Kinetics and Thermodynamics of the Formation of Inclusion Complexes between Cyclodextrins and DNA-intercalating Agents. Inclusion of Ellipticine in γ-Cyclodextrin

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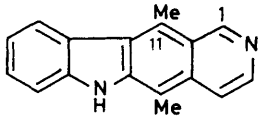
Ellipticine (E) seems to be included in two steps by two γ-cyclodextrin molecules. The first inclusion is detected by changes in the absorbance spectrum of ellipticine with added cyclodextrin. It occurs from the pyridine side of the molecule to form E–CD, the first inclusion complex, with a dissociation constant $K_1 = 9.80 \times 10^{-5}$ mol dm^{-3} and a *T*-jump-measured second-order rate constant $k_1 = 3.6 \times 10^6$ dm^3 mol^{-1} s^{-1}. E–CD is then included by another γ-cyclodextrin molecule to form E–CD$_2$, the second inclusion complex. As evidenced by changes in the absorbance and fluorescence spectra of E, this second inclusion occurs from the indole side of the molecule with a dissociation constant $K_2 = 1.85 \times 10^{-3}$ mol dm^{-3} and with a second-order rate constant $k_2 > 10^7$ dm^{-3} mol^{-1} s^{-1}. These results may be of interest in chemotherapy based on the use of planar DNA-intercalating agents.

Ellipticine—a natural plant alkaloid[1]—and its synthetic derivatives are DNA-intercalating planar pyridocarbazole systems[1,2] which are known to inhibit the replication of DNA and the transcription of RNA both *in vitro* and *in vivo*.[3] Some of these derivatives display antitumour properties and exhibit high cytotoxic activity against various tumour cells including breast cancers, renal-cell carcinoma, small and non-small lung cancer cells, and osteolitic breast cancer metastasis.[4]



The two major problems in the use of neutral ellipticines as pharmaceuticals are their toxicity and their lack of solubility in aqueous media.[5,6] This lack of solubility can be overcome by quaternization of N-2 which, however, inhibits the antitumour activity while decreasing the toxicity.[2] We therefore tried to improve the solubilization of ellipticine in water by using cyclodextrins which are good molecular receptors for planar and aromatic rings,[7] and, therefore, are used to solubilize their guest molecules.[8] Cyclodextrins are oligosaccharides formed by α(1–4)-linked glucose units arranged into a left-threaded screw of 6, 7, or 8 glycopyranose units.[7] These molecular receptors or hosts have an internal cavity which can include guest molecules, of adapted shape and size,[7] by Van der Waals' interactions, hydrogen bonding, and hydrophobic interactions.[9] Moreover, cyclodextrins and especially their derivatives are involved in several catalyses where they act as enzyme-like models.[10] This makes the interaction of planar drugs with those polysaccharides an interesting problem, investigation of which can lead to new approaches in drug design and in therapy where the cyclodextrins could, eventually, be used as specific drug carriers.[7]

In this work, we report a kinetic and thermodynamic study of the inclusion of ellipticine (E) in γ-cyclodextrin or cyclo-octa-amylose (CD).

Experimental

Chromatographically pure ellipticine and methylellipticinium were provided by Dr E. Lescot from the Gustave-Roussy Institute. Buffer solutions (pH 9.18) were Beckmann NBS standards. KCl (Merck suprapur), HCl (Merck titrisol), ethanol (Aldrich gold label), and α-, β-, and γ-cyclodextrin (Janssen) were pure-grade reagents and were used without further purification.

