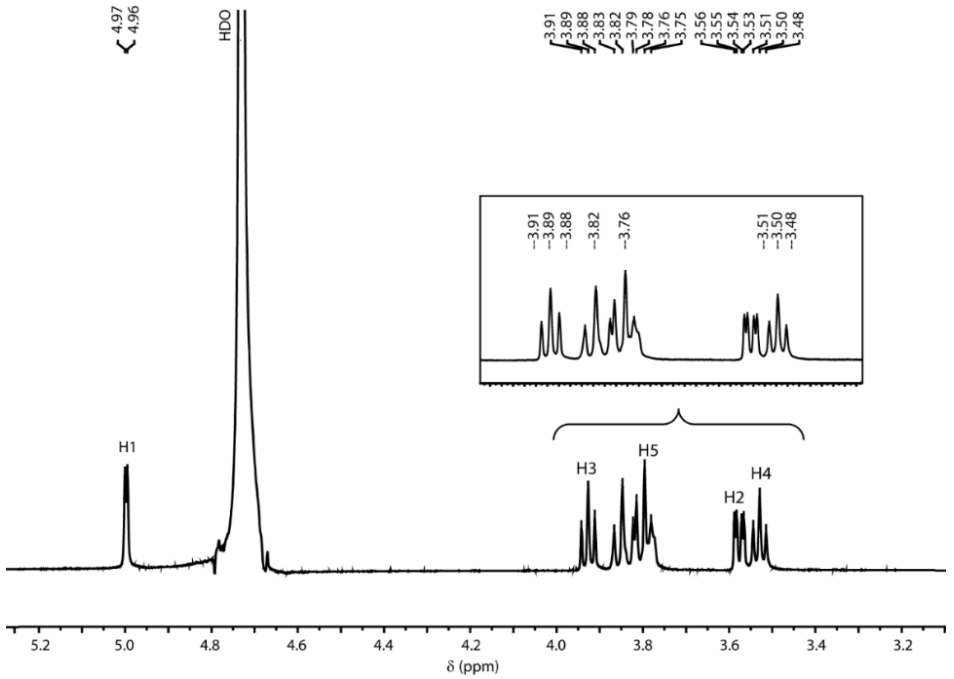


Fig.S7. : 1H-NMR spectra of α-Cyclodextrin in D_2O at 298.15K

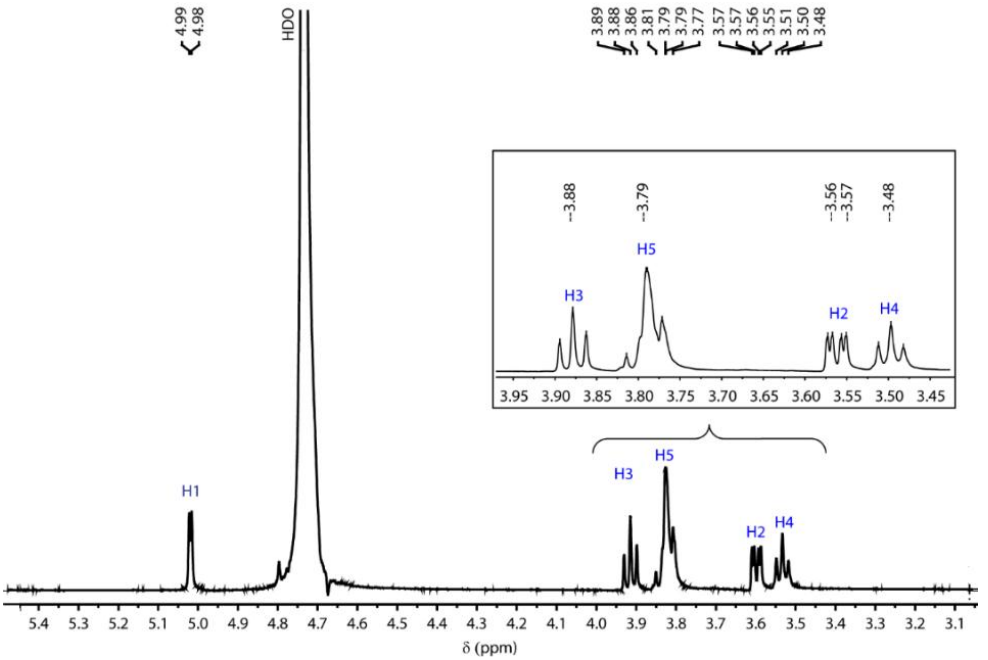


Fig .S8.: ^{1}H-NMR spectra of β-Cyclodextrin in D_2O at 298.15K

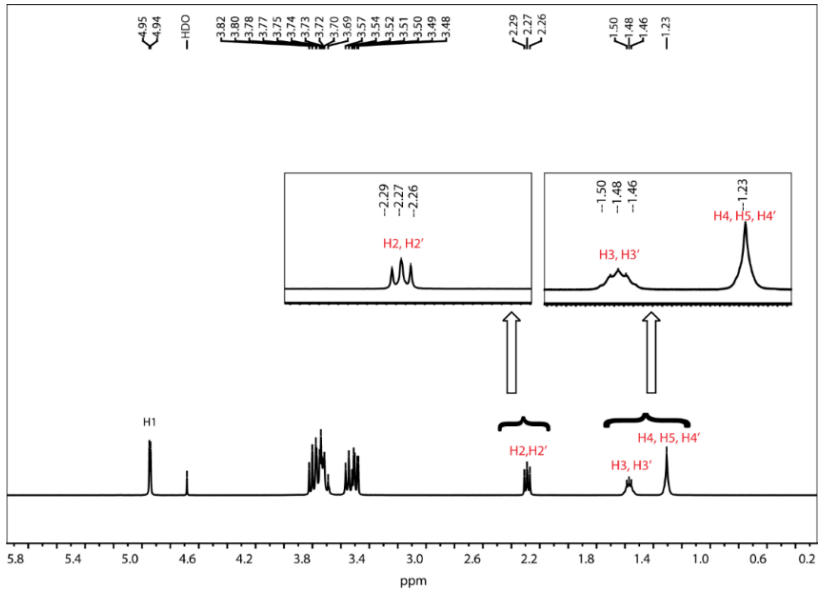


