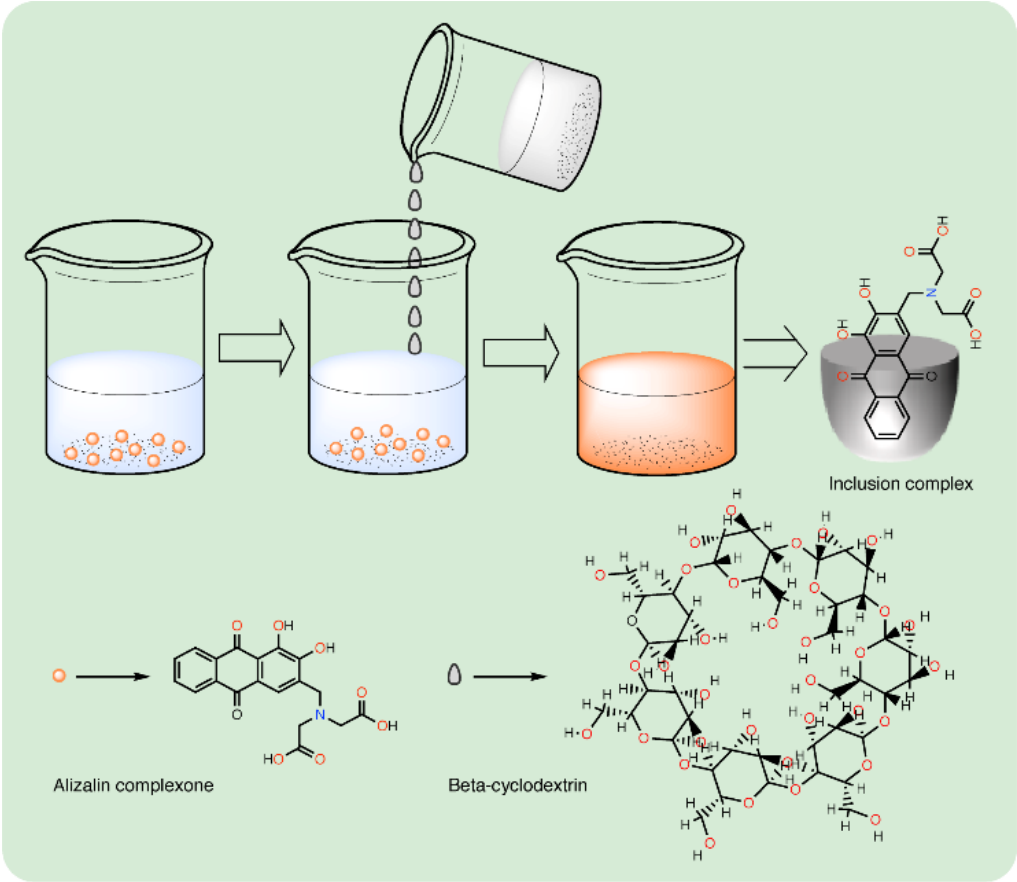
CHAPTER-V

Synthesis and Characterization of Host Guest Inclusion Complexation of Cyclic Oligosaccharide with an Industrially Potent Dye in Different Phases by Physicochemical Contrivance



ABSTRACT

β-CDs are known to absorb dyes and can, therefore, be used to bring down the loss of dye in savage water, thus it becomes capable to modify dye uniformity and prevent the running of dyes during washing. In this paper, an industrially important dye Alizarin complexone has been incorporated inside the cavity of β-Cyclodextrin and the inclusion complex formed was characterised by 1H NMR, 2D ROESY, UV-Visible Spectroscopy, Fluorescence Spectroscopy, HRMS and SEM study. Such inclusion phenomena protect the dye molecule from external hazards like environmental degradation, oxidation, photolytic cleavage and can be used to remove the dye present in savage water.

Chapter V

1. INTRODUCTION

β-Cyclodextrin (βCD) has a truncated cone-shaped structure with a cavity size of 7.9 Å depth and top and bottom diameters of 6.0 and 6.5 Å respectively [9],[169]. The hydrophilic outer surface and a hydrophobic interior of CD molecule make it enable of encapsulating different guest molecules inside the hollow cavity [170] [14, 171]. When dissolved in an aqueous medium, the cavity of the β-Cyclodextrin molecule gets occupied by 8-9 water molecules, which are excluded from the cavity after its complexation with a well-fitted guest molecule [172]. The presence of the α-1,4-linkage of each glucopyranose unit makes all of the hydrophilic 2-, 3-, and 6-hydroxyl groups to be located exterior of the hydrophobic cavity [172]. According to the literature survey [14, 173], it has been found that the most probable binding mode of both native and modified cyclodextrins (CDs) with different guest molecules deals with the encapsulation of the less hydrophilic part of the guest molecule inside the CD cavity, whereas the more hydrophilic portion stays outside the primary or secondary rim of the cavity. First and foremost, the size and shape of the CD cavity are regulated by the covalently bonded glucose units. Therefore, the cavity permits more pronounced van der Waals interactions than nonpolar organic media, in which solvent molecules are able to move freely, and consequently, a more exothermic heat effect is observed [174]. Secondly, the dipole-dipole interaction, dipole-induced dipole interaction, and ion-dipole interactions between host and guest play vital roles and their extent of interaction entirely depends upon complexation of CD with lipophilic inorganic ions (e.g., PF_6^-, ClO_4^-, or SCN^-) [175] and with neutral and charged aromatics (e.g., substituted phenols) [14]. Such interactions combinely contribute to the large negative enthalpies of complexation. In most of the cases, hydrophobic interactions, hydrogen bonding along with van der Waals force serve as the key factors for the stable supramolecular network[175, 176]. The intracavity interactions between CD and guest is considered as a “nonclassical” hydrophobic model, in which the change in enthalpy (ΔH^0) and entropy (ΔS^0) can either be positive or negative[176] [177] rather than a “classical” one, in which both ΔH^0 and ΔS^0 are positive [178, 179]. The process of inclusion complexation can modify both the photochemical and photophysical properties of the guest molecules because of the formation of a host-guest. As a consequence of such alteration in the physical, chemical and biochemical properties of guest molecules, and their application capability can also be improved in the fields such as drug delivery technology [180, 181] [182], food industry[183] ,medicine [171, 180], agriculture [184], textile industry [185] etc. The magnitude of binding constant is one of the determining factors for the widespread application

