Photo Physical and Computational Studies on the Complexation of Acetanilide with B-Cyclodextrin

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Abstract
Inclusion complex of acetanilide (AA) with β-cyclodextrin (β-CD) has been investigated by UV and Fluorescence techniques. The red shift in λmax and enhanced absorption in β-CD medium confirms the –NH-CO-CH_3 aromatic side chain and part of benzene ring of AA is inserted in the β-CD cavity. The formation of complex has been confirmed by Benesi-Hildebrand plot obtained from the results of UV and fluorescence studies. The pKa values of prototropic species, stability constant K1:1 and the thermodynamic parameters (ΔG, ΔH and ΔS) of inclusion process were determined. The results indicated that the inclusion process was an exergonic and spontaneous process. The solid AA:β-CD complex was investigated by FTIR and XRD methods. The AA:β-CD inclusion complex obtained by molecular docking studies was in good correlation with the results obtained through experimental methods.
Keywords: β-cyclodextrin, acetanilide, pH effects, patchdock server, inclusion complex.

I. INTRODUCTION

Acetanilide (AA) is an aromatic primary amide of ethanoic acid. AA is an odourless solid chemical of leaf or flake-like appearance, melts at 114.3˚C and soluble in ethanol, diethyl ether, acetone and benzene. AA was the first aniline derivative serendipitously found to posses analgesic as well as antipyretic properties and was introduced into medical practice under the name of antifebric by A. Cahn and P. Hepp in 1886 [1]. AA synthetic organic compound introduced in therapy as a fever reducing drug. It's effectiveness in relieving pain was discovered soon thereafter, and it was used as an alternative to aspirin for many years in treating such common complaints as headache, menstrual cramps and rheumatism. Excessive or prolonged use of AA causes toxic side effect, it interferes with the function of hemoglobin, the oxygen-carrying pigment of the blood. The acute effects of AA exposure have been examined in mice rats, guinea pigs, rabbits, cats and dogs [2]. After several conflicting results over the ensuing fifty years, it was established in 1948 that AA was mostly metabolized to paracetamol in the human body, and that it was the paracetamol that was responsible for the analgesic and antipyretic properties [3-6]. AA is used as a precursor in the synthesis of penicillin and other pharmaceuticals [7].
Cyclodextrins (CDs) are cyclic oligosaccharides obtained from enzymatic hydrolysis of starch. The β-CD is the most abundant natural oligomers and corresponds to the association of seven glucose units with cavity, which exhibits a hydrophobic character whereas the exterior is strongly hydrophilic. Their ability to form host-guest complexes has led to the use of CDs in a number of fields [8,9]. CDs have been used in the pharmaceutical industry, as solubilizers, diluents and as tablet ingredients which improve the chemical stability, solubility, bioavailability and pharmacokinetic properties of drugs.
In this paper, we report the photo physical and computational studies on the complexation of AA and β-CD at different conditions. UV and fluorescence studies, to estimate the stoichiometry and binding constant of AA:β-CD inclusion complex. Further it was also supported by FTIR, XRD, semiemprical method and molecular docking studies.

II. MATERIALS AND METHODS

A. Materials

Acetanilide and β-CD were obtained from 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 range 2.0–12.0 were prepared by adding the appropriate amount of NaOH and H_3PO_4. A modified Hammett's acidity scale (H_0) [10] for the solutions below pH~2 (using a H_2SO_4– H_2O mixture) and Yagil basicity scale (H_-) [11] for solutions above pH~12 (using a NaOH–H_2O mixture) were employed. The solutions were prepared just before taking measurements. The concentration of β-CD was varied from zero to 1.2×10^{-3} mol dm^{-3}. From the stock solution 2, 4, 6, 8, 10 and 12 $\times10^{-3}$ mol dm^{-3} of β-CD 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

