SUPRAMOLECULAR CHEMISTRY OF
BETA– AND GAMMA–
CYCLODEXTRIN DIMERS

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Thesis submitted for the degree of
Doctor of Philosophy
in
The University of Adelaide
School of Chemistry and Physics

October 2010
CHAPTER 1

INTRODUCTION
1.1. Cyclodextrins

1.1.1. Introduction and General Overview of Cyclodextrin Chemistry

Cyclodextrins, CDs (Figure 1.1), also known as Schardinger dextrins, cycloamyloses and cycloglucoamyloses, belong to the family of cyclic oligosaccharides. The three most common and practically important natural CDs are α-, β- and γCD, which are comprised of 6, 7 and 8 α-1,4-linked D-glucopyranose units, respectively. They are produced through the degradation of starch by the enzyme cyclodextrin glycosyl transferases (CGTases),¹ which are isolated from bacteria, mainly Bacillus macerans.²³

![Diagram of CDs](image)

**Figure 1.1.** Schematic structure representation of βCD (A), the chair conformation of α-1,4-linked D-glucopyranose unit (B) and a side view truncated torus representation of CDs and the main structural features (C).

Cyclodextrins and their modified forms are at the centre of supramolecular chemistry. The interest in CD research mainly arises from their ability to act as robust hosts to a wide variety of guest species, as a consequence of their hydrophobic annuli being able to form host–guest complexes in aqueous solution.⁴⁻⁵ Consequently, many thousands of tonnes of CDs are produced and used in a variety of industries annually.⁶⁻⁹ Some of the major uses of CDs are in the field of pharmaceuticals, as drug carriers, solubilisers and ingredients to improve the stability, bioavailability and pharmacokinetic properties of drugs.¹⁰⁻¹⁴ They are
also used in the food, cosmetics, personal care and toiletry industries, mainly for stabilisation, odour control, flavour protection and flavour delivery in lipsticks, water solubility and enhanced thermal stability of oils.\textsuperscript{15,16} Additionally, they are used in the agricultural and chemical industries, as additives in agricultural chemicals or catalysts to remove or detoxify waste materials;\textsuperscript{8} and in analytical sciences, such as in enantiomer separations by high-performance liquid chromatography (HPLC) or gas chromatography (GC), or as chiral agents in nuclear magnetic resonance (NMR) and circular dichroism (CD) studies.\textsuperscript{4,17}

1.1.2. Structure and Properties of Cyclodextrins

The three major cyclodextrins, $\alpha$-, $\beta$- and $\gamma$CD are crystalline, homogeneous, nonhygroscopic substances, which are torus-like macro-rings built up from 6, 7 and 8 glucopyranose units, respectively,\textsuperscript{4} although larger cyclodextrins comprised of 9–13 units have also been isolated and identified.\textsuperscript{17} The glucose units possess a $^4C_1$ chair conformation, which give CDs the overall shape of a truncated cone, with the wider end formed by the secondary 2- and 3-hydroxyl groups and the narrow end by the primary 6-hydroxyl groups (Figure 1.1, C). The number of glucose units determines the dimension and size of the CD annulus (Figure 1.2). The annulus is lined by the hydrogen atoms and the glycosidic oxygen bridges. The non–bonding electron pairs of the glycosidic oxygen bridges are directed toward the inside of the annulus producing a high electron density.\textsuperscript{4} The CD annulus is relatively hydrophobic, while its exterior is hydrophilic. Hydrogen bonds are formed between the 2-hydroxyl and the 3-hydroxyl groups of adjacent glucose units making the structure of CDs quite rigid.\textsuperscript{17} Among the three CDs, $\beta$CD is the most rigid due to the complete belt of hydrogen bonds, which coincides with it being the least soluble in water. The hydrogen-bond belt is incomplete in $\alpha$CD while the structure of $\gamma$CD is noncoplanar and more flexible, which evidently makes it the most water soluble of the three.\textsuperscript{4}

The main physicochemical properties of CDs are summarised in Table 1.1. The conformation of CDs in solution is almost identical to that in the crystalline state.\textsuperscript{17} Cyclodextrins are stable in alkaline solutions, but they are susceptible to acid hydrolysis.\textsuperscript{18}
Table 1.1. Characteristics of α-, β- and γCD.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>α</th>
<th>β</th>
<th>γ</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of glucose units</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Molecular weight (g mol$^{-1}$)</td>
<td>972.85</td>
<td>1134.99</td>
<td>1297.14</td>
<td></td>
</tr>
<tr>
<td>Solubility in water (mol dm$^{-3}$), 25 °C</td>
<td>0.121</td>
<td>0.0163</td>
<td>0.168</td>
<td>19</td>
</tr>
<tr>
<td>Cavity diameter, narrow–wide (Å)</td>
<td>4.7–5.3</td>
<td>6.0–6.5</td>
<td>7.5–8.3</td>
<td>4, 17</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>
<td>4, 17</td>
</tr>
<tr>
<td>pK$_a$ values, 25 °C</td>
<td>12.33</td>
<td>12.20</td>
<td>12.08</td>
<td>20, 21</td>
</tr>
</tbody>
</table>

Figure 1.2. Approximate geometric dimensions of α-, β- and γCD.$^4$

1.1.3. Cyclodextrin Host–Guest Complexes

The most important characteristic of CDs is their ability to form host–guest complexes with a great variety of guest molecules (Figure 1.3).$^{4, 18}$ In water, the slightly apolar CD annulus is occupied by water molecules which can be readily replaced by appropriate guest molecules that are more hydrophobic. This host–guest complexation process is largely enthalpy–driven.$^{4, 22}$
Figure 1.3. Schematic representation of the host–guest complex formation between a hydrophobic guest and CD. The small circles represent water molecules.

The formation of a 1:1 host–guest complex in solution is an equilibrium process between the dissociated and associated species (Eqn. 1.1), which is characterised by the complex stability constant, $K_1$ (Eqn. 1.2). This is the simplest and most frequent case, however 2:1, 1:2 or 2:2 stoichiometries exist for CD host–guest complexes.\(^\text{4,23-31}\)

$$\text{CD} + \text{Guest} \underset{K_1}{\overset{}{\rightleftharpoons}} \text{CD.Guest} \quad (1.1)$$

$$K_1 = \frac{[\text{CD.Guest}]}{([\text{CD}][\text{Guest}])} \quad (1.2)$$

Three-component CD complexes have been reported, where third species such as alcohols or amines act as a “space regulator” to optimise the fit of the guest to the CD annulus. As a result, 1:1:1 complexes are formed, as exemplified by $\gamma$CD/pyrene/n-butanol,\(^\text{32}\) (B\(\beta\)CD or $\gamma$CD)/pyrene/tert-butanol,\(^\text{33}\) B\(\beta\)CD/acridine/alcohol,\(^\text{34}\) or $\gamma$CD/(pyrene or naphthalene)/aliphatic amines ternary complexes.\(^\text{35}\) More unusual stoichiometries have also been reported, such as a 2:1:1 complex of $\gamma$CD/1-pyrene butyrate/n-hexanefulfonate.\(^\text{36}\)

Much research interest has centred on the nature of the interactions during the host–guest complexation as well as the structure of the CD complexes.\(^\text{37}\) X-ray crystallography, nuclear magnetic resonance (NMR) spectroscopy, optical spectroscopy (UV–vis, fluorescence, circular dichroism) and molecular modelling methods have been frequently used in these structural studies.
The earliest crystallographic studies were carried out on αCD/substituted benzene complexes by Harata et al., where it was shown that the phenyl ring fitted tightly in the αCD annulus. For the larger βCD and γCD, there is more flexibility in the way the guest can interact with the CD annulus, resulting in greater structural variety for similar guest species. For example, 4-tert-butyltoluene orientates differently from 4-tert-butylbenzyl alcohol and 4-tert-butylbenzoic acid upon complexation with βCD. In the first case, the tert-butyl group is directed toward the primary hydroxyl rim and the methyl group toward the second hydroxyl end, while in the last, the tert-butyl moiety is near the secondary face.

Nuclear magnetic resonance (NMR) spectroscopy has been widely used to elaborate the CD host–guest complex phenomenon. Demarco and Thakkar were the first to observe \(^1\)H chemical shift variations of the CD H\(_3\) and H\(_5\) resonances in the presence of various aromatic guest molecules, an indication that host–guest complexation in the CD annulus had taken place. Two-dimensional \(^1\)H NMR techniques, such as Rotating Frame Overhauser Enhancement Spectroscopy (ROESY) or Nuclear Overhauser Enhancement Spectroscopy (NOESY), have also provided a great deal of details about the CD complexation structure. In such NMR spectra, any NOE cross-peaks observed between the CD resonances (H\(_3\), H\(_5\) or H\(_6\)) and those of the guests are indicative of the two sets of protons being less than 400 pm apart, which infers complexation in the CD annulus has occurred. In addition to \(^1\)H NMR, \(^13\)C NMR spectroscopy has also been used to study CD complexes.

Optical spectroscopy techniques, such as UV–vis, fluorescence and circular dichroism (CD) spectroscopy, are powerful tools to elucidate the CD complexation phenomena. A chromophore present within the guest or the CD molecule can exhibit some variations in its optical spectra upon experiencing a more hydrophobic environment due to complexation. These changes can be in the absorbance or fluorescence intensity, shifts of the absorbance or emission maxima or the presence of induced circular dichroism of achiral guest molecules due to complexation within the chiral CD environment. These methods are the most commonly used for studies of CD/aromatic guest complexation.

Finally, theoretical calculations have also been widely applied to predict the structures of CD complexes. Both quantum-based and empirical force field methods have been used for this purpose, among which molecular mechanics calculations are the most
common. However, due to the lack of solvent involvement, these calculations are generally interpreted together with experimental findings.

1.1.4. Cyclodextrin Complexation Stability and Thermodynamics

There are many factors that contribute to the stability of CD complexes. The most common factors are believed to be the displacement of water molecules from the CD annulus by the guest species and its consequent desolvation; the relief of conformational strain energy of the free CD; hydrophobic and van der Waals interactions; electrostatic interactions, mainly dipole-dipole; hydrogen bonding; induction forces; and London dispersion forces.

There is no obvious correlation between complex stability and structural features of the guest molecules. However, it is generally accepted that a tight fit of the guest to the CD annulus conduces to strong complexation. For instance, αCD complexes more strongly to straight-chain guests than βCD, while adamantyl guests will complex better in βCD by comparison with αCD. For charged guest species, the most probable complexation mode involves the insertion of the less polar (more hydrophobic) part of the guest into the CD annulus, while the more polar charged group remains exposed to the bulk water outside the annulus. Generally, neutral guests have higher affinities than corresponding charged species of similar type.

The thermodynamic parameters associated with complexation are often calculated using the van’t Hoff or Arrhenius equations, based on the dependence of complexation equilibrium or rate constants on the temperature. The linear enthalpy–entropy compensatory relationship (Eqn. 1.3) has been long studied in an attempt to elaborate the mechanism for the combined driving forces behind the CD complexation. In such an equation, the slope α indicates to what extent the enthalpic gain induced by any alterations in host, guest or solvent is cancelled by the accompanying entropic loss. On the other hand, the intercept $T\Delta S^o$ represents the inherent complex stability ($\Delta G^o$) obtained when $\Delta H^o = 0$. This means that if $T\Delta S^o$ is positive, the complex is stabilised even in the absence of enthalpic stabilisation.

$$T\Delta S^o = \alpha\Delta H^o + T\Delta S_0^o$$

(1.3)
In the analysis of a large amount of data, the $T\Delta S^0$ values were found to be linearly correlated with the $\Delta H^0$ values for all complex systems of $\alpha$-, $\beta$- and $\gamma$CD (Figure 1.4).\textsuperscript{22} The slope $\alpha$ increases gradually from 0.79 to 0.80 to 0.97 and the intercept $T\Delta S^0_0$ also increases from 8 to 11 to 15 kJ mol$^{-1}$, as the CD ring size increases from $\alpha$- to $\beta$- to $\gamma$CD. This trend is consistent with greater ring flexibility and a larger number of water molecules in and around the CD annulus as the ring size increases. The large slopes of almost unity and high intercept values indicate that while the complexation is enthalpy–driven, any enthalpic gain is almost cancelled out by the loss in entropy due to the substantial conformation changes of both the CD and the guest and other factors as a result of complexation.\textsuperscript{22}

![Figure 1.4](image.png)

**Figure 1.4.** Individual enthalpy–entropy compensation plots for natural $\alpha$- (○), $\beta$- (▲) and $\gamma$CD (■).\textsuperscript{22}

### 1.2. Modified Cyclodextrins

#### 1.2.1. Approaches to Selective Modification of Cyclodextrins

Although native CDs themselves are of much interest as hosts for many guest species, there are a lot of limitations in their structural features in terms of size, shape and availability of chemically useful functional groups.\textsuperscript{57} Therefore, it is a logical step to think
about modifying them in such ways that the size and shape can be altered and new functional groups are introduced for greater functionality and host–guest specificity.\textsuperscript{4,5,18,57}

All modifications of CDs are done at the hydroxyl groups, and since these are nucleophilic, the initial reaction involves an electrophilic attack on these groups. However, due to their abundance and similarity, these hydroxyl groups compete for the reagent and make selective modification difficult.\textsuperscript{57} Of the three types of hydroxyl groups, those at the 6-position are the most basic and often most nucleophilic, those at the 2-position are the most acidic ($pK_a \sim 12.1$), and those at the 3-position are the most inaccessible.\textsuperscript{58,59}

Monosubstitution at the 6-position is most commonly achieved by nucleophilic attack on mono-6-sulfonyl-CDs. The most popular route for mono-6-CD sulfonation (notably $\beta$CD) is through the reaction of the CDs with $p$-toluenesulfonyl chloride in aqueous alkaline solution, with reasonable yield and purity.\textsuperscript{60} Subsequent nucleophilic displacement of the tosyl group by suitable nucleophiles affords monoiodo-, azido-, thio-, hydroxyamino- or alkylamino CDs.\textsuperscript{57} Modification at the wide rim often involves sulfonation at the 2-position by $p$-toluenesulfonyl chloride in DMF with dibutyltin oxide and triethylamine as catalysts.\textsuperscript{61} The elimination of the tosyl group at the 2-position affords the 2,3-manno-epoxide CDs,\textsuperscript{62} which can subsequently be subject to further nucleophilic attack to afford 3-substituted CDs. These are the most common and frequently used routes to obtain mono-6- and mono-3-substituted CDs with various functional groups, although there are many other methods to achieve mono- and poly-substitutions of CDs at either the 2-, 3- or 6-position.\textsuperscript{57}

1.2.2. Linked Cyclodextrin Dimers

Covalently linked CD dimers (or bisCDs) have attracted much attention as promising versatile receptors for molecular recognition and building blocks for functional materials.\textsuperscript{5,63-66} When the two adjacent CDs are joined together through a linker, the complexation ability to guest molecules, especially those with two or more hydrophobic complexation sites, can be enhanced substantially more than the simple statistical advantage, due to possible cooperative complexation.\textsuperscript{5,66} Furthermore, the linker can be tailored with appropriate functional groups to further enhance the complex stability and selectivity.\textsuperscript{66}

There are three possibilities for linking two CDs to form a linked CD dimer, i.e. head-to-head, in which the linker is substituted on the 6-position of both cavities; tail-to-tail,
where the linker are substituted on either the 2- or 3-position; and head-to-tail, where the linker is on the 6-position of one CD and on 2- or 3-position on the other CD (Figure 1.5). There can be other types of CD dimers of course, for instance those where the CDs are linked by more than one bridge.\(^{63}\)

![Figure 1.5](image)

**Figure 1.5.** Schematic representation of the main types of linked CD dimers, where X is a functionalised linker.

The first report on the synthesis of a cyclodextrin dimer was in 1972, whereby two cyclodextrin cavities were linked on the secondary face by a terephthalate group.\(^{67}\) But it was not until the 1980s when systematic studies on cyclodextrin dimers started to emerge. Breslow et al. were among the earliest research groups who started investigating cyclodextrin dimers in the early 1980s. Since then, they have reported several series of \(\beta\)CD dimers, in which the two CD cavities are linked by disulfide,\(^{68,71}\) diester,\(^{68}\) dithioether,\(^{69,70,72}\) bipyridine,\(^{73-77}\) or cleavable\(^{78}\) linkers. Several disulfide, diester and dithioether linked \(\beta\)CD dimers were found to complex very strongly to appropriate guests, with complexation constants exceeding \(10^8\) dm\(^3\) mol\(^{-1}\).\(^{68,69}\) Some of the Cu\(^{2+}\) complexes of bipyridyl linked \(\beta\)CD dimers were found to mimic the properties of metalloenzymes in the hydrolysis of esters, with the rate acceleration as high as \(1.45 \times 10^7\).\(^{73-77}\) The hydrolysis of the CD dimers were significantly improved by comparison with the hydrolysis through a single CD complexing group.\(^{75}\) More significantly, Breslow et al. were able to synthesise cyclodextrin dimers with cleavable carbon-carbon double bond linkers. These dimers could act as photosensitisers, which could potentially be utilised in photodynamic tumour therapy.\(^{78}\)

Other studies in the early 1980s on cyclodextrin dimers were reported by Tabushi et al., with a diamine linked \(\beta\)CD dimer;\(^{79}\) Harada, et al., with the bis(\(\beta\)CD) succinate and bis(\(\beta\)CD) glutarate;\(^{80}\) and Fujita et al.\(^{81}\) Recently, Fujita et al. reported the synthesis of a
head-to-tail βCD dimer linked by 2,2’-dipyridyl disulfide, a single sulfur atom linked βCD dimer - the shortest linker possible, and a disulfide linked βCD dimer.

Liu et al. have reported extensively on the synthesis of several series of βCD dimers, with organoselenium linkers, diamine linkers, bipyridine linkers, and amide linkers. They studied the host–guest complex phenomena of these hosts with various guest molecules, including dyes, oligopeptides and steroids by various methods such as 2D NMR spectroscopy, circular dichroism, UV-visible spectroscopy, fluorescence spectroscopy and thermodynamic studies. In most cases, the complexation constants of the CD dimers with guest molecules were significantly higher than those of natural βCD. This was attributed to the effect of cooperative complexing by two adjacent cyclodextrin annuli, as well as multiple recognition, as a result of size-fit, shape-fit, charge-fit, hydrophobicity-fit and chirality-fit factors.

Reinhoudt et al. have studied CD dimers as potential sensitisers or photoswitchable hosts. They reported on βCD dimers linked by dipropanolamine as receptors for the development of steroid sensors, an EDTA-linked cyclodextrin dimer as sensitisers for lanthanide(III) luminescence, and dithienylethene or bis(phenylthienyl)ethene-linked cyclodextrin dimers as photoswitchable hosts. All of these dimeric cyclodextrin-based molecules have high potential for utilisation.

It can be seen that the vast majority of CD dimers reported in the literature are based on βCD. Despite the fact that γCD dimers could provide many potential uses, owing to the bigger and more flexible γCD annulus, the number of reports on γCD dimers has been very limited. This is probably due to the fact that γCD is relatively scarce and expensive compared to βCD, and that mono-substitution on either end of γCD is rather difficult. Ishamaru et al. reported the synthesis of a 2,2’-dimethyl benzene linked γCD dimer, with the linker substituted on the 2-position. Hamada et al. reported the syntheses of bis-dansyl and bis-pyrene modified γCD dimers, whereby two dansyl or pyrene groups were appended onto the aminoethyl linker substituted on the 6-position of both γCD cavities. These dimers were studied for their fluorescence sensing ability on bile acids and endocrine disruptors.

As previously mentioned, the most important feature of CD dimers is their intramolecular cooperative complexation ability. This is evident when the complexation
constant, $K_1$, of the 1:1 host–guest complex formed between the linked CD dimer with a
guest exceeds the statistical advantage (i.e. double) over $K_1$ of the corresponding single
native CD complex. Among many guest species, organic dyes possessing two or more
hydrophobic regions are the most commonly used as spectral probes for the studies of CD
dimers.\(^5,6,3,6,3,6,6,8,7,8,11,0\) The $K_1$ for the CD dimer complexes with dyes are as high as $10^{8}$ dm\(^3\) mol\(^{-1}\) \(^6,6\) and in some cases with appropriate geometries, $K_1$ can exceed $10^{11}$ dm\(^3\) mol\(^{-1}\) \(^6,4\). These are considered a substantial enhancement over native CD complexes, where $K_1$ are
normally in the order of $10^{4}$ dm\(^3\) mol\(^{-1}\) or less.\(^6,4\)

Recently, several CD trimers\(^11,1,1,5\) and tetramers\(^11,6,1,1,7\) have been synthesised with the
aims to form polymeric networks, complex large guest species like porphyrins or make
functional molecular devices.

### 1.3. Cyclodextrins and Polymers

Supramolecular polymers, an emerging field combining supramolecular chemistry and
polymer science, are polymeric arrays of monomeric units bound together by noncovalent
interactions such as hydrogen bonds, coordination bonds, electrostatic interactions,
hydrophobic interactions, and host–guest interactions.\(^11,8\) Unlike traditional covalently
linked polymers, supramolecular polymers are directional, reversible and highly
controllable through the association of bifunctional monomers and through external stimuli
such as temperature or radiation.\(^11,8,1,2,0\) Based on the origin of the interactions,
supramolecular polymers can be divided into two major categories, main-chain
supramolecular polymers, where the noncovalent interactions occur on the polymer
backbone,\(^1,2,0\) and side-chain supramolecular polymers, where the interactions occur via
functionalised recognition units on the side chain.\(^1,2,1\) Much interest has focused on the
research and development of new polymeric hydrogel materials based on side-chain
supramolecular polymers because of potential applications in drug delivery, biosensing,
tissue engineering, functional nanodevices and biological coating technologies.\(^1,2,2,1,2,8\)

Among the various noncovalent interactions, host–guest interaction is one of the most
important because of its ubiquitousness in biological systems.\(^1,2,9\) Studies on supramolecular
polymer networks formed by self-assembled binary association between host species, such
as CDs and hydrophobic guest species, as substituents on polymer side-chains, therefore,
can provide great insight into the biological processes of nature. It is also important to
understand and control the supramolecular assembly at the molecular and macroscopic levels in order to produce hydrogels which exhibit predictable and controllable character and constitute new materials.

