CHAPTER VI

STUDY ON HOST-GUEST INCLUSION COMPLEXATION OF A DRUG IN CUCURBIT [6] URIL

6.1. Introduction

Encapsulation of drug and its controlled delivery is very important in pharmaceutical associated investigations, [1, 2] thus here we have studied the host guest inclusion complexation of pyridine-2-aldoxime drug inside into CB [6] in aqueous saline medium by various physicochemical contrivance. Supramolecular materials are proficient to perform several functions; particularly the targeted drug-delivery vehicle is of great interest now a day [3, 4]. Molecular recognition guided by supramolecular interactions is an outstanding well-built construction principle of molecular assemblies between host and guest with extraordinary physicochemical properties [5,6]. The macrocyclic molecule CB [6] is a barrel shaped cavity (scheme1) with six methylene-bridged glycoluril units, possesses a hydrophobic core (5.5 Å width and 6.0 Å height) and polar carbonyl groups surrounding the portals [7]. The uredio-carbonyl groups on both rims are suitable for complexing with cationic organic guests through ion–dipole and hydrogen bonding interactions [8, 9]. The association constant shown by Shih et al. for 1:1 complexes of n-alkyl diammonium ion with CB[6] was in the order of 10^8 M^{-1}[10]. Therefore, CB [6] has excellent complexation properties towards cationic guest molecules [11]. The CB[6] macromolecule is sparingly soluble in pure water and in common organic solvents, but it is soluble in the range of 10^{-2} M in strongly acidic aqueous solutions (e.g., 1:1 formic acid and water) and also in neutral saline water solutions[12-14]. Due to these exceptional properties CB[6] behaves as a promising host constituent for drug molecules in controlled delivery and nanoscale materials for nanoassemblies [15,16]. Besides this application, CB[6] is often utilized for the construction of supramolecular architectures, such as (pseudo) rotaxanes, polyrotaxanes and molecular necklaces[17-20].

Parlidoxime or 2-pyridine aldoxime methochloride (2-PAM) is an oxime base drug molecule. In drug industry it has immense importance for nerve stimuli

because of its prompt functionality. It is an important drug and acts as a nerve agent for the treatment of organophosphorus poisoning in the nervous system [21, 22]. It has a suitable charged pyridine moiety and an oxime part resides at C-2 position of pyridine ring (Scheme 1). The cationic pyridine moiety helps the 2-PAM for the formation of inclusion with CB [6] through non covalent interactions.

The aim of this work is to develop a reliable approach for the formation of 1:1 inclusion complex (IC) between the drug (2-PAM) molecule and CB[6] in aqueous saline environment. For this purpose, ^{1}H NMR and 2D ROESY NMR, UV-Vis spectroscopy, IR spectroscopy, surface tension and conductivity experiments have been performed in saline water medium at 298.15K. Due to poor aqueous solubility of CB[6] the present work has been carried out in 0.45% (w/v) of saline solution i.e., 77mM saline[13,14] solution, which is compatible with physiological systems.

6.2. Experimental Section

6.2.1. Materials

Pyridine-2-aldoxime methochloride and cucurbit [6]uril of puriss grade were procured from Sigma-Aldrich Company, Germany and used as purchased. Purity of Pyridine-2-aldoxime methochloride and cucurbit [6]uril were 0.99 and 0.98 respectively. The required sodium chloride salt was procured from Merck, having 0.99 purity.

6.2.2 Preperation of solid inclusion complex

CB[6] (10 mg, 0.01mmol) and NaCl (59 mg, 1.0 mmol) were dissolved in distilled water (2 ml) upon gentle heating. The solution of 2-PAM (3.4mg, 0.02mmol) was carefully added to the solution of CB[6] and stirred overnight at 40^{0}C. The resulting solution was filtered off and the filtrate was kept for few days until the formation of crystalline solid inclusion complex was observed. The sample was dried over oven at 120^{0}C temperatures. Then the powdered inclusion complex was used for experimental analysis.

6.2.3. Apparatus and procedure

Prior to the start of the experimental work solubility of all the studied materials have been precisely checked in triply distilled and degassed water (specific conductivity is1.0μS·cm^{-1}). The 77mM of saline water solution has been prepared for the all experimental measurement. All the stock solutions were prepared by mass (weighed by Mettler Toledo AG-285 with uncertainty 0.0003 g), and then the working solutions were obtained by mass dilution at 298.15K.

