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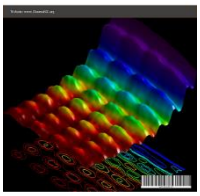

Exploring Existence of Host-Guest Inclusion Complex of β-Cyclodextrin of a Biologically Active Compound with the Manifestation of Diverse Interactions

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Abstract

The host–guest interaction of p-nitro benzaldehyde as guest β-Cyclodextrins have been investigated which have significant applications in the field of medicine such as controlled drug delivery. The 1H NMR study confirms the formation of inclusion complex while surface tension and conductivity studies support the formation inclusion complex with 1:1 stoichiometry. The stoichiometry of the inclusion complex was also supported with Job's plot method by UV-Visible spectroscopy. FT-IR spectra and SEM study also support the inclusion process. Association constants of the inclusion complexes have been calculated using the Benesi–Hildebrand method, while the thermodynamic parameters have been estimated with the help of van't Hoff equation.

Keywords:

B-Cyclodextrin (B-CD);
Para Nitro-Benzaldehyde (PNB);
Inclusion Complex (IC);
Scanning Electron Microscope (SEM).

Article History:

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1- Introduction

Cyclodextrins (CD) are macro cyclic oligosaccharides composed of repeating α-D-glucopyranose units. Three types of CDs are α, β and γ-CD with 6, 7 and 8 glucopyranoside units, respectively (Figure 1) [1, 2]. The cyclic orientation of these units gives conical or truncated cone structure with a hydrophobic interior and a hydrophilic exterior. This unique structure permits CD to form host–guest inclusion complexes with different sized guest molecules [3]. The chemical stability of guest molecule can be explained on the basis of the van der Waals attraction, hydrogen bonding and hydrophobic attractions, etc. [4]. Due to its wide applicability, CDs used in different areas, such as the cosmetics, food, medicine, hygiene, nano materials and agro industries [5]. Inclusion complex of CDs have been used in aqueous solubility enhancement of poorly soluble compounds to increase their bioavailability. Among CD family ,β-cyclodextrin is the most widely used one due to the low cost.

p-nitro benzaldehyde (PNB) is a very useful compound in organic synthesis. It also has applications in medicinal chemistry. (Scheme2). In this present work we attempt to ascertain the formation and nature of inclusion complex β-CD with PNB in aqueous environment by spectroscopic and physicochemical studies. Our aim is to explore the formation, carrying and controlled release of PNB by forming IC with CD without any chemical and biological modification of the guest molecule.

2- Experimental Section

2-1- Source and Purity of Samples

P-nitro benzaldehyde and β-cyclodextrin of puriss grade were bought from Sigma Aldrich, Germany and used as purchased. The mass fraction purity of PNB and β-cyclodextrin are ≥ 0.99 and ≥ 0.98 respectively.

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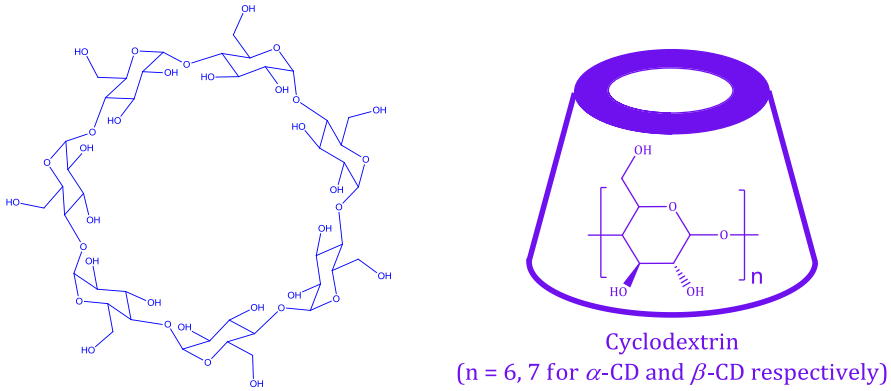

Figure 1. Structure of cyclodextrin molecules.