Cyclodextrins have been utilised for the construction, study and control of many polymer systems. Since the early 1990s, Harada et al.\textsuperscript{130-137} have reported the selective formation of host–guest complexes between CDs and various hydrophilic and hydrophobic polymers. For example, poly(ethylene glycol) formed a complex with $\alpha$CD but not with $\beta$CD,\textsuperscript{130-132} while poly(propylene glycol) complexed only with $\beta$CD not $\alpha$CD.\textsuperscript{133,134} Similarly, polyethylene was complexed strongly by $\alpha$CD,\textsuperscript{135} while polyisobutylene was only complexed by $\beta$CD and $\gamma$CD.\textsuperscript{136,137}

Polymers incorporating CDs as substituents on side chains have been found to complex with polymers containing hydrophobic substituents to form polymeric hydrogels through host–guest complexation. Wenz et al.\textsuperscript{138,139} reported the study of the binary interactions between the $\beta$CD side-groups and those of adamantane and 4-\textit{tert}-butylanilide substituted on poly[[\textit{N}-vinyl-2-pyrrolidinone)-co-(maleic anhydride)] or poly[(maleic anhydride)-\textit{alt-} (isobutene)] by microcalorimetry. The complexation resulted in a large increase in viscosity.

Harada et al. studied the interactions between a $\beta$CD bearing polymer and poly(acrylamide)s bearing 1- and 2-naphthylmethyl side chains by viscometry.\textsuperscript{140} It was shown that the association between $\beta$CD and 2-naphthylmethyl groups led to a remarkably larger increase in viscosity by comparison with the $\beta$CD/1-naphthylmethyl interaction. Harada and co-workers also showed that stimuli-responsive hydrogels could be constructed.\textsuperscript{141,142} In one case, a hydrogel system consisting of $\alpha$CD, dodecyl-modified poly(acrylic acid)s and 4,4'-azodibenzoic acid exhibited gel-to-sol and sol-to-gel transitions upon irradiation of UV or visible light, respectively.\textsuperscript{141} In another case, a ternary mixture of $\beta$CD, dodecyl-modified poly(acrylic acid)s and ferrocenecarboxylic acid (FCA) exhibited a gel-like behaviour in the reduced state of FCA, and was sol-like when FCA was in the oxidised state.\textsuperscript{142}
Inter-polymer strand cross-linking between poly(acrylic acid)s bearing azobenzene linked by dodecamethylene and two kinds of poly(acrylic acid)s carrying αCD, attached through either the 3- or the 6-position, showed contrasting changes in viscosity upon UV irradiation (Figure 1.6). The mixture of the 6-position-modified αCD polymer showed an enhanced viscosity due to the azobenzene being interlocked into αCD upon photoisomerisation from trans to cis. On the other hand, the viscosity of the mixture of the 3-position-modified αCD polymer decreased due to the dissociation of the host–guest complex upon photoisomerisation.

Formation of host–guest complexes between adamantyl and alkyl derivatives and βCD has frequently been exploited to design self-assembled polymeric networks, due to their high complexation constants. For example, a mixture of βCD 3% substituted poly(acrylate)s (PAA) with octadecyl 3% substituted PAA, or with adamantyl 3% substituted PAA (Figure 1.7), showed maximum viscosity enhancement over simple hydrophobic association in the absence of the βCD polymer at 1:1 molar ratio. This indicated that binary interactions between the βCD and the guests were responsible for network formation. Other examples include the substitution of βCD and adamantane onto...
hyaluronic acid, as either monovalent or divalent substituents (Figure 1.8).\textsuperscript{148,149} The 2:2 host–guest βCD/adamantane complexation constant (Figure 1.8, right) was found to be higher than that of the 1:1 host–guest interaction (Figure 1.8, left). For these interactive systems, reversible control of the assembly process can be achieved through changing the substituents, the extent of substitution, varying the host–guest mole ratio, adjusting the tether length between the polymer backbone and substituents, and changing polymer concentration, ionic strength, pH and temperature.\textsuperscript{150-152}

**Figure 1.8.** Schematic representation for network formation due to monovalent (1:1) and bivalent (2:2) βCD/adamantane interactions.\textsuperscript{148}

Covalently linked CD dimers have been used as cross-linkers in the formation of polymer networks.\textsuperscript{128} Kretschmann et al.\textsuperscript{153} reported mixtures of a terephthalimide linked βCD dimer and adamantyl-containing \(N,N'\)-dimethylacrylamide or \(N\)-isopropylacrylamide copolymers, which formed stable gels within seconds (Figure 1.9). Bistri et al.\textsuperscript{154} reported the syntheses of βCD dimers with single or double oligo(ethylene oxide) linkers and their complexation with adamantane-grafted chitosan was studied by viscometry. The singly bridged βCD dimer did not form a self-assembled network, while only 0.5 molar equivalent of the doubly linked βCD dimer was required to obtain maximum viscosity. This indicated that cross-linking by the βCD dimer was crucial in network formation and the cross-linking ability was affected by the dimer’s architecture.
Figure 1.9. Hydrogel formation between adamantyl substituted $N,N'$-dimethylacrylamide or $N$-isopropylacrylamide copolymers and terephthalimide linked $\beta$CD dimer.$^{128,153}$

1.4. Aims of This Research

The aims of this research are to advance the understanding of the supramolecular chemistry of linked cyclodextrin dimers, in which the differences in size, shape and geometry are expected to affect their complexation behaviour toward various guest species.

Chapter 2 describes the complexation of dimerising cationic pyronines B and Y, PB$^+$ and PY$^+$, by $\beta$CD and two succinamide-linked $\beta$CD dimers, in which the two $\beta$CD units are linked either head-to-head or tail-to-tail. The complexation constants are determined by UV–vis, fluorescence and $^1$H NMR spectroscopy. The modes of complexation, dimerisation and fluorescence quenching are studied in light of the structural differences and the 1D and 2D $^1$H NMR spectroscopic data.

Chapter 3 describes the preparation of two new head-to-head and tail-to-tail succinamide-linked $\gamma$CD dimers. The competitive equilibria between the dimerisation and host–guest complexation of hematoporphyrin, HP$^{2-}$, by $\gamma$CD and the new linked $\gamma$CD dimers are quantified by UV–vis and fluorescence spectroscopy. The thermodynamics of the dimerisation and complexation processes, and the nature of interaction between HP$^{2-}$ and the $\gamma$CD and $\gamma$CD dimer hosts are studied.
Chapter 4 describes the preparation of new 3% randomly 1-naphthalene substituted poly(acrylate) polymers. The complexation of the 1-naphthyl substituents by βCD, γCD and their succinamide-linked dimers are quantified by fluorescence spectroscopy. The competition between 1-naphthyl substituent aggregation and host–guest complexation by the linked CD dimers and the 1-naphthyl substituents in forming inter–polymer strand cross–links is examined in aqueous solution at the macroscopic level by rheology and at the molecular level by 2D $^1$H NOESY NMR and fluorescence spectroscopy.
1.5. References


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CHAPTER 2

SUPRAMOLECULAR CHEMISTRY OF PYRONINES B AND Y, β-CYCLODEXTRIN AND LINKED β-CYCLODEXTRIN DIMERS†

† The material in this chapter has been published in:

2.1. Introduction

Since the discovery of the first synthetic dye, mauveine, by W. H. Perkin in 1856, many thousands of synthetic dyes have been prepared and have replaced traditional natural dyes in a great variety of applications.\(^1\) Dyes can be classified based on their applications, e.g. acid dyes or anionic dyes that are used for fibres such as silk and wool; basic dyes or cationic dyes which are applied to acrylic fibres; or solvent dyes for wood staining, etc. Dyes can also be divided into classes by the nature of the chromophore, e.g. azo chromophore; anthraquinone chromophore; phthalocyanine chromophore or fluorescent dyes, etc.\(^1\)

Beside their industrial applications, organic dyes play an important role as being models in the study of many supramolecular systems, in which the interactions with appropriate host molecules, such as cyclodextrins in their native and modified forms,\(^2-6\) can provide insight into the more complex biological phenomena.

Often in supramolecular systems, several competing equilibria exist, as exemplified by host–guest complexation and guest aggregation. The secondary bonding between interacting host and guest species modifies the chemical and physical behaviour of both in proportion to the strength of the interaction. The quantitative study into these interesting processes is a step toward the understanding and potentially replicating those that occur in biological systems.
2.1.1. Structure and Properties of Pyronines B and Y

The cationic pyronines B and Y, 3,6-bis(diethylamino)xanthylium and 3,6-bis(dimethylamino)xanthylium chloride salts, \( \text{PB}^+ \) and \( \text{PY}^+ \) (Figures 2.1 and 2.2), belong to the family of water–soluble xanthene dyes. They are planar and symmetrical molecules, which exist in aqueous solution as four resonance structures (a)–(d) (Figure 2.3), where the singly positive charge is evenly distributed among the two N atoms and the aromatic centre. Like many other xanthene dyes, the photophysical properties of \( \text{PB}^+ \) and \( \text{PY}^+ \) are influenced by their aggregation in solutions.\(^7\)\(^-\)\(^10\) These are typified by the deviation from Beer’s law in their absorption spectra at high concentrations.\(^8\)\(^,\)\(^11\)

**Figure 2.1.** Pyronine B and Y, \( \text{PB}^+ \) and \( \text{PY}^+ \)

**Figure 2.2.** MM2 energy-minimised molecular models of \( \text{PB}^+ \) and \( \text{PY}^+ \). Carbon, nitrogen and oxygen atoms are shown in gray, blue and red, respectively. Hydrogen atoms and lone pairs are not shown.
Figure 2.3. Resonance structures (a)–(d) of PB\(^+\), R = CH\(_2\)CH\(_3\) and PY\(^+\), R = CH\(_3\).

Figure 2.4. UV–vis absorbance spectra (left ordinate, blue lines) and relative fluorescence spectra (right ordinate, red lines) of PB\(^+\) (solid line, 6.0 \times 10^{-6} and 6.0 \times 10^{-7} mol dm\(^{-3}\), respectively) and PY\(^+\) (dashed line, 9.0 \times 10^{-6} and 9.0 \times 10^{-7} mol dm\(^{-3}\), respectively) in aqueous hydrochloric acid (1.00 \times 10^{-4} mol dm\(^{-3}\), \(I = 0.10\) mol dm\(^{-3}\) NaCl) at 298.2 K.

Commercially available samples of PB\(^+\) (Eastman Kodak, \(\sim 46\) % pure, and Sigma, 95 % pure) are in the form of the PB\(_2\)Fe\(_2\)Cl\(_8\) salt,\(^{12}\) which can be purified by recrystallisation.\(^{9,13}\) The molar absorptivity of PB\(^+\) is therefore well defined, \(\varepsilon (553\text{ nm}) = 1.07 \times 10^{5}\) mol\(^{-1}\) dm\(^{3}\) cm\(^{-1}\).\(^{13}\) On the other hand, the commercial PY\(^+\) chloride salt samples (Merck, BDH Chemicals, Eastman Kodak and Sigma) are normally \(\sim 60\) % pure and purification by recrystallisation, chromatographic or extraction methods is rather difficult.\(^{14}\) Attempts in the literature to purify or synthesise PY\(^+\) resulted in varying molar absorptivities (Table 2.1).\(^{7,8,10,11,14}\) In the present study, the molar absorptivities, \(\varepsilon (553\text{ nm}) = 1.07 \times 10^{5}\) mol\(^{-1}\) dm\(^{3}\) cm\(^{-1}\) for PB\(^+\) (Sigma, 95 % pure)\(^{13}\) and \(\varepsilon (547\text{ nm}) = 8.1 \times 10^{4}\) mol\(^{-1}\) dm\(^{3}\) cm\(^{-1}\) for PY\(^+\)
(Sigma, \(\sim 60\%\) pure)\textsuperscript{14} are used. The typical UV–vis absorbance and fluorescence spectra of PB\textsuperscript{+} and PY\textsuperscript{+} are shown in Figure 2.4.

**Table 2.1.** Molar absorptivities of PB\textsuperscript{+} and PY\textsuperscript{+} at their absorption maxima in aqueous solution.

<table>
<thead>
<tr>
<th>Species</th>
<th>(\varepsilon) (mol(^{-1}) dm(^3) cm(^{-1}))</th>
<th>Source</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB\textsuperscript{+}</td>
<td>(5.4 \times 10^{4})</td>
<td>Eastman Kodak’s recrystallised</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>(1.07 \times 10^{5})</td>
<td>Sigma’s reagent recrystallised</td>
<td>13</td>
</tr>
<tr>
<td>PY\textsuperscript{+}</td>
<td>(1.2 \times 10^{4})</td>
<td>Merck’s reagent crude</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(7.0 \times 10^{4})</td>
<td>Merck’s reagent recrystallised from ethanol</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(5.5 \times 10^{4})</td>
<td>Eastman Kodak C8707 extracted from chloroform</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(8.1 \times 10^{4})</td>
<td>Eastman Kodak C8707 EDTA treated</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>(5.7 \times 10^{4})</td>
<td>BDH’s reagent extracted from methanol</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>(1.07 \times 10^{5})</td>
<td>synthesised by authors</td>
<td>10, 11</td>
</tr>
<tr>
<td></td>
<td>(8.1 \times 10^{4})</td>
<td>Sigma’s filtered</td>
<td>14</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Claimed to be ~88 % pure after the recrystallisation.

The dimerisation of PB\textsuperscript{+} and PY\textsuperscript{+} (Figure 2.5) has been studied by UV–vis and temperature–jump spectroscopy in the concentration ranges of up to \(1 \times 10^{-3}\) mol dm\(^{-3}\)\textsuperscript{8,10,13-15}. With the exception of very high values of \(K_d\) obtained by Bordello et al.,\textsuperscript{15} the \(K_d\) values for the dimerisation of PB\textsuperscript{+} and PY\textsuperscript{+} are in order of \(\sim 1 \times 10^{3}\) dm\(^3\) mol\(^{-1}\) (Table 2.2). As a result, the UV–vis absorption of PB\textsuperscript{+} and PY\textsuperscript{+} generally obeys Beer’s law at concentration ranges of up to \(\sim 1.6 \times 10^{-5}\) mol dm\(^{-3}\),\textsuperscript{11} and therefore spectrophotometric absorption measurements can be used for concentration determinations below this value.
Figure 2.5. Dimerisation of \( \text{PB}^+ \), \( R = \text{CH}_2\text{CH}_3 \) and \( \text{PY}^+ \), \( R = \text{CH}_3 \).

Table 2.2. Dimerisation constants, \( K_d \), of \( \text{PB}^+ \) and \( \text{PY}^+ \) in aqueous solution.

<table>
<thead>
<tr>
<th>Species</th>
<th>( K_d ) (dm(^3) mol(^{-1}))</th>
<th>( T ) (K)</th>
<th>pH</th>
<th>Method</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>(( \text{PB}^+ ))(_2)</td>
<td>1300</td>
<td>298.2</td>
<td>5.7</td>
<td>temperature–jump spectrophotometry</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>293.2</td>
<td>4.0</td>
<td>UV–vis spectroscopy</td>
<td>15</td>
</tr>
<tr>
<td>(( \text{PY}^+ ))(_2)</td>
<td>1243</td>
<td>293.2</td>
<td>3–4</td>
<td>UV–vis spectroscopy</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>298.2</td>
<td>3.0</td>
<td>UV–vis spectroscopy</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1100</td>
<td>298.2</td>
<td>6.1</td>
<td>temperature–jump spectrophotometry</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>293.2</td>
<td>4.0</td>
<td>UV–vis spectroscopy</td>
<td>15</td>
</tr>
</tbody>
</table>
2.1.2. Complexation of PB\(^+\) and PY\(^+\) by βCD

In aqueous solution, the complexation of PB\(^+\) and PY\(^+\) by βCD often occurs in the presence of the competing dimerisation equilibrium of the dyes,\(^{13,16,17}\) as shown in Eqns. 2.1 and 2.2 for PB\(^+\). Analogous equations apply for PY\(^+\). The extent of this competition depends on the equilibrium constants \(K_1\) and \(K_d\) and the concentration ranges being studied.

\[
\beta\text{CD} + \text{PB}^+ \rightleftharpoons \beta\text{CD.PB}^+ \quad (2.1)
\]

\[
2\text{PB}^+ \rightleftharpoons K_d (\text{PB}^+)_2 \quad (2.2)
\]

**Table 2.3.** Equilibrium constants, \(K_1\), for the 1:1 host–guest complexation of PB\(^+\) and PY\(^+\) by βCD in aqueous solution.

<table>
<thead>
<tr>
<th>Species</th>
<th>(K_1) (dm(^3) mol(^{-1}))</th>
<th>(T) (K)</th>
<th>pH</th>
<th>Method</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>βCD.PB(^+)</td>
<td>4000</td>
<td>298.2</td>
<td>5.7</td>
<td>UV–vis spectroscopy</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>7300</td>
<td>298.2</td>
<td>5.7</td>
<td>temperature–jump spectrophotometry</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>293.2</td>
<td>4.0</td>
<td>UV–vis spectroscopy</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2100</td>
<td>293.2</td>
<td>4.0</td>
<td>fluorimetry</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>293.2</td>
<td>4.0</td>
<td>time-resolved fluorescence</td>
<td>17</td>
</tr>
<tr>
<td>βCD.PY(^+)</td>
<td>5200(^a)</td>
<td>298.2</td>
<td>6.1</td>
<td>UV–vis spectroscopy</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>4200(^b)</td>
<td>298.2</td>
<td>6.1</td>
<td>temperature–jump spectrophotometry</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>390</td>
<td>293.2</td>
<td>4.0</td>
<td>UV–vis spectroscopy</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>293.2</td>
<td>4.0</td>
<td>fluorimetry</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>360</td>
<td>293.2</td>
<td>4.0</td>
<td>time-resolved fluorescence</td>
<td>17</td>
</tr>
</tbody>
</table>

\(^a\) The sequential \(K_2\) for a 2:1 host–guest complex was also reported as 170 dm\(^3\) mol\(^{-1}\).

\(^b\) The sequential \(K_2\) for a 2:1 host–guest complex was also reported as 270 dm\(^3\) mol\(^{-1}\).
The complexation of PB\(^+\) and PY\(^+\) by \(\beta\)CD has been studied by temperature-jump visible spectrophotometry,\(^{13,14}\) UV–vis spectroscopy,\(^{13,17}\) fluorimetry\(^{17}\) and time-resolved fluorescence spectroscopy.\(^{17}\) The values of equilibrium constants and the conditions under which the experiments were carried out are summarised in Table 2.3. There are generally disagreements between the \(K_1\) obtained by Schiller \textit{et al.}\(^{13,14}\) and those obtained by Reija \textit{et al.}\(^{17}\) The much larger \(K_1\) values by Schiller \textit{et al.} were probably due to strong approximations and the absence of \(K_d\) in the data analysis while the experimental conditions (high dye concentrations and high ionic strength \(I = 1.0\) mol dm\(^{-3}\) NaCl) favour dye aggregations.\(^{18}\) The results by Reija \textit{et al.},\(^{17}\) while appear to be more accurately determined, lack substantial evidence to support their proposed model for the interaction between these dyes and \(\beta\)CD and for the resultant fluorescence quenching mechanism.

### 2.1.3. Aims of This Study

The aims of this study are to reinvestigate the complexation of PB\(^+\) and PY\(^+\) by \(\beta\)CD as well as for the first time quantifying the complexation of these dyes by two succinamide-linked \(\beta\)CD dimers, \(N,N'\)-bis((2\(^A\)S,3\(^A\)S)-3\(^A\)-deoxy-\(\beta\)-cyclodextrin-3\(^A\)-yl) succinamide, \(33\beta\)CD\(_2\)suc, and \(N,N'\)-bis(6\(^A\)-deoxy-\(\beta\)-cyclodextrin-6\(^A\)-yl) succinamide, \(66\beta\)CD\(_2\)suc, by UV–vis and fluorescence spectroscopy. The competition between dye dimerisation and complexation is investigated by \(^1\)H NMR spectroscopy. The mode of complexation, dimerisation and fluorescence quenching are discussed in light of the structural differences and the 1D and 2D \(^1\)H NMR spectroscopic data.
2.2. Synthesis of Succinamide-Linked βCD Dimers

The bis(4-nitrophenyl) succinate linker required for the synthesis of the βCD dimers was prepared from the reaction of succinyl chloride with 4-nitrophenol in dichloromethane using a method similar to those in the literature (Figure 2.6).\(^{19-21}\)

![Figure 2.6. Synthetic scheme for bis(4-nitrophenyl) succinate.](image)

6\(^{A}\)-Amino-6\(^{A}\)-deoxy-β-cyclodextrin, 6βCDNH\(_2\),\(^{22}\) and 3\(^{A}\)-amino-3\(^{A}\)-deoxy-(2\(^{A}\)S,3\(^{A}\)S)-β-cyclodextrin, 3βCDNH\(_2\),\(^{23}\) were prepared according to literature methods with some modifications. Treatment of these amino-β-cyclodextrins with bis(4-nitrophenyl) succinate in pyridine afforded the two linked βCD dimers, \(N,N'-\text{bis}(6\(^{A}\)-deoxy-β-cyclodextrin-6\(^{A}\)yl) succinamide, 66βCD\(_2\)suc, and \(N,N'-\text{bis}(2\(^{A}\)S,3\(^{A}\)S)-3\(^{A}\)-deoxy-β-cyclodextrin-3\(^{A}\)yl) succinamide, 33βCD\(_2\)suc (Figure 2.7).\(^{19}\) In 33βCD\(_2\)suc, the C\(_{2}\)\(^{A}\) and C\(_{3}\)\(^{A}\) carbons of the substituted βCD A ring were inverted to form an altropyranose unit.\(^{24}\)

![Figure 2.7. Synthetic scheme for the succinamide-linked βCD dimers.](image)
2.3. UV–vis and Fluorescence Studies

2.3.1. UV–vis Spectrophotometric Titrations

The variations of the UV–vis spectra of PB$^+$ ($6.0 \times 10^{-6}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K with increasing concentrations of βCD ($0 – 6.5 \times 10^{-3}$ mol dm$^{-3}$), 3βCD$_2$suc ($0 – 2.0 \times 10^{-3}$ mol dm$^{-3}$) and 6βCD$_2$suc ($0 – 2.0 \times 10^{-3}$ mol dm$^{-3}$) are shown in the following pages and those for analogous PY$^+$ systems ($9.0 \times 10^{-6}$ mol dm$^{-3}$) appear in the Appendix, section 2.9.1. A red shift of the absorption maxima of both PB$^+$ and PY$^+$ occurs together with a decrease in the observed absorbance as the concentration of each host increases. The isosbestic point occurs near 556 nm (Figures 2.8, 2.10 and 2.12) consistent with PB$^+$ existing predominantly in the free and 1:1 complexed states with either the βCD or βCD dimer hosts in an equilibrium characterised by $K_1$ (Eqn. 2.1). Likewise, the isosbestic point near 553 nm (Figures 2.33, 2.35 and 2.37, Appendix) denotes the equilibrium of a 1:1 host–guest complexation of PY$^+$ by either the βCD or βCD dimer hosts.

