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Original Article

STUDIES ON THE PREPARATION AND CHARACTERIZATION OF β-CYCLODEXTRIN-PICRIC ACID INCLUSION COMPLEXES

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Abstract

The objective of the present work is to analyze the effect of Beta Cyclodextrin (β-CD) on the absorption spectra of picric acid (PA) in buffer solutions of different pH values 4 and 9. The study reveals that in both pH, PA forms 1:1 inclusion complex. Complexes of PA with β-CD of 1:1 and 1:2 molar ratios (host: guest) were prepared and formation of solid inclusion complexes was confirmed by means of FT-IR and 1H NMR spectroscopy. The results obtained confirmed the inclusion of PA into β-CD cavity. The significance of this work lies in the increase of PA bioavailability by complexation with β-CD and hence is a good pathway to make it potentially useful for its application as Anti-Fungal and Anti-microbial agent.

Keywords: β - Cyclodextrin, Picric acid, FT-IR, 1H NMR and Inclusion Complexes

1. Introduction

There has been great interest for PA, an aromatic nitro compound in various fields like synthesis of a staining agent and as an antiseptic. PA (2, 4, 6-trinitrophenol) is a pale yellow, odorless crystalline solid that has been used as a military explosive, as a yellow dye and as an antiseptic. In homeopathy, it is used as a medicine for treating ‘burn out’ or exhaustion. PA is slightly soluble in water. In addition to its use in explosives, PA has been used in the synthesis of chloropicrin, or nitro trichloro methane (CCl3NO2), a powerful insecticide. It also acts as Anti-Fungal [1] and Anti-microbial [2] agents.

Cyclodextrins can increase the solubility of nitro phenolic compounds [3, 4]. Cyclodextrins form inclusion complexes, with different guest molecules having suitable polarity and dimensions [5, 6]. Cyclodextrins are cyclic oligosaccharides, which are connected at 1 and 4 carbon atoms. With six to eight α-D-glucopyranose units they are called as α-, β- and γ-Cyclodextrins respectively. The special characteristic of Cyclodextrins is the ability to form an inclusion complex having apolar nature [7]. The inclusion complex formation occurs through various interactions, such as hydrogen bonding, Van der Waals interaction, hydrophobic interactions and also electrostatic attraction. The physical, chemical and biochemical properties of guest molecules will be modified and their applications can be therefore enhanced [8].

PA forms crystalline picrates with various organic molecules through ionic, hydrogen bonding and π-π interactions [9]. Bonding involves not only electrostatic interactions but also formation of molecular complexes [10]. Taking into account the importance of aromatic nitro compounds herein we have prepared and studied inclusion complexes of PA with β-CD and confirmed the inclusion phenomenon by various physical measurements.

In this article, we focus on the interaction between β-CD and PA at different conditions. The results show that β-CD and PA form an inclusion complex, which increases the solubility of PA in water. The binding constant of the inclusion complex at different pH shows that the inclusion complex becomes unstable under alkaline condition and catalyzes its decomposition [11, 12]. The mode of inclusion of PA into β –CD cavity has been illustrated in Fig 1.
Fig. 1. Proposed model of the inclusion equilibrium for Picric acid-β-Cyclodextrin complex

2. Materials and methods

2.1. Materials.

β-Cyclodextrin (Hi-Media Chemical Reagents Company) ,Picric acid (PA, Merck Chemical Reagents Company) and other chemical reagents were of analytical reagent grade and used as commercial. Double distilled water was used to prepare all solutions.

2.2. Instruments

The UV–Vis spectrum was recorded on an Ellico VL 222 Double-beam Bio Spectro Photometer equipped with a stopped quartz cell with 1.0 cm optical path length. The pH values were measured on an Ellico pH meter. FT-IR spectra were obtained on Shimadzu FT-IR spectrometer using KBr pelleting. Proton NMR was recorded on a Bruker model spectrometer operating at a proton frequency 400 MHZ using DMSO-d6 as a solvent.

2.3. Preparation of inclusion complex

To a saturated solution of β-CD in water, equimolar amount of PA dissolved in minimum amount of ethanol was added and magnetically stirred for 48 hrs at room temperature. The resultant crystalline precipitate was filtered , washed with diethyl ether to remove any uncomplexed substrate and dried in an air oven for 4 hrs at 60°C. The dried crystalline powder was used for spectral studies. Higher order complex (1:2 host: guest) was also prepared using the above procedure with excess amount of PA. For UV studies, a constant volume of 10−3 M aqueous solution of PA was mixed with different concentration of β-CD aqueous solution with appropriate buffer solution and shaken intensively with a magnetic stirrer for 24 hrs at 25°C and the samples analyzed spectrophotometrically. The solid complex of β-CD and PA has been studied by FT-IR and 1HNMR.