Stock Solutions.—To avoid the protonation of ellipticine which occurs with a p*K* of ~7.4, the cyclodextrin solutions (1×10^{-4} to 5×10^{-2} mol dm^{-3}) were prepared in NBS pH 9.18 buffer, the ionic strength of which was adjusted to 0.2 mol dm^{-3} with KCl. Fresh solutions of ellipticine (1 mg cm^{-3}) were prepared in ethanol, and were afterwards injected—by microcaps—into a u.v. cell containing the cyclodextrin solution (final ellipticine concentrations ranging from 1×10^{-6} to 1×10^{-5} mol dm^{-3}). This technique was used to avoid precipitation and the possible adsorption of ellipticine at the inner surface of the u.v. cell.[6] Above a certain concentration (~5–6 $\times 10^{-6}$ mol dm^{-3}) the absorbance of ellipticine decreases (by *ca.* 25% at 10^{-5} mol dm^{-3}) as a function of the stirring rate of the solution. This absorbance decrease does not affect the general aspect of the u.v. spectrum of ellipticine.[6] Under our experimental conditions ($< 5 \times 10^{-6}$ mol dm^{-3} of ellipticine), the absorbance of ellipticine alone (without any cyclodextrin) may decrease by *ca.* 1–6%. In the presence of γ-cyclodextrin (for [CD] $\geqslant 1 \times 10^{-4}$ mol dm^{-3}) this absorbance decrease becomes negligible. Moreover, the u.v. spectrum of ellipticine alone was measured in buffer containing 4% alcohol where the absorbance was found to be unaffected. The spectrum is, however, slightly modified in a 10% alcohol solution. However, all the spectra of ellipticine with γ-cyclodextrin used for the static measurements were recorded in alcohol-free aqueous media. In the fluorescence experiments, the intensity of fluorescence (I_F) of ellipticine is very weak compared with that of the final ellipticine–cycloedextrin complex. Therefore, the uncertainity in the I_F-value of ellipticine hardly affects our results.

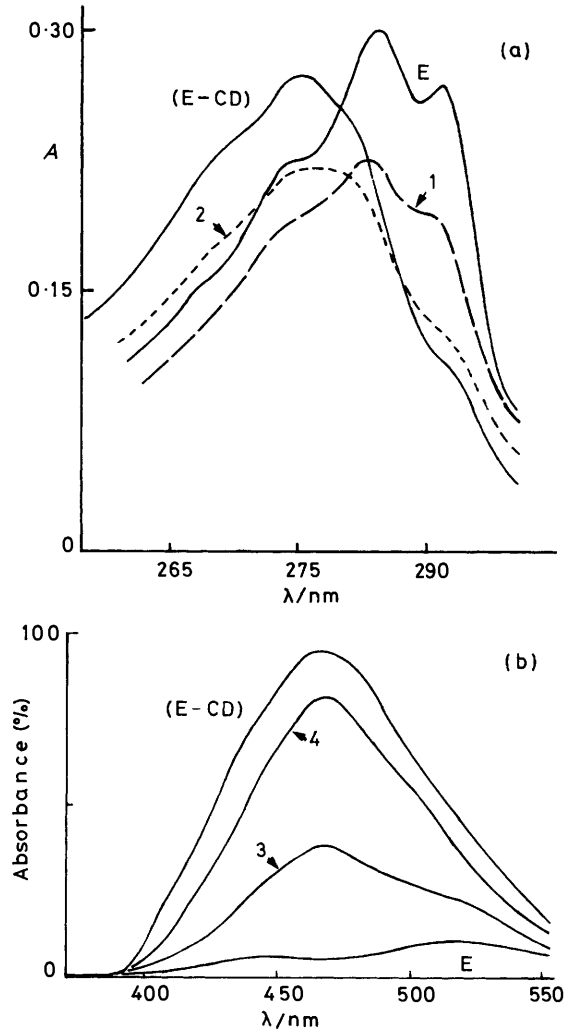


Figure 1. (a) The u.v. spectra of E in the presence of CD in a pH 9.18 buffered solution. $c_1 = 4.5 \times 10^{-6}$ mol dm^{-3}, $c_1 = 1 \times 10^{-4}$ mol dm^{-3} for curve 1 and 1×10^{-3} mol dm^{-3} for curve 2. (b) The emission fluorescence spectra ($\lambda_{ec.}$ 280 nm) of E in the presence of CD in the same buffer as that in (a). $c_1 = 4 \times 10^{-6}$ mol dm^{-3}, $c_2 = 1 \times 10^{-3}$ mol dm^{-3} for curve 3 and 5×10^{-3} mol dm^{-3} for curve 4. The ionic strength throughout was 0.2 mol dm^{-3} and the temperature 25 ± 0.5 °C.

