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The use of fluorescence quenching measurements to study the cyclodextrin inclusion complexes of 2-aceotonaphthone

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THE USE OF FLUORESCENCE QUENCHING MEASUREMENTS TO STUDY THE CYCLODEXTRIN INCLUSION COMPLEXES OF 2-ACETONAPHTHONE

by

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ABSTRACT

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2-Acetonaphthone (2-AN) is an unusual molecule in that it will only fluoresce in the presence of strong hydrogen bond donors such as water. Less than a 10 mol % addition of a weaker hydrogen bonding molecule such as ethanol results in significant quenching of the 2-AN fluorescence in water. We have used this behavior to monitor the binding of 2-AN to the ethanol-like interior of the α–,β–, and γ-Cyclodextrin (CD) cavities. The addition of CD's to aqueous solutions of 2-AN causes a dramatic quenching of the 2-AN fluorescence. Because the quenching is static, we attribute the observed behavior to the formation of a temperature dependent equilibrium between the CD, 2-AN and their corresponding 1:1 complex. The equilibrium constant (K) can be determined by fluorescence quenching measurements. We have obtained data which characterizes the temperature dependency of the formation constant and used it to calculate the enthalpies and entropies of complex formation. Further studies involving the use of fluorinated alcohols to modify the cyclodextrin cavity as well as methods for the analysis of complexes with other than 1:1 stoichiometries will be discussed.

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TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

INTRODUCTION

Cyclodextrins are toroidal shaped, cyclic oligomers of D(+)-glucopyranose molecules joined together by α–1,4-glycosidic bonds. Cyclodextrins are unique because they are able to form inclusion complexes with a variety of aromatic molecules by incorporating the aromatic moiety within their toroidal internal cavity. The three most commonly used cyclodextrins are α–, β– and γ-cyclodextrins composed of 6, 7 and 8 glucose units, respectively. Some of their physical properties are presented in Table 1.

Because of their unique properties, cyclodextrin inclusion complexes have been extensively studied. The formation of such inclusion complexes during a chromatographic separation is known to significantly enhance the selectivity of the separatory method. As such, cyclodextrins have been used in both the bonded and the mobile phases of HPLC separations.[1] Moreover, because complexation imposes certain geometrical requirements on the included molecule, cyclodextrins have become useful catalysts for several photochemical reactions.[2,3] Thus cyclodextrins are important tools in organic synthesis as well.

The binding forces involved in cyclodextrin inclusion complexes are not completely understood. Because the internal cavity of the cyclodextrin is nonpolar, it is thought that the hydrophobic effect partially contributes to the thermodynamic factors which permit complex formation. However, in most

TABLE 1

Some Physical Properties of the Cyclodextrins

TABLE 1

CD	Number of glucose units	Molecular weight (g/mol)	Internal diameter (Å)
α-CD	6	972.86	4.5 - 6.0
β-CD	7	1135.01	6.0 - 8.5
γ-CD	8	1297.15	8.0 - 10.5

cases, there is also a very large positive entropy associated with complex formation. The structure of the included molecule is a fundamental factor in determining the different degrees to which these two thermodynamic forces compete in the overall binding process. Several other factors must also be taken into account when analyzing the energies which favor complex formation:[1] these are:

(1) Van der Waals interactions between the cyclodextrin and its guest

(2) Hydrogen bonding to hydroxyl groups on the cavity's ridge

(3) Release of previously included water molecules upon complexation

(4) The effect of complex formation on the strain energy of the macromolecular ring.

The goal of this project is to understand, more completely, the ways in which cyclodextrins bind substituted naphthalenes. In doing so, the effects of varying the substituent's structure on the thermodynamics of complex formation will be discussed.

Molecular fluorescence spectroscopy is a popular technique for studying cyclodextrin inclusion complexes. In most cases, cyclodextrins cause fluorescence enhancement upon the inclusion of fluorophores.[2,3] This occurs for a number of reasons. Molecules are more fluorescent in nonpolar rather than in aqueous media. Thus, the etahanol-like environment of the cyclodextrin cavity is likely to cause an increase in fluorescence quantum yield upon complexation.[3] Also cyclodextrins also protect the

fluorophore from bimolecular quenchers such as molecular oxygen. Furthermore, the bound fluorophore suffers a decrease in its vibrational degrees of freedom which may reduce the probability that radiationless decay will occur through vibratory modes.

Because of the potential applications of CD inclusion complexes to the many fields of chemistry, a wide variety of literature on the subject has become available. Articles on cyclodextrin chemistry have appeared in the literature as early as 1950. Several recent articles describe the use of molecular fluorescence spectroscopy to study the formation of cyclodextrin complexes. Most of these articles indicate that cyclodextrins cause an increase in fluorescence quantum yields and a decrease in Stern - Volmer quenching constants because the included fluorophore is protected from bimolecular quenchers.[4] These properties were then used to determine the dissociation constants of such inclusion complexes. In addition Street et al. have described methods in which the pyrene I/III fluorescence band ratios are used to measure the dielectric constants of the CD cavities.[5]

In order to fully understand the ways in which substituted naphthalenes form inclusion complexes with cyclodextrins, a review of the relevant literature published over this last decade (1980-1989) is warranted. This review will be used to draw comparisons to the results we report for the formation of cyclodextrin inclusion complexes with 2-acetonaphthone.

Kano et al. investigated the 1:1 inclusion complexes formed between naphthalene and the three cyclodextrins. The strongest inclusion complex of naphthalene is formed with β-CD while the weakest is formed with γ-CD.

Although α-CD does not form a 1:1 inclusion complex with naphthalene, it is possible that some naphthalene may bind to the external surface of the α-cyclodextrin molecule.[4] This type of size dependent behavior is typical of the so called lock and key mechanism used to describe enzyme substrate interactions. It indicates that the strongest inclusion complex for a given substrate is formed in the cyclodextrin cavity which is most similar in size to the substrate.

Perhaps one of the most comprehensive studies of 1:1 cyclodextrin inclusion complexes is that conducted by Catena and Bright.[6] They report the formation constants, as well as the enthalpies and entropies of complexation for the 1:1 inclusion complexes formed between β-CD and eight different anilinonaphthalenesulfonates (ANS). The relative differences in complex strength were attributed to steric factors as well as the different abilities of each guest to form hydrogen bonds with the hydroxyl groups lining the outer ridges of the β-CD cavity. For example, the sulfonate functionalities of 2,6-ANS and 2,7- ANS readily form a hydrogen bond with the hydroxyl groups lining the β-CD cavity. Their binding constants and enthalpies of formation are much larger than those of 2,8-ANS, which is sterically inhibited from forming a tightly bound complex with β-CD.[6] Generally, any 1-substituted naphthalene will form a weaker cyclodextrin inclusion complex than its 2-substituted analog.

From the aforementioned trends in their data, Catena and Bright established what they call the equatorial mechanism for cyclodextrin

inclusion complex formation (Figure 1). In this mechanism, the long axis of the naphthalene moiety aligns itself parallel to the central axis of the cyclodextrin cavity. This is energetically favored over the axial approach in which the long axis of the naphthalene moiety aligns itself perpendicular to the central axis of the cavity. Based on the dimensions of the naphthalene moiety and the cyclodextrin cavity, a complex formed in the axial manner would generate too much ring strain in the CD macrocycle to be energetically favored over the equatorial mechanism.[6]

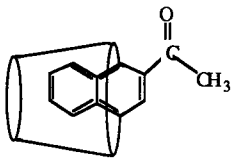
Kano et al. also studied the static fluorescence quenching of pyrene and naphthalene by several amine quenchers in aqueous cyclodextrin solutions.[4] Their purpose was to use bimolecular fluorescence quenching measurements to learn how cyclodextrins catalyze cycloaddition reactions such as the

Diels-Alder reaction. An extensive analysis of naphthalene fluorescence quenching by various amines has shown that most amine quenchers are less potent in the presence of β-CD than in the presence of γ-CD, while α-CD has the smallest affect on the strength of the quencher. This is because the naphthalene is not as tightly bound to the α and γ-CD cavities as it is to the β-CD cavity. Consequently, it is easier for the quencher to interact with naphthalene bound to the α or γ-cyclodextrin cavity. In addition, it is expected that bulkier quenchers will have smaller Stern-Volmer quenching constants in the presence of a given cyclodextrin. Indeed, trimethylamine has a K_{SV} of 195 $\underline{M}^{-1}$ in the presence of γ-CD, while the larger quencher,

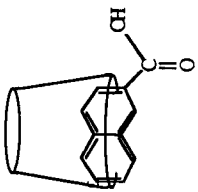
FIGURE 1

Axial and Equatorial Mechanisms for
Cyclodextrin Inclusion Complex Formation

FIGURE 1



Equatorial Mechanism



Axial Mechanism

triethylamine, has a K_{SV} of only 36 M^{-1}. [4]

Cyclodextrins have also been used to determine the points of collision involved in a bimolecular quenching process. For example Yorozu et al. investigated the fluorescence of 2–naphthol in solutions of the three cyclodextrins.[7] Circular dichroism was used to determine differences in the geometrical configurations of the three resulting inclusion complexes. The 2-naphthol molecule forms structurally similar complexes with β and γ-CD, both of which differ from the complex formed with α-CD. Furthermore the strongest complex is formed with β-CD followed by α-CD and then γ-CD. Yet the rate of proton dissociation is suppressed more by α-cyclodextrin than by the other two cyclodextrins. Thus the hydroxyl substituent must be included in the α-CD cavity but not in the β or γ-CD cavities.[7] The differing structures of these complexes were then used to explain the role of the hydroxyl substituent in NaI induced, bimolecular quenching. Since 2-naphthol is more efficiently quenched by I^- in the presence of β and γ-CD than in the presence of α-CD, it is concluded that collision with the hydroxyl substituent is necessary to achieve bimolecular quenching.

Cox et al. found that 1-α-naphthyl-3-(dimethylamino)propane forms a charge transfer exciplex in the presence of β-CD.[8] In solutions of .01 M β-CD, a decrease in the naphthalene-like fluorescence band (334 nm) with increasing pH is accompanied by a simultaneous increase in the exciplex emission band (500 nm). This is attributed to the increased quenching strength of the deprotonated amine group relative to its quenching strength in the protonated form. They determined the equilibrium constants for the

formation of the 1:1 complexes formed between both the protonated and deprotonated amine and β-CD. The relatively small binding constant of the protonated species, 250 M^{-1}, is attributed to its increased solubility in water over that of the deprotonated species (K= 630 M^{-1}).[8]

Substituted naphthalenes do not always form 1:1 complexes with cyclodextrins. It is sometimes favorable for the cyclodextrin to form higher order complexes by including two guests to form a 1:2 complex, or by incorporating another cyclodextrin (complexed or not) into the 1:1 complex to form a 2:1 or even 2:2 complex. However, the formation of higher order inclusion complexes is not understood as well as the formation of 1:1 complexes.This behavior is particularly typical of γ-cyclodextrin, where the cavity is significantly larger than its substituted naphthalene guests.

