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Journal of Photochemistry and Photobiology A: Chemistry 114 (1998) 151–157

Journal of Photochemistry and Photobiology A:Chemistry

The fluorescence enhancement of 1-anilinonaphthalene-8-sulfonate (ANS) by modified β-cyclodextrins

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Received 17 October 1997; received in revised form 26 November 1997; accepted 4 December 1997

Abstract

The fluorescence enhancement of the probe molecule 1-anilino-8-naphthalenesulfonate (ANS) by a number of modified β-cyclodextrins has been studied. Alkyl- and hydroxyalkyl-substituted β-cyclodextrins show significantly greater enhancement of ANS fluorescence than does the parent unmodified β-cyclodextrin (β-CD). In the cases of methyl-β-cyclodextrin (Me-β) and hydroxypropyl-β-cyclodextrin (HP-β), enhancements by a factor of 120 and 180, respectively, were observed for ANS fluorescence, compared to a factor of only 8.4 in the case of β-CD. The binding constant for formation of the 1:1 ANS:CD complex was determined to be 370 ± 80 M^{-1} for Me-β and 430 ± 70 M^{-1} for HP-β. The large increase in enhancement ability was shown to be a result of the relatively less polar environment experienced by the ANS probe incorporated in the modified as compared to the unmodified cyclodextrin cavities. The dielectric constants of the HP-β and Me-β cavities experienced by the included ANS were found to be 22 and 25, respectively. These polarities are similar to that of ethanol, whereas that of β-CD was found to be 54, a polarity similar to that of a 3:1 methanol:water mixture.

Keywords: Fluorescence enhancement; 1-Anilinonaphthalene-8-sulfonate; β-cyclodextrin

1. Introduction

There has been extensive interest in the physical and spectroscopic properties of guest–host inclusion complexes of organic molecules in cyclodextrin hosts [1–4]. Cyclodextrins (CDs) are cyclic amylose polymers, consisting of 6 (α), 7 (β), or 8 (γ) sugar units, with an overall truncated cone shape [5], as illustrated in Fig. 1 for β-CD. The hydroxyl groups around the top and bottom rims give CDs significant solubility in aqueous solution. The cyclodextrin molecules have an internal cavity, accessible to other molecules by an opening of 5.7, 7.8, and 9.5 Å for α, β, and γ, respectively [3]. This internal cavity is relatively nonpolar, therefore in aqueous solutions, hydrophobic molecules tend to become included in the CD cavity, forming supramolecular guest–host complexes [1–5]. Cyclodextrins may be referred to as 'molecular buckets', because of their shape and ability to act as a container for other molecules. The variation in cavity size among the three naturally occurring cyclodextrins allows for a certain degree of selectivity in the complexation of organic molecules of various sizes. This ability of cyclodextrins to form selective guest–host complexes has many practical applications to a wide range of fields, including molecular separations and chromatography [3,6], molecular sensing and recognition [7], solubilization of guest molecules [8], and enhancement of guest fluorescence (vide infra). As a result, cyclodextrins have been extensively studied as particularly useful supramolecular hosts in a variety of applications.

It has been well established that a wide range of organic molecules in aqueous solution exhibit enhanced fluorescence when incorporated into CD hosts [3,4]. There are a number of reasons for this, including protection from fluorescence quenchers (e.g., O_2), the loss of rotational freedom (which can result in less efficient nonradiative decay and hence greater fluorescence), and the relatively nonpolar environment provided to the probe molecule by inclusion within the CD cavity [3]. The latter is significant because many fluorescent probe molecules show significantly greater fluorescence in nonpolar than in polar environments. This fluorescence enhancement by cyclodextrin complexation has been exploited to increase the sensitivity of fluorescence-based trace detection techniques, such as the fluorescence detection of illicit drugs [9,10] and polychlorinated biphenyls (PCBs) [11].