Equation 2.3 describes the observed absorbance, $A$, at any given wavelength when the complexation equilibrium (Eqn. 2.1) exists in the solution, where $\varepsilon_{PB}$ and $\varepsilon_{\beta CD.PB}$ represent the molar absorbances of the free PB$^+$ and 1:1 complex βCD.PB$^+$. Analogous equations apply for the other five systems.

\[
A = \varepsilon_{PB}[PB^+] + \varepsilon_{\beta CD.PB}[\beta CD.PB^+] \tag{2.3}
\]

\[
K_1 = [\beta CD.PB^+]/([\beta CD][PB^+]) \tag{2.4}
\]

An algorithm analogous to Eqn. 2.3 for the formation of the 1:1 complexes, βCD.PB$^+$, 3βCD$_2$suc.PB$^+$ and 6βCD$_2$suc.PB$^+$, and the analogous PY$^+$ systems best-fits the data at 0.5 nm intervals in the range 500–590 nm using the SPECFIT/32 protocol to yield the equilibrium constants, $K_1$, which appear in Table 2.4.

Under the conditions of the UV–vis and fluorescence (section 2.3.2) studies, the formation of the dimerised (PB$^+$)$_2$ and (PY$^+$)$_2$ species is negligible, as will be discussed in section 2.4.
Figure 2.8. UV–visible absorbance spectra of PB$^+$ alone ($6.35 \times 10^{-6}$ mol dm$^{-3}$) and in the presence of increasing concentrations of βCD (ranging from 0.00 to 6.50 × 10$^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid (1.00 × 10$^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The arrow indicates the direction of absorbance change as [βCD]$_{\text{total}}$ increases. An isosbestic point occurs at 558 nm. $\lambda_{\text{max}} = 553$ nm ($\varepsilon = 1.07 \times 10^5$ dm$^3$ mol$^{-1}$ cm$^{-1}$) and 555 nm ($\varepsilon = 9.82 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$) for the free and complexed PB$^+$ species, respectively.

Figure 2.9. UV–visible absorbance variation of PB$^+$ ($6.35 \times 10^{-6}$ mol dm$^{-3}$) with βCD (0.00 to 6.50 × 10$^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid (1.00 × 10$^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles represent experimental data at 552 nm and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 500–590 nm.
Figure 2.10. UV–visible absorbance spectra of PB$^+$ alone ($6.51 \times 10^{-6}$ mol dm$^{-3}$) and in the presence of increasing concentrations of 33βCD$_2$suc (ranging from 0.00 to 1.97 $\times$ 10$^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The arrow indicates the direction of absorbance change as [33βCD$_2$suc]$_{total}$ increases. An isosbestic point occurs at 556 nm. $\lambda_{max} =$ 553 nm ($\varepsilon = 1.07 \times 10^5$ dm$^3$ mol$^{-1}$ cm$^{-1}$) and 557 nm ($\varepsilon = 1.01 \times 10^5$ dm$^3$ mol$^{-1}$ cm$^{-1}$) for the free and complexed PB$^+$ species, respectively.

Figure 2.11. UV–visible absorbance variation of PB$^+$ ($6.51 \times 10^{-6}$ mol dm$^{-3}$) with 33βCD$_2$suc (0.00 to 1.97 $\times$ 10$^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles represent experimental data at 552 nm and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 500–590 nm.
Figure 2.12. UV–visible absorbance spectra of PB$^+$ alone ($6.38 \times 10^{-6}$ mol dm$^{-3}$) and in the presence of increasing concentrations of 66βCD$_2$suc (ranging from 0.00 to $2.01 \times 10^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The arrow indicates the direction of absorbance change as [66βCD$_2$suc]$_{\text{total}}$ increases. An isosbestic point occurs at 558 nm. $\lambda_{\text{max}} = 553$ nm ($\varepsilon = 1.07 \times 10^5$ dm$^3$ mol$^{-1}$ cm$^{-1}$) and 556 nm ($\varepsilon = 9.83 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$) for the free and complexed PB$^+$ species, respectively.

Figure 2.13. UV–visible absorbance variation of PB$^+$ ($6.38 \times 10^{-6}$ mol dm$^{-3}$) with 66βCD$_2$suc (0.00 to $2.01 \times 10^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles represent experimental data at 552 nm and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 500–590 nm.
2.3.2. Fluorimetric Titrations

The fluorescence variations of PB$^+$ ($6.0 \times 10^{-7}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K with increasing concentrations of $\beta$CD ($0 – 7.0 \times 10^{-3}$ mol dm$^{-3}$), $33\beta$CD$_2$suc ($0 – 1.5 \times 10^{-3}$ mol dm$^{-3}$) and $66\beta$CD$_2$suc ($0 – 2.0 \times 10^{-3}$ mol dm$^{-3}$) are shown in the following pages and those for analogous systems of PY$^+$ ($9.0 \times 10^{-7}$ mol dm$^{-3}$) appear in the Appendix, section 2.9.2. A red shift of the fluorescence maxima of both PB$^+$ and PY$^+$ occurs together with a decrease in the observed fluorescence intensity as the concentration of either host increases consistent with the formation of the 1:1 host–guest complexes.

The observed fluorescence intensity, $I_F$, at any given wavelength for the $\beta$CD.PB$^+$ complex is equal to the mole fraction–weighted sum of the fluorescence intensities of the free and complexed PB$^+$, as shown in Eqn. 2.5. Analogous equations apply for the other five systems.

$$I_F = I_{F(PB)}([PB^+]/[PB^+]_{total}) + I_{F(\beta CD.PB)}([\beta CD.PB^+]/[PB^+]_{total})$$ (2.5)

An algorithm analogous to Eqn. 2.5 for the formation of the 1:1 complexes, $\beta$CD.PB$^+$, $33\beta$CD$_2$suc.PB$^+$ and $66\beta$CD$_2$suc.PB$^+$, and the analogous PY$^+$ systems best-fits the data at 0.5 nm intervals in the range 540–650 nm for PB$^+$ and 530–630 nm for PY$^+$, using the SPECFIT/32 protocol$^{25}$ to yield the equilibrium constants, $K_1$, which are shown in Table 2.4.
**Figure 2.14.** Emission spectra of PB⁺ alone (6.02 × 10⁻⁷ mol dm⁻³) and in the presence of increasing concentrations of βCD (ranging from 0.00 to 7.00 × 10⁻³ mol dm⁻³) in aqueous hydrochloric acid (1.00 × 10⁻⁴ mol dm⁻³, I = 0.10 mol dm⁻³ NaCl) at 298.2 K. Excitation wavelength λₑₓ = 515 nm with excitation and emission slit widths of 5 nm. The arrow indicates the direction of relative fluorescence emission change as [βCD]ₜ₉ₐ₅ increases. λₘ₉ₐ₅ = 568 nm (641 a.u.) and 572 nm (269 a.u.) for the free and complexed PB⁺ species, respectively.

**Figure 2.15.** Relative fluorescence variation of PB⁺ (6.02 × 10⁻⁷ mol dm⁻³) with βCD (0.00 to 7.00 × 10⁻³ mol dm⁻³) in aqueous hydrochloric acid (1.00 × 10⁻⁴ mol dm⁻³, I = 0.10 mol dm⁻³ NaCl) at 298.2 K. Emission was measured at 568 nm. The circles represent experimental data and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 540–650 nm.
Figure 2.16. Emission spectra of PB$^+$ alone ($6.19 \times 10^{-7}$ mol dm$^{-3}$) and in the presence of increasing concentrations of 33βCD$_2$suc (ranging from 0.00 to $1.54 \times 10^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. Excitation wavelength $\lambda_{\text{ex}} = 515$ nm with excitation and emission slit widths of 5 nm. The arrow indicates the direction of relative fluorescence emission change as [33βCD$_2$suc]$_{\text{total}}$ increases. $\lambda_{\text{max}} = 568$ nm (656 a.u.) and 572 nm (431 a.u.) for the free and complexed PB$^+$ species, respectively.

Figure 2.17. Relative fluorescence variation of PB$^+$ ($6.19 \times 10^{-7}$ mol dm$^{-3}$) with 33βCD$_2$suc (0.00 to $1.54 \times 10^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. Emission was measured at 568 nm. The circles represent experimental data and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 540–650 nm.
Figure 2.18. Emission spectra of PB$^+$ alone ($6.28 \times 10^{-7}$ mol dm$^{-3}$) and in the presence of increasing concentrations of 66$\beta$CD$_2$suc (ranging from 0.00 to $1.97 \times 10^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. Excitation wavelength $\lambda_{ex} = 515$ nm with excitation and emission slit widths of 5 nm. The arrow indicates the direction of relative fluorescence emission change as [66$\beta$CD$_2$suc]$_{total}$ increases. $\lambda_{max} = 568$ nm (662 a.u.) and 572 nm (354 a.u.) for the free and complexed PB$^+$ species, respectively.

Figure 2.19. Relative fluorescence variation of PB$^+$ ($6.28 \times 10^{-7}$ mol dm$^{-3}$) with 66$\beta$CD$_2$suc (0.00 to $1.97 \times 10^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. Emission was measured at 568 nm. The circles represent experimental data and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 540–650 nm.
2.4. 1D $^1$H NMR Studies

At higher concentrations in the range of $10^{-4} - 10^{-2}$ mol dm$^{-3}$ in aqueous solution, the aggregation of PB$^+$ and PY$^+$ to form the dimerised species becomes significant.$^{18}$ Therefore, it is possible to study the competitive complexation of the dimerising PB$^+$ and PY$^+$ by the βCD and βCD dimer hosts at these concentrations. Although UV–vis spectroscopy is a powerful technique in characterising this competing aggregation–complexation process, the strongly absorbing nature of PB$^+$ and PY$^+$ normally prevents the measurements at concentrations above $1 \times 10^{-3}$ mol dm$^{-3}$, which can make the data fittings less reliable due to the small $K_d$ of these dyes.$^8,10,13-15$

$^1$H NMR spectroscopy permits the study of equilibrium processes at higher concentrations than in the case with UV–vis spectroscopy.$^{26}$ The $^1$H resonances of particular protons within the PB$^+$ and PY$^+$ molecule change as that part of the molecule experiences a change in environment due to dimerisation or complexation. Since the equilibrium is a fast–exchange process on the NMR time–scale, the chemical shift, $\delta$, for the proton in question is a mole fraction–weighted average over all of the chemical species in which the proton is present, as typified by the PB$^+$/βCD system in Eqns. 2.6 and 2.7.

$$\delta_{\text{exp}} = \delta_{\text{PB}}[\text{PB}^+] + \delta_{\text{PB}_2}[(\text{PB}^+)_2]$$
(2.6)

$$\delta_{\text{exp}} = \delta_{\text{PB}}[\text{PB}^+] + \delta_{\text{PB}_2}[(\text{PB}^+)_2] + \delta_{\text{βCD.PB}}[\text{βCD.PB}^+]$$
(2.7)

By monitoring the chemical shift variations of one or several protons of PB$^+$ and PY$^+$ over a range of concentrations of the dyes alone or in the presence of either βCD or the βCD dimer hosts, it is possible to quantify the equilibrium constants for the dimerisation and complexation by fitting Eqns. 2.6 or 2.7 to the experimental data using the HypNMR 2003 program.$^{26,27}$
2.4.1. Dimerisation of PB$^+$ and PY$^+$

The $^1$H (300 MHz) resonances of H$_1$–H$_6$ of PB$^+$ and H$_1$–H$_5$ of PY$^+$ were monitored in D$_2$O ($1.00 \times 10^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K at concentrations ranging from $2.0 \times 10^{-4}$ to $2.0 \times 10^{-2}$ mol dm$^{-3}$. The H$_1$–H$_6$ resonances of PB$^+$ systematically shift upfield as [PB$^+$]$_{total}$ increases, consistent with the formation of the (PB$^+$)$_2$ dimer, as do the H$_1$–H$_5$ resonances of PY$^+$. These upfield shifts are the greatest for the PB$^+$ and PY$^+$ aromatic H$_1$–H$_4$ resonances with the ethyl and methyl proton resonance shifts being significantly less.

Accordingly, simultaneous fitting of Eqn. 2.6 to the $\delta$ variations of H$_1$–H$_4$ of both PB$^+$ and PY$^+$ (Figures 2.20 and 2.21) was carried out to give the dimerisation constants, $K_d = 100 \pm 10$ mol dm$^{-3}$ and $260 \pm 10$ mol dm$^{-3}$ for PB$^+$ and PY$^+$, respectively (Table 2.4).

The calculated chemical shifts of all protons of PB$^+$ and PY$^+$ in their monomer and dimer states are given in Table 2.5 and their significance is discussed in section 2.6.

The $K_d$ for (PB$^+$)$_2$ and (PY$^+$)$_2$ are generally five to ten times less than those reported in the literature.$^8,10,13-15$ However, when considering the other $K_d$ being derived from UV–vis spectroscopy in which strong approximations were made in the fitting due to the low maximum concentrations studied, as well as the variation in molar absorptivities due to variation in the purity of PY$^+$, some discrepancies in the values of $K_d$ obtained can be expected. For (PY$^+$)$_2$, $K_d$ is 2.6 times that of (PB$^+$)$_2$ which suggests that of the factors likely to cause differences in dimerisation: hydrophobic attraction, charge repulsion, hydration changes and steric hindrance, the last is the most obvious with the bulkier ethyl groups of PB$^+$ causing greater steric hindrance than the methyl groups of PY$^+$. 
Figure 2.20.
Figure 2.20. (continued) Left ordinate: variation of the $^1$H (300 MHz) chemical shift of the aromatic H$_1$ proton (A), aromatic H$_2$ proton (B), aromatic H$_3$ proton (C) and aromatic H$_4$ proton (D) of PB$^+$ with [PB$^+$]$_{\text{total}}$ ranging from $2.00 \times 10^{-4}$ mol dm$^{-3}$ to $2.00 \times 10^{-2}$ mol dm$^{-3}$ in D$_2$O (1.00 $\times$ 10$^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles are the experimental data and the solid curve $a$ is the best fit of the algorithm for dimerisation of PB$^+$ to the chemical shift variations of protons H$_1$–H$_4$. Right ordinate: speciation relative to [PB$^+$]$_{\text{total}}$, curve $b$ is the percentage of [PB$^+$] and curve $c$ is twice the percentage of [(PB$^+$)$_2$].
Figure 2.21.
Figure 2.21. (continued) Left ordinate: variation of the $^1$H (300 MHz) chemical shift of the aromatic H$_1$ proton (A), aromatic H$_2$ proton (B), aromatic H$_3$ proton (C) and aromatic H$_4$ proton (D) of PY$^+$ with [PY$^+$]$_{\text{total}}$ ranging from $2.00 \times 10^{-3}$ mol dm$^{-3}$ to $1.81 \times 10^{-2}$ mol dm$^{-3}$ in D$_2$O ($1.00 \times 10^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles are the experimental data and the solid curve $a$ is the best fit of the algorithm for dimerisation of PY$^+$ to the chemical shift variations of protons H$_1$–H$_4$. Right ordinate: speciation relative to [PY$^+$]$_{\text{total}}$, curve $b$ is the percentage of [PY$^+$] and curve $c$ is twice the percentage of [(PY$^+$)$_2$].
2.4.2. Complexation Studies

The complexations of PB$^+$ and PY$^+$ (2.00 × 10$^{-3}$ mol dm$^{-3}$) by βCD, 33βCD$_2$suc and 66βCD$_2$suc over the concentration range 0 – 5.00 × 10$^{-3}$ mol dm$^{-3}$ were studied under the same conditions as the dimerisations (section 2.4.1), under which a significant proportion of the dyes exists as dimers (23.8 % for PB$^+$ and 38.8 % for PY$^+$). A downfield chemical shift occurred for the PB$^+$ H$_1$-H$_4$ and H$_6$ protons and PY$^+$ H$_1$-H$_5$ protons, consistent with the change from the aqueous environment of their uncomplexed states to the more hydrophobic environment of their complexed states (the PB$^+$ H$_5$ protons are obscured due their chemical shift being similar to those of βCD protons). The overall observed downfield shift is the sum of the two opposite shifts due to dimerisation and complexation. The best fits to these data for the all six complex systems were obtained for an algorithm representing Eqns. 2.6 and 2.7, as shown in following figures for PB$^+$ (those for PY$^+$ can be found in the Appendix, section 2.9.3). The derived equilibrium constants, $K_1$, for the 1:1 host–guest complexation appear in Table 2.4.

The calculated chemical shifts of all protons of PB$^+$ and PY$^+$ in their complexed states are given in Table 2.5, and their significance is discussed in section 2.6.
Figure 2.22.
Figure 2.22. (continued) Left ordinate: variation of the $^1$H (300 MHz) chemical shift of the aromatic H$_1$ proton (A), aromatic H$_2$ proton (B), aromatic H$_3$ proton (C), aromatic H$_4$ proton (D) and H$_6$ proton (E) of PB$^+$ ($2.75 \times 10^{-3}$ mol dm$^{-3}$) with [βCD]$_{\text{total}}$ (ranging from 0 to $4.60 \times 10^{-3}$ mol dm$^{-3}$) in D$_2$O ($1.00 \times 10^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles are the experimental data and the solid curve $a$ is the best fit of the algorithm incorporating PB$^+$, (PB$^+$)$_2$ and βCD.PB$^+$ to the chemical shift variations of protons H$_1$–H$_4$ and H$_6$. Right ordinate: speciation relative to [PB$^+$]$_{\text{total}}$, curve $b$ is the percentage of [PB$^+$], curve $c$ is twice the percentage of [(PB$^+$)$_2$] and curve $d$ is the percentage of [βCD.PB$^+$].
Figure 2.23.
Figure 2.23. (continued) Left ordinate: variation of the $^1$H (300 MHz) chemical shift of the aromatic H$_1$ proton (A), aromatic H$_2$ proton (B), aromatic H$_3$ proton (C), aromatic H$_4$ proton (D) and H$_6$ proton (E) of PB$^+$ ($2.15 \times 10^{-3}$ mol dm$^{-3}$) with [33$\beta$CD$_2$suc]$_{total}$ (ranging from 0 to $5.00 \times 10^{-3}$ mol dm$^{-3}$) in D$_2$O ($1.00 \times 10^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles are the experimental data and the solid curve $a$ is the best fit of the algorithm incorporating PB$^+$, (PB$^+$)$_2$ and 33$\beta$CD$_2$suc.PB$^+$ to the chemical shift variations of protons H$_1$–H$_4$ and H$_6$. Right ordinate: speciation relative to [PB$^+$]$_{total}$. Curve $b$ is the percentage of [PB$^+$], curve $c$ is twice the percentage of [(PB$^+$)$_2$] and curve $d$ is the percentage of [33$\beta$CD$_2$suc.PB$^+$].
Figure 2.24.
Figure 2.24. (continued) Left ordinate: variation of the $^1$H (300 MHz) chemical shift of the aromatic H$_1$ proton (A), aromatic H$_2$ proton (B), aromatic H$_3$ proton (C), aromatic H$_4$ proton (D) and H$_6$ proton (E) of PB$^+$ (2.00 $\times$ 10$^{-3}$ mol dm$^{-3}$) with [66$\beta$CD$_2$suc]$_{total}$ (ranging from 0 to 5.00 $\times$ 10$^{-3}$ mol dm$^{-3}$) in D$_2$O (1.00 $\times$ 10$^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles are the experimental data and the solid curve $a$ is the best fit of the algorithm incorporating PB$^+$, (PB$^+$)$_2$ and 66$\beta$CD$_2$suc.PB$^+$ to the chemical shift variations of protons H$_1$–H$_4$ and H$_6$. Right ordinate: speciation relative to [PB$^+$]$_{total}$, curve $b$ is the percentage of [PB$^+$], curve $c$ is twice the percentage of [(PB$^+$)$_2$] and curve $d$ is the percentage of [66$\beta$CD$_2$suc.PB$^+$].
2.5. 2D $^1$H ROESY NMR Studies

2D $^1$H ROESY NMR (600 MHz, 300 ms mixing time) spectra were recorded for PB$^+$ and PY$^+$ (2.00 $\times$ $10^{-3}$ mol dm$^{-3}$) in the presence of either double the concentration of native $\beta$CD or the same concentration of either of the linked $\beta$CD dimers in D$_2$O solution (1.00 $\times$ $10^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. Under these conditions, the 1:1 host–guest complexes are the dominant species, with $\sim$70 % of PB$^+$ and $\sim$40 % of PY$^+$ exist in their complexed form.

