CHAPTER VII

EXPLORING INCLUSION COMPLEXES OF CYCLODEXTRINS WITH QUINOLINONE BASED GASTRO PROTECTIVE DRUG FOR ENHANCING BIOAVAILABILITY AND SUSTAINED DISCHARGEMENT

ABSTRACT

Solid rebamipide based inclusion complexes were achieved by freeze-dry method and characterized by FTIR, UV-vis, ^{1}H-NMR, 2D-ROESY, fluorescence spectroscopy, SEM and conductance. The enzyme substituted emission spectrum of the two comparative inclusion complexes with β-CD and HP-β-CD in the diverse solvent systems determined the controlled release of the drug were the mid of interest. Amylase increased the stability of the inclusion complexation, proved that if it is taken together with the inclusion complex, the effectiveness and impact of the inclusion complexes will have a prolonged effect in the body. It could significantly improve the bioavailability of rebamipide.

Keywords: β-Cyclodextrin; Hydroxypropyl-β-Cyclodextrin; Drug release assay; Molecular recognition; Supramolecular Chemistry.

1. INTRODUCTION:

In recent ages, supramolecular chemistry has become a major influence in the field of macrocyclic chemistry. Although low solubility is a common issue for various drug molecules but encapsulation process makes the host-guest complexes more soluble in water. This study basically focuses on the host–guest inclusion phenomena of low water soluble rebamipide (RB) in comparison with two types of cyclodextrin for the prevention of different types of diseases like gastric cancer [1], mucosal protection against gastro duodenal ulcers [2], treatment of gastritis [3], inflammatory disorder [4], analgesic activity, antinociceptive activity, dry eye disease, allergic conjunctival diseases, attenuated cartilage degeneration [5]. In our present exertion, we have taken rebamipide (RB) as the guest molecule, β-

cyclodextrin (β-CD) and Hydroxypropyl-β-cyclodextrin (HP-β-CD) as the two analogous host molecules as the articles about the study on selecting inclusion materials of mucosal drug are few (Scheme 1).

Rebamipide is chosen as the guest molecule having various impact which is still to be explored in the fields of supramolecular chemistry for it's sustain release of drug delivery in the body. Rebamipide ($C_{19}H_{15}ClN_2O_4$) belongs to the class of quinolinone family. (±)-2-(4-chlorobenzoylamino)-3-(2-oxo-1H-quinolinon-4-yl) propionic acid (MW: 370.786 g/mol) is the IUPAC name of the compound [6]. Historical facts tell that RB was known by the trade name of Mucosta, a gastro protective drug, developed in Japan, Rebagen in South Korea (Republic of Korea), China and India, Rebagit in Russia.

Cyclodextrins (CDs) are the category of cyclic oligosaccharides which have in recent times been recognized as useful matrices. Owing to its hydrophobic cavity, CD can interact with suitably sized molecules to result in the formation of inclusion complexes. This polysaccharide is a sort of novel functional macromolecule which possesses the cumulative effects of inclusion, size specificity, controlled release capability and transport properties of CD over and above the biocompatibility, non-toxicity and biodegradability of it. Recently, encapsulation of various cyclic oligosaccharides such as cyclodextrins having good cavity size with organic molecule have been carried out to validate its inclusion phenomena and it's various thermodynamic stabilities [7].

Apart from cyclodextrins, other several host molecules can also be used e.g, 18-crown-6, Calixarenes, cucurbit[n]urils such as, 18-crown-6, cucurbit[6]uril have been used as a host and drug molecules are encapsulated within their cavity to assort various interactions and emphasize the inclusion process [8, 9]. However, in this work, among the various types of cyclodextrin, β-CD and HP-β-CD are taken as the host molecules for both the size and solubility factor matching with the model drug molecule. In support to various physicochemical, spectroscopic techniques for the two complexes in solution phase in both pure (DMSO) and mixed solvent (1:1–

C_2H_5OH:H_2O) have been investigated for their potential use in controlled drug delivery applications (Scheme 2).

2. EXPERIMENTAL SECTION:

2.1 Materials

Rebamipide was bought from TCI Chemicals (India) Pvt. Ltd. Both the Cyclodextrins (i. e., β-CD and HP-β-CD) was bought from Sigma Aldrich Germany and used as purchased. The mass fraction purities of RB, β-CD and HP-β-CD were ≥0.99, 0.98 and 0.98, respectively.

2.2 Instruments

All the Stock solutions of rebamipide, β-CD and HP-β-CD were prepared by weighing (Mettler Toledo AG-285 with uncertainty 0.0001 g) and dilution and kept in a slightly heating water bath.

Conductivities of both the solutions were studied by Mettler Toledo Seven Multi conductivity meter having uncertainty 1.0 μSm^{-1}. It was carried out in a thermostated water bath at 298.15 K with uncertainty ±0.01 K. Double distilled water was used with specific conductance 6.0 μSm^{-1}. Moreover, the conductivity cell was calibrated using 0.01 M aqueous KCl solution.

UV–visible spectra were recorded by Agilent 8453 Spectrophotometer. The temperatures were controlled with a digital thermostat. All the absorption spectra were recorded at 298± 0.1K, 303± 0.1K, 308± 0.1K.

Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer FT-IR spectrometer by means of the KBr disk technique. Samples were prepared as KBr disks with 1 mg of solid inclusion complex and 100 mg of KBr. FTIR measurements were performed in the scanning range of (4000–400 cm^{-1}) with resolution of 4 cm^{-1} at room temperature.

Differential Scanning Calorimetry (DSC) spectra were recorded by Perkin Elmer Pyris 6 DSC calibrated by pyris manager software. The samples were heated in the temperature range 30-300^{0}C in an inert nitrogen atmosphere at a heating rate of 10^{0}C/min. The samples were taken in an aluminum container at about 1.2 mg in all cases.

Steady state fluorescence Spectra were recorded on spectrofluorimeter from photon Technology International (PTI, U.S.). The measurements were done at an excitation of 230 nm by using quartz cuvette having 1 cm path length.

^{1}H-NMR and 2D-Rotating-frame Overhauser Effect Spectroscopy (ROESY) spectra were recorded at 400 MHz Bruker AVANCE at 298.15 K in d_6-DMSO using a 5mm probe. Signals are quoted as δ values in ppm by means of residual protonated solvent signals as an internal standard (δ 2.50 ppm). Acquisition parameters consisting of spectral width of 2000 MHz, number of scan 8, acquisition time 3.27 sec. Data were reported as chemical shift.

Scanning electron microscopy (SEM) morphological images were obtained using JEOL JSM IT 100 scanning electron microscope (SEM). The images were captured at an excitation voltage of 2.5kV, 3.0kV, 5kV, 2.5kV and 4.0kV whereas magnification of 6000, 1400, 1400, 5500, 2700× for RB, β-CD, HP-β-CD, IC-1 and IC-2 respectively.

2.3 Sample preparation of Solid Inclusion Complex of Rebamipide with Cyclodextrins:

To prepare a complex between Cyclodextrins (i.e., β-CD and HP-β-CD) and drug, freeze drying method is the most popular method to form the inclusion complex in a solution of cyclodextrin. In this method, the guest is dissolved into a solvent solution of cyclodextrin to form the inclusion complex in a crystalline form. However, since the chosen pharmaceutical drugs have a very low solubility in water, we investigated the preparation of inclusion complexes between Cyclodextrins and the guest (RB) in organic solvent, DMSO. As reported, 1mM

solution of 25 ml RB (9.26 mg) and 1mM of 25ml β-CD (28.37 mg) as well as HP-β-CD (38.53 mg) were prepared. Then the solutions were mixed (added drop wise) in two different beaker and stirred for 12 hrs at 55^0C. Finally, the solution was freeze dried for 24 h to obtain inclusion complex.

3. RESULTS AND DISCUSSION:

Rebamipide, the guest molecule selected in these assay is moderately soluble in organic solvent (i.e., DMSO) and partially in the binary solvent of C_2H_5OH and water. As our aim is to prepare a host-guest inclusion complex which have sustain release in the body, therefore all the measurements were done by slightly increasing the temperature. Thereby increasing the temperature it was noted that all the physicochemical and spectroscopic evidences deep-rooted the formation of the various types (1:1 or 2:1 or 2:2) inclusion complexes in stable equilibrium.

Two types of solvents were used in order to show compare their stability and specificity during the formation of inclusion Complexation in various aforementioned ratios. Slightly increased temperature was used to totally dissolve both the host and the guest molecule.

Rebamipide is an insoluble drug in water and in most common organic solvents due to intermolecular electrostatic attraction between ammonium and carboxyl group. Therefore in order to increase its applicability we have tried diverse solvents to increase its solubility two fold. There are various publications based on amino acid based surfactants [10] which has increased the worth of amino acids. Here in these work we have tried an innovative way to work on derivative amino acid (i.e., rebamipide) based cyclodextrins in pure DMSO and mixed solvent of C_2H_5OH and water. In various previous publications [11, 12] we found that after inclusion in cyclodextrin there is a chance of aggregation or vesicle formation with various imperative guest molecule. Here as we have taken aromatic amino acids, therefore, there is much probability of it to behave as a surfactant. Therefore we have performed the surface tension and conductivity experiments. Different

concentrations of rebamipide based cyclodextrin solutions were prepared and measured respectively.

3.1 Conductance study:

Ion mobility increases as the temperature is raised, which leads to an increase in the limiting conductance. As the solubilization is precise at high temperature, therefore we had studied the inclusion Complexation by conductance method at the higher temperature. With the adding of cyclodextrin appears an extreme point on the dependence, which becomes more harshly when the temperature increases. At high concentrations of cyclodextrin (>1.60 mM), cyclodextrin-rebamipide–DMSO interaction becomes more intensive, which leads to the formation of bulky solvate shell affecting negatively on the ion mobility, thereby forming an inclusion complex.

From the previous publications, we have studied appearance of the extreme point on these curves indicates the presence of competing interactions in the system. We have examined two systems i.e., (HP-β-CD+RB+DMSO) and (β-CD+ RB+DMSO), where the prominent results were observed. Thereby, over viewing the fact of encapsulation with the formation of break point, Sharp break point is prominent for β-CD-Rebamipide solution in DMSO (Fig. 1a) which shows the formation of 1:1 complex and in HP-β-CD-Rebamipide-DMSO solution (Fig. 1b), there is the uniformity in the values which shows that if we increase the cyclodextrin concentration, a linear increase in the specific conductance up to the HP-β-CD concentration at which “micelle like” formation began, i.e., up to the break point (i.e., CAC in case of surfactant) (Table S1 and S2 of supporting information). After the CAC was attained, conductance further increased linearly, but with a lower slope than before the CAC and the break in the conductance-concentration titration curve provides the CAC of the HP-β-CD [13-19].

3.2 Job Plot: Elucidation of Stoichiometry Behaviour of Cyclodextrins: Rebamipide Inclusion Complex:

Job's method, commonly known as the continuous variation method, is a very proficient and successful method to determine stoichiometry of any host-guest inclusion complexes. So, due to this reason this method was applied here by using UV-visible spectroscopy [20]. Here, two sets of solutions were prepared of RB with β-CD and HP-β-CD in 50% ethanolic solution, respectively, by varying the mole fractions of the guest (RB) in the range 0-1 (Tables S3, S4, S5 and S6, supplementary data). Job's plots of the mentioned sets were plotted of using ΔA×R against R, where ΔA means the difference in absorbance of RB without and with CD and R=[RB]/[RB]+[CD]).The whole process of taking absorbance values was done at respective λ_{max} by maintaining 298.15 K temperature. The stoichiometry of an IC is obtained by taking the value of R at the maximum point on the curve, for example, if the ratio of guest to host is 1:2 for R∽0.33, 1:1 for R∽0.5, 2:1 for R∽0.66 and so on. Here, in this work we got R∽0.5 as maximum in each plot, indicating a 1:1 stoichiometry of the corresponding inclusion complexes (Fig. 2).

