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Evaluation of spectroscopic, molecular modeling and UV protective cotton fabric studies over inclusion complexes of *p*-aminobenzoic acid with β-Cyclodextrin

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The inclusion complex of β-cyclodextrin (β-CD) and *p*-aminobenzoic acid (PABA) has been prepared using the co-precipitation method. β-CD and PABA ratio have been accurately weighed with 1:1 M ratio. The concentration β-CD has been varied from zero to 16×10^{-3} mol dm^{-3}. The inclusion complexation between PABA and β-CD has been monitored by using UV-Vis and fluorescence spectral analysis methods. The stoichiometry and binding constant of the PABA:β-CD inclusion complex has been determined by using Benesi-Hildebrand relation. The formation of inclusion complex is predicted by semi empirical quantum mechanical calculations and are further evaluated by using FT-IR spectral data and molecular docking analysis. In addition, ultra violet protective factor of the PABA treated cotton fabric and PABA:β-CD treated cotton fabric has been investigated. A mechanism has also been proposed for this inclusion complex.

Keywords: β-Cyclodextrin, FT-IR, *p*-Aminobenzoic acid, Inclusion complex, Cotton fabric, Benesi-Hildebrand relation

Ultra violet radiation (particularly UVB radiation) causes many problems to human such as darkening the skin, photo ageing, sunburn, premature ageing, skin cancer, DNA damages, DNA mutations. The scientists are focusing the research work to increase the ultra violet protection factor (UPF) of the cotton fabric by ultra violet protective clothing using UV absorbers or UV blockers. These are protecting our skin by increasing the UPF cotton fabric, mostly used UV absorber in current scenario is sunscreen agent's benzophenone and its derivatives. It blocking both UVA and UVB radiation, but its biggest drawback is harmful to skin and our body. On the other hand, the literature survey revealed that *p*-aminobenzoic acid (PABA) which is one of the compounds under investigation is a cyclic amino acid, belongs to the vitamin *B* group, and is used as a protective drug against solar insolation and in diagnostic tests for the state of the gastrointestinal tract in medicine. So, these properties imbibed the author to focus on the present study. It is come to understood that *p*-aminobenzoic acid(PABA) is UVB absorber, which is used in sunscreens and it is less harmful than benzophenone, but there is no evidence of using PABA for UV protective clothing which only protect from UVB radiation[1-10]. Hence, the present study would try to enhance the *p*-aminobenzoic acid's ultraviolet protective factor using β-cyclodextrin by forming the inclusion complex.

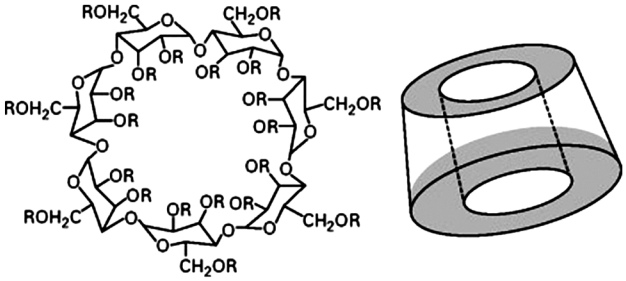
β-Cyclodextrin (β-CD) is an another compound under study which is cone-shaped molecule (Scheme 1). It is hydrophilic at the outer surface of the cavity because of many hydroxyl groups, but hydrophobic inside the cavity. So β-CD is soluble in water and a variety of hydrophobic guest molecules can be encapsulated in its non-polar cavity. Such a characteristic feature has been widely applied in the fields of drug-controlled release, separation and adsorption. At the same time, since β-CD is environmentally sensitive hydrogel, it plays vital role in textile industries. Therefore, the present study has been undertaken by the authors to show the anomalous behaviour of both the compounds. The following figure shows the molecular structure of β-CD.

Experimental Section

Materials

The procurement details are provided in the Table 1.

