CHAPTER IV

STUDY TO EXPLORE COMPLEXATION OF CROWN ETHER WITH ANTIDEPRESSANT DRUG PREVALENT IN AQUEOUS SYSTEM BY PHYSICOCHEMICAL CONTRIVANCE

4.1. Introduction

The drug molecule Nortriptyline [3-(10, 11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-*N*-methyl-1-propanamine] hydrochloride (NTPH) (Scheme 1) belongs to the class of medicines known as tricyclic antidepressant (TCAs). Depression is fetching one of the most imperilling diseases disturbing human health and quality of living [1-5]. Compared to the other TACs drug molecules, NTPH shows various advantages. The antidepressant effects of TCA are thought to be due to an overall increase in serotonergic neurotransmission and in depressed individuals, NTPH exerts a positive effect on mood. TCAs can block histamine-H1 receptors, α1-adrenergic receptors which accounts for their sedative, hypotensive effects respectively. NTPH exerts less sedative side effects compared to the tertiary amine TCAs. NTHCL has also neuro protective effects and it is used as key models of chronic neuro-degeneration. It expanded as strong inhibitor of mitochondrial permeability transition (MPT) in both isolated [6] and brain. MPT results due to openings of protein pores that are formed in the inner membrane of mitochondria and allow free diffusion of molecules having molecular weight less than 1500Da, resulting mitochondrial swelling and cell death [7]. NTPH can also inhibit the release of cytochrome C and capsize activation in tissue. As, 15-C-5s are secure and friendly for human health and considered as safe drug carrier in human body [8], so, formulating inclusion complex of NTPH with 15-C-5s could potentially introduce a new prospect and hope in drug delivery systems and also in research field[9].

Beside many other host molecules, crown ethers (CEs) are used as significant hosts in host-guest chemistry. Here the host-guest interaction imitates natural systems as well as builds various materials [10-15]. CEs are macromolecular heterocyclic compounds with essential repeating unit -CH2CH2O-[16]. A number of investigators are working on fabrication of crown-ether-based stimuli-responsive materials that have exclusive characters of ion recognize ability [17-19]. A variety of current supramolecular ingredients, for instance rotaxanes are made on these unique recognition properties of CEs [20, 21]. Binding of cations with CEs having high selectivity and affinity has found marked importance in chemistry [22, 23]. Formation of molecular assemblies has vast implication for the building of molecular machines having plausible use as analogous to sophisticated machines of natural systems [24, 25]. Hence, fundamental investigations of the interactions between CEs and cationic species are important for their advanced applications [26, 27]

In this study formation of the complex of the crown ether and NTPH has been studied in aqueous medium for the probable applications in supramolecular host-guest chemistry.

4.2. Experimental

4.2.1. Source and purity of samples:

The above mentioned drug and Crown ether were purchased from Sigma-Aldrich, Germany. The mass fraction purity of drug and ether were ≥0.98.

4.2.2. Apparatus and procedure

Mass of the solid guest and hosts were taken using Mettler Toledo AG-285 with uncertainty of ±0.0001g and the solutions were prepared by mass solution at 298.15 K. Precautions were taken to reduce the evaporation during mixing.

Surface tensions of the prepared solutions were measured by platinum ring detachment technique using a Tensiometer (K9, KRUSS; Germany) at 298.15 K and accuracy was ±0.1 mN m^{-1}. Temperature was maintained by using

circulating thermostated water through a double-walled glass vessel containing the solution.

Conductivities of the prepared solutions were studied using Mettler Toledo Seven Multi conductivity meter with uncertainty of ±1.0 μSm^{-1}. Measurement was performed in a thermostated water bath at 298.15 K with uncertainty ±0.01 K. The conductivity cell was calibrated by freshly prepared 0.01 M aqueous KCl solution.

^1H NMR spectra of the solid inclusion complex prepared were recorded in D_2O using Bruker ADVANCE 400 MHz instrument. Signals are presented as values in ppm using residual protonated solvent signal at 4.79 ppm in D_2O as internal standard and all the Data are reported as chemical shift.