3.3 Determination of binding constant of RB/β-CD in aqueous ethanol by UV–Vis spectroscopy:

The binding constant between β-CD, HP-β-CD and each RB has been evaluated via UV–Vis spectroscopy [21]. The Benesi–Hildebrand technique represents one of the most common strategies to determine binding constants based on absorption spectra for inclusion complex. In order to have an accurate estimation of binding constants of the inclusion complexes under investigation, changes in the absorption intensity of the RB at different wavelength, were monitored as a function of the CD's concentration and non-linear regression estimation of the K_a (Fig. 3a) was chosen.

From the UV-vis titration, Association constant value for RB/β-CD was found to be $2.03 \times 10^4\ M^{-1}$, $3.34 \times 10^4\ M^{-1}$, $1.39 \times 10^4\ M^{-1}$, at 298.15K, 303.15K, and 308.15K,

respectively (Table S7 & Fig. S1). The increase in association constant values (K_a) with increasing temperature indicated the increasing nature of interaction for inclusion complexation but then again association constant value went to decrease sharply indicating weakest interaction between guest and host [22].

3.4 Determination of binding constant of RB/HP-β-CD in aqueous ethanol solution by UV–Vis spectroscopy:

The evaluation of K_a by direct spectroscopic methods relies on analytical differences between the free and complexed amino acid. The binding constant between HP-β-CD and RB also has been evaluated via UV–vis spectroscopy. The Benesi–Hildebrand technique represents one of the most common strategies to determine binding constants based on absorption spectra for inclusion complex. In order to have an accurate estimation of binding constants of the inclusion complexes under investigation, changes in the absorption intensity of the RB at different wavelength, were monitored as a function of the CD's concentration and non-linear regression estimation of the K_a (Fig. 3b) was chosen.

Here, the association constant was found to be 2.73 $\times 10^4$ M^{-1}, 5.72 $\times$ 10^4 M^{-1}, 1.18 $\times$ 10^4 M^{-1}, at 298.15K, 303.15K, and 308.15K, respectively (Table S8 & Fig. S2). However, similar nature of association constant value is observed in case of RB/HP-β-CD system but interesting fact is that interaction between RB and HP-β-CD is greater than RB and β-CD system (Fig. 4).

3.5 Evaluation of Thermodynamic Parameters:

The thermodynamic and structural characteristics during Complexation could be used to estimate the solubilizing and mode of action of cyclodextrin. To calculate the basic thermodynamic parameters, the enthalpy and entropy values, we have used the van't Hoff's equation as follows [23],

$$\mathbf{lnKa} = -\frac{\mathbf{\Delta H^\circ}}{\mathbf{RT}} + \frac{\mathbf{\Delta S^\circ}}{\mathbf{R}}$$

Where H^0 and S^0 are the enthalpy and entropy during complex formation, respectively, T is the temperature, and R is the gas constant.

The Gibbs energy change was calculated by equation given below [24],

$$\Delta G^\circ = -RTlnKa$$

The thermodynamic parameters obtained from the van't Hoff plot using the above-mentioned equations were shown in Table 1a & 1b. From the given tables, the negative values in the Gibbs energy change (ΔG) and the enthalpy change (ΔH) indicated that the interaction of RB and β-CD, HP-β-CD were spontaneous and exothermic. Greater negative enthalpy change (ΔH) for RB/ HP-β-CD was usually an indication of quite strong molecular interactions caused by both van der Waals forces and formation of hydrogen bonds between host and guest than that of RB/β-CD system. These interactions were may be due to incorporation hydrophobic guest into the cyclodextrin cavity host [25]. Similarly, higher negative entropy change (ΔS) for RB/HP-β-CD indicates that inclusion process is more entropy favored for RB/HP-β-CD than that of RB/β-CD system.

3.6 Fourier Transform Infrared Spectroscopy (FTIR) Study:

FT-IR is a prominent technique used to confirm the formation of an inclusion complex as there will be variation of the shape, shift and intensity of the FT-IR absorption peak before and after the formation of inclusion complex [26]. The FT-IR spectra of RB, β-CD, HP-β-CD, IC-1, IC-2 are presented in Fig. 5. The FT-IR spectrum of RB consisted of the sharp absorption bands appear in the 3416 cm^{-1} for O-H stretching from –COOH, 3269 cm^{-1} (N-H) stretching of CONH group stretch, (-C=O) stretching of CONH appear in the region 1646 cm^{-1} and aromatic ring appear in the region 1539 cm^{-1}. In both cases of β-CD and HP-β-CD, the IR spectra can be characterized by the intense band at 3397 and 3410 cm^{-1} for O-H stretching frequencies and C-H stretching appears at 2922 and 2928 cm^{-1} respectively. No characteristic peak is observed from 1500 cm^{-1} to 400 cm^{-1} for the two Host molecules. After the formation of the inclusion complex, in both cases of β-CD and

HP-β-CD, the peak assigned for absorption of N-H stretching of CONH group has been disappeared. However, the spectra of the IC-1 i.e., inclusion complex of RB and β-CD correspond simply to the superposition of the spectra of the β-CD. All these phenomena indicate that only amide group and the acid group attached with tertiary carbon atom has been inserted into the cavity of cyclodextrin ring.

3.7 Differential Scanning Calorimetry (DSC) Analysis:

The pure drug RB and its Inclusion complexes with β-CD and HP-β-CD i.e, IC-1 and IC-2 were analyzed by DSC method as it can be used as recognition tool. According to the theory, if guest molecules are encapsulated into the cyclodextrin host cavities, their physical characteristics such as melting point, boiling point should be shifted to a different temperature or may get disappeared [27]. As shown in Fig. 6, RB showed a sharp characteristic endothermic melting peak at 305^0C, according to the previous literature. β-CD and HP-β-CD showed a single endothermic peak at about 88^0C and 60^0C which is due to the release of water from the β-CD and HP-β-CD respectively. However, when the guest molecules formed inclusion complex with β-CD i.e, IC-1, the endothermic peaks were shifted to 271^0C (RB) and 81^0C (β-CD). In case of IC-2, endothermic peaks were shifted to 259^0C (RB) and 70^0C (HP-β-CD). This Phenomena gave a strong evidence that inclusion complex has been formed.

3.8 ^{1}H-NMR and 2D-NMR Study:

The formation of IC can be explained on the light of the 1H NMR spectroscopy study. This method based on the changes of chemical shifts of protons due to encapsulation of guest molecule into the Cyclodextrin cavity [28]. In both β-CD and HP-β-CD structure, the H3 (near to wider opening side) and H5 (close to narrow rim side) are located inner part of the Cyclodextrin cavity and it is expected that when a hydrophobic moiety entered into the cyclodextrin cavity, there will be an upfield shift for those protons. It is clearly observed from Table 2a & 2b (in case of HP-β-CD) that for H3 and H5, a large upfield shift has been occurred than that of β-CD. The considerable changes of chemical shifts (Δδ) suggested that the RB monomer

entered into the nano hydrophobic hole of CD. Spectra are shown in Fig. 7a & 7b, For HP-β-CD, The upfield shift of H3 (**Δδ=-0.07 ppm**) is much greater than the H5 shifting (**Δδ=-0.04 ppm**) whereas, in case of β-CD, downfield shift in both cases of H3 (**Δδ=0.09 ppm**) and the H5 (**Δδ=0.04 ppm**) was observed, where, **Δδ= $\delta_{complex}$-δ_{free}**. On the other hand, minor chemical shifts are observed for H1, H2, H4, H6 and H7 that are not part of the interior hydrophobic hole of β-CD and HP-β-CD. This observation also confirmed that encapsulation of the guest moiety into hydroxypropyl derivative of cyclodextrin moiety was more prominent than that of the beta-cyclodextrin and the association constant values in solution phase for both the cases agreed with this report.

Similarly, further support for Complexation, ^{1}H NMR of RB has also been performed in Complexed form (Table. 2c). A significant upfield shift of Quinolone moiety rather than aromatic (holding –Cl) Protons has been observed in case of IC-2 as most of the aromatic peaks got disappeared which tells that Quinolone part are situated in hydrophobic hollow space. However, In case of IC-1, Here also upfield shift of Quinolone moiety rather than aromatic ring (holding –Cl) Protons has been observed but not that extent as in IC-2. Considering all these experimental data, one can conclude that the extent of formation of IC using RB is much more prominent in case of HP-β-CD over β-CD.

Two-dimensional (2D) NMR spectroscopy now-a-days has been extensively used to get some important information about the spatial proximity between host and guest molecule since two protons located closer than 4Å in space are expected to produce cross-peaks in ROESY spectra [29]. The 2D ROESY spectrum for the inclusion complex RB/β-CD (Fig. 8) showed the aromatic protons of RB have cross peaks to the H-3, and H-5 protons of β-CD, indicating the deep insertion of the benzene ring into the host cavity. Similarly, in case of RB/HP-β-CD also (Fig. 8), a cross peak has been observed between aromatic protons of RB and H-3 and H-5 proton of the HP-β-CD as well as two distinct cross peaks with methine proton at δ 4.92-4.93 (ppm) and methylene proton appeared at δ4.39-4.43 (ppm) which

indicate the insertion of aromatic ring and interaction of those protons with H-3 and H-5 proton of the host molecules.

3.9 Scanning Electron Microscope (SEM) Study:

SEM is an ideal method to visualize significant changes in the surface morphology of various substances such as host, guest as well as inclusion complex [30]. The SEM images of the samples are presented in Fig. 9. Pure Rebamipide appeared as needle shape crystal with small dimensions. The morphology revealed that β-CD appeared as rectangular shaped whereas HP-β-CD was appeared as spherical particles. After the formation of the RB/β-CD inclusion complex i.e, IC-1, surface morphology had been changed in different irregular shape in which the original morphology of both components disappeared and this comparison indicate the formation of the inclusion complex. However, when RB/HP-β-CD inclusion complex i.e, IC-2 was formed, some of the characteristic RB crystals were still present in the surface but shape of the surface morphology has been changed which indicate that inclusion complex was formed.

3.10 Release behavior in different solvents and an enzyme:

In case of IC-1, as simple hydrophobic interaction rather inclusion phenomena took place, fluorescence intensity was relatively high than IC-2 in Fig. 10(a). And it was largest when EtOH+H2O used as a solvent may be due to formation of hydrogen bond between EtOH+H2O with β-CD and Guest (RB) was relatively free. But upon addition of enzyme, it started to interact with guest and consequently intensity got weaker (red line). However, when DMSO was used as a solvent, intensity went to decrease which possibly due to polar aprotic nature of the solvent (green line) as DMSO is not hydrogen bond donor. Again it was decrease more when enzyme was used in the solution mixture.