^{1}H NMR and 2D ROESY NMR spectra were recorded on Bruker Avance 300 MHz spectrometer using saline D_2O at 298.15K. Signals are quoted as δ values in ppm using residual protonated solvent signals as internal standard (D_2O: δ 4.79 ppm). Data are reported as chemical shift.

UV-visible spectra were recorded by JASCO V-530 UV/VIS Spectrophotometer, with an uncertainty of wavelength resolution of ±2 nm. The temperature was controlled by an automated digital thermostat.

Fourier-transform infrared (FT-IR) was carried out on a Perkin Elmer FT-IR spectrometer (Spectra RXI) with sample prepared as KBr pellets. The spectra were acquired in the frequency range 4000−400 cm^{-1} at a resolution of 4 cm−1 with a total of 16 scans.

The surface tension and conductivity study of this work has been performed as described earlier [1, 2].

6.2.4 ^{1}HNMR data

δ (300MHz) values for 2-PAM: 4.35 (3H,s), 7.62-7.67 (1H,m), 8.01-8.09 (1H,m), 8.43-8.19 (1H,m), 8.30 (1H,s), 8.35-8.38 (1H,m).

δ (300MHz) values for CB[6]: 4.43 (1H,d,J=15.6Hz), 5.74 (1H,s), 5.77 (1H,d,J=15.6Hz).

δ (300MHz) values for IC: 4.45 (1H,d,J=15.6), 4.50 (3H,s),5.75 (1H,s), 5.81 (1H,d,J=15.6), 7.61-7.65 (1H,m), 8.00-8.06 (1H,m), 8.10-8.16 (1H,m), 8.29 (1H,s), 8.31-8.36 (1H,m).

6.2. Result and Discussion

6.3.1. NMR study

The binding interaction between 2-PAM and CB[6] complex can be deduced via the chemical shift of proton of the CB[6] molecule[16]. The hydrophobic cavity provides a potential inclusion site for the cationic guest molecules, and the polar carbonyl groups at the portals allow the CB [6] to bind charged molecules by charge–dipole and hydrogen bonding interactions [23-25]. In the structure of CB [6], there are twelve methylene (CH_2) groups and twelve tertiary C-H groups. The two H atoms of CH_2 are diastereotropic and show signals at 5.77 ppm (H1) and 4.43 ppm (H2) respectively. The tertiary C-H shows signal at 5.74 ppm (H3). Here the H1 hydrogen is projected toward inside of the cavity, while H2 and H3 are projected outward (Scheme 1). In figure 1 chemical shift of the H1 and that of the CH_3 group of 2-PAM is observed, which indicate interaction between them. Therefore, entering of the guest molecule in the CB[6] cavity may be confirmed by ^{1}H NMR study. The chemical $\text{shift } (\Delta\delta) \text{ of H1 of CB}$ [6] and CH_3 of 2-PAM are tabulated in table1 [26, 27].

6.3.2. 2D ROESY NMR

^{1}H-^{1}H ROESY is useful for determining which signals arise from protons that are close to each other in space, even if they are not bonded. A ROESY spectrum yields through-space correlations via spin-spin relaxation. So, ^{1}H-^{1}H ROESY spectra can further confirm the structure of IC. Thus it provides information about the proximity of the non bonded protons, which are close within 4 Å in space. In this study CB[6] and 2-PAM were allowed to interact in saline D_2O environment and the spectrum has been shown in figure 2, where the 2D ROESY spectra of IC shows no correlations observed between the protons of pyridine ring. The result is the same with that of the ^{1}H NMR, showing that no interaction existed between the ring proton of pyridine and CB[6]. Two cross peaks are found (marked in red circles), which clearly indicate the special correlation between the CH_3 protons of 2-PAM and the H1 proton of CB [6]. This is an inevitable phenomenon since CH_3 protons of 2-PAM locate an adjacent position in the CB [6]. They resonates at 4.50 and 5.81 ppm and thus it clearly suggests the methyl group of the 2-PAM was inside in the

CB[6]cavity and the pyridinium ring protrudes outside. This study confirms the inclusion phenomenon (Scheme 2) of the above host and guest in the studied medium [28].

