Journal of Inclusion Phenomena and Macrocyclic Chemistry **48:** 173–180, 2004.

Higher-Order Cyclodextrin Complexes: The Naphthalene System

SALLY SAU[1], BINDIYA SOLANKI[1], RICARDO ORPRECIO[1], JAN VAN STAM[2] and CHRISTOPHER H. EVANS[1,*]

[1]*Department of Chemistry and Biology, Ryerson University, 350 Victoria St., Toronto, ON, Canada, M5B-2K3;* [2]*Department of Physical Chemistry, Karlstad University, SE-651 88, Karlstad, Sweden*

(Received: 24 June 2003; in final form: 2 December 2003)

Key words: binding constants, cyclodextrins, fluorescence spectroscopy, host–guest inclusion complexes, naphthalene excimer

Abstract

Naphthalene excited dimer (excimer) fluorescence is observed in the presence of β- and γ-cyclodextrin (CD) at elevated naphthalene (NAP) concentrations (100 μM) but not at low NAP concentrations (5 μM). This is attributed to formation of 2:2 CD:NAP complexes in the former situation. Complexes of NAP with hydroxypropyl β-CD are exclusively 1:1 and no excimer emission is observed. Complexes of NAP with α-CD do not show excimer emission either but the complex stoichiometry is 2:1 CD:NAP in this case. The formation constants for both the 1:1 and 2:2 β-CD:NAP complexes have been determined and they have been found to depend on the ionic strength of the salting out agent NaCl. $K_{1:1} = 377 \pm 35\ M^{-1}$ in the absence of salt and $657 \pm 60\ M^{-1}$ at 1 M NaCl. The corresponding values for $K_{2:2}$ are $(1.0 \pm 0.2) \times 10^4$ and $(4.0 \pm 1.0) \times 10^4\ M^{-1}$, respectively. Stern-Volmer fluorescence quenching studies of the 1:1 and 2:2 species by water-based quenchers (NaI and CsBr) show that both types of complexes protect the fluorophore from the quencher. However, the more completely encapsulated NAP in the 2:2 complex is protected to a greater extent. This is also the case for the 2:2 γ-CD:NAP complex. This protective effect is reflected in the observed rate constants for NAP quenching. For example, $k_Q = 7.1 \times 10^9\ M^{-1}\ s^{-1}$ for NaI in the absence of CD. This is reduced to $2.1 \times 10^9\ M^{-1}\ s^{-1}$ for the 1:1 complex and $1.2 \times 10^9\ M^{-1}\ s^{-1}$ for the 2:2 complex when 10 mM β-CD is present. A similar pattern is observed for CsBr as quencher. The 2:2 complexes are disrupted in the presence of additives such as linear alcohols and surfactants. The implications of these results for application of CDs for drug stabilization are discussed.

Introduction

Drug stability is of major importance to the pharmaceutical industry. It is also a significant scientific challenge to develop systems/conditions that stabilize drugs in a form in which they can be successfully delivered to a patient. Various methods have been proposed relying on control of ambient conditions (temperature, pH etc.) that may vary over time [1,2]. A more robust approach is to encapsulate drugs in a medium that protects them from chemical reactions. In this paper we use model systems to explore the potential of cyclodextrins as encapsulating agents.

Cyclodextrins (CDs) are oligosaccharides consisting of various numbers of α-1,4-linked glucose units [3]. The most commonly encountered sizes are the 6-, 7- and 8-member cycles, known as α-, β- and γ-CD, respectively. The CD structure is such that the molecule possesses a molecular-scale cavity (a 'molecular bucket'). Unsubstituted CDs are moderately water soluble due to hydroxyl groups lining the rims of the cavity, the cavity interiors, on the other hand, are rather hydrophobic. These properties give the CDs the unique characteristic of being able to form host-guest inclusion complexes in water with organic guest molecules [4].

The most common form of CD:guest complex is the so-called 1:1 complex, in which one CD molecule hosts one guest molecule within its cavity [4-6]. It is well established that such CD-complexed species exhibit chemical and physical properties that may be quite different from that observed in homogeneous solution [5-11]. These differences may be changes in product type, product distribution or rate of reaction. In particular, rates may be increased or decreased upon complexation, depending upon the type of reaction and the reagents involved.

In recent years much effort has been made to explore and develop pharmaceutical applications of CDs [11-18], including the preparation of a variety of derivatized systems with better toxicity profiles [12]. It has been pointed out that CDs show promise as drug stabilizers [1, 11, 19]. Drugs encapsulated in 1:1 CD complexes often show reduced rates of reaction towards water-based reagents as a result of compartmentalization [1, 10, 19, 20]. Reaction inhibition has also been observed with other, non-pharmaceutical, guests [7, 21-26]. CDs offer clear advantages over other stabilizing

* Author for correspondence. E-mail: cevans@ryerson.ca

excipients as they also enhance the aqueous solubility and bio-availability of drugs [16, 18].

Improved chemical stability in CD host–guest systems arises from encapsulation. It stands to reason that the more complete the encapsulation, the greater the isolation of the guest and the more fully protected it will be. One possible approach is to develop systems forming higher-order complexes. By 'higher-order complexes' we refer to host–guest systems comprised of two CD and one guest (2:1 complexes) or two CD and two guests (2:2 complexes).