UV–visible spectroscopic study was carried out using JASCO V-530 UV/VIS Spectro-photometer with wavelength accuracy of ±0.5nm. Spectra were recorded at (297.15±1)K.

4.2.3. Preparation of Solid Inclusion Complex:

The drug molecule and the crown ether were taken in 1:1 molar ratio and they were dissolved in triply distilled water separately. The mixtures were stirred over magnetic stirrer to make homogeneous. After both the homogeneous mixtures were prepared, the NTPH solution was then added into crown ether solution slowly with continuous stirring and after completion of the addition the NTPH solution the mixture was stirred for 48 h continuously. After that the mixture was allowed to cool at lower temperature and a solid was observed. The precipitate was filtered and washed for several times. Finally, the dry powder was obtained after drying in oven at 40^0C for 24h. The solid inclusion complex with crown ether was prepared following the same procedure. These solids were further analysed and characterised by means of FTIR, NMR spectroscopic methods.

4.3. Result and discussion:

4.3.1. JOB Plot:

Using the Job's continuous variation method the stoichiometry of the inclusion complexes was determined [28-30]. By the measurement of absorbance of a set of solutions prepared of the NTPHs and host in water in the mole fraction range of 0–1 (TableS1.). Calculating ΔA × R values we plotted it against R, where ΔA signifies the difference in absorbance of the NTPH in the pure form and complexed form and R is [NTPH]/([NTPH] + [host]). λ_{max} was found at 252 nm at 298.15K. The ratio of guest and host i.e., stoichiometry is obtained from the value of R at the maxima on the Job' Plot such as R ≈ 0.33, for 1:2 IC, R≈ 0.5 for 1:1 IC, R≈ 0.66 for 2:1 IC etc. In this experiment of the drug and the host the maxima in the Job's plots were obtained at R≈0.5 which is the clear indication of formation of IC of 1:1 stoichiometry (Figure1).

4.3.2. Association Constant

The UV absorption spectra of NTPH in aqueous 15-C-5 medium were carried out in at 298.15K. The spectral data of NTPH in various concentration of 15-C-5 in different temperature has been listed in a table. The Figure2. depicts the absorption spectra of NTPH (2×10^{-4} M) in absence and presence of 15-C-5 solutions. The strong absorption peaks of NTPH (2×10^{-4} M) appears at 208 nm and with addition of 15-C-5 blue shift was found. In each case the absorbance intensities of NTPH gradually increase with increasing the concentration of 15-C-5. This confirms the inclusion of the guest molecule into the cavity of 15-C-5 [31, 32].

Various non-covalent interactions act as main driving forces throughout the complexation processby stimulating the dissolution of the guest molecule (NTPH). From Job's plot a clear indication is obtained that NTPH molecule forms 1:1 IC with 15-C-5. Hence, the IC formed between NTPH and 15-C-5 can be expressed as

15-C-5 +NTPH⇄15-C-5···NTPH (IV.1)

For 1:1 complexation processes, the association constant (*K*) can been obtained from the double reciprocal plot using Benesi - Hildebrand equation [33]. The absorption values were used in the following Benesi - Hildebrand equation (IV.2)[34].

$$\frac{1}{A-A_0}=\frac{1}{K_a(A"-A_0)}.\frac{1}{[Host]}+\frac{1}{(A"-A_0)} \qquad \text{(IV.2)}$$

D depicts the plot of $1/(A-A_0)$ against $1/[15\text{-C-}5]$ for NTPH. A good linear correlation was obtained. The values of *K* are evaluated by using the equation (IV.3) from the slop values of straight lines. The resultant association constant of NTPH in neutral medium has been found $5.58X10^3$, which is a significant value for describing the association of the two molecules.

$$K=\frac{1}{Slope(A"-A_0)} \qquad \text{(IV.3)}$$

4.3.3.Spontaneity of Inclusion Complex formation

Free energy change (ΔG), the thermodynamic parameter, defines the spontaneity of a process. This can easily be estimated from the values of association constant (*K*) by using the following equation (IV.4) [35, 36]

$$\Delta G=-RT\ln K_a \qquad \text{(IV.4)}$$

The ΔG values for the binding partners (NTPH and 15-C-5) are negative (−ΔG), which indicates that the host-guest IC proceeded spontaneously at 298.15 K and the complexation is an exergonic process. The value of ΔG was found -21.38 kJ mol^{-1}, which is negative and indicates the spontaneity of the process.