Naphthalene itself, forms both 1:1 and 2:2 complexes with β-CD.[4] An excimer emission band at longer wavelengths is a characteristic feature of the $(\beta\text{-CD:naphthalene})_2$ complex. Hamai also studied the inclusion complexes formed between naphthalene and β-CD.[9] In his conclusions, he raised two unique, and currently unanswered questions about the forces which unite two 1:1 complexes to form a 2:2 complex. The first is whether or not cyclodextrins form dimers when they are alone in aqueous solution. Secondly, If cyclodextrins do not naturally form dimers in aqueous solution, then is the dimer formation the first step of a precipitation process initiated because the inclusion complex is less soluble than the free cyclodextrin?

Ueno et al. studied the formation and structure of inclusion complexes

formed between α–, β– and γ–cyclodextrins and 1-naphthylacetate (1–NA).[10] The normal fluorescence band of 10^{-2} M 1–NA is not dependent upon the concentration of α–cyclodextrin, while it is actually enhanced by β– and γ–cyclodextrins. Neither the normal or excimer fluorescence bands of a 10^{-4} M solution of 1–NA are affected by [γ-CD]. However, excimer emission of 1-NA at 10^{-2} M is enhanced by increases in [γ-CD]. The inability of γ-CD to form a 1:1 complex at 10^{-4} M 1-NA, suggests that γ-CD has a greater tendency to include two 1–NA molecules and form a 1:2 complex, γ-CD:(NA*NA), in which the excited monomer may either combine with a nearby 1–NA molecule to form the excimer, which fluoresces at longer wavelengths, or simply emit normal, albeit enhanced, fluorescence.

Later, the same group published a study of 1–naphthyloxyacetic acid (1–NOA) with β and γ-cyclodextrins.[11] They observed a much stronger fluorescence enhancement from β-CD than from γ-CD. However, they found that the addition of cyclohexanol to solutions containing the aforementioned inclusion complexes causes an increase in fluorescence enhancement in the case of γ-CD, while it actually suppresses the enhancement generated by the β-CD complex. They interpreted these results by a spacefilling model in which cyclohexanol binds to the cyclodextrin cavity and decreases the cavity size. The 1–NOA then fits tightly into the smaller γ-CD cavity and the entire complex is bound together in a stronger arrangement than it originally was. However, in the case of β-CD, which cannot accommodate both the cyclohexanol and the 1–NOA simultaneously, there is a competition for the

β-CD cavity between the cyclohexanol and the 1–NOA. This displaces some of the 1–NOA from the cavity and consequently suppresses the fluorescence enhancement found in the absence of cyclohexanol.

The modification of cyclodextrin cavities is a fairly common theme in the study of cyclodextrin inclusion complexes. There are two ways in which the cavities can be modified. The easiest is to use a third modifying molecule such as a small alcohol, which is readily included by the cyclodextrin cavity.[10,12] Secondly, the structure of the cyclodextrin itself can be altered. For example, it is possible to alkylate the hydroxyl groups which line the lip of the CD cavity.[2] Nelson et al. have investigated both of these techniques. They established that t-butyl alcohol is intimately involved with the pyrene:cyclodextrin inclusion complex because it increases the fluorescence lifetime of the guest.[12] Furthermore, they suggest there is a specific modifier concentration at which maximum fluorescence enhancement is observed. The concentration at which this maximum occurs is an indication of the relative strength of the CD:modifier complex.[12] Finally, methylated β-CD was used to establish the importance of forming a hydrogen bond between the alcohol modifier and the cyclodextrin. The ability of t-butyl alcohol to augment pyrene fluorescence enhancement was inhibited when the modifying alcohol was unable to form hydrogen bonds at the ridge of the alkylated β-cyclodextrin cavity.[12]

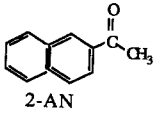
We chose to study inclusion complexes formed between cyclodextrins and 2-acetonaphthone (2-AN) which is a unique molecule because it will only fluoresce in protic solvents. In order for 2-AN to fluoresce, the solvent

must establish a hydrogen bond with the its carbonyl group. The formation of this hydrogen bond causes an inversion of the first (S_1) and second (S_2) singlet excited states, in which the singlet (n,π^*) state, originally S_1, is blue shifted to become the S_2 level while the singlet (π,π^*) state, originally S_2, is red shifted to become the S_1 level.[13] Since radiative decay is more probable when the lowest lying singlet excited state is a (π,π^*) state, the fluorescence quantum yield for 2-AN is dependent on the hydrogen bond donating ability of the solvent. We observed this phenomenon during some earlier studies in which we found that small additions of ethanol to aqueous solutions of 2-AN caused dramatic quenching of 2-AN fluorescence with an accompanying blue shift in the wavelength of the fluorescence maximum. Since the internal cavity of the cyclodextrin presents an apolar, ethanol-like environment to its occupant,[3] we thought that 2-AN fluorescence would be quenched upon the formation of an inclusion complex.

There are two types of fluorescence quenching which might occur in this situation. The first is static quenching, which occurs when the fluorophore forms a ground state complex that inhibits its ability to fluoresce. However, dynamic quenching may also occur if the fluorophore undergoes some type of excited state reaction which results in quenching. In the case of static quenching, a mechanism relating the binding constant to the quencher concentration may be derived.

A mechanism which relates the binding constant to the cyclodextrin concentration can be derived, assuming that the formation of a 1:1 complex

between a particular cyclodextrin and 2-AN results in static fluorescence quenching. The process of complex formation, in which a cyclodextrin is united with the 2-AN to form a 1:1 complex, is depicted below.



2-AN

$$+ \quad CD \rightleftharpoons 2\text{-AN:C D}$$

The equilibrium expression for this process is defined in equation (1).

$$K = \frac{[2\text{-AN:C D}]}{[2\text{-AN}]\ [CD]} \qquad (1)$$

We can express the equilibrium concentrations of the three species involved in equation (1) by known quantities such as fluorescence intensity, F, or the analytical concentration, C. The equilibrium concentration of the cyclodextrin is approximately equal to its analytical concentration because $C_{2\text{-AN}}$ (10^{-5} M) is small enough to render the amount of complex formed insignificant relative to C_{cd} (10^{-3}M).

$$[CD] = C_{cd} \qquad (2)$$

The equilibrium concentration of 2-AN ([2-AN]) can be determined by its steady state fluorescence intensity. This is given by equation (3), in which F is the steady state fluorescence intensity of a cyclodextrin containing 2-AN solution and k is a constant which depends on the physical properties of the

fluorophore as well as the geometry of the instrument.

$$[2\text{-AN}] = \frac{E}{k} \qquad (3)$$

The analytical concentration of 2-AN is given by equation (4), where F^o is the fluorescence intensity of a pure, aqueous solution of 2-AN.

$$C_{2\text{-AN}} = \frac{F^\circ}{k} \qquad (4)$$

The equilibrium concentration of the complex, presented in equation (5), is simply the total 2-AN concentration less its equilibrium concentration.

$$[2\text{-AN:CD}] = C_{2\text{-AN}} - [2\text{-AN}] \qquad (5)$$

Substituting equations (3) and (4) into equation (5) yields equation (6), which expresses the equilibrium concentration of the complex in terms of two easily measured parameters.

$$[2\text{-AN:C D}] = \left[\frac{F^\circ}{k} - \frac{E}{k} \right] \qquad (6)$$

Equations (3), (4), and (6) may now be substituted into equation (1). Rearrangement yields the linear relationship expressed in equation (7).

$$\left[\frac{F^{\circ}}{F} - 1\right] = K\, C_{CD} \qquad (7)$$

Thus, the binding constant, K, is the slope of (Fo/F) - 1 Vs. C_{cd}. This type of relationship, where fluorescence intensity is expressed as a function of quencher concentration, is commonly referred to as a Stern-Volmer plot.

If a set of binding constants is obtained over a range of temperatures, the Van't Hoff equation, (8), can be used to determine the enthalpies an entropies of complex formation.

$$\ln K = \frac{-\Delta H}{R}\left[\frac{1}{T}\right] + \frac{\Delta S}{R} \qquad (8)$$

Using the methods described above, binding constants and thermodynamic parameters for the formation of cyclodextrin inclusion complexes with 2-AN were obtained for three different cyclodextrins at four temperatures. While many others have conducted studies of the binding of substituted naphthalenes to cyclodextrins, we are not aware of any which employ a quenching mechanism similar to the one we have used to elucidate the binding of 2-AN to cyclodextrins.

EXPERIMENTAL

REAGENTS

One of the two fluorophores studied, 2-acetonaphthone, was purchased from Aldrich Chemical Co and was twice recrystallized from both n-hexane and ethanol. Another fluorophore, β-(1,1,1)-trifluoroacetylacetylnaphthalene (TFAAN), was purchased from Molecular Probes Inc. of Eugene, Oregon. Spectroscopic grade ethanol (ETOH), acetonitrile (MeCN), cyclohexane, trifluoroethanol (TFE), hexafluoroisopropanol (HFIP), chloroform, deuterium oxide, deuterated acetone (d-ACE) and deuterated dimethylsulfoxide (d-DMSO) were purchased from Aldrich. The salts $NaClO_4$ and $MgSO_4$ and α–, β– and γ-cyclodextrin were used as supplied by Aldrich Chemical Co.

INSTRUMENTATION

All fluorescence spectra were taken on a Perkin-Elmer (PE) Lambda 5B Spectrofluorometer (LS-5B). The LS-5B Spectrofluorometer was equipped with a Perkin Elmer thermostated cell container for temperature control. Dry nitrogen gas (Union carbide) at 5 psi was pumped into the sample compartment to prevent moisture from condensing on the quartz cells at low temperature. The temperature of the water pumped through the cell holder was controlled by the Flexicoil system from FTS Systems INC. The cell temperature was measured by an Omega model 871digital thermometer. Fluorescence emission spectra were corrected for the wavelength dependence of the emission monochromator and the detector

system using the procedure described in the manual, and then recorded on a PE R100A chart recorder. Aqueous solutions of 2-acetonaphthone were excited at 340 nm and their emission spectra were recorded from 360 to 570 nm using a scan speed of 120 nm/min. A slitwidth of 10 was used for both the excitation and emission monochromators . This required a pen response of 3. The PE LS-5B could be interfaced to a PE-3600 data station in case mathematical manipulation of the spectrum was required. The PECLS software package was used in conjunction with the PE 3600 data station in order to subtract one spectrum from another. However, spectra recorded with the use of the PE3600 data station were not corrected for the wavelength dependencies of the emission monochromator and the detection system.

