Introduction
INTRODUCTION

1.1 Cyclodextrins

Continuing inquiries into the fashion in which the systems have been found to provide so called restricted microenvironment or cavities of molecular dimensions (of the order of 10 Å) capable of sequestering and controlling the chemistry of reactive molecules.[1] There are many reasons that make the cyclodextrins one of the most important among all the host type macromolecules, which have the ability to complex guest molecules without forming a covalent bond. Cyclodextrins are α (1,4) linked glucopyranose rings forming truncated cone shaped compounds and a wide variety of organic molecules can be complexed in the hydrophobic interior.[2] CD's are often found as building blocks of supramolecular system and in self assemblies.[3] The ability of CD's to form inclusion complexes, in which the physicochemical properties of the guest molecule change with respect to the free molecule, has led to their widespread industrial use and applications in green chemistry.[4] The inclusion complexes result from the specific non-covalent interactions between the guest molecules and CD was rationalised in terms of the spectral shift and the fluorescence quantum yield. Further the forces which play a considerable amount of changes are assumed to be hydrogen bonding,[5] van der Waals forces[6] or hydrophobic interaction,[7] where as the thermodynamic and structural studies of CD complexes have been extensively studied.[8] Cyclodextrins are produced in tons from natural precursors by relatively simple enzymes conversion and can be modified
chemically for different tasks. Cyclodextrins appear to pose no risk when used together with nutrients. Molecular encapsulation using cyclodextrins is already widely utilised in many industrial products, technologies and analytical methods, and as ingredients for drugs, food or cosmetics. The aim of understanding the dynamics of complexation requires a fundamental approach in measuring the dynamics of the inclusion complex, which is challenging and requires ultrafast techniques.

a. Discovery and nomenclature

In 1891, Villiers\[^{9}\] isolated about 3 g of a crystalline substance from 1000 g starch by digesting starch with a particular enzyme (*Bacillus macrons*), and determined its composition as \((C_6H_{10}O_5)_{2-3}H_2O\). This new compound was named “cellulosine” because it was resistant against acidic hydrolysis and did not show reducing properties. Villiers observed two distinct types of “cellulosines”, which were later referred to as \(\alpha\)- and \(\beta\)- cyclodextrins. In 1904, Schardinger\[^{10}\] described the properties of crystalline dextrins that seemed to be identical with the “cellulosines” of Villiers. In all his experiments, the major crystalline product was the so-called \(\alpha\)-dextrin. He found that the iodine reaction was suitable to distinguish between the \(\alpha\)- and \(\beta\)-dextrins.\[^{11}\] In 1936, Freudenberg and co-workers postulated the cyclic structure of the crystalline Schardinger dextrins\[^{12}\] and in 1948, they discovered \(\gamma\)-dextrin and elucidated its structure.\[^{13}\] Three years later Cramer, in his concept of the “cavity in solution”, developed a model for the complexing ability of the cyclodextrins, which was widely accepted in 1957.\[^{14}\] For a long time, the most common and commercially available
cycloextrins were α-cyclodextrin (cyclohexaamylose), β-cyclodextrin (cycloheptaamylose), and γ-cyclodextrin (cyclooctaamylose), which are referred to as α-CD, β-CD, and γ-CD respectively. The larger cyclodextrins (δ-, ε-CD etc.), which were observed by French \cite{15} in the early 1950s, are not regular cylinder shaped structures. They have collapsed, and their real cavity is even smaller than in γ-CD.

b. **Structural features**

Cyclodextrins are macrocyclic oligosaccharides, formed by α-1,4-linked glucopyranose subunits, and appear like toroidal macro rings with a cavity in the center (Figure 1.1).\cite{15} Crystal structure analyses of cyclodextrins have proven that all glucose residues in the ring possess the thermodynamically favoured \textit{4}C$_1$ chair conformation with all substitutions in the equatorial position. The external surface of a cyclodextrin contains secondary hydroxyl groups situated on one of the two rims of the ring, whereas primary hydroxyl groups are placed on the other rim. The inner surface of the cavity is lined by the hydrogen atoms and ether-like oxygen.\cite{16} The overall appearance of a cyclodextrin molecule is less than that of a ring, but rather of a truncated cone with the wide "open" side formed by secondary hydroxyl groups, whereas the primary hydroxyl groups are located on the narrower "closed" side. The cavity diameters (maximum values) are 5.3, 6.5, and 8.3 Å for α-CD, β-CD, and γ-CD respectively. Table 1.1 summarises the most important structural features of α-, β-, and γ-cyclodextrin.
Figure I.1  Structural features of $\alpha$-, $\beta$-, and $\gamma$-cyclodextrin

Table I.1  Structural and some physical features of $\alpha$-, $\beta$-, and $\gamma$-Cyclodextrin.\textsuperscript{17,18}

