LINKED BETA-CYCLODEXTRIN TRIMERS: From Molecular Recognition to Polymer Network Hydrogels

Hanh-Trang Nguyen
(Nguyễn Thị Hạnh Trang)

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

November, 2013
CONTENTS

ABSTRACT v
DECLARATION vii
ACKNOWLEDGEMENT viii
PUBLICATIONS AND PRESENTATIONS ix
ABBREVIATIONS xi

CHAPTER 1. INTRODUCTION FROM CYCLODEXTRINS TO NANOMATERIALS AND POLYMER HYDROGELS 1
1.1. CYCLODEXTRINS 2
  1.1.1. Introduction and General Overview of Cyclodextrin Chemistry 2
  1.1.2. Host-guest Complexation 5
  1.1.3. Cyclodextrin Modification 8
  1.1.4. Metallo-Cyclodextrins 8
  1.1.5. Cyclodextrin Oligomers 9
  1.1.6. CD Based Metal-Organic Frameworks 13
  1.1.7. Linked CD Metal Nanoparticles 14
  1.1.8. Cyclodextrin Based Molecular Devices 15
1.2. POLYMER HYDROGELS 17
  1.2.1. General 17
  1.2.2. Cross-links by Hydrogen Bonding 18
  1.2.3. Cross-links by Hydrophobic Interaction 20
  1.2.4. Cross-links through Metal-Ligand Interaction 21
  1.2.5. Cross-links by Host-Guest Interaction 23
1.3. CYCLODEXTRIN BASED POLYMER HYDROGELS 23
  1.3.1. General 23
  1.3.2. Interaction of CD Substituted Polymers and Guest Substituted Polymers 24
  1.3.3. Interaction of Guest Substituted Polymers and Linked CD Dimers 27
1.4. RESEARCH OBJECTIVES 28
  1.4.1. General 28
  1.4.2. Aims of This Research 29
1.5. REFERENCES 31
5.1.1. General 200
5.1.2. Aims of This Study 201
5.2. SYNTHESIS 203
5.3. 2D NOESY $^1$H NMR SPECTROSCOPY 203
5.4. FLUORESCENCE TITRATION STUDIES 207
  5.4.1. Isothermal Titration Calorimetry Results 211
  5.4.2. Entropy – Enthalpy Linear Relationship 218
5.5. DYNAMIC LIGHT SCATTERING 219
5.6. TIME RESOLVED FLUORESCENCE STUDIES 223
5.8. RHEOLOGICAL STUDY 227
5.9. SUMMARY AND CONCLUSIONS 229
5.10. REFERENCES 233
5.11. APPENDIX 238
  5.11.1. 2D NOESY $^1$H NMR (600 MHz) Spectra 238
  5.11.2. Fluorimetric Titration Data 242
  5.11.3. Isothermal Titration Calorimetry Data 247
  5.11.4. Light-Scattering - Hydrodynamic Diameter Distributions 251
  5.11.5. Time Resolved Fluorescence Data 257

CHAPTER 6. EXPERIMENTAL 261
6.1. GENERAL 262
  6.1.1. Instrumental 262
  6.1.2. Materials 265
6.2. EXPERIMENTAL 266
  6.2.1. Syntheses 266
  6.2.2. Sample preparation 272
6.3. REFERENCES 274

APPENDIX 277
ABSTRACT

The thesis describes the construction and characterisation of a variety of polymer network hydrogels based on β-cyclodextrin (β-CD) trimers and modified poly(acrylate)s.

Chapter 1 extensively reviews in cyclodextrin (CD) fields from its history from beginning in 1981 until 2013. The most significant work is highlighted as well as the field of polymer hydrogel, including the novel field of CD based polymer hydrogel.

In Chapter 2, a UV-vis and ¹H NMR spectroscopic study of the host-guest complexation by β-cyclodextrin (β-CD), 1,3,5-N,N,N-tris-(6⁵-deoxy-6⁵-β-cyclodextrin)-benzene (β-CD₃bz), and 1,3,5-N,N,N-tris(6⁵-(2-aminoethyl)amino-6⁵-deoxy-6⁵-β-cyclodextrin)-benzene (β-CDen₃bz) of cationic crystal violet (CV⁺) and pyronine B (PB⁺) and zwitterionic rhodamine B (RB) in aqueous phosphate buffer at pH 7.0 and I = 0.10 mol dm⁻³ is described. The complexation constants and the associated ΔH₁₁ and TΔS₁₁ for all nine complexes coincide with an entropy-enthalpy compensation plot for the formation of a wide range of β-CD and modified β-CD host-guest complexes reported in the literature. Crystal violet also forms (β-CD)₂.CV⁺, (β-CD₃bz)₂.CV⁺ and (β-CDen₃bz)₂.CV⁺ complexes characterized by 10²K₂₁ (298.2 K) = 2.14, 4.57 and 3.86 dm³ mol⁻¹ and analogous (β-CD)₂.PB⁺, (β-CDen₃bz)₂.PB⁺ and (β-CDen₃bz)₂.RB complexes also form, but the (β-CD₃bz)₂.PB⁺, (β-CD)₂.RB, and (β-CD₃bz)₂.RB complexes were not detected. The effects of the structures of the hosts and guests on the complexation processes are discussed.

In Chapter 3 the characterisation stability of constants and thermodynamic patterns in polymer hydrogels based on host-guest complexation of the linked β-CD trimers with the dodecyl (C12) and octadecyl (C18) 3% randomly substituted poly(acrylate)s PAAC12 and PAAC18 in aqueous solution are discussed. These studies compare hydrophobic interactions of the C12 and C18 substituted poly(acrylate)s and their interaction with β-CD and linked β-CD trimers. The complexation processes were studied by 2D NOESY ¹H NMR spectroscopy, ITC, dynamic light scattering and rheology. These data are used to establish the extent to which these interactions influence hydrogel formation in more concentrated solutions.

In Chapter 4 the supramolecular chemistry of polymer hydrogel based on host-guest chemistry of the linked β-cyclodextrin trimers and four adamantyl substituted
poly(acrylate)s with different linker tether lengths is discussed. 2D NOESY $^1$H NMR spectroscopy, isothermal titration calorimetry and rheological studies show that the β-CD groups of the two linked β-cyclodextrin trimers, β-CD$_3$bz and β-CDen$_3$bz, complex the adamantyl substituents and their tethers in 3.0% substituted poly(acrylate)s to form intra- and inter-poly(acrylate)s strand cross-links in aqueous solution. The structures of the linked-β-cyclodextrin trimers and the length of the tether between the adamantyl substituent and the poly(acrylate) backbone have substantial effects on the complexation constants, $K_{11}$, and the associated thermodynamic parameters. This is partially shown for the complexation by β-CD$_3$bz of the adamantyl substituents as tether length varies from -CONH- ($3.45 \times 10^5$) through -CONH(CH$_2$)$_n$NHCO- where $n = 2$ ($2.09 \times 10^5$), 6 ($3.17 \times 10^5$) or 12 ($7.46 \times 10^5$) in $0.13 - 0.37$ wt.% substituted poly(acrylate)s solutions and the figures in brackets are the $K_{11}$ in dm$^3$ mol$^{-1}$ at 298.2 K. For the same sequence of substituted poly(acrylate)s the variation of viscosity is: 0.03, 3.78, 3.48, and 2.03 Pa s$^{-1}$ at 500 s$^{-1}$ shear rate at 298.2 K for 5.0 wt.% substituted poly(acrylate)s solutions in which the β-CD groups of β-CD$_3$bz and the adamantyl substituents are equimolar at $1.5 \times 10^{-2}$ mol dm$^{-3}$. The eight data sets for the β-CD$_3$bz and β-CDen$_3$bz systems are discussed in terms of host-guest interactions between the host β-CD groups and the guest adamantyl substituents of the substituted poly(acrylate)s and are compared with those for the analogous β-CD systems.

In chapter 5, the supramolecular chemistry of the poly(acrylate)s hydrogels based on host-guest complexations of the linked β-CD$_3$bz and β-CDen$_3$bz trimers with the dansyl substituent guests (DS) attached through tethers of three different lengths containing 2, 6 and 12 methylene groups in 3.0% randomly substituted, PAADSen, PAADShn and PAADSddn are discussed. The six systems have been characterized at the molecular level by 2D NOESY $^1$H NMR, isothermal titration calorimetry, fluorescence spectroscopy and time-resolved fluorescence, and at the macroscopic level by dynamic light scattering and rheology. The data gathered are consistent with individual dansyl substituents forming aggregates and being complexed by the linked β-CD$_3$bz and β-CDen$_3$bz trimers in within poly(acrylate)s strands in dilute solutions and between poly(acrylate)s strands in hydrogels. The trends in β-CD$_3$bz and β-CDen$_3$bz complex stability constants fluorescence life times and viscosities of six systems are discussed.

Chapter 6 describes the experimental methodology deployed in the study.
DECLARATION

This is to declare that the work presented within this thesis is original and was carried out at the University of Adelaide during the period 2010-2013. This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is given.

I give consent to this copy of my thesis, when deposited in the University of Adelaide Library, being made available for loan and photocopying, subject to the provisions of Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses program (ADT) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Hanh-Trang Nguyen
06 / 11 / 2013
ACKNOWLEDGEMENT

It would not have been possible to write this PhD thesis without the help and support of the kind people, who have been very helpful to me during the course of my PhD. I would like to express my thanks to these people here.

I would like to express my special appreciation and thanks to my supervisor, Professor Stephen Lincoln, for allowing me to carry out this thesis and for encouraging my research. I also thank for his scientific advice and his encouragements for over last few years. He has always been a dedicated supervisor during the course of my PhD.

I am most grateful to Dr. Tara Pukala for her good comments and suggestions for my thesis.

I wish to thanks Dr. Duc-Truc Pham for his advice and a lot of help. I also would like to thanks everyone in the Lincoln Group (Hillary, Tien, Hamish, Noby, Jianjia, Liang, Yang, Charlotte, Annelie, Luke, Andrew and Quan) for their friendship and supports through the years.

I would especially like to thank the research group of Prof. Xuhong Guo, in particular Dr. Jie Wang at East China University of Science and Technology, Shanghai, China; the research group of Dr. Tak Kee, in particular Mr Scott Clafton at the University of Adelaide; the research group of Prof. Christopher Easton, Australian National University and the research group of Prof. Robert Prud’homme, Princeton University, USA for collaboration.

I would like to acknowledge the financial, academic and technical support of the Chemistry Department, the University of Adelaide, particularly in the award of a Postgraduate Research Studentship that provided the necessary financial support for this research. I also wish to thank Mr Phil Clements who ran my numerous 1D and 2D 600 MHz NMR spectra.

A special thanks to my family, relatives and friends, who have encouraged me throughout my studies. In particular, thanks to my parents, my brother, my husband and my little kids for their endless love and support.
PUBLICATIONS AND PRESENTATIONS

Journal Articles:


- **Nguyen, Hanh-Trang**: Pham, Duc-Truc; Lincoln, Stephen Frederick; Wang, Jie; Guo, Xuhong; Easton, Christopher J.; Prud’homme, Robert K. Host–Guest Chemistry of Linked β-Cyclodextrin Trimers and Adamantyl Substituted Poly(acrylate)s in Aqueous Solution. *Polymer Chemistry, 2013*, 4(3), 820-829. DOI: 10.1039/C2PY20746J

- **Nguyen, Hanh-Trang**: Pham, Duc-Truc; Lincoln, Stephen Frederick; Wang, Jie; Guo, Xuhong; Easton, Christopher J.; Prud’homme, Robert K. Complexation of Hydrophobe Substituted Poly(acrylate)s by β-Cyclodextrin Dimers and Trimers in Aqueous Solution and Hydrogels. *Ready to submit, 2013*.

Conference Items:


- **Nguyen, Hanh-Trang**: Pham, Duc-Truc; Lincoln, Stephen Frederick; Wang, Jie; Guo, Xuhong; Easton, Christopher J.; Prud’homme, Robert K. Supramolecular Chemistry of Beta-Cyclodextrin Trimers and Adamantyl Substituted Polyacrylates. *16th International Cyclodextrin Symposium*. Tianjin, China 6-10 May, 2012: pp. IL-01.
• Nguyen, Hanh-Trang; Pham, Duc-Truc; Wang, Jie; Guo, Xuhong; Lincoln, Stephen Frederick; Easton, Christopher J. Synthesis of β-cyclodextrin trimers for novel polymer networks. *Asian Cyclodextrin Conference* (6th: 2011: Canberra, Australia) ACC2011
ABBREVIATIONS

1. General

Å    angström (10^{-10} m)
Ar   aryl
\(d\) Density (g cm\(^{-3}\))
\(\delta\) chemical shift (ppm)
\(\Delta G^0\) Gibbs free energy
\(\Delta H^0\) enthalpy change
\(\Delta S^0\) entropy change
\(\varepsilon\) molar absorptivity (mol\(^{-1}\) dm\(^3\) cm\(^{-1}\))
Eqn. equation
et al. et alia
GC-MS Gas chromatography- mass spectrometry
Hz   Hertz
\(I\) ionic strength (mol dm\(^{-3}\))
\(I_F\) fluorescence intensity
ITC  isothermal titration calorimetry
\(J\) coupling constant (Hz)
\(K\) stability constant (dm\(^3\) mol\(^{-1}\))
\(K_d\) dimerisation constant (dm\(^3\) mol\(^{-1}\)) e.g. 2 RB \(\rightleftharpoons\) (RB)\(_2\)
\(K_{11}\) stability constant for 1:1 (host:guest) complexes (dm\(^3\) mol\(^{-1}\)), e.g. \(\beta\)-CD + Dye \(\rightleftharpoons\) \(\beta\)-CD.Dye
\(K_{12}\) stepwise stability constant for 1:2 (host:guest) complexes (dm\(^3\) mol\(^{-1}\))
e.g. \(\beta\)-CD. Dye + Dye \(\rightleftharpoons\) \(\beta\)-CD. Dye\(_2\)
\(K_{21}\) stepwise stability constant for 2:1 (host:guest) complexes (dm\(^3\) mol\(^{-1}\))
e.g. \(\beta\)-CD. Dye + \(\beta\)-CD \(\rightleftharpoons\) \(\beta\)-CD\(_2\). Dye
\(m/z\) mass/charge ratio
MS mass spectrometry
2. Chemicals

α-, β-, γ-CD  α-, β-, γ-cyclodextrin
6β-CDTs  6^λ-O-(4-methylbenzenesulfonyl)-β-cyclodextrin
6β-CDNH₃  6^λ-amino-6^λ-deoxy-β-cyclodextrin
6β-CDN₃  6^λ-azido-6^λ-deoxy-β-cyclodextrin
6β-CDen  6^λ-(2-aminoethyl)amino-6^λ-deoxy-β-cyclodextrin
β-CD₂ₓ  covalent linked β-cyclodextrin dimer, where x is a linker
66β-CD₂su  N,N'-bis(6^λ-deoxy-6^λ-β-cyclodextrinyl)-succinamide
66β-CD₂ur  N,N'-bis(6^λ-deoxy-6^λ-β-cyclodextrinyl)-urea
β-CD₃ₓ  covalent linked β-cyclodextrin trimer, where x is a linker
β-CD₃bz  1,3,5-N,N,N-tris(6^λ-deoxy-6^λ-β-cyclodextrinyl)-benzene
β-CDen₃bz  1,3,5-N,N,N-tris(6^λ-(2-aminoethyl)amino-6^λ-deoxy-6^λ-β-
cyclodextrinyl)-benzene
en  1,2-diamino ethane
hn  1,6-diamino hexane
ddn  1,12-diamino dodecane
C12  dodecyl
C18  octadecyl
AD   adamantane
DS   Dansyl
CV+  Crystal Violet
PB+  Pyronine B
RB   Rhodamine B
ADNH2 1-ami-no-adamantane
ADen 1-(2-aminoethyl)amino-adamantane
ADhn 1-(6-aminohexyl)amino-adamante
ADddn 1-(6-aminododecyl)amino-adamantane
DSen 1-(2-aminoethyl)amino-dansyl
DShn 1-(6-aminohexyl)amino-dansyl
DSddn 1-(6-aminododecyl)amino-dansyl
PAA  poly(acrylic acids) or poly(acrylate)s
PAAC12 PAA with 3% substituents C12
PAAC18 PAA with 3% substituents C18
PAAAD PAA with 3% substituents AD
PAAADen PAA with 3% substituents ADen
PAAADhn PAA with 3% substituents ADhn
PAAADddn PAA with 3% substituents ADddn
PAADSen PAA with 3% substituents DSen
PAADShn PAA with 3% substituents DShn
PAADSddn PAA with 3% substituents DSddn
NMP  N-methyl-2-pyrrolidinone
TFA  trifluoroacetic acid
THF  tetrahydrophuran
This page intentionally left blank.
CHAPTER 1

INTRODUCTION

FROM CYCLODEXTRINS TO NANOMATERIALS AND POLYMER HYDROGELS
1.1. CYCLODEXTRINS

1.1.1. Introduction and General Overview of Cyclodextrin Chemistry

Cyclodextrins, (CDs) (Figure 1.1) are naturally occurring macrocyclic oligosaccharides composed of α-1,4-linked D-glucopyranose in the 4\textsuperscript{1}C\textsubscript{1} conformation. CDs are produced through the degradation of starch by the enzyme CD trans glycosylase, which is obtained from bacteria exemplified by Bacillus macerans and Bacillus circulans.\textsuperscript{1} The number of glucose units per CD ring varies from 6-13,\textsuperscript{2} as the enzyme produces a range of oligosaccharides.\textsuperscript{3} A CD composed of 5 D-glucopyranose units has not been observed probably because the strain in the five-membered is too great. The three most common and practically important natural CDs contain 6, 7 and 8 units and are known as α-CD, β-CD and γ-CD, respectively. CDs larger than 9 units have been prepared, but most of them are less well studied as they are unstable.\textsuperscript{4,5} In this section the basic understanding of native and modified CDs, their host-guest complexation chemistry, and the CD research frontiers, where fascinating developments are occurring, are reviewed.

![Figure 1.1](image.png)

**Figure 1.1.** (a) Schematic structure of α-CD, β-CD, γ-CD and (b) a simplified representation that is used in this thesis.

The general structure of CDs is shown in Figure 1.1. The D-glucopyranose residues are labelled A-H in a clockwise direction when viewed from the entrance to the annulus defined by the primary hydroxy groups. Thus, in a CD in which a single primary hydroxy is substituted by another group, the carbon at which the substitution occurs is designated as C6\textsuperscript{A} where A identifies the substituted D-glucopyranose unit and 6 identifies the position of the carbon in that D-glucopyranose unit.
X-ray studies reveal that CDs have a slightly conical macrocyclic structures which form a shallow truncated cone (Figure 1.2). The narrow end is delineated by the primary hydroxy groups on the C6 of each D-glucopyranose unit and the wider end by the secondary hydroxy groups on the C2 and C3 of each D-glucopyranose unit.\textsuperscript{6-8}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{(a) X-ray solid-state structure\textsuperscript{6} and (b) space filling molecular model of $\beta$-CD.}
\end{figure}

The outer surface of a CD is hydrophilic due to the presence of hydroxy groups, which also determines their solubility in water.\textsuperscript{7} The interior of the CD annulus is hydrophobic due to the presence of hydrogen atoms, glycosidic oxygen bridges and the nonbonding electron pairs directed toward its interior. As each D-glucopyranose unit in the CD structure possesses five chiral carbon atoms, the CD macrocycle is homochiral. The structure of CD is quite rigid because of the intramolecular hydrogen bonds formed between the 2-hydroxy and 3-hydroxy groups of adjacent D-glucopyranose units (Figure 1.3). As $\beta$-CD has a complete belt-like set of hydrogen bonds on the face composed of coplanar secondary hydroxy groups, it is the most rigid structure and the least soluble among the three CDs: $\alpha$-CD, $\beta$-CD and $\gamma$-CD. The hydrogen-bond belt is incomplete in $\alpha$-CD while the structure of $\gamma$-CD is noncoplanar and more flexible, which coincides with it being the most soluble of the three CDs.\textsuperscript{7} Other physical properties of CDs are summarised in Table 1.1.
Figure 1.3. A side-view truncated torus representation of CDs and the chair conformation of the α-1,4-linked D-glucopyranose unit.

Table 1.1. Selected physicochemical properties of the native CDs

<table>
<thead>
<tr>
<th>Property</th>
<th>α-CD</th>
<th>β-CD</th>
<th>γ-CD</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of D-glucopyranose units</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Anhydrous molecular weight (g mol⁻¹)</td>
<td>972.85</td>
<td>1134.99</td>
<td>1296.42</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>9</td>
</tr>
<tr>
<td>pKₐ values (25 °C)</td>
<td>12.33</td>
<td>12.20</td>
<td>12.08</td>
<td>10,11</td>
</tr>
<tr>
<td>Annular diameter,</td>
<td>4.7; 5.3</td>
<td>6.0; 6.5</td>
<td>7.5; 8.3</td>
<td>2,7</td>
</tr>
<tr>
<td>Narrow end (C6); wide end (C3) (Å)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depth of annulus (Å)</td>
<td>7.9 ± 0.1</td>
<td>7.9 ± 0.1</td>
<td>7.9 ± 0.1</td>
<td>2,7</td>
</tr>
</tbody>
</table>

CDs and their modified forms are at the centre of supramolecular chemistry. Their ability to form host-guest complexes with a wide range of guest species, which often increases the solubility and stability of the guests, has led to the use of CDs in variety of industries. Many thousands of tonnes of CD are produced and used in industrial cyclodextrin production annually.¹²⁻¹⁵ For example, CDs have been used in the pharmaceutical industry, as drug carriers, solubilisers and ingredients to improve the stability, bioavailability and pharmacokinetic properties of drugs.¹⁶⁻²² They are also used in the food, cosmetic, toiletry and tobacco industries, mainly for the stabilisation of flavours and fragrances and the elimination of undesired tastes.²³⁻²⁵ They are used in the chemical industries as additives in agricultural chemicals or for stabilisation of organic dyes.²⁶⁻²⁸ In addition, they have been used in the analytical sciences, such as in enantiomer separations by high performance liquid chromatography or gas chromatography, or as chiral agents in nuclear magnetic resonance and circular dichroism studies.²,⁷,²⁹,³⁰
1.1.2. Host-Guest Complexation

The ability to form host-guest complexes with a great variety of guest species is one of the most important properties of CDs (Figure 1.4). Since Schardinger first discovered this property in 1911 and Freudenberg reported the first complexation in the CD annulus in 1939, many studies of host-guest complexation of CDs have been reported. \(^1\)\(^{12}\)\(^{33-38}\)

In general, hydrophobic (non-polar) guests form a stronger host-guest complex with CDs than hydrophilic (polar) guests, and highly hydrophilic molecules are either complexed very weakly or not at all. Thus, aromatic compounds have commonly been employed as guests in CD host-guest complexes and generally fit best in \(\beta\)-CD. \(^{39}\) Similarly, 1-adamantanecarboxylate is one of the most strongly complexed guests with \(\beta\)-CD, consistent with a close match between the annulus and guest diameters. The stability constant for this complex is of the order of \(10^4\) \(\text{dm}^3\) \(\text{mol}^{-1}\), and has been used in many molecular recognition systems. \(^{40,41}\)

There are several explanations for the interaction between CDs and guests. The major driving forces are hydrophobic interactions which consist largely of London dispersion forces. Other factors are also believed to contribute including: the enthalpy-driven displacement of ‘high-energy’ water molecules from the CD annulus; Van der Waals interactions between the CD and the guest; the relief of conformational strain energy possessed by the free CD upon guest complexation; electrostatic, polar and ionic interactions including dipole-dipole and hydrogen-bonding; and induction forces and dispersion forces. \(^{39,42}\)

Figure 1.4. The formation of a host-guest complex between a hydrophobic guest molecule and a CD host in an aqueous environment.
Qualitative characterisation of host-guest complexation are typically achieved by 2D nuclear magnetic resonance (NMR) spectroscopy. In 2D $^1$H Rotating frame Overhauser Enhancement Spectroscopy (ROESY) and Nuclear Overhauser Enhancement Spectroscopy (NOESY) experiments, a NOE cross-peak arising from the dipolar interaction a proton of the CD (H3, H5 and H6) with a proton of the guest will be observed if such protons are closer than 4Å, which implies that the guest is complexed in the annulus. The H3,5,6 resonances are readily distinguished from the H2,4 resonances of CD by their chemical shifts, and cross-peaks arise from the interactions of the H3,5,6 annular protons (e.g. 3.8 - 4.0 ppm in D$_2$O) with guest protons. However, in most modified CD systems the H3,5,6 proton resonances are not readily differentiated from the H2,4 protons, as monosubstitution of CD causes all D-glucopyranose units to become inequivalent and their H2-6 resonances to occur over an increased chemical shift range with some resonance superimposition occurring.\textsuperscript{43}

Quantitative assessments of host-guest complexation are gained through stability constants determined by various techniques. The stability constants for complexation of $\alpha$-CD, $\beta$-CD and $\gamma$-CD with many hydrophobic guests can be determined by UV-Vis, fluorescence, NMR, and circular dichroic spectroscopy, isothermal titration calorimetry (ITC) and temperature-jump studies.\textsuperscript{41} The key issues that have to be addressed when carrying out such experiments, together with the advantages and disadvantages for the most common methods: NMR, UV-Vis, fluorescence and ITC and data fitting methodologies and softwares, are discussed in the literature.\textsuperscript{44,45}

The most common stoichiometry of the complexation of a CD host and a guest (G) is a ratio of 1:1 CD.G (stability constant $K_{11}$) (Figure 1.4). Higher order complexation in the ratios of 1:2, CD.G$_2$; 2:1, CD$_2$.G and 2:2 CD$_2$.G$_2$ are characterised by the sequential stability constants, $K_{12}$, $K_{21}$, and $K_{22}$, $K_{22}'$, respectively (Figure 1.5). Although the equilibria characterized by $K_{22}$ and $K_{22}'$ produce the same complex, CD$_2$.G$_2$, this is achieved through different routes and there is no necessity for $K_{22}$ and $K_{22}'$ to be of similar magnitude.\textsuperscript{1}
Figure 1.5. Multiple complexation equilibria for CD complexes of different stoichiometries.

Most of the thermodynamic data for the complexation of a large variety of guest species by CDs conform to a linear free energy relationship:

\[ T\Delta S = \alpha \Delta H + T\Delta S_0 \]  \hspace{1cm} (1.1)

where \( T \) is the temperature, \( \Delta S \) is the entropy change, \( \Delta S_0 \) is entropy change when \( \Delta H \), the enthalpy change is zero, and \( \alpha \) is the slope of the linear plot of \( T\Delta S \) against \( \Delta H \).\(^{41}\) Such plots at 298.2 K are shown in Figure 1.6 and yield \( \alpha = 0.79 \) and \( T\Delta S_0 = 12 \text{ kJ mol}^{-1} \) for \( \alpha \)-CD, \( \alpha = 0.80 \) and \( T\Delta S_0 = 11 \text{ kJ mol}^{-1} \) for \( \beta \)-CD and \( \alpha = 0.97 \) and \( T\Delta S_0 = 15 \text{ kJ mol}^{-1} \) for \( \gamma \)-CD. These positive \( T\Delta S_0 \) for \( \alpha \)-CD, \( \beta \)-CD and \( \gamma \)-CD values indicate that the host-guest complexes are entropically stabilized at \( \Delta H_0 = 0 \), the intercept value, where the corresponding entropy change is \( \Delta S_0 \). While Equation 1.1 does not reflect a necessary relationship between \( T\Delta S \) and \( \Delta H \), its widespread observation is usually taken as an indication of a variation in the relative importance of the solvational and structural changes within the systems studied.\(^{41}\)

Figure 1.6. Individual enthalpy-entropy compensation plots for natural \( \alpha \)-CD (○), \( \beta \)-CD (▲) and \( \gamma \)-CD (■).\(^{41}\)
1.1.3. Cyclodextrin Modification

Modifications to a CD usually alters its annular size, shape, charge, polarity and inherent symmetry to a greater or lesser extent. 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 α-CD, β-CD and γ-CD, respectively) renders selective modification challenging. The OH6 are the most basic and usually the most nucleophilic, the OH2 are the most acidic and the OH3 are sterically the most difficult to access. Substitution of OH6 in α-CD and β-CD are most readily achieved through 6^A-O-(4-methylbenzenesulfonyl)-cyclodextrin (Figure 1.7), while substitution of OH2 and OH3 is often achieved through 2^A-O-(4-methylbenzenesulfonyl)-cyclodextrin. However, using the literature reaction of α-CD or β-CD and 4-toluenesulfonyl chloride in aqueous solution for γ-CD results in polysubstitution at C6, probably because of the larger γ-CD annulus. Larger arenesulfonyl chlorides have been used to achieve mono C6^A substitution, but the yields are low and the cost of the arenesulfonyl chlorides is greater than that of 4-toluenesulfonyl chloride. A modification of the method of Murakami for the synthesis of 2^A-O-(4-methylbenzenesulfonyl)-β-cyclodextrin has been used as the economical simultaneous route to 2^A- and 6^A-O-(4-methylbenzenesulfonyl)-γ-cyclodextrin. Alternatively, mono-halide, aldehyde, and alkene variants of CD may be prepared.

![Figure 1.7. Synthesis of mono-6-substituted CDs. Nu: nucleophilic compound.](image)

Mono-substituted CDs may be complexed with metal ions or linked to other supramolecules to form nanomaterials and nanodevices. They may be linked to other CDs, nanoparticles or polymers for many potential applications as discussed in following sections.

1.1.4. Metallo-Cyclodextrins

CDs are able to form three types of complexes with metal ions: (i) host-guest complexes, (ii) metal complexes formed through bonding with OH groups of native CDs
and (iii) metal complexes formed through bonding with functional groups of modified CDs. In a metal host-guest complex, CDs act as second sphere coordination ligands, where the annulus includes the metal ion to form adducts. However, not many studies on molecules of this type have been reported.\(^{53}\) When native CDs coordinate metal ions through OH groups, they generally act as weak ligands and do not form strong complexes with metal ions in acidic and neutral solutions.\(^{54}\) However, deprotonated CDs represent stronger metal ion complexing ligands. Consequently, most complexes between native CDs and metal ions form mainly at basic pH. Metal ion-\(\beta\)-CD complexation studies indicate that at least the doubly deprotonated anion \(\beta\)-CD\(_2\)\(^-\) is involved in complex formation with two hydroxy groups adjacent to C2 and C3.\(^{55,56}\)

An excellent way to improve the complexing ability of CDs is to modify CDs with polyamines or carboxyl functional groups. Thus, a great number of metal complexes of modified CDs have been characterized in order to increase the potential applications of CD chemistry. Among them, chiral recognition and metalloenzyme mimicking systems are especially well developed. For example, copper(II) complexes of \(\beta\)-CD functionalized with homocarnosine (HC) substituted at either C6 or C3 in \(\beta\)-CDHC6 and \(\beta\)-CDHC3, respectively, were investigated by Bellia and co-workers.\(^ {57}\) It appears that the formation of different copper(II) complex species at different pH values occur. In the \(\beta\)-CDHC3 copper(II) complex, shown in Figure 1.8, coordination of the a secondary hydroxyl group on a C2 of the functionalized \(\beta\)-CD occurs. The antioxidant activity of both \(\beta\)-CDHC6 and \(\beta\)-CDHC3 was determined through pulse radiolysis measurements from which it was shown that both complexes have a high catalytic activity with the superoxide anion radical.

**Figure 1.8. The CDHC3 copper(II) complex.**\(^ {57}\)

### 1.1.5. Cyclodextrin Oligomers

Cyclodextrins may be linked together to form CD dimers, trimers, tetramers, hexamers and oligomers. A substantial range of linked CD dimers\(^ {52}\) has been synthesized with a variety of linkers, from short linkers such as urea, oxalamine, succinamine,\(^ {58}\) to longer
linkers$^{52,59-61}$ with a number of coordination centres for metal binding (Figure 1.9). The CDs dimers may be linked at the primary faces C6$^A$ (head) or secondary faces C2$^A$ and C3$^A$ (tail).$^{62,63}$ Statistically, there are six types of CD dimers linked through carbons on adjacent CDs which may be formed: C6$^A$-C6$^A$, C6$^A$-C3$^A$, C6$^A$-C2$^A$, C3$^A$-C3$^A$, C3$^A$-C2$^A$, and C2$^A$-C2$^A$. $^{52,62-64}$

![Figure 1.9. Schematic representation of examples of linked CD dimers. The linkers are: a) urea,$^{58}$ b) oxalamide,$^{58}$ c) succinamide$^{58}$ and longer linker dimers d)$^{59,60}$, and e).$^{52}$](image)

One of the most important properties of the linked CD dimers is their ability to form host-guest complexes with long ditopic guests; sometimes called cooperative complexation. A long guest possessing two hydrophobic groups at either end tends to be complexed with one hydrophobic group in each CD of the linked CD dimer.$^{65-69}$ The stability constants of these host-guest complexes is sometimes increased by 10-100 times in comparison with that of same guest with native CD.$^1,52$ An example of a particularly sophisticated linked CD dimer is shown in Figure 1.10. Here a ferric porphyrin is complexed in both annuli of the linked CD dimer and coordinated by the linker such that it can also complex oxygen and function as a myoglobin mimic.$^{70}$

![Figure 1.10. Dioxygen binding by the metallo-CD myoglobin mimic, where TMβ-CD is the per-O-methylated CD.$^{70}$](image)
Several linked CD trimers have been synthesized with linear, triangular and star-shaped structures (Figure 1.11).\textsuperscript{71-76} However, there have been very few reports of their complexing behaviour. Hamada \textit{et al}.\textsuperscript{77} synthesized a fluorescent linear β-CD trimer incorporating two dansyl groups as linkers between CDs which complexed guest species (Figure 1.11a). Some linear and star-shaped β-CD trimers (Figure 1.11b) were also prepared and studied as complexing agents for appropriate trimericaminoacid amides.\textsuperscript{71} Another linked β-CD trimer (Figure 1.11c) has been synthesized and studied by Kuroda \textit{et al}.\textsuperscript{78} This β-CD trimer complexed anthracene-derivatives over three times as strongly as the analogous β-CD dimer with the same linker. This was attributed to the presence of the third β-CD complexing site in the trimer.\textsuperscript{78,79}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Schematic representation of linked β-CD trimers \textit{a}) linear trimer,\textsuperscript{77} \textit{b}) star-shaped trimer,\textsuperscript{72} and \textit{c}) triangular trimer.\textsuperscript{78}}
\end{figure}

Recently, some linked CD tetramers have been synthesized with the structures shown in Figure 1.12. These linked CD tetramers have been used to complex large species such asporphyrins or to make functional molecular devices.\textsuperscript{59,60,80-83}
Figure 1.12. Schematic representation of linked CD tetramers with different structures a) bis(cyclodextrin)-copper(II),\textsuperscript{59,60} b) porphyrin CD tetramer,\textsuperscript{80,84} and c) cyclic β-CD tetramers (tetraplex).\textsuperscript{83}

Through complexation with metal ions, linked CD dimers may be act as bidentate ligands to form a tris metal complex which constitutes a CD hexamer.\textsuperscript{85,86} For example, Nelissen and co-workers synthesized and characterized tris(bipyridine)ruthenium(II) complexes bearing six β-CD ligand substituents (Figure 1.13a) and their complexation of viologen derivatives as guests in the β-CD annuli.\textsuperscript{86,87} Linked β-CD oligomers possessing more than six β-CDs have also prepared as exemplified by a linked CD octamer (Figure 1.13b).\textsuperscript{88}
1.1.6. CD Based Metal-Organic Frameworks

Metal-organic frameworks (MOFs) represent an extensive class of porous crystalline materials in which organic ligands form links between metal ion-containing clusters. Success in controlling the functionality and structure of MOFs has led to numerous applications, most notably gas adsorption, storage of clean gas fuels, catalysis, separation, and drug delivery. Some CD-MOFs have been reported. For example, γ-CDs are linked by potassium ions, in aqueous media at ambient temperature and pressure, to form a CD-MOF with a body-centered cubic structure which has the empirical formula \([(C_{48}H_{80}O_{40})(KOH)_2]\), (Figure 1.14). The interactions of CD-MOFs with several organic dyes have also been reported. Completely edible CD-MOFs can be prepared entirely from edible ingredients: through combining food-grade γ-CD with salt substitute (KCl) or potassium benzoate (food additive E212) in bottled water and Everclear grain spirit (EtOH).
Figure 1.14. A schematic representation of the structure of the $\gamma$-CD-MOF, showing the alternating coordination of $K^+$ ions and representation of cubic orientation of the six $\gamma$-CD. The cuboidal orientation of the six $\gamma$-CD tori, illustrating the 1.7 nm sized pore at the center of each $(\gamma$-CD)$_6$ repeating motif. The surfaces of the $\gamma$-CD units are portrayed in red, blue, yellow, purple, green, and orange.

1.1.7. Linked CD Metal Nanoparticles.

In recent years, CDs have increasingly been used in the preparation of metal nanoparticles such as those of silver, gold, titianium, palladium, and platinum (Figure 1.15). Among the various organic and biomolecules used to functionalize the nanoparticles, such as sulfur-based compounds, proteins or DNA, CDs are regarded as one of the best choices because of their high solubilising ability, low toxicity, and specific recognition of many model substrates. Moreover, linked CD metal nanoparticles possess dual properties, in particular, the electronic, magnetic, or catalytic properties of the metal/semiconductor core and the molecular recognition ability of the CDs in the organic shell. The molecular complexation properties of the shell-immobilised CDs may also partly drive the formation of these assemblies.
Figure 1.15. CD based nanoparticles a) silver or gold nanoparticle,92,100 b) Pt nanoparticle97 and c) TiO$_2$ nanoparticle.95

Linked β-CD TiO$_2$ nanoparticles synthesized by Shown et al. (Figure 1.15c)95 are water-dispersible and have an average particle diameter of 4.4 ± 1 nm. Pyrene fluorescence was enhanced by increasing concentrations of β-CD-modified TiO$_2$ nanoparticles and the sensitization effect was triply stronger than the case of β-CD only. The sensitization behaviour of pyrene fluorescence in the presence of TiO$_2$ nanoparticles arises from an increase in the extinction coefficient of pyrene, demonstrating the charge transfer between pyrene and metal oxide nanoparticle.

1.1.8. Cyclodextrin Based Molecular Devices

In nature, molecular devices are part of extremely complicated biological systems and function in a highly sophisticated manner.102 In nanochemistry, molecular devices are based on supramolecular assemblies held together by mechanical forces and maybe controllable. Simple examples of molecular devices are rotaxanes and catenanes which are unusual supramolecular assemblies held together by mechanical restraints (Figure 1.16). A rotaxane consists of a macrocycle threaded onto a linear molecule in a similar manner to the mounting of a wheel on an axle. Accordingly, the name rotaxane is derived from the Latin for wheel and axle, rota and axis, respectively. A catenane consists of macrocycles joined as links in a chain, and the name catenane is derived from the Latin for chain, catena. One or two cyclodextrins can be incorporated into a catenane structure. Many studies have reported the synthesis of interlocked molecules, such as rotaxanes and catenanes, because of their unique structures and properties.28,64,103-109
Several controllable molecular devices have been prepared. Easton et al. reported a molecular device called a molecular pump.\textsuperscript{110} Kaneda et al. synthesized and characterized $N,N'$-p-xylylene-linked oligo-Janus [2]-rotaxanes based on a permethylated $\alpha$-cyclodextrin, which exhibited a contractible and extendable nature upon photoisomerization\textsuperscript{111}

An example of a controllable molecular device is a “molecular muscle”. In nature, the contraction and expansion of muscle fibers, caused by the simultaneous sliding of the stacked filaments of myosin and actin upon chemical stimulation by ATP hydrolysis, enables our controlled movement.\textsuperscript{112} Molecular mimics of muscle fibers having a ditopic axle, comprising both a stilbene and aliphatic group for CD complexation have been reported.\textsuperscript{113} Intercoversion between the trans- and cis-stilbene isomers causes the CDs to move between the alternative complexation sites (Figure 1.17). Intermediate and contracted states of the rotaxane (E,E), (E,Z) and (Z,Z) have been isolated and fully characterized by 2D NMR spectroscopy, thus directly establishing the changes in geometry and function similar to that of a muscle fiber.
Figure 1.17. (a) Representation of the switching between extended and contracted structures in a rotaxane linked α-CD dimer. (b) Structural formula of rotaxane linked α-CD dimer and its operation as a photo-chemically driven molecular muscle.\textsuperscript{113,114}

1.2. POLYMER HYDROGELS

1.2.1. General

Polymers consist of main-chain and side-chain classes. Main-chain polymers are composed of monomer subunits linearly linked to form a polymer backbone. In contrast, side-chain polymers have substituents attached to the polymer backbone, and such substituents may be capable of functionalization to incorporate molecular recognition character.\textsuperscript{115,116} In biological systems such as antigens, proteins and DNA, molecular recognition through the sequence of side chain interactions plays an important role in constructing supramolecular structures.\textsuperscript{117}

Polymer hydrogels (also called aquagels) are a network of polymer chains that are water-soluble. Polymer hydrogels have been extensively studied because of their potential applications in a broad range of fields.\textsuperscript{118-123} For example, Gupta \textit{et al.}\textsuperscript{124} reviewed the use of hydrogels in controlled drug delivery and the effects of pH, light, ionic strength and temperature. Lee and Mooney\textsuperscript{119} also reviewed the application of hydrogels for tissue engineering, which showed an exciting and revolutionary strategy to treat patients requiring a new organ or tissue. This involves the engineering of man-made organs and
tissues, with one such strategy utilising a combination of a patient’s own cells combined with hydrogel scaffolds.

Based on the origin of the interactions, polymer hydrogels are divided into two classes: the covalent hydrogels with permanent cross-links (covalent bonding) and the physical hydrogels with reversible cross-links (non-covalent bonding) such as hydrogen bonding, metal coordination interactions, hydrophobic interactions and host-guest interaction.\textsuperscript{125-127} Physical hydrogels are particularly promising as ideal candidates for biomaterials due to their high water content, tissue-like flexibility and biocompatibility.\textsuperscript{128-131} These polymer networks have tuneable properties and are sometimes called “smart” networks. The cross-links may be switchable and the hydrogels may undergo gel-to-sol or sol-to-gel transitions when triggered by external stimuli such as light, heat, pH change and redox-response.\textsuperscript{132-135} Some examples of physical hydrogels with different cross-links are mentioned as follow.

1.2.2. Cross-links by Hydrogen Bonding

In nature, perhaps the best known example of network cross-links by hydrogen bonding is the complementarily of base pairs in DNA. Some polymer networks use hydrogen bonding as a major driving force for inter-polymer interactions such as poly(carboxylic acids) and non-ionic polymers. The energy of a single hydrogen bond is comparatively low (2-167 kJ.mol\textsuperscript{-1}) and its length is in 1.2–3.0 Å range.\textsuperscript{136,137} However, when there is a simultaneous formation of a large number of inter-macromolecular hydrogen bonds, the strength of the interaction becomes significant. The most commonly used poly(carboxylic acids) for preparing interpolymer complexes are poly(acrylic acid) and poly(methacrylic acid). These polyacids have been complexed with various classes of water-soluble non-ionic polymers, such as poly(oxyethylene). Their properties have also been widely studied by a variety of techniques including viscometry, turbidity and pH measurements which are also used to characterise the nature of the complexes formed.\textsuperscript{137-141}

Varghese \textit{et al.}\textsuperscript{142} has prepared a self-healing hydrogel based on a polymer network with flexible pendant side chains carrying an optimal balance of hydrophilic and hydrophobic moieties. This allows the side chains to mediate hydrogen bonds across the hydrogel interface with minimal steric hindrance and hydrophobic collapse. The self-healing reported was rapid, occurring within seconds of the insertion of a crack into the hydrogel or attachment of two separate hydrogel pieces. The healing is reversible and can
be switched on and off through changes in pH which allows external control over the healing process (Figure 1.18).

Figure 1.18. Structure of the pendant side chains in self-healing hydrogel: a) healing hydrogel at low pH; b) unhealed hydrogels at high pH; c) schematic explanation for the healed hydrogels process.\textsuperscript{142}

At low pH, the terminal carboxyl groups are mostly protonated, which allows them to form hydrogen bonds with other terminal-carboxyl groups or amide groups across the interface, thus allowing the hydrogels to fuse (Figure 1.18a). At pH above their pKa (4.4 for 6-aminocaproic acid, the parent amino acid from which the acryloyl-6-aminocaproic acid monomer is synthesized) the acryloyl-6-aminocaproic acid carboxyl groups are deprotonated and exhibit significant electrostatic repulsion, which prevents hydrogen bonding (Figure 1.18b). Schematic shown in Figure 1.18c explanation for why the healed hydrogels exhibit a mechanically stronger fuse line compared to the bulk after healing for small timescales, and vice versa at very long times. Darker gray represents the toughened regions of the hydrogels due to protonation. The lighter gray represents the deprotonated (softer) regions of the hydrogels, which protonates and toughens with increasing exposure to low-pH solution.\textsuperscript{142}
1.2.3. Cross-links by Hydrophobic Interaction

The simplest functionalization of hydrogels is the self-functionalization exhibited by hydrophobic substituents through hydrophobe aggregation. This may occur as intra- or inter-strand functionalization or self-association.\(^{116}\) An example of self-association occurs where hydrophobic substituents form inter-polymer backbone clusters, or cross-links, with 10-30 groups per cross-link (Figure 1.19), and the number of substituents in the crosslink changes with the concentration of the polymer.\(^{143,144}\)

Figure 1.19. Association of hydrophobic substituents in a polymer network. The yellow strands represent the polymer backbone, the blue cylinders represent hydrophobic substituents and those enclosed in a green circle represent hydrophobic aggregation forming cross-links.\(^{144}\)

A simple reversible hydrophobic cross-link formation is shown in Figure 1.20.\(^{135}\) Dodecyl (C12) substituted poly(acrylic acid)s form a hydrophobic cross-linked polymer network. When addition of β-CD, β-CD interacts with C12 side chains in PAAC12 to form inclusion complexes under semidilute conditions, interpolymer hydrophobic associations of C12 side chains are prevented by addition of β-CD, resulting in a decrease in the viscosity of the viscoelastic aqueous solution of PAAC12. Upon addition of ferrocenecarboxylic acid (FCA) to the binary mixture of β-CD and PAAC12 (FCA), the mixture exhibits a sol-gel transition due to competitive interaction of C12 and FCA with β-CD. In the reduced state of FCA, the ternary mixture exhibits a gel-like behaviour. On the other hand, in the oxidized state of FCA, the mixture exhibits a low-viscosity and exhibits a sol-like behaviour.
There have been several studies of cross-linked polymer networks in which metal-ligand interactions, induced changes in the cross-links, the polymer network and its viscosity. A simple example of such a polymer network was reported by Peng et al.\textsuperscript{145} This system involves a reversible gel-sol/sol-gel transition in an aqueous poly(acrylate)s system switched by alternation between the ferrous/ferric ion redox states conjugated as a consequence of photo-reduction and oxidation. Thus, Fe(III) forms inter-polymer cross-links by coordinating carboxylate groups, yielding a hydrogel. Under photochemical stimulus, Fe(III) is reduced to Fe(II) in the presence of citrate, causing the cross-links to break and the system to transition to a fluid solution. Upon reaction with oxygen, Fe(II) oxidises to Fe(III) and the cross-links reform, returning the fluid solution to a hydrogel state (Figure 1.21).
Figure 1.21. Viscosity changes of poly(acrylate)s and Fe(III) controlled by photo-reduction and oxidation.\textsuperscript{145}

Recently, a self-assembly of macroscopic materials through metal-ligand interactions was reported in which a gel assembly was formed using polyacrylamide modified with Fe-porphyrin and L-histidine moieties.\textsuperscript{146} In this report, metal-ligand interactions were utilized to assemble macroscopic gels. Cubic gels containing Fe-porphyrin and L-histidine mixed in solution at pH 9.0 were stuck together in a few minutes (Figure 1.22). The stress values for the assembly increased as the concentration of either Fe-porphyrin or L-histidine in the gels increased. In contrast, the 2H-porphyrin gel (without Fe) and the L-histidine gel did not yield an assembly. However, when metalation of Fe (III) to 2H-Por in the gel, the gel forms an assembly with the L-His gel. These results indicate that metal-ligand interactions are responsible for self-assembly of the gels.

Figure 1.22. Metal-ligand interaction of Fe-porphyrin or L-histidine as cross-linked polymer network.\textsuperscript{146}
1.2.5. Cross-links by Host-Guest Interaction

Among the various non-covalent interactions including hydrogen bonding, metal-ligand and hydrophobic interactions, host-guest interaction is one of the most important because of its common occurrence in biological systems. Polymer networks arise through supramolecular assembly when a substituent shows molecular recognition with other substituents. This network may occur when one polymer bears one type of substituent which recognizes a different substituent on a second polymer. Alternatively, both polymers may be multiply substituted and bear several different substituents (Figure 1.23). Interestingly, many materials in nature are based on non-covalent interactions involving multiple substituents, whereas the majority of synthetic systems employ a single substituent capable of showing molecular recognition.

![Figure 1.23. Single and multiple functionalization, the open symbols represent receptor or host substituents and the square, circular and triangular substituents represent guest substituents.](image)

Among the most common host molecules, such as calixarenes, cucurbiturils, porphyrins and crown ethers, cyclodextrins are the most promising host for development of polymer hydrogels due to their availability, solubility and selectivity.

1.3. CYCLODEXTRINBASED POLYMER HYDROGELS

1.3.1. General

The host-guest interactions between a CD (host) and a hydrophobic guest are an ideal reversible, selective and controllable interaction for the cross-links in polymer network hydrogels. Since the first report that α-CD can form host-guest complexes with poly(ethylene glycol) (PEG) based on inter-molecularly cross-linked α-CD-PEG precursor polyrotaxanes (sliding gels), many CD based polymer hydrogels have been prepared in a
controllable configurations. Polymer network hydrogels can be formed from the interaction of CD-substituted polymers and guest-substituted polymers as well as guest-substituted polymers with linked CD dimers. The interactions of CD-substituted polymers with linked guest dimers are less well studied as most linked guest dimers are weakly soluble in water.\textsuperscript{144,154-164}

1.3.2. Interaction of CD Substituted Polymers and Guest Substituted Polymers

Recent studies have generated a range of CD-based hydrogels which exhibit predictable characteristics. New materials have been constructed from a range of adamantane (guest) and CD (host) substituted poly(acrylate)s which interact in a controlled manner.\textsuperscript{165-167} The basis for these studies is shown in Figure 1.24.\textsuperscript{168} The β-cyclodextrin substituent (Figure 1.24a) acts as a receptor for a hydrophobic substituent. The receptor and hydrophobe form a host-guest complex, which constitutes a cross-link between polymers. The hydrophobic adamantyl substituent (Figure 1.24b), which has an excellent fit to the β-cyclodextrin annulus, forms a cross-link in this way. This interaction leads to controlled aggregation to form a hydrogel (Figure 1.24c). Studies investigating the effects of changing the length of the tether (-CH\textsubscript{2}-CH\textsubscript{2}-) from 2, 6 and 12 carbons and the percentage adamantane substitution at 3%, 6% and 10% have also been conducted.\textsuperscript{144,166,169,170}

![Figure 1.24](image-url)

**Figure 1.24.** Host-guest interactions of adamantyl- and β-cyclodextrin-substituted polymers forming cross-links in poly(acrylate)s systems.\textsuperscript{168}

A polymer network formed by ditopic β-CD substituent/adamantyl substituent (β-CD/AD) cross-linked complexes has also been prepared with the intention of comparing the viscoelastic behaviour in an aqueous medium with systems resulting from cross-linking monotopic complexes.\textsuperscript{165} The stability constants characterising the double, or ditopic, cross-links (Figure 1.25) are much higher than those for monotopic cross-links as shown
by isothermal titration calorimetry, ITC, as expected on the basis of a chelate effect similar to that observed in metal complexes. The complexation enthalpy of the ditopic cross-link is twice that of the monotopic cross-link. The rheology results also show that viscosity of ditopically cross-linked hydrogels is higher than that of the monopically cross-linked hydrogels.\textsuperscript{165}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1_25.png}
\caption{Representation of polymer networks in which ditopic $\beta$-CD substituent/adamantyl substituent complexes form intra-polymer links.\textsuperscript{165}}
\end{figure}

The host-guest interactions in polymer systems are affected by the length of the tether attaching the substituent to the polymer backbone.\textsuperscript{35,167,171} Variations in the strength of interactions of the $\beta$-CD substituted poly(acrylate)s and adamantyl substituted poly(acrylate)s were studied by using substituted poly(acrylate)s with tether lengths of zero, two, six and twelve methylene groups. In the longer tether systems, the adamantyl substituent and the tether compete for host-guest complexation in the substituent $\beta$-CD annulus to form isomeric inter-strand linkages in the polymer network. As the tether length shortens, steric interactions with the poly(acrylate)s backbone increasingly inhibit intermolecular host-guest complexation and the relative sizes of the adamantyl and $\beta$-CD substituents become important in determining the formation of the cross-links.\textsuperscript{35,167,171}

Interestingly, Harada and co-worker\textsuperscript{172} recently demonstrated that molecular-recognition events can be used to direct the assembly of macroscopic objects into larger aggregated structures. Polyacrylamide-based gels functionalized with either CDs or small hydrocarbon-group guest moieties were synthesized. Cubic pieces of host and guest gels were shown to adhere to one another through the mutual molecular recognition of the CDs and hydrocarbon groups on their surfaces. By changing the size and shape of the host and
guest units, different gels can be selectively assembled and sorted into distinct macroscopic structures that are on the order of millimetres to centimetres in size (Figure 1.26).