All absorption spectra were taken on a Perkin-Elmer Lambda 3B UV/VIS Spectrophotometer and recorded on a PE R100A chart recorder. Nuclear magnetic resonance spectra were obtained from a Varian 200 MHz FT NMR Spectrometer.

In addition, the measurement of excited state lifetimes was conducted by Dr. Douglas R. James of Photon Technology International INC. (PTI), in Ontario,Canada. These measurements were performed on the PTI LS-100 fluorescence lifetime fluorometer using a Nitrogen gas discharge lamp as the excitation source. The 2-AN molecule was excited at 337 nm and emission was recorded at 430 nm for 25 scans.

PROCEDURE

1. Preparation of fluorophore solutions

A saturated solution of 2-AN in deionized water was prepared by placing 2-AN crystals in a 1L volumetric flask filled to 75% of its capacity with water. The solution was stirred gently overnight with a magnetic stirring bar. It was then filtered by vacuum filtration through a 0.45 μm Milipore filter and transferred into a brown glass container for storage. The cocncentration of this solution was obtained from its absorbance of .84 at 340 nm, the exciting wavelength. We made three such solutions, one in July, another in August and a third in January. Using a molar absorptivity of 1650 L mol^{-1} cm^{-1} obtained from the literature, we calculated the concentration of these 2-AN stock solutions to be 5.1×10^{-4} M.[14] A saturated solution of TFAAN was prepared by the same procedure.

All spectral analyses were conducted on 1:10 dilutions of the 2-AN stock solution. Their absorbance at the exciting wavelength, 340 nm, was .08 and corresponded to a calculated concentration of 5×10^{-5} M. These solutions were prepared by placing 1.00 ml of the stock 2-AN, as measured with an Eppendorf digital pipette, into a 10 ml volumetric and then filling to the mark with solvent. Both fluorescence and absorption were measured in 1 cm cells, except in the case of several absorption spectra which required the use of a 10 cm cell.

2. Mixed solvent studies

The fluorescence emission spectrum of 2-AN was studied in mixed

aqueous and organic solvents . The mixed aqueous solvents were composed of either ETOH/H_2O mixtures or MeCN/H_2O mixtures. The mixed organic solvents consisted of solutions of 2-AN in TFE containing small additions of ETOH. The organic solvents were prepared directly in the fluorescence cell by adding small aliquots of ETOH to a solution of 2-AN in TFE. In these cases, fluorescence quenching is attributed to ETOH or MeCN only when the decrease in fluorescence intensity is greater than that expected from diluting the fluorescent solution. Ranges of MeCN and ETOH used in these experiments are presented in Table 2. In addition, we also studied the emission spectrum of 2-AN in 1M $NaClO_{4(aq)}$, a water structure breaker, and 1M $MgSO_{4(aq)}$ a structure maker.

3. Cyclodextrin Inclusion complexes

A stock solution of each cyclodextrin was prepared by placing the appropriate mass of the cyclodextrin into a 25 ml volumetric flask which was subsequently filled with deionized water. The masses and concentrations of each cyclodextrin used to prepare the stock solutions are presented in Table 3. Solutions of 2-AN in water with varying CD concentrations were prepared by pipetting the appropriate amount of the CD stock solution into a 10 ml volumetric flask containing 1.00 ml of the 2-AN stock solution. The total 2-AN concentration of these solutions is estimated to be $5x10^{-5}$ M. These solutions were then transferred into a fluorescence cell and cooled to near 5.0 C°. Their fluorescence emission spectrum was obtained and then the temperature was increased until the

TABLE 2

Composition of the Solvents Used toStudy
The Fluorescence of 2-AN in Mixed Solvent Systems

TABLE 2

Solvent	Range of $\chi_{quencher}$
ETOH/H2O	0.00 to 0.24
MeCN/H2O	0.00 to 0.26
ETOH/TFE	0.00 to 0.17

TABLE 3

Experimental Cyclodextrin Concentrations

TABLE 3

Cyclodextrin	Stock concentration (M)	Working range (M)
α	.025	.0025 to .01
β	.010	.0005 to .002
γ	.010	.0010 to .0040

emission spectrum of each solution was recorded at four different temperatures spanning a 35 c° range.

A Basic program on the Apple II computer was used for linear regression analysis of the data. The experimental error in the equilibrium constants was determined from the standard deviation in the slope of the Stern-Volmer plot. The standard deviations in the slope and y-intercept of the Van't Hoff plot were used to calculate the experimental error in the enthalpies and entropies of complex formation.

4. Excited State Lifetime Measurements

In November 1989, five solutions were packaged and mailed to PTI of Onatrio, Canada. The components of these five solutions are listed in Table 4. Each solution was prepared as previously described. Their absorption at 340 nm was recorded and they were transfered into a Kimble 20 ml glass vial, capped and shipped to Canada. The solutions were then analyzed by Dr. James on March 16,1990.

TABLE 4

Compositions of The Solvents Used
in the Fluorescence Lifetime Analysis
of 2-Acetonaphthone

TABLE 4

The following five solutions were mailed to PTI for fluorescence Lifetime analysis:

Solution	A_{340}
1. 2-AN in H_2O	.096
2. 2-AN in .0075 M α-CD	.099
3. 2-AN in .0010 M β-CD	.094
4. 2-AN in .0020 M γ-CD	.096
5. 2-AN in MeCN χ_{MeCN} = .018	.092

RESULTS

The fluorescence of 2-AN in mixed solvent systems

The fluorescence of 2-AN in ethanol/water and acetonitrile/water solvents was studied as a follow up to the LoParo work.[15] The emission spectra of 2-AN in the afforementioned solvents, presented in Figures 2 and 3, demonstrate that both ethanol and acetonitrile quench 2-AN fluorescence and produce a simultaneous blue shift in the wavelength of maximum emission. The results from the spectral studies of 2-AN in mixed solvent systems are listed in Tables 5 through 7. Acetonitrile (MeCN) proved to be a stronger quencher than ethanol. As the mole fraction of MeCN was increased from 0 to 0.26, the intensity of 2-AN fluorescence decreased to 1.6% of its unquenched intensity while the maximum emission wavelength blueshifted by approximately 15nm. (Table 5) In comparison, Table 6 shows that an increase in the mole fraction of ethanol over nearly the same range (0 to 0.24) causes a similar spectral shift but only decreases the fluorescence intensity to12% of its unquenched value. This is depicted by Figure 3.

Figure 4 shows that ethanol quenches 2-AN fluorescence in mixed organic solvents as well. From Table 8, it is apparent that increasing the ethanol mole fraction of an ETOH/TFE solvent from 0 to 0.17 quenches the intensity of 2-AN fluorescence to 47% of its original intensity while

FIGURE 2

Fluorescence Emission Spectra of
Aqueous 2-AN Quenched by Acetonitrile

FIGURE 2

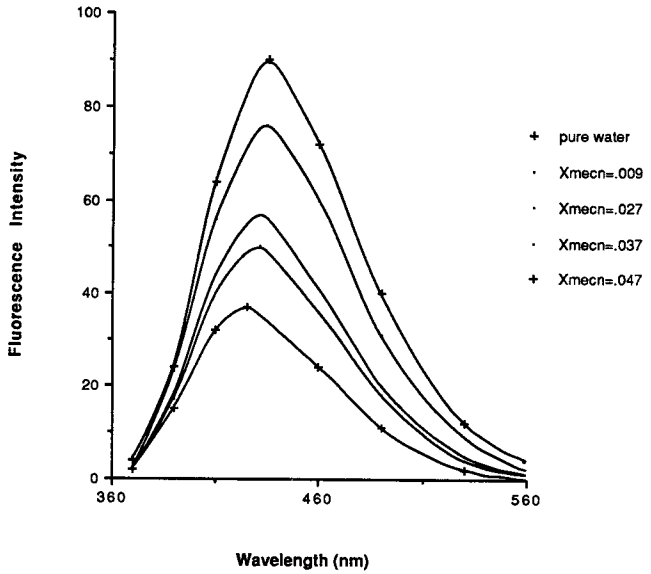

100
80
60
40
20
0
360
460
560
Fluorescence Intensity
Wavelength (nm)
pure water
Xmecn=.009
Xmecn=.027
Xmecn=.037
Xmecn=.047

TABLE 5

Results of the Spectral Studies
of 2-AN in CH_3CN/H_2O Solvents

TABLE 5

Mol Fraction CH_3CN	Average F/F°	λ_{max} (nm)
0.000	1.00	435 ± 1
0.009	0.84 ± .01	433 ± 1
0.018	0.75 ± .01	432 ± 1
0.027	0.64 ± .01	431 ± 1
0.037	0.58 ± .01	430 ± 1
0.047	0.44 ± .01	426 ± 1
0.068	0.31 ± .01	426 ± 1
0.079	0.29 ± .01	427 ± 1
0.129	0.12 ± .01	425 ± 1
0.187	0.047 ± .01	421 ± 1
0.256	0.016 ± .01	420

FIGURE 3

Fluorescence Emission Spectra
of Aqueous 2-AN Quenched by Ethanol

FIGURE 3

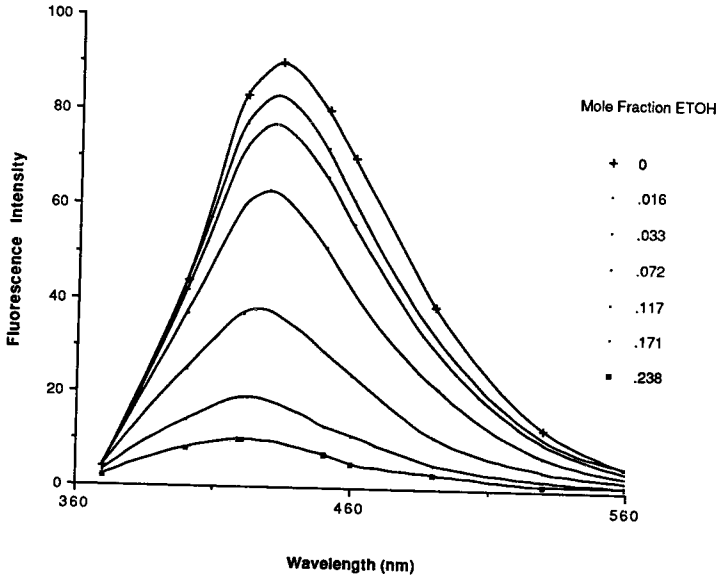

100
80
60
40
20
0
360
460
560
Fluorescence Intensity
Wavelength (nm)
Mole Fraction ETOH
0
.016
.033
.072
.117
.171
.238