The NOE cross–peaks arising from interactions between the $\beta$CD H$_3$, H$_5$ and H$_6$ annular protons and the H$_6$ and H$_4$ protons of PB$^+$ (those arising from the PB$^+$ H$_5$ protons are obscured due their chemical shift being similar to those of $\beta$CD protons) seen in Figure 2.25 are consistent with the two sets of interacting protons being within 400 pm of each other and the ethyl groups of PB$^+$ and the attached aromatic ring being partially within the $\beta$CD annulus of the dominant $\beta$CD.PB$^+$ host–guest complex. Because the ratio of methyl protons to aromatic protons is 6:1 the cross–peaks arising from the methyl protons will be the more intense if all other factors are the same. Similar spectra are observed for the 33$\beta$CD$_2$suc.PB$^+$ (Figure 2.26) and 66$\beta$CD$_2$suc.PB$^+$ (Figure 2.27) host–guest complexes. Similar cross–peaks are also observed for the analogous PY$^+$ solutions but are weaker consistent with the PY$^+$ complexes exhibiting lower $K_1$ values (Figures 2.28 – 2.30).
Figure 2.25. 2D $^1$H ROESY NMR (600 MHz) spectrum of PB$^+$ (2.0 $\times$ 10$^{-3}$ mol dm$^{-3}$) with two molar equivalent $\beta$CD in D$_2$O (1.00 $\times$ 10$^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I$ = 0.10 mol dm$^{-3}$ NaCl) at 298.2 K with a mixing time of 300 ms. Cross–peaks were observed between H$_6$ of PB$^+$ and H$_3$, H$_5$ of $\beta$CD; between aromatic H$_4$ of PB$^+$ and H$_5$ of $\beta$CD.

Figure 2.26. 2D $^1$H ROESY NMR (600 MHz) spectrum of PB$^+$ (2.0 $\times$ 10$^{-3}$ mol dm$^{-3}$) and equimolar 33$\beta$CD$_2$suc in D$_2$O (1.00 $\times$ 10$^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I$ = 0.10 mol dm$^{-3}$ NaCl) at 298.2 K with a mixing time of 300 ms. Cross–peaks were observed between H$_6$ of PB$^+$ and H$_3$–H$_5$ of $\beta$CD; between H$_4$ of PB$^+$ and H$_3$–H$_5$ of $\beta$CD.
Figure 2.27. 2D $^1$H ROESY NMR (600 MHz) spectrum of PB$^+$ ($2.0 \times 10^{-3}$ mol dm$^{-3}$) and equimolar 66βCD$_2$suc in D$_2$O ($1.00 \times 10^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K with a mixing time of 300 ms. Cross–peaks were observed between H$_6$ of PB$^+$ and H$_3$–H$_5$ of βCD.

Figure 2.28. 2D $^1$H ROESY NMR (600 MHz) spectrum of PY$^+$ ($1.8 \times 10^{-3}$ mol dm$^{-3}$) with two molar equivalent βCD in D$_2$O ($1.00 \times 10^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K with a mixing time of 300 ms. Cross–peaks were observed between H$_5$ of PY$^+$ and H$_3$, H$_5$ of βCD.
Figure 2.29. 2D $^1$H ROESY NMR (600 MHz) spectrum of PY$^+$ (1.8 x 10$^{-3}$ mol dm$^{-3}$) and equimolar 33βCD$_2$suc in D$_2$O (1.00 x 10$^{-4}$ mol dm$^{-3}$ HCl, I = 0.10 mol dm$^{-3}$ NaCl) at 298.1 K with a mixing time of 300 ms. Cross–peaks were observed between H$_5$ of PY$^+$ and H$_3$–H$_5$ of βCD.

Figure 2.30. 2D $^1$H ROESY NMR (600 MHz) spectrum of PY$^+$ (1.8 x 10$^{-3}$ mol dm$^{-3}$) and equimolar 66βCD$_2$suc in D$_2$O (1.00 x 10$^{-4}$ mol dm$^{-3}$ HCl, I = 0.10 mol dm$^{-3}$ NaCl) at 298.1 K with a mixing time of 300 ms. Cross–peaks were observed between H$_5$ of PY$^+$ and H$_3$–H$_5$ of βCD; between H$_2$–H$_4$ of PY$^+$ and H$_3$–H$_5$ of βCD.
2.6. Discussion

The equilibrium constants, $K_1$, for the 1:1 complexation of PB$^+$ and PY$^+$ by βCD and the two linked βCD dimers determined by UV–vis, fluorescence and $^1$H NMR spectroscopy are summarised in Table 2.4. There is reasonable agreement between the $K_1$ derived through all three techniques. The $K_1$ for the βCD.PB$^+$ and βCD.PY$^+$ complexes are comparable to those obtained by Reija et al.$^{17}$ but are much smaller than those reported by Schiller et al.$^{13,14}$ although there were issues in their experimental conditions as discussed in section 2.1.2.

**Table 2.4.** Equilibrium constants for the dimerisation of PB$^+$ and PY$^+$ ($K_d$) and the 1:1 host/guest complexes ($K_1$), determined by UV–vis, fluorescence and $^1$H NMR spectroscopy.

<table>
<thead>
<tr>
<th>Complex</th>
<th>UV–visible$^a$ $K_1$/dm$^3$ mol$^{-1}$</th>
<th>Fluorescence$^a$ $K_1$/dm$^3$ mol$^{-1}$</th>
<th>$^1$H NMR$^b$ $K_1$/dm$^3$ mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(PB$^+$)$_2$</td>
<td>–</td>
<td>–</td>
<td>100 ± 10$^c$</td>
</tr>
<tr>
<td>(PY$^+$)$_2$</td>
<td>–</td>
<td>–</td>
<td>260 ± 10$^c$</td>
</tr>
<tr>
<td>βCD.PB$^+$</td>
<td>1660 ± 80</td>
<td>2000 ± 100</td>
<td>1500 ± 150</td>
</tr>
<tr>
<td>33βCD$_2$suc.PB$^+$</td>
<td>4200 ± 200</td>
<td>4600 ± 200</td>
<td>4400 ± 200</td>
</tr>
<tr>
<td>66βCD$_2$suc.PB$^+$</td>
<td>4500 ± 200</td>
<td>4900 ± 200</td>
<td>5400 ± 200</td>
</tr>
<tr>
<td>βCD.PY$^+$</td>
<td>370 ± 20</td>
<td>320 ± 30</td>
<td>320 ± 70</td>
</tr>
<tr>
<td>33βCD$_2$suc.PY$^+$</td>
<td>760 ± 40</td>
<td>830 ± 40</td>
<td>500 ± 50</td>
</tr>
<tr>
<td>66βCD$_2$suc.PY$^+$</td>
<td>780 ± 40</td>
<td>740 ± 40</td>
<td>500 ± 50</td>
</tr>
</tbody>
</table>

$^a$ In aqueous $1.00 \times 10^{-4}$ mol dm$^{-3}$ hydrochloric acid at $I = 0.10$ mol dm$^{-3}$ (NaCl) and 298.2 K. $^b$ In $1.00 \times 10^{-4}$ mol dm$^{-3}$ hydrochloric acid D$_2$O solution at $I = 0.10$ mol dm$^{-3}$ (NaCl) and 298.2 K. $^c$ $K_d$ for the dimerisation of PB$^+$ and PY$^+$.

The $K_1$ for the complexation of PB$^+$ and PY$^+$ by 33βCD$_2$suc and 66βCD$_2$suc are slightly more than twice the $K_1$ for complexation by βCD. This indicates that 33βCD$_2$suc and 66βCD$_2$suc have little more than a statistical advantage in forming host–guest complexes.
over βCD. However, PB$^+$ is complexed approximately 5 times more strongly than PY$^+$, consistent with the ethyl groups extending the hydrophobicity of PB$^+$ by a greater amount to interact more strongly with the hydrophobic annuli of βCD, 33βCD$_2$suc and 66βCD$_2$suc than do the methyl groups of PY$^+$. The differing stereochemistries of 33βCD$_2$suc and 66βCD$_2$suc have little effect on complexation despite the inversions at the $C_2^A$ and $C_3^A$ carbons of both of the substituted altropyranose units of the former. The simplest explanation of these observations is that the dominant 33βCD$_2$suc.PB$^+$ and 66βCD$_2$suc.PB$^+$ complexes and their PY$^+$ analogues have either guest complexed in a single βCD annulus in a similar way to that in βCD.PB$^+$ and βCD.PY$^+$.

A similar relationship holds for the $K_1$ characterising the analogous BNS$^-$ and TNS$^-$ systems (Figure 2.31),$^{28,29}$ where the more hydrophobic t-butyl group of BNS$^-$ interacts more strongly than does the methyl group of TNS$^-$. The magnitudes of $K_1$ for the PB$^+$ and PY$^+$ systems are one to four orders of magnitude less than $K_1$ for the BNS$^-$ and TNS$^-$ systems. This reflects the structural differences between the two types of guest and may indicate that while the positive charge of PB$^+$ and PY$^+$ is delocalised over two dialkylamino groups, the negative charge of BNS$^-$ and TNS$^-$ is largely localized on the sulfonate group. It is noticeable that BNS$^-$ and TNS$^-$ complex substantially more strongly than do PB$^+$ and PY$^+$ and that the $K_1$ of 66βCD$_2$suc.BNS$^-$ and 66βCD$_2$suc.TNS$^-$ are consistent with substantial cooperativity in complexation between the two linked βCD annuli.

![Figure 2.31. Structures of TNS$^-$ and BNS$^-$](image)

The $^1$H NMR chemical shifts of PB$^+$, PY$^+$ and their corresponding dimers and complexes of βCD, 33βCD$_2$suc and 66βCD$_2$suc were derived from the fitting of appropriate equilibrium algorithms (Eqns. 2.6 and 2.7) to the chemical shift variation data in D$_2$O (1.00 × 10$^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl at 298.2 K) and appear in Table 2.5. For PB$^+$, the protons H$_1$–H$_6$ experienced an upfield shift of 1.247, 1.030, 0.849, 1.161, 0.397 and 0.251 ppm, respectively when dimerising. For PY$^+$ the upfield shift of the H$_1$–H$_5$ protons in (PY$^+$)$_2$ is 0.973, 0.082, 0.713, 1.057 and 0.033 ppm,
respectively. The greater upfield shifts for the PB$^+$ and PY$^+$ aromatic H$_1$-H$_4$ resonances compared with the ethyl and methyl proton resonance shifts are consistent with H$_1$–H$_4$ experiencing an increased electron density in (PB$^+$)$_2$ and (PY$^+$)$_2$ which reflects their positioning in the aromatic $\pi$ electron density of the adjacent PB$^+$ or PY$^+$ as approximately shown in Figure 2.5 on page 33. The ethyl and methyl groups experience a lesser change in electron density as they are further from the aromatic $\pi$ electron density.

The downfield shifts of H$_1$ and H$_6$ of PB$^+$ in $\beta$CD.PB$^+$, 33$\beta$CD$_2$suc.PB$^+$ and 66$\beta$CD$_2$suc.PB$^+$ from H$_1$ and H$_6$ of free PB$^+$ are 0.174 and 0.094, 0.165 and 0.087, and 0.190 and 0.082 ppm, respectively, determined simultaneously with the derivation of $K_1$ from the observed $\delta$ data. The downfield shifts of H$_1$ and H$_5$ of PY$^+$ in $\beta$CD.PY$^+$, 33$\beta$CD$_2$suc.PY$^+$ and 66$\beta$CD$_2$suc.PY$^+$ from H$_1$ and H$_5$ of free PY$^+$ are 0.078 and 0.016, 0.212 and 0.097, and 0.165 and 0.032 ppm, respectively. Because the H$_1$ and H$_6$ of PB$^+$ and the H$_1$ and H$_5$ of PY$^+$ are the most distant from each other in the two pyronines they are likely to exhibit the largest change in $\delta$ due to differences in interaction upon complexation by $\beta$CD, 33$\beta$CD$_2$suc and 66$\beta$CD$_2$suc. In the first three cases the downfield shift of H$_1$ is about twice that of H$_6$, and in the second three cases the downfield shift of H$_1$ varies from two to five times that of H$_5$. This is consistent with an equilibrium existing between $\beta$CD.PB$^+$ isomers (d) and (f), in which one pair of PB$^+$ ethyl groups reside in the $\beta$CD annulus and isomer (e) in which the PB$^+$ xanthene entity is centred in the $\beta$CD annulus (Figure 2.32) such that both H$_1$ and H$_6$ experience the electronic environment of the $\beta$CD annulus. (The resulting inequivalence of the two N-diethyl groups is not observed in the $^1$H spectra as the complex lifetimes are short on the $^1$H NMR time-scale.) Alternatively, a dominant $\beta$CD.PB$^+$ complex with a structure midway between either (d) and (e) or (e) and (f) or both may exist. Similar possibilities exist for the complexation of PB$^+$ in single $\beta$CD annuli of 33$\beta$CD$_2$suc.PB$^+$ and 66$\beta$CD$_2$suc.PB$^+$ and for the analogous PY$^+$ systems.
Table 2.5. $^1$H NMR chemical shifts of PB$^+$ and PY$^+$ and their dimers and complexes of βCD, 33βCD$_2$suc and 66βCD$_2$suc derived from the fitting of appropriate equilibrium algorithms to chemical shift variation data in D$_2$O (1.00 $\times$ 10$^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K.

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<th>Species</th>
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<tr>
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<td>$\delta$ ppm</td>
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<td>7.114</td>
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<td>2.904$^b$</td>
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<tr>
<td>βCD.PY$^+$</td>
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<td>7.815</td>
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<td>66βCD$_2$suc.PY$^+$</td>
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<td>6.956</td>
<td>6.494</td>
<td>3.270</td>
<td>N/A$^d$</td>
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</table>

$\delta$ referenced to external trimethylsilylpropiosulfonic acid in D$_2$O. $^a$ Observed chemical shifts at the lowest concentration of [PB$^+$]$_{total}$ or [PY$^+$]$_{total}$. $^b$ Observed chemical shifts at the highest concentration of [PB$^+$]$_{total}$ or [PY$^+$]$_{total}$. $^c$ The H$_5$ proton resonance was not detectable due to overlapping with βCD signals. $^d$ The H$_6$ proton is not present in PY$^+$. 

65
The deductions from the $^1$H NMR data concerning the complexation of PB$^+$ and PY$^+$ are relevant to interpretation of the resulting fluorescence quenching. Both the model for complexation (Figure 2.32) and the alternative model where a dominant βCD.PB$^+$ complex with a structure midway between either (d) and (e) or (e) and (f) or both may exist are likely to alter the symmetry of the charge distribution represented by the PB$^+$ and PY$^+$ resonance structures (a)–(d) (Figure 2.3, page 31) through which all bonds share partial double bond character. Complexation by βCD, 33βCD$_2$suc and 66βCD$_2$suc results in part of PB$^+$ and PY$^+$ being in the hydrophobic βCD annulus and part in the aqueous environment. As both dialkylamino groups cannot be simultaneously in the βCD annulus both PB$^+$ and PY$^+$ experience an environmental asymmetry which is likely to introduce an asymmetry in charge distribution. As complexation causes fluorescence quenching the resulting change in charge distribution must increase the probability of non-radiative decay of the excited states of PB$^+$ and PY$^+$.

Two studies in a range of solvents are consistent with the most probable non-radiative decay for PB$^+$ and PY$^+$ occurring through a two-state mechanism where the fluorescent planar states resembling (a) and (c) are in equilibrium with non-emissive states resembling (b) and (d) (Figure 2.3, page 31) in which nitrogen assumes a tetrahedral stereochemistry such that $S_1$-$S_0$ internal conversion occurs.$^{30,31}$ A similar pathway for non-radiative...
transitions of excited electronic states of PB$^+$ and PY$^+$ to their ground states is a plausible explanation for the decrease in fluorescence shown by PB$^+$ and PY$^+$ upon complexation by βCD, 33βCD$_2$suc and 66βCD$_2$suc.

A model has also been proposed by Reija et al. for the decreased fluorescence of PB$^+$ and PY$^+$ upon complexation by βCD.$^{17}$ It is postulated that the xanthene entities of PB$^+$ and PY$^+$ are completely complexed inside the βCD annulus to form a non-emissive charge-transfer excited state where the positive charge is located at the centre of the xanthene entity as a consequence of stabilisation by the electron rich environment generated by the ether oxygens of the βCD annulus. This accentuates structural change of the amino groups towards a tetrahedral stereochemistry which engenders non-radiative deactivation. This model was proposed in the absence of evidence for the substantial interaction of the dialkylamino groups of PB$^+$ and PY$^+$ with the interior of the βCD annulus shown to occur in the present study. In view of this it is apparent that Reija’s model represents a component of the two alternative models proposed here for introducing asymmetry into the charge distribution in PB$^+$ and PY$^+$ upon complexation by βCD, 33βCD$_2$suc and 66βCD$_2$suc and consequent fluorescence quenching.

2.7. Conclusion

The complexation of PB$^+$ and PY$^+$ by βCD, 33βCD$_2$suc and 66βCD$_2$suc has been characterised in aqueous solution by UV–vis, fluorescence and $^1$H NMR spectroscopy. By comparison with the stabilities of the βCD.PB$^+$ and βCD.PY$^+$ complexes those of the 66βCD$_2$suc.PB$^+$, 33βCD$_2$suc.PB$^+$ and the analogous PY$^+$ complexes are slightly more than twice as stable consistent with a statistical enhancement in stability and little cooperativity between the two linked βCD annuli in complexing PB$^+$ and PY$^+$. However, PB$^+$ is complexed ~5 times more strongly than PY$^+$ consistent with the greater hydrophobicity of the PB$^+$ ethyl groups interacting more strongly with the hydrophobic annuli of βCD, 33βCD$_2$suc and 66βCD$_2$suc than do the methyl groups of PY$^+$. The fluorescence quenching of PB$^+$ and PY$^+$ in the complexes is attributed to a change in their charge distribution such that non-emissive relaxation of the excited state occurs through a dialkylamino group assuming a tetrahedral stereochemistry in the complexes than is the case in free PB$^+$ and PY$^+$. The dimerisations of PB$^+$ and PY$^+$ which occurs at the higher concentrations required for $^1$H NMR studies have also been characterised.
2.8. References


2.9. Appendix

2.9.1. UV–vis Titrations for Pyronine Y

![Absorbance spectra](image)

**Figure 2.33.** UV–visible absorbance spectra of PY$^+$ alone ($8.99 \times 10^{-6}$ mol dm$^{-3}$) and in the presence of increasing concentrations of βCD (ranging from 0.00 to $9.37 \times 10^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The arrow indicates the direction of absorbance change as [βCD]$_{total}$ increases. An isosbestic point occurs at 553 nm. $\lambda_{max} = 546$ nm ($\varepsilon = 8.10 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$) and 550 nm ($\varepsilon = 7.19 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$) for the free and complexed PY$^+$ species, respectively.

![Absorbance variation](image)

**Figure 2.34.** UV–visible absorbance variation of PY$^+$ ($8.99 \times 10^{-6}$ mol dm$^{-3}$) with βCD (0.00 to $9.37 \times 10^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles represent experimental data at 546 nm and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 500–590 nm.
**Figure 2.35.** UV–visible absorbance spectra of PY$^+$ alone ($9.10 \times 10^{-6}$ mol dm$^{-3}$) and in the presence of increasing concentrations of 33βCD$_2$suc (ranging from 0.00 to 2.50 $\times$ 10$^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The arrow indicates the direction of absorbance change as [33βCD$_2$suc]$_{\text{total}}$ increases. An isosbestic point occurs at 552 nm. $\lambda_{\text{max}} = 546$ nm ($\varepsilon = 8.10 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$) and 553 nm ($\varepsilon = 7.41 \times 10^4$ dm$^3$ mol$^{-1}$ cm$^{-1}$) for the free and complexed PY$^+$ species, respectively.

**Figure 2.36.** UV–visible absorbance variation of PY$^+$ ($9.10 \times 10^{-6}$ mol dm$^{-3}$) with 33βCD$_2$suc (0.00 to 2.50 $\times$ 10$^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles represent experimental data at 546 nm and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 500–590 nm.
Figure 2.37. UV–visible absorbance spectra of PY\textsuperscript{+} alone (9.18 \times 10^{-6} \text{ mol dm}^{-3}) and in the presence of increasing concentrations of 66\textbeta{CD}\textsubscript{2}suc (ranging from 0.00 to 2.74 \times 10^{-3} \text{ mol dm}^{-3}) in aqueous hydrochloric acid (1.00 \times 10^{-4} \text{ mol dm}^{-3}, I = 0.10 \text{ mol dm}^{-3} \text{ NaCl}) at 298.2 K. The arrow indicates the direction of absorbance change as [66\textbeta{CD}\textsubscript{2}suc]\textsubscript{total} increases. An isosbestic point occurs at 552 nm. $\lambda_{\text{max}} = 546 \text{ nm}$ ($\varepsilon = 8.10 \times 10^{4} \text{ dm}^{3} \text{ mol}^{-1} \text{ cm}^{-1}$) and 551 nm ($\varepsilon = 7.46 \times 10^{4} \text{ dm}^{3} \text{ mol}^{-1} \text{ cm}^{-1}$) for the free and complexed PY\textsuperscript{+} species, respectively.