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1010-6030/98/$19.00
PII S1010-6030(98)00210-X

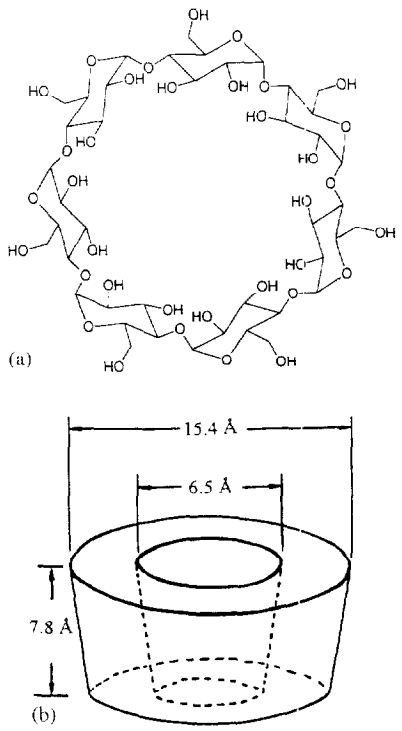

Fig. 1. (a) Chemical structure of β-cyclodextrin. (b) Idealized 3-dimensional structure and dimensions of β-cyclodextrin.

The naturally occurring α-, β-, and γ-cyclodextrins can be modified by replacing the hydrogens of the 1° and 2° –OH groups by other functional groups, such as alkyl or hydroxyalkyl groups [12,13]. These modified cyclodextrins offer a number of improvements over the unmodified forms, including increased aqueous solubility. In addition, the nonpolar cavity of the parent β-cyclodextrin has been extended to varying degrees; this should result in improved fluorescence enhancement abilities of these modified cyclodextrins. There have been a small number of studies on the fluorescence enhancement abilities of modified cyclodextrins, which have clearly shown the increased fluorescence enhancement compared with the unmodified counterparts [14–24]. In this paper, we systematically investigated the fluorescence enhancement of 1-anilino-8-naphthalenesulfonate (ANS) in a variety of modified β-cyclodextrins, listed in Table 1. ANS is a well-known fluorescence probe, which shows extreme sensitivity of its fluorescence (both wavelength maximum and quantum yield) to the polarity of its local environment [25,26]. This probe has been used extensively to study unmodified cyclodextrins [27–31], and in a very few cases, modified CDs [20,23]. The large changes observed in the fluorescence wavelength maximum and intensity of ANS upon incorporation into the modified cyclodextrins allowed for a detailed spectroscopic investigation of the effect of inclusion on the fluorescent probe. In addition, information on certain physical properties of the modified cyclodextrins themselves, such as cavity polarity, was obtained.

2. Experimental

8-Anilino-1-naphthalene sulfonic acid (ANS), α-CD, β-CD, Me-β, and HP-β were obtained from Aldrich Chemical, and used as received. γ-CD, Me-β, HE-β, HP-β, Su-β, and CM-β were obtained from Cerestar USA, and were used as received. The abbreviations used, the average molecular weights, and the degree of substitution (out of a maximum of 21 hydroxyl substitution sites) for the various modified β-CDs obtained from the two sources are given in Table 1. The degree of substitution was calculated by dividing the difference between the reported molecular weight of the modified β-CD and that of unsubstituted β-CD by the molecular weight of the substituting group minus 1 hydrogen. Solutions of ANS were either 1.0×10^{-4} M or 5.0×10^{-5} M in aqueous phosphate buffer ($pH=6.80\pm0.10$); observed enhancement results were the same at the two ANS concentrations.

Absorption spectra were measured on a Hewlett Packard 8453 diode array spectrometer. Fluorescence spectra were obtained on a Perkin-Elmer LS-5 luminescence spectrometer, with excitation and emission monochrometer bandpasses set at 3 nm and an excitation wavelength of 340 nm in 1 cm^2 quartz fluorescence cells. Solutions were not oxygen-purged, as preliminary investigations showed a negligible effect of