There are a number of CD:guest systems that spontaneously form such higher order complexes. Pyrene forms both 1:1 and 2:1 complexes with β-CD as judged by absorption and fluorescence measurements of equilibrium constants [27, 28]. It also has been reported to form 2:2 complexes with γ-CD [29-31] although only 1:1 complexes seem to form at low pyrene concentrations [32]. 2-Anthracene sulfonate and 2-anthracene carbonate form 2:2 complexes with β-CD [33]. Alpha-terthienyl forms 2:2 complexes with γ-CD as evidenced by the direct observation of excimer-type fluorescence from this chromophore in the presence of the CD host [34]. A similar situation pertains for oxadiazole complexes with γ-CD [35–37]. However, it is higher order complexes of CDs with naphthalene and its derivatives that have received most attention. A very early report from Ueno *et al.* [38] on the 2:2 complex of β-CD with 1-naphthaleneacetate was followed by a series of articles from Hamai [39–43] and, more recently, Bohne *et al.* [24, 44] and Grabner *et al.* [45] have established that naphthalene and its derivatives readily form 2:2 complexes with β-CD if the naphthalene and CD concentrations are sufficiently high. These are relatively strong complexes with reported formation constants on the order of 4000 M^{-1} [24, 39, 42]. In the majority of these systems the presence of the 2:2 complex is indicated by observation of naphthalene excited dimer (i.e., excimer) fluorescence. Other systems, such as 6-bromo-2-naphthol and α-CD, for example, form 2:1 host;guest complexes [41].

Information on the dynamics of higher order CD complexes is limited. Early time-resolved fluorescence data from Yorozu *et al.* [29] indicate that the pyrene excimer fluorescence observed in the presence of γ-CD is generated within the duration of the 0.8 ns excitation pulse. This suggests the 2:2 complex is preformed and little or no motion of the pyrenes is required to achieve the geometry needed for excimer fluorescence to occur. More recently contributions from De Feyter *et al.* [34], Bohne *et al.* [24, 44] and Grabner *et al.* [45] have all indicated rapid formation of excimer emitting complexes. De Feyter's and Grabner's articles indicate the excimer is formed within the excitation pulse ($<$1 ns) while Bohne and co-workers were able to determine the rate of formation of 1:1 complexes between β-CD and naphthyl ethanols (about 3.5×10^8 s^{-1}). This latter work also reports that dissociation of the 2:2 complexes is very slow, taking place on the millisecond time scale. Thus, self-assembling higher order complexes involving aromatic hydrocarbons and CDs form very rapidly but seem to dissociate very slowly, i.e. they are to be regarded as stable complexes.

A current goal in our research is to develop improved systems for chemical stabilization based on encapsulation by CDs. Although a significant body of information is available on higher order CD complexes, very little attention has been paid to the issue of to what extent such systems may offer protection to a reactive guest. In Hamai's original paper [30] some efforts were made to assess the extent of protection of a naphthalene guest by the β-CD host in the 2:2 complex. Hamai reported a variation in Stern-Volmer constant for fluorescence quenching by water-based quenchers (I^- and IO_3^-) in the order free naphthalene $>$ 1:1 complex $>$ 2:2 complex. More recently Funasaki and co-workers have reported that the rate of basic hydrolysis of the anticholinergic drugs propantheline bromide and oxyphenonium bromide is strongly reduced via formation of 2:1 β-CD:drug complexes while 1:1 complexes of these drugs with α- and γ-CD do not strongly suppress hydrolysis [11]. These reports suggest that 2:n CD:drug complexes do indeed offer additional protection for the guest. However, many issues remain unexamined. One particularly important question is whether the additional protection can be maintained in the presence of additives such as salts and surfactants. This latter point is of interest as drug formulations invariably contain excipients such as buffer salts, anti-microbial agents, surfactants etc. The present contribution presents results on the β-CD:naphthalene system under conditions of 2:2 complex formation. This system is a useful model and starting point for the design and development of more robust and general CD-based encapsulating systems.

Experimental

Most reagents were purchased from Sigma-Aldrich. Naphthalene (NAP) of scintillation grade, was used as received as were the sodium chloride (BDH, AnalR), sodium iodide, cesium bromide (Merck), sodium dodecyl sulfate (SDS) and buffer salts (disodium hydrogen *ortho*-phosphate, potassium dihydrogen phosphate, BDH) and sodium hydroxide (BDH, AnalR). α-Cyclodextrin (α-CD) and hydroxy propyl β-CD (HPCD) were used as received while β-CD was recrystallized twice from water. All alcohols were of spectroscopic or HPLC grade and used without additional purification. Water was conductivity grade (MilliQ Academic Model V 2.04). Phosphate buffer solutions (pH 7.4) were prepared according to standard guidelines [46].

Samples for fluorescence analysis were prepared as follows. A saturated solution of NAP in water was stirred overnight and then filtered. The filtered solution was ca. 0.2 mM in NAP based on its absorption spectrum. This solution was diluted with water to produce aqueous NAP stock which was used to prepare all subsequent solutions. Appropriate CD stocks were prepared by weighing the required mass of CD (or CD plus SDS) into a volumetric flask and dissolving it with the NAP stock. This was subsequently diluted with the NAP stock to obtain solutions with a constant NAP concentration but the desired variable concentration of CD (or

CD plus SDS). If an alcohol was to be added, the neat alcohol was injected via a Hamilton microlitre syringe directly into 10 mL of the CD:NAP solution. The CD samples were stirred overnight to obtain equilibrium. Quencher stock solutions in water were prepared just before use by dissolving an appropriate mass of quencher in the appropriate CD/NAP or CD/NAP/alcohol solution. Quenching experiments were performed by injecting small aliquots of quencher stock into 2 mL samples of CD:NAP solutions, with or without additive, immediately prior to measurement. Exposure to light was kept to a minimum during all sample preparation and handling. All samples were air saturated.