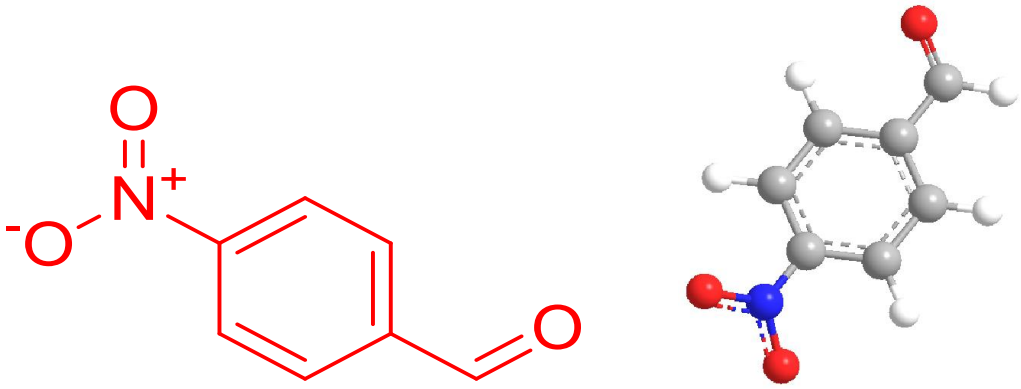

Figure 2. Two dimensional and three dimensional structure of PNB

2-2- Apparatus and Procedure

The guest molecule PNB and the β-CDs are freely soluble in triply distilled, deionized and degassed water. The stock solutions of PNB and aqueous CD were prepared by mass at 298.15 K. Mettler Toledo AG-285(uncertainty 0.0001 g) was used for weighing.

UV–visible spectra were taken by JASCO V-530 UV/VIS Spectrophotometer, with an uncertainty in wavelength as ±2 nm. The temperature during experiment is kept constant using a digital thermostat.

^{1}H NMR spectra were taken in D_2O at 300 MHz with help of Bruker Advance instrument at 298.15 K. Signals are mentioned as δ values in ppm. The internal standard is D_2O (protonated signal at 4.79 ppm). Data are cited as chemical shift.

The surface tension study was performed with platinum ring detachment technique using a Tensiometer (K9, KRSS; Germany). The temperature is maintained at 298.15 K by circulation of thermostated water through a double wall glass vessel containing the solution. The accuracy of the instrument is about ±0.1mN m^{-1}.

Conductivities of the solutions are measured using a Mettler Toledo Seven Multi conductivity meter having uncertainty 1.0 μSm^{-1} having uncertainty ±0.01 K. Thermostated water bath kept the temperature constant at 298.15 throughout the experiment. HPLC grade water was used with specific conductance 6.0l Sm^{-1}. The conductivity cell was calibrated using 0.01 M aqueous KCl solution.

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

The solid inclusion complex [β-CD+PNB] has been prepared taking 1:1 molar ratio of both the PNB and CD. In this case 1.0 mmol CD was dissolved in 20 mL water and 1.0 mmol PNB was dissolved in 20 mL ethanol and stirred separately for 4 hrs. Then the ethanol solution of PNB was added drop wise to the aqueous CD solution. The mixture was then allowed to stir for 72 hrs at 50–55°C. It was filtered at this temperature, then cooled to 5°C and kept for 12 hrs. The resulting suspension was filtered and the white polycrystalline powder was found, which was washed with ethanol and dried in air.

3- Result and Discussion

3-1- Job Plot Demonstrates the Stoichiometry of the Host–Guest Inclusion Complex

The stoichiometry of the host guest inclusion complex can be predicted with the help of Job's method of continuous variation using UV-Visible spectra [6]. The difference of absorbance of PNB in presence and absence of β-CD was calculated and designated as ΔA. Then a plot is constructed taking ΔA × R against R where R = [PNB]/([PNB]+[CD]). (Figure 3)Job plots are constructed with variation of mole fraction of PNB in the range of 0-1[7, 8]. Absorbance values were measured at 293 nm at 298K for a series of solutions. (Table 1) The stoichiometry of the inclusion complex can be found with the value of R at the point of maximum deviation. (R=0.5 for 1:1 complexes; R=0.33 for 1:2complexes; R=0.66 for 2: 1 complexes) [9, 10]. In this study the plot show maxima at R=0.5 which means that the inclusion complex was formed with 1:1 molar ratio of PNB and β-CD.

