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# Cyclodextrin Complexes of Polyaromatic Hydrocarbons in the Presence of Aliphatic Alcohols

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**Abstract.** Pyrene, as a fluorescence probe, has been used to study the cyclodextrin complexation process in the presence of different alcohols. The complex formation as well as the hydrophobicity of the cyclodextrin interior can be significantly influenced by the introduction of alcohols. The alcoholic alkyl group and the primary and secondary hydroxyl groups of the cyclodextrin proved to be the determining factors in the complex formation.

**Key words:** Complex formation, ternary complex, fluorescence enhancement and quenching, Stern-Volmer equation.

## 1. Introduction

Cyclodextrins have been used as complexing agents in a number of various areas of chemistry. Thus, chemical applications of cyclodextrins have expanded into many different research areas such as separations, organic synthesis, stereoselectivity and spectroscopy. An increasing number of recent studies have also reported unique interactions for cyclodextrins with various molecules. Most of these interactions have been attributed to the ability of cyclodextrins to form inclusion complexes with different species.

Several studies have examined this complexation with cyclodextrins and the ability of the complexes to enhance fluorescence and phosphorescence of selected lumophores [1, 2]. This important enhancement phenomenon of cyclodextrins with selected lumophores is ascribed mainly to the compartmentalization and shielding of the excited singlet and triplet species from non-radiative decay processes that can occur in bulk solution. To date, binary cyclodextrin-fluorophore systems have primarily been studied to evaluate luminescence enhancement. Although heavy atoms have been introduced as third components to promote the excited triplet formation in phosphorescence studies, this component is not usually an active participant in determining the complexation properties of the cyclodextrin molecule [3, 4]. Thus, an alternative approach for obtaining information about the compexation process would be a study of the properties of the cyclodextrins in the presence of a third component which readily participates in the complexation process [5].

Pyrene has been found to be a useful fluorescence probe for studying the microenvironment within the cyclodextrin cavity, since it is very sensitive to different quenching processes and microenvironmental changes. Recent studies in our laboratory have described the quenching of pyrene in the presence of cyclodextrins

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[6, 7]. This manuscript describes a systematic study of the formation of ternary complexes of pyrene, aliphatic alcohols and cyclodextrins in aqueous media. In these complexes, the quenching observed during complexation with cyclodextrins is diminished and the pyrene fluorescence is enhanced. In this study, different saturated alcohols in conjunction with 2,6-di-*O*-methyl- and 2,3,6-tri-*O*-methyl-$\beta$-cyclodextrins were used to evaluate the role of the alkyl chain size of various aliphatic alcohols and primary and secondary OH groups of cyclodextrins in the formation of the ternary complex.

The acquired data were analyzed using a modified Stern-Volmer (MSV) equation which has been described previously in the literature [7]. A systematic comparison of the MSV constants is provided along with a special consideration of the changes in the microenvironment in the cyclodextrin cavity. A possible complexation arrangement of the ternary pyrene-cyclodextrin-alcohol complex is proposed.

## 2. Experimental Section

### 2.1. REAGENTS

The alcohols, 1-propanol, 2-propanol and *t*-butanol were obtained from Fisher Scientific company (Fair Town, NJ). Methanol was obtained from American Burdick and Jackson (Muskegon, MI) and ethanol was purchased from Midwest Grain Products (Atchison, KS). All alcohols were ACS grade. Pyrene stock solutions were prepared using the appropriate alcohols and were diluted with deionized water (Continental Water Systems, Atlanta, GA) or with the appropriate concentration of cyclodextrin solution (10-fold) before each experiment. The presence of 10% aliphatic alcohol had increased pyrene solubility such that a concentration of $1.25 \times 10^{-6}$ can be achieved. The compounds, 99+% purity pyrene, $\beta$-, di-*O*-methyl-$\beta$-, tri-*O*-methyl-$\beta$- and $\gamma$-cyclodextrins were obtained from Aldrich Chemical Company and used without further purification. Solutions in equilibrium with the air were used in this study.