Another important observation was that solvent as well as enzyme had a great impact on the binding capacity between host and guest in inclusion complex. When DMSO was used in case of IC-2 showing in Fig. 10(b), it has the largest fluorescence intensity (blue line) but when enzyme was added intensity got

decreased. It may be due to binding of the enzyme with HP-β-CD so that guest molecule was unable to come out of the cavity (orange line). However, when same IC-2 was taken in EtOH+H2O co-solvent, intensity was also lower than that using DMSO solvent (grey line). Fluorescent intensity was lowest when enzyme was added in the same co-solvent (yellow line).Therefore, in all cases, it was found that DMSO was much more effective solvent that EtOH+H2O co-solvent. For higher soluble guest such as HP-β-CD, enzyme can be used for better binding availability.

3.11 Sustained release nature of inclusion complexes:

Just like other experiment in this research work, biological assessment has also been done in two different solvent. One is ethanol-water mixture (1:1) and the other one is DMSO. This work has been carried out with human salivary which contains α-amylase as an enzyme. It has been reported that α-amylase has the ability to hydrolyze water-soluble beta cyclodextrin at a slower rate compared with the corresponding unsubstituted beta cyclodextrin [31].

The release of RB from the inclusion complexes were determined by fluorescence emission spectroscopy where excitation was done at 230 nm [32]. IC-1 i.e. inclusion complex of RB/β-CD when dissolved in DMSO, its fluorescence intensity shown in Fig. 11, was gradually decreasing up to 3 hrs but 3 to 4 hrs there was a sharp increase which indicate that the guest molecule was released very quickly. However, with increase in time, intensity was again decreasing gradually. When enzyme was used in the same solution, similar nature of fluorescence curve was obtained but here intensity in that time gap 3 to 4 hrs was quite lower which may be due to interaction between enzyme and the host cyclodextrin molecules that inhibits to release from the host cavity.

Similarly, when IC-1 dissolved in EtOH and water solvent mixture, there was a gradual decrease in the fluorescence intensity shown in Fig. 11, which probably due to the non release of the part of the guest molecule. All the spectra were shown in Fig. S3 of supporting information. It should be noted that solvent is highly polar in

nature where as aromatic part of the guest molecule is hydrophobic in nature. Hence, it preferred to stay inside the host. Again, same process had been carried out with ethanol-water with enzyme mixture. Result showed that there was a steep rise in the 1st hr but then intensity went down gradually which indicate that part of the RB guest molecule was still in the cavity of β-CD. So, all these facts suggest that RB/β-CD inclusion complex showed controlled release nature in DMSO solvent and DMSO with enzyme as well ethanol-water mixture with enzyme but not in case of ethanol-water mixture alone.

In case of IC-2 i.e. inclusion complex of RB/HP-β-CD when dissolved in DMSO, somewhat similar release profile was observed as in case of IC-1. When enzyme was used in the same solution, similar nature of fluorescence curve was obtained which indicate that enzyme did not affect its release nature.

Similarly, when IC-2 dissolved in EtOH and water solvent mixture, there was a gradual increase in the fluorescence intensity which probably due to the high release of the part of the guest molecule (Fig. 12). The spectra were shown in Fig. S4 of supporting information. Although It should be noted that solvent is highly polar in nature where as aromatic part of the guest molecule is hydrophobic in nature but it may happen that when inclusion complex was adding in co-solvent, EtOH and H_2O molecules got attached with Hydroxy propyl chain of the HP-β-CD and it open up its wider part of HP-β-CD. Hence, it preferred to go outside the host. Again, same process had been carried out with ethanol-water with enzyme mixture. Result showed that there was no change in intensity which indicates that part of the RB guest molecule was still in the cavity of β-CD. So, all these facts suggest that RB/HP-β-CD inclusion complex showed controlled release nature in DMSO solvent and DMSO with enzyme as well ethanol-water mixture but not in case of ethanol-water mixture with enzyme.

4. CONCLUSIONS:

In the present work, RB/β-CD and RB/HP-β-CD complexes with greater water solubility were successfully prepared. The FT-IR, DSC, ^{1}H NMR, and SEM

results confirmed the formation of the RB/β-CD and RB/HP-β-CD inclusion complexes. Job's plot confirmed the 1:1 stoichiometry of the guest and host molecules. The high association constant (K_a) value obtained by RB/HP-β-CD complex suggests that the inclusion complexes formed between RB and HP-β-CD showed greater interaction than RB/β-CD inclusion complex. ^{1}H-NMR confirmed the above statement by revealing the greater upfield shift of H3 & H5 proton in case of HP-β-CD. Conductance study revealed that Rebamipide molecules tend to aggregate in presence of β-CD and HP-β-CD in different solvents. 2D-NMR indicates that aromatic nucleus has been inserted into the cavity of β-CD and aromatic rings as well as methine and methylene group have been inserted into the cavity of HP-β-CD molecules. Finally, from the released kinetics study, it revealed that both β-CD and HP-β-CD inclusion complexes are a promising strategy for making it sustained release of the guest molecule. This study enriches the field the supramolecular construct and may find various applications in biology as well as therapeutic and analytical chemistry.

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Notes

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TABLES

Complex	T(K)	ΔG KJ mol-1	ΔH^0/kJ mol-1	ΔS^0/J mol-1 K-1
	298.15	-25.141		
RB/β-CD	303.15	-25.087	-28.344	-10.742
	308.15	-25.033		

Table 1a: Energy values of RB/β-CD inclusion Complexation

Complex	T(K)	ΔG KJ mol^{-1}	ΔH^0/kJ mol-1	ΔS^0/J mol-1 K-1
	298.15	-26.277		
RB/HP-β-CD	303.15	-25.660	-63.09	-123.47
	308.15	-25.043		

Table 1b: Energy values of RB/HP-β-CD inclusion Complexation

β-CD protons	Free β-CD δ (ppm)	IC-1 δ (ppm)	Δδ (ppm)
H1	5.71-5.78	5.71-5.78	0.00
H2	3.29-3.32	3.34	-
H3	3.65	3.74	**0.09**
H4	3.33-3.37	3.29-3.31	-
H5	3.61	3.65	**0.04**
H6	3.63	3.62	-0.01

Table 2a: ^{1}H-NMR Chemical shift data of free β-CD and its variation in complex

HP-β-CD protons	Free HP-β-CD δ (ppm)	IC-2 δ(ppm)	Δδ (ppm)
H1	5.01	4.93	0.08
H2	3.23	-	-
H3	3.78	3.71	**-0.07**
H4	3.13	-	-
H5	3.55	3.51	**-0.04**
H6	3.49	3.48	0.01
Me	0.99	1.12	-0.13

Table 2b: ^{1}H-NMR Chemical shift data of free HP-β-CD and its variation in complex

Pure Rebamipide	RB In IC-1	RB in IC-2
8.94-8.96 (d, 1H, J = 8Hz, aliphatic −N**H**−CO)	8.81-8.83 (d, 2H, J = 8Hz, aliphatic −N**H**−CO)	8.50-8.52 (d, 2H, J = 8Hz, aliphatic N**H**-CO)
7.80-7.82 (d, J = 8Hz, 2H, Arm H of benzene moiety)	7.82-7.84 (2H, J = 8Hz, Arm H of benzene moiety)	7.72-7.74 (d, 2H, J = 8Hz, Arm H of benzene moiety)
7.65-7.67 (d, J = 8Hz, 2H, Arm H of benzene moiety)	7.75-7.77 (2H, J = 8Hz, Arm H of benzene moiety)	_
7.43-7.46 (d, 2H, Arm **H** of quinolone moiety)	7.47-7.51 (2H, Arm **H** of quinolone moiety)	_
7.49-7.53 (t, Arm **H** of quinolone moiety)	7.31-7.29(d, Arm **H** of quinolone moiety)	_
7.24-7.32 (m, 2H, Arm **H** of quinolone moiety)	7.21-7.25 (2H, Arm **H** of quinolone moiety)	_
6.47 (d, 1H, **H** adjacent to C=O group)	6.43 (1H, **H** adjacent to C=O group)	_
4.68-4.74 (m, methine H)	4.63 (S, methine H)	4.92-4.93 (m, methine H)
4.24 (S, 1H, -CH_2-)	_	4.39-4.43 (S, 1H, -CH2-)
3.47-3.51 (d, 1H, -C**H**(H)-, geminal)	_	_
3.17-3.23 (t, 1H, -CH(**H**)-, geminal)	_	_

Table 2c: ^{1}H-NMR Chemical shift data of free RB and its variation in complex

Vol. of β-CD (ml)	Total Vol.(ml)	Conc. Of Rebamipide(mM)	Conc. Of β-CD(mM)	Conc. Ratio of RB & β-CD	Conductivity (µS/ppm)
0	10	10	0	0	1.58
1	11	9.0909	0.9091	0.1000011	1.6
2	12	8.333	1.667	0.200048002	1.62
3	13	7.692	2.308	0.300052002	1.64
4	14	7.142	2.858	0.40016802	1.67
5	15	6.666	3.334	0.500150015	1.69
6	16	6.25	3.75	0.6	1.7
7	17	5.882	4.118	0.700102006	1.73
8	18	5.555	4.445	0.800180018	1.75
9	19	5.263	4.737	0.900057002	1.76
10	20	5	5	1	1.77
11	21	4.761	5.239	1.100399076	1.77
12	22	4.545	5.455	1.200220022	1.77

Table S1. Conductance of RB with β-CD in DMSO

Vol. of HP-β-CD(ml)	Total Vol.(ml)	Conc. Of Rebamipide(mM)	Conc. Of HP-β-CD(mM)	Conc. Ratio of RB & HP-β-CD	Conductance(μS/ppm)
0	10	10	0	0	1.58
1	11	9.0909	0.9091	0.1000011	2.57
2	12	8.333	1.667	0.200048002	3.18
3	13	7.692	2.308	0.300052002	3.8
4	14	7.142	2.858	0.40016802	4.24
5	15	6.666	3.334	0.500150015	4.46
6	16	6.25	3.75	0.6	4.87
7	17	5.882	4.118	0.700102006	5.14
8	18	5.555	4.445	0.800180018	5.3
9	19	5.263	4.737	0.900057002	5.56
10	20	5	5	1	5.72
11	21	4.761	5.239	1.100399076	5.9
12	22	4.545	5.455	1.200220022	6.04
13	23	4.347	5.653	1.300437083	6.17
14	24	4.166	5.834	1.400384061	6.34
15	25	4	6	1.5	6.43
16	26	3.846	6.154	1.600104004	6.54
17	27	3.7	6.3	1.702702703	6.58
18	28	3.5714	6.4286	1.8000224	6.59

Table S2. Conductance of RB with HP-β-CD in DMSO

RB (ml)	β-CD (ml)	RB (μM)	β-CD (μM)	[RB] /([RB]+[β-CD])	Absorbance(A)	ΔA	ΔA*[RB]/([RB]+[β-CD])
4	0	100	0	1	2.0966506	0	0
3.6	0.4	90	10	0.9	1.89721632	0.19943428	0.179490852
3.2	0.8	80	20	0.8	1.686665535	0.409985065	0.327988052
2.8	1.2	70	30	0.7	1.486929359	0.609721241	0.426804869
2.4	1.6	60	40	0.6	1.218173504	0.878477097	0.527086258
2	2	50	50	0.5	1.065342903	1.031307697	0.515653849
1.6	2.4	40	60	0.4	0.842918396	1.253732204	0.501492882
1.2	2.8	30	70	0.3	0.631968498	1.464682102	0.439404631
0.8	3.2	20	80	0.2	0.408406258	1.688244343	0.337648869
0.4	3.6	10	90	0.1	0.187841415	1.908809185	0.190880919
0	4	0	100	0	0.096532165	2.000118435	0