Steady-state fluorescence measurements were carried out at 20 ± 1 °C with a Perkin-Elmer LS-50B instrument. Instrument control, data collection and preliminary data processing were carried out by a micro-computer interfaced to the fluorimeter. Samples for fluorescence measurements were contained in 1 × 1 cm^2 standard (Hellma) quartz cuvettes. The spectra were recorded with excitation at 280 nm. Spectra were scanned between 300 and 500 nm. The band pass was typically 2 nm. The emission spectra were uncorrected. Fluorescence intensities were estimated from peak heights at 336 and 420 nm. Absorption spectra were recorded with a Perkin-Elmer Lambda 40 UV-VIS spectrophotometer.

Results and discussion

Evidence for the 2:2 napthalene:β-cyclodextrin complex

Figure 1 shows the variation in NAP fluorescence ([NAP] = 100 μM) as a function of increasing β-CD concentration (0–10 mM). In the absence of the host, a fluorescence band is observed centered near 337 nm. This is the normal fluorescence of naphthalene in water. As CD is added the intensity of this band increases (plateauing between 8 and 10 mM) as expected because of redistribution of the fluorescent molecule to the less polar, more protected environment of the CD cavity. However, in contrast to observations made at low (10 μM or less) NAP concentrations, an additional broad, featureless emission band appears centered near 420 nm. The intensity of this band increases with increasing NAP concentration. This band is not detected in water in the absence of β-CD.

This type of behavior has been observed before [24, 39-45] and the 420 nm feature attributed to NAP excimer-like fluorescence arising from host-guest complexes involving two β-CD and two NAP molecules, according to Equations (1) (equilibrium constant K_1) and (2) (equilibrium constant K_2).

$$\mathrm{NAP} + \mathrm{CD} \rightleftharpoons (\mathrm{CD{:}NAP}) \qquad (1)$$

$$(\mathrm{CD{:}NAP}) + (\mathrm{CD{:}NAP}) \rightleftharpoons (\mathrm{CD_2{:}NAP_2}) \qquad (2)$$

To confirm this we carried out two simple experiments to provide evidence that the 420 nm feature is indeed due to

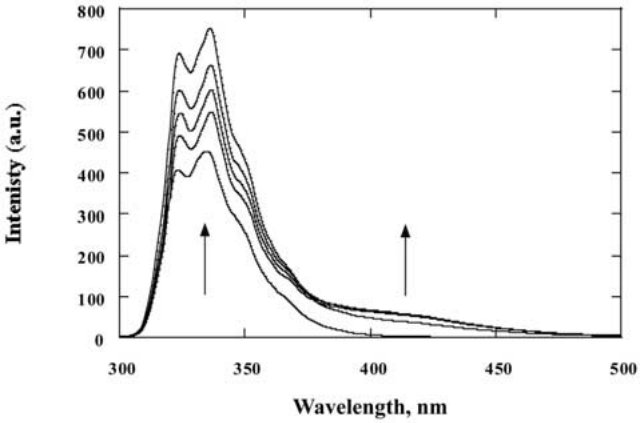

Figure 1. Variation in naphthalene fluorescence intensity recorded in the presence of increasing concentrations of β-cyclodextrin (0, 2, 4, 8 and 10 mM). λ_{ex} = 280 nm, [Naphthalene] = 100 μM.

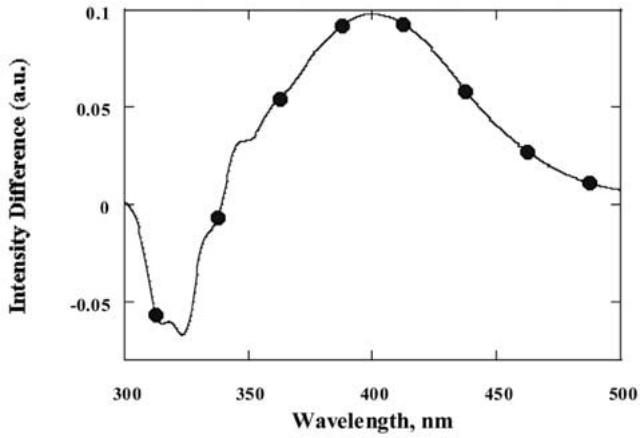

Figure 2. Spectrum recording the difference between the fluorescence of 5 μM naphthalene in a 10 mM β-cyclodextrin solution and that of 100 μM naphthalene at the same cyclodextrin concentration. The spectra were normalized at 337 nm prior to taking the difference. λ_{ex} = 280 nm.

an excimer-like arrangement of NAP in the presence of β-CD. Figure 2 is a fluorescence difference spectrum obtained by measuring NAP fluorescence under conditions for which the 420 nm feature is not observed (5 μM NAP, 10 mM β-CD) and under conditions for which it is (100 μM NAP, 10 mM β-CD). The intensities of the two spectra were normalized at 337 nm and the difference spectrum calculated. The difference spectrum in the region between 380 nm and 500 nm matches closely the well-known NAP excimer spectrum [47]. This supports the assignment of the 420 nm feature to NAP molecules in an excimer-like arrangement. The negative band centered near 325 nm is a result of the presence of the excited dimer and is analogous to what is found in transient absorption of similar species [34].

