



## Characterization of Cyclodextrin Inclusion Complexes – A Review

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### Abstract:

Cyclodextrins are cyclic oligosaccharides which have recently been recognized as useful pharmaceutical excipients. The molecular structure of these glucose derivatives generates a hydrophilic exterior surface and a non polar cavity interior. Such cyclodextrins can interact with appropriate size drug molecules which lead to the formation of inclusion complexes. A critical review of the literature was carried out to characterize the formation of inclusion complexes by different techniques in the solid and in the solution state. The characterization of inclusion complexes was done with a purpose to determine the interaction of drug molecules with cyclodextrins which confirm the formation of inclusion complexes.

**Key words:** Cyclodextrin, Inclusion, Complexes

### Introduction:

Cyclodextrins (CDs) are cyclic oligosaccharides containing six ( $\alpha$ -CD), seven ( $\beta$ -CD) or eight ( $\gamma$ -CD)  $\alpha$ -1,4-linked glucopyranose units, with a hydrophilic hydroxyl group on their outer surface and a hydrophobic cavity in the center. Owing to lack of free rotation about the bonds connecting the glucopyranose units, the cyclodextrins are not perfectly cylindrical molecules but are toroidal or cone shaped. Based on the architecture, the primary hydroxyl groups are located on the narrow side of the torus while the secondary hydroxyl groups are located on the wider edge (Figure 1).

The most common cyclodextrins are  $\alpha$ -cyclodextrin( $\alpha$ CD),  $\beta$ -cyclodextrin( $\beta$ CD) and  $\gamma$ -cyclodextrin( $\gamma$ CD) which consist of six, seven and eight glucopyranose units respectively. But due to steric factors, cyclodextrins having fewer than six glucopyranose units cannot exist, cyclodextrins containing nine, ten, eleven, twelve and thirteen glucopyranose units, which are designated  $\delta$ -,  $\epsilon$ -,  $\zeta$ -,  $\eta$ - and  $\theta$ -cyclodextrin, respectively have been reported. The chemical and physical properties of the four most common cyclodextrins are given in Table 1.

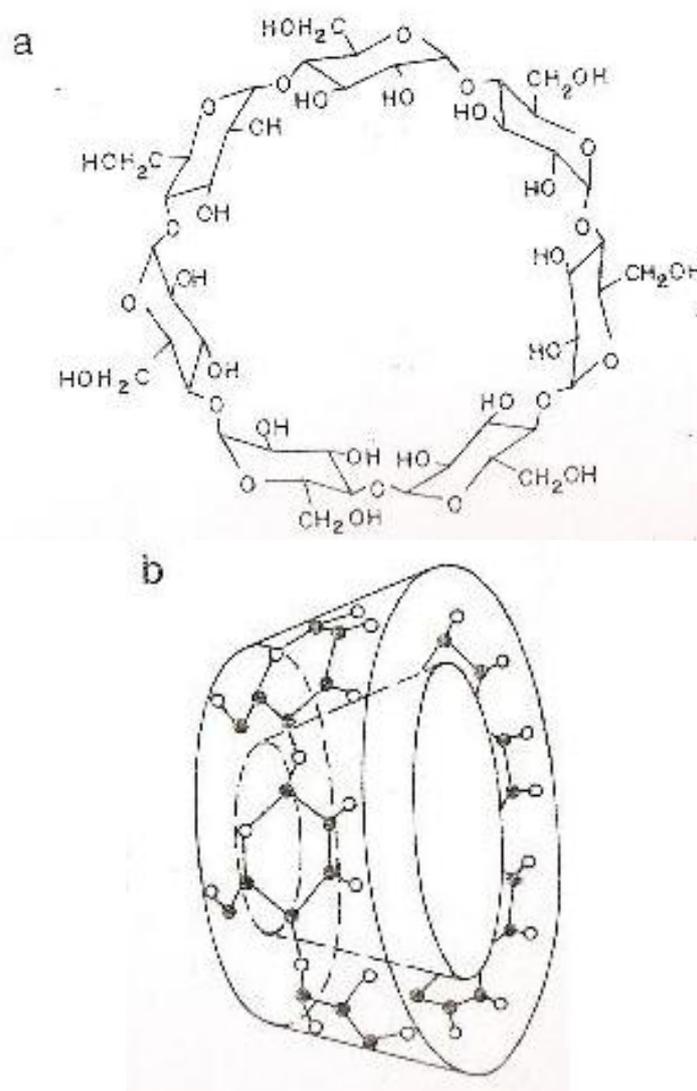
CDs are capable of forming inclusion complexes with many drugs by taking up a

