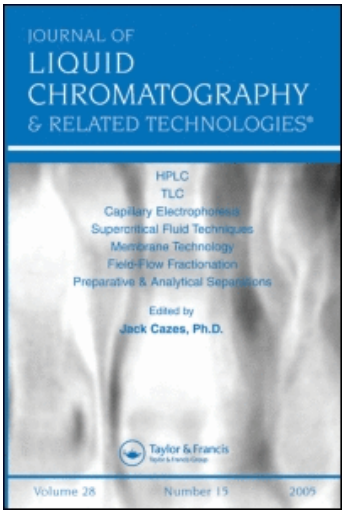

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STOICHIOMETRY AND FORMATION CONSTANTS OF SIX PAHs WITH γ-CYCLODEXTRIN, DETERMINED BY HPLC USING A CYANO STATIONARY PHASE

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ABSTRACT

The interaction of γ-cyclodextrin with a series of six polycyclic aromatic hydrocarbons (PAHs) (naphthalene, fluorene, anthracene, phenanthrene, fluoranthene, and pyrene) was studied by high performance liquid chromatography (HPLC), using a cyano bond silica column. In order to assess the retention mechanism, two models were tested. In the first one, the interaction of the CD-PAH complex with the column was neglected, while in the second one the adsorption of this complex was taken into account. For all the PAHs, the stoichiometry of the

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inclusion complexes between each molecule and the γ-cyclodextrin was found to be 1 : 1 and the formation constants were calculated from the retention data. As well, the thermodynamic parameter variations were obtained using the van't Hoff plots. The enthalpy–entropy compensation was analysed in order to provide further information about the interaction mechanism between the PAHs and the macrocycle.

INTRODUCTION

Cyclodextrins (CD) are torus-shaped molecules formed from the α-1,4 linkage of glucopyranose units. The interior of the cyclodextrin cavity is fairly non polar due to the glucosidic oxygen and hydrogen atoms in the cavity. The three most common cyclodextrins are α-, β-, and γ-, which respectively contain 6, 7, and 8 monomeric glucopyranose units, and have various cavity diameters. The CDs are of particular interest due to their ability to form host–guest complexes with different guest species (1).

Polycyclic aromatic hydrocarbons (PAHs) are an important class of environmental pollutants. Some of them are known to be mutagenic and carcinogenic (2).

Previous studies have described HPLC methods where a co-modifier had been added to the eluent in order to define the stoichiometry and the formation constants of the CD-PAH complexes (3,4). However, the presence of this co-modifier led to a modification in the interaction between the macrocycle and the PAHs. In that chromatographic condition, the "true" inclusion mechanism could not be observed. Thus, in order to clarify this "true" inclusion mechanism of PAHs into the CD, a study has been recently carried out using a cyano phase, more polar than a C18 phase, and a modifier-free hydro-organic phase, with varying concentrations of β-CD (from 0 to 7.5 mM) (5).

Two interaction models were derived in order to describe the PAHs behavior in this chromatographic system. The first one considered the interaction of the CD-PAH complex with the stationary phase negligible (*i*); the second one reflected on this interaction (*ii*). It was concluded, that model (*ii*) described more accurately the retention behavior of the five PAHs in this chromatographic system.

The aim of the present paper is to extend the experimental chromatographic approach to the study of PAHs inclusion in γ-CD. Most of the studies concerning the stoichiometry and the apparent formation constants for the complexes with γ-CD have been carried out using spectroscopic methods, such as fluorescence and absorbance measurements (6). Only a few of them have reported the use of liquid chromatography to characterise the inclusion mechanism of PAHs into the γ-CD

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(7). This paper describes a study using a cyano bonded silica column and γ-CD as a mobile phase additive. The two interaction models previously described were tested in order to determine the stoichiometry and the association constants of the γ-CD-PAHs inclusion complexes. As well, enthalpy–entropy compensation was examined in relation to the interaction mechanism.

EXPERIMENTAL

Apparatus

The LC system consists of a Waters (Milford, Massachusetts) 600E multisolvent delivery system equipped with an in-line degasser, a Jasco AS-95 automatic injector (Kyoto, Japan), a Shimadzu SPD-6AV (Kyoto, Japan) ultraviolet detector set at 254 nm, and a Shimadzu CR-3A Chromatopac integrator. A Nucleosil 250 mm × 4 mm CN column (5 µm, particle size) supplied by Macherey-Nagel (Dueren, Germany) was used at controlled temperature in an Interchim Igloocil oven (Montluçon, France).

