Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **15**: 359–368, 1993.
© 1993 *Kluwer Academic Publishers. Printed in the Netherlands.*

UV Spectroscopic Study of the Interaction between α-, β-, and γ-Cyclodextrins and Pyridine Derivatives

M. COTTA RAMUSINO,* L. RUFINI and C. MUSTAZZA
Laboratorio di Chimica del Farmaco, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy

(Received: 15 March 1993; in final form: 22 June 1993)

Abstract. Complex formation between α-, β- and γ-cyclodextrins and some pyridine derivatives (hydroxy, O- and N-methyl) have been investigated by UV spectroscopy. Inclusion processes do not exert any noticeable influence on the UV absorption spectra of most of the guests which do not give rise to tautomeric equilibria or which show a strong prevalence for one of the tautomeric forms. In contrast, the equilibrium between the lactim and the lactim forms of 3-hydroxypyridine (which are present in comparable amounts in aqueous solution) was shown to be markedly affected by the inclusion process with a preferential complexation of the latter tautomer.

Key words: Cyclodextrin complexes, pyridine derivatives, tautomeric equilibria, UV spectroscopy.

1. Introduction

Cyclodextrins (CD) are cyclic oligosaccharides composed of $(1 \rightarrow 4)$ linked $\alpha - D$ glucopyranosyl residues. α-, β- and γ-Cyclodextrins contain six, seven and eight units, respectively [1], and possess a toroidal shape with an hydrophobic cavity which can act as host to a wide range of substrates; in particular they are good molecular receptors for aromatic rings [2].

The present study is concerned with the investigation, by means of UV absorption spectroscopy, of the inclusion complexes in aqueous solution between α-, β- and γ-cyclodextrins and various pyridine derivatives.

2-, 3-, and 4-Hydroxypyridines and their O- and N-methyl derivatives have been chosen as guest compounds because their nucleus is included in a wide range of drugs of different pharmacological action [3]. Moreover 2-, 3-, and 4-hydroxypyridines are known to give rise to tautomeric equilibria in solution which markedly affect their UV spectrum. These equilibria play a relevant role in many biological processes and are influenced to a great extent by the polarity of the surrounding medium.

2. Experimental

UV absorption spectra were recorded at $25 \pm 0.5°C$ on a Cary 2200 spectrophotometer using 1 cm matched quarz cuvettes.

* Author for correspondence.

α- and γ-Cyclodextrins (Fluka) and β-cyclodextrin (Sigma) were used without further purification. Their water content was determined by thermogravimetric analysis with a Perkin–Elmer TGA 7 instrument and the appropriate corrections were made to the concentrations of the relative solutions.

2-Hydroxypyridine (Aldrich), 3-hydroxypyridine (B. H. Shilling), 4-hydroxypyridine (Schuchard), 2-methoxypyridine (Fluka), and N-methyl-2-pyridone (Aldrich) were purified by sublimation or distillation; N-methyl-3-pyridone, 3-methoxypyridine and N-methyl-4-pyridone were prepared according to the following references: N-methyl-3-pyridone [4]; 3-methoxypyridine [5]; N-methyl-4-pyridone [6]; their identity was confirmed by ^{1}H NMR spectroscopy.

All solutions were made in phosphate buffer, pH 6.8 (0.025M K_2HPO_3 + 0.025M KH_2PO_3, from Fluka) and in citrate buffer ($Na_3C_6H_5O_7 \cdot 2\,H_2O$ 0.015M, from Carlo Erba, brought to pH 6.8 with 0.1N HCl). These buffer concentrations were chosen in order to have solutions of approximately equal ionic strength.

3. Results and Discussion

The UV absorption spectra of the three monohydroxypyridines and their N- and O-methyl derivatives have been studied both from an experimental [7–9] and a theoretical [10] point of view.

The positions of the absorption bands are markedly solvent dependent, with a red shift usually observed in going from a more polar to a less polar medium. Moreover the spectra are also influenced by the tautomeric equilibria of the monohydroxypyridines (Figure 1).

