Spectroscopic Study of Inclusion Complex of the Ethidium Bromide with B-Cyclodextrin in Solution NMR

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Abstract- The present study was conducted with the aim to form an inclusion complex of a toxic carcinogenic mutagenic agent Ethidium-bromide (EtBr) with the host molecule β-cyclodextrin (β-CD). The spectroscopy study of this inclusion complex in absence and presence of β-CD was carried out by Nuclear magnetic resonance (NMR). The binding constant and stoichiometry of the complexes were determined by Scott's method. The inclusion complex of EtBr with β-CD was further confirmed by 2D ^{1}H-^{1}H NMR and further by molecular docking technique. The binding constant and stoichiometry ratio were found to be 301 M^{-1} and 1:1, respectively which indicate the successful formation of the inclusion complex between EtBr and β-CD.

Index Terms- β-cyclodextrin, inclusion complexes, ^{1}H NMR, ^{1}H-^{1}H NMR, Molecular Docking.

I. Introduction

Cyclodextrins are extensively used in industries as well as analytical chemistry. The potential uses of cyclodextrins in products and technologies are numerous because its presence in drug not only enhances the solubility, bioavailability, stability, shelf life but also reduces side effects and gastrointestinal drug irritation.[1, 2, 3, 4] . Cyclodextrins are cyclic oligosaccharides, having hydrophilic outer surface and hydrophobic central cavity, which belong to the family of cage molecule[5].

Natural Cyclodextrins are α-cyclodextrin, β-cyclodextrin and γ-cyclodextrin, which consist of 6,7and 8 D-glucopyranose units respectively, and have α-D-glucopyranose (α-1, 4) linkage with the cavity[6]. Ethidium Bromide (3, 8-diamino-5-ethyl-6-phenylphenanthridium bromide, EtBr) is a common intercalating agent and guest molecule in the present work. Ethidium-bromide is an anti-tumor and antiviral compound. Because of its mutagenic and carcinogenic nature it is less therapeutically preferred in the study of molecular biology[7,8]. NMR Spectroscopy is generally used to analyze the structural elucidation of compounds in an aqueous form. NMR gives direct information about structure of CD-complex that which part of guest molecule is engulfed within the host molecule[9]. In inclusion complex, a complete guest molecule or a part of it is held within the hydrophobic cavity of the host cyclodextrin molecule and during the formation of inclusion complex no new covalent bond is formed or broken. The main driving force of complex formation is to release enthalpy-rich water molecules from the cavity, present in the solution to attain apolar-polar association which forms more stable state[10],[11]. Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex [12].

Ethidium Bromide is harmful, if swallowed and very toxic by inhalational route. It is irritant to the eyes, skin and respiratory system[13].The formation of inclusion complex increases the drug potency with minimal drug toxicity. Furthermore cyclodextrin entrapment of drugs at the molecular level wards off their direct contact with biological membranes thereby reducing their side effects and local irritation with no drastic loss of therapeutic benefits.

The structural assignment of β-CD with Ethidium Bromide has not been reported so far. EtBr offers an excellent potential for development into an antiviral or antitumour drug delivery system, after complexation with β-CD because its safety issues are overcome. This will enhance its commercial use in Indian market and pharmaceutical companies can expect a huge boost in their revenues. in order to overcome the safety problem of EtBr the present investigation was carried out with the aim to elucidate structural details of interaction between β-CD and Ethidium-Bromide in aqueous form, estimate the extent of binding and stoichiometry of the inclusion complexes.

II. Experimental

Material and Methods

Materials

Ethidium-Bromide and β-cyclodextrin were gifted by Sigma-Aldrich. For molecular docking, structure of Ethidium-Bromide and β-cyclodextrin were taken from Pubchem database (Id 5KCB).

Methods

Sampling In present investigation,5mM Ethidium-Bromide (3.94mg) of constant concentration solution was prepared in heavy water(D_2O) at 28°c and β-cyclodextrin solution of ten different mass were added, having lowest and highest concentration of 1mM and 10mM respectively [14].

