Retention Study of Dichlorophenol Isomers by High-Performance Liquid Chromatography Using a Mobile Phase Modified with β-Cyclodextrin

N. Morin

Laboratoire de Chimie Physique et Minérale, Faculté de Médecine et de Pharmacie, Place Saint Jacques, 25030 Besançon Cedex, France

E. Peyrin, S. Cornet, C. Guinchard, and **Y.C. Guillaume***

Laboratoire de Chimie Analytique, Faculté de Médecine et de Pharmacie, Place Saint Jacques, 25030 Besançon Cedex, France

Abstract

The retention mechanism of a series of 6 positional isomers of dichlorophenol in high-performance liquid chromatography (HPLC) are investigated using β-cyclodextrin as a mobile phase additive. The values of enthalpy and entropy of the transfer of an isomer from the mobile to the stationary phase and the association of an isomer with β-cyclodextrin are determined. These data demonstrate the leading role of enthalpy in relation to entropy in the association process. Enthalpy–entropy compensation reveal that the retention mechanism is independent of the chloro group position on the phenol ring. The stoichiometry of the inclusion complexes between the dichlorophenol isomers and β-cyclodextrin is determined as 1:3. The association constants of the inclusion complexes are calculated.

Introduction

Cyclodextrins (CDs), which are torus-shaped cyclic oligosaccharides consisting of six or more α-(1,4)-linked D-glucopyrannose units, are one of the well known host molecules capable of forming an inclusion complex (host–guest complex) with a wide variety of organic molecules or so-called guest molecules (1). In high-performance liquid chromatography (HPLC), CDs have been extensively chemically bonded to a stationary phase (2,3) and added to the mobile phase (4–7). Guillaume and Guinchard (8) examined the retention mechanism of six dichlorophenol isomers in gas chromatography using α-, β-, and γ-CD stationary phases. Paleologou et al. (9) studied the liquid chromatographic (LC) retention behavior of monoaromatic chlorophenols on a β-cyclodextrin column with respect to mobile phase composition, pH, temperature, and ionic strength. The results suggested that the unique selectivity of this column is because of an inclusion complex formation that provides the physical basis for the resolution of positional isomers. Under certain chromatographic conditions, however, the more highly chlorinated congeners appear to be excluded from the CD cavity and interact with secondary hydroxyls on the periphery of the CD moieties.

In this paper, the thermodynamic behavior of a series of six positional dichlorophenol isomers is investigated by HPLC over a range of column temperatures (between 20 and 55°C) and of β-cyclodextrin concentrations in the mobile phase (between 0 and 2.5mM). The shapes of van't Hoff plots were used to assess changes in the chromatographic process in relation to temperature and β-CD concentration. The stoichiometry of the complexes formed between the six dichlorophenols and the β-CD and their association constants were determined.

Experimental

Apparatus

The HPLC system consisted of a Waters (Saint-Quentin, Yvelines, France) HPLC pump 501, an Interchim Rheodyne (Montluçon, France) model 7125 injection valve fitted with a 20-µL sample loop, and a Shimadzu (Touzart-Matignon, Vitry sur Seine, France) SPD-10A variable wavelength ultraviolet (UV) spectrophotometer detector (Nogent sur Marne, France). A Lichrocart (Merck, Darmstadt, Germany) RP_{18} column (125 × 4-mm i.d., 5-µm particle size) was used with controlled temperature in an Interchim (TM number 701) oven. The mobile phase rate was fixed at 1 mL/min, and the wavelength was fixed at 230 nm.

Solvents and samples

HPLC-grade methanol (Carlo Erba, Val de Reuil, France) was used without further purification. Water was obtained from an Elgastat option I water purification system (Odil, Talant, France) fitted with a reverse-osmosis cartridge. The mobile phase used for these studies was a methanol–water (65:35, v/v) mixture with various β-CD concentrations (0, 0.5, 1, 1.5, 2, and 2.5mM). β-CD

* Author to whom correspondence should be addressed.

was a gift from the Roquette Laboratories (Lestrem, France). The chromatographed compounds were positional isomers. 2,3-dichloro- (1), 2,4-dichloro- (2), 2,5-dichloro- (3), 2,6-dichloro- (4), 3,4-dichloro- (5), and 3,5-dichloro- (6) phenols were obtained from Merck (Nogent sur Marne, France). The chemical structures of these compounds are given in Figure 1. For the chromatographic investigation, the samples were dissolved in pure water to obtain a concentration of 1 mg/L. Each solute was injected, and the retention times were measured using a Merck D2500 chromatointegrator. All experiments were repeated three times at each temperature and β-CD concentration. Sodium nitrate (Merck) was used as a dead-time marker.