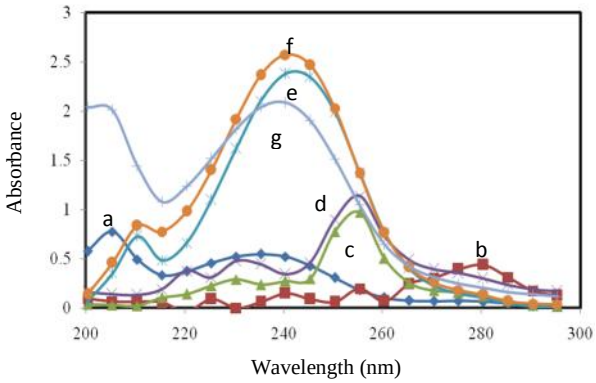
The pH values were measured using Elico pH meter LI-120. The UV spectra were recorded with Elico SL 159 spectrophotometer. The Fluorescence spectra were recorded using Spectrofluorometer, Perkin Elmer, USA. The IR spectra of all samples were recorded using Alpha-T FTIR Spectrometer (Bruker optics) equipped with OPUS version 6.5 by KBr pellet method. Powder X-ray diffraction spectra were taken by XPert PRO PANalytical diffractometer. The most probable structure of the AA:β-CD inclusion complex was determined by molecular docking studies using PatchDock server [12].

III. Results and Discussion

A. Effect of solvents

The absorption spectra of AA have been studied in of various solvents of different polarities and hydrogen bonding abilities. The relevant spectral data of benzanilide [13] and the spectra of AA in selected solvents are depicted in Fig. 1. The spectral shifts observed in the absorption spectrum of AA molecule in protic and aprotic solvents are consistent with the characteristic behaviour of $\rangle NH$ group [13]. Solvatochromic shifts of AA reveals that the absorption maxima are red shifted from cyclohexane to *2*-propanol but blue shifted in methanol and water. The red shift observed in the absorption spectra moving from cyclohexane to acetonitrile is due to the dispersive interactions and the most proton accepting nature of acetonitrile. However, methanol and water can act as proton donor solvents and thus produce a blue shift in the absorption spectra. So, the above shifts are mainly due to the interactions of the solvents with the $\rangle NH$ moiety of AA. The interactions of solvents with the lone pair of the $\rangle NH$ moiety will lead to a blue shift and the solvents interaction with hydrogen atom of the $\rangle NH$ moiety will lead to a red shift. The data infers that the absorption maxima of AA are blue shifted as compared to benzanilide.

Figure 1 Absorption spectra of AA in selected solvents at 303 K; concentration of AA 1×10^{-4} M (a) cyclohexane (b) ethylacetate (c) acetonitrile (d) 2-proponal (e) methanol and (f) water.



B. Effect of pH with and without β-cyclodextrin

The absorption spectra of different prototropic species of AA have been studied at different acid-base concentration in the H_0/pH/H_- range of –2.76 to 15.4 in the presence and absence of β-CD. The relevant data are given in Table 1 and the absorption spectra of various prototropic species. There are two prototropic species (neutral and monoanion) in AA. In the case of without β-CD medium the λ_{max} obtained for pH range –4.89-14.02, resemble the spectra observed in non-aqueous solvents and thus can be assigned to the neutral species [14]. On increasing the base concentration (above H_- 14.02) the absorption maximum is blue shifted. The blue shift confirms the formation of

TABLE 1 Various prototropic maxima (absorption spectra) and pKa values of AA in with and without β-CD medium

Species	With β-Cyclodextrin		Without β-Cyclodextrin	
	$\lambda^{max}_{(nm)}$	pKa	$\lambda^{max}_{(nm)}$	pKa
Neutral	240.0	-0.26 – 12.99	239.0	-4.89 – 14.02
Monoanion	224.9	14.02	226.0	14.37 – 15.4

monoanion due to the deprotonation of $\rangle NH$ moiety and a greater degree of interaction between the ionic substituent and the aromatic ring. The effect of pH on AA in the β-CD medium have been studied at different acid-base concentration in the H_0/pH/H_- range of –0.26 to 14.02. In the pH range –0.26-12.99 absorption maxima resemble the spectra observed in non-aqueous solvents and thus can be assigned to the neutral species [14]. Further

increase on base concentration (above H_- 12.99) the absorption maximum is blue shifted. The blue shifted spectra may be attributed to the formation of monoanion and a greater degree of interaction of ionic substituent with aromatic ring, but no clear isosbestic point is observed in the absorption spectra. The pK_a value for monoanion-neutral equilibrium of AA in β-CD medium (Table 1) differs appreciably than in aqueous medium and this confirms the encapsulation of AA molecule in the β-CD cavity. Further it is also confirmed by other methods as discussed below.