Nuclear Magnetic Resonance The above samples were added to Bruker 800MHz NMR instrument for ^{1}H NMR (1-D). ^{1}H-^{1}H ROESY spectrum (2-D) was also obtained from the instrument.

Calculation of binding constant Binding constant was determined by Scott's method from above NMR data. The Benesi-Hildebrand equation used for the calculation of binding constant was:

$$\frac{[\beta CD]}{\Delta\delta_{obs}} = \frac{[\beta CD]}{\Delta\delta_{max}} + \frac{1}{K.\Delta\delta_{max}}$$

where, $\Delta\delta_{obs}$ - observed chemical shifts difference between complexed and the pure β −
CD .
[βCD] - Fractional molar concentration of βCD in the EtBr aqueous solution.
.$\Delta\delta_{max}$ - Saturated value of chemical shift obtained from Scott's plots.

Molecular docking The molecular docking was carried out using AutoDockVina[15]. Ethidium-Bromide and β-cyclodextrin were used as ligand and receptor in docking studies respectively. The grid coordinates used were X = -6.736 Å, Y= -7.692Å, and Z= 4.168Å. The dimension of docking grid box was 40Å X 40Å X40Å with grid spacing of 0.375Å.

III. Results and Discussion

The results of the present investigation were found to be as follows:

Inclusion complex between β-CD and EtBr: The work in this research was centered on the change in proton chemical shifts of inclusion complex relative to free guest. In present study, chemical shifts of H_3 and H_5 protons of β-cyclodextrin shifted towards the upfield direction while that of drug protons shifted towards the downfield direction relative to the respective pure host/guest proton[16]. Similar to our work,[17] has also reported mixed shifts. This may be due to the fact that the protons which are positioned inside the cavity of β-cyclodextrin shows maximum shielding compared to other protons due to the presence of aromatic compound entering in the cavity. Furthermore the results also indicated that H_3 and H_5 protons show significant chemical shift compared to other protons.

Structural analysis of complex

^{1}H NMR NMR spectra of pure β-cyclodextrin (lower panel) and two of their inclusion complexes (middle and upper panels) are shown in fig.1

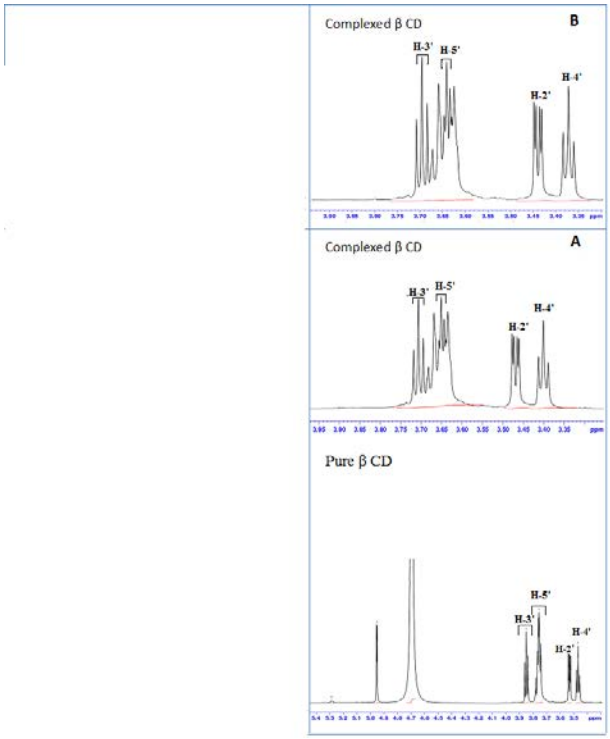

Fig.1: A part of ^{1}H NMR spectra showing protons of β-cyclodextrin in the absence as well as presence of varying amount of EtBr. Molar ratios (A = 1.67 and B = 1.25) in comparison to pure β-cyclodextrin.