Table S3. Job plot of RB with β-CD in 50% aqueous ethanol

RB (ml)	HP-β-CD (ml)	RB (µM)	HP-β-CD (µM)	[RB]/ ([RB]+[HP-β-CD])	Absorbance (A)	ΔA	ΔA* [RB]/([RB]+[HP-β-CD])
4	0	100	0	1	2.137899876	0	0
3.6	0.4	90	10	0.9	1.840091705	0.29780817	0.268027353
3.2	0.8	80	20	0.8	1.671793938	0.466105938	0.37288475
2.8	1.2	70	30	0.7	1.445265293	0.692634583	0.484844208
2.4	1.6	60	40	0.6	1.229182243	0.908717632	0.545230579
2	2	50	50	0.5	1.007203579	1.130696297	0.565348148
1.6	2.4	40	60	0.4	0.805520058	1.332379818	0.532951927
1.2	2.8	30	70	0.3	0.635123253	1.502776623	0.450832987
0.8	3.2	20	80	0.2	0.403303146	1.734596729	0.346919346
0.4	3.6	10	90	0.1	0.19769001	1.940209866	0.194020987
0	4	0	100	0	0.3713801	1.766519776	0

Table S4. Job plot of RB with HP-β-CD in 50% aqueous ethanol

RB (ml)	β-CD (ml)	RB (µM)	β-CD (µM)	[RB] /([RB]+[β-CD])	Absorbance(A)	ΔA	ΔA*[RB] /([RB]+[β-CD])
4	0	100	0	1	1.194057941	0	0
3.6	0.4	90	10	0.9	1.085681915	0.108376026	0.097538424
3.2	0.8	80	20	0.8	1.020839863	0.173218079	0.138574463
2.8	1.2	70	30	0.7	0.986588001	0.20746994	0.145228958
2.4	1.6	60	40	0.6	0.943848133	0.250209808	0.150125885
2	2	50	50	0.5	0.882666645	0.311391296	0.155695648
1.6	2.4	40	60	0.4	0.848176346	0.345881596	0.138352638
1.2	2.8	30	70	0.3	0.770879688	0.423178253	0.126953476
0.8	3.2	20	80	0.2	0.707821369	0.486236572	0.097247314
0.4	3.6	10	90	0.1	0.730592728	0.463465214	0.046346521
0	4	0	100	0	0.810363293	0.383694649	0

Table S5. Job plot of RB with β-CD in DMSO

RB (ml)	β-CD (ml)	RB (μM)	β-CD (μM)	[RB] /([RB]+[β-CD])	Absorbance(A)	ΔA	ΔA* [RB]/([RB]+[β-CD])
4	0	100	0	1	0.949682236	0	0
3.6	0.4	90	10	0.9	0.880939331	0.068742905	0.061868614
3.2	0.8	80	20	0.8	0.846073151	0.103609085	0.082887268
2.8	1.2	70	30	0.7	0.764143639	0.185538597	0.129877018
2.4	1.6	60	40	0.6	0.620417118	0.329265118	0.197559071
2	2	50	50	0.5	0.532864571	0.416817665	0.208408833
1.6	2.4	40	60	0.4	0.451699257	0.497982979	0.199193192
1.2	2.8	30	70	0.3	0.426976852	0.522705383	0.156811615
0.8	3.2	20	80	0.2	0.359044075	0.590638161	0.118127632
0.4	3.6	10	90	0.1	0.305735111	0.643947124	0.064394712
0	4	0	100	0	0.37138	0.578302236	0

Table S6. Job plot of RB with HP-β-CD in DMSO

temp /K	[RB] /μM	[β-CD] /μM	A_o	A	ΔA	1/[β-CD]/M^{-1}	1/ΔA	Intercept	Slope	Association Constant(K_a)
	50	30	1.0614	1.1122	0.0508	33333.33	19.68504			
	50	40	1.0614	1.1259	0.0645	25000	15.50388			
298.15	50	50	1.0614	1.1318	0.0704	20000	14.20455	7.2887	0.0003574	2.03×10^4
	50	60	1.0614	1.1372	0.0758	16666.67	13.19261			
	50	70	1.0614	1.1388	0.0774	14285.71	12.9199			
	50	30	1.0561	1.0864	0.0303	33333.33	33.0033			
	50	40	1.0561	1.0892	0.0331	25000	30.21148			
303.15	50	50	1.0561	1.0936	0.0375	20000	26.66667	16.7536	0.0005015	3.34×10^4
	50	60	1.0561	1.0961	0.04	16666.67	25			
	50	70	1.0561	1.0983	0.0422	14285.71	23.69668			
	50	30	1.0418	1.0584	0.0166	33333.33	60.24096			
	50	40	1.0418	1.0597	0.0179	25000	55.86592			
308.15	50	50	1.0418	1.0645	0.0227	20000	44.05286	18.4714	0.00132	1.39×10^4
	50	60	1.0418	1.0674	0.0256	16666.67	39.0625			
	50	70	1.0418	1.0688	0.027	14285.71	37.03704			

Table S7. Calculation of Association of RB/β-CD in ethanolic solution

[RB] /µM	[HPβCD] /µM	A_o	A	ΔA	1/[HPβCD] /M^{-1}	1/ΔA	Intercept	Slope	Association Constant(K_a)
50	30	1.0561	1.0819	0.0258	33333.33	38.7596899			
50	40	1.0561	1.0852	0.0291	25000	34.3642612			
50	50	1.0561	1.0875	0.0314	20000	31.8471338	17.6995	0.000648	2.73×10^4
50	60	1.0561	1.0916	0.0355	16666.66	28.1690141			
50	70	1.0561	1.0942	0.0381	14285.71	26.2467192			
50	30	1.0614	1.0735	0.0121	33333.33	82.6446281			
50	40	1.0614	1.0747	0.0133	25000	75.1879699			
50	50	1.0614	1.0752	0.0138	20000	72.4637681	52.4352	0.000916	5.72×10^4
50	60	1.0614	1.0763	0.0149	16666.66	67.114094			
50	70	1.0614	1.0768	0.0154	14285.71	64.9350649			
50	30	1.0718	1.0811	0.0093	33333.33	107.526882			
50	40	1.0718	1.0827	0.0109	25000	91.7431193			
50	50	1.0718	1.0843	0.0125	20000	80	28.6933	0.00243	1.18×10^4
50	60	1.0718	1.0864	0.0146	16666.66	68.4931507			
50	70	1.0718	1.0882	0.0164	14285.71	60.9756098			

Table S8. Calculation of Association of RB/HP-β-CD in ethanolic solution

FIGURES

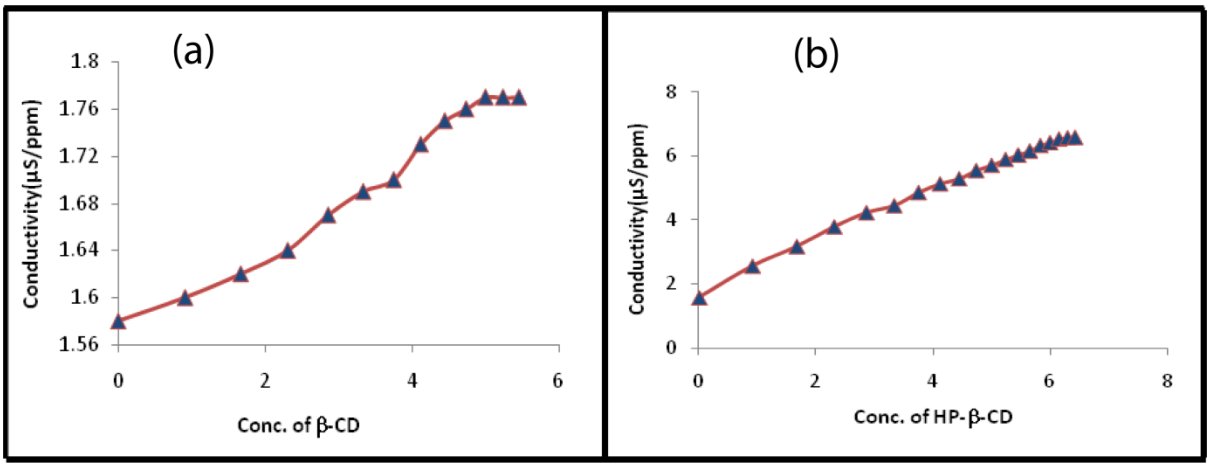


Figure1. Plot of Molar Conductance (Λ) against concentration of (a) β-Cyclodextrin added in 10mM for Rebamipide in DMSO solution at 308.15K and (b) HP-β-Cyclodextrin in 10mM for Rebamipide in DMSO at 308.15K.

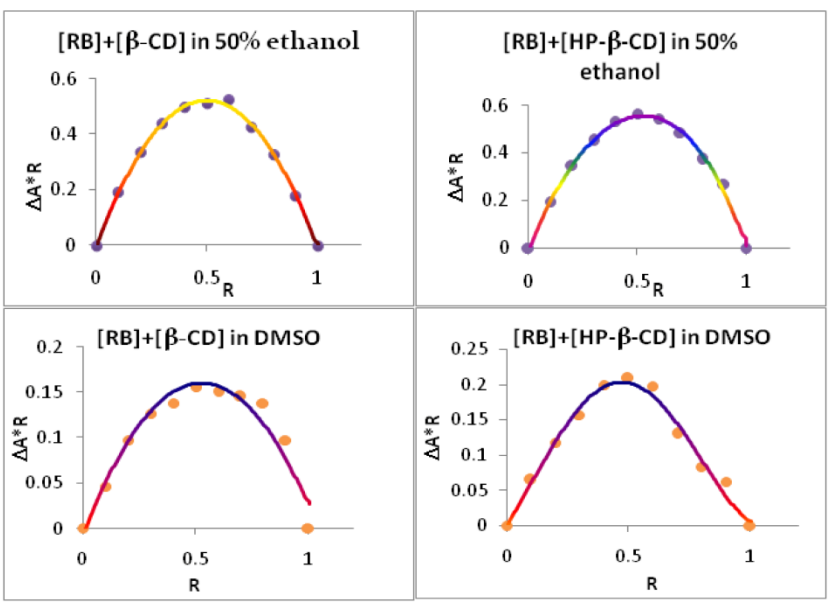


Figure2: Job plot of RB/β-CD and RB/HP-β-CD systems in 50% ethanol at 298.15K (above) RB/β-CD and RB/HP-β-CD systems in pure DMSO at 298.15K (below)