Figure 3 shows the effect of raising the pH on the observed fluorescence (100 μM NAP, 10 mM β-CD). At pH 7 both the 337 and 420 nm bands are observed. At pH 13 the 420 nm band is completely suppressed. The pK_a for β-CD is about 12 [48]. At high pH the hydroxyl groups of the CD are ionized. The resulting electrostatic repulsion makes it impossible for the 2:2 CD:NAP complexes to exist. This accounts for the loss of the 420 nm signal. These experiments

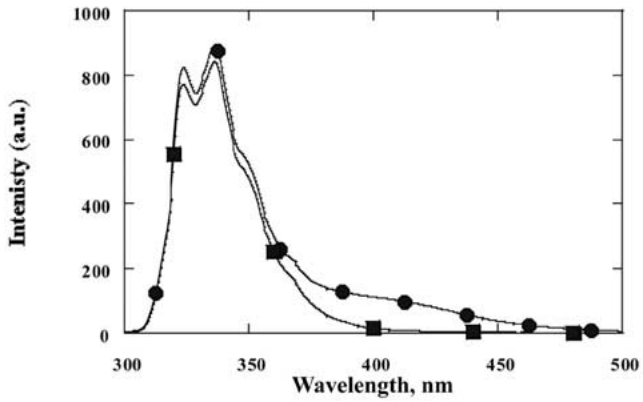

Figure 3. Fluorescence spectra recorded for 100 μM naphthalene in the presence of 10 mM β-cyclodextrin at pH 7 (●) and pH 13 (■). λ_{ex} = 280 nm.

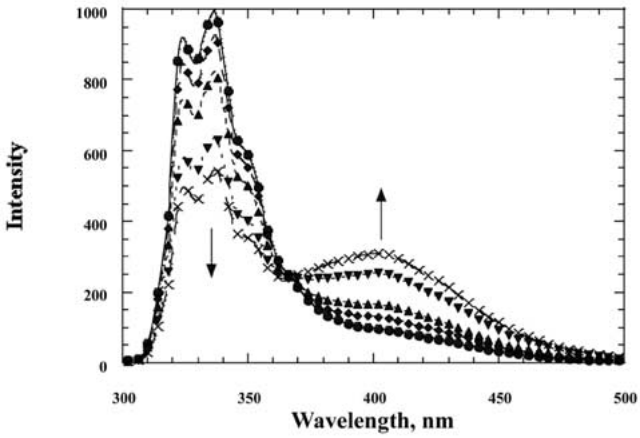

Figure 4. Changes in the relative importance of the monomer and excimer type complexes of naphthalene with β-cyclodextrin as a function of added NaCl. λ_{ex} = 280 nm, [Naphthalene] = 100 μM, [β-Cyclodextrin] = 10 mM. [NaCl] = 0, 0.5, 1, 2, 4 and approximately 7 M.

confirm that the 420 nm band is excimer-like fluorescence from NAP and arises from 2:2 β-CD:NAP complexes. They also confirm that no 1:2 CD:NAP complexes are formed.

Other cyclodextrin systems

Attempts were made to observe the NAP excimer fluorescence in other CD systems. Spectral measurements of 100 μM NAP in the presence of 10 mM hydroxypropyl-β-CD (HPBCD) showed no sign of the excimer fluorescence. This may simply mean that the hydroxypropyl groups interfere with the formation of a 2:2 complex with NAP so that the complexes that do form are exclusively 1:1 (Again, the absence of excimer emission confirms that 1:2 CD:NAP complexes are not present). We measured binding isotherms for the HPBCD:NAP system over a NAP concentration range of 5 to 100 μM. The binding isotherms were treated by a non-linear fitting method assuming a purely 1:1 complexation model [49]. The resulting binding constants were found to be independent of the NAP concentration (Average $K_1 = 670 \pm 92\ M^{-1}$). This type of concentration independence is generally taken to be good evidence of simple, 1:1 complexation [50] and is in contrast to our earlier report on the β-CD:NAP system where K_1 varies strongly with [NAP] [26]. A Benesi–Hildebrand double-reciprocal analysis of the binding isotherm data for 100 mM NAP and HPBCD gave a linear plot (correlation >98%). This is also evidence for 1:1 association [4]. We thus conclude that no 2:2 species (and in fact no 1:2 species) are involved in HPBCD:NAP complexation.

When varying concentrations (0 to 10 mM) of α-CD were added to a 100 μM NAP solution no excimer emission was detected. This is in agreement with a recent report by Grabner *et al.* [45] In our study a Benesi–Hildebrand analysis of the fluorescence data did not yield a linear plot but rather a strongly upward curving plot. Replotting the data as a function of $[\alpha\text{-CD}]^{-2}$ yielded a linear plot (correlation >99%) indicating the involvement of higher-order complexes. Earlier reports from Hamai indicate that 2-substituted naphthalenes form 2:1 α-CD:NAP complexes [41, 51], which cannot exhibit excimer emission. We propose that the situation is similar for the α-CD complex with NAP itself. The α-CD cavity is about the right size to comfortably accommodate a benzene ring [48] so it is not surprising that two such cavities would be required to effectively complex NAP.

Finally we investigated the γ-CD:NAP system. In this case a broad, featureless emission band at longer wavelengths (near 420 nm) was also observed. In an earlier report we [34] examined the γ-CD:α-terthienyl system using steady-state and time-resolved spectroscopic techniques. We showed that 2:2 complexes formed such that excimer emission of the thiophene compound could be detected. A similar situation has been reported for 2,5-diphenyloxazole in the presence of γ-CD [35-37]. In analogy with these systems, we conclude the longer wavelength band observed for 100 μM NAP in the presence of 10 mM γ-CD is due to excimer emission from a 2:2 complex.

Impact of ionic strength on the 2:2 complex with β-CD

Figure 4 shows a series of fluorescence spectra for the β-CD:NAP system recorded at various concentrations of NaCl, a salt that does not quench NAP fluorescence. Clearly ionic strength has a profound impact on the relative concentration of the complex species present in the β-CD:NAP system. Host-guest binding can be either increased or decreased by salts, depending on the nature of the host (charged vs. uncharged CD), the guest (charged or uncharged, size compared to CD cavity) and the salt (salting in or salting out species, tendency to bind to the CD cavity) [52-54].