### 2.2. APPARATUS AND SOLUTIONS

A Perkin-Elmer 650-10S fluorometer interfaced to an Apple $II^+$ computer is used for fluorescence measurement. Total fluorescence was calculated from the digitized data using an integration program. The integrated fluorescence intensity values were used for determination of the intensity ratios of different alcohol-pyrene-cyclodextrin systems [7]. Fluorescence from pyrene dimers was not observed in any of the experiments. No spectral shifts were observed. Small spectral shifts would not affect the calculation of the total peak areas. Temperature regulation to better than 0.1 °C was achieved using a Brinkman RM6 bath and circulator.

### 2.3. DATA INTERPRETATION

We have previously used a modified form of the Stern-Volmer equation, derived in our laboratory, to describe fluorescence quenching and enhancement [8]. This derivation assumes that the quantum yield of the complex changes. In other words, the quantum yield of the fluorophore decreases in case of quenching and increases

when fluorescence enhancement is observed. If we designate $d$ and $e$ as the factors for quantum yield change of the complexed and free forms of the fluorophore, respectively, we obtain the modified Stern-Volmer equation [7]:

$$\frac{I_0 - I}{I} = \frac{(1-e) + K_{EQ}C(1-d)}{e + K_{EQ}Cd} \tag{1}$$

where $I_0$ and $I$ are the fluorescence intensities of free pyrene solution and complex pyrene, respectively. The parameter $K_{EQ}$ is the equilibrium constant of the complexation and $C$ is the cyclodextrin concentration. In many cases, the change in quantum yield of the free form is negligible. Thus, a simplified form of the equation can be used.

$$\frac{I_0 - I}{I} = \frac{K_{EQ}C(1-d)}{1 + K_{EQ}Cd} \tag{2}$$

All data sets were analyzed by Equation (2) by using Zimmerman's [8] curve fitting method for determining $K_{EQ}$ and $d$ values.

## 3. Results and Discussion

The quenching phenomenon of pyrene in the presence of cyclodextrin has been described previously [6, 7]. These previous studies were conducted in pure aqueous solutions. In mixed alcohol/aqueous systems as reported here fluorescence enhancement has been observed. Typical Stern-Volmer plots of data for fluorescence in the presence of different alcohols are presented in Figure 1. The non-linearity and