Reagents

Naphthalene (I), fluorene (II), phenanthrene (III), anthracene (IV), fluoranthene (V), and pyrene (VI) were from Aldrich (Steinheim, Germany). Stock standard solutions of PAHs in methanol (0.5 mg mL^{-1}) were prepared and stored at 4°C, in glass bottles, in the dark. Working standard PAHs solutions were adjusted to 2.5 µg mL^{-1} for naphthalene, fluorine, and fluoranthene, 0.25 µg mL^{-1} for phenanthrene and anthracene, and 10 µg mL^{-1} for pyrene. LC-purity methanol was the analytical reagent (SDS, Villeurbanne, France). LC grade high quality water was obtained from Stillplus HP system (Oxon, U.K.). The potassium iodide (RP grade, Merck, Germany) was used to determine the void volume of the column.

The mobile phase consisted of a mixture of 35/65 (v/v) methanol/water with various γ-cyclodextrin concentrations (0; 1; 2.5; 5, and 7.5 mM). The flow rate was set at 1 mL min^{-1}. The γ-CD used in this study was provided by Wacker Chimie S.A. (Germany). The γ-CD was injected into the chromatographic system using a modifier-free mobile phase. The macrocycle was eluted roughly in the void volume ($k' = -0.12$) indicating that the interaction between the CD and the stationary phase could be considered as negligible under the operating conditions.

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Model Description

Various models have been used to describe the chromatographic retention of a solute when a CD is added to the mobile phase. These models assume that CD does not interact with the stationary phase. In most cases, the interaction of the solute–CD complex with the stationary phase is also not taken into account (3,8–10). In this case, the retention behavior of PAHs is based on the partitioning of the sample between the mobile and the stationary phases. The solute retention is split into two main physicochemical processes, i.e., the solute complexation by γ-CD and the transfer of the free solute from the hydro-organic mobile phase to the stationary phase. The values of the equilibrium constants for the complex formation, K_i, are obtained from the ratio (slope/intercept) of a plot of the retention factor reciprocal (k') against the concentration of cyclodextrin in the mobile phase, for each eluting solute. Equation 1 is representative of the model without adsorption of the complex on the stationary phase (3,8–11).

$$k' = \frac{k'_0}{1 + K_i[\mathrm{CD}]^x} \tag{1}$$

where k'_0 is the solute retention factor without CD in the mobile phase and [CD] is the concentration of γ-CD in the mobile phase. The term x corresponds to the complex stoichiometry.

However, it has also been previously demonstrated that the complex between the solute and CDs (particularly with β-CD) is able to interact strongly with the stationary phase (5,11).

The values of the equilibrium constants for the complex formation, K_i, are obtained with Equation (2) which represents the model with the stationary phase and the adsorbed complex (5,11).

$$k' = \frac{k'_0 + k'_c K_i[\mathrm{CD}]^x}{1 + K_i[\mathrm{CD}]^x} \tag{2}$$

where k'_c is the solute retention factor of the complex.

Temperature Studies

For each compound, the retention factor was determined at the following temperatures: 25°C, 30°C, 35°C, and 40°C. The chromatographic system was allowed to equilibrate for at least 1 hour prior to each experiment. At each temperature and for each γ-CD concentration, 25 µL of each solute were injected separately in triplicate and the retention times measured.

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Thermodynamic Relationships

$\Delta H^{\circ}_{S.CD}$ and $\Delta S^{\circ}_{S.CD}$ are, respectively, the standard enthalpy and entropy of the inclusion complex formation between the molecule S and the cyclodextrin. These energies can be calculated using the following thermodynamic relationship:

$$\ln K_i = \frac{-\Delta H^{\circ}_{S.CD}}{RT} + \frac{\Delta S^{\circ}_{S.CD}}{R} \quad (3)$$

where T is the temperature, R is the gas constant.

For a linear plot, the slope and intercept are, respectively, $-\Delta H^{\circ}_{S.CD}/R$ and $\Delta S^{\circ}_{S.CD}/R$.

Enthalpy–Entropy Compensation

The investigation of the enthalpy–entropy compensation temperature is a thermodynamic approach to the analysis of physico-chemical data. This approach has been previously used in chromatographic procedures to analyze and compare the retention mechanism for a group of compounds (12). The enthalpy–entropy compensation can be described by the following relation:

$$\Delta H^{\circ}_{S.CD} = \beta \Delta S^{\circ}_{S.CD} + (\Delta G^{\circ}_{S.CD})_{\beta} \quad (4)$$

where $(\Delta G^{\circ}_{S.CD})_{\beta}$ is the Gibbs free energy of solute inclusion at a compensation temperature β (both are constant). In accordance with Equation (4), when enthalpy–entropy compensation is observed for a group of compounds in a particular chemical interaction, all the compounds have the same free energy $(\Delta G^{\circ}_{S.CD})_{\beta}$ at a temperature β. Therefore, if enthalpy–entropy compensation is observed for the six PAHs, all will have the same interaction mechanism with CD at a compensation temperature β.