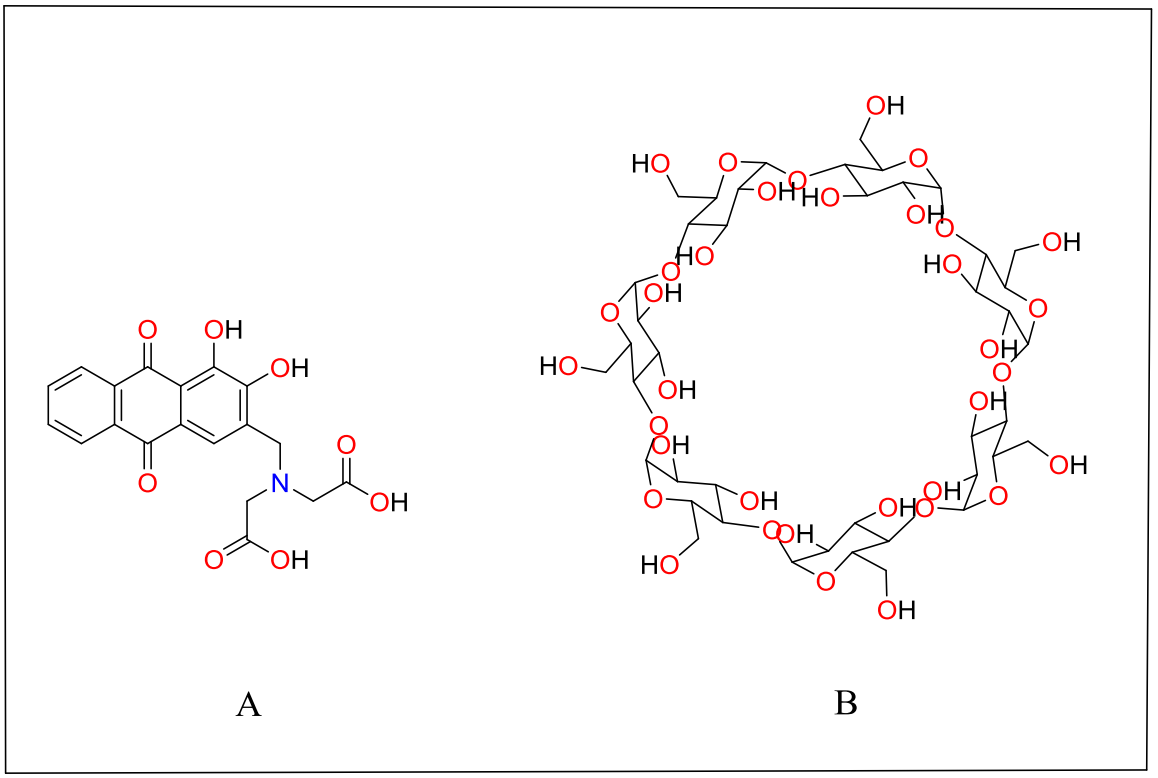
of CDs in the field of analytical chemistry [9]. In the textile industry, cyclodextrins act as defoaming agents by forming inclusion complexes with different detergent molecules [169]. CDs are also well known to remove dyes from aqueous solutions. The pattern of absorption and desorption of these dyes on CD molecules is precisely related to the extent of complexation strength of cyclodextrins [113].

Dyes have been the topic of utmost interest of the researchers in recent years because of increasingly demanding restrictions on the organic content of industrial sewage. Dyes are the most common water pollutants and often found in trace quantities in industrial wastewater. Many dyes do not degrade simply due to their complex aromatic structure and thus persist in the environment, which in turn causes serious ecotoxicological effects. Therefore, it would be advantageous to develop an inclusion complex of such dyes with CD molecules to eliminate them from the aqueous medium. Alizarin-3-methyliminodiacetic acid or Alizarin complexone (AC) is a very well-known reagent for the spectrophotometric determination of metals and it can form deeply coloured metal complexes [109]. In this paper, we have synthesised the inclusion complex of AC with β-CD molecule and the host-guest interactions have been studied with the help of UV-Visible Spectroscopy, Fluorescence Spectroscopy, 1H NMR Spectroscopy, 2D ROESY, HRMS and SEM analysis. We proposed that beta-cyclodextrin can be used to remove trace amount of AC from water.

The structures of the compounds used in this work are given in **Scheme1.**

Keywords

Alizarin-3-methyliminodiacetic acid or Alizarin complexone (AC), β-Cyclodextrin (β-CD), Fluorescence Study, Inclusion complex(IC), Job Plot, HRMS.



Scheme1. Molecular Structure of (A) Alizarin-3-methyliminodiacetic acid or Alizarin complexone and (B) β-Cyclodextrin.

2. EXPERIMENTAL SECTION

2.1. Materials used

Source and Purity of Samples

Alizarin-3-methyliminodiacetic acid or Alizarin complexone and β-Cyclodextrin were collected from Sigma-Aldrich, Germany and used without further purification. The mass fraction purity of the amino acids was ≥0.98.

The details of the chemicals used in this work are listed in **Table.1**; no further purification was performed. Distilled water was used for the preparation of all solutions.

Table. 1. Details of the chemicals used.

Name of chemicals	Source	CAS no	Purification method	Mass purity
Alizarin-3-methyliminodiacetic acid	Sigma-Aldrich, Germany	56-84-8	Used as purchased	≥98%
β- cyclodextrin	Sigma-Aldrich, Germany	7585-39-9	Used as purchased	≥99%
Distilled Water	Sigma-Aldrich, Germany	7732-18-5	Used as purchased	≥99%
Dimethyl sulfoxide-d6	Sigma-Aldrich, Germany	2206-27-1	Used as purchased	≥99%, ≤0.25% water)
Lithium hydroxide	Sigma-Aldrich, Germany	1310-65-2	Used as purchased	≥98%

2.2. Apparatus and procedure

The solubilities of the chosen compounds were precisely checked and it was found that the dye molecule taken is sparingly soluble in distilled water but it gets soluble in 70% methanol. The solutions used in this work were prepared by mass with the help of Mettler Toledo AG-285 having uncertainty ±0.0003g. Freshly prepared solutions were used every time. The uncertainty in molarity of the solutions was evaluated to ±0.0001 mol kg^{-3}.

Agilent 8453 UV-VIS spectrophotometer was used to measure the UV spectral pattern and JULABO F-32 thermostat was used to maintain the experimental temperature. Fluorescence study was performed with the help of QuantaMaster 40 spectrofluorometer.

For 1H NMR study, spectra were recorded at 400 MHz BRUKER AVANCE using D_2O. Signals were denoted as δ values in ppm using residual protonated solvent signals as internal standard (D_2O: δ 4.79 ppm). The differences in chemical shifts were recorded to study the interaction between AC and β-CD molecules.The Mass Spectroscopic analysis was done in Agilent Accurate-Mass Q-TOFLC/MS6520. Scanning Electron Microscope (SEM) study was performed in JEOL JSM-IT100 instrument.

2.3. Synthesis of the inclusion compound

Because of low solubility of AC in water, 0.5 mmol of AC was dissolved in 70% methanol (15mL) and β-CD.12H_2O (0.5 mmol) was dissolved in water (20 mL). The methanolic solution of AC was added dropwise to the aqueous solution of β-CD. The temperature was kept constant at 55°C. After 48 hr of continuous stirring at 55^0C, a light yellow coloured compound was obtained. After cooling down to room temperature, the precipitate was washed successively with pure methanol & distilled water. The crude solid was dried in vacuum drying oven to obtain perfectly dried AC.β-CD inclusion complex.

3. RESULT AND DISCUSSION

3.1 FTIR Spectroscopy

FTIR is a reliable technique to track the formation of the inclusion complex in both the solid and liquid state. We checked FTIR spectra for pure AC, pure β-CD and the inclusion complex formed. IR spectroscopic data obtained are given in **Table.S1**. **Fig.1**.shows that pure β-CD has a broad band at 3408.32cm^{-1}. On the other hand the guest molecule exhibits a broad band at 3465.73 cm^{-1} due to the O-H stretching vibration. In AC.β-CD complex, this band was found at 3451.45 cm^{-1}. Such change in frequency might happen due to the changed environment of the O-H groups inside the cyclodextrin molecule. In pure β-CD, the C-H stretching vibrations were found at 2943.65cm^{-1} and in case of AC compound, aliphatic C-H bond stretching frequency appeared at 2956.30cm^{-1} but in the IC C-H stretching frequency got shifted to 2929.49 cm^{-1}. This implies that the band of both the molecule got merged to generate a single band. Bending vibrations of –C-H (–CH_2) and O-H and C-O-C involving α-1,4 linkage for CD were obtained at 1402.42, 1161.56 and 974.23 cm^{-1} respectively and in inclusion complex all those got shifted to 1380.46, 1148.34 and 974.23 cm^{-1} respectively. The band due to aromatic C=O (in case of guest) stretching frequency was observed at 1632.74 cm^{-1} and in IC it was found at 1632.74 cm^{-1}, may be due to the insertion of the aromatic part of the guest molecule. This fact is in good agreement with the results

obtained from both 1H-NMR spectroscopic study and 2D ROESY spectrum. The stretching frequency (symmetric) of C=O and C-O (–COOH group) were found at 1432.25 cm^{-1} and 1285.17 cm^{-1} respectively but both stretching frequencies were observed at 1448.89 cm^{-1} and 1292.56 cm^{-1} respectively in case of the IC formed. Similarly, in inclusion complex, the stretching frequency of aromatic C-H bond was obtained at 791.87 cm^{-1} whereas, in case of pure AC, it was found at 797.72 cm^{-1}. This is simply due to the insertion of the aromatic portion of the AC and interaction of the aromatic protons with inner protons in the hydrophobic environment[186] [187].

