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Spectroscopic Studies of Solid State Cyclodextrin Complexation Reactions With Various Guest Molecules.

Michelle Butterfield Young
Louisiana State University and Agricultural & Mechanical College

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SPECTROSCOPIC STUDIES OF SOLID STATE CYCLODEXTRIN COMPLEXATION REACTIONS WITH VARIOUS GUEST MOLECULES

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in The Department of Chemistry

by

Michelle Butterfield Young
B.S., Morris Brown College, 1990
M.S. Emory University, 1992
August 1999
Dedication

In loving memory of my husband, Richard Young. You were my anchor and support and I could always count on you providing the silver lining in a dark cloud. You taught me to trust in God for all things and to believe in myself. When I wanted to prematurely shut the door on this journey, you encouraged me to press on. Thank you! I will always cherish our memories. By the grace of God, I made it.

and

To my beautiful daughter, Brittany Samaria Young, who had to endure mommy’s irritable moments after pulling all-nighters. I know that you do not understand this now, but one day you will know that with God, all things are possible. Sweetie, this chapter is over.
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I would like to acknowledge those who have encouraged, supported, and motivated me through this tedious journey. I thank all of you for your undying support.

First and foremost, I give honor and reverence to God for His many blessings. I know that none of this would have been possible without You. Thank you for allowing the Holy Spirit to lead and guide me through the years. Praise the Lord!

To my mother for always encouraging me to continue on to reach my goal. You have always been there to support me throughout the years. It is finally over!

To my brothers and sisters: Reverend Gloria O'Berry, Anita Meyer, Reverend Warren Butterfield, Reverend Steven Butterfield, and Ernest Butterfield for all the love and support you have given me throughout this educational journey. Your baby sister has finally finished!

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To the rest of the Warner Research Group for your support.
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Abstract

The research presented in this dissertation involves spectroscopic studies of cyclodextrin complexation reactions with various guest molecules. Chapter 1 provides an introduction that forms the basis for the remaining chapters. The topics covered in the introduction include the structures and properties of cyclodextrins with a brief section specifically dedicated to solid cyclodextrin. In addition, the environmental applications of cyclodextrins are discussed. The last part of the introduction concerns the theory and applications of fluorescence spectroscopy.

In Chapter 2, the extraction of volatile polycyclic aromatic hydrocarbons (PAHs) from air by use of solid cyclodextrin is presented. Naphthalene was chosen as the model compound due to its high vapor pressure. The effect of various alcohols on the gas-solid complexation reactions of PAHs as well as quantitative studies are also reported in this chapter. These studies with the solid cyclodextrin are compared to those examined in aqueous solutions.

Chapter 3 pertains to the effect of cyclodextrins on excited state proton transfer reactions of carboxylic acid compounds. Several naphthoic and anthroic acids including 2-naphthoic acid, 1-hydroxy-2-naphthoic acid, 9-anthroic acid, and 2-anthroic acid are studied in order to propose mechanisms for these compounds in monomeric and polymeric cyclodextrins. The inner cavity diameters of the cyclodextrins play a major role in the proposed mechanisms.

Chapter 4 reports the effect of the Cyclodextrin Host Isolation Matrix (CHIM) method on excited state proton transfer reactions. These studies involve solid samples...
of the compounds mentioned in Chapter 3 with the addition of 3-hydroxy-2-naphthoic acid. The CHIM method mimics the spectroscopy of isolated molecules. The results obtained from the solid samples are compared to those obtained from aqueous solutions reported in the previous chapter. Dual fluorescence is reported for the anthroic acids and an explanation for this phenomenon is given.
Chapter 1. Introduction

Cyclodextrins

In 1891, A. Villiers discovered and isolated cyclodextrins (CDs) from the enzymatic degradation of starch by bacteria [1]. However, Schardinger was the first to give a detailed account and characterize cyclodextrins as cyclic sugars. Thus, these cyclic sugars are sometimes referred to as Schardinger dextrins [2,3]. Cyclodextrins are a series of cyclic oligosaccharides formed by the linkage of glucopyranose units through α-(1,4) glycosidic bridges (Figure 1.1). These molecules have a torus shape with the primary hydroxyl groups lining the smaller edge of the cavity and the secondary hydroxyl groups lining the larger edge of the cavity (Figure 1.2). The cyclodextrin interior cavity is hydrophobic due to the glycosidic oxygens, whereas, the exterior of the cyclodextrin cavity is hydrophilic due to the hydroxyl groups. Nuclear magnetic resonance and infrared spectra studies indicate the formation of intra-molecular hydrogen bonds in solution between the secondary hydroxyl groups of adjacent glucopyranose units [4-6]. It is suggested that these bonds serve to restrict rotation of the glucopyranose units, contributing to the structural rigidity of cyclodextrin molecules.

The most widely used cyclodextrins are α-, β-, and γ- cyclodextrins with six, seven, and eight glucopyranose units, respectively. Cyclodextrins composed of glucopyranose units greater than eight units are known to exist, but they are highly flexible and do not favor complexation. Due to the extensive steric strain for CDs with five or less glucopyranose units, they are highly unlikely to exist. The cavity diameters are
approximately 5.7, 7.8, 9.5 Å for α-, β-, and γ-cyclodextrins, respectively. The variations in cavity size enables each CD to selectively incorporate hydrophobic guests based on size and geometry. The important physical properties of the most common cyclodextrins are summarized in Table 1.1.

The average bond distances between the O(2) and O(3) atoms of adjacent glucopyranose units for α-, β-, and γ-cyclodextrins are 3.00, 2.86, and 2.81 Å, respectively. Therefore, α-CD is the most flexible of the CDs in solution due to the weak intramolecular hydrogen bond interactions. Conversely, the intramolecular hydrogen bonds increase in strength for β-CD. Furthermore, the aqueous solubility of β-CD decreases relative to that of α- and γ-cyclodextrins. The odd number (7) of glucopyranose units seems to allow dimerization of the molecule in solution causing this decreased solubility.

<table>
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<th>Physical Property</th>
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<th>γ-CD</th>
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<tr>
<td># of glucose units</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Molecular weight, g</td>
<td>972</td>
<td>1135</td>
<td>1297</td>
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<tr>
<td>Cavity diameter, Å</td>
<td>5.7</td>
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</tr>
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<td>7.8</td>
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The use of cyclodextrins is restricted due to their limited solubility. This is particularly true for β-CD. Therefore, many attempts have been made to modify β-CD
chemically to improve its solubility. Many β-cyclodextrin derivatives have been synthesized due to their higher aqueous solubility than the native β-CD. The β-CD derivatives have been synthesized by substitution of the primary and secondary hydroxyl groups with different functional groups [7,8]. Monosubstituted 6-O-p-toluenesulfonyl-cyclodextrin is normally used as the starting material in the preparation of cyclodextrin derivatives. Methylation of heptakis-(2,6-di-O-methyl-)β-CD and heptakis-(2,3,6-tri-O-methyl-)β-CD have been reported [9,10]. The water solubilities of these two compounds are 57 and 31 g/100 mL for the di- and tri-methylated CDs, respectively [11]. Methylation of cyclodextrins involves all the hydroxyl groups at positions C2, C3, and C6 or all C2 secondary and C6 primary hydroxyl groups. Hydroxypropylation of cyclodextrins leads to the formation of heterogeneous products with various degrees of substitution [11]. These derivatives have amorphous structures with water solubility of more than 50 g/100 mL at 25 °C [11]. Similarly, water soluble cyclodextrin polymers have been synthesized. The most common procedure to produce these polymers involves the use of epichlorohydrin. The resulting CD polymers are a polydisperse mixture containing CD units joined by repeating glyceryl linkers (-CH₂-CHOH-CH₂-)ₙ. All three of the CD polymers (α-CDP, β-CDP and γ-CDP) are very water soluble and commercially available.

The study of cyclodextrin inclusion complexes has attracted interest over the past decades [12]. An inclusion complex can be defined as a guest molecule occupying the interior space of the host. Various property changes of the included guest is a result of cyclodextrin complexation. Depending on the host/guest system, complexation usually
Figure 1.1 The chemical structures of α-, β-, and γ-cyclodextrins
Figure 1.2 The cone representation of cyclodextrins
occurs in a manner that allows maximum exposure of the hydrophobic portion of the guest to the apolar host cavity. Effective complexation usually occurs by a number of factors [13]: (1) favorable energetics must exist in order to initiate CD/guest interactions; (2) the guest must have some affinity for the CD cavity; and (3) the protective guest must be a compatible size for the CD cavity so that full or partial inclusion can occur.

Water molecules play an important role in the driving force of CD complex formation with hydrophobic molecules in aqueous solutions [7]. High energy water molecules are released from the cyclodextrin cavity upon guest inclusion. Replacement of high energy water with a hydrophobic guest is favored because the cyclodextrin ring strain is reduced. Thus, complexation results from this decrease in the cyclodextrin ring strain upon inclusion of the guest molecule. Moreover, hydration of the cyclodextrin complex is energetically favored over the hydration of individual components. These considerations are not obvious when generally speaking about the properties of solid cyclodextrin.

**Solid Cyclodextrin**

Cyclodextrin inclusion complexes may also occur in the solid state. These complexes are different than those formed in solution. In solution, the guest molecule is included in the cavity and the whole complex is surrounded by a hydrate shell of water molecules. However, in the solid state, the guest molecule can either form a complex within the CD cavity or within the intra-molecular cavities formed by the crystal lattice of the CD molecule. It should be noted that solid CD includes water molecules in its cavity.
The number of water molecules included in the solid CD depends on the kind of CD and ranges from 6 to 17 water molecules [7]. Therefore, a driving force for CD solid complex formation, in which water molecules can be released from the CD cavity, is still possible, even in solid cyclodextrin. One additional note is that solid cyclodextrin complexes are seldom of stoichiometric composition.

Clathration [14] is a special type of complexation that occurs in the solid state, in which the guest molecule is retained in the host by crystal lattice forces. However, CDs exhibit complexation in both the solution phase and the solid state. The structures of solid CDs have been described as cage- or channel-type structures [7,14]. In the channel-type structure, the CD molecules are vertically stacked, with the guest molecules embedded into these channels. In contrast, the cavity of one CD in a cage-type structure is blocked on both sides by adjacent CD molecules, thereby leading to isolated cavities. A considerable amount of work regarding the use of solid CDs as host matrices has involved room-temperature solid surface luminescence analyses [15-17]. These analyses have explored cyclodextrin-salt mixtures as solid matrices. In addition, studies which involved the grinding of solid CD with solid pyrene or solid PPO reported the formation of inclusion complexes [18]. Otherwise, complex formation studies of CDs in the solid state have not received much attention from researchers [14].

Solid CD complexes can be formed with a variety of solid, liquid, or gaseous guest molecules. For example, liquids can be transformed into solid substances and formulated as dosable pills. Volatile substances, such as aromas, can be stabilized by forming solid
complexes. These complexes remain stable for long periods of time, whereas in aqueous media, these substances are liberated especially upon heating.

In the early 1990s, Procter and Gamble released its first commercial cyclodextrin-based product when it relaunched Bounce fabric softener sheets for the dryer [19]. The reformulated Bounce sheet contains a cyclodextrin/fragrance complex. During the drying process, this complex transfers to the fabric along with the softener. The fragrance is released when the fabric becomes wet from water or perspiration. Otherwise, the fragrance is not noticeable when locked inside the cyclodextrin cavity on dry fabric.

More recently, Procter and Gamble released another cyclodextrin-containing product called Febreze [19]. Febreze is a spray that was created to remove odors from fabrics such as clothing, carpet, or furniture. In this particular product, some of the cyclodextrins release fragrance while others are not complexed with fragrance. Instead, when Febreze is used on fabrics, the free cyclodextrins bind with odor-causing molecules such as tobacco, masking the odors.

Several intermolecular forces are involved in the formation of cyclodextrin inclusion complexes. These include van der Waals forces, hydrogen bonding, and hydrophobic interactions. Van der Waals or London dispersion forces are weak attractive forces which occur from dipole-dipole interactions. Hydrogen bonding usually involves interaction between a hydrogen atom and another very electronegative atom resulting in strong dipole-dipole interactions. Van der Waals forces and hydrogen bonding are not crucial in establishing stable cyclodextrin complexes. Hydrophobic interactions, on the
other hand, are the contributing factors needed for both formation and stabilization of cyclodextrin inclusion complexes. These interactions result when the entropy is changed due to the release of high energy water molecules from the CD cavity. The stability of CD inclusion complexes is also a function of the hydrophobicity of the guest molecule, and is considered to be proportional to the apolar character of the included molecules [7].

Complex formation between a guest molecule and cyclodextrin in solution can be described by the following dynamic equilibrium

\[ C + G \rightleftharpoons C-G \]  

(1.1)

\[ K_f = \frac{[C-G]}{[C][G]} \]  

(1.2)

where [C], [G], and [C-G] represent the respective equilibrium concentrations of cyclodextrin, guest molecule, and complex. The stability of the complex can be described by the association constant, \( K_f \), shown in equation 1.2. The parameter, \( K_f \), is usually expressed in L/mol. Values for association constants can be determined spectroscopically using the Benesi-Hildebrand [20] method by monitoring changes in spectral intensity upon the addition of cyclodextrin. The Benesi-Hildebrand method will be discussed in more detail in Chapter 3.

Equation 1.1 indicates a 1:1 stoichiometric ratio between the cyclodextrin and the guest, but other stoichiometric complexes such as 2:1 or 1:2 cyclodextrin:guest complexes also exist. The following equilibria describe a 2:1 inclusion complex

\[ 2C + G \rightleftharpoons C-G + C \rightleftharpoons C_2G \]  

(1.3)
This type of complex has been reported for the methyl orange:α-CD complex [21]. Two isosbestic points are observed in the absorption spectra of methyl orange upon increasing α-CD concentration. The first isosbestic point is observed at low α-CD concentrations and indicates formation of the 1:1 methyl orange:α-CD complex. The second isosbestic point appears upon further addition of α-CD and indicates formation of the 2:1 complex. These complexes are favorable for guest molecules that are too large to be fully included by one CD molecule. The portion of the guest that is not included may be surrounded by a second CD molecule, provided the geometry and size are comparable to the cyclodextrin.

There is a correlation between enthalpy, ΔH, and entropy, ΔS for complex formation. If little heat is generated during complexation, then ΔH is small and ΔS is large implying a higher degree of disorder after complex formation. Additionally, if ΔH is large, the entropy is negative resulting in a higher order of the complexation system. Calorimetric measurements are used to determine real values for ΔH and ΔS [22,23].

The procedure for the preparation of cyclodextrin guest complexes in water depends upon the guest properties and varies among experimentalists. One preparation method reported in the literature describes the dissolution of the guest analyte in an aqueous solution of cyclodextrin [24,25]. However, the guest analyte is normally too small to be weighed due to its low aqueous solubility. Therefore, an alternative method is to prepare the guest analyte in an organic solvent and transfer an aliquot of this stock solution to another container. The organic solvent is then evaporated and the residue is
diluted with an appropriate amount of aqueous cyclodextrin. In many instances, overnight equilibration of the cyclodextrin:guest solution is needed for adequate formation of an inclusion complex. In addition, turbid cyclodextrin:guest solutions occur especially those prepared with β-CD. The turbidity may occur from precipitation of either cyclodextrin or the complex from the aqueous solution. The preparation for solid cyclodextrin:guest complexes will be discussed in Chapter 2.

Many spectroscopic techniques have been used to analyze inclusion complexes. Normally, changes in the absorbance or fluorescence spectrum of the guest upon addition of cyclodextrin have been utilized to investigate the formation of an inclusion complex. Circular dichroism can also be employed to examine complex formation by addition of cyclodextrin. The chirality of CD can cause achiral compounds to exhibit induced circular dichroism signals upon CD complexation. NMR studies are used to obtain information on the location and orientation of the guest analyte with regards to the CD cavity.

The major interest in cyclodextrins arises from their ability to form inclusion complexes with a wide range of guest analytes. The varied practical applications of cyclodextrins have increased over the years as reflected in the increasing number of patents and publications involving inclusion complexes. Cyclodextrin complexation often results in changes in various properties of the included guest or guests. Thus, cyclodextrins have been utilized as microencapsulators in the food, pharmaceutical, and environmental industries.
Cyclodextrins in the Food Industry

Cyclodextrins have been used in the food industry for a wide range of applications. For example, CDs have been used in separations to remove caffeine from tea [26] and as additives in various food products [27]. Specifically, CDs have been incorporated as additives for fish and other nitrogen containing food products [27]. The stability of certain food ingredients has been increased due to the use of CD additives as preservatives. In this application, β-CD minimized the degradation of rice products by lessening the odor developed by the products during storage [28]. Similarly, CDs have been used to protect food ingredients in fruits and spices from the environmental effects of light, oxygen, and heat. The spices and fruit flavors were changed into powders using CDs which increases the stability of the products when subjected to the rigors of industrial food processing, with a higher shelf life than their liquid counterparts [29-31]. Hamilton and Heady [32] reported in a patent that unpleasant tastes of instant beverages associated with overextraction or overboiling can be removed by use of cyclodextrin. Suzuki [33] found that the bitter taste of protein hydrolyzates can also be reduced using cyclodextrin. The above examples show the wide applicability of the use of cyclodextrins in the food industry.

Szejtli [34] encapsulated various oils such as sage, raspberry, lemon, cinnamon, garlic, and onion by obtaining odorless powders with minimized hygroscopicity. The powdered oil showed more stability than the normal oils, losing less than five percent of their active ingredients after a two-year period [11]. Vinegar and other liquids have also
been prepared in powder form from CDs to produce food products with enhanced flavor [35-37].

Cyclodextrins are also used in the cosmetic industry as liquid body deodorants and breath freshners to mask unpleasant odors [11]. Cosmetic products often contain high concentrations of emulsifying agents and perfumes, which can have irritating side effects. Therefore, cyclodextrins can be used in this aspect to decrease undesirable effects of such agents. Cyclodextrins have also been used to stabilize the liposoluble vitamins such as vitamins A, K, E, and D in various formulations [11]. Furthermore, cyclodextrins can form complexes with fatty acids and have been used as additives in products to treat acne [11].

**Pharmaceutical Applications of Cyclodextrins**

The encapsulation of drugs in the cyclodextrin cavity offers many possibilities in the pharmaceutical industry. The physical and chemical properties of a cyclodextrin-encapsulated drug can differ significantly from the properties of the same drug in a free state, leading to numerous applications of cyclodextrins in the pharmaceutical industry.

The main requirement for any compound to be useful in the pharmaceutical is sufficient proof of its nontoxicity, supported by data from extensive clinical studies. Toxicity studies have shown that cyclodextrins can be administered orally, rectally, or dermally without harmful effects. The effects of oral administration of β-CD revealed no toxic, carcinogenic or mutagenic consequences [38]. Radio-labeling studies were also conducted to determine the degree of absorption of β-CD from the intestinal tract. Although glucose, maltose, and starch were found to be metabolized, β-CD was not
detected in the bloodstream following oral administration. These and other metabolic,
toxicological and pharmacokinetic investigations of cyclodextrins [39,40] have
convincingly demonstrated their nontoxicity, presenting unlimited possibilities for their use
in the pharmaceutical industry.