whole drug molecule, or some part of it, into the cavity. Such molecular encapsulation will affect many of the physicochemical properties of drugs, such as their aqueous solubility and rate of dissolution. Among the various approaches, preparation of inclusion complexes with cyclodextrin have proven to be successful in enhancing the solubility of poorly water soluble drugs<sup>1,2</sup>.

The formation of inclusion complexes with a wide variety of guest molecules is one of the most interesting properties of cyclodextrins. Molecular encapsulation may occur both in solid and in solution state. In solid state, guest molecules can be enclosed within the cavity or may be aggregated to the outside of the cyclodextrin molecule and in solution state; there is equilibrium between complexed and non-complexed guest molecules. A guest molecule experiences changes in the physicochemical properties when it gets incorporated within the cyclodextrin cavity. Changes in the physicochemical properties provide methods to characterize whether guest molecules are really included in the cyclodextrin cavity<sup>3</sup>.

### Techniques for characterization of inclusion complexation:

The complexation depends largely on the dimensions of the cyclodextrins and the particular sterical arrangement of the functional groups of the molecules, which



**Figure 1(a):** The chemical structure and **(b)** the toroidal shape of the  $\beta$ -cyclodextrin molecule.

**Table 1:** Chemical and physical properties of  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ -cyclodextrin

Physicochemical properties	$\alpha$	$\beta$	$\gamma$	$\delta$
No. of glucopyranose units	6	7	8	9
Molecular weight	972	1135	1297	1459
Central cavity diameter ( $\text{Å}^\circ$ )	4.7-5.3	6.0-6.5	7.5-8.3	10.3-11.2
Water solubility at $25^\circ\text{C}$ (g/100 mL)	14.5	1.85	23.2	8.19

leads to a relatively hydrophilic outside and a hydrophobic inside cavity of the molecule. Inclusion complexes formed between the guest and cyclodextrin molecules can be

characterized both in the solid and solution state by the following techniques:-

(A) Inclusion complexation in the solid state characterized by

- (i) Thermo-analytical methods.
- (ii) Scanning Electron Microscopy (SEM).
- (iii) X-ray diffractometry and single crystal X-ray structure analysis.
- (iv) Wettability and dissolution tests.
- (v) Infra-Red (I R) spectroscopy.
- (vi) Thin Layer Chromatography (T L C)
- (B) Inclusion complexation in solution state characterized by
  - (i) Electrochemistry.
  - (a) Polarography.
  - (b) Conductivity
  - (c) Polarimetry
  - (ii) Solubility studies.
  - (iii) Spectroscopy methods.
    - (a) Nuclear Magnetic Resonance (NMR) spectroscopy.
    - (b) Electron Spin Resonance (ESR).
    - (c) Ultraviolet/Visible (UV/VIS) spectroscopy.
    - (d) Fluorescence spectroscopy.
    - (e) Circular Dichroism (CD) spectroscopy.
  - (iv) pH-Potentiometric Titration.
  - (v) Microcalorimetry.

**(A) Inclusion complexation in the solid state characterized by :**

**(i) Thermo-analytical methods :-**

Thermo-analytical methods<sup>3-14</sup> determine whether the guest substance undergoes some change before the thermic degradation of cyclodextrin. The change of the guest substance may be melting, evaporation, decomposition, oxidation or polymorphic transition. The change of the guest substance indicates the complex formation. The effect of cyclodextrins on the thermogram obtained by DTA and DSC were observed for broadening, shifting and appearance of new peaks or disappearance of certain peaks. Changes in the weight loss were evaluated to provide supporting evidence for the formation of inclusion complexes. The nature of the drug and cyclodextrins used and method of preparation of complex have been found to influence the above finding

considerably. If the interaction between the drug and the excipient is weak, the shift in the endothermic peak is very small.