Fig.S9. : ^{1}H-NMR spectra of AA.α-CD complex in D_2O at 298.15K

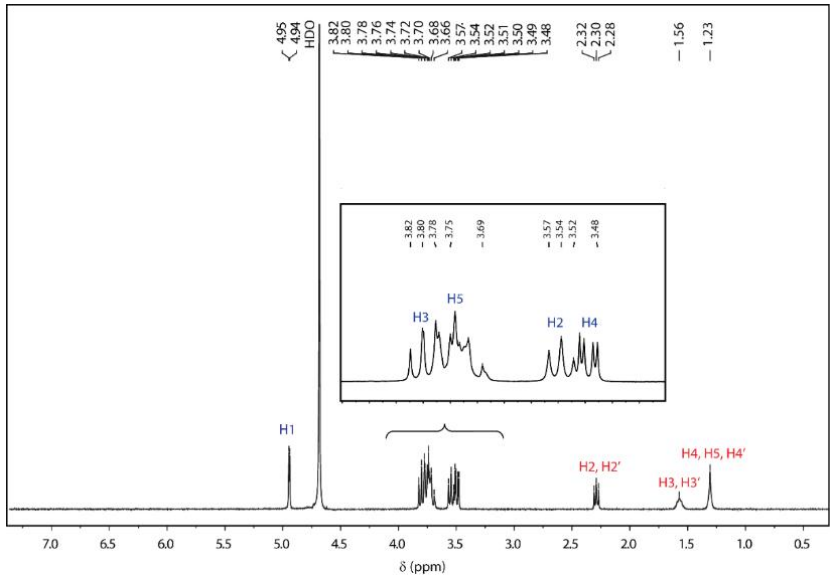


Fig.S10. : ^{1}H-NMR spectra of AA.β-CD complex in D_2O at 298.15K

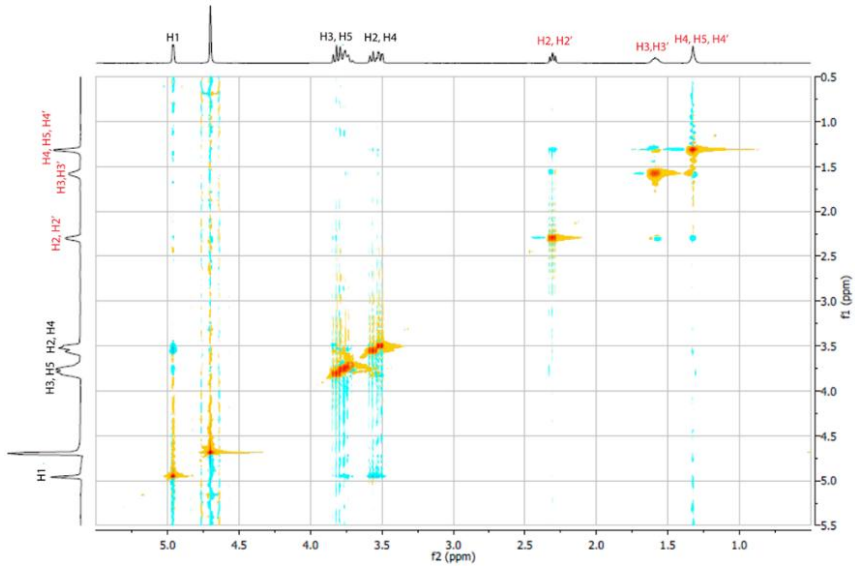


Fig.S11. 2D ROESY NMR Spectra of AA.α-CD inclusion complex