Cyclodextrins have also been shown to improve the solubility of drugs that are
poorly soluble in water. The encapsulation of such drugs in the hydrophobic cavity of
cyclodextrin promotes their dissolution in aqueous media. Hamada [41] investigated the
effect of monomeric glucose and different cyclodextrins upon the solubility of several
nonsteroidal anti-inflammatory drugs such as infomethacin, whose size is comparable with
the diameter of the β-CD cavity. The presence of glucose had no effect and α-CD only
slightly increased their solubility in water. However, β-CD proved to be quite effective
in the enhancement of the solubility of the drugs, which is most likely due to the formation
of an inclusion complex between the drug and the β-CD.

The bioavailability of a drug is the rate of its absorption from the dosage form into
circulation. The formation of a drug:cyclodextrin inclusion complex results in dispersion
of the drug molecules in a sugar matrix, which readily disintegrates under physiological
conditions. As a result, poorly water soluble drugs can be available in relatively high
concentrations in the plasma when they are encapsulated in the cyclodextrin cavity. The
effect of cyclodextrin inclusion on the bioavailability of digoxin was demonstrated by
Uekama et al. [42]. Their results indicated that the presence of γ-CD increases the rate
of absorption of the drug from the plasma.
Environmental Applications of Cyclodextrins

The use of cyclodextrins for environmental purposes is widespread. For example, cyclodextrins have been used in the analysis of polycyclic aromatic hydrocarbons. Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants that have received considerable attention because of their carcinogenic and mutagenic effects [43]. Due to the extensive amount of data suggesting the hazards of these compounds, many PAHs are on the Environmental Protection Agency (EPA) list [44]. The structures of the sixteen PAHs on the EPA's list are shown in Figure 1.3.

Significant levels of PAHs are found in the atmosphere but are more prominent in polluted urban areas. These compounds are often emitted into the atmosphere by way of combustion processes [43]. For example, a partial combustion of fuel in an internal combustion engine results in the formation of trace amounts of PAH compounds [45]. Due to their wide range of vapor pressures, some atmospheric PAHs exist exclusively in the gas phase, while others exist as adsorbed particulate matter. The volatility of these organic compounds controls their transport in the workplace and in the environment in general.

It is well established that CDs will size-selectively complex with PAHs through host-guest interactions and cause significant changes in the spectral properties of these PAH compounds[46]. Elliot et al. [47] reported a procedure to size selectively extract naphthalene, pyrene, and benzo(a)pyrene from an oil-in-water microemulsion system. The α-, β-, and γ-cyclodextrins were used in the extraction process and the ability of each CD
Figure 1.3 Structures of PAHs on the EPA Priority Pollutant List
to extract the PAHs was evaluated by measuring the fluorescence intensity of each PAH before and after extraction with that CD.

Cyclodextrins are also useful tools in providing protective environments for PAHs [48-50]. Husain et al. [51] developed a procedure to separate PAHs utilizing cyclodextrins and organic modifiers. The use of organic modifiers along with a size-compatible CD resulted in shorter retention times for the PAHs in this chromatographic system. Volatile pesticide compounds have been stabilized with the use of CDs by converting the pesticide compounds into microcrystalline complexes [52,53]. These complexes improve the potency of these pesticides as well as restrict the evaporating pollutant to selected areas. One example of this includes a class of herbicides, called thiolcarbamate esters [54]. These herbicides are effective for the protection of rice crops but are harmful to the environment. Cyclodextrin complexation of these herbicides caused a decrease in the loss of these compounds to the surrounding atmosphere [54].

Moreover, β-CD has been used to stabilize pyrethroids through inclusion and have been used to treat tea plantations and rice paddies in Japan [10]. Pyrethroids are excellent insecticides since they are highly effective and have a low toxicity to humans. However, these insecticides are susceptible to degradation by UV light and oxygen. Therefore, β-CD has been used to protect these compounds from such effects.

Pollutants present in the liquid phase can also be stabilized using cyclodextrins and the ability of CDs to form inclusion complexes with such molecules. Trichlorfon, a highly toxic, noncrystallizable insecticide can be converted to dichlorvos (DDVP) [55]. This
insecticide can form a water soluble inclusion complex with β-CD. This complex can then be easily extracted from the liquid phase. The β-CD causes a decrease in the toxicity of the substance.

It is apparent that cyclodextrins can be widely used for environmental purposes. The above environmental applications represent a sampling of the ability of cyclodextrins to influence certain properties of various harmful pollutants.

**Theory and Applications of Fluorescence Spectroscopy**

In 1852, G. G. Stokes [56] provided a detailed account of the phenomenon of fluorescence. He discovered the relationship between fluorescence intensity and concentration which led to the fluorescence process being thought of as an emission process. Since then, fluorescence spectroscopy has found many applications in various areas of research. Many review articles have been published on this technique over the years [57-60].

Luminescence measurements are highly sensitive and offer selectivity by choice of excitation and emission wavelengths as compared to absorption measurements. In addition, lasers and fiber optics have increased the sensitivity and selectivity of the fluorescence technique [61-64]. This section presents an overview of fluorescence spectroscopy in order to provide a sufficient background for forthcoming chapters.

Spectroscopy is a method of analysis that measures the amount of electromagnetic radiation that is absorbed, emitted, or scattered by a sample. Electromagnetic radiation is a form of energy that possesses wave properties and properties of discrete particles of
light (photons). Electromagnetic radiation is transmitted at high speed and can be recognized as light, radiant heat, x-ray, ultraviolet, microwave, and radio radiations. Processes that involve the absorption of electromagnetic radiation are explained by the particle nature of the electromagnetic radiation. However, processes that do not involve the absorption of electromagnetic radiation such as diffraction, reflection, and refraction of light are explained by the wave properties of the electromagnetic radiation. The dual nature of electromagnetic radiation is related by the Einstein-Planck equation

$$E = hv,$$

(1.4)

where $E$ is the energy of a single photon of the electromagnetic radiation in Joules, $v$ is the frequency of the wave in Hertz, and $h$ is Planck's constant ($6.634 \times 10^{-34}$ J sec). The equation relates the frequency of radiation (wave theory) to the energy of a quantum of radiation (particle theory). The frequency and wavelength are related as follows

$$\lambda = \frac{c}{v},$$

(1.5)

where $\lambda$ is the wavelength in m, $c$ is the speed of light in vacuum ($2.9979 \times 10^8$ m/s) and $v$ is the frequency in Hertz.

The electromagnetic spectrum is divided into regions according to energy. Figure 1.4 shows the various regions of the electromagnetic spectrum and some spectroscopic methods used in each region. Absorption of light by a molecule in the ultraviolet and visible regions is the process by which a molecule is promoted from a lower energy state to a higher energy state. Other absorption methods include infrared, atomic absorption, nuclear magnetic resonance, and x-ray absorption. Emission methods commonly
employed in molecular spectroscopy include fluorescence, phosphorescence, and x-ray emission.

![Figure 1.4 The electromagnetic spectrum](image)

The Jablonski diagram shown in Figure 1.5 can be used to illustrate the various processes associated with the absorption and emission of radiation. The diagram is composed of ground, first excited, and second excited singlet electronic states denoted by $S_0$, $S_1$ and $S_2$, respectively. The first excited triplet state is denoted by $T_1$. The total angular momentum determines the multiplicity of the various states for typical organic
molecules and is given by

\[ M = 2S + 1, \tag{1.6} \]

where \( S \) is the spin quantum number. For most organic molecules, \( S = 0 \) because they normally have an even number of electrons. Therefore, \( M = 1 \) and is referred to as the singlet state. All electrons in the molecule are spin paired in the singlet state. In the triplet state, an electron has flipped its spin in an excited state, providing a spin quantum number of one and therefore a multiplicity of three (triplet).

![Jablonski Diagram](image)

**Figure 1.5 The Jablonski diagram**

**Absorption**

The absorption process promotes an organic molecule from the lowest energy level or ground state \( (S_0) \) to a higher energy level known as the excited state \( (S_1 \text{ or } S_2) \). This process is depicted vertically in the Jablonski diagram and occurs in approximately \( 10^{-15} \)
seconds. According to the Franck-Condon principle, this time scale is too fast for nuclear rearrangement to occur. Therefore, the molecule reaches the higher energy state without movement of its atomic nuclei. This phenomenon is the basis for the Franck-Condon principle. This principle also states that if the probability of a specific transition is largest in the absorption, then the probability of the reciprocal transition is also largest in the emission. The transitions characterizing the Franck-Condon principle are shown in Figure 1.6.

![Diagram showing the Franck-Condon principle](image)

**Figure 1.6 Franck-Condon principle**
The absorption of energy is illustrated by the following equation

\[
\begin{align*}
S_0 + \text{hv} \quad &\longrightarrow (S_1)_n \text{ or } (S_2)_n \\
&\text{at rate } k_a
\end{align*}
\]

(1.7)

where \( n \) is the vibrational level occupied by the molecule; \( k_a \) is the rate constant for absorption; \( \nu \) is the frequency of the absorbed energy, and \( h \) is Planck’s constant.

According to the equation, absorption usually occurs from the lowest vibrational level of the ground state to a vibrational level in the excited state. The energy of the absorbed photon determines the electronic and vibrational levels to which a molecule is excited. The distribution of molecules in the lowest (0) and first (1) vibrational levels of \( S_0 \) can be described using the Boltzmann distribution and the ratio, \( R \), of molecules in each state as

\[
R = e^{-\Delta E/(kT)},
\]

(1.8)

where \( \Delta E \) is the energy difference between these two levels, \( k \) is the Boltzmann constant, and \( T \) is the temperature in Kelvin. A typical value for \( R \) at room temperature is 0.01. Therefore, most of the molecules are present in the lowest vibrational level with absorption of light mainly occurring from this energy level.

**Vibrational Relaxation**

Vibrational relaxation is a nonradiative process that occurs when a molecule deactivates from a higher vibrational level of an electronic excited state to a more stable vibrational level within that electronic excited state. Excess energy is lost due to collisions between the molecules in the excited state and collisions between the excited molecules and the solvent. This process usually occurs in approximately \( 10^{-12} \) seconds and normally...
continues in succession until the lowest vibrational level is reached. Vibrational relaxation can be illustrated by equation 1.9 as follows

\[ S_1 \rightarrow (S_1)_0 + \Delta \text{,} \quad \text{(1.9)} \]

where \( \Delta \) is the heat and \( k_{vr} \) is the vibrational relaxation rate constant.

**Internal Conversion**

Internal conversion is not well understood, but it involves the conversion of electronic energy of the molecule to heat. This radiationless transition occurs if a molecule has been excited to the \( S_2 \) state and undergoes relaxation to the \( S_1 \) state. This transition also results from solute/solvent interactions and/or solute/solute interactions. The rate of internal conversion is typically \( 10^{-8} \) seconds for the transition between \( S_1 \) and \( S_0 \). However, the rate of internal conversion is much faster for the smaller energy transition between \( S_1 \) and \( S_2 \). Therefore, the latter can compete with fluorescence and intersystem crossing for many molecules. Internal conversion can be represented as follows

\[ S_2 \rightarrow S_1 + \Delta \text{,} \quad \text{(1.10)} \]

where \( k_{ic} \) is the rate of internal conversion.

**Radiative Deactivation**

Fluorescence is a radiative relaxation process that occurs when a molecule releases energy in the form of photons from the lowest vibrational level of the excited singlet state to the various vibrational levels of the ground state. The energy difference between the excited and ground states is equivalent to the energy emitted by the molecule. The fluorescence process can be described as
where $k_F$ is the rate of fluorescence and $v$ is the frequency of the emitted photon. The value for $k_F$ is typically $10^9$ seconds.

**Intersystem Crossing**

Intersystem crossing is a nonradiative process that involves a molecule changing from a singlet state to a triplet state. In this process, an electron spin is reversed. Intersystem crossing can be expressed as

$$k_{ISC} \quad (S_1)_0 \rightarrow (T_1)_n + hv, \quad (1.12)$$

where $k_{ISC}$ is the rate of intersystem crossing and $T_1$ is the first excited triplet state. The rate of intersystem crossing is on the order of $10^8$ seconds and is much slower compared to the rate of internal conversion. Therefore, this process has a very low probability of occurrence when compared to internal conversion. Intersystem crossing can be enhanced when the sample contains a heavy atom such as bromine or iodine.

**Phosphorescence**

Phosphorescence is a radiative process between two states of different multiplicity. It is usually observed between the excited triplet state and the ground singlet state. This transition is a spin-forbidden process that rarely occurs. Phosphorescence may be described as

$$k_P \quad (T_1)_0 \rightarrow (S_0)_n + hv, \quad (1.13)$$

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where $k_p$ is the rate of phosphorescence. This rate is extremely slow, occurring in approximately $10^{-6}$ to 10 seconds. Phosphorescence has longer lifetimes longer emission wavelengths as compared to fluorescence.

**Characteristics of Fluorescence Emission**

Emission spectra represent the data obtained from fluorescence measurements. The fluorescence emission spectrum is a plot of relative intensity of radiation emitted versus wavelength (nm) or wavenumber (cm$^{-1}$) and occurs when a sample is excited at a fixed wavelength. In contrast, an excitation spectrum is the relative efficiency of exciting radiation at various wavelengths to produce emission at a given wavelength. It can be obtained by fixing the emission wavelength. The shape of the excitation spectrum is independent of monitored emission wavelength. The excitation spectrum is typically identical to the absorption spectrum.

The emission spectrum is independent of the excitation wavelength because fluorescence typically occurs from the lowest vibrational level in the first excited state. In addition, fluorescence usually occurs at longer wavelengths compared to absorbance wavelengths. This is the basis for the Stoke's shift phenomenon, which is attributed to loss of vibrational energy in the excited molecule prior to fluorescence. Moreover, solvent effects and excited state reactions contribute to Stoke's shift. Figure 1.7 illustrates the Stoke's shift for a hypothetical compound.

The spectra of fluorescence and absorption are usually observed to be mirror images of each other. Similarities exist in the vibrational levels of $S_0$ and $S_1$. This mirror image rule applies to many polycyclic aromatic hydrocarbons and is depicted in Figure 1.7.
Deviations from the mirror image rule may occur due to nuclear rearrangements, formation of excimers, or excited state proton transfer reactions.

Figure 1.7 Stokes Shift and Mirror Image
Kinetics of Fluorescence

The quantum yield of fluorescence, $\Phi_F$, is defined as the ratio of the number of photons emitted to the number of photons absorbed. The value for $\Phi_F$ is between zero and unity and depends upon whether the nonradiative or radiative processes dominate. The quantum yield may be expressed as

$$\Phi_F = \frac{k_F}{k_F + k_{IC} + k_{ISC} + k_{VR} + k_Q[Q]}, \quad (1.14)$$

where $k_F$, $k_{IC}$, $k_{ISC}$, $k_{VR}$ are the first order rate constants for fluorescence, internal conversion, intersystem crossing, and vibrational relaxation, respectively. The term, $k_Q$, is the rate of quenching (described later in the text) and $[Q]$ is the concentration of quencher molecules. The above equation describes the efficiency of fluorescence in the presence of all the deactivation processes.

The fluorescence lifetime of a molecule is the average amount of time a molecule spends in the excited state prior to emission and is given by

$$\tau = \frac{1}{k_F + k_{IC} + k_{ISC} + k_{VR} + k_Q[Q]} \quad (1.15)$$

The intrinsic or radiative lifetime, $\tau_0$, is the lifetime of a molecule in the absence of radiationless processes and can be expressed as

$$\tau_0 = \frac{1}{k_F} \quad (1.16)$$

Using equations 1.13, 1.14, and 1.15, the quantum yield can then be expressed as a ratio of the fluorescence and intrinsic lifetimes

$$\Phi_F = \frac{\tau}{\tau_0} \quad (1.17)$$

Equation 1.16 shows that a decrease in the fluorescence lifetime causes a decrease in the quantum yield of a molecule.
Factors That Affect Fluorescence

The fluorescence of a molecule is dependent upon many factors which include solvent, structure, temperature, and pH. These fluorescence characteristics make it a powerful tool for structure elucidation and impurity detection of many molecules. In addition, the environment of the fluorophore can be manipulated in order to improve the detection limit. This section discusses the important factors that affect fluorescence.

The solvent plays a major role in the fluorescence characteristics of a fluorophore. The changes that occur from solvent effects are due to dipole-dipole interactions between the solvent and the fluorophore. The dipole moment of the molecule in the excited state is usually larger than that of the molecule in the ground state. Therefore, absorption of a photon by a fluorophore results in the formation of an instantaneous dipole that changes the microenvironment of the fluorophore. By reorientation of the solvent molecules surrounding the fluorophore before fluorescence emission. This process is known as solvent relaxation. The new solvent arrangement normally possesses a lower energy than the energy of the system prior to reorganization. As a result, a spectral shift to longer wavelengths is observed in the fluorescence emission spectrum. The extent of the shift is determined by the polarity of the solvent. In many cases, the maximum of the emission wavelength increases with increasing solvent polarity.

Structural effects are also important for luminescing compounds. Fluorescence is considered a selective process because many molecules do not fluoresce or phosphoresce. Highly conjugated structures with increased mobility of \( \pi \) electrons show strong
fluorescence. The increased electron mobility causes a high degree of resonance stability. Therefore, most aromatic hydrocarbons are intensely fluorescent because of the \( \pi-\pi^* \) transitions.

Aromatic molecules that contain heteroatoms such as N, O, and S are often nonfluorescent or weakly fluorescent. However, phosphorescence is generally observed with these molecules. There is a relatively small energy gap between \( S_1 \) and \( T_1 \) in heteroatom-containing molecules and therefore, increased intersystem crossing occurs. This small energy gap is representative of \( n-\pi^* \) transitions. Benzophenone and pyrimidine contain \( n-\pi^* \) transitions and these compounds exhibit phosphorescence [65].

Substituents have significant effects on fluorescence. Electron-donating substituents such as -OH, -OCH\(_3\), and -NH\(_2\) usually enhance fluorescence because the probability between the lowest excited singlet state and ground state is increased. Conversely, electron-withdrawing substituents such as -NO\(_2\), -COOH, and -Cl decreases the fluorescence by introducing a low lying \( n-\pi^* \) state [65]. The presence of a heavy atom substituent such as -Br or -I into an aromatic system generally enhances phosphorescence. This effect is known as the internal heavy atom effect and is a result of the mixing of molecular singlet and triplet states. Heavy atoms cause an increase in the rate of intersystem crossing.

Aromatic molecules which have rigid, planar structures generally fluoresce with higher yield [65]. Fluorescein is an example of a rigid molecule which shows fluorescence. The extent of fluorescence usually increases with the number of \( \pi \) rings contained by the molecule [66]. Aliphatic molecules normally do not fluoresce.
Temperature and viscosity changes affect the fluorescence process. As the temperature increases, the viscosity decreases. In addition, there is an increase in the number of molecular collisions at higher temperatures which causes a decrease in fluorescence. The opposite is true regarding the viscosity. As the viscosity of the solvent increases, the number of molecular collisions of the excited state decreases. This enhances the fluorescence yield.

The effect of pH on fluorescence is often due to the protonation of a basic group or the dissociation of an acidic group on the fluorophore. As a result of these processes, the rates of nonradiative processes that compete with fluorescence are changed as well as the relative separation of the ground and excited state molecules. Thus, the quantum yield of emission is changed, causing a shift in the fluorescence spectrum. Protonation of electron-withdrawing groups causes a spectral shift to longer wavelengths (red shift) whereas protonation of electron-donating groups produces a spectral shift to shorter wavelengths (blue shift). In contrast, dissociation of electron-withdrawing groups causes a blue shift while dissociation of electron-donating groups produces a red shift. Therefore, the control of pH is necessary for fluorescence measurements of pH sensitive molecules.