6.3.3. UV-Vis Study

The 1:1 binding stoichiometry has been confirmed by the continuous variation method (Job's method) for the studied systems. For this UV-visible method, the mole fractions of the guest and the host were varied in aqueous saline solution, while the total concentrations of both the guest and the host were kept constant (Table S1). If the Job plot exhibits a maximum at 0.5 (the concentration ratio of CB[6] to the sum of the concentrations of CB[6] and the 2-PAM), it is indicative of a 1:1 binding stoichiometry between the host and the guest molecules [29,30]. The Job plot for this complex system were obtained by the ΔA x R Vs R plotting (where, ΔA is the difference in absorbance of the 2-PAM with and without CB [6] and R=[2PAM]/([CB6] + [2-PAM])), as monitored using UV-visible spectroscopy (Figure 3) at 292 nm, reached a maximum at a ratio of 0.5 for [2-PAM]/([CB6]+[2-PAM]). This behaviour thus denoted that the 1:1 complexes between CB[6] and 2-PAM represented the principal species (Scheme 2) in this concentration range.

The binding constant of a host–guest complex is often considered as an essential parameter to evaluate the non-covalent binding strength between the host and the guest molecules. The binding constant (K_a) has been calculated for the IC by UV visible spectroscopy as a result of changes in molar extinction coefficient (Δε) of the 2-PAM when complexed with CB[6] molecule, which is due to the encapsulation-induced [29] environment change from the bulk surroundings to macrocyclic microenvironments (Table S2).The double reciprocal Benesi–Hildebrand method was employed for calculating the binding constant (K_a) for 1:1 host-guest IC. The experimental fitting curve of the absorbance reciprocal at 292 nm against the concentration reciprocal is once again in good concurrence [29-31] with a 1 : 1 binding stoichiometry model and provides a binding constant, K_a of 2.21x 10^5 M^{-1} (Table 2 and Figure 4).

6.3.4. FT-IR Study

FT-IR spectroscopy is an important tool to investigate the inclusion mechanism of the host-guest complexes. The FT-IR spectra of CB[6], [2-PAM] and IC are presented in figure 5. The stretching vibration of interacting groups of pure compound and IC are compared, and are reported in table 3 within the range of wave number 400-4000 cm^{-1}.

Inspection of the IR spectrum of the CB[6], the peak around 1742 cm^{-1} is attributed to the carbonyl stretching vibration and the band at 1627 cm^{-1} arises from the O–H bending vibration of water. The bending vibration of methylene group of CB[6] appears at 1476 cm^{-1} and the out-of plane wagging vibration appears at 1294 cm^{-1}. The asymmetric and symmetric C–N stretching vibrations of the glycoluril ring show peak at 1414cm^{-1} and 1376 cm^{-1} respectively. The combination vibration of C–N stretching, C–C stretching, and C–H bending of the glycoluril ring appears at 1327 cm^{-1}; the combination vibration of C–N stretching and N–C–N bending of the glycoluril ring appears at 1235 cm^{-1}; and the combination vibration of C–N stretching and C–H bending of the glycoluril ring appears at 1190 cm^{-1} respectively. The C–C stretching vibration of the glycoluril ring appears at 967 cm^{-1}. In-plane and out-of-plane deformation vibrations of the glycoluril ring appear at 805, 758, and 673 cm^{-1} respectively. The combination vibration of C–C stretching and C–H bending of the glycoluril ring appears at 1149 cm^{-1} and the N–C–N bending vibration appears at 452 cm^{-1} [32, 33].

After examination of the data presented in Figure 5 in the region of 4000-2000 cm^{-1} that the weak peak positioned at 3694 cm^{-1} can be assigned O-H stretching vibration specific for OH attached to -N═CH group, substituted in pyridine ring. In 2-PAM spectrum the most intense bands in the region of 3100cm^{-1}–2700 cm^{-1} correspond to C-H asymmetric stretching vibrations in CH_3, H-C═ in the aromatic ring. The bands positioned at 2369, 2345 cm-1 are specific for N-O bond in 2-PAM.

The shifting of characteristic vibration band of CB [6] is an indication of inclusion of a guest (2-PAM) into the CB [6] cavity. In the analysis of IR spectra of IC, the noticeable change of C=O stretching vibration arise at 1730 cm^{-1} from 1742 cm^{-1}, reveals that the 2-PAM resides inside in the cavity of CB[6]. The other characteristics stretching vibrations for the IC are also shifted and are tabulated in table3. Thus, the

IR spectrum scrutiny is also in a good agreement of the inclusion phenomena (Scheme 2) and supported the above NMR and UV-Vis spectroscopic studies.