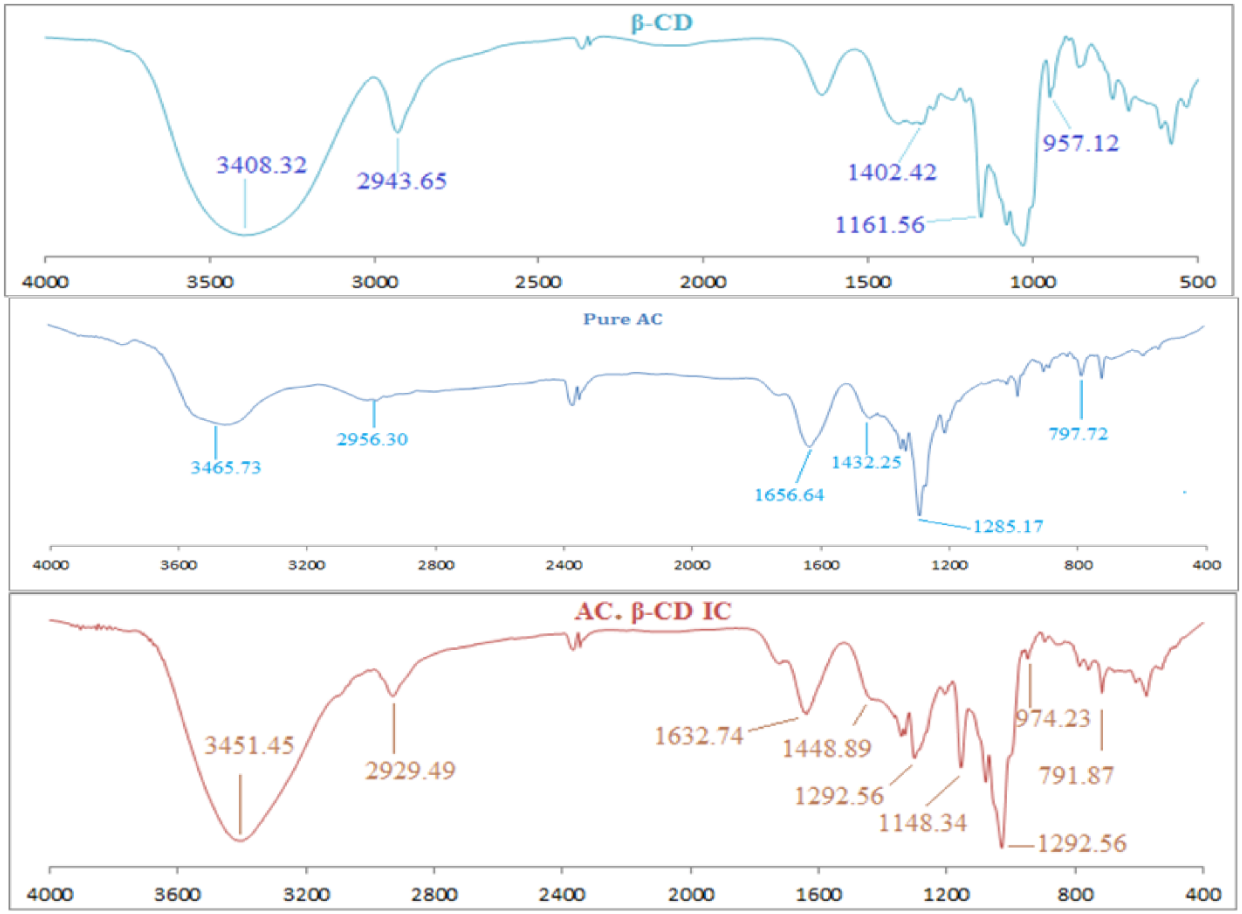


Fig.1. FT-IR spectra of free β-CD, AC and their 1:1 inclusion complex (AC.βCD) and 298.15K.

3.2. UV-Vis Spectroscopy

3.2.1 Job's Plot

We applied "Job's method of continuous variation" to determine the stoichiometry of the prepared inclusion complex [188-190]. All the solutions were prepared in 70% methanol (v/v), because of the low solubility of the guest molecule in water. A set of solutions of different concentrations ranging from 20 μM to 200 μM were prepared and the change in intensity along

with the absorption pattern of the solutions were recorded (**Table. S5**); The maximum absorption wavelength (λ_{max}) was found at 426 nm. The difference in absorbance ΔA was plotted against R and we found R-value at 0.5 (**Fig.2**.), which indicates 1:1 stoichiometry. ΔA denotes the difference in absorbance of the pure AC and in pure form and AC.β-CD complex and R represents [AC]/([AC]+[CD]). The value of R is a measure of the stoichiometry of host-guest inclusion complexes such as R ≈ 0.33, R≈ 0.5 and R≈ 0.66 stands for 1:2, 1:1 and 2:1 host: guest stoichiometry respectively.

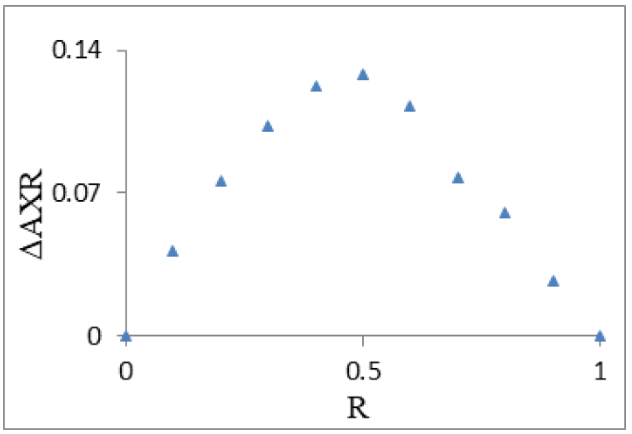


Fig.2. Job Plot performed by UV-Vis spectroscopic study for AC.β-CD system at 298.15K.

3.2.2. Association Constant and Thermodynamic Parameters

The UV-Visible spectroscopic study is the most commonly applied technique for the determination of the association constant (K_a) of the host-guest inclusion complex[191]. During the process of inclusion, when the guest (chromophore) molecule gets encapsulated into the cavity of CD molecule from the more polar bulk solvent system, a considerable change in the molar extinction coefficient (Δε) of the chromophore takes place [192]. It reflects in the change of the absorption pattern. UV-Vis absorption (ΔA) of AC (at λ_{max} = 426 nm) was recorded at three different temperatures ranging in between 293.15K to 313.15K and the association constants (K_a) at those temperatures were also calculated **(Table.S2, S3 and S4.)** [193]. The double reciprocal plots were made on the basis of Benesi–Hildebrand equation for IC of 1:1 stoitiometry. We divided the intercept by the slope obtained from the straight line of the double reciprocal plot and thereby calculated association constants (K_a) at three different temperatures [194] [189] [162] (**Fig.S1**).

$$\frac{1}{\Delta A} = \frac{1}{K_a[AC]_0\Delta\varepsilon}.\frac{1}{[CD]_0} + \frac{1}{\Delta\varepsilon[AC]} \quad (1)$$

With the help of van't Hoff equation (2), various thermodynamic parameters such as enthalpy (ΔH^0), entropy (ΔS^0) and free energy change (ΔG^0) were calculated (**Table.2**.) and a linear relationship was obtained between ln K_a and 1/T [195] (**Fig.S2**.).

$$\ln K_a = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (2)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (3)$$

Table.2. Association constants (K_a) obtained by the Benesi–Hildebrand method at 293.15Ka, 303.15Ka, 313.15Ka, and corresponding Gibb's free energy change (ΔG^0), enthalpy change (ΔH^0) and entropy change (ΔS^0) of AC.β-CD system.