![Figure 1.26](image)

**Figure 1.26.** (a) Molecular recognition between CD and (b) hydrocarbon-guest residues leads to self assembly at a macroscopic level.172

Polymer networks based on host-guest interactions of CD-substituted polymers with some special guest-substituted polymers have been show to form reversible hydrogels. For example, interaction of CD-substituted polymers with a ferrocene substituted polymer formed redox-driven hydrogels.148 A hydrogel actuator consisting of a poly(acrylamide) network cross-linked by \(N,N'\)-methylenebis(acrylamide) and a complex of \(\beta\)-CD-ferrocene shows an expansion-contraction process in response to oxidation and reduction of the ferrocenemoieties, leading to macroscale expansion and contraction of the hydrogel (Figure 1.27).173

![Figure 1.27](image)

**Figure 1.27.** Redox generated hydrogel actuator based on the CD-ferrocene interaction.173

Other studies have demonstrated photo-responsive controllable hydrogels formed by interaction of CD-substituted polymers with azobenzene-substituted polymers.132,133 For
example, a photo-responsive supramolecular hydrogel with α-CD as a host molecule and an azobenzene derivative as a photo-responsive guest molecule exhibits reversible macroscopic deformations in both size and shape when irradiated by ultraviolet light at 365nm or visible light at 430 nm (Figure 1.28). When ultraviolet irradiation is applied, which induces isomerisation from the trans to cis azobenzene, the complex between α-CD and the azobenzene units dissociates to expand α-CD-azobenzene gels. After visible light irradiation causes isomerization from the cis to trans azobenzene, complexation between α-CD and the azobenzene units regenerates, shrinking the α-CD-azobenzene gel.

Figure 1.28. Schematic representation of the expansion-contraction of α-CD-azobenzene gel irradiated by ultraviolet and visible light.174

1.3.3. Interaction of Guest Substituted Polymers and Linked CD Dimers

Covalent CD dimers have been used as cross-linking agents to form polymer networks with guest substituted polymers (Figure 1.29). Kretschmann et al. reported thermo-responsive polymer networks formed by mixtures of linked β-CD dimers and copolymers of adamantyl-containing monomer and N-isopropylacrylamide or N,N-dimethylacrylamide. They found that the mixtures formed gels within seconds.164

Figure 1.29. Schematic representation of the polymer network formed by linked CD dimers and guest-substituted polymers.
The properties of the hydrogels assembled from linked β-CD dimers and adamantyl-substituted poly(acrylate)s can be controlled by the adamantyl-substituted poly(acrylate)s tether length. Increasing the length of the adamantyl tether from zero to six methylene groups progressively decreases the steric hindrance between adjacent polymer backbones. The ease of host-guest complexation increases and the polymer network formation strengthens. The length of the linked β-CD dimer linker can also affect hydrogel properties. The complexation between adamantyl substituted poly(acrylate)s with zero methylene groups and the longer linked β-CD succinamide dimer has a higher viscosity and stronger polymer network than the complexation with the shorter linked β-CD urea dimer.\textsuperscript{167}

1.4. RESEARCH OBJECTIVES

1.4.1. General

While many polymer networks are prepared, as far as we know, there is no reported polymer network formed by β-CD trimers. Furthermore, most studies for host-guest interaction in polymer hydrogels are studied qualitatively, without determination of stability constant values and their associated thermodynamic parameters.\textsuperscript{34,153,162}

To gain further understanding of inter-polymer interactions, it has been decided to study new polymer networks from the interaction of some hydrophobic guest-substituted poly(acrylate)s with linked β-CD trimers as indicated in the general scheme shown in Figure 1.30.
Figure 1.30. Schematic representation of the polymer network formed by linked CD trimers and guest-substituted polymers.

The host-guest complexation of β-CD trimers will be studied through interaction with small guests and different hydrophobic guest substituted polymers in solution by different methods including 2D NMR, ITC, fluorescence titration, rheology and size measurement by dynamic light scattering, depending on the properties of the hydrophobic guest.

1.4.2. Aims of This Research

The aims of this research are to advance the understanding of the supramolecular chemistry of linked CD trimers. The complexation behaviour will be studied and polymer networks based on linked CD trimers will be constructed.

Chapter 2 describes the preparation of two new linked β-CD trimers. This chapter also studies the host-guest complexation by β-CD and two linked β-CD trimers of cationic crystal violet (CV⁺), pyrodanine B (PB⁺) and zwitterionic rhodamine B (RB). The host-guest complexation and thermodynamics are studied by UV-vis and 1H 2D NOESY NMR spectroscopy. The molecular models of complexation are prepared. The effects of the structures of the host and guests on the complexation processes are discussed.

Chapter 3 describes the characterisation stability of constants and thermodynamic patterns in host-guest complexation of the β-CD trimers with n-dodecyl (C12) and n-octadecyl (C18) substituted-poly(acrylate)s, PAAC12 and PAAC18, systems in dilute aqueous solutions. This studies compare hydrophobic interactions of the C12 and C18
substituted-poly(acrylate)s and their interaction with β-CD and linked β-CD trimers. The complexes are studied by $^1$H 2D NOESY NMR spectroscopy, ITC, size measurement by dynamic light scattering and rheological analysis. The data obtained is used to establish the extent to which this pattern imposes on hydrogel formation in more concentrated solutions.

Chapter 4 describes the host-guest chemistry of linked β-CD trimers and adamantyl substituted poly(acrylate)s with different linker tether lengths. The complexes are studied by $^1$H 2D NOESY NMR spectroscopy, ITC and rheological analysis. Effects on the complexation constants and the associated thermodynamic parameters by structures of the linked-CD trimers and the length of the tether between the adamantyl substituent and the poly(acrylate)s backbone are studied.

Chapter 5 describes the host-guest chemistry of linked β-CD trimers and dansyl substituted poly(acrylate)s with different linker tether lengths. Complexation of the dansyl substituent by the linked trimers is quantified by $^1$H 2D NOESY NMR spectroscopy, ITC, fluorescence spectroscopy, dynamic light scattering and rheological studies.
1.5. REFERENCES


44. P. Thordarson. in *Supramolecular chemistry*, John Wiley & Sons, Ltd, **2012**.


This page intentionally left blank.
CHAPTER 2

COMPLEXATION OF

CRYSTAL VIOLET, PYRONINE B AND RHODAMINE B

BY LINKED β-CYCLODEXTRIN TRIMERS *

*The material in this chapter has been published in:
2.1. INTRODUCTION

The host-guest chemistry of the most common naturally occurring homochiral cyclic oligomers formed by 6, 7 and 8 α-1,4-linked D-glucopyranose units, α-, β- and γ-cyclodextrin, α-CD, β-CD and γ-CD, respectively, has been extensively explored\(^1\)-\(^8\) as has been reviewed in Chapter 1 of this thesis. Because of their ability to act as hosts in the complexation of a great range of guest species in water, cyclodextrins and their modified forms are the most widely employed class of supramolecular agents and have found many practical uses, particularly in the food\(^9\)-\(^13\) and pharmaceutical industries.\(^14\)-\(^18\) The complexation constants and the thermodynamic parameters of the host-guest complexation of native α-CD, β-CD and γ-CD with a large variety of guest species have been reported.\(^8\) Studies of host-guest complexation by linked CD dimers show them to complex ditopic long guests in a cooperative process. In these cases, a long guest possessing two hydrophobic groups at either end tends to be complexed with one hydrophobic group in each CD of the linked CD dimer.\(^19\)-\(^23\) The stability constants of the host-guest complex for linked CD dimers with guests is sometimes increased by 10-100 times, and the entropy of complexation is more negative due to the loss of conformational freedom by comparison with that of the corresponding monomer CD.\(^7\),\(^24\) A substantial range of linked β-CD dimers have been synthesized and their complexation constants and thermodynamic parameters have been reviewed.\(^24\) However, only a few linked β-CD trimers have been synthesized,\(^25\)-\(^27\) and only a single paper reported the host-guest complexation of a linked β-CD trimer which complexed an anthracene-derivative >3 times as strongly as a linked β-CD dimer with the same linker and 5 times as strongly as native β-CD.\(^28\)

Organic dyes have been extensively used in host-guest chemistry where the dyes form a guest interaction with appropriate host molecules.\(^1\)-\(^8\) Since the first synthetic dye was discovered by Perkin in 1856,\(^29\) synthetic dyes quickly replaced the traditional natural dyes, and many thousands of synthetic dyes have been prepared and are used in a broad range of fields.\(^29\),\(^30\) Based on their application, dyes can be classified into many classes such as acid dyes (or anionic dyes) which are applied to fibres (silk, wood, nylon); basic dyes (cationic dyes) that are used for acrylic fibres; or solvent dyes that are used for wood staining. By the nature of their chromophore, dyes can also be divided into classes:
acridine dyes, anthraquinone dyes, azo dyes, diazonium dyes, nitro dyes, thiazole dyes, xanthene dyes or fluorene dyes.\(^\text{29}\)

### 2.1.1. Structure and Properties of Crystal Violet

The cationic crystal violet, CV\(^+\), (tris(4-(dimethylamino)phenyl)methyl)ium ion, also known as methyl violet 10B, hexamethyl pararosaniline chloride, or pyocyanin(e),\(^\text{31}\) is a symmetrical triphenylmethane dye. It was first synthesis by Alfred Kern Crystal in 1883. Crystal violet has antibacterial, antifungal, and anthelmintic properties and was formerly important as a topical antiseptic and has been the subject of interest in various fields of science.\(^\text{32}\) It has been studied in complexes with a variety of molecules such as nucleic acids,\(^\text{33}\) synthetic polymers,\(^\text{34}\) cyclodextrin\(^\text{35}\) or used as a biological stain.\(^\text{36}\) The structure and molecular models of CV\(^+\) are shown in Figure 2.1.

![Figure 2.1](image)

**Figure 2.1.** (a) Chemical structure of CV\(^+\), (b) space filling model and (c) MM2 energy-minimized molecular model of CV\(^+\) using the PM7 method (MOPAC2012). Carbon and nitrogen atoms are shown in gray and blue, respectively. Hydrogen atoms and lone pairs are not shown.

### 2.1.2. Structure and Properties of Pyronine B

The cationic pyronine B, PB\(^+\), ([6-(diethylamino)xanthen-3-ylidene]-diethylazanium ion), is a water-soluble xanthene dye which is sensitive to its molecular environment and has found applications in biological and synthetic polyelectrolyte systems.\(^\text{37}\) The structure and molecular models of PB\(^+\) are shown in Figure 2.2.
2.1.3. Structure and Properties of Rhodamine B

The zwitterionic rhodamine B, RB, ([9-(2-carboxyphenyl)-6-(diethylamino) xanthen-3-ylidene]-diethylamine), also belongs to the family of xanthene dyes, and is used extensively in biotechnology applications as a staining fluorescent dye. Rhodamine dyes are generally toxic, and are soluble in water, methanol and ethanol. Rhodamine B has been used to investigate the properties of molecules adsorbed on various organic and inorganic substrates. The structure and molecular models of RB are shown in Figure 2.3.

\[ \text{2.1.4. Dimerisation of CV}^+, \text{ PB}^+ \text{ and RB} \]

In aqueous solution, CV\(^+\), PB\(^+\) and RB have a tendency to aggregate to form dimers with increasing concentration:

\[ \text{2 Dye } K_d \rightleftharpoons (\text{Dye})_2 \]  \hspace{1cm} (2.1)
where $K_d$ is the dimerisation constant. The dimerisation and inclusion complexation equilibria of CV$^+$, PB$^+$ and RB in aqueous solution (Figure 2.4) have been studied by UV-Vis and temperature-jump spectrophotometry. The $K_d$ values for the dimerisation of CV$^+$, PB$^+$ and RB are shown in Table 2.1.

![Figure 2.4. Dimerisation of CV$^+$, PB$^+$ and RB](image)

**Table 2.1.** Dimerisation constants, $K_d$, of CV$^+$, PB$^+$ and RB in aqueous solution at 298.2K.

<table>
<thead>
<tr>
<th>Species</th>
<th>$K_d$ (dm$^3$ mol$^{-1}$)</th>
<th>pH</th>
<th>Method</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>(CV$^+$)$_2$</td>
<td>600</td>
<td>6.5</td>
<td>Temperature-jump spectrophotometry</td>
<td>40</td>
</tr>
<tr>
<td>(CV$^+$)$_2$</td>
<td>180</td>
<td>7.2</td>
<td>UV-visible spectroscopy</td>
<td>41</td>
</tr>
<tr>
<td>(PB$^+$)$_2$</td>
<td>1300</td>
<td>5.7</td>
<td>Temperature-jump spectrophotometry</td>
<td>42</td>
</tr>
<tr>
<td>(RB)$_2$</td>
<td>1800</td>
<td>6.4</td>
<td>Temperature-jump spectrophotometry</td>
<td>43</td>
</tr>
</tbody>
</table>

### 2.1.5. Complexation of CV$^+$, PB$^+$ and RB by β-CD

In aqueous solution, the complexation of CV$^+$, PB$^+$ and RB by β-CD often occurs in the presence of the competing dimerisation equilibrium of the dyes.$^{40-43}$ The dye forms complexes of stoichiometries 1:1 (host:guest) with β-CD as shown in Equation 2.2. The complexation of CV$^+$, PB$^+$ and RB by β-CD have been studied and the equilibrium
constants, \( K_{11} \), for the 1:1 complexation at varied temperature and pH have been determined by UV-Vis\textsuperscript{42,44-46}, fluorescence\textsuperscript{35,44} and \(^1\)H NMR spectroscopy\textsuperscript{46} as shown in Table 2.2.

\[
\beta\text{-CD} + \text{Dye} \xrightleftharpoons[K_{11}]{\text{Dye}} \beta\text{-CD.Dye} \tag{2.2}
\]

**Table 2.2.** Equilibrium constants, \( K_{11} \), for the 1:1 host-guest complexation of CV\(^+\), PB\(^+\) and RB by \( \beta\text{-CD} \) in aqueous solution.

<table>
<thead>
<tr>
<th>Species</th>
<th>( K_{11} \text{(dm}^3\text{mol}^{-1}) )</th>
<th>T (K)</th>
<th>pH</th>
<th>Method</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \beta\text{-CD.CV}^+ )</td>
<td>4800</td>
<td>298.2</td>
<td>6.5</td>
<td>UV-visible spectroscopy</td>
<td>\textsuperscript{47}</td>
</tr>
<tr>
<td></td>
<td>5850</td>
<td>298.2</td>
<td>6.0</td>
<td>Spectrofluorometric titration</td>
<td>\textsuperscript{35}</td>
</tr>
<tr>
<td>( \beta\text{-CD.PB}^+ )</td>
<td>1500</td>
<td>298.2</td>
<td>4.0</td>
<td>(^1)H NMR spectroscopy</td>
<td>\textsuperscript{46}</td>
</tr>
<tr>
<td></td>
<td>1660</td>
<td>298.2</td>
<td>4.0</td>
<td>UV-visible spectroscopy</td>
<td>\textsuperscript{46}</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>293.2</td>
<td>4.0</td>
<td>UV-visible spectroscopy</td>
<td>\textsuperscript{44}</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>293.2</td>
<td>4.0</td>
<td>Time-resolved fluorescence</td>
<td>\textsuperscript{44}</td>
</tr>
<tr>
<td></td>
<td>2100</td>
<td>293.2</td>
<td>4.0</td>
<td>Fluorimetry</td>
<td>\textsuperscript{44}</td>
</tr>
<tr>
<td></td>
<td>4000</td>
<td>298.2</td>
<td>5.7</td>
<td>UV-visible spectroscopy</td>
<td>\textsuperscript{42}</td>
</tr>
<tr>
<td>( \beta\text{-CD.RB} )</td>
<td>4240</td>
<td>298.2</td>
<td>7.2</td>
<td>Fluorimetry</td>
<td>\textsuperscript{48}</td>
</tr>
<tr>
<td></td>
<td>5900</td>
<td>298.2</td>
<td>6.4</td>
<td>Kinetic spectrophotometry</td>
<td>\textsuperscript{43}</td>
</tr>
</tbody>
</table>

**2.1.6. Aims of this study**

The aims of this study are to synthesize two new linked \( \beta\text{-CD} \) trimers, 1,3,5-\( N,N,N \)-tris(6\(^A\)-deoxy-6\(^A\)-\( \beta \)-cyclodextrin)-benzene, and 1,3,5-\( N,N,N \)-tris(6\(^A\)-(2-aminoethyl)amino-6\(^A\)-deoxy-6\(^A\)-\( \beta \)-cyclodextrin)-benzene, \( \beta\text{-CD}_{3}\text{bz} \) and \( \beta\text{-CDen}_{3}\text{bz} \), respectively, and investigate the complexation of CV\(^+\), PB\(^+\) and RB by \( \beta\text{-CD} \) as well as for the first time quantifying the complexation of these dyes by two linked \( \beta\text{-CD} \) trimers, by UV-Vis and 2D ROESY \(^1\)H NMR spectroscopy.

It is anticipated that the present study will provide insight into the complexing characteristics of \( \beta\text{-CD}_{3}\text{bz} \) and \( \beta\text{-CDen}_{3}\text{bz} \), and that complexation by linked \( \beta\text{-CD} \) trimers
can be ‘tuned’ by controlling the length of the linker between \( \beta \)-cyclodextrin annuli. This will help to understand the behaviour of \( \beta \)-CD\textsubscript{3}bz and \( \beta \)-CDen\textsubscript{3}bz in subsequent studies where the trimers act as tritopic hosts while forming intra- and inter-molecular cross-links in and between polymer strands through complexing hydrophobe substituents 3% randomly substituted onto poly(acrylate)s to form networks and hydrogels (see Chapter 4).

### 2.2. SYNTHESIS AND MOLECULAR MODELLING OF LINKED \( \beta \)-CD TRIMERS

#### 2.2.1. Synthesis of Two Linked \( \beta \)-CD Trimers

The 1,3,5-trinitrophenyl-benzene required for the synthesis of the \( \beta \)-CD trimers was prepared from the reaction of 1,3,5-benzenetricarbonyltrichloride with 4-nitrophenol in dichloromethane in presence of triethylamine under \( \text{N}_2 \). The product was recrystallised from dichloromethane, washed with water and dried to give a yield of 82%. 6\(^\text{A}\)-Amino-6\(^\text{A}\)-deoxy-\( \beta \)-CD, 6\( \beta \)-CDNH\(_2\), and 6\(^\text{A}\)-(1-(2-Aminoethyl)amino)-6\(^\text{A}\)-deoxy-\( \beta \)-CD, 6\( \beta \)-CDen, were prepared according to literature methods.\(^{49,50}\) Reaction of these amino-\( \beta \)-CDs with 1,3,5-trinitrophenyl-benzene in pyridine afforded the two linked \( \beta \)-CD trimers, 1,3,5-\( N,N,N \)-tris(6\(^\text{A}\)-deoxy-6\(^\text{A}\)-\( \beta \)-cyclodextrin)-benzene, \( \beta \)-CD\textsubscript{3}bz, and 1,3,5-\( N,N,N \)-tris(6\(^\text{A}\)-(2-aminoethyl)amino-6\(^\text{A}\)-deoxy-6\(^\text{A}\)-\( \beta \)-cyclodextrin)-benzene, \( \beta \)-CDen\textsubscript{3}bz (Figure 2.5). The products were purified in 65-68% yield. The structures of compounds were confirmed by TLC, \(^1\)H NMR, \(^{13}\)C NMR, MS and elemental analysis (see Chapter 6- experimental chapter).

The two linked \( \beta \)-CD trimers were prepared, as shown in Figure 2.5, incorporating three equivalent \( \beta \)-CDs attached at the 1, 3 and 5 positions of the benzene center through either an amido link (\( \beta \)-CD\textsubscript{3}bz) or a longer (2-aminoethyl)amido link (\( \beta \)-CDen\textsubscript{3}bz) such that their \( \beta \)-CD groups are seperated by 7 and 13 atoms, respectively. Each of the three \( \beta \)-CD groups may form host-guest complexes with single dye molecules, or two of the three \( \beta \)-CD groups may cooperatively form host-guest complexes.
2.2.2. Molecular Modelling of β-CD Trimers

Molecular models of the linked β-CD trimers, β-CD₃bz and β-CDen₃bz, were constructed and energy-minimised using the MM2 molecular mechanics method using ChemBio3D Ultra 11.0 software, followed by geometry optimisation by the PM7 semiempirical method using MOPAC2012. The models are displayed as space-filling representations (Figure 2.6) with the hydrogen atoms and lone pairs omitted to give a clearer view. Two models are shown for each linked β-CD trimer. The energies where calculated by the PM7 method (heats of formation in the gas phase at standard states) of the 2 β-CD trimers models are -50590.4 EV or -51026.8 EV and -52524.7 EV or -52523.9 EV for β-CD₃bz and β-CDen₃bz, respectively (Figure 2.6). The length of linker from N (-C₆A) to N (-C₆A) in β-CD₃bz is 7.49 Å where that of β-CDen₃bz is 12.5 Å.
Figure 2.6. Space-filling representation of β-CD₃bz and β-CDen₃bz constructed and energy-minimised using the MP7 method (MOPAC2012). Two models are shown for each compound. Carbon, nitrogen and oxygen atoms are shown in gray, blue and red, respectively. Hydrogen atoms and lone pairs are not shown.

2.3. COMPLEXATION OF CV⁺, PB⁺ AND RB BY β-CD AND LINKED β-CD TRIMERS

2.3.1. UV-Vis Studies

2.3.1.1. Equilibria and Data Analysis

The equilibria in which β-CD, β-CD₃bz and β-CDen₃bz complex CV⁺, PB⁺ and RB have been characterized in aqueous phosphate buffer at pH 7.0 and I = 0.10 mol dm⁻³ by UV-Vis spectroscopy. The UV-Vis spectra of solutions were determined against reference solutions in matched 1 cm quartz cells and were recorded at 0.25 nm intervals using a Cary 5000 UV-Vis spectrophotometer over the range 400-700 nm for CV⁺ and 500-600 nm for PB⁺ and RB. Solutions were freshly prepared in aqueous phosphate buffer at pH 7.0 and
$I = 0.10 \text{ mol dm}^{-3}$. Samples were 2.0 cm$^3$ in volume and were thermostated at 278.2, 288.2, 298.2 and 308.2 K for 30 min before starting the titration. The titration solutions were all $5.00 \times 10^{-6} \text{ mol dm}^{-3}$ in either CV$^+$, RB or PB$^+$ and were titrated using sequential injections (5 mm$^3$ each) of either $\beta$-CD (1.41 $\times$ $10^{-3}$ mol dm$^{-3}$), $\beta$-CD$_3$bz (2.81 $\times$ $10^{-3}$ mol dm$^{-3}$) or $\beta$-CDen$_3$bz (1.36 $\times$ $10^{-3}$ mol dm$^{-3}$) also in aqueous phosphate buffer pH 7.0, $I = 0.10$ mol dm$^{-3}$. The reference solutions were similarly titrated. Typical molar absorbance changes of solutions of CV$^+$, PB$^+$ and RB and $\beta$-CD$_3$bz, and the best fits of algorithms describing the complexation processes at 298.2 K appear in Figures 2.7 - 2.9.

All changes in the UV-Vis spectra, the best-fits of the algorithms describing the complexation equilibria to the UV-Vis absorbance changes, the derived UV-Vis spectra of the equilibrium species and speciation plots for the three systems $\beta$-CD.CV$^+$, $\beta$-CD$_3$bz.CV$^+$ and $\beta$-CDen$_3$bz.CV$^+$ are shown in Figures 2.27 - 2.32 in the Appendix, section 2.6, at the end of this Chapter. The derived UV-vis spectra of the $\beta$-CD$_3$bz.CV$^+$ and ($\beta$-CD$_3$bz)$_2$.CV$^+$ species are quite similar probably as a result of the 4-(dimethylamino)-phenyl chromophore experiencing a similar environmental change upon complexation in the $\beta$-CD annuli of both complexes. A similar situation occurs for CV$^+$ complexation by $\beta$-CD, and $\beta$-CDen$_3$bz.

Similar changes arise in the PB$^+$ and RB UV-Vis spectra at 278.2, 288.2, 298.2, and 308.2 K through complexation by $\beta$-CD, $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz. All changes in the UV-Vis spectra, the best-fits of the algorithms describing the equilibria to the UV-Vis absorbance changes, the derived UV-Vis spectra of the equilibrium species, and speciation plots for the three system are shown in Figures 2.32 to 2.44 in the Appendix, section 2.6, at the end of this Chapter.
Figure 2.7. (a) Molar absorbance change of CV⁺ (5.00 × 10⁻⁶ mol dm⁻³) in phosphate buffer, pH 7.0, I = 0.10 mol dm⁻³ with sequential injections (5 mm⁻³ each) of β-CD₃bz (2.81 × 10⁻³ mol dm⁻³) into both the sample and reference cells at 298.2K. The arrows indicate the direction of molar absorbance change as the molar ratio of [β-CD₃bz]/[CV⁺] increases. (b) Molar absorbance variation at 590nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-650 nm.
Figure 2.8. (a) Molar absorbance change of \( PB^+ \) (5.01 \( \times \) \( 10^{-6} \) mol dm\(^{-3} \)) in phosphate buffer, pH 7.0, \( I = 0.10 \) mol dm\(^{-3} \) with sequential injections (5 mm\(^2 \) each) of \( \beta\text{-CD}_3\text{bz} \) (2.81 \( \times \) \( 10^{-3} \) mol dm\(^{-3} \)) into both the sample and reference cells at 298.2K (isosbestic point 558 nm). The arrows indicate the direction of molar absorbance change as the molar ratio of \([\beta\text{-CD}_3\text{bz}]/[PB^+]\) increases. (b) Molar absorbance variation at 550 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation over the wavelength range 500-570 nm.
Figure 2.9. (a) Molar absorbance change of RB ($5.01 \times 10^{-6}$ mol dm$^{-3}$) in phosphate buffer, pH 7.0, $I = 0.10$ mol dm$^{-3}$ with sequential injections (5 mm$^3$ each) of $\beta$-CD$_{3}$bz ($2.81 \times 10^{-3}$ mol dm$^{-3}$) into both the sample and reference cells at 298.2K (isosbestic point 565 nm). The arrows indicate the direction of molar absorbance change as the molar ratio of $\left[[\beta\text{-CD}_{3}\text{bz}] / [RB]\right]$ increases. (b1) Molar absorbance variation at 546 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation over the wavelength range 500-570 nm.
2.3.1.2. Data Analysis

Equilibria 2.3 and 2.4, where Dye is either CV$^+$ or PB$^+$ or RB, were investigated by UV-Vis for the host-guest ratio 1:1 and 2:1 for β-CD$_3$bz.Dye system as well as were the analogous equilibria for the β-CD.Dye and β-CDen$_3$bz.Dye systems. The complexation constants, $K_{11}$ and $K_{21}$, are defined in Equations 2.5 and 2.6, respectively. An algorithm for the formation of a 1:1 β-CD$_3$bz.Dye complex alone (Equation 2.3) fitted the UV-Vis data poorly in some cases, and it was necessary to add to the algorithm a term for the formation of a 2:1 (β-CD$_3$bz)$_2$.Dye complex (Equation 2.4) to obtain a good fit.

$$\beta\text{-CD}_3\text{bz} + \text{Dye} \rightleftharpoons \beta\text{-CD}_3\text{bz}.\text{Dye} \quad (2.3)$$
$$\beta\text{-CD}_3\text{bz}.\text{Dye} + \beta\text{-CD}_3\text{bz} \rightleftharpoons (\beta\text{-CD}_3\text{bz})_2.\text{Dye} \quad (2.4)$$

$$K_{11} = \left[ \beta\text{-CD}_3\text{bz}.\text{Dye} \right] / \left[ \left[ \beta\text{-CD}_3\text{bz} \right] \left[ \text{Dye} \right] \right] \quad (2.5)$$
$$K_{21} = \left[ \left( \beta\text{-CD}_3\text{bz} \right)_2.\text{Dye} \right] / \left[ \left[ \beta\text{-CD}_3\text{bz}.\text{Dye} \right] \left[ \beta\text{-CD}_3\text{bz} \right] \right] \quad (2.6)$$

When $[\text{Dye}]_{\text{tot}}$ and $[\beta\text{-CD}_3\text{bz}]_{\text{tot}}$ are the initial concentrations:

$$[\text{Dye}]_{\text{tot}} = [\text{Dye}] + [\beta\text{-CD}_3\text{bz}.\text{Dye}] + [(\beta\text{-CD}_3\text{bz})_2.\text{Dye}] \quad (2.7)$$

It follows that:

$$[\beta\text{-CD}_3\text{bz}]_{\text{tot}} = [\beta\text{-CD}_3\text{bz}] + [\beta\text{-CD}_3\text{bz}.\text{Dye}] + [(\beta\text{-CD}_3\text{bz})_2.\text{Dye}] \quad (2.8)$$

The measured molar absorbance at a particular wavelength is given by Equation 2.9:

$$A(\lambda) = \varepsilon_{\text{Dye}}[\text{Dye}] + \varepsilon_{(\beta\text{-CD}_3\text{bz}.\text{Dye})} \left[ \beta\text{-CD}_3\text{bz}.\text{Dye} \right] + \varepsilon_{(\beta\text{-CD}_3\text{bz})_2.\text{Dye}} \left[ \left( \beta\text{-CD}_3\text{bz} \right)_2.\text{Dye} \right] \quad (2.9)$$

where $\varepsilon_{\text{Dye}}$, $\varepsilon_{(\beta\text{-CD}_3\text{bz}.\text{Dye})}$, and $\varepsilon_{(\beta\text{-CD}_3\text{bz})_2.\text{Dye}}$ represent the respective molar absorbances. Thus, for the CV$^+$ system, $K_{11}$ and $K_{21}$ and the molar absorbances of β-CD$_3$bz.CV$^+$ and (β-CD$_3$bz)$_2$.CV$^+$ may be determined through the best-fitting of an algorithm derived through Equations 2.3 to Equations 2.9 to the data in the range 500–650 nm using a nonlinear least-squares program HypSpec.$^{53,54}$ Similar procedures may be used to derive $K_{11}$ and $K_{21}$ for the β-CD.CV$^+$ and β-CDen$_3$bz.CV$^+$ systems.

The UV-Vis data were best-fitted over the range 500–570 nm for each of the PB$^+$ and RB systems. The UV-Vis changes characterising the complexation of PB$^+$ by β-CD and β-CDen$_3$bz, and RB by β-CDen$_3$bz, are consistent with the formation of both 1:1 and 2:1 complexes while those observed for the complexation of PB$^+$ by β-CD$_3$bz and of RB by
β-CD and β-CD₃bz are consistent with the formation of 1:1 complexes alone. However, it is possible that the differences between the UV-Vis spectra of either PB⁺ or RB in the 1:1 and 2:1 complexes in these three systems are insufficient for detection of the 2:1 complexes. All data and fitting results are summarised in Table 2.3 to Table 2.9 and are discussed below.

Dimerisation of CV⁺, PB⁺ and RB is characterized by $K_d$ (298.2 K) = $6 \times 10^2$, $1.3 \times 10^3$ and $1.8 \times 10^3$ dm³ mol⁻¹, respectively, determined by UV-Vis spectroscopy under similar experimental conditions.⁴⁰,⁴²,⁴³ Thus, the monomers in the dimers (CV⁺)$_2$, (PB⁺)$_2$ and (RB)$_2$ constitute only 0.30, 0.87 and 0.63%, respectively of the CV⁺, PB⁺ and RB $5.0 \times 10^{-6}$ mol dm⁻³ total concentrations studied here and do not contribute significantly to the observed UV-Vis changes.

### 2.3.1.3. Thermodynamic Studies

The relationship between the Gibbs free energy change ($\Delta G_{11}$), the enthalpy change ($\Delta H_{11}$), the product of the temperature and the entropy change ($T\Delta S_{11}$) for the complexation constants ($K_{11}$) are given by the following equations:

$$\Delta G_{11} = -RT\ln K_{11} \tag{2.10}$$

where $R$ is the ideal gas constant.

and

$$\Delta G_{11} = \Delta H_{11} - T\Delta S_{11} \tag{2.11}$$

From Equation 2.10 and Equation 2.11,

$$\ln K_{11} = -\Delta H_{11}/RT + \Delta S_{11}/R \tag{2.12}$$

The plot of $\ln K_{11}$ versus $1/T$ according to Equation 2.12 is a van't Hoff plot, of which the slope and the intercept represent $-\Delta H_{11}/RT$ and $\Delta S_{11}/R$, respectively.

Using the van’t Hoff plots, $\Delta H_{11}$ and $T\Delta S_{11}$ were calculated from the slopes and intercepts for the complexation of CV⁺, PB⁺ and RB by β-CD and linked β-CD trimers. The derived $K_{11}$ at 298.2 K and $\Delta H_{11}$ and $T\Delta S_{11}$ for nine system are summarized in Table 2.9. The van’t Hoff plots for the formation of β-CD.CV⁺, β-CD₃bz. CV⁺, β-CD₃bz.CV⁺, β-CD.PB⁺, β-CD₃bz. PB⁺, β-CD₃bz.PB⁺, β-CD.RB, β-CD₃bz.RB and β-CD₃bz.RB are shown in Figures 2.10, 2.11 and 2.12, as is discussed in section 2.3.2. Because of the high error estimates for $K_{21}$ characterizing the 2:1 complexes of CV⁺, PB⁺ and RB (Table 2.9) analogous van’t Hoff plots were not considered.
2.3.1.3. Discussion

The equilibrium constants, $K_{11}$ and $K_{21}$, for the 1:1 and 1:2 complexations of CV$^+$, PB$^+$, RB by $\beta$-CD and linked $\beta$-CD trimers are determined by UV-Vis. The derived $K_{11}$ and $K_{21}$ at each temperature studied, and the corresponding $\Delta H_{11}$ and $T\Delta S_{11}$ derived for the formation of $\beta$-CD.Dye, $\beta$-CD$_3$bz.Dye, and $\beta$-CDen$_3$bz.Dye from van’t Hoff plots appear in Table 2.3 to 2.8. The errors shown for $K_{11}$ and $K_{21}$ are data fitting errors. The maximum experimental errors for $K_{11}$ and $K_{21}$ are estimated to be ±5% and ±10%, respectively and the errors shown for $\Delta H_{11}$ and $T\Delta S_{11}$ are those from fitting the van’t Hoff equation to the UV-Vis data.

Table 2.3. Complexation constants ($K_{11}$) and thermodynamic parameters for the complexation of $\beta$-CD, $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz with CV$^+$ at different temperatures by UV-Vis titration in aqueous phosphate buffer at pH 7.0 and $I = 0.10$ mol dm$^{-3}$.

<table>
<thead>
<tr>
<th>$T$ K</th>
<th>Host</th>
<th>$10^3K_{11}$ dm$^3$ mol$^{-1}$</th>
<th>$\Delta H_{11}$ kJ mol$^{-1}$</th>
<th>$T\Delta S_{11}$ kJ mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>278.2</td>
<td>$\beta$-CD</td>
<td>6.61 ± 0.08</td>
<td>-11.7 ± 0.6</td>
<td>8.7 ± 0.7</td>
</tr>
<tr>
<td>288.2</td>
<td>$\beta$-CD</td>
<td>5.62 ± 0.06</td>
<td>-11.7 ± 0.6</td>
<td>9.0 ± 0.7</td>
</tr>
<tr>
<td>298.2</td>
<td>$\beta$-CD</td>
<td>4.68 ± 0.04</td>
<td>-11.7 ± 0.6</td>
<td>9.3 ± 0.7</td>
</tr>
<tr>
<td>308.2</td>
<td>$\beta$-CD</td>
<td>4.07 ± 0.04</td>
<td>-11.7 ± 0.6</td>
<td>9.6 ± 0.7</td>
</tr>
<tr>
<td>278.2</td>
<td>$\beta$-CD$_3$bz</td>
<td>204 ± 0.9</td>
<td>-27.2 ± 1.4</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>288.2</td>
<td>$\beta$-CD$_3$bz</td>
<td>141 ± 0.7</td>
<td>-27.2 ± 1.4</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>298.2</td>
<td>$\beta$-CD$_3$bz</td>
<td>86.3 ± 0.5</td>
<td>-27.2 ± 1.4</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>308.2</td>
<td>$\beta$-CD$_3$bz</td>
<td>67.8 ± 0.3</td>
<td>-27.2 ± 1.4</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>278.2</td>
<td>$\beta$-CDen$_3$bz</td>
<td>12.3 ± 0.2</td>
<td>-17.9 ± 0.9</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>288.2</td>
<td>$\beta$-CDen$_3$bz</td>
<td>8.91 ± 0.09</td>
<td>-17.9 ± 0.9</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>298.2</td>
<td>$\beta$-CDen$_3$bz</td>
<td>7.08 ± 0.08</td>
<td>-17.9 ± 0.9</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>308.2</td>
<td>$\beta$-CDen$_3$bz</td>
<td>5.75 ± 0.06</td>
<td>-17.9 ± 0.9</td>
<td>4.2 ± 0.3</td>
</tr>
</tbody>
</table>
Table 2.4. Complexation constants ($K_{21}$), $\lambda_{\text{max}}$, and $\epsilon$ for the complexes $\beta$-CD$_2$.CV$^+$, (β-CD$_3$bz)$_2$.CV$^+$ and (β-CDen$_3$bz)$_2$.CV$^+$ at different temperatures by UV-Vis titration in aqueous phosphate buffer at pH 7.0 and $I = 0.10$ mol dm$^{-3}$.

<table>
<thead>
<tr>
<th>$T$ K</th>
<th>Host</th>
<th>$K_{21}$ dm$^3$ mol$^{-1}$</th>
<th>$\lambda_{\text{max}}$ nm</th>
<th>$10^{-4} \epsilon$ mol dm$^3$ cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>278.2</td>
<td>$\beta$-CD$_2$.CV$^+$</td>
<td>352 ± 32</td>
<td>601</td>
<td>8.54</td>
</tr>
<tr>
<td>288.2</td>
<td>$\beta$-CD$_2$.CV$^+$</td>
<td>219 ± 20</td>
<td>601</td>
<td>8.50</td>
</tr>
<tr>
<td>298.2</td>
<td>$\beta$-CD$_2$.CV$^+$</td>
<td>214 ± 19</td>
<td>601</td>
<td>8.39</td>
</tr>
<tr>
<td>308.2</td>
<td>$\beta$-CD$_2$.CV$^+$</td>
<td>126 ± 11</td>
<td>601</td>
<td>8.51</td>
</tr>
<tr>
<td>278.2</td>
<td>(β-CD$_3$bz)$_2$.CV$^+$</td>
<td>955 ± 86</td>
<td>602</td>
<td>9.14</td>
</tr>
<tr>
<td>288.2</td>
<td>(β-CD$_3$bz)$_2$.CV$^+$</td>
<td>741 ± 67</td>
<td>602</td>
<td>9.07</td>
</tr>
<tr>
<td>298.2</td>
<td>(β-CD$_3$bz)$_2$.CV$^+$</td>
<td>457 ± 41</td>
<td>602</td>
<td>8.95</td>
</tr>
<tr>
<td>308.2</td>
<td>(β-CD$_3$bz)$_2$.CV$^+$</td>
<td>372 ± 33</td>
<td>602</td>
<td>8.87</td>
</tr>
<tr>
<td>278.2</td>
<td>(β-CDen$_3$bz)$_2$.CV$^+$</td>
<td>631 ± 57</td>
<td>602</td>
<td>8.92</td>
</tr>
<tr>
<td>288.2</td>
<td>(β-CDen$_3$bz)$_2$.CV$^+$</td>
<td>468 ± 42</td>
<td>602</td>
<td>8.49</td>
</tr>
<tr>
<td>298.2</td>
<td>(β-CDen$_3$bz)$_2$.CV$^+$</td>
<td>386 ± 35</td>
<td>602</td>
<td>8.77</td>
</tr>
<tr>
<td>308.2</td>
<td>(β-CDen$_3$bz)$_2$.CV$^+$</td>
<td>209 ± 19</td>
<td>602</td>
<td>8.56</td>
</tr>
</tbody>
</table>

Figure 2.10. Plots of ln$K_{11}$ determined by UV-vis absorption spectroscopy at 278.2, 288.2, 298.2 and 308.2 K against $10^3/T$ for the complexation of CV$^+$ by $\beta$-CD (■, $y = 1.40x + 3.76$, $R^2 = 0.9982$), β-CD$_3$bz (●, $y = 3.27x + 0.47$, $R^2 = 0.9886$) and β-CDen$_3$bz (▲, $y = 2.16x + 1.64$, $R^2 = 0.9942$). The solid lines represent the best fit of the van’t Hoff equation to the $K_{11}$ data.
Table 2.5. Complexation constants \( (K_{11}) \) and thermodynamic parameters for the complexation of \( \beta\text{-CD}, \beta\text{-CD}_{3}\text{bz} \) and \( \beta\text{-CDen}_{3}\text{bz} \) with \( \text{PB}^+ \) at different temperatures by UV-Vis titration in aqueous phosphate buffer at pH 7.0 and \( I = 0.10 \text{ mol dm}^{-3} \).

<table>
<thead>
<tr>
<th>( T ) K</th>
<th>Host</th>
<th>( 10^3K_{11} ) dm(^3) mol(^{-1} )</th>
<th>( \Delta H_{11} ) kJ mol(^{-1} )</th>
<th>( T\Delta S_{11} ) kJ mol(^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>278.2</td>
<td>( \beta\text{-CD} )</td>
<td>2.19 ± 0.03</td>
<td>-12.5 ± 0.7</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>288.2</td>
<td>( \beta\text{-CD} )</td>
<td>1.88 ± 0.02</td>
<td>-12.5 ± 0.7</td>
<td>5.5 ± 0.6</td>
</tr>
<tr>
<td>298.2</td>
<td>( \beta\text{-CD} )</td>
<td>1.58 ± 0.02</td>
<td>-12.5 ± 0.7</td>
<td>5.7 ± 0.6</td>
</tr>
<tr>
<td>308.2</td>
<td>( \beta\text{-CD} )</td>
<td>1.29 ± 0.02</td>
<td>-12.5 ± 0.7</td>
<td>5.9 ± 0.6</td>
</tr>
<tr>
<td>278.2</td>
<td>( \beta\text{-CD}_{3}\text{bz} )</td>
<td>125 ± 0.6</td>
<td>-26.9 ± 1.3</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>288.2</td>
<td>( \beta\text{-CD}_{3}\text{bz} )</td>
<td>90.0 ± 0.4</td>
<td>-26.9 ± 1.3</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>298.2</td>
<td>( \beta\text{-CD}_{3}\text{bz} )</td>
<td>60.8 ± 0.2</td>
<td>-26.9 ± 1.3</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>308.2</td>
<td>( \beta\text{-CD}_{3}\text{bz} )</td>
<td>40.4 ± 0.3</td>
<td>-26.9 ± 1.3</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

Table 2.6. Complexation constants \( (K_{21}) \), \( \lambda_{\text{max}} \) and \( \varepsilon \) for the complexes \( \beta\text{-CD}_{2}\text{PB}^+ \) and \( (\beta\text{-CDen}_{3}\text{bz})_2\text{PB}^+ \) at different temperatures by UV-Vis titration in aqueous phosphate buffer at pH 7.0 and \( I = 0.10 \text{ mol dm}^{-3} \).

<table>
<thead>
<tr>
<th>( T ) K</th>
<th>Host</th>
<th>( K_{21} ) dm(^3) mol(^{-1} )</th>
<th>( \lambda_{\text{max}} ) nm</th>
<th>( 10^4\varepsilon ) mol dm(^{-3} ) cm(^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>278.2</td>
<td>( \beta\text{-CD}_{2}\text{PB}^+ )</td>
<td>257 ± 23</td>
<td>557</td>
<td>15.1</td>
</tr>
<tr>
<td>288.2</td>
<td>( \beta\text{-CD}_{2}\text{PB}^+ )</td>
<td>240 ± 22</td>
<td>557</td>
<td>14.9</td>
</tr>
<tr>
<td>298.2</td>
<td>( \beta\text{-CD}_{2}\text{PB}^+ )</td>
<td>195 ± 18</td>
<td>557</td>
<td>14.3</td>
</tr>
<tr>
<td>308.2</td>
<td>( \beta\text{-CD}_{2}\text{PB}^+ )</td>
<td>158 ± 14</td>
<td>557</td>
<td>13.7</td>
</tr>
<tr>
<td>278.2</td>
<td>( (\beta\text{-CDen}_{3}\text{bz})_2\text{PB}^+ )</td>
<td>398 ± 57</td>
<td>559</td>
<td>15.0</td>
</tr>
<tr>
<td>288.2</td>
<td>( (\beta\text{-CDen}_{3}\text{bz})_2\text{PB}^+ )</td>
<td>379 ± 42</td>
<td>559</td>
<td>14.7</td>
</tr>
<tr>
<td>298.2</td>
<td>( (\beta\text{-CDen}_{3}\text{bz})_2\text{PB}^+ )</td>
<td>362 ± 35</td>
<td>559</td>
<td>14.6</td>
</tr>
<tr>
<td>308.2</td>
<td>( (\beta\text{-CDen}_{3}\text{bz})_2\text{PB}^+ )</td>
<td>331 ± 19</td>
<td>559</td>
<td>14.2</td>
</tr>
</tbody>
</table>
Figure 2.11. Plots of \( \ln K_{11} \) determined by UV-vis absorption spectroscopy at 278.2, 288.2, 298.2 and 308.2 K against \( 10^3/T \) for the complexation of \( PB^+ \) by \( \beta\text{-CD} \) (■, \( y = 1.50x + 2.29, R^2 = 0.9893 \)), \( \beta\text{-CD}_3\text{bz} \) (●, \( y = 3.24x + 0.13, R^2 = 0.9940 \)) and \( \beta\text{-CDen}_3\text{bz} \) (▲, \( y = 1.99x + 1.25, R^2 = 0.9952 \)). The solid lines represent the best fit of the van’t Hoff equation to the \( K_{11} \) data.

Table 2.7. Complexation constants (\( K_{11} \)) and thermodynamic parameters for the complexation of \( \beta\text{-CD}, \beta\text{-CD}_3\text{bz} \) and \( \beta\text{-CDen}_3\text{bz} \) with RB at different temperatures by UV-Vis titration.

<table>
<thead>
<tr>
<th>( T ) K</th>
<th>Host</th>
<th>( 10^3 K_{11} ) dm(^3) mol(^{-1} )</th>
<th>( \Delta H_{11} ) kJ mol(^{-1} )</th>
<th>( T\Delta S_{11} ) kJ mol(^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>278.2</td>
<td>( \beta\text{-CD} )</td>
<td>5.95 ± 0.08</td>
<td>-13.5 ± 0.7</td>
<td>6.6 ± 0.6</td>
</tr>
<tr>
<td>288.2</td>
<td>( \beta\text{-CD} )</td>
<td>4.54 ± 0.05</td>
<td>-13.5 ± 0.7</td>
<td>6.8 ± 0.6</td>
</tr>
<tr>
<td>298.2</td>
<td>( \beta\text{-CD} )</td>
<td>3.96 ± 0.06</td>
<td>-13.5 ± 0.7</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td>308.2</td>
<td>( \beta\text{-CD} )</td>
<td>3.33 ± 0.04</td>
<td>-13.5 ± 0.7</td>
<td>7.3 ± 0.6</td>
</tr>
<tr>
<td>278.2</td>
<td>( \beta\text{-CD}_3\text{bz} )</td>
<td>152 ± 0.8</td>
<td>-25.8 ± 1.4</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>288.2</td>
<td>( \beta\text{-CD}_3\text{bz} )</td>
<td>107 ± 0.5</td>
<td>-25.8 ± 1.4</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>298.2</td>
<td>( \beta\text{-CD}_3\text{bz} )</td>
<td>76.9 ± 0.3</td>
<td>-25.8 ± 1.4</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>308.2</td>
<td>( \beta\text{-CD}_3\text{bz} )</td>
<td>50.6 ± 0.3</td>
<td>-25.8 ± 1.4</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>278.2</td>
<td>( \beta\text{-CDen}_3\text{bz} )</td>
<td>7.94 ± 0.09</td>
<td>-16.8 ± 0.8</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>288.2</td>
<td>( \beta\text{-CDen}_3\text{bz} )</td>
<td>6.17 ± 0.07</td>
<td>-16.8 ± 0.8</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>298.2</td>
<td>( \beta\text{-CDen}_3\text{bz} )</td>
<td>4.68 ± 0.04</td>
<td>-16.8 ± 0.8</td>
<td>4.3 ± 0.4</td>
</tr>
<tr>
<td>308.2</td>
<td>( \beta\text{-CDen}_3\text{bz} )</td>
<td>3.98 ± 0.05</td>
<td>-16.8 ± 0.8</td>
<td>4.4 ± 0.4</td>
</tr>
</tbody>
</table>
Table 2.8. Complexation constants ($K_{21}$), $\lambda_{\text{max}}$ and $\varepsilon$ for the complexes ($\beta$-CDen$_3$bz)$_2$.RB at different temperatures by UV-Vis titration in aqueous phosphate buffer at pH 7.0 and $I = 0.10$ mol dm$^{-3}$.

<table>
<thead>
<tr>
<th>$T$ K</th>
<th>Host</th>
<th>$K_{21}$ dm$^{-3}$ mol$^{-1}$</th>
<th>$\lambda_{\text{max}}$ nm</th>
<th>$10^{-4}$ $\varepsilon$ mol dm$^{-3}$ cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>278.2</td>
<td>($\beta$-CDen$_3$bz)$_2$.RB</td>
<td>182 ± 19</td>
<td>558</td>
<td>8.38</td>
</tr>
<tr>
<td>288.2</td>
<td>($\beta$-CDen$_3$bz)$_2$.RB</td>
<td>140 ± 15</td>
<td>558</td>
<td>8.24</td>
</tr>
<tr>
<td>298.2</td>
<td>($\beta$-CDen$_3$bz)$_2$.RB</td>
<td>128 ± 12</td>
<td>558</td>
<td>8.12</td>
</tr>
<tr>
<td>308.2</td>
<td>($\beta$-CDen$_3$bz)$_2$.RB</td>
<td>126 ± 13</td>
<td>558</td>
<td>8.02</td>
</tr>
</tbody>
</table>

Figure 2.12. Plots of ln$K_{11}$ determined by UV-vis absorption spectroscopy at 278.2, 288.2, 298.2 and 308.2 K against $10^3/T$ for the complexation of RB by $\beta$-CD (■, $y = 1.62x + 2.86$, $R^2 = 0.9849$), $\beta$-CD$_3$bz (●, $y = 3.09x + 0.82$, $R^2 = 0.9938$) and $\beta$-CDen$_3$bz (▲, $y = 2.02x + 1.72$, $R^2 = 0.9933$). The solid lines represent the best fit of the van’t Hoff equation to the $K_{11}$ data.