<table>
<thead>
<tr>
<th>Cyclodextrins</th>
<th>$\alpha$</th>
<th>$\beta$</th>
<th>$\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of glucose units</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>972</td>
<td>1135</td>
<td>1297</td>
</tr>
<tr>
<td>Solubility in water (g/100ml)</td>
<td>14.5</td>
<td>1.85</td>
<td>23.2</td>
</tr>
<tr>
<td>Cavity diameter, Å</td>
<td>4.7-5.3</td>
<td>6.0-6.5</td>
<td>7.5-8.3</td>
</tr>
<tr>
<td>Height of torus, Å</td>
<td>7.9 ± 0.1</td>
<td>7.9 ± 0.1</td>
<td>7.9 ± 0.1</td>
</tr>
<tr>
<td>Approx. volume of cavity, Å</td>
<td>174</td>
<td>262</td>
<td>427</td>
</tr>
</tbody>
</table>
c. Solubility in polar solvents

Because of the presence of many hydroxyl groups, cyclodextrins are soluble in polar solvents.\textsuperscript{[19]} Studies of cyclodextrin chemistry have therefore mostly been performed in aqueous media. Of the three common cyclodextrins $\beta$-CD has the lowest water solubility (see Table 1.1), this is possibly due to intramolecular hydrogen bond formation between the C2-OH group of one glucopyranoside unit with the C3-OH group of the adjacent glucopyranose. In $\beta$-CD, a complete secondary belt is formed by these hydrogen bonds; thus the $\beta$-CD molecule presents a rather rigid structure. The hydrogen bond belt is incomplete in the $\alpha$-CD molecule, because one glucopyranose unit is in a distorted position. Consequently, instead of the six possible hydrogen bonds, only four can be established fully. $\gamma$-CD is the most soluble among the three cyclodextrins\textsuperscript{[17]} due to its flexible non-coplanar structure.

d. Cyclodextrin inclusion complexes

In contrast to the hydrophilic outer lining, the inner surface of cyclodextrins is hydrophobic. In an aqueous solution, the internal cavity of a cyclodextrin is filled with water molecules which are energetically unstable (polar - nonpolar interaction), and are readily displaced by appropriate “guest molecules” which are less polar than water (figure 1.2).\textsuperscript{[14]} In this way, aqueous solutions of cyclodextrins can form complexes with a wide range of solid, liquid, and even gaseous guest molecules.\textsuperscript{[20]} Cyclodextrins can also form inclusion compounds in the solid state.\textsuperscript{[21]}
Figure I.2 Schematic representation of inclusion of the guest inside the host (CD)

i. Stoichiometric ratios and driving force

There is a wide variety of guest molecules forming inclusion complexes with cyclodextrins, ranging from dyes,\textsuperscript{[21-25]} drugs,\textsuperscript{[26]} small anions,\textsuperscript{[27]} carboxylic acids,\textsuperscript{[28]} to alcohols.\textsuperscript{[29]} Such substrates usually form inclusion complexes with 1:1 host:guest stoichiometries (figure I.3). However, the other stoichiometries have been reported \textsuperscript{[24,25,30]} and among which 1:2 is the most common one. The stoichiometries 2:1 and 2:2 and even more complicated ones have also been reported.\textsuperscript{[31-34]}

Much of the works on cyclodextrin inclusion complexes have been focused on the determination of the formation constants of such complexes. The two main components for the driving force of inclusion are the repulsive forces between the included water molecules and the nonpolar cyclodextrin cavity, and the bulk water and the nonpolar guest molecule. Studies on cyclodextrin chemistry indicate that the interactive forces mainly responsible for the formation of cyclodextrin inclusion complexes are van der Waals- forces,\textsuperscript{[35]} hydrophobic interaction,\textsuperscript{[36,37]} strain energy of the macro cyclic
ring,\textsuperscript{[38]} dipolar interactions,\textsuperscript{[39]} and hydrogen bonding.\textsuperscript{[40]} Covalent bonding is inoperative in the inclusion complexes of cyclodextrins.

![Diagram](image)

**Figure 1.3** Schematic representation of production of a CD inclusion complex

ii. Methods of detecting the inclusion process

By forming an inclusion complex with a guest molecule, a cyclodextrin may change some of the physical and chemical properties of the guest molecule; by these changes the complex formation is recognised and studied. For example, spectrophotometric determinations,\textsuperscript{[22]} which rely on the difference in absorptivity of the free and complexed substrate, conductance measurements,\textsuperscript{[42,43]} which depend on the difference in mobility of the free and complexed forms. When achiral guests are inserted into the chiral cyclodextrin cavity, they become chiral and show strong induced Cotton
effects.\cite{43} Sometimes the maximum of the UV absorption is shifted by several nm and the fluorescence intensity is enhanced, because the fluorescing molecule is transferred from the aqueous media into a non-polar surrounding.\cite{44} In NMR spectra, the chemical shifts of the anisotropically shielded atoms are significantly modified.\cite{45}