The spectra of Figure 4 indicate that higher ionic strength favors the 2:2 complex over the 1:1 complex. In the absence of NaCl the monomer to excimer ratio (I_m/I_{ex} where the intensities are recorded at 337 and 420 nm, respectively) was 15 while in the presence of 4 M NaCl it was 1.8. Thus, the salt shifts the overall equilibrium described by equations 1 and 2 to the right. This can be put on a more quantitative basis by analyzing the observed changes in terms of K_1 and K_2. Values of K_1 can be obtained from non-linear regression analysis of the binding isotherms under excimer-free conditions (i.e. at low [NAP]). Under these conditions

$$K_1 = \frac{[CD{:}NAP]}{[NAP][CD]_0} \quad (3)$$

and the binding isotherm is defined as [26, 49]

$$\Delta I = K_1 \left(\frac{\Delta I[NAP]_0[CD]_0}{1 + K_1[CD]_0} \right), \quad (4)$$

where ΔI refers to the difference between the fluorescence intensity at total cyclodextrin concentration $[CD]_0$ and that observed in the absence of CD, $[NAP]_0$ is the total naphthalene concentration and Δi reflects the maximum value of ΔI. Note that $[CD]_0$, rather than [CD], is used in these equations as it is always true in our systems that $[CD] \gg [NAP]$ so that at all conditions [CD] is essentially equal to $[CD]_0$. From these calculations $K_1 = 377 \pm 35\ M^{-1}$ at 5 μM NAP in the absence of NaCl but $657 \pm 60\ M^{-1}$ at 1M NaCl. Clearly increased ionic strength has a significant impact on the 1:1 binding process.

A value for K_2 can be obtained from the variation of the excimer fluorescence band intensity with CD concentration. Analysis [24, 39] of equilibria (1) and (2) leads to the following expression for the excimer concentration, $[CD_2{:}NAP_2]$, as a function of total cyclodextrin concentration, $[CD]_0$

$$[CD_2{:}NAP_2] = \frac{[NAP]_0 - [NAP]_{aq} - K_1[CD]_0[NAP]_{aq}}{2} \quad (5)$$

Here $[NAP]_{aq}$ refers to the concentration of free NAP. This concentration is given by [24, 39]

$$[NAP]_{aq} = \frac{-(1 + K_1[CD]_0) + \sqrt{(1 + K_1[CD]_0)^2 + 8K_1^2K_2[CD]_0^2[NAP]_0}}{4K_1^2K_2[CD]_0^2} \quad (6)$$

Expressions (5) and (6) were used to simulate a series of curves showing the variation of normalized $[NAP_2{:}CD_2]$ as a function of $[CD]_0$ for various values of K_2 and a fixed value of K_1 (Figure 5). Normalized experimental values of excimer fluorescence intensity (measured at 420 nm with $[NAP]_0 = 100\ \mu M$) were compared to the simulated curves. The best fit yielded the value of K_2. The value of K_1 used in these tests was $377 \pm 35\ M^{-1}$ [26]. This approach yields somewhat "soft" values of K_2 [24] as adequate fits are obtained for a relatively wide range of K_2 values. The values of K_2 determined at 0M and 1M NaCl are $(10 \pm 2) \times 10^3$ and $(40 \pm 10) \times 10^3\ M^{-1}$, respectively.

The values of both K_1 and K_2 increase markedly at elevated NaCl concentrations. This can be explained in terms of the salting out effect [55]. NaCl is a well know a salting out agent. When a hydrophobic molecule like NAP is dissolved in water, water molecules tend to avoid it. The water molecules create an open volume in their bulk structure into which the NAP can fit. When a salting out agent is added, its ionic charges become hydrated. This results in a volume contraction of the water meaning there will be less "empty" volume available for the hydrocarbon. In the absence of CD this contraction would normally lead to the precipitation of the NAP. When CD cavities are present, they provide a hydrophobic volume into which the NAP can redistribute. This eliminates the volume requirement for placing NAP in water. As NAP redistributes more strongly to the CD environment a larger population of 1:1 complexes are formed which in turn interact to form 2:2 complexes. The net effect is a greater yield of 2:2 complexes. Thus, in the presence of elevated [NaCl], the NAP would be expected to be more fully isolated from the aqueous medium.

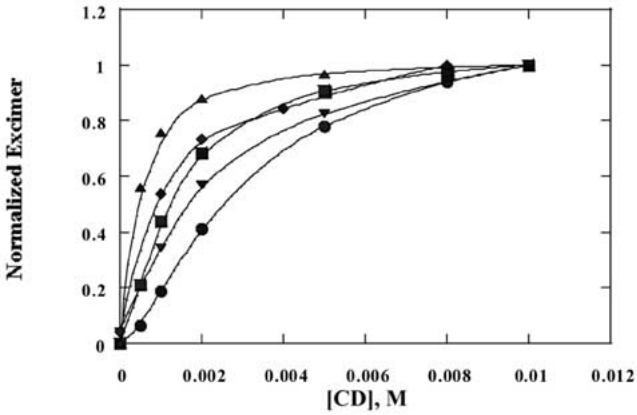

Figure 5. Comparison of simulated (Equations (5) and (6)) and experimental measures of the naphthalene excimer concentration ([Naphthalene] = 100 μM). Simulated with $K_1 = 377\ M^{-1}$ and K_2 values of 4000 (●), 40 000 (■) and 400 000 M^{-1} (▲). Experimental excimer intensities (420 nm) at 0 M (▼) and 1 M (diamond) NaCl.