6.3.5. Surface Tension study

2-PAM has a cationic pyridine part and a side chain oxime residue makes it considerable surface active material in saline water medium while CB[6] does not show any remarkable change in surface tension (γ) compared to pure saline water. Here, γ values of 10.0mM solution of 2-PAM has been measured with the increasing concentration of CB[6] at 298.15K in the saline solution (Table S3 & S4). The observed increasing trend (Figure 6a) of γ with increasing concentration of CB[6] may be because of deduction of the drug molecules (surface active) from the surface of the solution and inclusion into the hydrophobic cavity of CB [6] to form host-guest complexes[34]. From the plot it also indicates that there is a break point at certain concentrations after which the line becomes linear. Identification of a single break point in surface tension curve not only indicates formation of IC but also gives information about its 1:1stoichiometry [35, 36] (Scheme2) of host and guest in the IC. The γ value and the corresponding concentrations of CB [6] at break point have been listed in Table 4.

6.3.6 Conductivity Study

The conductivity measurement, in addition, provides analogous indications of the 1:1 IC. The specific conductivity (κ) of 2-PAM in the saline water solution of CB[6] have been measured to get the information about inclusion complex as well as the stoichiometry of the complexes as it contains charged structures [37]. Conductivity of the 10.0mM solution of the studied drug have been carried out with the increment addition of in 10.0mM CB [6] stock solution at 298.15K. The decrease in conductivity (Figure 6b) is due to the inclusion of 2-PAM molecules one by one into the cavity of CB[6]. The noticeable change in solution conductivity with the rise in concentration (table S4) of CB [6] corresponds to the formation of ICs between 2-PAM and CB[6] molecules. At a definite concentration of CB[6], a remarkable change, i.e., a discernible break point is observed in conductivity curve [38]

suggesting the formation of 1:1 IC. The break in the conductivity curve occurred at a concentration (Table 5) of near about 5 mM concentration for CB[6] in the studied solvent systems; signify that the stoichiometry of the inclusion complex is almost 1:1 molar ratio[37, 39]. Although dynamic equilibrium exists between the host and guest molecules at that break point, however, the maximum inclusion occurs in this molar ratio. Therefore, the shifting of equilibrium favors for the formation of IC. The single break point obtained in this experiment is a good correlation for the IC as seen from all the above studies.

6.4 Conclusion

In summary, the studies on the binding interactions of 2-PAM with CB[6] provides evidence for the 1:1 stoichiometric inclusion mechanism between the drug and CB[6]. The structural viability of the guest in accordance with CB[6], and the position of the positive charge on the guest encourage inclusion route. The supramolecular assembly of the drug, envisioned in this work, is very potent and promising for pharmacological applications and as well as for designing tunable artificial molecular devices or nano-materials. The low cytotoxicity and bio-adaptability of CB[6]-decorated stimuli responsive drug systems investigated here may have potential applications in bio-systems for therapeutics.

Tables

Table 1. Change in chemical shifts of interacting potons of 2-PAM and CB[6] with IC.

Groups	Free molecule(δ) /ppm	Inclusion complex(δ) /ppm	Chemical shift change($\Delta\delta$) /ppm
-CH_3 (2-PAM)	4.35	4.50	0.15
-H1 (CB[6])	5.77	5.81	0.04

Table 2. Assoctian constant of IC at 298.15K

Inclusion complex	Association costant (K_a) /M^{-1}
2-PAM@CB[6]	2.2×10^5

Table 3. Comparision of FT-IR stretching vibration of CB[6], 2-PAM with IC.

Groups (cm^{-1})	Free molecule (cm^{-1})	2-PAM@CB[6] (cm^{-1})	Difference in IR band (cm^{-1})
CB[6]			
ν (C=O)	1742	1730	12
δ (CH2)	1476	1473	3
ν_{asym} (C-N)	1414	1413	1
ν_{sym}(C-N)	1376	1374	2
ν (C-N)+ ν (C-C)+ δ (C-H)	1327	1325	2
ω (CH2)	1294	1288	4
ν (C-N)+ δ (N-C-N)	1235	1231	4
ν (C-N)+ δ (C-H)	1190	1181	9
ν (C-C)+ δ (C-H)	1149	1152	3
ν (C-C)	967	965	2
In plane def of glycoluril ring	800	805	5
Out of plane def of glycoluril	758	765	7

ring			
Out of plane def of glycoluril ring	673	657	16
δ (N-C-N)	452	438	14
2-PAM			
ν (OH attached to -N═CH)	3694	3691	3
ν_{sym} (═C-H)	3080	3071	9
ν_{asym} (═C-H)	3023	3020	3
ν_{sym} (N-O)	3369	2374	5
ν_{asym} (N-O)	2345	2349	4

ν, Stretching; δ, Scissoring; ω, wagging; asym, asymmetric ;sym, symmetric; def, deformation .