In most of the cases the reactivity of the included molecule in cyclodextrin cavity decreases, \textit{i.e.} the guest is stabilised, but in many cases the cyclodextrin behaves as an artificial enzyme, accelerating various reactions and modifying reaction pathways.\cite{46} The analysis of x-ray data give the most reliable information about the structure of cyclodextrin complexes.\cite{47}

e. **Practical use of cyclodextrins**

By appropriately orienting the guest molecule inside the cavity, cyclodextrins can act as catalysts in chemical reactions such as hydrolysis and oxidation.\cite{46,48} The major part of research papers on cyclodextrins, almost 25\% deals with their pharmaceutical applications.\cite{49} Many drug molecules are ideal guest molecules for cyclodextrins, because their polarity, molecular mass and structure enable them to get included into the cyclodextrin cavity. The inclusion of drugs in cyclodextrins can enhance drug solubility, retards the evaporation and may cover bad taste.\cite{16} Cyclodextrin can also be used to mask or eliminate unpleasant odours and provide protection against oxidation and proteolysis.\cite{50} Biologically active molecules can be modified in their action if administered in an encapsulated molecular form.\cite{51}
Cyclodextrins are widely used in food, cosmetic, and toiletry production. 70% of all cyclodextrins produced are used in this field,[52] at the same time only 7% of cyclodextrin related research papers are dedicated to this field. There are some applications of cyclodextrins in pesticide formulation. The effects that can be obtained in this area are essentially the same as in the drug formulation, *viz.* enhancement of stability, absorption and persistency.

f. Cyclodextrin derivatives

The natural cyclodextrins form the basis for a practically unlimited number of derivatives, because of the many hydroxyl groups (both of the primary and secondary types) present in the macromolecule. These groups are the most common reaction sites and have been derivatised extensively. Cyclodextrins are derivatised mainly to modify the complex solubility, complex properties (stability constants, guest selectivities), or to introduce groups with specific functions (*e.g.* catalytic). For example, methylated cyclodextrins in general exhibit greater stability than their parent cyclodextrins. Methylation also increases solubility by affecting the distribution of the hydrogen bond system. Increased solubility by appropriate substitution is also well exemplified by the hydroxypropyl, acetyl and sulfopropyloxy derivatives. Among the three main cyclodextrins, β-cyclodextrin is the least soluble in water, which reduces its use both as solubilising agent and carrier of drugs or other organic compounds; therefore most of the cyclodextrin derivatives known presently are derived from β-cyclodextrin.[49]
g. Picosecond and femtosecond studies in cyclodextrin nanocavities

The invention and development of laser technology and other related ultrafast techniques has opened and established new areas in molecular science called femtochemistry and femtobiology.\textsuperscript{[53-57]} The presence of the hydrophobic environment inside the CD cavity and the restricted movement of the guest molecule inside these cavities often influence the photophysical and photochemical properties of the latter.\textsuperscript{[58-61]} In recent years, many investigators have employed such properties of the CD inclusion complexes to understand the mechanistic details of many processes such as fluorescence and phosphorescence enhancement, excimer/exciplex formation, photocleavage, charge and proton transfer, energy hoping and cis-trans isomerisation. The presence of the hydrogen bonding, electron accepting and electron donating groups and twisting groups influences the electronic properties of the encapsulated guest. Both enthalpy and entropy terms determine the energetic balance between the free and encapsulated guest. The fluorescence spectroscopy has been found to be an excellent technique for characterising the inclusion of guest inside the nanocavity. Time-resolved anisotropy experiments gave the rotational time of the guest inside the nanocavity and the overall rotation of the complex. The effect of the charge and size of the guest on the behavior of the confined geometry offered by CD complexes has been investigated using different probes like oxazine 725, oxazine 118 and resorufin in the presence of the CD.\textsuperscript{[62]} The molecular restriction to rotation was examined in a picosecond study of trans-stilbene photoisomerisation in CD.\textsuperscript{[63]} The hydophobic nanocavities provided by CD's
were used to study the photodynamics of a caged (1-(2-naphthyl)-2-ethenyl-(2-benzothiazolium) iodide (1,2-NEB). The photochemistry and dynamics of 1'-hydroxy-2'acetonaphthone (HAN) has been studied in CD nanocavities\textsuperscript{[65,66]} and the stoichiometry of the formed inclusion complex depends on the nature and size of the cage.

1.2 Fluorescence spectroscopy

Fluorescence is the most important property that is used for studying the structure and dynamics of complex systems. Any electronically excited molecule comes back to its ground state either by radiative or non-radiative mechanisms. The fluorophores following the radiative mechanism, emit photons and the process is called fluorescence emission. The fluorescence photons have the information about wavelength, time, polarisation and intensity or the number of photons at a given wavelength. Each of the above parameter of the fluorescence photon gives information about the local environment surrounding the fluorophore under investigation. So the fluorescence intensity, spectrum, polarisation and their time dependence are important parameters that one can use for the characterisation\textsuperscript{[67]} of the complex systems.