Inclusion Complex	ka ($10^{-3}M^{-1}$)			ΔG^0 kJ mol^{-1}	ΔH^0 kJ mol^{-1}	ΔS^0 mol^{-1} K^{-1}
	293.15K	303.15K	313.15			
AC.β-CD	4.06	3.02	1.86	-2.59	-2.97	-12.80

The negative enthalpy (ΔH^0) value indicates the inclusion process to be an exothermic one and the negative entropy value states the process to be entropy controlled [196]. When the guest molecule enters into the hollow cavity of CD,it leads to the decrease of the entropy value and hence negative entropy value was obtained, which in turn increases the disorderness of the whole system. This ultimately leads the whole process of inclusion as thermodynamically favourable. The negative ΔG^0 value signifies the overall process is spontaneous one [196].

3.3. Fluorescence Spectroscopy

With the help of fluorescence spectroscopy we can determine the change in fluorescence intensity of the inclusion complex even if it is present in a trace amount (micromolar range) in solution. This technique is proved to be very sensitive in case of determining very large association constant with high accuracy [197]. In this paper, we recorded the variation in the intensity of the emission spectra with different concentration of the β-CD molecule ranging from 20 μM-200 μM, while the concentration of the guest was kept constant. The emmission maxima

was recorded at 492 nm. The association constant can be determined according to the Benesi–Hildebrand double reciprocal equation (eqn. 4).

$$\frac{1}{F_i - F_0} = \frac{1}{K_a[F_{max} - F_0]} \cdot \frac{1}{[CD]^n} + \frac{1}{[F_{max} - F_0]} \quad (4)$$

The graph of 1/ΔA against 1/[CD] gives a linear plot with a regression value of 0.9906 **(Fig. 3).** From the double reciprocal plot, we calculated the association constant at 298.15 K. The association constant calculated was 2.0×10^3, which is in good agreement with the association constant calculated from UV-Vis spectroscopic study (**Table.S6. & Table.S7.**). The stoichiometry of the inclusion complex can also be predicted from fluorescence spectroscopy and it is entirely based upon the linearity of the plot. The plot of 1/ΔA v/s 1/[CD] follows a linear trend with a regression value of 0.9906 but the plot of 1/ΔA against $1/[CD]^2$ gives a nonlinear plot having a regression value of 0.9272 (**Fig.3.**). The nonlinear plot completely ruled out the possibility of 1:2 inclusion complexation formation [198]. Hence, fluorescence study supports the phenomena of inclusion as explained earlier as well as it confirms the result obtained from Job plot.

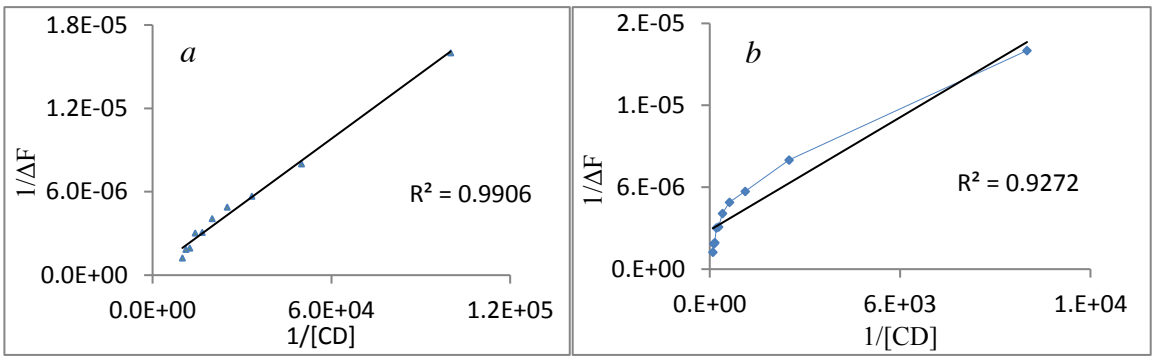


Fig.3. Benesi-Hildebrand double reciprocal plot showing linearity of *(a)* 1/ΔF against 1/[CD] and *(b)* 1/ΔF against $1/[CD]^2$ at 298.15K.

3.4 $^{1H\ NMR}$ analysis

NMR spectra of the D_2O (NMR solvent) was taken first to check its purity. Due to very low solubility of AC in D_2O solvent, of 1-1.5 mg of LiOH base was used to get the whole sample dissolved. For the IC, we used the same procedure. All measurements were performed at 298.15 K using a 400 MHz spectrometer. Whenever a guest molecules gets encapsulated inside the hollow cavity of CD molecule, there happens some specific changes in the chemical shift values of the protons in the IC[199] [200]. From the structure of β-CD molecule, it has been observed that H3 and H5 protons are placed inside the hollow cavity whereas other protons such as H1,

H2 and H4 are present at the exterior of the cyclodextrin molecule [189]. More precisely, H3 proton is located near the wider rim and H5 proton is closer to the narrower rim. When AC molecule enters through the –OH groups of less polar part interacts with the with the H3 and H5 protons, which gives rise to the upfield chemical shift of H3 and H5 protons. Because of the location of H3 proton (placed near the wider rim) compared to H5 proton, it will undergo more upfield shift rather than the H5 one and H1, H2, H4 will exhibit negligible upfield shifts. In inclusion complex the H3 proton has been shifted to 0.06 ppm (upfield), whereas H5 proton shifts to 0.03 ppm (upfield) only. Such upfield shifts for H3 and H5 protons might be explained in terms of magnetic anisotrophic effect originating from the aromatic ring of guest molecules (**Fig.S3**.) [201].In the inclusion complex, peaks for aromatic protons of the guest molecule experienced some extent of upfield shift and the peak due to presence of H5 proton (guest molecule) got disappeared. Upfield shift of both CD protons (H3 and H5) and guest protons (H1, H2, H3 & H4) indicates that the less polar part of the guest molecule has been encapsulated in the cavity of β-CD without any covalent bond formation.

1H NMR data

β- Cyclodextrin (β-CD): 1H NMR (in D_2O): δ/ppm 3.49(t, 6H, *J* = 9.2 Hz), 3.56-3.61 (dd, 6H, *J* = 9.6, 3.2 Hz), 3.79-3.81 (m, 18H), 3.88(t, 6H, *J* = 9 Hz), 4.97-4.98 (d, 7H, *J* =3.6 Hz)

Alizarine Complexone (AC): 1H NMR (in D_2O): δ/ppm 7.29-7.38 (d, 4H, J= 3.6 Hz) , 4.06 (s, 1H) , 3.66 (s, 4H)

AC.βCD IC 1H NMR (in D_2O) : δ/ppm 3.49(t, 6H, *J* = 9.2 Hz), 3.56-3.61 (dd, 6H, *J* = 9.6, 3.2 Hz), 7.21-7.26 (d, 4H, J= 3.6 Hz), 3.62(s, 4H), 3.74-3.77 (m, 18H), 3.82(t, 6H, *J* = 9 Hz), 4.97-4.98 (d, 7H, *J* =3.6 Hz)

3.5 2D ROESY

2D ROESY NMR is a technique for investigating the spatial proximity between the host and the guest moieties by observation of intermolecular dipolar cross-correlations. In NOESY or ROESY spectroscopy, when two protons are located in space closer than 4Å able to produce a nuclear overhauser effect (NOE) cross peaks. **Fig.4.** shows the 2D spectrum of AC.βCD inclusion complex in which only a single intermolecular NOE cross-peak was observed. The correlation of H1, H2, H3 and H4 protons from AC with H3 and H5 protons from βCD indicated the insertion of the benzene ring of AC into the cavity of βCD through the wider rim. No other cross peaks between

H5, H6, H7, H8 of AC with the protons of βCD were observed. These results further confirmed that the AC.βCD inclusion complex was successfully formed in the solution phase. Possible modes of interactions in the inclusion complex is shown in **Scheme 2**.