Table 1. Data for the Job plot performed by UV-Vis spectroscopy for PNB-*β*-CD system at 298.15K[a]

PNB(mL)	*β*-CD (mL)	PNB (μM)	*β*-CD (μM)	[PNB]/([PNB]+[*β*-CD])	Absorbance (A)	ΔA	ΔA×[PNB]/([PNB]+[*β*-CD])
0.0	1.0	0	100	0.0	0.0000	0.7420	0.0000
0.1	0.9	10	90	0.1	0.0636225	0.6783	0.0678
0.2	0.8	20	80	0.2	0.1380672	0.6039	0.1208
0.3	0.7	30	70	0.3	0.2318311	0.5101	0.1530
0.4	0.6	40	60	0.4	0.2906623	0.4513	0.1805
0.5	0.5	50	50	0.5	0.3502874	0.3917	0.1958
0.6	0.4	60	40	0.6	0.4292407	0.3127	0.1876
0.7	0.3	70	30	0.7	0.4997888	0.2422	0.1695
0.8	0.2	80	20	0.8	0.5812507	0.1607	0.1286
0.9	0.1	90	10	0.9	0.6672149	0.0747	0.0673
1.0	0.0	100	0	1.0	0.7419558	0.0000	0.0000

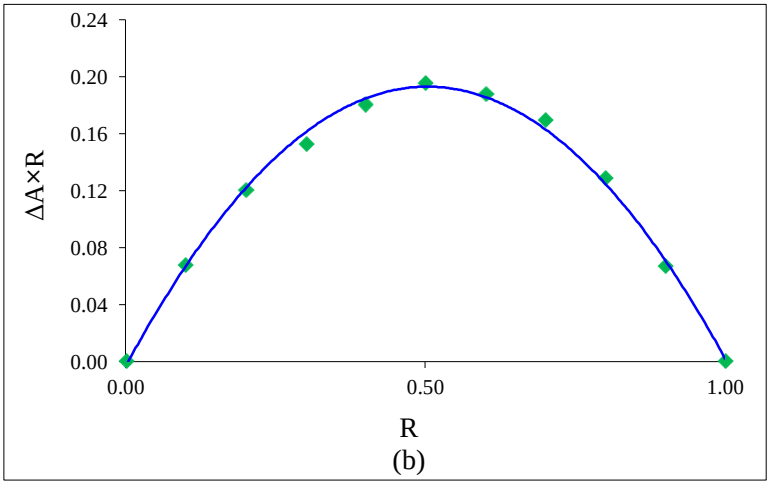

Figure 3. Job plot of (b) PB-β-CD system at λ_{max}=243 nm at 298.15 K .R= [PNB]/([PnB] + [CD]) ,ΔA=absorbance difference of PNB without and with β-CD

3-2- 1H-NMR Study

1H-NMR study gives us valuable information about the formation of inclusion complex between PNB and β-CD [11]. When the guest PNB molecule inserts into the hydrophobic cavity of β-CD molecule the protons of both PNB and β-CD show considerable chemical shift. The positions of different protons in the CD molecule are shown in scheme. The H3 and H5 protons are placed in the cavity while H1, H2 and H4 are located outside the cavity. (Figure 4) H3 is situated near the wider rim and H5 is close to the narrower rim [12, 13]. The respective δ values of PNB, β-CD and inclusion complex are mentioned in Table 2. The protons of PNB and β-CD show considerable upfield shift in the spectra of inclusion complex. (Figure 5) From NMR data it can be concluded that the aromatic protons of PNB interacts more with the H3 protons of β-CD in comparison to H5 perhaps due to the reason that the PNB molecule enters in the hydrophobic cavity from the wider rim. All the protons of both host and guest show considerable chemical shift due to

change of environment after forming inclusion complex. The H6 proton remains unaffected suggesting PNB enters from wider end.