The mechanisms by which electronically excited molecules come to ground state are given by the Jablonski diagram as shown in Figure 1.5. The absorption of a photon takes the molecule from ground state (singlet state, $S_0$) to either first excited state (singlet state $S_1$) or second excited state ($S_2$). Then the excited molecule relaxes to the lowest vibronic level of the first excited
state through internal conversion (IC). Then it can relax from the singlet excited state to the ground state via three-mechanisms. First, by emitting a photon (radiative process), second without emitting a photon (non-radiative mechanism) and third it goes to a triplet state ($T_1$) by intersystem crossing (ISC) which is also a non-radiative process. The transition from triplet ($T_1$) to ground singlet state is forbidden and hence is a very slow process relative to fluorescence. Emission from $T_1$ is called phosphorescence, and is generally shifted to longer wavelength relative to the fluorescence. The excited state can also be deactivated by a quenching reaction, in which a quencher Q quenches the excited state of the fluorophore through an excited state reaction.

Figure 1.4  Jablonski diagram showing the energy levels and various processes in an electronically excited molecule.
a. Fluorescence spectra and intensity

Generally, the wavelength of maximum fluorescence intensity is shifted to longer wavelength relative to the wavelength of its absorption maximum. The difference between these two wavelengths is known as Stokes’ shift. The Stokes’ shift arises because of the relaxation from the initially excited state to the ‘ground’ vibronic level of the $S_1$ which involves in loss of energy. The further loss of energy is due to the transitions from $S_1$ to the higher vibrational levels of the ground state $S_0$. The Stokes’ shift is further increased because of general solvent effects. The energy difference between absorption maximum ($v_a$) and emission maximum ($v_f$) is given by Lippert equation (equation 1)\cite{68}. The energy difference of a fluorophore is a function of the refractive index ($n$) and dielectric constant ($\varepsilon$) of the solvent.

$$v_a - v_f = \frac{2}{hc} \left( \frac{\varepsilon - 1}{2\varepsilon + 1} - \frac{n^2 - 1}{2n^2 + 1} \right) \frac{(\mu^* - \mu)}{a^3} \text{ + const} \quad (1)$$

where $h$ is the Planck’s constant, $c$ is the speed of the light, $a$ is the radius and $\mu$ and $\mu^*$ are the ground and excited state dipole moments respectively.

The fluorescence emission spectrum is generally independent of excitation wavelength\cite{67}. This is because of the rapid relaxation to the lowest vibrational level of $S_1$ prior to emission, irrespective of excitation to any higher electronic and vibrational levels. Excitation on the extreme red edge of the absorption spectrum frequently results in a red-shifted emission. The fluorescence emission spectrum is dependent on the excitation wavelength.
and fluorescence excitation spectrum is dependent on the emission wavelength. Also fluorescence excitation spectrum observed for a given emission wavelength differs from that of the absorption spectrum for many system. The fluorescence emission spectrum is generally a mirror image of the absorption spectrum ($S_0$ to $S_1$ transition). This symmetric nature is lost when the structure or the geometry of the excited state is different from that of the ground state.

Fluorescence intensity is a measure of the fluorophores ability to decay through radiative mechanism. Fluorescence quantum yield ($\phi$) is the probability with which an excited fluorophore emits a photon and is related to the radiative and non-radiative rates of the deactivation of the excited state as shown below.

$$\phi = \frac{k_r}{k_r + k_{IC} + k_{ISC}}$$ (2)

where $k_r$ is the radiative rate of the fluorescence and $k_{IC}$, $k_{ISC}$, $k_q$ represent the rate constants for internal conversion and intersystem crossing respectively.

b. **Fluorescence intensity decay and lifetime**

Time resolved fluorescence methods give the kinetic information on the various processes involved in the deactivation of the excited state. With the advent of lasers as excitation light sources, it is possible to have the time resolution of the order of femtoseconds for the excited processes under investigation. The typical fluorescence intensity decay is a plot of
fluorescence intensity as a function of time. For a simple system having a single fluorophore the fluorescence intensity decay, \( I(t) \) is a single exponential and is given as

\[
I(t) = I_0 e^{-\frac{t}{\tau}}
\]  

(3)

where \( I_0 \) is the initial intensity and \( \tau \) is the fluorescence lifetime. The fluorescence lifetime, \( \tau \), represents the average time spent by the fluorophore in the excited state before coming to the ground state. The fluorescence lifetime is related to the radiative and the nonradiative rates. The fluorescence quantum yield of the excited state is given as equation (4)

\[
\tau = \frac{1}{k_r + k_{nr}} = \frac{\phi}{k_r}
\]  

(4)

For a complex system having multiple fluorescent species, is not fitted to one exponential system. Then the fluorescence intensity decay is given as

\[
I(t) = \sum_{i=1}^{n} B_i e^{-\frac{t}{\tau_i}}
\]

(5)

where \( B_i \) and \( \tau_i \) are the \( i^{th} \) pre-exponential factor and the lifetime in the multiexponential decay respectively. Pre-exponential factors are generally positive but can be negative whenever there is an excited state kinetics. A negative pre-exponential factor indicates the formation of an emissive species in the excited state reaction.
c. Fluorescence anisotropy