Figure 2.38. UV–visible absorbance variation of PY\textsuperscript{+} (9.18 \times 10^{-6} \text{ mol dm}^{-3}) with 66\textbeta{CD}\textsubscript{2}suc (0.00 to 2.74 \times 10^{-3} \text{ mol dm}^{-3}) in aqueous hydrochloric acid (1.00 \times 10^{-4} \text{ mol dm}^{-3}, I = 0.10 \text{ mol dm}^{-3} \text{ NaCl}) at 298.2 K. The circles represent experimental data at 546 nm and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 500–590 nm.
2.9.2. Fluorimetric Titrations for Pyronine Y

**Figure 2.39.** Emission spectra of PY$^+$ alone ($9.19 \times 10^{-7}$ mol dm$^{-3}$) and in the presence of increasing concentrations of βCD (ranging from 0.00 to $9.65 \times 10^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. Excitation wavelength $\lambda_{\text{ex}} = 500$ nm with excitation and emission slit widths of 5 nm. The arrow indicates the direction of relative fluorescence emission change as $[\beta CD]_{\text{total}}$ increases. $\lambda_{\text{max}} = 563$ nm (816.5 a.u.) and 572 nm (365 a.u.) for the free and complexed PY$^+$ species, respectively.

**Figure 2.40.** Relative fluorescence variation of PY$^+$ ($9.19 \times 10^{-7}$ mol dm$^{-3}$) with βCD (0.00 to $9.65 \times 10^{-3}$ mol dm$^{-3}$) in aqueous hydrochloric acid ($1.00 \times 10^{-4}$ mol dm$^{-3}$, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. Emission was measured at 563 nm. The circles represent experimental data and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 530–630 nm.
Figure 2.41. Emission spectra of PY\(^+\) alone (9.56 \times 10^{-7} \text{ mol dm}^{-3}) and in the presence of increasing concentrations of 33βCD\(_2\)suc (ranging from 0.00 to 2.49 \times 10^{-3} \text{ mol dm}^{-3}) in aqueous hydrochloric acid (1.00 \times 10^{-4} \text{ mol dm}^{-3}, I = 0.10 \text{ mol dm}^{-3} \text{ NaCl}) at 298.2 K. Excitation wavelength \(\lambda_{\text{ex}} = 500\) nm with excitation and emission slit widths of 5 nm. The arrow indicates the direction of relative fluorescence emission change as [33βCD\(_2\)suc]\(_{\text{total}}\) increases. \(\lambda_{\text{max}} = 563\) nm (819 a.u.) and 572 nm (400 a.u.) for the free and complexed PY\(^+\) species, respectively.

Figure 2.42. Relative fluorescence variation of PY\(^+\) (9.56 \times 10^{-7} \text{ mol dm}^{-3}) with 33βCD\(_2\)suc (0.00 to 2.49 \times 10^{-3} \text{ mol dm}^{-3}) in aqueous hydrochloric acid (1.00 \times 10^{-4} \text{ mol dm}^{-3}, I = 0.10 \text{ mol dm}^{-3} \text{ NaCl}) at 298.2 K. Emission was measured at 563 nm. The circles represent experimental data and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 530–630 nm.
Figure 2.43. Emission spectra of \( \text{PY}^+ \) alone \( (9.62 \times 10^{-7} \text{ mol dm}^{-3}) \) and in the presence of increasing concentrations of \( 66\beta\text{CD}_2\text{suc} \) (ranging from 0.00 to \( 2.59 \times 10^{-3} \text{ mol dm}^{-3} \)) in aqueous hydrochloric acid \( (1.00 \times 10^{-4} \text{ mol dm}^{-3}, I = 0.10 \text{ mol dm}^{-3} \text{ NaCl}) \) at 298.2 K. Excitation wavelength \( \lambda_{\text{ex}} = 500 \text{ nm} \) with excitation and emission slit widths of 5 nm. The arrow indicates the direction of relative fluorescence emission change as \([66\beta\text{CD}_2\text{suc}]_{\text{total}}\) increases. \( \lambda_{\text{max}} = 563 \text{ nm} \) (820 a.u.) and 572 nm (375 a.u.) for the free and complexed \( \text{PY}^+ \) species, respectively.

Figure 2.44. Relative fluorescence variation of \( \text{PY}^+ \) \( (9.62 \times 10^{-7} \text{ mol dm}^{-3}) \) with \( 66\beta\text{CD}_2\text{suc} \) (0.00 to \( 2.59 \times 10^{-3} \text{ mol dm}^{-3} \)) in aqueous hydrochloric acid \( (1.00 \times 10^{-4} \text{ mol dm}^{-3}, I = 0.10 \text{ mol dm}^{-3} \text{ NaCl}) \) at 298.2 K. Emission was measured at 563 nm. The circles represent experimental data and the solid line represents the best fit of the algorithm for a 1:1 complexation model in the range 530–630 nm.
2.9.3. $^1$H NMR Titrations for Pyronine Y

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

Figure 2.45.
Figure 2.45. (continued) Left ordinate: variation of the $^1$H (300 MHz) chemical shift of the aromatic H$_1$ proton (A), aromatic H$_2$ proton (B), aromatic H$_3$ proton (C), aromatic H$_4$ proton (Fig. A51) and H$_5$ proton (D) of PY$^+$ (2.00 $\times$ 10$^{-3}$ mol dm$^{-3}$) with [βCD]$_{total}$ (ranging from 0 to 4.50 $\times$ 10$^{-3}$ mol dm$^{-3}$) in D$_2$O (1.00 $\times$ 10$^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles are the experimental data and the solid curve $a$ is the best fit of the algorithm incorporating PY$^+$, (PY$^+$)$_2$ and βCD.PY$^+$ to the chemical shift variations of protons H$_1$–H$_5$. Right ordinate: speciation relative to [PY$^+$]$_{total}$, curve $b$ is the percentage of [PY$^+$], curve $c$ is twice the percentage of [(PY$^+$)$_2$] and curve $d$ is the percentage of [βCD.PY$^+$].
Figure 2.46.
Figure 2.46. (continued) Left ordinate: variation of the $^1$H (300 MHz) chemical shift of the aromatic H$_1$ proton (A), aromatic H$_2$ proton (B), aromatic H$_3$ proton (C), aromatic H$_4$ proton (D) and H$_5$ proton (E) of PY$^+$ (2.00 $\times$ 10$^{-3}$ mol dm$^{-3}$) with [33$\beta$CD$_2$suc]$_{\text{total}}$ (ranging from 0 to 5.00 $\times$ 10$^{-3}$ mol dm$^{-3}$) in D$_2$O (1.00 $\times$ 10$^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles are the experimental data and the solid curve $a$ is the best fit of the algorithm incorporating PY$^+$, (PY$^+$)$_2$ and 33$\beta$CD$_2$suc.PY$^+$ to the chemical shift variations of protons H$_1$–H$_5$. Right ordinate: speciation relative to [PY$^+$]$_{\text{total}}$, curve $b$ is the percentage of [PY$^+$], curve $c$ is twice the percentage of [(PY$^+$)$_2$] and curve $d$ is the percentage of [33$\beta$CD$_2$suc.PY$^+$].
Figure 2.47.
Figure 2.47. (continued) Left ordinate: variation of the $^1$H (300 MHz) chemical shift of the aromatic H$_1$ proton (A), aromatic H$_2$ proton (B), aromatic H$_3$ proton (C), aromatic H$_4$ proton (D) and H$_5$ proton (E) of PY$^+$ (2.00 × $10^{-3}$ mol dm$^{-3}$) with [66βCD$_2$suc]$_{\text{total}}$ (ranging from 0 to 5.00 × $10^{-3}$ mol dm$^{-3}$) in D$_2$O (1.00 × $10^{-4}$ mol dm$^{-3}$ hydrochloric acid, $I = 0.10$ mol dm$^{-3}$ NaCl) at 298.2 K. The circles are the experimental data and the solid curve $a$ is the best fit of the algorithm incorporating PY$^+$, (PY$^+$)$_2$ and 66βCD$_2$suc.PY$^+$ to the chemical shift variations of protons H$_1$–H$_5$. Right ordinate: speciation relative to [PY$^+$]$_{\text{total}}$, curve $b$ is the percentage of [PY$^+$], curve $c$ is twice the percentage of [(PY$^+$)$_2$] and curve $d$ is the percentage of [66βCD$_2$suc.PY$^+$].
CHAPTER 3

DIMERISATION AND COMPLEXATION OF HEMATOPORPHYRIN BY $\gamma$-CYCLODEXTRIN AND LINKED $\gamma$-CYCLODEXTRIN DIMERS†

† Publication associated with part of the material in this chapter:
3.1. Introduction

Porphyrsins are among the most common candidates as photosensitizers for photodynamic therapy (PDT) for the treatment of cancers and non-cancerous diseases.\(^1\)\(^,\)\(^2\) The most commonly used and studied photosensitizer to date is the commercially available Photofrin\(^\circ\), also known as hematoporphyrin derivative (HPD),\(^3\)\(-\)\(^5\) which is a mixture of different oligomers of hematoporphyrin (di- and mono-) acetates. Haematoporphyrin IX (hematoporphyrin), protoporphyrin IX and its deuteron- and meso- analogues are also known for their photodynamic activities and are the subject of intensive research among other new generation photodynamic sensitizers.\(^1\)\(^,\)\(^6\)\(-\)\(^10\) Water-soluble porphyrins and metallloporphyrins have also been studied as potential models for myoglobin, hemoglobin\(^11\)\(-\)\(^15\) as well as agents for artificial photosynthesis.\(^16\)\(-\)\(^21\)

However, one of the issues with water-soluble porphyrins is their tendency to aggregate in aqueous solutions which impacts on their functionality. One solution for this issue is to encapsulate the porphyrins inside hydrophobic host species such as cyclodextrins. Consequently, native cyclodextrins and their modified forms have been studied as host species in supramolecular complexes of porphyrins as models for various biological applications.\(^15\)\(^,\)\(^22\)
3.1.1. Structure and Properties of Hematoporphyrin

Hematoporphyrin, HP, 7,12-bis(1-hydroxyethyl)-3,8,13,17-tetramethyl-21H,23H-porphine-2,18-dipropanoic acid, also known as hematoporphyrin IX, haematoporphyrin, photody or sensibion (Figures 3.1 and 3.2), belongs to a class of water–soluble porphyrins formed by the acid hydrolysis of hemoglobin. The first crystalline of HP was isolated by Nencki and Zaleski in 1900.\textsuperscript{23}

![Figure 3.1. Hematoporphyrin, HP](image)

![Figure 3.2. MM2 energy-minimised molecular models of HP. Carbon, nitrogen and oxygen atoms are shown in black, dark blue and dark red, respectively. Hydrogen atoms and lone pairs are not shown.](image)

In aqueous solutions, volumetric titration of HP yields two $pK_a$ values in the low pH region ($pK_{a1} \approx 2.1$ and $pK_{a2} \approx 1.8$) and two $pK_a$ values in the near neutral pH region ($pK_{a3} \approx \ldots$)
5.9 and $pK_{a4} \approx 6.7$), which are associated with the protonation of the imino nitrogens in the porphyrin ring and the dissociation of the carboxylic acid groups, as shown in the following equilibria:\textsuperscript{24}

$$[H_2(HP)]^{2+} \overset{pK_{a1}}{\rightleftharpoons} [H_1(HP)]^+ \overset{pK_{a2}}{\rightleftharpoons} HP \overset{pK_{a3}}{\rightleftharpoons} [H_{-1}(HP)]^- \overset{pK_{a4}}{\rightleftharpoons} [H_{-2}(HP)]^{2-} \quad (3.1)$$

A typical speciation plot of HP is shown in Figure 3.3.\textsuperscript{24} It should be noted that both Eqn. 3.1 and Figure 3.3 represent an oversimplification of the actual species existing in solution. While the ionic configurations of the $[H_2(HP)]^{2+}$ and $[H_{-2}(HP)]^{2-}$ species are unambiguous, the other three species can exist as zwitterionic forms.\textsuperscript{24}

![Figure 3.3. Speciation of different species of HP relative to [HP]$_{total}$ (5.0 × 10$^{-3}$ mol dm$^{-3}$) as a function of pH in aqueous solution.](), The species include $[H_2(HP)]^{2+}$ (red), $[H(HP)]^+$ (blue), HP (green), $[H_{-1}(HP)]^-$ (orange) and $[H_{-2}(HP)]^{2-}$ (black).

The commercial sample of HP for this study was obtained from Sigma as the 95% pure dihydrochloride salt and was used as received. A typical UV–vis absorption spectrum of a fresh solution of HP (5.1 × 10$^{-7}$ mol dm$^{-3}$) in pH 10.0 bicarbonate buffer (Figure 3.4) shows a very intense Soret band in the 350–400 nm region and four weak Q bands in the visible region (500–620 nm). For the purpose of this study, all quantitative measurements focus on the larger variations in the Soret band region of the absorption spectrum of HP. Figure 3.4 (red line) shows a typical fluorescence spectrum of HP (2.1 × 10$^{-7}$ mol dm$^{-3}$) in pH 10.0 bicarbonate buffer when excited at 390 nm, with the excitation and emission slits
being 5 nm and 10 nm, respectively. All measurements in this study are carried out in pH 10.0 bicarbonate buffer, in which HP exists exclusively as $[\text{H}_2\text{(HP)}]^2^-$ (Figure 3.3), where both carboxylic acid groups are deprotonated. For simplification, from here on $[\text{H}_2\text{(HP)}]^2^-$ will be referred to as HP$^{2^-}$.

**Figure 3.4.** UV–vis molar absorbance (left ordinate, blue line) and relative fluorescence (right ordinate, red line) spectra of HP$^{2^-}$ ($5.1 \times 10^{-7}$ and $2.1 \times 10^{-7}$ mol dm$^{-3}$, respectively) in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) at 298.2 K.

The 1D $^1$H NMR spectra (600 MHz) of HP in D$_2$O and DMSO-$d_6$ are shown and assigned in Figure 3.5. The assignments of the resonances (Table 3.1) are consistent with those found in the literature.$^{25,26}$
**Figure 3.5.** $^1$H NMR spectra (600 MHz) of HP at 298.2 K in DMSO-$d_6$ and $D_2$O.

**Table 3.1.** $^1$H NMR chemical shifts, $\delta$ (ppm), of HP protons at 298.2 K in $D_2$O and DMSO-$d_6$.

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<th>$H_{12}$</th>
<th>$H_6$</th>
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<td>3.69</td>
<td>2.12</td>
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3.1.2. Dimerisation of HP$^{2-}$ in Aqueous Solution

Hematoporphyrin is known to aggregate strongly in aqueous solution in a monomer–dimer equilibrium (Eqn. 3.2) existing at concentrations from around $5 \times 10^{-7}$ to $2.5 \times 10^{-4}$ mol dm$^{-3}$, and higher order aggregates can exist at higher concentrations.$^{27}$ Typical evidence for the aggregation process includes the presence of two fluorescing species having two different lifetimes in the fluorescence decay of HP and deviation from Beer’s law in the absorption spectra.$^{28}$ The dimerisation constants of HP under various conditions have been determined by UV–vis spectroscopy and appear in Table 3.2.$^{29-33}$

Table 3.2. Dimerisation constants, $K_d$, and photophysical properties for the dimerisation of HP in aqueous solution determined by UV–vis spectroscopy.

<table>
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<th>Species</th>
<th>$K_d$ dm$^3$ mol$^{-1}$</th>
<th>$\lambda_{\text{max}}$ nm</th>
<th>$\varepsilon$ mol$^{-1}$ dm$^3$ cm$^{-1}$</th>
<th>pH</th>
<th>$T$ K</th>
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</tr>
</thead>
<tbody>
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<td>–</td>
<td>394</td>
<td>$1.55 \times 10^5$</td>
<td>7.2$^a$</td>
<td>RT</td>
<td>32</td>
</tr>
<tr>
<td>HP$_2$</td>
<td>$1.7 \times 10^5$</td>
<td>$\sim 12^{b,d}$</td>
<td>$\sim 11^{c,d}$</td>
<td>298.2</td>
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<tr>
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<td>RT</td>
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<td>$1.7 \times 10^5$</td>
<td>$10.0^{d}$</td>
<td>298.2</td>
<td>33</td>
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</tbody>
</table>

$^a$ At this pH, HP exists predominantly as a mixture of the monoanionic and dianionic species (Figure 3.3). $^b$ In 0.02 mol dm$^{-3}$ sodium hydroxide. $^c$ In 0.002 mol dm$^{-3}$ sodium hydroxide. $^d$ At high pH values, HP exists predominantly as the dianionic HP$^{2-}$ species.

In order to understand the nature of the dimerisation process and the factors affecting this process, Karns et al.$^{29}$ studied the thermodynamics of the dimerisation of HP$^{2-}$ and several of its derivatives under alkaline conditions (0.02 mol dm$^{-3}$ sodium hydroxide) and found that the dimerisation was enthalpy–driven ($K_d = 1.7 \times 10^5$ dm$^3$ mol$^{-1}$, $\Delta H^o = -25.5$ kJ mol$^{-1}$ and $\Delta S^o = 11.8$ J mol$^{-1}$ K$^{-1}$ for HP$^{2-}$) and that the addition of alcohol decreased the dimerisation constant. The dimerisation equilibrium was not very dependent on the ionic strength if sodium chloride was used as supporting electrolyte. However, salts such as lithium sulphate or disodium phosphate affected the dimerisation in a rather complicated manner.$^{29}$ These observations were supported by Margalit et al.$^{34}$ On the other hand, the dimerisation equilibrium is significantly effected by pH,$^{35}$ due to different protonation states of HP.$^{24,36}$
### 3.1.3. Complexation of HP$^{2-}$ by α-, β- and γCD

\[
\begin{align*}
2\text{HP}^{2-} & \xrightarrow{K_d} (\text{HP}^{2-})_2 \\
\gamma \text{CD} + \text{HP}^{2-} & \xrightarrow{K_1} \gamma \text{CD}.\text{HP}^{2-}
\end{align*}
\] (3.2) \hspace{1cm} (3.3)

Equilibria 3.2 and 3.3 represent the dimerisation and competitive complexation processes that dominate the γCD/HP$^{2-}$ system in aqueous solution and analogous equilibria apply to the other CD/HP$^{2-}$ systems.

The complexation of HP$^{2-}$ by αCD and γCD was first studied by Hirai \textit{et al.} by UV–vis spectroscopy.\textsuperscript{30} Later, Hamai studied the complexation of HP$^{2-}$ by βCD, γCD and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-βCD) using fluorescence spectroscopy.\textsuperscript{33} The equilibrium constants, $K_1$, and the conditions of the experiments are summarised in Table 3.3.

#### Table 3.3. Equilibrium constants, $K_1$, and photophysical properties for the complexation of HP$^{2-}$ by αCD, βCD, TM-βCD and γCD in aqueous solution.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$K_1$ (dm$^3$ mol$^{-1}$)</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Method</th>
<th>pH</th>
<th>$T$ (K)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>αCD. HP$^{2-}$</td>
<td>35</td>
<td>395</td>
<td>UV–vis</td>
<td>~11$^a$</td>
<td>293.2</td>
<td>30</td>
</tr>
<tr>
<td>βCD. HP$^{2-}$</td>
<td>200</td>
<td>not stated</td>
<td>Fluorescence</td>
<td>10.0</td>
<td>298.2</td>
<td>33</td>
</tr>
<tr>
<td>TM-βCD. HP$^{2-}$</td>
<td>938</td>
<td>not stated</td>
<td>Fluorescence</td>
<td>10.0</td>
<td>298.2</td>
<td>33</td>
</tr>
<tr>
<td>γCD. HP$^{2-}$</td>
<td>73</td>
<td>396</td>
<td>UV–vis</td>
<td>~11$^a$</td>
<td>293.2</td>
<td>30</td>
</tr>
<tr>
<td>γCD. HP$^{2-}$</td>
<td>95.7</td>
<td>not stated</td>
<td>Fluorescence</td>
<td>10.0</td>
<td>298.2</td>
<td>33</td>
</tr>
</tbody>
</table>

$^a$ In 0.002 mol dm$^{-3}$ sodium hydroxide.

It is interesting that according to Hamai, HP$^{2-}$ was complexed more strongly by βCD than γCD, while it is expected that the wider annulus of γCD should accommodate HP$^{2-}$ more comfortably than βCD. Our preliminary UV–vis spectroscopy studies show that at the same concentration, γCD induces a larger spectral change in HP$^{2-}$ than βCD (Figure 3.6) indicating a stronger interaction. Overall the UV–vis changes are small.
Figure 3.6. UV–vis molar absorbance spectra of HP\textsuperscript{2−} alone (5.0 × 10\textsuperscript{-6} mol dm\textsuperscript{-3}, black line) and in the presence of βCD (6.0 × 10\textsuperscript{-3} mol dm\textsuperscript{-3}, blue line) and γCD (6.0 × 10\textsuperscript{-3} mol dm\textsuperscript{-3}, red line) in pH 10.0 bicarbonate buffer (I = 0.10 mol dm\textsuperscript{-3}) at 298.2 K.

3.1.4. Aims of This Study

The work in this chapter aims to quantitatively study and understand the competitive equilibrium processes between the dimerisation and host–guest complexation of HP\textsuperscript{2−} by γ-cyclodextrin and the newly synthesised linked γCD dimers, \(N,N′\text{-bis((2\textsuperscript{A}S,3\textsuperscript{A}S)-3\textsuperscript{A}-deoxy-γ-cyclodextrin-3\textsuperscript{A}-yl)succinamide, 33γCD}_2\text{suc, and }N,N′\text{-bis(6\textsuperscript{A}-deoxy-γ-cyclodextrin-6\textsuperscript{A}-yl)succinamide, 66γCD}_2\text{suc, in which a succinamide linker joins two γCDs through either the }C_3^\textsuperscript{A} \text{ or } C_6^\textsuperscript{A} \text{ carbons of altropyranose and glucopyranose units, respectively.}^{37}\) γ-Cyclodextrin hosts were chosen in favour of βCD because the larger annuli are expected to complex HP\textsuperscript{2−} more strongly. The competing equilibrium constants, characterised by UV–vis and fluorescence spectroscopy, thermodynamic parameters as well as molecular modelling are expected to provide insight into the nature of the interactions between HP\textsuperscript{2−} and the cyclodextrins.
3.2. Synthesis and Molecular Modelling of Succinamide–Linked γCD Dimers

3.2.1. Synthesis

The modification of native CDs may be achieved through substitution of either a OH₂, OH₃ or OH₆ but their similarity and number (6, 7, and 8 of each in αCD, βCD and γCD, respectively) renders selective modification challenging. The OH₆ are the most basic and usually the most nucleophilic, the OH₂ are the most acidic (pKₐ ~ 12.1) and the OH₃ are sterically the most difficult to access. Substitution of OH₆ in βCD is usually most readily achieved through 6^A-O-(4-methylbenzenesulfonyl)-β-cyclodextrin, while substitution of OH₂ and OH₃ is often achieved through 2^A-O-(4-methylbenzenesulfonyl)-β-cyclodextrin. However, using the literature reaction of βCD and 4-toluenesulfonyl chloride in aqueous solution for γCD results in polysubstitution at C₆ probably because of the larger γCD annulus, which makes purification difficult.