4.3.4.Surface tension study reveals the inclusion and also thestoichiometric ratio of the inclusion complexes

Surface tension (γ) was measured at 298.15 K for aqueous 15-C-5 molecule, which was found to be almost constant with increasing molarity [37]. Surface tensions (γ) were observed with corresponding concentrations of the drug in different molarities of 15-C-5(Table2).The plausibility of formation of an

inclusion complex can be predicted from the surface tension study, wherein the formation of an inclusion complex has been confirmed from the break point in the curve of surface tension vs. concentration.

The 1:1 and 1:2 stoichiometry of the host: guest inclusion complexes have been confirmed from the appearance of single and double by the break point in the γvs. conc. curve. The value of γ and the corresponding concentration of drug at the break point have been determined from the two intersecting straight lines indicating the feasibility of inclusion with increasing amount of 15-C-5in solution[38].However, the plots for NTPH with 15-C-5 clearly indicate single break point (Figure3. Table 1) at a specific concentrations, i.e., after a certain point surface tension becomes relatively steady with increasing concentration of the nucleosides. This is the indication of formation of an inclusion complex, which occurs by the insertion of NTPH into the cavity of 15-C-5.The NTPH enter the cavity of 15-C-5, which is geometrically allowed.

4.3.5. Conductivity

Conductivity study is a convincing method for exploring complexation in solution not only because it affords information for minute alteration of concentrations of the charged particles, but also it offers data for the various interactions among the particles taking place in the solution system [39]. Conductivity of a solution with NTPH and added crown ether (CE) provides valuable information for the complexation process between the CE and NTPH in solution [40]. In our work complexation has been explored between NTPH and CE 15-C-5 in aqueous medium.

Thus to acquire data about complexation, conductivity of the NTPH solution with initial concentration of 10.0 mM have been measured with increasing concentration of the CEs at 293.15K and presented in Tables 3 with increasing CE concentration. The plot of conductance has been depicted in Figure4. in which CE concentration is shown in abscissa and conductance is shown in ordinate. A gradual decrease in conductance is observed with increasing concentration of CE with a break point near 5mM concentration (Table3, Table1, Figure4.), which signifies the capture of the NTPH cation by the CE, because NTPH being strong

electrolyte can't form ion pair in the studied solution system [41]. So the complexation processes of NTPH ion with the CE have been illustrated by decrease in conductance in Figure4. become approximately plateau as the CE/NTPH mole ratio exceed 1.0, evidently suggesting development of adequately stable 1:1 NTPH-CE complex in aqueous solution [42].

4.3.6. 1H NMR

The complexation of NTPH with the CE, namely, 15-C-5has been explored by 1H NMR spectroscopic study in aqueous solution at 298.15K. Figure5. represents 1H NMR spectra of the complex of NTPH with 15-C-5, which describes slight downfield shift of the aliphatic protons of CE (C-H proton of free CE appears at $\delta 3.35$ and it is found in complex at $\delta 3.39$).The signal due to aryl protons are nearly unshifted and little broadening. On the other hand the protons of guest molecules of the aliphatic chain show a slight change of the in their signals while present in the complex (α, β and γ protons of free NTPH appears at $\delta 5.745$–5.794, δ 2.765–2.866 and $\delta 2.38$–2.444 respectively, which are found for the complex at $\delta 5.73$–5.77, $\delta 2.68$–2.74 and $\delta 2.35$–2.42 respectively. This result clearly reveals the existence of some sort of association between the electron rich oxygen atoms of the 15-C-5 and the ammonium ion (scheme 2)[43,44]. The aromatic part of the NTPH shows no change of their signals indicating their free state in the solvent medium.