Distilled water was used to prepare all solutions and all other chemicals used were of analytical grade. The concentration β-CD varied from zero to 16×10^{-3} mol dm^{-3}. From the stock solution 2,4,



Scheme 1 — Structure of β-Cyclodextrin (β-CD)

Table 1 — Procurement details of components

S. No.	Name of the Component	CAS RN	Supplier name
1	β-CD	7585-39-9	HiMedia Laboratories Pvt. Ltd.
2	*p*-Aminobenzoic acid	150-13-0	Simson Laboratories, Mumbai
3	Fabric	Not Applicable	B. I. Txtile Mills Pvt. Ltd., Mumbai

6,8,10,12,14 and 16×10^{-3} mol dm^{-3} of β-CD were prepared using double distilled water.

Instruments

The UV–Visible spectrum was performed with analytic Jena, Germany, spectrophotometer. Fluorescence spectrum was recorded using Perkin Elmer LS-45 fluorescence spectrophotometer. The IR spectra of powder samples of β-CD, PABA and the solid inclusion complex were measured between 4000 cm^{-1} and 400 cm^{-1} on Alpha-T FTIR spectrometer (Bruker optics) equipped with OPUS version 6.5 by KBr pellet method.

By molecular docking studies and Patch Dock server, the most probable structure of the PABA: β-CD inclusion complex was determined. The 3D structural data of β-CD and PABA was obtained from crystallographic databases. Patch Dock server is an online source for the docking of guest molecule PABA in to the host molecule (β-CD) cavity by submitting the 3D coordinate data of PABA and β-CD molecules. Docking was performed with complex type configuration settings. Patch Dock server follows a geometry-based molecular docking algorithm to find the docking transformations with good molecular shape complementarily. Patch Dock algorithm separates the Connolly dot surface representation of the molecules into concave, convex and flat patches. The patches are used for geometric fit evaluation and atomic de-solvation energy scoring function. RMSD (root mean square deviation) clustering is applied to the docked solutions to select the non-redundant results and to discard redundant docking structures.

UPF was measured using UV-VIS double beam spectrophotometer as per the American standard (ASTMD6603–2000) and AATCC test method.

Methods of preparation of β-CD-PABA inclusion complex

Preparation of inclusion complexes in solutions state

The 250 mL of stock solution of β-CD prepared in 16×10^{-2}M concentration using double distilled water. Then it will be diluted to 14 to 2×10^{-3} M concentrations. Then the 10 mL of guest, *p*-aminobenzoic acid solution prepared in 1×10^{-4}M by using ethanol as solvent. 8 test tubes were taken and labelled them from 0 to 16×10^{-3}M and 10 mL of each concentration β-CD solutions are taken in respective test tubes. 0.2 mL of *p*-aminobenzoic acid solution is added in each test tube and shaken well. Then the mixture is subjected to UV spectrometer and fluorescence spectrometer analysis.

Preparations of inclusion complexes in solid state

The inclusion complex of β-CD and PABA was prepared using the co-precipitation method. β-CD and PABA ratio was accurately weighed with 1:1 M ratio. In 50 mL beaker, 1g of β-CD was dissolved in 20 mL of distilled water, allowed it stirring for half an hour and 0.98g of PABA was dissolved in 10 mL of ethanol and also allowed it stirring for half an hour. Both the solutions were mixed and keep it in a magnetic stirrer continuously for 48 hours. Then the solution was kept in a refrigerator for 24 hours and dried in an oven at 50°C for 12 hours, the yellow powdered product was obtained with 1.696g yield. This is solid inclusion complex of PABA and β-CD.

Ultra violet protective clothing on cotton fabric using inclusion complex of β-CD and PABA and without β-CD (only PABA)

Pretreatment of cotton fabric

This pre-treatment consists of three process *i.e.* resizing, scouring, leaching.

Resizing

The cotton fabric is taken and cut the fabric in the size of 20cm×20cm. then 1000 mL 0.1N sulphuric acid prepared and then fabric is immersed in 1000 mL of 0.1N sulphuric acid and kept it for 4 hours, the fabric is washed in cold water for three times, then washed with hot water and dried it in an oven.