TABLE 6

Results of the Spectral Studies
of 2-AN in ETOH/H_2O Solvents

TABLE 6

Mol Fraction Ethanol	Average F/F°	λ_{max} (nm)
0.00	1.00	433 ± 1
0.02	0.95 ± .03	432 ± 1
0.03	0.89 ± .02	430 ± 2
0.07	0.75 ± .04	430 ± 2
0.12	0.42 ± .01	425 ± 2
0.17	0.22 ± .01	422 ± 2
0.24	0.12 ± .01	417 ± 2

FIGURE 4

Fluorescence Emission Spectra of
2-AN inTFE Quenched by Ethanol

FIGURE 4

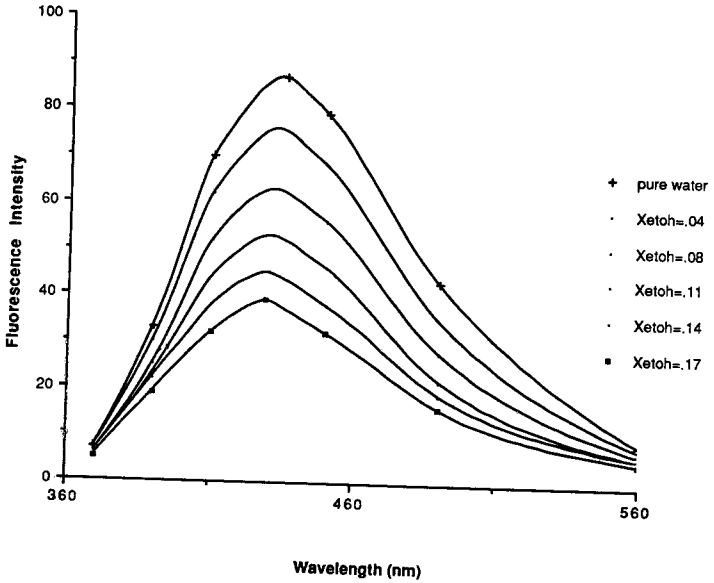

100
80
60
40
20
0
Fluorescence Intensity
360
460
560
Wavelength (nm)
pure water
Xetoh=.04
Xetoh=.08
Xetoh=.11
Xetoh=.14
Xetoh=.17

TABLE 7

Results of the Spectral Studies
of 2-AN in ETOH/TFE Solvents

TABLE 7

Mol Fraction Ethanol	λ_{max} (nm)	Average F/F°
0.000	1.00	434 ± 1
0.040	0.88 ± .03	433 ± 0
0.076	0.78 ± 0.6	432 ± 1
0.110	0.64 ± .03	430 ± 1
0.142	0.60 ± .04	420 ± 0
0.171	0.47 ± .02	428 ± 1

simultaneously blueshifting the wavelength maximum by 6nm.

In order to determine whether the structure of the water lattice was responsible for the observed behavior of 2-AN, we decided to characterize the fluorescence of 2-AN in the presence of 1M $MgSO_4$, a known structure maker, and 1M $NaClO_4$, a structure breaker. (Figure 5) These salts are so designated because, in their presence, the structure of a water lattice at a given temperature is either similar to a water lattice at a lower temperature, if the salt is a structure maker, or is similar to a water lattice at a higher temperature, in the case of a structure breaker. Our results, presented in Table 8, demonstrate a slight increase in the fluorescence of 2-AN in 1M solutions of both salts.

Cyclodextrin inclusion complexes of 2-AN.

The fluorescence of 2-AN in aqueous solutions of α–,β– and γ-cyclodextrin was investigated over a temperature range of 30 C°. At concentrations below 4×10^{-3} M, each cyclodextrin quenched 2-AN fluorescence without causing any apparent spectral shifting or broadening. This type of behavior is indicative of the formation of a ground state inclusion complex between the cyclodextrin and the 2-AN, in which the fluorescence of the bound 2-AN is completely quenched. The strength of the complex formed is proportional to the degree of quenching. For a given cyclodextrin, the amount of quenching increases as temperature decreases, indicating the formation of a stronger complex at lower

FIGURE 5

Fluorescence Emission Spectra of
2-AN in the Presence of 1M
$MgSO_4$ and 1M $NaClO_4$

FIGURE 5

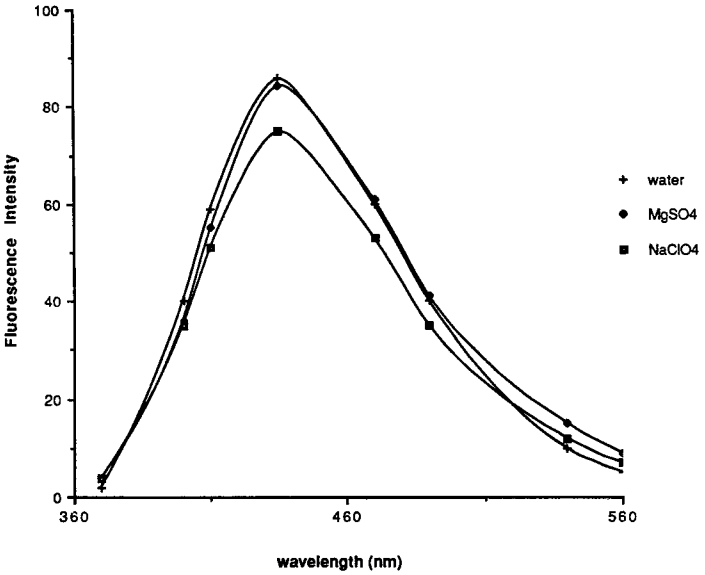

100
80
60
40
20
0
Fluorescence Intensity
360
460
560
wavelength (nm)
water
MgSO4
NaClO4

TABLE 8

Results from the Spectral Studies

of 2-AN in the Presence of 1M $NaClO_4$ and 1M $MgSO_4$

TABLE 8

Sample	λ_{max} (nm)	F_{max}
2-AN in H_2O	433	3.38
2-AN in $MgSO_4$	434	4.37
2-AN in $NaClO_4$	432	3.94

temperatures. Table 9 shows the extent of 2-AN quenching caused by a given concentration of the cyclodextrins. The equilibrium constants at several different temperatures for the formation of cyclodextrin inclusion complexes of 2-AN are listed in Table 10.

From the data in Table 9, it is apparent that the strongest quencher of 2-AN fluorescence is β-cyclodextrin. Several fluorescence emission spectra of 2-AN in the presence of various concentrations of β-CD are presented in Figure 6. Increasing the concentration of β-CD from 0 to 1.75×10^{-3} M decreases the fluorescence intensity of 2-AN to 54% of its unquenched intensity. As shown in Figure 6, there is no accompanying evidence of spectral broadening or shifting over this concentration range. The equilibrium constants at four different temperatures were determined from the Stern-Volmer plots and were then used in the Van't Hoff equation to obtain the changes in enthalpy and entropy resulting from complex formation. The Stern-Volmer plot and the Van't Hoff plot for β-CD are shown in Figures 7 and 8, respectively. The room temperature equilibrium constant for the β-CD:2-AN complex, as listed in Table 10, is 575±14 M^{-1}. The corresponding changes in enthalpy and entropy for complex formation, presented in Table 11, are -10.7±.6 KJ mol^{-1} and 16±2 e.u. respectively.

The next most potent quencher of 2-AN fluorescence is γ-cyclodextrin. Figure 9 demonstrates how a 2.00 x 10-3 M solution of γ-CD decreases the

TABLE 9

Extent of 2-AN fluorescence Que. ng
caused by the Cyclodextrins

TABLE 9

Cyclodextrin	[CD] (M)	F/F°	Temperature (C°)
β-CD	0.002	.47	21.0
γ-CD	0.002	.90	23.9
α-CD	0.0025	.93	21.8

‡ F is the maximum fluorescence intensity of a solution of 2-AN containing a cyclodextrin. F° is the fluorescence intensity of a solution of pure, aqueous 2-AN. Since both solutions have the same absorbace at the exciting wavelength, the term (F/F°) measures the extent of quenching caused by the cyclodextrin.

FIGURE 6

Fluorescence Emission Spectra of

2-AN Quenched by β-Cyclodextrin

FIGURE 6

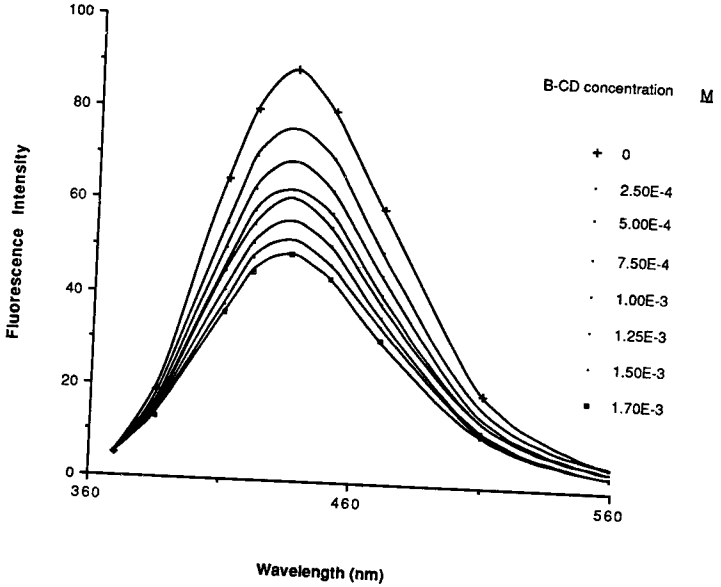

100
80
60
40
20
0
360
460
560
Fluorescence Intensity
Wavelength (nm)
B-CD concentration M
0
2.50E-4
5.00E-4
7.50E-4
1.00E-3
1.25E-3
1.50E-3
1.70E-3