The formation of inclusion complex of Salbutamol with cyclodextrins by various methods was evaluated using DSC. The DSC endotherm of Salbutamol at 158°C was shifted to 150°C in the physical mixture showing a weak interaction. But the freeze dried complex showed no peak around 157°C indicating the formation of a true inclusion complex<sup>15</sup>. Formation of inclusion complex of Azelaic acid with Hydroxypropyl-β-cyclodextrins(HPβCD) by physical mixture, co-evaporation and freeze dried was evaluated by DSC. The thermogram of Azelaic acid revealed an endothermic peak at around 105°C, corresponding to its melting point. The thermogram of physical mixture demonstrated the melting point of Azelaic acid, indicating that an inclusion complex could not be obtained by simple blending the drug and HPβCD. The inclusion complex formed by co-evaporation method did not exhibit the melting endothermic peak of Azelaic acid, indicating that Azelaic was incorporated in the HPβCD cavity<sup>16</sup>.

**(ii) Scanning Electron Microscopy (SEM) :-**

Scanning Electron Microscopy<sup>6, 9, 10, 67</sup> is used to study the microscopic aspects of the raw material (cyclodextrin and the guest substances, respectively) and the product obtained by co-precipitation /evaporation<sup>15-18</sup>. The difference in crystallization state of the raw material and the product seen under electron microscope indicates the formation of the inclusion complexes<sup>19-21</sup>, even if there is a clear difference in crystallization state of the raw material and the product obtained by co-precipitation. This method is inadequate to affirm inclusion complex formation<sup>4,7,8</sup>. The particle morphology of Ketoprofen, β-cyclodextrin(βCD), its physical mixtures and solid complexes were evaluated by SEM

photographs. Ketoprofen appeared as plate like crystals, tending to form aggregates. HP $\beta$ CD consisted of shrunken, cylindrical particles, whereas  $\beta$ CD appeared as irregularly shaped crystals. The physical mixtures showed particles of HP $\beta$ CD,  $\beta$ CD embedded with Ketoprofen particles and a comparable morphology with pure compounds, taken separately. In contrast, a drastic change in the morphology and change in the crystalline nature was observed in 1:1 freeze-dried, coprecipitated and kneaded products of both HP $\beta$ CD and  $\beta$ CD, it was revealed that there was an apparent interaction in the solid state<sup>6</sup>.

SEM analysis was performed to investigate the morphologies of pure drug and carriers and their combinations. The results showed that the typical polyhedron-shaped drug crystals of Ketoprofen were recognizable in physical mixtures but such polyhedron-shaped drug crystals were no longer recognizable in the complexes of Ketoprofen with  $\beta$ CD and HP $\beta$ CD prepared by co-evaporation and sealed-heating methods which demonstrated the formation of amorphous aggregates<sup>9</sup>.

Lipospheres containing complex between HP $\beta$ CD and butyl methoxybenzoylmethane(BMOBM) (i.e the sunscreen agent) were characterized by SEM. The results indicated that the lipospheres loaded with BMOBM alone showed a spherical shape and a smooth surface, while the ones containing the sunscreen agent complexed with HP $\beta$ CD were mainly irregular and exhibited uneven surfaces. Hence, the microparticle morphology was affected by the incorporation of the cyclodextrin<sup>10</sup>.

**(iii) X-ray diffractometry and single crystal X-ray structure analysis:-**

Powder X-ray diffractometry<sup>4, 5</sup> may be used to detect inclusion complexation in the solid state. When the guest molecules are liquid since liquid have no diffraction

pattern of their own, then the diffraction pattern of a newly formed substance clearly differs from that of uncomplexed cyclodextrin. This difference of diffraction pattern indicates the complex formation<sup>17-20</sup>. When the guest compound is a solid substance, a comparison has to be made between the diffractogram of the assumed complex and that of the mechanical mixture of the guest and cyclodextrin molecules<sup>16, 22-25</sup>. Comparison of the diffractogram is only possible if the cyclodextrin as well as the guest molecules are treated under identical conditions as that of the assumed complex because cyclodextrin inclusion complex preparation processes such as freeze drying and grinding, may change the crystallinity of the pure substances and this may lead to different diffraction patterns<sup>3, 8</sup>. A diffraction pattern of a physical mixture is often the sum of those of each component, while the diffraction pattern of cyclodextrin complexes are apparently different from each constituent and lead to a "new" solid phase with different diffractograms<sup>26 - 29</sup>. Diffraction peaks for a mixture of compounds are useful in determining the chemical decomposition and complex formation. The complex formation of drug with cyclodextrin alters the diffraction patterns and also changes the crystalline nature of the drug. The complex formation leads to the sharpening of the existing peaks, appearance of a few new peaks and shifting of certain peaks. For example: the inclusion complexes prepared by neutralization method for Naproxen<sup>30</sup>. On the other hand, formation of amorphous complexes leads to the disappearance of certain peaks or the peaks become less sharpen than those of the pure compound or physical mixture. For example, the spray dried complexes of Acetaminophen, Indomethacin, Piroxicam and Warfarin with  $\beta$ CD and the freeze dried complexes of Naproxen with  $\beta$ CD<sup>30, 31</sup>.

