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Supramolecular Complex of 2-Hydroxypropyl-β-cyclodextrin with D- and L-tryptophan

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Cyclodextrins are known by their properties of molecular recognition. In the present work, it was established, by using high-performance liquid chromatography, that complexes between 2-hydroxypropyl-β-cyclodextrin (HPCD) and D- and L-tryptophan are readily formed in solution. Association constants of $(2 \pm 1) \times 10$ and of $(9 \pm 2) \times 10^{-1}$ for D and L-isomers, respectively, were calculated from UV electronic spectroscopy experiments. Solid state complexes were characterized by Fourier-transform infrared spectroscopy, which showed that the subtracted/deconvoluted spectra present wavenumber changes of the NH_3^+ asymmetric angular deformation and of the COO^- asymmetric stretching.

Keywords: Cyclodextrins; Molecular recognition; Fourier-self deconvolution; FTIR

INTRODUCTION

Cyclodextrins (CDs) present the possibility of behaving as hosts in molecular complexation with guest organic molecules. This is of fundamental importance in increasing water solubility and for the enantiomeric separation of some substances of biochemical interest [1]. β-Cyclodextrin is so far the most studied compound of this family. It has a toroidal-shape structure, composed by seven D-glucose units, with primary hydroxyl groups around the minor cavity diameter, while secondary groups, more acidic, lie around the greater cavity opening [2]. The 2-hydroxypropyl-β-cyclodextrin form is obtained by hydroxyalkylation of the most acidic secondary OH groups located at position 2 in D-glucose [3]. This derivative presents greater water solubility than the β form, due to amorphisation [2]. The most relevant aspect about all cyclodextrin structures is their hydrophobic-hydrophylic environments inside-outside the cavity, respectively.

The enormous importance of the interaction of cyclodextrins not only in separation science but also as a host for a series of pharmaceuticals lead us to better understand the thermodynamic of the interactions of β-Cyclodextrin and a model DL-amino acid, tryptophan.

To quantify the supramolecular interaction in solution between the organic guest and the cyclodextrin host several approaches have already been described. In general, this is done calculating the equilibrium constant of the complex, which can be obtained by Scott and Scatchard plots [4,5] and solubility diagrams [6], using UV electronic spectroscopy [7,8], affinity capillary electrophoresis (ACE) [9] and/or high performance liquid chromatography (HPLC) [10].

For the case in which both host and guest absorb in the same UV region, it is suitable to use an azo dye as a probe [11,12]. The progressive addition of the guest molecule enables in-solution dissociation of the CD-azo complex: this gives, in the visible region, an absorbance which is significantly different from that of the adduct. This allows, with the help of appropriate calculations, to estimate the stability constant.

In the solid state, Fourier-transform infrared spectroscopy (FTIR) has been established as the best technique to show complex formation [13].

Cyclodextrins are known by their properties of molecular recognition, which are useful for enantiomeric separations of amino and hydroxyl acids, for example. Past reports have already showed the interactions in solution between some amino acids, such as tryptophan, and cyclodextrins [14,15].

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No solid state characterization of the complexes has been so far carried out.

Basically, the objective of the present work is to quantify the supramolecular interactions in solution between D- and L-tryptophan (the "guests") and 2-hydroxypropyl-β-cyclodextrin (the "host"), as well as to study the production of the corresponding supramolecular complexes in the solid state.

EXPERIMENTAL

HPLC was used to evaluate the response of D- and L-tryptophan in the presence of variable cyclodextrin concentrations in the mobile phase.

In-solution stability constants were quantified by electronic spectroscopy.

Solid state characterization of inclusion compounds was carried out using Fourier-transform infrared spectroscopy (FTIR).

Chemicals

2-Hydroxypropyl-β-cyclodextrin (HPCD), with a substitution degree of 0.6, was purchased from Fluka. D-Tryptophan and methyl orange were supplied by Sigma Chemical Company while L-Tryptophan was supplied by Riedel-de Haën. Acetonitrile (MeCN) HPLC-grade solvent came from Grupo Química.

HPLC

A reverse phase C8 column (Lichrosorb RP-8) was used (Shimadzu LC 10 AS liquid Chromatograph). UV detection was at 254 nm and the flow rate of mobile phase was 1.0 mL min^{-1}.