NTPH

1H NMR (400 MHz, D2O): δ = 2.528 (3H, s); 2.960–3.025 (4H, m); 5.745–5.794 (1H, t, J = 7.5); 2.38–2.444 (2H, m); 2.765–2.866 (2H, m); 7.051–7.303 (8H, m).

15-C-5

1H NMR (400 MHz, D2O): δ = 3.35 (20H, s)

NTPH & 15-C-5 complex:

1H NMR (400 MHz, D2O): δ = 2.504 (3H, s); 2.953–3.025 (4H, m);3.39 (20H, s) 5.73–5.77 (1H, t, J = 7.5); 2.35–2.42 (2H, m); 2.68–2.74 (2H, m); 7.040–7.303 (8H, m),

4.4. Conclusion

The experimental findings suggest that the drug molecule binds with the crown ether (15-C-5) to form a complex. The oxygen atoms of the host molecule bind the positive centre of the guest NTPH to form a complex, which is confirmed by the 1H NMR study. One guest molecule binds with one host molecule to form complex of 1:1 stoichiometry, which was indicated by the surface tension and conductance study and further it was confirmed by the Job's plot from UV-Vis study. The Formation and the feasibility of the complex were found to be positive. The calculated ΔG^0 value was again found to be negative which revealed that the process of the formation of the complex to be thermodynamically feasible.

4.5. REFERENCES

References of CHAPTER IV are given in BIBLIOGRAPHY (Page No. 290-292)

Tables

Table1. Values of surface tension (γ) and conductance (*k*) at the break point with corresponding concentration of 15-C-5 for NTPH at 298.15K[a]

Conc. of 15-C-5/mM	γ/mN m^{-1}	Conc. of 15-C-5/mM	κ/mS.cm^{-1}
5.16	68.52	5.17	0.45

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

Table2. Data for the Surface tension study of aqueous 15-C-5+NTPH (concentration of stock solution of NTPH = 10mM, concentration of stock solution of CE = 10mM) at 298.15K[a].

Volm .of drug	vol. of 15-C-5	conc. Of drug	conc.of A 15-C-5	ST/mN.m-1
10	0	10	0	65.2
10	1	9.091	0.909	65.9
10	1	8.333	1.667	66.2
10	1	7.692	2.307	66.6
10	1	7.143	2.857	66.9
10	1	6.667	3.333	67.3
10	1	6.25	3.75	67.5
10	1	5.882	4.117	67.7
10	1	5.556	4.444	67.9
10	1	5.263	4.736	68.2
10	1	5	5	68.4
10	1	4.762	5.238	68.5
10	1	4.545	5.454	68.5
10	1	4.348	5.652	68.6
10	1	4.167	5.833	68.6
10	1	4	6	68.6
10	1	3.846	6.153	68.6
10	1	3.704	6.296	68.6
10	1	3.571	6.428	68.6
10	1	3.448	6.551	68.6

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

Table3. Data for the Conductivity study of aqueous 15-C-5+NTPH (concentration of stock solution of NTPH = 10mM, concentration of stock solution of CD = 10mM) at 293.15K[a].

15-C-5 added (mL)	conc of NTPHCL (mM)	conc of 15-C-5 (mM)	Conductance (mS m^{-1})
0	10.000	0.000	0.81
1	9.091	0.909	0.74
2	8.333	1.667	0.69
3	7.692	2.308	0.65
4	7.143	2.857	0.62
5	6.667	3.333	0.59
6	6.250	3.750	0.55
7	5.882	4.118	0.53
8	5.556	4.444	0.50
9	5.263	4.737	0.48
10	5.000	5.000	0.46
11	4.762	5.238	0.45
12	4.545	5.455	0.44
13	4.348	5.652	0.44
14	4.167	5.833	0.44
15	4.000	6.000	0.43
16	3.846	6.154	0.43
17	3.704	6.296	0.43
18	3.571	6.429	0.43
19	3.448	6.552	0.42
20	3.333	6.667	0.42