**Single crystal X-ray structure analysis** may be used to determine the detailed inclusion structure and mode of interaction. The interaction between the host and guest molecules can be identified and the precise geometrical relationship can be established. This information obtained during the analysis lead to know about the formation of inclusion complexes<sup>6,7</sup>.

**(iv) Wettability and Dissolution tests :-**

The wetting of the solid phase by a solvent is always the first step of any dissolution process. Cyclodextrin complexations of the lipophilic drug often improve the wettability in water considerably, but also simple addition of  $\beta$ -cyclodextrin to non-wettable solid enhances their wettability. Three methods to characterize the wettability of solid cyclodextrin formulations include the measurement of the contact angles, powder sedimentation studies which may be carried out by layering equal amounts of the samples onto the surface of water, following their sedimentation photographically and the last method demonstrates the upward migration of a coloured front of three open tubes containing the guest compound, a mixture of the guest compound with cyclodextrin and the inclusion complex, respectively, as function of the time. When an assumed complex is dispersed in water, very rapid dissolution rate tests are based on this observation. The most often used dissolution tests are the rotating disk method<sup>14</sup> and the dispersed amount technique<sup>32-35</sup>.

In the rotating disk method, the solid cyclodextrin formulations are pressed into tablets with exact identical surfaces for the samples and these tablets are placed on a rotating disc apparatus in an aqueous solution. At appropriate intervals samples are removed and analyzed for the guest content. The dispersed amount technique is a similar method but instead of a tablet, a powder is used.

The dissolution rates have been found to be affected to different extents by the methods employed for complexation with  $\beta$ CD or its derivatives. For example, when freeze drying method was employed in the case of Indomethacin, the dissolution rate was increased to 1.6 fold. But in the kneading method, the solubility was not much affected although the dissolution was increased from 39 % to 95 % in 3 minutes at 1:2 M ratio of Indomethacin with  $\beta$ CD<sup>31</sup>.

**(v) Infra-Red (IR) spectroscopy :-**

Infra-Red spectroscopy is used to estimate the interaction between cyclodextrin and the guest molecules in the solid state<sup>6,7</sup>. Cyclodextrin bands often change only slightly upon complex formation and if the fraction of the guest molecules encapsulated in the complex is less than 25%, bands which could be assigned to the included part of the guest molecules are easily masked by the bands of the spectrum of cyclodextrin<sup>3,12,13,19-21,28,29,36</sup>. The technique is not generally suitable to detect the inclusion complexes and is less clarifying than other methods<sup>14,17,18</sup>. The application of the Infra-red spectroscopy is limited to the guests having some characteristic bands, such as carbonyl or sulfonyl groups<sup>23,26,37</sup>.

Infra-red spectral studies give information regarding the involvement of hydrogen in various functional groups. This generally shifts the absorbance bands to the lower frequency, increases the intensity and widens the band caused by stretching vibration of the group involved in the formation of the hydrogen bonds. Hydrogen bond at the hydroxyl group causes the largest shift of the stretching vibration band. It has been observed that cleavage of the hydrogen bonding due to inclusion complexation results in the shift of absorbance bands to higher frequency. For example, a shift of the aromatic carbon stretching at 1272 to 1296  $\text{cm}^{-1}$  in case of  $\beta$ CD complex, and the stretch of the ester

function from 1183 to 1206  $\text{cm}^{-1}$  in case of dimethyl- $\beta$ -cyclodextrin was reported<sup>38</sup>. Formation of hydrogen bond has resulted in lengthening of bond O-H, N-H etc due to reduction of elasticity. Accordingly, the frequencies of the stretching vibrations were decreased. For example, when Piroxicam was complexed with  $\beta$ CD, the band at 1180  $\text{cm}^{-1}$  got shifted to 1154  $\text{cm}^{-1}$ <sup>39</sup>.