RESULTS AND DISCUSSION

Determination of the Stoichiometry and the Association Constants for the PAHs–γ-CD Complexes

Using the solute retention time and the void time, the k' values were determined for all the γ-CD concentrations at a temperature equal to 25°C. In this chromatographic system, the retention factor of the six PAHs decreased when the γ-CD concentration increased (Figure 1). Such a phenomenon was not observed for the most hydrophobic compounds, such as pyrene, when a C18 stationary

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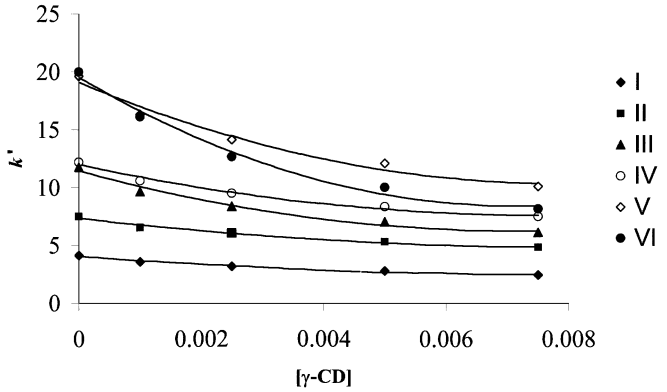

Figure 1. Plots of k' vs. [γ-CD] for all the compounds (assuming 1 : 1 stoichiometry, model with complex adsorption).

phase-hydro-organic mobile phase chromatographic system was used (3,4). This behavior difference can be explained by the fact that the PAH affinity for the cyano stationary phase is lower than for a C18 stationary phase. The competition of PAHs between the weak apolar stationary phase and the cavity interior of CD is effective. Therefore, the use of a more polar stationary phase allows the study of the "pure" stoichiometry and apparent formation constants of very hydrophobic molecules, i.e., without the use of a co-modifier.

It has been previously demonstrated, that PAHs such as pyrene and anthracene, form 1 : 1 complexes with γ-CD at concentrations below their aqueous solubility (13). In liquid chromatography with addition of cyclopentanol in the mobile phase, naphthalene, phenanthrene, and anthracene form 1 : 1 complexes with γ-CD (14). Therefore, the retention data were analyzed for all the PAHs using the two interaction models assuming a 1 : 1 stoichiometry ($x = 1$ in Equations (1) and (2)). Model without complex adsorption and model with complex adsorption were fitted to experimental data. A software "table curve 2D" was used in order to determine the best fits. The values of the non linear regression coefficients R and F were calculated (Table 1). Using the F values, it appears clearly that the behaviors of PAHs were well described by the model, taking into account the adsorption of complex on the stationary phase (Figure 2). Our results confirm that the F value constitutes a more discriminating parameter than the R value when assessing the significance of model equations (15).

Similar results concerning the adsorption of complexes have been observed in the previous study using β-CD as mobile phase additive (5). Additionally, the retention data for the smallest PAH, i.e., naphthalene, was analyzed using the complex adsorption model with 2 : 1 stoichiometry ($x = 2$). However, the F value

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Table 1. Correlation Coefficients R and F Values for the Plots from Equations (1) and (2)*

	Model Without Complex Adsorption		Model With Complex Adsorption	
	R	F	R	F
I	0.980	102	0.994	445
II	0.974	77	0.995	350
III	0.981	105	0.997	780
IV	0.964	53	0.994	268
V	0.984	154	0.996	465
VI	0.996	642	0.999	462

*Assuming a 1 : 1 stoichiometry for all compounds.

was lower than the F value for the complex adsorption model, assuming 1 : 1 stoichiometry (data not shown). This is consistent with an inclusion process involving only one molecule of naphthalene with one molecule of γ-CD. From the different fits obtained for each PAH, the apparent K_i value and the retention factor k'_c of each species S.γ-CD were calculated. Table 2 presents the structures for all

