INCLUSION COMPLEXES OF ACETANILIDE WITH 18-CROWN-6: SPECTROSCOPIC AND MOLECULAR MODELING STUDIES

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Abstract— The inclusion complexes of Acetanilide (AA) with 18-Crown-6 (18C6) were studied by UV and fluorescence techniques. The absorption and emission of AA in 18C6 medium confirms the –NH-CO-CH₃ aromatic side chain AA is inserted into the 18C6 cavity. The stoichiometry and binding constant of 1:1 inclusion complex was calculated using the Benesi– Hildebrand plot derived UV and fluorescence studies and the thermodynamic parameters (ΔG) of inclusion process were also determined. The results indicated that the inclusion process was an exergonic and spontaneous process. The complex was investigated by semiempirical and molecular docking methods. The AA: 18C6 inclusion complex obtained by molecular docking studies was in good correlation with the results obtained through experimental methods.

Index terms- 18-Crown-6, Acetanilide, Inclusion complex, Patchdock server.

I. INTRODUCTION

Acetanilide (AA) (Fig.1a) is an odourless, white flake solid or crystalline powder (pure form), soluble in hot water alcohol, ether, chloroform, acetone, glycerol, and benzene, melting point 114.3°C and boiling point 304° C, can undergo self-ignite at 545° C, but is otherwise stable under most conditions. Acetanilide was the first <u>aniline</u> derivative serendipitously found to possess<u>analgesic</u> as well as <u>antipyretic</u> properties, and was quickly introduced into medical practice under the name of Antifebrin by A. Cahn and P. Hepp in 1886(1). Acetanilide which can be obtained by acetylation of aniline undergoes nitration at low temperature and yields highly the para-nitro products.

18-Crown-6 (18C6) (1,4,7,10,13,16hexaoxacyclooctadecane) is a white crystalline hygroscopic solid. Crown ethers are used as phase transfer catalysts. 18-Crown-6 is a binds to a variety of small cations. It wraps around metal cations, providing an octahedral cavity. It is also having ability to form host-guest complex formation

In this paper, we report the photo physical and computational studies on the complexation AA, 18C6 at different conditions. In addition to the UV and Fluorescence studies, we have utilized the complexation behavior of AA for the stoichiometry and binding constant of AA:18C6 inclusion complex. Further it was also supported by semi empirical method and molecular docking studies.

II. MATERIALS AND METHODS

A. Materials

Acetanilide, 18C6 were obtained from Aldrich, HiMedia Laboratories and used without further purification. Triply distilled water was used to prepare all solutions and spectrograde solvents were used. Solutions in the pH 7 were prepared by adding the appropriate amount of NaOH and H₃PO₄. The concentration of 18C6 was varied from zero to 1.2×10^{-3} mol dm⁻³. From the stock solution 2, 4, 6, 8, 10 and 12 $\times 10^{-3}$ mol dm⁻³ of 18C6 were prepared using pH~ 7 buffers. The concentrations of the solutions were of the order 10⁻⁴ mol dm⁻³. All experiments were carried out at 30°C. The solid inclusion complex was also prepared by coprecipitation method.

B. Methods

The pH values were measured using Elico pH meter LI-120. The UV spectra were recorded with Specord 200+ spectrophotometer, Germany. The Fluorescence spectra were recorded using Spectrofluorometer, Perkin Elmer, USA. The most probable structure of the AA:18C6 inclusion complex was determined by molecular docking studies using Patch Dock server [2].

III. RESULTS AND DISCUSSION

A. Hosts-Guest Interaction of AA with 18C6

The absorption spectral data of AA in different concentrations of 18C6 recorded in pH~7 are compiled in Table 1. In AA upon increasing the concentration of 18C6 a slight blue shift is observed in the absorption maxima. No clear isosbestic point is observed in absorption spectrum. The absorption spectra show slight change in absorption maxima even in the presence of highest concentration of 18C6 used $(12x10^{-3} \text{ M})$ in pH~7. This behavior has been attributed to the enhanced dissolution of AA molecule through the hydrophobic

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interaction between guest molecule (AA) and non-polar cavity of 18C6 [3-4] as reported by others also [6-7] since, this indicates the formation of 1:1, host–guest inclusion complex of AA: 18C6.

The binding constant for the formation of AA:18C6 complex has been determined by analyzing the changes in the intensity of absorption maxima with the 18C6 concentration. In the case of inclusion complex formed between AA and 18C6, the equilibrium can be written as,

The binding constant 'K' and stoichiometric ratios of the inclusion complex of AA can be determined according to the Benesi–Hildebrand [5] relation assuming the formation of a 1:1 host–guest complex.

A cetanili de + 18C6 \Box^{K} A cetanili de....18C6

$$\frac{1}{A - A_0} = \frac{1}{\Delta \varepsilon} + \frac{1}{K \text{ [Acetanilide]}_0 \Delta \varepsilon \text{ [18C6]}_0}$$

Where, A and A_0 is the difference between the absorbance of AA in the presence and absence of 18C6, $\Delta\epsilon$ is the difference between the molar absorption coefficient of AA the inclusion complex, [AA]₀ and [18C6]₀ are the initial concentration of AA and 18C6 respectively. The plot of 1/A-A₀ verses 1/[18C6] for AA in pH~1 and pH~7. For both pH solutions, a good linear correlation was obtained, confirming the formation of a 1:1 inclusion complex. From the intercept and slope values of this plot, the binding constant 'K' was evaluated. The 'K' value for AA, neutral condition 982.47 M⁻¹, at pH~7 at 303K.