Figures

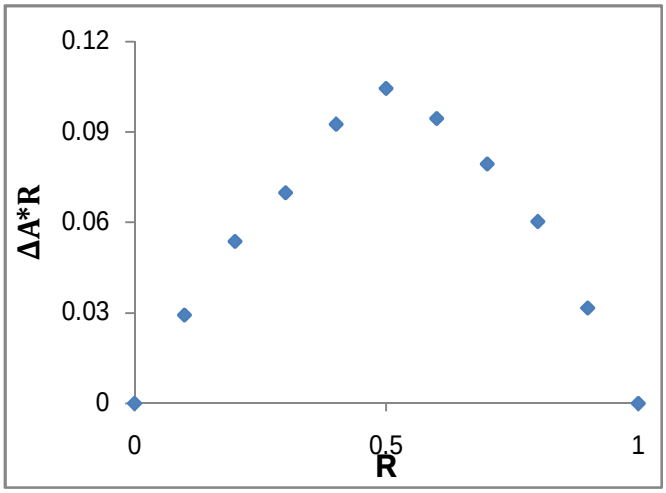


Figure1. Job plot of NTPH-15-C-5 system at λ_{max}= 239 nm at 298.15K. R=[NTPH]/([NTPH] + [CE]), ΔA= absorbance difference of NTPH without and with CE.

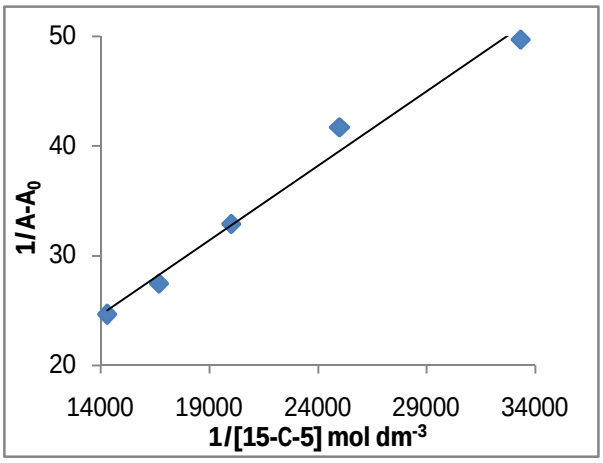


Figure2. Benesi-Hildebrand double reciprocal plot for the effect of 15-C-5 and NTPH at 298.15K.

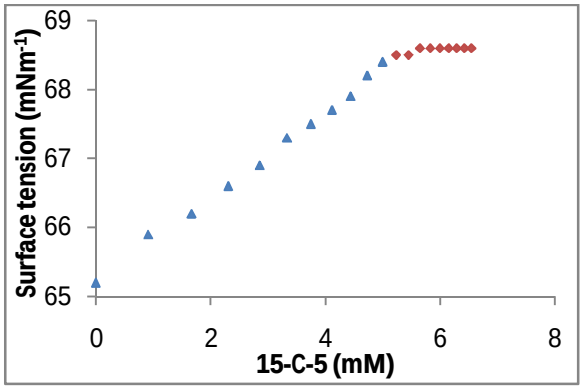


Figure3. Plot of surface tension with increasing concentration of 15-C-5 with NTPH at 298.15 K.