Table 4. Value of surface tension at break point with corresponding concentration of CB[6] and 2-PAM at 298.15K

Conc. of CB[6] /mM	Conc. of 2-PAM /mM	Surface Tension /mN.m^{-1}
4.95	5.05	66.36

Table 5. Value of conductivity at break point with corresponding concentration of CB[6] and 2-PAM at 298.15K

Conc. of CB[6] /mM	Conc. of 2-PAM /mM	Coductivity /mS.cm^{-1}
4.78	5.22	8.24

Figures

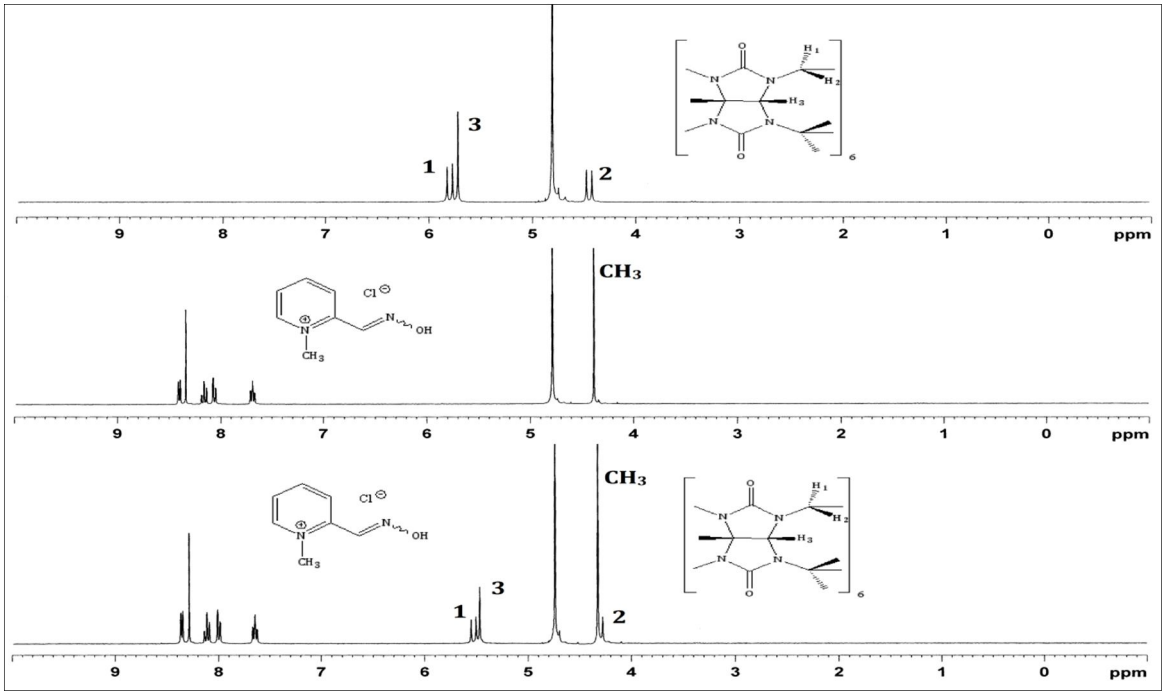


