A Systematic Study of Pyrene Inclusion Complexes with α-, β-, and γ-Cyclodextrins

4 **AUTHORS**, INCLUDING:

Isiah Warner
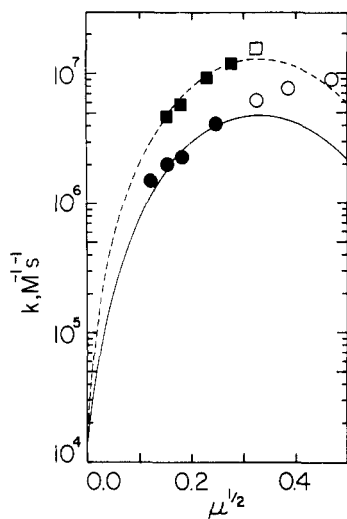Louisiana State University

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Figure 4. Variation of the rate constant k for the $Fe(4,4'-Me_2bpy)_3^{2+,3+}$ exchange reaction with the square root of the ionic strength in acetonitrile at 25 °C. Points represent observed k values from ref 3; ($\bullet$,$\bigcirc$) PF_6^- is the anion; ($\blacksquare$,$\square$) ClO_4^- is the anion; ($\bullet$,$\blacksquare$) used for least-squares analysis. Lines represent calculated functions (eq 14 and 11 with $k_0 = 1.3 \times 10^4$ M^{-1} s^{-1} and $S = -0.5$ M^{-1}): (—) PF_6^- is the anion ($k_1 K_1 = 6.7 \times 10^7$ M^{-1} s^{-1}, $K_1 = 280$ M^{-1}, $a^0 = 6.1$ Å); (---) ClO_4^- is the anion ($k_2 K_2 = 3.4 \times 10^8$ M^{-1} s^{-1}, $K_2 = 890$ M^{-1}, $a^0 = 5.2$ Å). K_1, K_2, and a^0 values are from ref 4. The value of $f_P = 0.1$ was used for the calculated curves.

paper, was used with $a^0 = 6.1$ Å and $K_1 = 280$ M^{-1} for PF_6^- and $a^0 = 5.1$ Å and $K_2 = 890$ M^{-1} for ClO_4^-.[4] The ratio of $k_2 K_2 / k_1 K_1 \approx 5$ is close to the value of ~4 for the $FeL'_3^{3+} + OsL'_3^{2+}$ reaction. The observations that the ratios are >1 can be attributed[4] to the greater degree of association of ClO_4^- than of PF_6^- with FeL'_3^{3+}. At the higher ionic strengths the functions bend over and do not represent the data well.

It is concluded, therefore, for the type of systems investigated and reported here and elsewhere,[3,4] that the $S\mu$ term is a useful approximation to the extended terms[7–9] of the Debye–Hückel activity-coefficient equation for moderate and low electrolyte concentrations ($\mu \le$ ~0.05 M). For higher concentrations, more complex functions for the extended terms are probably required. Also, the approximation of ion association being limited to ion-pair formation between the +3 reactant ion and a single anion may not be valid at the higher anion concentrations. Reaction paths involving several anions may become important due to formation of ion triplets and ion pairs between the +2 reactant and an anion.

The foregoing discussion illustrates the difficulty of extrapolation to zero ionic strength of rate data for electron-transfer reactions between highly charged ions measured at relatively high electrolyte concentrations ($\mu > 0.05$ M), conditions under which many such reactions have been measured.[32] Not only should ion association be taken into account, but extended forms of the activity-coefficient equation should probably be used.

Reliable extrapolations of experimental data to zero ionic strength are necessary for meaningful comparisons of data with theory,[33–35] as was done in previous papers.[4,27] Of course, theoretical predictions at zero ionic strength could be corrected for electrolyte effects for comparison with data under conditions of measurement, but the corrections would need to account for ion association and for deviation of activity coefficients from simple equations, as discussed above.