The bands of deformation vibrations were less sensitive to hydrogen bonding, which resulted in a slight increase in the frequency of deformation vibrations. In amides, the absorption of the N-H group was prone to change due to involvement in hydrogen bonding. For example, shift from 3465-3300  $\text{cm}^{-1}$  and 1568 to 1555  $\text{cm}^{-1}$  in case of Acetaminophen complexed with  $\beta$ CD was reported<sup>39</sup>. Carbonyl function is very susceptible for hydrogen bonding in which case the absorption frequency of the carbonyl group observed at around 1700  $\text{cm}^{-1}$  shows slight decrease in its intensity. For example, the complete disappearance of the carbonyl band around 1680  $\text{cm}^{-1}$  in case of Indomethacin and 80% reduction in intensity of the same band in case of Naproxen was reported when complexed with  $\beta$ CD<sup>40</sup>.

(vi) **Thin Layer Chromatography(T L C):**

In Thin Layer Chromatography, the Rf values of a guest molecule diminishes to considerable extent and this helps in identifying the complex formation between guest and host molecule<sup>41</sup>. Inclusion complexation between guest and host molecules is a reversible process. Consequently, the complex may separate completely in guest and host molecules during the chromatographic process and only the spots of the guest and host molecules are found on the TLC-plate<sup>42</sup>. The Rf value of Diclofenac sodium and Indomethacin were different from those of the complex formed using  $\beta$ CD on

concurrent crystallization from water/organic solvent system<sup>42</sup>.

**(B) Inclusion complexation in solution characterized by :**

(i) **Electrochemistry:-**

(a) **Polarography :-**

Polarography is a suitable method to study inclusion complexation if the electron distribution of a complexed electroactive guest molecule in aqueous solution is different from that in the non-complexed state in aqueous solution<sup>19,43</sup>.

(b) **Conductivity:-**

Conductivity measurement may be used to characterize inclusion complexation. Anionic surfactants having different polar heads, different tail configurations, the same  $\text{Na}^+$  counter ion and their solution conductivities are dramatically affected by inclusion complex formation with cyclodextrins. When these ionic surfactant form inclusion complexes with cyclodextrins, the amphiphilicity of the former often leads to strong associative species that dramatically affect solution conductivities<sup>37</sup>.

(c) **Polarimetry :-**

Specific rotation  $[\alpha]$  is the inherent characteristic property of an optically active species. A polarimetric study was conducted as a supporting tool for the complex formation because  $\beta$ CD is optically active in nature ( $\alpha$ -[D]-glucopyranose units). Celecoxib shows no optical activity in the methanol:water solution and in pure methanol at 25° C. Physical mixtures of Celecoxib and  $\beta$ CD were insoluble in the methanol:water (1:1) mixture; therefore, their optical rotation was not recorded. The specific rotation of  $\beta$ CD is  $162.5 \pm 0.5^\circ$  in water at 25° C. Each complex is characterized by its specific rotation. Sharp changes in the specific rotation of complexes were observed, which suggest the possibility of the interaction of  $\beta$ CD with Celecoxib to form inclusion complexes<sup>19</sup>.