The inner filter effect caused by instrumental artifacts that absorb a portion of excitation or emission energy produces a reduction in the observed fluorescence intensity. This effect can be caused by self-absorption of the fluorophore itself at higher concentrations.
**Fluorescence Linearity**

The observed fluorescence intensity is directly proportional to the amount of light absorbed, i.e.

\[ I_F = \Phi_F \Delta I = \Phi_F (I_0 - I_t), \quad (1.18) \]

where \( I_F \) is the fluorescence intensity, \( \Phi_F \) is the quantum yield, \( I_0 \) is the incident light intensity, and \( I_t \) is the transmitted light intensity. Beer-Lambert’s law can be used to relate fluorescence intensity to concentration using \( I_t = I_0 e^{-ebc} \). Therefore, equation 1.18 becomes

\[ I_F = \Phi_F (I_0 e^{-ebc}) = \Phi_F I_0 (1 - e^{-ebc}), \quad (1.19) \]

where \( \epsilon \) is the molar absorptivity, \( b \) is the path length of the sample cell in cm, and \( c \) is the concentration of the absorbing species in moles/liter. For dilute solutions, i.e., \( ebc \leq 0.01 \), equation 1.19 can be rewritten as approximately

\[ I_F = 2.303 \Phi_F I_0 \epsilon bc \quad (1.20) \]

Hence, the fluorescence intensity is proportional to the quantum yield, incident radiation, and absorbance of the sample. As a result, at low sample concentrations, a linear plot is obtained for fluorescence intensity versus concentration. Deviations from linearity often occur at high sample concentrations due to the inner filter effect. Therefore, the sample should be diluted prior to analysis to reduce the problems associated with the inner filter effect which may cause an attenuation in the measured fluorescence.

**Fluorescence Quenching**

Fluorescence quenching is the process that refers to a decrease in the fluorescence intensity of a fluorophore. It is due to interaction between the fluorophore and a quencher
molecule. There are many types of quenching processes including dynamic quenching, static quenching, energy transfer, and excited state reactions. Quenching studies are used to gather information on the localization of fluorophores in solution.

Dynamic quenching, also known as collisional quenching, is a result of collisional encounters between the quencher and fluorophore. The Stern-Volmer equation [67] describes dynamic quenching as follows

\[ \frac{F}{F_0} = k_Q \tau_0 [Q] + 1 = k_{sv}[Q] + 1, \quad (1.21) \]

where \( F_0 \) and \( F \) are the fluorescence intensities in the absence and presence of the quencher, \( Q \), respectively; \( k_Q \) is the bimolecular quenching constant; \( \tau_0 \) is the lifetime of the fluorophore in the absence of quencher; \([Q]\) is the quencher concentration, and \( k_{sv} \) is the Stern-Volmer quenching constant. Since \( F/F_0 \) is proportional to \( [Q] \), a plot of \( F/F_0 \) versus \([Q]\) normally results in a straight line. Equation 1.20 can be rewritten in terms of the lifetimes of the fluorophore to give

\[ \frac{\tau_0}{\tau} = k_Q \tau_0 [Q] + 1, \quad (1.22) \]

where \( \tau_0 \) is the lifetime in the absence of quencher and \( \tau \) is the lifetime in the presence of quencher. One of the characteristics of dynamic quenching is a decrease in the lifetime given by the following equation

\[ \frac{F_0}{F} = \frac{\tau_0}{\tau} \quad (1.23) \]

Consequently, a decrease in fluorescence intensity is equivalent to a decrease in lifetime.

Static quenching is due to formation of a nonfluorescent complex between the fluorophore and the quencher in the ground state. The following equations describe the static quenching process
\[ F + Q \rightarrow FQ \]  \hspace{1cm} (1.24)

\[ K_s = \frac{[FQ]}{[F][Q]}, \]  \hspace{1cm} (1.25)

where \( K_s \) is the association constant for complex formation, \([FQ]\) is the concentration of the complex, \([F]\) is the fluorophore concentration and \([Q]\) is the quencher concentration.

The total concentration of the fluorophore, \( F_0 \), may be written as

\[ [F]_0 = [F] + [FQ] \]  \hspace{1cm} (1.26)

The fluorescence intensities, \( F_0 \) and \( F \), can replace the fluorophore concentrations and upon rearrangement, equation 1.25 becomes

\[ \frac{F_0}{F} = k_s [Q] + 1 \]  \hspace{1cm} (1.27)

Equation 1.26 is the Stern-Volmer equation for static quenching. Fluorescence lifetime measurements are used to distinguish between static and dynamic quenching. The only observed fluorescence in static quenching comes from the uncomplexed fluorophore. Therefore, for static quenching, \( \tau_0/\tau = 1 \) whereas \( \tau_d/\tau = F_0/F \) for dynamic quenching.

Absorption spectra, temperature, and viscosity can also be used to distinguish between static and dynamic quenching.

Energy transfer is the process that refers to the transfer of excitation energy from the donor to a nearby molecule or acceptor. As a result of this process, the fluorescence intensity of the donor molecule is quenched because it has transferred its energy to the acceptor. In addition, energy transfer occurs during the lifetime of the donor molecule and may cause the acceptor to fluoresce. The following equations express the energy transfer process

\[ D + hv \longrightarrow D^* \]  \hspace{1cm} (1.28)
\[ D^* + A \rightarrow D + A^*, \quad (1.29) \]

where \( * \) denotes excited electron states and \( D \) and \( A \) are donor and acceptor molecules, respectively. There are two types of energy transfer: (1) resonance excitation transfer and (2) exchange mechanism.

Resonance excitation transfer results from dipole-dipole interactions and is a nonradiative process. The excited donor molecule can be considered as an electric dipole that generates an electrical field. The donor and acceptor molecules are not in contact with one another. However, the rate of energy transfer depends on the distance between the molecule, the orientation of the dipoles of the molecules, and the degree of overlap of the emission wavelength of the donor molecule with the absorption wavelength of the acceptor molecule. This rate is given by

\[ k_{FT} = \frac{1}{\tau_D} \left( \frac{R_0}{R} \right)^6, \quad (1.30) \]

where \( k_{FT} \) is the rate constant for resonance energy transfer, \( \tau_D \) is the lifetime of the excited donor, \( R \) is the mean distance between the centers of the donor and acceptor dipoles, \( R_0 \), the Föster radius, is a constant for a given donor-acceptor pair [68].

In contrast to resonance excitation transfer, exchange mechanism is a radiative process whereby the donor and acceptor electron clouds are in direct contact. There are collisions between the donor and acceptor molecules and the mechanism is diffusion-controlled. Thus, the rate is also dependent on the viscosity.

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Excited State Reactions

Excited state reactions are a result of excited state molecules interacting with other molecules to form complexes that are stable only in the excited states. These reactions cause shifts in the fluorescence spectrum as well as intensity and lifetime changes. Proton transfer is an example of an excited state reaction. These reactions may compete with radiative deactivation processes of excited state molecules. However, if the excited-state proton transfer reaction is too slow to compete with fluorescence for deactivation, then the fluorescence intensity of the emitting base or acid will depend on the absorbance at the excitation wavelength. The excitation wavelength will then depend on the pKₐ of the base or acid in the ground state.

On the other hand, if the excited-state proton transfer reaction competes with fluorescence, then the quantum yield will have a different pH dependence. The fluorescence intensity caused by this pH dependence will occur in a pH region different than the pH region in which the fluorescence intensity depends on the absorbing species of interest.

Aromatic acids and bases are known to exhibit different acid-base equilibrium in the excited electronic energy states relative to the ground state [69,70]. It is generally acknowledged that electron transfer to or from the heteroatom within an aromatic molecule is enhanced when the molecule is in the S₁ or T₁ state. This phenomenon applies to molecules with both electron donating and withdrawing substituents [69a]. As a result,
the acid-base equilibrium constant of the excited state (pKₐ(calc)) is no longer the same as that of the ground state (pKₐ).

The excited state proton transfer reactions normally consist of two types: intermolecular and intra-molecular. Both inter- and intra-molecular proton transfer require the presence of a hydrogen bond. In addition, these reactions involve protonation or deprotonation of a molecule in the excited state.

Phenol is an example of a molecule that is more acidic in the excited state than in the ground state. Therefore, it undergoes excited state intermolecular proton transfer (ESIₐ,PT). This is evidenced by the fact that fluorescence emission can be observed from the phenolate anion at a very low pH, although the undissociated phenol is the only absorbing species in the ground state.

Excited state intra-molecular proton transfer (ESIᵢ,PT) involves proton transfer between different moieties of the same molecule. The ESIᵢ,PT molecules often show dual emission: a normal emission at lower wavelengths and a Stokes-shifted tautomer emission. These emissions are dependent on solvent polarity, pH, hydrogen-bonding character of solvent, and structure of the molecules in both the ground and excited states [71,72]. Roberts, et al. [73] recently examined the effect of organized media on the ESIᵢ,PT reaction of 10-hydroxybenzo[h]quinoline. They studied this molecule by monitoring the large Stokes-shifted tautomer emission using fluorescence spectroscopy. Both inter- and intra-molecular proton transfer may be used to provide information on the hydrogen-bonding character of various media. In addition, the different conformations of a molecule
in the ground and excited states can be understood. These excited-state proton transfer reactions will also be discussed in Chapter 3.

**Fluorescence Instrumentation**

A schematic diagram of a conventional fluorescence spectrophotometer is given in Figure 1.8. The basic components of a fluorescence instrument consist of an excitation source, monochromators or filters, a sample compartment, a detector, and a recorder. In the following section, a brief description of the different components of a fluorescence spectrophotometer is given.

The most common excitation sources used in a fluorometer are mercury and xenon arc lamps. Mercury lamps are relatively inexpensive with long lifetimes and are useful when high intensities are needed. However, these lamps provide line emission and are only appropriate if the mercury lines are at wavelengths suitable for excitation of the molecule. Xenon arc lamps are commonly used in commercial fluorometers due to their continuous light output in the ultraviolet/visible wavelength regions. These lamps are less intense than mercury lamps but they allow a wide range of selectivity in excitation wavelength choices. In addition, xenon lamps have lifetimes of approximately 2000 hours and exhibit decreases in intensity over time.

Monochromators are devices used to select the appropriate excitation and emission wavelengths. These devices consist of diffraction gratings or quartz prisms. A quartz prism monochromator is very expensive and therefore, most modern instruments employ a diffraction grating monochromator. Monochromators are chosen in order to reduce the
amount of scattered light. Monochromators also consist of entrance and exit slits used to control resolution and the amount of light reaching the sample. The settings for the slits are usually optimized because the amount of intensity that passes through the monochromator is proportional to the square of the slit width. Therefore, large slit widths produce relatively high intensities with loss in spectral resolution.

The sample compartment is located so that the fluorescence from the sample is measured at right angles to the direction of the exciting light. The right angle geometry aids in minimizing the interference from stray exciting light. Therefore, the compartment is located between the excitation and emission monochromators. Quartz cuvettes (1 cm²) are typically used for analyte solutions. Fluorescence cuvettes are polished on all four sides due to the right angle sample emission.

The most commonly used detector in fluorescence instruments is the photomultiplier tube (PMT). The PMT is a device that produces a current proportional to the light intensity and its response is also dependent on the wavelength of light being detected. Amplification of the PMT signal occurs through collision of the emitted photons with the photocathode, which produces electrons that collide with the PMT dynodes. Many PMTs have optimal and relatively flat responses between 300-600 nm. For wavelengths greater than 600 nm, special red sensitive PMTs are available to provide better sensitivity. The resulting amplified signal is digitized and transported to a computer for data acquisition and analysis.
Excitation Source

Excitation Monochromator

Sample Holder

Emission Monochromator

Detector

Data Station or PC

Figure 1.8 A schematic diagram of a fluorescence spectrophotometer

Scope of This Work

This dissertation addresses the use of fluorescence spectroscopy to study cyclodextrin complexes with various guest molecules. Chapter 2 focuses on the extraction of volatile polycyclic aromatic hydrocarbons from air using solid cyclodextrin. The spectral changes observed upon addition of various alcohols will be discussed along with quantitative measurements for the air sampling system. In Chapter 3, the effect of cyclodextrins on excited state transfer proton reactions of various carboxylic acid compounds is examined. Several carboxylic acid compounds have been analyzed using
spectroscopic techniques. Chapter 4 highlights the Cyclodextrin Host Isolation Matrix (CHIM) method for various guest molecules. This method is used to gather spectroscopic information from the excited state. Chapter 5 provides an overall summary and future studies.

References


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Chapter 2. Extraction of Volatile PAHs from Air By Use of Solid Cyclodextrin

There has been a continuing interest in ambient air sampling and detection of polycyclic aromatic hydrocarbons (PAHs) [1]. The identification and quantification of PAHs are often complex, time-consuming, and often inaccurate. Hence, there is a need for an air sampling method which minimizes these problems.

PAHs are an important class of environmental pollutants found in urban atmospheres. Many of these compounds are on the priority pollutant list of the Environmental Protection Agency (EPA) [2,3]. These compounds pose a potential threat to humans due to their tendency to cause cancer and/or other long-term health related problems. Trace amounts of these compounds are found in the environment as a result of gasoline and diesel consumption, power plant emissions, crude oil refining byproducts, as well as several natural resources [4-10]. For example, elevated levels of PAHs are likely to occur in close proximity to natural fires such as forest fires. Furthermore, complex mixtures of these compounds are found in petroleum products.

Astronomers are also concerned about the role of PAH compounds in the chemistry of the interstellar medium [11-14]. PAH molecules have been identified in meteorites [15] and interplanetary dust particles [16] by mass spectrometry. Zhang and his colleagues [17] reported the emission spectra of gaseous PAHs in the far-infrared region. They suggested that the spectra may be useful in the assignment of unidentified spectral features from astronomical objects.
The potential hazards of PAHs increase significantly when they react with other common air pollutants in the environment. For example, two pollutants that may not have any known mutagenic or carcinogenic effect can become health hazards when they react to form a new compound. Coal furnace stack gas may contain over 1000 µg/m^3 of PAH compounds and cigarette smoke almost 100 µg/m^3 [18]. The analysis of these compounds in the atmosphere is an important task because of the potential health hazards.

Many researchers have studied the effect of air pollution on human health [19-21]. Epidemiological studies have indicated that incidences of lung cancer are higher in urban than in rural areas due to the greater amount of mutagenic and/or carcinogenic compounds found in particulate matter in urban air [22]. Other researchers have also shown a potential relationship between the general location of industrial pollutant sources and geographical distribution of certain types of cancer including gastric, bladder and liver, and lung cancers [23-26]. Specifically, Blot et al. [27] found that incidences of skin, nasal, and lung cancer were higher among workers in oil refining areas. PAHs are associated with many of these industrial areas.

Chuang, et al. [28] used a quiet sampler to determine the pollutant levels of indoor and outdoor air. They also measured the concentrations of PAHs, PAH derivatives, and nicotine in air in selected residences in Columbus, Ohio. The residences were chosen based on electric/gas heating system, electric/gas cooking appliances, and the absence/presence of environmental tobacco smoke. They found that naphthalene was the most abundant PAH indoors. In addition, the average concentrations of most of the PAHs
were higher indoors than outdoors. Furthermore, the findings indicated that homes with gas heating systems had higher indoor pollutant levels than homes with electric heating systems.

Numerous mechanisms exist for the destruction and chemical alteration of PAH compounds. Atmospheric PAHs undergo oxidation and photochemical reactions under high volume air sampling conditions [29]. For example, destruction and chemical alteration of PAHs are possible in the presence of oxidants such as HO\(^{+}\), \(O_3\), \(NO_2\), \(N_2O_5\), and \(HNO_3\) [18]. Direct photolysis of PAHs is also possible. However, PAHs adsorbed to particles are much more resistant to reactions [18]. Due to their reactivities and volatilities, losses of PAHs during sampling are almost unavoidable. In addition, the concentrations of PAHs in air are low and many of these compounds are unstable and volatile. These phenomena add significant error to the accurate detection of PAHs by air sampling. For this reason, most air sampling methods tend to focus on sampling the less volatile PAHs. Therefore, an air sampling method that provides a more accurate and reliable detection scheme for quantification of PAHs in air is needed.

High-volume air samplers are widely used in the sampling of semivolatile organic compounds [30-33]. These samplers consist of a filter to collect particles, a sorbent to collect vapor-phase material, and a blower motor to move the air through the filter and sorbent. The high-volume samplers allow high flow rates which permit large volumes of air to be collected in a reasonable amount of time. Diffusion denuders, on the other hand, are low flow-rate samplers used in the collection of volatile inorganic compounds [34-39].
The low flow limits the use of denuders for the collection of semivolatile compounds. Many semivolatile compounds such as PAHs are relatively nonpolar, and therefore these compounds are difficult to collect because long sampling times are needed.

Several studies have reported an examination of different sampling methods for the analysis of PAHs in air [40-43]. Glass fiber filters are often used since they allow high flow rates [42]. However, the sample obtained by the filter is not always representative of the total amount of PAH present in the air because some of these compounds, due to their high vapor pressures, will pass through the filter. Other sampling media include polyurethane foam, Chromosorb polymers, and carbonaceous adsorbents [40]. In order to improve the collection efficiency of the filters, Thrane and Mikalsen [40] examined glass fiber filters in combination with plugs of polyurethane foam. An increase in the collection efficiency of these filters was observed with increased air pollution levels. It was observed that polyurethane foam removes the PAHs from the air through a trapping mechanism [42]. It should be noted that many problems associated with the collection of PAH air samples have been reported [44]. In particular, significant losses due to the volatility of PAHs during the collection time is often reported. Many low volatile PAHs also undergo significant volatility losses during long collection times on filters. Losses of PAHs during sampling due to their volatility have been demonstrated and reported in the literature for a number of years. Therefore, various approaches have been tried in order to improve sampling methods for PAH compounds. Furthermore, the addition of cyclodextrins (CDs) on the glass fiber filters can be efficient in minimizing the loss of PAHs.
Smolková et al. [45-47] reported on the use of CDs in a gas-solid chromatographic system. They verified the existence of the formation of inclusion compounds with sorbates in the gaseous state. However, their measurements were performed at temperatures from 50 to 80°C. More recently, Armstrong et al. [48,49] used CDs as stationary phases for the gas-solid chromatographic separation of light hydrocarbons and inorganic gases at ambient to elevated temperatures. They showed that these cyclodextrin stationary phases provided a practical and efficient means for separating a wide variety of gases. Although Smolková provided the evidence for the existence of the formation of inclusion complexes of CDs with substances present in the gaseous phase, this study examines gas:solid complexation of solid cyclodextrin and gaseous analytes at room temperature.

**Part 1. Solid Cyclodextrin and PAHs**

Cyclodextrins (CDs) are widely used for many purposes and in particular as organized media for many types of chemistries [50, 51]. As mentioned in Chapter 1, the CDs are cyclic oligosaccharides formed by an \( \alpha-(1,4) \) linkage of glucopyranose units. The most commonly used CDs are \( \alpha-, \beta-, \) and \( \gamma- \) cyclodextrins with six, seven, and eight glucopyranose units and approximate inner cavity diameters of 5.7, 7.8, and 9.5Å, respectively. Cyclodextrins incorporate guest molecules based on size and hydrophobicity [51]. It is well established that CDs will size selectively complex with polycyclic aromatic hydrocarbons (PAHs) through host:guest interactions [52]. In aqueous solutions, various inclusion complexes are formed with PAHs [53]. In addition, solid CDs have been used to enhance the stability of volatile pharmaceuticals [51]. However, most of these
complexes were initially prepared in aqueous solutions. The focus of this work is to use solid CD to reduce the volatility of PAHs. Fluorescence and absorbance measurements are used to examine the gas-solid interaction of the PAHs with the solid CD.