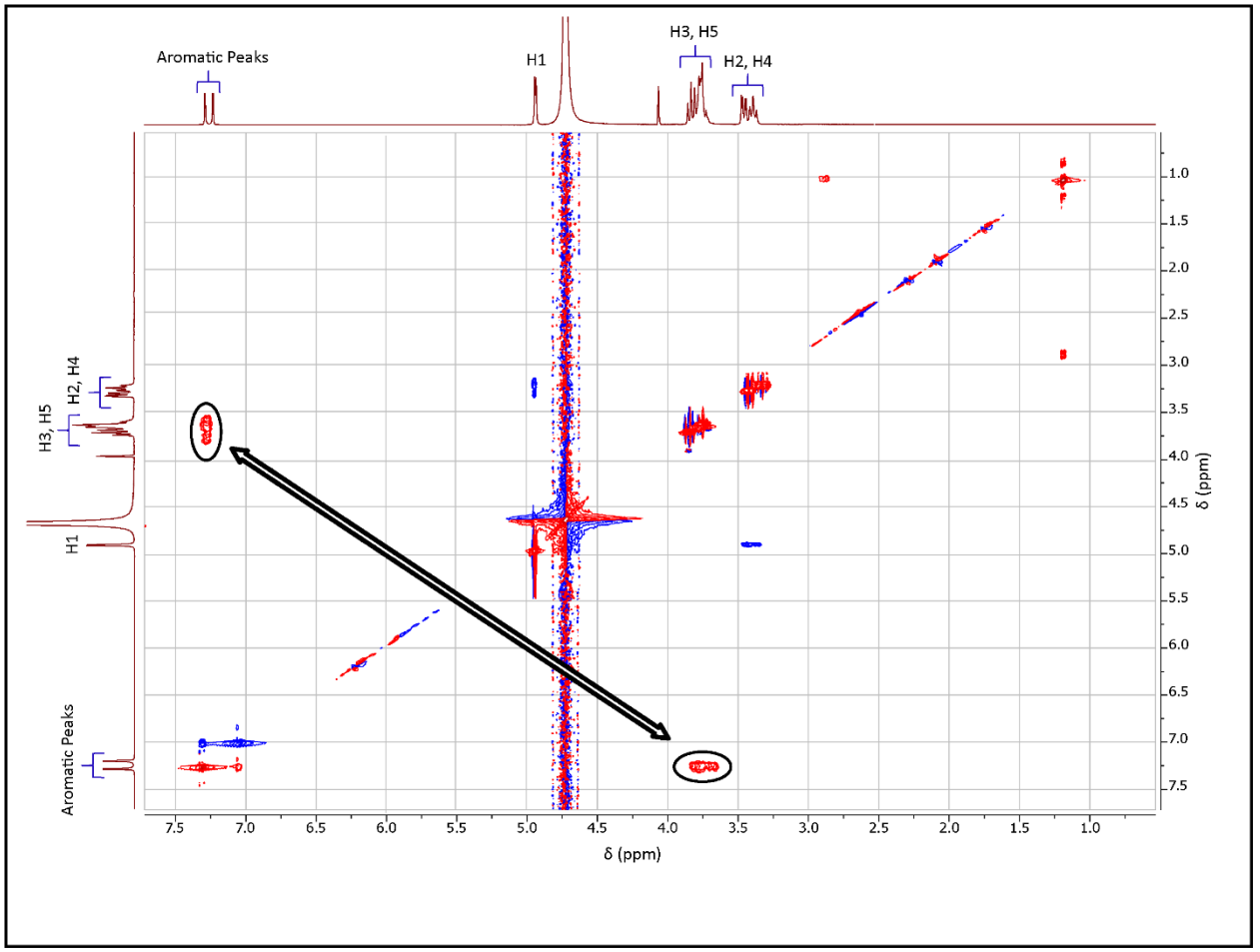
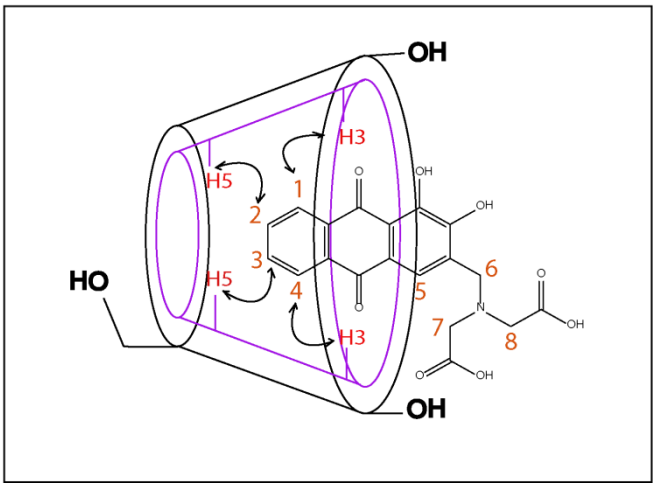


Fig.4. 2D ROESY spectrum of AC.β-CD inclusion complex in D_2O.



Scheme 2. Possible modes of interactions between host and guest in AC.β-CD inclusion complex.

3.6 HRMS Study

AC.β-CD inclusion complex was prepared in methanol and were introduced into the ion source of the mass spectrometer with a flow rate of 25mL/min with the help of a syringe pump. The detection was performed in the positive ion mode. The peaks were given in m/z (% of basis peak).

The peak obtained at 1520.4555 and 1542.4379 in $[M+H]^+$ and $[M+Na]^+$ mode respectively indicates the formation of 1:1 AC.βCD IC. These values are quite close to the calculated values of m/z 1520.4573 and 1542.4392 for $[M+H]^+$ and $[M+Na]^+$ respectively and the spectra have been revealed in **Fig.S4** [187].

Table.3. The calculated values and obtained m/z values of the solid inclusion complex in methanol

Ions	Exact Mass (calculated) $[M+H]^+$	m/z values Obtained	Exact Mass (calculated) $[M+Na]^+$	m/z values Obtained
[AC+βCD]	1520.4573	1520.4555	1542.4392	1542.4379

3.7. Scanning Electron Microscope (SEM) Study

The scanning electron microphotographs of pure βCD, pure AC, AC: βCD physical mixtures and AC.βCD inclusion complex are given in **Fig.5**. Scanning electron microscopy (SEM) analysis was performed on a JEOL JSM-IT100 to find out whether any morphological change took place in case of the AC.β-CD inclusion complex. After dissolving in isopropanol, the sample solution was dropped onto freshly cleaved cover glass, and dried at 65°C and kept in vaccum desicator for 6-8 hours. After complete drying, a few nm-thick layer of gold was spread onto the surface of cover glass. From the SEM images it can be seen that pure β-CD particles appeared as three-dimensional particles with a parallelogram shape [202] and pure Alizarin compound appeared as three dimensional irregular shaped component. In the physical mixture, the presence of both AC and β-CD has been found. On the other hand, morphograph of AC.β-CD inclusion complex showed presence of homogeneous aggregates of small block shaped crystals, which seem to be different from those of the pure compounds and the physical mixture[202]. Under high resolution, the IC appeared as bar shaped crystals with well-developed faces. Such sharp change

in the surface morphologies indicates the presence of a new phase; this fact can be attributed to the molecular encapsulation of AC molecule inside β-CD.