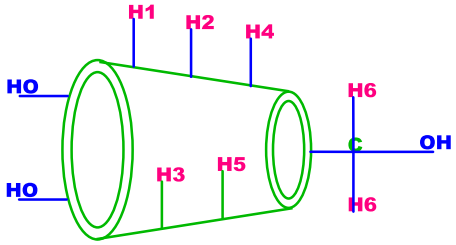

Figure 4. Truncated conical structure of β-Cyclodextrin.

Table 2. ^{1}H NMR data of PNB, β-CD and inclusion complex

β-Cyclodextrin	(500 MHz, Solv: D_2O) δ=3.49-3.54 (6H, t, *J* = 9.2 Hz), 3.57-3.60 (6H, dd, *J* = 9.6, 3.2 Hz), 3.79-3.84 (18H, m), 3.87-3.92 (6H,t, *J* = 9.2 Hz), 5.00-5.01 (6H, d, *J* = 3.6 Hz)
PNB	(400 MHz, Solv: D_2O) δ=7.9-8.0 (2H,m),8.1-8.2 (2H,m), 8.2-8.3(1H,d)
PNB+β-CD complex	(1:1 molar ratio, 300 MHz, Solv: D_2O) : δ=3.3-3.5(6H, t), 3.5-3.9(6H, dd), 4.5-5.0(18H, m), 7.9-8.1(2H,m), 8.1-8.3(2H,m), 9.8 (1H,d)