(32) See, for example, Cannon, R. D. *Electron Transfer Reactions*: Butterworths: London, 1980; p 208.
(33) Marcus, R. A. *J. Chem. Phys.* **1965**, *43*, 679.
(34) Sutin, N. *Prog. Inorg. Chem.* **1983**, *30*, 441–498.
(35) Sutin, N. *Acc. Chem. Res.* **1982**, *15*, 275.

CONDENSED PHASES AND MACROMOLECULES

A Systematic Study of Pyrene Inclusion Complexes with α-, β-, and γ-Cyclodextrins

G. Patonay, A. Shapira, P. Diamond, and I. M. Warner*

Department of Chemistry, Emory University, Atlanta, Georgia 30322 (Received: September 17, 1985)

The quenching process of pyrene in the presence of cyclodextrins is described. A modified Stern–Volmer (MSV) equation is derived for a more accurate description of this process. This approach proved useful for the interpretation of pyrene–cyclodextrin systems at cyclodextrin concentrations below 1×10^{-3} M. In contrast to the standard Stern–Volmer approximation, the predictions of the MSV equation are in good agreement with the experimental data. The oxygen concentration in solution is observed to play a key role in this quenching process.

Introduction

A number of recent studies have reported unique interactions for α-, β-, and γ-cyclodextrins with various molecules. Many of these interactions can be attributed to the ability of cyclodextrins to complex with different inorganic and organic species within their inner cavities. For example, the cyclodextrins have been used as fluorescence or phosphorescence enhancers,[1–3] to purify

structural isomers,[4] or for increasing solubility.[5]

An important parameter for understanding each complexation process is the formation constant of the cyclodextrin–guest system. The measurement of the formation constant of a cyclodextrin–

(1) Turro, N. J.; Sidney Cox, G.; Li, X. *Photochem. Photobiol.* **1983**, *37*, 149.

(2) Nakajima, A. *Spectrochim. Acta* **1983**, *39A*, 913.
(3) Herkstroeter, W. G.; Martic, P. A.; Farid, S. *J. Chem. Soc., Perkin Trans. 2* **1984**, 1453.
(4) Armstrong, D. W.; Stine, G. Y. *J. Am. Chem. Soc.* **1983**, *109*, 2504.
(5) Szejtli, J. *Cyclodextrins and Their Inclusion Complexes*; Akademiai Kiado: Budapest, Hungary, 1982.

guest system can be a fairly simple task. It can be achieved by using chromatographic,[4] spectroscopic,[2] or other methods.[5] Although the spectroscopic approach seems to be the most powerful, the experimentalist has to be extremely careful to avoid certain sources of error. For example, if the luminescence enhancement phenomenon is examined, the sample preparation can be a crucial step.[6]

The enhancement phenomenon of cyclodextrins with selected fluorophores are ascribed mainly to the compartmentalization and shielding of the excited singlet species from quenching and non-radiative decay processes that can occur in bulk solution. To date, only detailed studies of the enhancement phenomenon of cyclo-dextrins have been reported for cyclodextrins and polynuclear aromatic compounds although a recent study has described the quenching of pyrene in the presence of β-cyclodextrin.[6] Thus, an alternative approach for obtaining information about the complexation process would be a study of the properties of the cyclodextrins as quenchers of pyrene. Since pyrene is highly sensitive to different quenching processes, it has proven to be a very useful fluorescence probe for studying microenvironments.

This paper describes a systematic study of the quenching phenomenon of the cyclodextrins with pyrene in aqueous solution. The study is conducted over a wide range of concentrations and temperatures to provide detailed information about the complexation process. A modified Stern–Volmer (MSV) equation is derived which provides a more accurate description of the quenching process of pyrene in the presence of cyclodextrins than the conventional Stern–Volmer (SV) equation. A systematic comparison of the MSV with experimental results is provided along with special cases of the derived equation.