Many groups explain the principle underlying the fluorescence depolarisation.\(^{69-85}\) The fluorescence emission, emitted from the samples excited with polarised light is also polarised. This polarisation is due to the photoselection of the fluorophores according to their orientation relative to the detection of the polarised excitation. This photoselection is proportional to the square of the cosine of the polarised excitation angle between the absorption dipole of the fluorophore and the axis of polarisation of the excited light. The orientational anisotropic distribution of the excited fluorophore population relaxes by rotational diffusion of the fluorophores. The polarised fluorescence emission becomes depolarised by such process. The fluorescence anisotropy measurement reveals the average angular displacement of the fluorophore, which occurs between absorption and subsequent emission of a photon. The steady state fluorescence anisotropy \(r\) defined as in following equation.

\[
\frac{I_\| - I_\perp}{I_\| + 2I_\perp}
\]  

where, \(I_\|\) and \(I_\perp\) represent the fluorescence intensities when the orientation of the emission polarised is parallel and perpendicular to the orientation of the excitation polarised respectively. The fluorescence anisotropy \(r\) is a measure of the average depolarisation during the lifetime of the excited fluorophore under steady state condition. But the time resolved measurement of fluorescence anisotropy using ultrafast polarised excitation source (laser)
give insight into the time dependent depolarisation. The time dependent fluorescence anisotropy or fluorescence anisotropy decay $r(t)$ is defined as follows,

$$r(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)}$$ \hspace{1cm} (7)

where $I_{\parallel}(t)$ and $I_{\perp}(t)$ are the fluorescence intensity decays collected with the polarisation of the emission polariser with the polarisation of the emission polariser kept parallel and perpendicular to the polarisation of the excitation source respectively. For a fluorophore in a simple solvent, the fluorescence depolarisation is simply due to rotational motion of the excited fluorophore and the decay parameters depend on the size and shape of the fluorophore. For spherical fluorophores, the fluorescence anisotropy decay has single rotational correlation time and is show in equation

$$r(t) = \sum_j r_0 \exp \left( -\frac{t}{\tau_r} \right)$$ \hspace{1cm} (8)

where $r_0$ is initial anisotropy (anisotropy at time $t=0$ or anisotropy observed in the absence of any depolarising processes) and $\tau_r$ is the rotational correlation time. The initial anisotropy $r_0$ is related to the angle $\beta$ between the absorption and emission dipoles of the fluorophore under study and is given as

$$r_0 = \frac{2}{5} \left[ \frac{3\cos^2 \beta - 1}{2} \right]$$ \hspace{1cm} (9)
where the value $r_0$ can vary between 0.4 and -0.2 as the angle $\beta$ varies between 0° and 90° respectively. The rotational correlation time $\tau_r$ of the fluorophore is governed by the viscosity ($\eta$), temperature (T) of the solution, molecular volume (V) of the fluorophore and the Boltzmann constant (k). This is given by the Stokes-Einstein relation as shown below (10)

$$\tau_r = \frac{\eta V}{kT}$$  \hspace{1cm} (10)

The time resolved anisotropy decay measurements provide information on the average angular displacement of the fluorophore during the excited state lifetime of the fluorophore. Due to the process of photoselection\textsuperscript{[86]}, fluorescence emission is polarised to some extent. The origin of this phenomenon is based on the existence of transition moments for absorption and emission, which lie along specific direction within the fluorophore structure. The dependence of fluorescence anisotropy upon rotational motion has resulted in numerous applications. However, this theory relates anisotropy decay to the shape of the molecule. The rotating fluorophore need not be symmetric about any axis. Hence, non-spherical molecules are being described as a general ellipsoid or an ellipsoid of revolution\textsuperscript{[87]}. Two cases are possible, the prolate ellipsoid, in which the symmetry axis is longer than the other two equal axes ($a>b=c$) and an oblate ellipsoid, in which the symmetry axis is shorter than the other two equal axes ($a<b=c$).

The theory for the rotational diffusion of ellipsoids and measurements by the fluorescence anisotropy can be traced to the classical reports by
Perrin\textsuperscript{[75]} and the theory has been summarised in several reviews\textsuperscript{[75 - 85]}. The theory of non-spherical molecules is usually described in terms of prolate and oblate ellipsoids. The anisotropy decay of an ellipsoid of revolution can display two or three correlation times, which are functions of two diffusion rotational coefficient ($D_\parallel$ and $D_\perp$). The amplitudes of the anisotropy depend on the orientation of the transition moments. If one of the transition moments is directed along any of the symmetry axis of the ellipsoid, then the decay becomes biexponential. Zewail\textsuperscript{[74]} group has approximated the decay constant as $(3D_\perp + 3D_\parallel)^{-1}$. It is also useful to define a set of rotational correlation times ($\tau_{r_1}$ and $\tau_{r_2}$), which are functions of rotational diffusion coefficients\textsuperscript{[72]} as shown in equations (11), (12) and (13).

\begin{align*}
\tau_{r_1}^{-1} &= 6D_\perp \quad (11) \\
\tau_{r_2}^{-1} &= 2D_\perp + 4D_\parallel \quad (12) \\
\tau_{r_3}^{-1} &= 5D_\perp + D_\parallel \quad (13)
\end{align*}