Figure 1. ^{1}H NMR spectra of CB[6], 2-PAM, and IC in saline D_2O at 298.15K

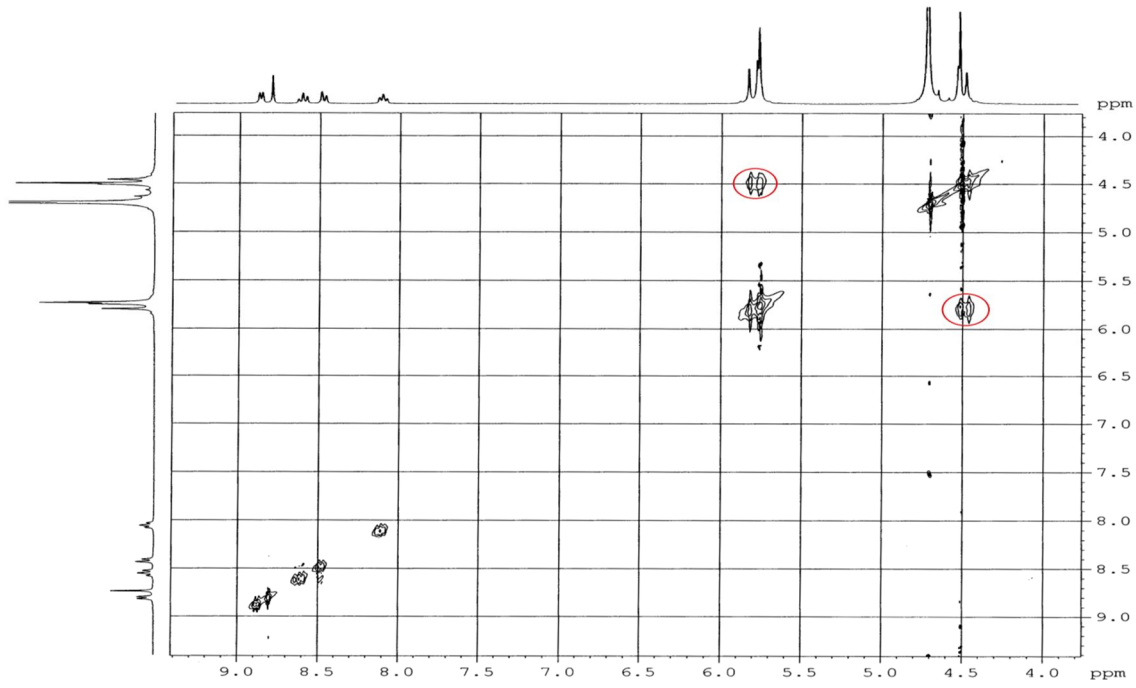


Figure 2. 2D NMR (ROESY) spectra of IC in saline D_2O at 298.15K

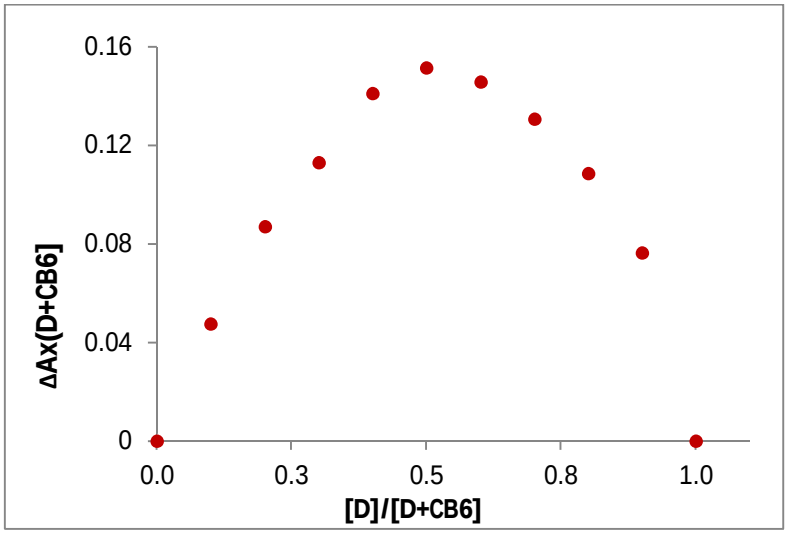


Figure 3. Job's plot of CB[6] and 2-PAM in saline D_2O at 298.15K

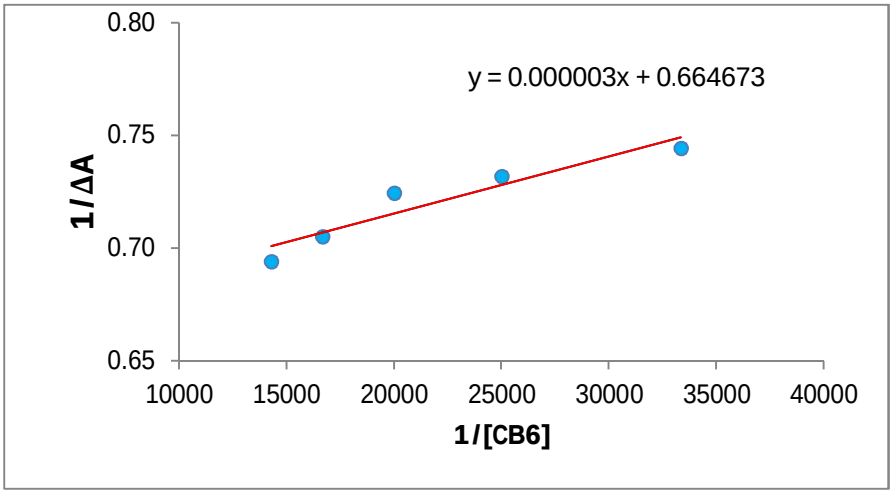


Figure 4. Benesi-Hildebrand double reciprocal plot of CB[6] and 2-PAM in saline D_2O at 298.15K