**Experimental**

Apparatus. The design of the laboratory air sampling system is shown in Figure 2.1. In this system, a gas cylinder of compressed air is attached to the air inlet. Solid cyclodextrin was spread over a glass fiber filter through which air flows. The PAHs examined in the initial phases of this study were sprinkled directly onto the filter with solid cyclodextrin which allowed solid-solid contact of PAH and cyclodextrin. Therefore, later studies used naphthalene in a cup which was placed onto a raised platform over the filter with cyclodextrin. This latter approach prevented solid-solid contact of PAH and cyclodextrin. In order to capture vapor which is not extracted by the filters, the air passes through two additional one liter liquid traps of organic solvent. Fused silica frits were used to create small bubbles in order to increase the interacting surface area of the air with the solvent in the traps. Flow rates between 200-300 ml/min were used.

Materials. Cyclodextrins were obtained from American Maize Products (Hammond, IN) and were used as received. Naphthalene (99%), phenanthrene (98%), acenaphthene (99%), and acenaphthylene (75%) were obtained from Aldrich Chemical Company, (Milwaukee, WI) and were used as received. Glass fiber filters were obtained from Sierra Instruments, (Carmel Valley, CA). Cyclohexane (HPLC Grade) was used without further purification.
Method. The glass fiber filter was cut and weighed initially and an amount of β-cyclodextrin ranging from 200 - 1200 mg was uniformly spread onto the filter. A known amount of PAH was placed on the filter as described above. The first and second traps were filled with 400 ml and 250 ml of cyclohexane, respectively. The vaporous PAH is carried with the flowing air and passes through the solid cyclodextrin which can extract the PAH from the air stream through formation of an inclusion complex. Naphthalene, the most volatile of the PAHs examined, was sampled for ten hours. After sampling, the filter content was placed in cyclohexane solution for extraction of the included PAH from the

![HIGH VOLUME AIR SAMPLING SYSTEM](image)

**Figure 2.1 Laboratory designed air sampling apparatus**
solid cyclodextrin. Since cyclodextrins are not soluble in nonpolar organic solvents, this produces a cyclodextrin precipitate with the PAH extracted into the organic solvent. The solvent from each trap was then removed in vacuo (Büchi RE 111 rotavapor). Afterwards, a solution was prepared that was 10% of its initial volume. An appropriate volume of solution was pipetted into a 10 ml flask and the flask was filled to the mark with cyclohexane before measurement. All measurements were performed at room temperature. Fluorescence measurements were acquired using a Spex Model F2T211 spectrofluorometer equipped with a thermostated cell housing and a thermoelectrically cooled Hamamatsu R928 photomultiplier tube. Excitation and emission bandwidths of 2.0 and 8.0 nm were used, respectively. Excitation wavelengths of 275, 303, 252, and 303 nm were used for naphthalene, acenaphthene, phenanthrene, and acenaphthylene, respectively. Absorption measurements were obtained with a Shimadzu UV-3101 PC UV-vis-NIR scanning spectrophotometer using a 1-cm path length cell.

Results and Discussion

The formation of a solid CD:PAH complex is not always straightforward. It should be noted that water molecules play an active role in the driving force of CD complex formation with hydrophobic molecules in aqueous solution [51]. The water molecules included inside the CD cavity and those water molecules surrounding the PAH molecule are not energetically favored in the free CD. Therefore, the CD:PAH complex formed in aqueous solutions releases these water molecules which in turn stabilizes the
inclusion complex. It should be noted that water molecules are also released in solid CD. Thus, solid CD should form complexes with gas phase PAHs.

Figure 2.2 displays the relative fluorescence emission intensities of the filter contents for several representative PAHs in the absence and presence of solid β-CD. It is interesting to observe the increase in the fluorescence emission intensities with the addition of solid β-CD on the filter which confirms a gas-solid interaction between the

Figure 2.2 Fluorescence intensities of several PAHs: (□) without β-CD and (■) with β-CD

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PAH and the solid CD. As expected, absorbance measurements also support the gas-solid interaction. This gas-solid interaction led to the focus on one compound in order to examine it more thoroughly. Naphthalene was chosen as a model compound because of its high vapor pressure. This compound is very volatile in comparison to other solid PAHs. As a result, it is easier to introduce gas phase naphthalene into the air stream than other PAHs. Naphthalene is allowed to interact with the solid CD and the formation of a CD:Naphthalene complex should be observed.

Figure 2.3 Influence of increasing amounts of β-CD on fluorescence intensity of naphthalene (filter): (a) 200, (b) 600, and (c) 1000 mg of β-CD

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Figure 2.3 shows the fluorescence emission intensity of entrapped naphthalene from the air as the amount of solid CD increases. The enhancement in the fluorescence emission intensity as the amount of solid CD increases supports the conclusion of gas-solid complexation of naphthalene with solid β-CD. The increase in the amount of solid CD on the filter correlates qualitatively with an increase of the CD concentrations in aqueous solutions. Thus, higher concentrations yield more PAH/cyclodextrin complex. Using the same flow rate and time, larger amounts of solid CD on the filter should yield more naphthalene/CD complex. Therefore, an increase in the fluorescence emission intensity is expected.

As a complementary measurement, the liquid trap solutions were analyzed by use of fluorescence spectroscopy. Examination of the fluorescence emission spectrum in Figure 2.4 reveals a decrease in the fluorescence intensities with an increase in the amount

![Graph](image)

**Figure 2.4** Influence of decreasing amounts of β-CD on fluorescence intensity of naphthalene (trap1):(a) 1200, (b) 800, and (c) 200 mg of β-CD
of CD. This observation suggests that smaller amounts of the PAH are extracted by the organic solvent in the trap which indicates that larger amounts of the PAH interact with CD. This is in good agreement with the data observed from the filter extracts (Figure 2.3).

Figure 2.5 shows that the fluorescence emission intensity of the solid extract increases linearly as the amount of cyclodextrin on the filter increases. Figure 2.6 shows that the fluorescence intensity of the PAH in the organic solvent in the trap decreases linearly as the amount of cyclodextrin increases. These complementary data allow the suggestion that a complexation between gaseous naphthalene and solid cyclodextrin occurs.

![Figure 2.5 Filter linear plot of fluorescence intensity versus amount of β-CD](image-url)
In an attempt to examine whether the β-CD:PAH complex is an association or an inclusion complex, a comparison study was performed with α-cyclodextrin. In the presence of α-CD, weak complexation is expected because naphthalene is too bulky to fit into the 5.0 Å cavity of α-cyclodextrin. In addition, the surface area of α-CD which is proportional to the number of glucose units for the same weight of α and β-CD is similar to that of β-CD. Therefore, if the interaction is merely association, the fluorescence intensities should be close to those measured with β-CD.
Figure 2.7 shows that there is a marked decrease in the fluorescence intensities when using α-cyclodextrin relative to β-CD. This significant decrease in the fluorescence intensity of the extracted naphthalene supports the suggestion that the cavity of the α-cyclodextrin is too small to fully include the naphthalene molecule. Therefore, solid β-cyclodextrin interacts with gaseous naphthalene by formation of an inclusion complex.

Figure 2.7 Linear plot for the naphthalene complex with: (a) β-CD and (b) α-CD

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Part II. Effect of Various Alcohols and Quantitative Studies on Gas-Solid Complexation Reactions of PAHs

Several investigations have reported the influence of alcohols as third components in cyclodextrin complexes. Ueno et al. [54] reported that the addition of cyclohexanol and γ-cyclodextrin to α-(naphthyloxy) acetic acid showed a significant fluorescence intensity enhancement compared to the addition of γ-cyclodextrin alone. This enhancement was attributed to the formation of a ternary 1:1:1 γ-cyclodextrin:α-(naphthyloxy) acetic acid:cyclohexanol complex.

The effect of alcohols on cyclodextrin complexes has also been studied extensively in our laboratory using steady-state fluorescence and fluorescence lifetime measurements [55-58]. Changes in the fluorescence properties of pyrene in the presence of alcohols upon cyclodextrin complexation were demonstrated in these studies. The fluorescence intensity and lifetime are significantly enhanced for CD:pyrene complexes in the presence of specific alcohols such as tert-butanol [56].

Muñoz de la Peña et al. [59] examined the addition of linear, branched, or cyclic alcohols to the β-cyclodextrin:pyrene complex. The results showed a stronger association of pyrene with β-cyclodextrin in the presence of each alcohol than in the absence of alcohol. In addition, the formation constants increased by one to three orders of magnitude in the alcohol solutions.

Schuette et al. [60] characterized the cyclodextrin:pyrene complex interaction with chiral alcohols and diols by fluorescence spectroscopy. They observed a stronger hydrophobicity for complexes capped by chiral alcohols relative to complexes formed with
a similar achiral modifier, suggesting the importance of the alcohol chiral center. Moreover, they found that the diols induced a more hydrophobic environment than their alcohol equivalents with the β-CD:pyrene complex.

Patonay and colleagues [61] studied the cyclodextrin complexation process in the presence of different aliphatic alcohols using pyrene as the fluorescence probe. In this study, it was found that complex formation as well as the hydrophobicity of the cyclodextrin interior was significantly influenced by the introduction of alcohols such as methanol, ethanol, propanol, iso-propanol, and tert-butanol.

While most of the work has focused on the fluorescence probe, pyrene, Nelson and Warner [62] also examined naphthalene in the presence of alcohols and iodide. Their results showed that CD-complexed naphthalene in the presence of benzyl alcohol is virtually shielded from iodide quenching. This quenching elimination is important because it allows the CD inclusion complexes to selectively protect the appropriate sized guest molecules.

Another study by Schuette and Warner [63] showed the importance of the addition of an alcohol. However, in this study, the interaction of the CD:perylene complex with 1-pentanol was examined. Again, a significant enhancement in the spectral characteristics of the probe molecule was seen upon addition of the alcohol.

It has been well established that molecules can be included into the CD cavity based on their size, geometry, and hydrophobicity. In addition, it has been shown that third components significantly modify CD complexation by enhancing the formation
constant and slowing down the in/out rate of the complex [62]. As a result, ternary complex stability may be governed by the size and polarity of the alcohol. For example, Muñoz de la Peña et al. [59] were able to show that in the case of linear alcohols, the formation constant increased with an increase in the alkyl chain length. Also, the branched alcohols produced higher formation constants than the linear alcohols. It has been suggested that alcohols primarily function as cavity extenders or space regulators [64, 65]. Therefore, the stability of the ternary complex is related to the proper sizing of the geometry and/or volume of the alcohol as well as the ability of the alcohol to occupy the residual void space in the complex.

In addition, a number of researchers have incorporated alcohol guest molecules into studies which assess the importance of certain CD features to host/guest binding. Specifically, Hingerty and Saenger [66] employed methanol as a substrate to perturb the internal binding of α-CD. Methanol was able to relax the distorted α-CD. Interactions of the methanol:water structure with α-CD are considered to result from Van der Waals forces. However, these forces are normally weak in β-CD:alcohol complexes. In other studies using alcohols, the stabilizing forces in the formation of CD complexes are frequently attributed to hydrophobic interactions [67-70].

Figure 2.8 shows two possible configurations proposed for the β-CD:pyrene:cyclopentanol complex [71]. Both of these configurations assume that the hydrophobic portion of the alcohol is located inside the cavity and that the hydroxyl group
of the alcohol is oriented toward the exterior of the cavity. This configuration increases the chance of hydrogen bond formation between the CD and the alcohol. In the type I

\begin{center}
\includegraphics{type_i_config}
\end{center}

\textit{Type I Configuration}

\begin{center}
\includegraphics{type_ii_config}
\end{center}

\textit{Type II Configuration}

\textbf{Figure 2.8 Two proposed configurations for $\beta$-CD:pyrene:cyclopentanol complex}

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arrangement, pyrene is positioned axially inside the β-cyclodextrins, and the alcohol caps the open edge of each cyclodextrin. Thus, the alcohol acts as a space filler in the remaining void volume not occupied by the complexed pyrene. In the type II configuration, the pyrene molecule is tilted in order to accommodate the alcohol moiety. In this aspect, the alcohol acts as a space regulator such that its coinclusion is regulated from shifting or rotating the included pyrene molecule. The second configuration allows a stronger association between the alcohol and the protons located in the interior of the β-CD.

The role of alcohols in cyclodextrin systems are generally categorized as space-regulatory, competitive, organizational, or protective. Their role depends upon the nature of the guest molecule included in the CD and the extent of its interaction with the CD structure. Optimum interaction with the CD cavity is typically governed by guest geometry. Therefore, alcohols are chosen based on their polarity and spatial distribution. The polarity is evaluated by increasing alkyl character as seen in linear alcohols and the spatial distribution is examined by branching which appears in cyclic alcohols.

Previous investigations have focused mainly on incorporating longer or more bulky alcohols in CD:guest solutions since no major influences have been seen in the presence of methanol. Coinclusion of the alcohol usually enhances formation of the complex which gives rise to larger association constants. The studies to date have involved the effect of alcohols in aqueous cyclodextrin systems with the popular fluorescence probe, pyrene.
**Experimental**

Instrumentation. Fluorescence measurements were acquired using a Spex Model F2T211 spectrofluorometer equipped with a thermostated cell housing and a water cooled Hamamatsu R928 photomultiplier tube. Excitation and emission bandwidths of 2.0 and 8.0 nm were used, respectively. An excitation wavelength of 275 nm was used for naphthalene.

Materials. Cyclodextrins were obtained from American Maize Products (Hammond, IN) and were used as received. Isopropanol, n-propanol, n-butanol, and tert-butanol were of HPLC and/or spectroscopic grade and were used without further purification.

Method. The cyclodextrins were placed in a vial and the various alcohols were added to the vial. The vial was placed in a heated dessicator for drying. The heated dessicator was set at a temperature just below the boiling point of the respective alcohol. After the cyclodextrins were dried, the vial was removed from the dessicator and capped. Varying amounts of cyclodextrin ranging from 200 - 1200 mg were taken from the vial and the procedure described in the previous section was followed. Modifications to the procedure included sampling naphthalene for five hours instead of ten hours and the solvent from trap 1 was removed in vacuo and reduced to 25% of its initial volume.

**Results and Discussion**

These examples illustrate the significance of the addition of the alcohols as third components in the aqueous CD complexes. These alcohols are potentially useful for the
improved detection of environmental contaminants such as PAHs. Therefore, in this section, the effect of various alcohols on the gas:solid complexation reactions of PAHs as well as the quantitative studies of these reactions are investigated. Fluorescence measurements were performed to qualitatively assess the effect of various alcohols on the naphthalene:β-CD complex.

Figure 2.9 shows the fluorescence emission intensity of naphthalene and 200 mg β-CD from the filter with (a) no alcohol, (b) n-butanol (NBA), (c) isopropanol (IPA), (d) n-propanol (NPA), and (e) tert-butanol (TBA). It is evident that the fluorescence emission intensity is enhanced for β-CD pre-treated with various alcohols. However, the graph illustrates that TBA shows the greatest enhancement. This enhancement leads to the suggestion that the tert-butanol displaces the water molecules from the CD cavity, possibly forming hydrogen bonds with the CD hydroxyl groups. This is in good agreement with results obtained in aqueous solutions when TBA is used as a comodifier in the β-CD:pyrene complex. In that study, various alcohols including methanol, ethanol, 1-propanol, 2-propanol, and tert-butanol were examined [61]. Although all the alcohols exhibited similar effects, the largest relative change in peak intensities were noted for the cyclodextrin:pyrene complex in the presence of tert-butanol. The data here show the same trend where the fluorescence intensities are enhanced in the presence of the different alcohols, but tert-butanol shows the most significant enhancement. According to the peak ratios in the investigation by Patonay et al., the hydrophobicity in the cyclodextrin environment was comparable to that of cyclohexane when tert-butanol was present in the
Figure 2.9 Fluorescence emission intensity of naphthalene and 200 mg β-CD from the filter with (a) no alcohol, (b) n-butanol (NBA), (c) isopropanol (IPA), (d) n-propanol (NPA), and (e) tert-butanol (TBA)
The proposed structure for this ternary complex, \( \beta \)-cyclodextrin:naphthalene:tert-butanol, is shown in Figure 2.10. It is possible for the hydroxyl groups of the alcohol to bind to the cyclodextrin hydroxyl groups through hydrogen bonding. Therefore, the cyclodextrin interior cavity is even more hydrophobic. The \( \beta \)-CD has seven of each type of hydroxyl group at the cavity edges and all of these are potential hydrogen bonding sites. A similar structure was proposed for the ternary \( \gamma \)-cyclodextrin:pyrene:tert-butanol system [61]. The primary and secondary hydroxyl groups of the cyclodextrin were shown to be crucial in the formation of the ternary complex in the presence of tert-butanol.

![Figure 2.10 Proposed structure for the \( \beta \)-CD:naphthalene:tert-butanol complex](image)

The data obtained for each alcohol with varying amounts of CD from the filter and trap are shown in Figures 2.11 and 2.12, respectively. The amount of CD is varied from 200 - 1200 mg. Figure 2.11 shows that for each amount of CD present on the filter, the relative intensity is enhanced in the presence of each alcohol. Again, the most significant enhancement is upon addition of tert-butanol. The tert-butanol is the bulkiest of the
Figure 2.11 Plot of naphthalene:CD complex from filter with (○) no alcohol, (●) NBA, (△) IPA, (■) NPA, and (□) TBA.

Figure 2.12 Plot of naphthalene:CD complex from trap with (○) no alcohol, (●) NBA, (△) IPA, (■) NPA, and (□) TBA.
alcohols examined and it is again assumed that it participates as a third component. The bulkiness of the tert-butanol group allows it to participate in hydrogen bonding with the hydroxyl groups present on the cyclodextrin resulting in the higher emission intensities. As a matter of fact, as the amount of cyclodextrin on the filter is increased, the emission intensities between cyclodextrin alone and tert-butanol increase as well. It is obvious that the other linear alcohols such as n-butanol and n-propanol do not allow the same degree of protection. However, it does appear that the addition of each alcohol changes the microenvironment of the cyclodextrin cavity but to lesser degrees. These results are similar to those obtained for the aqueous β-CD:pyrene complex whereby the tert-butanol group showed the most significant impact on the system. Furthermore, Figure 2.11 illustrates that there is less error associated with the smaller amounts of CD (200 - 600 milligrams) present on the filter when compared to the larger amounts (1000 and 1200 milligrams). It is also interesting to note that there exists minimal error when using cyclodextrin alone on the filter. The possible errors that may occur upon addition of the alcohols include not fully saturating the cyclodextrin or over drying.

It is expected that the results from the trap would be in direct contrast to those obtained from the filter. In other words, since the fluorescence intensities increased as the amount of CD was increased on the filter in the presence of each alcohol, it is assumed that the fluorescence intensities would decrease for the trap as the amount of CD is increased. Figure 2.12 illustrates this particular point. The general trend does depict that the fluorescence intensities do decrease as the amount of CD is increased. Therefore, the
results obtained are expected. However, the errors observed with the trap solution suggest that there may be losses of the vaporous PAH upon traveling from the filter to the trap.