Temperature studies

Isomer retention factors were determined at the temperature values of 20, 25, 30, 35, 40, 45, 50, and 55°C. The chromatographic system was allowed to equilibrate at each temperature for at least 1 h prior to each experiment. To study this equilibration, the retention time of the 2,3-dichlorophenol was measured every hour for 7 h and again after 22, 23, and 24 h. The maximum relative difference in the retention times of this compound between these different measurements was always 0.2%, making the chromatographic system sufficiently equilibrated for use after 1 h.

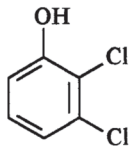
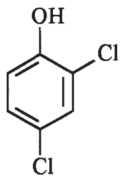
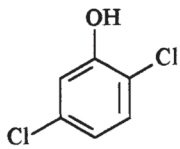
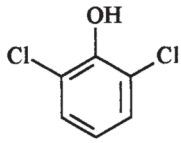
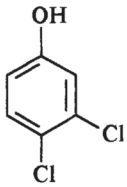
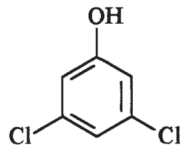
Compound number	Nomenclature	Chemical structure
1	2,3-dichlorophenol	
2	2,4-dichlorophenol	
3	2,5-dichlorophenol	
4	2,6-dichlorophenol	
5	3,4-dichlorophenol	
6	3,5-dichlorophenol	

Figure 1. The six dichlorophenol isomer structures.

Methods

Thermodynamic relationships

Solute retention is usually expressed in terms of the retention factor k' by the following well-known equations (10,11):

$$\ln k' = \frac{-\Delta H^\circ_{M.Ls}}{RT} + \Delta S^{\circ *}_{M.Ls} \qquad \text{Eq. 1}$$

$$\Delta S^{\circ *}_{M.Ls} = \frac{\Delta S^\circ_{M.Ls}}{R} + \ln \phi \qquad \text{Eq. 2}$$

where $\Delta H^\circ_{M.Ls}$ and $\Delta S^\circ_{M.Ls}$ are the enthalpy and entropy, respectively, of transfer of the solute molecule M from the mobile phase to the stationary phase ligand Ls, T is the temperature, R is the gas constant, and ϕ is the phase ratio of the column (volume of the stationary phase divided by the volume of the mobile phase). $\ln k'$ versus $1/T$ is called a van't Hoff plot. For a linear plot, the slope and intercept were $-\Delta H^\circ_{M.Ls}/R$ and $\Delta S^{\circ *}_{M.Ls}$, respectively.

Enthalpy–entropy compensation

Investigation of the enthalpy–entropy compensation temperature is a thermodynamic approach to the analysis of physicochemical data. Mathematically, enthalpy–entropy compensation can be expressed by the following formula (12):

$$\Delta H^\circ_{M.Ls} = \beta \Delta S^\circ_{M.Ls} + (\Delta G^\circ_{M.Ls})_\beta \qquad \text{Eq. 3}$$

where $(\Delta G^\circ_{M.Ls})_\beta$ is the Gibbs free energy of a physicochemical interaction at a compensation temperature β (both are constant). In accordance with Equation 3, when enthalpy–entropy compensation is observed with a group of compounds in a particular chemical interaction, all of the compounds have the same free energy $(\Delta G^\circ_{M.Ls})_\beta$ at temperature β. Therefore, if enthalpy–entropy compensation is observed for the six dichlorophenols, all will have the same net retention at the compensation temperature β, although their temperature dependence may differ. Rewriting Equation 3 using Equation 1,

$$\ln(k')_T = \ln k'_0 - \left(\frac{-\Delta H^\circ_{M.Ls}}{R}\right) \times \left[\left(\frac{1}{T}\right) - \left(\frac{1}{\beta}\right)\right] \qquad \text{Eq. 4}$$

where

$$\ln k'_0 = -\left[\frac{(\Delta G^\circ_{M.Ls})_\beta}{R\beta}\right] + \ln \phi \qquad \text{Eq. 5}$$

Equation 4 shows that if a plot of $\ln(k')_T$ versus $-\Delta H^\circ_{M.Ls}$ is linear, then the six dichlorophenols are retained by an essentially identical interaction mechanism.

Chemical equilibria

HPLC is a very convenient method of studying the equilibria taking place in solutions, especially in the mixtures of water and organic modifier. The retention behavior of dichlorophenols in HPLC is based on the partitioning of the solutes between the mobile and stationary phases. The equilibrium of the species M with the stationary phase ligand Ls is as follows:

$$M + Ls \rightleftharpoons M.Ls \qquad \text{Eq. 6}$$

The solute molecule M association with β-CD in the mobile phase is represented by the following equilibrium:

$$x\,M + y\,\beta\text{-CD} \rightleftharpoons M_x.\beta\text{-CD}_y \qquad \text{Eq. 7}$$

where x and y are the number of molecules of each species, M and β-CD, respectively, implicated in the complexation process. Equation 7 can be rewritten:

$$M + y/x\,\beta\text{-CD} \rightleftharpoons M.\beta\text{-CD}_{y/x} \qquad \text{Eq. 8}$$

The equilibrium constant K for Equation 8 is as follows:

$$K = \frac{[M.\beta\text{-CD}_{y/x}]}{[M][\beta\text{-CD}]^{y/x}} \qquad \text{Eq. 9}$$

where [M], [β-CD], and [M.β-$CD_{y/x}$] are the M, β-CD, and complex concentrations, respectively.