Modified Stern–Volmer Equation

A variety of processes can result in decreased fluorescence intensity of a given fluorophore. Two of the most important of these processes are dynamic and static quenching. Many quenching processes can be accurately described by using a mathematical relationship between the fluorescence intensity change and the concentration of the quencher. Knowledge of this relationship provides important information about the quenching process itself. In this paper, we are primarily concerned with quenching which results from complex formation in the ground state, i.e., static quenching. Thus, the one-to-one complexation reaction for pyrene (P) and α-, β-, or γ-cyclodextrin (C) may be represented by[7]

$$P + C \rightleftharpoons PC \tag{1}$$
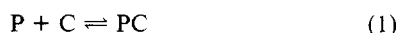
The equilibrium expression for this reaction is given by

$$K_{eq} = [PC]/[P][C] \tag{2}$$

where [PC], [P], and [C] are respectively the concentrations of the inclusion complex, the free pyrene, and the free cyclodextrin. If [P_0] is defined as the initial concentration of pyrene, then the complexed fraction, a, can be defined as

$$a = [PC]/[P_0] \tag{3}$$

The fluorescence intensities of the free pyrene solution (I_0) and complexed pyrene (I) are then given by

$$I_0 = K[P_0] \qquad I = K(1 - a)[P_0] \tag{4}$$

where K is a proportionality factor for instrumental artifacts such as detector response and geometry of detection. The ratio of the fluorescence intensities can be calculated as

$$\frac{I_0 - I}{I} = \frac{Ka[P_0]}{(1 - a)K[P_0]} = \frac{a}{1 - a} = K_{EQ}C \tag{5}$$

This equation gives the well-known Stern–Volmer relationship for static quenching. In the above derivation, it is assumed that quenching occurs as a result of the formation of a nonfluorescent

complex between the fluorophore and the quencher.[7] Although the formation of a ground-state dark complex is an obvious approach, an equally plausible explanation is a change in the quantum yield of the complex. In our derivation, we do not assume the existence of a dark complex, but a general change in the quantum yield. Obviously, the formation of a dark complex will be a special case of quantum yield change. Furthermore, we can assume even a more general case where not only the complexed form has a different quantum yield, but the free pyrene is also affected by interactions with free and complexed cyclodextrin (static or dynamic interactions). Thus, a quantum yield change is also predicted for free pyrene. Considering this alternative approach, a more general description of the pyrene–cyclodextrin system is possible. If we designate d and e as the factors for the quantum yield change of the complexed and free forms, respectively, then the fluorescence intensities of the free pyrene and complexed pyrene are respectively given by

$$I_0 = K[P]_0 \qquad I = K[P_0]\{e(1 - a) + ad\} \tag{6}$$

The ratio of the fluorescence intensities can then be expressed as

$$\frac{I_0 - I}{I} = \frac{(1 - a)(1 - e) + a(1 - d)}{e(1 - a) + ad} \tag{7}$$

Since $a/(1 - a) = K_{EQ}C$ we can arrange eq 7 to give

$$\frac{I_0 - I}{I} = \frac{(1 - e) + K_{EQ}C(1 - d)}{e + K_{EQ}Cd} \tag{8}$$

Equation 8 provides our modified Stern–Volmer equation (MSV) in the presence of cyclodextrins. This equation incorporates both types of quantum yield changes (increase or decrease) for the complexed and noncomplexed pyrene.

It is interesting to examine special cases when the quantum yield change of the noncomplexed or complexed pyrene reaches special values of interest (i.e., 0 or 1).

Case I. No Quantum Yield Change in Free Pyrene. If there is no quantum yield change in free pyrene, then $e = 1$ and eq 8 is reduced to

$$\frac{I_0 - I}{I} = \frac{K_{EQ}C(1 - d)}{1 + K_{EQ}Cd} \tag{9}$$

Since the formation constants for pyrene in cyclodextrin systems are on the order of a few hundred,[4-7] eq 9 describes a fairly linear relationship in the concentration range of $<10^{-3}$ M.

Case II. No Quantum Yield Change of Complexed Pyrene. For this special case where $d = 1$, the fluorescence intensity ratio is given by

$$\frac{I_0 - I}{I} = \frac{1 - e}{e + K_{EQ}C} \tag{10}$$

This equation describes a nonlinear relationship between $(I_0 - I)/I$ and pyrene concentration.

Case III. Nonfluorescent Complex Formation. If a dark complex is formed ($d = 0$), we obtain the traditional SV equation (eq 5).