3. Results and Discussion

3.1 UV/Visible absorption spectra

The complexation between PA and β-CD has been established by UV/Visible spectrophotometric method. The significant difference in λmax values and absorbance confirms the stabilization of PA in the CD cavity. The UV spectra of PA with β-CD at two different pH values 4 and 9 using buffers are given in Figures 2 and 3. PA has an exponent of acidity pKa = 0.38, the study was developed separately for the two pH ranges, at pH 4 and at pH 9. The absorption intensities were increased by increasing the concentration of CD. The red-shift with increase of CD concentration suggested the formation of complex. At pH 4 no significant spectral shift is observed in PA. Further, as acid concentration is increased, a large red shifted spectrum is observed, indicating that protonation takes place in the OH group. When the pH is increased to 9, complexes give a new red shifted spectrum. This is because of the formation of mono anion / deprotonation takes place in the OH group [13]. The shifts can be due to direct interaction between β-CD and PA or due to expulsion of solvating water molecules from β-CD cavity [14].

Fig. 2. UV Spectrum of PA and β-Cyclodextrin at pH=4; [Guest]=1x10−3 M; [Host]=1x10−3 M

Apparent binding constants (kb) were calculated from Benesi-Hildebrand plots shown in fig: 4 and fig: 5. The Benesi –Hildebrand plots are plots of ([H] [G]/ΔA) Vs ([H] + [G]).

Fig. 3. UV Spectrum of PA and β-Cyclodextrin at pH=9; [Guest]=1x10−3 M; [Host]=1x10−3 M

Fig. 4. Benesi- Hildebrand plot for PA- β-Cyclodextrin 1:1 complex at pH=4; ΔA – difference in absorbance; Binding Constant kb= slope / Intercept
Fig. 5. Benesi–Hildebrand plot for PA- beta cyclodextrin 1:1 complex at pH=9; ΔA – difference in absorbance; Binding Constant kb= slope / Intercept

Binding constants are higher at pH 4 compared to pH 9 as given in Table 1. The effect of pH on host-guest inclusion interaction indicates that the conformations of guest are dissimilar at different pH values or the polarity of the guest changes. All the plots exhibited good linearity. This implied that the formation of 1:1(β-CD – PA) inclusion complex. The inclusion interaction of β-CD with PA was in the order: pH4.0 > pH9.0. One of the major factors affecting the inclusion interaction is the hydrophobic nature of the guest. At pH 4.0, the neutral (uncharged) form of PA is predominant, which is more hydrophobic than the anion form, so it is easier to form the inclusion complex with β-CD.

Table 1. Binding constants of complexes of PA with β-Cyclodextrin at pH=4 and pH=9 From UV data

<table>
<thead>
<tr>
<th>pH</th>
<th>Ks(M⁻¹cm³) of complexes of PA with β-CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>8070</td>
</tr>
<tr>
<td>9</td>
<td>6850</td>
</tr>
</tbody>
</table>

3.2 Fourier Transform Infrared spectra:

After purified and dried, inclusion complexes were obtained as yellowish crisp granulate. The IR spectra of β-Cyclodextrin, Picric acid and β-cyclodextrin – Picric acid complexes (1:1&1:2 molar ratios) are shown Figure 6. The formation of inclusion complex of β-CD and a guest substance is accompanied by changes in their IR spectra as compared with the individual components [15, 16]. Table 2 illustrates the IR peaks of Picric acid and its β-CD complex. Fig 6 shows the FT-IR spectra of PA, β-CD and β-CD- PA 1:1 & 1:2 complexes. The broad peak of OH in β-CD becomes sharp and intense in the inclusion complex. The infrared spectrum of the inclusion complex shows specific absorption peaks for β-Cyclodextrin at 3365 cm⁻¹(OH stretching H bonded), 2922 cm⁻¹(OH stretching), 1635 cm⁻¹(OH bending), 1271 cm⁻¹(OH bending) and 1029 cm⁻¹(C-O-C stretching). Weak absorption peaks found at 1155 cm⁻¹ due to phenolic oxygen atom and those at 1334 cm⁻¹ attributed to NO₂ stretching, at 530 cm⁻¹ to NO₂

Fig. 6. FT-IR spectra of (A) Picric acid (B) β-CD (C) 1:1 β-CD- PA Inclusion complex and (D) 1:2 β-CD- PA Inclusion complex

scissoring vibrations of PA. C-H out of plane bending of aromatic ring at 851 cm⁻¹, led us to the conclusion that PA is included in the β-CD. The sharp signal at 1334 cm⁻¹, due to NO₂ group drastically reduces its intensity and sharpness in the complex. In the inclusion complexes the stretching peaks of aromatic nitro group intensities are reduced and shifted from1344 cm⁻¹ to 1334 cm⁻¹. This is because the nitro groups are included into the β-CD cavity. The N=O stretching frequency appeared at 1633 cm⁻¹ in PA but it shifted to 1635 cm⁻¹ due to the inclusion of the nitro groups into the β-CD cavity. The small shift might be owing to the effect of inner microenvironment and non-covalent interaction of β-CD hydroxyls on PA. The spectra of all the complexes made on a small scale were similar to the spectra of the corresponding samples made on a large scale. In all the inclusion complexes the prominent and characteristics peaks of PA are appeared indicating intactness of PA in complexes.