The ~20 fold greater values of $K_{11}$ for the three $\beta$-CD$_3$bz systems by comparison with those for the three $\beta$-CD systems are consistent with cooperative complexation of CV$^+$, PB$^+$ and RB as ditopic guests in adjacent substituent $\beta$-CD annuli of $\beta$-CD$_3$bz, whereas these guests can only be monotopically complexed by $\beta$-CD. This is consistent with the ~2 fold more negative value of $\Delta H_{11}$ for the $\beta$-CD$_3$bz systems by comparison with those of the $\beta$-CD systems. While the complexation by $\beta$-CD of CV$^+$, PB$^+$ and RB and the consequent decrease of independent species from two to one in the host-guest complex is expected to result in a $T\Delta S_{11}$ decrease, the displacement of water from the $\beta$-CD annulus appears to
offset this such that the overall $T\Delta S_{11}$ are positive.$^{8,55}$ By comparison, $T\Delta S_{11}$ for the β-CD$_3$bz systems are less positive, consistent with the ditopic complexation of CV$^+$, PB$^+$ and RB substantially decreasing the flexibility of the β-CD$_3$bz host-guest complexes by comparison with their β-CD analogues.

While β-CDen$_3$bz appears capable of complexing CV$^+$, PB$^+$ and RB as ditopic guests, the $K_{11}$ characterizing its 1:1 host-guest complexes are less than twice the magnitude of those for the analogous β-CD complexes consistent with β-CDen$_3$bz gaining a modest statistical advantage over β-CD in forming host-guest complexes. For the complexing of CV$^+$, PB$^+$ and RB by β-CDen$_3$bz both $\Delta H_{11}$ and $T\Delta S_{11}$ are midway between those for the analogous complexes by β-CD and β-CD$_3$bz. This may indicate a degree of cooperativity in ditopic complexation by β-CDen$_3$bz. The origin of these effects is the ethylene linker units joining the three β-CD substituents to the central benzene in β-CDen$_3$bz which both increase the distance between the β-CD substituents and overall flexibility by comparison with β-CD$_3$bz, and result in substantially lower $K_{11}$ values for the 1:1 complexes of β-CDen$_3$bz by comparison with those of β-CD$_3$bz.

The data in Table 2.9 may be considered together with similar data collected for host-guest complexation by β-CD,$^8$ mono-substituted β-CD$^8$ and linked β-CD dimers$^{24}$ with a large variety of guests when $T\Delta S_{11}$ is plotted against $\Delta H_{11}$ in Figure 2.13. Such entropy-enthalpy compensation plots yield linear least squares fits of $T\Delta S_{11}$ against $\Delta H_{11}$ to Equation 2.13 at 298.2 K for each of the three literature data sets and yield $\alpha = 0.80$ and $T\Delta S_{11,0} = 11 \text{ kJ mol}^{-1}$ for β-CD, $\alpha = 0.99$ and $T\Delta S_{11,0} = 17 \text{ kJ mol}^{-1}$ for mono-substituted β-CD, and $\alpha = 0.89$ and $T\Delta S_{11,0} = 23.5 \text{ kJ mol}^{-1}$ for linked β-CD dimers. The positive $T\Delta S_{11,0}$ values are consistent with the host-guest complexes being entropically stabilized at the intercept value where $\Delta H_{11,0} = 0$ and the corresponding entropy change is $\Delta S_{11,0}$.

$$T\Delta S_{11} = \alpha \Delta H_{11} + T\Delta S_{11,0} \quad (2.13)$$

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 $T\Delta S_{11,0}$ represents the inherent complex stability ($\Delta G_{11}$) obtained when $\Delta H_{11,0} = 0$. 

Although there is no necessary relationship between $T\Delta S_{11}$ and $\Delta H_{11}$, this relationship has been observed for a variety of complexations and is usually taken to indicate that compensatory variations in the relative importance of structural and solvation changes occur.\textsuperscript{8,24,56-58}

**Table 2.9.** Complexation constants, $K_{11}$ and thermodynamic parameters at 298.2 K for the formation of host-guest complexes determined by UV-Vis titration.\textsuperscript{A}

<table>
<thead>
<tr>
<th>Host</th>
<th>Guest</th>
<th>$10^3K_{11}$ \textsuperscript{B} (dm$^3$ mol$^{-1}$)</th>
<th>$\Delta H_{11}$ \textsuperscript{C} (kJ mol$^{-1}$)</th>
<th>$T\Delta S_{11}$ \textsuperscript{C} (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-CD</td>
<td>CV$^+$</td>
<td>4.68 ± 0.07</td>
<td>-11.7 ± 0.6</td>
<td>9.3 ± 0.7</td>
</tr>
<tr>
<td>$\beta$-CD</td>
<td>PB$^+$</td>
<td>1.66 ± 0.02</td>
<td>-11.0 ± 0.5</td>
<td>7.3 ± 0.7</td>
</tr>
<tr>
<td>$\beta$-CD</td>
<td>RB</td>
<td>3.96 ± 0.06</td>
<td>-13.5 ± 0.7</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz</td>
<td>CV$^+$</td>
<td>86.3 ± 0.4</td>
<td>-27.2 ± 1.4</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz</td>
<td>PB$^+$</td>
<td>60.8 ± 0.2</td>
<td>-26.9 ± 1.3</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz</td>
<td>RB</td>
<td>76.9 ± 0.3</td>
<td>-25.8 ± 1.4</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>$\beta$-CD$_{Den3}$bz</td>
<td>CV$^+$</td>
<td>7.08 ± 0.08</td>
<td>-17.9 ± 0.9</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>$\beta$-CD$_{Den3}$bz</td>
<td>PB$^+$</td>
<td>2.80 ± 0.02</td>
<td>-16.9 ± 0.8</td>
<td>2.8 ± 0.3</td>
</tr>
<tr>
<td>$\beta$-CD$_{Den3}$bz</td>
<td>RB</td>
<td>4.97 ± 0.04</td>
<td>-17.0 ± 0.8</td>
<td>4.0 ± 0.4</td>
</tr>
</tbody>
</table>

\textsuperscript{A} In aqueous phosphate buffer at pH 7.0 and I = 0.10 mol dm$^{-3}$.

\textsuperscript{B} The errors shown for $K_{11}$ and $K_{12}$ are data fitting errors. The maximum experimental errors for $K_{11}$ and $K_{12}$ are estimated to be ±5% and ±10%, respectively.

\textsuperscript{C} The errors shown for $\Delta H_{11}$ and $T\Delta S_{11}$ are those from fitting the van’t Hoff equation to the UV-Vis data.

The new $T\Delta S_{11}$ and $\Delta H_{11}$ data for $\beta$-CD, $\beta$-CD$_3$bz and $\beta$-CD$_{Den3}$bz from Table 2.9 are plotted in Figure 2.13 from which it is seen that they fall well within the range of the literature data. Hence, it appears that guest complexation within the $\beta$-CD host annuli of the nine new systems studied largely determines the thermodynamics irrespective of whether $\beta$-CD, $\beta$-CD$_3$bz or $\beta$-CD$_{Den3}$bz is the host involved. These observations make an interesting contrast with the complexation by $\beta$-CD$_3$bz and $\beta$-CD$_{Den3}$bz of the adamantyl substituents linked through linkers of different lengths to a 3% substituted poly(acrylate)s backbone.\textsuperscript{59} In these poly(acrylate)s systems the $T\Delta S_{11}$ and $\Delta H_{11}$ lie well within the data spread shown in Figure 2.13 but the $T\Delta S_{11}$ are all negative and the $\Delta H_{11}$ are
correspondingly more negative than those observed in the present study. It was proposed that the formation of the intra- and inter-strand cross-links arising from the complexation of the adamantyl substituents substantially decreased the freedom of motion and entropy in each system, while the complexation of the adamantyl substituents in the \( \beta \)-CD annuli of \( \beta \)-CD\(_{3} \)bz and \( \beta \)-CD\(_{3} \)bz largely constrained the relative magnitudes of \( T \Delta S_{11} \) and \( \Delta H_{11} \) to be within the linear relationship shown in Figure 2.13. The present study of \( \beta \)-CD\(_{3} \)bz and \( \beta \)-CD\(_{3} \)bz appears to be in accord with this interpretation.

**Figure 2.13.** A plot of \( T \Delta S_{11} \) against \( \Delta H_{11} \) for the 1:1 complexes formed by the hosts \( \beta \)-CD, \( \beta \)-CD\(_{3} \)bz and \( \beta \)-CD\(_{3} \)bz with the guests \( CV^+ \), \( PB^+ \) and \( RB \) characterized in this study, together with analogous data from the literature\(^8,24 \) for \( \beta \)-CD, mono-substituted \( \beta \)-CD and \( \beta \)-CD dimers.

The \( K_{21} \) (298.2 K) characterizing the complexation of a second \( CV^+ \) in (\( \beta \)-CD)\(_2 \).\( CV^+ \), (\( \beta \)-CD\(_{3} \)bz)\(_2 \).\( CV^+ \) and (\( \beta \)-CD\(_{3} \)bz)\(_2 \).\( CV^+ \) (Table 2.4) are much smaller than the analogous \( K_{11} \) (Table 2.9). This decreased stability is as expected in general terms for the complexing of a second guest in a host-guest complex. The more significant decrease for (\( \beta \)-CD\(_{3} \)bz)\(_2 \).\( CV^+ \) infers the dominance of ditopic complexation in \( \beta \)-CD\(_{3} \)bz.\( CV^+ \) in contrast to monotopic complexation in \( \beta \)-CD.\( CV^+ \). Similar characteristics apply to the detected 2:1 complexes in the \( PB^+ \) and \( RB \) systems. However, as \( PB^+ \) can only act as a ditopic guest, both \( \beta \)-CD\(_{3} \)bz in (\( \beta \)-CD\(_{3} \)bz)\(_2 \).\( PB^+ \) are consequently required to act as monotopic hosts. If the sterically hindered 2-carboxyphenyl group of \( RB \) acts as a guest along with both diethylamino groups, \( RB \) may act as a tritopic guest in (\( \beta \)-CD\(_{3} \)bz)\(_2 \).\( RB \), otherwise it will
act as a ditopic guest with both $\beta$-CDen$_3$bz acting as monotopic hosts through their diethylamino groups. The earlier mentioned uncertainty of detection levels for some 2:1 complexes of PB$^+$ and RB preclude a more detailed discussion.

2.3.2. 2D ROESY $^1$H NMR Studies

The complexation behaviour of CV$^+$, PB$^+$ or RB with either $\beta$-CD, $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz were studied by 2D ROESY $^1$H NMR. Spectra were recorded on a Varian Inova 600 spectrometer operating at 599.957 MHz using a standard pulse sequence with a mixing time of 300 ms. Solutions for host-guest complexation studies in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) contained either CV$^+$, PB$^+$ or RB ($2 \times 10^{-3}$ mol dm$^{-3}$) and either $\beta$-CD ($6 \times 10^{-3}$ mol dm$^{-3}$), $\beta$-CD$_3$bz ($2 \times 10^{-3}$ mol dm$^{-3}$) or $\beta$-CDen$_3$bz ($2 \times 10^{-3}$ mol dm$^{-3}$). Solutions were allowed to equilibrate at the thermostated probe temperature of $298.2 \pm 0.1$ K for 30 min in 5 mm NMR tubes prior to recording their spectra. In ROESY experiments, a NOE (nuclear Overhauser enhancement) cross-peak between a proton of the $\beta$-CD annulus and a proton of the guest will be observed if the protons are closer than 400 pm through space, which infers that the guest is complexed in the annulus.$^{60}$

Spatial information on the interactions between CV$^+$, PB$^+$ and RB and $\beta$-CD, $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz is provided by 2D ROESY $^1$H NMR as a consequence of dipolar interactions between protons within 400 pm of each other generating characteristic cross-peaks.$^{60}$ This is exemplified in Figure 2.14 where NOE interactions between the CV$^+$ H1 and H2 aromatic and H3 methyl protons with the H3,5 and 6 protons (3.78-3.94 ppm) inside the annulus of $\beta$-CD occur, consistent with the 4-(dimethylamino)phenyl groups of CV$^+$ entering the annulus in the $\beta$-CD.CV$^+$ and ($\beta$-CD)$_2$.CV$^+$ complexes. Similar cross-peaks are seen in the analogous spectra of CV$^+$ in the presence of $\beta$-CD$_3$bz (Figure 2.15) and $\beta$-CDen$_3$bz (Figure 2.16) and a similar interpretation applies for the complexes formed. However, the monosubstitution of each of the $\beta$-CD substituents in the trimers causes all of their glucopyranose units to be inequivalent and characterized by different chemical shifts such that the H2-6 chemical shift envelopes are more complex than is the case for $\beta$-CD.
Figure 2.14. 2D ROESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $2.0 \times 10^{-3} \text{ mol dm}^{-3}$ in CV$^+$ and $6.0 \times 10^{-3} \text{ mol dm}^{-3}$ in $\beta$-CD in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10 \text{ mol dm}^{-3}$) at 298.2K. Rectangles A and B enclose the cross-peaks arising from interaction between the annular H3,5,6 protons of $\beta$-CD and H1,2 aromatic and H3 methyl protons of CV$^+$, respectively.

Figure 2.15. 2D ROESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $2.0 \times 10^{-3} \text{ mol dm}^{-3}$ in CV$^+$ and $2.0 \times 10^{-3} \text{ mol dm}^{-3}$ in $\beta$-CD$_3$bz in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10 \text{ mol dm}^{-3}$) at 298.2K. Rectangles A and B enclose the cross-peaks arising from interaction between the annular H3,5,6 protons of $\beta$-CD and H1,2 aromatic and H3 methyl protons of CV$^+$, respectively.
Figure 2.16. 2D ROESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $2.0 \times 10^{-3}$ mol dm$^{-3}$ in CV$^+$ and $2.0 \times 10^{-3}$ mol dm$^{-3}$ in $\beta$CDen$_3$bz in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. Rectangles A and B enclose the cross-peaks arising from interaction between the annular H3,5,6 protons of $\beta$-CD and H1,2 aromatic and H3 methyl protons of CV$^+$, respectively.

The 2D ROESY $^1$HNMR spectra of solutions of either PB$^+$ or RB with $\beta$-CD, $\beta$-CD$_3$bz or $\beta$CDen$_3$bz show cross-peaks arising from the H6 methyl protons of PB$^+$ and the H9 methyl protons of RB of interacting with the H3,5,6 annular protons of either $\beta$-CD, $\beta$-CD$_3$bz or $\beta$CDen$_3$bz. (Figure 2.17 to Figure 2.22) The spectrum of the $\beta$CDen$_3$bz.PB$^+$ solution (Figure 2.19) also shows a cross-peak arising from the H3,5,6 protons of the $\beta$-CD substituents and the H4 proton of PB$^+$, and the spectra of the $\beta$CD$_3$bz.RB Figure 2.21 and $\beta$CDen$_3$bz.RB solutions (Figure 2.22) show cross-peaks arising from the H3,5,6 protons of the $\beta$-CD substituents and the H6,7 protons of RB. While these data are consistent with the complexation of CV$^+$, PB$^+$ and RB in the annuli of $\beta$CD$_3$bz and $\beta$CDen$_3$bz, they do not distinguish between complexation in a single annulus and simultaneous complexation in two or more annuli.
Figure 2.17. 2D ROESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $2.0 \times 10^{-3}$ mol dm$^{-3}$ in PB$^+$ and $6.0 \times 10^{-3}$ mol dm$^{-3}$ in $\beta$-CD in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. Rectangle enclose the cross-peaks arising from interaction between the annular H3,5,6 protons of $\beta$-CD and the H6 proton of PB$^+$.

Figure 2.18. 2D ROESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $2.0 \times 10^{-3}$ mol dm$^{-3}$ in PB$^+$ and $2.0 \times 10^{-3}$ mol dm$^{-3}$ in $\beta$-CD$_3$bz in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. Rectangle enclose the cross-peaks arising from interaction between the annular H3,5,6 protons of $\beta$-CD and the H6 proton of PB$^+$. 
Figure 2.19. 2D ROESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $2.0 \times 10^{-3}$ mol dm$^{-3}$ in PB$^+$ and $2.0 \times 10^{-3}$ mol dm$^{-3}$ in β-CD$^{3}$bz in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. Rectangles A and B enclose the cross-peaks arising from interaction between the annular H3,5,6 protons of β-CD and H4 proton and the H9 proton of PB$^+$, respectively.

Figure 2.20. 2D ROESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $2.0 \times 10^{-3}$ mol dm$^{-3}$ in RB and $6.0 \times 10^{-3}$ mol dm$^{-3}$ in β-CD in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. Rectangle enclose the cross-peaks arising from interaction between the annular H3,5,6 protons of β-CD and the H9 proton of RB.
Figure 2.21. 2D ROESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $2.0 \times 10^{-3}$ mol dm$^{-3}$ in RB and $2.0 \times 10^{-3}$ mol dm$^{-3}$ of $\beta$-CD$_{3}$$\text{bz}$ in D$_{2}$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. Rectangles A and B enclose the cross-peaks arising from interaction between the annular H3,5,6 protons of $\beta$-CD and H6,7 proton and the H9 proton of RB, respectively.

Figure 2.22. 2D ROESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $2.0 \times 10^{-3}$ mol dm$^{-3}$ in RB and $2.0 \times 10^{-3}$ mol dm$^{-3}$ of $\beta$-CD$_{3}$$\text{bz}$ in D$_{2}$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. Rectangles A and B enclose the cross-peaks arising from interaction between the annular H3,5,6 protons of $\beta$-CD and H6,7 proton and the H9 proton of RB, respectively.
2.3.3. Molecular Modelling Studies

The aim of this work is to help understand the nature of the interactions in the complexation of CV\(^+\), PB\(^+\) and RB by β-CD and the linked β-CD trimers. The molecular modelling focuses on the predominant 1:1 complexes and provides a visual conception of the relative sizes of the host and guest species. The complex geometries were optimised using the PM7 semiempirical method using MOPAC2012.\(^{52}\) For each complex, two alternative models were constructed, with either 4-(dimethylamino)phenyl or diethylamino groups being the main complexation site within the β-CD groups. The models are displayed in space-filling representations with the hydrogen atoms and lone pairs omitted to give a clearer views. (Figures 2.23 to 2.26). Gas phase molecular modelling\(^ {51,52}\) indicates that ditopic complexation by β-CD\(_3\)bz and β-CDen\(_3\)bz occurs through the two complexing annuli presenting their primary hydroxy faces to the CV\(^+\), PB\(^+\) and RB, as shown in Figures 2.24 to 2.26.

In the monotopic β-CD complexes (Figure 2.23) the energy differences for CV\(^+\) 4-(dimethylamino)phenyl and PB\(^+\) and RB diethylamino groups entering the β-CD annulus through either the primary or secondary hydroxyl face are too small for a significant preference for either complexation to arise. The interactions involved in gas-phase complexation are likely to make a significant contribution to the overall complexation energies in aqueous solution.
Figure 2.23. Space-filling representation of $\beta$-CD.CV$^+$, $\beta$-CD.PB$^+$ and $\beta$-CD.RB constructed and energy-minimised using the MP7 method (MOPAC2012). Two models are shown for each compound. Carbon, nitrogen and oxygen atoms are shown in gray, blue and red, respectively. Hydrogen atoms and lone pairs are not shown.
Figure 2.24. Space-filling representation of β-CD₃bz.CV⁺ and β-CDen₃bz.CV⁺ constructed and energy-minimised using the MP7 method (MOPAC2012). Carbon, nitrogen and oxygen atoms are shown in gray, blue and red, respectively. Hydrogen atoms and lone pairs are not shown.

Figure 2.25. Space-filling representation of β-CD₃bz.PB⁺ and β-CDen₃bz.PB⁺ constructed and energy-minimised using the MP7 method (MOPAC2012). Carbon, nitrogen and oxygen atoms are shown in gray, blue and red, respectively. Hydrogen atoms and lone pairs are not shown.
Figure 2.26. Space-filling representation of $\beta$-CD$_3$bz,RB and $\beta$-CDen$_3$bz,RB constructed and energy-minimised using the MP7 method (MOPAC2012). Carbon, nitrogen and oxygen atoms are show in gray, blue and red, respectively. Hydrogen atoms and lone pairs are not shown.

2.4. CONCLUSIONS

The host-guest complexation of CV$^+$, PB$^+$, and RB by $\beta$-CD, $\beta$-CD$_3$bz, and $\beta$-CDen$_3$bz of has been characterised in aqueous solution by UV-Vis and 2D ROESY $^1$H NMR spectroscopy. Equilibria represented by Equation 2.4 (host-guest complex ratio 1:1) and Equation 2.5 (host-guest complexes ratio 2:1) were investigated and were found to dominate the $\beta$-CDen$_3$bz/CV$^+$, $\beta$-CDen$_3$bz/PB$^+$, $\beta$-CDen$_3$bz/RB system as do the analogous equilibria for the $\beta$-CD/CV$^+$, $\beta$-CD/PB$^+$ and $\beta$-CD$_3$bz/CV$^+$. However, for the $\beta$-CD$_3$bz/PB$^+$, $\beta$-CD/RB, and $\beta$-CD$_3$bz/RB systems the equilibria analogous to Equation 2.4 dominated and equilibria analogous to Equation 2.5 were not detected. The derived complexation constants $K_{11}$ and $K_{21}$ are defined in Equations 2.6 and 2.7, respectively. Using van't Hoff equations and plots for the dependence of equilibrium constants on temperature, the thermodynamic parameters, $\Delta G_{11}$, $\Delta H_{11}$ and $T\Delta S_{11}$, have been quantified. The stabilities of $\beta$-CD$_3$bz.CV$^+$ and the corresponding PB$^+$ and RB complexes are ~20-fold greater than those of the analogous $\beta$-CD complexes, consistent with cooperative complexation of CV$^+$, PB$^+$, and RB as ditopic guests by $\beta$-CD$_3$bz. In contrast, the stabilities of $\beta$-CDen$_3$bz.CV$^+$ and the corresponding PB$^+$ and RB complexes are only ~two-fold greater than those of the
analogous β-CD complexes, consistent with β-CDen₃bz possessing a small statistical advantage over β-CD in forming complexes. This difference in the stabilities of the β-CD₃bz.CV⁺ and β-CDen₃bz.CV⁺ and the corresponding PB⁺ and RB complexes is attributed to a good match of the distance between β-CD substituent annuli in the 1:1 complexes, as exemplified by β-CD₃bz.CV⁺ and the complexing groups of the guest species for ditopic host-guest complexation. Correspondingly, the greater distance between β-CD substituent annuli in β-CDen₃bz.CV⁺ and its analogues diminishes the intensity and probability of such ditopic interactions (The length calculated by molecular modeling of the linker from N(-C₆A) to N(-C₆A) in β-CD₃bz is 7.49 Å whereas that of β-CDen₃bz is 12.5 Å, Section 2.2.2.) These conclusions are consistent with the variation of ΔH₁₁ and TΔS₁₁ for the nine 1:1 host-guest complexes. Six 2:1 complexes are also formed and their K₂₁ values are substantially less than the K₁₁ values, characterizing their 1:1 precursor complexes as a consequence of the statistically based decrease expected for the second host complexation, and in the case of 2:1 β-CD₃bz complexes the ditopic complexation of β-CD₃bz in the 1:1 complexes.
2.5. REFERENCES

52. MOPAC2012. (Stewart computational chemistry: colorado springs, CO), 2012.
54. HypSpec, Protonic Software, 2 Templegate Avenue, Leeds LS15 0HD, UK.

82
2.6. APPENDIX

2.6.1. UV-Vis Titration Data for CV⁺

Figure 2.27. (a1,a2) Molar absorbance change of CV⁺ (5.00 × 10⁻⁶ mol dm⁻³) in phosphate buffer, pH 7.0, I = 0.10 mol dm⁻³ with sequential injections (5 mm³ each) of β-CD (1.41 × 10⁻² mol dm⁻³) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of [β-CD]/[CV⁺] increases. (b1,b2) Molar absorbance variation at 590 nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-650 nm. (c1,c2) Calculated molar absorbance of free and complexed CV⁺. (d1,d2) Speciation plot with [CV⁺]total = 100%. Note: a1-d1: data at 278.2K. a2-d2: data at 288.2K.
Figure 2.28. (a1,a2) Molar absorbance change of CV\(^+\) (5.00 \times 10^{-6} \text{ mol dm}^{-3}) in phosphate buffer, pH 7.0, I = 0.10 \text{ mol dm}^{-3} with sequential injections (5 mm\(^3\) each) of \(\beta\text{-CD} \) (1.41 \times 10^{-2} \text{ mol dm}^{-3}) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of [\(\beta\text{-CD}]/[CV^+]\) increases. (b1,b2) Molar absorbance variation at 590nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-650 nm. (c1,c2) Calculated molar absorbance of free and complexed CV\(^+\). (d1,d2) Speciation with [CV\(^+\)]\(_{\text{total}}\) = 100\%. Note: a1-d1: data at 298.2K. a2-d2: data at 308.2K.
Figure 2.29. (a1,a2) Molar absorbance change of CV⁺ (5.00 × 10⁻⁶ mol dm⁻³) in phosphate buffer, pH 7.0, I = 0.10 mol dm⁻³ with sequential injections (5 mm³ each) of β-CD₃bz (2.81 × 10⁻³ mol dm⁻³) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of [β-CD₃bz]/[CV⁺] increases.
(b1,b2) Molar absorbance variation at 590nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-650 nm.
(c1,c2) Calculated molar absorbance of free and complexed CV⁺.
(d1,d2) Speciation with [CV⁺]total = 100%. Note: a1-d1: data at 278.2K. a2-d2: data at 288.2K.
Figure 2.30. (a1,a2) Molar absorbance change of CV⁺ (5.00 × 10⁻⁶ mol dm⁻³) in phosphate buffer, pH 7.0, \( I = 0.10 \) mol dm⁻³ with sequential injections (5 mm mol⁻³ each) of β-CD₃bz (2.81 × 10⁻³ mol dm⁻³) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of \([β-CD₃bz]/[CV⁺]\) increases. (b1,b2) Molar absorbance variation at 590nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-650 nm. (c1,b2) Calculated molar absorbance of free and complexed CV⁺. (d1,d2) Speciation with \([CV⁺]_{total} = 100\%\). Note: a1-d1: data at 298.2K, a2-d2: data at 308.2K.
Figure 2.31. (a1,a2) Molar absorbance change of CV⁺ (5.00 × 10⁻⁶ mol dm⁻³) in phosphate buffer, pH 7.0, I = 0.10 mol dm⁻³ with sequential injections (5 mm² each) of β-CDen₃bz (1.36 × 10⁻² mol dm⁻³) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of [β-CDen₃bz]/[CV⁺] increases. (b1,b2) Molar absorbance variation at 590nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-650 nm. (c1,c2) Calculated molar absorbance of free and complexed CV⁺. (d1,d2) Speciation with [CV⁺]total = 100%. Note: a1-d1: data at 278.2K. a2-d2: data at 288.2K.
Figure 2.32. (a1,a2) Molar absorbance change of CV$^+$ ($5.00 \times 10^{-6}$ mol dm$^{-3}$) in phosphate buffer, pH 7.0, $I = 0.10$ mol dm$^{-3}$ with sequential injections (5 mm$^3$ each) of $\beta$-CDen$_3$bz (1.36 $\times$ 10$^{-2}$ mol dm$^{-3}$) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of $[\beta$-CDen$_3$bz]/[CV$^+$] increases. (b1,b2) Molar absorbance variation at 590nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-650 nm. (c1,c2) Calculated molar absorbance of free and complexed CV$^+$. (d1,d2) Speciation with [CV$^+$]$_{total} = 100\%$. Note: a1-d1: data at 298.2K. a2-d2: data at 308.2K.
2.6.2. UV-Vis Titration Data for PB⁺

**Figure 2.33.** (a1,a2) Molar absorbance change of PB⁺ (5.01 × 10⁻⁶ mol dm⁻³) in phosphate buffer, pH 7.0, I = 0.10 mol dm⁻³ with sequential injections (5 mm³ each) of β-CD (1.41 × 10⁻² mol dm⁻³) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of [β-CD]/[PB⁺] increases. (b1,b2) Molar absorbance variation at 550 nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-570 nm. (c1,c2) Calculated molar absorbance of free and complexed PB⁺. (d1,d2) Speciation with [PB⁺]total = 100%. Note: a1-d1: data at 278.2K, a2-d2: data at 288.2K.
Figure 2.34. (a1,a2) Molar absorbance change of \( \text{PB}^+ \) (5.01 × 10^{-6} \text{ mol dm}^{-3}) in phosphate buffer, pH 7.0, \( I = 0.10 \text{ mol dm}^{-3} \) with sequential injections (5 mm$^3$ each) of \( \beta\text{-CD} \) (1.41 × 10^{-2} \text{ mol dm}^{-3}) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of \([\beta\text{-CD}]/[\text{PB}^+]\) increases. (b1,b2) Molar absorbance variation at 550 nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-570 nm. (c1,c2) Calculated molar absorbance of free and complexed \( \text{PB}^+ \). (d1,d2) Speciation with \([\text{PB}^+]\)total = 100%. Note: a1-d1: data at 298.2K, a2-d2: data at 308.2K.
Figure 2.35. (a1,a2) Molar absorbance change of PB$^+$ (5.01 × 10$^{-6}$ mol dm$^{-3}$) in phosphate buffer, pH 7.0, $I = 0.10$ mol dm$^{-3}$ with sequential injections (5 mm$^3$ each) of $\beta$-CD$_3$bz (2.81 × 10$^{-3}$ mol dm$^{-3}$) into both the sample and reference cells (isosbestic point 558 nm). The arrows indicate the direction of molar absorbance change as the molar ratio of $[\beta$-CD$_3$bz]/[PB$^+$] increases. (b1,b2) Molar absorbance variation at 550 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation over the wavelength range 500-570 nm. (c1,c2) Calculated molar absorbance of free and complexed PB$^+$. (d1,d2) Speciation with $[PB^+]_{\text{total}} = 100\%$. Note: a1-d1: data at 278.2K. a2-d2: data at 288.2K.
Figure 2.36. (a1,a2) Molar absorbance change of PB⁺ (5.01 x 10⁻⁶ mol dm⁻³) in phosphate buffer, pH 7.0, I = 0.10 mol dm⁻³ with sequential injections (5 mm³ each) of β-CD₃bz (2.81 x 10⁻³ mol dm⁻³) into both the sample and reference cells (isosbestic point 558 nm). The arrows indicate the direction of molar absorbance change as the molar ratio of [β-CD₃bz]/[PB⁺] increases. (b1,b2) Molar absorbance variation at 550 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation over the wavelength range 500-570 nm. (c1,c2) Calculated molar absorbance of free and complexed PB⁺. (d1,d2) Speciation with [PB⁺] total = 100%. Note: a1-d1: data at 298.2K. a2-d2: data at 308.2K.
Figure 2.37. (a1,a2) Molar absorbance change of \( \text{PB}^+ \) (5.01 \( \times \) 10\(^{-6} \) mol dm\(^{-3} \)) in phosphate buffer, pH 7.0, \( I = 0.10 \) mol dm\(^{-3} \) with sequential injections (5 mm\(^3\) each) of \( \beta\text{-CDen}_{bz} \) (1.36 \( \times \) 10\(^{-2} \) mol dm\(^{-3} \)) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of \( [\beta\text{-CDen}_{bz}] / [\text{PB}^+] \) increases.

(b1,b2) Molar absorbance variation at 550 nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-570 nm.

(c1,c2) Calculated molar absorbance of free and complexed \( \text{PB}^+ \).

(d1,d2) Speciation with \( [\text{PB}^+]_{\text{total}} = 100\% \). Note: a1-d1: data at 278.2K. a2-d2: data at 288.2K.
Figure 2.38. (a1,a2) Molar absorbance change of $\text{PB}^+$ ($5.01 \times 10^{-6}$ mol dm$^{-3}$) in phosphate buffer, pH 7.0, $I = 0.10$ mol dm$^{-3}$ with sequential injections (5 mm$^3$ each) of $\beta$-CDen$_3$bz (1.36 $\times$ 10$^{-2}$ mol dm$^{-3}$) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of $[\beta$-CDen$_3$bz]/[$\text{PB}^+$] increases. (b1,b2) Molar absorbance variation at 550 nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-570 nm. (c1,c2) Calculated molar absorbance of free and complexed $\text{PB}^+$. (d1,d2) Speciation with $[\text{PB}^+]_{\text{total}} = 100\%$. Note: a1-d1: data at 298.2K. a2-d2: data at 308.2K.
2.6.3. UV-Vis Titration Data for RB

![Graphs showing UV-Vis titration data for RB](image)

**Figure 2.39.** (a1,a2) Molar absorbance change of RB \(5.01 \times 10^{-6} \text{ mol dm}^{-3}\) in phosphate buffer, pH 7.0, \(I = 0.10 \text{ mol dm}^{-3}\) with sequential injections \(5 \text{ mm}^{-3}\) each) of \(\beta\)-CD \(1.41 \times 10^{-2} \text{ mol dm}^{-3}\) into both the sample and reference cells (isosbestic point 530 nm). The arrows indicate the direction of molar absorbance change as the molar ratio of \([\beta\text{-CD}]/[RB]\) increases. (a1,a2) Molar absorbance variation at 560 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation over the wavelength range 500-570 nm. (c1,c2) Calculated molar absorbance of free and complexed RB. (d1,d2) Speciation with \([RB]_{\text{total}} = 100\%\). Note: a1-d1: data at 278.2K. a2-d2: data at 288.2K.
Figure 2.40. (a1,a2) Molar absorbance change of RB (5.01 × 10^{-6} mol dm^{-3}) in phosphate buffer, pH 7.0, I = 0.10 mol dm^{-3} with sequential injections (5 mm^3 each) of β-CD (1.41 × 10^{-2} mol dm^{-3}) into both the sample and reference cells (isosbestic point 530 nm). The arrows indicate the direction of molar absorbance change as the molar ratio of [β-CD]/[RB] increases. (b1,b2) Molar absorbance variation at 560 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation over the wavelength range 500-570 nm. (c1,c2) Calculated molar absorbance of free and complexed RB. (d1,d2) Speciation with [RB]_{total} = 100%. Note: a1-d1: data at 298.2K. a2-d2: data at 308.2K.
Figure 2.41. (a1,a2) Molar absorbance change of RB (5.01 × 10^{-6} mol dm^{-3}) in phosphate buffer, pH 7.0, I = 0.10 mol dm^{-3} with sequential injections (5 mm^3 each) of β-CD, bz (2.81 × 10^{-3} mol dm^{-3}) into both the sample and reference cells (isosbestic point 565 nm). The arrows indicate the direction of molar absorbance change as the molar ratio of [β-CD,bz]/[RB] increases. (b1,b2) Molar absorbance change as the molar ratio of [β-CD,bz]/[RB] increases. (c1,c2) Calculated molar absorbance of free and complexed RB. (d1,d2) Speciation with [RB]_{total} = 100%. Note: a1-d1: data at 278.2K. a1-d2: data at 288.2K.
**Figure 2.42.** (a1,a2) Molar absorbance change of RB \((5.01 \times 10^{-6} \text{ mol dm}^{-3})\) in phosphate buffer, pH 7.0, \(I = 0.10 \text{ mol dm}^{-3}\) with sequential injections \((5 \text{ mm}^3 \text{ each})\) of \(\beta\-CD\_bz\ \((2.81 \times 10^{-3} \text{ mol dm}^{-3})\) into both the sample and reference cells (isosbestic point 565 nm). The arrows indicate the direction of molar absorbance change as the molar ratio of \([\beta\-CD\_bz]/[RB]\) increases. (b1,b2) Molar absorbance variation at 546 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation over the wavelength range 500-570 nm. (c1,c2) Calculated molar absorbance of free and complexed RB. (d1,d2) Speciation with \([RB]_{\text{total}} = 100\%\). Note: a1-d1: data at 298.2K. a2-d2: data at 308.2K.
Figure 2.43. (a1,a2) Molar absorbance change of RB (5.01 × 10⁻⁶ mol dm⁻³) in phosphate buffer, pH 7.0, I = 0.10 mol dm⁻³ with sequential injections (5 mm³ each) of β-CDen₃bz (1.36 × 10⁻² mol dm⁻³) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of [β-CDen₃bz]/[RB] increases. (b1,b2) Molar absorbance variation at 546 nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-570 nm. (c1,c2) Calculated molar absorbance of free and complexed RB. (d1,d2) Speciation with [RB]total = 100% Note: a1-d1: data at 278.2K a1-d2: data at 288.2K.
Figure 2.24. (a1,a2) Molar absorbance change of RB (5.01 × 10⁻⁶ mol dm⁻³) in phosphate buffer, pH 7.0, I = 0.10 mol dm⁻³ with sequential injections (5 mm³ each) of β-CDen₃bz (1.36 × 10⁻² mol dm⁻³) into both the sample and reference cells. The arrows indicate the direction of molar absorbance change as the molar ratio of [β-CDen₃bz]/[RB] increases. (b1,b2) Molar absorbance variation at 546 nm and the line representing the best fit of an algorithm for 1:1 and 2:1 host-guest complexation over the wavelength range 500-570 nm. (c1,c2) Calculated molar absorbance of free and complexed RB. (d1,d2) Speciation with [RB]_total = 100%. Note: a1-d1: data at 298.2K. a2-d2: data at 308.2K.
CHAPTER 3

COMPLEXATION OF HYDROPHOBIC SUBSTITUTED POLY(ACRYLATE)S BY β-CYCLODEXTRIN DIMERS AND TRIMERS IN AQUEOUS SOLUTION
3.1. INTRODUCTION

3.1.1. General

Interactions between water soluble hydrophobe substituted polymers result in the formation of small aggregates in dilute solution and hydrogels in more concentrated solutions which, together with aggregates and hydrogels incorporating other species, find actual or potential applications in paint, cosmetics, pharmaceutical, food, oil recovery, water treatment and tissue engineering technologies. These interactions also have a substantial intrinsic interest, and as a consequence, the chemistry of hydrophobe substituted water soluble polymers is the subject of substantial research. This has shown that the interactions occurring in hydrophobe-substituted water soluble polymers is a balance between hydrophobic, hydrophilic and hydrophobe-hydrophobe receptor associations, some of which are illustrated for substituted poly(acrylate)s in Figure 3.1.

Thus, when hydrophobes such as the dodecyl and octadecyl entities are substituted onto a poly(acrylate)s strand (Figure 3.1 A) they tend to aggregate to form both intra- (Figure 3.1 B) and inter-strand linkages such that at a sufficiently high concentration a hydrogel network forms (Figure 3.1 C). This is exemplified by the 3% random substitution of poly(acrylate)s by dodecyl (C12) and octadecyl (C18) groups to give the substituted poly(acrylate)s PAAC12 and PAAC18. In aqueous solutions, hydrophobic interactions between the C12 and C18 subtituents of the PAAC12 and PAAC18 result in intra- and inter-strand associations, depending on the polymer concentration. A 0.5 wt.% solution of PAAC18 exhibits a low viscosity consistent with the formation of small dispersed aggregates, while increasing the concentration to ≥1 wt.% results in the formation of a hydrogel. However, PAAC12 only forms a hydrogel at concentrations ≥3 wt.%, thereby demonstrating that the greater length and hydrophobicity of the C18 substituent enables it to form stronger associations than does the C12 substtituent at the same concentration. Thus, this spontaneous cross-linking between substituted poly(acrylate)s strands conveniently allows variation of viscosity up to the formation of hydrogels as concentration is varied.
Figure 3.1. Interaction of hydrophobe substituted polymers exemplified by dodecyl and octadecyl substituted poly(acrylate)s alone (A-C) and in the presence of cyclodextrins (D), cyclodextrin-substituted poly(acrylate)s (E) and linked cyclodextrin dimers in aqueous solution (F).

When a water soluble hydrophobe-receptor, such as α-, β- or γ- cyclodextrin (α-CD, β-CD or γ-CD) is added to aqueous solutions of PAAC12 and PAAC18 competitive interactions occur, resulting in hydrophobe disaggregation and the formation of host-guest complexes between the cyclodextrin hydrophobe-receptor acting as the host either C12 or C18 acting as the guest and a decrease in viscosity (Figure 3.1D)\(^{14,16}\). In contrast, the addition of a poly(acrylate)s randomly substituted with α-CD or β-CD results in the formation of inter-strand cross links formed by complexation of the C18 substituents of PAAC18 which are stronger than those formed by hydrophobic aggregation of C18 as shown in Figure 3.1 E\(^{14,16,18}\). A similar situation occurs when small ditopic linked β-CD
dimers, exemplified by \(N,N'-\text{bis}(6^A\text{-deoxy}-6^A\text{-\beta-cyclodextrin})\)-urea, \(\beta\text{-CD}_{2\text{ur}}\), and \(N,N'-\text{bis}(6^A\text{-deoxy}-6^A\text{-\beta-cyclodextrin})\)-succinamide, \(\beta\text{-CD}_{2\text{su}}\), are added to aqueous solutions of PAAC12 and PAAC18. An increase in viscosity occurs as a consequence of the ditopic linked \(\beta\text{-CD}\) dimers complexing either the C12 or C18 substituents of adjacent strands to form inter-strand cross-links as shown in Figure 3.1 F.\(^{19}\)

### 3.1.2. Aims of This Study

The aims of the research described in this chapter are to characterise the structural thermodynamic and macroscopic aspects of the host-guest interactions between the substituents of the dodecyl (C12) and octadecyl (C18) substituted poly(acrylate)s, PAAC12 and PAAC18 (Figure 3.2a), by the two linked \(\beta\text{-CD}\) trimers\(^{20}\) (Figure 3.2b), 1,3,5-\(N,N,N\)-tris-(6\(^A\text{-deoxy}-6^A\text{-\beta-cyclodextrin})\)-benzene, \(\beta\text{-CD}_{2\text{bz}}\), and 1,3,5-\(N,N,N\)-tris(6\(^A\text{-}(2\text{-aminoethyl})\text{amino}-6^A\text{-\beta-cyclodextrin})\)-benzene, \(\beta\text{-CD}_{2\text{en}_{3\text{bz}}}\), in aqueous solution, and to make comparisons with the analogous host-guest interactions of the linked \(\beta\text{-CD}\) dimers, \(\beta\text{-CD}_{2\text{ur}}\) and \(\beta\text{-CD}_{2\text{su}}\)\(^{21,22}\) (Figure 3.2c), and \(\beta\text{-CD}\) with PAAC12 and PAAC18.

![Figure 3.2](image-url). (a) The 3\% hydrophobe substituted poly(acrylate)s, PAAC12 and PAAC18, (b) the linked trimers \(\beta\text{-CD}_{3\text{bz}}\) and \(\beta\text{-CD}_{2\text{en}_{3\text{bz}}}\) and (c) the linked dimers \(\beta\text{-CD}_{2\text{ur}}\) and \(\beta\text{-CD}_{2\text{su}}\).
3.2. SYNTHESIS

The preparations of 3.0 (± 0.1) % dodecyl (C12) and octadecyl (C18) substituted poly(acrylate)s, PAAC12 and PAAC18, β-CD2ur and β-CD2su (Figures 3.3) were carried out as described in the literature and β-CD3bz and β-CDen3bz trimers were synthesized as reported in Chapter 2. The host-guest interactions of β-CD2ur, β-CD2su, β-CD3bz and β-CDen3bz with PAAC12 and PAAC18 were investigated at the molecular level using 2D NOESY ¹H NMR spectroscopy and isothermal titration calorimetry (ITC), and at the macroscopic level using dynamic light scattering and rheology.

![Reaction Scheme](image)

**Figure 3.3.** Synthetic scheme for preparation of (a) 3% randomly substituted PAAC12 and PAAC18, (b) the linked dimer β-CD2ur and (c) the linked dimer β-CD2su.

3.3. 2D NOESY ¹H NMR SPECTROSCOPY

Host-guest interactions between the 3% dodecyl (C12) and octadecyl (C18) substituted poly(acrylate)s, PAAC12 and PAAC18, with β-CD3bz and β-CDen3bz were studied by 2D NOESY ¹H NMR spectroscopy. Spectra were recorded on a Varian Inova 600 spectrometer operating at 599.957 MHz using a standard pulse sequence with a mixing time of 0.3 s. The solutions were made up in D₂O phosphate buffer at pH 7.0 and I = 0.10 mol dm⁻³. Each solution was 3.0 × 10⁻³ mol dm⁻³ in either the C12 or C18 substituents of PAAC12 or PAAC18 and 3.0 × 10⁻³ mol dm⁻³ in either β-CD or the β-CD groups of β-CD3bz or β-CDen3bz, such that the β-CD groups and C12 or C18 substituents were
equimolar. Solutions were allowed to equilibrate at the thermostated probe temperature of 298.2 K for 30 min in 5 mm NMR tubes prior to recording their spectra.

The 2D NOESY $^1$H NMR spectra of PAAC12 and PAAC18 show strong cross-peaks arising from interactions between the protons of their C12 and C18 substituents, respectively, and the H3, H5, and H6 annular protons of either β-CD, β-CD$_3$bz or β-CD$_{3n}$bz, consistent with host–guest complexation. These data do not distinguish between

**Figure 3.4.** 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $3.0 \times 10^{-3}$ mol dm$^{-3}$ in β-CD and 1.0 wt.% in 3% substituted PAAC12 such that the dodecyl groups substituent concentration is $3.0 \times 10^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, I = 0.10 mol dm$^{-3}$) at 298.2K. The rectangles enclose the cross-peaks arising from interaction between the annular H3,5,6 protons of β-CD and protons of the dodecyl 2-11 methylene protons of PAAC12. A schematic representation of the complexation between β-CD and PAAC12 is shown above the spectrum.
single substituent complexation and the simultaneous complexation of either C12 or C18 substituents to form intra- and inter-strand cross-links. Two examples of the spectra recorded are shown in Figures 3.4 - 3.5 and the other spectra are shown in Figures 3.23 - 3.26 in Section 3.8.1 (Appendix). Analogous 2D NOESY $^1$H NMR spectra of PAAC12 and PAAC18 in the presence of $\beta$-CD$_2$ur and $\beta$-CD$_2$su have been reported previously.$^{19,22}$

**Figure 3.5.** 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $1.0 \times 10^{-3}$ mol dm$^{-3}$ in $\beta$-CD$_3$bz and 1.0 wt.% in 3% substituted PAAC18 such that the octadecyl groups substituent concentration is $3.0 \times 10^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. The rectangles enclose the cross-peaks arising from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CD$_3$bz and the octadecyl 2-17 methylene protons of PAAC18. A schematic representation of the complexation between $\beta$-CD$_3$bz and PAAC18 is shown above the spectrum.
3.3. DYNAMIC LIGHT SCATTERING

Dynamic light scattering experiments were carried out at 298.2 K using a Malvern Nano-ZetaSizer. The instrument settings were automatically determined by Malvern dispersion technology software. The solutions were prepared in filtered (0.2 μm) degassed Millipore Milli-Q purified water and all were 0.3 wt.% in either PAAC12 or PAAC18 alone or in the presence of either β-CD or the β-CD substituents of either β-CD2ur, β-CD2su, β-CD3bz and β-CDen3bz at concentrations equal to those of the C12 and C18 substituents of PAAC12 and PAAC18, respectively. The light scattering showed aggregates, which are likely to be dynamic in nature, spread over small ranges of hydrodynamic diameter to exist in all solutions studied. The distribution of the hydrodynamic diameters determined for each solution are shown in Figures 3.6 and 3.7 for PAAC12 or PAAC18 alone, respectively.

![Figure 3.6](image)

**Figure 3.6.** Hydrodynamic diameter distributions of 0.3 wt.% of PAAC12 ([C12] = 9.15 × 10^{-4} mol dm^{-3}) in filtered (0.2 μm) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 1.26 ± 0.03 μm, mean distribution width 0.32 μm.
Figure 3.7. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC18 ([C18] = 8.92 × 10^{-4} \text{ mol dm}^{-3}) in filtered (0.2 \mu m) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 0.87 ± 0.06 \mu m, mean distribution width 0.23 \mu m.

The differing effects of \( \beta \)-CD entities on the mean hydrodynamic diameter and the mean distribution width are illustrated by Figure 3.8 in which it is seen that interaction with \( \beta \)-CD\(_{2s}\) substantially decreases both parameters for PAAC12, whereas interaction with \( \beta \)-CD substantially increases both parameters for PAAC18 as seen in Figure 3.9.

Figure 3.8. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC12 ([C12] = 9.15 × 10^{-4} \text{ mol dm}^{-3}) with one molar equivalent of \( \beta \)-CD units of \( \beta \)-CD\(_{2s}\) in filtered (0.2 \mu m) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 0.68 ± 0.03 \mu m, mean distribution width 0.13 \mu m.
Figure 3.9. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC18 ([C18] = 8.92 × 10^{-4} mol dm^{-3}) with one molar equivalent of β-CD in filtered (0.2 μm) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 1.68 ± 0.05 μm, mean distribution width 0.57 μm.

The remaining eight hydrodynamic diameter distributions are shown in Figures 3.27 – 3.34 in Section 3.8.2 (Appendix). All of the mean hydrodynamic diameters and mean distribution widths are collected in Table 3.1, and the mean hydrodynamic diameters are plotted in Figure 3.10.

Figure 3.10. Mean hydrodynamic diameters of PAAC12 and PAAC18 alone and in the presence of one molar equivalent β-CD and one molar equivalent of β-CD units of β-CD_{2ur}, β-CD_{2su}, β-CD_{3bz} and β-CD_{en3bz} in filtered (0.2 μm) degassed Millipore Milli-Q purified water in the pH range 7.0 – 8.0 and 298.2 K measured by dynamic light scattering.
Table 3.1. Mean hydrodynamic diameters of PAAC12 and PAAC18 aggregates alone and in the presence of either $\beta$-CD, $\beta$-CD$_2$ur, $\beta$-CD$_3$su, $\beta$-CD$_3$bz or $\beta$-CDen$_3$bz.$^a$

<table>
<thead>
<tr>
<th>System</th>
<th>Mean hydrodynamic diameter (μm)</th>
<th>Mean distribution width$^b$ (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAAC12</td>
<td>1.26 ± 0.03</td>
<td>0.32</td>
</tr>
<tr>
<td>$\beta$-CD/PAAC12</td>
<td>1.29 ± 0.15</td>
<td>0.32</td>
</tr>
<tr>
<td>$\beta$-CD$_2$ur/PAAC12</td>
<td>1.04 ± 0.03</td>
<td>0.37</td>
</tr>
<tr>
<td>$\beta$-CD$_2$su/PAAC12</td>
<td>0.68 ± 0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAAC12</td>
<td>0.88 ± 0.06</td>
<td>0.41</td>
</tr>
<tr>
<td>$\beta$-CDen$_3$bz/PAAC12</td>
<td>0.77 ± 0.10</td>
<td>0.20</td>
</tr>
<tr>
<td>PAAC18</td>
<td>0.87 ± 0.06</td>
<td>0.23</td>
</tr>
<tr>
<td>$\beta$-CD/PAAC18</td>
<td>1.68 ± 0.05</td>
<td>0.57</td>
</tr>
<tr>
<td>$\beta$-CD$_2$ur/PAAC18</td>
<td>0.69 ± 0.01</td>
<td>0.20</td>
</tr>
<tr>
<td>$\beta$-CD$_2$su/PAAC18</td>
<td>0.86 ± 0.07</td>
<td>0.27</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAAC18</td>
<td>0.72 ± 0.04</td>
<td>0.42</td>
</tr>
<tr>
<td>$\beta$-CDen$_3$bz/PAAC18</td>
<td>0.81 ± 0.17</td>
<td>0.26</td>
</tr>
</tbody>
</table>

$^a$ Where either $\beta$-CD or the $\beta$-CD unit of $\beta$-CD$_2$ur, $\beta$-CD$_3$su, $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz are equimolar to either the C12 or C18 substituents of either PAAC12 or PAAC18 aggregates in filtered (0.2 μm) degassed Millipore Milli-Q purified water in the pH range 7.0 – 8.0 and 298.2 K. $^b$ The mean width of the hydrodynamic diameter distribution is equal to $(D_{V,0.9} - D_{V,0.1})/D_{V,0.5}$ where $D_{V,0.5}$ is the diameter where 50% of the distribution is above and 50% is below, $D_{V,0.9}$ is the diameter where 90% of the distribution is below this value and $D_{V,0.1}$ is the diameter where 10% of the distribution is below this value.