Degree of encapsulation: impact of quenchers

Inorganic salts that quench NAP fluorescence are convenient tools to probe the relative importance of the various complex species that may form in the presence of β-CD in an aqueous medium [10, 22, 26]. We decided to carry out a quenching study on the NAP plus β-CD system under conditions for which the both the monomer and excimer fluorescence could be observed. Stern-Volmer plots were obtained, using NaI and CsBr as quenchers, both in the absence and presence of additives intended to model biomolecules or pharmaceutical excipients (linear alcohols and the surfactant SDS). In light of the role of ionic strength on complexation observed above, the quencher concentrations never exceeded 20 mM. Under these conditions the Stern-Volmer plots were all linear. The results of this study are tabulated in Table 1.

In the absence of additives, NaI quenching of the monomeric NAP fluorescence is inhibited, compared to that observed in water, by all of the CDs tested (α-, β-, HPBCD and γ). This is due to partial encapsulation of the fluorophore by the CD cavity in a 1:1 complex [10, 22, 24-26] thus protecting it from the iodide which is strongly associated with the bulk water environment (The binding constant for I^- with β-CD is only 18 M^{-1} [56]) The least protection is provided by α-CD which binds NAP relatively weakly due to the size mismatch between the α-CD cavity and the NAP (*vide supra*). Similar trends are observed for CsBr quenching of NAP (Table 1). Again, CsBr is associated primarily with the aqueous environment.

Table 1. NaI and CsBr quenching of naphthalene (100 μM) flourescence in the presence of cyclodextrins (10 mM)

CD Type	Additive	Im/Iex[b]	NaI				CsBr			
			K_{sv},m[c]	K_{sv},ex[c]	k_Q,m[d]	k_Q,ex[d]	K_{sv},m[c]	K_{sv},ex[c]	k_Q,m[d]	k_Q,ex[d]
None	–	[a]	292	–	7.5	–	58	–	1.5	–
β-CD	–	15	130	111	2.1	1.2	14.1	8.8	0.23	0.09
	10 mM propanol	17	251	95			18	9		
	100 mM propanol	142	85	–			8.2	–		
	10 mM pentanol	56	119	88			15.3	8.8		
	4 mM SDS	56	205	127			15	10.0		
HP-β-CD	–	[a]	171	–			8.6	–		
α-CD	–	[a]	214	–			23	–		
γ-CD	–	25	141	35			30	4.8		

[a]No excimer observed.
[b]Ratio of Im (monomer, 337 nm) to Iex (excimer, 420 nm) emission intensity.
[c]Stern-Volmer constants, K_{SV} in M^{-1}, for quenching of the monomer (1:1) and excimer (2:2) complexes. Errors as $\pm$10%.
[d]Rate constants ($k_Q \times 10^9$, M^{-1} s^{-1}) for quenching of the monomeric (or free if no CD present) and excimeric NAP excited states.

Table 1 also presents quenching data for the 2:2 β-CD:NAP complex. The ability of NaI to quench monomeric NAP is reduced by a factor of 292/130 = 2.3 times compared to free NAP. The quenching of the 2:2 complex is somewhat less efficient or 130/111 = 1.2 times less than the quenching of the 1:1 complex. The trend is similar for the CsBr quenching. The monomeric β-CD complex protects the NAP from CsBr by a factor of 58/14.1 = 4.1 times whereas the 2:2 complex is protected by an additional factor of 14.1/8.8 = 1.6.

The recent article from Grabner's group [45] reports unquenched fluorescence lifetimes for the three NAP species under consideration in the present analysis: the free NAP, the 1:1 β-CD:NAP complex; the 2:2 β-CD:NAP complex. The reported values are τ_{free} = 39 ns, $\tau_{1:1}$ = 61 ns and $\tau_{2:2}$ = 91 ns in the presence of 10 mM β-CD. These values have been combined with our measured Stern-Volmer constants (where $K_{SV} = k_Q\tau$) to generate values for the quenching rate constants, k_Q, shown in Table 1. The rate constants clearly support the view that the 2:2 β-CD complex provides additional protection of the NAP moiety compared to the 1:1 complex. This additional protection arises most likely because of the more complete encapsulation, and therefore isolation, of the NAP in the 2:2 complex.

Similar trends are observed for the complexes of NAP with γ-CD in the presence of NaI. The γ-CD protects the monomeric form of NAP by a factor of 292/141 = 2.1 times but the 2:2 form is protected by an additional factor of 141/35 = 4.0 times. The situation is very similar for CsBr quenching in the γ-CD system.

We have established that 2:2 complexation is a viable mode to protect a model compound from reagents (i.e., quenchers in this case) which are strongly associated with the aqueous environment. Can this protective encapsulation be maintained in the presence of additives? To answer this question we extended our quenching study to systems containing linear alcohols and the surfactant sodium dodecyl sulfate (SDS). Figure 6 is a plot of the observed monomer to excimer fluorescence intensity ratio (Im/Iex) as a function of added alcohol (propanol or pentanol) or of added SDS. In each case the additive reduces the importance of the 2:2 β-CD:NAP complex. The figure clearly shows that the longer the alkyl chain of the additive the more efficiently it breaks up the 2:2 complex. It should be noted that all the SDS data shown were collected under conditions where [Micelle] < [NAP]. Even at 10 mM SDS (the largest [SDS] used) the micelle concentration is only about 30 μM based on a cmc = 8.0 mM [57] and an aggregation number of 63 [58] for SDS. This means that the vast majority of the SDS present is in the form of non-micellized molecules.