D- and L-Tryptophan were injected in duplicate from 1×10^{-4} mol L^{-1} solutions of pure isomers, prepared dissolving the amino acids in the mobile phase (60:40, H_2O:MeCN), two other mobile phases were used containing 0.8 and 2.4 mM HPCD in 60:40, H_2O:MeCN. All mobile phases presented pH 5. Before each injection, the column was balanced with the mobile phase for 1 hour. The column void time was obtained from the injection of a $NaNO_2$ solution.

Electronic Spectroscopy

Methyl orange was dissolved in a 10^{-1} mol L^{-1} H_2SO_4 solution to have a 10^{-5} mol L^{-1} concentration. HPCD was then added, giving a 5 mmol L^{-1} solution. Aliquots from the latter were taken and mixed with appropriate amounts of D- and L-tryptophan. The spectra of all these solutions were acquired in the 200–800 nm interval at 25°C. Solutions with variable HPCD concentrations (5 to 14 mmol L^{-1}) and constant methyl orange amounts (1×10^{-5} mol L^{-1}) were also analyzed in the same wavelength interval. The equipment used for the experiments was a HP8452A spectrometer.

Preparation of Solid State Complexes

This was done in two steps: i) dissolution in water of equimolar quantities of Trp and HPCD, with microfiltration (Millipore 0.22μ) of the resulting solution; and ii) rotoevaporation at 90°C. Mechanical mixtures were obtained by mixing equimolar contents of HPCD and D/L-Trp using an agate mortar and pestle.

FTIR

Spectra were acquired from Nicolet Magna equipment, with 32 accumulations and 2 cm^{-1} resolution. Each KBr pellet was made with 1%w/w of analyte. Using the OMNIC software, the spectra of the complexes and of the mechanical mixtures were subtracted from the HPCD signal and compared to those of D/L - Trp. All spectra were submitted to Fourier self-deconvolution.

RESULTS AND DISCUSSION

Before determining in-solution stability constants of inclusion compounds, the ready response of both amino acid isomers to the HPCD presence in the mobile phase was verified by means of HPLC. As duplicate injections were made, one-way ANOVA tests were applied to the data collected [16]. Capacity factors were calculated by the following expression:

$$k = \frac{t - t_0}{t_0} \quad (1)$$

where t is the retention time and t_0 is the column void time. The latter was obtained by reading the retention time of an injected $NaNO_2$ solution, which is an analyte that does not interact with the stationary phase of the column.

The null hypothesis was *H_0: there is no effect on D- and L- Trp capacity factors due to the presence of HPCD in the mobile phase*. As Table I shows, the null hypothesis was rejected, since the calculated F-test values are higher than the critical F [16], confirming HPCD- D- and L-Trp in-solution interaction.

In order to explain this result, the effect of polarity has to be taken into account. It can be verified that the effect prevailed: the main point of interaction between each Trp isomer and the stationary phase is the hydrophobic indole moiety. The presence of HPCD may disturb this interaction, insofar as the cyclodextrin cavity competes with the side chain of Trp for the interaction. It must be assumed that there are negligible HPCD interactions with the stationary

TABLE I Capacity factor data and ANOVA parameters calculated from HPLC experiments

	D-Tryptophan			L-Tryptophan		
HPCD concentration (mM)	0	0.8	2.4	0	0.8	2.4
Capacity factors	1.164	1.157	1.112	1.184	1.158	1.138
	1.162	1.143	1.096	1.177	1.154	1.111
Mean value	1.163	1.150	1.104	1.181	1.156	1.125
One-tailed F test* (Fcrit)		25.61657 (9.552)			11.44434 (9.552)	

* at 95% of confidence level

phase, since it presents hydrophilic character outside the cavity. Those assumptions explain why inclusion complexes flow faster than each Trp isomer.

It can also be seen in these experiments that acetonitrile, which acts as an organic modifier of the mobile phase, does not prevent the formation of inclusion compounds, in contrast to what has been described when using methanol [17]. Methanol can auto-ionize and it is known as a proton donor solvent, hence, with acidic character [18,19]. Furthermore, it can make hydrogen bonds (H-bonds) to the superficial OH groups and oxygen atoms of cyclodextrins. On the other hand, acetonitrile acts as a proton acceptor, due to its basic character [18], which limits its interaction with HPCD. This explains why acetonitrile interaction with cyclodextrins does not prevent the formation of inclusion compounds with D- and L-tryptophan.