Table 1
The modified β-cyclodextrins used in this work

Modified cyclodextrin (modifying group)	Source	Abbreviation	Average molar mass	Average degree of substitution
Methyl-β-cyclodextrin ($-CH_3$)	Cerestar	Me-β-1	1330	13.9
	Aldrich	Me-β-2	1310	12.5
Hydroxyethyl-β-cyclodextrin ($-CH_2CH_2OH$)	Cerestar	HE-β	1420	6.5
Hydroxypropyl-β-cyclodextrin ($-CH_2CHOHCH_3$)	Cerestar	HP-β-1	1510	6.5
	Aldrich	HP-β-2	1540	7.0
Sulfated β-cyclodextrin ($-SO_3^-Na^+$)	Cerestar	Su-β	2560	14
Carboxymethyl-β-cyclodextrin ($-CH_2CO_2^-Na^+$)	Cerestar	CM-β	1450	3.9

purging on the observed fluorescence intensity ($<5\%$). Fluorescence enhancements (F/F_0) were determined as the ratio of the integrated area under the corrected fluorescence spectrum (I_F vs. $\bar{\nu}$) of ANS in the presence and absence of the CD of interest.

3. Results

3.1. Fluorescence enhancement

The relative fluorescence spectra of ANS in the absence of CD and in the presence of 10 mM β-CD, HP-β-1, HP-β-2, and Me-β-1 are shown in Fig. 2. On this scale, the fluorescence of ANS in the absence of added CD is extremely low, barely registering above the baseline. Addition of 10 mM β-CD resulted in a significant enhancement of the ANS fluorescence, as is well-known from the literature [27,29]. However, addition of some of the modified β-CDs resulted in a significantly greater fluorescence enhancement of the ANS fluorescence. The values of the observed fluorescence enhancements of ANS in all of the CDs studied are listed in Table 2. The enhancement by α-CD of only 2.1 can be attributed to the relatively small cavity size of this CD, which is apparently unable to accommodate the naphthyl fluorophore moiety of ANS. The value of 8.4 for β-CD agrees well with the early literature value of 10 [27]. In the case of γ-CD, the observed ANS enhancement is 11; its larger cavity size does not result in a significant increase in the ANS fluorescence enhancement.

In the case of the modified CDs, significant differences were observed in the ANS fluorescence enhancements relative to that of β-CD. For the two anionic CDs, Su-β and CM-β, the observed enhancement was decreased by approximately a factor of 2. This can be explained by the fact that ANS is an anionic probe in the phosphate buffer, hence Coulombic repulsion between the cyclodextrin host and the guest probe species would result in less favorable complex formation. Such a decrease in fluorescence for these anionic CDs relative to the unmodified β-CD might not be observed in the case of a neutral probe molecule.

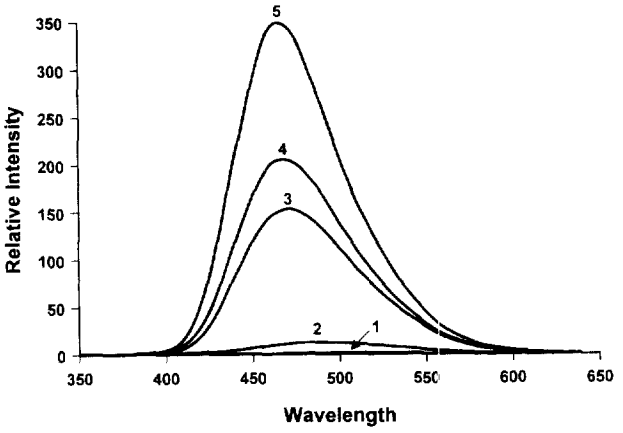

Fig. 2. Relative fluorescence spectra of ANS in various cyclodextrin solutions (10 mM in phosphate buffer): **1** no CD; **2** β-CD; **3** HP-β-1; **4** Me-β-1; **5** HP-β-2.