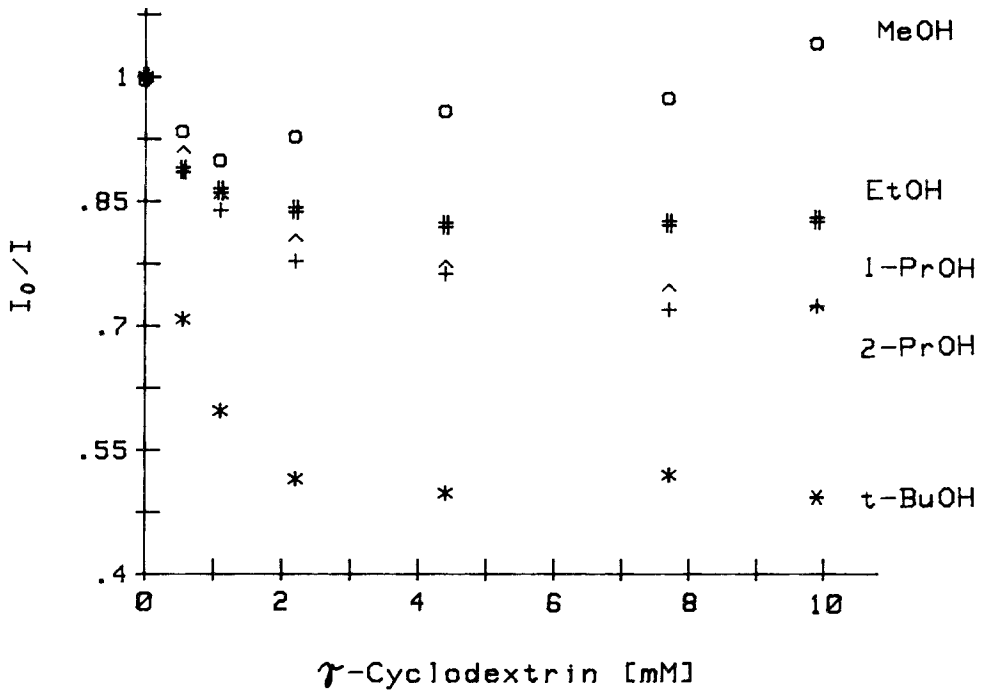


Fig. 1. Stern-Volmer plots of pyrene in the presence of different alcohols. ($1.25 \times 10^{-6}$ M pyrene in 10% alcohol). □ MeOH; # EtOH; ∧ 1-PrOH; + 2-PrOH; * $t$-BuOH.

Table I. Calculated MSV constants of γ-CD in the presence of different alcohols at 24 °C (Pyrene concentration = $1.25 \times 10^{-6}$ M.)

| Alcohol | $K_{EQ}$ | $d$ |
|---|---|---|
| Methanol | 545 | 1.29 |
| Ethanol | 1820 | 1.24 |
| 1-Propanol | 514 | 1.44 |
| 2-Propanol | 803 | 1.42 |
| $t$-Butanol | 1540 | 2.08 |

negative slope of the data indicate a fluorescence enhancement phenomenon. The calculated MSV constants of γ-cyclodextrin in the presence of different alcohols are provided in Table I. The curve fitting was evaluated using $\chi^2$ values as an indication of "goodness-of-fit" [8]. As noted in Table I, the greatest fluorescence intensity enhancement is achieved in the presence of $t$-butanol. The $K_{EQ}$ is the stability constant for the complex: whereas $d$ is an indication of whether the microenvironment is favorable for deactivation of the fluorophore by emission. Consequently, there is no apparent relationship between the two parameters.

Previous studies have used pyrene to evaluate the hydrophobicity of the cyclodextrin cavity since the fluorescence vibronic structure of pyrene depends on the hydrophobicity of the environment [9]. Such can also be done for our data since the pyrene intensities exhibit drastic changes with various cyclodextrin concentration (Table II). The largest relative change in peak intensities is noted for the pyrene/cyclodextrin complex in the presence of $t$-butanol. It is interesting to compare these peak ratios to observed peak ratios of pyrene in solvents of varying hydrophobicity [9]. According to the peak ratios, the hydrophobicity in the cyclodextrin microenvironment is comparable to that of cyclohexane when $t$-butanol is present in the bulk solvent. Other alcohols exhibit similar effects, but the peak ratios indicate a much less hydrophobic environment when compared to the $t$-butanol (Table III). In addition, complexation with γ-cyclodextrin in pure aqueous solvent also changes the polarity of the pyrene microenvironment (Table III). However, the peak ratios indicate a much less hydrophobic environment when alcohols are not present.

Table II. Peak ratios of pyrene fluorescence in the presence of 10% $t$-butanol and γ-cyclodextrin ($1.25 \times 10^{-6}$ M Pyrene)

| γ-cyclodextrin [mM] | III[a] | $V$[a] |
|---|---|---|
| 0 | 0.729 | 0.944 |
| 0.55 | 1.159 | 1.240 |
| 1.1 | 1.307 | 1.345 |
| 2.2 | 1.425 | 1.423 |
| 4.4 | 1.497 | 1.473 |
| 7.7 | 1.518 | 1.487 |
| 9.9 | 1.528 | 1.496 |

[a] Normalized relative to peak I.