Absorbance Spectrophotometry.—Two spectrophotometers (Cary 118 and C 210) equipped with sample cells thermostatted at 25 (±0.5) °C were used.

Fluorescence Spectrophotometry.—A Perkin-Elmer LS-5 luminescence spectrophotometer equipped with a cell carrier thermostatted at 25 (±0.5) °C was used.

T-Jump Kinetics.—For *T*-jump experiments two Messanlagen and Studiengesellshaft *T*-jump spectrophotometers were used; one was equipped with a fluorescence detector. The buffered γ-cyclodextrin solutions (in concentrations ranging from 1×10^{-4} to 5×10^{-2} mol dm^{-3}) were introduced into the sample cell [thermostatted at 20 (±0.5) °C]. The required amounts of ellipticine ($3–9 \times 10^{-6}$ mol dm^{-3}) were microinjected directly into the cyclodextrin solution. A 5 °C *T*-jump was performed by discharging a condenser of 0.05 μF charged at 22 kV into the sample cell. The signals were acquired on a PDP 11 computer and analysed according to published procedures.[11] In the fluorescence experiments the best emission was observed with an excitation wavelength of 292 nm and a u.v. long-pass filter was placed before the detection photomultiplier.

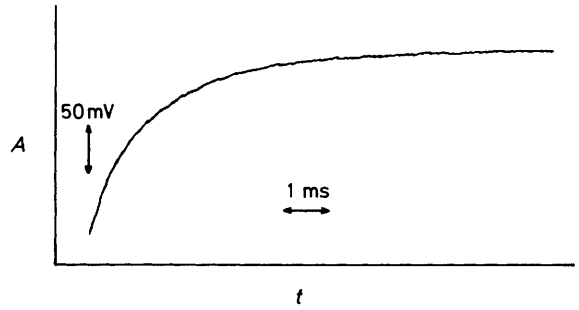


Figure 2. Absorbance variation at λ 292 nm when a pH 9.18 buffered solution of E and CD was submitted to a fast temperature jump from 20 to 25 °C and at 0.2 mol dm^{-3} ionic strength ($c_1 = 8.05 \times 10^{-6}$ mol dm^{-3} and $c_2 = 2.05 \times 10^{-4}$ mol dm^{-3}).

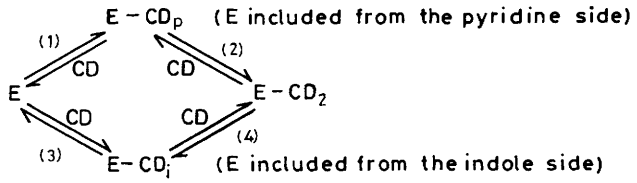
Results and Discussion

The solubility of ellipticine is increased in the presence of γ-cyclodextrin from around 1×10^{-5} mol dm^{-3} to $> 1 \times 10^{-3}$ mol dm^{-3}. Furthermore, this increase in solubility is accompanied by major modifications in its u.v. and fluorescence spectra. Moreover, it should be noted that the spectra of ellipticine methylated at N-2 are not modified in the presence of α-, β-, or γ-cyclodextrin (cyclohexa-, cycloheptа-, and cyclooctamylose), thus showing that the quaternary ellipticinium cation does not seem to interact with cyclodextrins.

Interaction of Ellipticine with γ-Cyclodextrin.—Spectrophotometric detection. At high concentrations of α- and β-cyclodextrin ($> 10^{-3}$ mol dm^{-3}) the fluorescence spectrum of ellipticine is modified, while its absorbance spectrum is slightly affected. However, with γ-cyclodextrin two types of important modifications in the absorbance spectrum of ellipticine are observed (Figure 1). The first occurs, at low [CD], as a slight hypsochromic shift accompanied by a general decrease in absorbance; the second occurs, at higher [CD], as a large hypsochromic shift accompanied by an increase in absorbance at λ 271 nm. Moreover, in the presence of γ-cyclodextrin, a marked increase in the fluorescence emission spectrum of E, accompanied by a hypsochromic shift ~50 nm, is observed (Figure 1). In acidic media, however, the absorbance and the fluorescence spectra of ellipticine are unchanged by the cyclodextrin, which indicates that there is probably no noticeable interaction between cyclodextrin and EH^+ (ellipticine protonated at N-2 with a p*K* of *ca.* 7.4).