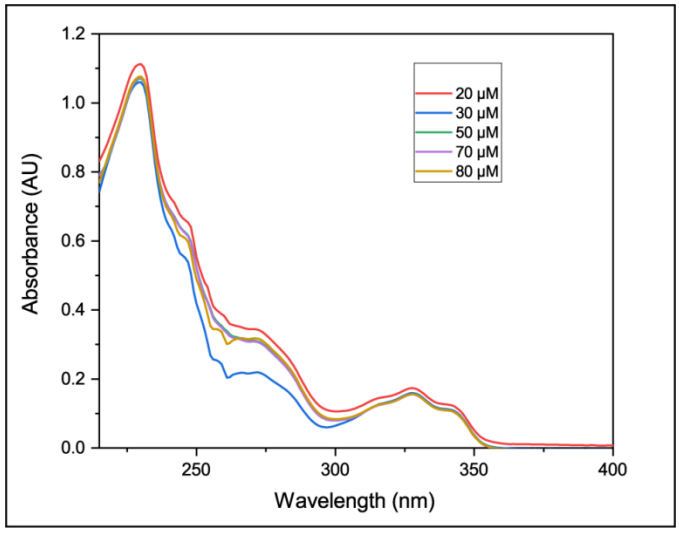


Figure 3a: Variation of UV-vis spectra in different micromolar concentration of β-CD and RB in 50% ethanolic solution at 298.15K

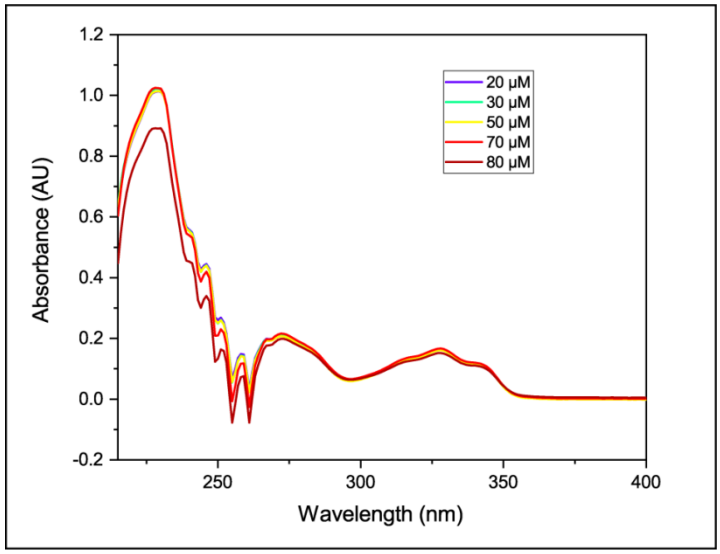


Figure 3b: Variation of UV-vis spectra in different micromolar concentration of HP-β-CD and RB in 50% ethanolic solution at 298.15K

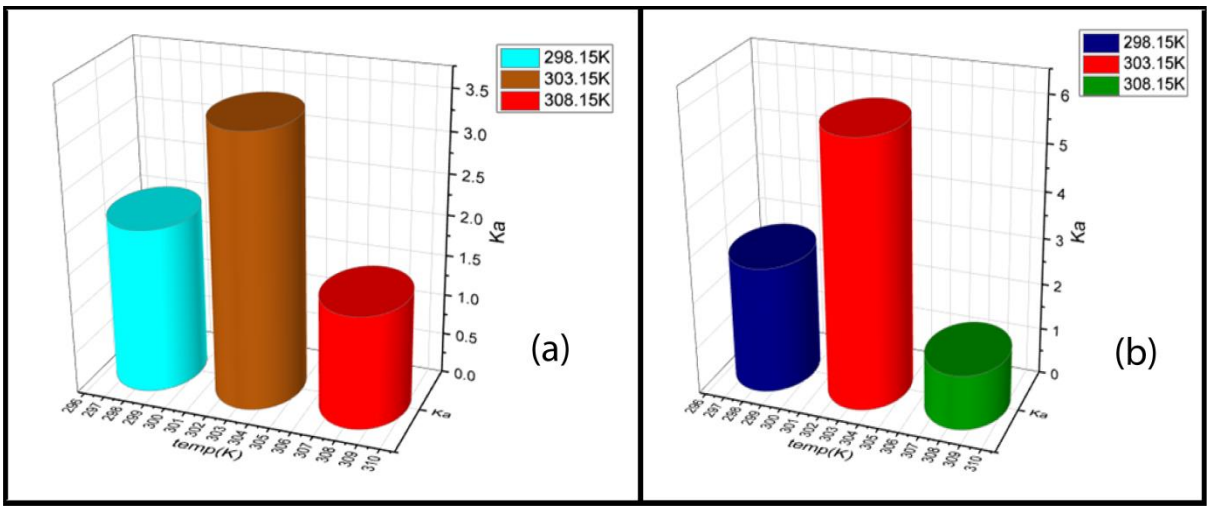


Figure 4: 3D graphical representation of association constant value of (a) RB/β-CD and (b) RB/HP-β-CD in aqueous ethanolic solution at three different temperatures

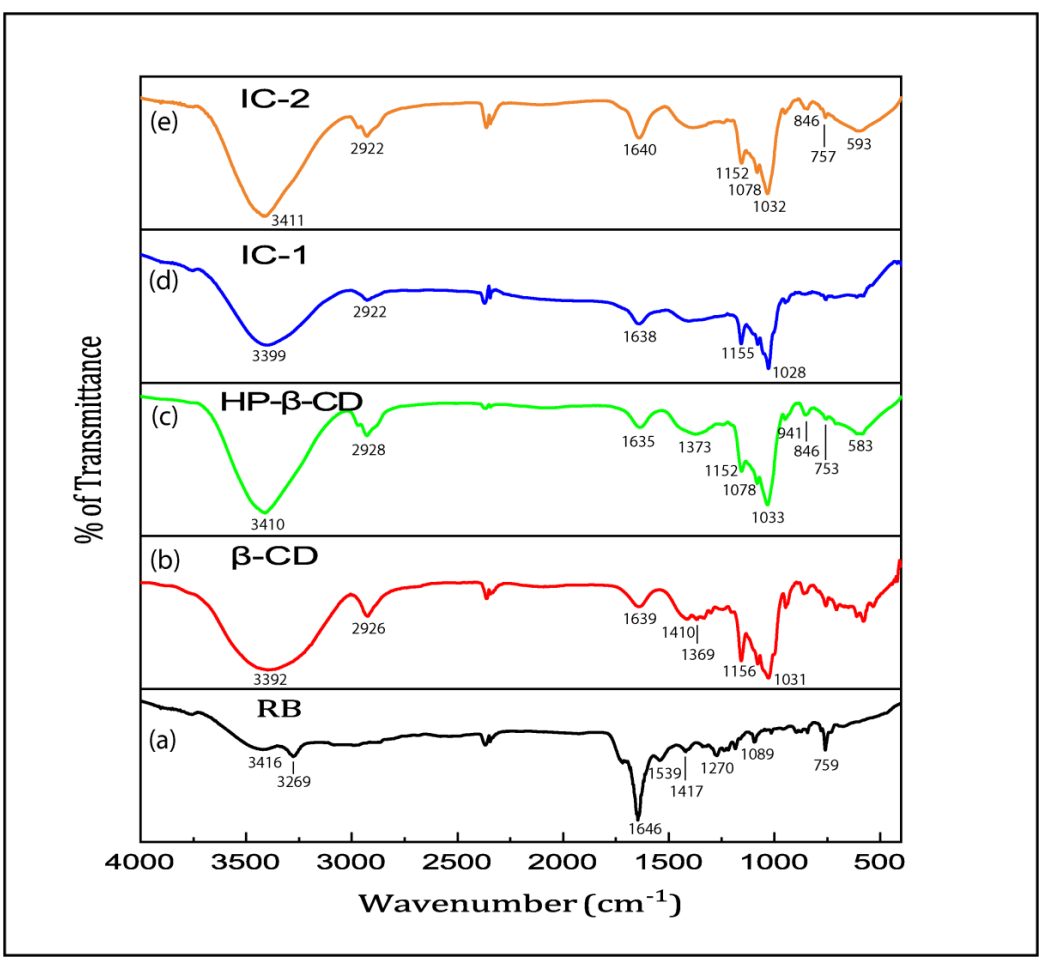


Figure 5: FT-IR spectra of (a) RB, (b) β-CD, (c) HP-β-CD, (d) inclusion complex of RB/ β-CD and (e) inclusion complex of RB/ HP-β-CD.

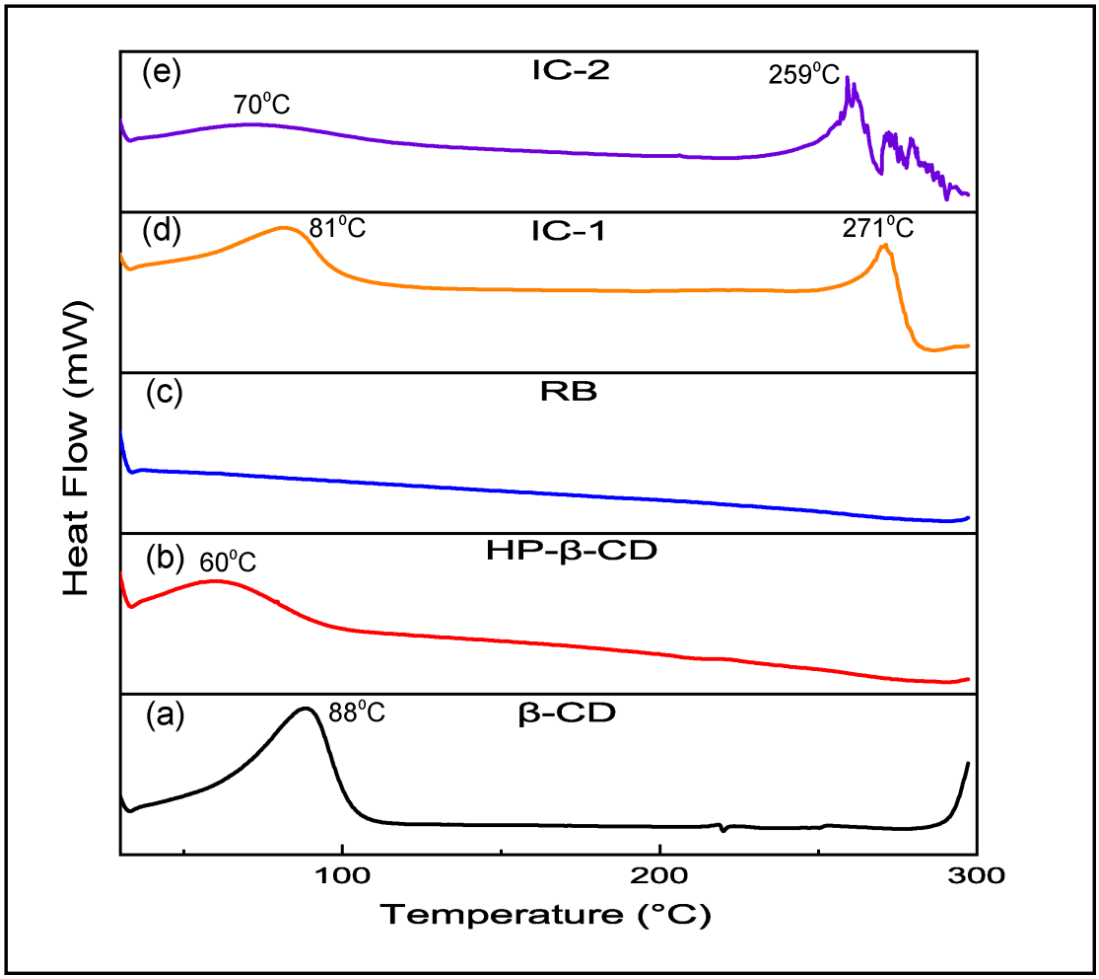


Figure 6: DSC thermograms of (a) β-CD, (b) HP-β-CD, (c) RB, (d) IC-1 and (e) IC-2.