For the determination of stability constants, methyl orange was used as a probe in the electronic spectroscopy experiments in the visible region. This method has already been described in the literature [11,12]. Addition of cyclodextrin to a methyl orange solution causes a decrease in the maximum absorbance value at 508 nm (Fig. 1). Increasing quantities of amino acid added to the HPCD-azo solution progressively raises the absorbance and hence it can be assumed that no ternary complex is formed in solution. Then, the following competition equilibrium will exist:

$$\beta\text{HPCD}-\text{M}+[\text{D}-\text{orL}-\text{Trp}] \rightarrow \beta\text{HPCD} - [\text{D}-\text{orL}-\text{Trp}] + \text{M} \quad (2)$$

where M represents methyl orange. The stability equilibria will be:

$$\beta\text{HPCD} + [\text{D}-\text{orL}-\text{Trp}] \rightarrow \beta\text{HPCD} - [\text{D}-\text{orL}-\text{Trp}] \quad (3)$$

$$\beta\text{HPCD} + \text{M} \rightarrow \beta\text{HPCD}-\text{M} \quad (4)$$

The concentration of free HPCD in these equilibria can be calculated by the following expression [11,12]:

$$[\text{HPCD}] = \frac{Abs_M - Abs}{K_4(Abs - Abs_{HPCD-M})} \quad (5)$$

where *Abs*, Abs_M *and* $Abs_{HPCD\text{-}M}$ are absorbance values at 508 nm of tryptophan-methyl orange-HPCD, methyl orange and HPCD-methyl orange solutions, respectively; and K_4 is the stability constant of the HPCD-methyl orange complex. This constant can be calculated from the following Scott expression:

$$b\frac{[HPCD]_0}{\Delta Abs} = \frac{[HPCD]_0}{[M]\Delta\epsilon} + \frac{1}{[M]\Delta\epsilon K_4} \quad (6)$$

where *b* is the path length, *Δabs* the absorbance change, *[M]* the total methyl orange concentration, $\Delta\epsilon$ the molar absorptivity change and $[HPCD]_0$ the total cyclodextrin concentration. The Scott plot of HPCD-methyl orange complex can be found in Fig. 2. The K_4 value of $(1.9 \pm 0.1) \times 10^2$ can be calculated from the slope/y-intercept quotient.

Association constants of both tryptophan isomers with HPCD can be calculated using the following expression [11,12]:

$$K_3 = \frac{[HPCD]_0 - [HPCD]}{[HPCD]([Trp] - [HPCD]_0 + [HPCD])} \quad (7)$$

where *[HPCD]* can be calculated using (5) and *[Trp]* is the total tryptophan concentration. K_3 values of $(2 \pm 1) \times 10$ and $(9 \pm 2) \times 10^{-1}$ were obtained for D- and L-isomers, respectively, which shows that the

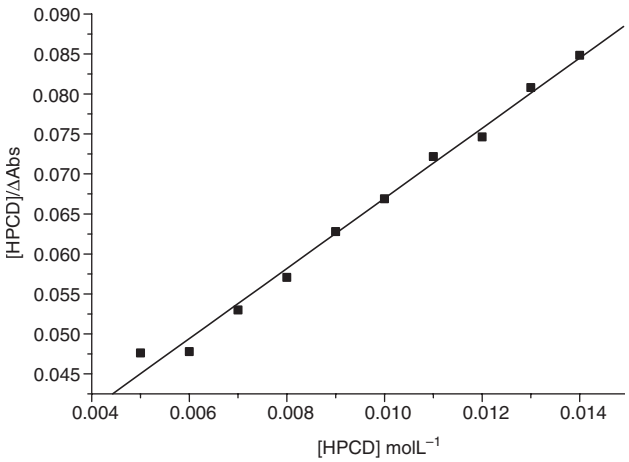

FIGURE 1 Scott plot at 25°C using increasing cyclodextrin concentrations for solutions with fixed content of methyl orange (10^{-5} mol L^{-1}).