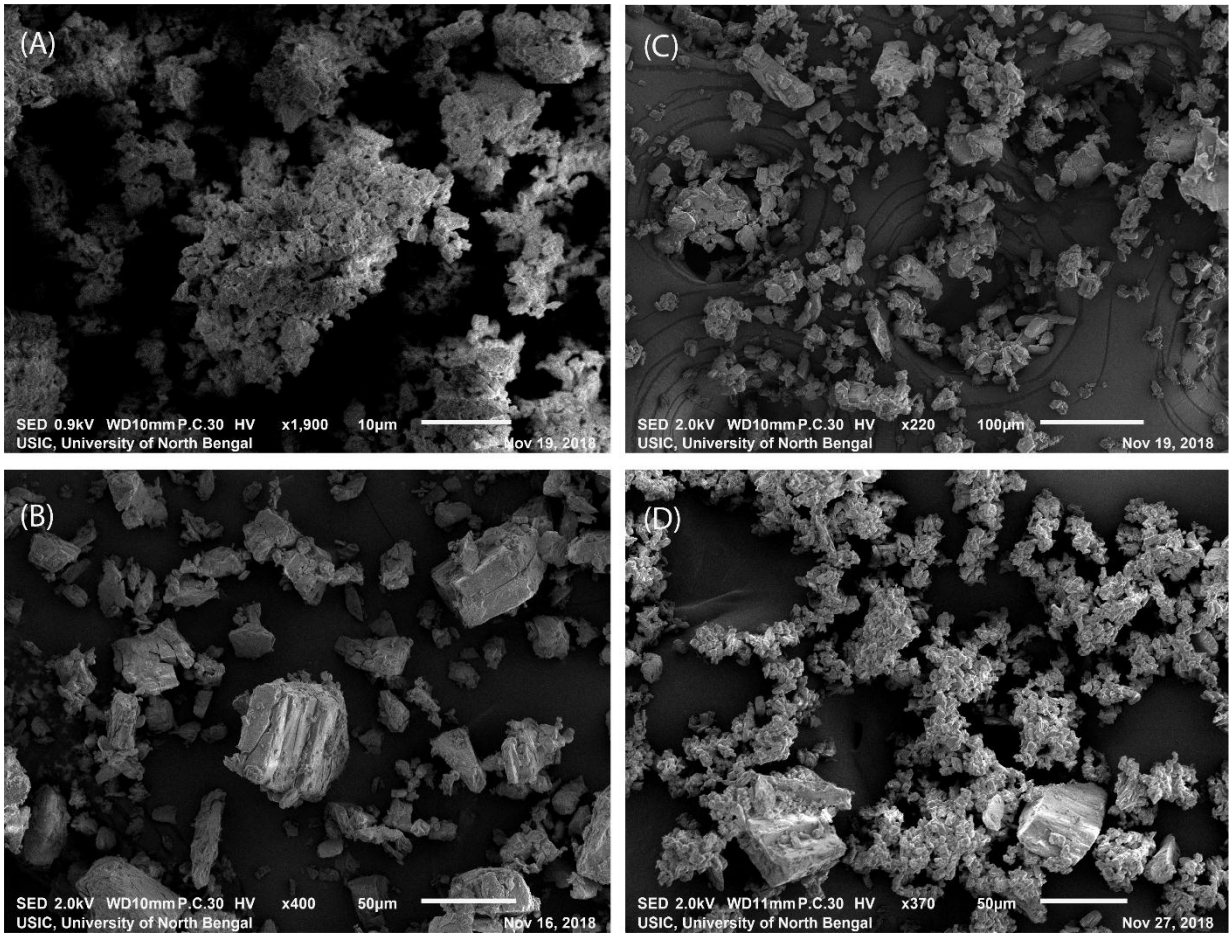


Fig.5. SEM images of (A) pure Alizarine Complexone, (B) pure βCD , (C) AC.βCD inclusion complex and (D) Physical mixture of AC and βCD.

CONCLUSION

In this paper, the results obtained from FTIR spectroscopic study shows that the less polar part of the guest molecule that is the aromatic ring was included inside the hollow cavity of β-CD and the rest part of the the molecule remained outside. From Job's plot, The R value was found at 0.5 (approximately) indicating formation of 1:1 stoichiometry. The upfield chemical shifts experienced by H3 and H5 protons (of β-CD molecule) and H1, H2, H3, H4 protons (of AC molecule) further supports the fact. The large value of association constatnts obtained from both UV-Visible and fluorescence spectroscopic study confirmed the formation of inclusion complex which is in good agreement with the results obtained from 2D ROESY. SEM study too showed significant morphological changes between pure compounds and IC formed. Such inclusion

phenomena led to an improved dye strength and deepen the shades of dyes.This can also enhance possibility of dyeing at lower temperatures.

Supporting Informations

Synthesis and Characterization of Host Guest Inclusion Complexation of Cyclic Oligosaccharide with an Industrially Potent Dye in Different Phases by Physicochemical Contrivance

Table. S1. Stretching frequencies obtained from FTIR spectroscopic study of pure β-CD, AC, AC.β-CD complex

Functional Groups	Wave number (cm-1)		
	B-CD	AC	AC.β-CD
stretching of O-H	3408.32		3451.45
stretching of –C-H from $–CH_2$	2943.65		2929.49
bending of –C-H from $–CH_2$ and bending of O-H	1402.42		1380.46
bending of C-O-C	1161.56		1148.34
vibration involving α-1,4 linkage	974.23		957.12
Streching of Aromatic O-H		3431.73	3451.45
Streching due to Aliphatic C-H bond		2956.3	2929.49
Streching due to aromatic C=O		1632.74	1632.74
Symmetric streching due to carboxylic C=O		1432.25	1440.89
Streching due to C-O from -COOH		1285.17	1292.56
Aromatic C-H		779.72	781.87

Table.S2. Data for Benesi-Hildebrand double reciprocal plot performed by UV-Visible Spectroscopic Study for of AC.β-CD system at 293.15K

a_0	a_1	Δa	1/Δa	[CD]	1/[CD]	1/Δa
0.21351	0.22159	0.00808	4.512839	1.0E-05	100000	123.7624
0.21351	0.23123	0.01772	4.324698	2.0E-05	50000	56.43341
0.21351	0.24785	0.03434	4.034698	3.0E-05	33333	29.12056
0.21351	0.24827	0.03476	4.027873	4.0E-05	25000	28.7687

0.21351	0.249	0.03549	4.016064	5.0E-05	20000	28.17695
0.21351	0.25089	0.03738	3.985811	6.0E-05	16667	26.75227
0.21351	0.25144	0.03793	3.977092	7.0E-05	14286	26.36436
0.21351	0.25365	0.04014	3.94244	8.0E-05	12500	24.91281
0.21351	0.25991	0.0464	3.847486	9.0E-05	11111	21.55172
0.21351	0.29573	0.08222	3.381463	1.0E-04	10000	12.16249

Table. S3. Data for Benesi-Hildebrand double reciprocal plot performed by UV-Visible Spectroscopic Study for of AC.β-CD system at 303.15K

a_0	a_1	Δa	1/Δa	[CD]	1/[CD]	1/Δa
0.20273	0.21348	0.01075	4.68428	1.0E-05	100000	93.02326
0.20273	0.23029	0.02756	4.342351	2.0E-05	50000	36.28447
0.20273	0.25195	0.04922	3.969041	3.0E-05	33333	20.31694
0.20273	0.25848	0.05575	3.868771	4.0E-05	25000	17.93722
0.20273	0.26079	0.05806	3.834503	5.0E-05	20000	17.22356
0.20273	0.26116	0.05843	3.82907	6.0E-05	16667	17.1145
0.20273	0.2616	0.05887	3.82263	7.0E-05	14286	16.98658
0.20273	0.26198	0.05925	3.817085	8.0E-05	12500	16.87764
0.20273	0.26229	0.05956	3.812574	9.0E-05	11111	16.78979
0.20273	0.26229	0.05956	3.812574	1.0E-04	10000	16.78979