In absence of β–CD, all aromatic protons were found merged and resonating at 6.233 - 7.915 ppm, while in the presence of β–CD resonation position was changed. From the fig.1, the change in chemical shift of inclusion complexes relative to pure β-Cyclodextrin is clearly indent. The change in observed chemical shift relative to pure β-cyclodextrin showed upfield shifts; while downfield shift changes were observed relative to the EtBr which means the formation of guest-host inclusion complex[3].

^{1}H-^{1}H Rotating frame Overhauser Effect Spectroscopy (ROESY) It is useful for determining the cross peaks/signals arising from protons that are close to each other in space even if they are not attached with the bond.^{1}H-^{1}H ROESY plot of the inclusion complexes are depicted in Fig. 2.

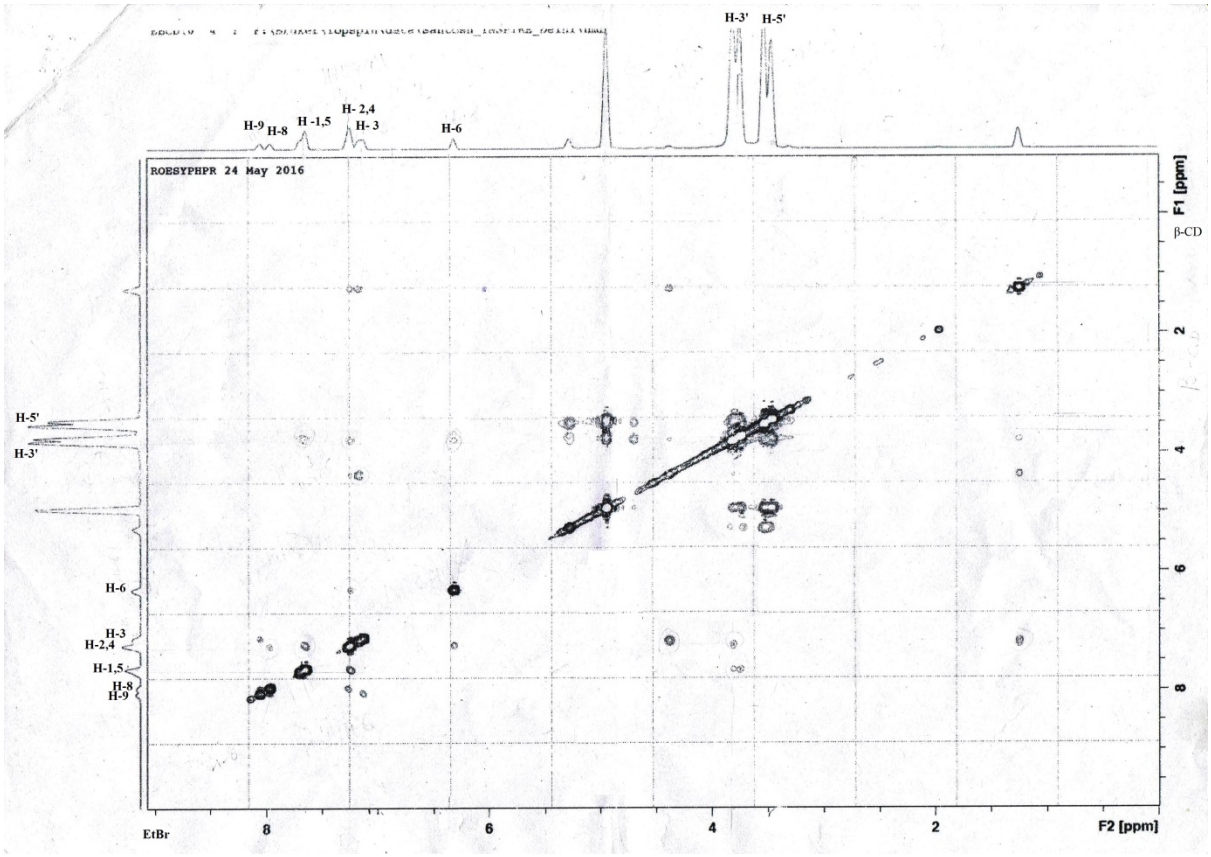