FIGURE 7

Stern - Volmer Plot for 2-AN

Quenched by β-Cyclodextrin

at 20.6 c°

FIGURE 7

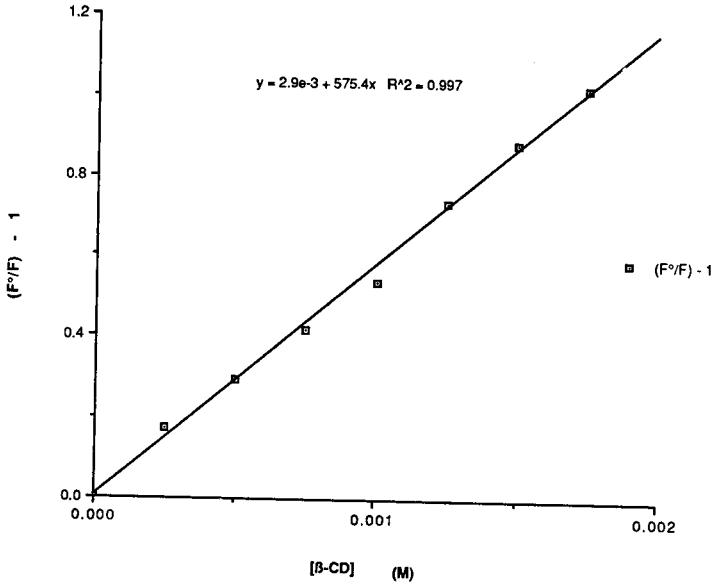


FIGURE 8

Van't Hoff Plot for the formation of the

β-CD:2-AN inclusion complex

FIGURE 8

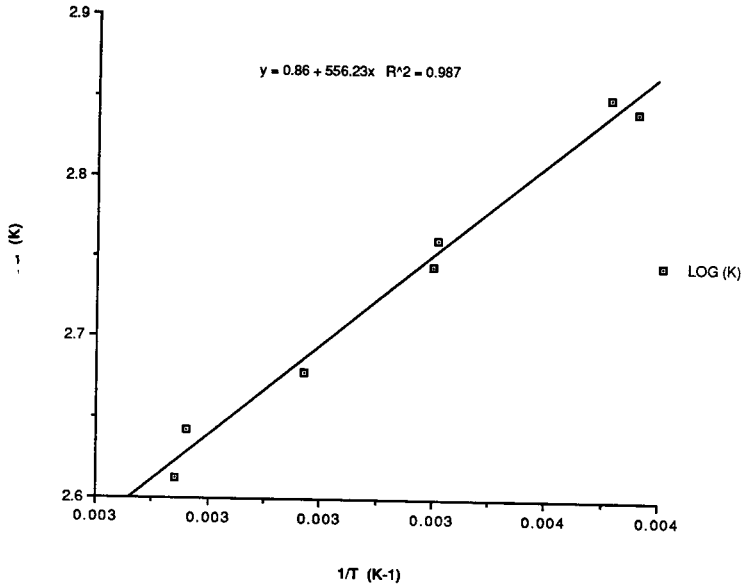

y = 0.86 + 556.23x R^2 = 0.987
2.9
2.8
2.7
2.6
0.003
0.003
0.003
0.003
0.004
0.004
LOG (K)
1/T (K-1)

TABLE 10

Binding Constants for the Cyclodextrin
Inclusion Complexes of 2-AN

TABLE 10

Binding Constants

Temperature (C°)	β-CD (M^{-1})	γ-CD (M^{-1})	α-CD (M^{-1})
5.1	----	84 ± 3	----
7.8	----	----	39 ± 3
8.0	706 ± 30	----	----
20.6	575 ± 14	----	----
21.1	----	----	36 ± 1
23.9	----	57 ± 3	----
31.2	----	----	29 ± 5
31.3	476 ± 14	----	----
32.1	----	52 ± 2	----
40.5	----	----	24 ± 2
41.2	438 ± 6	----	----
41.6	----	31 ± 6	----

TABLE 11

Thermodynamic Parameters for the
Formation of Cyclodextrin Inclusion Complexes
of 2-Acetonaphthone

TABLE 11

Cyclodextrin	ΔH (KJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
b-CD	-10.7 ± .6	16 ± 2
g-CD	-18 ± 5	-28 ± 15
a-CD	-7 ± 2	6.8 ± .8

fluorescence of 2-AN to only 90% of its unquenched intensity. The room temperature binding constant for the γ-CD:2-AN complex, as listed in Table 10, is 57±3 $\underline{M}^{-1}$. The corresponding changes in enthalpy and entropy for complex formation are -19±5 KJ/mol and -28±15 e.u., respectively (Table 11). A representative Stern-Volmer plot for γ-CD as well as a Van't Hoff plot are shown in Figures 10 and 11, respectively.

Upon increasing [γ-CD] to 4 x 10^{-3} $\underline{M}$ some spectral broadening was observed as measured by the spectral width at half-height ($W_{1/2}$). An 8nm increase in $W_{1/2}$ was observed over the range of γ-CD studied (0 to 4.00 x 10^{-3} $\underline{M}$). The longer wavelength fluorescence intensities of the 2-AN emission spectrum were found to increase with γ-CD concentration and an isoemissive point at approximately 510 nm was observed. These results are presented in Table 12. By subtracting the emission spectrum of 2-AN in pure water from that of 2-AN in 4.00 x 10^{-3} $\underline{M}$ γ-CD, we were able to resolve another broad emission band around 500nm. This emission band is shown in Figure 12. It is possible that this band may be emission from a 2-AN excimer formed within the γ-CD cavity. In determining the 1:1 binding constant, we could not confine our measurements to lower γ-CD concentrations because the quenching was not extensive enough to yield consistent results.

Finally, the weakest quencher of 2-AN fluorescence is α-cyclodextrin. Figure 13 and Table 9 demonstrate that a solution of 2.00 x 10^{-2} $\underline{M}$ α-CD decreases the fluorescence intensity of 2-AN to 47% of its original value

FIGURE 9

Fluorescence Emission Spectra of

2-AN Quenched by γ-Cyclodextrin

[redacted]

FIGURE 9

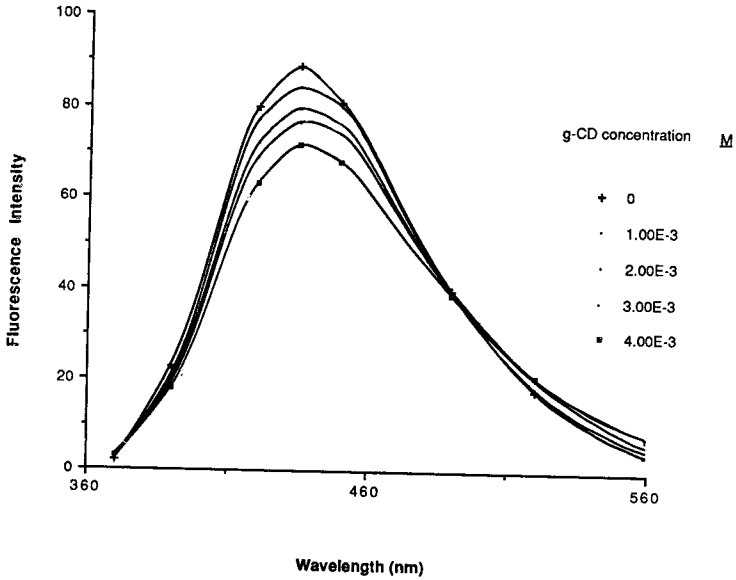

100
80
60
40
20
0
360
460
560
Fluorescence Intensity
Wavelength (nm)
g-CD concentration M
0
1.00E-3
2.00E-3
3.00E-3
4.00E-3

FIGURE 10

Stern - Volmer Plot for 2-AN

Quenched by γ-Cyclodextrin

at 23.9 c°

FIGURE 10

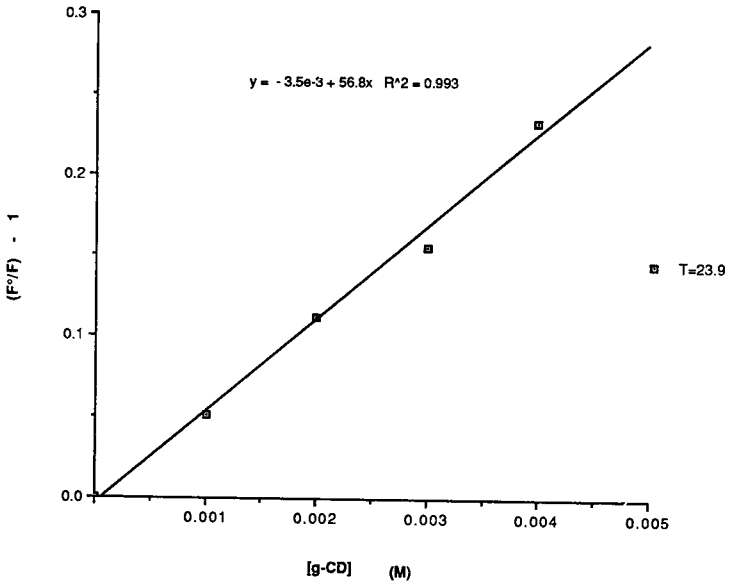

y = - 3.5e-3 + 56.8x R^2 = 0.993
(F°/F) - 1
0.3
0.2
0.1
0.0
0.001
0.002
0.003
0.004
0.005
[g-CD] (M)
T=23.9

FIGURE 11

Van't Hoff Plot for the formation of the

γ-CD:2-AN inclusion complex

FIGURE 11

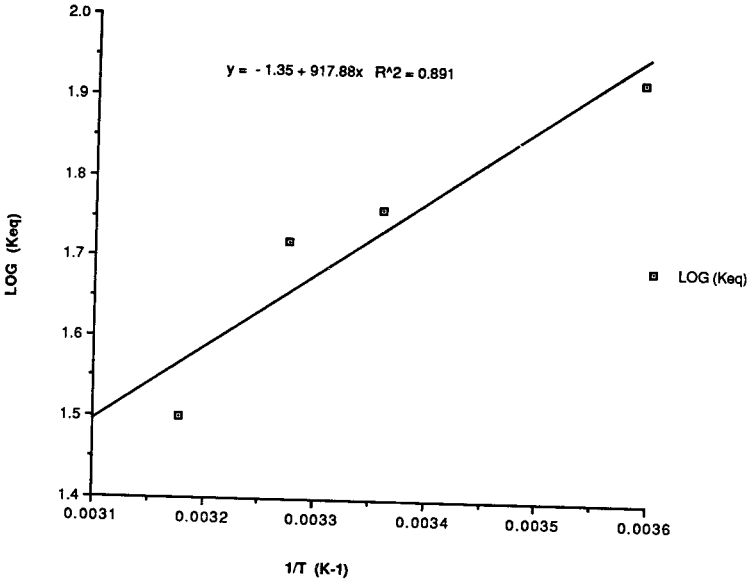

y = - 1.35 + 917.88x R^2 = 0.891
LOG (Keq)
1/T (K-1)
2.0
1.9
1.8
1.7
1.6
1.5
1.4
0.0031
0.0032
0.0033
0.0034
0.0035
0.0036
LOG (Keq)

TABLE 12

Width at Half-Height(W1/2) for the Fluorescence Emission Spectra of 2-AN in the Presence of γ-CD