**(ii) Solubility studies: -**

In the solubility studies<sup>5, 42</sup>, changes in solubility of the guest are plotted as a function of the cyclodextrin concentration, if the solubility of a potential guest increases with increasing cyclodextrin concentration; complex formation in solution is indicated<sup>44-46</sup>. Vinpocetine aqueous solubility was evaluated in complexed and uncomplexed forms. Solubility studies were performed to evaluate the drug pH solubilization profile and to assess the effect of multicomponent complexation on Vinpocetine solubility. The solubility studies reported clearly illustrated the impact of PH on Vinpocetine solubility and dissolution. From the solubility values obtained, it was predictable that the extent of Vinpocetine dissolution in the gastric environment would be high but the PH values generally found in the upper regions of the gastrointestinal tract, the solubility and dissolution of pure Vinpocetine will not be sufficient for complete dissolution of the doses administered. Vinpocetine solubility in multicomponent form was found to be higher than pure Vinpocetine. Vinpocetine solubility in uncomplexed form (approx. 5 µg/ml) increased to approximately 80 mg/ml in multicomponent complex, which corresponds to a 16,000 fold higher increase on Vinpocetine aqueous solubility<sup>46</sup>. Solubility studies of curcuminoids with CDs showed that curcuminoids with side groups on the phenyl moiety have higher affinity for the hydroxypropyl- $\gamma$ -cyclodextrin (HP $\gamma$ CD) than for the  $\beta$ CDs and the relative affinity of the larger HP $\gamma$ CD cavity increases with the curcuminoid molecule size<sup>45</sup>.

**(iii) Spectroscopic methods:****(a) Nuclear Magnetic Resonance (NMR) spectroscopy:-**

The most direct evidence for the inclusion of a guest into a cyclodextrin cavity in solution is obtained by <sup>1</sup>H-NMR spectroscopy<sup>7, 19, 67</sup>. <sup>1</sup>H-NMR may also be used to determine the

direction of penetration of guest molecules into the cyclodextrin cavity<sup>20, 29</sup>. The H-3 and H-5 atoms of cyclodextrin, which are directed towards the interior of the cyclodextrin will show a significant upfield shift if inclusion does indeed occur and the H-1, H-2 and H-4 atoms, located on the exterior of the cavity will show only marginal upfield shifts. The spectrum of the guest molecule may also be changed upon inclusion complex formation<sup>46-58</sup>.

A similar method to investigate inclusion complex formation is <sup>13</sup>C-NMR spectroscopy<sup>31</sup>. It is often used to gain insight into the inclusion modes of inclusion complexes in aqueous solution. The cyclodextrin induced change in the <sup>13</sup>C-chemical shift result predominantly from the electrical environment effect of the cyclodextrin cavity and in general <sup>13</sup>C inclusion shift may be mainly divided into hydrophobic and Vander waals interaction shifts<sup>47</sup>.

The <sup>1</sup>H-NMR chemical shift for each proton of  $\beta$ CD was evaluated for the formation of inclusion complex between Salbutamol and  $\beta$ CD at different molar ratios of 1:0.5, 1:1 and 1:2 of Salbutamol and  $\beta$ CD. The shift corresponding to each of the protons of  $\beta$ CD for 1:1 M ratio was maximum compared to other ratios, which was observed to be the limiting factor for the formation of the inclusion complex. The downfield shift of aromatic protons of Salbutamol was more to those of aliphatic protons. This suggested that the aromatic ring of Salbutamol interacted more strongly with  $\beta$ CD than the aliphatic chain<sup>59</sup>.

In case of the inclusion of Bropirime with  $\beta$ CD in the ratio 1:1 M, formed by freeze drying and co-precipitation techniques, the <sup>1</sup>H-NMR signals of H-3 and H-5 protons of  $\beta$ CD were shifted up field more compared to the signals of H-1, H-2 and H-4 protons. On increasing the drug concentration in the complex, one group of signals shifted up

field and the other group signals downfield. This was an indication of partial inclusion of the drug (only the phenyl moiety) in the  $\beta$ CD cavity<sup>42</sup>.

(b) *Electron Spin Resonance (ESR):-*

Electron Spin Resonance<sup>41</sup> is a useful method to investigate inclusion complexation with radicals in aqueous solutions. The hyperfine coupling constant of radicals is known to be sensitive to the polarity of the medium. If the hyperfine coupling constant alters, the movement of a radical to an environment less polar than water is indicated and confirms the inclusion complex formation. For example, the inclusion complexes were prepared between Miconazole and cyclodextrins by freeze drying and kneading method and evaluated by Electron Spin Resonance. The electron microscopic pictures showed that the physical appearance and size of the complexes formed were completely different from that of Miconazole or corresponding cyclodextrins alone. The particle size of the inclusion complexes was much smaller than the parent cyclodextrins. The size of the HP $\beta$ CD and  $\alpha$ -cyclodextrin( $\alpha$ CD) was 256.7 and 50  $\mu$ m, respectively, while that of the corresponding miconazole complexes was 2.3 and 5  $\mu$ m<sup>60</sup>.