Depending upon the shape of the ellipsoid of rotation and the orientation of the transition moments, a variety of anisotropy decay can be predicted\textsuperscript{[88-90]}. If both the transition moments are perpendicular to the long axis then in that case the anisotropy decays with two correlation times. The decay is more rapid in one case because the faster rotation in one axis displaces the transition moment. This results in rapid randomisation along that axis. The other decay is slower which will result in complete depolarisation. For the prolate ellipsoids one correlation time is longer and is determined by
the rotation that displaces the long axis of the prolate ellipsoid. The ability to
detect these correlation times depends on the amplitude, which in turn
depends on the angles with respect to the symmetry axis.

1.3 Importance of acridinedione dyes

Acridinedione dyes have been developed recently as one of the
efficient laser dyes\textsuperscript{[91]} and these dyes have structural similarity with NADH.
Reduced nicotinamide adenine dinucleotide (NADH) is an important
coenzyme in biological system.\textsuperscript{[92]} These dyes have been shown to mimic the
NADH analogs largely because of its tricyclic structure, which is capable of
protecting the enamine moiety.\textsuperscript{[93]} Drugs such as nifedipine, nimoldipine,
nisoldipine fall into this class and found to have enormous application in
medicine such as calcium antagonists, antihypertensive agents and anti-
inflammatory drugs.\textsuperscript{[94]}

Acridinedione is a bifunctional molecule, due to this nature it act as
both an electron donor and acceptor. Due to the presence of bifunctional
 group, this molecule undergoes various interesting reactions in the excited
state. This dye shows lasing around 480 nm under Nd-YAG and nitrogen
laser excitation and its lasing efficiency are comparable to coumarin 102.\textsuperscript{[91]}
Derivatives of acridinedione dyes were used as useful tool for the estimation
of aldehydes.\textsuperscript{[95]} Acridinedione dyes were also used as photoinitiators for
polymerisation of acrylates and methyl acrylates.\textsuperscript{[96]} These dyes were also
used as photosensitiser for the onium salt decomposition.\textsuperscript{[97]}
a. Photophysical and photochemical properties

The longest wavelength absorption band (360 - 400 nm) of acridinedione is sensitive to solvent polarity and is due to intramolecular charge transfer. The shift in the absorption maximum on varying the substituent on the nitrogen and in the ninth position has been reported.\textsuperscript{[98]} These dyes emit around 430 - 470 nm on excitation in the long wavelength absorption band.

The ground and excited state dipole moments of acridinedione dyes were reported.\textsuperscript{[99]} These dyes were reported to be more polar in the excited state than the ground state. The change in the emission maximum and fluorescence quantum yields on varying the solvents and changing substituents have been investigated.\textsuperscript{[98]}

Triplet-triplet absorption spectra for the ADD dyes have been reported in methanol, which shows that the triplet absorption maximum is dependent on substituent in the ninth position and on nitrogen.

Photochemical studies

Laser flash photolysis of acridinedione dyes resulted in transient absorption maximum at 480 and 620 nm region.\textsuperscript{[100-101]} The transient at 480 nm region was assigned as anion radical, which has lifetime of 1-3 microseconds. This was further supported by the pulse radiolysis\textsuperscript{[102,103]} experiments and the electrochemical reduction.\textsuperscript{[104]} The transient at 620 nm is assigned as triplet-triplet absorption as it is quenched by oxygen which is an
well known triplet quencher. The acid base property of ADD dyes in the
ground and excited state was studied by Venkatachalapathy et al.\textsuperscript{[105]} In the
excited state these dyes have higher acidity and undergo proton transfer
reactions with amines having pK\textsubscript{a} value more than seven.\textsuperscript{[102]}

Acridinedione dyes undergo photoionisation in the excited state and
results in the formation of cation radical. The cation radical absorbs at 440
and 680 nm, which is confirmed by the pulse radiolysis of acridinedione in
presence of one electron oxidant.\textsuperscript{[106,107]} Recently, Marcinek et al.\textsuperscript{[108]} reported
that the cation radical undergo keto-enol tautomerisation. The enol form of
the cation radical absorbs at 550 nm. The nature of the photoproducts depends
on the substitution in the 9\textsuperscript{th} position and in the nitrogen.\textsuperscript{[100]} Recent findings
on the photochemistry of acridinedione dyes in micelles reveal the
involvement of different intermediates based on the pH of the medium.\textsuperscript{[109]} A
carbon centered radical is established under neutral and alkaline conditions
and a enol form of the cation radical is observed under acidic condition.
Presence of equilibrium between enol form of the cation radical and carbon
centered is also established. This study forms as a model for the NADH
oxidation under different environments.

b. Electrochemical studies

Srividya et al. have reported\textsuperscript{[104]} that all the acridinediones undergo
irreversible oxidation around +1.0 V and they have observed that the shape of
the cyclic voltammograms and the oxidation potential were influenced by
oxygen. The cyclic voltammograms depend upon the substitution on nitrogen
and in the 9th position. The N-H compounds yielded tetrahydroacridinediones and N-substituted compounds gave the acridinium salt.\textsuperscript{[100]} Radicals formed during electrochemical oxidation has been trapped and identified. They have proposed a suitable mechanism for electrochemical oxidation based on the product and intermediates formed.