Table 2
Fluorescence parameters of ANS in various cyclodextrin solutions (10 mM in phosphate buffer)

Cyclodextrin	$\lambda_{F,max}$ (nm)	$\bar{\nu}_{F,max}/10^3$ cm^{-1}	Fluorescence enhancement
α	505	19.80	2.1 ± 0.2
β	492	20.32	8.4 ± 0.8
γ	492	20.32	11 ± 1
Su-β	497	20.12	5.6 ± 0.3
CM-β	492	20.32	5.5 ± 0.2
HP-β-1	471	21.23	87 ± 7
HE-β	469	21.32	92 ± 6
Me-β-2	467	21.41	100 ± 12
Me-β-1	467	21.41	120 ± 14
HP-β-2	465	21.52	180 ± 20

In the case of the alkyl and hydroxyalkyl substituted CDs, extremely large increases in the fluorescence enhancement of ANS were observed. HE-β showed an enhancement of ANS fluorescence of 92. Me-β showed a larger enhancement, with values of 120 and 100, respectively, for Me-β-1 and Me-β-2; the former is a factor of 14 times larger than the enhancement observed for unmodified β-CD. An even greater enhancement of 180 was observed with HP-β-2; this is a factor of 21 times larger than the enhancement observed for unmodified β-CD. However, HP-β-1 showed an enhancement of only 86. For the two modified CDs obtained from the two different sources, the differences observed can be related to the calculated degree of substitution: the sample with the higher degree of substitution gave the greater observed enhancement (Me-β-1 and HP-β-2). In the remainder of this work, unless otherwise indicated, the modified β-CD samples of each type used was that which gave the largest observed enhancement (i.e., Me-β refers to Me-β-1 and HP-β refers to HP-β-2).

3.2. Binding constant for the ANS:Me-β-CD and ANS:HP-β-CD complex

For a 1:1 ANS:CD complex, the binding equilibrium constant K can be defined as follows:

$$\mathrm{ANS}+\mathrm{CD}\rightleftharpoons\mathrm{ANS{:}CD} \quad (1)$$

$$K=\frac{[\mathrm{ANS{:}CD}]}{[\mathrm{ANS}][\mathrm{CD}]} \quad (2)$$

The numerical value of K can be obtained from observed fluorescence enhancement F/F_0 as a function of added cyclodextrin concentration ($[\mathrm{CD}]_0$): [32,33]

$$F/F_0=1+(F_\infty/F_0-1)\frac{[\mathrm{CD}]_0K}{1+[\mathrm{CD}]_0K} \quad (3)$$

where F is the integrated fluorescence intensity in the presence of CD, F_0 is the integrated fluorescence intensity in the

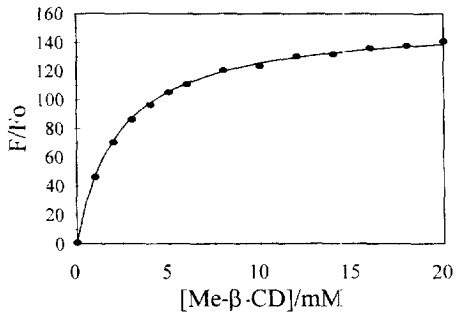

Fig. 3. Observed fluorescence enhancement of ANS as a function of Me-β-CD concentration. Solid line: best fit to Eq. (3): $K = 370\ M^{-1}$.

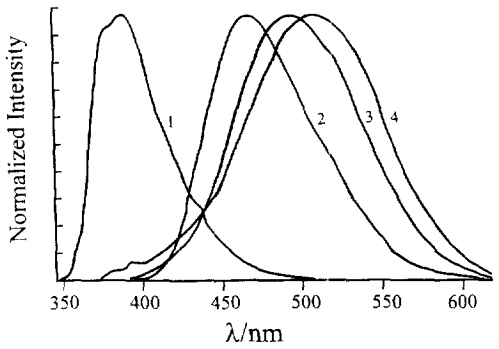

Fig. 4. Scaled fluorescence spectra of ANS in various cyclodextrin solutions (10 mM in phosphate buffer) and solvents: **1** cyclohexane; **2** Me-β-CD; **3** β-CD; **4** H_2O.

absence of CD, and F_∞ is the integrated fluorescence intensity when *all* of the ANS probe molecules have been complexed by CD molecules.