Kinetic phenomena. The methods and techniques of chemical relaxation were used in this study.[12] When a pH 9.18 buffered solution of ellipticine and γ-cyclodextrin was submitted to a fast *T*-jump, a single kinetic phenomenon was observed in the 275–320 nm range. This phenomenon is always an exponential increase of absorbance with relaxation time ranging from 1×10^{-4} to 2×10^{-3} s (Figure 2). Its amplitude was [CD]- and λ-dependent. It becomes negligible at λ < 275 nm and λ > 320 nm. When the same solution of ellipticine and cyclodextrin was submitted to the same *T*-jump but with fluorescence detection, a very fast decrease in the fluorescence intensity ($< 15 \times 10^{-6}$ s) was observed, the amplitude of which was [CD]-dependent. Both kinetic phenomena disappeared in acidic media where no modification in either the absorbance or the fluorescence spectra of EH^+ was observed in the presence of γ-cyclodextrin.

Mechanism of Inclusion of Ellipticine in γ-Cyclodextrin.—The u.v. spectra of ellipticine in the presence of γ-cyclodextrin



General mechanism I for inclusion of one ellipticine molecule in two γ-cyclodextrin hosts with K_1 = [E][CD]/[E–CD$_p$] and K_3 = [E][CD]/[E–CD$_i$].

Table 1. A multilinear regression of $\Delta(I_F)^{-1}$ against $[CD]^{-1}$, $[CD]^{-2}$, and $([CD]\Delta I_F)^{-1}$ gave, respectively, $(2.75 \pm 1.56) \times 10^{-7}$ mol dm^{-3}, $7.109 \pm 10.42) \times 10^{-10}$ mol dm^{-3}, $-(4.98 \pm 13.22) \times 10^{-5}$ mol dm^{-3}, and an intercept of $(1.50 \pm 0.94) \times 10^{-4}$ mol dm^{-3}, with r 0.999 36 and F 1 941.90. A multilinear regression of $\Delta(I_F)^{-1}$ against $[CD]^{-1}$ and $[CD]^{-2}$ gave, respectively, $(3.31 \pm 0.44) \times 10^{-6}$ mol dm^{-3} and $(3.25 \pm 0.48) \times 10^{-10}$ mol dm^{-3}, and an intercept of $(1.25 \pm 0.60) \times 10^{-3}$ mol dm^{-3} with r 0.999 31 and F 2 711.02.

10^4[CD]/mol dm^{-3}	ΔI_F
1	15
2	36
2.97	66
3.95	94
4.92	111
6.84	151
9.68	194
14.3	252
18.6	309
23.1	356
27.2	387
31.3	414
35.3	441
42.3	464
50	468

showed the occurrence of at least two phenomena involving the cyclodextrin and ellipticine. However, the monotonic fluorescence increase of Figure 1 did not directly confirm this hypothesis. Notwithstanding this, we will consider three possible explanations of the spectroscopic behaviour of ellipticine in the presence of γ-cyclodextrin. (i) In the first case ellipticine is included by the cyclodextrin either from the pyridine side or from the indole side. (ii) In the second case ellipticine is first included from the pyridine side to produce a mono-inclusion complex which in turn is included from the indole side by a second cyclodextrin molecule, producing a double-inclusion complex as reported for several other guest molecules.[7,13–15] (iii) In this final case—the combination of the two previous ones—the solution would contain four forms of ellipticine; (a) free ellipticine, (b) the pyridine-side mono-included complex, (c) the indole-side mono-included complex, and (d) the doubly included complex.

Case (i). *Double mono-inclusion of E by a γ-cyclodextrin molecule.* The double mono-inclusion of ellipticine in γ-cyclodextrin is described by steps (1) and (3) of the general mechanism I of inclusion for one ellipticine in two γ-cyclodextrin host molecules.

If ellipticine is included in γ-cyclodextrin from the pyridine and from the indole sides [step (1) + step (3)], then [E–CD$_i$]/[E–CD$_p$] = K_1/K_3. This should be manifested by a monotonic modification of absorbance. However, in Figure 1, two different modifications of absorbance are detected. Therefore, the double mono-inclusion of ellipticine can be rejected.