TABLE 12

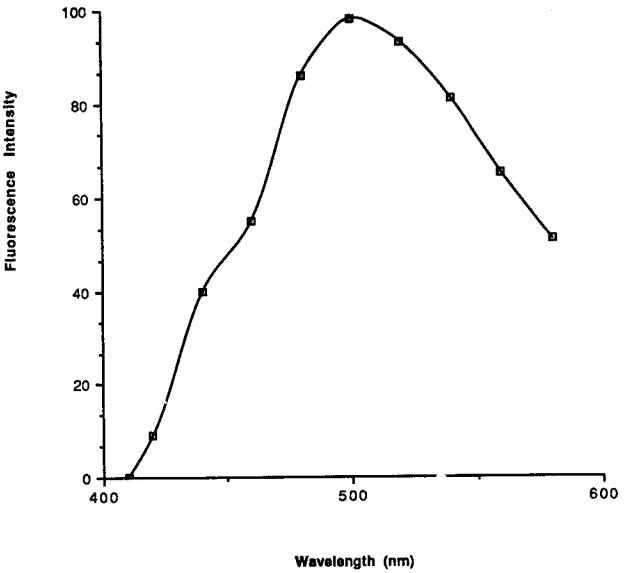
[g-CD] (M)	$W_{1/2}$ (nm)	λ_{max} (nm)
0.000	81.0	434
0.001	84.0	434
0.002	86.5	434
0.003	88.5	434
0.004	90.0	434

FIGURE 12

Fluorescence Emission Spectrum of

the 2-AN Excimer Formed in .004 M γ-CD

Figure 12


Fluorescence Intensity
100
80
60
40
20
0
400
500
600
Wavelength (nm)

with no accompanying evidence of spectral shifting or broadening. Large negative intercepts, shown in Figure 14, began to occur at [α-CD] >10^{-2} M. Thus all data used to determine the equilibrium constant was obtained over the range from 0 to 1.00 x 10^{-2} M α-CD, where upward curvature in the Stern-Volmer plot is minimal. The room temperature binding constant for the α-CD:2-AN complex is19±4 M^{-1}. This corresponds to an enthalpy change of -7±2 KJ/mol and an entropy change of 6.1±.8 e.u. The corresponding Stern-Volmer and Van't Hoff plots are presented in Figures 15 and 16, respectively.

Figures 17 and 18 show the effects of trifluoroethanol (TFE) and Hexafluoroisopropanol (HFIP) on the fluorescence of aqueous 2-AN respectively. Both TFE and HFIP are stronger hydrogen bond donors than water and therefore enhance the fluorescence of aqueous 2-AN. Even though TFE enhances 2-AN fluorescence in water, it quenches the fluorescence of 2-AN in 3.00 x 10^{-3} M γ-CD. While the isoemissive point observed in the fluorescence emission spectra of 2-AN quenched by unmodefied γ-CD (Figure 9) is still present when 2-AN is quenched by the TFE modified-γ-CD (Figure 17), the increase in the intensities of the longer wavelength emission components of 2-AN fluorescence become less prevalent.

We used the quenching of 2-AN fluorescence observed in the presence of the TFE-modified γ-CD to determine the binding constant for the

FIGURE 13

Fluorescence Emission Spectra of

2-AN Quenched by α-Cyclodextrin

FIGURE 13

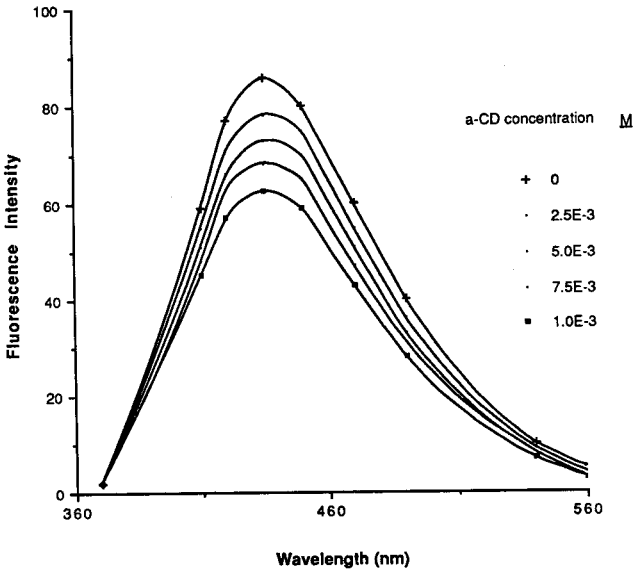

100
80
60
40
20
0
Fluorescence Intensity
360
460
560
Wavelength (nm)
a-CD concentration M
0
2.5E-3
5.0E-3
7.5E-3
1.0E-3

FIGURE 14

Stern - Volmer Plot, Showing LargeNegative Intercept,

for theRoom Temperature Quenching of 2-AN by α-CD

FIGURE 14

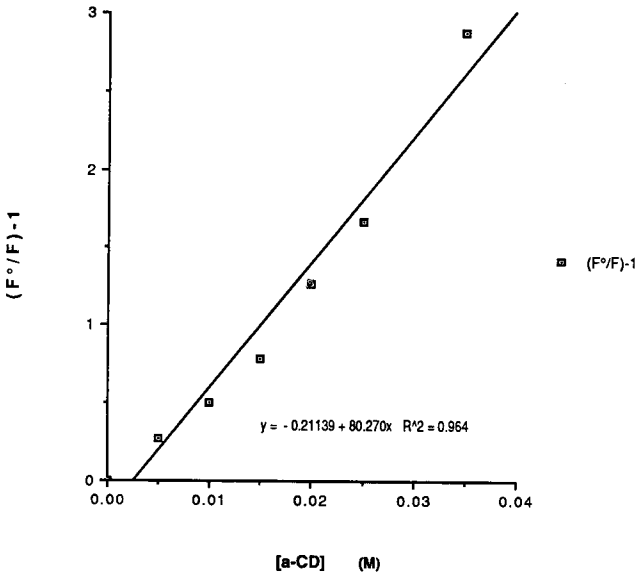


FIGURE 15

Stern-Volmer Plot for 2-AN

Quenched by α-Cyclodextrin at 21.8 c°

Figure 15

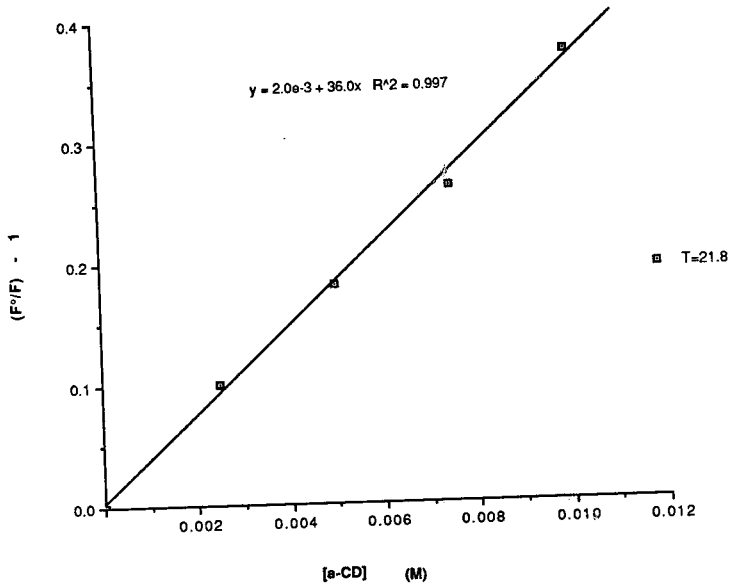

y = 2.0e-3 + 36.0x R^2 = 0.997
T=21.8
(F°/F) - 1
[a-CD] (M)
0.0
0.1
0.2
0.3
0.4
0.002
0.004
0.006
0.008
0.010
0.012

FIGURE 16

Van't Hoff Plot for the formation of the

α-CD:2-AN inclusion complex

FIGURE 16

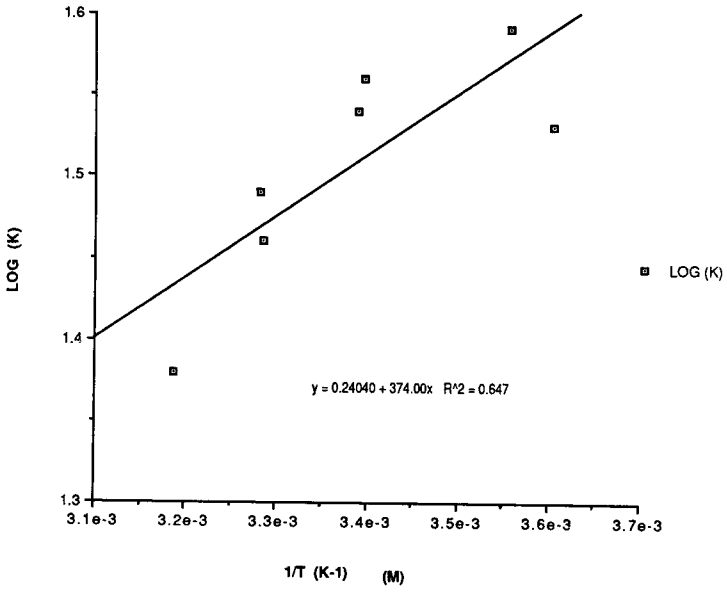

1.6
1.5
1.4
1.3
LOG (K)
y = 0.24040 + 374.00x R^2 = 0.647
LOG (K)
3.1e-3
3.2e-3
3.3e-3
3.4e-3
3.5e-3
3.6e-3
3.7e-3
1/T (K-1) (M)

FIGURE 17

Fluorescence Emission Spectra of 2-AN in

a) H_2O + 1 vol % TFE

b) H_2O

c) .003 M γ-CD

d) .003 M γ-CD + 1 vol % TFE

FIGURE 17

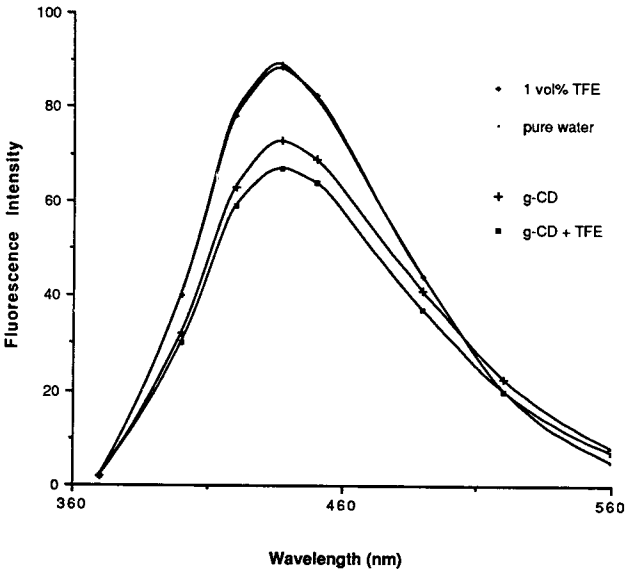

100
80
60
40
20
0
Fluorescence Intensity
360
460
560
Wavelength (nm)
1 vol% TFE
pure water
g-CD
g-CD + TFE