Table 2. FT-IR frequencies (ν cm⁻¹) of free picric acid and PA-β-CD complex

<table>
<thead>
<tr>
<th>Type of vibration</th>
<th>Free PA</th>
<th>Complex of PA-β-CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>H bonded O-H stretch</td>
<td>3441</td>
<td>3365</td>
</tr>
<tr>
<td>N=O stretching frequency</td>
<td>1633</td>
<td>1635</td>
</tr>
<tr>
<td>N-O symmetric stretch (nitro group)</td>
<td>1344</td>
<td>1334</td>
</tr>
<tr>
<td>C-H bend in aromatic rings</td>
<td>918</td>
<td>851</td>
</tr>
</tbody>
</table>

*FT-IR recorded in KBr disc

3.3. ¹H NMR spectra

Direct evidence for the formation of inclusion complex can be obtained from ¹H NMR [17]. Fig.7 depicts the NMR spectra of PA, β-CD and inclusion complexes. The significant shifts shown by these ¹H NMR spectra
strongly confirmed the formation of inclusion complexes. The values of chemical shifts, for different protons in β-CD, PA and PA-β-CD inclusion complex are listed in Tables 3 and 4.

Table 3. $^1$H chemical shift of β-CD protons in free and complex state a

<table>
<thead>
<tr>
<th>Proton</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>H4</th>
<th>H5</th>
<th>H6</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-CD</td>
<td>4.85</td>
<td>3.45</td>
<td>3.78</td>
<td>3.4</td>
<td>3.71</td>
<td>3.73</td>
</tr>
<tr>
<td>Complex</td>
<td>4.80</td>
<td>3.33</td>
<td>3.59</td>
<td>3.34</td>
<td>3.56</td>
<td>3.66</td>
</tr>
</tbody>
</table>

a Chemical shifts expressed in ppm

Table 4. $^1$H chemical shift of PA in free and complex state b

<table>
<thead>
<tr>
<th>Proton</th>
<th>phenolic H</th>
<th>phenyl H</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>5.82</td>
<td>8.65</td>
</tr>
<tr>
<td>Complex</td>
<td>4.46</td>
<td>8.59</td>
</tr>
<tr>
<td>$\Delta \delta$</td>
<td>1.36</td>
<td>0.06</td>
</tr>
</tbody>
</table>

b Chemical shifts expressed in ppm

It can be seen from the $^1$H NMR that in inclusion complex, a great upfield shift has occurred for H3 and H5 located in the cavity of β-CD. The changes of chemical shift of H3 and H5 suggest that the PA monomer has completely entered into the hydrophobic cavity of β-CD. The phenyl ring of PA has upfield shifted the signals of protons (H3 and H5). On the contrary, the chemical shifts of H1, H2, H4 and H6, which are on the outer surface of β-CD and the narrow side of β-CD, are only slightly affected by the guest molecule. Similarly, the chemical shifts of aromatic H of PA located in the hydrophobic cavity of β-CD are in upfield significantly because of the interaction between PA and β-CD. Also when PA monomer enters into the hydrophobic cavity of β-CD, the change of the micro-environment of PA protons leads to the splitting of the phenyl ring proton signals [18].

Fig 7 $^1$H NMR of (A) β-CD, (B) Picric acid,(C)1:1 β-CD-PA Inclusion complex and (D) 1:2 β-CD- PA Inclusion complex

4. Conclusions

In this work, the inclusion complexes of picric acid and β-Cyclodextrin have been prepared and intermolecular interactions between them studied. The significant difference in FT-IR, $^1$H NMR and UV-Visible spectra of the complexes confirm the molecular interactions. In addition, the observed upfield chemical shift of H3 and H5 proton of β-CD suggest that PA interacts with internal protons of β-CD and reveal clear evidence for inclusion phenomena. Overall, the findings from this study strongly support the possibility of using β-CD to significantly enhance the solubility of Picric acid in aqueous systems and enlarge the range of applications of this antimicrobial and antifungal agent. Furthermore, the bitter tasting picric acid could be ‘sugar coated’ by the natural sugar derivative β-CD in the complexed form thereby increasing its efficacy as an antimicrobial and an insecticide.

References


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