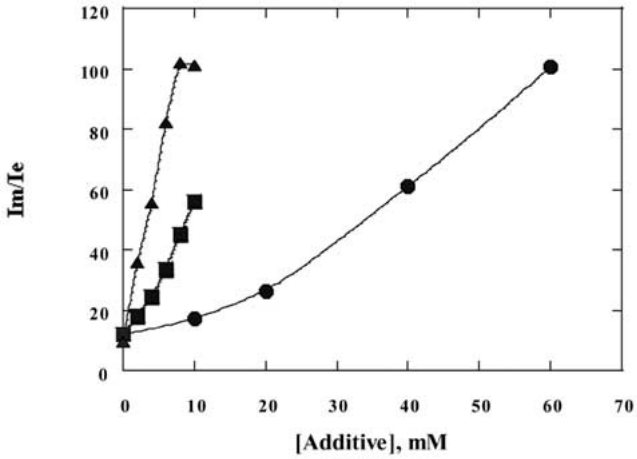

Figure 6. Variation of naphthalene monomer to excimer fluorescence intensity as a function of additive concentration. (●) = 1-Propanol, (■) = 1-Pentanol, (▲) = SDS. λ_{ex} = 280 nm, [Naphthalene] = 100 μM, [β-Cyclodextrin] = 10 mM.

The disruption of the 2:2 complex indicated in Figure 6 is reflected in the quenching data (Table 1) observed in the presence of the additives. 10 mM propanol has little impact on the Im/Iex value but increases K_{SV} for the monomer

complexes compared to that in the absence of the alcohol. This is true for both NaI and CsBr. Propanol has no impact on K_{SV} in the absence of β-CD. In an earlier study [26] of NAP binding to β-CD under 1:1 conditions only (i.e. very low [NAP]), we established that propanol lowers the binding of NAP to the CD to a modest extent. We attribute the increase in the K_{SV} values observed in the present study to an alcohol-mediated redistribution of NAP out of the CD cavities into the aqueous environment. This leaves the NAP relatively more exposed to the quenchers. However, that fraction of NAP that remains in a 2:2 complex is quenched to the same extent as in the absence of propanol. At 100 mM propanol the 2:2 complex no longer contributes to the observed NAP fluorescence indicating that the alcohol has completely broken up this complex. Interestingly the value of K_{SV} for the 1:1 complex has dropped relative to that observed at 10 mM propanol. As in an earlier report from our group, we attribute this to the formation of ternary complexes involving one unit each of the CD, NAP and propanol [26]. The alcohol causes partial redistribution of the NAP into the aqueous environment but the fraction that remains complexed in the ternary system is less quenchable than free or 1:1 complexed NAP.

In the presence of 10 mM pentanol the excimer complex is still observable, although its contribution is much reduced compared to the no additive case (Im/Iex = 56 as compared to 15 in the absence of pentanol). Again the 2:2 complex offers superior protection of the NAP from both NaI and CsBr. The behavior of 4 mM SDS is quite similar, i.e. it breaks up much of the 2:2 complex but that which remains protects the NAP from quenchers.

In conclusion, the present contribution establishes that higher-order CD complexes do provide enhanced protection of organic guests from water-based reagents via improved encapsulation. However, it also shows that the stability of higher-order systems built upon rather weak supramolecular association is very sensitive to ambient conditions such as ionic strength and the presence of alcohols and SDS. As a result we conclude that weakly bound higher-order complexes are probably not suitable for drug stabilization from a practical point of view. Rational design of CDs that experience electrostatic attraction is an alternate approach to developing more robust higher-order encapsulation systems. Studies of such systems are currently being pursued by us.

Acknowledgements

Financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Ryerson University Summer Academic Research Assistant Program is gratefully acknowledged.