1.4 Theoretical concepts of molecular simulation techniques

Molecular Mechanics (MM) calculations are derived from classical energy calculations based on simple potential energy functions considering various strain energy and non-bonded interactions to describe realistic structure. The application of molecular simulation technique to model intermolecular interactions allows one to quantify the energy parameters involved in the interaction and the conformational changes that ADR/β-CD complexes undergoes during interaction.

van der Waals interactions acts as the main driving force for the interaction between CD and guest compounds. The type of molecules that can be complexed in the hydrophobic cavity of CD’s depends mainly on the geometric factors such as shape and size. Computer modeling studies of cyclodextrin complexes are an important area to the understanding of the mechanism of complex formation.

In MM, the structure of the molecule is described by treating the molecule as a series of points (atoms) connected by springs (bonds), where each spring is characterised by an equilibrium bond length, bond angle and
corresponding force constant derived from Hooke’s law. The spring
deformation describes the ability of bonds to stretch, bend and twist. Non-
bonded atoms interact through van der Waals attraction, steric repulsion and
electrostatic attraction / repulsion. The energy associated with a given
conformation of a molecule is predicted as a sum of stretching, bending,
torsion and non-bonded interactions present in the molecule.

a. Molecular mechanics force field

Force fields enable the potential energy of the molecular system to be
calculated rapidly and accurately. It employs a combination of internal
coordinates to describe the bonded interactions and inter atomic distances to
describe non-bonded interactions (van der Waals and electrostatic). The
functional forms used to fit potential energy surface (PES) range from simple
quadratic forms to Morse functions, Fourier expansions, Lennard–Jones
potentials etc. The force-field interpolates and extrapolates from the empirical
data of the small set of molecules used to parameterise the force field to a
large set of molecules and structures. In MM the total energy associated with
a given conformation of a molecule is given by

$$E_{\text{Total}} = E_{\text{Valence}} + E_{\text{Crossterm}} + E_{\text{Nonbond}}$$

(14)

The energy of valence interactions is accounted from bond stretching
($E_{\text{Bond}}$), valence angle bending ($E_{\text{Angle}}$), dihedral angle torsion ($E_{\text{Torsion}}$) and out
of plane ($E_{\text{Outp}}$) interactions. Interaction between atom pairs involved (Urey-
Bradley term – $E_{UB}$) is also accounted here and the expression is
\[ E_{\text{Valence}} = E_{\text{Bond}} + E_{\text{Angle}} + E_{\text{Torsion}} + E_{\text{Oop}} + E_{\text{UB}} \] (15)

The accuracy of force field is improved by the inclusion of cross terms originating from the bond or angle distortions caused by nearer atoms. The cross terms normally included in the force field is stretch-stretch, stretch-bend-stretch, bend-bend, torsion-stretch, torsion-bend-bend, bend-torsion-bend, stretch-torsion-stretch interactions.

Non-bonded interactions arise from electrostatic, hydrogen bonding and van der Waals interactions. Most of the theoretical studies of biomolecular systems have been developed based on potential energy functions of the ‘molecular mechanics’ type.\[^{110}\]

b. Classification of force field

The quality of the force field and its ability to predict the molecular properties relies on the analytical expression adopted for the potential energy surfaces. Force fields are classified as follows: second generation force fields, broadly applicable force field, classical force fields and special purpose force fields.

Second generation force fields are consistent force fields such as CFF91, PCFF, CFF, COMPASS and Merck molecular force field\[^{111-122}\]. The family of consistent force fields have been parameterised for organic compounds containing H, C, N, O, S, P, halogen atoms, ions, alkali metal cations and several divalent metal cations. The Merck molecular force field
(MMFF93) is developed specially for the study of receptor-ligand interactions between biomolecules and other chemical structures. The Extensive systematic force field (ESFF), Universal force field (UFF) and Dreiding force field are broadly applicable force fields \[^{123, 124}\]. ESFF has been designed to cover the properties of all the elements of periodic table and conformational energies. The UFF is an excellent general-purpose force field generated based on the elements hybridisation and connectivity. VALBOND is a combination of VALBOND theory augmented with UFF.\[^{125}\] Dreiding force field is an all-purpose force field, which allows reasonable prediction for a very large number of structures.