Case (ii). *Two-step inclusion of ellipticine by two γ-cyclodextrin molecules.* The inclusion of ellipticine by two γ-cyclodextrin molecules is depicted in mechanism II.

$$E + CD \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\text{–}CD \quad (1)$$

$$E\text{–}CD + CD \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E\text{–}CD_2 \quad (2)$$

Mechanism II, with K_2 = [E–CD][CD]/[E–CD$_2$].

In the case of mechanism II, and under our experimental conditions where the Beer–Lambert law is obeyed by the absorbance spectra, the intensity of fluorescence (I_F) can be expressed as equation (3) where γ_1, γ_2, and γ_3 are the

$$I_F = \gamma_1[E] + \gamma_2[E\text{–}CD] + \gamma_3[E\text{–}CD_2] \quad (3)$$

fluorescence coefficients of E, E–CD, and E–CD$_2$, respectively. The analytical concentrations of ellipticine and of cyclodextrin can be expressed as shown in equation (4).

$$c_1 = [E] + [E\text{–}CD] + [E\text{–}CD_2] \quad (4)$$

Since, under our experimental conditions, $c_1 \ll c_2$ and [CD] = c_2, we can easily derive equation (5) where ΔI_F = $I_F - (I_F)_0$ (I_F is the fluorescence intensity and $(I_F)_0$ is the fluorescence intensity of E without any CD), $\Gamma_1 = \gamma_2 - \gamma_1$, and $\Gamma_2 = \gamma_3 - \gamma_1$.

$$\frac{1}{\Delta I_F} = \frac{1}{\Delta(I_F)_0} + \frac{K_2}{\Delta(I_F)_0}\frac{1}{[CD]} + \frac{K_1K_1}{\Delta(I_F)_0}\frac{1}{[CD]^2} - \frac{\Gamma_1K_2}{\Gamma_1}\frac{1}{\Delta I_F[CD]} \quad (5)$$

A multiple linear regression of the data against equation (5) (Table 1) gives $\Gamma_1K_2/\Gamma_2 = -(5 \pm 13) \times 10^{-5}$ mol dm^{-3} which, within the limits of uncertainty, shows that $\Gamma_1 \sim 0$. This indicates that $\gamma_1 \sim \gamma_2$. Thus the first inclusion slightly affects the fluorescence, while the second inclusion greatly affects the fluorescence. On the other hand, it is known that the fluorescence is particularly affected when indoles interact with hydrophobic media.[16] This seems to indicate that the inclusion of ellipticine in γ-cyclodextrin first occurs from the pyridine side of the molecule and then from the indole side. This was confirmed by the fact that cyclodextrins do not interact with the ellipticinium cation methylated at N-2 and do not interact either with ellipticine protonated at N-2. Thus it seems that the positive charge at the N-2 position inhibits at least the first interaction phenomenon between ellipticine and γ-cyclodextrin. A multilinear regression of the data against equation (5), but without $\Gamma_1K_2/\Gamma_2\Delta I_F[CD]$, gives (Table 1) $K_2 = (1.85 \pm 0.25) \times 10^{-3}$ mol dm^{-3} and $K_1 = (9.80 \pm 1.35) \times 10^{-5}$ mol dm^{-3}. Thus, it seems that ellipticine is included by two γ-cyclodextrin molecules in two steps; (i) the inclusion of the pyridine side and then (ii) the inclusion of E–CD from the indole side, which affects the fluorescence. However, this cannot exclude the third possibility: the double mono-inclusion of ellipiticine.