In aqueous solutions the equilibrium constants are reported to be 910 for 2-hydroxypyridine [11], 1.1 (buffered solution, pH 7.0) for 3-hydroxypyridine [12] and 1910 for 4-hydroxypyridine [11]. We therefore started a preliminary study using α-, β- and γ-cyclodextrins as host compounds to investigate the influence of the inclusion process on the UV absorption spectra of the guest species.

Two different buffers (phosphate and citrate) were always used and the results compared to rule out any influence of the buffer on the inclusion process. The pH

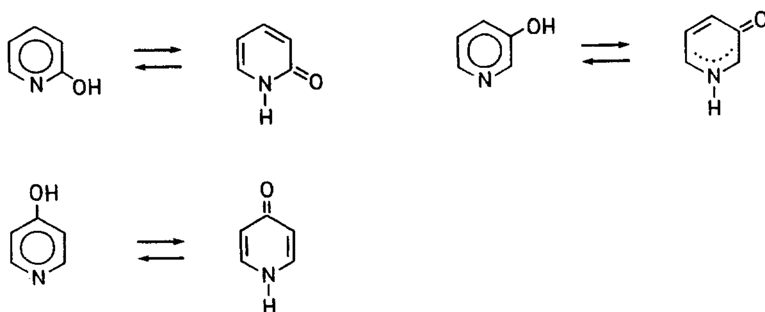


Fig. 1. Tautomeric equilibria of monohydroxypyridines in aqueous solution.

of the solutions was chosen taking into account the pK_a values of the considered pyridine derivatives [13] so as to minimize the presence of charged species whose UV absorption bands partially overlap those of the neutral species [9].

We first dealt with solutions containing 2-hydroxypyridine and 4-hydroxypyridine. Since in both cases the lactam tautomer is by far the most predominant form in aqueous solution (more than 99.9% of the total) any change in the UV spectrum following CD addition should be due to a perturbation of the electronic transitions of the lactam tautomer rather than to a shift in the tautomeric equilibrium.

In our experimental conditions only very small changes could be detected in the UV absorption spectra of 2- and 4-hydroxypyridines upon addition of up to 5.0×10^{-2} M (α-CD), 1.0×10^{-2} M (β-CD) and 3.8×10^{-2} M (γ-CD) of the host compound (guest concentrations were in the range $0.5 - 2.0 \times 10^{-4}$ M). These modifications were of the same order of magnitude as the uncertainty associated with the experimental measurements. But even if no significant spectral changes have been observed, inclusion processes cannot be ruled out solely on the basis of these observations.

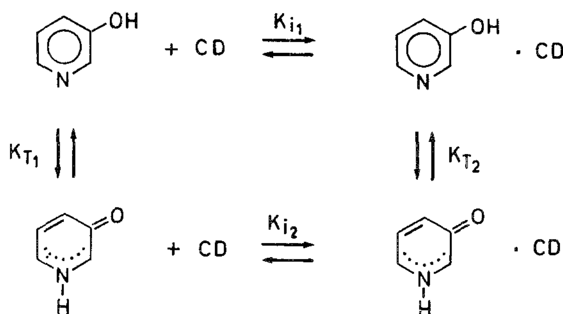
A preliminary study with ^{1}H NMR spectroscopy does confirm that 2- and 4-hydroxypyridine are actually included in β-CD. In fact the resonances of the protons lining the host cavity are shifted upfield as a result of interaction with the guest compounds.

We then turned our attention to the behaviour of 3-hydroxypyridine where the lactim and lactam tautomer are present in comparable amounts in aqueous solution.

Major changes were observed in this case on addition of α-CD (Figure 2) and β-CD (Figure 3); the effect of γ-CD was much smaller and consequently no attempt was made at analyzing the data.

Taking into account the findings with 2- and 4-hydroxypyridine, where no effect was observed upon addition of CD, these changes can only be attributed to a perturbation of the tautomeric equilibrium presented by 3-hydroxypyridine.

The processes which take place in aqueous solutions of 3-hydroxypyridine in the presence of cyclodextrins can be described by Scheme 1.



Scheme 1. Complex formation and tautomeric equilibria of 3-hydroxypyridine in buffered solution (pH 6.8) in the presence of cyclodextrins.