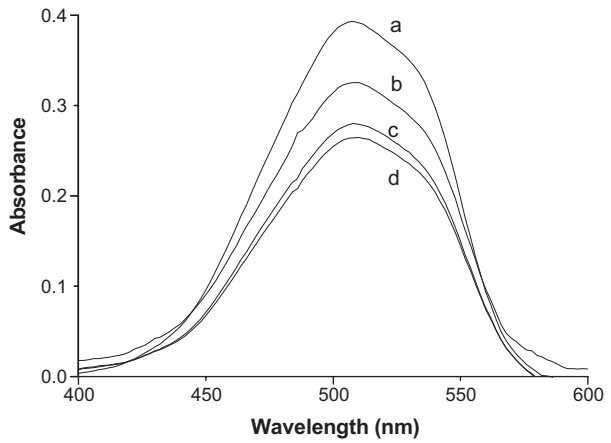

FIGURE 2 Electronic spectra of 10^{-5} methyl orange solutions containing HPCD in the following concentrations: (a) 0, (b) 5×10^{-3}, (c) 1.0×10^{-2} and (d) 1.4×10^{-2} mol L^{-1}.

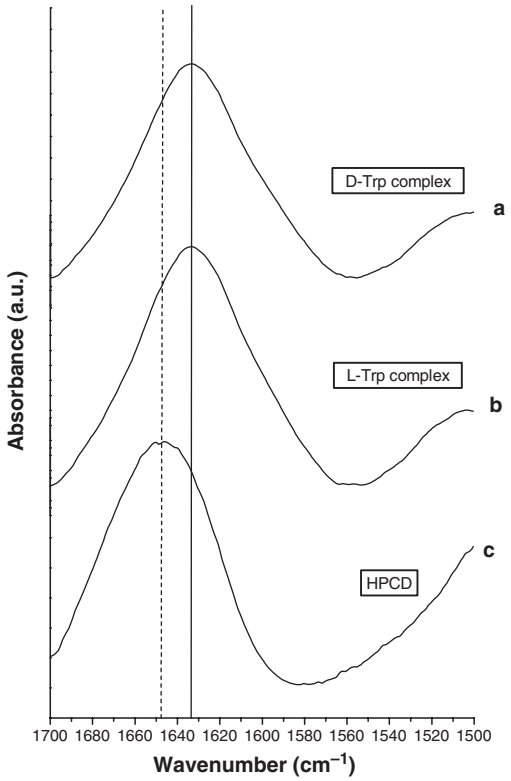

FIGURE 3 Non-subtracted and non-deconvoluted FTIR spectra for (a) D-Trp complex (b) L-Trp complex and (c) HPCD. Dotted and continuous lines indicate the maxima of HPCD and complex bands, respectively.

former leads to an interaction of one order of magnitude stronger than that of the latter.

FTIR spectra in the 1700–1500 cm^{-1} range for HPCD and for both complexes using the procedures described in the experimental section are compared in Fig. 3. A band shift from 1646 to 1633 cm^{-1} is observed when considering the HPCD and the complexes spectra, respectively. This signals that some chemical modification occurs in this wavenumber region when the inclusion compound is obtained.

In order to confirm this assumption, we have subtracted Fig. 4a and b from c and the results, as well as the amino acid FTIR spectra, were submitted to the Fourier self-deconvolution tool in OMNIC software. Self-deconvoluted spectra are presented in Fig. 4. It can be observed that the spectra of mechanical mixtures reproduced aminoacid signals quite well.

Table II presents the main bands in Fig. 4 assigned with help of the literature [20,21]. For the complexes, the COO^- asymmetric stretching band was shifted to higher wavenumber values (Fig. 4a and d and Table II). This can be explained by the complexation process, which prevents the Trp molecules from interacting with each other. Hence the COO^- is kept from interacting with a neighboring NH_3^+ group, which increases the vibration energy for the stretching mode of the former. For the NH_3^+ asymmetric angular deformation, the band at $\sim$1660 cm^{-1} vanishes when complexation occurs (Fig. 4a and d). However, intense bands appear at 1635 cm^{-1}, and they may be related to the new chemical environment of amino acid isomers, in which NH_3^+ forms H-bonds with the superficial OH groups of HPCD. This result corroborates the disappearance of intermolecular $COO^- \cdots NH_3^+$ interaction stated above. Appreciable wave number changes (i.e., beyond 4 cm^{-1}) are also observed for the intramolecularly coupled $COO^- \cdots NH_3^+$ vibration as a result of the band shifts cited above (Table II).

Finally, it can be observed that the complexes have similar spectra. This means that both isomers have in-solution interaction with cyclodextrin (although in different magnitudes, as already seen) and form complexes in the solid state.