Case (iii). *The double mono-inclusion of E by one and two cyclodextrin molecules.* The double mono-inclusion of one ellipticine in cyclodextrin is described by the general mechanism of inclusion. In this case, we consider that ellipticine is included in γ-cyclodextrin from the pyridine side [step (1)] or from the indole side [step (3)] and that both E–CD$_p$ and E–CD$_i$ are included by another cyclodextrin molecule to form the final E–CD$_2$ complex [steps (2) and (4)]. The value of K_2 reported for

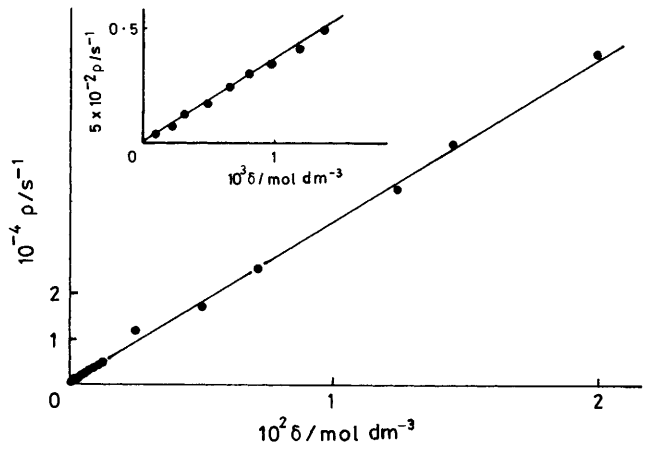


Figure 3. Plot of ρ against δ, where $\rho = (K_2 + [CD])^{-1}/K_2$ and $\delta = k_1(K_2 + [CD])[CD]/K_2 + k_{-1}$. Intercept = $(1.95 \pm 6.70) \times 10^2$ s^{-1}; slope = $(3.60 \pm 0.10) \times 10^6$ dm mol^{-3} s^{-1}; r 0.9987 for 15 experimental points.

reaction (2) (inclusion of E–CD from the indole side) is in good agreement with the dissociation constant of inclusion of indole in β-cyclodextrin (5×10^{-3} mol dm^{-3}).[14] Therefore, K_3 (the dissociation constant of inclusion of E from the indole side) probably has the same order of magnitude as K_2, which means that $K_3 \gg K_1$, showing that $[E–CD_i] \ll [E–CD_p]$. Therefore, we cannot detect steps (3) and (4) by the techniques used here. Furthermore, the average distance between the 5-methyl and the 11-methyl groups in ellipticine is *ca.* 7 Å,[17] while the diameters of the cavities of α-, β-, and γ-cyclodextrins are of 4.7–5.2, 6–6.6, and 7.5–8.3 Å, respectively.[7] Therefore, the cavities of α- and β-cyclodextrin are too small to include ellipticine from the pyridine side. However, they should be capable of including ellipticine from the indole side.[13–15] Even if it takes place at higher cyclodextrin concentrations, the inclusion of ellipticine from the indole side in α- and β-cyclodextrins does occur and is revealed by modification in the fluorescence spectrum of ellipticine. Thus, the two methyls at C-5 and C-11 are responsible for the fact that the inclusion of ellipticine in γ-cyclodextrin first occurs from the pyridine side. As shown by the fluorescence spectra and by the non-inclusion of methylellipticinium EMe^+ and ellipticinium EH^+, both bearing a positive charge at N-2. However, even if they justify mechanism II, all these results are based on statistical evidence which needs to be confirmed kinetically.

Kinetic Analysis of the Inclusion of One Molecule of E in One or Two γ-Cyclodextrin Molecules.—The amplitude *vs.* λ plot of the fast *T*-jump phenomenon detected by absorbance, at values of [CD] ranging from 1×10^{-4} to 1×10^{-3} mol dm^{-3}, seems to be proportional to the absorbance decrease detected in the same concentration range (Figure 1) while the very fast *T*-jump fluorescence variation at $[CD] > 1 \times 10^{-3}$ mol dm^{-3} is probably associated with the second absorbance variation of Figure 1. If the very fast *T*-jump phenomenon detected by fluorescence is attributable to the inclusion of E–CD in cyclodextrin [reaction (2)], the fast *T*-jump absorbance phenomenon of Figure 2 can, then, be ascribed to the first inclusion of ellipticine in cyclodextrin [reaction (1)], the rate equation of which can be expressed as equation (6).

$$-d[E]/dt = K_1[E][CD] - k_{-1}[CD] \quad (6)$$

We can use substitution methods[12] to derive the reciprocal relaxation time associated with reaction (1) (τ^{-1}). Under our experimental conditions [E], [E–CD], and $[E–CD_2] \ll [CD]$. From the conservation of mass, equations (7) and (8) follow.