In the case of β-CD, ANS is known to form only a 1:1 complex [29]. This must also be shown to be true in the case of the modified β-CDs, however, before Eq. (3) can be used to obtain K. This can be determined by a double reciprocal plot (also known as a Benesi–Hildebrand-type plot [34]) of F_0/F vs. 1/[CD]; this plot will be linear if only a 1:1 complex is formed, but will show curvature if complexes of other stoichiometry are being formed [29,30,32–34]. The enhancement was measured as a function of [CD] from 0 to 20 mM, and an excellent linear plot was obtained for both Me-β ($R = 0.9998$) and HP-β ($R = 0.9991$), thus confirming the 1:1 complex as being the only significant complex in this concentration range. The data were therefore fitted to Eq. (3), using nonlinear least squares analysis, with F_∞/F_0 and K as the fit parameters. Fig. 3 shows the plot of the fluorescence enhancement F/F_0 as a function of Me-β concentration; the solid line shows the best fit of this data with Eq. (3), for which the result was $K = 370 \pm 80\ M^{-1}$ (the given error range is the statistical deviation from the nonlinear fit; this value of K was well reproducible within this range in three independent trials). Similar plots were obtained for HP-β from both sources, yielding values of $K = 280 \pm 80\ M^{-1}$ and $430 \pm 70\ M^{-1}$ for HP-β-1 and HP-β-2, respectively.

3.3. Cavity polarity of the modified cyclodextrins

Fig. 4 compares the positions of the normalized fluorescence spectra of ANS in water, 10 mM β-CD, 10 mM Me-β, and cyclohexane. The spectrum in β-CD is blue-shifted relative to that in water, indicating the relatively less polar environment of the β-CD cavity relative to the bulk water solution. However, the spectrum in Me-β is significantly more blue-shifted, with the emission maximum moving closer to that in the nonpolar cyclohexane. The relative polarity of the CD cavities can be determined by comparing the emission maximum of the ANS:CD inclusion complexes (listed in Table 2) with that of ANS in solvents of varying polarity (listed in Table 3). Fig. 5 shows a plot of $\bar{\nu}_{F,max}$ vs. dielectric constant for the solvents listed in Table 3; an excellent correlation is obtained ($R = 0.991$). Only polar protic solvents or solvent mixtures[1] were used, as a different correlation (i.e., a different slope) was obtained with aprotic polar solvents. Similar results were observed in a previous β-CD polarity study using diphenylamine as the probe [35]. Also shown on Fig. 5 are the values of $\bar{\nu}_{F,max}$ for β-CD and Me-β plotted on the line of best fit; these allow for the determination of the dielectric constant for the cavities of these two CDs: $\varepsilon = 54$ for β-CD and $\varepsilon = 25$ for Me-β. Correlations of $\bar{\nu}_{F,max}$ with other solvent polarity parameters, such as Z and E_T, were also attempted; none of these were as satisfactory as the correlation obtained with solvent dielectric constant. In the case of HP-β-CD, the emission maximum was

[1] For the mixed methanol:water solvents, the dielectric constant was calculated based on the mole fractions and dielectric constants of the two solvents: $\varepsilon_{mix} = \chi_{MeOH}\varepsilon_{MeOH} + \chi_{H_2O}\varepsilon_{H_2O}$.

Table 3
Solvent polarity parameters and absorption and emission maxima of ANS in various polar protic solvents

Solvent	Z	E_T	ε	λ_A/nm	λ_F/nm	$\bar{\nu}_{F,max}/10^3\ cm^{-1}$	$\bar{\nu}_A - \bar{\nu}_F/cm^{-1}$
H_2O	94.6	63.1	78.4	356	520	19.23	8859
3:1 H_2O:MeOH	93.2	62.[illegible]	72.4	360	508	19.69	8093
1:1 H_2O:MeOH	91.4	61.[illegible]	64.0	367	495	20.2	7046
1:3 H_2O:MeOH	89.9	59.8	52.3	371	489	20.44	6504
Ethylene glycol	85.1	56.6	37.7	370	479	20.88	6150
Methanol	83.6	55.5	32.7	373	475	21.05	5757
Ethanol	79.0	51.9	24.5	374	466	21.46	5279
1-Butanol	77.7	50.2	20.4	377	464	21.55	4973
2-Propanol	76.3	48.6	19.9	375	462	21.64	5022
2-Butanol	–	47.1	16.6	375	462	21.64	5022