(c) *Ultraviolet/Visible (UV/VIS) Spectroscopy:-*

The complexation causes a change in the absorption spectrum of a guest molecule<sup>4, 7, 13</sup>. During the spectral changes, the chromophore of the guest is transferred from an aqueous medium to the non-polar cyclodextrin. These changes must be due to a perturbation of the electronic energy levels of the guest caused either by direct interaction with the cyclodextrin, by the exclusion of solvating water molecules or by a combination of these two effects<sup>28, 33</sup>. Small shifts are observed on the UV spectra of the included guests, the method is often used to detect inclusion complexation<sup>61-63</sup>.

Hypsochromic or bathochromic shift or increase in the absorptivity without change in the  $\lambda_{\max}$  have been considered as evidence for interaction between cyclodextrin and the drug in the formation of the complex. Hydrogen bonding can be considered as the main force behind the inclusion complex formation. As hydrogen bonding lowers the energy of 'n' orbitals, a hypsochromic shift (blue shift) is observed. For example, 1.1 nm shift was observed when Hydrocortisone butyrate was complexed with (2, 6-di-O-methyl)- $\beta$ -cyclodextrin<sup>38</sup>. Cleavage of the existing hydrogen bonds in the compound can lead to a bathochromic shift due to complexation. For example, 1.2 nm shifts was observed on complexing 1, 8-dihydroxy anthraquinone with gamma cyclodextrin<sup>64</sup>. An increase or decrease in the absorption intensity of UV band without change in its  $\lambda_{\max}$  is also reported in certain inclusion complexes. For example, the absorption intensity was increased when Bropiramine was complexed with  $\beta$ -cyclodextrin and decreased in the case of purine nucleosides when complexed with  $\beta$ -cyclodextrin<sup>65</sup>.

(d) *Fluorescence spectroscopy:-*

When fluorescent molecules in aqueous solution are included in cyclodextrins, fluorescence spectra may be influenced which indicates the formation of inclusion complexes<sup>62, 66</sup>. The inclusion complex formation generally leads to the change of excitation and emission wavelength of the drug<sup>42</sup>. The effect of inclusion between 1,8-dihydroxyanthraquinone with  $\alpha$ CD,  $\beta$ CD and  $\gamma$ -Cyclodextrin( $\gamma$ CD) on the fluorescence spectra of the drug was studied which indicates that the inclusion complex formation leads to the change of excitation and emission wavelength of the drug. A red shift from 571 to 595 nm of the emission band was observed along with an increase in the intensity of emission when 1, 8-dihydroxyanthraquinone complexed with  $\gamma$ CD by hydrogen bond formation<sup>64</sup>. The

fluorescence emission spectra confirmed that the solubility of the complex formed using  $\beta$ CD and  $\gamma$ CD was more than that of plain drug. Binding of the chromophore in a monomeric form with  $\beta$ CD and  $\gamma$ CD was also confirmed. The spectral change was maximum in  $\gamma$ CD, less in  $\beta$ CD and none in  $\alpha$ CD indicating that the substrate was included inside the cavity completely in case of  $\gamma$ CD due to larger cavity size, and partly into the  $\beta$ CD cavity and no inclusion in case of  $\alpha$ CD<sup>42</sup>.

(e) *Circular Dichroism (CD) spectroscopy:* Circular Dichroism<sup>7, 8</sup> is a useful method to detect cyclodextrin inclusion complexes in aqueous solution. When an achiral guest molecule is included within the asymmetric locus of the cyclodextrin cavity which consists of chiral glucose units, new Circular Dichroism (CD) bands can be induced in the absorption bands of the optically inactive guest<sup>28, 61</sup>. Not only achiral guest molecules but also chiral guest molecules may show changes in circular Dichroism (CD) spectra upon the formation of inclusion complexes with cyclodextrin<sup>63</sup>. For example, the cyclodextrin spectra of  $\beta$ CD complexes with naphthalene derivatives found a remarkable difference in the spectra between 1-substituted and 2-substituted naphthalene complexes, indicating that the steric effects of substituents on the formation of the complex is so strong that the complexation mode may be different for these guest molecules. They suggested that a positive CD band suggests an axial inclusion, while a negative CD band suggests an equatorial inclusion. According to this proposal, 2-substituted naphthalene is estimated to be included axially in the  $\beta$ CD cavity<sup>41</sup>.