Table. S4. Data for Benesi-Hildebrand double reciprocal plot performed by UV-Visible Spectroscopic Study for of AC.β-CD system at 313.15K

a_0	a_1	Δa	1/Δa	[β-CD]	1/[CD]	1/Δa
0.18932	0.19738	0.00806	5.066369	1.0E-05	100000	124.06948
0.18932	0.20765	0.01833	4.815796	2.0E-05	50000	54.55537

0.18932	0.22572	0.0364	4.430268	3.0E-05	33333	27.47253
0.18932	0.22773	0.03841	4.391165	4.0E-05	25000	26.03489
0.18932	0.22791	0.03859	4.387697	5.0E-05	20000	25.91345
0.18932	0.22914	0.03982	4.364144	6.0E-05	16667	25.11301
0.18932	0.2311	0.04178	4.327131	7.0E-05	14286	23.93490
0.18932	0.23153	0.04221	4.319095	8.0E-05	12500	23.69107
0.18932	0.25182	0.0625	3.97109	9.0E-05	11111	16.00000
0.18932	0.25715	0.06783	3.888781	1.0E-04	10000	14.74274

Table.S5. Data for Job plot performed by UV-Visible Spectroscopic Study for of AC.β-CD system at 293.15K

drug conc. [D] (μm)	β-CD (μm)	[D]/ ([D]+ [β-CD])	A @λmax 426nm	ΔA (0.49208-A)	ΔAx[D]/ ([D]+[β-CD])
0	100	0.0	0.00000	0.49208	0.000000
10	90	0.1	0.07021	0.42187	0.042187
20	80	0.2	0.1095	0.38258	0.076516
30	70	0.3	0.14786	0.34422	0.103266
40	60	0.4	0.18492	0.30716	0.122864
50	50	0.5	0.23481	0.25727	0.128635
60	40	0.6	0.30459	0.18749	0.112494
70	30	0.7	0.38116	0.11092	0.077644
80	20	0.8	0.41641	0.07567	0.060536
90	10	0.9	0.46208	0.03000	0.027000
100	0	1	0.49208	0.00000	0.000000

Table.S6. Data for Benesi-Hildebrand double reciprocal plot performed by fluorescence Spectroscopic Study for of AC.β-CD system at 293.15K

[β-CD] (μM)	F_0	F	ΔF	1/[β-CD] (M-1)	1/ΔF
10	1692264	1754720.38	62456.38	100000.00	1.60112E-05
20	1692264	1817184.25	124920.25	50000.00	8.00511E-06
30	1692264	1867867.00	175603.00	33333.33	5.69466E-06
40	1692264	1896432.25	204168.25	25000.00	4.89792E-06
50	1692264	1937050.88	244786.88	20000.00	4.08519E-06
60	1692264	2015943.00	323679.00	16666.67	3.08948E-06
70	1692264	2022118.63	329854.63	14285.71	3.03164E-06
80	1692264	2203450.50	511186.50	12500.00	1.95623E-06
90	1692264	2231364.00	539100.00	11111.11	1.85494E-06
100	1692264	2490871.00	798607.00	10000.00	1.25218E-06

Table.S7. Data for Benesi-Hildebrand double reciprocal plot (assuming the stoichiometry to be 1:2) performed by fluorescence Spectroscopic Study for of AC.β-CD system at 293.15K

[β-CD] (μM)	$[β\text{-CD}]^2$ (μM)	F_0	F	ΔF	1/[β-CD]2 (M-1)	1/ΔF
10	100	1692264	1754720.38	62456.38	10000.00	1.60112E-05
20	400	1692264	1817184.25	124920.25	2500.00	8.00511E-06
30	900	1692264	1867867.00	175603.00	1111.11	5.69466E-06
40	1600	1692264	1896432.25	204168.25	625.00	4.89792E-06
50	2500	1692264	1937050.88	244786.88	400.00	4.08519E-06
60	3600	1692264	2015943.00	323679.00	277.78	3.08948E-06

70	4900	1692264	2022118.63	329854.63	204.08	3.03164E-06
80	6400	1692264	2203450.50	511186.50	156.25	1.95623E-06
90	8100	1692264	2231364.00	539100.00	123.46	1.85494E-06
100	10000	1692264	2490871.00	798607.00	100.00	1.25218E-06

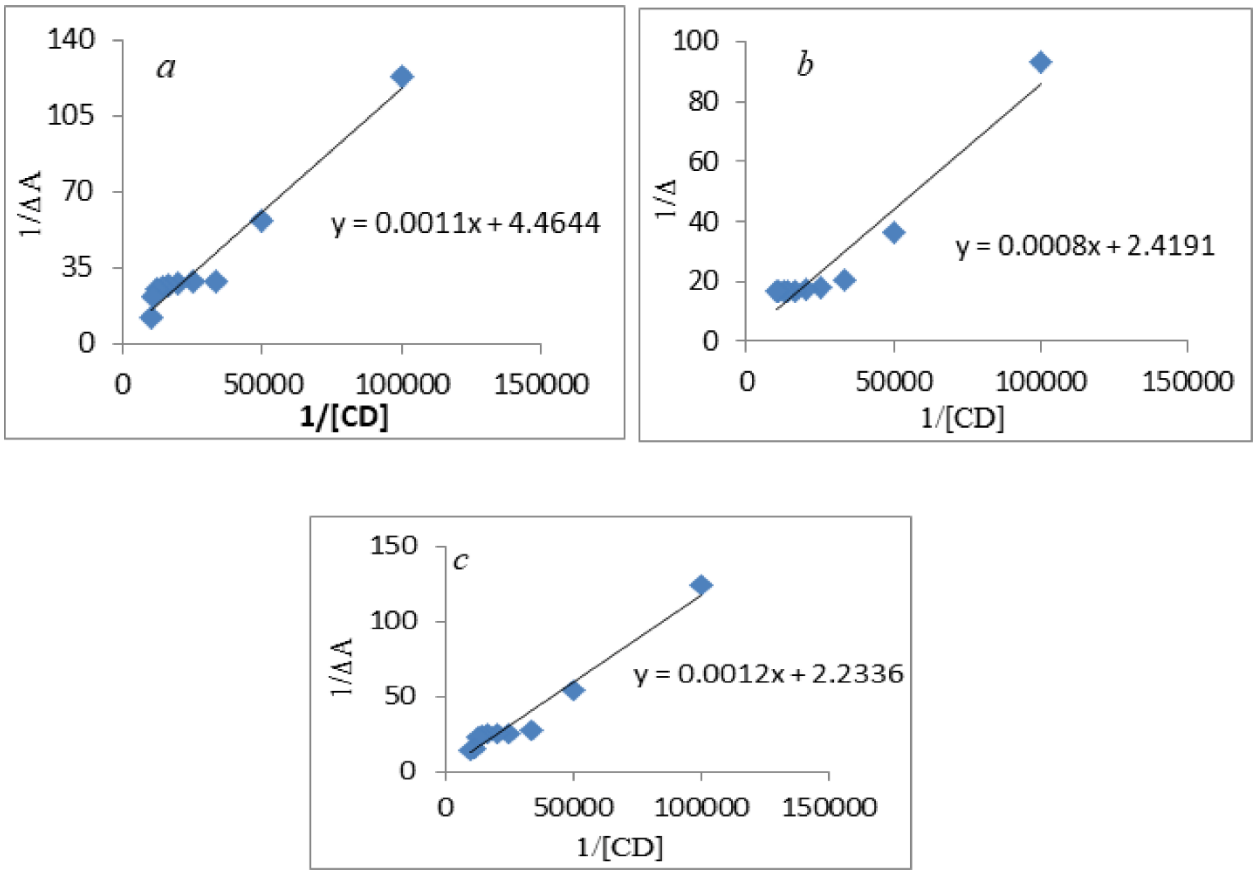


Fig.S1. Benesi-Hildebrand double reciprocal plot for the effect of β-CD on the absorbance of AC at three different temperatures (*a*) 293.15 K ,(*b*) 303.15 K and (*c*) 313.15 K.