The effect of 18C6 on the fluorescence spectra of AA (Table 1) is different from absorption spectra and more pronounced than the relative effect on the absorption spectra. In AA, there is no significant change is

| S. | Concentration of | Acetanilide | | | |
|---------------------------------------|---------------------|-------------|------|---------|-----------|
| No. | 18-Crown-6 | λ | log | λ | Flu. |
| | (M) | max(nm) | ε | max(nm) | Intensity |
| | 0 | | | | |
| 1 | Without | 240 | 3.38 | 486.5 | 18.261 |
| | 18C6 | | | | |
| 2 | 0.002 | 240 | 3.42 | 486.5 | 47.348 |
| 3 | 0.004 | 240 | 3.46 | 486.5 | 38.091 |
| 4 | 0.006 | 239 | 3.48 | 486.0 | 20.298 |
| 5 | 0.008 | 240 | 3.42 | 486.0 | 18.648 |
| 6 | 0.01 | 240 | 3.39 | 486.5 | 17.532 |
| 7 | 0.012 | 239 | 3.39 | 486.5 | 17.142 |
| Binding constant (M ⁻¹⁾ | | 982.47 | | 65.82 | |
| $\Delta G (kJ mol^{-1})$ | | -17.35 | | -10.54 | |

Table 1: Absorption and Flurosence maxima (nm) and log ε of acetanilide (0.001 M) at different concentrations of 18C6 in pH~7 solutions

observed in emission maxima (~486 nm) at pH~7. The emission intensity of AA in pH~7 is increases when the 18C6 concentration is increased, whereas the intensity is increased. (Figure 1) shows the Benesi–Hildebrand plot of observed changes in the fluorescence intensity with increasing concentration of 18C6. It is seen from this plot that the emission intensity of AA initially increases with 18C6 concentration and then saturates to a limiting value at 0.012M 18C6, indicating the maximum inclusion of AA molecule in the 18C6 cavity

and then saturates to a limiting value at 0.012M 18C6, indicating the maximum inclusion of AA molecule in the 18C6 cavity. The binding constant for the formation of complex has been determined by analyzing the changes in the intensity of emission maxima with the 18C6concentration using the Benesi-Hildebrand[5] relation assuming the formation of a 1:1 host –guest complex.

$$\frac{1}{I - I_0} = \frac{1}{I' - I_0} + \frac{1}{K [I' - I_0] [18 C 6]_0}$$

Where, $[18C6]_0$ represents the initial concentration of 18C6, "I₀"and "I" are the fluorescence intensities in the absence and presence 18C6 respectively, and I' is the limiting intensity of florescence. The 'K' value was estimated from the slope and intercept of the Benesi–Hildebrand plot which shows a good linear correlation supporting the assumption of 1:1, AA: 18C6 inclusion complex. The binding constant

'K' value for AA in neutral condition 65.82 M⁻¹, at pH~7 at 303K.

B. The thermodynamics of inclusion process

The thermodynamic parameters ΔG for the binding of guest molecule to 18C6 cavity can be calculated from the binding constant 'K' by using the following equation,

$$\Delta G = -RT \ln K$$

The thermodynamic parameters ΔG for the binding of guest molecules (AA) to 18C6 cavity are given in Table1. The negative value of ΔG suggests that the inclusion process proceeded spontaneously at 303K. Considering the above discussions, the

possible inclusion mechanism is proposed. Naturally, the inclusion complex formation between AA with 18C6, are possible with the -NH-CO-CH₃ aromatic side chain and part of benzene ring of AA is inserted in the 18C6 cavity as shown in figure 2.

Figure 1. The Fluorescence spectra of Acetanilide (pH~7) in different 18C6 concentrations (mol dm⁻³): (1) 0.0 M, (2) 0.002 M, (3) 0.004 M, (4) 0.006 M, (5) 0.008 M, (6) 0.010M and (7) 0.012 M.(**Inside-** Benesi–Hildebrand plot of $1/F-F_0$ vs. 1/[18C6] for Acetanilide in pH~7 solution)



C. Semi empirical quantum mechanical calculations

The internal diameter of the 18C6 is approximately 3.2 Å (Scheme 1). In AA the vertical distance between H_{13} - H_{18} is 8.1 Å and this is higher than the height of 18C6. The shorizontal distance between H_{11} - H_{15} is 4.3 Å and



this is also higher than the internal diameter of 18C6.Since, the height of AA is higher than that of 18C6, the -NH-CO-CH₃ aromatic side chain and part of benzene ring of AA slight insertion in the 18C6 cavity is possible as shown in (Scheme 1)

D. Molecular docking study of inclusion process

The 3D structure of 18C6, AA obtained from crystallographic databases are shown in figure 2. The guest molecule, AA was docked into the cavity 18C6 using PatchDock server. The PatchDock server gave several possible docked models for the most probable structure based on the energetic parameters; geometric shape complementarity score [8], approximate interface area size and atomic contact energy [9] of the model (figure 2c) AA:18C6inclusion complex.

Figure 2. Ball and stick representation of (a) 18C6, (b) AA, (c) AA:18C6 1:1 inclusion complex. oxygen atoms are shown as red balls, carbon atoms as golden balls and sticks, nitrogen atoms as blue balls and hydrogen atoms are shown in grey balls and sticks.



The docked AA:18C6 model (figure 2c) with the highest geometric shape complementarity score 2036 approximate interface area size of the complex 241.60 $Å^2$ and atomic contact energy -78.15,kcal/mol was the highly probable and energetically favourable model.

IV. CONCLUSIONS

In summary, the inclusion complex with 1:1 molar ratio was formed between18C6 and AA. The -NH-CO-CH₃ aromatic side chain and part of benzene ring of AA was inserted in the 18C6 cavity. Thermodynamic parameter values show the inclusion processes are spontaneous. UV, Fluorescence, semi empirical and molecular docking results confirms the formation of AA: 18C6 inclusion complex. The inclusion complex formation which was also confirmed by molecular docking studies.

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