Scouring

1000 mL of water is added with 3g of sodium hydroxide, 1g of sodium carbonate, 0.2g of EDTA and 2 mL of liquid soap. Then the resized fabric is soaked in the beaker and the beaker is subjected to heat at 100°C in hot plate for 2hours, then wash the fabric in water and dried it in oven.

Bleaching

1000 mL of water is added with 3g of sodium hydroxide, 0.2g of EDTA and 4 mL of hydrogen peroxide. Then the scoured fabric is soaked in the beaker, the beaker is kept in hot plate at 80°C for 2 hours, then wash the fabric and dried well in oven.

Preparation of inclusion complex solution β-CD-PABA

The oil bath with magnetic stirrer is taken and filled with sufficient amount of silicone oil. In a three neck flask one neck is fitted with condenser and another is fitted with thermometer and the third neck is sealed. Through the sealed neck, add 25 mL of deionized water and add 0.25g of β-CD put a magnetic pellet, switch on the oil bath, the temperature was maintained at 80°C followed by add o.25g of PABA at 80°C and stirred for 4hours till the complete dissolution of the guest compound occurs. The inclusion complex solution is filtered. This experiment is same for UV protective clothing by only PABA (without β-CD).

Finishing treatment of the fabric by inclusion complex and PABA (without β-CD)

100 mL of hot distilled water is added with 8g of the citric acid and 6g of sodium hypophosphite, and then the inclusion complex solution (β-CD-PABA) is added and stirred it for half an hour at 80°C. This is called finishing bath. Then the cotton fabric is dipped in the finishing bath two times and dipped in distilled water one time. And finally coated fabric dried at 100°C for 5 min in an oven. This experiment also is continued for only guest clothing.

Results and Discussion

UV spectral Analysis

The β-CD and PABA inclusion complex formation was analyzed by the UV spectroscopy. The absorbance maximum of PABA shows peak at 267 nm. There is red shift occurs in the wavelength of PABA: β-CD inclusion complex when increasing the β-CD concentration. Fig. 1 show the absorption

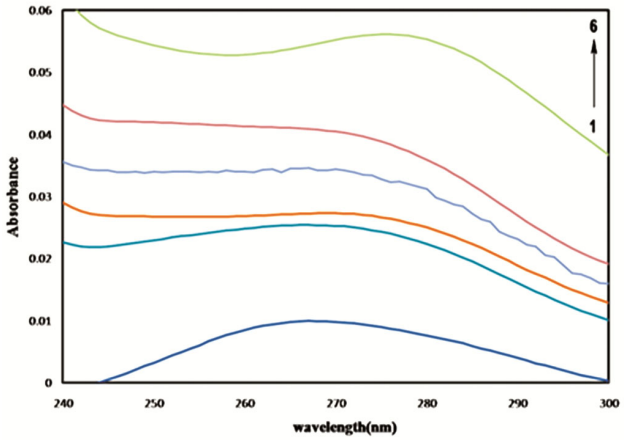


Fig. 1 — UV-Vis spectra of PABA (10^{-4}M) in different β-CD concentration (M): (1) 0, (2) 0.008, (3) 0.010, (4) 0.012, (5) 0.014 and (6) 0.016

spectrum of PABA with β-CD. There is a linear increase in the absorbance intensity of PABA with the increase in the concentration of β-CD from 0 M to 16×10^{-2} M**.** It indicates the formation of β-CD: PABA inclusion complex. This increase in absorbance shows that, the increasing dissolution leads inclusion of PABA into the hydrophobic cavity of β-CD.