Fig.2:^{1}H -^{1}H ROESY spectra of inclusion complex, showing all the cross correlation peaks between host and the guest protons. Analysis of the figure revealed that H_3 of 3.851 ppm of β-Cyclodextrin correlated with H_1 and H_5 at 7.616 ppm of EtBr and the same H_3 combines with H_2 and H_4 at 7.144 ppm [18].

Binding constant of Inclusion complex and Stoichiometry

The association constant and stoichiometry of the complexes were studied by Benesi-Hildebrand equation[19]. Nine different ratios of β-Cyclodextrin and Ethidium Bromide were used in the present study.

Scott's plots of H_3 and H_5 protons showed straight lines of $\frac{[\beta CD]}{\Delta\delta_{obs}}$ versus [βCD] curve (fig. 3)

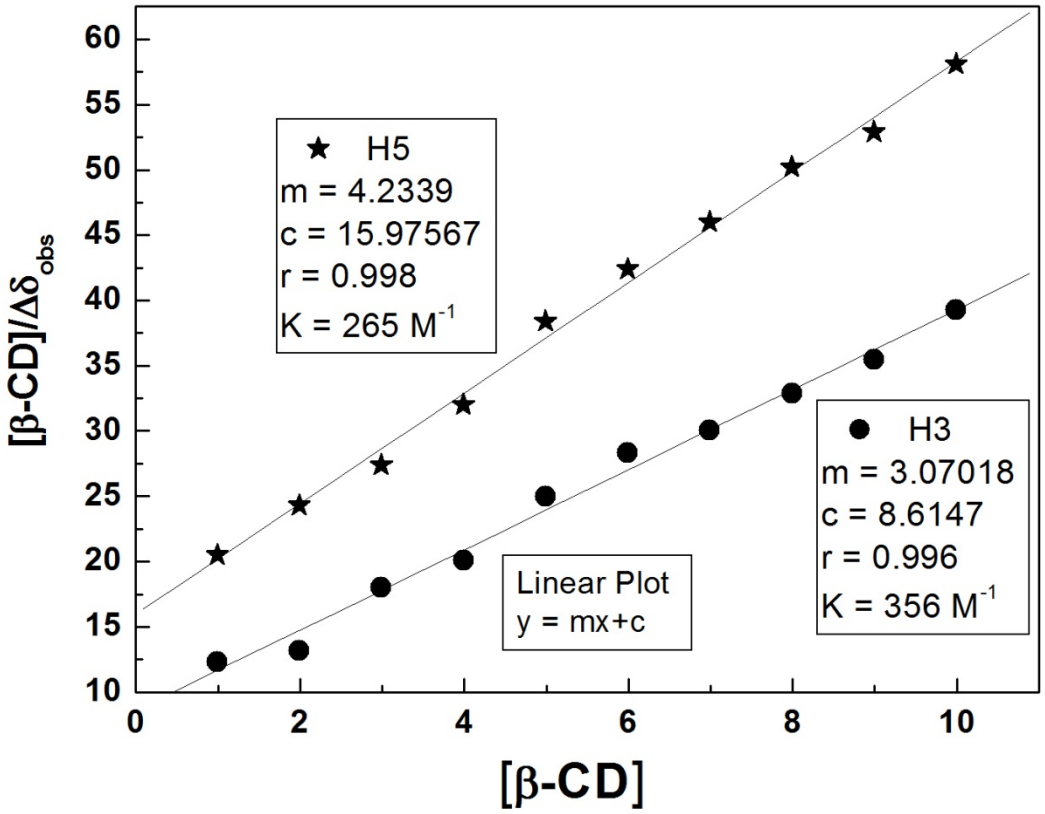

Fig.3: Scott's plots of inclusion complex showing the stoichiometry in the 1:1.