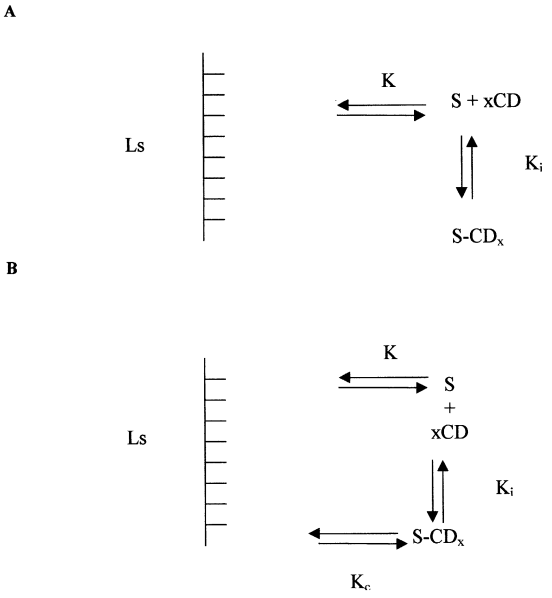

Figure 2. A. Chemical equilibria for model without adsorption of complex in stationary phase. B. Chemical equilibria for model with adsorption of complex in stationary phase.

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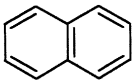
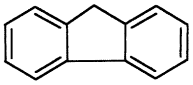
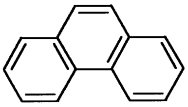
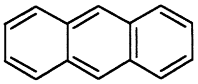
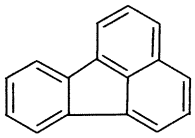
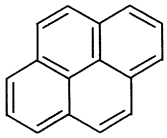
Table 2. The Six Polycyclic Aromatic Hydrocarbon Structures

Compound No.	Nomenclature	Chemical Structure
I	Naphthalene	
II	Fluorene	
III	Phenanthrene	
IV	Anthracene	
V	Fluoranthene	
VI	Pyrene	

the species and Table 3 presents these values for all the species. For example, the estimated apparent binding constant for the formation of 1 : 1 γ-CD : pyrene is 294 M^{-1}. This value is in very good accordance with the estimates from other studies, i.e., 250 M^{-1}(16) and 300 M^{-1} (13). For anthracene, the formation constant between γ-CD and anthracene ($K_i = 335\ M^{-1}$) is similar to the estimate of Blyshak et al., i.e., $241 \pm 94\ M^{-1}$ (17).

Influence of the CD Cavity Size

It is well known that the formation of the inclusion complex depends strongly upon shape, size, and spatial geometry of the solute and diameter of the CD cavity. The K_i values can be considered as a measure of how a solute fits

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Table 3. Retention Parameters for the γ-CD–PAH (I, II, III, IV, V and VI) Complexes in Methanol–H_2O (35–65; v/v) Using Model with Complex Adsorption on Stationary Phase and Assuming a 1 : 1 Stoichiometry

PAH	k'_0 $T=25°C$	k'_c $T=25°C$	K_i(γ-CD) $T=25°C$	K_i(β-CD) $T=25°C$
I	4.13	1.63	267	ND
II	7.50	3.51	258	397
III	11.75	4.01	332	529
IV	12.20	5.83	335	602
V	19.63	5.83	272	1454
VI	20.01	2.87	294	2.3×10^5*

*(M^{-2}).
k'_0: Solute retention factor without γ-CD in the mobile phase.
k'_c: Complex retention factor.
K_i(γ-CD): Equilibrium constant between S and γ-CD (M^{-1}).
K_i(β-CD): Equilibrium constant between S and β-CD (M^{-1}) (from Ref. 5).

inside the CD cavity. In order to investigate the role of the CD cavity size on the interaction with PAHs, the K_i (and stoichiometry) values obtained for PAHs–γ-CD complexes were compared to the association constants (and stoichiometry) previously determined for PAHs–β-CD complexes (5).

Pyrene formed a 1 : 1 complex with γ-CD, while a 2 : 1 complex has been observed with β-CD (5,8,18). In the case of β-CD, which has a cavity diameter of 7.8 Å, pyrene (8.2 Å wide and 10.4 Å long) is assumed to be too bulky to fit entirely into the β-CD cavity. So, the part of pyrene exposed to the aqueous environment is considered to be more important than the part included inside the CD. Thus, it is more likely that more than one β-CD participates in the complex formation as previously shown (16,19). Conversely, γ-CD has a larger cavity diameter (9.5 Å) and is able to induce a better inclusion of pyrene. Then, the part of the pyrene exposed to the aqueous environment is expected to be relatively weak to that included inside the CD. So, the likelihood that more than one γ-CD participates in the complex formation is reduced. This can explain the 1 : 1 stoichiometry obtained with the γ-CD.