The equilibrium of β-CD: PABA inclusion complex formation between β-CD and PABA inclusion complex can be written as follows,

PABA + β-CD PABA…… β-CD

The binding constant K and 1:1 stoichiometry of β-CD: PABA inclusion complex was determined by using Benesi-Hildebrand relation (B-H plot).

$$\frac{1}{A - A_0} = \frac{1}{\Delta\varepsilon} + \frac{1}{K\,[\ \text{PABA}\ ]_0\,\Delta\varepsilon\,[\beta-\text{CD}]_0}$$

Where, A and A_0 is the difference between the absorbance of PABA in the presence and absence of β-CD, $\Delta\varepsilon$ is the difference between the molar absorption coefficient of PABA and the inclusion complex, $[PABA]_0$ and $[\beta\text{-CD}]_0$ are the initial concentration of PABA and β-CD respectively. The plot of $1/A\text{-}A_0$ *vs*. $1/[\beta\text{-CD}]$ for PABA, shows a good linear correlation (Fig. 2) which confirms the formation of 1:1 inclusion complex. From the intercept and slope values of the plot, the binding constant 'K' is evaluated as 31.41 M^{-1}.

$$K = \frac{1}{Slope(A - A_0)}$$

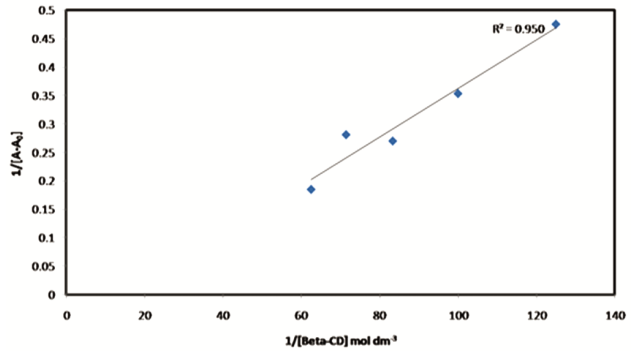


Fig. 2 — Benesi–Hildebrand plot of 1/A-A_0 *vs*. 1/[β-CD] for PABA: β-CD complex

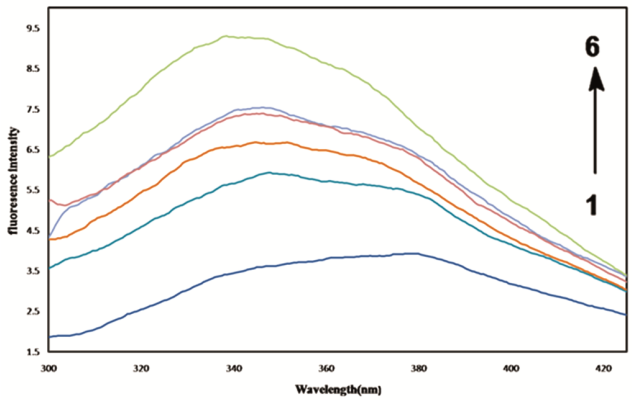


Fig. 3 — The Fluorescence spectra of PABA (10^{-4}M) in different β-CD concentration (M): (1) 0,(2) 0.008, (3) 0.010, (4) 0.012, (5) 0.014 and (6) 0.016

Fluorescence Spectral Analysis

The effect of β-CD on the fluorescence spectra of PABA is analyzed by fluorescence spectroscopy. The emission spectrum of PABA is shown in Fig. 3; there is an increase in the fluorescence intensity with the increasing concentration of β-CD from 0 to 0.016 M. This shows that the PABA is encapsulated into the β-CD cavity indicates the formation of PABA: β-CD inclusion complex. The binding constant for the complex formation was determined by using Benesi-Hildebrand relation the 1:1 stoichiometry ratio of PABA: β-CD inclusion complex determined.

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

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 of 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, PABA: β-CD inclusion complex. The binding constant '*K*' is found to be 46.15 M^{-1}.

