Host–guest interaction of 2,4-dinitrophenylhydrazine:β-Cyclodextrin inclusion complex

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Abstract — The inclusion complex of 2,4-dinitrophenylhydrazine (DNPH) and β-cyclodextrin (β-CD) has been investigated by using fluorescence spectral analysis technique. The formation of this complex has been confirmed by Benesi-Hildebrand Plot. The stability constant $K_{1:1}$ and the 1:1 stiochiometric of complexation were determined. Further it was also confirmed semi empirical methods and Molecular Modeling studies. It was found that the NO_2 group containing benzene ring may be present in the cavity of β-CD.

I.INTRODUCTION

Cyclodextrins (CDs) are cyclic oligosaccharides obtained from enzymatic hydrolysis of starch. The β-cyclodextrin 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 [1,2]. 2,4-Dinitrophenylhydrazine (DNPH, Brady's reagent) is the chemical compound $C_6H_3(NO_2)_2NHNH_2$. DNPH is relatively sensitive to shock and friction; it is a shock explosive so care must be taken with its use. It is a red to orange solid, usually supplied wet to reduce its explosive hazard. It is a substituted hydrazine, and is often used to qualitatively test for carbonyl groupsassociated with aldehydes and ketones. The hydrazone derivatives can also be used as evidence toward the identity of the original compound.

II. MATERIALS AND METHODS

A. Materials

2,4-Dinitrophenylhydrazine (DNPH) and β-CD were obtained from Aldrich, HiMedia Laboratories and used without further purification. Distilled water was used to prepare all solutions and spectrograde solvents were used. The concentration of β-CD was varied from zero to 1.6×10^{-3} mol dm^{-3}. From the stock solution 2, 4, 6, 8, 10, 12, 14 and 16 $\times10^{-3}$ mol dm^{-3} of β-CD were prepared using double distilled water. All experiments were carried out at 30°C.

B. Methods

The fluorescence spectra were recorded using spectrofluorometer, Perkin Elmer, USA. The most probable structure of the DNPH:β-CD inclusion complex was determined also by molecular docking studies using the PatchDock server [3]. The 3D structural data of β-CD and DNPH were obtained from crystallographic databases. The guest molecule (DNPH) was docked in to the host molecule (β-CD) cavity using PatchDock server by submitting the 3D coordinate data of DNPH and β-CD molecules.

Docking was performed with complex type configuration settings. PatchDock server follows a geometry-based molecular docking algorithm to find the docking transformations with good molecular shape complementarity. PatchDock algorithm separates the Connolly dot surface representation [4,5] of the molecules into concave, convex and flat patches. These divided complementary patches are matched in order to generate candidate transformations and evaluated by geometric fit and atomic desolvation energy scoring [6] 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.

III. RESULTS AND DISCUSSION

A. Fluorescene spectral analysis

The effect of β-CD on the fluorescence spectra of DNPH is shown in Fig. 1. The emission intensity of DNPH decreases when the β-CD concentration is increased. Fig. 1 shows 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 DNPH initially decreases with β-CD concentration and then saturates to a limiting value at 0.016M β-CD, indicating the maximum inclusion of DNPH molecule in the β-CD cavity. The binding constant for the formation of complex has been determined by analyzing the changes

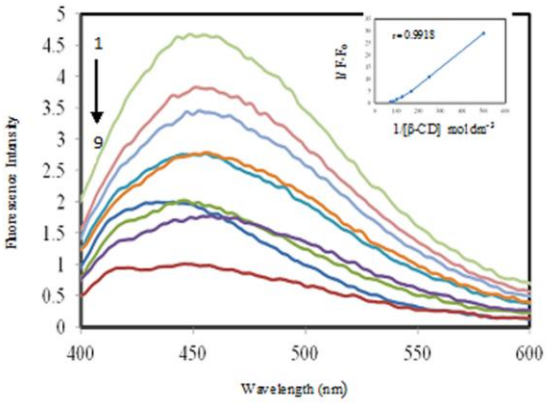
in the intensity of emission maxima with the β-CD concentration using the Benesi- Hildebrand [7] relation assuming the formation of a 1:1 host –guest complex.

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

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 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, DNPH:β-CD inclusion complex. The binding constant 'K' is evaluated as 153.14 M^{-1}. The possible 1:1 inclusion complex mechanism has been suggested as shown in Scheme 1.

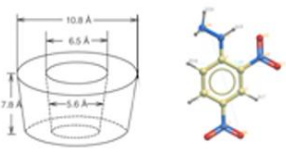
Fig. 1: The fluorescence spectra of DNPH (10^{-4}M) in different β-CD concentrations (M): (1) 0.0, (2) 0.002 , (3) 0.004 , (4) 0.006 , (5) 0.008 , (6) 0.010 (7) 0.012 (8) 0.014 and (9) 0.016 .

[Inside: Benesi Hildebrand plot of 1/ F-F_0 VS 1/[β-CD] for DNPH in distilled water]



B.Semiempirical quantum mechanical calculations

The internal diameter of the β-CD is approximately 6.5 °A and its height is 7.8 °A (Scheme 1). Considering the shape and dimensions of β-CD, it is clear that the DNPH molecule cannot be completely included in the β-CD cavity. Because, the overall height of DNPH is 8.2°A (i.e., the vertical distance between $H_{20} - O_8$), but the overall height of β-CD is only 7.8 Å. Hence, it is possible to locate half of the DNPH molecule inside the β-CD cavity as shown in Scheme 1.

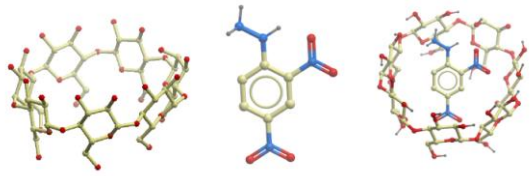


β-CD DNPH

Scheme 1: DNPH:β-CD inclusion complex

C. Molecular docking study of inclusion process

The 3D structure of β-CD and DNPH obtained from crystallographic databases are shown in Fig. 2a and 2b. The guest molecule, DNPH was docked into the cavity of β-CD using PatchDock server. The PatchDock server program 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 DNPH:β-CD inclusion complex. The docked DNPH:β-CD 1:1 model (Fig. 2c) with the highest geometric shape complementarity score 2558, approximate interface area size of the complex 269.1 $Å^2$ and atomic contact energy -214.9 kcal/mol is the highly probable and energetically favourable model.



(a) **(b)** **(c)**

Fig. 2: Ball and stick representation of (a) β-CD (b) DNPH (c) DNPH:β-CD inclusion complex; the oxygen atoms are shown as red ball, carbon as golden yellow balls and hydrogen atoms as grey balls.

IV. CONCLUSION

In summary, the inclusion complexation between 2,4-dinitrophenylhydrazine (DNPH) and β-cyclodextrin (β-CD) has been studied using fluorescence spectral method. The formation of 1:1 molar ratio complex formed between β-CD and DNPH was determined by Benesi-Hildebrand plot. Further, the inclusion complexation between DNPH and β-CD was investigated using semi empirical quantum mechanical calculations and molecular docking analysis. Based on the investigations, a mechanism for the complexation between DNPH and β-CD is proposed.

V. REFERENCES

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