Larger arenesulfonyl chlorides have been used to achieve mono C₆^A substitution, among which 2,4,6-triisopropylbenzenesulfonyl chloride was claimed to be the best candidate to give the pure mono-substituted 6^A-O-(2,4,6-triisopropylbenzenesulfonyl)-γ-cyclodextrin. However, repeated attempts to follow this procedure gave low yields of the mono-6^A-γCD, and scaling up of the reaction presented difficult purification problems. Furthermore, the high cost of 2,4,6-triisopropylbenzenesulfonyl chloride by comparison with 4-toluenesulfonyl chloride makes it a relative uneconomical way to obtain multigram quantities of the substituted γCD.

A modification of the method by Murakami for the synthesis of 2^A-O-(4-methylbenzenesulfonyl)-β-cyclodextrin was selected as the simplest and most economical simultaneous route to 2^A- and 6^A-O-(4-methylbenzenesulfonyl)-γ-cyclodextrin although the yields are modest. The reaction of γCD and 4-toluenesulfonyl chloride in dimethylformamide (DMF) in the presence of dibutyltin oxide gave a mixture of 2^A- and 6^A-O-(4-methylbenzenesulfonyl)-γ-cyclodextrin (2γCDTs and 6γCDTs) which were readily separated on a Diaion HP-20 column in 5-10% yields of each. The relative ease of purification allowed multigram quantities of both 2γCDTs and 6γCDTs to be prepared.
Also it was possible to recover large amounts of unreacted $\gamma$CD through column chromatography for reuse. The reaction scheme is shown in Figure 3.7.

Bis(4-nitrophenyl) succinate required in the preparation of $33\gamma$CD$_2$suc and $66\gamma$CD$_2$suc was synthesised by reaction of succinyl chloride with 4-nitrophenol as described in chapter 2.

The first step in the synthesis of previously unreported ($2^A$S,$3^A$S)-$3^A$-amino-$3^A$-deoxy-$\gamma$-cyclodextrin ($3\gamma$CDNH$_2$) was achieved through reaction of $2\gamma$CDTs with aqueous ammonium bicarbonate to give $2^A$,$3^A$-manno-epoxide-$\gamma$-cyclodextrin ($23\gamma$CDO) which was isolated through chromatography on Diaion HP-20 and BioRex 70 ($H^+$) in 78.6 % yield. The product was then reacted with ammonium hydroxide (25 %, 60 cm$^3$) at 60 °C for 4 hours to give $3\gamma$CDNH$_2$, after chromatographic separation on BioRex 70 ($H^+$), in 56.7 % yield. Carbon-13 and $^1$H NMR spectra indicate that the C$_2^A$ and C$_3^A$ carbons of a glycopyranose unit in $\gamma$CD moiety are inverted to form an altropyranose ring during the synthesis, as is also the case for $3\beta$CDNH$_2$. The new $6^A$-amino-$6^A$-deoxy-$\gamma$-cyclodextrin ($6\gamma$CDNH$_2$) was converted from $6\gamma$CDTs by treatment with ammonium hydroxide over five days and was isolated through chromatography on BioRex 70 ($H^+$) in 18 % yield.

The $33\gamma$CD$_2$suc and $66\gamma$CD$_2$suc linked $\gamma$CD dimers were synthesised through a general method with yields of 75.7 and 91.6 %, respectively. The succinate diester was stirred with 2.5 equivalent of either $3\gamma$CDNH$_2$ or $6\gamma$CDNH$_2$ in pyridine for 48 hours at room temperature to give $33\gamma$CD$_2$suc and $66\gamma$CD$_2$suc which were separated on Biores 70 ($H^+$).
Figure 3.7. Synthetic scheme for preparation of succinamide-linked $\gamma$CD dimers.
3.2.2. Molecular Modelling

Models of the newly synthesised linked γCD dimers, $33\gamma\text{CD}_2\text{suc}$ and $66\gamma\text{CD}_2\text{suc}$, were constructed and energy-minimised using the MM2 molecular mechanics method using the ChemBio3D® Ultra 11.0 software, followed by geometry optimisation by the PM6 semi-empirical method using MOPAC2009. The final model renderings with the ChemBio3D® interface are shown in Figure 3.8 along their Z (left) and Y (right) axes, respectively.

The calculated energies (heats of formation in the gas-phase at standard states) of the two γCD dimers are $-14,827.1 \text{ kJ mol}^{-1}$ and $-14,912.4 \text{ kJ mol}^{-1}$ for $33\gamma\text{CD}_2\text{suc}$ and $66\gamma\text{CD}_2\text{suc}$, respectively. The inversions of the $C_2^A$ and $C_3^A$ carbons on both of the substituted altropyranose units of $33\gamma\text{CD}_2\text{suc}$ appear to lead to a slight distortion of the annuli and cause the succinamide linker to bend toward the space between the two annuli bringing them closer together by comparison with those of $66\gamma\text{CD}_2\text{suc}$.

![Figure 3.8](image-url)

Figure 3.8. Space-filling representations of $33\gamma\text{CD}_2\text{suc}$ and $66\gamma\text{CD}_2\text{suc}$ constructed and energy-minimised using the PM6 method. Carbon, nitrogen and oxygen atoms are shown in gray, blue and red, respectively. Hydrogen atoms and lone pairs are not shown.
3.3. UV–vis Spectroscopy and Thermodynamic Studies of the Dimerisation of HP$^{2-}$

The monomer–dimer equilibrium of HP$^{2-}$ shifts as temperature changes from 278.2 to 338.2 K (Figure 3.9) as predicted by Le Chatelier's principle. An isosbestic point at 381 nm is consistent with HP$^{2-}$ existing predominantly in the monomer and dimer forms. A decrease of the absorption band near 374 nm and increase in the region near 394 nm as the temperature increases coupled with an overall bathochromic shift indicate a shift toward the formation of the monomer. The increase in absorbance at the maximal wavelength 394 nm as a function of temperature (278.2–338.2 K, Figure 3.10) follows an asymptotical curve, which suggests that at temperatures higher than 323.2 K, the dimerised (HP$^{2-}$)$_2$ species dissociate almost completely in favour of the HP$^{2-}$ monomer species.

![Figure 3.9](image1)

**Figure 3.9.** Variation of UV–vis absorbance of HP$^{2-}$ (4.09 × 10$^{-6}$ mol dm$^{-3}$) as the temperature was varied from 278.2 to 328.2 K (in 5 K increments) in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$). The arrows indicate the direction of absorbance changes as the temperature increases.

![Figure 3.10](image2)

**Figure 3.10.** Observed absorbance at 394 nm of HP$^{2-}$ (4.09 × 10$^{-6}$ mol dm$^{-3}$) as a function of temperature.
3.3.1. UV–vis Spectroscopic Titrations

The dimerisation constant of HP$^{2-}$ was determined by monitoring the absorbance variation of HP$^{2-}$ over concentrations in the range $3.25 \times 10^{-6} – 7.74 \times 10^{-6}$ mol dm$^{-3}$ in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) by serially diluting (20 times $\times$ 0.100 cm$^3$ addition of the pH 10.0 bicarbonate buffer) an initial sample of HP$^{2-}$ in the concentration range $7.58 \times 10^{-6}$ to $7.74 \times 10^{-6}$ mol dm$^{-3}$. Absorbance spectra were recorded at 0.5 nm intervals over the range 300–450 nm (Figure 3.11).

Equation 3.4 describes the observed absorbance at any given wavelength when a dimerisation equilibrium (Eqn. 3.2) exists in the solution, where $A$, $\varepsilon_{\text{HP}}$ and $\varepsilon_{\text{HP}_2}$ represent the total absorbance, molar absorbance of the monomer HP$^{2-}$ and molar absorbance of the dimer (HP$^{2-}$)$_2$.

$$A = \varepsilon_{\text{HP}}[\text{HP}^2] + \varepsilon_{\text{HP}_2}[(\text{HP}^2)_2] \quad (3.4)$$

$$K_d = [(\text{HP}^2)_2]/[\text{HP}^2]^2 \quad (3.5)$$

The dimerisation constant, $K_d$, of HP$^{2-}$, as well as the molar absorptivities, $\varepsilon_{\text{HP}}$ and $\varepsilon_{\text{HP}_2}$, for the monomer and dimer species, respectively, were derived by simultaneously fitting the dimerisation algorithm (Eqns. 3.4 and 3.5) to the absorbance variation (Figure 3.11) over the range 350–410 nm at 0.5 nm intervals, using the HypSpec protocol (Figure 3.12), and are shown in Table 3.4.

The dimerisation titrations were carried out at five temperatures (278.2, 288.2, 298.2, 308.2 and 318.2 K, Figure 3.11) to obtain the five dimerisation constants, $K_d$, and are shown in Table 3.4. The variation in the volume of water as the temperature changes from 278.2 K ($d_{\text{water}} = 0.999965$ g cm$^{-3}$) to 318.2 K ($d_{\text{water}} = 0.99025$ g cm$^{-3}$) accounts for approximately 1% in experimental error which is incorporated with fitting and other experimental errors in the overall errors for $K_d$ values (Table 3.4). A van’t Hoff plot of the relationship between $K_d$ and temperature was used to calculate the enthalpy and entropy changes for the dimerisation (section 3.3.2).
Figure 3.11.
Figure 3.11. (continued) Variation in the observed molar absorptivity of HP$^{2-}$ in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) upon sequential dilution (0.100 cm$^{3}$ each, 20 times) to approximately $3.25 \times 10^{-6}$ mol dm$^{-3}$ at 278.2 K (A, $[\text{HP}^{2-}]_{\text{initial}} = 7.59 \times 10^{-6}$ mol dm$^{-3}$, isosbestic points 354 nm, 371 nm and 419 nm), 288.2 K (B, $[\text{HP}^{2-}]_{\text{initial}} = 7.74 \times 10^{-6}$ mol dm$^{-3}$, isosbestic points 355 nm, 370 nm and 419 nm), 298.2 K (C, $[\text{HP}^{2-}]_{\text{initial}} = 7.58 \times 10^{-6}$ mol dm$^{-3}$, isosbestic points 350 nm, 376 nm and 419 nm), 308.2 K (D, $[\text{HP}^{2-}]_{\text{initial}} = 7.74 \times 10^{-6}$ mol dm$^{-3}$, isosbestic points 347 nm, 375 nm and 416 nm) and 318.2 K (E, $[\text{HP}^{2-}]_{\text{initial}} = 7.59 \times 10^{-6}$ mol dm$^{-3}$, isosbestic points 347 nm, 379 nm and 417 nm). The arrows indicate the direction of molar absorbance changes as $[\text{HP}^{2-}]_{\text{total}}$ decreases.
Figure 3.12.
Figure 3.12. (continued) Left ordinate: variation in the observed molar absorptivity of HP$^{2-}$ in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) at 278.2 K (A, [HP$^{2-}$]$_{\text{initial}} = 7.59 \times 10^{-6}$ mol dm$^{-3}$), 288.2 K (B, [HP$^{2-}$]$_{\text{initial}} = 7.74 \times 10^{-6}$ mol dm$^{-3}$), 298.2 K (C, [HP$^{2-}$]$_{\text{initial}} = 7.58 \times 10^{-6}$ mol dm$^{-3}$), 308.2 K (D, [HP$^{2-}$]$_{\text{initial}} = 7.74 \times 10^{-6}$ mol dm$^{-3}$) and 318.2 K (E, [HP$^{2-}$]$_{\text{initial}} = 7.59 \times 10^{-6}$ mol dm$^{-3}$). The circles represent experimental data at 393 nm and the solid curve $a$ is the best fit of the algorithm for dimerisation of HP$^{2-}$ in the range 350–410 nm. Right ordinate: speciation relative to [HP$^{2-}$]$_{\text{total}}$, curve $b$ is the percentage of [HP$^{2-}$] and curve $c$ is twice the percentage of [(HP$^{2-})_2$].
3.3.2. Thermodynamic Studies

UV–vis titrations at five temperatures ranging from 278.15 to 318.15 K were performed to obtain the dimerisation constants, $K_d$, of HP$^{2-}$ as shown in section 3.3.1.

The relationship between the Gibbs free energy ($\Delta G^0$), enthalpy ($\Delta H^0$) and entropy ($\Delta S^0$) for the dimerisation constants ($K_d$) are given by the following van’t Hoff equations:

$$\Delta G^0 = -RT\ln K_d \quad (3.6)$$

with

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \quad (3.7)$$

and from 3.6 and 3.7,

$$\ln K_d = -\Delta H^0/RT + \Delta S^0/R \quad (3.8)$$

where $R$ is the gas constant and $T$ is the absolute temperature. The plot of $\ln K_d$ versus $1/T$ according to Eqn. 3.8 is a van’t Hoff plot, of which the slope and the intercept represent $-\Delta H^0/R$ and $\Delta S^0/R$, respectively.

The van’t Hoff plot for HP$^{2-}$ (Figure 3.13) of $\ln K_d$ versus $1/T$ is linear with a correlation coefficient, $R^2 = 0.992$. The slope of $-\Delta H^0/R = 6,958$ and intercept of $\Delta S^0/R = -11.62$, which correspond to an enthalpy change of $\Delta H^0 = -58 \pm 3$ kJ mol$^{-1}$, and an entropy change of $\Delta S^0 = -97 \pm 10$ J K$^{-1}$ mol$^{-1}$. The derived values of $K_d$, $\Delta G^0$, $\Delta H^0$ and $T\Delta S^0$ at all temperatures appear in Table 3.4.

The $K_d$ at 298.2 K found in the present study is within the range of values found in the literature (Table 3.2), as is the Gibbs free energy by comparison with that obtained by Karns et. al.$^{29}$ The thermodynamic parameters indicate that the dimerisation is enthalpy–driven with a negative entropy, which is common for the aggregation of porphyrins in aqueous solution.$^{29,34,51}$
Figure 3.13. The van’t Hoff plot for HP\textsuperscript{2−} on the effect of temperature (ranging from 278.2 to 318.2 K at 10 K increments) on the dimerisation constant ($K_d$) of HP\textsuperscript{2−} as determined by UV–vis absorption spectroscopy and the line of best-fit for a linear relationship, $R^2 = 0.992$.

Table 3.4. Dimerisation constants ($K_d$) and thermodynamic parameters for the dimerisation of HP\textsuperscript{2−} in pH 10.0 bicarbonate buffer ($I = 0.10 \text{ mol dm}^{-3}$) at different temperatures.$^a$

<table>
<thead>
<tr>
<th>$T$ K</th>
<th>$10^{-4} \times K_d$ $^b$</th>
<th>$\Delta G^a$ $^c$</th>
<th>$\Delta H^a$ $^c$</th>
<th>$T\Delta S^{a,d}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>278.2</td>
<td>62.0 ± 4.0</td>
<td>$-31.0 \pm 1.6$</td>
<td>$-58 \pm 3$</td>
<td>$-27 \pm 5$</td>
</tr>
<tr>
<td>288.2</td>
<td>26.0 ± 1.0</td>
<td>$-30.0 \pm 1.5$</td>
<td>$-58 \pm 3$</td>
<td>$-28 \pm 5$</td>
</tr>
<tr>
<td>298.2</td>
<td>14.3 ± 0.7</td>
<td>$-29.0 \pm 1.5$</td>
<td>$-58 \pm 3$</td>
<td>$-29 \pm 5$</td>
</tr>
<tr>
<td>308.2</td>
<td>4.9 ± 0.3</td>
<td>$-28.1 \pm 1.4$</td>
<td>$-58 \pm 3$</td>
<td>$-30 \pm 4$</td>
</tr>
<tr>
<td>318.2</td>
<td>2.8 ± 0.1</td>
<td>$-27.1 \pm 1.4$</td>
<td>$-58 \pm 3$</td>
<td>$-31 \pm 4$</td>
</tr>
</tbody>
</table>

$^a$ $\varepsilon_{\text{HP,394nm}} = 1.58 \times 10^5 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$ and $\varepsilon_{\text{HP\textsuperscript{2−},376nm}} = 8.94 \times 10^4 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$ for the monomer HP\textsuperscript{2−} and dimer (HP\textsuperscript{2−})\textsubscript{2} species, respectively. $^b$ Errors calculated from the fitting of UV–vis data taken into account other experimental errors. $^c$ Errors calculated from the slope of the van’t Hoff plot. $^d$ Errors are the sum of the errors in $\Delta G^0$ and $\Delta H^0$. 

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3.4. Complexation of HP²⁻ by βCD, γCD and Linked γCD Dimers

3.4.1. Complexation of HP²⁻ by βCD

It is expected from preliminary UV–vis study that βCD does not complex HP²⁻ very strongly as indicated by very small changes in the absorbance spectra. The absorbance variations of HP²⁻ (5.00 × 10⁻⁶ mol dm⁻³) in pH 10.0 bicarbonate buffer (I = 0.10 mol dm⁻³) were carefully monitored at 298.2 K over the range 300 – 450 nm, in the absence and presence of increasing concentrations of βCD (ranging from 2.00 × 10⁻⁴ to 8.00 × 10⁻³ mol dm⁻³). Selected molar absorbance spectra are shown in Figure 3.14 and the molar absorbance variations at 395 nm are shown in Figure 3.15. A slight increase with a red shift of the molar absorbance at the peak near 394 nm and a slight decrease of the band near 374 nm are consistent with the dissociation of the dimer (HP²⁻)₂ into HP²⁻ monomers due to complexation of HP²⁻ by βCD. However, the absorbance changes are quite small, and as a result a reliable fitting to derive the complexation constant, K₁, has not been obtained.

![Figure 3.14. Variation in the observed molar absorptivity of HP²⁻ (5.00 × 10⁻⁶ mol dm⁻³) in the absence and presence of different concentrations of βCD (2.53 × 10⁻³, 3.53 × 10⁻³, 6.01 × 10⁻³, 6.45 × 10⁻³ and 6.96 × 10⁻³ mol dm⁻³) in pH 10.0 bicarbonate buffer (I = 0.10 mol dm⁻³) at 298.2 K. The arrows indicate the direction of molar absorbance changes as the concentration of [βCD]_total increases.](image-url)
Figure 3.15. Variation in the observed molar absorptivity at 395 nm of HP\textsuperscript{2-} (5.00 × 10\textsuperscript{-6} mol dm\textsuperscript{-3}) in the absence and presence of different concentrations of βCD (ranging from 4.93 × 10\textsuperscript{-4} to 7.25 × 10\textsuperscript{-3} mol dm\textsuperscript{-3}) in pH 10.0 bicarbonate buffer (I = 0.10 mol dm\textsuperscript{-3}) at 298.2 K. The solid line is an arbitrary trend line to show the trend in absorbance variations only.

The more sensitive fluorescence spectra of HP\textsuperscript{2-} (2.20 × 10\textsuperscript{-7} mol dm\textsuperscript{-3}) in pH 10.0 bicarbonate buffer (I = 0.10 mol dm\textsuperscript{-3}) at 298.2 K were recorded in the absence and presence of increasing concentrations of βCD (ranging from 2.00 × 10\textsuperscript{-4} to 8.00 × 10\textsuperscript{-3} mol dm\textsuperscript{-3}). Selected fluorescence spectra are shown in Figure 3.16 and the fluorescence variations at 613 nm are shown in Figure 3.17. The fluorescence intensity of HP\textsuperscript{2-} decreases and a slight red shift of emission maxima occurs as the concentration of βCD increases. Similarly to the UV–vis absorbance variations, these fluorescence changes are too small for a reliable fitting for $K_1$ derivation.

The results of these UV–vis and fluorescence titrations suggest that the complexation of HP\textsuperscript{2-} by βCD under the conditions of this study is weak.
Figure 3.16. Variation in the observed fluorescence of HP\(^{2-}\) (2.20 \(\times\) 10\(^{-7}\) mol dm\(^{-3}\)) in the absence and presence of different concentrations of \(\beta\)CD (2.08 \(\times\) 10\(^{-4}\), 6.08 \(\times\) 10\(^{-4}\), 2.28 \(\times\) 10\(^{-3}\) and 7.25 \(\times\) 10\(^{-3}\) mol dm\(^{-3}\)) in pH 10.0 bicarbonate buffer \((I = 0.10\) mol dm\(^{-3}\)) at 298.2 K. The arrows indicate the direction of fluorescence changes as the concentration of \([\beta\text{CD}]_{\text{total}}\) increases. The excitation wavelength \(\lambda_{\text{ex}} = 390\) nm and the excitation and emission slit widths are 5 and 10 nm, respectively.