$$K = \frac{1}{Slope(I - I_0)}$$

Fourier Transform Infrared Spectra Analysis

The FT-IR spectra of β-CD, PABA and β-CD: PABA inclusion complex shown in Fig. 4 and corresponding wavelengths are tabulated in Table 2; β-CD: PABA inclusion complex are prepared by co-precipitation method and it was characterized by FT-IR spectroscopy. The inclusion complex formation between β-CD and PABA is by non-covalent interactions such as hydrophobic interactions, Vander Waals interaction and hydrogen bonding. The inclusion complex is an encapsulation of guest molecule in to β-CD cavity[11]. The included part of PABA molecule's vibrations frequency is shifted. The FT-IR spectrum of β-CD observed band at 3385 cm^{-1}, 2926 cm^{-1}, 1158 cm^{-1}, and 1030 cm^{-1} which correspond to the symmetric and anti-symmetric stretching of OH, C-H, C-C and bending vibration of C-O frequencies respectively. The FT-IR spectrum of PABA observed two sharp and strong bands at 3467cm^{-1} and 3372cm^{-1} for 1° N-H symmetric and asymmetric stretching, but in β-CD: PABA inclusion complex the N-H stretching frequency are decreasing and shifted to 3372cm^{-1} and 3368cm^{-1} respectively, then the two sharp peaks are become combined together. In pure PABA C-N stretching at 1167cm^{-1} is shifted to 1164cm^{-1} in β-CD: PABA inclusion complex. PABA the peak observed at 1524cm^{-1} (N-H bending), in complex, it observed at 1525cm^{-1} with reduced intensity. These IR spectral vibration shows that, the NH_2 group part of PABA is encapsulated into hydrophobic cavity of β-CD and the decreasing wave number due to the vibration of 1° NH_2 group is restricted by an interaction (hydrogen bonding) between PABA N-H group and 1° hydroxyl rim of β-CD. In pure PABA NH_2 has intra hydrogen bond stretching at 3232cm^{-1}, in β-CD: PABA inclusion complex it will increase to 3235cm^{-1} due to the hydrogen bonding which will be disappeared when PABA inclusion in β-CD. and in pure PABA, the broad peaks at 2970 cm^{-1}, 2672 cm^{-1},2551 cm^{-1}shows the O-H stretching of carboxylic group but, in β-CD: PABA inclusion complex the frequency is decreases to 2925cm^{-1}, 2670cm^{-1}, 2550cm^{-1} with reduced