C. Effect of β-CD

The UV-Visible absorption spectral data of AA in different concentrations of β-CD recorded in pH~7 are complied in Table 2. At pH~7, AA exists in the neutral form; to check the formation of monocation in the acidic condition the absorption spectra was recorded. The absorption peaks of AA (1×10^{-4} M) in pH~7 appears at ~240 nm. Upon increasing the concentration of β-CD in pH no clear isosbestic point is observed in the absorption spectra. In presence of β-CD, at pH~7 there is no any change in the $\lambda_{max.}$ The absorption maxima and the spectral shape of AA molecule in pH~7 solutions are almost same and it infers that monocation is not formed.

The absorption spectra show only a very slight change in absorption maxima even in the presence of the highest concentration of β-CD used ($12x10^{-3}$ M) in pH-7. This behavior has been attributed to the enhanced dissolution of AA molecule through the hydrophobic interaction between guest molecule (AA) and non-polar cavity of β-CD [15-17] as reported by others also [18,19]. These results indicate that AA molecule is entrapped in the β-CD cavity to form 1:1 inclusion complex. In pH~7, the binding constant for the formation of AA:β-CD complex has been determined by analyzing the changes in the intensity of absorption maxima with the β-CD concentration. In the case of inclusion complex formed between AA and β-CD, the equilibrium can be written as,

$$\text{Acetanilide} + \quad -\text{CD} \overset{K}{\quad} \text{Acetanilide....} \quad -\text{CD}$$

The binding constant '*K*' and stoichiometric ratios of the inclusion complex of AA can be determined according to the Benesi–Hildebrand [20] relation assuming the formation of a 1:1 host–guest complex.

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

Where, A and A_0 is the difference between the absorbance of AA in the presence and absence of β-CD, $\Delta\varepsilon$ is the difference between the molar absorption coefficient of AA and the inclusion complex, $[AA]_0$ and $[\beta\text{-CD}]_0$ are the initial concentration of AA and β-CD respectively. The plot of $1/A\text{-}A_0$ verses $1/[\beta\text{-CD}]$ for AA in pH~7. For pH~7 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 in neutral condition (49.62 M^{-1} at pH~7) at 303K. The binding constants are sensitive to change of pH values, which reveal that selective inclusion associated with the species form (neutral and acidic condition) of AA.

TABLE 2 Absorption and fluorescence maxima (nm) of AA ($1x10^{-3}$ M) at different concentrations of β-CD in pH~7 solutions.

S. No.	Concentration of β-Cyclodextrin (M)	UV		Fluorescence	
		$\lambda_{max(nm)}$	log ε	$\lambda_{Flu\ (nm)}$	Flu. Intensity
1	0 Without β-CD	240.7	4.03	485.5	49.696
2	0.002	240.2	4.04	485.5	52.582
3	0.004	240.5	4.04	485.5	54.142
4	0.006	240.3	4.05	485.5	64.617
5	0.008	240.4	4.07	485.5	70.573
6	0.01	241.0	4.08	485.5	73.118
7	0.012	240.6	4.09	485.5	75.758
Binding constant (M^{-1})		49.62		114.69	
a*G (kJ mol^{-1})*		-9.83		-11.94	
a*H (kJ mol^{-1})*		-4222.25		-4222.25	
a*S (kJ mol^{-1} k^{-1})*		-13.90		-13.89	

The effect of β-CD on the fluorescence spectra of AA, (Table 2) is different from absorption spectra and more pronounced than the relative effect on the absorption spectra. In AA, there is no significant change is observed in emission maxima (~486 nm) at pH~7. The emission intensity of AA in pH~7 is increases when the β-CD concentration is increased, whereas the intensity is increased. The Benesi–Hildebrand plot of observed changes in the fluorescence intensity with increasing concentration of β-CD. It is seen from this plot that the emission intensity of AA, initially increases with β-CD concentration and then saturates to a limiting value at 0.012M β-CD, indicating the maximum

inclusion of AA molecule in the β-CD cavity. The binding constant for the formation of complex has been determined by analyzing the changes in the intensity of emission maxima with the β-CD concentration using the Benesi-Hildebrand [20] relation assuming the formation of a 1:1 host –guest complex.