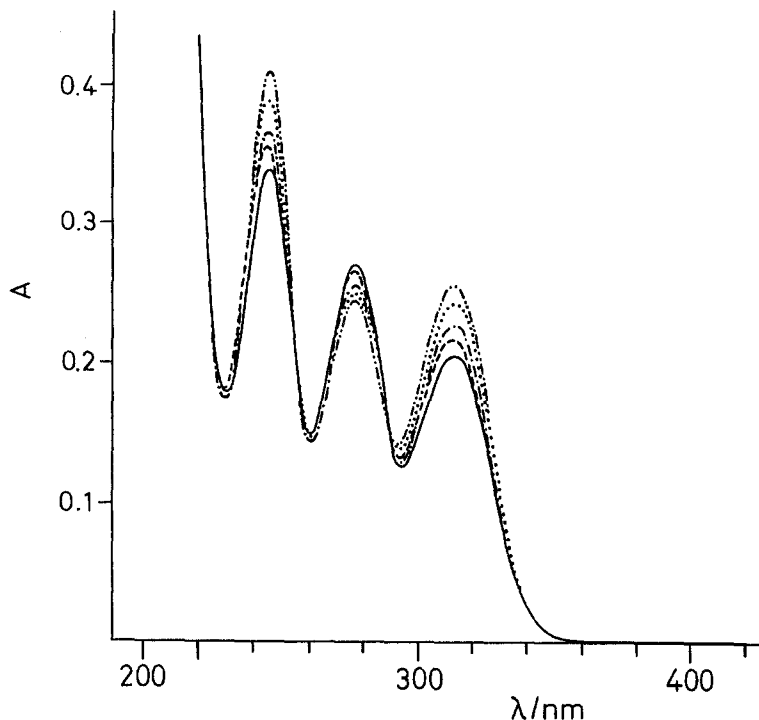


Fig. 2. UV absorption spectra of 3-hydroxypyridine (1.00×10^{-4} M) in phosphate buffer solution (pH = 6.8) at 25°C in the presence of various concentrations of α-cyclodextrin: —··—··— 1.67×10^{-2} M; ······ 2.50×10^{-2} M; —·—·— 3.30×10^{-2} M; – – – – 4.20×10^{-2} M; ——— 5.00×10^{-2} M.

A 1:1 inclusion mechanism has been assumed throughout this study for the following reasons:

(1) 3-hydroxypyridine presents only one interaction moiety (the aromatic ring) and its molecular dimensions seems to preclude inclusion in more than one CD molecule;
(2) the guest compound is not known to give rise to autoassociation processes in the concentration range considered here ($0.5 - 1.0 \times 10^{-4}$ M).

We then assumed that the molar absorptivities and the λ_{max} of the complexed guest do not differ from those of the free guest.

To test this hypothesis we examined the effect of CD addition on the UV absorption spectra (in aqueous solution) of 2-methoxypyridine, N-methyl-2-pyridone, 3-methoxpyridine, N-methyl-3-pyridone and N-methyl-4-pyridone. These N- and O-methyl derivatives of monohydroxypyridine should indeed be able to penetrate the CD cavity to form inclusion complexes (as confirmed by preliminary ^{1}H NMR