$$\Delta[E–CD_2] + \Delta[E–CD] + \Delta[E] = 0 \quad (7)$$

$$\Delta[CD] + 2\Delta[E–CD_2] + \Delta[E–CD] = 0 \quad (8)$$

Since reaction (2) is very fast compared with reaction (1) we can consider that reaction (2) is in a constant equilibrated state during reaction (1). Thus, equation (9) holds.

$$\Delta[E–CD_2] = [E–CD]\Delta[CD]/K_2 + [CD]\Delta[E–CD]/K_2 \quad (9)$$

From equations (6)–(9) we can derive the reciprocal relaxation time equation (τ^{-1}), which can be expressed as equation (10).

$$\tau^{-1} = k_1[CD] + k_{-1}K_2/(K_2 + [CD])$$

$$(K_2 + [CD])\tau^{-1}/K_2 = k_1(K_2 + [CD])[CD]/K_2 + k_{-1} \quad (10)$$

A linear least-squares regression of the data against equation (10) gives (Figure 3) $k_1 = (3.60 \pm 0.10) \times 10^6$ dm^3 mol^{-1} s^{-1}, while k_{-1} is too small to be accurately measured: $k_{-1} = (1.95 \pm 6.70) \times 10^2$ s^{-1}.

Thus, it seems that the relaxation process detected by absorbance is associated with the first inclusion of ellipticine in cyclodextrin and subsequently the very fast fluorescence decrease can be ascribed to the inclusion of E–CD in γ-cyclodextrin. Furthermore, the amplitude of the fast ($< 15 \times 10^{-6}$ s) fluorescence decrease cannot be detected for $[CD] < 1 \times 10^{-3}$ mol dm^{-3}. Therefore, the second-order rate constant of reaction (2), k_2, is $> 1 \times 10^7$ mol dm^{-3} s^{-1}, which is comparable with the second-order rates of inclusion of some other molecules in α- and β-cyclodextrins.[18,19] The value of k_1 is lower than k_2 because, in reaction (1), the steric hindrance of the two methyl groups at C-8 and C-11 inhibits the rate of inclusion in γ-cyclodextrin as reported for nitrophenols in α-cyclodextrin.[18] Furthermore, the variation of fluorescence when $E–CD_2$ is formed is much greater than that of the absorbance (Figure 1). This is probably why we detect the kinetics of reaction (2) by fluorescence only. These results confirm mechanism II, showing that the inclusion of ellipticine in γ-cyclodextrin probably occurs in two steps involving two cyclodextrins hosts for one ellipticine guest molecule.

Spectra of Absorbance of E, E–CD, and E–CD$_2$.—Knowing K_1 and K_2, then for $c_2 \gg c_1$ we can easily show that equations (11)–(13) hold where, $Q = ([CD]^2 + K_2[CD] + K_1K_2)$.

$$[E] = K_1K_2c_1/Q \quad (11)$$

$$[E–CD] = K_2[CD]c_1/Q \quad (12)$$

$$[E–CD_2] = [CD]^2c_1/Q \quad (13)$$

These three relationships give the concentrations of E, E–CD, and $E–CD_2$ at any [CD]. This allows us to determine the spectrum of E–CD from the u.v. spectrum measured at a known [CD]. We can, therefore, simulate the u.v. spectrum for a known concentration of ellipticine at any known concentration of cyclodextrin. Figure 4 shows a very good agreement between the simulated and measured spectra of ellipticine in the presence of γ-cyclodextrin, giving, therefore, further evidence in support of the K_1 and K_2 values measured here.

Table 2. The rate and equilibrium constants of the double inclusion of one ellipticine in two γ-cyclodextrin molecules.