FIGURE 18

Fluorescence Emission Spectra of 2-AN in

a) H_2O + 1 vol % HFIP

b) H_2O

c) .003 M γ-CD

d) .003 M γ-CD + 1 vol % HFIP

FIGURE 18

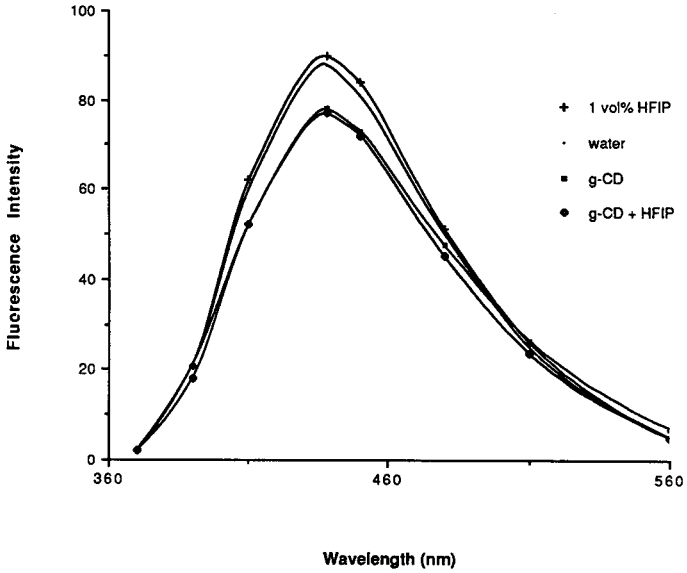
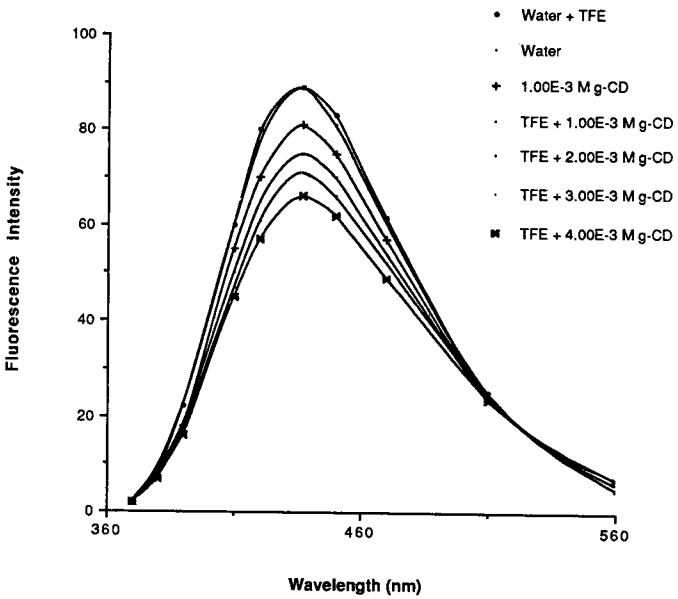

100
80
60
40
20
0
360
460
560
Flucrescence Intensity
Wavelength (nm)
1 vol% HFIP
water
g-CD
g-CD + HFIP

FIGURE 19

Fluorescence Emission Spectra of 2-AN

in the Presence of γ-CD + 1 Vol % TFE

FIGURE 19


Water + TFE
Water
1.00E-3 M g-CD
TFE + 1.00E-3 M g-CD
TFE + 2.00E-3 M g-CD
TFE + 3.00E-3 M g-CD
TFE + 4.00E-3 M g-CD
Fluorescence Intensity
100
80
60
40
20
0
360
460
560
Wavelength (nm)

associated inclusion complex (Figure 19). The Stern Volmer plot for the modified γ-CD is presented in Figure 20. Upon a 1 vol% addition of TFE to solutions of γ-CD, the room temperature equilibrium constant for the corresponding 1:1 complex is increased from 57±3 $\underline{M}^{-1}$ in the unmodified γ-CD, to 86±6 $\underline{M}^{-1}$ in the TFE- modified γ-CD. As demonstrated by Figure 18, HFIP has no effect on the inclusion complex formed between 2-AN and γ-CD.

The results of the fluorescence lifetime experiments are presented in Table 13. The measured lifetimes of the 2-AN singlet excited state are on the order 1 ns in each of the five environments studied. Except for the cases of 2-AN in 1.00 x10^{-3} $\underline{M}^{-1}$ β-CD and 2-AN in pure water, all the decay curves fit a single exponential equation. However, the former two cases exhibit dual exponential decay.

FIGURE 20

Stern - Volmer Plot for 2-AN quenched by γ-CD

in the Presence of 1 Vol % TFE at 21.0 c°

FIGURE 20

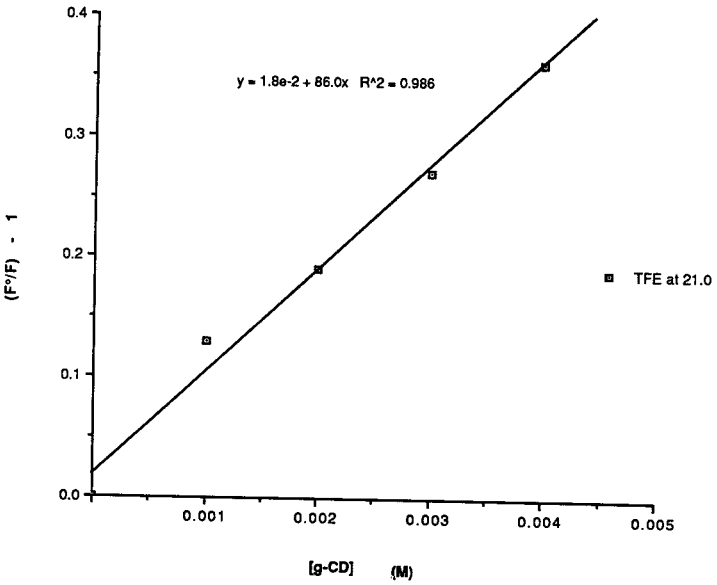

y = 1.8e-2 + 86.0x R^2 = 0.986
TFE at 21.0
(F°/F) - 1
[g-CD] (M)
0.0
0.1
0.2
0.3
0.4
0.001
0.002
0.003
0.004
0.005

TABLE 13

Results of the Fluorescence Lifetime Analysis
of 2-AN in Five Different Environments

TABLE 13

Sample	A_1	τ_1 (ns)	A_2	τ_2 (ns)
2-AN in H_2O	0.627	1.158	0.373	2.232
2-AN in MeCN	1.000	1.109	---	---
2-AN in α–CD	1.000	1.290	---	---
2-AN in β-CD	0.716	1.598	0.284	3.230
2-AN in γ-CD	1.000	1.237	---	---

DISCUSSION

Studies of the Luminescent Properties of 2-AN in Mixed Solvents

Since the amount of quenching induced by small additions of ethanol or acetonitrile is much greater than what is predicted om the changes in solvent dielectric constant, we are forced to conc :e that the microenvironment of the fluorophore is significantly different from that of the bulk solution. Others have made the same observations in similar systems with different fluorophores. Belyakova and Vuks studied the fluorescence of salicylic acid in alcohol/water solvents. They suggest that the alcohol causes a change in the structure of the water lattice which, in effect, weakens the hydrogen bond between solvent and fluorophore.[16] Easteal reports the same occurrence in water- acetonitrile solutions.[17] Both groups report that the formation of the salicylate dimer results in the appearance of a new band around 465 nm at higher concentrations of the fluorophore.

Because the fluorescence intensity of 2-AN varies linearly with concentration over a 20 fold range with no accompany g spectral shifting or broadening, we are confident that our measurements are not plagued by the formation of 2-AN aggregates.

In order to determine whether the alcohol mediated change in the structure of the water lattice structure was responsible for the aforementioned behavior, we studied the emission spectra of 2-AN in 1M $MgSO_4$, a water structure maker and 1M $NaclO_4$, a water structure breaker. Table 8 shows that both salts increase the fluorescence of 2-AN. Thus, changes in the solvent structure alone can not account for the

behavior of 2-AN in mixed solvents. Rather, the slight increase in 2-AN fluorescence in the presence of both salts is attributed to either a change in the refractive index of the solution, or the salting out of dissolved oxygen.

Perhaps the most comprehensive explanation of the effects of dilute alcohols on the fluorescence properties of carbonyl substituted aromatics, was reported by Dederen et al. in their studies on the fluorescence of pyrenecarboxaldehyde (PCHO) in mixed water- alcohol solutions.[18] They observed a large blue shift in the emission maximum at small additions of the alcohol. They also confirmed the existence of two excited states by fitting fluorescence lifetime decay curves to a dual exponential decay. The two excited states were identified as PCHO fluorescing from an alcohol dominated environment and PCHO fluorescing from a primarily aqueous environment. Thus they also conclude that the microenvironment of the fluorophore is different from that of the bulk solution.

Unfortunately, we do not have enough confidence in the data to make any conclusions on the basis of the fluorescent lifetime analysis of 2-AN in mixed solvent systems. That aqueous 2-AN exhibits dual exponential decay is in direct contradiction to the study in which we found 2-AN fluorescence to vary linearly with concentration. In addition, contrary to the results of Dederen et al., our data shows that 2-AN in acetonitrile exhibits single exponential decay. Although fluorescence lifetime analysis could be a useful technique in studying this problem, we are not yet confident in our results.

Both the trends in our data, and in the literature, point to the possibility that the fluorophore is selectively solvated by a pocket of the

organic additive within the solvent. Fore example, in the case of 2-AN in the ethanol/water solvents, the ethanol would form a pocket around the 2-AN which results in the quenching of fluorescence as the 2-AN--H_2O hydrogen bond is broken. This is supported by the data in Table 7, which demonstrates that ethanol quenching is more extensive in mixed aqueous rather than in mixed organic environments. There is a stronger tendency for the naphthone to be solvated by ethanol in the aqueous system rather than in the organic system because of the more pronounced differences in the polarities among the aqueous components. It is possible that this idea of 'selective solvation' may be some type of preliminary step in the formation of a micelle.