Case IV. Nonfluorescent Free Pyrene. If the outside pyrene does not fluoresce ($e = 0$), we have

$$\frac{I_0 - I}{I} = \frac{1 + K_{EQ}C(1 - d)}{K_{EQ}Cd} \tag{11}$$

Equation 11 gives a nonlinear relationship between $(I_0 - I)/I$ and pyrene concentrations. It is interesting to note that if the quantum yield change of the free pyrene is not negligible, a nonzero intercept is predicted for the data. A positive intercept indicates a quantum yield decrease for the free pyrene. Extrapolation of the MSV equation to zero concentration of the cyclodextrin gives

$$\frac{I_0 - I}{I} = \frac{1 - e}{e} \tag{12}$$

Thus, knowing the intercept, the quantum yield change of the free pyrene (e) can easily be calculated by using eq 12. In addition,

(6) Patonay, G.; Rollie, M. E.; Warner, I. M. *Anal. Chem.* **1985**, *57*, 569.
(7) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum: New York, 1983.

TABLE I: Total Fluorescence of 5×10^{-8} M Pyrene Solution in the Presence of β-Cyclodextrin

β-cyclo-dextrin concn, mM	rel total fluores-cence	β-cyclo-dextrin concn, mM	rel total fluores-cence
0	100	1.12	73.7
0.298	94.2	1.27	70.4
0.430	87.7	1.41	69.8
0.623	82.2	1.53	67.4
0.917	77.9		

knowledge of the value of a allows determination of $K_{EQ}(1 - d)$ or $K_{EQ}d$. We can designate these values as MSV constants. Unfortunately, K_{EQ} cannot be calculated by using the MSV equation alone unless one of these parameters is known. For example, the quantum yield change of the complexed pyrene can be predicted by using K_{EQ} values from other sources.[4,8]

Experimental Section

Fluorescence measurements were obtained by using a Perkin-Elmer 650-10S fluorescence spectrophotometer interfaced to an Apple II+ computer. This combination allowed storage of the entire emission or excitation spectrum on disk in digitized form with 12-bit resolution using 256 equally spaced points for spectral representation. Each of these data points consists of an average of three consecutive measurements. Storage of the entire emission or excitation spectrum in digital form provides several advantages, especially if the spectrum undergoes changes. For example, the experimentalist can calculate the true quantum efficiency ratios. Quantum efficiency ratios were calculated by integration of the fluorescence spectral area. If one were to use only fluorescence intensity at a given wavelength to calculate these changes, it should be obvious that the value obtained is wavelength dependent if the spectrum undergoes changes.

Temperature regulation to better than 0.1 °C was achieved by using a Brinkman RM6 bath and circulator. All solutions were equilibrated for 2 h in the bath before transferring to the fluorescence cell. The cell holder was also thermostated at the desired temperature by using the circulator.

Pyrene solutions were prepared fresh each day with deionized water (Continental Water Systems, Atlanta, GA). The compounds, 99+ purity pyrene and α, β-, and γ-cyclodextrins were obtained from Aldrich Chemical Co. and used without further purification. The procedure for preparation of solutions has been described previously.[6] Solutions in equilibrium with the air and deaerated with ultrapure N_2 were used in this study.

The total fluorescence has been calculated from the digitized data by using an integration program. Broad excimerlike fluorescence was not observed. Thus, the possible presence of pyrene aggregates in the solution was discounted. Very small (<1 nm) spectral shifts were observed. This spectral shift does not affect the calculation of the total peak area. Little or no absorption by the cyclodextrin solutions was observed at the excitation/emission wavelengths of pyrene.

Results and Discussion

Typical data for the change in the total fluorescence of 5×10^{-8} M pyrene solution in the presence of β-cyclodextrin at 25 °C is presented in Table I. Sample solutions in equilibrium with the air were used in this experiment. As is obvious from Table I, increasing β-cyclodextrin concentration results in decreasing total fluorescence intensity. This suggests the presence of some quenching process. The formation of a dark complex would be an obvious approach to explaining this quenching process. However, this possibility may be discounted by examining special cases of our MSV equation.