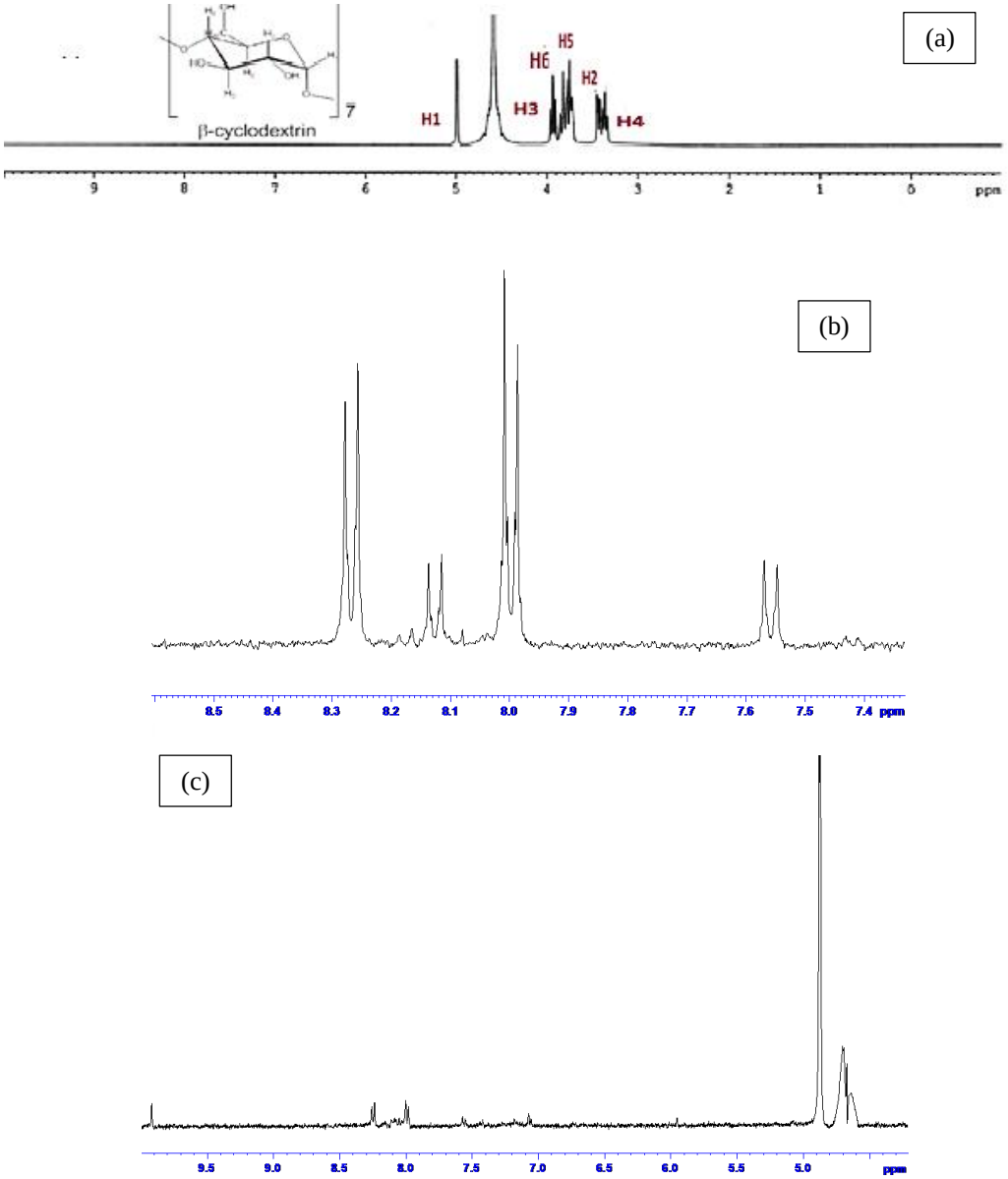

Figure 5. H NMR Spectra of (a) β-CD (b) PNB and (c) 1:1 molar ratio of β-CD + PNB in D_2O in 298.15 K.

3-3- Surface Tension Study Confirms the Stoichiometry of the Inclusion Complex

Cyclodextrin is freely soluble in water. The aqueous solution of β-CD does not show any notable change with increasing concentration [14]. The guest molecule p-nitro benzaldehyde shows surfactant like behavior due to its structure. This means that the presence of PNB in water reduces the surface tension value (γ) of water. In this experiment the γ values of PNB has been measured with increasing concentration of host β-CD at 298.15 K. (Table 3) The aqueous solution of PNB shows a regular increase with increasing concentration of host β-CD. Perhaps this occurs due to the removal of surface active PNB molecules from the solution into the hydrophobic cavity of β-CD after forming the inclusion complex. The surface tension plot also contains a sharp break point which is indication of the formation of inclusion complex with 1:1 stoichiometry (Figure 6) [15, 16]. Appearance of more number of break points in the surface tension curves reveal more complex stoichiometry such as 1:2, 2:1, 2:2 etc. the value of γ at the break point with corresponding concentration of β-CD is listed in table.

Table 3. Data for surface tension and conductivity study of aqueous PNB-*β*-CD system at 298.15K[a]

Volm of B-CD (mL)	Total volm (mL)	Conc of PNB (mM)	Conc of β-CD (mM)	Surface tension (mN m^{-1})	Conductuvity (mS m^{-1})
0	10	10.000	0.000	54.4	22.2
1	11	9.091	0.909	56.1	21.2
2	12	8.333	1.667	57.6	20.3
3	13	7.692	2.308	58.8	19.6
4	14	7.143	2.857	60.1	19.1
5	15	6.667	3.333	61.1	18.5
6	16	6.250	3.750	61.9	18
7	17	5.882	4.118	62.7	17.6
8	18	5.556	4.444	63.5	17.1
9	19	5.263	4.737	64.2	16.7
10	20	5.000	5.000	64.7	16.2
11	21	4.762	5.238	64.9	16
12	22	4.545	5.455	65.1	15.9
13	23	4.348	5.652	65.3	15.8
14	24	4.167	5.833	65.5	15.7
15	25	4.000	6.000	65.7	15.6
16	26	3.846	6.154	65.8	15.5
17	27	3.704	6.296	65.9	15.4
18	28	3.571	6.429	66.0	15.3
19	29	3.448	6.552	66.1	15.2
20	30	3.333	6.667	66.2	15.1