Table III. Peak ratios of pyrene fluorescence in the presence of different alcohols and $\gamma$-cyclodextrin. ($\gamma$-Cyclodextrin concentration = $7.7 \times 10^{-3}$ M; Pyrene concentration = $1.25 \times 10^{-6}$ M.)

| Alcohol | III[a] | V[a] |
|---|---|---|
| Methanol | 0.893 | 1.059 |
| Ethanol | 0.994 | 1.129 |
| 1-Propanol | 1.111 | 1.202 |
| 2-Propanol | 1.059 | 1.173 |
| *t*-Butanol | 1.518 | 1.487 |

[a] Normalized relative to peak I.

Similarly, the peak ratios are indicating less hydrophobicity when no cyclodextrin is present in the 10% alcoholic pyrene solution (Table II and Table III). These facts provide a strong indication that the alcohol actually participates in the complexation process as a third component. This theory is also supported by a study of the lifetime changes of pyrene in the alcohol-cyclodextrin system [10].

To determine the possible complexation arrangement of the pyrene-alcohol-cyclodextrin system, some of the cyclodextrin groups must be blocked. Since one possible arrangement of the ternary complex can be derived from interactions between the OH group of the alcohol and the cyclodextrin primary and secondary hydroxyl groups, the use of di-*O*-methyl- and tri-*O*-methyl-cyclodextrins can supply a wealth of information on the complexation arrangement. Di-*O*-methyl- and tri-*O*-methyl-$\gamma$-cyclodextrins are not commercially available. This is unfortunate since the $\gamma$-cyclodextrin cavity size is the most favorable for complexation with pyrene. Nevertheless, $\beta$-cyclodextrin is also able to form a stable complex with pyrene [2]. Accordingly, commercially available *O*-methylated $\beta$-cyclodextrins (2,6-di-*O*-methyl-$\beta$-cyclodextrin and 2,3,6,-tri-*O*-methyl-$\beta$-cyclodextrin) were used in these studies.

Table IV is a summary of the data obtained using these different substituted $\beta$-cyclodextrins complexes of pyrene in the presence of 10% *t*-butanol. Similar interactions of pyrene with $\beta$-cyclodextrin and di-*O*-methyl-$\beta$-cyclodextrin can be noted, since their $K_{EQ}$ and $d$ values as computed using the MSV are similar. Moreover, the peak ratios of pyrene indicate increased hydrophobicity over pure aqueous pyrene/cyclodextrin complexes, equivalent to that of pure ethanol. At the same time, the $K_{EQ}$ and $d$ values of tri-*O*-methyl-$\beta$-cyclodextrin indicate the presence of a quenching process as opposed to an enhancement phenomenon of $\beta$-cyclodextrin and

Table IV. Calculated MSV constants of different $\beta$-cyclodextrins at 24 °C in the presence of 10% *t*-butanol ($1.25 \times 10^{-6}$ M Pyrene.)

| Cyclodextrin | $K_{EQ}$ | $d$ |
|---|---|---|
| $\beta$-cyclodextrin | 6240 | 1.38 |
| Di-*O*-methyl-$\beta$-cyclodextrin | 5890 | 1.36 |
| Tri-*O*-methyl-$\beta$-cyclodextrin | 1670 | 0.96 |

Table V. Peak ratios of pyrene fluorescence in the presence of 10% *t*-buOH and different β-cyclodextrins ($1.25 \times 10^{-6}$ M Pyrene.)

| Cyclodextrin | Concentration [mM] | III[a] | V[a] |
|---|---|---|---|
| β-cyclodextrin | 2.32 | 0.934 | 1.091 |
| Di-*O*-methyl-β- | 2.25 | 0.956 | 1.109 |
| Tri-*O*-methyl-β- | 2.51 | 0.777 | 0.981 |

[a] Normalized relative to peak I.

di-*O*-methyl-β-cyclodextrin. Furthermore, the hydrophobicity of the cavity as indicated by the peak ratios is much less, equivalent to that of glacial acetic acid. It should be noted that the peak ratios of the β-cyclodextrin complex in the absence of *t*-butanol are 0.797 and 0.965, respectively. This value is very close to that of the tri-*O*-methyl-β-cyclodextrin complex in the presence of *t*-butanol.