Case I (eq 8) describes a fairly linear relationship in the concentration range used in this study. This linearity explained why our data provide a fairly good fit with the traditional Stern–Volmer equation. One of the advantages of eq 8 and 9 when compared

(8) Hirai, H.; Toshima, N.; Venoyama, S. *Polym. J.* **1981**, *13*, 607.
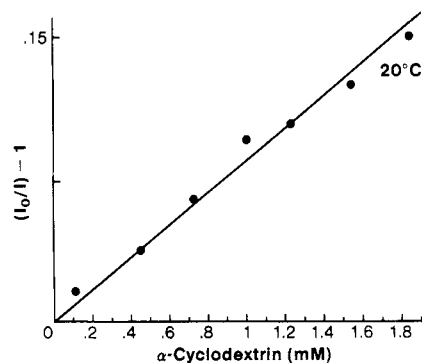
Figure 1. Quenching of pyrene in the presence of α-cyclodextrin.
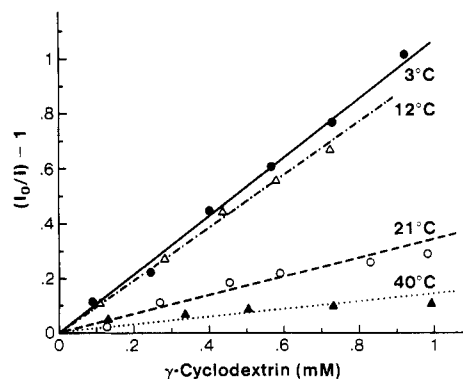
Figure 2. Quenching of pyrene in the presence of γ-cyclodextrin at different temperatures.

TABLE II: Modified Stern–Volmer Constants of Cyclodextrin–Pyrene Systems at Various Temperatures[a]

T, °C	K, M^{-1}		
	α-CD	β-CD	γ-CD
3	286		1029
12	163		942
21	78	277	399
30	~10	65	81
40	~0	~20	53
52	~0	~0	~0

[a] Pyrene concentration: 5×10^{-7} M.

to eq 5 is that the spectral change can be explained. Case II and case IV give a nonlinear relationship and can be ruled out on the basis of our observed data. The form of the MSV given by eq 10 and 11 deviates significantly from the observed data such that we can assume that the quantum yield change of the complexed pyrene is not negligible and the free pyrene is not dark. Thus, the only possible explanation is by use of the form of the MSV given by eq 8 or 9.

At higher concentrations of pyrene, a similar trend as in Table I was noted. The modified Stern–Volmer equation (MSV, eq 8) was used to evaluate the data. Extrapolation of the MSV plot to zero cyclodextrin concentration should provide an estimate of the quantum yield change of the free pyrene at different temperatures for α-, β-, and γ-cyclodextrins according to eq 12. It is interesting that this value does not change drastically with variations in temperature or the size of the cyclodextrin cavity, although the run to run reproducibility is poor. It should also be noted that this value is assumed to be unrelated to the quantum yield change of the pyrene incorporated inside of the cyclodextrin cavity.

Figure 1 provides a Stern–Volmer plot in the presence of α-cyclodextrin at room temperature. Since the plot represents a good linear relationship, it is reasonable to assume that the $K_{EQ}Cd$ term is negligible in the denominator of the MSV equation. This is obvious if we estimate the MSV constants. Figure 2 provides a plot of the temperature dependence of pyrene quenching in the presence of γ-cyclodextrin.

TABLE III: Modified Stern–Volmer Constants of γ-Cyclodextrin–Pyrene System with N_2 Purging at Various Temperatures[a]

T, °C	K, M^{-1}	T, °C	K, M^{-1}
a. Deoxygenated N_2		b. N_2	
3	513	4	751
12	500	14	638
20	330	25	272

[a] Pyrene concentration: 5×10^{-7} M.