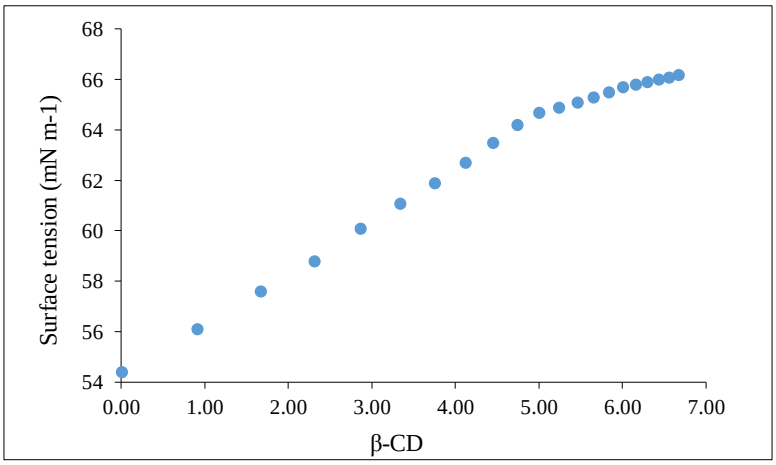

Figure 6. Variation of surface tension of aqueous PNB solution with increasing concentration of β-CD at 298.15K.

3-4- Conductivity Study Again Proves the Stoichiometry of the Inclusion Complex

The formation of inclusion complex is again confirmed with the help of conductivity study [17, 18]. The guest

molecule PNB exists in ionic form in aqueous solution having a measureable value of κ. In this study we have added aqueous CD solution to the aqueous solution of PNB; it has been found that κ value shows a regular declining trend. It is again probably due to insertion of PNB molecules from the solution to the hydrophobic cavity of CD. At certain concentration of both the host and guest molecules a single break point appeared in the conductivity plot, this confirms the formation of inclusion complex. (Figure 7) The value of κ with corresponding concentration of β-CD is listed in Table 3 [19]. The appearance of single breakpoint again suggests the stoichiometry of the inclusion complex 1:1.

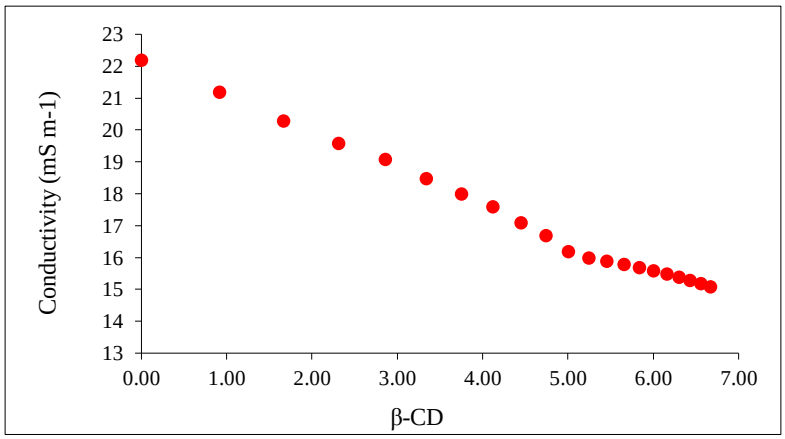

Figure 7. Variation of conductivity of aqueous PNB solution with increasing concentration β-CD at 298.15K.

3-5- Association Constants and Thermodynamic Parameters

The association constant K_a of the inclusion complex of PNB with β-CD can be calculated from UV visible spectra. When the PNB molecule enters into the cavity of β-CD the environment of the guest changes, it results in the change of molar extinction coefficient ε [20]. The value of binding constant has been determined from the double reciprocal plots on the basis of Benesi-Hildebrand method which can be stated as:

$$\frac{1}{\Delta A} = \frac{1}{\Delta\varepsilon[PNB]K_a} \times \frac{1}{[CD]} + \frac{1}{\Delta\varepsilon[PNB]} \quad (1)$$