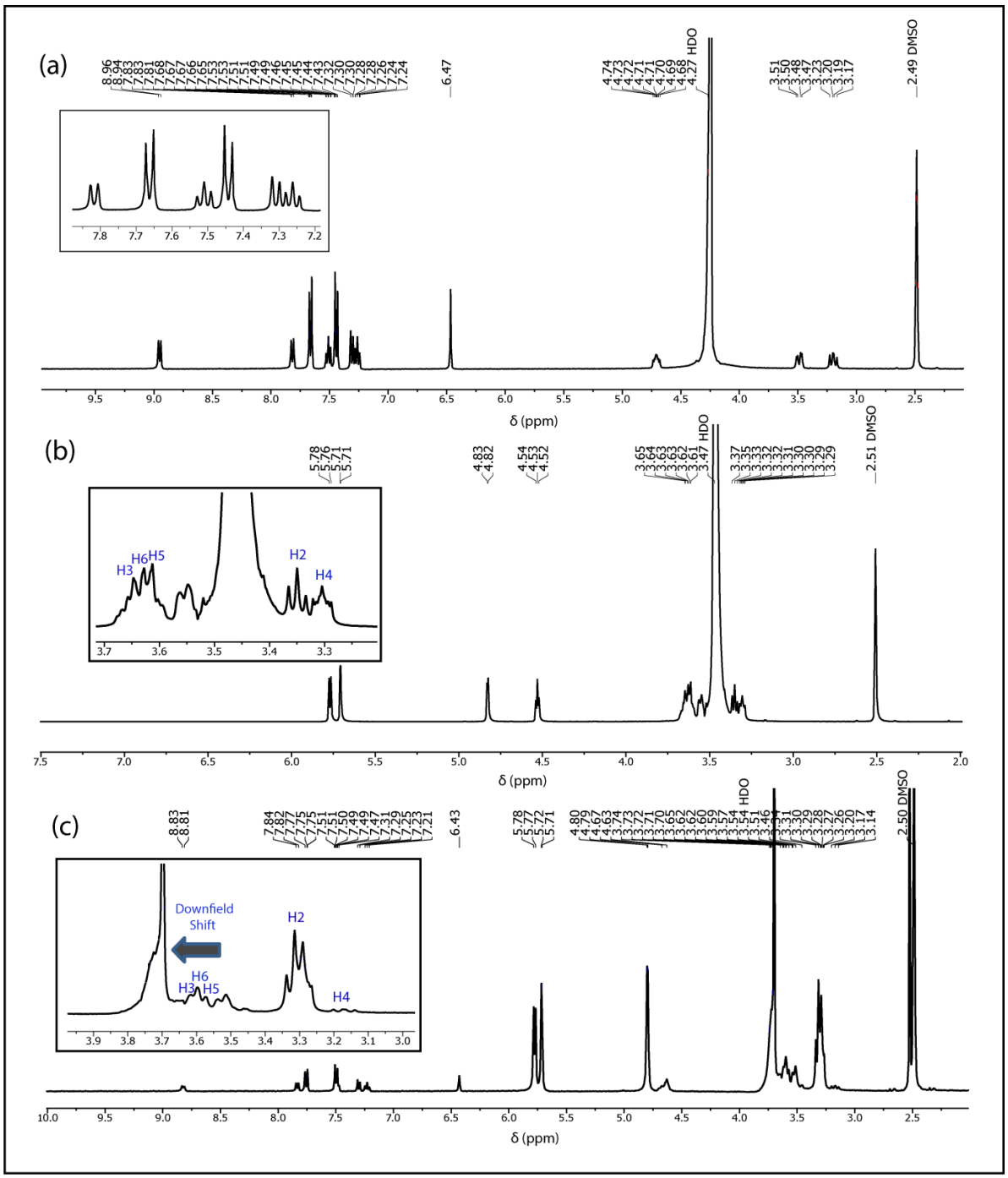


Figure 7a: ^{1}H-NMR spectra of (a) RB (b) β-CD and (c) RB/ β-CD (IC-1) inclusion complex

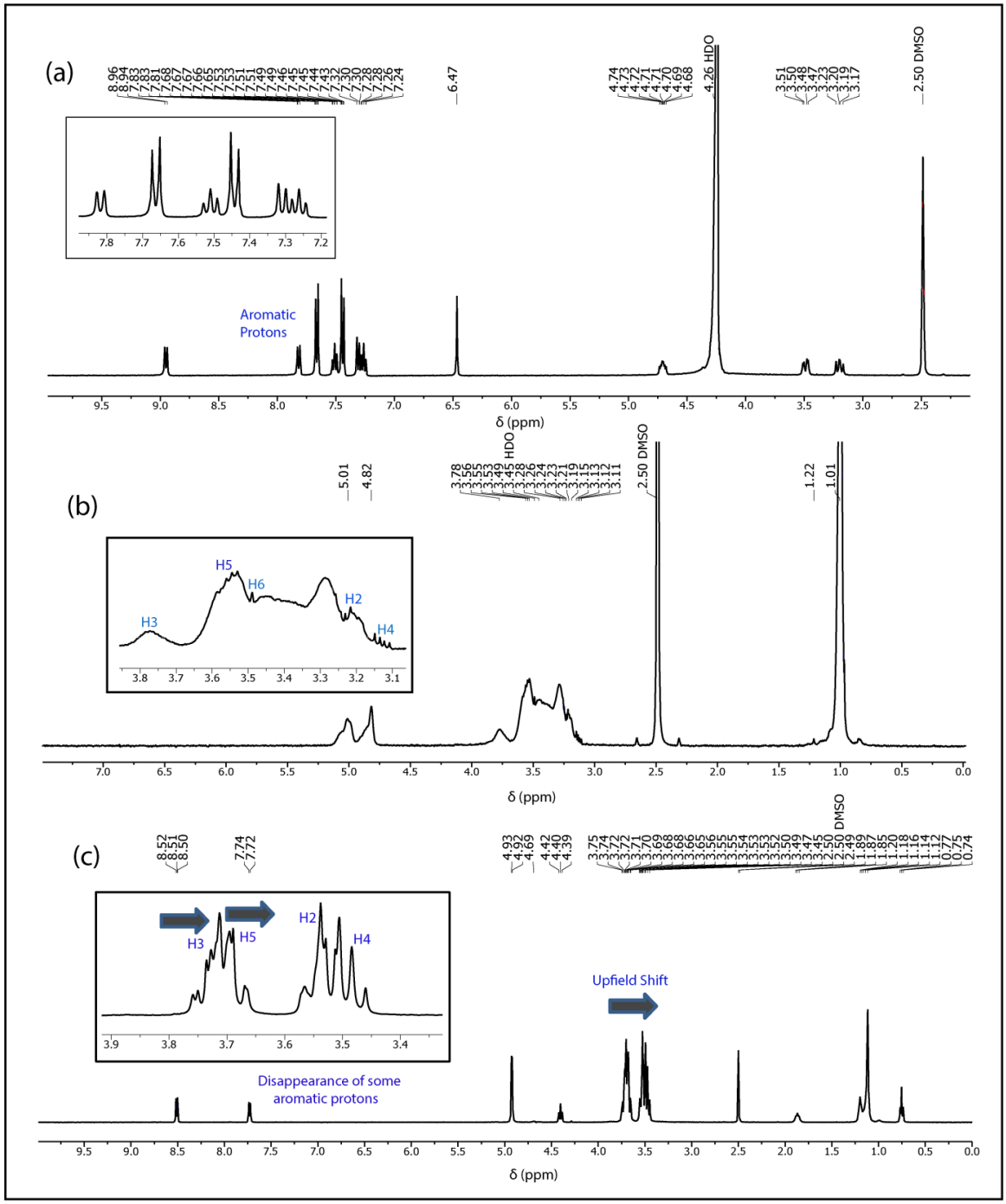


Figure 7b: ^{1}H-NMR spectra of (a) RB (b) HP-β-CD and (c) RB/ HP-β-CD (IC-2) inclusion complex.

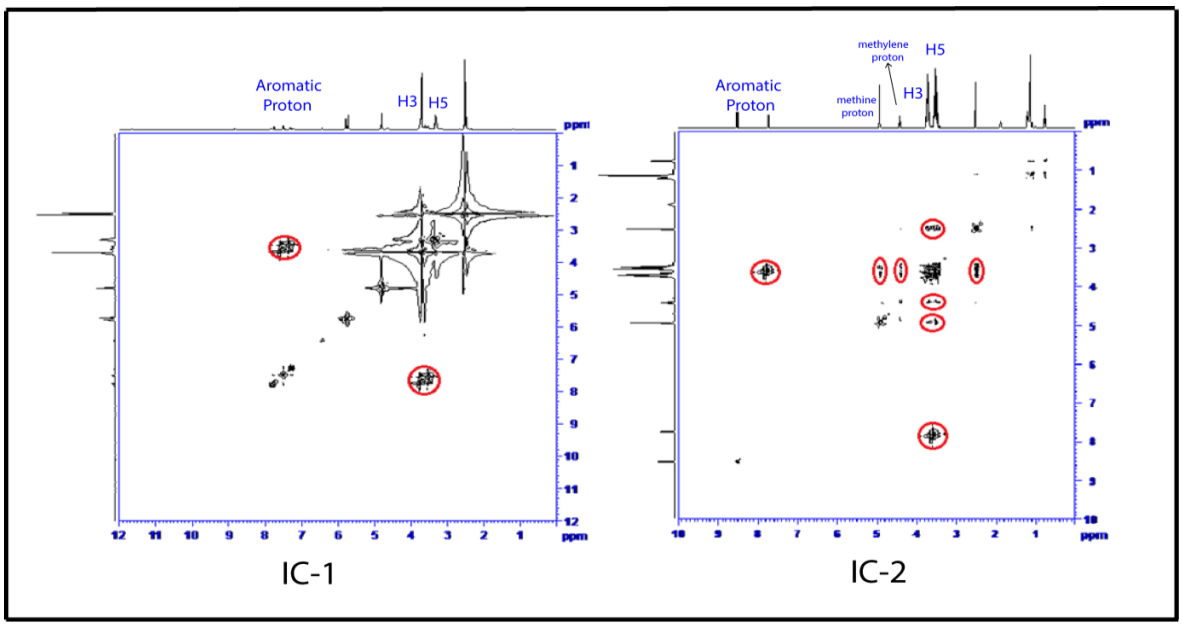


Figure 8: 2D ROESY spectra of IC-1 (left) & IC-2 (Right)