Cyclodextrin Inclusion Complexes of 2-Acetonaphthone

That all three cyclodextrins quench 2-AN fluorescence is a preliminary indication that an inclusion complex exists between the cyclodextrin and 2-AN in which fluorescence of the bound 2-AN is completely quenched. By definition, this quenching must be static. However, the possibility of dynamic quenching is eliminated by emperical calculations based on the excited state lifetime of 2-AN which show that the quencher concentration required to achieve the reported levels of quenching via a dynamic mechanism, are two orders of magnitude larger than the experimental cyclodextrin concentrations. Since the excited state lifetime of 2-AN is approximately 10^{-9} sec, the product $k_q[Q]$ must be on the order of 10^9 sec^{-1} to compete with alternate paths for excited-state decay. Given a maximum probable k_q value of 10^{10} L mol^{-1} sec^{-1}, the product

$k_Q[Q]$ will only be 10^7 at quencher concentrations of 10^{-3} M, which are near the upper limit of our Stern-Volmer plots. Perhaps the strongest evidence supporting the static quenching mechanism, and the validity of our proposed method to determine binding constants, is the linearity of the Stern-Volmer plots.

The strongest cyclodextrin inclusion complex of 2-AN is formed with β-Cyclodextrin. This is in agreement with trends in the literature for other substituted naphthalenes. In general, most naphthalenes form the strongest complex with β-CD. The α-CD cavity is too small, while the γ-CD cavity is too large to tightly accommodate the naphthalene moiety. The inherent experimental error in the measured binding constant is primarily determined by the magnitude of fluorescence quenching induced by each cyclodextrin, which is proportional to the strength of the corresponding inclusion complex. Because β-CD formed the strongest complex with 2-AN, the relative error in the β-CD:2-AN binding constant is much smaller than that in the γ-CD:2-AN or α-CD:2-AN binding constants.

The thermodynamic forces which drive the process of complex formation are dependent on the size and functionality of the included guest. For example, the binding of 2,7-ANS and 2,8-ANS to β-CD is characterized by the highly favorable ΔH terms of -18 and -13.2 KJ mol^{-1} respectively, while the change in entropy upon complexation for both molecules is negative.[6] Clearly, the driving force for the formation of these two complexes is the enthalpy term. In contrast, 2,6-ANS exhibits thermodynamicaly favorable changes in both enthalpy (-16.2 KJ mol^{-1}) and entropy (8.8 e.u) upon complexation with β-CD.[6]

Cyclodextrin inclusion complexes of 2-AN are very similar to the latter case in which both thermodynamic driving forces favor complex

formation. From Table 11 it is apparent that the changes in enthalpy for complexation with all three cyclodextrins are thermodynamically favorable. However, the large positive ΔS in the case of β-CD is clearly responsible for the strong binding in the β-CD complex relative to the two weaker cyclodextrin complexes.

The likely primary factors contributing to the enthalpy of complex formation are the hydrophobic effect and the ability of the carbonyl group of 2-AN to form hydrogen bonds with the hydroxyl groups lining the ridges of the cyclodextrin cavity.

The physical processes which determine the entropy of complexation are somewhat more complex. There are actually two competitive effects which determine the change in entropy upon complexation. Because 2-AN is significantly less polar than water, water molecules are more attracted to each other than to 2-AN. This results in the formation of a more ordered water lattice upon the addition of 2-AN. The first effect, which partially determines the change in entropy of complexation, is the destruction of this highly ordered water lattice as the cyclodextrin removes 2-AN from the bulk solution. This gives rise to an increase in entropy. After the 2-AN is included by the cyclodextrin it suffers a decrease in its rotational, vibrational and its translational degrees of freedom. This is the second effect, and it generates a negative change in entropy. The extent to which each of these two effects occurs establishes the magnitude of the overall change in the entropy of complex formation.

The large entropy of complexation in the case of the β-CD:2-AN complex arises because the increase in entropy brought about by destruction of the water lattice is large enough to overcome the decrease in

entropy due to the reduced kinetic freedom of the included molecule. The opposite is true in the case of the α-CD:2-AN complex. Here, the small size of the α-CD binding site renders the decrease in entropy imparted by the loss of *kinetic* freedom large enough to dominate the increase in entropy generated by the destruction of the water lattice. In addition, α–CD, due to the small size of its internal cavity, may leave 2-AN partially exposed to the aqueous phase. This would reduce the disruption of the water structure upon binding and therefore decrease the magnitude of the associated increase in entropy.

The negative entropy of formation for the γ-CD is somewhat more difficult to explain. The large degree of experimental error in the binding constants, which is primarily the result of the small extent of quenching and the possible existence of higher order complexes, makes it difficult to draw conclusions about the thermodynamic forces governing the formation of the γ-CD:2-AN complex. Given the relative size of the γ-CD cavity, it is unlikely that the kinetic freedom of 2-AN included by γ-CD would be any more inhibited than in 2-AN included by α-CD. However, it is possible that some water molecules may still occupy the γ-CD cavity even after 2-AN has been included. If the motion of these water molecules is restricted or if they are forced into a more ordered orientation within the cavity because of the presence of the 2-AN, then a new effect responsible for decraeses in entropy may arise. This would also contribute to the scattering in the Van't Hoff plot because the Van't Hoff equation does not account for the changing effects of the $(H_2O)_n$:γ-CD equilibrium on the formation of the γ-CD:2-AN complex with increasing temperature.

With the use of CPK molecular models, it can be shown that, in

addition to 2-AN, the γ-CD cavity is large enough to accommodate a fluorinated alcohol such as TFE. We chose to modify the γ-CD cavity with fluorinated alcohols because a 1% solution (by volume) of these molecules causes a slight enhancement of the free 2-AN fluorescence (Figure 17). Thus, the possibility exists that fluorescence enhancement would occur if TFE would form a hydrogen bond with the included 2-AN. However, the opposite was observed. The addition of TFE actually quenched the fluorescence of 2-AN in the presence of γ-CD. This indicates that the bound 2-AN and TFE are oriented in such a way that they cannot hydrogen bond to each other. The larger binding constant of the TFE-modified γ-CD, $86 \pm 6\ \underline{M}^{-1}$, indicates that it forms a stronger complex with 2-AN than the unmodified γ-CD. This is because the 2-AN is more tightly bound by the smaller binding site of the modified γ-CD. Because, HFIP bears no effect on fluorescence in the presence of $3 \times 10^{-3}\ \underline{M}$ γ-CD we conclude that it is too bulky to share the γ-CD cavity with 2-AN. Again, this is confirmed by CPK molecular models.

The observed spectral broadening and nonlinear Stern-Volmer plots suggests that 2-AN can form cyclodextrin inclusion complexes having other than 1:1 stoichiometries. The upward curving Stern-Volmer plot in the case of α–CD is indicative of higher order complexes involving more than one cyclodextrin. Because it is not likely that α–CD would include more than one acetylnaphthalene, the 2:1 complex, $(\alpha\text{–CD})_2$:AN is the most plausible structure of the component causing the upward curvature of the Stern-Volmer plot. This can be shown by treating the formation of such a complex in a manner similar to the mechanism previously described for the 1:1 complex. Mass balancing the total 2-AN concentration followed by substitution of the appropriate fluorescence intensity measurements

results in a modified version of equation (7) which is second order in C_{CD}, equation (8).

$$(F^{\circ}/F) = 1 + k_{1:1}C_{CD} + 2\,K_{1:1}\,K_{2:1}\,(C_{CD})^2 \qquad (8)$$

It is this $(C_{CD})^2$ term which gives rise to the observed curvature in the Stern-Volmer plot. In this case, a second order polynomial regression analysis would reveal binding constants for both the 1: 1 and 2:1 complexes.

The isoemissive point observed in Figure 9, and the emission spectrum of the 2-AN excimer formed in γ-CD (Figure 12), indicate the presence of the 1:2 or 2:2 complexes, γ-CD:$(AN)_2$ or $(\gamma\text{-CD})_2$:$(AN)_2$. The formation of such complexes is deemed plausible on the basis of structural studies involving CPK molecular models. In addition, the diminishing appearance of the isoemissive point in the TFE- modified γ-CD , which is less likely to incorporate two acetylnaphthalenes than the unmodified γ-CD, further demonstrates the ability of γ-CD to form higher order inclusion complexes with 2-AN. Due to the variety of complexes formed between 2-AN and γ-CD, and the small extent of quenching observed, it is very difficult to interpret the quenching data for this case.

However, a mechanism defining the formation of complexes involving more than one 2-AN can be used to explain variations in the Stern-Volmer plots. Again, balancing the total mass of 2-AN and substituting with expressions for fluorescence intensity results in another variation of the Stern-Volmer equation which is now first order in [2-AN]. Equation (9) is the modified Stern-Volmer equation for the formation of a 2:2 complex from two 1:1 complexes. Equation (10) is the modified

Stern-Volmer equation for the 2:1 complex formed as 2-AN is included by a 1:1 complex.

$$(F°/F) = 1 + K_{1:1}C_{CD} + 2(K_{1:1})^2 K_{2:1} [2\text{-AN}](C_{CD})^2 \quad (9)$$

$$(F°/F) = 1 + k_{1:1}C_{CD} + 2 K_{1:1} K_{2:1} C_{CD}[2\text{-AN}] \quad (10)$$

According to equations (8), (9) and (10), the 1:2 and 2:2 complexes can be distinguished from the 2:1 complex because the Stern-Volmer plots for the former two cases are dependent on [2-AN] while the same plot for the latter case does not depend on [2-AN]. If the fluorescence constant, k, is known, then some quantitative information about the binding constants may be derived because [2-AN] =F/k.

It is possible that more insight into the inclusion complexes of γ-CD may be obtained from fluorescence lifetime analysis. As was previously mentioned, we are unable to make any conclusions based on the PTI lifetime analysis. Decay curves for the emission at 430nm, the maximum wavelength of normal 2-AN fluorescence, were analyzed. However, in the case of γ-CD, an isoemissive point is observed at approximately 510 nm and the excimer emission spectrum has a maximum beyond 500 nm. Even though the weakness of the signal at these longer wavelengths would probe the limitations of the instrument, it is possible that some meaningful results could be obtained by monitoring the excited-state decay curves at these wavelengths.

In the future, the fluorescence of 2-AN in the presence of α-CD and γ-CD should be reinvestigated and the data should be analyzed by the methods described above. This may help to reduce the experimental error inherent in the previously measured binding constants. In addition, lifetime analysis of 2-AN in mixed solvent systems should be continued. In

particular, the temporal properties of the longer wavelength emission of 2-AN in the presence of γ-CD should be investigated, since 2-AN excimer emission is observed beyond 500 nm. However, this is a nontrivial problem given the magnitude of the signal at this wavelength and the weak intensity of the excimer emission. Also, the concentrations of β-CD and 2-AN should be varried in order to find evidence for the existence of higher order complexes formed between β-CD and 2-AN. Finally, cyclodextrin inclusion complexes of other carbonyl substituded naphthalenes, which exhibit luminescence properties similar to those of 2-AN, should be investigated.

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