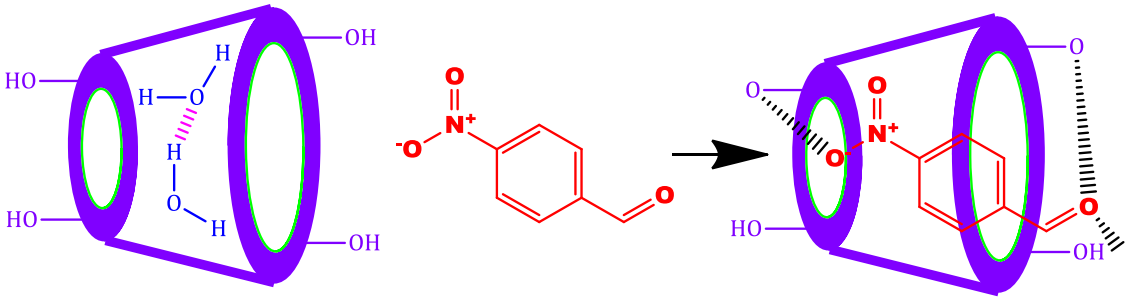

Figure 8. Plausible Schematic representation of mechanism for the formation of 1:1 inclusion complex of PNB with *β* Cyclodextrin.

The equation is valid in case of 1:1 host-guest IC only [21, 22]. We get the value of binding constant by dividing the intercept by the slope from double-reciprocal plots. The Ka value is calculated at three different temperatures. The thermodynamic parameters ΔG°, ΔH° and ΔS° for the formation of IC is measured from the famous Van't Hoff equation with the help of association constant K_a [23, 24].

$$\ln K_a = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} \quad (2)$$

$\ln K_a$ varies linearly with 1/T. The ΔH° value depends upon the equilibrium constant at different temperatures. (Table 4) The ΔG° value for the formation of inclusion complex is negative which indicates the process to be thermodynamically favourable. The negative value of ΔH° shows that the process is exothermic. The entropy value ΔS° is also negative. The randomness of the molecules decrease due to insertion in the cavity of CD, as a result the ΔS° value is negative. The higher negative value of ΔG° overcomes the effect and the whole process is spontaneous.

Table 4. Association constant (Ka) and thermodynamic parameters ΔH∘, ΔS∘ and ΔG∘ of different PNB -Cyclodextrin inclusion complexes.

	Temp (K)[a]	Ka×10^{-3}(M^{-1})[b]	ΔH°(kjmol^{-1})[b]	ΔS°(Jmol^{-1}K^{-1})[b]	ΔG°(298.15 K) (kJmol^{-1})[b]
PNB+β-CD	298.15	1.36	-20.75	-9.74	-17.85
	303.15	1.18			
	308.15	1.01			

3-6- FT-IR Study

The encapsulation of PNB by β-CD is confirmed by FT-IR study [25, 26]. There are many changes in the position of peaks in the solid inclusion complexes. The various frequencies of PNB, β-CD and PNB+β-CD are reported in Table 5. All the spectra are shown in the Figure 9. The spectra of PNB is identified by various characteristic peaks of –CHO, -NO_2, aromatic protons at 1707, 1535, 3424 etc. Broad characteristic peaks of –OH at about 3349 cm^{-1} is present in the spectrum of β-CD. In the spectra of solid inclusion complexes many peaks of the PNB are either absent or shifted due to change of environment after insertion. The –O-H frequency of β-CD are shifted to lower region probably due to involvement of the –O-H groups of the host molecules in hydrogen bonding with the PNB molecule. No additional peaks are recognized in the solid inclusion complex which means no chemical reaction occurred between the molecule PNB and CD [27].

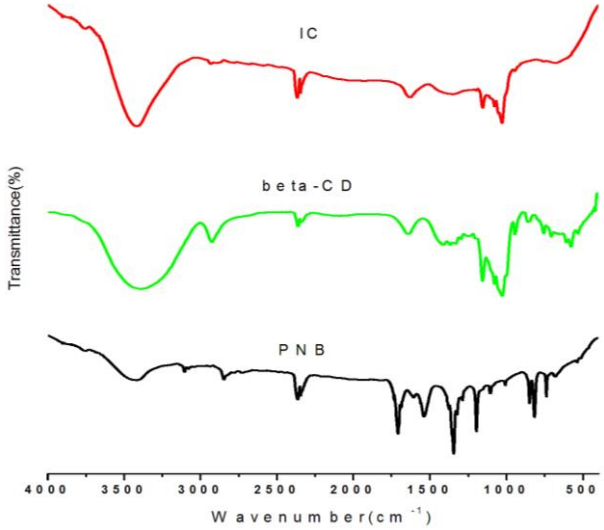

Figure 9. FT-IR spectra of β-CD, PNB, PNB- β-CD inclusion complex.