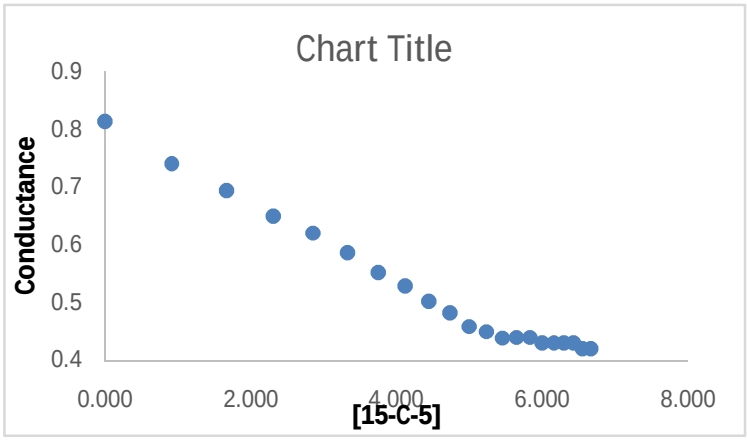


Figure4. Variation of conductance of NTPH with 15-C-5 at 298.15K

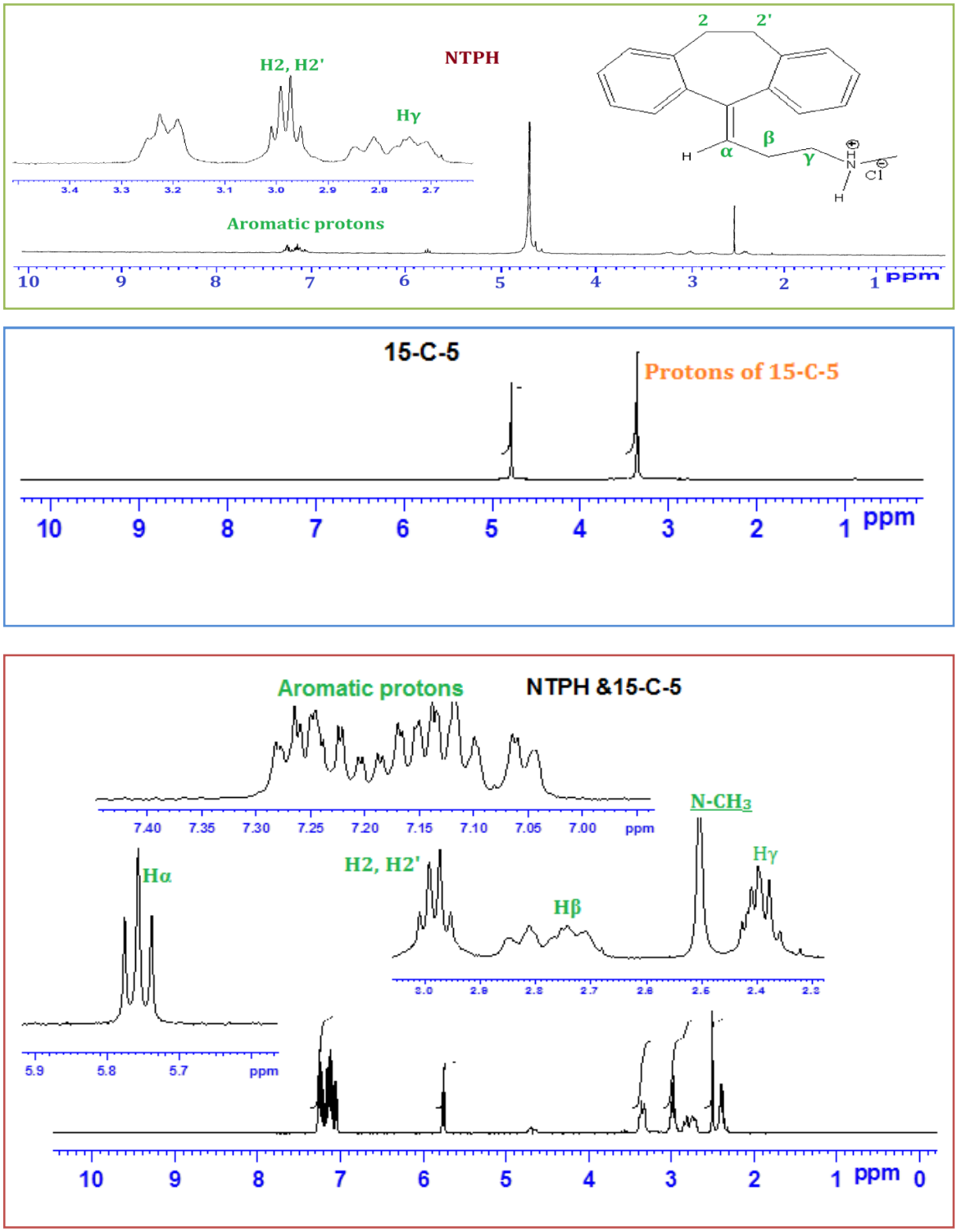
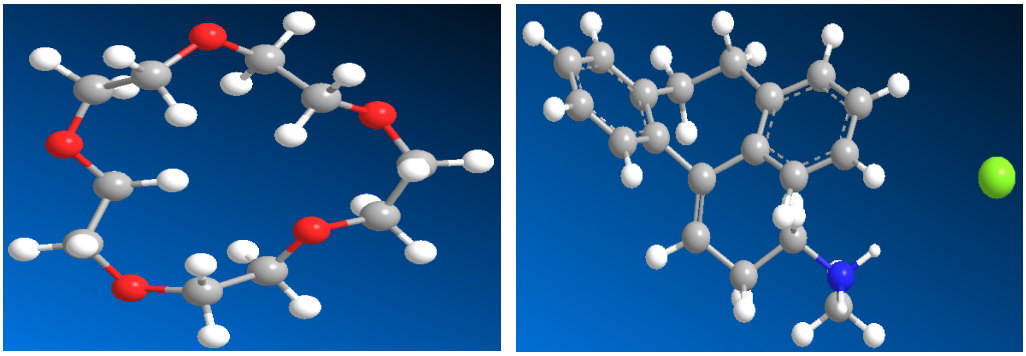