The standard AMBER force field, Homan’s carbohydrate force field, CHARMM force field and Consistent Valance force field (CVFF) come under classical force fields. The CHARMM force field has been widely used in molecular simulations.\[^{126,127}\] The force field CVFF is designed with some unharmonic and cross term enhancements and is being used as a traditional default force field in the Discover program of MSI.\[^{128}\] In this work, molecular simulation calculation using the default force field CVFF has been carried out.

c. Molecular minimisation algorithms

After the application of force field, a stable conformation is achieved by the use of certain optimisation algorithms. Energy minimisation algorithm use input from the potential energy function, which is an explicit and
differentiable function of Cartesian coordinates. This information is used to generate a new set of coordinates in an effort to reduce the potential energy until the molecular structure is minimised. Energy minimisation methods can therefore be used to refine molecular structures in the sense of eliminating the worst steric conflicts and adjusting bond lengths and bond angles to values nearer to their respective optima, and they can provide information on the relative energies of different conformations.

The optimisation methods can be classified by its order, which is defined by the higher order derivative used in that method. The zeroth order method (Grid method), first order method (Steepest Descent and Conjugate Gradient) and the second order method (Newton-Raphson) have been widely used in molecular simulations.

d. Basic principles of molecular mechanics

Molecular mechanics is used to describe (i) the structure and stability of a molecular system, (ii) the free energy of different states of a molecular system and (iii) the reaction processes with molecular systems in terms of interactions at the atomic level based on the force field. To accomplish this, MM codes must generate an equation for each molecule that relates its potential energy to its nuclear coordinates. This equation defines a potential hypersurface of 3N+1 dimensions where, 3N dimensions are used to specify the positions of each of the N atoms in the molecule and the extra dimension defines the potential energy. Each point on the potential surface corresponds to the geometry of the molecule. Minima on the potential surface correspond
to the stable conformation of the molecule. MM codes are able to locate these minima and thereby identify stable conformations. The potential energy associated with each stable conformation is used to assign their relative stability.

In MM models, the equation that relates a molecule's potential energy to its conformation is composed of a sum of terms that represent different types of energy contributions. The types of term that are included in a potential energy equation (16) often differ from one force field parameter to the other. In the simplest model, the total potential energy is broken down into four components:

$$U_{\text{total}} = \sum U_r + \sum U_0 + \sum U_\phi + \sum U_{\text{vdw}}$$  

(16)

The sum represents contributions of the potential energy, $U_{\text{total}}$, due to bond stretching and compression terms, $U_r$, valence angle bending terms, $U_0$, internal rotational or torsional terms, $U_\phi$, and van der Waals interactions, $U_{\text{vdw}}$.

e. Force field parameters

The terms in the potential energy equation are described by simple analytical expressions with adjustable parameters. These are called potential functions. A set of potential functions and its corresponding set of parameters together, are called as force fields. Force fields are categorised by the type of potential functions they contain. The force fields commonly used for describing molecules employ a combination of internal coordinates and terms
(bond distances, bond angles, torsions, etc) to describe part of the potential energy surface due to interactions between bonded atoms and nonbonded terms to describe the van der Waals and electrostatic interactions between atoms. The actual coordinates of a model combined with the force field data create the energy expression for the model. This energy expression is the equation that describes the potential energy surface of a particular model as a function of its atomic coordinates.

The Universal force field \[^{130}\] is an excellent general-purpose force field. It was parameterised for the full periodic table and has been carefully validated for main-group compounds, organic molecules and metal complexes. Allinger’s MM2 force field can also be applied for organic molecules. The Extensible and Systematic force field (ESFF) supports 879 atom types covering all the elements of the periodic table up to Rn.

f. Minimisation

Minimisation is an important method for exploring the potential energy surface to find the configurations, which are stable points on the surface. It is performed in two steps. First, the energy expression must be defined and evaluated for a given conformation. Next, the conformation is adjusted to lower the value of the energy expression. A minimum may be found after one adjustment or may require many thousands of iteration depending on the nature of the algorithm, the form of the energy expression and the size of the model. The minimisers used are discover and discover_3 module in Insight II package. The popular and widely used minimisation algorithms are steepest
descent and conjugate gradient method. In the steepest descent method, the line search direction is defined along the direction of the local downhill gradient. The exclusive reliance of the steepest descent method on the gradient is its both weakness and strength. Convergence is slow near the minimum because the gradient approaches zero, but the method is extremely robust, even for the systems that are far from harmonic. Therefore, this method is often used when the gradients are large and the configurations are far from the minimum. This is commonly the case with initial relaxation of poorly refined models. The more advanced algorithms like conjugate gradient are often designed to begin with the steepest descent as the first step. In this algorithm, the time per iteration may be longer than for the steepest, but more efficient convergence to the minimum is achieved by conjugate gradients.

Of the available computational approaches ab. initio methods, semi empirical quantum mechanics methods are used for the complex studies. However, these studies are not satisfactory while the molecular mechanics can easily handle structures as large as cyclodextrin complexes. There is not much information in the literature concerning the inclusion complex phenomenon of $\beta$-CD complexes and only a few papers are available for molecular modeling of $\beta$-CD inclusion complexes.$^{[131-134]}$ Molecular modeling has been recently proposed as a powerful tool for obtaining information about the three-dimensional structures and the interaction energies of the inclusion complexes.$^{[135-137]}$ This has been substantiated by carrying out the molecular modeling studies and calculating the binding energies.