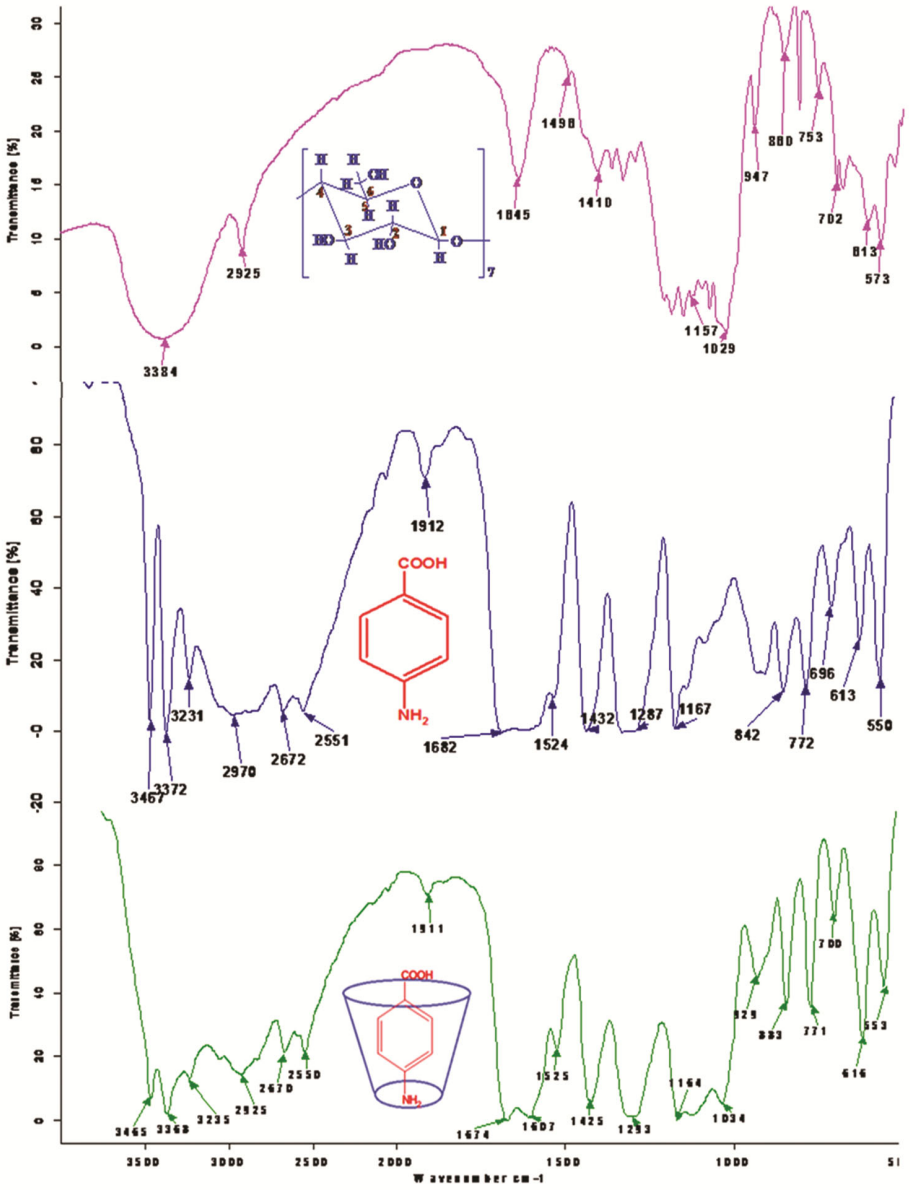


Fig. 4 — FTIR spectra of (a) β-CD, (b) PABA, (c) β-CD: PABA

Table 2 — Wave numbers (cm^{-1}) and assignments for the bands observed in the FTIR spectra of PABA, PABA: β-CD inclusion complex

Peak assignments	Wave number (cm^{-1})		
	β-CD	Pure PABA	PABA:β-CD complex
O-H stretching	3384 cm^{-1}	–	–
N-H Asymmetric stretching	–	3467cm^{-1}	3465cm^{-1}
N-H symmetric stretching	–	3372cm^{-1}	3368 cm^{-1}
N-H hydrogen bond stretching	–	3232cm^{-1}	3235 cm^{-1}
C-H aliphatic stretching	2925 cm^{-1}	–	–
O-H (COOH) stretching	–	2970cm^{-1}	2925 cm^{-1}
O-H (COOH) stretching	–	2672cm^{-1}	2670 cm^{-1}
O-H (COOH) stretching	–	2551 cm^{-1}	2550 cm^{-1}
Para-substitution stretching	–	1912cm^{-1}	1911 cm^{-1}

(*Contd.*)

Table 2 — Wave numbers (cm^{-1}) and assignments for the bands observed in the FTIR spectra of PABA, PABA: β-CD inclusion complex (*Contd.*)

C=O stretching	–	1682 cm^{-1}	1674 cm^{-1}
	---	–	1607 cm^{-1}
N-H bending	–	1524cm^{-1}	1525 cm^{-1}
C=C aromatic stretching	–	1432cm^{-1}	1425cm^{-1}
C-N stretching	–	1287cm^{-1}	1294 cm^{-1}
C-C stretching	1157 cm^{-1}	–	–
C-O stretching	1029 cm^{-1}	–	–
O-H bending	947 cm^{-1}	–	929 cm^{-1}
Para-sub. bending Out of plan	–	842 cm^{-1}	843 cm^{-1}
N-H bending out of plan (wagging)	–	772 cm^{-1}	771 cm^{-1}
C-H aromatic out of plan bending	–	696cm^{-1}	700cm^{-1}