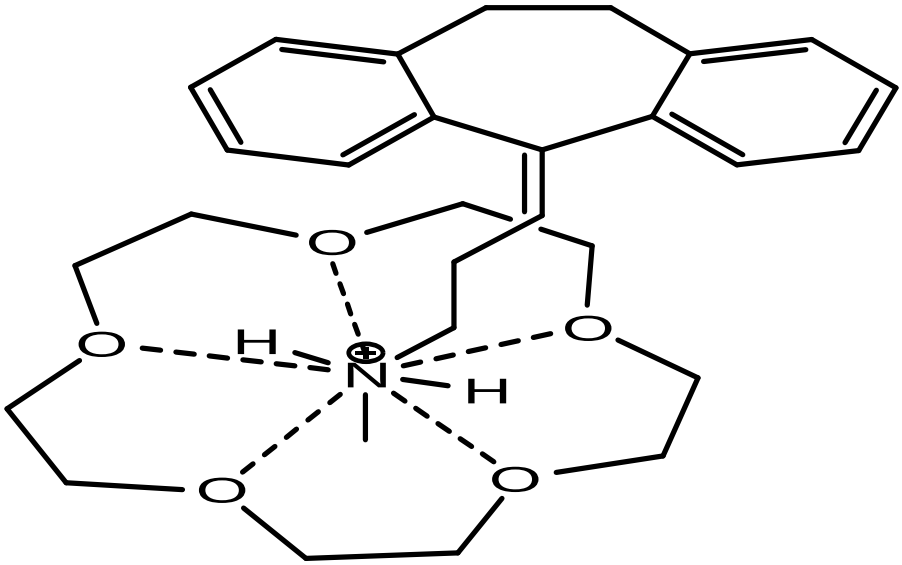
Figure5. 1H NMR spectra of *15-C-5*, NTPH and *15-C-5* & NTPH complex of 1:1 molar ratio in D2O at 298.15K.

Schemes



(a) (b)

Scheme1. Ball & stick representation of (c) 15-C-5 and (c) NTPH; gray for carbon and red for oxygen, white for hydrogen, blue for nitrogen



Scheme2. Schematic representation of the plausible interactions taking place in the complex.