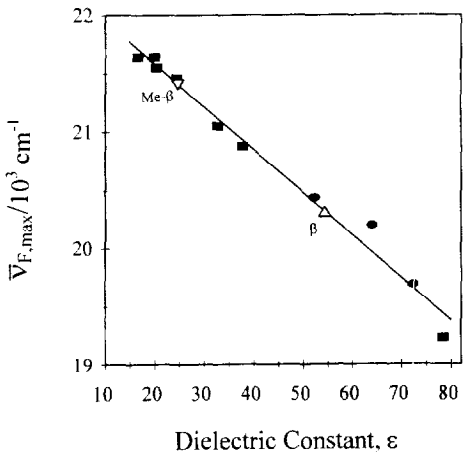

Fig. 5. Plot of fluorescence maximum $\bar{\nu}_{F,max}$ vs. dielectric constant, ε, for ANS in various solvents and aqueous cyclodextrin solutions: (■) pure solvents; (●) MeOH:H_2O mixtures; (△) β-CD; (▽) Me-β-CD.

slightly blue-shifted with respect to Me-β, as shown in Table 2. From the correlation shown in Fig. 5, a value of $\varepsilon = 22$ was obtained for the cavity polarity of HP-β.

4. Discussion

A significant increase in ANS fluorescence enhancement was obtained using the alkyl- and hydroxyalkyl-substituted β-cyclodextrins; the largest enhancement observed was a factor of 180 for ANS in HP-β. Of the previous literature reports on fluorescence enhancement by modified CDs, only three were found that gave numerical values for the observed enhancements. Frankewich et al. [16] measured the enhancement of a wide range of probe molecules (not including ANS) in HE-β, HP-β, and Me-β; the maximum enhancement observed was a factor of 48 for aflatoxin B1 in HP-β. Reeuwijk et al. [20] observed enhancements of 3.1 and 21 for ANS in β-CD and HP-β, respectively. Penn et al. [21] observed an extremely large enhancement of 245 for 2,6-ANS in Me-β. However, in all three of these cases, the enhancement was measured as the ratio of the fluorescence intensities in the presence and absence of CD *at a single emission wavelength*. Thus, these reported enhancements include not only the increase in fluorescence quantum yield upon incorporation into the CD, but also the effect of the blue-shift in the emission spectrum of the probe molecules. By choosing the emission wavelength near the maximum of the spectrum of the probe–CD complex, the largest possible enhancements were obtained. This is completely legitimate in these studies, which were concerned with the application of fluorescence enhancement by modified CDs to chromatographic separations and detection, thus, the authors were interested in obtaining the maximum possible enhancement. However, these reported enhancements do not accurately reflect the effect of CD complexation on the photophysics of the probe molecule, which is of fundamental interest in the understanding of the guest–host interactions in these complexes. The method of calculating enhancement as the ratio of the integrated emission spectrum (in wavenumbers) used in the present study eliminates the effect of the blue shift of the spectrum. Since the same ANS concentrations were used, and since the ANS absorption spectrum does not change appreciably upon incorporation into CDs, these enhancements are direct measurements of the *relative* quantum yields of the ANS probe in the various CDs. Thus, this work shows that the fluorescence quantum yield of ANS increases significantly upon complexation with CDs, with the largest quantum yield being observed in the alkyl-substituted CDs Me-β and HP-β.