References

1. T. Loftsson and M.E. Brewster: *J. Pharm. Sci*. **85**, 1017 (1996).
2. G. Thomas: *Medicinal Chemistry: an Introduction*, J. Wiley & Sons, Toronto (2000).
3. W. Saenger, J. Jacob, K. Gessler, T. Steiner, D. Hoffmann, H. Sanbe, K. Koizumi, S.M. Smith, and T. Takaha: *Chem. Rev*. **98**, 1787 (1998).
4. K.A. Connors: *Chem. Rev*. **97**, 1325 (1997).
5. O.S. Tee: *Adv. Phys. Org. Chem*. **29**, 1 (1994).
6. T. Nagai: *Comprehensive Supramolecular Chemistry, Vol. 3*, Pergamon/Elsevier: Oxford (1996).
7. C.H. Evans and T. Gunnlaugsson: *J. Photochem. Photobiol. A: Chem*. **78**, 57 (1994).
8. R. Breslow: *Acc. Chem. Res*. **28**, 146 (1995).
9. P. Bortolus and S. Monti: In D.C. Neckers, D.H. Volman and G. von Bünau (eds.), *Advances in Photochemistry*, John Wiley & Sons, New York (1996), Vol. 27, p 1.
10. M. Partyka, H.A. Bao, and C.H. Evans: *J. Photochem. Photobiol. A: Chem*. **140**, 67 (2001).
11. N. Funasaki, S. Ishikawa, and S. Neya: *J. Pharm. Sci.* **90**, 740 (2001).
12. K. Uekama, F. Hirayama, and T. Irie: *Chem. Rev*. **98**, 2045 (1998).
13. V.J. Stella, V.M. Rao, E.A. Zabbou, and V. Zia: *Adv. Drug Delivery Rev*. **36**, 3 (1999).
14. T. Irie and K. Uekama: *Adv. Drug Deliv. Rev.* **36**, 101 (1999).
15. D.C. Bibby, N.M. Davies, and I.G. Tucker: *Int. J. Pharmaceutics* **197**, 1 (2000).
16. T. Loftsson, A. Magnusdottir, M. Masson, and J.F. Sigurjonsdottir: *J. Pharm. Sci.* **91**, 2307 (2002).
17. J. Luengo, A. Aránguiz, J. Sepúlveda, L. Hernández, and C. Von Plessing: *J. Pharm. Sci*. **91**, 2593 (2002).
18. V.M. Rao and V. Stella: *J. Pharm. Sci*. **92**, 927 (2003).
19. M. Masson, T. Loftsson, S. Jonsdottir, H. Fridriksdottir, and D.S. Petersen: *Int. J. Pharmacutics* **164**, 45 (1998).
20. T. Loftsson: *Drug Stability* **1**, 22 (1995).
21. M.V. Encinas, E.A. Lissi, and A.M. Rufs: *Photochem. Photobiol*. **57**, 603 (1993).
22. C.H. Evans, S. De Feyter, L. Viaene, J. van Stam, and F.C. De Schryver: *J. Phys. Chem*. **100**, 2129 (1996).
23. J. van Stam, S. De Feyter, F. C. De Schryver, and C.H. Evans: *J. Phys. Chem.* **100**, 19959 (1996).
24. T.C. Barros, K. Stefaniak, J.F. Holzwarth, and C. Bohne: *J. Phys. Chem. A* **102**, 5639 (1998).
25. M. Christoff, L.T. Okano, and C. Bohne: *J. Photochem. Photobiol. A: Chem*. **134**, 169 (2000).
26. C.H. Evans, M. Partyka, and J. van Stam: *J. Inclusion Phenom*. **38**, 381 (2000).
27. A. Muñoz de la Peña, T.T. Ndou, J.B. Zung, K.L. Greene, D.H. Live, and I.M. Warner: *J. Am. Chem. Soc*. **113**, 1572 (1991).
28. H.C.B. Yang: *J. Phys. Chem*. **100**, 14533 (1996).
29. T. Yorozu, M. Hoshino, and M. Imamura: *J. Phys. Chem*. **86**, 4426 (1982).
30. K. Kano, I. Takenoshita, and T. Ogawa: *Chem. Lett*. 321 (1982).
31. W.G. Herkstroeter, P.A. Martic, and S. Farid: *J. Chem. Soc., Perkin Trans. II* 1453 (1984).
32. J.B. Zung, A. Muñoz de la Peña, T.T. Ndou, and I.M. Warner: *J. Phys. Chem*. **95**, 6701 (1991).
33. T. Tamaki, T. Kokubu, and K. Ichimura: *Tetrahedron* **43**, 1485 (1987).
34. S. De Feyter, J. van Stam, F. Imans, L. Viaene, F.C. De Schryver, and C.H. Evans: *Chem. Phys. Lett*. **277**, 44 (1997).
35. R.A. Agbaria and D. Gill: *J. Phys. Chem*. **92**, 1052 (1988).
36. R.A. Agbaria and D. Gill: *J. Photochem. Photobiol. A* **78**, 161 (1994).
37. K.A. Agnew, T.D. McCarley, R.A. Agbaria, and I.M. Werner: *J. Photochem. Photobiol. A* **91**, 205 (1995).
38. A. Ueno, K. Takahashi, and T. Osa: *J. Chem. Soc., Chem. Commun*. 921 (1980).
39. S. Hamai: *Bull. Chem. Soc. Jpn*. **55**, 2721 (1982).
40. S. Hamai: *J. Phys. Chem.* **94**, 2595 (1990).
41. S. Hamai, and N. Mononobe: *J. Photochem. Photobiol A: Chem.* **91**, 217 (1995).
42. S. Hamai: *Bull. Chem. Soc. Jpn.* **69**, 2469 (1996).
43. S. Hamai: *Bull. Chem. Soc. Jpn*. **69**, 543 (1996).
44. R.S. Murphy, T.C. Barros, B. Mayer, G. Marconi, and C. Bohne: *Langmuir* **16**, 8780 (2000).
45. G. Grabner, K. Rechthaler, B. Mayer, and G. Kohler: *J. Phys. Chem. A* **104**, 1365 (2000).
46. C. Bob: *Handbook of Chemistry and Physics*, 63rd edn. ed., CRC Press: Boca Raton (1982).

47. N.J. Turro, M. Aikawa, and A. Yekta: *J. Am. Chem. Soc.* **101**, 772 (1979).
48. J. Szejtli: *Cyclodextrins and Their Inclusion Complexes*, Akademiai Kiado: Budapest (1982).
49. Y. Liao, and C. Bohne: *J. Phys. Chem*. **100**, 734 (1996).
50. K.A. Connors: *Binding Constants* Wiley: New York (1987).
51. S. Hamai: *J. Incl. Phenom. Molec. Recog*. **27**, 57 (1997).
52. J. Dey, E.L. Roberts, and I.M. Warner: *J. Phys. Chem. A* **102**, 301 (1998).
53. Z. Yi, C. Zhao, Z. Huang, H. Chen, and H. Yu: *Phys. Chem. Chem. Phys*. **1**, 441 (1999).
54. M. Ghosh, R. Zhang, R.G. Lawler, and C.T. Seto: *J. Org. Chem*. **65**, 735 (2000).
55. R. Breslow: *Acc. Chem. Res.* **24**, 159 (1991).
56. R.P. Rohrbach, L.J. Rodriguez, E.M. Eryring, and J.F. Wojcik: *J. Phys. Chem.* **81**, 944 (1977).
57. H. Nakamura, A. Sano, and K. Matsuura: *Analytical Sciences* **14**, 379 (1998).
58. K. Kalyanasundaram: *Photochemistry in Microheterogeneous Systems*, Academic Press (1987).