intensity and C=O stretching of PABA at 1682cm^{-1} is splitted into two peaks at 1674cm^{-1} and 1607cm^{-1} in β-CD: PABA inclusion complex. It shows that the COOH carboxylic group of the PABA interacts with the 2° hydroxyl rim of β-CD. In PABA, the aromatic C=C stretching vibration at 1432cm^{-1} changes to 1425cm^{-1} in complex. In pure PABA observed weak peaks at 1912cm^{-1}, 842cm^{-1}(out of plan) for *para*-substituted ring, but in complex it has reduced intensity peaks at 1911cm^{-1}, 843cm^{-1}(out of plan). The aromatic C-H stretching of PABA observed at 696cm^{-1} but in complex it shifted to 700cm^{-1} with reduced intense weak peak. It shows the aromatic ring is encapsulated in β-CD cavity. From the FT-IR spectral data changing vibration frequency of C=O and NH_2 groups in β-CD: PABA inclusion complex, give idea about β-CD: PABA inclusion complex formation. From this we can speculate that the NH_2 group part of the PABA is inclusion into hydrophobic cavity of β-CD and its positioning near to 1° hydroxyl rim of β-CD, make hydrogen bond interaction with 1° rim O-H groups of β-CD. And the carboxylic group of PABA is positioning at near to 2° hydroxyl rim of β-CD, make hydrogen bond interaction with 2° rim O-H groups of β-CD. This will be coincided with the Docking analysis.

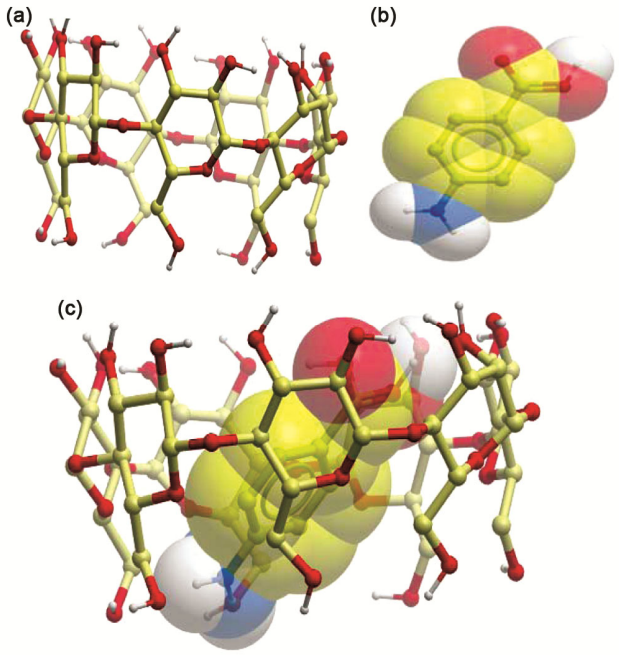


Fig. 5 — Ball and stick representation of (a) β-CD (b) PABA (c) PABA: β-CD inclusion complex, the oxygen atoms are shown as red ball, carbon as golden yellow balls and hydrogen atoms as grey balls.

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, it is clear that the PABA molecule can be included in the β-CD cavity, but not completely. Because, the overall height of PABA is 8 Å (*i.e.*, the vertical distance between H15 – O11), but the overall height of β-CD is only 7.8 Å. Hence, it is possible to PABA which has bi functional groups, which are positioning near to hydroxyl rims β-CD as interpreted using experimental data (FT-IR) and as shown in Scheme 1.

Molecular docking study of inclusion process

The feasible structure of PABA: β-CD inclusion complex was obtained from molecular docking studies. The 3D structure of β-CD and PABA were shown in Fig. 5 and Fig. 6. PABA was docked into β-CD cavity using Patch Dock server, obtained different feasible models based on the energetic parameters; geometric shape complementarity score, approximate interface area size and atomic contact energy of the PABA: β-CD inclusion complex.