Aggregations with mean hydrodynamic diameters in the range 0.68 – 1.68 μm are detected as is seen in Figure 3.10 and Table 3.1. For PAA12 and PAA18 alone the amount of strand aggregation is dependent on the extent of intra- and inter-strand aggregation of the hydrophobic C12 and C18 substituents which results in the mean hydrodynamic diameters of the PAAC12 aggregate (1.26 μm) being larger than that of PAAC18 (0.87 μm). This is either because the extent or strength (or both) of aggregation between the C12 substituents is less than between the C18 substituents (as is also observed at the much higher PAAC12 and PAAC18 3.3 wt.% concentration at which hydrogels form, as discussed in Section 3.5), or because fewer strands are involved in forming the PAAC18 aggregate. This situation is reversed in the presence of $\beta$-CD and $\beta$-CD$_2$ur, $\beta$-CD$_2$su, $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz as the extent of C12 and C18 substituent complexation varies.
The complexation of PAAAC12 with β-CD, equimolar to the C12 substituents (9.15 × 10^{-4} mol dm^{-3}), increases the hydrodynamic diameters of the aggregates of β-CD/PAAC12 slightly to 1.29 μm and the mean width of the distribution is unchanged at 0.32 μm. However, the complexation of PAAAC18 with β-CD, equimolar to the C18 substituents (8.92 × 10^{-4} mol dm^{-3}), increases the hydrodynamic diameters of the aggregates of β-CD/PAAC18 to 1.68 μm as well as the mean width of the distribution from 0.23 μm to 0.57 μm. These results are consistent with β-CD complexing a portion of the C12 and C18 substituents thereby decreasing the number of inter-strand cross-links and thereby the extent and tightness of aggregation with a consequent increase in aggregate size.

In contrast, the complexation of linked β-CD dimers and β-CD trimers with either PAAC12 or PAAC18 decreases the hydrodynamic diameter to 0.68-1.04 μm for PAAC12 and 0.69-0.86 for PAAC18, consistent with the formation of new cross-links being between by β-CD dimers or β-CD trimers with either C12 or C18 substituents. The number of polymer strands in these aggregates may vary from system to system, and their formation does not preclude the existence of independent PAAC12 and PAAC18 strands and their complexed forms outside the aggregates. It is assumed that similar aggregation occurs, but perhaps to a lesser extent, in the 0.2 wt.% PAAC12 and PAAC18 solutions studied by isothermal titration calorimetry as discussed in Section 3.4 below.

When 2.0 wt.% solutions of either PAAC12 or PAAC18 were subjected to dynamic light scattering experiments irreproducible hydrodynamic diameters distributions were obtained consistent with a greater extent of inter-strand cross-linking occurring between poly(acrylate)s strand to yield aggregates with hydrodynamic diameter greater than the instrumental detection limit of 6 μm.

The numbers of PAAC12 and PAAC18 strands in the aggregates are likely to be substantial as is shown through calculations for strands of 250 kD weight average masses from the summations of the volumes of the atoms composing the strand, 28.2 and 29.2 nm^3, respectively, to which is added the volume of a hydration sheath of water molecules in direct contact with the individual PAAC12 and PAAC18 strands. This sheath is composed of a hydrophilic component formed by the hydrogen bonding of water to the carboxylate groups, and a hydrophilic component composed of water molecules immediately adjacent to the alkyl components of PAAC12 and PAAC18 which are partially protected from hydrogen bonding interactions in the region immediately adjacent
to the alkyl group and consequently exchange with bulk water less rapidly than would otherwise be the case.\(^{25,26}\) Dielectric relaxation studies of the hydration of formate, acetate, propanoate and butanoate anions indicate that their carboxylate groups are hydrated by 5-6 water molecules and the methyl, propyl and butyl groups by \(\sim 17\) and \(\sim 27\) water molecules, respectively, in dilute solutions.\(^{25,26}\)

On the basis of these observations, an estimate of the hydration of the PAAC12 and PAAC18 strands may be made by assigning hydration numbers of 11 to each \(-\text{CH}_2\text{CO}_2^-\) unit (5 to the carboxylate group and 6 to the methylene group) and 72 and 108 to each the hydrophobic hydration shells of the C12 and C18 groups, respectively. Thus, the volume of the average 250 kD molecular weight strands of PAAC12 and PAAC18 and their hydration shells are 395 and 592 nm\(^3\), respectively, which compare with the spherical volumes of \(1.04 \times 10^9\) and \(3.4 \times 10^8\) nm\(^3\) for the PAAC12 and PAAC18 aggregates of 1.26 and 0.87 mean hydrodynamic diameter, respectively, and corresponding volumes of 1.042 and 0.342 μm\(^3\). While these estimates of the volumes of the PAAC12 and PAAC18 strands are very approximate, it is clear that a large number of them are likely to be contained in the aggregates, which are probably dominantly composed of water as are the hydrogels discussed in Section 3.5.

### 3.4. ISOTHERMAL TITRATION CALORIMETRY

Isothermal titration calorimetry, ITC, measurements were made with a MicroCal VP isothermal titration calorimeter.\(^ {27}\) Solutions were prepared in aqueous phosphate buffer at pH 7.0 and \(I = 0.10\) mol dm\(^{-3}\) and were degassed and thermostated at 298.2 K immediately prior to titration. The titrations were carried out under concentration conditions\(^ {28}\) where the product of the total C12 or C18 substituent concentration, the complexation constant, \(K\), and the number of either β-CD or linked β-CD dimer and trimer complexing each substituent, \(N\), yielded either a single sigmoidal variation, or a combination of such variations, of heat released against titrant added for each system. The initial cell volume 1.46 cm\(^3\) of 2 wt.% solution of either PAAC12 or PAAC18 were titrated with 10 mm\(^3\) aliquots of either β-CD or a linked β-CD dimer or trimer from a computer-controlled micro-syringe at intervals of 210 s. The concentration correction for displaced volume effects which occur with each injection were calculated by Origin 7.0 MicroCal protocol.\(^ {27}\) The concentrations of the β-CD or linked β-CD dimer or trimer titrant solutions varied as
indicated in the captions of Figures 3.11 – 3.14 below and in Figures 3.35 – 3.40 in Section 3.8.3 (Appendix)

The contributions of heats of dilution to the ITC data were determined by titrating the phosphate buffer pH 7.0 and \( I = 0.10 \text{ mol dm}^{-3} \) solution into either PAAC12 or PAAC18 in 2 wt.% solutions made up in the same buffer, and by titrating similarly buffered \( \beta \)-CD or linked \( \beta \)-CD dimer or trimer solutions of the same concentrations used in the complexation studies into phosphate buffer at pH 7.0 and \( I = 0.10 \text{ mol dm}^{-3} \) at 298.2 K. The heat changes observed for the PAAC12 or PAAC18, \( \beta \)-CD, \( \beta \)-CD\(_{2}\)ur, \( \beta \)-CD\(_{2}\)su and \( \beta \)-CD\(_{3}\)bz solutions were less than 1% of those observed for the complexation titrations. However, in the case of the \( \beta \)-CD\(_{3}\)bz solutions the heat change contributed up to 10% of the heat evolved during the host-guest complexation titrations (Figures 3.18 and 3.37 in Section 3.8.3 (Appendix)), and corrections were made for this in the derivation of the complexation parameters.

Algorithms for complexation according to Equations 3.1 - 3.7 below in Sections 3.4.1 and 3.4.2 by either \( \beta \)-CD or the individual \( \beta \)-CD groups or either linked \( \beta \)-CD dimers, \( \beta \)-CD\(_{2}\)ur, \( \beta \)-CD\(_{2}\)su or linked \( \beta \)-CD trimers, \( \beta \)-CD\(_{3}\)bz or \( \beta \)-CD\(_{3}\)en of the C12 and C18 substituents of PAAAs and to the experimental data points provided the best fit using the Origin 7.0 MicroCal protocol\(^{27} \) to yield \( K_{xy} \), \( \Delta H_{xy} \) and \( T\Delta S_{xy} \) for the range of complexation stoichiometries observed.

3.4.1. Isothermal Titration Calorimetry of the PAAC12 Systems

The complexation mode of the C12 and C18, substituents of the substituted poly(acrylate)s PAAC12 and PAAC18, respectively, by \( \beta \)-CD, \( \beta \)-CD\(_{2}\)ur, \( \beta \)-CD\(_{2}\)su, \( \beta \)-CD\(_{3}\)bz, and \( \beta \)-CD\(_{3}\)en varies significantly with the identities of the substituted poly(acrylate)s and the \( \beta \)-CD species. The simplest patterns are observed for the PAAC12 systems for which C12 complexation is characterized by a single ITC heat release phase irrespective of the number of \( \beta \)-CD annuli changing from one in \( \beta \)-CD, to two in \( \beta \)-CD\(_{2}\)ur and \( \beta \)-CD\(_{2}\)su, to three in \( \beta \)-CD\(_{3}\)bz and \( \beta \)-CD\(_{3}\)en. The ITC profile for the complexation of the C12 of PAAC12 by \( \beta \)-CD is shown in Figure 3.11. A best fit of the algorithm for a single complexation process (Equation 3.1) to the heat release data is consistent with complexation of a single C12 in the \( \beta \)-CD annulus and the derived \( N = 1.01 \) which is the ratio of one \( \beta \)-CD to the number of C12 complexed. The derived complexation constant
$K_{11} = 8.25 \times 10^3$ mol dm$^{-3}$ characterizes the equilibrium shown in Equation 3.1, and the corresponding $\Delta H_{11} = -15.07$ kJ mol$^{-1}$ and $T\Delta S_{11} = 7.29$ kJ mol$^{-1}$.

\[
\beta\text{-CD} + \text{C12} \rightleftharpoons \beta\text{-CD.C12}
\]

\[
K_{11} = \frac{[\beta\text{-CD.C12}]}{[\beta\text{-CD}][\text{C12}]}
\]  
(3.1)

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure311a.png}
\caption{(a) ITC data for titration of $\beta$-CD ($6.61 \times 10^3$ mol dm$^{-3}$) into 0.2 wt.% PAAC12 ($[\text{C12}] = 6.10 \times 10^4$ mol dm$^{-3}$) in aqueous phosphate buffer at pH 7.0 ($I = 0.10$ mol dm$^{-3}$) at 298.2 K. The top section shows the heat evolution with time as the titration proceeds. The bottom section shows the heat evolved with the addition of each aliquot of $\beta$-CD solution. The solid curve represents the best fit of an algorithm for 1:1 $\beta$-CD:C12 complexation. (b) Speciation plot showing the variation in percentage of C12 substituent complexed and the changing proportions of the complex $\beta$-CD.C12 as $[\beta$-CD]$_{\text{total}}$/[C12]$_{\text{total}}$ increases.}
\end{figure}
Figure 3.12. (a) ITC data for titration of β-CD₃bz (2.11 × 10⁻³ mol dm⁻³) into 0.2 wt.% PAAC12 ([C12] = 6.10 × 10⁻⁴ mol dm⁻³) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm⁻³) at 298.2 K. The top section shows the heat evolution with time as the titration proceeds. The bottom section shows the heat evolved with the addition of each aliquot of β-CD₃bz solution. The solid curve represents the best fit of an algorithm for the initial 1:1 β-CD₃bz:C12 complexation which is followed by rapid successive second and third C12 complexations. (b) Speciation plot showing the variation in percentage of C12 substituent complexed, and the changing proportions of the complexes β-CD₃bz:C12 as [β-CD₃bz]_total/[C12]_total increases.
Monophasic ITC data profiles consistent with equilibria analogous to that in Equation 3.1 also characterize the \( \beta\text{-CD}_{bz}/\text{PAAC12} \) system, as shown in Figure 3.12, and also the \( \beta\text{-CD}_{2\text{ur}}/\text{PAAC12} \), \( \beta\text{-CD}_{2\text{su}}/\text{PAAC12} \) and \( \beta\text{-CD}_{3\text{bz}}/\text{PAAC12} \) systems as shown in Figures 3.35 - 3.37, respectively, in Section 3.8.3 (Appendix). The derived \( K_{11}, \Delta H_{11}, T\Delta S_{11} \) and \( N \) data are collected in Table 3.2. The \( K_{11} \) and \( \Delta H_{11} \) for the \( \beta\text{-CD}_{2\text{ur}}/\text{PAAC12} \), \( \beta\text{-CD}_{2\text{su}}/\text{PAAC12} \), and \( \beta\text{-CD}_{3\text{bz}}/\text{PAAC12} \) systems are substantially greater than those for the \( \beta\text{-CD}/\text{PAAC12} \) system, and their \( T\Delta S_{11} \) are negative whereas \( T\Delta S_{11} \) for the \( \beta\text{-CD}/\text{PAAC12} \) system is positive. These differences are consistent with cooperative complexing of C12 occurring, except in the \( \beta\text{-CD}/\text{PAAC12} \) system. The effect of this is seen in the much greater heat release accompanying the addition of each aliquot of \( \beta\text{-CD}_{3\text{bz}} \) solution (Figure 3.12) than is the case for each aliquot of \( \beta\text{-CD} \) solution (Figure 3.11) consistent with much stronger complexing of C12 by \( \beta\text{-CD}_{3\text{bz}} \). Thus, \( [\text{C12}]_{\text{complexed}}/[\text{C12}]_{\text{free}} = 0.60 \) when \( [\beta\text{-CD}]_{\text{total}}/[\text{C12}]_{\text{total}} = 0.5 \) whereas \( [\text{C12}]_{\text{complexed}}/[\text{C12}]_{\text{free}} = 0.95 \) when \( [\beta\text{-CD}_{3\text{bz}}]_{\text{total}}/[\text{C12}]_{\text{total}} = 0.5 \) during the ITC titrations.

\[
\begin{array}{|c|c|c|c|c|}
\hline
\text{System} & 10^4K_{11}^b & \Delta H_{11} & T\Delta S_{11} & N^c \\
\hline
\beta\text{-CD}/\text{PAAC12} & K_{11} & 0.825 \pm 0.04 & -15.07 \pm 0.15 & 7.29 \pm 0.36 & 1.01 \pm 0.10 \\
\beta\text{-CD}_{2\text{ur}}/\text{PAAC12} & K_{11} & 5.78 \pm 0.3 & -27.70 \pm 1.39 & -0.52 \pm 0.03 & 0.43 \pm 0.04 \\
\beta\text{-CD}_{2\text{su}}/\text{PAAC12} & K_{11} & 4.43 \pm 0.22 & -27.71 \pm 1.39 & -1.19 \pm 0.06 & 0.46 \pm 0.05 \\
\beta\text{-CD}_{3\text{bz}}/\text{PAAC12} & K_{11} & 14.9 \pm 0.75 & -47.26 \pm 0.47 & -17.73 \pm 0.89 & 0.27 \pm 0.03 \\
\beta\text{-CD}_{3\text{en3 bz}}/\text{PAAC12} & K_{11} & 1.47 \pm 0.07 & -91.67 \pm 0.92 & -67.88 \pm 3.39 & 0.32 \pm 0.03 \\
\hline
\end{array}
\]
\[
^a\text{In aqueous phosphate buffer at pH 7.0 and } T = 0.10 \text{ mol dm}^{-3} \text{ at 298.2 K.} \quad ^bK_{11} \text{ is defined through Equation 3.1 and analogous equilibria. The errors shown for } K_{11} \text{ and the associated parameters are the data fitting error. When experimental error is also included, it is estimated that the overall error in } K_{11} \text{ is } \leq \pm 5\%. \quad ^cN \text{ is the ratio of one } \beta\text{-CD} \text{ or } \beta\text{-CD-dimer or -trimer to the number of C12 substituents complexed.}
\]

The cooperative complexation process proposed for the \( \beta\text{-CD}_{3\text{en3 bz}}/\text{PAAC12} \) system has some similarity to the complexation of a tridentate ligand by a metal ion characterized by a single complexation constant.\textsuperscript{29} Using this analogy, it follows that the complexation of the first C12 substituent of a PAAC12 strand by \( \beta\text{-CD}_{3\text{bz}} \) causes the second and third C12 substituents to complex in the second and third annuli of \( \beta\text{-CD}_{3\text{bz}} \) in rapid succession as shown in Figure 3.13. On average, thirty-two acrylate units are interposed between the C12
substituent sites, but the proximity of the $\beta$-CD$_3$bz-complexed C12 substituents in the PAAC12 strand cannot be deduced from the ITC data. Under these circumstances $N = 0.33$ should be observed which compares with the derived $N = 0.27$. This difference may be a consequence of $\beta$-CDen$_3$bz complexing three C12 of one PAAC12 strand in most cases, complexing two C12 of one PAAC12 strand and one C12 of an adjacent strand in some cases, and experimental error.

The change in $T\Delta S_{11}$ from $7.29$ to $-17.73$ kJ mol$^{-1}$ for the $\beta$-CD/PAAC12 and $\beta$-CD$_3$bz/PAAC12 systems, respectively, indicates that there is an additional decreasing entropy factor arising from the dominantly tritopic complexing of C12 by $\beta$-CD$_3$bz. The positive entropy contribution arising from displacement of water from the $\beta$-CD annulus

Figure 3.13. The tritopic complexation of C12 substituents of PAAC12 by $\beta$-CD$_3$bz. The orientations of the complexed C12 substituents show are intended to indicate that they probably fold inside the $\beta$-CD annuli, but they are not intended to be prescriptive.
outweighs the negative contribution arising from the combination of β-CD and a C12 into a single entity as observed for many β-CD systems,\textsuperscript{30} is likely to also apply to the complexation of C12 in each of the β-CD\textsubscript{3}bz annuli. However, the additional decrease in entropy arising from the constraint on movement of the C12 substituents and within a PAAC12 strand through tritopic complexation by β-CD\textsubscript{3}bz causes the overall $T\Delta S_{11}$ to be substantially negative. There is an accompanying increase in $\Delta H_{11}$ from -15.07 to -47.2 kJ mol\textsuperscript{-1} in the β-CD/PAAC12 and β-CDen\textsubscript{3}bz/PAAC12 systems, respectively, consistent with the $\Delta H_{11}$ for C12 complexing in a single β-CD annulus being approximately additive in the β-CD\textsubscript{3}bz/PAAC12 system.

Similar reasoning applies to the dominantly ditopic β-CD\textsubscript{2}ur/PAAC12 and β-CD\textsubscript{2}su/PAAC12 systems which also show substantially greater $K_{11}$, $\Delta H_{11}$ and negative $T\Delta S_{11}$ values (Table 3.2). Their respective $N$ values of 0.43 and 0.46 are less than the ideal value of 0.5, and may also reflect the complexation of C12 of one PAAC12 strand and one C12 of an adjacent strand in a minority of cases together with experimental error.

The β-CDen\textsubscript{3}bz/PAAC12 system is characterised by $N = 0.32$ which indicates predominantly tritopic complexation, but the combination of the largest $\Delta H_{11}$ and most negative $T\Delta S_{11}$ for the PAAC12 systems (Table 3.2) produces a $K_{11}$ which is one tenth that for the β-CD\textsubscript{3}bz/PAAC12 system and only 1.8 times that of the β-CD/PAAC12 system. Thus, there appears to be an additional factor affecting complexation in the β-CDen\textsubscript{3}bz/PAAC12 system which is associated with the increased length of the link between β-CD and the benzene centre in β-CDen\textsubscript{3}bz by comparison with that in β-CD\textsubscript{3}bz. When β-CDen\textsubscript{3}bz is titrated into poly(acrylate)s (which is identical to PAAC12 except for the 3\% C12 substitution) solution, heat is evolved which is attributable to dissociation of β-CDen\textsubscript{3}bz aggregates upon dilution (Figure 3.37 in Section 3.8.3 (Appendix). While the details of this aggregation on the C12 complexation cannot be discerned from the ITC data, it is probable that it arises from interactions between the hydrophobic tris-aminoethyl-benzene cores of adjacent β-CDen\textsubscript{3}bz which are less sterically crowded than the core of β-CD\textsubscript{3}bz.
3.4.2. Isothermal Titration Calorimetry of the PAAC18 Systems

There is a substantial change in the ITC profiles of the PAAC18 systems by comparison with those of the PAAC12 systems. This difference is attributable to the six additional methylene groups in C18 substituent rendering its hydrophobic extent greater than that of the C12 substituent. Consequently, aggregation of PAAC18 is much stronger than is that of PAAC12 as shown by rheological studies.\textsuperscript{19} Thus, competitive C18 aggregation and more extensive hydrophobic interaction of C18 with the interior of \( \beta \)-CD annuli are likely to be reflected in the ITC data for the PAAC18 systems. Of those studied, only the \( \beta \)-CD/PAAC18 system ITC profile is monophasic (Figure 3.38 in Section 3.8.3 Appendix) and is characterised by \( N = 0.53 \). This infers that two C18 enter the \( \beta \)-CD annulus in the dominant complex, in either or both of two modes. In the first mode two C18 complex from either end of the \( \beta \)-CD annulus sequentially and aggregate therein as shown in Figure 3.14. This is akin to \( \beta \)-CD acting in a similar way to a metal ion complexing a bidentate ligand where the first complexation stage is slow and rate determining and the second step occurs much more rapidly such that only a single complexation process and a single complexation constant are observed.\textsuperscript{29} For an analogous process to occur for the \( \beta \)-CD/PAAC18 system both C18 substituents would have to be attached to the same PAAC18 strand. (The \( \beta \)-CD annulus has a depth of 790 pm and internal diameters of 600 and 650 pm at the narrow and wide ends, respectively, such that two octadecyl substituents of a cross-sectional diameter of 310 pm could fit into the \( \beta \)-CD annulus.)
In the second mode both C18 enter the β-CD annulus from the same end. A plausible mechanism is shown in Figure 3.15 involving a preformed C18 substituent dimer which could either be composed of C18 substituents from the same PAAC18 strand or from two different strands. Irrespective of which of the two mechanisms operate, the overall result is a substantially increased $\Delta H_{11}$ (Table 3.3) by comparison with that of the β-CD/PAAC12 system (Table 3.2) probably because of the combination of the hydrophobic interactions between the two C18 and with the interior of the β-CD annulus. The associated $T\Delta S_{11}$ is also strongly negative which is largely attributable to the simultaneous complexation of two C18 substituents and the consequent restriction of motion of PAAC18 as discussed earlier for the ditopic β-CD$_{2su}$/PAAC12 and β-CD$_{2su}$/PAAC12 and tritopic β-CD$_3bz$/PAAC12 and β-CD$_3bz$/PAAC12 systems. (This contrasts with the positive $T\Delta S_{11}$ observed for the monotopic β-CD/PAAC12 system.) The magnitude of $K_{11}$ for the β-CD/PAAC18 system is less than that of any of the C12 systems because the substantial $\Delta H_{11}$ and large negative $T\Delta S_{11}$ offset each other.

Figure 3.14. The first possible mode of ditopic complexation of C18 substituents of PAAC18 by β-CD where the complexation of the first C18 engenders a faster sequential complexation of the second C12 in a similar manner to the complexation of a bidentate ligand by a metal ion.
Table 3.3. Thermodynamic Parameters Derived from ITC Data$^a$

<table>
<thead>
<tr>
<th>System</th>
<th>$10^4 K_{xy}^{b}$ (dm$^3$ mol$^{-1}$)</th>
<th>$\Delta H_{xy}$ (kJ mol$^{-1}$)</th>
<th>$T \Delta S_{xy}$ (kJ mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-CD/PAAC18</td>
<td>$K_{11}$ 0.297 ± 0.01</td>
<td>-68.23 ± 0.68</td>
<td>-48.41 ± 2.42</td>
</tr>
<tr>
<td>$\beta$-CD$_2$ur/PAAC18</td>
<td>$K_{11}$ 2200 ± 200</td>
<td>-81.21 ± 8.12</td>
<td>-39.29 ± 3.93</td>
</tr>
<tr>
<td>$\beta$-CD$_2$ur/PAAC18</td>
<td>$K_{12}$ 88.6 ± 4.4</td>
<td>-37.76 ± 3.78</td>
<td>-3.81 ± 0.38</td>
</tr>
<tr>
<td>$\beta$-CD$_2$su/PAAC18</td>
<td>$K_{11}$ 84.1 ± 8.4</td>
<td>-138.68 ± 13.87</td>
<td>-104.86 ± 10.5</td>
</tr>
<tr>
<td>$\beta$-CD$_2$su/PAAC18</td>
<td>$K_{12}$ 0.114 ± 0.005</td>
<td>-18.41 ± 1.84</td>
<td>-0.96 ± 0.10</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAAC18</td>
<td>$K_{11}$ 57.9 ± 2.8</td>
<td>-154.0 ± 1.5</td>
<td>-120.96 ± 6.05</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAAC18</td>
<td>$K_{12}$ 6.29 ± 0.3</td>
<td>-46.88 ± 0.5</td>
<td>-19.47 ± 0.97</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAAC18</td>
<td>$K_{11}$ 13.4 ± 0.67</td>
<td>-209 ± 2</td>
<td>-180 ± 9</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAAC18</td>
<td>$K_{21}$ 7.19 ± 0.6</td>
<td>1612 ± 16</td>
<td>1640 ± 80</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAAC18</td>
<td>$K_{12}$ 16.5 ± 0.9</td>
<td>-5039 ± 50</td>
<td>-5010 ± 250</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAAC18</td>
<td>$K_{22}$ 7.39 ± 0.4</td>
<td>9225 ± 90</td>
<td>9253 ± 460</td>
</tr>
</tbody>
</table>

$^a$In aqueous phosphate buffer at pH 7.0 and $I = 0.10$ mol dm$^{-3}$ at 298.2 K. $^b$ $K_{xy}$ is defined through Equations 3.2 - 3.7. The errors shown for $K_{xy}$ and the associated parameters are the data fitting error. When experimental error is also included it is estimated that the overall error in $K_{xy}$ is $\leq \pm 5\%$.

**Note:** Only the $\beta$-CD/PAAC18 system ITC profile is monophasic and is characterised by $N = 0.53$. Other $N$ numbers for the titration of either $\beta$-CD-dimers or -trimers to C18 could not be determined.

Figure 3.15. The second possible mode of ditopic complexation of C18 substituents of PAAC18 by $\beta$-CD where a preformed C18 substituent dimer is complexed.
The β-CD$_2$ur/PAAC18 system is characterized by a biphasic ITC profile (Figure 3.16) consistent with the sequential formation of the complexes β-CD$_2$ur.C18 and β-CD$_2$ur.C18$_2$ according to Equations 3.2 and 3.3, respectively, and characterized by complexation constants $K_{11}$ and $K_{12}$ (Table 3.3). An algorithm derived from Equations 3.2 and 3.3 best fits the ITC data and the derived speciation is shown in Figure 3.16b. It should be noted that when overlap of the heat release profiles of two complexation processes occur the simple interpretation of $N$ applied to a single complexation cannot be reliably applied. In the early stages of the ITC profile β-CD$_2$ur.C18$_2$ is the dominant complex as a consequence of the low [β-CD$_2$ur]$_{total}$/[C18]$_{total}$ ratio, and reaches a maximum concentration corresponding to 94% of [C18]$_{total}$ complexed at [β-CD$_2$ur]$_{total}$/[C18]$_{total}$ = 0.5. As this ratio further increases [β-CD$_2$ur.C18$_2$] decreases and [β-CD$_2$ur.C18] increases to become the dominant complex at [β-CD$_2$ur]$_{total}$/[C18]$_{total}$ = 1, at which stage 71% of [C18]$_{total}$ is complexed (Figure 3.16b). This strong complexation of C18 reflects the large $K_{11}$ and $K_{12}$ values characterizing the β-CD$_2$ur/PAAC18 system.

\[
\begin{align*}
K_{11} & 
\beta\text{-CD}_2\text{ur} + \text{C18} \rightleftharpoons \beta\text{-CD}_2\text{ur.C18} \\
K_{11} & = \frac{[\beta\text{-CD}_2\text{ur.C18}]}{([\beta\text{-CD}_2\text{ur}][\text{C18}]}) \quad (3.2) \\
K_{12} & 
\beta\text{-CD}_2\text{ur.C18} + \text{C18} \rightleftharpoons \beta\text{-CD}_2\text{ur.C18}_2 \\
K_{12} & = \frac{[\beta\text{-CD}_2\text{ur.C18}_2]}{([\beta\text{-CD}_2\text{ur.C18}][\text{C18}]}) \quad (3.3)
\end{align*}
\]
Figure 3.16. (a) ITC data for titration of \(\beta\text{-CD}_2\text{ur} \ (3.27 \times 10^{-3} \text{ mol dm}^{-3})\) into 0.2 wt.% \(\text{PAAC18} \ ([\text{C18}] = 5.95 \times 10^{-4} \text{ mol dm}^{-3})\) in aqueous phosphate buffer at pH 7.0 \((I = 0.10 \text{ mol dm}^{-3})\) at 298.2 K. The top section shows the heat evolution with time as the titration proceeds. The solid curve represents the best fit of an algorithm for the successive formation of 1:1 \(\beta\text{-CD}_2\text{ur}:\text{C18}\) and 1:2 \(\beta\text{-CD}_2\text{ur}:\text{C18}_2\) complexes. (b) Speciation plot showing the variation in percentage of C18 substituent complexed, and the changing proportions of the complexes \(\beta\text{-CD}_2\text{ur}.\text{C18}\) and \(\beta\text{-CD}_2\text{ur}.\text{C18}_2\) as \([\beta\text{-CD}_2\text{ur}]_{\text{total}}/[\text{C18}]_{\text{total}}\) increases.
The $\beta$-CD$_{2\text{su}}$/PAAC18 and $\beta$-CD$_{3\text{bz}}$/PAAC18 systems are also characterized by biphasic ITC profiles (Figures 3.39 and 3.40 in Section 3.8.3 (Appendix)) but their $K_{11}$ and $K_{12}$ values are much smaller than those of the $\beta$-CD$_{2\text{ur}}$/PAAC18 system (Table 3.3). Thus, the shorter urea link of three atoms, including both nitrogen atoms, between the two $\beta$-CD annuli of $\beta$-CD$_{2\text{ur}}$ favours stronger complexation of C18 in comparison with $\beta$-CD$_{2\text{su}}$ and $\beta$-CD$_{3\text{bz}}$ in which there are six and seven atoms between the annuli, respectively. A possible cause is that C18 is of sufficient length to be complexed simultaneously in both annuli of $\beta$-CD$_{2\text{ur}}$ as shown in Figure 3.17, whereas the increased linker lengths in $\beta$-CD$_{2\text{su}}$ and $\beta$-CD$_{3\text{bz}}$ decrease the extent of interaction between the C18 and a second $\beta$-CD annulus. The $K_{11}$ and $\Delta H_{11}$ values for the $\beta$-CD$_{2\text{ur}}$/PAAC18 and $\beta$-CD$_{2\text{su}}$/PAAC18 systems are much greater than those of their PAAC12 analogues, and their $T\Delta S_{11}$ values are substantially more negative.

As a consequence of its tritopic nature it is likely that the complexation modes of $\beta$-CD$_{3\text{bz}}$ differ in detail from those of ditopic $\beta$-CD$_{2\text{ur}}$ and $\beta$-CD$_{2\text{su}}$, however it is clear from the $\beta$-CD$_{3\text{bz}}$/PAAC18 ITC titration that there are two dominant complexes formed involving equilibria analogous to those in shown in Equations 3.2 and 3.3. It is probable that the complexation of $\beta$-CD$_{3\text{bz}}$ characterized by $K_{11}$ involves the complexation of a C18 substituent in two of the $\beta$-CD groups of $\beta$-CD$_{3\text{bz}}$ simultaneously, and that the complexation characterized by $K_{12}$ involves the complexation of a C18 substituent in two of the $\beta$-CD groups of $\beta$-CD$_{3\text{bz}}$ simultaneously and the complexation of a second C18 substituent by third $\beta$-CD group of $\beta$-CD$_{3\text{bz}}$, possibly with a simultaneous occupation of a $\beta$-CD by the ends of both complexed C18 substituents. However, the available data does not reliably permit a more detailed analysis than this.
Figure 3.17. The equilibria proposed for the ditopic complexation of C18 substituents in β-CD2ur.PAAC18 and β-CD2ur.(PAAC18)$_2$.

The ITC profile for the β-CD$_{en3}$bz/PAAC18 system is also multiphasic, but is more complicated than those of the preceding systems, as shown in Figure 3.18a. It is consistent with the formation of four dominant complexes according to the equilibria shown in Equations 3.4 - 3.7. An algorithm derived from Equations 3.4 - 3.7 best fits the ITC data and the derived speciation plot is shown in Figure 3.18b, from which it is unsurprisingly apparent that the proportion of β-CD$_{en3}$bz in these complexes increases with increase in the [β-CD$_{en3}$bz]$_{total}$/[C18]$_{total}$ ratio. The greater linker length between the β-CD groups and the benzene centre of β-CD$_{en3}$bz increases the stoichiometric variety in the β-CD$_{en3}$bz/C18 system by comparison with that the β-CD$_3$bz/PAAC18 system, probably as a consequence of the increased flexibility of β-CD$_{en3}$bz. The stoichiometric variety of the β-CD$_{en3}$bz/C18 system is also much greater than that of the β-CD$_{en3}$bz/PAAC12 system in which a single complexation process dominates. Thus, it is clear that increases in the flexibility of β-CD$_{en3}$bz and the length of C18 combine to produce more complexation stoichiometries, but an attempt to identify the nature of the complexes formed on the basis of the current data would be speculative and is not attempted.
A) ITC data for titration of β-CDen_3bz (2.03 \times 10^{-3} \text{ mol dm}^{-3}) into 0.2 wt.% PAAC18 ([C18] = 5.95 \times 10^{-4} \text{ mol dm}^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC data set where (i) is the data for dilution of β-CDen_3bz and (ii) is titration of β-CDen_3bz into 0.2 wt.% PAAC18. The bottom section shows the heat evolved with the addition of each aliquot of β-CDen_3bz solution. The solid curve represents the best fit of an algorithm for the formation of the β-CDen_3bz.C18, β-CDen_3bz.C18_2, (β-CDen_3bz)_2.C18 and (β-CDen_3bz)_2.C18_2 complexes.

B) Speciation plot showing the variation in percentage of C18 complexed, and the changing proportions of the complexes β-CDen_3bz.C18, β-CDen_3bz.C18_2, (β-CDen_3bz)_2.C18 and (β-CDen_3bz)_2.C18_2 as [β-CDen_3bz]_total/[C18]_total increases.

Figure 3.18. (a) ITC data for titration of β-CDen_3bz (2.03 \times 10^{-3} \text{ mol dm}^{-3}) into 0.2 wt.% PAAC18 ([C18] = 5.95 \times 10^{-4} \text{ mol dm}^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC data set where (i) is the data for dilution of β-CDen_3bz and (ii) is titration of β-CDen_3bz into 0.2 wt.% PAAC18. The bottom section shows the heat evolved with the addition of each aliquot of β-CDen_3bz solution. The solid curve represents the best fit of an algorithm for the formation of the β-CDen_3bz.C18, β-CDen_3bz.C18_2, (β-CDen_3bz)_2.C18 and (β-CDen_3bz)_2.C18_2 complexes. (b) Speciation plot showing the variation in percentage of C18 complexed, and the changing proportions of the complexes β-CDen_3bz.C18, β-CDen_3bz.C18_2, (β-CDen_3bz)_2.C18 and (β-CDen_3bz)_2.C18_2 as [β-CDen_3bz]_total/[C18]_total increases.
Qualitative inspection of the ITC profile for the β-CDen₃bz/C18 system shows an exothermic phase followed by an endothermic phase followed by an additional exothermic phase. Analysis of Equations 3.4 - 3.7 shows the $K_{11}$ and $K_{21}$ processes to be exothermic with negative entropy changes, and the $K_{12}$ and $K_{22}$ processes to be endothermic with positive entropy changes. However, it is seen from Figure 3.18b that the β-CDen₃bz.C18₂ species ($K_{12}$) dominates over the [β-CDen₃bz]_{total}/[C18]_{total} range studied with (β-CDen₃bz)₂.C18₂ ($K_{21}$) making a moderate contribution and β-CDen₃bz.C18 ($K_{11}$) and (β-CDen₃bz)₂.C18 ($K_{21}$) making much smaller contributions. This lessens the reliability of the magnitudes of the $\Delta H_{xy}$ and $T\Delta S_{xy}$ values in Table 3.2 to the extent that they are probably at best semi-quantitative.

\[
K_{11} = \frac{[\beta-CDen₃bz.C18]}{[\beta-CDen₃bz][C18]}
\]

\[
K_{12} = \frac{[\beta-CDen₃bz.C18₂]}{[\beta-CDen₃bz][C18]}
\]

\[
K_{21} = \frac{[(\beta-CDen₃bz)₂.C18]}{[\beta-CDen₃bz][\beta-CDen₃bz.C18]}
\]

\[
K_{22} = \frac{[(\beta-CDen₃bz)₂.C18₂]}{[(\beta-CDen₃bz)₂.C18][C18]}
\]

3.4.3. Entropy – Enthalpy Linear Relationship

The thermodynamic data in Tables 3.2 and 3.3 may be placed in context with similar data from the literature for host-guest complexation of a large variety of guests by β-CD, mono-substituted β-CD and linked β-CD dimers and trimers (Chapter 2 and 4) by plotting $T\Delta S_{11}$ at 298.2 K against $\Delta H_{11}$ as shown in Figure 3.19. Such plots are described by Equation 3.8.

\[
T\Delta S_{11} = \alpha \Delta H_{11} + T\Delta S_{11,0}
\]

It is seen from Figure 3.19a that much of the data obtained in this thesis falls within the range of the literature data, with the complexation by β-CD₂ur, β-CD₂su, β-CD₃bz and
β-CD$_3$bz of the adamantyl, dodecyl (C12) and octadecyl (C18) substituents appearing in the more negative $\Delta H_{11}$ and $T\Delta S_{11}$ region of the plot.

Linear least squares fit of $T\Delta S_{11}$ against $\Delta H_{11}$ of Equation 3.8 at 298.2 K to the literature data yield $\alpha = 0.80$ and $T\Delta S_{11,0} = 11$ kJ mol$^{-1}$ for β-CD, $\alpha = 0.99$ and $T\Delta S_{11,0} = 17$ kJ mol$^{-1}$ for the mono-substituted β-CD systems,$^{30}$ and $\alpha = 0.89$ and $T\Delta S_{11,0} = 23.5$ kJ mol$^{-1}$ for linked β-CD dimers.$^{32}$ For the new data obtained in this thesis, $\alpha = 0.91$ and $T\Delta S_{11,0} = 21.6$ kJ mol$^{-1}$ ($R^2 = 0.9853$). The positive $T\Delta S_{11,0}$ values indicate that the host-guest complexes are entropically stabilized at the intercept value where $\Delta H_{11} = 0$ and the corresponding entropy change is $\Delta S_{11,0}$. Hence, it appears that guest complexation within the β-CD host annuli of the nine new systems studied largely determines the thermodynamics irrespective of whether β-CD, β-CD$_3$bz or β-CDen$_3$bz is the host involved. A linear relationship between $T\Delta S$ and $\Delta H$ has been observed for a range of complexations and is commonly taken to indicate that compensatory variations in the relative importance of structural and solvation changes occur.$^{30-32,34,35}$
Figure 3.19. a) A plot of $T\Delta S_{11}$ against $\Delta H_{11}$ for the 1:1 complexes formed by the hosts $\beta$-CD, $\beta$-CD$_{2u}$, $\beta$-CD$_{3u}$, $\beta$-CD$_{3b}z$ and $\beta$-CD$_{3b}z$ with the guest dyes and adamantyl substituted poly(acrylates)s (characterized in Chapters 2 and 4, respectively) and with PAAC12 and PAAC18 determined in this Chapter 3, together with similar data for 1:1 $\beta$-CD, mono-substituted $\beta$-CD and linked $\beta$-CD dimers from the literature. b) A similar plot for the data in this thesis alone. The solid line represents the best-fit of Equation 3.8 to the data. (Data for the $\beta$-CD$_{3b}z$/PAAC18 system is excluded from both plots as it may be subject to higher error.)
3.5. RHEOLOGY

Rheological measurements were carried out with a Physica MCR 501 (Anton Parr GmbH) stress-controlled rheometer with a 25 mm cone and plate geometry. Temperature was controlled at 298.2 ± 0.1 K by a Peltier plate. All studies were carried out in 0.10 mol dm\(^{-3}\) aqueous NaCl solution adjusted to pH 7.0 with 0.10 mol dm\(^{-3}\) aqueous NaOH. The variation of the viscosities with shear rate of 3.3 wt.% aqueous solutions of either PAAC12 or PAAC18 in the presence of either β-CD\(_3\)bz or β-CDen\(_3\)bz are shown in Figure 3.20, and are plotted together with zero shear viscosities from a previous study\(^{19}\) in Figure 3.21.

![Viscosity variations with shear rate of 3.3 wt.% aqueous solutions of PAAC12 and PAAC18 with β-CD\(_3\)bz and β-CDen\(_3\)bz at pH = 7.0 and [NaCl] = 0.10 mol dm\(^{-3}\) at 298.2 K.](image)

Figure 3.20. Viscosity variations with shear rate of 3.3 wt.% aqueous solutions of PAAC12 and PAAC18 with β-CD\(_3\)bz and β-CDen\(_3\)bz at pH = 7.0 and [NaCl] = 0.10 mol dm\(^{-3}\) at 298.2 K.

It is apparent from both Figures 3.20 and 3.21 that the viscosity characteristics of PAAC12 and PAAC18 differ substantially over the range of conditions studied. It has previously been shown that the zero-shear viscosities of solutions increase in the sequence PAAC12 (0.016) < β-CD/PAAC12 (0.03) < β-CD_{2ur}/PAAC12 (0.12) < β-CD_{2su}/PAAC12 (0.25).\(^{19}\) In this study the β-CD\(_3\)bz/PAAC12 (0.12) and β-CDen\(_3\)bz/PAAC12 (0.8) solutions show small increases in zero-shear viscosity compared with that of PAAC12 alone, but similar values for β-CD\(_3\)bz/PAAC12 (0.12) and β-CD_{2ur}/PAAC12 (0.12) and a slightly decreased value for β-CDen\(_3\)bz/PAAC12 (0.08) by comparison with the β-CD_{2ur}/PAAC12 (0.12) and β-CD_{2su}/PAAC12 (0.25) systems (Figure 3.21) (zero shear viscosities in Pa.s are shown in brackets). In contrast, the zero-shear viscosities of solutions
determined in a previous study\textsuperscript{19} and this study decrease in the sequence: PAAC18 (645) > \(\beta\)-CD\textsubscript{2ur}/PAAC18 (339) > \(\beta\)-CD\textsubscript{2su}/PAAC18 (110) > \(\beta\)-CD\textsubscript{en3bz}/PAAC18 (38.2) > \(\beta\)-CD\textsubscript{3bz}/PAAC18 (6.40) > \(\beta\)-CD/PAAC18 (0.07) such that in all cases the PAAC12 solutions are less viscous than the analogous PAAC18 solutions. This is consistent with the greater extent of the hydrophobic C18 substituent dominating inter-strand cross-link formation, both through C18 aggregation and multiple C18 complexation.

![Figure 3.21](image)

**Figure 3.21.** Variation of zero shear viscosities from a previous study\textsuperscript{19} at 3.3 wt.% of PAAC12 (0.016) and PAAC18 (645) and in presence of \(\beta\)-CD: \(\beta\)-CD/PAAC12 (0.03) and \(\beta\)-CD/PAAC18 (0.07); \(\beta\)-CD\textsubscript{2ur}: \(\beta\)-CD\textsubscript{2ur}/PAAC12 (0.12) and \(\beta\)-CD\textsubscript{2ur}/PAAC18 (339); \(\beta\)-CD\textsubscript{2su}: \(\beta\)-CD\textsubscript{2su}/PAAC12 (0.25) and \(\beta\)-CD\textsubscript{2su}/PAAC18 (110); and this study \(\beta\)-CD\textsubscript{3bz}: \(\beta\)-CD\textsubscript{3bz}/PAAC12 (0.12) and \(\beta\)-CD\textsubscript{3bz}/PAAC18 (6.40) and \(\beta\)-CD\textsubscript{en3bz}: \(\beta\)-CD\textsubscript{en3bz}/PAAC12 (0.08) and \(\beta\)-CD\textsubscript{en3bz}/PAAC18 (38.2) at pH = 7.0 and [NaCl] = 0.10 mol dm\textsuperscript{-3} at 298.2 K.

The small increase in the viscosity of PAAC12 solution caused by \(\beta\)-CD contrasts with the large decrease in PAAC18 solution viscosity caused by \(\beta\)-CD which, nevertheless, remains greater than that of the \(\beta\)-CD/PAAC12 solution. This suggests that the extent of inter-strand cross-linking in the PAA12 solution is substantially less than that for PAAC18 such that 1:1 complexation by \(\beta\)-CD of C12 substituents has little effect on viscosity whereas 1:2 complexation of two C12 substituents by \(\beta\)-CD forms inter-strand cross-links and increases viscosity. In contrast 1:1 complexation of C18 substituents result in a substantial decrease in viscosity which, nevertheless, remains greater than that of the \(\beta\)-CD/PAAC12, possibly due to 1:2 complexation of two C18 substituents by \(\beta\)-CD.
forming inter-strand cross-links. It is pertinent that the formation of 1:2 \( \beta\text{-CD}_{18} \) complexes were detected through ITC titrations in 0.2 wt.% solutions (Section 3.4.2).

All of the remaining PAAC12 systems have higher zero-shear viscosities than PAAC12 alone, consistent with inter-strand cross-link formation through ditopic or tritopic complexation of C12 substituents. Interestingly, the variation in magnitudes of zero-shear viscosities of the PAAC12 solutions does not closely reflect the variation of \( K_{11} \) determined by ITC (Section 3.4.2). This may be a consequence of the \( K_{11} \) relating predominantly to intra-strand complexations whereas the viscosity data relates to inter-strand interaction.

The \( \beta\text{-CD}_{2\text{ur}}/\text{PAAC18} \), \( \beta\text{-CD}_{2\text{su}}/\text{PAAC18} \), \( \beta\text{-CD}_{3\text{bz}}/\text{PAAC18} \) and \( \beta\text{-CDen}_3\text{bz}/\text{PAAC18} \) solutions all show substantially greater zero-shear viscosities than the analogous PAAC12 solutions as a consequence of the stronger ditopic and tritopic complexation of the C18 substituent, which is also reflected in the corresponding \( K_{11} \) magnitudes (Tables 3.2 and 3.3). The variations in zero-shear viscosity of these solutions qualitatively reflect the variation in \( K_{11} \), but not that of \( K_{12} \) which characterizes the formation of linked \( \beta\text{-CD} \) dimer or trimer with two C18 substituents. As the ITC titrations and rheological studies were carried out on 0.2 % and 3.3 wt.% solutions, respectively, it is probable that the relative proportions of intra- and inter-strand C18 substituent formation varies substantially. This renders attempts to further relate the equilibrium and rheology data subject to considerable uncertainty; and consequently no further such relationships are sought.

3.6. CONCLUSIONS

Host-guest interactions between the aggregation of the dodecyl substituents of 3% randomly substituted poly(acrylate)s, PAAC12, and of the octadecyl substituents of the PAAC18 analogue, and their complexation by \( \beta\text{-CD} \), the linked dimers, \( \beta\text{-CD}_{2\text{ur}} \) and \( \beta\text{-CD}_{2\text{su}} \), and the linked trimers, \( \beta\text{-CD}_{3\text{bz}} \) and \( \beta\text{-CDen}_3\text{bz} \) have been studied in aqueous solution by 2D NOESY \(^1\text{H} \) NMR, dynamic light scattering, ITC, and rheology. This study completes data collection for the interactions of PAAC12 and PAAC18 with \( \beta\text{-CD} \) and the linked \( \beta\text{-CD} \) dimers, which have previously only been studied by 2D NOESY \(^1\text{H} \) NMR and rheology.
Patterns in the relative magnitudes of the effects of β-CD and the linked β-CD dimers and trimers on the complexation constants for the PAAC12 and PAAC18 host-guest complexes and the viscosities of hydrogel networks are multifold. They are consistent with a combination of the lengths of the C12 and C18 substituents and their complexation by β-CD being the dominant factors controlling these interactions. They are also consistent with the length and flexibility of the linkers in the β-CD dimers and trimers which control the extent and strength of the intra- and inter-strand interactions formed through either C12 or C18 substituent aggregation or host-guest complexation as exemplify in Figure 3.22 for a hydrogel network. In principle, this provides insight for the design of new aqueous polymer networks and hydrogels with potential for practical applications.

**Figure 3.22.** Schematic illustration of cross-linking in a poly(acrylate)s network through aggregation and host-guest complexation by β-CD\(_{3}\)bz of the C18 substituents (blue) attached to the poly(acrylate)s backbone (black) of PAAC18. Variation in the number of C18 substituents in particular aggregates is unknown, and those shown are illustrative only. It is possible that some C18 substituents remain disaggregated and that others are singly complexed by β-CD\(_{3}\)bz.
3.7. REFERENCES


23. Malvern Instruments, Worcestershire, United Kingdom

24. Zetasizer Software 6.34, Malvern Instruments, Worcestershire, United Kingdom.


27. MicroCal, 22 Industrial Drive East, Northampton, MA 01060, USA.


3.8. APPENDIX

3.8.1. 2D NOESY $^1$H NMR Spectra

Figure 3.23. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $1.0 \times 10^{-3}$ mol dm$^{-3}$ in $\beta$-CD$_3$bz and 1.0 wt.% in 3% substituted PAAC12 such that the dodecyl groups substituent concentration is $3.0 \times 10^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. The rectangles enclose the cross-peaks arising from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CD$_3$bz and the dodecyl 2-11 methylene protons of PAAC12. A schematic representation of the complexation between $\beta$-CD$_3$bz and PAAC12 is shown above the spectrum.
Figure 3.24. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $1.0 \times 10^{-3}$ mol dm$^{-3}$ in $\beta$-CDen$_3$bz and 1.0 wt. % in 3% substituted PAAC12 such that the dodecyl groups substituent concentration is $3.0 \times 10^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. The rectangles enclose the cross-peaks arising from interaction between the annular $\beta$-CD H3, 5, 6 protons of $\beta$-CDen$_3$bz and the dodecyl 2-11 methylene protons of PAAC12. A schematic representation of the complication between $\beta$-CD$_3$bz and PAAC12 is shown above the spectrum. A schematic representation of the complication between $\beta$-CD$_3$bz and PAAC12 is shown above the spectrum.
Figure 3.25. 2D NOESY $^1H$ NMR (600 MHz) spectrum (mixing time 300 ms) of a solution
$3.0 \times 10^{-3}$ mol dm$^{-3}$ in $\beta$-CD and 1.0 wt.% in 3% substituted PAAC18 such that the
octadecyl groups substituent concentration is $3.0 \times 10^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0
phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. The rectangles enclose the cross-peaks
arising from interaction between the annular H3,5,6 protons of $\beta$-CD and the octadecyl 2-
17 methylene protons of PAAC18. A schematic representation of the complexation between
$\beta$-CD$_3$bz and PAAC12 is shown above the spectrum.
Figure 3.26. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $1.0 \times 10^{-3}$ mol dm$^{-3}$ in $\beta$-CD$_{en3}$bz and 1.0 wt.% in 3% substituted PAAC18 such that the octadecyl groups substituent concentration is $3.0 \times 10^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. The rectangles enclose the cross-peaks arising from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CD$_{en3}$bz and the octadecyl 2-17 methylene protons of PAAC18. A schematic representation of the complexation between $\beta$-CD$_{3}$bz and PAAC18 is shown above the spectrum.
3.8.2. Light-Scattering – Hydrodynamic Diameter Distributions

Figure 3.27. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC12 ([C12] = 9.15 × 10^{-4} mol dm^{-3}) with one molar equivalent of β-CD in filtered (0.2 μm) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 1.29 ± 0.15 μm, mean distribution width 0.32 μm.

Figure 3.28. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC12 ([C12] = 9.15 × 10^{-4} mol dm^{-3}) with one molar equivalent of β-CD units of β-CD_{2ur} in filtered (0.2 μm) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 1.04 ± 0.03 μm, mean distribution width 0.37 μm.
Figure 3.29. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC18 ([C18] = 8.92 × 10^{-4} mol dm^{-3}) with one molar equivalent of β-CD units of β-CD₂ur in filtered (0.2 μm) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 0.69 ± 0.01 μm, mean distribution width 0.20 μm.