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

Where, $[\beta\text{-CD}]_0$ represents the initial concentration of β-CD, "I_0" and "I" are the fluorescence intensities in the absence and presence β-CD 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:β-CD inclusion complex. The binding constant 'K' value is AA evaluated as 114.69 M^{-1}.

D. The thermodynamics of inclusion process

The thermodynamic parameters ΔG, ΔH and ΔS for the binding of guest molecule to β-CD cavity can be calculated from the binding constant '*K*' by using the following equation,

At constant temperature,

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

$$\Delta S = \frac{\Delta H - \Delta G}{T}$$

The thermodynamic parameters ΔG, ΔH and ΔS for the binding of guest molecule to β-CD cavity are given in Table 2. The negative value of ΔG suggests that the inclusion process proceeded spontaneously at 303K. ΔH and ΔS are also negative in the experimental temperature range, which indicates that the inclusion process is an exergonic and enthalpy controlled process. The negative enthalpy change (ΔH) arose from the Vander Waal's interaction, while the negative entropy change (ΔS) is the steric barrier caused by molecular geometrical shape and the limit of β-CD cavity to the freedom of shift and rotation of guest molecule. The experimental results indicate that the inclusion reaction of β-CD with AA was an exothermic reaction accompanied with negative ΔS. In this case, the actions that enthalpy and entropy change played were on the contrary. The conclusion of these thermodynamic parameters is that changes in ΔH are largely compensated for by changes in ΔS. Considering the above discussions, the possible inclusion mechanism is proposed. Naturally, the inclusion complex formation between AA and β-CD are possible with the –NH-CO-CH_3 aromatic side chain and part of benzene ring of AA is inserted in the β-CD cavity as shown in Fig 4.

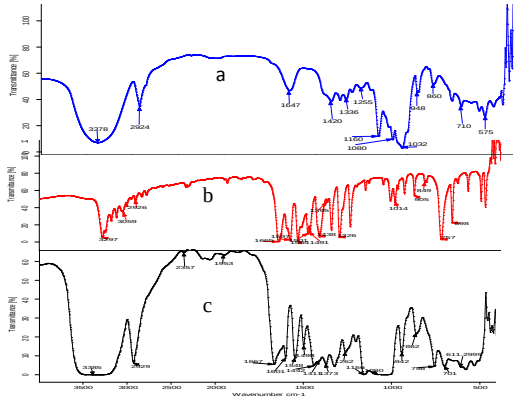


Figure 2 FT-IR Spectra of (a) β-CD, (b) Acetanilide (c) Acetanilide:β-CD solid complex in KBr.

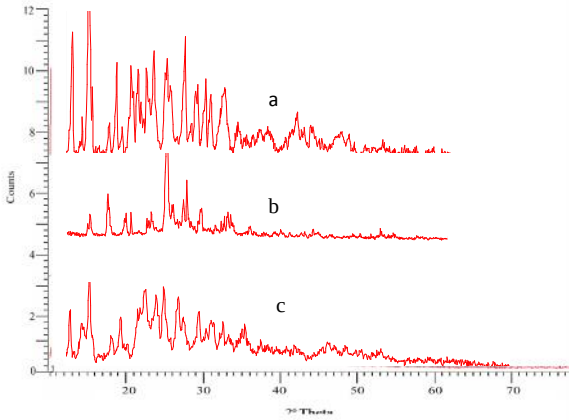


Figure 3 XRD pattern of (a) β-CD (b) Acetanilide (c) Acetanilide: β-CD solid complex

E. FTIR spectral studies

In AA, the characteristic –CH stretching vibration of $-CH_3$ group appear at 2926 cm^{-1}, but disappears in the inclusion complex (Fig. 2). The –CO group absorption appearing at 1665 cm^{-1} in AA whereas it is shifted to 1667 cm^{-1} in inclusion complex. The –NH stretching absorption appearing at 3297 cm^{-1} disappears and the –NH bending vibration peak at 1597 cm^{-1} shifted to 1601 cm^{-1}. In AA, the absorption peak appearing at 1501 cm^{-1} for C=C of aromatic ring shifted to 1498 cm^{-1}. Further the absorption intensity in inclusion complex was significantly greater than AA. These results indicate that the aromatic side chain and part of the benzene ring of AA is entrapped in to the β-CD cavity. The AA:β-CD solid inclusion complex did not show any new peaks, indicating that no chemical bonds were formed between AA and β-CD which was also confirmed by molecular docking studies.