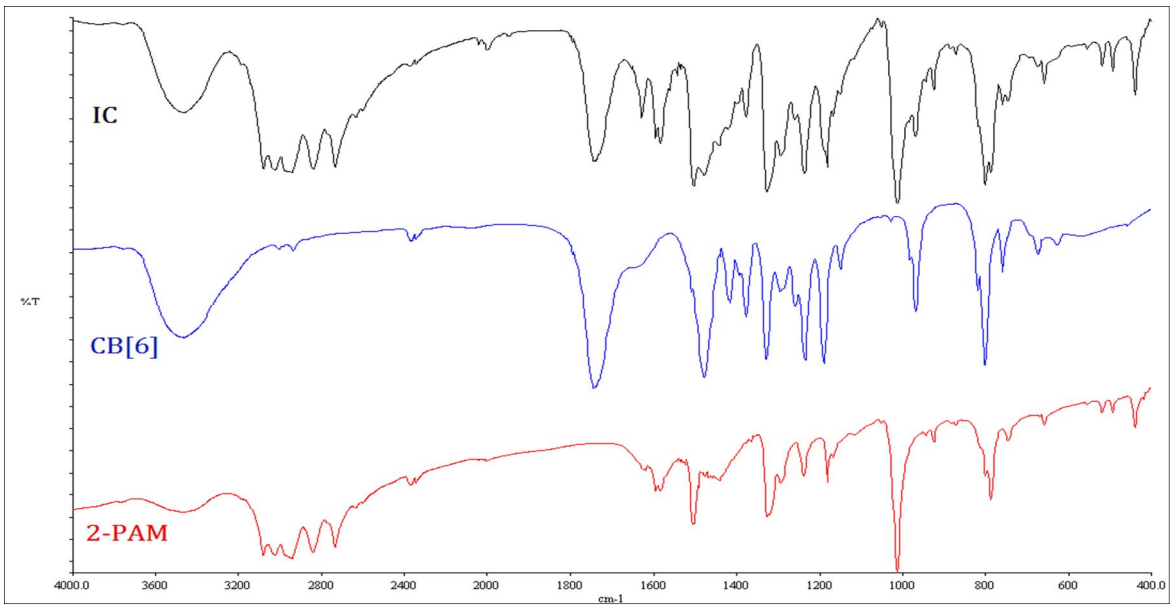


Figure 5. FT-IR spectra of 2-PAM, CB[6] and IC.