Figure 3.30. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC18 ([C18] = 8.92 × 10^{-4} mol dm^{-3}) with one molar equivalent of β-CD units of β-CD₂su in filtered (0.2 μm) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 0.86 ± 0.07 μm, mean distribution width 0.27 μm.
Figure 3.31. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC12 ([C12] = 9.15 × 10^{-4} \text{mol dm}^{-3}) with one molar equivalent of β-CD units of β-CD₃bz in filtered (0.2 \text{μm}) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 0.88 ± 0.06 \text{μm}, mean distribution width 0.41 \text{μm}.

Figure 3.32. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC18 ([C18] = 8.92 × 10^{-4} \text{mol dm}^{-3}) with one molar equivalent of β-CD units of β-CD₃bz in filtered (0.2 \text{μm}) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 0.72 ± 0.04 \text{μm}, mean distribution width 0.42 \text{μm}. 
Figure 3.33. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC12 ([C12] = 9.15 × 10^{-4} mol dm^{-3}) with one molar equivalent of β-CD units of β-CDen₃bz in filtered (0.2 μm) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 0.77 ± 0.10 μm, mean distribution width 0.20 μm.

Figure 3.34. Hydrodynamic diameter distributions of 0.3 wt.% of PAAC18 ([C18] = 8.92 × 10^{-4} mol dm^{-3}) with one molar equivalent of β-CD units of β-CDen₃bz in filtered (0.2 μm) degassed Millipore Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 0.81 ± 0.17 μm, mean distribution width 0.26 μm.
3.8.3. Isothermal Calorimetric Titration (ITC) Data

Figure 3.35. (a) ITC data for titration of $\beta$-CD$_{2ur}$ ($3.27 \times 10^{-3}$ mol dm$^{-3}$) into 0.2 wt.% PAAC12 ([C12] = $6.10 \times 10^{-4}$ mol dm$^{-3}$) in aqueous phosphate buffer at pH 7.0 ($I = 0.10$ mol dm$^{-3}$) at 298.2 K. The top section shows the heat evolution with time as the titration proceeds. The bottom section shows the heat evolved with the addition of each aliquot of $\beta$-CD$_{2ur}$ solution. The solid curve represents the best fit of an algorithm for the initial 1:1 $\beta$-CD$_{2ur}$:C12 complexation which is followed by rapid successive second C12 complexations. (b) Speciation plot showing the variation in percentage of C12 substituent complexed, and the changing proportions of the complexes $\beta$-CD$_{2ur}$.C12 as $[\beta$-CD$_{2ur}]_{total}/[C12]_{total}$ increases.
Figure 3.36. (a) ITC data for titration of $\beta$-CD$_{2}$su ($3.19 \times 10^{-3}$ mol dm$^{-3}$) into 0.2 wt.% PAAC12 ([C12] = $6.10 \times 10^{-4}$ mol dm$^{-3}$) in aqueous phosphate buffer at pH 7.0 ($I = 0.10$ mol dm$^{-3}$) at 298.2 K. The top section shows the heat evolution with time as the titration proceeds. The bottom section shows the heat evolved with the addition of each aliquot of $\beta$-CD$_{2}$su solution. The solid curve represents the best fit of an algorithm for the initial 1:1 $\beta$-CD$_{2}$su:C12 complexation which is followed by rapid successive second C12 complexations. (b) Speciation plot showing the variation in percentage of C12 substituent complexed, and the changing proportions of the complexes $\beta$-CD$_{2}$su.C12 as [β-CD$_{2}$su]$_{total}$/[C12]$_{total}$ increases.
Figure 3.37. a) ITC data for titration of $\beta$-CDen$_3$bz (2.03 $\times$ 10$^{-3}$ mol dm$^{-3}$) into 0.2 wt.% PAAC12 ([C12] = 6.10 $\times$ 10$^{-4}$ mol dm$^{-3}$) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm$^{-3}$) at 298.2 K. The top section shows the raw ITC data set where (i) is the data for dilution of $\beta$-CDen$_3$bz and (ii) is titration of $\beta$-CDen$_3$bz into 0.2 wt.% PAAC12. The bottom section shows the heat evolved with the addition of each aliquot of $\beta$-CDen$_3$bz solution. The solid curve represents the best fit of an algorithm for the initial 1:1 $\beta$-CDen$_3$bz:C12 complexation which is followed by rapid successive second and third C12 complexations. (b) Speciation plot showing the variation in percentage of C12 substituent complexed, and the changing proportions of the complexes $\beta$-CDen$_3$bz:C12 as [\$\beta$-CDen$_3$bz]$_{total}$/[C12]$_{total}$ increases.
Figure 3.38. (a) ITC data for titration of $\beta$-CD ($6.61 \times 10^{-3}$ mol dm$^{-3}$) into 0.2 wt.% PAAC18 ([C18] = $5.95 \times 10^{-4}$ mol dm$^{-3}$) in aqueous phosphate buffer at pH 7.0 ($I = 0.10$ mol dm$^{-3}$) at 298.2 K. The top section shows the heat evolution with time as the titration proceeds. The bottom section shows the heat evolved with the addition of each aliquot of $\beta$-CD solution. The solid curve represents the best fit of an algorithm for 1:1 $\beta$-CD:C18 complexation. (b) Speciation plot showing the variation in percentage of C18 substituent complexed, and the changing proportions of the complexes $\beta$-CD.C18 as $[\beta$-CD]$_{total}$/[C18]$_{total}$ increases.
Figure 3.39. (a) ITC data for titration of $\beta$-CD$_{2}$su ($3.19 \times 10^{-3}$ mol dm$^{-3}$) into 0.2 wt.% PAAC18 ([C18] = $5.95 \times 10^{-4}$ mol dm$^{-3}$) in aqueous phosphate buffer at pH 7.0 ($I = 0.10$ mol dm$^{-3}$) at 298.2 K. The top section shows the heat evolution with time as the ITC proceeds. The bottom section shows the heat evolved with the addition of each aliquot of $\beta$-CD$_{2}$su solution. The solid curve represents the best fit of an algorithm for the successive formation of 1:1 $\beta$-CD$_{2}$su:C18 and 1:2 $\beta$-CD$_{2}$su:C18$_{2}$ complexes. (b) Speciation plot showing the variation in percentage of C18 substituent complexed and the changing proportions of the complexes, $\beta$-CD$_{2}$su:C18 and $\beta$-CD$_{2}$su:C18$_{2}$ as [$\beta$-CD$_{2}$su]$_{total}$/[C18]$_{total}$ increases.
Figure 3.40. (a) ITC data for titration of $\beta$-CD,bz (2.11 $\times$ 10^{-3} mol dm^{-3}) into 0.2 wt.% PAAC18 ([C18] = 5.95 $\times$ 10^{-4} mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the heat evolution with time as the titration proceeds. The bottom section shows the heat evolved with the addition of each aliquot of $\beta$-CD_{2su} solution. The solid curve represents the best fit of an algorithm for the successive formation of 1:1 $\beta$-CD,bz:C18 and 1:2 $\beta$-CD,bz:C18_{2} complexes. (b) Speciation plot showing the variation of the percentage of C18 substituent complexed and the changing proportions of the complexes $\beta$-CD,bz.C18 and $\beta$-CD,bz.C18_{2} as $[\beta$-CD,bz]_{total}/[C18]_{total} increases.
This page intentionally left blank.
CHAPTER 4

HOST-GUEST CHEMISTRY OF LINKED $\beta$-CYCLODEXTRIN TRIMERS AND ADAMANTYL SUBSTITUTED POLY(ACRYLATE)S IN AQUEOUS SOLUTION*
4.1. INTRODUCTION

4.1.1. General

Hydrophobic interactions between host and guest substituents on polymers to form host-guest complexes in aqueous solution substantially influence interactions within and between polymer strands.1-9 Understanding these interactions at the molecular level gives insight into polymer network and hydrogel formation at the macroscopic level. In turn, hydrogels are potentially deployable in a range of applications including drug delivery,10,11 electro-optics12 and tissue engineering.13

In chapters 1 and 3 of this thesis studies of several polymer network hydrogels based on poly(acrylate)s, PAAs, have been reviewed14-23 and those formed by the 3% dodecyl and octadecyl randomly substituted poly(acrylate)s PAAC12 and PAAC18 have been studied in some detail in the main body of Chapter 3. As an extension of this research, adamantyl (AD) substituted poly(acrylate)s have been chosen for study as the AD group complexes particularly strongly in the β-CD annulus because of a good size match, whereas complexation by the smaller α-CD and larger γ-CD is weaker.24 Adamantyl (AD) substituted poly(acrylate)s appear not to aggregate particularly strongly as is deduced from the observation that despite strong complexation of the AD substituents by β-CD (Figure 4.1A,B) this does not greatly change the solution viscosity.22 However, AD substituted PAAs form a strong hydrogel network when mixed with β-CD substituted PAAs (Figure 4.1C), and the viscosity of these hydrogels can be controlled by the varying the length of the tether binding the AD or β-CD substituents to the poly(acrylate)s backbone.21,22 Hydrogels are also formed when AD substituents of substituted poly(acrylate)s are complexed by linked β-CD dimers (Figure 4.1D).3,25 However, few studies which examine the interactions of AD substituted poly(acrylate)s and linked β-CD entities at both the molecular and macroscopic levels, and it is the object of this Chapter to expand knowledge in this area.
**Figure 4.1. Interactions of adamantyl (AD) substituted poly(acrylate)s with cyclodextrins, cyclodextrin-substituted poly(acrylate)s and linked cyclodextrin dimers in aqueous solution.**

### 4.1.2. Aims of This Study

The research described in this chapter seeks to extend the range and understanding of substituted poly(acrylate)s host-guest complexation particularly with respect to the associated thermodynamic patterns in dilute aqueous solutions and the formation of hydrogel network formation in more concentrated solutions. The β-CD annulus is retained as the host species in the linked β-CD trimers, 1,3,5-N,N,N-tris-(6<sup>α</sup>-deoxy-6<sup>α</sup>-β-cyclodextrin)-benzene (β-CD<sub>3</sub>bz) and 1,3,5-N,N,N-tris(6<sup>α</sup>-(2-aminoethyl)amino-6<sup>α</sup>-deoxy-6<sup>α</sup>-β-cyclodextrin)-benzene (β-CDen<sub>3</sub>bz) in which three β-CDs, are attached through the
C6\textsuperscript{A} carbon of the substituted D-glucopyranose unit either through an amido link or through a longer 2-(aminoethyl)amido link to the 1,3,5 sites of benzene, respectively. (Figure 4.2).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4_2.png}
\caption{Schematic representation of (a) $\beta$-CD; (b) linked $\beta$-CD\textsubscript{3bz} trimer and (c) linked $\beta$-CDen\textsubscript{3bz} trimer.}
\end{figure}

As mentioned earlier the adamantyl (AD) group is selected as the guest species as a consequence of its ability to form strong host-guest complexes with $\beta$-CD and its modified forms (Figure 4.1) in aqueous solution as shown in a range of studies.\textsuperscript{3,20-30} In this study, poly(acrylate)s randomly 3\% substituted with AD substituents through an amide tether in PAAAD and -CONH(CH\textsubscript{2})\textsubscript{n}NHCO- tethers, where $n = 2$, 6 or 12 in PAAADen, PAAADhn and PAAADdddn, respectively, yields four AD substituted poly(acrylate)s (Figure 4.3). These, in combination with $\beta$-CD, $\beta$-CD\textsubscript{3bz} and $\beta$-CDen\textsubscript{3bz}, yield twelve systems in which to study variations in host-guest interactions as molecular characteristics are varied.

This study first seeks to characterize the structural and thermodynamic aspects of the host-guest interactions between the linked $\beta$-CD trimers and the substituents of the AD substituted PAAs in aqueous solution using 2D NOESY \textsuperscript{1}H NMR spectroscopy and isothermal titration calorimetry (ITC). A comparison of these new thermodynamic data with those characterizing $\beta$-CD host-guest interactions which show a linear relationship\textsuperscript{31} between $T\Delta S_{11}$ and $\Delta H_{11}$ as discussed in Chapters 2 and 3 should provide insight into the interactions controlling the linked $\beta$-CD trimer/AD substituent host-guest interactions. The extent to which these factors impact on the formation of hydrogel networks in more
concentrated aqueous solutions is them assessed from rheological studies. The size measurement by dynamic light scattering was not attempted as adamantyl substituted poly(acrylate)s appear not to aggregate particularly strongly as is deduced from the observation that despite strong complexation of the AD substituents by β-CD this does not greatly change the solution viscosity.22

Figure 4.3. Schematic representation of 3% randomly AD substituted poly(acrylate)s: PAAAD, PAAADen, PAAADhn and PAAADddn.

4.2. SYNTHESIS

The 3.0 (±0.1) % random substituted poly(acrylate)s, 1-amino-adamantane-poly-(acrylate)s, 1-(2-aminoethyl)-adamantane-1-carboxamide poly(acrylate)s, 1-(6-aminohexyl)-adamantane-1-carboxamide poly(acrylate)s and 1-(12-aminododecyl)-adamantane-1-carboxamide poly(acrylate)s, PAAAD, PAAADen, PAAADhn and PAAADddn were synthesized as previously described22,32,33 (Figure 4.4). The linked β-CD₃bz and β-CD₄bz trimers were synthesized as reported in Chapter 2.
4.3. 2D NOESY $^1$H NMR SPECTROSCOPY

The host-guest interactions between the AD substituents of PAAAD, PAAADen, PAAADhn and PAAADddn with β-CD$_3$bz and β-CDen$_3$bz were studied using 2D NOESY $^1$H NMR spectroscopy. Spectra were recorded on a Varian Inova 600 spectrometer operating at 599.957 MHz using a standard pulse sequence with a mixing time of 0.3 s. Solutions for host-guest complexation studies in D$_2$O (pD 7.0, phosphate buffer, $I = 0.10$ mol dm$^{-3}$) contained AD substituted poly(acrylate)s ($3.0 \times 10^{-3}$ mol dm$^{-3}$) and β-CD$_3$bz ($1.0 \times 10^{-3}$ mol dm$^{-3}$) or β-CDen$_3$bz ($1.0 \times 10^{-3}$ mol dm$^{-3}$) in 1 cm$^3$ D$_2$O such that the β-CD groups and AD substituents of the substituted poly(acrylate)s were equimolar. Solutions were allowed to equilibrate at the thermostated probe temperature of 298.2 ± 0.1 K for 30 min in 5 mm NMR tubes prior to recording their spectra.

The spectra of D$_2$O solutions containing either PAAAD, PAAADen, PAAADhn or PAAADddn and β-CD$_3$bz or β-CDen$_3$bz in which the AD substituent and β-CD group concentrations are equimolar show strong cross-peaks consistent with interaction between the AD H2-4 protons and annular H3,5,6 protons of β-CD$_3$bz and β-CDen$_3$bz for all eight systems as seen in Figure 4.5 to Figure 4.7 and Figures 4.15 - 4.19 in the Appendix, Section 4.8.1. However, the β-CD group annular H3,5,6 and H2,4 proton resonances of
β-CD₃bz and β-Cデン₃bz are not readily differentiated in the systems studied as monosubstitution of β-CD causes all d-glucopyranose units to become inequivalent and their H2-6 resonances to superimpose. In contrast, the H3,5,6 resonances are readily distinguished from the H2,4 resonances of β-CD, and cross-peaks arising from the interactions of the H3,5,6 annular protons with AD substituent protons are clearly observed. No such cross-peaks arise for the β-CD H2,6 protons, confirming AD complexation in the β-CD annulus. Consequently, it is a plausible assumption that cross-peaks observed in this study between the AD substituted PAAs dominantly reflect host-guest complexation in the β-CD group annuli of the linked β-CD trimer as opposed to a more general interaction.

Figure 4.5. (left) 2D NOESY ¹H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution β-CD₃bz (1.0 × 10⁻³ mol dm⁻³) and 1.0 wt.% in 3% substituted PAAAD [AD] = 3.0 × 10⁻³ mol dm⁻³ in D₂O (pD 7.0 phosphate buffer, I = 0.10 mol dm⁻³) at 298.2K. The rectangles enclose the cross-peaks arising from interaction between the annular β-CD H3,5,6 protons of β-CD₃bz and the AD substituent protons (AD H2-4) of PAAAD. (right) Schematic representation of the complexation between β-CD₃bz and PAAAD.
Figure 4.6. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $\beta$-CD$_3$bz (1.0 $\times$ 10$^{-3}$ mol dm$^{-3}$) and 1.0 wt.% in 3% substituted PAAADhn [AD] = 3.0 $\times$ 10$^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, $I$ = 0.10 mol dm$^{-3}$) at 298.2K. Rectangles A and B enclose the cross-peaks arising dominantly from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CD$_3$bz and the AD substituent protons (AD H2-4) and the tether hexyl 2-4 methylene protons (hn-CH$_2$) of PAAADhn, respectively. Above: Schematic representation of the complexation between $\beta$-CD$_3$bz and PAAADhn.
Figure 4.7. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution of β-CD$_3$bz (1.0 × 10$^{-3}$ mol dm$^{-3}$) and 1.0 wt.% in 3% substituted PAAADddn [AD] = 3.0 × 10$^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, I = 0.10 mol dm$^{-3}$) at 298.2K. Rectangles A and B enclose the cross-peaks arising dominantly from interaction between the annular β-CD H3,5,6 protons of β-CD$_3$bz and the AD substituent protons (AD H2-4) and the tether dodecyl 2-11 methylene protons (ddn-CH$_2$) of PAAADddn, respectively. Above: Schematic representation of the complexation between β-CD$_3$bz and PAAADddn.
Additional cross-peaks arising from the interaction of the annular H3,5,6 protons of β-CD3bz and the protons of the tether hexyl 2-4 methylene protons of PAAADhn are also observed, consistent with competitive complexation between the AD substituent and the hexyl component of its tether. Analogous cross-peaks arising from the interaction of the annular H3,5,6 protons of β-CD3bz with the tether hexyl-CH2NH- protons of PAAADhn are also just distinguishable from the main axis component of the spectrum (Figure 4.6). Similar cross-peaks are observed for the β-CDen3bz/PAAADhn system (Figure 4.18, Appendix) consistent with an analogous duality of host-guest complexation occurring. Cross-peaks also arise from the interaction of the annular H3,5,6 protons of β-CD3bz and β-CDen3bz with the tether dodecyl 2-11 methylene protons and tether dodecyl -CH2NH protons of PAAADddn (Figure 4.7 and Figure 4.19, Section 3.8.1, Appendix). This behaviour is consistent with competitive host-guest complexation of the AD substituent and its dodecyl tether occurring. Two possible complexation modes of the dodecyl tether of PAAADddn are envisaged in Figure 4.7 and Figure 4.19 (Section 3.8.1, Appendix), in contrast to the analogous PAAADhn systems where it seems likely on stereochemical grounds that complexation of the shorter hexyl tether dominantly involves passage of the AD substituent through the β-CD group annulus (Figure 4.6 and Figure 4.18, Section 3.8.1, Appendix). The spectra of the β-CD3bz/PAAAD, β-CD3bz/PAAADen, β-CDen3bz/PAAAD and β-CDen3bz/PAAADen systems (Figures 4.5, and Figures 4.15, 4.16, 4.17, Section 3.8.1, Appendix) show cross-peaks arising from the interaction of the annular H3,5,6 protons of the β-CD groups and those of the AD substituents only, as anticipated for their shorter adamantyl tethers.

The cross-peaks arising from host-guest complexation in the linked β-CD trimer/AD substituted poly(acrylate)s systems may result from complexation either within a single AD substituted poly(acrylate)s strand, between two or more such strands or both. While the 1H NMR studies do not distinguish between these possibilities in the 1.0 wt.% substituted poly(acrylate)s solutions studied, the rheological studies of the more concentrated 5.0 wt.% solutions discussed below indicate the occurrence of substantial inter-strand cross-linking.

4.4. ISOTHERMAL TITRATION CALORIMETRY (ITC)

ITC measurements were made with a MicroCal VP isothermal titration calorimeter. Solutions were prepared in aqueous phosphate buffer (pH 7.0, I = 0.10 mol dm⁻³) and were
degassed and thermostated at 298.2 K immediately prior to titration. The titrations were carried out under concentration conditions where the product of the total AD substituent concentration ([AD]_{total}), the complexation constant, $K_{11}$, and the number of either β-CD, β-CD$_3$bz or β-CDen$_3$bz complexing each AD substituent, $N$, yielded a sigmoidal variation of heat released against titrant added for each system. The concentrations pertaining to twelve of the titrations appear in the figure captions from Figure 4.20 to Figure 4.31 in the Appendix, section 4.8, at the end of this chapter. The initial cell volume 1.46 cm$^3$ of each of the four AD substituted poly(acrylate)s with concentrations ranging from 0.13 to 0.37 wt.% were titrated by adding 10 mm$^3$ aliquots of either β-CD, β-CD$_3$bz or β-CDen$_3$bz solutions from a computer-controlled micro-syringe at intervals of 210 s. The concentration correction for displaced volume effects which occur with each injection were calculated by Origin 7.0 MicroCal protocol. The concentrations of four AD substituted poly(acrylate)s and the β-CD groups are varied as indicated in the figure captions. The contributions of heats of dilution were determined by titrating the buffer solution into either buffered PAAAD, PAAADen, PAAADhn or PAAADddn solutions of the appropriate concentration, and by titrating either β-CD, β-CD$_3$bz or β-CDen$_3$bz at the appropriate concentrations into buffer solution. The heat changes observed for the PAAAD, PAAADen, PAAADhn or PAAADddn, β-CD and β-CD$_3$bz solutions were less that 1% of those observed for the host-guest complexation titrations. However, in the case of the β-CDen$_3$bz solutions the heat change contributed up to 10% of the heat evolved during the host-guest complexation titrations (Figures 4.28–Figure 4.31 in Section 4.8, Appendix), and corrections were made in the derivation of the complexation parameters. An algorithm for complexation according to Equations 4.1–4.3 below by either β-CD or the individual β-CD groups of either β-CD$_3$bz or β-CDen$_3$bz with the AD substituents of either PAAAD, PAAADen, PAAADhn or PAAADddn to the experimental data points provided the best fit using the Origin 7.0 MicroCal protocol to yield $K_{11}$, $\Delta H_{11}$ and $T\Delta S_{11}$. The raw ITC data and the best fit for all twelve systems are shown in Figures 4.20 to Figure 4.31 in the Appendix, Section 4.8, at the end of this Chapter.

The complexation constants, $K_{11}$, (defined in Equation 4.1 - 4.3) for complexation between β-CD, β-CD$_3$bz and β-CDen$_3$bz host and the PAAAD, AD substituent guests and the enthalpy of complexation, $\Delta H_{11}$, and the product of temperature and entropy of complexation, $T\Delta S_{11}$, were determined by ITC. The $K_{11}$ values for the other nine systems
are similarly defined. The parameters derived for all twelve systems are shown in Table 4.1 and described below.

\[ K_{11} = \frac{[\beta\text{-CD}.\text{AD}]}{[\beta\text{-CD}][\text{AD}]} \quad (4.1) \]

\[ K_{11} = \frac{[\beta\text{-CD}_{3}\text{bz}.\text{AD}]}{[\beta\text{-CD}_{3}\text{bz}][\text{AD}]} \quad (4.2) \]

\[ K_{11} = \frac{[\beta\text{-CD}_{3}\text{en}.\text{AD}]}{[\beta\text{-CD}_{3}\text{en}][\text{AD}]} \quad (4.3) \]

An algorithm for Equation 4.1 for complexation by \( \beta\text{-CD} \) host with the AD substituents guest of PAAAD and analogous algorithms for PAAADen, PAAADhn and PAAADddn, best fit the experimental variation of the heat change per injection with a molar ratio of \( N = [\beta\text{-CD}]/[\text{AD}] = 0.77, 0.86, 0.85 \) and 0.83, respectively. These \( N \) values are less than the optimum value of unity, and may indicate that for the \( \beta\text{-CD}/\text{PAAAD} \) system steric interactions between the \( \beta\text{-CD} \) and the PAAAD poly(acrylate)s backbone hinder the complexing of AD substituents. Lengthening the tether of the AD substituents in PAAADen, PAAADhn and PAAAddn is likely to diminish such steric effects whilst increasing substituent intra-strand hydrophobic aggregation which may compete with complexation by \( \beta\text{-CD} \) and the \( \beta\text{-CD} \) groups of \( \beta\text{-CD}_{3}\text{bz} \) and \( \beta\text{-CD}_{3}\text{en} \). This behaviour, previously observed, occurs in host-guest interactions between some ditopic \( \beta\text{-CD} \) and AD hyaluronic acid derivatives.\(^{25}\) In more concentrated solutions some evidence for an increase in substituent tether length increasing inter-strand substituent aggregation arises from the rheology data as is discussed below.
Figure 4.8. ITC data for 0.13 wt.% PAAAD ([AD] = 4.00 × 10^{-4} mol dm^{-3}) with β-CD (1.06 × 10^{-2} mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of β-CD solution. The solid curve represents the best fit of an algorithm for the 1:1 β-CD:AD complexation analogous to that shown in Equation 4.1.
Figure 4.9. ITC data for 0.20 wt.% PAAADhn ([AD] = 6.00 × 10^{-4} mol dm^{-3}) with β-CD_{3bz} (2.10 × 10^{-3} mol dm^{-3}) (or β-CD group = 6.30 × 10^{-3} mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The bottom section shows the heat evolved with the addition of each aliquot of β-CD_{3bz} solution. The solid curve represents the best fit of an algorithm for the initial 1:1 β-CD_{3bz}:AD complexation analogous to that shown in Equation 4.2 which is followed by rapid successive second and third AD complexations.
Figure 4.10. ITC data for 0.20 wt.% PAAADhn ([AD] = 6.00 × 10^{-4} mol dm^{-3}) with β-CDen₃bz (2.17 × 10^{-3} mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (l = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot where (a) is the data for dilution of β-CDen₃bz and (b) is that for this titration. The bottom section shows the heat evolved with the addition of each aliquot of β-CDen₃bz solution. The solid curve represents the best fit of an algorithm for the initial 1:1 β-CDen₃bz:AD complexation analogous to that shown in Equation 4.3 which is followed by rapid successive second and third AD complexations.
Algorithms for Equation 4.2 and Equation 4.3 for complexation by β-CD₃bz and β-CDen₃bz of the PAAAD guest AD substituents, and analogous algorithms for PAAADen, PAAADhn and PAAADddn best fit the experimental heat change data with a molar ratio of \( N = [\beta-\text{CD}_3\text{bz}]/[\text{AD}] = 0.22, 0.28, 0.27 \) and 0.29, respectively, and \( N = [\beta-\text{CDen}_3\text{bz}]/[\text{AD}] = 0.26, 0.31, 0.30 \) and 0.32, respectively. In the optimal case, \( N \) should be \( 1/3 \) if each of the three \( \beta-\text{CD} \) groups of \( \beta-\text{CD}_3\text{bz} \) and \( \beta-\text{CDen}_3\text{bz} \) complex a single AD substituent. This value is more closely approached by the more flexible \( \beta-\text{CDen}_3\text{bz} \) trimer in its interactions with PAAADen, PAAADhn and PAAADddn (Table 4.1). The steric and hydrophobic AD substituent aggregation effects discussed for the \( \beta-\text{CD} \) systems are still expected to apply, and the steric effect still appears to be more important in the \( \beta-\text{CD}_3\text{bz}/\text{PAAAD} \) and \( \beta-\text{CDen}_3\text{bz}/\text{PAAAD} \) systems as evidenced by \( N \) being significantly less than \( 1/3 \). Typical raw ITC data and the best fit for \( \beta-\text{CD}_3\text{bz}/\text{PAAADhn} \) and \( \beta-\text{CDen}_3\text{bz}/\text{PAAADhn} \) systems are shown in Figures 4.9 and Figures 4.10.

On a statistical basis, the concentrations of the substituted poly(acrylate)s in the range 0.13 – 0.37 wt.% are below those at which significant strand overlap occurs and accordingly the data for the linked \( \beta-\text{CD} \) trimers probably refer dominantly to intra-strand interactions.\(^{17} \) It is anticipated that most of the substituents in a 3% AD substituted poly(acrylate)s strand are on average sufficiently far apart for their complexation by \( \beta-\text{CD} \) to be largely independent of the complexation state of adjacent AD substituents. The four \( \beta-\text{CD} \) systems characterized yield the smallest \( K_{11} \) and \( \Delta H_{11} \) values and the only positive \( T\Delta S_{11} \) values in Table 4.1. Although a negative \( T\Delta S_{11} \) might be expected for the complexation of \( \beta-\text{CD} \) and the AD substituent, it appears that this is offset by the positive entropy change arising from the expulsion of 15 - 25 water molecules from the \( \beta-\text{CD} \) annulus.\(^{27,31} \)
Table 4.1. Thermodynamic parameters derived from ITC data

<table>
<thead>
<tr>
<th>System</th>
<th>$K_{11} \times 10^{3}$</th>
<th>$\Delta H_{11}$</th>
<th>$T\Delta S_{11}$</th>
<th>$N^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$-CD-PAAAD</td>
<td>3.35 ± 0.13</td>
<td>-3.00 ± 0.06</td>
<td>17.12 ± 0.16</td>
<td>0.77 ± 0.09</td>
</tr>
<tr>
<td>$\beta$-CD-PAAADen</td>
<td>8.77 ± 0.24</td>
<td>-20.81 ± 0.12</td>
<td>1.72 ± 0.18</td>
<td>0.86 ± 0.07</td>
</tr>
<tr>
<td>$\beta$-CD-PAAADhn</td>
<td>14.4 ± 0.06</td>
<td>-15.45 ± 0.09</td>
<td>8.29 ± 0.18</td>
<td>0.85 ± 0.06</td>
</tr>
<tr>
<td>$\beta$-CD-PAAADddn</td>
<td>5.77 ± 0.18</td>
<td>-16.58 ± 0.17</td>
<td>4.89 ± 0.24</td>
<td>0.83 ± 0.05</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz-PAAAD</td>
<td>34.5 ± 0.03</td>
<td>-27.00 ± 0.06</td>
<td>-1.10 ± 0.08</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz-PAAADen</td>
<td>209 ± 1.3</td>
<td>-74.93 ± 0.06</td>
<td>-44.56 ± 0.08</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz-PAAADhn</td>
<td>317 ± 2.0</td>
<td>-66.64 ± 0.04</td>
<td>-35.24 ± 0.06</td>
<td>0.27 ± 0.03</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz-PAAADddn</td>
<td>74.6 ± 1.8</td>
<td>-67.10 ± 0.23</td>
<td>-39.28 ± 0.29</td>
<td>0.29 ± 0.02</td>
</tr>
<tr>
<td>$\beta$CDen$_3$bz-PAAAD</td>
<td>3.70 ± 0.13</td>
<td>-37.67 ± 1.27</td>
<td>-17.31 ± 1.36</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>$\beta$CDen$_3$bz-PAAADen</td>
<td>40.6 ± 1.5</td>
<td>-71.56 ± 1.22</td>
<td>-45.25 ± 1.32</td>
<td>0.31 ± 0.02</td>
</tr>
<tr>
<td>$\beta$CDen$_3$bz-PAAADhn</td>
<td>38.7 ± 1.4</td>
<td>-64.47 ± 1.45</td>
<td>-38.28 ± 1.52</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>$\beta$CDen$_3$bz-PAAADddn</td>
<td>25.0 ± 1.1</td>
<td>-53.58 ± 0.72</td>
<td>-28.48 ± 0.81</td>
<td>0.32 ± 0.04</td>
</tr>
</tbody>
</table>

a In aqueous phosphate buffer at pH 7.0 and $I = 0.10$ mol dm$^{-3}$ at 298.2 K. b $K_{11}$ is defined through Equations 4.1 – 4.3 and analogous equivalent expressions. The errors shown for $K_{11}$ and the associated parameters are the data fitting error. When experimental error is also included it is estimated that the overall error in $K_{11}$ is $\leq \pm 5\%$. c $N$ is the number of either $\beta$-CD, $\beta$-CD$_3$bz or $\beta$CDen$_3$bz complexing each AD substituent in the dominant complexation interaction.

The ITC data for the $\beta$-CD$_3$bz systems are consistent with $\beta$-CD$_3$bz complexing up to three AD substituents in rapid succession. The $\Delta H_{11}$ values for the $\beta$-CD$_3$bz systems are substantially larger than the those of the $\beta$-CD systems and the $T\Delta S_{11}$ values range from close to zero to strongly negative, with the consequence that the $\beta$-CD$_3$bz $K_{11}$ values for a particular substituted poly(acrylate)s are the largest seen in Table 4.1. This is consistent with some cooperativity between the $\beta$-CD groups of $\beta$-CD$_3$bz in the complexation process. Within a single PAA strand, complexation of the first AD substituent by $\beta$-CD$_3$bz is likely to restrain the adjacent AD substituents such that another one or two are sequentially complexed. Such sequential complexation is characterized by a single $K$ in much the same way as is the complexation of a multidentate ligand by a metal ion.$^{36}$ However, in 3% substituted PAA the loop formed by the poly(acrylate)s sub-units between two immediately adjacent complexed AD substituents contains about 66 atoms in the
poly(acrylate)s backbone on average, to which must be added the number of atoms in each substituent tether. Thus, the total number of atoms in the loop is much greater than the 5-7 atoms usually found in the chelate rings of metal complexes. Nevertheless, the restriction of motion within the poly(acrylate)s loops formed by this complexation appears to cause a greater decrease in $T\Delta S_{11}$ than the increase anticipated for the expulsion of water from the annuli of the $\beta$-CD groups of $\beta$-CD$_3$bz.

A similar explanation applies to the increase in $\Delta H_{11}$ and the more negative $T\Delta S_{11}$ for the $\beta$-CD$_{en3}$bz systems by comparison with the analogous data for the $\beta$-CD systems (Table 4.1). However, the $K_{11}$ values characterizing the $\beta$-CD$_{en3}$bz systems show a much smaller increase over those of the $\beta$-CD systems than is the case for the $\beta$-CD$_3$bz systems, which indicates that the distance between the $\beta$-CD groups in $\beta$-CD$_3$bz and $\beta$-CD$_{en3}$bz is also important in determining the thermodynamics of host-guest complexation. It was noted above that while the heats of dilution for the substituted poly(acrylate)s, $\beta$-CD and $\beta$-CD$_3$bz contributed less than 1% contribution to the titration heat changes, the heat of dilution of $\beta$-CD$_{en3}$bz contributed up to 10% (Figures 4.28-4.31 in Section 4.8, Appendix). This probably arises from association of $\beta$-CD$_{en3}$bz molecules as a consequence of decreased steric crowding between the three $\beta$-CD groups by comparison with that in $\beta$-CD$_3$bz. This association may involve two $\beta$-CD$_{en3}$bz assuming conformations such that their tris-(2-aminoethyl)-aminobenzene centres undergo $\pi$-$\pi$ interaction. Alternatively, a $\beta$-CD group of one $\beta$-CD$_{en3}$bz may hydrogen bond within the cavity formed by the three $\beta$-CD groups of a second $\beta$-CD$_{en3}$bz. $^1$H NMR 300 MHz studies in which [$\beta$-CD$_{en3}$bz] was systematically diluted from $1.20 \times 10^{-2}$ to $2.23 \times 10^{-3}$ mol dm$^{-3}$ in D$_2$O phosphate buffer at pH 7.0 and $I = 0.10$ mol dm$^{-3}$ detected a 0.05 ppm shift of the aromatic proton resonance (Table 4.2) and similarly small shifts of other resonances, but these were too small to quantify any $\beta$-CD$_{en3}$bz association. Whatever its nature, such association is likely to compete with the complexation of the AD substituents of the poly(acrylate)s and contribute to the lowering of the $K_{11}$ values for the $\beta$-CD$_{en3}$bz systems.
Table 4.2. Variation in chemical shift of the aromatic $^1H$ resonance of $\beta$-CDen$_3$bz with concentration change.$^{a,b}$

<table>
<thead>
<tr>
<th>[β-CDen$_3$bz] mol dm$^{-3}$</th>
<th>$\delta$ ppm$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.20 \times 10^{-2}$</td>
<td>8.44</td>
</tr>
<tr>
<td>$9.46 \times 10^{-3}$</td>
<td>8.46</td>
</tr>
<tr>
<td>$7.44 \times 10^{-3}$</td>
<td>8.47</td>
</tr>
<tr>
<td>$5.84 \times 10^{-3}$</td>
<td>8.47</td>
</tr>
<tr>
<td>$4.59 \times 10^{-3}$</td>
<td>8.48</td>
</tr>
<tr>
<td>$3.61 \times 10^{-3}$</td>
<td>8.48</td>
</tr>
<tr>
<td>$2.83 \times 10^{-3}$</td>
<td>8.49</td>
</tr>
<tr>
<td>$2.23 \times 10^{-3}$</td>
<td>8.49</td>
</tr>
</tbody>
</table>

$^a$In D$_2$O phosphate buffer at pH 7.0, $I = 0.01$ mol dm$^{-3}$ and 298.2 K. $^b$Chemical shift downfield of the methyl resonance of sodium-3(trimethylsilyl)propanesulfonate at 0.0 ppm.$^{37}$ Determined at 300 MHz.

A trend is seen in Figure 4.11 in which the smallest $K_{11}$ values for complexation of the AD substituents of the substituted poly(acrylate)s by $\beta$-CD, $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz are observed for the PAAAD systems. This is consistent with the short tether between the AD substituent and the PAA backbone, causing significant steric hindrance to complexation by $\beta$-CD and the $\beta$-CD groups of $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz. The decrease in this hindrance resulting from lengthening of the tether in PAAADen causes substantial increases in $K_{11}$ for complexation by $\beta$-CD, $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz. A further increase in tether length in PAAAdhn increases $K_{11}$ for complexation by $\beta$-CD and $\beta$-CD$_3$bz but causes a leveling out for $\beta$-CDen$_3$bz. Thereafter, the large increase in the length of the 1,12-(aminododecyl)amido tether in PAAADddn causes $K_{11}$ to decrease for complexation by all three hosts coincident with anticipated increases in substituent intra-strand hydrophobic aggregation competing with complexation of the substituents by $\beta$-CD and the $\beta$-CD groups of $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz. However, the changes in the thermodynamic parameters are insufficiently systematic to permit confident assignment of these effects to specific changes in $\Delta H_{11}$ and $T\Delta S_{11}$. 

171
The variation of $K_{11}$ (298.2 K) with the hosts $\beta$-CD, $\beta$-CD$_3$bz and $\beta$-CD$_{en}$$_3$bz and the AD substituents on PAAAD, PAAADen, PAAADhn and PAAADddn.

The $\Delta H_{11}$ and $T\Delta S_{11}$ data in Table 4.1 may be viewed in a broader context when plotted together with similar data collected for host-guest complexation by $\beta$-CD, 27 monosubstituted $\beta$-CD, 31 and linked $\beta$-CD dimers 38 of a large variety of guest species much smaller in size than the poly(acrylate)s of this study. Entropy-enthalpy compensation plots yield linear least squares fits of these $T\Delta S_{11}$ against $\Delta H_{11}$ to Equation 4.4 at 298.2 K as shown in Figure 4.12 (yield $\alpha = 0.80$ and $T\Delta S_{11,0} = 11$ kJ mol$^{-1}$ for $\beta$-CD; $\alpha = 0.99$ and $T\Delta S_{11,0} = 17$ kJ mol$^{-1}$ for mono substituted $\beta$-CD, and $\alpha = 0.89$ and $T\Delta S_{11,0} = 23.5$ kJ mol$^{-1}$ for linked $\beta$-CD dimers). 38 The positive $T\Delta S_{11,0}$ values indicate that the host-guest complexes are entropically stabilized at $\Delta H_{11,0} = 0$, the intercept value, where the corresponding entropy change is $\Delta S_{11,0}$.

$$T\Delta S_{11} = \alpha\Delta H_{11} + T\Delta S_{11,0} \quad (4.4)$$

While Equation 4.4 does not reflect a necessary relationship between $T\Delta S_{11}$ and $\Delta H_{11}$, its widespread observation is usually taken as an indication of a variation in the relative importance of the solvational and structural changes within the systems studied. 26,39 Also plotted in Figure 4.12 are the $T\Delta S_{11}$ and $\Delta H_{11}$ data from Table 4.1, where the new data points for $\beta$-CD fall within the range of the literature data for $\beta$-CD and the data for $\beta$-CD$_3$bz and $\beta$-CD$_{en}$$_3$bz fall within the range of the literature data generally. (For the eight systems based on $\beta$-CD$_3$bz and $\beta$-CD$_{en}$$_3$bz $\alpha = 0.88$ and $T\Delta S_{11,0} = 19.5$ kJ mol$^{-1}$). This
indicates that although the variation of the thermodynamic parameters derived in this study are substantially influenced by the AD substituted poly(acrylate)s as discussed above, they remain within the entropy-enthalpy compensation relationship (Equation 4.4) characterizing host-guest complexation in β-CD-based systems. This suggests that complexation in the β-CD annulus substantially controls the thermodynamics of host-guest complexation within the substituted poly(acrylate)s systems studied. There are few other thermodynamic studies of host-guest complexation in aqueous polymer systems with which comparison may be made. One such study yields $\Delta H_{11} = -3.24$ kJ mol$^{-1}$ and $T\Delta S_{11} = -16.62$ kJ mol$^{-1}$ for the 1:1 host-guest complexation of substituents of the 1-(2-aminoethyl)amidoadamantyl substituents of the 3% randomly substituted poly(acrylate)s PAAADen by the $6^A$-(1-(2-aminoethyl)amino)-$6^A$-β-cyclodextrin in aqueous solution at 298.2 K$^{23}$ which lie within the scatter of the $\Delta H_{11}$ and $T\Delta S_{11}$ data plotted in Figure 4.12.

![Figure 4.12](image-url)  

**Figure 4.12.** Plot of $T\Delta S_{11}$ against $\Delta H_{11}$ from the literature for β-CD$^{27}$ monosubstituted β-CD$^{31}$ and β-CD dimers$^{38}$ and for the β-CD, β-CD$_3$bz and β-CDen$_3$bz systems characterized in this study.

### 4.5. RHEOLOGY

Rheological measurements were carried out with a Physica MCR 501 (Anton Parr GmbH) stress-controlled rheometer with a 25 mm cone and plate geometry. Temperature was controlled at 298.2 K by a Peltier plate. All studies were carried out in 0.10 mol dm$^{-3}$ aqueous NaCl solution at pH 7.0 adjusted with 0.10 mol dm$^{-3}$ aqueous NaOH.
Figure 4.13. Variation in viscosity with shear rate of a) PAAAD, PAAADen, PAAADhn and PAAADddn alone and in mixtures with b) $\beta$-CD$_3$bz or c) $\beta$-CDen$_3$bz (where the AD substituent concentration and the $\beta$-CD groups of either $\beta$-CD$_3$bz or $\beta$-CDen$_3$bz are equimolar) in aqueous 0.10 mol dm$^{-3}$ aqueous NaCl at pH 7.0 and 298.2 K. The AD-substituted poly(acrylate)s concentrations are 5.0 wt.% and the concentrations of their AD substituents are $1.5 \times 10^{-2}$ mol dm$^{-3}$.

The variation of viscosity with AD substituted PAAs and solution composition is shown in Figure 4.13a, which indicates that over the shear rates studied, there is little viscosity variation for a given system. The small increase in viscosity as tether length increases in the sequence: PAAAD (0.010), PAAADen (0.012), PAAADhn (0.015) and PAAADddn (0.016) (viscosities in Pa.s at a 500 s$^{-1}$ shear rate are shown in brackets) is attributable to increasing hydrophobic association of the AD substituents as tether length increases to produce weak cross-linking between adjacent substituted poly(acrylate)s strands. Upon addition of $\beta$-CD$_3$bz, the viscosities of all four systems increase generally: $\beta$-CD$_3$bz/PAAAD (0.03), $\beta$-CD$_3$bz/PAAADen (3.78), $\beta$-CD$_3$bz/PAAADhn (3.48) and
β-CD₃bz/PAAADddn (2.03) as seen in Figure 4.13b. Upon addition of β-CDen₃bz viscosity increases also occur: β-CDen₃bz/PAAAD (0.03), β-CDen₃bz/PAAADen (1.01), β-CDen₃bz/PAAADhn (0.68) and β-CDen₃bz/PAAADddn (0.49) as seen in Figure 4.13c.

The trends in viscosity for β-CD₃bz and β-CDen₃bz with the substituted poly(acrylate)s are broadly similar as seen in Figure 4.14. Consistent with the conclusions drawn from the ITC data, the shortness of the tether in PAAAD and the consequent steric hindrance by the poly(acrylate)s backbone appears to be a dominant factor precluding optimal complexation of the AD substituent by either β-CD₃bz or β-CDen₃bz. This is in contrast to PAAADen where the two methylene groups lengthen the tether to allow stronger complexation of the AD group by either β-CD₃bz or β-CDen₃bz and a substantial viscosity increase. Further lengthening of the tethers in PAAADhn and PAAADddn results in successive decreases in viscosity. This behaviour probably arises due to corresponding increases in the extent of hydrophobic association between the AD substituent tethers and competition with the formation of inter-strand cross-links through AD substituent complexation by β-CD groups.

The higher viscosities of the β-CD₃bz/(PAAADen-PAAADddn) systems by comparison with the analogous β-CDen₃bz systems are probably largely attributable to competition between the aggregation of β-CDen₃bz and the formation of inter-strand cross-links, which is absent from the β-CD₃bz systems. Some of the differences in viscosity may also be attributed to the shorter inter-strand cross-links formed and the reduced flexibility of the networks formed in the β-CD₃bz systems by comparison with those formed in β-CDen₃bz systems where the inter-strand cross-links are longer. There may also be differences in the ratios of intra- to inter-strand cross-links which could contribute to the overall effect. This trend in viscosity is qualitatively reflected in the previously discussed variations of \( K_{11} \) (298.2 K) of the host-guest complexes formed by β-CD₃bz by comparison with those formed by β-CDen₃bz in relatively dilute 0.13 - 0.37 wt.% AD-substituted poly(acrylate)s systems (Figure 4.14). Qualitatively, it appears that the factors affecting the complexation constants of the host-guest complexes in 0.13 - 0.37 wt.% solutions impact on the relative viscosities of the 5.0 wt.% solutions in a similar way, and that differences between the complexation constant and viscosity profiles probably arise from differences in the relative proportions of inter-strand cross-linking, which is likely to be much more prevalent in the 5.0 wt.% solutions.
Figure 4.14. Variation of 500 s$^{-1}$ shear rate viscosities of 5.0 wt.% AD-substituted poly(acrylate)s solutions alone or in the presence of either β-CD$_3$bz or β-CDen$_3$bz in 0.10 mol dm$^{-3}$ aqueous NaCl at pH 7.0 and 298.2 K.

4.6. CONCLUSION

In this study, twelve host-guest complex systems of β-CD, β-CD$_3$bz and β-CDen$_3$bz with the AD-substituent guests attached through tethers of four different lengths in 3% randomly substituted PAAs have been characterized at the molecular and macroscopic levels. Each of the three β-CD groups β-CD$_3$bz and β-CDen$_3$bz in may form host-guest complexes with single AD substituents of the substituted poly(acrylate)s PAAAD, PAAADen, PAAADhn and PAAADddn. In principle, one linked β-CD trimer may interact with a single AD substituted PAAs strand to form intra-strand cross-links between three AD substituents in dilute solution. In more concentrated solution the linked β-CD trimers interact with multiple strands to form an inter-strand cross-linked network in a hydrogel. We have used 2D $^1$H NOESY NMR spectroscopy, isothermal titration calorimetry and rheology to explore these possibilities and the factors controlling host-guest complexation and network formation at the molecular and macroscopic levels in the eight linked β-CD trimer/substituted poly(acrylate)s systems. The analogous four β-CD systems, in which host-guest complexation occurs, but significant cross-linking is unlikely, are used as convenient reference systems. The 2D $^1$H NOESY NMR and ITC studies were carried out in D$_2$O and aqueous phosphate buffer at pD or pH 7.0, respectively, at $I = 0.1$ mol dm$^{-3}$ and
298.2 K. The rheological studies were carried out in 0.10 mol dm\(^{-3}\) sodium chloride at pH 7.0 and 298.2 K under which conditions the systems were in their anionic poly(acrylate)s state. Several salient points arise.

First, the shortest tether length between the AD substituent and the poly(acrylate)s in PAAAD coincides with significant steric crowding between the individual complexing β-CD or β-CD groups of the trimers and the poly(acrylate)s backbone, which results in the lowest \(K_{11}\) values observed for complexing the poly(acrylate)s AD substituents by β-CD, β-CD\(_3\)bz and β-CD\(_{3}\)en bz in 0.13 – 0.37 wt.% solutions. For the last two hosts this also coincides with the lowest viscosity in 5.0 wt.% solution.

Second, the hydrophobic hexyl and dodecyl tethers compete with the AD group for complexation in the annuli of β-CD\(_3\)bz and β-CD\(_{3}\)en bz.

Third, β-CD\(_3\)bz yields the largest \(K_{11}\) (298.2 K) for complexation of AD substituents consistent with it exercising the greater cooperativity in host-guest complexation. It also forms the most viscous hydrogel, which indicates the formation of the tightest network structure.

Fourth, β-CD\(_{3}\)en bz generates substantially lower \(K_{11}\) values and hydrogel viscosities than does β-CD\(_3\)bz, thought to be largely as a consequence of its aggregation competing with AD poly(acrylate)s substituent complexation in intra- and inter-strand cross-link formation.