Table 5. Frequencies at FTIR spectra of PNB, β-CD and solid inclusion complexes

	Wave Number / cm^{-1}	Group
PNB	816.5	-C-H
	897.3	-C-C
	1346.7	-NO_2 symmetrical stretching
	1535.6	-NO_2 asymmetrical stretching
	1707	-CHO
	3424.3	=C-H
β-CD	3349.84	stretching of O-H
	2921.52	stretching of –C-H from –CH_2
	1412.36	bending of –C-H from –CH_2 and bending of O-H
	1157.57	bending of C-O-C
	1033.51	stretching of C-C-O
	938.53	skeletal vibration involving α-1,4linkage
PNB+β-CD	2944.6	stretching of O-H of β-CD
	2369.7	stretching of –C-H from –CH_2 of β-CD
	1627.8	-CHO of PNB
	1354.9	- NO_2 asymmetrical stretching of PNB
	1153.6	- NO_2 symmetrical stretching of PNB

3-7- Scanning Electron Study (SEM) Study

Scanning electron microscopy is a qualitative method used to visualize the surface structure of raw materials or the prepared products [28]. The SEM images of PNB, β-CD and inclusion complex are shown in Figure 10. As shown in the SEM pictures β-CD was observed as a plate shaped crystal. PNB appeared also as plate shaped. The inclusion complex was appeared in irregular shapes with variable size, some looks like needle. The size and shape of the new obtained complexes were different from PNB and the host molecule. These changes can be taken as proofs of the formation of new inclusion complexes by molecular encapsulation.

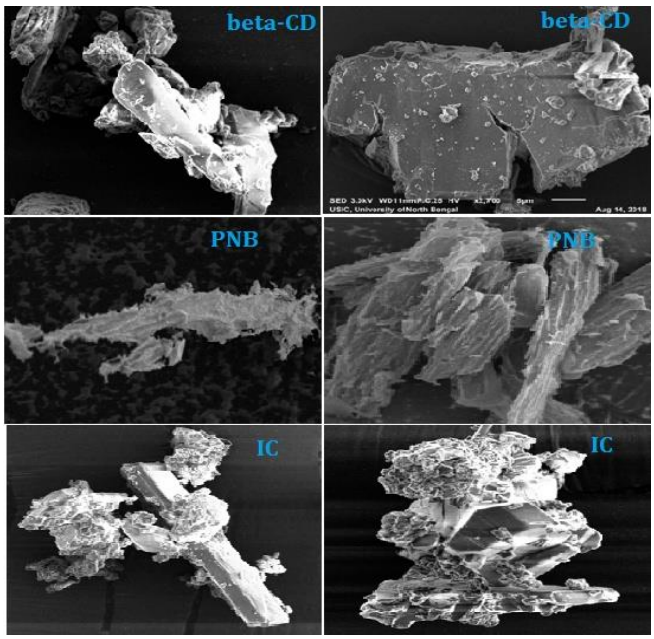

Figure 10. Scanning electron microphotographs (a) β-CD (b) PNB (c) PNB- β-CD inclusion complex.

4- Conclusion

This work confirms that p-nitro benzaldehyde forms inclusion complex with β-CD in the aqueous medium and in the solid state which can be used as regulatory releaser of this compound. ^{1}H NMR study and SEM study confirms the inclusion phenomenon and its mechanism. Surface tension, conductance and Job plot from UV-visible spectroscopy determines the 1:1 stoichiometric ratio of the IC. FT-IR spectra also supported the formation of IC. The association constants and thermodynamic parameters have been estimated ICs by reliable techniques. There is a drop in ΔS^{0}, which is overcome by higher negative value of ΔH^{0}, making the overall inclusion process thermodynamically favourable. This IC has various applications in the field of modern biochemistry and medical science.

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6- Conflict of Interest

The authors declare no conflict of interest.

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