# The Quenching of Lumichrome Fluorescence by β-Cyclodextrin: Evidence for Inclusion Complex

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The formation of inclusion complex between lumichrome and β-cyclodextrin was studied by fluorimetric and solubility methods. The accessibility of lumichrome to the iodide ion in presence of β-cyclodextrin was also studied. The equilibrium constant of formation of this inclusion complex, $K$, determined by fluorometric method and solubility method is 966 and 491 mol dm$^{-3}$, respectively.

Cyclodextrins (CD), because of their torus like shape, are known to form inclusion complexes with various other molecules[1—3] and thereby modify the photochemical and other properties of the molecules.[4—6]

Flavins are blue light absorbers[7] and are photobiologically active class of compounds which initiate phototropism,[8,9] sensitize photooxidation of eye lens proteins[10] etc. The photophysical properties of lumichrome (LC), an important member in flavin family, in microheterogeneous environment are receiving attention. In this paper we report the formation of inclusion complexes between LC and β-CD.

## Experimental

**Materials:** β-CD and LC purchased from Sigma Chemical Co., USA were used without further purification. Potassium iodide was obtained from E. Merck (India). Glass distilled water was used for preparing solutions.

**Measurements:** Fluorescence spectra were measured with a Perkin–Elmer MPF-44B Spectrofluorometer equipped with a Julabo F20-UC temperature controller. Aqueous solutions were used in all cases.

Life time measurements were performed on an applied photophysics single photon counting set up using a pulsed nitrogen lamp ($\lambda_{ex}=337$ nm).[11] The decay curve of the fluorescence intensity of LC was monitored at 464 nm at 24 °C both in the presence and in the absence of β-CD. Absorption spectra were recorded in Perkin–Elmer 5506 UV-vis Spectrophotometer.

To determine the solubilities, aqueous solutions of β-CD of different strengths were saturated with LC in 6 M HCl, followed by immediate neutralization of the mixture. pH of the solution was maintained at 6.2 using Elico LI 120 pH-meter. Each filtrate was diluted ten times and absorbance of the solution was measured at 350 nm.

## Results and Discussion

**(1) Solubility of LC in the Presence of β-CD.** The solubility of LC in water increases with increase in the concentration of β-CD in the solution as shown in Fig. 1. This is an indication of the formation of ground state complex between LC and β-CD.[12] The increase in solubility of LC in β-CD may be due to the fact that LC, having very low solubility in water seeks the hydrophobic environment inside β-CD torus. However, we did not find any significant change in absorption spectra of LC on the addition of β-CD.

**(2) The Fluorescence Property of LC in the Presence of β-CD.** The fluorescence of aqueous solutions of LC is quenched by the addition of β-CD as shown in Figs. 2 and 3. Figure 2 also shows that emission band of LC undergoes a small blue shift on complexation. The change of fluorescence intensity of LC by β-CD is in confirmity with the observation that the

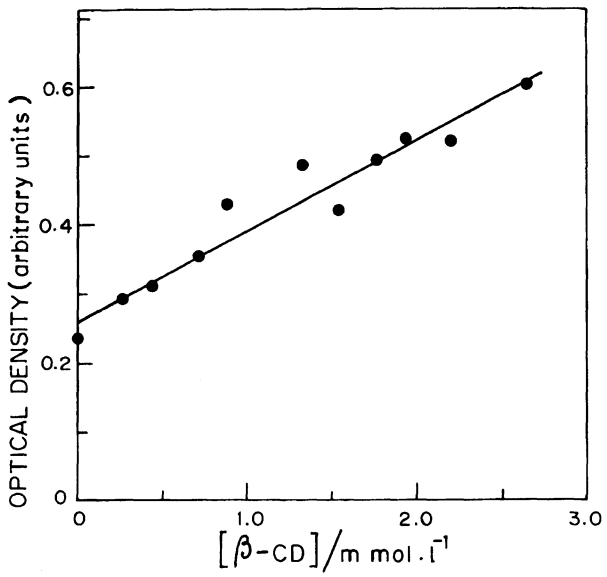


Fig. 1. Solubility of lumichrome in aqueous β-CD solution of different concentrations.