Figure 3.17. Variation in the observed fluorescence at 613 nm of HP\(^{2-}\) (2.20 \(\times\) 10\(^{-7}\) mol dm\(^{-3}\)) in the absence and presence of different concentrations of \(\beta\)CD (ranging from 2.08 \(\times\) 10\(^{-4}\) to 7.25 \(\times\) 10\(^{-3}\) mol dm\(^{-3}\)) in pH 10.0 bicarbonate buffer \((I = 0.10\) mol dm\(^{-3}\)) at 298.2 K. The solid line is an arbitrary trend line to show the trend in fluorescence variations only.
3.4.2. UV–vis Spectroscopic Studies of the Complexation of HP\(^2\) by \(\gamma\)CD and Linked \(\gamma\)CD Dimers

Upon the addition of \(\gamma\)CD, the dimerising HP\(^2\) experiences a larger change in the UV–vis absorbance by comparison with \(\beta\)CD, whereby the peak near 394 nm increases with a slight red shift coupled with a decrease in the 374 nm region (Figure 3.6, page 90). This is typical of the formation of a 1:1 host-guest \(\gamma\)CD.HP\(^2\) complex, as exemplified by Eqn. 3.3 on page 89. The equilibrium constant, \(K_1\), for this 1:1 complex is also dependent on temperature in a similar manner to \(K_d\) for the dimerisation of HP\(^2\), as shown in Figure 3.18. Therefore, it is possible to quantify the thermodynamic parameters for the complexation of HP\(^2\) by \(\gamma\)CD and the linked \(\gamma\)CD dimers using the van’t Hoff equation for the relationship between \(K_1\) and the temperature.

Figure 3.18 shows the temperature–dependent absorbance variation of a solution containing HP\(^2\) (4.96 \(\times\) \(10^{-6}\) mol dm\(^{-3}\)) and \(\gamma\)CD (1.00 \(\times\) \(10^{-3}\) mol dm\(^{-3}\)). There exists an isosbestic point at 377 nm which suggests that HP\(^2\) exists in two dominating environments as the temperature changes. It is expected that there should be two competing equilibria, i.e. dimerisation and complexation, as exemplified by Eqns. 3.2 and 3.3 on page 89. The absorbance change results from a combination of the complexation of HP\(^2\) by \(\gamma\)CD and the corresponding shift in the position of the HP\(^2\)/(HP\(^2\))\(_2\) equilibrium and the temperature dependences of both.

![Figure 3.18](image-url)
3.4.2.1. UV–vis Spectroscopic Titrations

The variations of molar absorbance spectra of HP\(^{2-}\) upon sequential injection (20 times × 0.100 cm\(^3\)) of \(\gamma\)CD solution (2.63 × 10\(^{-2}\) mol dm\(^{-3}\)) were studied at five temperatures (278.2, 288.2, 298.2, 308.2 and 318.2 K), and are shown in Figure 3.19. A larger increase in the molar absorbance at the peak near 394 nm and a significant decrease of the band near 374 nm are consistent with the dissociation of the (HP\(^{2-}\))\(_2\) dimer into the HP\(^{2-}\) monomers, which is induced by the complexation by \(\gamma\)CD. A red shift of the absorbance maximum is indicative of the formation of a \(\gamma\)CD.HP\(^{2-}\) complex (\(\lambda_{\text{max}} = 397\) nm, \(\varepsilon = 1.27 \times 10^5\) mol\(^{-1}\) dm\(^{-3}\) cm\(^{-1}\)).

Equation 3.9 describes the observed absorbance at any given wavelength when the dimerisation and complexation equilibria (Eqns. 3.2 and 3.3, page 89) coexist in the solution, where \(A\), \(\varepsilon_{\text{HP}}\), \(\varepsilon_{\text{HP}}\)\(_2\) and \(\varepsilon_{\text{CD,HP}}\) represent the total absorbance, molar absorbances of the monomer HP\(^{2-}\), dimer (HP\(^{2-}\))\(_2\) and 1:1 complex \(\gamma\)CD.HP\(^{2-}\).

\[
A = \varepsilon_{\text{HP}}[\text{HP}^{2-}] + \varepsilon_{\text{HP}}[(\text{HP}^{2-})_2] + \varepsilon_{\text{CD,HP}}[\gamma\text{CD.HP}^{2-}] \tag{3.9}
\]

\[
K_1 = [\gamma\text{CD.HP}^{2-}]/([\gamma\text{CD}][\text{HP}^{2-}]) \tag{3.10}
\]

The complexation constant, \(K_1\), of the \(\gamma\)CD.HP\(^{2-}\) complex, as well as the molar absorptivity, \(\varepsilon_{\text{CD,HP}}\), were derived by simultaneously fitting the algorithm incorporating Eqns. 3.5 (page 96), 3.9 and 3.10 to the absorbance variation (Figure 3.19) over the range 350–410 nm at 0.5 nm intervals, using the HypSpec protocol,\(^{48,49}\) with the known values of \(K_d\), \(\varepsilon_{\text{HP}}\) and \(\varepsilon_{\text{HP}}\)\(_2\) obtained in section 3.3.1, as shown in Figure 3.20.

Similar titrations and fittings were conducted to yield \(K_1\) for the 33\(\gamma\)CD\(_2\)Suc.HP\(^{2-}\) and 66\(\gamma\)CD\(_2\)Suc.HP\(^{2-}\) complexes at 278.2, 288.2 and 298.2 K (Figures 3.21 – 3.24), which appear in Table 3.5. At these low temperatures, the complexation of HP\(^{2-}\) by the two \(\gamma\)CD dimers resulted in larger absorbance variations, which were sufficient for \(K_1\) to be derived reliably through UV–vis titrations. However, at higher temperatures of between 308.2 and 318.2 K, decreased complexation prevented reliable fittings to the smaller absorbance variations over the concentration ranges studied.
3.4.2.1.1. UV–vis Titrations of HP\textsuperscript{2-} with γCD

Figure 3.19.

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Figure 3.19. (continued) Variation in the observed molar absorptivity of HP$^{2-}$ in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) upon sequential injection (0.100 cm$^3$ each, 20 times) of γCD solution ($2.63 \times 10^{-2}$ mol dm$^{-3}$) into both the sample and reference cells at 278.2 K (A, $[\text{HP}^{2-}]_{\text{initial}} = 7.55 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point 383 nm), 288.2 K (B, $[\text{HP}^{2-}]_{\text{initial}} = 7.60 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point 383 nm), 298.2 K (C, $[\text{HP}^{2-}]_{\text{initial}} = 7.55 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point 383 nm), 308.2 K (D, $[\text{HP}^{2-}]_{\text{initial}} = 7.60 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point 383 nm) and 318.2 K (E, $[\text{HP}^{2-}]_{\text{initial}} = 7.60 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point 388 nm). The arrows indicate the direction of molar absorbance changes as the mole ratio of $[\gamma\text{CD}]_{\text{total}}/[\text{HP}^{2-}]_{\text{total}}$ increases.
Figure 3.20.
Figure 3.20. (continued) Left ordinate: variation in the observed molar absorptivity of HP\(^2\)- in pH 10.0 bicarbonate buffer (\(I = 0.10 \text{ mol dm}^{-3}\)) upon sequential injection (0.100 cm\(^3\) each, 20 times) of \(\gamma\)CD solution (2.63 \(\times\) 10\(^{-2}\) mol dm\(^{-3}\)) into both the sample and reference cells at 278.2 K (A, [HP\(^2\)-]\(_{\text{initial}}\) = 7.55 \(\times\) 10\(^{-6}\) mol dm\(^{-3}\)), 288.2 K (B, [HP\(^2\)-]\(_{\text{initial}}\) = 7.60 \(\times\) 10\(^{-6}\) mol dm\(^{-3}\)), 298.2 K (C, [HP\(^2\)-]\(_{\text{initial}}\) = 7.55 \(\times\) 10\(^{-6}\) mol dm\(^{-3}\)), 308.2 K (D, [HP\(^2\)-]\(_{\text{initial}}\) = 7.60 \(\times\) 10\(^{-6}\) mol dm\(^{-3}\)) and 318.2 K (E, [HP\(^2\)-]\(_{\text{initial}}\) = 7.60 \(\times\) 10\(^{-6}\) mol dm\(^{-3}\)). The circles represent experimental data at 395 nm and the solid curve \(a\) is the best fit of the algorithm incorporating HP\(^2\)-, (HP\(^2\)-)\(_2\) and \(\gamma\)CD.HP\(^2\)- in the range 350–410 nm. Right ordinate: speciation relative to [HP\(^2\)-]\(_{\text{total}}\), curve \(b\) is the percentage of [HP\(^2\)-], curve \(c\) is twice the percentage of [(HP\(^2\)-)\(_2\)] and curve \(d\) is the percentage of \([\gamma\)CD.HP\(^2\)-].
3.4.2.1.2. UV–vis Titrations of HP$^{2-}$ by 33γCD$_2$suc

**Figure 3.21.** Variation in the observed molar absorptivity of HP$^{2-}$ in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) upon sequential injection (0.100 cm$^3$ each, 20 times) of 33γCD$_2$suc solution ($2.50 \times 10^{-3}$ mol dm$^{-3}$) into both the sample and reference cells at 278.2 K (A, [HP$^{2-}$]$_{initial} = 7.64 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point at 383 nm), 288.2 K (B, [HP$^{2-}$]$_{initial} = 7.62 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point 383 nm) and 298.2 K (C, [HP$^{2-}$]$_{initial} = 7.75 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point 383 nm). The arrows indicate the direction of molar absorbance changes as the mole ratio of [33γCD$_2$suc]$_{total}$/[HP$^{2-}$]$_{total}$ increases.
Figure 3.22. Left ordinate: variation in the observed molar absorptivity of HP$_2^-$ in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) upon sequential injection (0.100 cm$^3$ each) of 33γCD$_2$suc solution ($2.50 \times 10^{-3}$ mol dm$^{-3}$) into both the sample and reference cells at 278.2 K (A, [HP$_2^-$]$_{initial} = 7.64 \times 10^{-6}$ mol dm$^{-3}$), 288.2 K (B, [HP$_2^-$]$_{initial} = 7.62 \times 10^{-6}$ mol dm$^{-3}$) and 298.2 K (C, [HP$_2^-$]$_{initial} = 7.75 \times 10^{-6}$ mol dm$^{-3}$). The circles represent experimental data at 395 nm and the solid curve $a$ is the best fit of the algorithm incorporating HP$_2^-$, (HP$_2^-$)$_2$ and 33γCD$_2$suc.HP$_2^-$ in the range 350–410 nm. Right ordinate: speciation relative to [HP$_2^-$]$_{total}$, curve $b$ is the percentage of [HP$_2^-$], curve $c$ is twice the percentage of [(HP$_2^-$)$_2$] and curve $d$ is the percentage of [33γCD$_2$suc.HP$_2^-$].
3.4.2.1.3. UV–vis Titrations of HP$^{2-}$ by 66γCD$_2$suc

![Graph A](image)

![Graph B](image)

![Graph C](image)

**Figure 3.23.** Variation in the observed molar absorptivity of HP$^{2-}$ in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) upon sequential injection (0.100 cm$^3$ each, 20 times) of 66γCD$_2$suc solution ($2.50 \times 10^{-3}$ mol dm$^{-3}$) into both the sample and reference cells at 278.2 K (A, $[\text{HP}^{2-}]_{\text{initial}} = 7.12 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point at 383 nm), 288.2 K (B, $[\text{HP}^{2-}]_{\text{initial}} = 7.65 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point 383 nm) and 298.2 K (C, $[\text{HP}^{2-}]_{\text{initial}} = 7.64 \times 10^{-6}$ mol dm$^{-3}$, isosbestic point 383 nm). The arrows indicate the direction of molar absorbance changes as the mole ratio of [66γCD$_2$suc]$_{\text{total}}$/[HP$^{2-}$]$_{\text{total}}$ increases.
Figure 3.24. Left ordinate: variation in the observed molar absorptivity of HP$^2^-$ in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) upon sequential injection (0.100 cm$^3$ each, 20 times) of 66γCD$_2$suc solution ($2.50 \times 10^{-3}$ mol dm$^{-3}$) into both the sample and reference cells at 278.2 K (A, [HP$^2^-$]$_{\text{initial}} = 7.12 \times 10^{-6}$ mol dm$^{-3}$), 288.2 K (B, [HP$^2^-$]$_{\text{initial}} = 7.65 \times 10^{-6}$ mol dm$^{-3}$) and 298.2 K (C, [HP$^2^-$]$_{\text{initial}} = 7.64 \times 10^{-6}$ mol dm$^{-3}$). The circles represent experimental data at 395 nm and the solid curve $a$ is the best fit of the algorithm incorporating HP$^2^-$, (HP$^2^-$)$_2$ and 66γCD$_2$suc.HP$^2^-$ in the range 350–410 nm. Right ordinate: speciation relative to [HP$^2^-$]$_{\text{total}}$, curve $b$ is the percentage of [HP$^2^-$], curve $c$ is twice the percentage of [(HP$^2^-$)$_2$] and curve $d$ is the percentage of [66γCD$_2$suc.HP$^2^-$].
3.4.2.2. Thermodynamic Studies

The relationship between the Gibbs free energy ($\Delta G^o$), enthalpy ($\Delta H^o$) and entropy ($\Delta S^o$) for the complexation constants ($K_1$) are given by the following van’t Hoff equations:

$$\Delta G^o = -RT\ln K_1$$  \hspace{1cm} (3.11)

with

$$\Delta G^o = \Delta H^o - T\Delta S^o$$  \hspace{1cm} (3.12)

and from 3.11 and 3.12,

$$\ln K_1 = -\Delta H^o/RT + \Delta S^o/R$$  \hspace{1cm} (3.13)

where $R$ is the gas constant and $T$ is the absolute temperature. The plot of $\ln K_1$ versus $1/T$ according to Eqn. 3.13 is a van’t Hoff plot, of which the slope and the intercept represent $-\Delta H^o/R$ and $\Delta S^o/R$, respectively.

Using the van’t Hoff plots (Figure 3.25), $\Delta H^o$ and $\Delta S^o$ were calculated from the slopes and intercepts for the complexation of HP$^{2-}$ by $\gamma$CD and the linked $\gamma$CD dimers. The thermodynamic parameters as well as the equilibrium constants, $K_1$, and their respective molar absorptivities of the three complexes appear in Table 3.5.

**Figure 3.25.** The van’t Hoff plot for the effect of temperature (ranging from 278.2 to 298.2 K at 10 K increments) on the complexation constants ($K_1$) for HP$^{2-}$ by $\gamma$CD (■), 33$\gamma$CD$_2$suc (◊) and 66$\gamma$CD$_2$suc (●) as determined by UV–vis absorption spectroscopy and the best-fit lines. $R^2 = 0.998$, 0.982 and 0.986 for $\gamma$CD, 33$\gamma$CD$_2$suc and 66$\gamma$CD$_2$suc, respectively.
Table 3.5. Stability constants ($K_1$) and thermodynamic parameters for the complexation of HP$^{2-}$ by $\gamma$CD, 33$\gamma$CD$_2$suc and 66$\gamma$CD$_2$suc at different temperatures.

<table>
<thead>
<tr>
<th>$T$ K</th>
<th>Host</th>
<th>$K_1^a$ dm$^3$ mol$^{-1}$</th>
<th>$10^4 \times \varepsilon_{CD, HP}$ cm$^{-1}$ mol$^{-1}$</th>
<th>$\Delta G^b$ kJ mol$^{-1}$</th>
<th>$\Delta H^b$ kJ mol$^{-1}$</th>
<th>$T\Delta S^c$ kJ mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>278.2</td>
<td>$\gamma$CD</td>
<td>150 ± 8</td>
<td>12.7</td>
<td>$-11.7 \pm 0.6$</td>
<td>$-27.3 \pm 1.3$</td>
<td>$-15.6 \pm 1.9$</td>
</tr>
<tr>
<td>288.2</td>
<td>$\gamma$CD</td>
<td>106 ± 5</td>
<td>(397 nm)</td>
<td>$-11.1 \pm 0.5$</td>
<td>$-27.3 \pm 1.3$</td>
<td>$-16.2 \pm 1.8$</td>
</tr>
<tr>
<td>298.2</td>
<td>$\gamma$CD</td>
<td>73 ± 4</td>
<td></td>
<td>$-10.6 \pm 0.5$</td>
<td>$-27.3 \pm 1.3$</td>
<td>$-16.8 \pm 1.8$</td>
</tr>
<tr>
<td>308.2</td>
<td>$\gamma$CD</td>
<td>45 ± 2</td>
<td></td>
<td>$-10.0 \pm 0.5$</td>
<td>$-27.3 \pm 1.3$</td>
<td>$-17.3 \pm 1.8$</td>
</tr>
<tr>
<td>318.2</td>
<td>$\gamma$CD</td>
<td>36 ± 2</td>
<td></td>
<td>$-9.4 \pm 0.4$</td>
<td>$-27.3 \pm 1.3$</td>
<td>$-17.9 \pm 1.7$</td>
</tr>
<tr>
<td>278.2</td>
<td>33$\gamma$CD$_2$suc</td>
<td>860 ± 40</td>
<td>9.71</td>
<td>$-15.7 \pm 1.7$</td>
<td>$-27 \pm 3$</td>
<td>$-10 \pm 4$</td>
</tr>
<tr>
<td>288.2</td>
<td>33$\gamma$CD$_2$suc</td>
<td>640 ± 30</td>
<td>(395 nm)</td>
<td>$-15.4 \pm 1.7$</td>
<td>$-27 \pm 3$</td>
<td>$-10 \pm 4$</td>
</tr>
<tr>
<td>298.2</td>
<td>33$\gamma$CD$_2$suc</td>
<td>410 ± 20</td>
<td></td>
<td>$-15.0 \pm 1.6$</td>
<td>$-27 \pm 3$</td>
<td>$-11 \pm 4$</td>
</tr>
<tr>
<td>278.2</td>
<td>66$\gamma$CD$_2$suc</td>
<td>640 ± 30</td>
<td>13.6</td>
<td>$-15.1 \pm 1.7$</td>
<td>$-48 \pm 5$</td>
<td>$-33 \pm 7$</td>
</tr>
<tr>
<td>288.2</td>
<td>66$\gamma$CD$_2$suc</td>
<td>360 ± 20</td>
<td>(399 nm)</td>
<td>$-13.9 \pm 1.4$</td>
<td>$-48 \pm 5$</td>
<td>$-34 \pm 6$</td>
</tr>
<tr>
<td>298.2</td>
<td>66$\gamma$CD$_2$suc</td>
<td>159 ± 8</td>
<td></td>
<td>$-12.7 \pm 1.3$</td>
<td>$-48 \pm 5$</td>
<td>$-35 \pm 6$</td>
</tr>
</tbody>
</table>

$^a$ Errors calculated from the fitting of UV–vis data taken into account other experimental errors. $^b$ Errors calculated from the slopes of the van’t Hoff plots. $^c$ Errors are the sum of the errors on $\Delta G^0$ and $\Delta H^0$.

At 298.2 K, the $K_1$ for the 33$\gamma$CD$_2$suc.HP$^{2-}$ and 66$\gamma$CD$_2$suc.HP$^{2-}$ complexes are approximately six times and twice that of $\gamma$CD.HP$^{2-}$. This is consistent with moderate cooperativity in complexation between the two linked $\gamma$CD annuli being present in 33$\gamma$CD$_2$suc.HP$^{2-}$ but not in 66$\gamma$CD$_2$suc.HP$^{2-}$, where the advantage in forming a host-guest complex over $\gamma$CD is simply statistical (i.e. ~double). This suggests that the inversions of $C_2^A$ and $C_3^A$ carbons of both the substituted altropyranose units of 33$\gamma$CD$_2$suc have improved the stereochemistry of the dimer to cooperatively complex HP$^{2-}$ within the two $\gamma$CD annuli.

Equation 3.14 describes the linear enthalpy–entropy compensatory relationship of $T\Delta S^0$ vs. $\Delta H^0$ for the complexation of HP$^{2-}$ by $\gamma$CD,

$$T\Delta S^0 = \alpha\Delta H^0 + T\Delta S^0_0 \quad (3.14)$$

where the slope $\alpha$ indicates to what extent the enthalpic gain induced by any interactions of the host, guest or solvent is cancelled by the accompanying entropic
loss, whereas the intercept $T\Delta S^0_o$ represents the inherent complex stability ($\Delta G^0$) obtained when $\Delta H^o = 0$. This means that if $T\Delta S^0_o$ is positive, the complex is stabilised even in the absence of enthalpic stabilisation.$^{52}$

The thermodynamic parameters, $\Delta G^o$, $\Delta H^o$ and $T\Delta S^o$ for the complexation of HP$^{2-}$ by $\gamma$CD and the two $\gamma$CD dimers (Table 3.5) indicate that the complexation of HP$^{2-}$ by these hosts is exothermic and enthalpy–driven, with a negative entropy contribution. The enthalpy–entropy $\Delta H^o$–$T\Delta S^o$ relationship for all three complex systems is consistent with an enthalpy–entropy compensation plot for a large number of $\gamma$CD complexes (Figure 3.26),$^{52}$ which has a correlation coefficient of 0.94, a slope $\alpha = 0.97$ and intercept $T\Delta S^0_o = 16.3$ kJ mol$^{-1}$. The slope $\alpha$ is almost unity (0.97), meaning that only 3% of the enthalpic gain contributes to overall complex stability, whereas 97% of the enthalpic gain from the complexation is cancelled out by the entropy decrease caused by complex formation. This decrease in entropy is likely to arise from a combination of several factors, such as the decrease in species when the host and guest form a complex, combined with the displacement of water molecules from the $\gamma$CD annulus by HP$^{2-}$ and the subsequent desolvation of the latter, as well as conformational changes of both the host and the guest molecules. Nevertheless, the complexation of HP$^{2-}$ by $\gamma$CD and the two $\gamma$CD dimers is still favoured due to the ring flexibility and a large number of associated water molecules in and around the $\gamma$CD annuli.$^{52}$

![Figure 3.26.](image)

Figure 3.26. Enthalpy–entropy compensation plot for $\gamma$CD complexes (o) constructed using data from ref 52, and for the complexation of HP$^{2-}$ by $\gamma$CD (●), $33\gamma$CD$_2$suc (♦) and $66\gamma$CD$_2$suc (■) in aqueous solution at 298.2 K determined in the present study.
3.4.3. Fluorimetric Titration Studies of the Complexation of HP\textsuperscript{2-} by \(\gamma\)CD and Linked \(\gamma\)CD Dimers

The variations of the fluorescence spectra of HP\textsuperscript{2-} (2.10 \times 10^{-7} \text{ mol dm}^{-3}) in aqueous pH 10.0 bicarbonate buffer, \(I = 0.10 \text{ mol dm}^{-3}\) at 298.2 K with increasing concentrations of \(\gamma\)CD (0 – 3.73 \times 10^{-2} \text{ mol dm}^{-3}, 14 samples), 33\(\gamma\)CD\textsubscript{2}suc (0 – 2.01 \times 10^{-3} \text{ mol dm}^{-3}, 22 samples) and 66\(\gamma\)CD\textsubscript{2}suc (0 – 3.73 \times 10^{-3} \text{ mol dm}^{-3}, 16 samples) are shown in Figures 3.27, 3.29 and 3.31. The fluorescence intensity of HP\textsuperscript{2-} decreases and a slight red shift of emission maxima occurs as the concentration of each host increases. At this HP\textsuperscript{2-} concentration, there is less than 3\% of HP\textsuperscript{2-} existing in the dimerised state, according to the dimerisation constant, \(\textit{K}_d\), determined in section 3.3. Therefore, it is safe to assume that there is only one equilibrium for complexation of HP\textsuperscript{2-} by \(\gamma\)CD or either of the \(\gamma\)CD dimers that governs the variations in the observed fluorescence, as exemplified by Eqn. 3.3 on page 89.