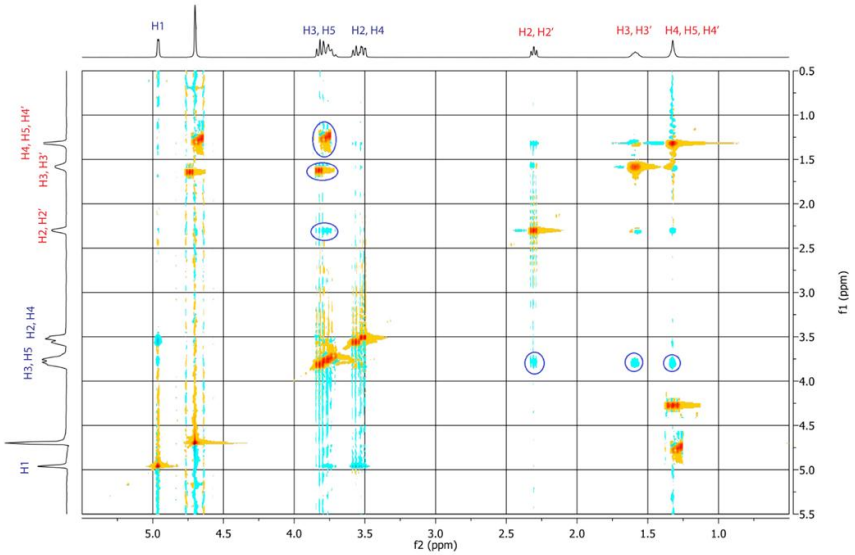


Fig.S12. 2D ROESY NMR Spectra of β-CD.AA inclusion complex

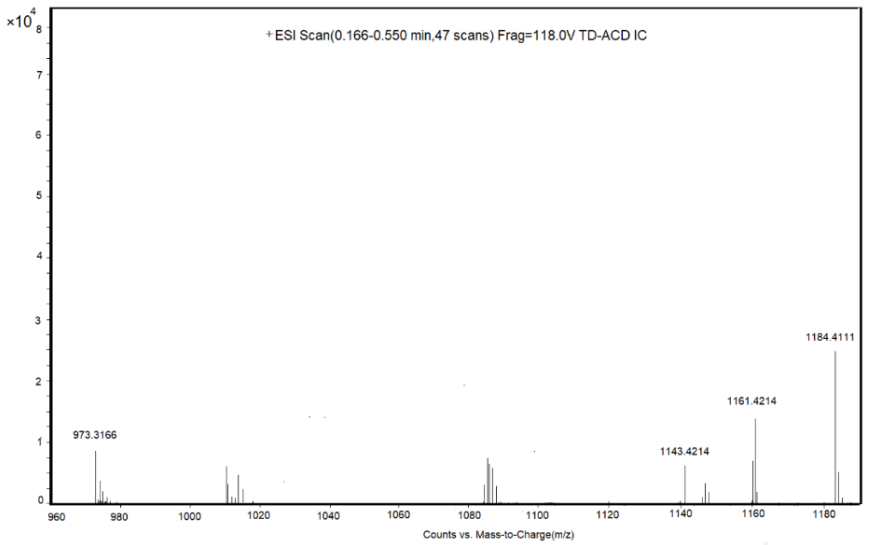


Fig.S13. : HRMS spectra of AA.α-CD inclusion complex

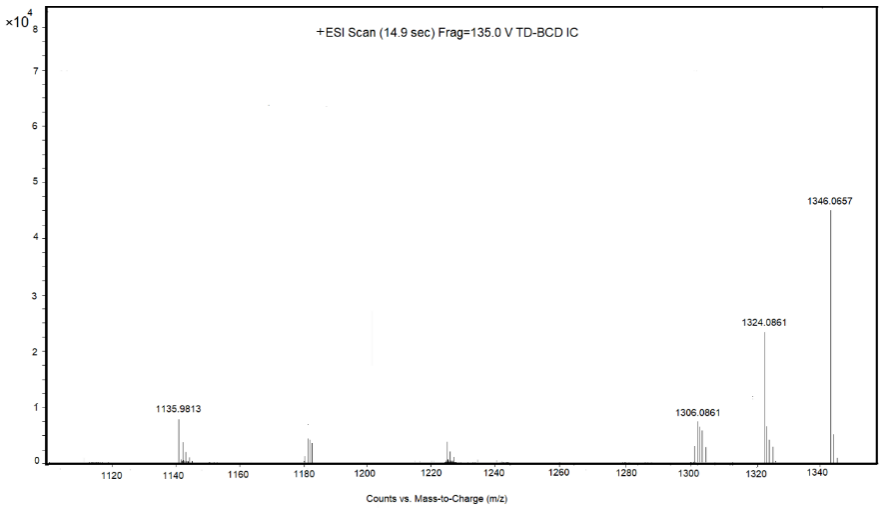


Fig.S14. : HRMS spectra of AA.β-CD inclusion complex