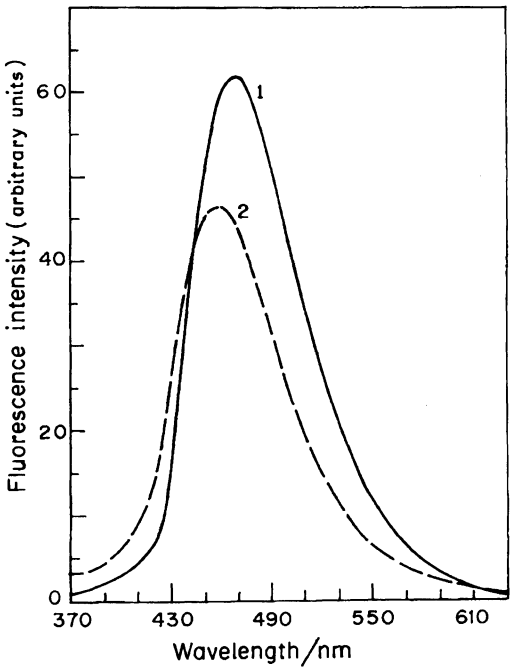


Fig. 2. Fluorescence spectra of lumichrome in (1) absence of β-CD : continuous line; (2) presence of $4\times10^{-3}$ mol dm$^{-3}$ β-CD : dashed line. $\lambda_{ex}=354$ nm.

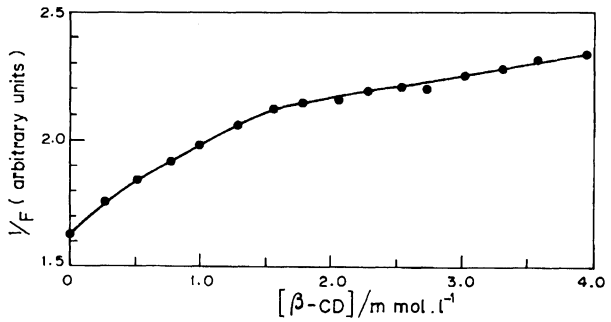


Fig. 3. Stern–Volmer type plot of lumichrome fluorescence quenching by β-CD in aqueous solution.

fluorescence intensity may increase[13,14] or decrease[15] on complexation with β-CD.

**(3) Life-Time Measurements.** The fluorescence life time of LC in aqueous solution is found to be 2.38 ns. This is in close agreement with the reported value of 2.4±0.3 ns.[16] In the presence of β-CD, the measured life times remain constant within experimental error. From these considerations we can conclude that only static interactions are involved.

**(4) The Accessibility of LC to the Quencher $I^-$ in the Presence of β-CD.** If LC forms an inclusion complex with β-CD, its accessibility to any quencher should be low. We have probed this by measuring the Stern–Volmer constant of quenching of LC fluorescence by iodide ion at different β-CD concentration, concentration range of iodide being 0—40 mmol dm$^{-3}$. A graph of $K_{sv}$ vs. β-CD concentration is plotted in Fig. 4. We observed that addition of β-CD lowers the $K_{sv}$ value i.e., restrict the accessibility of LC thus indicating formation of inclusion complex. We also observed that at lower concentrations (<2 mmol dm$^{-3}$) of β-CD, $K_{sv}$ decreases rapidly but at higher concentrations (>2 mmol dm$^{-3}$) the decrease of $K_{sv}$ slows down. This indicates that more and more LC molecules are being 'capped' into the inclusion complex as β-CD concentration is increased but after a β-CD concentration of 2 mmol dm$^{-3}$ very few LC molecules are left free and $K_{sv}$ thus becomes insensitive to the variation of β-CD concentration in that range.

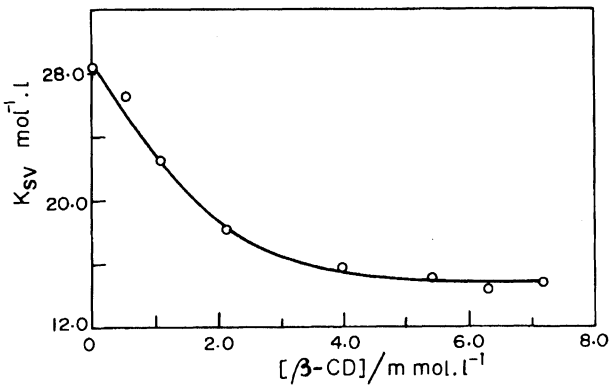


Fig. 4. The effect of β-CD on the $K_{sv}$ of lumichrome fluorescence quenching by iodide ion.