The values of the binding constant, K, of 280, 370, and 430 M^{-1} obtained for ANS in HP-β-1, ME-β and HP-β-2 are significantly larger than the values of 65 M^{-1} [31], 85 M^{-1} [30] and 110 M^{-1} [29] reported in the literature for ANS binding with β-CD. This increased binding efficiency is a result of the extension of the β-CD cavity by the alkyl or hydroxyalkyl groups, which results in a better fit for the relatively large ANS molecule. This is in agreement with a previous report of a similar increase in ANS binding constant upon attachment of side chains (other than alkyl or hydroxyalkyl) to β-CD [36]. There is a similar trend in the values of K and the observed enhancement factors: a CD with a higher observed ANS enhancement was also found to have a higher measured binding constant. However, these values of K for ANS in the modified β-CDs are still significantly smaller than the reported value of 1260 M^{-1} for ANS in γ-CD [31]. This clearly shows that it is not the efficiency of complex formation which is important in fluorescence enhancement, since the fluorescence of ANS in γ-CD is enhanced only by a factor of 11, whereas the fluorescence of ANS in HP-β is enhanced by a factor of 180.

The polarity of the CD cavity as experienced by ANS is much lower for the alkyl and hydroxyalkyl modified β-CDs than for β-CD. This makes sense from a consideration of the chemical structures: the rims of the cavity of β-CD are lined with hydroxyl groups, whereas for HP-β-CD and Me-β-CD many of these hydroxyl groups have been replaced by $-CH_3$ or $-CH_2-$ groups. The value of $\varepsilon = 54$ determined for β-CD indicates that this CD has a cavity polarity similar to 1:3 H_2O:MeOH, whereas the values of $\varepsilon = 22$ and 25 for HP-β-CD and Me-β-CD, respectively, are similar to that of ethanol. Only one previous report of a cavity polarity for a modified CD was found in the literature. Hansen et al. [19] reported that the polarity of the di-Me-β-CD cavity resembles that of a 3:1 EtOH:H_2O solvent mixture; this is in reasonable agreement with the values determined here. By contrast, there have been numerous reports in the literature on the cavity polarity of unmodified β-CD, however, with a fair degree of discrepancy. The value obtained here is in excellent agreement with a recent report based on the emission maxima of indoles [37] and a previous report based on the emission maximum of pyrene-3-carboxaldehyde [38], but is much higher than a number of other previous measurements, which report the β-CD cavity polarity to be similar to ethanol ($\varepsilon = 24.5$)

[35,39,40], 1-propanol (ε=20) [41], or ethyl acetate (ε=6) [42]. Part of the reason for such a wide range of β-CD cavity polarities is that the measurements have been based on the spectroscopic properties of a wide variety of probe molecules; clearly the cavity polarity experienced by each type of probe will be somewhat different depending, for example, on the probe's size and shape.

The observed ANS fluorescence enhancements by the various modified β-CDs correspond well with the relative cavity polarities determined. The enhancement was found to increase as the cavity polarity of the modified β-CD decreased. Thus, the ability of a particular modified β-CD to enhance ANS fluorescence depends directly on the polarity experienced by the ANS probe when it is complexed with the CD. The reason that the alkyl and hydroxyalkyl β-CDs show such greater fluorescence enhancement than the parent unmodified β-CD is simply that they provide a much less polar micro-environment. As was illustrated by the enhancement results for the modified β-CDs from the different sources, this will depend not only on the particular substituent involved, but also on the degree of substitution. In the present case, having more alkyl or hydroxyalkyl substituents per β-CD was found to give a larger enhancement, and in the case of HP-β-CD, a more blue-shifted ANS spectrum, which is indicative of a less polar CD cavity. In this latter case, where a large difference in enhancement by HP-β-CD from the two sources was observed, the difference in average degree of substitution between the two sources was very small, only 0.5 hydroxypropyl groups per cyclodextrin. It may be possible that the distribution of substituted hydroxypropyl groups may also be different between the two sources, i.e., a different ratio of primary to secondary sites modified in the two samples. This possibility is also indicated by the small but significant difference in the value of the ANS binding constant for this CD from these two sources (280 and 370 M^{-1}). Such a distribution could have an effect on the micro-environment experienced by the ANS in the CD cavity. Thus, it is essential that any modified cyclodextrins being used for fluorescence enhancement be well characterized.