Fifth, despite the structural dissimilarity between β-CD, β-CD\(_3\)bz and β-CD\(_{3}\)en bz, the \(T\Delta S_{11}\) and \(\Delta H_{11}\) data characterizing their complexation of the AD substituents of the four AD-substituted poly(acrylate)s in 0.13 - 0.37 wt.% solutions fit well within the linear \(T\Delta S_{11}\) and \(\Delta H_{11}\) compensation relationship observed for the complexation of a wide range of guest species by β-CD, mono-substituted β-CDs and linked β-CD dimers. This indicates that complexation in the β-CD annulus largely controls the thermodynamics of host-guest complexation within the substituted poly(acrylate)s systems studied.
4.7. REFERENCES


35. MicroCal, 22 Industrial Drive East, Northampton, MA 01060, USA.


4.8. APPENDIX

4.8.1. 2D NOESY $^1$H NMR Spectroscopy Data

Figure 4.15. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of a solution $\beta$-CD$_3$bz ($1.0 \times 10^{-3}$ mol dm$^{-3}$) and 1.0 wt.% in 3% substituted PAAADen [AD] = $3.0 \times 10^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, I = 0.10 mol dm$^{-3}$) at 298.2K. The rectangles enclose the cross-peaks arising from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CD$_3$bz and the AD substituent protons (AD H2-4) of PAAADen. Above: Schematic representation of the complexation between $\beta$-CD$_3$bz and PAAADen.
Figure 4.16. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300ms) of a solution of $\beta$-CDen$_3$bz (1.0 × 10$^{-3}$ mol dm$^{-3}$) and 1.0 wt.% in 3% substituted PAAAD [AD] = 3.0 × 10$^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, I = 0.10 mol dm$^{-3}$) at 298.2K. The rectangle encloses the cross-peaks arising dominantly from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CDen$_3$bz and the AD substituent protons (AD H2-4) of PAAAD. Above: Schematic representation of the complexation between $\beta$-CDen$_3$bz and PAAAD.
Figure 4.17. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of $\beta$-CDen$_3$bz (1.0 $\times$ 10$^{-3}$ mol dm$^{-3}$) in and 1.0 wt.% in 3% substituted PAAADen [AD] = 3.0 $\times$ 10$^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, $I$ = 0.10 mol dm$^{-3}$) at 298.2K. The rectangle encloses the cross-peaks arising dominantly from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CDen$_3$bz and the AD substituent protons (AD H2-4) of PAAADen. Above: Schematic representation of the complexation between $\beta$-CDen$_3$bz and PAAADen.
Figure 4.18. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of β-CDen$_3$bz ($1.0 \times 10^{-3}$ mol dm$^{-3}$) and PAAADhn [AD] = $3.0 \times 10^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. Rectangles A and B enclose the cross-peaks arising dominantly from interaction between the annular β-CD H3,5,6 protons of β-CDen$_3$bz and the AD substituent protons (AD H2-4) and the tether hexyl 2-4 methylene protons (hn-CH$_2$) of PAAADhn, respectively. Above: Schematic representation of the complexation between β-CDen$_3$bz and PAAADhn.
Figure 4.19. 2D NOESY 1H NMR (600 MHz) spectrum (mixing time 300 ms) of β-CDen₃bz (1.0 × 10⁻³ mol dm⁻³) and PAAADddn [AD]= 3.0 × 10⁻³ mol dm⁻³ in D₂O (pD 7.0 in phosphate buffer, I = 0.10 mol dm⁻³) at 298.2K. Rectangles A and B enclose the cross-peaks arising dominantly from interaction between the annular β-CD H3,5,6 protons of β-CDen₃bz and the AD substituent protons (AD H2-4) and the tether dodecyl 2-11 methylene protons (ddn-CH₂) of PAAADddn, respectively. Above: Schematic representation of the complexation between β-CDen₃bz and PAAADddn.
4.8.2. Isothermal Titration Calorimetry Data

**Figure 4.20.** ITC data for 0.13 wt.% PAAAD ([AD] = 4.00 × 10^{-4} mol dm^{-3}) with β-CD (1.06 × 10^{2} mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of β-CD solution. The solid curve represents the best fit of an algorithm for the 1:1 β-CD:AD complexation analogous to that shown in Equation 4.1.
Figure 4.21. ITC data for 0.33 wt.% PAAADen ([AD] = 1.00 × 10^{-3} mol dm^{-3}) with β-CD (8.22 × 10^{-3} mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of β-CD solution. The solid curve represents the best fit of an algorithm for the 1:1 β-CD:AD complexation analogous to that shown in Equation 4.1.
Figure 4.22. ITC data for 0.37 wt.% PAAADhn ([AD] = 1.10 × 10^{-3} \text{ mol dm}^{-3}) with β-CD (8.22 × 10^{-3} \text{ mol dm}^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 \text{ mol dm}^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows a solid curve representing the best fit of an algorithm. The bottom section shows the heat evolved with the addition of each aliquot of β-CD solution. The solid curve represents the best fit of an algorithm for the 1:1 β-CD:AD complexation analogous to that shown in Equation 4.1.
Figure 4.23. ITC data for 0.21 wt.% PAAADddn ([AD] = \(6.00 \times 10^{-4}\) mol dm\(^{-3}\)) with \(\beta\)-CD (1.06 \(\times\) \(10^{-2}\) mol dm\(^{-3}\)) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm\(^{-3}\)) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of \(\beta\)-CD solution. The solid curve represents the best fit of an algorithm for the 1:1 \(\beta\)-CD:AD complexation analogous to that shown in Equation 4.1.
Figure 4.24. ITC data for 0.13 wt.% PAAAD ([AD] = 4.00 × 10^{-4} mol dm^{-3}) with β-CD_{3bz} (2.10 × 10^{-3} mol dm^{-3}) (or β-CD group = 6.30 × 10^{-3} mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of β-CD_{3bz} solution. The solid curve represents the best fit of an algorithm for the initial 1:1 β-CD_{3bz}:AD complexation analogous to that shown in Equation 4.2 which is followed by rapid successive second and third AD complexations.
Figure 4.25. ITC data for 0.20 wt.% PAAADen ([AD] = 6.00 × 10^{-4} mol dm^{-3}) with β-CD_{3bz} (2.10 × 10^{-3} mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of β-CD_{3bz} solution. The solid curve represents the best fit of an algorithm for the initial 1:1 β-CD_{3bz}:AD complexation analogous to that shown in Equation 4.2 which is followed by rapid successive second and third AD complexations.
Figure 4.26. ITC data for 0.20 wt.% PAAADhn ([AD] = 6.00 × 10^4 mol dm⁻³) with β-CD₃bz (2.10 × 10⁻³ mol dm⁻³) (or β-CD group = 6.30 × 10⁻³ mol dm⁻³) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm⁻³) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of β-CD₃bz solution. The solid curve represents the best fit of an algorithm for the initial 1:1 β-CD₃bz:AD complexation analogous to that shown in Equation 4.2 which is followed by rapid successive second and third AD complexations.
Figure 4.27. ITC data for 0.21 wt.% PAAADddn ([AD] = 6.00 × 10⁻⁴ mol dm⁻³) with β-CD₃bz (2.10 × 10⁻³ mol dm⁻³) (or β-CD group = 6.30 × 10⁻³ mol dm⁻³) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm⁻³) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of β-CD₃bz solution. The solid curve represents the best fit of an algorithm for the initial 1:1 β-CD₃bz:AD complexation analogous to that shown in Equation 4.2 which is followed by rapid successive second and third AD complexations.
Figure 4.28. ITC data for 0.13 wt.% PAAAD ([AD] = \(4.00 \times 10^{-4}\) mol dm\(^{-3}\)) with \(\beta\text{-CDen}_3\text{bz}\) (\(2.17 \times 10^{-3}\) mol dm\(^{-3}\)) (or \(\beta\text{-CD group} = 6.51 \times 10^{-3}\) mol dm\(^{-3}\)) in aqueous phosphate buffer at pH 7.0 (\(I = 0.10\) mol dm\(^{-3}\)) at 298.2 K. The top section shows the raw ITC experimental plot where (a) is the data for dilution of \(\beta\text{-CDen}_3\text{bz}\) and (b) is that for this titration. The bottom section shows the heat evolved with the addition of each aliquot of \(\beta\text{-CDen}_3\text{bz}\) solution. The solid curve represents the best fit of an algorithm for the initial 1:1 \(\beta\text{-CDen}_3\text{bz}:\text{AD}\) complexation analogous to that shown in Equation 4.3 which is followed by rapid successive second and third AD complexations.
196

Figure 4.29. ITC data for 0.20 wt.% PAAADen ([AD] = 6.00 × 10^{-4} \text{ mol dm}^{-3}) with β-CDen₃bz (2.17 × 10^{-3} \text{ mol dm}^{-3}) (or β-CD group = 6.51 × 10^{-3} \text{ mol dm}^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 \text{ mol dm}^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot where (a) is the data for dilution of β-CDen₃bz and (b) is that for this titration. The bottom section shows the heat evolved with the addition of each aliquot of β-CDen₃bz solution. The solid curve represents the best fit of an algorithm for the initial 1:1 β-CDen₃bz:AD complexation analogous to that shown in Equation 4.3 which is followed by rapid successive second and third AD complexations.
Figure 4.30. ITC data for 0.20 wt.% \textit{PAAADhn} ([AD] = $6.00 \times 10^{-4}$ mol dm$^{-3}$) with \textit{\textbeta-CDen\textsubscript{3}bz} ($2.17 \times 10^{-3}$ mol dm$^{-3}$) (or \textit{\textbeta-CD} group = $6.51 \times 10^{-3}$ mol dm$^{-3}$) in aqueous phosphate buffer at pH 7.0 ($I = 0.10$ mol dm$^{-3}$) at 298.2 K. The top section shows the raw ITC experimental plot where (a) is the data for dilution of \textit{\textbeta-CDen\textsubscript{3}bz} and (b) is that for this titration. The bottom section shows the heat evolved with the addition of each aliquot of \textit{\textbeta-CDen\textsubscript{3}bz} solution. The solid curve represents the best fit of an algorithm for the initial 1:1 \textit{\textbeta-CDen\textsubscript{3}bz:AD} complexation analogous to that shown in \textit{Equation 4.3} which is followed by rapid successive second and third AD complexations.
Figure 4.31. ITC data for 0.21 wt.% PAAADdnn ([AD] = 6.00 × 10^{-4} mol dm^{-3}) with β-CDen_{3}bz (2.17 × 10^{-3} mol dm^{-3}) (or β-CD group = 6.51 × 10^{-3} mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot where (a) is the data for dilution of β-CDen_{3}bz and (b) is that for this titration. The bottom section shows the heat evolved with the addition of each aliquot of β-CDen_{3}bz solution. The solid curve represents the best fit of an algorithm for the initial 1:1 β-CDen_{3}bz:AD complexation analogous to that shown in Equation 4.3 which is followed by rapid successive second and third AD complexations.
CHAPTER 5

HOST-GUEST CHEMISTRY OF LINKED $\beta$-CYCLODEXTRIN TRIMERS AND DANSYL SUBSTITUTED POLY(ACRYLATE)S IN AQUEOUS SOLUTION
5.1. INTRODUCTION

5.1.1. General

Fluorophore substituted water-soluble polymers are a new class of hydrophobic substituted polymers. Photophysical studies of such fluorescent polymers may provide greater understanding of the properties of polymers at the molecular level. In recent studies, the photophysical properties of fluorescent polymers, randomly substituted with pyrenyl,1,2,6 naphthyl,7,9 and dansyl7,10,14 groups were investigated by steady-state and time-resolved fluorescence spectroscopy in aqueous and organic solvents. It was found that the fluorescence intensities and lifetimes of such fluorophores were sensitive to the local environment and this provided considerable insight into interactions occurring within the polymer solutions.1-14 Apart from such fundamental studies, fluorescent polymers have been utilised in scintillators,15 luminescent solar concentrators,16 laser resistant materials,17 fiber optic sensors18 and laser dyes.19

In chapters 3 and 4 of this thesis several polymer network hydrogels formed by the 3% dodecyl, octadecyl and adamantyl randomly substituted poly(acrylate)s have been studied. As an extension of this research, dansyl substituted poly(acrylate)s have been chosen for study as the dansyl group has environment-sensitive fluorescence properties that can be monitored when it aggregates or complexes in a β-CD annulus. A previous study found that a dansyl substituted poly(acrylate)s associated in water to produce polymer aggregation and entanglement (Figure 5.1A and B) which decreased the dansyl group fluorescence lifetime.20 When β-CD was added, the complexation of dansyl by β-CD disrupted the dansyl aggregation, causing a decrease in viscosity and increase in fluorescence lifetimes (Figure 5.1C). However, the addition of linked β-CD dimer N,N′-bis(6-A-deoxy-β-cyclodextrin-6-A-yl)urea (β-CD2ur) increased the viscosity of the dansyl substituted poly(acrylate)s solutions through host-guest complexation of dansyl substituents on adjacent strands to form interstrand linkages which are either stronger or more prevalent (or both) than dansyl substituent aggregation20 (Figure 5.1E). On the other hand, upon addition of a second polymer substituted with fluorophore receptors (such as β-CD substituted poly(acrylate)s), host-guest complexes with the dansyl groups of the dansyl substituted poly(acrylate)s are formed, and aggregation of the dansyl substituted poly(acrylate)s is disrupted as new cross-links form between the dansyl and β-CD substituents (Figure 5.1D). This typically results in a more viscous network. For example,
addition of 6^&-(2-aminoethyl)amino-6^&-deoxy-β-cyclodextrin 3% randomly substituted poly(acrylate)s to dansyl substituted poly(acrylate)s solutions results in higher viscosities than those of solutions containing to dansyl substituted poly(acrylate)s alone.\textsuperscript{21}

![Diagram](image_url)

**Figure 5.1.** Interaction of dansyl substituted poly(acrylate)s alone (A,B) and in the presence of cyclodextrins (C), cyclodextrin-substituted poly(acrylate)s (D) and linked cyclodextrin dimers (E) in aqueous solution.

### 5.1.2. Aims of This Study

To gain more insight into the interactions of fluorophore randomly substituted poly(acrylate)s,\textsuperscript{22-31} and particularly into the host-guest interaction of dansyl substituted poly(acrylate)s with cyclodextrins, this study utilised dansyl randomly substituted poly(acrylate)s where the dansyl substituent has fluorophore properties as well as an ability to form host-guest complexes with the β-CD trimers β-CD\textsubscript{3}bz and β-C\textsubscript{3}bz\textsubscript{3}bz (Figure 5.2a,b). The poly(acrylate)s studied are 3% randomly substituted by \textit{N}-(2-aminoethyl)-, \textit{N}-(6-aminohexyl)- and \textit{N}-(12-aminododecyl)-5-dansyl-sulfonamide, PAADSen, PAADShn.
and PAADSddn, in which the dansyl substituent tether length progressively increases from 2, to 6 and 12 methylene groups, respectively (Figure 5.2c). The host-guest complexes between PAADSen, PAADShn and PAADSddn and β-CD₃bz and β-CD₇bz were investigated at the macroscopic level by size measurement and rheological study as well as at the molecular level by 2D NOESY ¹H NMR, isothermal titration calorimetry, fluorescence spectroscopy and time resolved fluorescence spectroscopy. These methods detect the variation of dansyl substituent spectroscopic and complexation response to environmental change at the molecular level in dilute aqueous solution. The results gained were used to interpret macroscopic observations gained through rheological studies of variations in the viscosities in terms of network formation in more concentrated solutions.

![Chemical structures](image)

**Figure 5.2.** (a) β-CD₃bz; (b) β-CD₇bz trimers and (c) the 3% randomly dansyl substituted poly(acrylate)s, PAADSen, PAADShn and PAADSddn.

These studies facilitate understanding of the effect of tether length on the dansyl substituent and the effect of linker length on β-CD trimers during complexation in dilute aqueous solution where interactions occur predominantly within individual substituted poly(acrylate)s strands. In more concentrated solutions, aggregation of dansyl substituents
between substituted poly(acrylate)s strands occur to form networks, which may be strengthened through β-CD trimers simultaneously complexing dansyl substituents from adjacent substituted poly(acrylate)s strands.

More broadly, this work is part of a rapidly expanding field in which CD host-guest chemistry is generating a wide variety of aqueous and polymer networks hydrogels.32-38

5.2. SYNTHESIS

The 3.0 (±0.1) % randomly substituted poly(acrylate)s with either \( N-(2\text{-aminoethyl})-5\), \( N-(6\text{-amino-hexyl})-5\) or \( N-(12\text{-aminododecyl})-5\) dansylsulfonamide, PAADSen, PAADShn or PAADSddn, respectively, were prepared by literature methods20,22,27 as shown in Figure 5.3. The linked β-CD\(_3\)bz and β-CDen\(_3\)bz trimers were synthesized as reported in Chapter 2.

![Figure 5.3. Synthetic scheme for the preparation of 3% randomly substituted PAADSen, PAADShn and PAADSddn.](image)

5.3. 2D NOESY \(^1\)H NMR SPECTROSCOPY

Host-guest interactions between the dansyl substitutents of PAADSen, PAADShn and PAADSddn with β-CD\(_3\)bz and β-CDen\(_3\)bz were studied at a molecular level using 2D NOESY \(^1\)H NMR spectroscopy. The 2D NOESY \(^1\)H NMR spectra were recorded on a Varian Inova 600 spectrometer operating at 599.957 MHz using a standard pulse sequence with a mixing time of 0.3 s. Solutions for host-guest complexation studies in D\(_2\)O (pD 7.0, phosphate buffer, \( I = 0.10 \text{ mol dm}^{-3} \)) contained dansyl substituted poly(acrylate)s (3.0 \( \times \) 10\(^{-3} \) mol dm\(^{-3} \)) and β-CD trimers (1.0 \( \times \) 10\(^{-3} \) mol dm\(^{-3} \)) in 1 cm\(^3\) D\(_2\)O such that the β-CD groups and dansyl substituents of the substituted poly(acrylate)s was equimolar. Solutions
were allowed to equilibrate at the thermostated probe temperature of 298.2 ± 0.1 K for 30 min in 5 mm NMR tubes prior to recording their spectra.

The NOESY spectrum of a D$_2$O solution of PAADSen, PAADShn or PAADSddn with either β-CD$_3$bz or β-CD$_{13}$bz, equimolar in the β-CD trimers and dansyl substituents, show moderate cross-peaks arising from interaction between the β-CD annular H3,5,6 protons and the dansyl H2-4,6-8 aromatic protons for all six systems as seen in Figure 5.4 (in rectangle A) and Figure 5.18 to Figure 5.21 in the Appendix, Section 5.11.1. Similarly, in each system, strong cross-peaks arising from interaction between the β-CD annular H3,5,6 protons of linked trimers and the dansyl methyl (dansyl N-CH$_3$) protons were observed (rectangle B in Figure 5.4 and Figures 5.18 - 5.21 in the Appendix, Section 5.11.1). The spectra of D$_2$O solutions of either PAADShn or PAADSddn with either β-CD$_3$bz or β-CD$_{13}$bz show strong cross-peaks consistent with interaction between annular H3,5,6 protons of β-CD$_3$bz and β-CD$_{13}$bz and the hexyl or dodecyl tether protons, hn-CH$_2$, as shown in rectangle C in Figures 5.5 (and Figures 5.19 - 5.21 in the Appendix, Section 5.11.1). On the other hand, cross-peaks arising from interaction of the ethyl tether were not observed as shown in Figures 5.4 (and Figures 5.18 in the Appendix, Section 5.11.1). These data are consistent with both the hexyl tether (or dodecyl tether) and the dansyl substituent complexing within the β-CD annulus, whereas only complexation of the dansyl substituents within the β-CD annuli was detected in PAADSen. The absence of cross-peaks associated with the ethyl tether is consistent with the length of the ethyl tether being insufficient to allow its complexation by linked β-CD trimers because of steric hindrance arising from the poly(acrylate)s backbone.

Possible complexation modes for all six systems are also shown in Figure 5.4 and Figure 5.5 (and Figures 5.18 to Figures 5.21 in Section 5.11.1, Appendix). The cross-peaks arising from host-guest complexation in the linked β-CD trimer/dansyl substituted poly(acrylate)s systems may result from complexation either within a single dansyl substituted poly(acrylate)s strand, between two or more such strands, or both. Although the $^1$H NMR data are consistent with host-guest complexation occurring, they do not distinguish between these possibilities in the 1.0 wt.% substituted poly(acrylate)s solutions studied, however the rheological studies of the more concentrated 5.0 wt.% solutions discussed below indicate the occurrence of substantial inter-strand cross-linking.
**Figure 5.4.** 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of $\beta$-CDen$_3$bz (1.00 $\times$ 10$^{-3}$ mol dm$^{-3}$) and 1.0 wt.% in 3% substituted PAADSen [dansyl] = 2.95 $\times$ 10$^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. The rectangles A and B enclose the cross-peaks arising from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CDen$_3$bz with the dansyl substituent H2-4, 6-8 and dansyl substituent methyl (N-CH$_3$) protons of PAADSen, respectively. Above: Schematic representation of the complexation between $\beta$-CDen$_3$bz and PAADSen.
Figure 5.5. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of $\beta$-CDen$_3$bz (1.00 × 10$^{-3}$ mol dm$^{-3}$) and 1.0 wt.% in 3% substituted PAADShn [dansyl] = 2.95 × 10$^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, I = 0.10 mol dm$^{-3}$) at 298.2K. The rectangles A, B and C enclose the cross-peaks arising from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CDen$_3$bz with the dansyl substituent H2-4, 6-8, dansyl substituent methyl (N-CH$_3$) and hexyl tether (hn CH$_2$) protons of PAADShn, respectively. Above: Schematic representation of the complexation between $\beta$-CDen$_3$bz and PAADShn.
5.4. FLUORESCENCE TITRATION STUDIES

The host-guest interactions between the dansyl substituents of PAADSen, PAADShn and PAADSddn with tether length progressively increasing from 2, to 6 and 12 methylene groups, and their linked β-CD₃bz and β-CD₃en₃bz trimers, were further quantitatively studied at the molecular level by fluorescence spectroscopy. The calculated concentrations of dansyl substituents were 1.0 × 10⁻³ mol dm⁻³ in phosphate buffer pH 7.0, I = 0.10 mol dm⁻³ at 298.2 K. Variation in the fluorescence spectra were monitored (over the range 450-600 nm) in 0.5 nm intervals as the sample of either 0.0033 wt.% PAADSen, 0.0034 wt.% PAADShn or 0.0035 wt.% PAADSddn solutions were sequentially diluted with 2 mm³ or 10 mm³ aliquots of β-CD trimers (concentration of β-CD trimers are varied as shown in captions of Figure 5.6 and Figure 5.22 to Figure 5.26 in the Appendix, Section 5.11.2).

The model used for determining the stability constants assumes that the dansyl substituents in PAADSen, PAADShn and PAADSddn complexed by either β-CD or linked β-CD trimers form 1:1 host-guest complexes exemplified by β-CD₃bz.PAADSen. Thus, for β-CD₃bz complexes of the dansyl substituents of PAADSen according to Equation 5.1:

\[ \beta-\text{CD}₃\text{bz} + \text{dansyl} \rightleftharpoons \beta-\text{CD}₃\text{bz} \cdot \text{dansyl} \]  \hspace{1cm} (5.1)

The stability constant, \( K_{11} \), at equilibrium is given by

\[ K_{11} = [\beta-\text{CD}₃\text{bz} \cdot \text{dansyl}] / ([\beta-\text{CD}₃\text{bz}][\text{dansyl}]) \]  \hspace{1cm} (5.2)

Given that [dansyl]_{total} and [β-CD₃bz]_{total} are the initial concentrations of the two complexation partners

\[ [\text{dansyl}]_{\text{total}} = [\beta-\text{CD}₃\text{bz} \cdot \text{dansyl}]_{\text{total}} + [\text{dansyl}] \]  \hspace{1cm} (5.3)

\[ [\beta-\text{CD}₃\text{bz}]_{\text{total}} = [\beta-\text{CD}₃\text{bzDS}]_{\text{total}} + [\beta-\text{CD}₃\text{bz}] \]  \hspace{1cm} (5.4)

The fluorescence at a particular wavelength is given by Equation 5.5

\[ F = I_{\text{DS}}[\text{dansyl}] + I_{\beta-\text{CD}₃\text{bz} \cdot \text{dansyl}} [\beta-\text{CD}₃\text{bz} \cdot \text{dansyl}] \]  \hspace{1cm} (5.5)

where \( F \), \( I_{\text{dansyl}} \) and \( I_{\beta-\text{CD}₃\text{bz} \cdot \text{dansyl}} \) represent the observed fluorescence and apparent molar fluorescences of PAADSen and β-CD₃bz.dansyl, respectively. The value of \( K_{11} \) was determined by best fitting the variation of the fluorescence spectra in the range 450-600 nm at 0.5 nm intervals to an algorithm based on Equations 5.2-5.5 as the β-CD₃bz concentration was varied using the HypSpec protocol. The same protocol was used for the other five systems. The molar fluorescence spectra and fitting of an algorithm for a 1:1 host-guest complex of PAADSen with β-CD₃bz are shown in Figure 5.6. Analogous spectra and fittings for host-guest complex of PAADShn, and PAADSddn with β-CD
trimers are shown in Figures 5.22 - 5.26 (Section 5.11.2, Appendix). The fitting results are summarised in Table 5.1.

![Graph](image1)

**Figure 5.6.** Top: Fluorescence increase of a PAADSen solution ([dansyl] = 1.0 × 10^{-5} mol dm^{-3}) (corresponding to 0.0033 wt.% in PAADSen) with 25 sequential injections (10 mm^3 each) of β-CD₃bz solution (7.03 × 10^{-3} mol dm^{-3}) in phosphate buffer pH 7.0, I = 0.10 mol dm^{-3} at 298.2 K. The arrow indicates the increase in fluorescence with each addition of β-CD₃bz solution. Excitation wavelength λ_{ex} = 331 nm with excitation and emission slit widths of 5nm. Bottom: Fluorescence variation at 505 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation by β-CD₃bz of a dansyl subsituent to data at 0.5 nm intervals over the wavelength range 450-600 nm.
Table 5.1. Fluorescence titration data for the complexation of PAADSen, PAADShn and PAADSddn with $\beta$-CD$_3$bz and $\beta$-CD$_3$en in aqueous phosphate buffer at pH 7.0 and $I = 0.10$ mol dm$^{-3}$ at 298.2 K.

<table>
<thead>
<tr>
<th>System</th>
<th>Fluorescence Data</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>$I_{\text{max}}$ (a.u.)</td>
<td>$K_{11}$ (298.2 K)$^B$ (dm$^3$ mol$^{-1}$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dansyl substituent</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAADSen</td>
<td>556</td>
<td>68</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAADShn</td>
<td>550</td>
<td>76</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAADSddn</td>
<td>546</td>
<td>103</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Complexed dansyl substituent</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-CD/PAADSen$^A$</td>
<td>524</td>
<td>378</td>
<td>89 $\pm$ 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-CD/PAADShn$^A$</td>
<td>524</td>
<td>406</td>
<td>105 $\pm$ 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-CD/PAADSddn$^A$</td>
<td>542</td>
<td>121</td>
<td>55 $\pm$ 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66$\beta$-CD$_2$ur/PAADSen$^A$</td>
<td>524</td>
<td>199</td>
<td>3040 $\pm$ 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66$\beta$-CD$_2$ur/PAADShn$^A$</td>
<td>506</td>
<td>618</td>
<td>34200 $\pm$ 500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>66$\beta$-CD$_2$ur/PAADSddn$^A$</td>
<td>513</td>
<td>681</td>
<td>242000 $\pm$ 5000</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>This study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAADSen</td>
<td>520</td>
<td>205</td>
<td>3940 $\pm$ 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAADShn</td>
<td>518</td>
<td>214</td>
<td>41400 $\pm$ 200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAADSddn</td>
<td>516</td>
<td>433</td>
<td>1990000 $\pm$ 9000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-CD$_3$en/PAADSen</td>
<td>514</td>
<td>493</td>
<td>187000 $\pm$ 900</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-CD$_3$en/PAADShn</td>
<td>514</td>
<td>520</td>
<td>469000 $\pm$ 2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-CD$_3$en/PAADSddn</td>
<td>519</td>
<td>361</td>
<td>366000 $\pm$ 1500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^A$From previous study.$^{29}$

$^B$The errors shown for $K_{11}$ are data fitting errors. The total experimental errors are estimated to be $\pm$ 5%.

Table 5.2. $K_{11}$ (298.2 K) for the complexation of PAADSen, PAADShn, PAADSddn by $\beta$-CD$_3$bz or $\beta$-CD$_3$en in comparison to that by $\beta$-CD or $\beta$-CD$_2$ur

<table>
<thead>
<tr>
<th>Guest</th>
<th>$K_{11 \beta$-CD$_3$bz}</th>
<th>$K_{11 \beta$-CD$_3$en}</th>
<th>$K_{11 \beta$-CD}</th>
<th>$K_{11 \beta$-CD$_2$ur}</th>
<th>$K_{11 \beta$-CD$_3$en}</th>
<th>$K_{11 \beta$-CD$_2$ur}</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAADSen</td>
<td>48</td>
<td>44</td>
<td>2101</td>
<td>1</td>
<td>62</td>
<td>62</td>
</tr>
<tr>
<td>PAADShn</td>
<td>11</td>
<td>394</td>
<td>4467</td>
<td>1</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>PAADSddn</td>
<td>0.2</td>
<td>36182</td>
<td>6655</td>
<td>8</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
By comparison with the previous studies,\textsuperscript{20} complexation between the dansyl substituents of PAADS\textsubscript{en}, PAADShn and PAADS\textsubscript{ddn} by either β-CD\textsubscript{3}bz or β-CD\textsubscript{3}bz\textsubscript{n3} (this study) range from 44 to 36182 times stronger than complexation by β-CD\textsuperscript{20} (Table 5.1 and Table 5.2) consistent with cooperative complexation of the dansyl substituents by the β-CD annuli of β-CD\textsubscript{3}bz or β-CD\textsubscript{3}bz\textsubscript{n3} (as is also the case for complexation by 66β-CD\textsubscript{2}ur). This also causes substantial decreases in fluorescence $\lambda_{max}$ and increases in $I_{max}$ of the complexed dansyl substituent by comparison with that of the uncomplexed dansyl substituent (Table 5.1). Thus, $K_{11}$ increases consistently with increasing cooperativity in complexation of the dansyl substituent and its tether in the β-CD annuli of the β-CD\textsubscript{3}bz trimer as tether length increases and steric hindrance caused by the poly(acrylate)s backbone lessens (as is also observed for complexation by 66β-CD\textsubscript{2}ur). Consistent with this interpretation, the change in $\lambda_{max}$ and $I_{max}$ for PAADS\textsubscript{en} when complexed β-CD\textsubscript{3}bz with is much less than that for the PAADShn and PAADS\textsubscript{ddn} complexation.

The cooperative complexation of the dansyl substituents may arise from the simultaneous complexation of both the dansyl substituent and its tether by two β-CD annuli of β-CD\textsubscript{2}ur and β-CD\textsubscript{3}bz such that $K_{11}$ increases with the hydrophobicity and length of the tether as steric hindrance from the poly(acrylate)s backbone simultaneously decreases. Alternatively, cooperativity may occur through simultaneous complexation of two dansyl substituents by the two β-CD annuli of β-CD\textsubscript{2}ur and three dansyl groups by the three β-CD annuli of β-CD\textsubscript{3}bz. Such, complexation has been observed in the isothermal titration calorimetry (ITC) studies as is discussed in Section 5.5. Multiple complexation is also required for the formation of the hydrogels discussed in Section 5.8.

The $K_{11}$ for complexation of the dansyl substituents of PAADS\textsubscript{en}, PAADShn and PAADS\textsubscript{ddn} by β-CD\textsubscript{3}bz are similar to those for β-CD\textsubscript{2}ur\textsuperscript{20} (Table 5.1 and Table 5.2). This is consistent with a similar degrees of steric hindrance from the poly(acrylate)s backbones of substantially influencing complexation by both β-CD\textsubscript{3}bz and β-CD\textsubscript{2}ur. For β-CD\textsubscript{3}bz\textsubscript{n3}, the $K_{11}$ for the complexation of the dansyl substituents of PAADS\textsubscript{en} and PAADShn are substantially greater than those for their complexation by β-CD\textsubscript{3}bz and β-CD\textsubscript{2}ur consistent with the longer linkers of β-CD\textsubscript{3}bz decreasing steric hindrance from the poly(acrylate)s backbone. However, $K_{11}$ for the complexation of the dansyl substituents of PAADS\textsubscript{ddn} by
\( \beta \)-CD\(_{3}\)bz is substantially greater than that for \( \beta \)-CDen\(_{3}\)bz which suggests that variation of the tether length in the dansyl-substituted poly(acrylate)s and the flexibility variation in \( \beta \)-CD\(_{3}\)bz and \( \beta \)-CDen\(_{3}\)bz resulting from the difference in linker length both contribute to the magnitude of \( K_{11} \).

The shift in \( \lambda_{\text{max}} \) and the increase in \( I_{\text{max}} \) accompanying complexation of the dansyl substituent is consistent with a change from an aqueous environment to one in which fluorescence quenching by water is less prevalent. In turn, this is consistent with complexation in the \( \beta \)-CD annulus of either native \( \beta \)-CD or the \( \beta \)-CD annuli of \( \beta \)-CD\(_{2}\)ur, \( \beta \)-CD\(_{3}\)bz and \( \beta \)-CDen\(_{3}\)bz. However, the 2D NOESY \(^1\)H NMR spectra of the complexes of PAADS\(_{hn}\) and PAADS\(_{ddn}\) with \( \beta \)-CD\(_{3}\)bz and \( \beta \)-CDen\(_{3}\)bz show that the \( hn \) and \( ddn \) tethers are also complexed in the \( \beta \)-CD annuli of \( \beta \)-CD\(_{3}\)bz and \( \beta \)-CDen\(_{3}\)bz, under which circumstances the dansyl entity will remain virtually fully hydrated and unlikely to show a significant change in fluorescence. Consequently, the \( K_{11} \) determined by fluorescence dominantly reflect the complexation of the dansyl groups alone. This is an important observation, as the magnitudes of the \( K_{11} \) determined by isothermal titration calorimetry for complexation of PAADS\(_{hn}\) and PAADS\(_{ddn}\) by \( \beta \)-CD\(_{3}\)bz and PAADSen, PAADS\(_{hn}\) and PAADS\(_{ddn}\) by \( \beta \)-CDen\(_{3}\)bz are all substantially smaller than those determined by fluorescence. The only exception is \( K_{11} \) determined for the complexation of PAADSen by \( \beta \)-CD\(_{3}\)bz for which fluorescence and isothermal titration calorimetry yielded similar values. The possible origins of these differences are discussed in the next section.

5.5. ISOThERMAL TITRATION CALORIMETRIC STUDIES

5.5.1. Isothermal Titration Calorimetry Results

Isothermal titration calorimetry (ITC) measurements were made with a MicroCal VP isothermal titration calorimeter. Solutions were prepared in aqueous phosphate buffer at pH 7.0 and \( I = 0.10 \text{ mol dm}^{-3} \) and were degassed and thermostated at 298.2 K immediately prior to titration. The titrations were carried out under concentration conditions\(^{40,41}\) where the product of the total dansyl substituent concentration, the complexation constant, \( K_{11} \), and the number of the \( \beta \)-CD trimers complexing each substituent, \( N \), yielded a sigmoidal variation of heat released against titrant added for each system. For native \( \beta \)-CD, the complexation of the dansyl substituted poly(acrylate)s were too weak for the ITC to be carried out.
The initial cell volume 1.46 cm\(^3\) of each of the dansyl substituted poly(acrylate)s 0.2 wt.% were titrated by adding 10 mm\(^3\) aliquots of the \(\beta\)-CD trimer solutions from a computer-controlled micro-syringe at intervals of 210 s. The concentration correction for displaced volume effects which occur with each injection were calculated by Origin 7.0 MicroCal protocol.\(^{42}\) The concentrations of the dansyl substituted poly(acrylate)s and the \(\beta\)-CD trimers were varied as indicated in the figure captions below. The contributions of heats of dilution were determined by titrating buffer solution into either buffered PAADSen, PAADShn or PAADSddn solutions of the appropriate concentration, and by titrating either \(\beta\)-CD\(_3\)bz or \(\beta\)-CDen\(_3\)bz at the appropriate concentrations into buffer solution. The heat changes observed for the PAADSen, PAADShn, PAADSddn and \(\beta\)-CD\(_3\)bz solutions were less than 1% of those observed for the host-guest complexation titrations. However, in the case of the \(\beta\)-CDen\(_3\)bz solutions the heat change contributed up to 10% of the heat evolved during the host-guest complexation titrations as shown in Figures 5.8 (and Figures 5.29-5.30 in the Appendix, Section 5.11.3), and corrections were made in the derivation of the complexation parameters. An algorithm for complexation according to Equations 5.1 - 5.2 and 5.6 - 5.7 was fitted to the experimental data points using the Origin 7.0 MicroCal protocol\(^{42}\) to yield \(K_{11}\), \(\Delta H_{11}\) and \(\Delta S_{11}\). Figures 5.7 and Figure 5.8 show the raw ITC data and the best fit for systems PAADSddn/\(\beta\)-CD\(_3\)bz and PAADSddn/\(\beta\)-CDen\(_3\)bz, respectively. The raw ITC data and the best fit for others systems are shown in Figures 5.27-5.30 in the Appendix, Section 5.11.3. The thermodynamic parameters derived from the ITC data are shown in Table 5.3 and are discussed below.

\[
\beta\text{-CDen}_3\text{bz} + \text{dansyl} \rightleftharpoons \beta\text{-CDen}_3\text{bz.dansyl} \quad (5.6)
\]

\[
K_{11} = [\beta\text{-CDen}_3\text{bz.dansyl}] / ([\beta\text{-CDen}_3\text{bz}] [\text{dansyl}]) \quad (5.7)
\]

Algorithms derived from Equations 5.1-5.2 and 5.6-5.7 for complexation of \(\beta\)-CD\(_3\)bz and \(\beta\)-CDen\(_3\)bz by the dansyl substituents PAADSen, and analogous algorithms for PAAADhn and PAAADddn best fit the experimental heat change data with a molar ratio \(N = 0.31-0.34\) (Table 5.3) which indicates predominantly tritopic complexation. This result is consistent with the ratio of 1: 3 for host-guest complexation whereby each of the three \(\beta\)-CD groups of \(\beta\)-CD\(_3\)bz and \(\beta\)-CDen\(_3\)bz complex a single dansyl substituent. The complexation by \(\beta\)-CD\(_3\)bz of the first dansyl substituent poly(acrylate)s strand causes the second and third dansyl substituents to complex in the second and third annuli of \(\beta\)-CD\(_3\)bz.
in rapid succession. Such sequential complexation is characterized by a single $K_{11}$ in much the same way as is the complexation of a multidentate ligand by a metal ion\textsuperscript{43} where on average, thirty-two acrylate units are interposed between the dansyl substituent sites.

![Figure 5.7](image-url)

**Figure 5.7.** *ITC data for 0.21 wt.% PAADSddn ([dansyl] = 5.85 \times 10^{-4} \text{ mol dm}^{-3}) with $\beta$-CD$_3$bz (2.11 \times 10^{-3} \text{ mol dm}^{-3})$ (or $[\beta$-CD$] = 6.33 \text{ mol dm}^{-3})$ in aqueous phosphate buffer at pH 7.0 ($I = 0.10 \text{ mol dm}^{-3})$ at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of $\beta$-CD$_3$bz solution. The solid curve represents the best fit of an algorithm for the 1:1 \beta-CD$_3$bz:dansyl complexation analogous to that shown in Equation 5.2 which is followed by rapid successive second and third dansyl complexations.*
Figure 5.8. ITC data for 0.21 wt.% PAADSddn ([dansyl] = 5.85 × 10^{-4} mol dm^{-3}) with β-CDen_3bz (2.03 × 10^{-3} mol dm^{-3}) (or [β-CD] = 6.09 mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot where (a) is the data for dilution of β-CDen_3bz and (b) is that for this titration. The bottom section shows the heat evolved with the addition of each aliquot of β-CDen_3bz solution. The solid curve represents the best fit of an algorithm for the 1:1 β-CDen_3bz:dansyl complexation analogous to that shown in Equation 5.7 which is followed by rapid successive second and third dansyl complexations.
Table 5.3: Thermodynamic parameters calculated from ITC experimental data at 298.2 K for the complexation of dansyl substituted poly(acrylate)s with β-CD trimers

<table>
<thead>
<tr>
<th>Systems</th>
<th>$10^3 K_{11}$ (dm$^3$ mol$^{-1}$)</th>
<th>$\Delta H_{11}$ (kJ mol$^{-1}$)</th>
<th>$T\Delta S_{11}$ (kJ mol$^{-1}$)</th>
<th>$N^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-CD$_3$bz-PAADSen</td>
<td>4.4 ± 0.2</td>
<td>-34.0 ± 0.3</td>
<td>-13.2 ± 0.2</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>3.93 ± 0.20$^d$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-CD$_3$bz-PAADShn</td>
<td>9.5 ± 0.4</td>
<td>-56.1 ± 0.6</td>
<td>-33.4 ± 0.3</td>
<td>0.31 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>41.4 ± 0.20$^d$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-CD$_3$bz-PAADSddn</td>
<td>63.9 ± 2.5</td>
<td>-92.8 ± 0.9</td>
<td>-65.3 ± 0.6</td>
<td>0.34 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>1990 ± 9$^d$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-CDen$_3$bz-PAADSen</td>
<td>1.50 ± 0.06</td>
<td>-126 ± 7</td>
<td>-107.5 ± 0.9</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>187.0 ± 0.9$^d$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-CDen$_3$bz-PAADShn</td>
<td>3.6 ± 0.1</td>
<td>-153 ± 8</td>
<td>-132.6 ± 1.2</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>469 ± 2$^d$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-CDen$_3$bz-PAADSddn</td>
<td>8.4 ± 0.4</td>
<td>-190 ± 10</td>
<td>-167.5 ± 1.5</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>366.0 ± 1.5$^d$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$In aqueous phosphate buffer at pH 7.0 and $I = 0.10$ mol dm$^{-3}$ at 298.2 K. $^b K_{11}$ is defined through Equations 5.2 and 5.7. The errors shown for $K_{11}$ and the associated parameters are the data fitting error. When experimental error is also included it is estimated that the overall error in $K_{11}$ is ≤ ± 10%. $^c N$ is the molar ratio of β-CD trimer to one dansyl substituents complexed. $^d K_{11}$ determined by fluorescence.

The $\Delta H_{11}$ and $T\Delta S_{11}$ values shown in Table 5.3 for the β-CD$_3$bz/PAADSen, β-CD$_3$bz/PAADShn, β-CD$_3$bz/PAADSddn, β-CDen$_3$bz/PAADSen, β-CDen$_3$bz/PAADShn, β-CDen$_3$bz/PAADSddn systems become substantially more negative with increasing tether length.

The $K_{11}$ values characterizing the β-CDen$_3$bz systems are smaller than those for the β-CD$_3$bz systems, which indicates that the distance between the β-CD groups in β-CD$_3$bz and β-CDen$_3$bz is also important in determining the thermodynamics of host-guest complexation. It was noted above (and also explained in Section 4.4 in Chapter 4) that while the heats of dilution for the substituted poly(acrylate)s and β-CD$_3$bz contributed less than 1% contribution to the titration heat changes, the heat of dilution of β-CDen$_3$bz contributed up to 10% (Figures 5.8 and Figures 5.29-5.30 in Appendix, Section 5.11.3). This probably arises from association of β-CDen$_3$bz molecules as a consequence of
decreased steric crowding between the three β-CD groups by comparison with that in β-CD₃bz. This association may involve two β-CDen₃bz assuming conformations such that their tris-(2-aminoethyl)aminobenzene centres undergo π-π interaction. Alternatively, the greater flexibility of β-CDen₃bz by comparison with β-CD₃bz may allow a greater degree of hydrogen bonding of the β-CD groups of adjacent β-CDen₃bz molecules. Whatever its nature, such association is likely to compete with the complexation of the dansyl substituents of the poly(acrylate)s and contribute to the lowering of the apparent $K_{11}$ values for the β-CDen₃bz systems.

![Figure 5.9](image_url)  

**Figure 5.9.** $K_{11}$ for host-guest complexation of β-CD₃bz and β-CDen₃bz with the dansyl-substituted poly(acrylate)s determined by ITC.

The $K_{11}$ (Figure 5.9) values of β-CD₃bz/PAADSen, β-CD₃bz/PAADShn and β-CD₃bz/PAADSddn and analogous β-CDen₃bz systems increase systematically with increase in the length of the tether of the dansyl substituents and complexation of the dansyl group and the tether (hexyl and docecyl) in the β-CD annuli of the β-CD trimers. The increase in $K_{11}$ is probably due to the decrease in steric hindrance caused by the poly(acrylate)s backbone as tether length increases. However, compared to the $K_{11}$ values obtained by fluorescence, only the $K_{11}$ of the β-CD₃bz/PAADSen determined by both fluorescence and ITC are comparable. The longer the linker or tether, the greater difference between the $K_{11}$ values obtained by the two methods becomes, ranging from 5 to 100 times higher by fluorescence titration than by ITC (Table 5.3). This may be due to several factors.
First, while fluorescence titration measures the micro-environment change of the dansyl substituent when complexed by a β-CD annulus, ITC measures the sum of the heat released upon complexation of the dansyl group in a β-CD annulus and also the heat released upon complexation of the ethyl, hexyl or dodecyl tether in a β-CD annulus. It is unlikely that the heat release of both processes will be identical. Accordingly, the process characterized by the greater heat release will dominate the ITC data such that the derived parameters pertain dominantly to this process. As \( K_{11} \) determined by fluorescence and ITC for the β-CD₃bz-PAASen system are identical within experimental error it is likely that this \( K_{11} \) dominantly characterizes complexation of the dansyl group. However, the greater magnitude of \( K_{11} \) determined by fluorescence by comparison with \( K_{11} \) determined ITC for the β-CD₃bz-PAASHn and β-CD₃bz-PAASddn systems suggests that β-CD₃bz complexing of the hexyl and dodecyl tethers dominates the heat released and thereby the magnitude of the ITC derived \( K_{11} \). A determination of \( \Delta H_{11} \) and \( \Delta S_{11} \) for these two system by fluorescence for comparison with those determined by ITC might shed further light on this, but unfortunately insufficient time was available to accomplish this.

A second reason for the divergence of the \( K_{11} \) β-CD₃bz-PAASen and β-CD₃bz-PAASddn data may be that PAASen, PAASHn and PAASddn form small aggregates in solution, which are of substantially different size at the 60-fold different dansyl substituted poly(acrylate)s concentrations studied by fluorescence and ITC (Section 5.6). Thus competition between β-CD₃bz complexing the dansyl substituents and the formation of aggregates of the same substituted poly(acrylate)s may be reflected in the magnitude of the derived \( K_{11} \) as the aggregate size varies.

All of the \( K_{11} \) determined by fluorescence characterizing the β-CDɛ₃bz-PAADSen, β-CDɛ₃bz-PAADShn and β-CDɛ₃bz-PAADSDdn systems are substantially greater than those determined by ITC. This is coincident with the greater flexibility of β-CDɛ₃bz than β-CD₃bz alluded to earlier, which probably superimposes on the factors discussed above for the β-CDɛ₃bz systems. An additional factor which may impinge on the \( K_{11} \) determined by fluorescence characterizing the β-CDɛ₃bz systems being substantially greater than those determined by ITC is the aggregation of β-CDɛ₃bz detected by ITC. This aggregation may be greater at the higher concentrations studied by ITC and compete more with host-guest complexations than is the case at the lower concentrations of the fluorescence studies.
5.5.2. Entropy – Enthalpy Linear Relationship

The thermodynamic data in Table 5.3 may be placed in context with similar data for host-guest complexation by β-CD, mono-substituted β-CD, linked β-CD dimers and the results of trimer complexation in Chapters 2, 3 and 4 with a large variety of guests by plotting $T\Delta S_{11}$ against $\Delta H_{11}$ (Figure 5.10a). The entropy-enthalpy relationship obtained for

![Figure 5.10](image-url)

**Figure 5.10.** (a) Plot of $T\Delta S_{11}$ against $\Delta H_{11}$ for the 1:1 complexes formed by the hosts $\beta$-CD, mono modified $\beta$-CDs, $\beta$-CD dimers, $\beta$-CD$_3$bz and $\beta$-CDen$_3$bz with the guest dyes (Chapter 2), dodecyl (C12) or octadecyl (C18) substituted poly(acrylate)s (Chapter 3), adamantyl (AD) substituted poly(acrylate)s (Chapter 4), together with data for the dansyl substituted poly(acrylate)s of this study. (b) The extracted $\beta$-CD trimers data and linear fitting for all $\beta$-CD trimers data $y = 0.99x + 24.6$, $R^2 = 0.9945$. The fitting for complexation by the $\beta$-CD trimers of dansyl substituted poly(acrylate)s is $y = 1.01x + 22.9$, $R^2 = 0.9973$. 

218
the β-CD₃bz and β-CD₄enz systems of this study and other linked β-CD trimer studies (Chapters 2-4) yield a linear least squares fit of $T\Delta S_{11}$ against $\Delta H_{11}$ of Equation 5.6 at 298.2 K to give $\alpha = 0.99$ and $T\Delta S_{11,0} = 24.6$ kJ mol$^{-1}$, $R^2 = 0.9945$ (Figure 5.10b), while the fitting values for complexation by the β-CD trimers of the dansyl-substituted poly(acrylate)s is $\alpha = 1.01$ and $T\Delta S_{11,0} = 22.9$ kJ mol$^{-1}$. In comparison, the literature values are $\alpha = 0.80$ and $T\Delta S_{11,0} = 11$ kJ mol$^{-1}$ for β-CD, $\alpha = 0.99$ and $T\Delta S_{11,0} = 17$ kJ mol$^{-1}$ for mono-substituted β-CD,$^{45}$ and $\alpha = 0.89$ and $T\Delta S_{11,0} = 23.5$ kJ mol$^{-1}$ for linked β-CD dimers.$^{46}$ The slope ($\alpha$) can be used as a statistical representation for the degree of conformational change, and intercept ($T\Delta S_{11}$) shows the extent of the desolvation effect upon complexation.$^{46}$ The positive $T\Delta S_{11,0}$ values indicate that the host-guest complexes are entropically stabilized at the intercept value where $\Delta H_{11} = 0$ and the corresponding entropy change is $\Delta S_{11,0}$ (Equation 5.8).

$$T\Delta S_{11} = \alpha \Delta H_{11} + T\Delta S_{11,0} \quad (5.8)$$

As discussed in chapters 2-4, a linear relationship between $T\Delta S_{11}$ and $\Delta H_{11}$ has been observed for a range of complexes and is commonly taken to indicate that compensatory variations in the relative importance of structural and solvation changes occur.$^{45-47}$ The new $T\Delta S_{11}$ and $\Delta H_{11}$ data for β-CD₃bz and β-CD₄enz from Table 5.3 are plotted in Figure 5.10a from which it is seen that they fall well within the range of the literature data. Hence, it appears that guest complexation within the β-CD host annuli of β-CD trimers for the six new systems studied largely determines the thermodynamics, irrespective of whether β-CD₃bz or β-CD₄enz is the host involved.

It should be noted that the $T\Delta S_{11}$ and $\Delta H_{11}$ data discussed above may reflect competition between complexation by the β-CD trimers and aggregation into small aggregates and that this may affect the position of these data in the linear relationships.

### 5.6. DYNAMIC LIGHT SCATTERING

Dynamic light scattering experiments were carried out at 298.2 K using a Malvern Nano-ZetaSizer.$^{48}$ The instrument settings were automatically determined by Malvern dispersion technology software.$^{49}$ The solutions were prepared in filtered (0.2 μm) degassed Millipore Milli-Q purified water of either 0.21 wt.% (equal to the concentration used in the ITC studies) or 0.0033 wt.% in PAADSen, 0.0034 wt.% in PAADShn and 0.0035 wt.% in PAADSddn solutions (equal to the concentrations used in fluorescence
studies) for either PAADSen, PAADShn or PAADSddn alone and in the presence of \( \beta \text{-CD}_3 \text{bz} \) or \( \beta \text{-CDen}_3 \text{bz} \) at concentrations equal to the concentrations of dansyl substituents. The typical distribution of hydrodynamic diameters for the PAADSddn/\( \beta \text{-CDen}_3 \text{bz} \) system appears in Figure 5.11. Hydrodynamic diameter distributions for the remaining systems appear in Figures 5.31-5.36 in the Appendix, Section 5.11.4, at the end of this chapter.

Figure 5.11. Hydrodynamic diameter distributions of 0.21 wt.% of dansyl-substituted PAADSddn aggregates (\([\text{dansyl}] = 6.0 \times 10^{-4} \text{ mol dm}^{-3}\)) with \( \beta \text{-CDen}_3 \text{bz} \) in filtered (0.2\( \mu \text{m} \)) Milli-Q purified water at 298.2 K. Mean hydrodynamic diameter = 1.29 ± 0.13, mean distribution width 0.32\( \mu \text{m} \).

Aggregations with a mean hydrodynamic diameter in the region of \( \sim 1 \mu \text{m} \) under the ITC conditions (0.021 wt.%) and \( \sim 0.5 \mu \text{m} \) in fluorescence conditions (0.0033 wt.% in PAADSen, 0.0034 wt.% in PAADShn and 0.0035 wt.% in PAADSddn solutions) exist as is seen in Table 5.4 and Figure 5.12. This indicates a degree of aggregation between polymer strands which is probably dynamic in nature. Thus, for PAADSen, PAADShn and PAADSddn alone the extent of strand aggregation is dependent on the extent of intra- and inter-strand aggregation of the dansyl substituents and their concentration which results in the mean hydrodynamic diameter of the polymers in ITC conditions being about 3 times larger than that for the fluorescence conditions.
Table 5.4. Size of PAADSen, PAADShn and PAADSddn aggregates alone and with β-CD trimers

<table>
<thead>
<tr>
<th>System</th>
<th>Mean diameter (µm)</th>
<th>Mean width (µm)</th>
<th>Mean diameter (µm)</th>
<th>Mean width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At ITC concentration</td>
<td></td>
<td>At FL concentration</td>
<td></td>
</tr>
<tr>
<td>PAADSen</td>
<td>1.49 ± 0.15</td>
<td>0.25</td>
<td>0.57 ± 0.06</td>
<td>0.17</td>
</tr>
<tr>
<td>PAADShn</td>
<td>1.42 ± 0.14</td>
<td>0.26</td>
<td>0.45 ± 0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>PAADSddn</td>
<td>1.51 ± 0.15</td>
<td>0.42</td>
<td>0.53 ± 0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>β-CD₃bz/PAADSen</td>
<td>1.16 ± 0.12</td>
<td>0.38</td>
<td>0.31 ± 0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>β-CD₃bz/PAADShn</td>
<td>0.77 ± 0.08</td>
<td>0.37</td>
<td>0.23 ± 0.02</td>
<td>0.10</td>
</tr>
<tr>
<td>β-CD₃bz/PAADSddn</td>
<td>0.79 ± 0.08</td>
<td>0.40</td>
<td>0.32 ± 0.03</td>
<td>0.13</td>
</tr>
<tr>
<td>β-CD₃enz/PAADSen</td>
<td>1.20 ± 0.12</td>
<td>0.45</td>
<td>0.44 ± 0.04</td>
<td>0.17</td>
</tr>
<tr>
<td>β-CD₃enz/PAADShn</td>
<td>0.95 ± 0.10</td>
<td>0.26</td>
<td>0.56 ± 0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>β-CD₃enz/PAADSddn</td>
<td>1.29 ± 0.13</td>
<td>0.32</td>
<td>0.48 ± 0.05</td>
<td>0.11</td>
</tr>
</tbody>
</table>

a The mean diameter and mean width of second peaks for some systems are as given: β-CD₃bz/PAADSen (1.51 ± 0.15, 0.80 µm), β-CD₃bz/PAADShn (1.03 ± 0.10, 0.42 µm), β-CD₃bz/PAADSddn (1.09 ± 0.11, 0.39 µm)

b The mean width of the hydrodynamic diameter distribution is equal to $[D_{(0.9)} - D_{(0.1)}] / D_{(0.5)}$, where $D_{(0.5)}$ is the diameter where 50% of the distribution is above and 50% is below, $D_{(0.9)}$ is the diameter where 90% of the distribution is below this value and $D_{(0.1)}$ is the diameter where 10% of the distribution is below this value.