The quantitative data obtained for the air sampling system are summarized in Tables 2.1 and 2.2. These data were gathered by using the calibration plot for naphthalene shown in Figure 2.13. The data shown in the tables reflect the amounts in milligrams of naphthalene recovered from the filter and trap. In the absence of alcohol, the amount of naphthalene recovered is substantially less than when an alcohol is present. In addition, in most cases, the amount of naphthalene recovered increases as the amount of cyclodextrin increases. Again, the presence of tert-butanol within the cyclodextrin generally causes a considerable amount of naphthalene to be recovered when compared to the other alcohols. This phenomenon is in agreement with the results reported earlier in the chapter. It is apparent that the presence of the various alcohols within the cyclodextrin is important in recovering ample amounts of naphthalene from the system.

Table 2.1 Quantitative Measurements for Air Sampling System. Amount recovered from filter

<table>
<thead>
<tr>
<th>mg β-CD</th>
<th>no alcohol</th>
<th>NBA</th>
<th>IPA</th>
<th>NPA</th>
<th>TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>0.0064 mg</td>
<td>0.0256 mg</td>
<td>0.0385 mg</td>
<td>0.0897 mg</td>
<td>0.1089 mg</td>
</tr>
<tr>
<td>400</td>
<td>0.0320 mg</td>
<td>0.1538 mg</td>
<td>0.1089 mg</td>
<td>0.2179 mg</td>
<td>0.2499 mg</td>
</tr>
<tr>
<td>600</td>
<td>0.0577 mg</td>
<td>0.1666 mg</td>
<td>0.1987 mg</td>
<td>0.1923 mg</td>
<td>0.3781 mg</td>
</tr>
<tr>
<td>1000</td>
<td>0.1025 mg</td>
<td>0.2627 mg</td>
<td>0.3845 mg</td>
<td>0.5832 mg</td>
<td>0.5063 mg</td>
</tr>
<tr>
<td>1200</td>
<td>0.2179 mg</td>
<td>0.2884 mg</td>
<td>0.2499 mg</td>
<td>0.5477 mg</td>
<td>0.6280 mg</td>
</tr>
</tbody>
</table>
Table 2.2 Quantitative Measurements for Air Sampling System. Amount recovered from trap

<table>
<thead>
<tr>
<th>mg β-CD</th>
<th>no alcohol</th>
<th>NBA</th>
<th>IPA</th>
<th>NPA</th>
<th>TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>0.2692 mg</td>
<td>0.8459 mg</td>
<td>0.7178 mg</td>
<td>0.3589 mg</td>
<td>0.4358 mg</td>
</tr>
<tr>
<td>400</td>
<td>0.2179 mg</td>
<td>0.8331 mg</td>
<td>0.6024 mg</td>
<td>0.3461 mg</td>
<td>0.5768 mg</td>
</tr>
<tr>
<td>600</td>
<td>0.3717 mg</td>
<td>0.3204 mg</td>
<td>0.7434 mg</td>
<td>0.5511 mg</td>
<td>0.4870 mg</td>
</tr>
<tr>
<td>1000</td>
<td>0.3204 mg</td>
<td>0.5768 mg</td>
<td>0.4614 mg</td>
<td>0.1923 mg</td>
<td>0.5896 mg</td>
</tr>
<tr>
<td>1200</td>
<td>0.4358 mg</td>
<td>0.6665 mg</td>
<td>0.5383 mg</td>
<td>0.2435 mg</td>
<td>0.3589 mg</td>
</tr>
</tbody>
</table>

The efficiency of the air sampling system was calculated using the following equation

\[
\text{Eff} = \left[ \frac{W_1}{W_1 + W_2} \right] \times 100 \tag{2.1}
\]

where \( W_1 \) and \( W_2 \) are the amounts in milligrams from the filter and trap, respectively. The summary of those results are shown in Table 2.3. It can be seen that the efficiencies for the air sampling system are higher in the presence of cyclodextrin and an alcohol compared to cyclodextrin alone. In most cases, 200 milligrams of β-CD was not efficient in capturing the vaporous PAH which is reasonable since this is the smallest amount of CD present on the filter. In contrast, 1200 milligrams of β-CD on the filter was quite efficient in extracting the gaseous PAH from the air stream in a few cases. This is logical since it was shown in Part I of this chapter that the more CD present on the filter, the more naphthalene vapor is expected to be captured. Therefore, the efficiencies will vary for the amounts of CD between 200 and 1200 milligrams. Furthermore, it is clear from Table 2.3 that tert-butanol was very efficient for each amount of cyclodextrin present on the filter. The efficiency percentage for tert-butanol and CD increases from 20% for 200 mg of CD.
Figure 2.13 Calibration plot for naphthalene
to approximately 65% for 1200 mg of CD. However, the efficiency percentage for 1200 mg of CD for n-butanol, isopropanol, and n-propanol decreases which is not expected. This could be due to the relative error associated with this particular amount of coverage on the filter. The results reported here show the importance of the addition of an alcohol modifier to the CD in that the air sampling system is more efficient in extracting vaporous PAH when an alcohol is present with the CD.

Table 2.3 Efficiency of the Air Sampling System

<table>
<thead>
<tr>
<th>mg β-CD</th>
<th>no alcohol</th>
<th>NBA</th>
<th>IPA</th>
<th>NPA</th>
<th>TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>2.3%</td>
<td>2.9%</td>
<td>5.1%</td>
<td>20.0%</td>
<td>20.0%</td>
</tr>
<tr>
<td>400</td>
<td>12.8%</td>
<td>15.6%</td>
<td>15.3%</td>
<td>38.6%</td>
<td>30.2%</td>
</tr>
<tr>
<td>600</td>
<td>13.4%</td>
<td>34.2%</td>
<td>21.1%</td>
<td>25.9%</td>
<td>43.7%</td>
</tr>
<tr>
<td>1000</td>
<td>24.2%</td>
<td>31.3%</td>
<td>45.5%</td>
<td>75.2%</td>
<td>46.2%</td>
</tr>
<tr>
<td>1200</td>
<td>33.3%</td>
<td>30.2%</td>
<td>31.7%</td>
<td>69.1%</td>
<td>63.6%</td>
</tr>
</tbody>
</table>

Conclusion

The results reported herein suggest that gas-solid interaction of gaseous PAHs with solid cyclodextrin can be used to decrease the volatility of PAHs during air sampling. The enhanced fluorescence intensity of the extracted PAH due to the presence of increasing amounts of solid cyclodextrin on the glass fiber filter provides evidence for the formation of solid:gas complexes of β-CD:PAH. The complexation of cyclodextrins with volatile PAHs is significant and suggests a potential use for the improved detection of these volatile compounds in air sampling methods.

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In addition, the effect of various alcohols on the β-CD:Naphthalene complex was examined. It was shown that the addition of alcohols to the cyclodextrin enhanced the relative emission intensities for naphthalene. However, the tert-butanol showed the most significant changes in the β-CD:Naphthalene complex. The changes caused by the tert-butanol are thought to occur because of its bulkiness and ability to interact with the naphthalene forming a ternary complex. These results are in agreement with those obtained in aqueous media where it was shown that tert-butanol notably changed the microenvironment of the β-CD:pyrene complex. The tert-butanol was a third component in that ternary complex as well. Linear alcohols were also examined in aqueous media but they did not show the substantial fluorescence intensity increases as those found with tert-butanol. The linear alcohols may be able to penetrate deeper into the cavity preventing hydrogen bonding with the cyclodextrin hydroxyl groups. The orientation of the linear alcohols within the cyclodextrin cavity depends upon the void volume remaining after complexation with naphthalene and the geometrical considerations of the individual alcohols. Although there is no obvious trend with the linear alcohols, there still exists the increased emission intensities for all alcohols in the presence of cyclodextrin when compared to cyclodextrin alone.

The efficiency of the air sampling system was also calculated and it was found that the system is not very efficient when using cyclodextrin alone. The amount of naphthalene in milligrams recovered from the filter and trap appeared to increase as the amount of cyclodextrin on the filter increased. Also, the efficiency of the air sampling system was
increased when various alcohols were added to the cyclodextrin. This was the general trend observed. Specifically, the addition of various alcohols to the β-CD:Naphthalene complex enhanced the relative emission intensities thereby impacting the efficiency of the system.

References


[34] Ferm, M.; Sjodin, A. Atmos. Environ. 1985, 19, 979.


Chapter 3. The Effect of Cyclodextrins on Excited State Proton Transfer Reactions of Carboxylic Acid Compounds

Introduction

Excited state proton transfer (ESPT) reactions are widely studied by the use of fluorescence spectroscopy since many of these molecules fluoresce and ESPT typically occurs on the same time scale as fluorescence [1]. Aromatic acids and bases are known to exhibit different acid-base equilibrium in the excited electronic energy states relative to the ground state [2-3]. It is generally acknowledged that electron transfer to or from the heteroatom within an aromatic molecule is enhanced when the molecule is in the S₁ or T₁ state. This phenomenon applies to molecules with both electron donating and withdrawing substituents [2a]. As a result, the acid-base equilibrium constant of the excited state (pKₐ*) is no longer the same as that of the ground state (pKₐ). For example, a phenolic molecule is more acidic in the excited state than in the ground state. This is evidenced by the fact that fluorescence emission can be observed from the phenolate anion at a very low pH, although the undissociated phenol is the only absorbing species in the ground state.

ESPT has been used to probe the microenvironments of micellar media, cyclodextrins, and proteins [2a]. Cyclodextrins (CDs) have been shown to produce significant effects on excited state acid-base equilibrium [4], and CD polymers have been used to study host-guest binding interactions [5-7]. Therefore, cyclodextrins can be used to study the photochemistry and photophysics of guest molecules, and at the same time, the guest molecule can be used to probe the host characteristics of the cyclodextrins. The ESPT reactions normally consist of two types: inter-molecular and intra-molecular. Both
inter- and intra-molecular proton transfer require the presence of a hydrogen bond. One should note that the bimolecular character of inter-molecular proton transfer depends on the degree of protection from the bulk solution that is provided by cyclodextrin. In contrast, the unimolecular process of intra-molecular proton transfer is less affected by the bulk and is more dependent on whether the two protonation sites are included in the cavity of the cyclodextrin. A point of interest in the current study is to compare such induced effects of the cyclodextrins on the acid-base equilibrium of intra-molecular proton transfer relative to inter-molecular proton transfer.

**Inter-molecular Versus Intra-molecular**

As mentioned earlier, phenol is an example of a molecule that undergoes excited state intermolecular proton transfer (ESI\textsubscript{PT}) given by the following expressions

<table>
<thead>
<tr>
<th>Ground state</th>
<th>ROH $\rightarrow$ RO(^+) $+$ H(^-$) $\quad$ pK(_a) $\quad$ (3.1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ROH $+$ hv $\rightarrow$ ROH(^*) $\quad$ (3.2)</td>
</tr>
<tr>
<td></td>
<td>ROH(^*) $\rightarrow$ ROH $+$ hv(_1) $\quad$ (3.3)</td>
</tr>
<tr>
<td></td>
<td>ROH(^<em>) $\rightarrow$ RO(^-)</em> $+$ H(^+$) $\quad$ (3.4)</td>
</tr>
<tr>
<td></td>
<td>RO(^-)* $\rightarrow$ RO(^-) $+$ hv(_2) $\quad$ (3.5)</td>
</tr>
</tbody>
</table>

| Excited state      | ROH\(^*\) $\rightarrow$ RO\(^-\) $+$ H\(^+$) $+$ hv\(_2\) $\quad$ pK\(_a\)* $\quad$ (3.6). |

The excited state phenol molecule, ROH\(^*\), can emit photons (eqn 3.3) or produce an excited state phenolate ion (eqn 3.4). The excited phenolate ion, RO\(^-\)*, may also emit photons (eqn 3.6), but at a longer wavelength. The competition between these photoprocesses depends upon the pH of the microenvironment. Generally, for electron
donor groups, such as -OH and NH2, pK_a > pK_a*, whereas, for an electron acceptor group such as -COOH, pK_a < pK_a*.

Excited state intra-molecular proton transfer (ESIPT) involves proton transfer between different moieties of the same molecule. The ESIPT molecules often show dual emission: a normal emission at lower wavelengths and a Stokes-shifted tautomer emission. These emissions are dependent on solvent polarity, pH, hydrogen-bonding character of solvent, and structure of the molecules in both the ground and excited states [8,9].

Roberts, et al. [10] recently examined the effect of organized media on the ESIPT reaction of 10-hydroxybenzo[h]quinoline. They studied this molecule by monitoring the large Stokes-shifted tautomer emission using fluorescence spectroscopy. Both inter- and intra-molecular proton transfer may be used to provide information on the hydrogen-bonding character of various media. In addition, the different conformations of a molecule in the ground and excited states can be understood.

The CDs are water soluble cyclic oligosaccharides formed by an α-(1,4) linkage of glucopyranose units that are capable of forming inclusion complexes. The polymers of the CDs are even more water soluble. The most commonly used oligosaccharides are α-, β, and γ- CDs with six, seven, and eight glucopyranose units, respectively. These compounds possess a hydrophilic exterior which makes them soluble in water and an interior cavity which is less polar than water. Werner and Warner [5] reported that the binding site of CD polymers for naphthalene-based probes is more hydrophobic than that on the CD monomer. It was also concluded that the ratio of linker units to CD units in the
polymer, as well as the fluorophore itself governs the type of binding that exists between a fluorophore and the CD polymers [6]. As a result of forming an inclusion complex with CDs, excited state proton exchange between the guest molecule and the bulk aqueous solutions can be decreased to time scales much slower than the fluorescence lifetime scale. In addition, different microenvironments of the guest molecule caused by CD complexation results in a change in the photochemical pathways of ESPT reactions of the host-guest complex [11].

The effect of γ-CD complexation on the ESPT reaction of carbazole has been examined by Chattopadhyay, et al. [12]. It was concluded that inclusion in γ-CD enhances the rate of the forward deprotonation reaction by about 100%, (as compared with the bare chromophore), but with little effect on the reverse process. In addition, the ESPT reactions of carbazole and 2-naphthylamine in the presence of β-CD have been reported [11]. It was determined that the deprotonation rate of the CD inclusion complex is enhanced in the case of carbazole, whereas it decreases for guest molecules such as naphthylamine or naphthols. The location of the protonation site in the host-guest complex has a significant effect on the excited state proton transfer kinetics [1]. Therefore, the efficiency of ESPT reactions depend on the microenvironment of the molecule as well as the molecule itself [11].

Carboxylic acids are important both biologically and commercially. These compounds are found in proteins which play a significant role in virtually all biological processes [13]. Therefore, a study of the effect of α-, β-, and γ-CDs and their polymers on the ESPT reactions of various carboxylic acid compounds is of particular interest.
Part 1. Naphthoic Acids

Naphthoic acids are known to protonate in their lowest excited state at pH values which are far too high to produce ground-state protonation [14]. The 1- and 2- naphthoic acids are also known to be weaker acids in their lowest singlet electronic state than in the ground state. For example, Watkins investigated the photophysical properties of the naphthoic acids in aqueous solutions [15]. The 1- and 2- naphthoic acids were shown to undergo diffusion-controlled protonation in their lowest excited singlet state [15]. Other studies of the photophysical properties of 2-naphthoic acid in aqueous-ethanol solutions as a function of added sulfuric acid by means of steady-state and dynamic measurements indicated that as the amount of water present increases, the system appears to achieve a state of dynamic equilibrium in the excited state [16]. The authors concluded that the system behaves like a typical exciplex with a high alcohol content.

Experimental

Instrumentation. Steady-state fluorescence measurements were acquired by use of a Spex Model F2T211 spectrofluorometer in the photon counting mode equipped with a thermostated cell housing and a water cooled Hamamatsu R928 photomultiplier tube. Fluorescence emission spectra for 2-naphthoic acid were acquired using an excitation wavelength of 280 nm. Excitation and emission slit widths were set for a 5.1 nm bandpass.

Materials. The α-, β-, and γ-CDs were a gift from American Maize Products (Hammond, IN) and were used as received. The CD polymers were obtained from
Cyclolab R&D Laboratory Ltd. (Budapest, Hungary). The 2-naphthoic acid and 1-hydroxy-2-naphthoic acid were purchased from Aldrich Chemical Company (Milwaukee, WI) and were used without further purification.

**Method.** A stock solution of 0.1 M 2-naphthoic acid was prepared in ethanol. Aliquots of this solution were transferred into volumetric flasks and the ethanol was evaporated by purging with dry nitrogen. Aqueous stock solutions of the CDs and CD polymers were prepared. The pH values of the solutions of the acid and base were adjusted to 3.0 and 11.0 by addition of 0.2 N HCl or 0.1 N NaOH. All solutions were allowed to equilibrate overnight before analysis.

**Results and Discussion**

This study examines the changes in chemical properties of 2-naphthoic acid and 1-hydroxy-2-naphthoic acid in the photoexcited state by use of fluorescence spectroscopy and the inclusion of the molecules within α-, β-, and γ-CDs. Fluorescence spectroscopy is an effective technique for the study of photoexcited state changes that occur in a molecule. The CDs are capable of including different guest molecules, depending on the hydrophobicity and size of the guest, as well as the cavity size of the CD. As a result of CD complexation of the guest molecule, changes occur in the photochemical pathways of excited state proton transfer reactions of the host-guest complex. The rationale for the study outlined in this dissertation is to test the effect of CD complexation on the ESPT of 2-naphthoic acid compounds and to further characterize the excited state acid-base equilibria of this class of carboxylic acid derivatives.
2-naphthoic acid. Figure 3.1 shows the fluorescence spectra of 2-naphthoic acid in acidic aqueous solutions, (pH=3.4) in the absence and presence of \(\alpha\)-CD and \(\alpha\)-CD polymer. It is evident that the fluorescence maximum shifts to shorter wavelengths upon addition of \(\alpha\)-CD. These changes are observed only at low pH. In alkaline and neutral solutions, complexation with \(\alpha\)-CD does not shift the maxima but changes the relative intensities. In addition, there are no major changes observed in the absorption of 2-naphthoic acid. The reported pKa and pKa* of 2-naphthoic acid are 4 and 10, respectively. At pH=3.4, some of the naphthoic acid molecules are not protonated. However, the excited state population should be protonated given adequate equilibration time. Therefore, molecules in the excited state are not in equilibrium and must undergo protonation. It is clear that the size of the \(\alpha\)-CD cavity is too small to accommodate the entire molecule because only benzene type compounds are known to fully include in the \(\alpha\)-CD cavity [17]. Therefore, it is reasonable to conclude that the carboxyl group of 2-naphthoic acid could be included inside the \(\alpha\)-CD cavity, whereas the naphthyl group is exposed to the aqueous phase giving the observed structured fluorescence. Therefore, \(\alpha\)-CD blocks the protonation process, (Figure 3.2a), and thus, the blue shift of the fluorescence is reasonable. It has been reported that the hydroxyl group of 2-naphthol is included inside the \(\alpha\)-CD cavity while the naphthyl group is exposed to the aqueous phase [18].
Figure 3.1 Emission spectra of 2-naphthoic acid with α-CD compounds
(a) α-CD (b) acidic/no CD (c) neutral/no CD (d) α-CD polymer

Figure 3.3 depicts the fluorescence spectra of 2-naphthoic acid in acidic aqueous solutions (pH=3.4) in the absence of β-CD (3.3a) and presence of β-CD (3.3b), β-CD carboxylic acid derivative (3.3c), and β-CD polymer (3.3d). The fluorescence spectra of 2-naphthoic acid are all blue shifted upon addition of β-CD, but to a lesser extent than the shift caused by adding α-CD. Such an observation suggests that the carboxyl group is partially shielded from the bulk solution in the presence of β-CD. Therefore, the excited state equilibrium is not fully attainable even in β-CD. Moreover, a close inspection of the fluorescence spectra reveals that the β-CD polymer causes the largest blue shift amongst the β-CDs. The latter is an interesting observation for the purpose of studying the characteristics of the CD polymers as compared with native CDs. As with the α-CD, there
are no major changes that are observed in alkaline and neutral media. The β-CD inner cavity is capable of accommodating the naphthyl group with the carboxyl group outside the rim of the cavity (Figure 3.2b), giving rise to the broad fluorescence spectrum. Therefore, proton transfer can occur between the proton on the carboxyl group and the aqueous solution. Similar conclusions were drawn regarding complex formation of α- and β-naphthol with α- and β-CD [1].