For the same operating conditions ($T=25°C$ and mobile phase: 35/65 (v/v) methanol/water), the K_i values for the PAH–γ-CD are lower than the association constants observed for the PAH inclusion in the β-CD (Table 3). As well, the association constants for PAHs–β-CD complexes vary in relation to the size of the analyte, while no significant difference is observed for the K_i of PAH–γ-CD complexes (258–335 M^{-1}). The high K_i values for the PAH–β-CD complexes can be attributed to the fact that PAHs tightly bind to the small β-CD

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cavity. PAHs can interact strongly with the interior of the cavity in such a way that high van der Waals interaction energy is implied (5). This is consistent with the fact that these K_i values increase in the following order of fluorene, anthracene, and fluoranthene, according to their increasing hydrophobicity.

On the other hand, the weaker K_i values for the PAH–γ-CD inclusion can be interpreted as a consequence of the large diameter of the γ-CD cavity. It is expected that the compounds are too small to tightly bind to the γ-CD. The analytes appear to be able to move in such a way that they experience the same average environment. Thus, interactions between PAH and the cavity interior are expected to be similar for all the solutes, providing roughly identical values of association constants.

Enthalpy–Entropy Compensation

In order to gain further insight into the mechanistic picture of the PAH–γ-CD inclusion, plots of $\ln K_i$ against $1/T$ were constructed for all the PAHs. The van't Hoff plots were all linear with correlation coefficient higher than 0.95. The enthalpy–entropy compensation was examined using the values of $\Delta H^{\circ}_{\mathrm{S.CD}}$ and $\Delta S^{\circ}_{\mathrm{S.CD}}$ extracted from linear van't Hoff plots (Figure 3). If plots of $\Delta H^{\circ}_{\mathrm{S.CD}}$–$\Delta S^{\circ}_{\mathrm{S.CD}}$ are linear, then the solutes interact with CD by essentially identical mechanisms. In this case, the correlation coefficient for the linear fit was equal to 0.999. The values of the compensation temperature β and the Gibbs free energy of solute inclusion at this temperature $(\Delta G^{\circ}_{\mathrm{S.CD}})_{\beta}$, were equal to 295 K and 14.3 kJ/mol, respectively. These values are similar to the estimates previously calculated for the interaction between hydrophobic amino acid derivatives and macrocyclic antibiotic (20). The high degree of correlation observed for the plot $\Delta H^{\circ}_{\mathrm{S.CD}}$–$\Delta H^{\circ}_{\mathrm{S.CD}}$, allows interpretation as an enthalpy–entropy compensation.

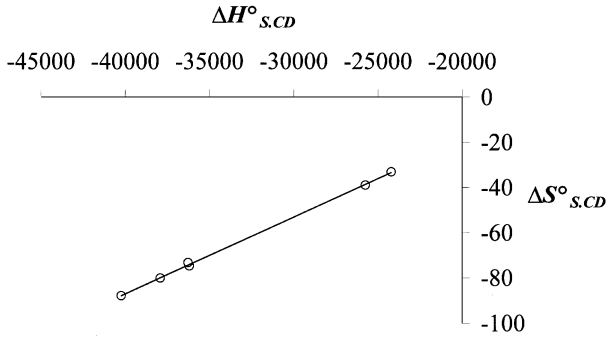

Figure 3. Plot of $\Delta S^{\circ}_{\mathrm{S.CD}}$ (J/mol K) vs. $\Delta H^{\circ}_{\mathrm{S.CD}}$ (J/mol).

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This result indicates that all the PAHs have probably the same inclusion mechanism, and confirms that the solute inclusion in γ-CD is independent of the molecular structure of PAHs.

CONCLUSION

In the present study, the inclusion complexes of very apolar compounds with γ-CD in HPLC was considered, with the use of a relatively polar stationary phase such as a cyano silica gel, which favors the solute inclusion phenomenon in the mobile phase, relative to the solute interaction with the stationary phase. The complex stoichiometry and the association constants were calculated using an interaction model, taking into account the adsorption of the complex on the stationary phase. From the data, it was predicted that one molecule of naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene interacted with one γ-CD molecule. The association constants for the PAH–γ-CD complexes were lower than those observed for the PAH–β-CD complexes. These results were interpreted as a consequence of the large γ-CD cavity size, which was responsible for a decrease in the van der Waals interactions between the analytes and the cavity interior.

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