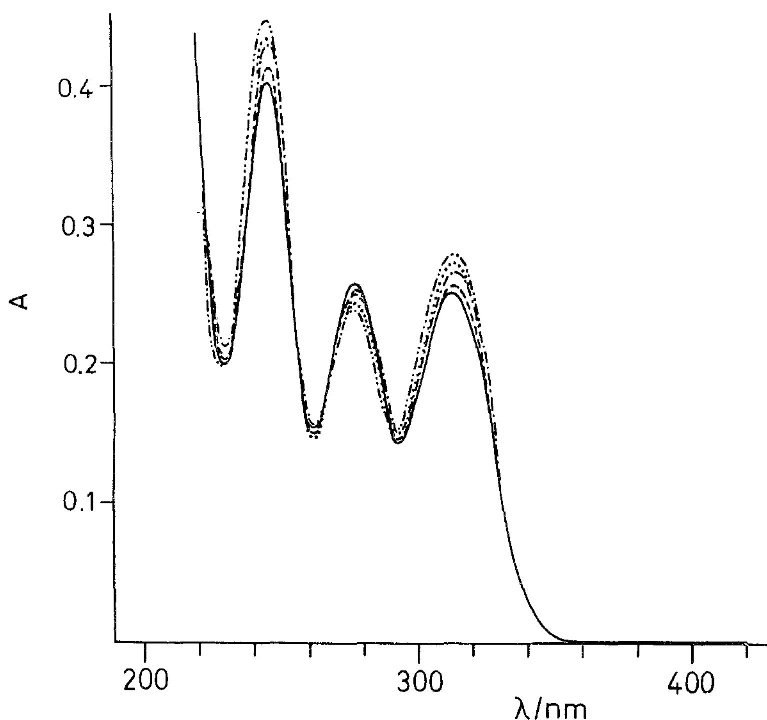


Fig. 3. UV absorption spectra of 3-hydroxypyridine (1.04×10^{-4} M) in phosphate buffer solution (pH = 6.8) at 25°C in the presence of various concentrations of β-cyclodextrin: –··–··– 3.00×10^{-3} M; ······ 4.50×10^{-3} M; –·–·– 6.00×10^{-3} M; – – – – 7.50×10^{-3} M; ——— 9.00×10^{-3} M.

studies) but they do not give rise to tautomeric equilibria because the exchangeable proton has been replaced by a methyl group.

As expected, no changes were observed in the UV spectra of these compounds following CD addition. We thus postulated that both λ_{max} and the molar absorptivities of 3-hydroxypyridine are not affected by the inclusion process.

Provided that CD concentrations are much larger than those of the guest compound, the following equation can be derived (see Appendix):

$$\frac{A \cdot 1/K_{T1}}{c\epsilon - A \cdot (1 + 1/K_{T1})} = \frac{1}{[\mathrm{CD}]} \cdot \frac{1}{K_{i1} - K_{i2}} + \frac{K_{i2}}{K_{i1} - K_{i2}} \tag{1}$$

where A and ϵ designate the absorbance value and the molar absorptivity, respectively, at $\lambda = 314$ nm. This wavelength corresponds to the first maximum in the UV absorption spectrum of 3-hydroxypyridine in aqueous solution, which is attributed to the long wavelength $\pi \rightarrow \pi^*$ transition of the lactam tautomer; c is the total concentration of the guest which was kept constant while the CD concentration was varied.

The value of ϵ at $\lambda = 314$ nm for the tautomeric mixture of 3-hydroxypyridine