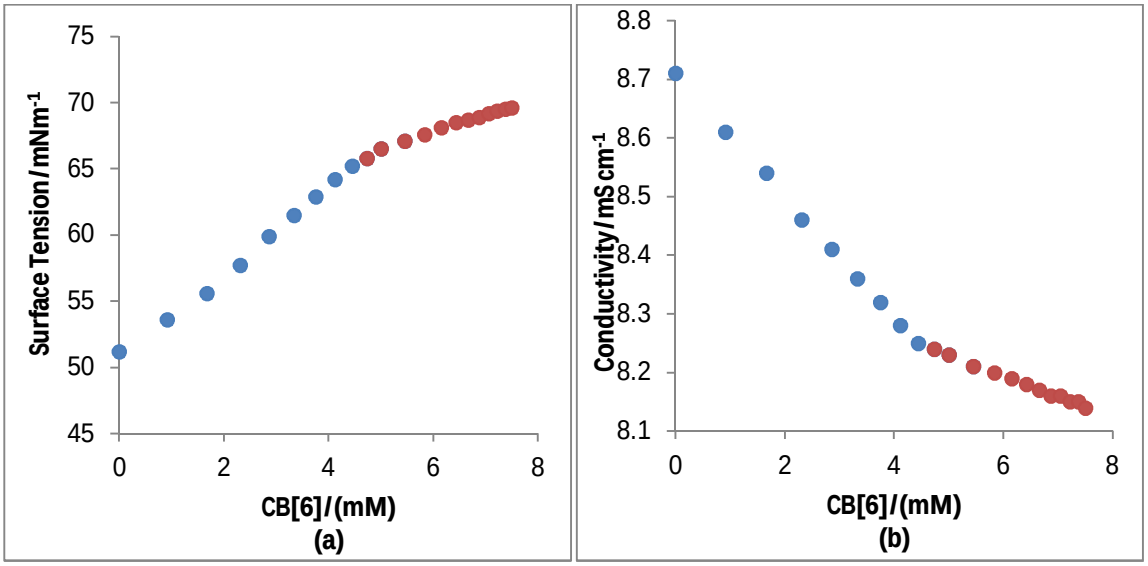
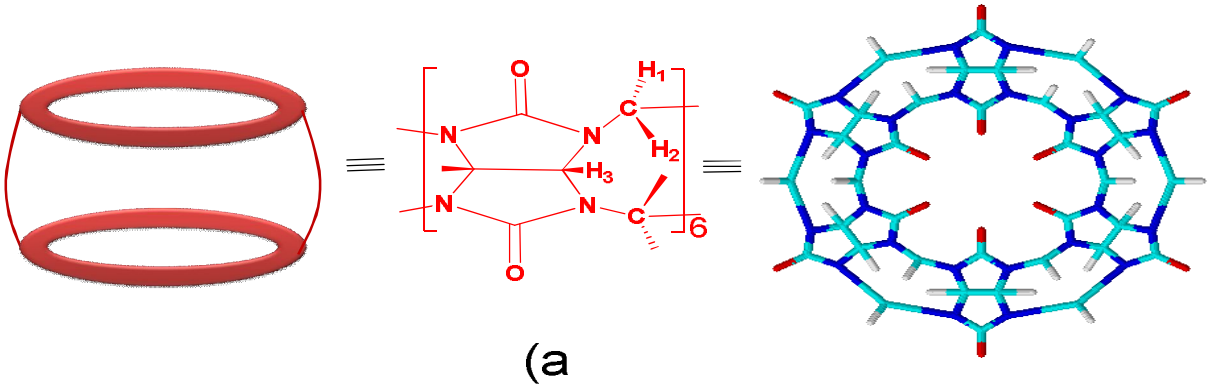
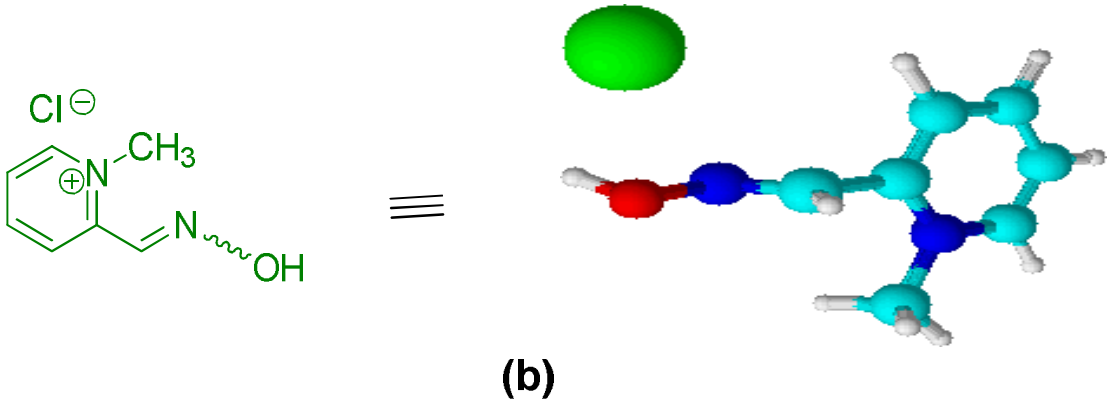


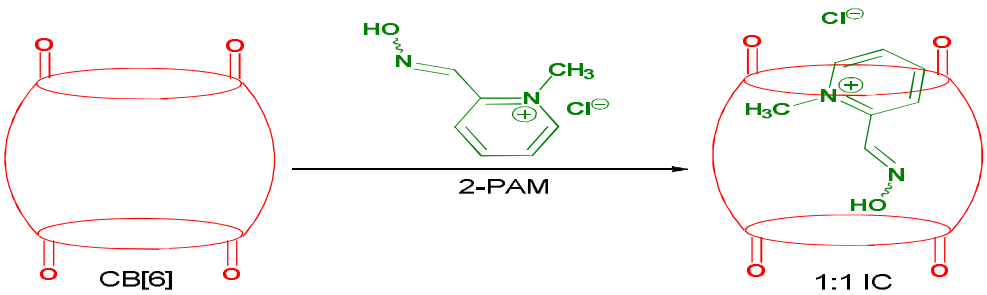
Figure 6: (a) Variation of surface tension of 2-PAM with increasing concentration of CB[6] and (b) Variation of conductivity of 2-PAM with increasing concentration of CB[6] at 298.15K.

Schemes





Scheme 1. Molecular structure of (a) CB[6] and (b) 2-PAM



Scheme 2. Plausible schematic representation of 1:1 molecular inclusion of CB[6] and 2-PAM