CONCLUSIONS

In the present work, supramolecular complexes of 2-hydroxypropyl-β-cyclodextrin and D/L-Tryptophan were described. They are formed in solution with stability constants of $(2 \pm 1) \times 10$ and $(9 \pm 2) \times 10^{-1}$ for D and L forms, respectively. We have also determined that the complexes occurred in the solid state, with two points of interaction: one superficial, with H-bonds between Tryptophan's NH_3^+ group and cyclodextrin OH groups; and another interaction between the indole moiety and CD cavity. This work represents a part of our continuous work on understanding interactions between chiral compounds [22] and can better clarify the thermodynamics associated with such interactions.

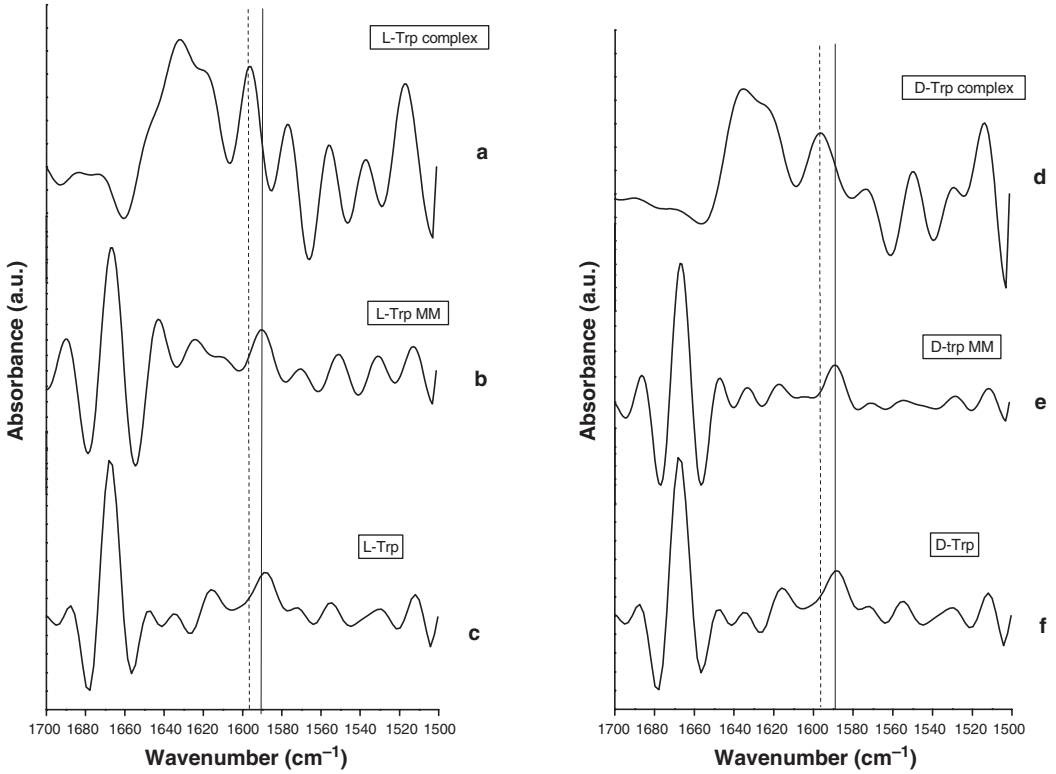

FIGURE 4 Fourier self-deconvoluted FTIR spectra of (a) L-Trp complex; (b) 1:1 L-Trp mechanical mixture; (c) L-Trp; (d) D-Trp complex; (e) 1:1 D-Trp mechanical mixture; and (f) D-Trp. a,b,d and e spectra were subtracted from HPCD signal before applying fourier self-deconvolution. Dotted and continuous lines indicate the position of the carboxylate asymmetric stretching band in complexes and in Trp isomers, respectively.

TABLE II Assignment of bands of interest shown by the self-deconvoluted FTIR spectra. Band intensities are in brackets

	δ_a NH_3^+	ν_a COO^-	δ_a NH_3^++ν_a COO^-
D-Trp complex	1635 cm^{-1} (s)	1597 cm^{-1} (m)	1626 cm^{-1} (s)
L-Trp complex	1632 cm^{-1} (s)	1597 cm^{-1} (m)	1620 cm^{-1} (m)
D-Tryptofan	1666 cm^{-1} (s)	1590 cm^{-1} (m)	1612 cm^{-1} (m)
L-Tryptophan	1668 cm^{-1} (s)	1590 cm^{-1} (m)	1614 cm^{-1} (m)

δ_a → assymmetric angular deformation; ν_a → assymmetric stretching; m → medium; s → strong.

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