Reaction	k/dm^3 mol^{-1} s^{-1}	k/s^{-1}	K/mol dm^{-3}
E + CD ⇌ E–CD	3.6×10^6	~350	9.80×10^{-5}
E–CD + CD ⇌ E–CD$_2$	$>10^7$		1.85×10^{-3}

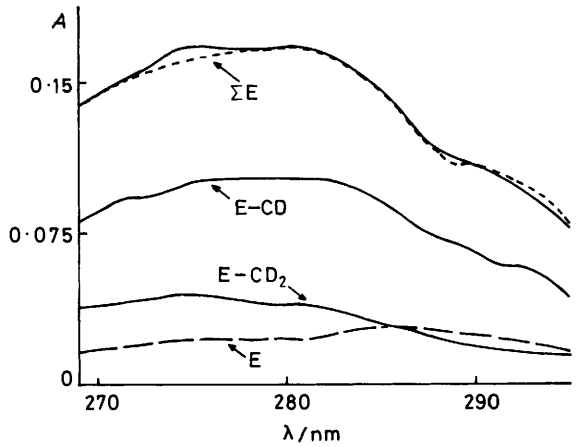


Figure 4. The theoretical spectrum, for $c_1 = 4.05 \times 10^{-6}$ mol dm^{-3} and $c_2 = 5.75 \times 10^{-4}$ mol dm^{-3}, calculated from the spectral contributions of E, E–CD, and E–CD$_2$ and compared with the experimental spectrum (— — —) measured under the same conditions.

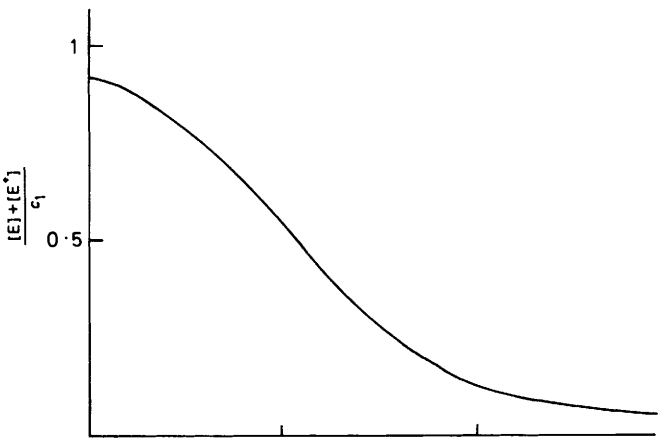


Figure 5. The variation of [E$^+$] and [E] with pH for $c_2 = 1 \times 10^{-3}$ mol dm^{-3}.

Analysis and Perspectives.—In the summary of our results (Table 2) we can see that the affinity of ellipticine for γ-cyclodextrin is high,[7,13–15,18] at least for the first inclusion. Although important, the average affinity of ellipticine for γ-cyclodextrin is lower than that of ellipticine for DNA.[6] We have shown that ellipticine is included in γ-cyclodextrin only in its neutral form and not in its ellipticinium form EH$^+$ (E protonated at N-2). Moreover, the pK for formation of EH$^+$ is between 6.9[6] and the value we obtained, ~7.4, while the physiological pH is 7.4. Thus, ellipticine and its neutral derivatives do not exist in these media in a single form. If the drug is included in an excess of γ-cyclodextrin and then injected into a physiological medium, an equilbrium would take place between the free drug and the included drug. In these media, this equilibrium largely favours the included form (Figure 5). However, in more acidic media free ellipticine is released from its cyclodextrin host (Figure 5). We can very cautiously speculate that this is the case in numerous tumours where a decrease in the pH of the extracellullar media occurs.[20] Although some tumours could have pHs <6, the pH decrease is usually *ca.* 0.5 pH units, putting the pH of the tumour cell in the range 6.5–6.9.[20] We, therefore, feel that the inclusion of ellipticine derivatives and other DNA-intercalating agents in γ-cyclodextrin should perhaps be investigated further, especially from a pharmaceutical standpoint.

Concluding Remarks.—In this work we have established, on the basis of thermodynamic and kinetic analysis, that the inclusion of ellipticine in two γ-cyclodextrin host molecules occurs in two steps, the first involving the pyridine residue and the second being the inclusion of this inclusion complex by another cyclodextrin from the indole side. This inclusion of an antitumour molecule may be of some importance in chemotherapy based on intercalating agents.

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Received 12*th May* 1988; *Paper* 8/01864B