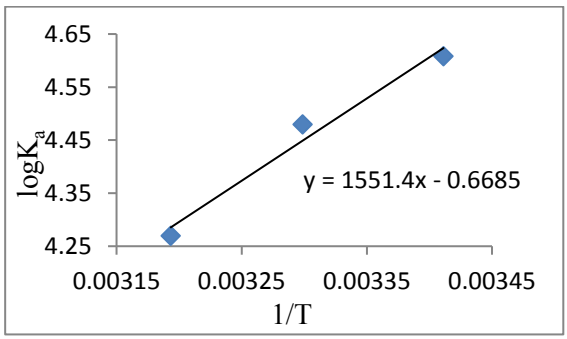


Fig.S2. Plot of logK_a vs 1/T for the interaction of β-CD with AC.

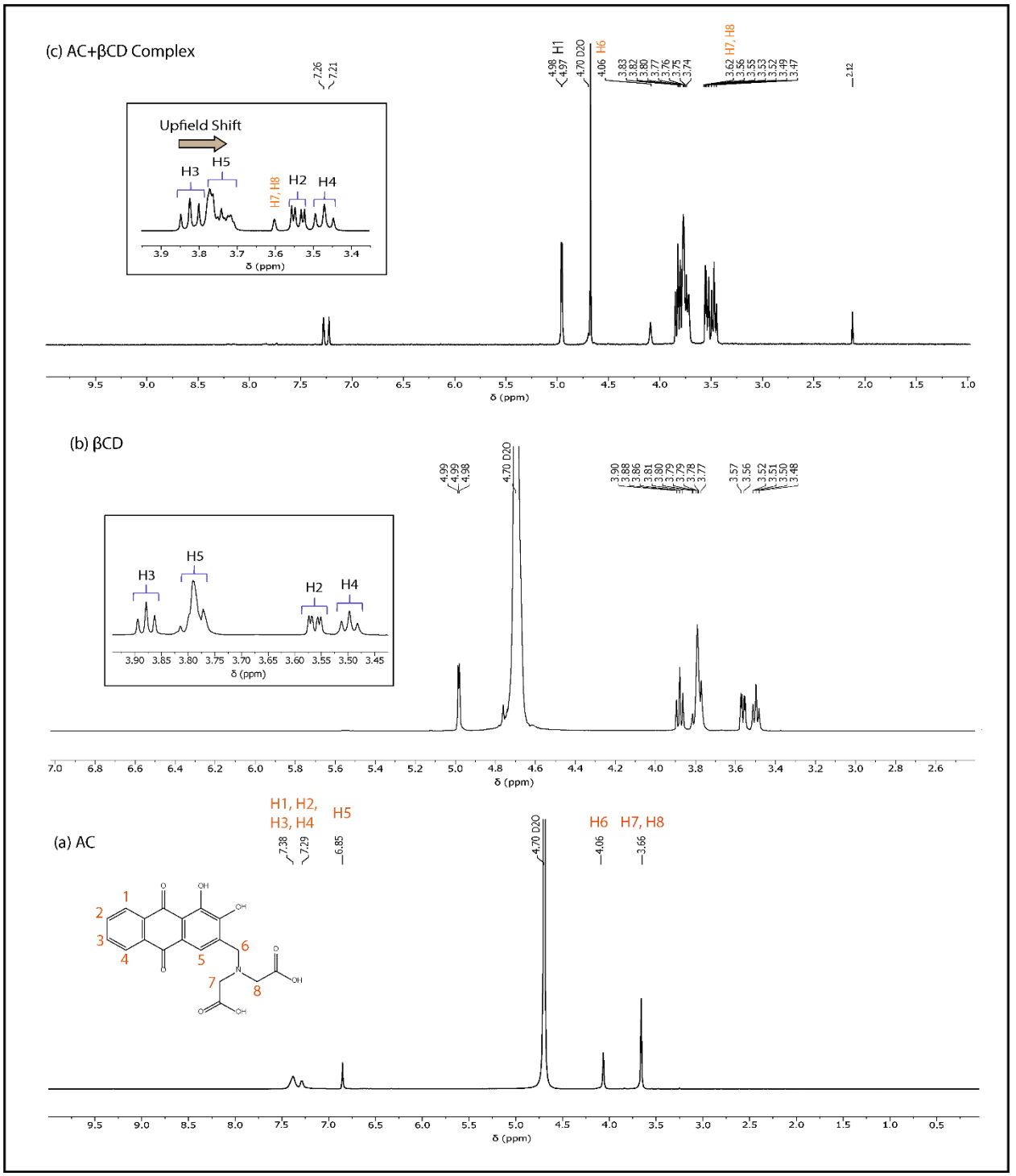


Fig.S3. 1H NMR spectra of (a) pure AC , (b) pure βCD and (c) (AC.βCD) system.

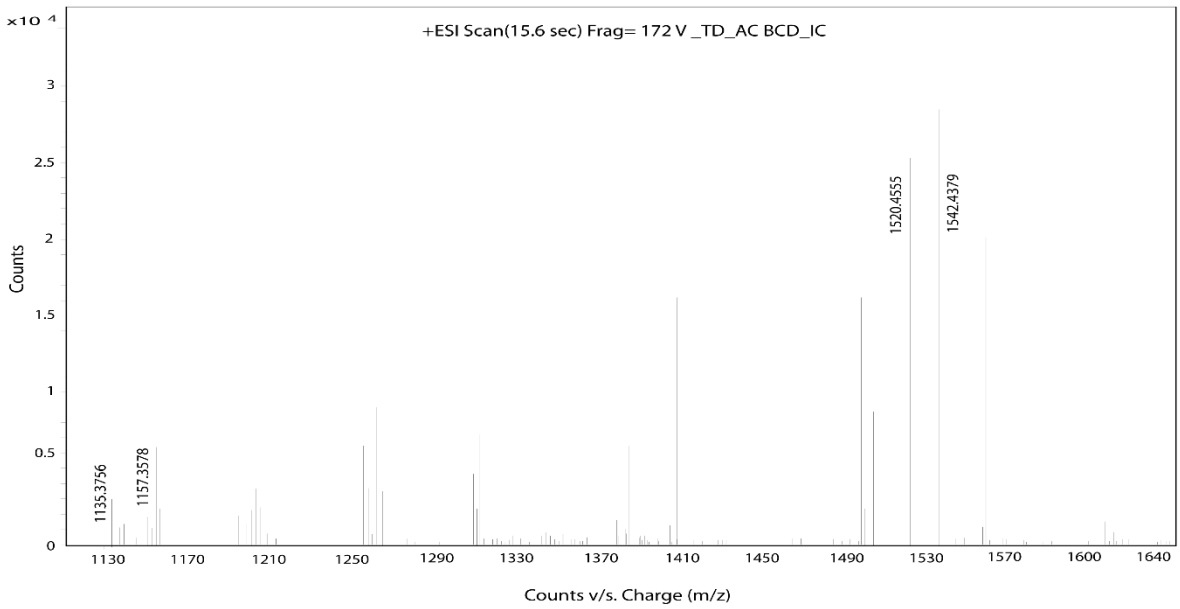


Fig. S4. HRMS Spectra of AC.β-CD inclusion complex.