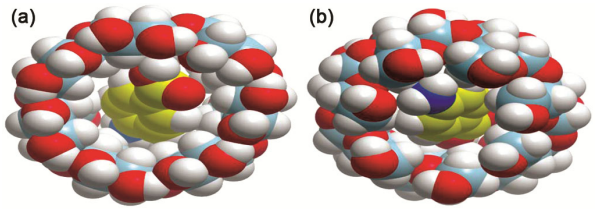


Fig. 6 — PABA: β-CD inclusion complex (a) front and (b) back views

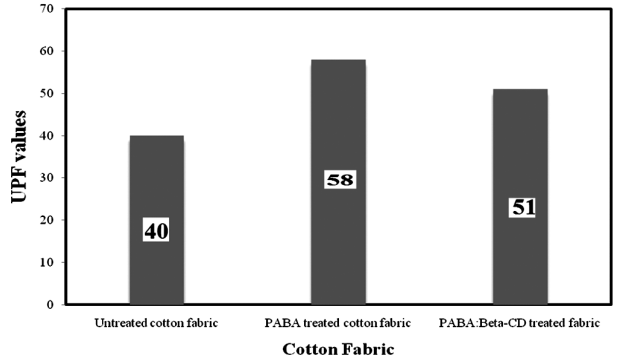


Fig. 7 — Ultra Violet Protective Factors values of fabric, PABA treated fabric, PABA:β-CD complex treated fabric

Table 3 — Ultraviolet Protective Factor Ratings

UPF Range	Protection Category	Effective UV-R Transmission (%)	UPF Rating
15-24	Good	6.7-4.2	15,20
25-39	Very Good	4.1-2.6	25,30,35
40-50+	Excellent	Less than 2.5	40,45,50+

From the obtained docked models, model with the highest geometric shape complementarity score 2252, approximate interface area size of the complex 232.30Å and atomic contact energy -166.87 kcal/mol was selected as highly feasible and relevant model for PABA: β-CD 1:1 inclusion complex (Fig. 6 and Fig. 7). The results obtained from docking were coincided with the experimental results of FT-IR[12-14].

Ultra violet protection factor determination of cotton fabric

The Table 3, shows the Ultraviolet Protective Factor (UPF) ratings (provided in literature), Fig. 7 shows the results of UPF testing on untreated cotton fabric, PABA treated cotton fabric and PABA: β-CD treated cotton fabric. The resized, scoured, and bleached untreated cotton fabric has 40 UPF. PABA treated cotton fabric has 58 UPF which confirmed that PABA is an UV absorber, it increases the cotton fabric UV protection. The PABA: β-CD inclusion complex treated with the cotton fabric and bindings with fabric are processed by means of cross-linking mechanism[15-17]. At higher temperatures, a dehydration reaction is carried out to form a five-member anhydride intermediate which can react easily with the hydroxyl groups of cellulose and/or β-CD *via* formation of ester derivatives. Part of citric acid acts as a cross-linking agent to provide the cellulose with anti-crease properties, while the rest of it acts as a bridge between PABA: β-CD inclusion complex and cellulose. The PABA: β-CD inclusion complex treated cotton fabric has 51 UPF values (Table 4).

Table 4 — Ultra Violet Protective Factors values of fabric, PABA treated fabric, PABA: β-CD complex treated fabric

S.No.	Fabric	UPF values
1	Untreated cotton fabric	40
2	PABA treated cotton fabric	58
3	PABA :β-CD inclusion complex treated cotton fabric	51

Conclusion

The inclusion complex formation between *p*-aminobenzoic acid (PABA) and β-cyclodextrin (β-CD) has been studied and identified using ultraviolet spectroscopy, fluorescence spectroscopy and fourier transform infrared spectra analysis. The ultraviolet spectroscopy and fluorescence spectroscopy data predicted that increasing the β-CD concentration, increases the absorbance and red shift was occurred, which confirms that the PABA: β-CD inclusion complexation takes place. The stoichiometry ratio of inclusion complex formed between β-CD and PABA was determined by using Benesi-Hildebrand plot. From the fourier transform infrared spectral data, the PABA: β-CD inclusion complex is characterized. The inclusion complex PABA: β-CD was investigated and identified by semi empirical quantum mechanical calculations and molecular docking analysis. From the above investigations, a new mechanism for the formation of PABA: β-CD inclusion complex is being proposed. Finally, this research work paves the way for the PABA treated cotton textile material preparation in textile industries to increase the ultraviolet protective factor (UPF) of cotton fabric and PABA: β-CD inclusion treated cotton textile material that will also enhance the ultraviolet protective factor of cotton fabric textile materials.

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