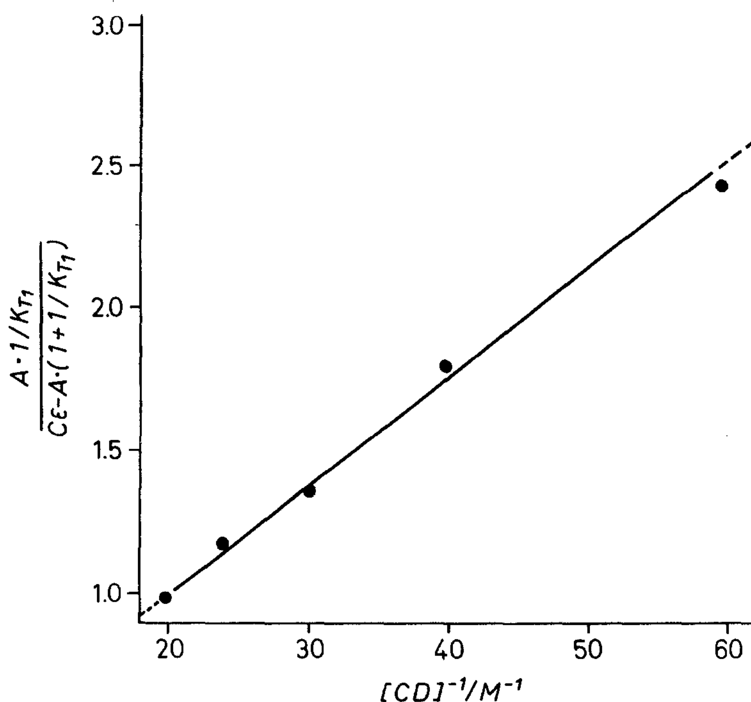


Fig. 4. Plot of $[A \cdot 1/K_{T1}]/[c\epsilon - A \cdot (1+1/K_{T1})]$ versus $[CD]^{-1}$ at $\lambda = 314$ nm for 3-hydroxypyridine solutions (phosphate buffer, pH = 6.8) in the presence of various α-cyclodextrin concentrations. The slope, intercept and correlation coefficient values are 0.0365M, 0.2988 and 0.990, respectively.

has been reported to be 3060 l mol^{-1} cm^{-1} [7, 8]. Taking into account a K_{T1} value of 1.1 (which implies a concentration of the lactam tautomer equal to 52.38%), this yields a molar absorptivity value for the lactam tautomer of 5840 l mol^{-1} cm^{-1}, which is also very similar to the ϵ of the first absorption maximum in the UV spectrum of N-methyl-3-pyridone. We therefore used this value throughout this study.

A plot of

$$\frac{A \cdot 1/K_{T1}}{c\epsilon - A \cdot (1 + 1/K_{T1})} \quad \text{versus} \quad \frac{1}{[\mathrm{CD}]}$$

gave a straight line whose slope and intercept are equal to

$$\frac{1}{K_{i1} - K_{i2}} \quad \text{and} \quad \frac{K_{i2}}{K_{i1} - K_{i2}},$$

respectively (Figures 4 and 5).

The very good fit obtained ($r > 0.98$) supports the hypothesis of 1:1 complex formation.

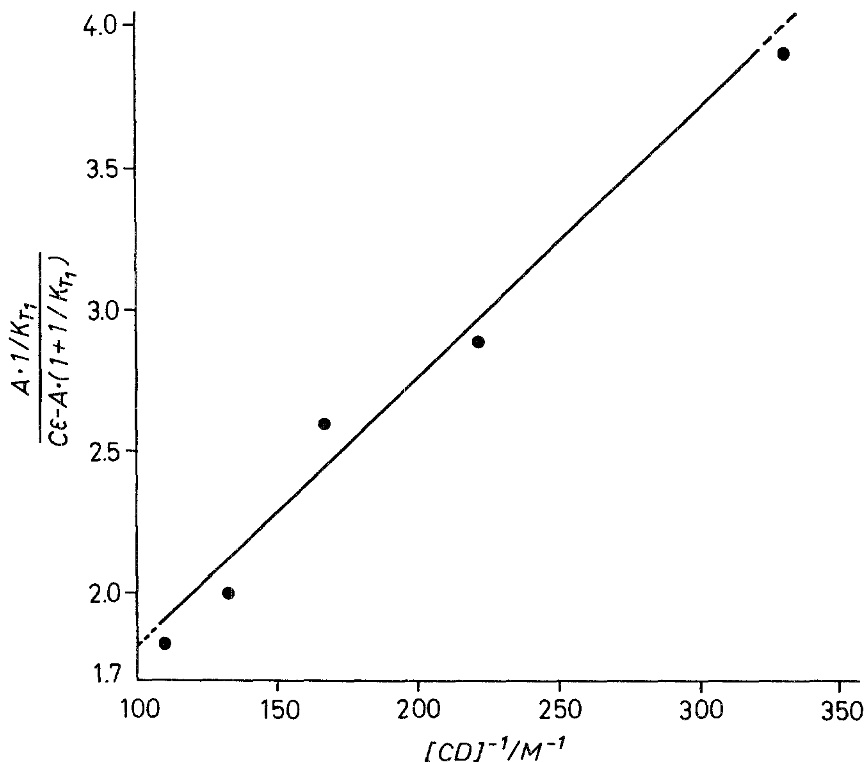


Fig. 5. Plot of $[A \cdot 1/K_{T1}]/[c\epsilon - A \cdot (1 + 1/K_{T1})]$ versus $[CD]^{-1}$ at $\lambda = 314$ nm for 3-hydroxypyridine solutions (phosphate buffer, pH = 6.8) in the presence of various β-cyclodextrin concentrations. The slope, intercept and correlation coefficient values are 0.0085:M, 0.965 and 0.986 respectively.