**(5) Determination of Equilibrium Constants.** In the absence of dynamic quenching the formation of inclusion complex between LC and β-CD may be expressed by Eq. 1 assuming 1 : 1 stoichiometry.

$$\text{LC} + \beta\text{-CD} \rightleftharpoons \text{LC–}\beta\text{-CD}. \quad (1)$$

The equilibrium constant ($K$) may be determined from the equation[17]

$$\frac{(1-\frac{F}{F_o})}{C_b^\circ} = (1-\frac{F_o'}{F})K - K(1-\frac{F}{F_o}), \quad (2)$$

Where $F_o$, $F_o'$, $F$ are the fluorescence intensities of LC solution, solution of totally complexes LC with β-CD, and experimental solution respectively and $C_b^\circ$ is the concentration of β-CD. A plot of $(1-\frac{F}{F_o})/C_b^\circ$ against $(1-\frac{F}{F_o})$ is shown in Fig. 5. The plot is found to be a straight line indicating the validity of our assumption. The equilibrium constant K=966 mol$^{-1}$ dm$^3$ at 22 °C is obtained from the slope of the line with correlation coefficient, $r=0.986$.

The equilibrium constant can also be determined from solubility data shown in Fig. 1. Since the molar absorbance of LC does not change on addition of β-CD, the change in optical density of LC on addition of β-CD is proportional to the concentration of the inclusion complex formed in solution. The equilibrium constant $K$, for the formation of inclusion complex may be obtained from the relation

$$OD_{mix} = OD_{LC} + OD_{LC}\, K\, C_b^\circ, \quad (3)$$

where $OD_{mix}$ and $OD_{LC}$ stand for the optical densities

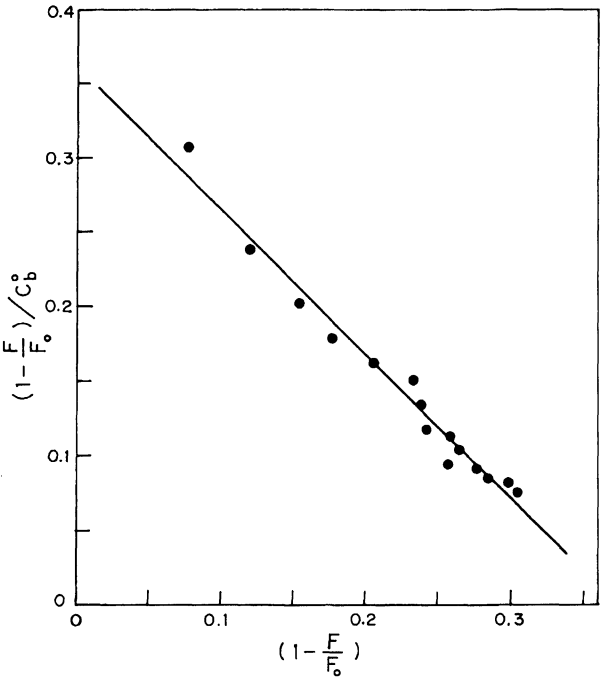


Fig. 5. Plot of $[1-(F/F_o)]/C_b^\circ$ against $(1-\frac{F}{F_o})$ according to Eq. 2.

of the saturated LC solution in the presence and in the absence of $\beta$-CD respectively and $C_b^\circ$ is the initial concentration of $\beta$-CD. The plot of OD values as a function of $\beta$-CD concentration gives a reasonably good straight line (Fig. 1) with correlation coefficient $r=0.965$. The value of $K$ estimated from the slope and the intercept is 491 $mol^{-1}\,dm^3$ at 26 °C. This is in the same order of magnitude with $K$ value determined fluorometrically.

These observations confirm that LC forms an inclusion complex with $\beta$-CD in aqueous solution.

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