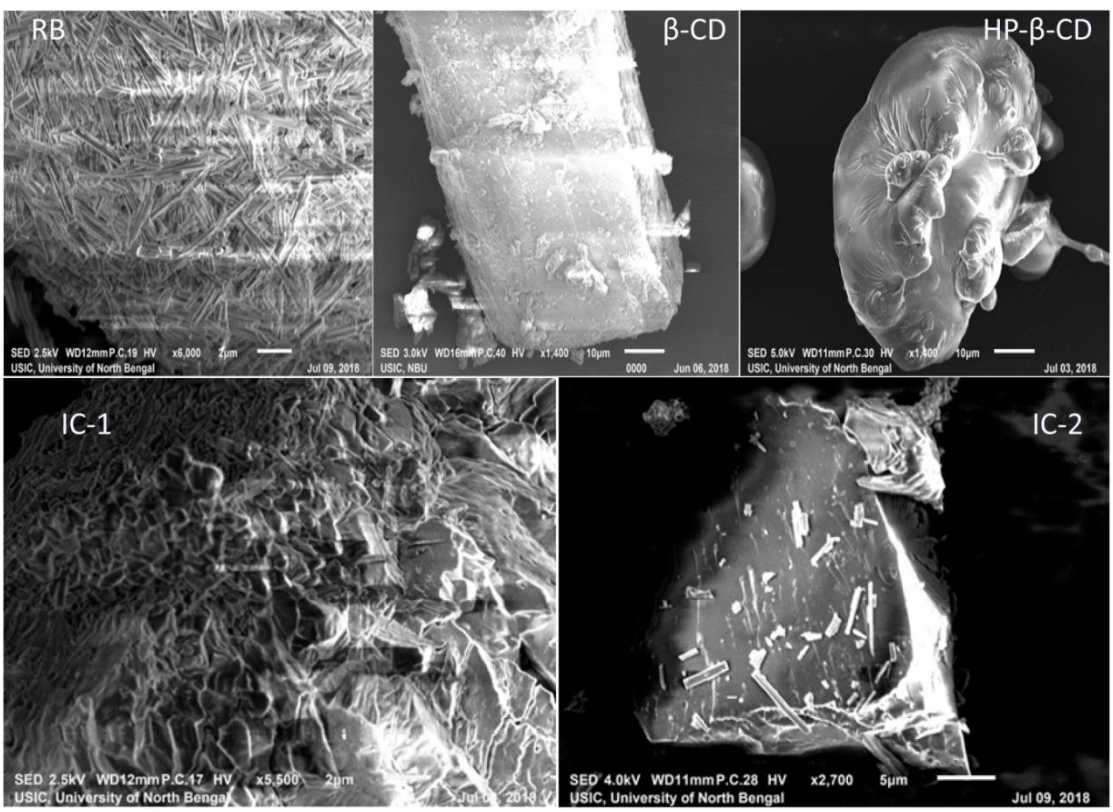


Figure 9: SEM microphotograph of RB, β-CD, HP-β-CD, IC-1 and IC-2 respectively.

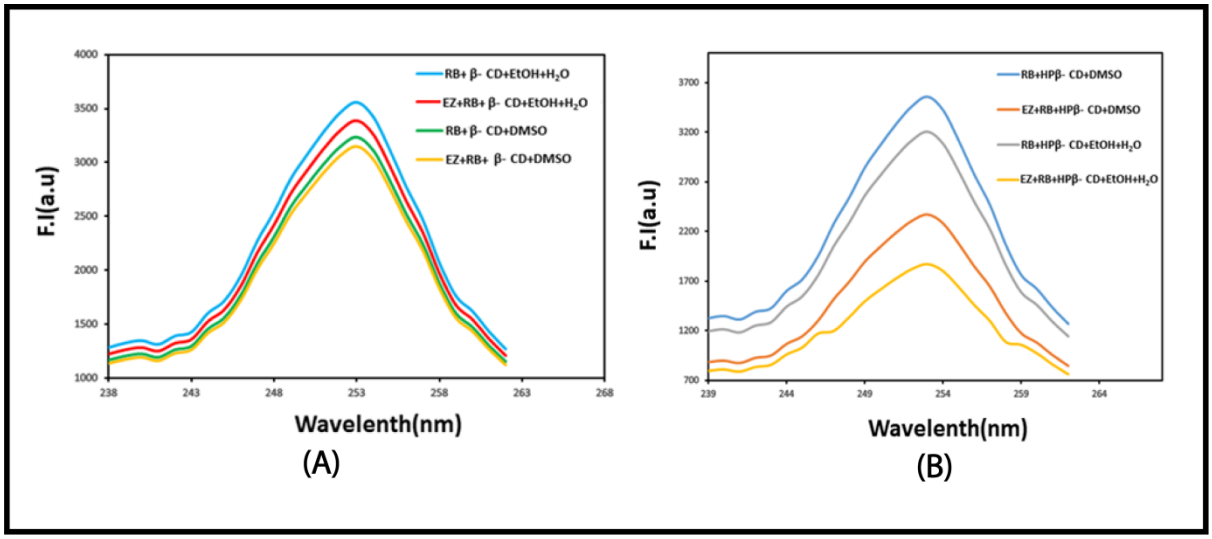


Figure 10: variation in fluorescence intensity of (a) RB in β-CD in EtOH+H_2O without enzyme (blue) and with enzyme (red) and in DMSO without enzyme (green) and with enzyme (yellow)
(b) RB in HP-β-CD in EtOH+H_2O without enzyme (blue) and with enzyme (red) and in DMSO without enzyme (green) and with enzyme (yellow)

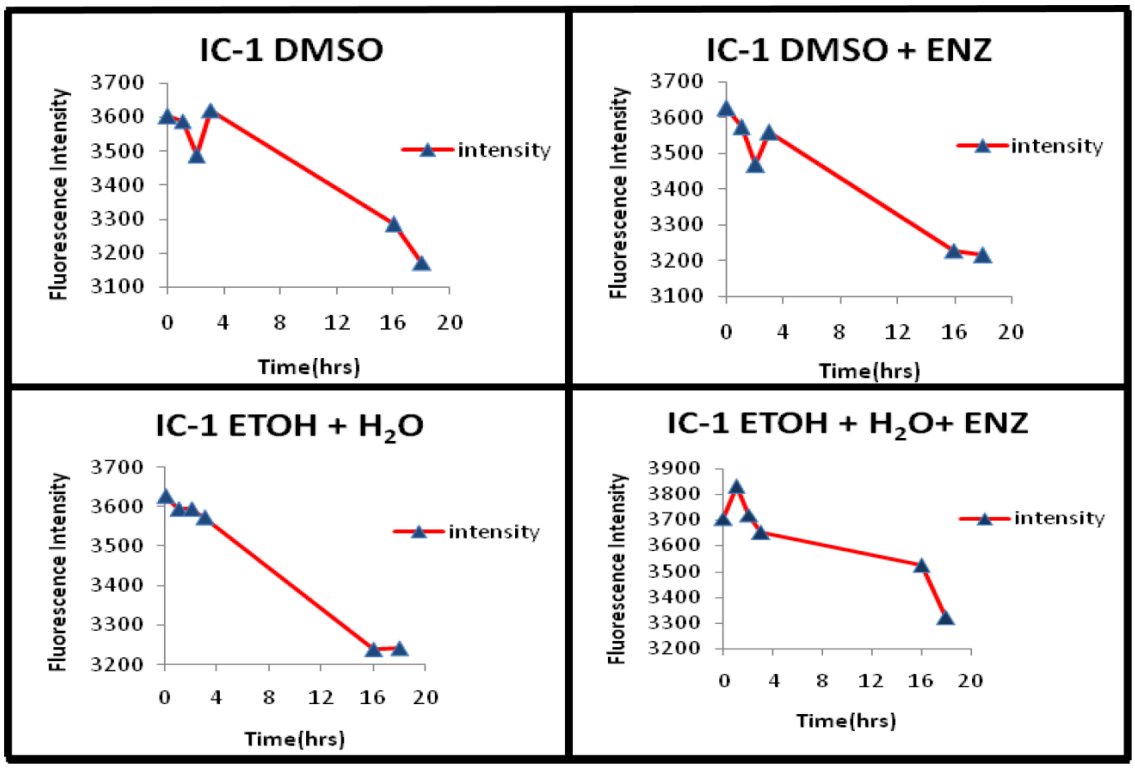


Figure 11: Variation in fluorescence intensity versus time of IC-1 in various solvent systems

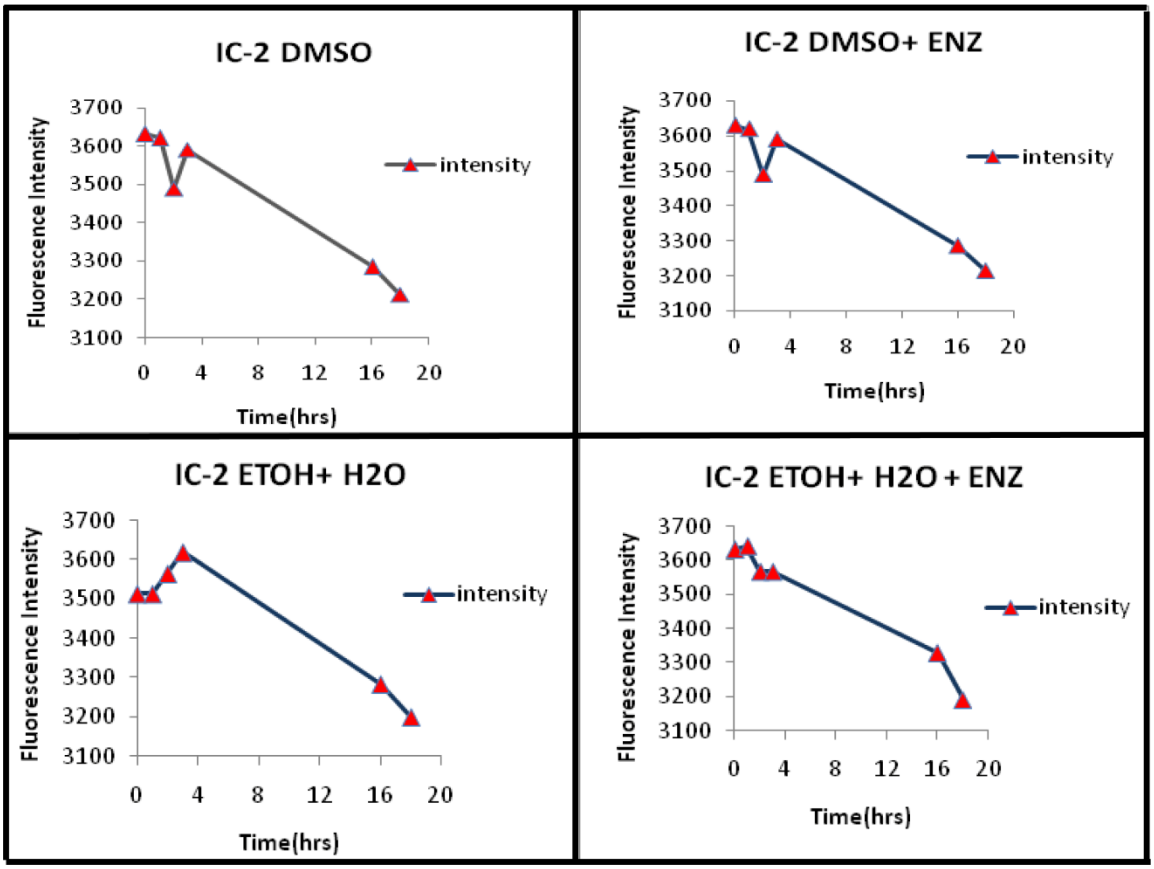


Figure 12: Variation in fluorescence intensity versus time of IC-1 in various solvent systems