Figure 5.12. Mean hydrodynamic diameters of PAADSen, PAADShn and PAADSddn alone and in the presence of β-CD₃bz and β-CD₃enz/bz in degassed water at pH 7.0 and 298.2 K measured by dynamic light scattering.
The number of strands in the PAADSen, PADShn and PAADSddn aggregates are likely to be substantial. Calculations using an average aggregate mass of 250 kD yielded the summations of the volumes of the atoms composing each strand are 52.5, 53.2 and 54.5 nm$^3$, respectively. However, these volumes do not include the hydration sheath of water molecules in direct contact with individual PAADSen, PAADShn and PAADSddn strands. This sheath is composed of two hydrophilic components, one formed by the hydrogen bonding of water to the carboxylate groups and another from water molecules immediately adjacent to the alkyl and dansyl components of the substituted poly(acrylate)s. The hydration sheath is partially protected from hydrogen bonding interactions in the region immediately adjacent to the alkyl group and consequently, exchange with bulk water occurs less rapidly than would otherwise be expected.$^{50,51}$ Dielectric relaxation studies of the hydration of formate, acetate, propanoate and butanoate anions indicate that their carboxylate groups are hydrated by 5-6 water molecules and the methyl, propyl and butyl groups by ~6, ~17 and ~27 water molecules, respectively, in dilute solutions.$^{50,51}$ On the basis of these observations, an estimate of the hydration of the PAADSen, PAADShn and PAADSddn strands may be made by assigning hydration numbers of 11 to each –CH$_2$CO$_2$– unit (5 to the carboxylate group and 6 to the methylene group), 48 to each dansyl group and 12, 36 and 72 to each the hydrophobic hydration shells of the en, hn and ddn tether, respectively. Thus, the volume of the average 250 kD molecular weight strands of PAADSen, PAADShn and PAADSddn and their hydration shells are 329, 460 and 656 nm$^3$, respectively, which compare with the spherical volumes of 1.73 × 10$^9$, 1.51 × 10$^9$ and 1.81 × 10$^9$ nm$^3$ for the PAADSen, PAADShn and PAADSddn aggregates of 1.49, 1.42 and 1.51 μm mean hydrodynamic diameter under the ITC study conditions, respectively. Similarly, for the fluorescence titration study, the spherical volumes of 9.80 × 10$^7$, 4.90 × 10$^7$ and 7.97 × 10$^7$ nm$^3$ were calculated for PAADSen, PAADShn and PAADSddn aggregates of 0.57, 0.45 and 0.53 μm mean hydrodynamic diameter. While these estimates are very approximate, it is clear that a large number of strands are likely to be contained in the aggregates which are probably dominantly composed of water as are the hydrogels discussed in Section 3.5.
Table 5.5. The possible number of polymer strands per aggregate at concentration of samples in ITC condition or in fluorescence condition

<table>
<thead>
<tr>
<th></th>
<th>Under the ITC conditions</th>
<th>Under the fluorescence condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAADSen</td>
<td>~4,500,000</td>
<td>~250,000</td>
</tr>
<tr>
<td>PAADShn</td>
<td>~2,900,000</td>
<td>~95,000</td>
</tr>
<tr>
<td>PAADSddn</td>
<td>~2,500,000</td>
<td>~110,000</td>
</tr>
</tbody>
</table>

If these assumptions are correct, the number of PAADSen strands in an aggregate formed under ITC conditions is about $4.5 \times 10^6$, which is approximately 18 times higher than that of PAADSen strands in an aggregate formed under the fluorescence titration conditions ($\sim 2.5 \times 10^5$) (Table 5.5). Similarly, the number of PAADShn strands in an aggregate formed under ITC conditions is about $2.9 \times 10^6$ approximately 31 times higher than that of PAADShn strands in an aggregate formed under the fluorescence titration conditions ($\sim 9.5 \times 10^4$); the number of PAADSddn strands in an aggregate formed under ITC conditions is about $2.5 \times 10^6$, approximately 22 times higher than that of PAADSddn strands in an aggregate formed under the fluorescence titration conditions ($\sim 1.1 \times 10^5$) (Table 5.5). These differences in the aggregates formed under the ITC conditions compared with those formed under the fluorescence titration conditions may produce some differences in the interaction of the poly(acrylate)s with the $\beta$-CD trimers and the apparent stability constant as well as thermodynamic parameters (See earlier discussion on page 217).

5.7. TIME RESOLVED FLUORESCENCE STUDIES

In previous studies, aqueous solutions of PAADSen, PAADShn and PAADddn in phosphate buffer pH 7.0, $I = 0.10 \text{ mol dm}^{-3}$ showed a bi-exponential decay in fluorescence.\textsuperscript{20} The time dependent fluorescence intensity, $I(t)$, is fitted to Equation 5.9:

\[
I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)
\]

where $A_1$ and $A_2$ are the amplitudes of the decay components assigned to the excited state aggregated and single dansyl substituents, respectively, and $\tau_1$ and $\tau_2$ are the corresponding time constants for the fluorescence decay. The assignment of $\tau_1$ is made on the basis that $\pi-\pi$ and other interactions where $\pi-\pi$ stacking is stereochemically prevented between dansyl substituents in the excited state aggregates will shorten fluorescence decay time constants, as has been shown for pyrene substituents in other polymer systems.\textsuperscript{2-4} The $\tau_2$
for the excited state single dansyl substituents of PAADSen and PAADShn are similar, but that for PAADSddn is twice as long possibly reflecting the greater length of the ddn tether. The longer tether allows greater freedom of motion and a shorter spatial residence time, and thereby a less effective fluorescence quenching and a longer $\tau_2$.

In this study, to ensure consistency with other methods used in this chapter, time-resolved fluorescence measurements were made on 1.0 wt.% substituted poly(acrylate)s solutions where the total dansyl substituent concentration was $2.95 \times 10^{-3}$ mol dm$^{-3}$ in phosphate buffer pH 7.0, $I = 0.10$ mol dm$^{-3}$ and 298.2 K. The time correlated single photon counting (TCSPC) technique was used.$^{52}$ Fluorescence decays were collected using a time-resolved fluorescence spectrometer (Ultrafast System Halcyone). Emission at 540 nm was collected through a double monochromator fitted with a slit with a 1 nm band pass. The instrument-response function of the apparatus had a full-width-at-half-maximum (fwhm) of 1 ns. The acquisition time window had a width of 62.5 ns with 0.05 ns time steps. A cuvette of 0.2 cm path length was used for the time-resolved measurements of the samples. Multi-exponential fits of the time-resolved fluorescence traces were obtained using the Ultrafast System Surface Explorer protocol.$^{53}$

The aqueous solution of PAADSddn in phosphate buffer pH 7.0, $I = 0.10$ mol dm$^{-3}$ and 298.2 K show a bi-exponential decay of fluorescence as shown in Figure 5.13 and similar bi-exponential decays are observed for aqueous solutions of PAADSen, PAADShn shown in Figure 5.37 in Section 5.11.5 (Appendix).

In the presence of the $\beta$-CD trimers, fluorescence time dependence for the PAADSen, PAADShn and PAADShn systems becomes tri-exponential:

$$I(t) = A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3)$$ (5.10)

where the $A_3\exp(-t/\tau_3)$ term in Equation 5.10 arises from the $\beta$-CD$_3$bz.dansyl and $\beta$-CDen$_3$bz.dansyl excited state host-guest complexes. A typical tri-exponential decay of fluorescence observed for the PAADSddn/$\beta$-CD$_3$bz system is shown in Figure 5.14. The exponential decay of fluorescence of other systems are shown in Figure 5.38 and Figure 5.39 in Section 5.11.5 (Appendix). The parameters in Table 5.6 are derived by fitting Equations 5.9 and 5.10, with the previously derived $\tau_1$ and $\tau_2$ as fixed parameters, to the fluorescence time dependencies. The overall fitting error is $\pm15\%$. 
Figure 5.13. Time dependent fluorescence at 540 nm of a 1.0 wt.% aqueous solution of PAADSddn in which the total dansyl substituent concentration is $2.95 \times 10^{-3}$ mol dm$^{-3}$, in phosphate buffer pH = 7.0, $I = 0.10$ mol dm$^{-3}$ at 298.2 K. The solid line represents the best fit of the data to an algorithm for the fluorescence biexponential decay $A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2)$ where $A_1$ and $A_2$ are the amplitudes of the decay components and $\tau_1$ and $\tau_2$ are the corresponding decay time constants. The resolution is 1 ns.

Figure 5.14. Time dependent fluorescence at 540 nm of a 1.0 wt.% aqueous solution of PAADSddn/$\beta$-CD$_{3}$bz in which the total dansyl substituent and $\beta$-CD$_{3}$bz concentration are equal at $2.95 \times 10^{-3}$ mol dm$^{-3}$, in phosphate buffer pH = 7.0, $I = 0.10$ mol dm$^{-3}$ at 298.2 K. The solid line represents the best fit of the data to an algorithm for the fluorescence triexponential decay $A_1\exp(-t/\tau_1) + A_2\exp(-t/\tau_2) + A_3\exp(-t/\tau_3)$ where $A_1$, $A_2$ and $A_3$ are the amplitudes of the decay components and $\tau_1$ and $\tau_2$ are the corresponding decay time constants. The resolution is 1 ns.
Table 5.6. Time dependent fluorescence data

<table>
<thead>
<tr>
<th>System</th>
<th>$A_1$</th>
<th>$\tau_1$ (ns)</th>
<th>$A_2$</th>
<th>$\tau_2$ (ns)</th>
<th>$A_3$</th>
<th>$\tau_3$ (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAADSen</td>
<td>0.89</td>
<td>2.60</td>
<td>0.11</td>
<td>6.62</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PAADShn</td>
<td>0.93</td>
<td>2.91</td>
<td>0.08</td>
<td>7.35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PAADSddn</td>
<td>0.80</td>
<td>3.55</td>
<td>0.20</td>
<td>8.29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAADSen</td>
<td>0.37</td>
<td>2.60</td>
<td>0.12</td>
<td>6.62</td>
<td>0.51</td>
<td>13.37</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAADShn</td>
<td>0.25</td>
<td>2.91</td>
<td>0.25</td>
<td>7.35</td>
<td>0.48</td>
<td>16.40</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAADSddn</td>
<td>0.09</td>
<td>3.58</td>
<td>0.15</td>
<td>8.29</td>
<td>0.75</td>
<td>17.65</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAADSSen</td>
<td>0.08</td>
<td>2.60</td>
<td>0.18</td>
<td>6.62</td>
<td>0.71</td>
<td>17.99</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAADShn</td>
<td>0.14</td>
<td>2.91</td>
<td>0.21</td>
<td>7.35</td>
<td>0.63</td>
<td>17.53</td>
</tr>
<tr>
<td>$\beta$-CD$_3$bz/PAADSddn</td>
<td>0.28</td>
<td>3.55</td>
<td>0.20</td>
<td>8.29</td>
<td>0.51</td>
<td>17.77</td>
</tr>
</tbody>
</table>

The overall error for $\tau_1$, $\tau_2$ and $\tau_3$ is 15%

For the three dansyl substituted poly(acrylate)s systems, a general trend is observed, whereby the time constants of fluorescence decay increase in the sequence: dimerized dansyl group ($\tau_1$) < monomer dansyl group ($\tau_2$) < $\beta$-CD trimers complexed dansyl group ($\tau_3$), consistent with the same trend in $I_{\text{max}}$ determined from the steady-state fluorescence measurements. It is probable that the dominant source of intermolecular fluorescence quenching arises from the interaction between water and the monomer dansyl group, and that this quenching is enhanced by intermolecular $\pi-\pi$ interactions in the dansyl group dimer. In contrast, the interactions between the dansyl group monomer and water is diminished by its complexation in the hydrophobic $\beta$-CD annulus and fluorescence quenching is inhibited.

Tri-exponential dansyl substituent fluorescence time dependences, $\tau_3$, according to Equation 5.9 are also observed in the presence of either $\beta$-CD$_3$bz or $\beta$-CD$_3$bz. There is a slight increase in $\tau_3$ from $\beta$-CD$_3$bz/PAADSSen < $\beta$-CD$_3$bz/PAADShn < $\beta$-CD$_3$bz/PAADSddn, and only a small difference in $\tau_3$ for the $\beta$-CD$_3$bz and dansyl substituted PAAs. The similarity of $\tau_3$ for $\beta$-CD$_3$bz or $\beta$-CD$_3$bz and the dansyl substituted poly(acrylate)s suggest that the excited state dansyl substituent environments are quite similar despite the significantly different stability of these complexes. In general, the two-fold increase in $\tau_3$ for the $\beta$-CD trimer complexed excited state dansyl substituents in comparison with the analogous $\tau_2$ is attributed to a change in the dansyl substituent from...
the aqueous environment to the hydrophobic β-CD annular environment, where quenching is less effective (similar to previous studies).\textsuperscript{20,54-56}

Overall, these time-dependent observations are consistent with the deductions made from the previously discussed \textsuperscript{1}H NMR and fluorescence studies. Unfortunately, the difference between the molar absorbances at the excitation wavelength 400 nm of the mono dansyl, aggregated dansyl and complexed dansyl, as reported previously,\textsuperscript{20} is not reliable for the determination of apparent complexation constants from \(A_1\), \(A_2\), and \(A_3\).

\textbf{5.8. RHEOLOGICAL STUDY}

Host-guest interactions at the macroscopic level between the dansyl substituents with different length on the poly(acrylate)s, PAADSen, PAADShn and PAADSddn, and the linked trimers, β-CD\(_3\)bz and β-CDen\(_3\)bz, are expected to affect the zero-shear viscosities of the solutions. Rheological measurements were carried out with a Physica MCR 501 (Anton Parr GmbH) stress-controlled rheometer with a 25 mm cone and plate geometry. Temperature was controlled at 298.2 K by a Peltier plate. All studies were carried out in 0.10 mol dm\(^{-3}\) aqueous NaCl solution at pH 7.0 adjusted with 0.10 mol dm\(^{-3}\) aqueous NaOH.

![Figure 5.15. Variation of the viscosity of 5.0 wt.% aqueous solutions of PAADSen, PAADShn and PAADSddn (\([\text{dansyl}] = 1.5 \times 10^{-2} \text{ mol dm}^{-3}\)) in the presence of β-CD\(_3\)bz and β-CDen\(_3\)bz at pH 7.0 and [NaCl]= 0.10 mol dm\(^{-3}\) at 298.2 K. The concentration of dansyl substituent and the β-CD groups of either β-CD\(_3\)bz or β-CDen\(_3\)bz are equimolar.](image)
Figure 5.16. Variation of 500 s\(^{-1}\) shear rate viscosities of 5.0 wt.% dansyl-substituted poly(acrylate)s solutions alone or in the presence of either β-CD\(_{3}\)bz or β-CDen\(_{3}\)bz in 0.10 mol dm\(^{-3}\) aqueous NaCl at pH 7.0 and 298.2K.

The viscosity variation with shear rate of aqueous solutions of 5 wt.% PAADSen, PAADShn or PAADSddn in the presence of either β-CD\(_{3}\)bz or β-CDen\(_{3}\)bz (where the mole ratio of the dansyl substituents to either β-CD\(_{3}\)bz or β-CDen\(_{3}\)bz is unity), are shown in Figure 5.15 in which the more viscous PAADSddn/β-CD\(_{3}\)bz and PAADSddn/β-CDen\(_{3}\)bz solutions show small decreases in viscosity with increasing shear rate. The zero shear rate variations are shown in Figure 5.16.

For the dansyl-substituted poly(acrylate)s system alone, results reported in a previous study\(^{20}\) suggest that the PAADSddn (0.081) solutions are much more viscous than the PAADShn (0.016) and PAADSen (0.015) solutions, consistent with the greater length of the ddn tether allowing more aggregation between substituents on adjacent strands (viscosities in Pa.s at a 500 s\(^{-1}\) shear rate are shown in brackets). Upon addition of β-CD\(_{3}\)bz, the viscosities of all four systems increase generally: β-CD\(_{3}\)bz/PAADSen (0.041), β-CD\(_{3}\)bz/PAADShn (0.176) and β-CD\(_{3}\)bz/PAADShn (7.57), consistent with the formation of crosslinks by complexation of the dansyl groups and by complexation of their tethers by the β-CD annuli of β-CD\(_{3}\)bz. Similarly, with the addition of β-CDen\(_{3}\)bz viscosity increases also occur: β-CDen\(_{3}\)bz/PAADSen (0.022), β-CDen\(_{3}\)bz/PAADShn (0.089) and β-CDen\(_{3}\)bz/PAADSddn (3.96) as shown in Figure 5.16. The addition of either β-CD\(_{3}\)bz or β-CDen\(_{3}\)bz increases the viscosity of all six solutions through host-guest complexation of
dansyl substituents on adjacent strands to form inter-strand linkages which are either stronger or more prevalent (or both) than substituent aggregation.

The viscosities of PAADSddn solutions with either β-CD₃bz or β-CD₆n₃bz are much higher than that of PAADShn and PAADSen. This behaviour probably arises due to the corresponding increase in the extent of hydrophobic association between the dansyl substituent tethers and competition with the formation of inter-strand cross-links through dansyl substituent complexation by β-CD groups. Consistent with the results from the fluorescence data, the short tether in PAADSen and the consequent steric hindrance by the poly(acrylate)s backbone could be a factor precluding optimal complexation of the dansyl group by either β-CD₃bz or β-CD₆n₃bz.

The larger viscosities of the β-CD₃bz/(PAADSen-PAADSddn) systems by comparison with the analogous β-CD₆n₃bz systems are probably largely attributable to competition between the aggregation of β-CD₆n₃bz and the formation of inter-strand cross-links, which is absent from the β-CD₃bz systems. This behaviour is also seen in the adamantyl system, described in Chapter 4. Some of the differences in viscosity may also be attributed to the shorter inter-strand cross-links formed, and the decreased flexibility of the networks formed in the β-CD₃bz systems. There may also be differences in the ratios of intra- to inter-strand cross-links which could contribute to the overall effect.

5.9. SUMMARY AND CONCLUSIONS

In this study, six host-guest complex systems of linked β-CD₃bz and β-CD₆n₃bz trimers with 3.0% dansyl randomly substituted poly(acrylate)s with different tethers lengths PAADSen, PAADShn and PAADSddn have been characterized at the molecular and macroscopic levels. The methods used at the molecular level were 2D NOESY ¹H NMR, isothermal titration calorimetry, fluorescence spectroscopy and time-resolved fluorescence, and at the macroscopic level size measurement by dynamic light scattering and rheology. The results showed the presence of dansyl substituents as monomers, intra- molecular aggregates or complexed intra-strand with β-CD trimers in dilute solution (Figure 5.17A and C), and in more concentrated solution, the formation of inter-strand cross links between trimers interacting with multiple strands of the polymer network hydrogels (Figure 5.17B and D).
Figure 5.17. **A.** Intra-strand aggregation of the dansyl groups of the 3% randomly substituted PAADSddn polyacrylate in dilute aqueous solution. **B.** Intra- and inter-strand aggregation of the dansyl groups of PAADSddn polyacrylate in concentrated aqueous solution. **C.** Intra-strand complexation of the dansyl groups and the tethers of a single PAADSddn strand by β-CD₃bz in dilute aqueous solution. **D.** Intra- and inter-strand complexation of the dansyl groups and the tethers of multiple PAADSddn strands by β-CD₃bz in concentrated aqueous solution to form a polyacrylate network and a hydrogel.
An increase in viscosity in the sequence PAADSen < PAADShn < PAADSddn was observed in the presence of either β-CD3bz or β-CDen3bz as complexation of dansyl substituents from adjacent poly(acrylate)s strands allowed formation of inter-strand linkages. For dilute aqueous solutions, steady-state and time-resolved fluorescence and 2D NOESY $^1$H NMR spectroscopy show the dansyl substituents to be complexed within the annulus of β-CD groups of the linked β-CD trimers in 1:1 host-guest complexation, characterized by apparent complexation constants, $K_{11}$, and fluorescence lifetimes for the six systems. Quantitative studies by ITC and fluorescence titration have shown different apparent complexation constants and together with more information from size measurements are consistent with the dynamics of the polymer systems. The cooperativity in dansyl substituent complexation by the three β-CD annuli of linked β-CD trimers results in increased 1:1 complex stability, coincident with the increasing dansyl substituent tether length decreasing poly(acrylate)s backbone steric hindrance.

The $T\Delta S_{11}$ and $\Delta H_{11}$ data characterizing complexation of β-CD3bz and β-CDen3bz with the dansyl substituents of the three dansyl substituted poly(acrylate)s fit well within the linear $T\Delta S_{11}$ and $\Delta H_{11}$ compensation relationship observed for the complexation of a wide range of guest species by β-CD, mono-substituted β-CDs and linked β-CD dimers as well as of dodecyl, octadecyl, and adamantyl substituted poly(acrylate)s by the linked β-CD trimers. This indicates that complexation in the β-CD annulus largely controls the thermodynamics of host-guest complexation within the substituted poly(acrylate)s systems studied. However, it should be noted that the $T\Delta S_{11}$ and $\Delta H_{11}$ data discussed above may reflect competition between complexation by the β-CD trimers and aggregate formation and that this may affect the position of these data in the linear relationships.
SUMMARY AND CONCLUSION OF THIS THESIS

In this thesis, syntheses of two new linked β-CD trimers and characterisation of their host-guest complexes with organic dyes and hydrophobic guests have been demonstrated.

The complexation studies described in Chapter 2 indicated that linked β-CD trimers generally form stronger host-guest complexes with organic dyes (crystal violet (CV⁺) and pyronine B (PB⁺) and zwitterionic rhodamine B (RB)) as compared to β-CD with those dyes, by approximately 20-fold. Using van't Hoff equations and plots for the dependence of equilibrium constants on temperature, the thermodynamic parameters, $\Delta G_{11}^\circ$, $\Delta H_{11}^\circ$ and $T\Delta S_{11}^\circ$, have been quantified.

The research was extended in Chapter 3, 4 and 5 with studies of six host-guest systems of three hosts β-CD, linked β-CD dimers and linked β-CD trimers with dodecyl or octadecyl substituted poly(acrylate)s; twelve host-guest systems of three hosts β-CD and linked β-CD trimers with four adamantyl substituted poly(acrylate)s; and six host-guest systems of two host linked β-CD trimers with three dansyl substituted poly(acrylate)s. Several data sets for the linked β-CD dimers if not available in literature were completed in this research.

From the results obtained in the research covered in this thesis, it can be concluded that host-guest complexation is important for the formation of polymer network hydrogels. First, with significant interaction of the β-CD annulus with alkyl chain substituted to the PAA backbone, the viscosity of the hydrogels of modified poly(acrylate)s with linked β-CD trimers can be 'tuned' when controlling the length of the tether of the substituents. Second, in some cases, the linked β-CD trimers may interact with substituents more effectively than that of linked β-CD dimers which may be due to the star shaped structures of linked β-CD trimers, allowing the formation of host-guest complexes from any direction. Third, the length of the linkers in β-CD trimers can also be useful to control the viscosity of the polymer network hydrogels.

In future studies it would be interesting to see if the polymer hydrogels studied herein could be used as topical drug delivery agents. This would require the determination of their capacity to accommodate a range of drugs, the rate of release of the drugs from the hydrogel, and the variation of the hydrogel viscosity under simulated topical conditions.
5.10. REFERENCES


234


42. MicroCal, 22 Industrial Drive East, Northampton, MA 01060, USA.


48. Malvern Instruments, Worcestershire, UK.

49. Zetasizer Software 6.34, Malvern Instruments, Worcestershire, United Kingdom.


52. *SPC Image*, Becker and Hickl, GmbH. Nahmitzer Damm 30, 12277 Berlin, Germany.

53. *Ultrafast Systems LLC*, 1748 Independence Blvd. Suite G-6, Sarasota, FL 34234, USA


5.11. APPENDIX

5.11.1. 2D NOESY $^1$H NMR (600 MHz) Spectra

Figure 5.18. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of $\beta$-CD$_3$bz ($1.00 \times 10^{-3}$ mol dm$^{-3}$) and 1.0 wt.% in 3% substituted PAADSen [dansyl] = 2.95 $\times$ 10$^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, I = 0.10 mol dm$^{-3}$) at 298.2K. The rectangles A and B enclose the cross-peaks arising from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CD$_3$bz with the dansyl substituent H2-4, 6-8, dansyl substituent methyl (N-CH$_3$) protons of PAADSen, respectively. Above: Schematic representation of the complexation between $\beta$-CD$_3$bz and PAADSen.
Figure 5.19. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of $\beta$-CD$_3$bz (1.00 $\times$ 10$^{-3}$ mol dm$^{-3}$) and 1.0 wt.% in 3% substituted PAADShn [dansyl] = 2.95 $\times$ 10$^{-3}$ mol dm$^{-3}$ in D$_2$O (pD 7.0 phosphate buffer, I = 0.10 mol dm$^{-3}$) at 298.2K. The rectangles A, B and C enclose the cross-peaks arising from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CD$_3$bz with the dansyl substituent H2-4, 6-8, dansyl substituent methyl (N-CH$_3$) and hexyl tether (hn CH$_2$) protons of PAADShn, respectively. Above: Schematic representation of the complexation between $\beta$-CD$_3$bz and PAADShn.
Figure 5.20. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of \( \beta\text{-CD},bz \) (1.00 \( \times \) \( 10^3 \) mol dm\(^{-3} \)) and 1.0 wt.% in 3% substituted PAADSddn [dansyl] = 2.95 \( \times \) \( 10^3 \) mol dm\(^{-3} \) in \( D_2\)O (pD 7.0 phosphate buffer, \( I = 0.10 \) mol dm\(^{-3} \)) at 298.2K. The rectangles A, B and C enclose the cross-peaks arising from interaction between the annular \( \beta\text{-CD} \) H3,5,6 protons of \( \beta\text{-CD},bz \) with the dansyl substituent H2-4, 6-8, dansyl substituent methyl \((N\text{-CH}_3)\) and dodecyl tether \((ddn \text{ CH}_2)\) protons of PAADSddn, respectively. Above: Schematic representation of the complexation between \( \beta\text{-CD},bz \) and PAADSddn.
Figure 5.21. 2D NOESY $^1$H NMR (600 MHz) spectrum (mixing time 300 ms) of $\beta$-CDen$_3$bz ($1.00 \times 10^{-3}$ mol dm$^-3$) and 1.0 wt.% in 3% substituted PAADSddn [dansyl] = $2.95 \times 10^{-3}$ mol dm$^-3$ in D$_2$O (pD 7.0 phosphate buffer, I = 0.10 mol dm$^-3$) at 298.2K. The rectangles A, B and C enclose the cross-peaks arising from interaction between the annular $\beta$-CD H3,5,6 protons of $\beta$-CDen$_3$bz with the dansyl substituent H2-4, 6-8, dansyl substituent methyl (N-CH$_3$) and dodecyl tether (ddn CH$_2$) protons of PAADSddn, respectively. Above: Schematic representation of the complexation between $\beta$-CDen$_3$bz and PAADSddn.
5.11.2. Fluorimetric Titration Data

**Figure 5.22.** Top: Fluorescence increase of a PAADShn solution for which the dansyl substituent concentration is $1.0 \times 10^{-5}$ mol dm$^{-3}$ (corresponding to 0.0034 wt.% in PAADShn) with 25 sequential injections (2 mm$^3$ each) of $\beta$-CD$_3$bz solution ($7.03 \times 10^{-3}$ mol dm$^{-3}$) in phosphate buffer pH 7.0, $I = 0.10$ mol dm$^{-3}$ at 298.2 K. The arrow indicates the increase in fluorescence with each addition of $\beta$-CD$_3$bz solution. Excitation wavelength $\lambda_{ex} = 331$ nm with excitation and emission slit widths of 5nm. Bottom: Fluorescence variation at 505 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation by $\beta$-CD$_3$bz of a dansyl subsituent to data at 0.5 nm intervals over the wavelength range 450-600 nm.
Figure 5.23. Top: Fluorescence increase of a PAADSddn solution for which the dansyl substituent concentration is $1.0 \times 10^{-5}$ mol dm$^{-3}$ (corresponding to 0.0035 wt.% in PAADSddn) with 35 sequential injections (10 mm$^3$ each) of $\beta$-CD$_3$bz solution ($3.51 \times 10^{-4}$ mol dm$^{-3}$) in phosphate buffer pH 7.0, $I = 0.10$ mol dm$^{-3}$ at 298.2 K. The arrow indicates the increase in fluorescence with each addition of $\beta$-CD$_3$bz solution. Excitation wavelength $\lambda_{ex} = 331$ nm with excitation and emission slit widths of 5nm. Bottom: Fluorescence variation at 505 nm, and the line representing the best fit of an algorithm for 1:1 host-guest complexation by $\beta$-CD$_3$bz of a dansyl substituent to data at 0.5 nm intervals over the wavelength range 450-600 nm.
Figure 5.24. Top: Fluorescence increase of a PAADSen solution for which the dansyl substituent concentration is $1.0 \times 10^{-5} \text{ mol dm}^{-3}$ (corresponding to 0.0033 wt.% in PAADSen) with 35 sequential injections (2 mm$^3$ each) of $\beta$-CDen$_3$bz solution ($1.36 \times 10^{-3} \text{ mol dm}^{-3}$) in phosphate buffer pH 7.0, $I = 0.10 \text{ mol dm}^{-3}$ at 298.2 K. The arrow indicates the increase in fluorescence with each addition of $\beta$-CDen$_3$bz solution. Excitation wavelength $\lambda_{ex} = 331 \text{ nm}$ with excitation and emission slit widths of 5nm. Bottom: Fluorescence variation at 505 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation by $\beta$-CDen$_3$bz of a dansyl substituent to data at 0.5 nm intervals over the wavelength range 450-600 nm.
Figure 5.25. Top: Fluorescence increase of a PAADShn solution for which the dansyl substituent concentration is $1.0 \times 10^{-5}$ mol dm$^{-3}$ (corresponding to 0.0034 wt.% in PAADShn) with 30 sequential injections (5 mm$^3$ each) of $\beta$-CDen$_3$bz solution ($3.39 \times 10^{-4}$ mol dm$^{-3}$) in phosphate buffer pH 7.0, $I = 0.10$ mol dm$^{-3}$ at 298.2 K. The arrow indicates the increase in fluorescence with each addition of $\beta$-CDen$_3$bz solution. Excitation wavelength $\lambda_{ex} = 331$ nm with excitation and emission slit widths of 5nm. Bottom: Fluorescence variation at 505 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation by $\beta$-CDen$_3$bz of a dansyl substituent to data at 0.5 nm intervals over the wavelength range 450-600 nm.
Figure 5.26. Top: Fluorescence increase of a PAADSddn solution for which the dansyl substituent concentration is $1.0 \times 10^{-5}$ mol dm$^{-3}$ (corresponding to 0.0035 wt.% in PAADSddn) with 25 sequential injections (5 mm$^3$ each) of $\beta$-CDen$_3$bz solution ($3.39 \times 10^{-4}$ mol dm$^{-3}$) in phosphate buffer pH 7.0, $I = 0.10$ mol dm$^{-3}$ at 298.2 K. The arrow indicates the increase in fluorescence with each addition of $\beta$-CDen$_3$bz solution. Excitation wavelength $\lambda_{ex} = 331$ nm with excitation and emission slit widths of 5nm. Bottom: Fluorescence variation at 505 nm and the line representing the best fit of an algorithm for a 1:1 host-guest complexation by $\beta$-CDen$_3$bz of a dansyl substituent to data at 0.5 nm intervals over the wavelength range 490-600 nm.
5.11.3. Isothermal Titration Calorimetry Data

![ITC data](image)

**Figure 5.27.** ITC data for 0.21 wt.% PAADSen ([DS] = 6.09 × 10^{-4} mol dm^{-3}) with β-CD₃bz (2.11 × 10^{-3} mol dm^{-3}) (or [β-CD] = 6.33 mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of β-CD₃bz solution. The solid curve represents the best fit of an algorithm for the 1:1 β-CD₃bz:DS complexation analogous to that shown in Equation 5.2 which is followed by rapid successive second and third dansyl complexations.
Figure 5.28. ITC data for 0.21 wt.% PAADShn ([dansyl] = 5.99 × 10^{-4} mol dm^{-3}) with β-CD₃bz (2.11 × 10^{-3} mol dm^{-3}) (or [β-CD] = 6.33 mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of β-CD₃bz solution. The solid curve represents the best fit of an algorithm for the 1:1 β-CD₃bz:DS complexation analogous to that shown in Equation 5.2 which is followed by rapid successive second and third dansyl complexations.
Figure 5.29. ITC data for 0.21 wt.% PAADSen ([dansyl] = 6.09 × 10^{-4} mol dm^{-3}) with β-CDen₃bz (2.03 × 10^{-3} mol dm^{-3}) (or β-CD group = 6.09 mol dm^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 mol dm^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot. The bottom section shows the heat evolved with the addition of each aliquot of β-CDen₃bz solution. The solid curve represents the best fit of an algorithm for the 1:1 β-CDen₃bz:DS complexation analogous to that shown in Equation 5.7 which is followed by rapid successive second and third dansyl complexations.
Figure 5.30. ITC data for 0.21wt.% PAADSnh ([dansyl] = 5.99 × 10^{-4} \text{ mol dm}^{-3}) with β-CDen_{3}bz (2.03 × 10^{-3} \text{ mol dm}^{-3}) (or β-CD group = 6.09 \text{ mol dm}^{-3}) in aqueous phosphate buffer at pH 7.0 (I = 0.10 \text{ mol dm}^{-3}) at 298.2 K. The top section shows the raw ITC experimental plot where (a) is the data for dilution of β-CDen_{3}bz and (b) is that for this titratin. The bottom section shows the heat evolved with the addition of each aliquot of β-CDen_{3}bz solution. The solid curve represents the best fit of an algorithm for the 1:1 β-CDen_{3}bz:DS complexation analogous to that shown in Equation 5.7 which is followed by rapid successive second and third dansyl complexations.
5.11.4. Light-Scattering - Hydrodynamic Diameter Distributions

Figure 5.31. Hydrodynamic diameter distributions of 0.21 wt.% of dansyl substituted poly(acrylate)s ([dansyl] = $6.0 \times 10^{-4}$ mol dm$^{-3}$) in filtered (0.2µm) Milli-Q purified water at 298.2 K (a) PAADSen; (b) PAADShn; (c) PAADSddn. Mean hydrodynamic diameter and mean distribution width are given in Table 5.4.
Figure 5.32. Hydrodynamic diameter distributions of 0.21 wt.% of dansyl substituted poly(acrylate)s ([dansyl] = 6.0 × 10⁻⁴ mol dm⁻³) in filtered (0.2µm) Milli-Q purified water at 298.2 K with (a) β-CD₃bz/PAADSen; (b) β-CD₃bz/PAADShn and (c) β-CD₃bz/PAADSddn. Mean hydrodynamic diameter and mean distribution width are given in Table 5.4.
Figure 5.33. Hydrodynamic diameter distributions of 0.21 wt.% of dansyl substituted poly(acrylate)s ([dansyl] = 6.0 × 10^{-4} mol dm^{-3}) in filtered (0.2µm) Milli-Q purified water at 298.2 K with (a) β-CDen₃bz/PAADSen; (b) β-CDen₃bz/PAADSbn and (c) β-CDen₃bz/PAADSddn. Mean hydrodynamic diameter and mean distribution width are appeared in Table 5.4.
Figure 5.34. Hydrodynamic diameter distributions of 0.0035 wt.% of dansyl substituted poly(acrylate)s ([DS] = 1.0 \times 10^{-5} \text{ mol dm}^{-3}) in filtered (0.2\mu m) Milli-Q purified water at 298.2 K alone: (a) PAADS\text{en}; (b) PAADS\text{hn} and (c) PAADS\text{ddn}. Mean hydrodynamic diameter and mean distribution width are given in Table 5.4.
Figure 5.35. Hydrodynamic diameter distributions of 0.0035 wt.% of dansyl substituted poly(acrylates) ([DS] = 1.0 × 10^{-5} mol dm^{-3}) in filtered (0.2µm) Milli-Q purified water at 298.2 K with (a) β-CD_bz/PAADS_{en}; (b) β-CD_bz/PAADS_{hn} and (c) β-CD_bz/PAADS_{ddn}. Mean hydrodynamic diameter and mean distribution width are given in Table 5.4.
Figure 5.36. Hydrodynamic diameter distributions of 0.0035 wt.% of dansyl substituted poly(acrylate)s (\([D_{S}] = 1.0 \times 10^{-5} \text{ mol dm}^{-3}\) in filtered (0.2µm) Milli-Q purified water at 298.2 K with (a) \(\beta-\text{CD}e_{3}\text{bz}/\text{PAADSen}\); (b) \(\beta-\text{CD}e_{3}\text{bz}/\text{PAADShn}\) and (c) \(\beta-\text{CD}e_{3}\text{bz}/\text{PAADSddn}\). Mean hydrodynamic diameter and mean distribution width are given in Table 5.4.
5.11.5. Time Resolved Fluorescence Data

Figure 5.37. Time dependent fluorescence at 540 nm of a 1.0 wt.% aqueous solution of (a) PAADSe, (b) PAADShn and (c) PAADSddn in which the total dansyl substituent concentration is $2.95 \times 10^{-3} \text{ mol dm}^{-3}$, in phosphate buffer pH = 7.0, $I = 0.10 \text{ mol dm}^{-3}$ at 298.2 K. The solid line represents the best fit of the data to an algorithm for the fluorescence biexponential decay $A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2)$ where $A_1$ and $A_2$ are the amplitudes of the decay components and $\tau_1$ and $\tau_2$ are the corresponding decay time constants. The resolution is 1 ns.
Figure 5.38. Time dependent fluorescence at 540 nm of a 1.0 wt.% aqueous solution of (a) PAADSe/β-CD₃bz, (b) PAADShn/β-CD₃bz and (c) PAADSddn/β-CD₃bz in which the total dansyl substituent and β-CD₃bz concentration are equal at 2.95 × 10⁻³ mol dm⁻³, in phosphate buffer pH = 7.0, I = 0.10 mol dm⁻³ at 298.2 K. The solid line represents the best fit of the data to an algorithm for the fluorescence triexponential decay $A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_3 \exp(-t/\tau_3)$ where $A_1$, $A_2$ and $A_3$ are the amplitudes of the decay components and $\tau_1$ and $\tau_2$ are the corresponding decay time constants. The resolution is 1 ns.
Figure 5.39. Time dependent fluorescence at 540 nm of a 1.0 wt.% aqueous solution of (a) PAADSen/β-CDen₃bz, (b) PAADShn/β-CDen₃bz and (c) PAADShdn/β-CDen₃bz in which the dansyl substituent and β-CDen₃bz concentration are equal at 2.95 × 10⁻³ mol dm⁻³, in phosphate buffer pH = 7.0, I = 0.10 mol dm⁻³ at 298.2 K. The solid line represents the best fit of the data to an algorithm for the fluorescence triexponential decay $A₁\exp(-t/\tau₁) + A₂\exp(-t/\tau₂) + A₃\exp(-t/\tau₃)$ where $A₁$, $A₂$ and $A₃$ are the amplitudes of the decay components and $\tau₁$ and $\tau₂$ are the corresponding decay time constants. The resolution is 1 ns.
This page intentionally left blank.
CHAPTER 6

EXPERIMENTAL

6.1. GENERAL

6.1.1. Instrumental

Routine 1D $^1$H and $^{13}$C NMR spectra were recorded on a Varian Gemini ACP-300 spectrometer at 300.145 and 75.4 MHz, respectively, unless otherwise stated. Solutions were prepared in either CDCl$_3$, D$_2$O or DMSO-$d_6$ and chemical shifts were referenced to either tetramethylsilane ($\delta_\text{H} 0.0$ ppm) and CDCl$_3$ ($\delta_\text{C} 77.0$ ppm) in CDCl$_3$, the residual solvent peak ($\delta_\text{H} 2.49$ ppm and $\delta_\text{C} 39.5$ ppm) in DMSO-$d_6$ or an external trimethylsilylpropiosulfonic acid in D$_2$O ($\delta$$_{\text{HOD}}$ 4.79 ppm). Chemical shifts are cited on the $\delta$ scale in parts per million, ppm, followed by multiplicity and assignment. The following abbreviations singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) refer to the multiplicity of the NMR resonances.

The 2D ROESY and NOESY $^1$H NMR spectra were recorded on a Varian Inova 600 (599.957 MHz) spectrometer, using a standard sequence with a mixing time of 300 ms.

Thin layer chromatography (TLC) was carried out on Merck Kieselgel 60 F$_{254}$ aluminium-backed sheets. For analysis of cyclodextrin derivatives, plates were developed with 7:7:5:4 v/v ethyl acetate/propan-2-ol/ammonium hydroxide/water. Compounds were visualised by drying the plate, dipping it into a 1% sulfuric acid in ethanol solution and heating it with a heat gun. To visualise amino bearing cyclodextrins, plates were dried prior to dipping into 0.5% ninhydrin in ethanol and heated with a heat-gun before dipping in 1% sulphuric acid in ethanol. For the modified cyclodextrins, whose preparations are described below, $R_c$ represents the $R_f$ of a substituted cyclodextrin relative to the $R_f$ of the parent cyclodextrin.

MALDI TOF mass spectra were acquired using a Bruker Ultrafle Xtreme MALDI TOF/TOF mass spectrometer (Bruker Daltonik GmbH) operating in linear mode under the control of Flex Control software (Version 3.3, Bruker Daltonik GmbH). External calibration was carried out using peptide standards (Bruker Daltonik GmbH), over a range from 800 to 4500 D, which was analysed under the same conditions as that of the sample. Spectra were obtained at various locations over the surface of the matrix spot at an intensity determined by the operator. The sample was dissolved in 1 cm$^3$ water and diluted to 100 µg/cm$^3$ with aqueous 0.1 % trifluoroacetic acid (TFA) solution. A 1 mm$^3$ portion was mixed with 1 mm$^3$ of sinapinic acid in water/acetonitrile/TFA (V/V/V) (10/90/0.1)
solution and 1 mm$^3$ of this mixture was applied to an 800 μm Anchor Chip target plate (Bruker Daltonik GmbH, Bremen, Germany) and air dried. Analysis was performed in both positive and negative ion mode. The MS spectra obtained were analysed using flex Analysis software (version 3.3, Bruker Daltonik GmbH) employing smoothing, background subtraction and peak detection algorithms.

Elemental analyses were performed by the Microanalytical Service of the Chemistry Department, University of Otago, New Zealand. Since cyclodextrin derivatives contain associated water molecules, fractional numbers of water molecules were added to the molecular formula to give the best fit to the micro analytical data.

Isothermal titration calorimetry (ITC) measurements were made with a MicroCal VP isothermal titration calorimeter. Solutions were prepared in aqueous phosphate buffer at pH 7.0 and $I = 0.10 \text{ mol dm}^{-3}$ and were degassed and thermostated at 298.2 ± 0.1 K immediately prior to titration. The initial cell volume was 1.46 cm$^3$. The concentration correction for displaced volume effects which occur with each injection (10 mm$^3$) were calculated by Origin 7.0 MicroCal protocol.$^1$ In each case, 1.46 cm$^3$ volume of the substituted poly(acrylate)s were titrated by adding 10 mm$^3$ aliquots of either β-CD, β-CD$_3$bz or β-CD$_n$bz solutions (whose concentrations appear in the figure captions of each of the titrations shown in each Chapter) from a computer-controlled micro-syringe at intervals of 210s.

UV-visible absorbance spectra were recorded using a Varian CARY 5000 UV-VIS-NIR spectrophotometer equipped with matched 1.0 cm path length quartz cells over a range of 400-700 nm wavelengths at 0.5 nm intervals. Each solution was run against a reference solution containing all components of the solution of interest except the absorbing species. Solutions were pre-equilibrated at 298.2 ± 0.2 K, unless otherwise stated, and maintained at this temperature during measurement using a thermostated cell block.

Fluorescence measurements were recorded using a Varian CARY Eclipse spectro fluorimeter equipped with a 1.0 cm path length quartz cell. Spectra were obtained over a range of desired wavelengths at 0.5 nm intervals, with both excitation and emission slit widths of 5 nm (unless stated otherwise), using a 1% transmittance emission filter. Emission spectra obtained were not corrected for instrumental factors. Solutions were pre-
equilibrated at 298.2 ± 0.2 K and maintained at this temperature during measurement by means of a thermostated cell block.

Time-resolved fluorescence measurements were made on 1.0 wt.% substituted poly(acrylate)s solutions where the total dansyl substituent concentration was 2.95 ×10⁻³ mol dm⁻³ in phosphate buffer at pH 7.0, I = 0.10 mol dm⁻³ and 298.2 K using the time correlated single photon counting (TCSPC) technique.² The laser source was a modelocked Ti-sapphire laser (Spectra Physics Tsunami), tunable from 720 to 1000 nm with a repetition rate of 80 MHz. The fundamental output from the Ti-sapphire oscillator was modulated by a Pockels cell (Model 350-160, Conoptics Inc) to reduce the repetition rate to about 16 MHz and was subsequently frequency doubled by focusing tightly into a 0.5-mm BBO crystal (Eksma Optics). The resulting blue light, which had a central wavelength of 400 nm, provided the excitation source. A half-wave plate before a vertical polarizer ensured the polarization of the excitation light. The fluorescence decays were collected using a time-resolved fluorescence spectrometer (Ultrafast System Halcyone). Emission at 540 nm was collected through a double monochromator fitted with a slit with a 1 nm band pass. The instrument–response function of the apparatus had a full-width-at-half-maximum of 1 ns. The acquisition time window had a width of 62.5 ns with 0.05 ns time steps. A cuvette of 0.2-cm path length was used for the time-resolved measurements of the samples. Multi-exponential fits of the time-resolved fluorescence traces were obtained using the Ultrafast System Surface Explorer protocol.³

Dynamic light scattering experiments to measure hydrodynamic diameter were determined at 298.2 K using a Malvern Nano-ZetaSizer.⁴ The instrument settings were automatically determined by Malvern dispersion technology software.⁵ In the particle size measurements, a solution of either 0.3 wt.% of a polymer was measured in the absence and presence of β-CD or modified β-CD. Samples were prepared in filtered (0.2 μm) Millipore Milli-Q purified water and were degassed immediately prior to measurement. The particle sizes are shown as hydrodynamic diameters (μm).

Rheological measurements were carried out at the State Key Laboratory of Chemical Engineering, East China University of Science and Technology, Shanghai, China using a Physica MCR 501 (Anton Parr GmbH) stress-controlled rheometer with a 25 mm cone and plate geometry. Temperature was controlled at 298.2 ± 0.1 K by a Peltier plate. Rheological samples were prepared by dissolution of the poly(acrylate)s in 0.10 mol dm⁻³
aqueous sodium chloride to ensure screening of the electrostatic interactions between the
carboxylate groups. The solution pH was adjusted to 7.0 with 0.10 mol dm$^{-3}$ aqueous
sodium hydroxide solution.

6.1.2. Materials

Squat column chromatography was carried out using Merck Kieselgel 60 F$_{254}$ thin layer
chromatography silica. Bio-Rex 70 resin was purchased from Bio-Rad Laboratories Inc.,
USA and was converted to the acid form using 3.0 mol dm$^{-3}$ hydrochloric acid. Diaion HP-
20 resin was purchased from Supelco.

Deionised water was prepared using a Milli-Q system to give a resistivity of $> 15$
MΩcm. An aqueous phosphate buffer (pH 7.0 and ionic strength 0.10 mol dm$^{-3}$) was
prepared from Na$_2$PO$_4$ (BDH) and KH$_2$PO$_4$ (Ajax) as describe in the literature.$^6$

Organic solvents: N,N-dimethylformamide (DMF) was obtained from Merck; diethyl
etherdichloromethane (DCM), tetrahydrofuran(THF), ethyl acetate and acetone from Chem
Supply; pyridine, ethanol and methanol from Ajax; N-methylpyrrolidin-2-one (NMP) from
Fluka, were of HPLC grade and were used without further purification.

β-Cyclodextrin (β-CD) was obtained from Nihon Shokuhin Kako Co. Hydrogen
peroxide was obtained from Chem-Supply, sodium hydroxide was obtained from Ajax,
1,2-diamino ethane (en), 1,6-diamino hexane (hn), 1,12-diamino-dodecane (ddn) were
obtained from Strem Chemicals; D$_2$O, d$_6$-DMSO, CDCl$_3$ were obtained from Cambridge
Isotope Laboratory. 4-Nitrophenol and 98%, 1,3,5-benzenetricarbonyltriclorid
was obtained from Sigma. Crystal Violet (CV$^+$) as the chloride salt (95%, BDH), Rhodamine B
(RB) as the chloride salt (95%, Sigma) and Pyronine B (PB$^+$) as (PB)$_2$Fe$_2$Cl$_8$ salt (95%,
Sigma) were twice recrystallised from water and dried to constant weight under high
vacuum prior to use. Poly(acrylic acid), PAA, ($M_w=250,000$, $M_w/M_n \approx 2$) 35 wt.% aqueous
solution (Sigma) was diluted to approximately 10 wt.% and freeze-dried to constant weight
to give a white solid. Other reagents used were obtained from Aldrich and were not further
purified before use unless otherwise stated.

1-Amino-adamantane, ADNH$_2$, was obtained by neutralizing ADNH$_2$HCl (Aldrich)
with NaOH followed by extraction into dichloromethane. 6$^\Lambda$-O-(4-methyl-
benzenesulfonyl)-β-cyclodextrin (β-CDtos),$^7$ 6$^\Lambda$-amino-6$^\Lambda$-deoxy-β-cyclodextrin

265
(β-CDNH₂)⁸ 6⁴-(2-aminoethyl)amino-6⁴-deoxy-β-cyclodextrin (β-CDen),⁹ N,N’-bis(6⁴-deoxy-β-cyclodextrin-6⁴-yl)urea (β-CD₂ur)¹⁰ and N,N’-bis(6⁴-deoxy-β-cyclodextrin-6⁴-yl)-sucinamide (β-CD₂su)¹⁰ were prepared as previously described. The modified cyclodextrins were dried to a constant weight over P₂O₅ and stored in the dark under refrigeration.