Based on the observations noted in the previous paragraph, we can infer that the primary and secondary hydroxyl groups of the cyclodextrin are crucial to formation of the ternary complex in the presence of *t*-butanol. The importance of at least

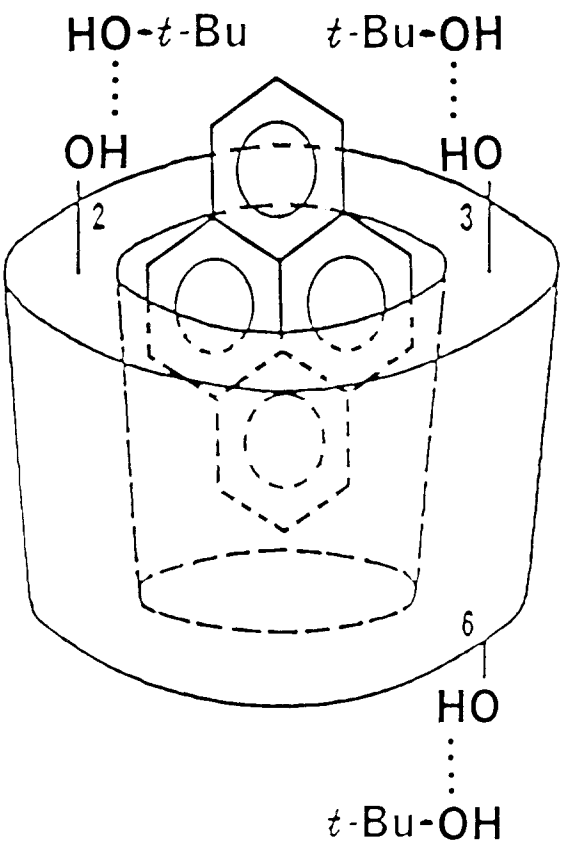


Fig. 2. Possible complexation arrangement of the ternary γ-cyclodextrin–pyrene–*t*-butanol system through the formation of H-bonding. (*Note*: there are 8 of each type of hydroxl group at the edge of the cyclodextrin molecule. All are potential H-bonding sites.)

some of these hydroxyl groups is indicated by the data obtained from the use of tri-*O*-methyl-*β*-cyclodextrin. One possible explanation is that the hydroxyl groups of the alcohols bind to the cyclodextrin hydroxyl groups through hydrogen bonding. Thus, an even more hydrophobic interior forms in the cyclodextrin cavity. Figure 2 is a simplified representation of this complex. For simplicity, only one of the 2,3- and 6-position hydroxyl groups is shown in this figure. In the case of *β*-cyclodextrin, there are seven of each type of hydroxyl group at the cavity edges and all of these are potential hydrogen bonding sites. Some earlier studies [5] have reported the participation of ethanol in the complexation process. It should be emphasized that our present observations do not contradict those studies [5] but represent additional insight into understanding the complex nature of cyclodextrin chemistry.

## 4. Conclusion

The use of fluorescent probes have proved to be one of the most informative approaches in cyclodextrin studies. Fluorescence probes can exhibit quenching or enhancement upon complexation with cyclodextrins. Several studies have evaluated the enhanced or quenched fluorescence of the cyclodextrin–pyrene complexes [2, 7]. However, very few publications have discussed different aspects of the complexation process when a third component participates in the complexation process. This present study has extended these studies to different substituted cyclodextrins and to solutions where different alcohols are present. The equilibrium constants and quantum yield changes of the complexed species have been calculated using a modified form of the Stern-Volmer equation. The participation of alcohols in the complexation process has been evaluated by using *O*-methyl substituted *β*-cyclodextrins. A possible complexation arrangement for this ternary complex has been proposed by assuming hydrogen bonds.

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