The apparent complex stability constants calculated in the forementioned way are reported in Table I. As can be seen, the buffer influence, if any, can be regarded as only very slight. The K_i values found are characteristic of weak intermolecular interactions and are of the same order of magnitude as reported elsewhere [14].

It is not surprising that the lactim tautomer of 3-hydroxypyridine has a greater affinity for the CD cavity than the lactam tautomer, given the enhanced stability of the former in less polar media [12]. Moreover the hydrogen atom of the OH group in the lactim tautomer might give rise to hydrogen bond interaction with the non bonding electron pairs of the glycosidic oxygen bridges lining the CD cavity.

The differences found in the K_i values for inclusion in α- and β-CD seem to confirm that the inclusion processes are very sensitive to the relative size of the cavity and the substrate. It is thus reasonable to assume that α-CD, which exhibits an internal diameter of 4.7–5.2 Å (as measured by CPK models), allows only limited penetration of the guest while β-CD (6.0–6.4 Å internal diameter) gives rise to a snug fit with the substrate. On the other hand the binding to γ-CD with its

TABLE I. Complex stability constants for the inclusion processes of 3-hydroxypyridine in α- and β-cyclodextrins at 25°C in aqueous solution.

Host		K_{i1} (M^{-1})	K_{i2} (M^{-1})	K_{T2}
α-CD	phosphate buffer	35.3±2.8	7.9±1.8	0.24±0.03
	citrate buffer	43.6±2.0	11.5±1.2	0.29±0.02
β-CD	phosphate buffer	230±41	113±30	0.54±0.04
	citrate buffer	229±11	104±8	0.50±0.01

larger cavity (7.5–8.3 Å) is poorer, in accord with previous observations [15].

4. Conclusions

In this work we have studied the influence exerted by CD inclusion on the tautomeric equilibria of some pyridine derivatives.

The equilibrium between the lactam and the lactim form of 3-hydroxypyridine was shown to be markedly affected by the inclusion process, with a preferential complexation of the lactim form. In contrast, the tautomeric equilibria of other pyridine derivatives which show a strong preference for one of the tautomeric forms are not much influenced by the inclusion process.

Further studies are in progress to shed more light on this kind of interaction in view of the relevant role that these tautomeric equilibria play in many biological processes.

Acknowledgements

We wish to thank Professor M. Cignitti for helpful discussion and M. T. Iela for performing TGA analysis to determine the water content of α-, β- and γ-cyclodextrins.

Appendix

Starting from the inclusion processes and tautomeric equilibria of 3-hydroxypyridine in buffered solution (pH 6.8) in the presence of CDs (see Scheme 1), the following relationship can be written:

$$[\mathrm{NHpy}]_f + [\mathrm{NHpy}]_c = A/\epsilon,$$

where $[\mathrm{NHpy}]_f$ and $[\mathrm{NHpy}]_c$ are the concentrations of the free and complexed lactam tautomers of 3-hydroxypyridine and A is the absorbance at $\lambda = 314$ nm

due to the free and complexed lactam forms which are assumed to have the same molar absorptivity (see text).

A/ϵ is equal to $[\mathrm{NHpy}]_\mathrm{f}$ + $[\mathrm{NYpy}]_\mathrm{f} \cdot [\mathrm{CD}] \cdot K_{i2}$, provided that the cyclodextrin concentration ([CD]) is much larger than that of the guest compound. Therefore

$$[\mathrm{NHpy}]_\mathrm{f} = \frac{A}{\epsilon \cdot (1 + [\mathrm{CD}] \cdot K_{i2})}.$$

If c is the total concentration of 3-hydroxypyridine, then

$$\begin{aligned} c &= [\mathrm{NHpy}]_\mathrm{f} + [\mathrm{OHpy}]_\mathrm{f} + [\mathrm{NHpy}]_\mathrm{c} + [\mathrm{OHpy}]_\mathrm{c} \\ &= [\mathrm{NHpy}]_\mathrm{f} \cdot (1 + 1/K_{T1} + [\mathrm{CD}] \cdot K_{i2} + [\mathrm{CD}] \cdot K_{i1}/K_{T1}), \end{aligned}$$

where $[\mathrm{OHpy}]_\mathrm{f}$ and $[\mathrm{OHpy}]_\mathrm{c}$ are the concentrations of the free and complexed lactim tautomers.