TABLE IV: Modified Stern–Volmer Constants of γ-Cyclodextrin–Pyrene System in the Presence of 2×10^{-3} M α-Cyclodextrin at Various Temperatures

T, °C	K, M^{-1}	T, °C	K, M^{-1}
3	531	30	~20
12	209	40	~0
21	102		

Table II shows the modified Stern–Volmer constants of pyrene in the presence of α-, β-, and γ-cyclodextrins at various temperatures. Table III contains the modified Stern–Volmer constants of the γ-cyclodextrin/pyrene system in the absence of oxygen (N_2 purging). All solutions were purged with ultrapure N_2 gas for 2 h. This ultrapure N_2 was further deoxygenated by both bubbling through Cr^{2+} solution[9] and/or used as received (Table III) in order to evaluate the effect of possible oxygen contamination of the N_2 gas.

As we discussed earlier, the formation of a dark complex is not probable due to the observed spectral changes in the presence of cyclodextrins, although the data do fit well to the traditional Stern–Volmer equation. Similarly the sole quenching process of he free pyrene fraction has to be ruled out due to the nonlinear behavior of eq 10 and 11. The most probable explanation is a decreased quantum yield of the complexed pyrene as well as some decreased yield of the free pyrene fraction.

It is interesting to note that the α-cyclodextrin exhibits a fairly strong quenching phenomenon in spite of its relatively small cavity size (5.7 Å). Although the α-cyclodextrin is not able to include the pyrene molecule (due to small cavity size), the MSV constants are fairly high (Table II). The larger the cavity size, the larger the MSV constants, indicating that the quenching process is likely related to the complexation process. Decreasing values of the MSV constants with increasing temperature suggest a static type of quenching. The α-cyclodextrin is able to reduce the effect of γ-cyclodextrin. This is evidenced by the much lower MSV constants in the presence of 2×10^{-3} M α-cyclodextrin (Table IV).

(9) Rollie, M. E.; Ho, C.-N.; Warner, I. M. *Anal. Chem.* **1983**, *55*, 2445.

Although the determination of the possible quenching mechanism present in the cyclodextrin–pyrene system is beyond the scope of this article, preliminary studies have been performed to evaluate this aspect. It is interesting to note that our data (Table III) demonstrates an important role for oxygen in the quenching process. The MSV constants are considerably lower if oxygen is not present. The behavior in the presence of oxygen can be explained if we assume an equilibrium such as

$$Cy + O_2 \rightleftharpoons CyO_2Py \rightleftharpoons Py + Cy \tag{13}$$
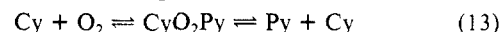
In this case, the oxygen can play an important role as an additional or primary quencher in the cyclodextrin cavity. Since the pyrene concentration in aqueous solution is much less than the oxygen concentrations, we can expect all of the complexed pyrene to be affected by oxygen. It should be noted that oxygen is probably present in the solid cyclodextrin as complexed oxygen. It is probably very difficult to remove such oxygen by purging. This bound oxygen can induce, at least partly, the observed quenching of pyrene. Another possible explanation is to assume that impurities other than O_2 can be present in the cyclodextrin cavity. Future studies will explore this possibility by successive recrystallization of the cyclodextrins.

Summary and Conclusion

Several studies have reported enhanced fluorescence of different fluorophores in the presence of cyclodextrins. To date, only the enhancement phenomenon of cyclodextrins has been evaluated with regard to the complexation process of different polynuclear aromatic compounds and cyclodextrins. An earlier study in our laboratory reported[6] the quenching of pyrene in the presence of β-cyclodextrin. The present study extends this earlier work to different cyclodextrins and to a wide temperature range. The results led to the derivation of a modified Stern–Volmer equation which provides a more accurate description of the quenching process of pyrene in the presence of cyclodextrins. The larger the cavity size, the larger the observed quenching. The equilibrium constants have been calculated for α-, β-, and γ-cyclodextrin-pyrene complexes at different temperatures. It is shown that the association constants cannot be determined based solely on the Stern–Volmer constants.

Registry No. α-Cyclodextrin, 10016-20-3; β-cyclodextrin, 7585-39-9; γ-cyclodextrin, 17465-86-0; pyrene, 129-00-0.