F. Powder X-ray diffraction spectra

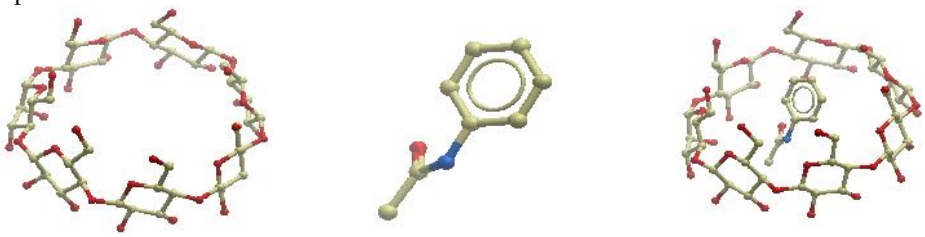
The formation of inclusion complex can be confirmed by X-ray diffractometry [21,22]. The Fig. 3 shows the powder X-ray diffraction spectra of β-CD, AA and AA:β-CD solid complex**.** The X-ray spectrum of the inclusion complex shown in Fig. 3c was evidently different from that of β-CD (Fig. 3a) and AA (Fig. 3b). The difference between the spectra of β-CD and inclusion complex is due to the interaction of β-CD with AA.

G. Semi empirical quantum mechanical calculations

The internal diameter of the β-CD is approximately 6.5 Å and its height is 7.8 Å. Considering the shape and dimensions of β-CD, AA cannot be completely embedded in the β-CD cavity. The ground state of AA molecule was optimized using AM1 method. The vertical distance between H_{13}-H_{18} is 8.1 Å and this is higher than the height of β-CD. The horizontal distance between H_{11}-H_{15} is 4.3 Å and is less than the internal diameter of β-CD. Since, the height of AA is higher than that of upper-lower rim of β-CD, the –NH-CO-CH_3 aromatic side chain and part of benzene ring of AA insertion in the β-CD cavity is possible as shown in Fig. 4

H. Molecular docking study of inclusion process

The 3D structure of β-CD and AA obtained from crystallographic databases are shown in Fig. 4a and Fig. 4b respectively. The guest molecule, AA was docked into the cavity of β-CD 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 [23], approximate interface area size and atomic contact energy [24] of the AA:β-CD inclusion complex. The docked AA:β-CD model (Fig. 4c) with the highest geometric shape complementarity score 2314, approximate interface area size of the complex 242.90 $Å^2$ and atomic contact energy -173.70 kcal/mol was the highly probable and energetically favourable model. The favourable computational model of AA:β-CD inclusion complex obtained through the PatchDock server was in good correlation with results obtained through experimental methods.



(a) β-CD (b) Acetanilide (c) 1:1, AA:β-CD inclusion complex

Figure 4 Ball and stick representation of (a) β-CD (b) AA (c) proposed 1:1, AA:β-CD inclusion complex, the oxygen atoms of –C=O groups are shown as red balls, nitrogen atom of –NH group is shown as blue ball, carbon as golden yellow balls and hydrogen atoms are not shown.

IV. CONCLUSIONS

In summary, the inclusion complex with 1:1 molar ratio was formed between β-CD and AA. The –NH-CO-CH_3 aromatic side chain and part of benzene ring of AA was inserted in the β-CD cavity. Thermodynamic parameter values show the inclusion processes are exothermic and spontaneous. UV, fluorescence, FTIR, XRD, semi empirical and molecular docking results confirms the formation of AA:β-CD inclusion complex. FTIR analysis indicates that no chemical bonds were created between AA and β-CD in the formed complex which was also confirmed by molecular docking studies. Computational model of AA:β-CD inclusion complex was in good correlation with the results obtained through experimental methods.

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