The constant $\frac{1}{\Delta\delta_{max}}$ is the slope of the straight line and $\frac{1}{K.\Delta\delta_{max}}$ is the intersection of the line on the $\frac{[\beta CD]}{\Delta\delta_{obs}}$ axis. It reflected the stiochiometry of an inclusion complex is 1:1. The association constant appeared on the Scott's plot using these values and it was found out to be 310 M^{-1} [Average of two values] [20].

Molecular Docking

Fig. 4 shows the effective ligand–receptor interaction studies done by molecular docking tool using Autodockvina in the present investigation. The model result suggested that there was involvement of one aromatic ring in complexation process. The molecular docking studies further confirmed that there were formation of host-guest complexes. Similar to above finding various other reports are available on the formation of inclusion complex using molecular docking technique [11].

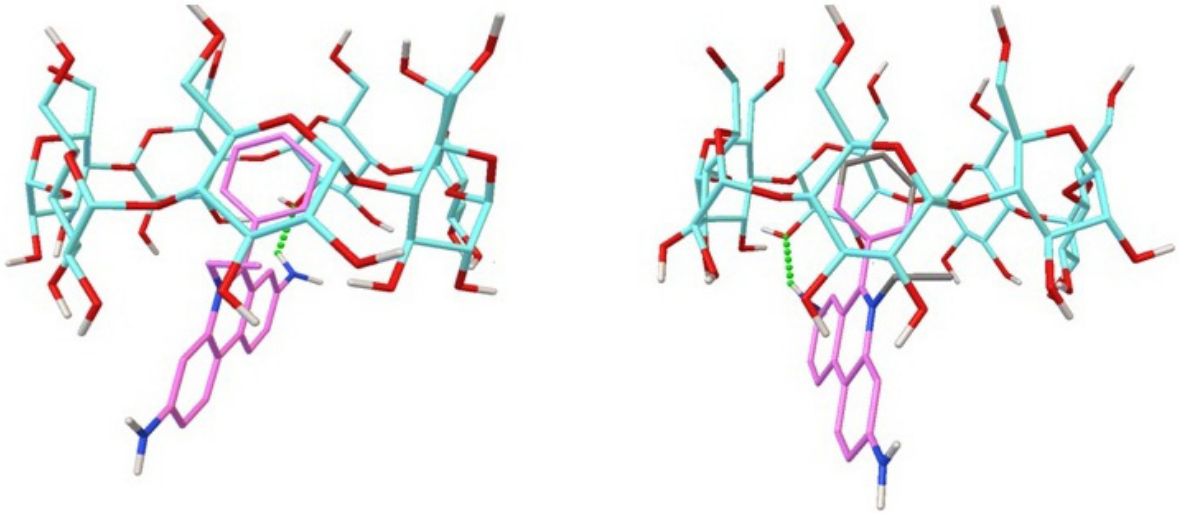

Fig.4: Molecular docking of Ethidium Bromide with β-cyclodextrin for two different binding affinities. The ocean green and red colors branches are of β-cyclodextrin, while pink and blue colours are of Ethidium Bromide, NH_2 of Ethidium Bromide is shown by grey colour.

IV. Conclusions

The study suggested the formation of hydrogen bond between EtBr and hydroxyl group of β-CD which was the main factor for non-covalent interaction and subsequent stabilization of complexes in an aqueous phase. NMR results suggested that guest-host inclusion complex was formed between β-cyclodextrin and Ethidium Bromide. The stoichiometry of the inclusion complex was in the ratio of 1:1 as obtained from Scott's plot. The interaction of host-guest inclusion was further clear in ROESY. The association constant was found to be 301 M^{-1}. Molecular docking study also supported the same results for the formation of inclusion complex. Structural elucidation of an inclusion complex by other techniques, viz. IR, Mass spectroscopy etc. and safety study are warranted in order to maximize its therapeutic benefits.

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