(iv) *pH-Potentiometric Titration:*

If the guest compound has a prototropic function, the potentiometric titration method can be used to detect inclusion complex formation<sup>41, 51</sup>. Due to the fact that cyclodextrin usually favour the unionized

guest molecules having a higher hydrophobicity, rather than the ionized ones, the pka value of an acidic guest molecule is usually increased, while those of basic ones is usually decreased by binding to cyclodextrin.

Potentiometric titrations were carried out at  $25.0 \pm 0.1^\circ\text{C}$ . The electrode system was calibrated in terms of hydrogen ion concentration by titrating 2 ml 0.005 M HCl with standardized 0.005 M NaOH. Both stock solutions contained calculated amounts of NaCl to ensure a constant ionic strength of 0.15 M during titration. For complex stability constant determinations, weighed amount of imatinib (1-1.5mM) was dissolved in 20% excess of HCl stock solution and titrated with 0.005 M NaOH under nitrogen stream. Measurements were performed in the presence of various  $\beta$ CD ranging from 0 to 13 mM. During the titration of an acidic (pH 2) Imatinib solution in the presence of cyclodextrin, five different protonation forms can interact with the host. The interaction of the fully protonated species can be neglected, since its mole fraction remains very low in the pH range studied. Hence four complex stability constant could be calculated from each titration curve by using known total host and guest concentrations and measuring the  $[\text{H}^+]$  at each step of titration. The obtained stability constants of the two CD:Imatinib complexes of 1:1 stoichiometry showed that the most stable inclusion complex is formed with the neutral, most apolar Imatinib species, due to hydrophobic interactions with the apolar cavity of cyclodextrin. The monoprotonated Imatinib species  $\text{HI}^+$  showed lower affinity to the host by one order of magnitude. The stability constants slightly increase as the charge of guest increases<sup>51</sup>.

(v) *Microcalorimetry:* -

Changes in thermodynamic properties due to inclusion complexation, can be measured by

microcalorimetry<sup>41</sup>. These changes in enthalpy and entropy are associated with the change in the behaviour of water structure within the cavity, removal of the water from the cavity, restructuring of water around the guest molecule and release of water into the bulk. Other contributions to the overall energies of reaction are due to the restriction in rotation around the glycosidal linkages of the cyclodextrin when the guest molecule enters the cavity<sup>24</sup>.

The Isothermal Titration Microcalorimetry (ITC) experiments were performed for the measurement of the enthalpy changes associated with the addition of successive aliquots of a 2.5 mM solution of guest compound (i.e. Ozonide) at a fixed temperature. Studies were performed with  $\beta$ CD to allow investigation of ionic influences on complexation. The initial ITC experiments with the Ozonide compounds were conducted at 37°C for the purpose of physiological relevance. However, the apparent stoichiometry (n) of the binding was slightly less than unity, due to a small degree of chemical degradation of the unbound Ozonide compounds. From these studies, the stoichiometry of binding interaction between the Ozonide compounds and  $\beta$ CD as confirmed to be 1:1. The calorimetric data for all species with  $\beta$ CD at both 25°C and 37°C indicated extremely strong binding as the association constants were in the order  $10^5$ - $10^6$ /M, which is two orders of magnitude greater than the established  $10^2$ - $10^4$ /m range quoted for other  $\beta$ CD complexes<sup>64</sup>.

#### Conclusion:

A survey of the literature related to use of cyclodextrins for obtaining inclusion complexes of cyclodextrins with practically insoluble drugs was conducted in order to characterize the formation of inclusion complexes by different techniques in the solid and in the solution state. The characterization of inclusion complexes in

the solid state was done by X-Ray Diffractometry, Infra Red spectroscopy, Thermo-analytical methods, Thin Layer chromatography and Scanning Electron Microscopy. The characterization of inclusion complexes in the solution state was done by UV/VIS spectroscopy, Fluorescence spectroscopy, Circular Dichroism spectroscopy, Electron Spin Resonance, pH-Potentiometric titration, Polarography, Conductivity, Microcalorimetry and solubility methods. The study of literature showed that these techniques confirmed the formation of inclusion complexes in the solid and in the solution state.

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