The effect of polarity on the quantum yield of ANS has previously been shown to be a result of the polarity dependence of the S_1–S_0 energy gap (ΔE_{10}), and therefore its effect on the rate of nonradiative decay [26]. The blue-shift in the fluorescence spectrum of ANS observed upon decrease in solvent polarity (cf. Table 3) indicates that ΔE_{10} increases as the polarity decreases. This will result in an exponential decrease in the rate constant k_{IC} for internal conversion (IC) from S_1 to S_0 [43,44]. This will result in an *increase* in the fluorescence quantum yield:

$$\phi_F = \frac{k_R}{(k_R + k_{NR})} \quad (4)$$

where k_R is the rate constant for radiative decay and k_{NR} is the rate constant for nonradiative decay, i.e., the total rate constant for IC and intersystem crossing (ISC). This can be rigorously tested by measuring both the absolute quantum yield ϕ_F (by comparison to a fluorescence standard) and the fluorescence lifetime τ_F of ANS in the various CDs. Since $\tau_F = (k_R + k_{NR})^{-1}$, measurement of ϕ_F and τ_F will allow for the separate calculation of k_R and k_{NR}. Therefore, this would determine conclusively whether the observed increase in ϕ_F of ANS upon incorporation into a modified CD is solely a result of a decrease in k_{NR}, as is the case in solvents of differing polarity [26]. Unfortunately, this is not as simple a procedure as it would be in homogeneous solution, because the fluorescence decay of probe molecules incorporated in CDs is well known to be very complicated, in fact requiring a distribution of lifetimes for proper fitting [45–48]. Thus, extracting a single lifetime from the measured fluorescence decay is not a straight forward procedure. An investigation of the proper technique for fitting these complex decays, and then using the fit results to obtain meaningful values for k_R and k_{NR} (which will necessarily be some sort of average) is currently underway in our laboratory. Once the proper procedure has been determined, it will then be possible to perform the above-described photophysical analysis of the fluorescence of ANS in modified β-CDs.

Although the large observed enhancements of ANS fluorescence have been explained solely in terms of the polarity of the CD cavities, there may also be a contribution from the effective microviscosity experienced by the included ANS. It has been shown that ϕ_F of 2,6-ANS increases in solvents of higher viscosity but similar bulk polarities [49], a result of the restriction of intramolecular rotation (i.e., of the anilino ring relative to the naphthalene moiety) and its effect on nonradiative decay. 1,8-ANS fluorescence has also been shown to be viscosity-dependent [50]. A contribution to the observed enhancement by such a microviscosity effect cannot be ruled out in the present case; future studies involving the measurement of time-resolved fluorescence spectra and anisotropy decays might allow for a determination of the importance of this effect.

5. Summary

ANS showed extremely large fluorescence enhancements when incorporated into modified β-cyclodextrins in aqueous solution; these enhancements (90–180) were much larger than the previously-reported enhancement of ANS by the parent unmodified β-cyclodextrin (10). Although the binding constant for the ANS–CD complex was found to be significantly larger for the modified β-CDs than that previously reported for β-CD, this was not the source of the increased enhancements, since the binding constants were still much smaller than that for ANS–γ-CD, which shows a relatively small enhancement, similar to that of β-CD. The results illustrated by Figs. 4 and 5 clearly show that ANS experiences a significantly less polar environment when incorporated in Me-β-CD than when incorporated in β-CD. The effect of this local polarity on the nonradiative decay rate is proposed to be the cause of the large observed increases in the ANS

fluorescence quantum yield, and thus the large fluorescence enhancements. This can be further investigated once proper techniques for analyzing the complex fluorescence decay curves for the guest–host complexes has been established.

Acknowledgements

Financial support for this work was provided by the U.P.E.I. Senate Committee on Research. The authors gratefully acknowledge Cerestar USA, for generously supplying samples of cyclodextrins and modified cyclodextrins free of charge.

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