Recombination of the two last relationships leads to the following equation:

$$\frac{c \cdot \epsilon \cdot (1 + [\mathrm{CD}] \cdot K_{i2})}{A} = 1 + 1/K_{T1} + [\mathrm{CD}] \cdot K_{i2} + [\mathrm{CD}] \cdot K_{i1}/K_{T1}.$$

Dividing both sides by $(1 + [\mathrm{CD}] \cdot K_{i2})$ gives:

$$\frac{c \cdot \epsilon}{A} - 1 = \frac{[\mathrm{CD}] \cdot K_{i1}/K_{T1} + 1/K_{T1}}{1 + [\mathrm{CD}] \cdot K_{i2}}.$$

This equation can be rearranged:

$$\frac{c \cdot \epsilon - A}{A/K_{T1}} = \frac{[\mathrm{CD}] \cdot (K_{i1} - K_{i2})}{1 + [\mathrm{CD}] \cdot K_{i2}} + 1$$

and

$$\frac{c \cdot \epsilon - A - A/K_{T1}}{A/K_{T1}} = \frac{[\mathrm{CD}] \cdot (K_{i1} - K_{i2})}{1 + [\mathrm{CD}] \cdot K_{i2}},$$

from which, taking the reciprocal of the left and right sides, Equation (1) can be obtained:

$$\begin{aligned} \frac{A/K_{T1}}{c \cdot \epsilon - A \cdot (1 + 1/K_{T1})} &= \frac{1 + [\mathrm{CD}] \cdot K_{i2}}{[\mathrm{CD}] \cdot (K_{i1} - K_{i2})} \\ &= \frac{K_{i2}}{K_{i1} - K_{i2}} + \frac{1}{[\mathrm{CD}]} \cdot \frac{1}{K_{i1} - K_{i2}}. \end{aligned}$$

References

1. W. Saenger: *Angew. Chem. Int. Ed. Engl.* **19**, 344 (1980).
2. J. M. El Hage Chanine, J. P. Bertigny and M. A. Schwaller: *J. Chem. Soc. Perkin Trans. 2*, 629 (1989).
3. S. El Gezawi, N. Omar, N. El Rabbat, H. Ueda and J. H. Perrin: *J. Pharm. Biomed. Anal.* **6**, 399 (1988).

4. L. Paoloni, M. L. Tosato and M. Cignitti: *Theoret. Chim. Acta (Berl.)* **14**, 221 (1968).
5. C. Finkentey, E. Langhals and H. Langhas: *Chem. Ber.* **116**, 2394 (1983).
6. L. Ruzicka and V. Fornasir: *Helv. Chim. Acta* **3**, 806 (1920).
7. S. F. Mason: *J. Chem. Soc.* 5010 (1957).
8. S. F. Mason: *J. Chem. Soc.* 1253 (1959).
9. D. E. Metzler, C. M. Harris, R. J. Johnson, D. B. Siano and J. A. Thomson: *Biochemistry* **12**, 5377 (1973).
10. M. Cignitti and L. Paoloni: *Theoret. Chim. Acta (Berl.)* **25**, 277 (1972).
11. P. Beak, F. S. Fry, J. Lee and F. Steele: *J. Am. Chem. Soc.* **98**, 171 (1976).
12. J. M. Sanchez Ruiz, J. Llor and M. Cortijo: *J. Chem. Soc. Perkin Trans. 2*, 2047 (1984).
13. S. F. Mason: *J. Chem. Soc.* 674 (1958).
14. J. Szejtli: *Cyclodextrins and their Inclusion Complexes,* Akademiai Kiado, Budapest (1982).
15. W. C. Cromwell, K. Byostroem and M. R. Eftink: *J. Phys. Chem.* **89**, 326 (1985).