Figure 3.4 shows the fluorescence spectra of 2-naphthoic acid in acidic solutions (pH~3.4) with γ-CD and γ-CD polymer. It is apparent that the spectral shift is not prominent in the presence of γ-CD. Yet, a slight blue shift is still observed. Therefore, γ-CD seems to provide the least degree of protection of the carboxyl group amongst the three CDs. Surprisingly, α-CD provides the highest degree of protection by shielding the carboxyl group from the bulk solution and inhibiting the ESPT. Similar results are observed with β-CD. It is apparent that the fluorescence is quenched when γ-CD and γ-CD polymer are added. This could be because both of these compounds can complex the 2-naphthoic acid with the carboxyl group still exposed to the aqueous phase (Figure 3.2c) resulting in a broad fluorescence spectrum with γ-CD and γ-CD polymer. There are no distinct spectral differences observed for 2-naphthoic acid in alkaline or neutral solutions.

It is worth noting that, in all cases, the fluorescence shift is more prominent in the presence of the CD polymer as compared with its corresponding CD. Werner, et al. [6] concluded that pyrene preferably forms a non-inclusion complex with CD polymers. However, the current observations suggest that the carboxyl group of 2-naphthoic acid is
better shielded from access to protons in the presence of the polymeric CD. It is apparent that the cavity of the CD still plays an important role in this shielding. This is particularly true when β-CD polymer is compared with γ-CD polymer.

![Diagram of cyclodextrins](image)

**Figure 3.2** Excited state proton transfer models depicting the effect of cyclodextrins (a) α-CD (b) β-CD (c) γ-CD on 2-naphthoic acid
Figure 3.3 Emission spectra of 2-naphthoic acid with β-CD compounds (a) acidic/no CD (b) β-CD (c) β-CD carboxylic acid derivative (d) β-CD polymer

Figure 3.4 Emission spectra of 2-naphthoic acid with γ-CD compounds (a) acidic/no CD (b) γ-CD (c) γ-CD polymer (d) neutral/no CD

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1-hydroxy 2-naphthoic acid (1-HNA). The ESPT characteristics of 2-naphthoic acid have already been discussed. In addition, the ESPT of 1-naphthol are well documented [19-21]. The reported pKa of the hydroxyl and the carboxyl protons are 2.7 and 12.9, respectively. Thus, at the wide pH range from 3 to almost 12, the ground state form of 1-HNA exists predominately as a protonated hydroxyl and deprotonated carboxyl group [22]. Therefore, excitation of 1-HNA at neutral pH is expected to yield an intramolecular proton transfer from the hydroxyl group (OH) to the carboxylate group (COO\(^{-}\)). Unlike the ESPT of 2-naphthoic which involves proton exchange with the bulk solution, the ESPT of 1-HNA is intra-molecular. Therefore, we anticipate that the ESPT in different conformations of the guest acid in the host cavity would be less pronounced for 1-HNA. Indeed, the observations of 1-HNA in the presence of different cyclodextrins show little dependence on the cyclodextrin cavity. Yet, the most prominent effect of adding CDs is observed in the case of \(\alpha\)-CD (Figure 3.5A). Apparently, \(\alpha\)-CD cannot include both the OH and COO\(^{-}\) groups simultaneously. The latter is a reasonable conclusion judging from the inner cavity diameter of \(\alpha\)-CD. However, the presence of \(\beta\)-CD shows a similar trend to that of \(\alpha\)-CD but to a much lesser extent (Figure 3.5B).

**Part 2. Anthrionic Acids**

The absorption and fluorescence spectra of various anthrionic acids have been investigated in the literature [23-27]. For example, Werner and Hercules [24] examined 1- and 2-anthrionic acids. They reported that excited-state rotation is not necessary to achieve a coplanar configuration, whereas it is necessary in the case of 9-anthrionic acid.
Figure 3.5A Emission spectra of 1-HNA with α-CD (a) acidic (b) neutral (c) basic

Figure 3.5B Emission spectra of 1-HNA with β-CD (a) acidic (b) neutral (c) basic
In another study, Suzuki, et al. [25] reported the concentration and temperature dependence of the absorption and fluorescence spectra of 1- and 2-anthroic acids. They were able to show that the 1- and 2-anthroic acids form hydrogen bonded dimers in the ground state.

9-anthroic acid. The fluorescence of 9-anthroic acid, (9-ACA), has been the subject of several studies, which have provided different interpretations concerning the origin of the dual emission of this compound [28, 29]. The effect of many solvents on the fluorescence of 9-ACA has also been examined. These include hydrophobic, hydrophilic, protic, and aprotic solvents [25]. The fluorescence of other anthracene substitutions such as 9-COOCH₃ has also been studied [29] and compared with that of 9-ACA. Various environmental factors have been examined, including the effects of pH, concentration, temperature, and viscosity [28, 29]. Fluorescence lifetime, quantum yield, and, therefore, radiative and non-radiative rate constants have been measured or estimated in different environments [29]. All of these studies were designed to determine whether the dual emission of 9-ACA is a result of unimolecular or multimolecular processes.

At alkaline pH, either in water or ethanol, a structured fluorescence (SF) spectrum (λ_max ~ 410 nm) of 9-ACA, similar to that of anthracene, is observed. In contrast, a structureless broad fluorescence (BF) at longer wavelengths (λ_max ~ 475 nm) predominates in acidic solutions [25, 29]. However, similar SF and BF were respectively observed at low and high concentrations of the compound in ethanol (EtOH). Acid-base equilibrium between different conformers was proposed as an explanation of the dual emission.
However, this explanation was challenged by authors who reasoned that the dual emission was the result of the formation of dimers in the ground state and tetramers in the excited singlet state [25].

The dependence of the dual emission on the concentration of 9-ACA has been used to suggest that the dual emission originates from the excimer (BF) and monomer (SF) of the 9-ACA [28]. However, the BF has also been observed at low concentrations. As an alternative explanation, a dimer was suggested to form in the ground state and, consequently, the excimer is formed without diffusion limitations [28]. Thus, the effect of pH was described as a factor which determines the formation of ground state hydrogen bonded dimers at low pH. At neutral or alkaline pH, 9-ACA is in the ionic form, and would not form dimers. Thus, only SF would be observed under such conditions. The effect of different solvents on the BF was explained in a manner similar to the effect of pH. However, a dual emission was observed [29] even at concentrations as low as $10^{-6}$ M.

The idea of unimolecular processes is based on the molecular properties of 9-ACA, which has been characterized as a weak acid [25, 28-29] with a $\text{pK}_a$ of 2.4-4. Two conformations can result from a twist of the two carboxyl species (protonated and unprotonated). It has been suggested [29] that the carboxylic group of 9-ACA is in a coplanar conformation (||) relative to the aromatic ring (Ar), giving rise to the BF. In addition, deprotonated 9-ACA has a perpendicular (⊥) conformation, which correlates with the SF. Hence, the dependence of the dual emission on pH is reasoned to be a result
of different conformations of the protonated and deprotonated forms. In addition, the pKₐ of 9-ACA in ethanol is expected to be above 8, a few orders of magnitude higher than that in water [25]. Yet, a significant dependence of the emission on the concentration was reported, and the BF was observed at high concentrations of 9-ACA in ethanol. Suzuki, *et al.* [25] suggested that the BF in ethanol is due to the fluorescence of an excited dimer that forms a tetramer in the excited singlet state. However, the interacting π-systems of the aromatic ring and the carboxyl group of 9-ACA was also proposed with a view of the molecule as constructed of these two separate moieties [26].

Both the theories of unimolecular and the multimolecular processes have been criticized by researchers supporting opposing points of view. However, neither of these interpretations explain all of the spectral observations associated with 9-ACA. For example, Werner and Hercules [29] argued for excimer formation by calculating the required diffusion constant which was found to be a few orders of magnitude higher than the actual one at low concentrations. In contrast, Suzuki, *et al.* [25] showed that the concentration effect cannot simply be due to the pH effect of a weak acid. Although all agree that deprotonated 9-ACA has a SF, which is similar to that of the parent anthracene compound, the controversy centers around the origin of the red shifted BF. In addition, it is suggested that the conformations in the excited singlet states of the protonated and deprotonated forms of 9-ACA are parallel and perpendicular, respectively. Other researchers have suggested both conformations for the ground state form of 9-COOH [26,29].

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The acidity in the excited state of 9-ACA and other anthroic and naphthoic acids has also been a subject of several studies [30, 31]. Excited singlet state $pK_a$ ($pK_a(S1)$ or $pK_{a^+}$) values ranging from 6.5 to 10 have been suggested [31]. In these studies, the differences between the emissions of the two forms (protonated and deprotonated) were attributed to different excited state conformations. However, a common perpendicular ground state conformation has also been suggested for both forms. In particular, the perpendicular conformation in the ground state of 9-ACA was assumed to explain the lack of an effect of the carboxyl group on the absorption of 9-ACA.

In this section, the question of the dual emission of 9-ACA by use of $\beta$-CD complexation is addressed. Cyclodextrins are known to enhance or inhibit processes by forming inclusion complexes with a wide variety of guest molecules [32, 33]. For example, it is well established that CDs can enhance excimer formation [34, 35], inhibit or slow down the rate of excited state proton transfer [1, 4], protect the included guest molecule from quenchers and thus also enhance fluorescence intensity. The CDs have also been shown to restrict the twisting of certain molecules. This results in enhanced fluorescence quantum yields and an increase in the rotational correlation time; and therefore, influences the fluorescence anisotropy [36]. The assumption here is that the internal cavity size of $\beta$-cyclodextrin would allow the inclusion of only one 9-ACA molecule into its cavity. Therefore, this would provide evidence in examining the origin of the dual emission. Under these conditions, the BF would exhibit a significant decrease in the presence of cyclodextrins if its origin is bimolecular. Specifically, since the $\beta$-CD
cavity has room for only one 9-ACA molecule, a decrease in the concentration of such dimers is expected upon the addition of β-CD. The data depict an opposite trend. In addition, solid samples of 9-ACA/β-CD, in which the crucial diffusion processes for forming tetramers are inhibited, show the opposite of what is expected for a BF process which has a multimolecular origin. These results will be discussed in chapter 4.

**Experimental**

**Instrumentation.** Steady-state fluorescence measurements were acquired by use of a Spex Model F2T211 spectrofluorometer in the photon counting mode equipped with a thermostated cell housing and a water cooled Hamamatsu R928 photomultiplier tube. Fluorescence emission spectra were acquired using an excitation wavelength of 330 nm. Fluorescence excitation spectra were obtained with emission wavelengths of 430 nm for the structured emission and 475 nm for the broad emission. Excitation and emission slit widths were set for bandpasses, 5.1 and 3.4 nm, respectively, for the emission spectra and 3.4 and 5.1 nm, respectively, for the excitation spectra. Absorption measurements were acquired by the use of a Shimadzu UV-3101 PC UV-VIS-NIR scanning spectrophotometer equipped with a 1-cm path length cell. All measurements were performed at room temperature.

**Materials.** The β-CD was obtained from American Maize Products (Hammond, IN) and used as received. The 9-anthoic and 2-anthoic acids (98%) were obtained from Aldrich Chemical Company, Milwaukee, WI and were used as received.

**Method.** A $1 \times 10^{-3}$ M stock solution of 9-anthoic carboxylic acid was prepared in ethanol. A 2.5 mL aliquot of this solution was transferred into a 250-mL volumetric
flask and the ethanol was evaporated by use of dry nitrogen purging. A 7.5 mL volume of 0.2 N HCl was added and the flask was filled to the mark with deionized water. The pH of the solution was measured to be 2.3. A 5 mL aliquot of the stock solution was taken and an amount of solid CD was added to achieve the desired concentration.

Results And Discussion

The rationale for this study is that β-CD is expected to enrich the monomers of 9-ACA at the expense of dimers which may form. The observations which suggest that the BF in aqueous solutions is not a result of aggregation of 9-ACA are discussed. Rather, fluorescence measurements of 9-ACA in liquid and solid solutions of β-CD supports the previously proposed dual conformation of the monomer [29].

Fluorescence. Figure 3.6 shows the fluorescence spectra of 9-ACA in acidic aqueous solutions (pH ~ 2.3) in the absence and presence of β-CD. It is evident that the intensity of the BF increases in the presence of β-CD. This observation contradicts the conclusion in which BF has been attributed to a dimer of 9-ACA. However, since only BF undergoes intensity changes in the presence of β-CD, this observation further suggests a complicated process.

A significant effect from the addition of β-CD to aqueous solutions of 9-ACA is observed only at low pH. In alkaline solutions where only SF exists, β-CD does not affect the SF and no additional fluorescence band appears. It should be noted that upon lowering the pH from neutral to ~2.3, by titration with HCl, the intensity of the SF decreases significantly as compared with the intensity of the BF which increases only slightly. In

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contrast, the absorption of 9-ACA does not show any significant change upon titration with HCl. Hence, the estimated quantum yield of the BF is about 8 fold lower than that of the SF.

Figure 3.6 Fluorescence spectra of 9-anthrocic acid at pH 2.4 at various concentrations of β-CD. The spectra in order from the bottom to top are at the following concentrations of β-CD in mmol: 0, 1, 1.5, 1.75, 2, 3, 4, 5, 6, 8, 10

Therefore, the relatively low intensity of the BF in aqueous solution results from a relatively low quantum yield of 9-ACA. It should be noted that β-CD enhances the intensity of the BF with no concomitant decrease in the SF intensity. Thus, β-CD does not affect the SF. This conclusion is also supported by the fact that the addition of β-CD to neutral or alkaline aqueous solutions of 9-ACA did not affect the fluorescence.
Cyclodextrins have been shown to produce significant effects on excited state acid-base equilibrium [1, 4]. As a result of forming an inclusion complex with CDs, excited state proton exchange of the guest molecule with the bulk aqueous solution can be decreased to time scales much lower than the fluorescence lifetime scale. However, this is not the case for 9-ACA because such a shift of the excited state equilibrium would enrich one form at the expense of the other. In addition, excited state deprotonation requires a $pK_a^* < pK_a$. This is also not the case for 9-ACA. In comparison, $pK_a^*$ and $pK_a$ of naphthoic acids are ~ 10 and ~ 4, respectively [37]. However, Vander Donckt et al. [30] reported slightly different values of $pK_a$, but significantly different values of $pK_a^*$ for several aromatic carboxylic acids. The reported $pK_a^*$ and $pK_a$ of 9-ACA are 6.5 and 3.0, respectively [30]. Thus, one can conclude that β-CD can at most inhibit protonation in the excited state, and thus, the intensity of BF should decrease. The data can be interpreted that the increased intensity of the BF is not at the expense of the SF. This observation rules out the possibility that β-CD inhibits excited state proton transfer. Therefore, β-CD seems to provide a microenvironment in which the radiative pathway of the BF becomes more competitive with the non-radiative pathways.

An examination of the fluorescence decay times of 9-ACA in the presence of β-CD failed to show any significant elongation of the fluorescence lifetimes. One should note that

$$\phi = k_r \tau$$

(3.7)

where $\phi$ and $\tau$ represent the quantum yield and the fluorescence lifetime, respectively, and
\( k_f \) and \( k_d \) are the radiative and non-radiative rate constants, respectively [38]. In addition

\[
\phi = k_f (k_f + k_d) \tag{3.8}
\]

and

\[
\tau = \frac{1}{k_f + k_d} \tag{3.9}
\]

The increased quantum yield at constant lifetime implies that \( k_f \) must also increase in order to maintain the equilibrium equation (3.7). Such increased \( k_f \) necessitates a decease in \( k_d \) in order for \( \tau \) to remain constant according to equation (3.9). Therefore, \( \beta \)-CD seems to enhance the intensity of BF by both reducing \( k_d \) and enhancing \( k_f \). This conclusion is consistent with CD constrained rotational degrees of freedom of the carboxyl group.

Association Constant. To further characterize this complex, Benesi-Hildebrand plots [39] are used to determine the stoichiometry of the guest and cyclodextrin(CD) molecules. These plots are also used to determine the binding constant of the guest with \( \beta \)-CD. The formation constant of 9-ACA with \( \beta \)-CD can be estimated by use of the increased fluorescence intensity of the BF. Assuming a 1:1 complex between 9-ACA and CD, we can write

\[
9 \text{-ACA} + \text{CD} \rightleftharpoons 9 \text{-ACA\text{-CD}} \tag{3.10}
\]

and the equilibrium constant \((K_c)\) for this complex is given by

\[
K_c = \frac{[9 \text{-ACA\text{-CD}}]}{[9 \text{-ACA}][\text{CD}]} \tag{3.11}
\]

where \([9 \text{-ACA}]\) is the equilibrium concentration of 9-ACA, \([\text{CD}]\) is the equilibrium concentration of cyclodextrin, and \([9 \text{-ACA\text{-CD}}]\) is the equilibrium concentration of the complex. It can be assumed that \([\text{CD}]\) is equal to the initial concentration \( \beta \)-CD. This is
verified by \( [CD]_o > [9-ACA]_o \), where \( [9-ACA]_o \) is the initial concentration of 9-ACA. With these assumptions, one can derive the following equation:

\[
\frac{1}{[9-ACA \cdot CD]} = \frac{1 + K_i [9-ACA]_o}{K_i [9-ACA]_o [CD]_o}
\] (3.12)

where \( [9-ACA]_o = [9-ACA] + [9-ACA \cdot CD] \) and \( [CD]_o = [CD] + [9-ACA \cdot CD] - [CD] \).

Since the intensity of the BF in the absence (\( I_o \)) and presence (\( I_i \)) of CD is proportional to the free and complexed compound, respectively. These values can be substituted into equation (3.11) to give

\[
\frac{1}{I - I_o} = \frac{1}{(I_i - I_o)} + \frac{1}{K_i (I_i - I_o)[CD]_o}
\] (3.13)

Therefore, a plot of \( 1/(I_i - I_o) \) vs \( 1/[CD]_o \) should give a straight line for a 1:1 complex.