The 3% dodecyl and octadecyl substituted poly(acrylate)s, PAAC12 and PAAC18, were prepared and characterized as previously described.¹¹,¹² The preparation of 3.0% randomly substituted onto the poly(acrylate)s backbone through an amide tether in 1-amino-adamantane-poly(acrylate)s, 1-(2-aminoethyl)-adamantane-1-carboxamide poly(acrylate)s, 1-(6-aminohexyl)-adamantane-1-carboxamide poly(acrylate)s and 1-(12-aminododecyl)-adamantane-1-carboxamide poly(acrylate)s, PAAAD, PAAADen, PAAADhn and PAAADddn were prepared as previously described.¹³-¹⁵ The 3% randomly substituted with either N-(2-aminoethyl)-, N-(6-aminohexyl)-5 or N-(12-aminododecyl)-5-dansyl-sulfonamide, PAADSen, PAADShn and PAADSddn were prepared by literature methods.¹⁶

6.2. EXPERIMENTAL

6.2.1. Syntheses

6.2.1.1. Preparation of 1,3,5-trinitrophenyl-benzene

![Figure 6.1. Synthesis of 1,3,5-trinitrophenyl-benzene.](image)

4-Nitrophenol (0.917g, 6.60 mmol) was dissolved in dichloromethane (DCM) (60 cm³) in a 250 cm³ round bottom flask after which 1,3,5-benzenetricarbonyltrichloride (0.5 g, 1.885 mmol) was added. The mixture was cooled to 0°C and triethylamine (TEA) (0.8 cm³, 5.65 mmol) was added dropwise over 15 min. The mixture was stirred for 3h at room temperature under N₂. The product precipitated out and was filtered off, recrystallized from DCM, washed with water and dried to a pale yellow solid. (Figure 6.1). Yield: 885 mg
(82%). $^1$H NMR: $\delta$ (DMSO-d$_6$) 9.12 (s, 3H, ArH); 8.46 (d, 6H, $J = 9.1$ Hz, 4NP H); 7.79 (d, 6H, $J = 9.1$ Hz, 4NP H). The $^1$H NMR (300 MHz) spectrum of 1,3,5-trinitrophenyl benzene is show in Figure 6.2.

![Figure 6.2. $^1$H NMR (300 MHz) spectrum of 1,3,5 trinitrophenyl benzene in $d^6$ dimethyl-sulfoxide.](image)

### 6.2.1.2. Preparation of 1,3,5-$N,N,N$-tris(6$^A$-deoxy-$6^A$-β-cyclodextrin)-benzene, β-CD$_3$bz.

![Figure 6.3. Synthesis of 1,3,5-$N,N,N$-tris(6$^A$-deoxy-$6^A$-β-cyclodextrin)-benzene, β-CD$_3$bz.](image)

6$^A$-Amino-6$^A$-deoxy-β-cyclodextrin (500 mg, 0.44 mmol) was dissolved in pyridine (20 cm$^3$) and stirred at room temperature for 15 min. 1,3,5-Trinitrophenylbenzene (72 mg, 0.125 mmol) was added to this solution with stirring over 1 h. The reaction mixture was stirred for a further 48 h at room temperature before being added dropwise to acetone (200 cm$^3$) with vigorous stirring. The resultant precipitate was collected by centrifugation, washed with acetone and diethylether and dried under vacuum. The product was dissolved in water and run down a BioRex 70 (H$^+$) column. The cream solid product was obtained by freeze drying the collected aqueous fractions containing the product followed by further drying over P$_2$O$_5$. Yield: 328 mg (68%).

TLC: $R_c = 0.39$. 

---
$^1$H NMR, $\delta_H (D_2O)$: 8.34 (s,3H, ArH); 5.16-5.08 (m, 21H, $\beta$-CD H1); 4.06-3.42 (m, 126H, $\beta$-CD H2-H6).

$^{13}$C NMR: $\delta_C(D_2O)$ 171.54 (amide C=O), 137.44 (Ar C-C=O), 131.80 (Ar C-H), 104.37 (C1), 85.94 (C4$^A$), 83.75-83.50 (C2-4), 75.72-74.17 (C2, C3, C5), 72.78 (C5$^A$), 62.97-62.58 (C6), 43.57 (C6$^A$).


Elemental analysis: C$_{135}$H$_{213}$N$_3$O$_{10.5}$2.2H$_2$O: C, 40.1; H, 6.6; N, 1.1. Found: C, 40.2; H, 6.5; N, 1.2.

The $^1$H NMR, $^{13}$C NMR (300 MHz) and MALDI mass spectra of $\beta$-CD$_3$bz are shown in Figure 6.4-Figure 6.7.

**Figure 6.4.** $^1$H NMR spectrum of $\beta$-CD$_3$bz in D$_2$O.

**Figure 6.5.** $^{13}$C NMR spectrum of $\beta$-CD$_3$bz in D$_2$O.
**Figure 6.6.** MALDI mass spectrum of $\beta$-CD$_3$bz, positive ion mode, singly charged species 3558.173 [M+H]$^+$, 3580.151 [M+Na]$^+$ and 3596.132 [M+K]$^+$.

**Figure 6.7.** MALDI mass spectrum of $\beta$-CD$_3$bz, negative ion mode, doubly charged species 1777.564 [(M-2H)$^2$].

### 6.2.1.3. Preparation of 1,3,5-$N,N,N$-tris(6$^A$-(2-aminoethyl)amino)-6$^A$-deoxy-6$^A$-$\beta$-cyclodextrin)-benzene, $\beta$-CDen$_3$bz

6$^A$-(1-(2-Aminoethyl)amino)-6$^A$-deoxy-$\beta$-cyclodextrin (500 mg, 0.425 mmol) was dissolved in pyridine (20 cm$^3$) and stirred at room temperature for 15 min. 1,3,5-trinitrophenylbenzene (72 mg, 0.125 mmol) was added to this solution with stirring over 1h. The reaction mixture was stirred for a further 48 h at room temperature before being added drop-wise to acetone (200 cm$^3$) with vigorous stirring. The resultant precipitate was collected by centrifugation, washed with acetone and diethylether and dried under vacuum.
The product was dissolved in water and run down a BioRex 70 (H⁺) column. The cream solid product was obtained by freeze drying the collected aqueous fractions containing the product followed by further drying over P₂O₅. (Figure 6.8).

Yield: 300 mg (65%).

TLC: \( R_c = 0.41 \). \(^1\)H NMR, \( \delta_H(D_2O): 8.36\) (s, 3H, Ar H); \( 5.15\text{--}5.08\) (m, 21H, \( \beta\)-CD H1); \( 4.06\text{--}3.42\) (m, 126H, \( \beta\)-CD H2-H6); \( 3.12\text{--}2.84\) (m, 12H, en H).

\(^1^3\)C NMR: \( \delta_C(D_2O)\) 168.76 (amide C=O), 134.77 (Ar C-C=O), 128.90 (Ar C-H), 101.71 (C1), 83.36 (C4\(^\Lambda\)), 81.13-80.79 (C2-4), 72.92-71.67 (C2, C3, C5), 70.16 (C5\(^\Lambda\)), 60.32-60.15 (C6), 48.79 (C-en), 47.42 (C6\(^\Lambda\)), 39.09 (C-en).

MALDI Mass spectrum m/z: 3686.159 \([\text{M+H}]^+\), 3708.148 \([\text{M+Na}]^+\), 3724.117 \([\text{M+K}]^+\) and 3684.259 \([\text{M-H}]^-\).

Elemental analysis: C\(_{141}\)H\(_{228}\)N\(_6\)O\(_{135}\).20H\(_2O\): C, 41.8; H, 6.6; N, 2.1. Found: C, 41.5; H, 6.5; N, 2.2. The \(^1\)H NMR, \(^1^3\)C NMR (300 MHz) and MALDI Mass spectrum spectra of \( \beta\)-CD\(_{3}\)bz are shown in Figure 6.9-Figure 6.11.

\[\text{Figure 6.9.} \quad ^1\text{H NMR spectrum of } \beta\text{-CD}_{3}\text{bz in } D_2O.\]

\[\text{Figure 6.10.} \quad ^{13}\text{C NMR spectrum of } \beta\text{-CD}_{3}\text{bz in } D_2O.\]
Figure 6.10. Mass spectrum of $\beta$-CDen$_3$bz, positive ion mode, singly charged species 3686.159 [M+H]$^+$, 3708.148 [M+Na]$^+$ and 3724.117 [M+K]$^+$. 

Figure 6.11. Mass spectrum of $\beta$-CDen$_3$bz, negative ion mode, singly charged species 3684.259 [M-H]$^-$. 

6.2.1.4. General method for preparation of 3% randomly substituted poly(acrylate)s

Poly(acrylic acid) 35 wt.% solution in water was diluted to 10 wt.% and freeze-dried to give poly(acrylic acid) as a white solid. The substituted poly(acrylate)s were synthesized by the literature method.$^{13,17}$ Poly(acrylic acid) (1.5g, 20.83 mmol of -COOH groups) were dissolved in 1-methylpyrrolidin-2-one (NMP) (50 cm$^3$) at 60°C and stirred for 24 hours. The substituents (0.625 mmol for 3% substitution) in NMP (5.0 cm$^3$) were added drop-wise followed by dicyclohexylidimide (0.8125 mmol) in NMP (5.0 cm$^3$). The reaction mixture was stirred at 60°-70° C for at least 48 hrs. The reaction mixture was cooled to room temperature and diluted with 40% w/w sodium hydroxide solution (50 cm$^3$). After vigorous shaking for 15-20 minutes, the precipitated crude substituted sodium poly(acrylate)s was collected by vacuum filtration. This was followed by washing with 30
cm$^3$ of NMP at 60 °C (three times) and repeated washing with 100 cm$^3$ of methanol until the solid became white. The crude product was dissolved in water (10 cm$^3$) and added drop-wise to form a precipitate in methanol (100 cm$^3$) (twice). The substituted sodium poly(acrylate)s were then dissolved in water (30 cm$^3$) and dialysed (cut-off 3500 g/mol) against deionised water for at least 4 days until the conductivity of the water outside the dialysis tube remained constant. The final substituted sodium poly(acrylate)s products were filtered and isolated after freeze-drying from aqueous solution. The degree of substitution degree (3.0 ± 0.1 %) was determined by NMR spectroscopy.

6.2.2. Sample preparation

6.2.2.1. Preparation of solution for 2D ROESY/NOESY $^1$H NMR (600MHz) Studies

Solutions for 2D ROESY $^1$H NMR were prepared in D$_2$O (pD 7.0 phosphate buffer, $I = 0.10$ mol dm$^{-3}$) at 298.2K. 1 cm$^3$ of each sample was added to a test tube containing the appropriate amount (2.0 × 10$^{-3}$ mol dm$^{-3}$) of either CV$, RB$, PB$^+$ and either 6.0 × 10$^{-3}$ mol dm$^{-3}$ β-CD or 2.0 × 10$^{-3}$ mol dm$^{-3}$ β-CD$_3$bz or β-CDen$_3$bz. When all of the solids were dissolved, a portion of each solutions was transferred to a 5 mm NMR tube.

Solutions for 2D NOESY $^1$H NMR host-guest complexation studies in D$_2$O (pD 7.0, phosphate buffer, $I = 0.10$ mol dm$^{-3}$) contained substituted poly(acrylate) (3.0 × 10$^{-3}$ mol dm$^{-3}$) and β-CD trimers (1.0 × 10$^{-3}$ mol dm$^{-3}$) in 1 cm$^3$ D$_2$O such that the β-CD groups and substituents (dodecyl, octadecyl, adamantyl and dansyl) of the substituted poly(acrylate)s were equimolar. Solutions were allowed to equilibrate at the thermostated probe temperature of 298.2 ± 0.1 K for 30 min in 5 mm NMR tubes prior to recording their spectra. The concentration of substituents (dodecyl, octadecyl, adamantyl and dansyl) are indicated in the Figure captions in chapters 3,4,5.

6.2.2.2. Preparation of solutions for UV-Vis titrations (Chapter 2)

All stock solutions were freshly prepared in aqueous phosphate buffer at (pH 7.0, $I = 0.10$ mol dm$^{-3}$). Solutions of the CV$^+$ (2.0 × 10$^{-5}$ mol dm$^{-3}$), RB (2.0 × 10$^{-5}$ mol dm$^{-3}$), PB$^+$ (2.0 × 10$^{-5}$ mol dm$^{-3}$) were prepared then diluted to 5.0 × 10$^{-6}$ mol dm$^{-3}$ with the hosts β-CD (1.41 × 10$^{-2}$ mol dm$^{-3}$), β-CD (2.81 × 10$^{-3}$ mol dm$^{-3}$) and β-CDen$_3$bz (1.36 × 10$^{-2}$ mol dm$^{-3}$). Samples was analysed at four different temperatures ranging from 278.2K to 308.2K in 10K increments.
6.2.2.3. Preparation of solution for isothermal titration calorimetry (Chapter 3,4)

Stock solutions of either β-CD, β-CD₃bz or β-CDen₃bz and substituted poly(acrylate)s were prepared in aqueous phosphate buffer at pH 7.0, \( I = 0.10 \) mol dm\(^{-3}\) with concentrations varied over wide ranges, as indicated in the Figure captions. 1.46 cm\(^3\) of each of the substituted poly(acrylate)s were titrated by adding 10 mm\(^3\) aliquots of either β-CD, β-CD₃bz or β-CDen₃bz solutions, whose concentrations also appear in the Figure captions, from a computer-controlled micro-syringe at intervals of 210s. The concentration correction for displaced volume effects which occur with each injection (10 mm\(^3\)) were calculated by Origin 7.0 MicroCal protocol.\(^1\)

6.2.2.4. Preparation of solution for fluorescence titrations (Chapter 5)

Fluorescence spectra were recorded for 0.0033 wt.% PAADSen, 0.0034 wt.% PAADShn and 0.0035 wt.% PAADSddn solutions, respectively. The calculated concentrations of DSen, DShn and DSddn substituents are 1.0 \( \times \) 10\(^{-5}\) mol dm\(^{-3}\) in phosphate buffer (pH 7.0, \( I = 0.10 \) mol dm\(^{-3}\)) at 298.2 K. Variation in the fluorescence spectra were monitored as the above solutions were sequentially diluted with 2 mm\(^3\) or 10 mm\(^3\) aliquots of β-CD trimers, as described in Figure captions, over the range 450-600nm in 0.5 nm intervals.

6.2.2.5. Preparation of solutions for dynamic light scattering (Chapter 3 and 5)

In Chapter 3, the calculated concentrations of C12 and C18 substituents are 9.15 \( \times \) 10\(^{-4}\) mol dm\(^{-3}\) and 8.92 \( \times \) 10\(^{-4}\) mol dm\(^{-3}\), respectively. Solutions of 0.3 wt.% of either PAAC12 or PAAC18 were prepared alone or in the presence of native β-CD and β-CD substituents of β-CD₂ur, β-CD₂su, β-CD₃bz and β-CDen₃bz with concentrations equal to the concentrations of C12 and C18 substituents in PAAC12 and PAAC18, respectively. All stock solutions were prepared in filtered (0.2 μm) Millipore Milli-Q purified water and were degassed immediately prior to measurement.

Similarly, in Chapter 5, a solution of 0.21 wt.% of either PAADSen, PAADShn or PAADSddn ([dansyl] = 6.0 \( \times \) 10\(^{-4}\) mol dm\(^{-3}\)) was prepared alone or in the presence of β-CD₃bz or β-CDen₃bz concentrations equal to the concentrations of dansyl substituents.
6.3. REFERENCES

1. MicroCal, 22 Industrial Drive East, Northampton, MA 01060, USA.
4. Malvern Instruments, Worcestershire, UK.
5. Zetasizer Software 6.3.4, Malvern Instruments, Worcestershire, United Kingdom.

This page intentionally left blank

NOTE:
This publication is included on pages 277 - 284 in the print copy of the thesis held in the University of Adelaide Library.

It is also available online to authorised users at:

http://dx.doi.org/10.1071/CH13172
Host-guest chemistry of linked β-cyclodextrin trimers and adamantyl substituted poly(acrylate)s in aqueous solution†

Hanh-Trang Nguyen,a Duc-Truc Pham,a Stephen F. Lincoln,a Jie Wang,b Xuhong Guo,b Christopher J. Eastonc and Robert K. Prud‘hommebd

1H NMR spectroscopy, isothermal titration calorimetry and rheological studies show that the β-cyclodextrin (β-CD) groups of two linked β-cyclodextrin trimers, β-CD-bz and β-CD-en-bz complex the adamantyl substituents and their ethers in 3.0 ± 0.1% substituted poly(acrylate)s to form intra- and inter-polymer (acrylate) strand cross-links in aqueous solutions. The structures of the linked-β-cyclodextrin trimers and the length of the tether between the adamantyl substituent and the poly(acrylate) backbone have substantial effects on the complexation constant, K, and the associated thermodynamic parameters. This is partially shown for the complexation by β-CD-bz of the adamantyl substituents as tether length varies from β-CO(NH-)(3.45 × 10⁶) through β-CO(NH(C₂H₅)₉-NHCO- where n = 2 (2.09 × 10⁶), 6 (3.17 × 10⁶) or 12 (7.46 × 10⁶) at 0.13–0.37 w/w % substituted poly(acrylate) solutions and the figures in brackets are the K in dm³ mol⁻¹ at 298.2 K. For the same sequence of substituted poly(acrylate) the variation of viscosity is 0.03, 3.78, 3.48, and 2.03 Pa s⁻¹ at 500 s⁻¹ shear rate at 298.2 K for 5.0 w/w % substituted poly(acrylate) solutions in which the β-CD groups of β-CD-bz and the adamantyl substituents are equimolar at 1.5 × 10⁻⁵ mol dm⁻³. The eight data sets for the β-CD-bz and β-CD-en-bz systems are discussed in terms of host-guest interactions between the host-β-CD groups and the guest adamantyl substituents of the substituted poly(acrylate)s and are compared with those for the analogous β-CD systems.

Introduction

Hydrophobic interactions between host and guest substituents to form host-guest complexes in aqueous polymer systems substantially influence interactions within and between polymer strands.1-3 Understanding these interactions at the molecular level gives insight into polymer network and hydrogel formation at the macroscopic level. In turn, this promises practical outcomes as such hydrogels are potentially deployable in a range of applications including drug delivery,4-8 material science,9 electro-optics10 and tissue engineering.11

In previous studies we have examined the formation of hydrogels formed by poly(acrylate)s bearing hydrophobic substituents which aggregate to produce hydrogel networks,12-14 the complexation of their hydrophobic substituents (the guests) by the hosts α-, β-, and γ-cyclodextrin (α-CD, β-CD, and γ-CD) to form host-guest complexes,15-18 poly(acrylate)s bearing hydrophobic substituents which are complexed by α-CD and β-CD substituents on a second poly(acrylate) to form hydrogels,18-23 and poly(acrylic)s bearing hydrophobic substituents which are complexed by linked β-CD and γ-CD dimers to form hydrogels.20,21 These studies, together with those reported by others,13,14,16-18 have substantially advanced the understanding of hydrogel formation.

In this study we seek to establish the thermodynamic pattern in host-guest complexation in selected substituted poly(acrylate) systems in dilute aqueous solutions and to establish the extent to which this pattern imposes on hydrogel formation in more concentrated solutions. We have selected as the host species β-cyclodextrin, β-CD, and the linked β-CD trimers, 1,3,5-N,N',N'-tris(6'-deoxy-6'-β-cyclodextrin)-benzene, β-CD-bz, and 1,3,5-N,N',N'-tris(6'-2-aminoethylamino-6'-deoxy-6'-β-cyclodextrin)-benzene, β-CD-en-bz, in which three β-CDs are attached through the C6' carbon of the substituted α-glucopyranose unit either through an amido link or through a longer 2-aminoethylamido link to the C1,3,5 sites of benzene (Fig. 1). As the guest species we have selected the hydrophobic adamantyl group which complexes strongly in the annulus of β-CD.
Hanh-Trang Nguyen

Appendix

Paper

Polymer Chemistry

formation of hydrogel networks in more concentrated aqueous solutions characterized by rheological studies.

Experimental

Materials

1,3,5-Benzene tricarbonyl chloride (Sigma), 4-nitrophenol (Sigma) and β-CD (Nihon Shokuhin Kako Co.) were used as supplied. Poly(acrylic acid) (Mw = 250 000, Mw/Mn ~ 2) was employed. Aldrich as a 35 wt% aqueous solution and freeze-dried to a constant weight. Adamantyl 3.0 ± 0.1% substituted poly(acrylate)s, 6'-amino-6'-deoxy-β-cyclodextrin (6'-CD-β-D)18 and 6'-[1-(2-aminoethyl)amino]-6'-deoxy-β-cyclodextrin (6'-CD-β-D)19 were prepared in a similar manner to that previously described.

Preparation of 1,3,5-trinitrophenyl-benzene

4-Nitrophenol (0.917 g, 6.60 mmol) was dissolved in dichloromethane (DCM) (60 cm³) in a 250 cm³ round bottom flask after which 1,3,5-benzenetricarbonyl chloride (0.5 g, 1.885 mmol) was added. The mixture was cooled to 0 °C and triethylamine (TEA) (0.8 cm³, 5.65 mmol) was added dropwise over 15 min. The mixture was stirred for 3 h at room temperature under N₂. The product precipitated out and was filtered off, recrystallized from DCM, washed with water and dried to a pale yellow solid. Yield: 885 mg (82%). 1H NMR: βH (DMSO-d₆) 9.12 (s, 3H, Ar βH); 8.46 (d, 6H, J = 9.1 Hz, 4NP H); 7.79 (d, 6H, J = 9.3 Hz, 4NP H).

Preparation of 1,3,5-N,N,N-tris(6'-deoxy-6'-β-cyclodextrin)-benzene, β-CD-bz

6'-Amino-6'-deoxy-β-cyclodextrin (500 mg, 0.44 mmol) was dissolved in pyridine (20 cm³) and stirred at room temperature for 15 min. 1,3,5-Trinitrophenyl-benzene (72 mg, 0.125 mmol) was added to this solution with stirring over 1 h. The reaction mixture was stirred for a further 48 h at room temperature before being added dropwise to acetone (200 cm³) with vigorous stirring. The resultant precipitate was collected by centrifugation, washed with acetone and diethylether and dried under vacuum. The product was dissolved in water and run down a Biotech 70 (H⁺) column. The cream solid product was obtained by freeze drying the collected aqueous fractions containing the product followed by further drying over P₂O₅. Yield: 328 mg (68%). TLC: Rₐ = 0.39, ¹H NMR: δH (DClO₃) 8.34 (s, 3H, Ar βH); 5.16-5.08 (m, 2H, β-CD H¹); 4.06-3.42 (m, 12H, β-CD H²⁻H⁶). ¹³C NMR: δC (DClO₃) 171.54 (amide C=O), 137.44 (Ar C=O−O), 131.80 (Ar C=H), 104.37 (C1), 85.94 (C4), 83.75-83.50 (C2-4), 75.72-74.17 (C2, C3, C5), 72.78 (C6), 62.97-62.58 (C6), 43.57 (C6'). MALDI Mass spectrum m/z: 3558.173 [M + H⁺], 3580.151 [M + Na⁺], 3596.132 [M + K⁺] and 1777.564 [M − 2H⁺]. Elemental analysis: C₃₁H₂₃N₃O₁₇; 22H₂O: C, 40.1; H, 6.6; N, 1.1. Found: C, 40.2; H, 6.5; N, 1.2.

Preparation of 1,3,5,N,N,N-tris(6'-[2-aminoethyl]amino)-6'-deoxy-6'-β-cyclodextrin-benzene, β-CD-bz

6'-[1-(2-Aminoethyl)amino]-6'-deoxy-β-cyclodextrin (500 mg, 0.425 mmol) was dissolved in pyridine (20 cm³) and stirred at...
room temperature for 15 min. 1,3,5-Trinitrophenyl-benzene (72 mg, 0.125 mmol) was added to this solution with stirring over 1 h. The reaction mixture was stirred for a further 48 h at room temperature before being added dropwise to acetone (200 cm³) with vigorous stirring. The precipitate was collected by centrifugation, washed with acetone and diethyl ether and dried under vacuum. The product was dissolved in water and run down a BioTeX 70 (H⁺) column. The aqueous product was obtained by freeze drying the collected aqueous fractions containing the product followed by further drying over P₂O₅. Yield: 300 mg (63%). TLC: R₅ = 0.41. ¹H NMR, δ (D₂O): 8.36 (s, 3H, Ar H); 5.15–5.08 (m, 21H, β-CD H⁺); 4.06–3.42 (m, 126H, β-CD H⁺); 3.12–2.64 (m, 12H, en H). ¹³C NMR, δ (D₂O): 168.76 (amide C=O); 134.77 (Ar C−C=O); 128.90 (Ar C−H); 101.71 (C1); 83.36 (C7); 51.13–40.79 (C2-4); 72.92–71.67 (C2, C3, C5); 70.16 (C5a); 60.32–60.15 (C6); 48.79 (Cen); 47.42 (C6c); 39.09 (C-en). MALDI Mass spectrum m/z: 3686.159 [M + H], 3708.148 [M + Na⁺], 3724.117 [M + K⁺] and 3684.259 [M − H⁻]. Elemental analysis: C₆₁H₅₂S₂N₄O₁₀S₃, 20H₂O; C, 41.6; H, 6.6; N, 2.1. Found: C, 41.5; H, 6.5; N, 2.2.

Characterization

Thin layer chromatography (TLC) was carried out on Merck Kieselgel 60 F₂₅₄ aluminium-backed sheets. For analysis of cycloextrin derivatives, plates were developed with 7:5:4 v/v ethyl acetate-propan-2-ol-ammonium hydroxide-water. Compounds were visualised by drying the plate, dipping it into a 1% sulfuric acid in ethanol solution and heating it with a heat gun. For the preparations described above R₅ represents the R₅ of a modified cycloextrin relative to the R₅ of the parent cycloextrin.

¹H NMR spectra were recorded on a Varian Gemini ACP-300 spectrometer operating at 300.145 MHz. The 2D ¹H NOESY NMR spectra were recorded on a Varian Inova 600 spectrometer operating at 599.957 MHz using a standard pulse sequence with a mixing time of 0.3 s. Solutions for host-guest complexation studies in D₂O (pD 7.0, phosphate buffer, t = 0.10 mol dm⁻³) contained an adamantyl substituted poly(acrylate) (10 mg) and β-CdH₃β (4 mg) in 1 cm³ D₂O such that the β-CD groups and adamantyl substituents of the substituted poly(acrylate) were equimolar. Solutions were allowed to equilibrate at the thermostat probe temperature of 298.2 ± 0.1 K for 30 min in 5 mm NMR tubes prior to recording their spectra. MALDI TOF mass spectra were acquired using a Bruker UltraFlexTM MALDI TOF/TOF mass spectrometer (Bruker Daltonik GmbH) operating in linear mode under the control of FlexControl software (Version 3.3, Bruker Daltonik GmbH). External calibration was performed using peptide standards (Bruker Daltonik GmbH), over a range from 800 to 4500 Da, which was analyzed under the same conditions. Spectra were obtained at various locations over the surface of the matrix spot at an intensity determined by the operator. The sample was dissolved in 1 cm³ water and diluted to 100 μg cm⁻³ with aqueous 0.1% trifluoroacetic acid (TFA) solution. A 1 mm³ portion was mixed with 3 mm³ of sinapinic acid in water/acetonitrile/TFA (10/90/0.1) solution and 1 mm³ of this mixture was applied to an 800 μm AnchorChip target plate (Bruker Daltonik GmbH, Bremen, Germany) and air dried. Analysis was performed in both positive and negative ion mode. The MS spectra obtained were analysed using flexanalysis software (version 3.3, Bruker Daltonik GmbH) employing smoothing, background subtraction and peak detection algorithms.

Isothermal titration calorimetry (ITC) measurements were made with a MicroCal VP isothermal titration calorimeter. Solutions were prepared in aqueous phosphate buffer at pH 7.0 and 1 mol dm⁻³ and were degassed and thermostated at 298.2 ± 0.1 K immediately prior to titration. The titrations were carried out under concentration conditions where the product of the total adamantyl substituent concentration, the complexation constant, and the number of either β-CD, β-CdH₃β or β-CdH₃β complexing each adamantyl substituent, N, yielded a sigmoidal variation of heat released against titrant added for each system. The concentrations pertaining to two of the titrations appear in the figure captions in the text, and those for the other systems appear in the captions to Fig. S17–S26 in the ESI. Two cm³ of each of the four adamantyl substituted poly(acrylate)s whose concentrations ranged from 0.13 to 0.37 wt% as indicated in the figure captions were titrated by adding 10 mm³ aliquots of either β-CD, β-CdH₃β or β-CdH₃β solutions, whose concentrations also appear in the figure captions, from a computer-controlled micro-syringe at intervals of 210 s. The contributions of heats of dilution were determined by titrating the buffer solution into either buffered PAAAD, PAAADn, PAAADH or PAAADDtn solutions of the appropriate concentration, and by titrating either β-CD, β-CdH₃β or β-CdH₃β at the appropriate concentrations into buffer solution. The heat changes observed for the PAAAD, PAAADn, PAAADDtn or PAAADDtn, β-CD and β-CdH₃β solutions were less that 1% of those observed for the host-guest complexation titrations. However, in the case of the β-CdH₃β solutions the heat change contributed up to 10% of the heat evolved during the host-guest complexation titrations of Fig. S23–S26), and corrections were made in the derivation of the complexation parameters. An algorithm for complexation according to eqns (1–4) below by either β-CD or the individual β-CD groups or either β-CdH₃β or β-CdH₃β of the adamantyl substituents of either PAAAD, PAAADn, PAAADH or PAAADDtn to the experimental data points provided the best fit using the Origin 7.0 Microcal protocol to yield K, ΔH and T₅₀.

Rheological measurements were carried out with a Physica MCR 501 (Anton Paar GmbH) stress-controlled rheometer with a 25 mm cone and plate geometry. Temperature was controlled at 298.2 ± 0.1 K by a Peltier plate. All studies were carried out in 0.10 mol dm⁻³ aqueous NaCl solution at pH 7.0 adjusted with 0.10 mol dm⁻³ aqueous NaOH.

Results and discussion

The linked β-CD trimers, prepared as shown in Fig. 2, incorporate three equivalent β-CDs attached at the 1, 3 and 5 positions of the benzene center through either an amido link (β-CdH₃β) or a longer [2 aminoethyl]amido link (β-CdH₃β) such that their β-CD groups are separated by 7 and 13 atoms.
respectively. Each of the three β-CD groups may form host-guest complexes with single adamantly substituted poly(acrylate) s PAAAD, PAAAden, PAAAdIn and PAAAdDIn shown in Fig. 1. In principle, one linked β-CD trimer may interact with a single adamantly substituted poly(acrylate) strand to form intra-strand cross-links between three adamantly substituents in dilute solution. In more concentrated solution the linked β-CD trimers interact with multiple strands to form an inter-strand cross-linked network in a hydrogel. We have used 2D $^1$H NOESY NMR spectroscopy, isothermal titration calorimetry (ITC) and rheology to explore these possibilities and the factors controlling host-guest complexation and network formation at the molecular and macroscopic levels in the eight linked β-CD trimer–substituted polycrylate systems. The analogous four β-CD systems, in which host-guest complexation occurs, but significant cross-linking is unlikely, are used as convenient reference systems. The 2D $^1$H NOESY NMR and ITC studies were carried out in D$_2$O and aqueous phosphate buffer at pH 7.0, respectively, at $J = 0.1$ mol dm$^{-3}$ and 298.2 K, and the rheological studies were carried out in 0.10 mol dm$^{-3}$ sodium chloride at pH 7.0 and 298.2 K under which conditions the systems were in their anionic poly(acrylate) state.

2D NOESY $^1$H NMR spectroscopy
The spectra of D$_2$O solutions of either PAAAD, PAAAden, PAAAdIn or PAAAdDIn and β-CD,βz or β-CDen,βz in which the adamantly substituent and β-CD group concentrations are equimolar show strong cross-peaks consistent with interaction between the adamantly H2-4 protons and annular H3,5,6 protons of β-CD,βz and β-CDen,βz for all eight systems as seen in Fig. 3 and S10–S16 (ESI). However, the β-CD group annular H3,5,6 proton resonances are not readily differentiated from those of the H2,4 protons in the systems studied as mono-substitution of β-CD causes all α-glucopyranose units to become inequivalent and their H2-6 resonances to superimpose. In contrast, the H3,5,6 resonances are readily distinguished from the H2,4 resonances of β-CD, and cross-peaks arising from the
interactions of the H3,5,6 annular protons with adamantyl substituent protons are clearly observed whereas no such cross-peaks arise for the β-CD H2,6 protons confirming adamantyl composition in the β-CD annulus. Consequently, it is a plausible assumption that cross-peaks observed in this study of the adamantyl substituted poly(acrylate)s dominantly reflect host–guest complexation in the β-CD group annuli of the linked β-CD trimer as opposed to a more general interaction.

Additional cross-peaks arising from interaction of the annular H3,5,6 protons of β-CDenz,bz and the protons of the tether hexyl 2-4 methylene protons of PAAAdn are also observed consistent with competitive complexation of the adamantyl substituent and the hexyl component of its tether. Analogous cross-peaks arising from interaction of the annular H3,5,6 protons of β-CDenz,bz with the tether hexyl-CH2NH-protons of PAAAdn are also just distinguishable from the main axis component of the spectrum. Similar cross-peaks are observed for the β-CDenz,bz–PAAAdn system (Fig. S12) consistent with an analogous duality of host-guest complexation occurring. Cross-peaks also arise from the interaction of the annular H3,5,6 protons of β-CDenz,bz and β-CDenz,bz with the tether dodecyl 2-11 methylene protons and tether-CH2NH-protons of PAAAdn (Fig. S13 and S16) consistent with competitive host-guest complexation of the adamantyl substituent and its dodecyl tether occurring. Two possible complexation modes of the dodecyl tether are envisaged in Fig. 4 in contrast to the analogous PAAAdn systems where it seems likely on stereochemical grounds that complexation of the shorter hexyl tether dominantly involves passage of the adamantyl substituent through the β-CD group annulus. The spectra of the β-CDenz,bz–PAAAd, β-CDenz,bz–PAAAd, β-CDenz,bz–PAAAden and β-CDenz,bz–PAAAden systems (Fig. S10, S11, S14 and S15) show cross-peaks arising from the interaction of the annular H3,5,6 protons of the β-CD groups and those of the adamantyl substituents only as anticipated for their shorter adamantyl tethers.

The cross-peaks arising from host-guest complexation in the linked β-CD trimer–adamantyl substituted poly(acrylate) systems may result from complexation either within a single adamantyl substituted poly(acrylate) strand, between two or more such strands or both. While the 1H NMR studies do not distinguish between these possibilities in the 1.0 wt% substituted poly(acrylate) solutions studied, the rheological studies of the more concentrated 5.0 wt% solutions discussed below indicate the occurrence of substantial inter-strand cross-linking.

**Isothermal titration calorimetry (ITC)**

The contribution constants, β, defined in eqns (1)–(3) for β-CD, β-CDenz,bz and β-CDenz,bz host complexation of the PAAAd adamantyl substituent guests and the associated ΔH and ΔS were determined by ITC. The K values for the other nine systems are similarly defined. The ITC data are typified by those for the β-CD–PAAAden and β-CDenz,bz–PAAAdn systems shown in Fig. 5 and 6, respectively. The parameters derived for all twelve systems, as described below, are shown in Table 1. (ITC data for the other ten systems appear in Fig. S17–S26.)

\[
\begin{align*}
\text{β-CD} + \text{PAAAd} & \rightleftharpoons K \text{β-CD} \cdot \text{PAAAd} \\
K & = [\text{β-CD} \cdot \text{PAAAd}] / ([\text{β-CD}] \cdot [\text{PAAAd}]) \\
\text{β-CD}_{\text{enz,} \text{bz}} + \text{PAAAd} & \rightleftharpoons K \text{β-CD}_{\text{enz,} \text{bz}} \cdot \text{PAAAd} \\
K & = [\text{β-CD}_{\text{enz,} \text{bz}} \cdot \text{PAAAd}] / ([\text{β-CD}_{\text{enz,} \text{bz}}] \cdot [\text{PAAAd}]) \\
\text{β-CD}_{\text{enz,} \text{bz}} + \text{PAAAd} & \rightleftharpoons K \text{β-CD}_{\text{enz,} \text{bz}} \cdot \text{PAAAd} \\
K & = [\text{β-CD}_{\text{enz,} \text{bz}} \cdot \text{PAAAd}] / ([\text{β-CD}_{\text{enz,} \text{bz}}] \cdot [\text{PAAAd}])
\end{align*}
\]

An algorithm for eqn (1) for complexation by β-CD of the PAAAd guest adamantyl substituents, and analogous algorithms for PAAAden (Fig. 5), PAAAdn and PAAAddn, best fit the experimental variation of the heat change per injection with a molar ratio of N = [β-CD] (adamantyl substituent) = 0.77, 0.86, 0.85 and 0.83, respectively. These N values are less than the optimum value of unity, and may indicate that for the β-CD–PAAAd system steric interactions between the β-CD and the PAAAd poly(acrylate) backbone hinder the complexing of adamantyl substituents. Lengthening the tether of the adamantyl substituents in PAAAden, PAAAdn and PAAAddn is likely to diminish such steric effects whilst increasing substituent intra-strand hydrophobic aggregation which may compete with complexation by β-CD and the β-CD groups of β-CDenz,bz and β-CDenz,bz as occurs in host–guest interactions between some ditopic β-CD and adamantyl hyaluronic acid derivatives. In more concentrated solutions some evidence for increase in
substituent tether length increasing inter-strand substituent aggregation arises from the rheology data discussed below.)

Algorithms for eqn (2) and (3) for complexation by β-CD,bz and β-CDen,bz of the PAAAD guest adamantyl substituents, and analogous algorithms for PAAADen (Fig. 6), PAAADn and PAAADddn, best fit the experimental heat change data with a molar ratio of $N = [\beta$-CD,bz]/[adamantyl substituent] = 0.22, 0.28, 0.27 and 0.29, respectively, and $N = [\beta$-CDen,bz]/[adamantyl substituent] = 0.26, 0.31, 0.30 and 0.32, respectively. In the optimal case $N$ should be 1/3 if each of the three β-CD groups of β-CD,bz and β-CDen,bz complex a single adamantyl substituent. This value is more closely approached by the more flexible β-CDen,bz trimer in its interactions with PAAADen, PAAADn and PAAADddn (Table 1). The steric and hydrophobic adamantyl substituent aggregation effects discussed for the β-CD systems are still expected to apply and the steric effect still appears to be more important in the β-CD,bz-PAAAD and β-CDen,bz-PAAAD systems as evidenced by $N$ being significantly less than 1/3.

On a statistical basis, the concentrations of the substituted poly(acrylate)s in the range 0.13–0.37 wt% are below those at which significant strand overlap occurs and accordingly the data for the linked β-CD trimers probably refer dominantly to intra-strand interactions.\textsuperscript{28} It is anticipated that most of the substituents in a 3.0 ± 0.1% adamantyl substituted poly(acrylate) strand are sufficiently far apart on average for their complexation by β-CD to be largely independent of the complexation state of adjacent adamantyl substituents. The four β-CD systems are characterized by the smallest $K$ and $\Delta H$ values and the only positive $\Delta S$ values in Table 1. Although a negative $\Delta S$ might be expected for complexation by β-CD of the adamantyl substituent it appears that this is offset by the positive entropy change arising from the expulsion of 15–25 water molecules from the β-CD annulus.\textsuperscript{28}

The ITC data for the β-CD,bz systems are consistent with β-CD,bz complexing up to three adamantyl substituents in rapid succession. The $\Delta H$ values for the β-CD,bz systems are substantially larger than those of the β-CD systems and the $\Delta S$ values range from close to zero to strongly negative with the consequence that the β-CD,bz $K$ values for a particular substituted poly(acrylate) are the largest in Table 1. This is consistent with some cooperativity between the β-CD groups of
β-CD-bz in the complexation process. Within a single poly-
(acrylate) strand, complexation of the first adamantyl substitu-
ent by β-CD-bz is likely to restrain the adjacent adamantyl substituents such that another one or two are sequentially complexed. Such sequential complexation is characterized by a single \( K \) in much the same way as is the complexation of a multidentate ligand by a metal ion. However, in 3.0% substituted poly(acrylate) the loop formed by the poly(acrylate) sub-units between two immediately adjacent complexed ada-
mantyl substituents contains about 66 atoms in the poly-
(acrylate) backbone on average to which must be added the number of atoms in each substituent tether. The total number of atoms in the loop is much greater than the 5–7 atoms usually found in the chelate rings of metal complexes. Never-
theless, the restriction of motion within the poly(acrylate) loops formed by this complexation appears to cause a greater decrease in \( T \Delta S \) than the increase anticipated for the expulsion of water from the annuli of the β-CD groups of β-CD-bz.

A similar explanation applies to the increase in \( \Delta H \) and the more negative \( T \Delta S \) for the β-CDene-bz systems by comparison with the analogous data for the β-CD systems (Table 1). However, the \( K \) values characterizing the β-CDene-bz systems show a much smaller increase over those of the β-CD systems than is the case for the β-CD-bz systems which indicates that the distance between the β-CD groups in β-CD-bz and β-
CDEne-bz is also important in determining the thermodynamics of host-guest complexation. It was noted in the experimental section that while the heats of dilution for the substituted poly(acrylates), β-CD and β-CD-bz contributed <1% contribution to the titration heat changes, the heat of dilution of β-
CDEne-bz contributed up to 10% (Fig. S23–S26). This probably arises from association of β-CDEne-bz molecules as a conse-
quence of decreased steric crowding between the three β-CD groups by comparison with that in β-CD-bz. This association may involve two β-CDEne-bz assuming conformations such that their tris-(2-aminoethyl)amino benzene centers undergo π–π interaction. Alternatively, a β-CD group of one β-CDEne-bz may hydrogen bond within the cavity formed by the three β-CD groups of a second a β-CDEne-bz. \(^{1}H\) NMR 300 MHz studies in which [β-CDEne\(_n\)] was systematically diluted from 1.20 × 10\(^{-2}\) to 2.23 × 10\(^{-3}\) mol dm\(^{-3}\) in D\(_2\)O phosphate buffer at pH 7.0 and \( T = 0.10\) mol dm\(^{-3}\) detected a 0.05 ppm shift of the aromatic proton resonance (Table S1) and similarly small shifts of other resonances but these were too small to quantify any β-CDEne-bz association.) Whatever its nature, such association is likely to compete with the complexation of the adamantyl substituents of the poly(acrylate)s and contribute to the lowering of the \( K \) values for the β-CDEne-bz systems.

A trend is seen in Fig. 7 in which the smallest \( K \) values for complexation of the adamantyl substituents by β-CD, β-CD-bz and β-CDEne-bz are observed for PAAAD consistent with the short tether between the adamantyl substituent and the poly-
(acrylate) backbone causing significant steric hinderance to complexation by β-CD and the β-CD groups of β-CD-bz and β-
CDEne-bz. The decrease in this hinderance resulting from lengthening of the tether in PAAADene causes substantial increases in \( K \) for complexation by β-CD, β-CD-bz and β-
CDEne-bz. A further increase in tether length in PAADdh increases \( K \) for complexation by β-CD and β-CD-bz but causes a leveling out for β-CDEne-bz. Thereafter, the large increase in the length of the 1,12-(aminododecy)lamido tether in PAADdhh causes \( K \) to decrease for complexation by all three hosts.

\( ^{6} \) In aqueous phosphate buffer at pH 7.0 and \( T = 0.10\) mol dm\(^{-3}\) at 298.2 K. \(^{3} \) \( K \) is defined through eqns (1)–(3) and their equivalents. The errors shown for \( K \) and the associated parameters are the data fitting errors. When experimental error is also included it is estimated that the overall error in \( K \) is ±15%. \(^{4} \) \( N \) is the number of either β-CD, β-CD-bz or β-CDEne-bz complexing each adamantyl substituent in the dominant complexation interaction.
coincident with anticipated increases in substituent intrastrand hydrophobic aggregation competing with complexation of the substituents by β-CD and the β-CD groups of β-CD₃bz and β-CD₆en₆bz. However, the changes in the thermodynamic parameters are insufficiently systematic to permit confident assignment of these effects to specific changes in ΔH and ΔS. The ΔH and ΔS data in Table 1 may be viewed in a broader context when plotted together with similar data collected for host-guest complexation by β-CD, mono-substituted β-CD and linked β-CD dimers of a large variety of guest species much smaller in size than the poly(acrylate)s of this study (Fig. 8). Entropy–enthalpy compensation plots yield linear least squares fits of these ΔS against ΔH to eqn (4) at 298.2 K yield α = 0.80 and ΔS0 = 11 kJ mol⁻¹ for β-CD, α = 0.99 and ΔS0 = 17 kJ mol⁻¹ for mono substituted β-CD, and α = 0.89 and ΔS0 = 33.5 kJ mol⁻¹ for linked β-CD dimers. The positive ΔS0 values indicate that the host-guest complexes are entropically stabilized at ΔH = 0, the intercept value, where the corresponding entropy change is ΔS0:

\[
\Delta S = \alpha \Delta H + \Delta S_0
\]

While eqn (4) does not reflect a necessary relationship between ΔS and ΔH, its widespread observation is usually taken as an indication of a variation in the relative importance of the solvational and structural changes within the systems studied. Also plotted in Fig. 8 are the ΔS and ΔH data from Table 1 from which it is seen that the new data for β-CD fall within the range of the literature data for β-CD and that the data for β-CD₃bz and β-CD₆en₆bz fall within the range of the literature data generally. (For the eight systems based on β-CD₃bz and β-CD₆en₆bz α = 0.88 and ΔS0 = 19.5 kJ mol⁻¹.) This indicates that although the variation of the thermodynamic parameters derived in this study are substantially influenced by the adamantyl substituted poly(acrylate)s as discussed above, they remain within the entropy–enthalpy compensation relationship (eqn (4)) characterizing host-guest complexation in β-CD-based systems. This suggests that complexation in the β-CD annulus substantially controls the thermodynamics of host-guest complexation within the substituted poly(acrylate) systems studied. There are few other thermodynamic studies of host-guest complexation in aqueous polymer systems with which comparison may be made. One such yields ΔH = –3.24 kJ mol⁻¹ and ΔS = –16.62 kJ mol⁻¹ for the 1:1 host-guest complexation by the 6'-O-(2-aminooethyl)amino)-6''-β-cyclodextrin of substituents of the 1-(2-aminooethyl)amidoadamantyl substituents of the 3.0 ± 0.1% randomly substituted poly(acrylate) PAAAD in aqueous solution at 298.2 K. While these data lie within the scatter of the ΔH and ΔS data plotted in Fig. 8 more data are required to determine to what extent such host-guest complexations between β-CD substituents on one poly(acrylate) and hydrophobic substituents on another fit the ΔS/ΔH linear relationship shown therein.

**Rheology**

The variation of viscosity with substituted poly(acrylate) identity and solution composition is shown in Fig. 9a from which it is seen that over the shear rates studied there is little viscosity variation for a given system. The small increase in viscosity as shear rate increases in the sequence: PAAAD (0.016), PAAADen (0.012), PAAADh (0.015) and PAAADDn (0.016).

![Fig. 9 Variation of the viscosity of (a) PAAAD, PAAADen, PAAADh and PAAADDn alone and in mixtures with (b) β-CD₃bz or (c) β-CD₆en₆bz where the adamantyl substituent concentration and the β-CD groups of either β-CD₃bz or β-CD₆en₆bz are equivalent in aqueous 0.10 mol dm⁻³ aqueous NaCl at pH 7.0 and 298.2 K with shear rate. The substituted poly(acrylate) concentrations are 5.0 wt% and the concentrations of their adamantyl substituents are 1.5 × 10⁻⁴ mol dm⁻³.](image-url)
(viscosities in Pa s at a 500 s⁻¹ shear rate are shown in brackets) is attributable to increasing hydrophobic association of the substituents as tether length increases to produce weak cross-linking between adjacent substituted poly(acrylate) strands. Upon addition of β-CD-bz, the viscosities of all four systems increase generally: β-CD-bz-PAAADn (0.03), β-CD-bz-PAAADden (3.78), β-CD-bz-PAAADbn (3.48) and β-CD-bz-PAAADdnn (2.03) as seen in Fig. 9b. Upon addition of β-CDen_bz viscosity increases also occur: β-CDen_bz-PAAAD (0.03), β-CDen_bz-PAAADen (1.01), β-CDen_bz-PAAADbn (0.68) and β-CDen_bz-PAAADdnn (0.49) as seen in Fig. 9c.

The trends in viscosity for β-CD-bz and β-CDen_bz with the substituted poly(acrylate)s are broadly similar as seen in Fig. 10. Consistent with the conclusions drawn from the FTC data, the shortness of the tether in PAAAD and the consequent steric hindrance by the poly(acrylate) backbone appears to be a dominant factor precluding optimal complexation of the adamantyl group by either β-CD-bz or β-CDen_bz in contrast to PAAADen were the two methylene groups lengthen the tether to allow stronger complexation of the adamantyl group by either β-CD-bz or β-CDen-bz and a substantial viscosity increase. Further lengthening of the tethers in PAAADbn and PAAADdnn results in successive decreases in viscosity probably due to corresponding increases in the extent of hydrophobic association between the adamantyl substituent tethers and competition with the formation of inter-strand cross-links through adamantyl substituent complexation by β-CD groups.

The larger viscosities of the β-CD-bz-(PAAADen - PAAADdnn) systems by comparison with the analogous β-CDen_bz systems are probably largely attributable to the competition between the aggregation of the β-CDen_bz and the formation of inter-strand cross-links which is absent from the β-CD-bz systems. Some of the differences in viscosity may also be attributable to the shorter inter-strand cross-links formed and the lesser flexibility of the networks formed in the β-CD-bz systems by comparison with those formed in the β-CDen_bz systems where the inter-strand cross-links are longer. There may also be differences in the ratios of intra- to inter-strand cross-links which could also contribute to the overall effect.

This trend in viscosity is qualitatively reflected in the previously discussed variations of K (298.2 K) of the host-guest complexes formed by β-CD-bz by comparison with those formed by β-CDen_bz in relatively dilute 0.13–0.37 wt% adamantyl-substituted poly(acrylate) systems (Fig. 6). Qualitatively, it appears that the factors affecting the complexation constants of the host-guest complexes in 0.13–0.37 wt% solutions impact on the relative viscosities of the 5.0 wt% solutions in a similar way and that differences between the complexation constant and viscosity profiles probably arise from differences in the relative proportions of inter-strand cross-linking which is likely to be much more prevalent in the 5.0 wt% solutions.

Conclusion

Twelve host-guest complex systems have been characterized at the molecular and macroscopic levels in this study of the complexation by the β-CD, β-CD-bz and β-CDen_bz hosts of the adamantyl substituent guests attached through tethers of four different lengths in 3.0 ± 0.1% randomly substituted poly(acrylate)s. Several salient points arise. First, the shortest tether length between the adamantyl substituent and the poly(acrylate) in PAAAD coincides with significant steric crowding between the individual complexes β-CD or β-CD groups of the trimers and the poly(acrylate) backbone which results in the lowest K values observed for complexing the poly(acrylate) adamantyl substituents by β-CD, β-CD-bz and β-CDen_bz in 0.13–0.37 wt% solutions. (For the last two hosts this also coincides with the lowest viscosity in 5.0 wt% solution.) Second, the hydrophobic hexyl and dodecyl tethers compete with the adamantyl group for complexation in the annull of β-CD-bz and β-CDen-bz. Third, β-CD-bz yields the largest K (298.2 K) for complexation of adamantyl substituents consistent with it exercising the greater cooperativity in host-guest complexation. It also forms the most viscous hydrogel which indicates the formation of the tightest network structure. Fourth, β-CDen_bz generates substantially lower K values and hydrogel viscosities than does β-CD-bz probably largely as a consequence of its aggregation competing with adamantyl poly(acrylate) substituent complexation in intra- and inter-strand cross-link formation. Fifth, despite the structural dissimilarities between β-CD, β-CD-bz and β-CDen-bz, the TAΔS and ΔH data characterizing their complexation of the adamantyl substituents of the four adamantyl-substituted poly(acrylate)s in 0.13–0.37 wt% solutions fit well within the linear TAΔS and ΔH compensation relationship observed for the complexation of a wide range of guest species by β-CD, monosubstituted β-CDs and linked β-CD dimers. This indicates that complexation in the β-CD annulus largely controls the thermodynamics of host-guest complexation within the substituted poly(acrylate) systems studied.

Acknowledgements

We gratefully acknowledge the Australian Research Council, NSFC Grant 20774028 and 20774030 for support of this work.
References

36. MicroCal, 22 Industrial Drive East, Northampton, MA 01060, USA.