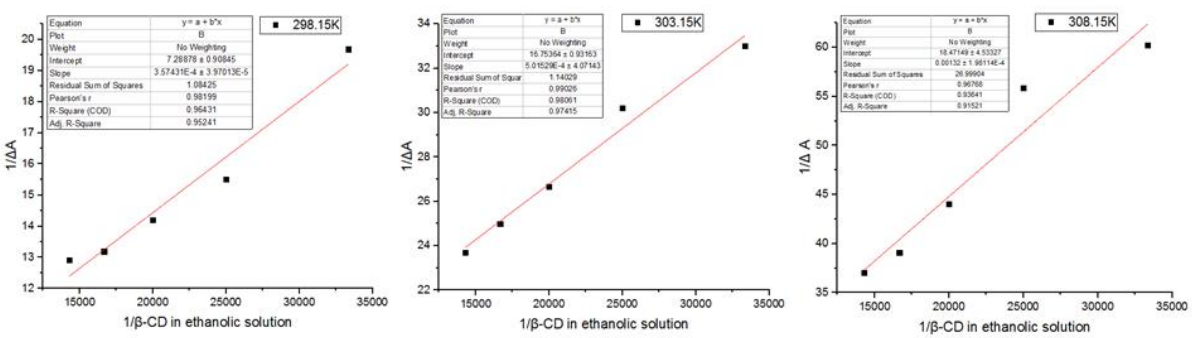


Figure S1: Plot of association constant for RB/β-CD in ethanolic solution at three different temperatures

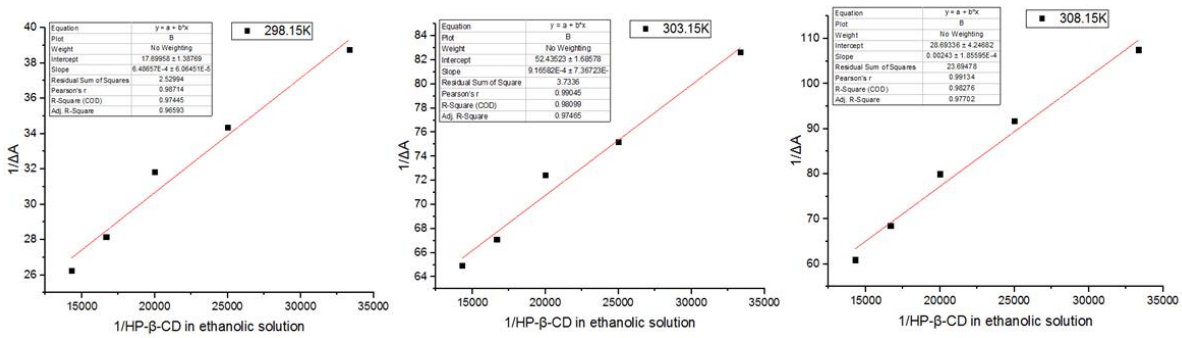


Figure S2: Plot of association constant for RB/HP-β-CD in ethanolic solution at three different temperatures

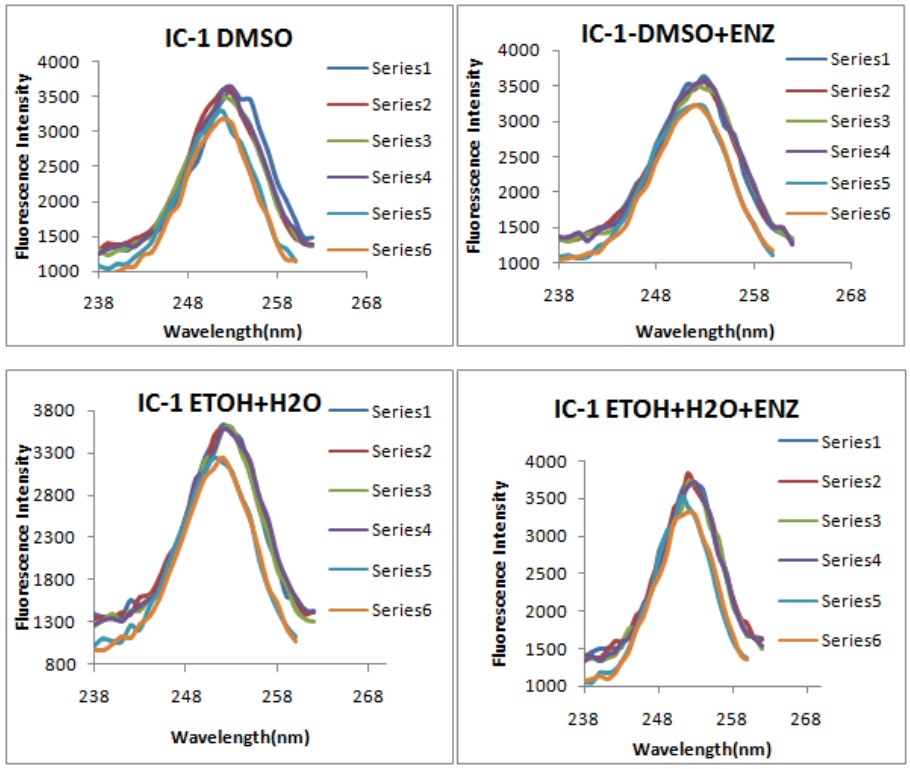


Figure S3: Variation in fluorescence intensity versus wavelength (nm) of IC-1 in various solvent systems

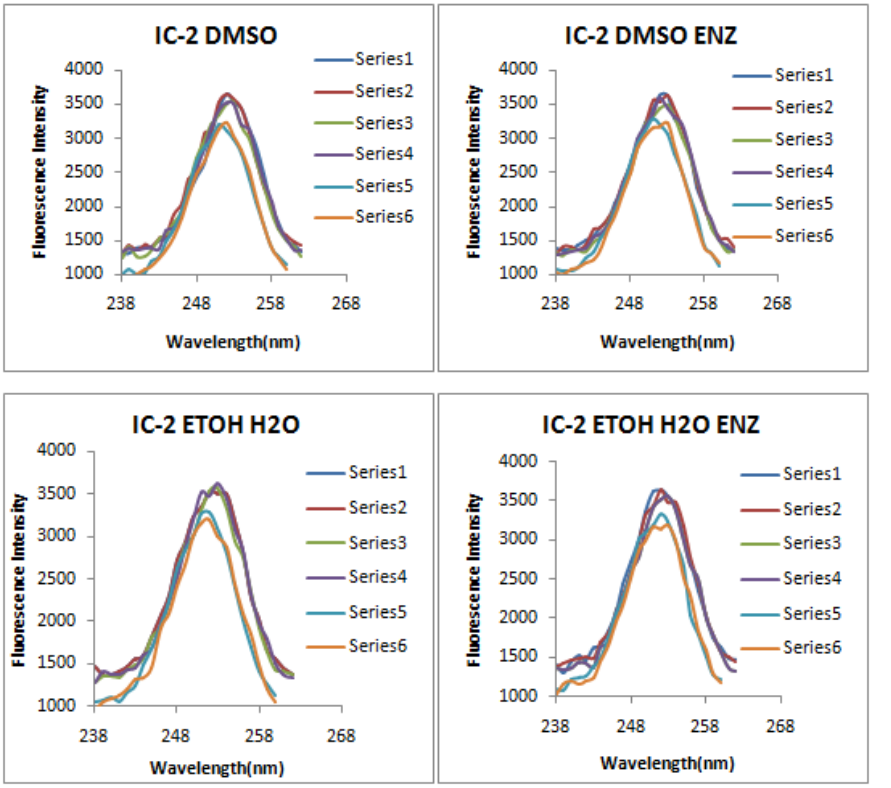
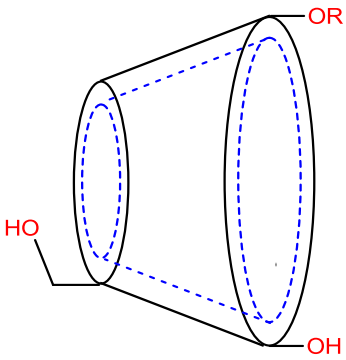
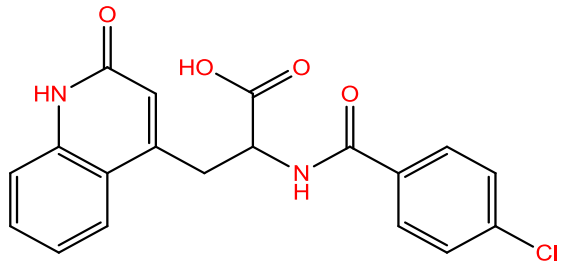


Figure S4: Variation in fluorescence intensity versus wavelength (nm) of IC-2 in various solvent systems

SCHEMES

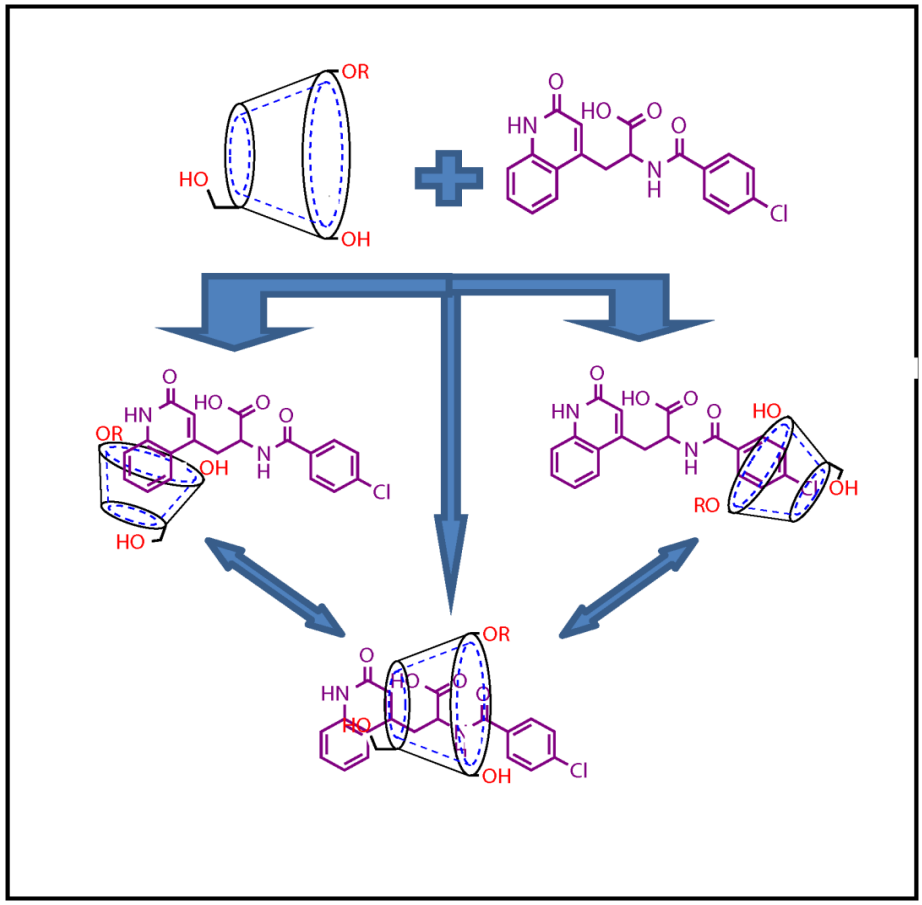




Beta-CD R=H
HP-beta-CD R= CH2CH(CH3)OH

Rebamipide

Scheme 1: Structures of rebamipide, β-CD and HP-β-CD



Scheme 2: Schematic representation of cyclodextrin molecules forming inclusion complex with rebamipide guest