A Benesi-Hildebrand plot of the data presented in Figure 3.6 is shown in Figure 3.7. Data are fit to a 1:1 stoichiometry. The plot shows a linear regression with a correlation coefficient of \( r = 0.996 \). However, Benesi-Hildebrand plots place more emphasis on the lower concentrations and, thus, the slope of the line is more sensitive to the ordinate values of the points having lower abscissa values [40]. Therefore, we also estimated the formation constant by use of a non-linear regression fit [41] to equation 3.12. Both methods gave similar estimates of \( 204 \, M^{-1} \) for the formation constant. This value is the formation constant of 9-ACA if one were to assume that only the BF complexes with \( \beta \)-CD. However, there is every reason to believe that both forms (BF and SF) can complex with \( \beta \)-CD. Therefore, the formation constant should be reevaluated.
with this hypothesis in mind. Under the assumptions that both forms are equally abundant and that each has similar association constants, it can be shown that the actual formation constant would be twice the calculated value. The assumption of equal abundance of both forms is justified by adjusting the pH to 2.4 which is equal to the reported value for the pKa of 9-ACA [29]. The second crude assumption is based on a fluorescence quenching study which is discussed later. Under these conditions, the formation constant is estimated to be ~ 400 M⁻¹. However, some deviation from the actual value is to be expected, depending on the validity of the above assumptions.

Figure 3.7 Benesi-Hildebrand plot of data from Figure 3.6. The estimated value from the plot is 204 M⁻¹.

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Fluorescence Quenching. A simple approach for estimating the relative formation constant of each form with β-CD is to use a fluorescence quencher. Acrylamide is soluble in water and is a well known fluorescence quencher [42] of aromatic hydrocarbons. Therefore, different concentrations of acrylamide into acidic aqueous solutions of 9-ACA/β-CD were titrated. Different association constants of the two forms of 9-ACA with β-CD, are expected to yield different quenching rates and, as a result, the relative SF and BF intensities are expected to vary with the concentration of acrylamide. Although a decrease in the total fluorescence intensity of both forms is observed as a function of increased concentration of acrylamide, the relative intensities of the two forms remain constant. This observation suggests that acrylamide has similar accessibility to both forms of 9-ACA. Under the conditions used, i.e. pH ~ pKₐ, both forms have similar abundance. If in addition, both forms have similar association constants, then similar portions of both forms should form complexes with β-CD. In this case, quenching of the two forms is indistinguishable. Consequently, the relative fluorescence intensities of both forms will remain constant. Thus, it is also apparent that both forms have similar association constants with β-CD. This similarity between the association constants also suggests similar ground state conformations of both forms.

Absorption And Excitation. The excitation spectra were monitored at two different fluorescence wavelengths, one in the SF region and the other in the BF region. Both the absorption and the excitation spectra possessed well-resolved vibronic fine
structure which is similar to that of anthracene, and are also similar to spectra reported in the literature [28-29].

Although β-CD has a significant effect on the BF, no effect is observed in the absorption spectra. In addition, the excitation spectra of both the SF and the BF are similar in structure in the absence and presence of β-CD. It is worth noting that the resolved vibronic excitation structure is more pronounced when excitation in the SF range is monitored. In this region, the vibronic structure of the excitation spectrum of BF in the presence of β-CD becomes less pronounced than that of the SF. However, no spectral shift and no total loss of vibronic structure was observed even in saturated solutions of β-CD. Thus, the only significant effect of β-CD is observed in the BF excitation spectrum. The increased intensity of the BF excitation spectrum reflects the increased fluorescence intensity of BF in the presence of β-CD. These observations are similar to excitation and absorption spectra reported in the literature for different solvents [28, 29].

Comparison of the vibronically structured excitation spectrum with the broad fluorescence at 470 nm suggests possible excited state kinetics. Broad absorption and structured fluorescence in biphenyl is known to be caused by its planarity in the excited state [43]. Ghoneim, et al. [26] attributed the broad fluorescence of biphenyl to the twisting of the phenyl rings in the ground state. The formation of an exciplex between the carboxyl group and anthracene has also been proposed as an explanation for the red shift and the loss of vibronic structure of the BF [29]. The model of 9-ACA is stabilized in the parallel conformation. This confirmation is interrupted by events of deprotonation, in
which the carboxyl group can twist to the perpendicular conformation. In this conformation, it is uncoupled from the aromatic group and does not affect the SF. The BF is broadened by the rate of either deprotonation or interruption of the perpendicular conformation.

Unimolecular Versus Bimolecular. In 9-ACA, the pH indirectly governs the process of twisting of the carboxyl group. In the parallel conformation, the carboxyl group which becomes part of the conjugated π-systems is not rigid and as a result the red shifted band is structureless. The ionic form is free to twist to the perpendicular conformation in the excited state, and thus no significant effect of carboxylate ion on the fluorescence of anthracene is observed. In addition, the conjugation of the carbonyl group with the aromatic π-system is not significant in the ground state, regardless of the conformation. This reflects the fact that changes are not observed in the absorption spectra[30] of both forms. These conclusions are valid in aqueous solutions, although this may not be the case in organic solvents.

Suzuki, et al. [25] have studied the dependence of the BF on the concentration of 9-ACA. In EtOH solutions, the BF intensity increases either by increasing the concentration of 9-ACA or by lowering the pH of the solution. These observations have been explained by others in aqueous solutions [29]. However, Suzuki, et al. [25] argued against this explanation and concluded that the BF is a result of the formation of dimers in the ground state and tetramers in the excited state. These observations do not allow this model to be extended to aqueous solutions. Therefore, this conclusion cannot be
generalized to include aqueous solutions. It is further suggested that the pH and the concentration effects seem to be two variations of the same phenomenon. Since the pH in aqueous solution has an indirect effect on the conjugation of 9-ACA, a similar indirect effect can be induced by the formation of dimers. In such dimers, the π-systems of the aromatic rings do not necessarily interact. However, a twist of the carboxylic group is restricted in such dimers. Therefore, the concentration and the pH would both induce restrictions on the twist of the carboxyl group.

It is particularly interesting to note that 9-ACA in neutral aqueous solution does not show dual fluorescence. In comparison, neutral solutions of β-naphthol, which has pKₐ ~ 9.5 and pKₐ⁺ ~ 3, does exhibit dual fluorescence. In neutral aqueous solution, β-naphthol exists in the protonated form but undergoes excited state deprotonation. The carboxylic acids of polycyclic aromatic hydrocarbons [30] typically have a pKₐ of about 4 and a pKₐ⁺ of about 7. Therefore, 9-anthroic acid is expected to undergo excited state protonation in neutral aqueous solution. However, only the SF of 9-COO⁻ appears in neutral aqueous solution. Two explanations can be given for this observation. Either excited state protonation of 9-ACA does not occur on the time scale of its fluorescence lifetime, or protonation in the excited state alone is not sufficient to yield the BF. The latter requires a reconformation of the carboxyl group. Thus, the ground state acid-base equilibrium, in which the protonated form of 9-ACA exists, would govern the BF. In addition, acid-base equilibria between different electronic configurations do not occur even on the time scale of the excited triplet state [37]. Therefore, different electronic
configurations of the excited states of deprotonated 9-ACA and protonated 9-ACA would also account for the absence of dual emission under neutral conditions. The latter implies that a twist of COO⁻ to the perpendicular conformation is much faster than capturing a proton from solution.

2-anthroic acid. Figure 3.8 shows the normalized fluorescence spectra of 2-anthroic acid in acidic aqueous solutions with (3.8a) acidic/no CD, (3.8b) α-CD, (3.8c) α-CD polymer, and (3.8d) neutral/no CD. The emission maxima are red-shifted for the acidic solution with no CD and α-CD with respect to the α-CD polymer and neutral solutions. Apparently, there are two forms that emit at \( \lambda_1(\text{max}) \sim 425 \text{ nm} \) and \( \lambda_2(\text{max}) \sim 470 \text{ nm} \). This is reasonable since the pH of the solutions is approximately 3.4 and the

![Graph showing fluorescence intensity vs. wavelength for different conditions](image-url)

**Figure 3.8** Emission spectra of aqueous solutions of 2-anthroic acid with (a) acidic/no CD (b) α-CD (c) α-polymmer (d) neutral/no CD

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reported pKₐ and pKₐ* are 4.2 and 6.6, respectively [23]. At this pH, two forms are expected to be present. Therefore, α-CD seems to have little effect on the system which is not surprising since it normally accommodates benzene type compounds. This is more evident in the solid samples which will be discussed in the next chapter.

Figure 3.9 depicts the normalized emission spectra of aqueous solutions of 2-anthroic acid with (3.9a) β-CD polymer, (3.9b) β-CD, (3.9c) β-CD carboxylic acid derivative, and (3.9d) acidic/no CD. The spectra in the β-CD and the β-CD derivative are very similar whereas the spectrum in the β-CD polymer is blue shifted with respect to those two. There seems to be only one form present for the β-CD compounds which suggests that these compounds provide some degree of protection for the protonation
site. At this pH (~3.4), two forms should still exist. The fact that there is only one form present implies that the β-CD compounds are slowing down or inhibiting the proton transfer process. Therefore, the carboxyl group should be included inside the β-CD cavity.

Figure 3.10 shows the normalized emission spectra of 2-anthroic acid in aqueous media with (3.10a) acidic/no CD, (3.10b) γ-CD, (3.10c) γ-CD polymer, and (3.10d) neutral/no CD. The most significant change occurs with the γ-CD polymer since its spectrum is broadened and slightly blue shifted with respect to no CD present and γ-CD. It is interesting to note that the fluorescence of 2-naphthoic acid was quenched when both γ-CD and γ-CD polymer were added. However, for 2-anthroic acid, the fluorescence is

Figure 3.10 Normalized emission spectra for aqueous solutions of 2-anthroic acid with (a) acidic/no CD, (b) γ-CD, (c) γ-CD polymer, (d) neutral/no CD
quenched only in the presence of γ-CD. The quenching is probably due to the fact that γ-CD complexes with the 2-anthroic acid.

Conclusion

This chapter proposes the use of monomeric and polymeric CDs to determine mechanisms of excited state proton transfer reactions. Conformations of the inclusion complexes with different CDs are suggested based on fluorescence measurements. It was shown that α-CD cannot include the entire 2-naphthoic acid molecule but can provide protection for the carboxyl group. The β-CD is capable of including the naphthyl group, and hence leaves the carboxyl group exposed to the bulk solution. Furthermore, γ-CD provides a larger cavity and the guest is even more accessible to the bulk solution than in β-CD. Moreover, a comparison between inter- and intra-molecular proton transfer in 1-hydroxy-2-naphthoic acid is shown to provide a good tool for studying structure-function relationships of the photochemically active guest molecules in the host-guest complexes.

For 9-anthroic acid, the data in the literature are complementary rather than contradictory. The observed BF is attributed to monomeric 9-ACA. The formation of ground state dimers and protonation may have similar effects on 9-ACA due to similar restrictions on the carboxyl group. In both cases, the conformation of protonated 9-ACA in the excited singlet state differs from that of the deprotonated form. Both forms of 9-ACA (protonated and deprotonated) have similar coplanar conformation in the ground state. However, it is postulated that only the deprotonated form can twist to a perpendicular conformation in the excited singlet state. Thus, the acid-base equilibrium

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has an indirect effect on the 9-ACA fluorescence by restricting the twist of the carboxyl group in the excited state.

Similar results were obtained for 2-anthroic acid with the different CDs along with their polymers. The α-CD is not capable of including the 2-anthroic acid whereas the β-CD can include the anthracene group. However, the γ-CD is able to fully include the entire molecule. Fluorescence measurements were used to study the effects of the CDs and 2-anthroic acid.

References


Chapter 4. The Effect of the Cyclodextrin Host Isolation Matrix (CHIM) Method on Excited State Proton Transfer Reactions

Introduction

Matrix isolation is a technique used for trapping species as isolated entities in an inert solid, or matrix, in order to investigate their properties, usually by spectroscopic methods. A suitable matrix must, at the temperature of the experiment, be an inert solid, rigid with respect to diffusion, and transparent in the spectral region of interest. Noble-gas crystals, reactive-gas crystals, ionic crystals, and molecular solids can all be used as matrices.

The technique of trapping at low temperatures in an inert solid matrix was originally developed as a means of studying unstable molecular species. Under these conditions the lifetime of a trapped species is considerably increased. The matrix cage severely restricts the occurrence of bimolecular collisions, while the cryogenic temperatures (usually liquid helium) employed, effectively prevent any reaction with an activation energy greater than a few kilojoules per mole. Thus, the trapped species can be studied at leisure using conventional spectroscopic techniques.

G. N. Lewis [1] in 1941, was the first to apply the matrix isolation method when he studied the phosphorescence of low concentrations of various aromatic molecules suspended in a rigid glassy medium at low temperatures. The use of argon or nitrogen as matrix supports for the isolation of molecules and the photoproduction of free radicals was simultaneously proposed by G. Porter [2] and G. C. Pimentel [3] in 1954. The subsequent
development of the technique, particularly in its adaptation to infrared studies, has been primarily pioneered by Pimentel and his associates.

In the matrix isolation technique, a Knudsen cell is used to produce a molecular beam of the high temperature species which is directed at a suitably cooled transparent window. Simultaneously, an excess of inert gas is directed at the same surface. Upon condensation, the high temperature species are trapped in the inert gas crystal lattice. The spectrum of the trapped species is then observed using a suitable instrument.

The isolation of monomeric solute molecules in an inert environment reduces intermolecular interactions, resulting in a sharpening of solute absorptions compared with other condensed phases. Most entrapped species are tightly held, preventing rotation with a few exceptions. The matrix isolation technique has found widespread application in most fields of spectroscopy, particularly infrared (IR), ultraviolet (UV), and electron spin resonance (ESR).

Fredin et al., [4] studied the infrared spectrum of carbon dioxide in solid nitrogen and solid argon. Nitrogen and argon were passed through glass spirals immersed in liquid nitrogen or oxygen, respectively. Concentration dependence and diffusion studies allowed the identification of a carbon dioxide dimer in solid argon. However, no clear evidence of dimer formation was obtained in solid nitrogen.

Milligan and Jacox [5] used matrix isolation to study the ultraviolet spectra of the free radical, formyl (HCO). This radical was the first polyatomic free radical to be stabilized in a matrix in sufficient concentration for direct observation of its infrared
spectrum. These investigators observed an absorption system in the 2100-2600 Å spectral region and assigned it to HCO. This was accomplished by using a carbon monoxide matrix.

The use of matrix isolation for trapping ion-neutral reaction products using ESR was studied by Knight et al., [6]. The ESR magnetic parameters, which reveal structural and electronic bonding characteristics, are useful in the identification of important chemical intermediates and potential interstellar species. Detailed studies probing the concentration effects of carbon monoxide, CO, and its cation, CO⁺, in neon matrices at 4 K demonstrated that ion-neutral reaction products can be stabilized and isolated for spectroscopic investigation. The ethylenedione cation radical, C₂O₂⁺, believed to be formed during deposition by the reaction of CO⁺ and CO, is a potentially important species in the chemistry of the upper atmosphere.

Most of the research efforts involving matrix isolation studies have focused on free radicals and unstable molecules [7-10]. However, the technique can also be used for special examples of matrix-isolated molecules such as clathrates [11]. Clathrates are stable crystal lattices in which guest molecules are entrapped within the cavities of these host lattices. The features which most distinguish these compounds from systems involving species dissolved in inert gas matrices are the stability of the guest crystal involved and the small degree of interaction between the host and guest molecules. Clathrates are of considerable interest since the structures of a number of these molecules have been elucidated by X-ray diffraction studies, which gives exact knowledge as to the size and
shape of the cavity occupied by the trapped species. Thus, the environment and interactions of the guest molecule can be described fairly precisely. An advantage of the clathrates is their ability to be studied at ambient temperatures due to their crystal stability.

Some of the best known examples of clathrates are β-quinol compounds with methane, carbon dioxide, and sulfur dioxide. The first infrared study of clathrates involving β-quinol with HCl, H2S, SO2 and CO2, was reported by Hexter and Goldfarb in 1957 [12]. Another study of the spectra of a large number of mono- and disubstituted benzenes clathrated in Ni11 or Co11 thiocyanate-γ-picoline4 cages was investigated by Casellato and Casu. Cyclodextrins can also be used as matrix-isolated molecules. The Cyclodextrin Host Isolation Matrix (CHIM) method [13] was first used by Agbaria et al. in 1994. This method provides a unique environment that can be applied to mimic the photophysics of the isolated molecule in the gas phase or at low temperatures. Agbaria et al. [13] used the CHIM method to study the photophysical characteristics of 1,N6-ethenoadenosine. Their data from β-CHIM with 1,N6-ethenoadenosine supported the reported observations of multiple emissions from 1,N6-ethenoadenosine. In the CHIM method, the solid samples reflect images of ground state equilibrium in which diffusion processes are most inhibited. Thus, the method is used to simulate the spectroscopy of an isolated molecule. The changes that occur are purely excited state phenomenon. To date, the CHIM method has not been used extensively by researchers.

In theory, static interactions between guest and host molecules may be greater in solid complexes than in dissolved ones. Solid complexes do not dissociate as they do in
solution. Thus, the motions of guest molecules in the toruses of solid complexes are expected to be attenuated. Additionally, the removal of mobile solvent molecules, usually water, around dissolved complexes probably imposes more constraints upon the direction of substrate inclusion in solid complexes.

The interaction of naproxen with β-cyclodextrin in solution and in the solid state has been examined [14]. The complex was prepared by freeze-drying, spray-drying, and kneading. Three-dimensional modeling showed that the naproxen was totally included in the interior of the β-cyclodextrin cavity. Infrared spectroscopy and differential scanning calorimetry were used to characterize the complex in the solid state.

This chapter focuses on the effect of the CHIM method on excited state proton transfer reactions involving several carboxylic compounds. These compounds include 2-naphthoic acid, 1-hydroxy-2-naphthoic acid, 3-hydroxy-2-naphthoic acid, and 2-anthroic acid. The results of the above-mentioned compounds obtained in aqueous solutions were discussed in Chapter 3 and those results will be compared to the solid samples in this chapter.

**Experimental**

**Instrumentation.** Front-face fluorescence measurements were acquired by use of a Spex Model F2T211 spectrofluorometer in the photon counting mode equipped with a thermostated cell housing and a water cooled Hamamatsu R928 photomultiplier tube. Fluorescence emission spectra for 2-naphthoic acid, 1-hydroxy-2-naphthoic acid, 3-hydroxy-2-naphthoic acid, and 2-anthroic acid were acquired using excitation wavelengths...
of 280, 340, 350, and 454, respectively. Excitation and emission slit widths were set for a 5.1 nm bandpass.

**Materials.** The α-, β-, and γ-CDs were a gift from American Maize Products (Hammond, IN) and were used as received. The CD polymers were obtained from CycloIab R&D Laboratory Ltd. (Budapest, Hungary). The 2-naphthoic acid, 1-hydroxy-2-naphthoic acid, 3-hydroxy-2-naphthoic acid, and 2-anthroic acid were purchased from Aldrich Chemical Company (Milwaukee, WI) and were used without further purification.

**Method.** Aqueous stock solutions of the different acids, the CDs, and the CD polymers were prepared. Aliquots of the stock solutions were transferred into appropriate containers in order to obtain concentrations of $1.0 \times 10^{-5}$ M and $1.0 \times 10^{-2}$ M for the acid and CD solutions, respectively. These solutions were allowed to equilibrate overnight. In order to obtain the solid samples, the aqueous solutions were lyophilized or freeze-dried. The solid samples were placed on a laboratory designed support for front-face fluorescence measurement analysis.