The observed fluorescence intensity, \(I_F\), at any given wavelength is therefore equal to the mole fraction–weighted sum of the fluorescence intensities of the free and complexed HP\textsuperscript{2-}, as shown in Eqn. 3.15.

\[
I_F = I_{F(HP)}([\text{HP}^2^-]/[\text{HP}^2^-]_{\text{total}}) + I_{F(\gamma\text{CD.HP})}([\gamma\text{CD.HP}^2^-]/[\text{HP}^2^-]_{\text{total}}) \quad (3.15)
\]

The algorithm for the formation of the 1:1 complex analogous to Eqn. 3.15 best–fits the data at 0.5 nm intervals in the range 600–700 nm (Figures 3.28, 3.30 and 3.32), to yield \(K_1 = 60 \pm 5, 400 \pm 10\) and \(187 \pm 9 \text{ dm}^3 \text{ mol}^{-1}\) for the \(\gamma\)CD.HP\textsuperscript{2-}, 33\(\gamma\)CD\textsubscript{2}suc.HP\textsuperscript{2-} and 66\(\gamma\)CD\textsubscript{2}suc.HP\textsuperscript{2-} complexes, respectively. There is reasonable agreement between the \(K_1\) derived by the fluorimetric study with those derived by UV–vis studies (section 3.4.2). The \(K_1\) values for complexation of HP\textsuperscript{2-} by \(\gamma\)CD (73 dm\textsuperscript{3} mol\textsuperscript{-1} by UV–vis and 60 dm\textsuperscript{3} mol\textsuperscript{-1} by fluorimetry) are similar to the reported \(K_1\) of 73 dm\textsuperscript{3} mol\textsuperscript{-1} by UV–vis for a 0.002 mol dm\textsuperscript{-3} sodium hydroxide solution of HP\textsuperscript{2-} at 293.15 K by Hirai \textit{et al}.,\textsuperscript{30} and \(K_1\) of 95.7 dm\textsuperscript{3} mol\textsuperscript{-1} by fluorimetry in pH 10.0 buffer by Hamai.\textsuperscript{33}
Figure 3.27. Variation in the observed fluorescence intensity of HP$^{2-}$ alone ($2.10 \times 10^{-7}$ mol dm$^{-3}$) in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) and in the presence of γCD (ranging from $3.88 \times 10^{-4}$ to $3.73 \times 10^{-2}$ mol dm$^{-3}$, 14 samples) at 298.2 K. The excitation wavelength $\lambda_{ex} = 390$ nm. The excitation and emission slit widths are 5 and 10 nm, respectively. The arrows indicate the direction of fluorescence changes as the concentration of [γCD]$_{total}$ increases.

Figure 3.28. Fluorescence intensity observed at 613 nm (circles) and the best fit of an algorithm for a 1:1 complexation model (Eqn. 3.15) to the experimental data in the range 600–700 nm. $\lambda_{max} = 613$ nm (358 a.u.) and 617 nm (255 a.u.) for the free and complexed HP$^{2-}$ species, respectively.
**Figure 3.29.** Variation in the observed fluorescence intensity of HP$^{2-}$ alone ($2.10 \times 10^{-7}$ mol dm$^{-3}$) in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) and in the presence of 33γCD$_2$suc (ranging from $8.39 \times 10^{-5}$ to $2.01 \times 10^{-3}$ mol dm$^{-3}$, 22 samples) at 298.2 K. The excitation wavelength $\lambda_{\text{ex}} = 390$ nm. The excitation and emission slit widths are 5 and 10 nm, respectively. The arrows indicate the direction of fluorescence changes as the concentration of [33γCD$_2$suc]$_{\text{total}}$ increases.

**Figure 3.30.** Fluorescence intensity observed at 613 nm (circles) and the best fit of an algorithm for a 1:1 complexation model (Eqn. 3.15) to the experimental data in the range 600–700 nm. $\lambda_{\text{max}} = 613$ nm (358 a.u.) and 615 nm (34 a.u.) for the free and complexed HP$^{2-}$ species, respectively.
Figure 3.31. Variation in the observed fluorescence intensity of HP$^{2-}$ alone ($2.10 \times 10^{-7}$ mol dm$^{-3}$) in pH 10.0 bicarbonate buffer ($I = 0.10$ mol dm$^{-3}$) and in the presence of 66γCD$_2$suc (ranging from $5.35 \times 10^{-4}$ to $3.73 \times 10^{-3}$ mol dm$^{-3}$, 16 samples) at 298.2 K. The excitation wavelength $\lambda_{ex} = 390$ nm. The excitation and emission slit widths are 5 and 10 nm, respectively. The arrows indicate the direction of fluorescence changes as the concentration of [66γCD$_2$suc]$_{total}$ increases.

Figure 3.32. Fluorescence intensity observed at 613 nm (circles) and the best fit of an algorithm for a 1:1 complexation model (Eqn. 3.15) to the experimental data in the range 600–700 nm. $\lambda_{max} = 613$ nm (358 a.u.) and 620 nm (122 a.u.) for the free and complexed HP$^{2-}$ species, respectively.
3.5. Molecular Modelling Studies

To help understanding the nature of the interactions in the complexation of HP\textsuperscript{2-} by γCD and the succinamide linked γCD dimers, molecular models of the complexes were constructed and their geometries optimised using the PM6 semi-empirical method. For each complex, two alternative models were constructed, with either the 2-carboxylatoethyl moiety or 1-hydroxyethyl moiety being the main complexation site within the γCD annulus (Figure 3.33). The formation energies, $E_{\text{complex}}$ (heats of formation in the gas-phase at standard states) and the corresponding complexation energies, $\Delta E$, for the γCD.HP\textsuperscript{2-}, 33γCD\textsubscript{2}suc.HP\textsuperscript{2-} and 66γCD\textsubscript{2}suc.HP\textsuperscript{2-} complexes, are summarised in Table 3.6.

For all three complex systems, the interactions through the 2-carboxylatoethyl moiety give the most stable complexes, consistent with its higher hydrophobicity interacting more strongly with the hydrophobic γCD annulus than the 1-hydroxyethyl moiety. The lowest energy for the 33γCD\textsubscript{2}suc.HP\textsuperscript{2-} complex (Figure 3.33, left) is consistent with it being the most stable complex, while γCD.HP\textsuperscript{2-} is the least stable complex. This data is consistent with the complexation stability and thermodynamic data obtained by UV–vis spectroscopy in section 3.4. Although hydration will affect the stability of the above complexes in aqueous solution it is expected that the interactions involved in the gas-phase studies constitute major contributions to the complex stabilities in aqueous solution.

Table 3.6. Energies of formation ($E_{\text{complex}}$) and complexation energies ($\Delta E$) of the γCD.HP\textsuperscript{2-}, 33γCD\textsubscript{2}suc.HP\textsuperscript{2-} and 66γCD\textsubscript{2}suc.HP\textsuperscript{2-} complexes obtained with the PM6 method.

<table>
<thead>
<tr>
<th>Host</th>
<th>Complexation site</th>
<th>$E_{\text{complex}}$\textsuperscript{a} \ (kJ mol\textsuperscript{-1})</th>
<th>$E_{\text{host}}$\textsuperscript{a} \ (kJ mol\textsuperscript{-1})</th>
<th>$\Delta E$\textsuperscript{b} \ (kJ mol\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>γCD</td>
<td>2-carboxylatoethyl</td>
<td>$-8658.7$</td>
<td>$-7417.0$</td>
<td>$-332.3$</td>
</tr>
<tr>
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<td>1-hydroxyethyl</td>
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<td>$-7417.0$</td>
<td>$-245.6$</td>
</tr>
<tr>
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<td>2-carboxylatoethyl</td>
<td>$-16108.2$</td>
<td>$-14827.1$</td>
<td>$-371.7$</td>
</tr>
<tr>
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<td>1-hydroxyethyl</td>
<td>$-15940.3$</td>
<td>$-14827.1$</td>
<td>$-203.8$</td>
</tr>
<tr>
<td>66γCD\textsubscript{2}suc</td>
<td>2-carboxylatoethyl</td>
<td>$-16185.6$</td>
<td>$-14912.4$</td>
<td>$-363.8$</td>
</tr>
<tr>
<td></td>
<td>1-hydroxyethyl</td>
<td>$-16147.3$</td>
<td>$-14912.4$</td>
<td>$-325.5$</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Calculated energies for the complexes and the CD hosts were obtained as heats of formation in the gas-phase at standard states.

\textsuperscript{b} $\Delta E = E_{\text{complex}} - (E_{\text{host}} + E_{\text{HP}})$, where $E_{\text{HP}} = -909.4$ kJ mol\textsuperscript{-1}.
\[ \Delta E = -332.3 \text{ kJ mol}^{-1} \quad \gamma\text{CD.HP}^2^- \]
\[ \Delta E = -245.6 \text{ kJ mol}^{-1} \quad \gamma\text{CD.HP}^2^- \]
\[ \Delta E = -371.7 \text{ kJ mol}^{-1} \quad 33\gamma\text{CD}_{2\text{suc}}.\text{HP}^2^- \]
\[ \Delta E = -203.8 \text{ kJ mol}^{-1} \quad 33\gamma\text{CD}_{2\text{suc}}.\text{HP}^2^- \]
\[ \Delta E = -363.8 \text{ kJ mol}^{-1} \quad 66\gamma\text{CD}_{2\text{suc}}.\text{HP}^2^- \]
\[ \Delta E = -325.5 \text{ kJ mol}^{-1} \quad 66\gamma\text{CD}_{2\text{suc}}.\text{HP}^2^- \]

**Figure 3.33.** Space-filling representations of \( \gamma\text{CD.HP}^2^- \), \( 33\gamma\text{CD}_{2\text{suc}}.\text{HP}^2^- \) and \( 66\gamma\text{CD}_{2\text{suc}}.\text{HP}^2^- \) constructed and energy–minimised using the PM6 method. Two models are shown for each complex, where either the 2-carboxylatoethyl moiety (left) or 1-hydroxyethyl moiety (right) is the main interaction site. Colours: \( \gamma\text{CD} \): gray – C, red – O, blue – N; HP\(^2^-\): black – C, brown – O, dark blue – N. Hydrogen atoms and lone pairs are not shown.
3.6. 2D $^1$H NOESY NMR Studies

2D $^1$H NOESY NMR spectra of the $\gamma$CD.HP$^{2-}$, 33$\gamma$CD$_2$suc.HP$^{2-}$ and 66$\gamma$CD$_2$suc.HP$^{2-}$ complexes were obtained to seek further evidence concerning the nature of the complexes, through NOE cross–peaks arising from interactions between the HP$^{2-}$ protons and those of H$_{3,5}$ protons of the annular $\gamma$CD. Such cross–peaks are indicative of two sets of interacting protons being within 400 pm of each other, which generally means complexation within the $\gamma$CD annulus has occurred. Based on the molecular modelling studies in section 3.5, cross–peaks are expected to arise from NOE interaction between the protons of HP$^{2-}$ 2-carboxylatoethyl moiety and/or 1-hydroxyethyl moiety and the $\gamma$CD H$_{3,5}$ protons.

Figure 3.34. $^1$H NMR (300 MHz) spectra of (a) HP$^{2-}$ alone (5.0 × 10$^{-3}$ mol dm$^{-3}$), (b) equimolar HP$^{2-}$ and $\gamma$CD, (c) equimolar HP$^{2-}$ and 33CD$_2$suc and (d) equimolar HP$^{2-}$ and 66$\gamma$CD$_2$suc in D$_2$O (pD 10.0 bicarbonate buffer, $I$ = 0.10 mol dm$^{-3}$) at 298.2 K.

Prior to 2D $^1$H NMR NOESY experiments, it is important to investigate the 1D $^1$H NMR spectra. Figure 3.34 shows the typical 1D $^1$H NMR spectra (300 MHz) of HP$^{2-}$ alone and in the presence of equimolar $\gamma$CD, 33CD$_2$suc and 66$\gamma$CD$_2$suc in D$_2$O (pD 10.0 bicarbonate buffer, $I$ = 0.10 mol dm$^{-3}$) at 298.2 K. As can be seen, the HP$^{2-}$ H$_{26,31}$ and H$_{37,38}$ resonances have similar chemical shifts to those of $\gamma$CD H$_{2-6}$ protons, therefore cross–peaks arising from these resonances will be obscured. There is no
apparent change in the chemical shifts of the HP$^{2-}$ aromatic protons. This suggests that either these aromatic protons do not experiencing significant changes in their environment, or that the changes in chemical shift are too small to be observed. The latter seems more likely because at the concentrations of these NMR studies, HP$^{2-}$ exists predominantly as the (HP$^{2-}$)$_2$ dimer (> 90%) and less than 5% of [HP$^{2-}$]$_{\text{total}}$ exists in its complexed form, based on the $K_d$ and $K_1$ values determined in sections 3.3 and 3.4. Since the observed chemical shifts are the mole fraction–weighted averages of all the species, the contribution of chemical shift changes due to complexation will be small.

A 2D $^1$H NMR NOESY spectrum for HP$^{2-}$ alone in D$_2$O (pD 10.0 bicarbonate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2 K was obtained and shown in Figure 3.35, in order to identify all the cross–peaks arising from self–interactions among HP$^{2-}$ protons. Various cross–peaks were observed among the HP$^{2-}$ resonances (as stated in the caption), either due to their close proximity in a single HP$^{2-}$ or as a result of HP$^{2-}$ dimerisation, which is significant at this concentration.

Figure 3.35. 2D 1H NOESY NMR (600 MHz) spectrum of HP$^{2-}$ alone (1.00 × 10$^{-2}$ mol dm$^{-3}$) in D$_2$O (pD 10.0 buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2 K with a mixing time of 300 ms. The cross–peaks enclosed in rectangles arise from self–interactions between H$_{24}$ and H$_{43,44}$, H$_{37,38}$, H$_{35,39}$; between H$_{18}$ and H$_{43,44}$, H$_{35,39}$; between H$_{12}$ and H$_{26,31}$; between H$_{6}$ and H$_{27,32}$, H$_{43,44}$, H$_{25,30}$, H$_{26,31}$; and between H$_{35,39}$ and H$_{43,44}$, H$_{26,31}$, H$_{37,38}$. 

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The 2D $^1$H NMR NOESY spectra (600 MHz) of HP$^{2-}$ ($5.00 \times 10^{-3}$ mol dm$^{-3}$) in the presence of the same concentration of $\gamma$CD, 33CD$_2$suc and 66$\gamma$CD$_2$suc in D$_2$O (pD 10.0 bicarbonate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2 K are shown in Figures 3.36, 3.37 and 3.38, respectively. As expected, the cross–peaks found in all three systems resemble those found in the spectrum of HP$^{2-}$ alone (Figure 3.35) which suggests that they arise largely from proton self–interactions within the HP$^{2-}$ alone. No cross–peaks were observed between the HP$^{2-}$ aromatic protons and those inside the $\gamma$CD annulus in any case. This is consistent with the 1D $^1$H NMR data (Figure 3.34), where there is no change in the chemical shifts of these resonances. Cross–peaks between the resonances of the HP$^{2-}$ 2-carboxylatoethyl moiety or 1-hydroxyethyl moiety ($H_{26,31}$ and $H_{37,38}$ protons) were not observed due to them being obscured by those of the $\gamma$CD $H_{2-6}$ resonances. The cross–peaks enclosed in the small rectangles (Figures 3.36 – 3.38) were not likely to arise from the through-space interactions between the HP$^{2-}$ $H_{43,44,27,32}$ protons and the $\gamma$CD $H_{3,5,6}$ protons, but arose from self-interactions between the HP$^{2-}$ $H_{43,44,27,32}$ protons and the HP$^{2-}$ $H_{26,31,37,38}$ protons. As mentioned earlier, at the concentrations of the NMR studies, the majority of HP$^{2-}$ (> 90%) exists in the dimerised state and only < 5% exists in the complexed form in all systems, due to the much higher $K_d$ by comparison with $K_1$ values. Therefore, any cross–peaks arising from the interactions by complexation would be much less intense, while those arising from self-interaction between HP$^{2-}$ protons are dominant.

In principle the concentration of $\gamma$CD, 33$\gamma$CD$_2$suc and 66$\gamma$CD$_2$suc could be increased so that more HP$^{2-}$ was complexed. However, the increase in the concentration required would produce a substantial increase in the relative amplitudes of the CD resonances and the diagonal to the extent that the cross–peaks of interest would be obscured.
Figure 3.36. 2D 1H NOESY NMR (600 MHz) spectrum of HP$^{2-}$ ($5.00 \times 10^{-3}$ mol dm$^{-3}$) and equimolar $\gamma$CD in D$_2$O (pD 10.0 bicarbonate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2 K with a mixing time of 300 ms. The cross–peaks enclosed in rectangles arise from self–interactions between the different protons of HP$^{2-}$. No clear interactions between HP$^{2-}$ protons and the $\gamma$CD H$_{3,5,6}$ protons were observed.

Figure 3.37. 2D 1H NOESY NMR (600 MHz) spectrum of HP$^{2-}$ ($5.00 \times 10^{-3}$ mol dm$^{-3}$) and equimolar 33$\gamma$CD$_2$suc in D$_2$O (pD 10.0 bicarbonate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2 K with a mixing time of 300 ms. The cross–peaks enclosed in rectangles arise from self–interactions between the different protons of HP$^{2-}$. No clear interactions between HP$^{2-}$ protons and the $\gamma$CD H$_{3,5,6}$ protons were observed.
Figure 3.38. 2D 1H NOESY NMR (600 MHz) spectrum of HP$^{2-}$ (5.00 × 10$^{-3}$ mol dm$^{-3}$) and equimolar 66γCD$_2$suc in D$_2$O (pD 10.0 bicarbonate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2 K with a mixing time of 300 ms. The cross-peaks enclosed in rectangles arise from self-interactions between the different protons of HP$^{2-}$. No clear interactions between HP$^{2-}$ protons and the γCD H$_{3,5,6}$ protons were observed.
3.7. Conclusion

The dimerisation and complexation of HP$^{2-}$ by $\gamma$CD, 33$\gamma$CD$_2$suc and 66$\gamma$CD$_2$suc to form 1:1 host-guest complexes have been characterised in aqueous solution by UV–vis spectroscopy. Using van’t Hoff equations and plots for the dependence of equilibrium constants on temperature, the thermodynamic parameters, $\Delta G^\circ$, $\Delta H^\circ$ and $\Delta S^\circ$, have been quantified. The dimerisation constant, $K_d$, for (HP$^{2-}$)$_2$ and its thermodynamic parameters are consistent with those reported in the literature. The complexation stabilities, $K_1$, for the 33$\gamma$CD$_2$suc.HP$^{2-}$ and 66$\gamma$CD$_2$suc.HP$^{2-}$ complexes are about six times and twice that of $\gamma$CD.HP$^{2-}$ complex, consistent with cooperativity between the two $\gamma$CD annuli exists in 33$\gamma$CD$_2$suc.HP$^{2-}$ but little in 66$\gamma$CD$_2$suc.HP$^{2-}$. The $K_1$ for the complexation of HP$^{2-}$ by the smaller $\beta$CD has not been obtained by either UV–vis absorption or fluorescence spectroscopy due to the very small observed spectral variations induced by the complexation over the concentration range studied. This suggests that $K_1$ for the $\beta$CD.HP$^{2-}$ complex is smaller than that for the $\gamma$CD.HP$^{2-}$ complex, consistent with the better fit of the larger $\gamma$CD annulus in complexing HP$^{2-}$.

The thermodynamic parameters for the complexation of HP$^{2-}$ by $\gamma$CD and the $\gamma$CD dimer hosts are consistent with those for a large number of $\gamma$CD complexes, since they fit well to the enthalpy-entropy compensation plot constructed from data found in the literature. The PM6–minimised complexation energies the three complexes: $\gamma$CD.HP$^{2-}$, 33$\gamma$CD$_2$suc.HP$^{2-}$ and 66$\gamma$CD$_2$suc.HP$^{2-}$ from the molecular modelling studies are consistent with the equilibrium and thermodynamic studies and suggest that the mode of complexation is most likely to arise from interaction between the HP$^{2-}$ 2-carboxylatoethyl moiety and/or 1-hydroxyethyl moiety and the $\gamma$CD annulus. The 2D $^1$H NOESY NMR spectra did not provide further unequivocal evidence for the interactions due to the HP$^{2-}$ H$_{26,31}$ and H$_{37,38}$ resonances being obscured by those of $\gamma$CD H$_{2-6}$ protons, and the dominance of the dimerised (HP$^{2-}$)$_2$ species by comparison with the complexed species under the $^1$H NMR conditions.
3.8. References


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