**Results and Discussion**

There is a considerable amount of literature involving acid-base properties of excited states [15-19]. It has been established that some organic molecules undergo a change in fluorescence emission wavelength due to the acid-base properties of the excited state species [20,21]. It is also well known that carboxylic acid compounds are weaker acids in the lowest excited singlet state than in the ground state [22]. The molecule, 2-naphthoic acid, is an example of such a molecule. These aromatic carboxylic acids are used as fluorescent probe molecules.
2-naphthoic acid. Broad fluorescence spectra and spectral shifts are characteristics of excited state proton transfer reactions. Figure 4.1 shows the normalized emission spectra of 2-naphthoic acid with α-CD (4.1a) and α-CD polymer (4.1b). From the spectra it can be seen that there are no shifts in the emission maxima or broad fluorescence. However, changes in the relative intensity are observed. The spatial restrictions of α-CD do not allow it to fully accommodate the 2-naphthoic acid compound. Therefore, excited state proton transfer cannot occur in α-CD, evidenced by the lack of a spectral shift in α-CD.

![Normalized emission spectra of 2-naphthoic acid with (a) α-CD and (b) α-CD polymer](image.png)

Figure 4.1 Normalized emission spectra of 2-naphthoic acid with (a) α-CD and (b) α-CD polymer
Figure 4.2 shows that when 2-naphthoic acid is complexed with β-CD, the spectrum is much broader and red-shifted, relative to the β-CD polymer, but blue shifted relative to that in aqueous solution (shown in Chapter 3). These are the only changes observed with this compound. Due to the broad spectrum and red spectral shift in the case of β-CD, it is reasonable to conclude that 2-naphthoic acid is not fully included inside the cavity allowing proton transfer to occur.

Figure 4.2 Emission spectra of 2-naphthoic acid with (a) β-CD and (b) β-CD polymer

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Figure 4.3 shows the emission spectra of 2-naphthoic acid with solid γ-CD and γ-CD polymer. Note that the broad spectrum observed in aqueous γ-CD (Figure 4.4) is now

![Graph showing emission spectra with different conditions](image)

**Figure 4.3** Emission spectra of 2-naphthoic acid with (a) γ-CD and (b) γ-CD polymer

![Graph showing emission spectra of aqueous 2-naphthoic acid](image)

**Figure 4.4** Emission spectra of aqueous 2-naphthoic acid with γ-CD compounds (a) acidic/no CD (b) γ-CD (c) γ-CD polymer (d) neutral/no CD
structured in the solid compound. This again suggests that \( \gamma \)-CD is blocking the protonation process. However, unlike \( \alpha \)-CD, it is assumed that \( \gamma \)-CD can completely include the entire 2-naphthoic acid compound.

The \( \alpha \)-hydroxynaphthoic acids can be used as chelating agents, fluorescent indicators, and in the manufacture of dyestuffs. Therefore, these compounds are of considerable analytical significance. Fluorescence spectroscopy provides a sensitive method for the detection and determination of these compounds. It is interesting to study the differences in the fluorescent properties of these two acids since they both contain two ionizing groups - the carboxyl group and the hydroxyl group. Thus, it is reasonable to conclude that 1-hydroxy-2-naphthoic acid and 3-hydroxy-2-naphthoic acid could undergo intra-molecular proton transfer in the excited state. The CHIM method affords an effective tool in describing the excited state spectroscopic changes that may occur.

1-hydroxy-2-naphthoic acid (1-HNA) and 3-hydroxy-2-naphthoic acid (3-HNA). Figures 4.5, 4.6, and 4.7 show the emission spectra of 1-HNA with \( \alpha \)-CD, \( \beta \)-CD, and \( \gamma \)-CD, respectively, in acidic (4.5a, 4.6a, 4.7a), neutral (4.5b, 4.6b, 4.7b) and basic (4.5c, 4.6c, 4.7c) media. Observations of the solid CHIM samples provide similarities to aqueous solutions. Specifically, 1-HNA in the presence of the three most common cyclodextrins show little dependence on the cyclodextrin cavity. The observations suggest that the intra-molecular ESPT is affected by the cyclodextrins. Such an effect of the CDs on the fluorescence of 1-HNA indicates that the bulk solution plays a role even in intra-molecular ESPT.
Figure 4.5 Emission spectra of 1-HNA with α-CD in (a) acidic, (b) neutral, and (c) basic media

Figure 4.6 Emission spectra of 1-HNA with β-CD in (a) acidic, (b) neutral, and (c) basic media
Figure 4.7 Emission spectra of 1-HNA with γ-CD in (a), neutral (b) acidic, and (c) basic media

Figure 4.8 shows the emission spectra of 3-HNA with α-CD in acidic (4.8a), neutral (4.8b), and basic (4.8c) media. It is interesting to note that the emission maxima undergoes a red shift upon going from an acidic medium to a neutral or basic medium. There appear to be two forms (protonated hydroxyl - OH and deprotonated carboxyl - COO⁻) present in the neutral and basic media, whereas in the acidic medium, one species is present. These results are similar for β-CD (Figure 4.9) and γ-CD (Figure 4.10). In addition, the longer wavelength form is significantly dominant in neutral medium for α-CD and β-CD, whereas, it is dominant in basic medium for γ-CD.
Figure 4.8 Emission spectra of 3-HNA with α-CD in (a) acidic, (b) neutral, and (c) basic media

Figure 4.9 Emission spectra of 3-HNA with β-CD in (a) acidic, (b) neutral, and (c) basic media

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Hirota [23] also found a long wavelength species present in basic solvents and he concluded that intra-molecular proton transfer did occur in the case of 3-HNA. Since the CHIM samples reflect excited state phenomenon, this conclusion is very reasonable. Similar results are obtained in the different media for 3-HNA with α-CD and β-CD suggesting the inner cavity diameter of these cyclodextrins may not be able to fully accommodate the 3-HNA compound and thereby promoting intra-molecular proton transfer. However, when 3-HNA is complexed with γ-CD, the relative intensity is decreased in acidic medium as compared to α-CD and β-CD and the deprotonated form is enhanced in basic medium. This observation suggests that the intra-molecular proton transfer...
transfer process is blocked which is reasonable when noting the larger inner cavity
diameter for γ-CD. The γ-CD cavity is able to fully include 3-HNA.

Dual fluorescence is two distinct fluorescence spectra from a single apparently
homogeneous sample. Dual fluorescence has often been characterized by the formation
of excimers and exciplexes which result from associations between excited and ground
state molecules [24, 25]. When molecular associations are absent, dual fluorescence is
attributed to different conformations of the excited state molecule [26]. The
anthracenecarboxylic acid compounds are examples of molecules which exhibit dual
fluorescence.

9-anthroic acid (9-ACA). The dual emission of 9-ACA was characterized using
the CHIM method. Figure 4.11 shows the emission spectra of 9-ACA in aqueous solution
(4.11a) and solid β-CHIM (4.11b). Although the dominant fluorescence in aqueous
solution is structured fluorescence only, a red-shifted broad fluorescence is observed in
solid β-CD. The broad fluorescence from the β-CHIM of 9-ACA is blue shifted relative
to that in aqueous solution (Figure 4.12). One expects the solid sample to reflect ground
state properties, and indeed both forms have similar broad fluorescence in β-CHIM.
Apparently, twisting of the carboxyl group is not possible in β-CHIM, and thus both forms
yield similar fluorescence. The broad fluorescence peaks in aqueous solution and solid 9-
ACA:β-CD are 475 and 445 nm, respectively. Relaxation processes and solvent effects
may be additional factors in shifting the broad fluorescence further to the red in aqueous
solutions.
Figure 4.11 Fluorescence emission spectra of 9-ACA in (a) aqueous solution (b) solid β-CHIM

Figure 4.12 Fluorescence spectra of 9-ACA in various concentrations of β-CD

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2-anthroic acid (2-ANA). There are significant changes that occur in the solid CHIM samples with 2-ANA. For example, Figure 4.13 shows the emission spectra of 2-anthroic acid with α-CD (4.13a) and α-CD polymer (4.13b). An additional band occurs at a longer wavelength. This additional band also occurs in 2-ANA with γ-CD (Figure 4.14a) but the maxima is slightly blue shifted relative to that in α-CD. These two bands indicate that two forms of the compound are present in the excited state. The values for the pKₐ and pKₐ' for carboxylic acids of polycyclic aromatic hydrocarbons are 4 and 7, respectively [27]. It is clear from the figures that the band at the shorter wavelength is decreased in γ-CD, and the overall relative intensities are lower for 2-ANA in γ-CD. This suggests that 2-ANA is included inside the cavity of γ-CD, whereas, it is too large to fit into the smaller cavity of α-CD. Therefore, 2-ANA appears to undergo inter-molecular

Figure 4.13 Emission spectra of 2-anthroic acid with (a) α-CD and (b) α-CD polymer
Figure 4.14 Emission spectra of 2-anthroic acid with (a) γ-CD and (b) γ-CD polymer

Figure 4.15 Emission spectra of 2-ANA with (a) β-CD derivative (b) β-CD (c) β-CD polymer
proton transfer with α-CD. This seems reasonable since both forms (protonated and deprotonated) of 9-anthroic acid were proposed to exist in CHIM samples. However, these changes are not as clear with β-CD as shown in Figure 4.15. The absence of the additional band at longer wavelengths suggests the blocking of the protonation process by β-CD.

Conclusion

This chapter examines the use of the Cyclodextrin Host Isolation Matrix (CHIM) method to determine the mechanisms of the excited state species of various carboxylic acid compounds. The results obtained with the solid samples were compared to those obtained in aqueous solutions reported in Chapter 3. The CHIM samples mimic the spectroscopy of isolated molecules. Therefore, it is reasonable to conclude that pharmaceutical as well as spectroscopic information can be gleamed from CHIM studies. In the case of α-CD, its inner cavity diameter is too small to accommodate any of the carboxylic acid compounds. However, the cavity of α-CD can be used to block the protonation process by capping the carboxyl group. In addition, inter-molecular and intra-molecular proton transfer were explored with the 1-hydroxy-2-naphthoic acid and 3-hydroxy-2-naphthoic acid compounds. From these studies, it is apparent that the cyclodextrin cavity as well as the size of the compound itself are major factors in predicting the orientation and conformation of the carboxylic acid compounds within the cyclodextrin cavity.
References


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Chapter 5. Summary and Future Research

Summary

The research presented in this dissertation has involved the area of fluorescence spectroscopy and cyclodextrins. Specifically, the application of cyclodextrins along with their polymers and various luminescent probes have been discussed. In addition, the comparison of studies involving solid cyclodextrin and aqueous solutions of cyclodextrin has been addressed. Methods that permit qualitative and quantitative assessment of such organized media is necessary in order to fully realize their wide applicability. The interactions involved in host:guest chemistry is the foundation of molecular recognition and can be used to create models on chemical binding mechanisms. This section of the dissertation will briefly summarize the research and discuss future concerns.

In Chapter 1, the chemical and physical structures of cyclodextrins were reviewed along with their environmental applications. There was also a section that discussed solid cyclodextrin. Similarly, the theoretical and instrumental aspects of fluorescence spectroscopy were discussed. The photochemical concepts of excited state inter- and intra-molecular proton transfer were introduced as well. This chapter was used as the basis for the research discussed in subsequent chapters.

Chapter 2 concerned the extraction of volatile polycyclic aromatic hydrocarbons (PAHs) from air by use of solid cyclodextrin. A comparison study in which β-cyclodextrin was compared by α-cyclodextrin provided evidence that β-cyclodextrin extracts vapor phase PAHs by adsorption interactions. Thus, solid cyclodextrin complexed with vapor...
phase PAHs and thereby reduced their volatilities. The gas-solid interaction of the PAHs with \( \beta \)-cyclodextrin and the effect of \( \beta \)-cyclodextrin on the volatilities of these compounds were discussed. Fluorescence and absorbance spectroscopies were used to examine the variables that affect formation of the PAH complexes with the solid cylcodextrin. In addition, the effect of various alcohols on the gas-solid complexation reactions of PAHs was examined and compared to the results obtained in aqueous solutions. Quantitative measurements for these studies were also presented.

The effect of cyclodextrins on excited state proton transfer reactions of carboxylic compounds was explored in Chapter 3. Fluorescence characteristics of 2-naphthoic acid in aqueous solutions and solid samples of \( \alpha \), \( \beta \), and \( \gamma \) cyclodextrins and corresponding polymers were examined in order to probe inter-molecular excited state proton transfer reactions. Cyclodextrins provided a tool which enables the shift of excited state acid-base equilibrium, depending on the conformation of the host-guest complex of the carboxylic acid with cyclodextrin. Cyclodextrins were utilized in this study in order to add insight to the understanding of excited state proton transfer of aryl carboxylic acids. In contrast, 2-naphthoic acid was used to compare the confined environment induced by the polymeric cyclodextrins relative to that of the monomeric cyclodextrins. An additional study of the intra-molecular proton transfer in 1-hydroxy-2-naphthoic acid was used to compare the induced microenvironment restraints of the cyclodextrins with those observed for inter-molecular proton transfer. The latter is a bimolecular process and is anticipated to depend on access of the acid to the bulk solution, whereas the former is a unimolecular process.
The anthroic acids, 9-anthroic and 2-anthroic acids, were also examined. Cyclodextrins and fluorescence spectroscopy were used to probe molecular processes. It was shown that the dual fluorescence of 9-anthroic acid is dependent on the pH, the solvent, and the concentration of the acid. It was also concluded that acid-base equilibrium was a more plausible explanation for the observed dual emission of 9-anthroic acid rather than formation of dimers. Similar results were obtained for 2-anthroic acid. Again, fluorescence measurements along with monomeric and polymeric cyclodextrins were used to study the excited state mechanisms of this compound.

Chapter 4 concerned the effect of the cyclodextrin host isolation matrix (CHIM) method on excited state proton transfer reactions. The CHIM method involves solid compounds and is based on excited state phenomena. Again, several compounds were examined including 2-naphthoic acid, 1-hydroxy-2-naphthoic acid, 3-hydroxy-2-naphthoic acid, 9-anthroic acid, and 2-anthroic acid. From the results obtained, it appeared that the interactions of the different carboxylic acid compounds with cyclodextrins were dictated by the structural orientation of the molecules as well as inter-/intra-molecular proton transfer. The different mechanisms for the interactions were proposed.

Future Research

The results of the studies discussed in this dissertation give insight into the use of cyclodextrins and fluorescence spectroscopy for various guest molecules. In the case of the extraction of volatile PAHs from air by use of solid cyclodextrin, the system presented was an approach for improved ambient air sampling. It would be interesting to explore
the effects of the solid cyclodextrin when α-, β-, and γ-cyclodextrins are combined on one filter or separated on different filters in a row. Cyclodextrins have been proposed as possible cigarette filters but the smokers did not like the fact that most of the nicotine was filtered out. Variations of the sampling methods proposed here can be potential uses for the improved detection of volatile compounds. In addition, a thorough study of each cyclodextrin with different PAHs is needed.

In regards to the effect of various alcohols on the gas-solid complexation reactions of PAHs, additional probes such as tert-butylamine and n-propylamine could be examined and compared to studies already performed in aqueous solutions. These amine probes were utilized as ternary components in aqueous solutions. Therefore, it would be reasonable to assume that they could be used with the solid cyclodextrin system as well.

A general examination of various third components with a wide class of fluorescent compounds would be very beneficial. This type of investigation may provide information which relates cyclodextrin with guest structure, physical, or chemical properties. In addition, a relationship between modifier and various guest properties may be found. Therefore, the behavior of new CD:guest systems could be predicted based on an evaluation of a wide range of third components and guests.

It would also be beneficial to explore gas-solid interactions of cyclodextrins and PAHs with nitrogen oxides, NOx and sulfur oxides, SOx reactions. The NOx and SOx are potentially dangerous compounds. Low levels of sulfur dioxide may affect the respiratory tract, producing irritation and increasing airway resistance, especially to people with
respiratory weaknesses and sensitized asthmatics. Therefore, exposure to the gas may increase the effort required to breathe. Similarly, nitrogen dioxide can be quite harmful to human health. Specifically, low levels of exposure to nitrogen dioxide causes inflammation of lung tissue whereas death occurs within two to ten days after exposure to high levels of nitrogen dioxide.

The combination of hydrocarbons, nitrogen oxides, and ultraviolet light are key ingredients necessary for the formation of photochemical smog. Moreover, the oxidation of atmospheric sulfur dioxide increases in the presence of hydrocarbons and nitrogen oxides. Therefore, it would be interesting to study the effect of solid cyclodextrin on the reactivities of these reactions. The formation of new products is expected when the polycyclic aromatic hydrocarbons combine with the nitrogen oxides and sulfur oxides. Since the solid cyclodextrin was capable of reducing the volatility of some of the PAHs, it is reasonable to assume the solid cyclodextrin could be used to reduce the reactivities of the PAH reactions with the NO$_x$ and SO$_x$.

For the excited state proton transfer reactions, solid state NMR and semiempirical calculations would be good tests to examine the proposed mechanisms reported here. The NMR measurements should be able to determine the configurations of the solid systems. Fluorescence lifetime measurements of the solid CHIM samples may provide additional information on these solid systems. Furthermore, other guest molecules where the two ionizing groups are in the meta position would allow different mechanisms and reactions to occur. It would be interesting to examine what effect, if any, occurs as a result of this
position. These are only a few suggestions as to the directions of future research for the studies discussed in this dissertation.
Vita

Michelle Butterfield Young was born Michelle Teresa Butterfield in Barnwell, South Carolina, on March 30, 1968. She is the sixth of six children born to Annie Mae Butterfield and the late Earnest James Butterfield. She attended Barnwell High School where she graduated salutatorian in 1986.

In the fall of 1986, she entered Morris Brown College in Atlanta, Georgia. While at Morris Brown College, Michelle worked under the direction of Dr. Leroy Frazier on a project involving the characterization of the alcohol derivative of sulfolane using infrared spectroscopy. She also participated in the Undergraduate Research Program in Chemistry at the Atlanta University Center Science Research Institute where she worked with Vernon Morris on the determination of the optimized geometries, relative energies, and harmonic frequencies of chlorine oxide isomers using ab-initio self-consistent field calculations. She also tutored students in general and organic chemistry in the Department of Chemistry at Morris Brown College. Michelle was a member of the Beta Kappa Chi Honor Society and the vice-president of Delta Sigma Theta Sorority, Incorporated. She was the 1990 Senior Class Treasurer. Michelle graduated Magna Cum Laude with a bachelor of science degree in Chemistry in May 1990.

Following college, she entered the graduate program as a Patricia R. Harris Fellow in the Department of Chemistry at Emory University in Atlanta, Georgia. She worked under the direction of Dr. M. C. Lin where she studied the kinetics of the reaction of CN radicals with allene, butadiene, propylene and acrylonitrile using laser-induced
fluorescence. Michelle received a master of science degree in Physical Chemistry in December 1992.

After graduating from Emory University, Michelle entered the graduate program as a Board of Regents Fellow in the Department of Chemistry at Louisiana State University. Her dissertation work involves spectroscopic studies of cyclodextrin complexation reactions with various guest molecules under the direction of Dr. Isiah M. Warner. Michelle served as secretary of the L.S.U. chapter of the National Organization for the Professional Advancement of Black Chemists and Chemical Engineers for the 1995-1996 term. She was also a member of the American Chemical Society. The list of her publications along with those in preparation appears below:


While at LSU, Michelle has given numerous presentations at professional meetings:


Michelle is currently pursuing her doctorate in chemistry. She will graduate in August 1999. Upon earning the degree of Doctor of Philosophy, she plans to begin her career as a forensic chemist with the Georgia Bureau of Investigation.
Candidate: Michelle Butterfield Young

Major Field: Chemistry

Title of Dissertation: Spectroscopic Studies of Solid State Cyclodextrin Complexation Reactions with Various Guest Molecules

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