The equilibrium of the species M.β-$CD_{y/x}$ with the stationary phase is as follows:

$$M.\beta\text{-CD}_{y/x} + Ls \rightleftharpoons M.\beta\text{-CD}_{y/x}.Ls \qquad \text{Eq. 10}$$

Neglecting the interaction of the complexes with the stationary phase, the retention factor k' of the species M is given by the following:

$$k' = \phi\left\{\frac{[M.Ls]}{[M] + [M.\beta\text{-CD}_{y/x}]}\right\} \qquad \text{Eq. 11}$$

where [M.Ls] is the M concentration in the stationary phase. Combining Equations 9 and 11, the following is obtained:

$$k' = \phi\left\{\frac{[M.Ls]}{[M] + K[M][\beta\text{-CD}]^{y/x}}\right\} \qquad \text{Eq. 12}$$

Taking the reciprocal (13) of Equation 12 leads to the following:

$$\frac{1}{k'} = \frac{1}{k'_0} + \frac{K[\beta\text{-CD}]^{y/x}}{k'_0} \qquad \text{Eq. 13}$$

where

$$\frac{1}{k'_0} = \frac{[M]}{\phi[M.Ls]} \qquad \text{Eq. 14}$$

Thus, the values of the association constants K between the M and β-CD molecules are obtained from the slope-to-intercept ratio of a plot of the reciprocal of the capacity factor k' of each eluting solute versus the concentration of β-CD incorporated in the mobile phase.

Enthalpy and entropy of association between the M solute molecule and the β-CD ($\Delta H^\circ_{M.b\text{-}CDy/x}$ and $\Delta S^\circ_{M.b\text{-}CDy/x}$, respectively) are determined by plotting the logarithm of the complex formation constant values as a function of the temperature reciprocal:

$$\ln K = \left(\frac{-\Delta H^\circ_{M.b\text{-}CDy/x}}{RT}\right) + \left(\frac{\Delta S^\circ_{M.b\text{-}CDy/x}}{R}\right) \qquad \text{Eq. 15}$$

For a linear plot, the slope and intercept are $-\Delta H^\circ_{M.b\text{-}CDy/x}/R$ and $\Delta S^\circ_{M.b\text{-}CDy/x}/R$, respectively. The Gibbs free energy is determined at a particular temperature using the following equations:

$$\ln K = \frac{-\Delta G^\circ_{M.b\text{-}CDy/x}}{RT} \qquad \text{Eq. 16}$$

$$\Delta G^\circ_{M.b\text{-}CDy/x} = \Delta H^\circ_{M.b\text{-}CDy/x} - T\,\Delta S^\circ_{M.b\text{-}CDy/x} \qquad \text{Eq. 17}$$

Results and Discussion

Elution order

The six dichlorophenols eluted in the order 2,6- < 2,3- < 2,5- < 2,4- < 3,4- < 3,5. A comparison of this elution order with that provided in the literature (9) showed that some differences appear because of the significant variations in experimental conditions (for example, the type of column used and the composition of the mobile phase; the method reported in Reference 9 utilized LC with a β-CD-bonded stationary phase). Two additional parameters affect the retention of dichlorophenols: the position of the chloro groups on the phenol ring and the solvation of the solutes in the mobile phase.

Because the phenol ring has chloro groups in the ortho position of the hydroxyl group, the solvation of the species by the methanol–water cluster (14) decreased, inducing a decrease in the affinity of the compound for the clusters constituting the mobile phase. Thus, the hydrophobic character of the compound increased, increasing its affinity for the apolar β-CD cavity. The affinity of the solute for the β-CD cavity (complexation by the β-CD dissolved in mobile phase) induced an increase of the complex affinity for the mobile phase, decreasing its retention time. Thus, the 3,5-isomer, which was the most solvated compound, was retained the most, whereas the 2,6-isomer, which has two chloro groups in ortho position of the hydroxyl group, was the least solvated and retained compound.

Enthalpy and entropy changes for the solute transfer from the mobile to the stationary phase

The van't Hoff plots were all linear for the six dichlorophenols at all β-CD concentrations. The capacity factor increased when the temperature decreased. The correlation coefficient r for all the fits was greater than 0.980. Figure 2 shows the van't Hoff plot for 2,4-dichlorophenol at a β-CD concentration equal to 2.5mM. A complete list of $\Delta H^\circ_{M.Ls}$ and $\Delta S^{\circ *}_{M.Ls}$ values for all solutes at

all β-CD concentrations is shown in Table I. Both the $\Delta H°_{M.Ls}$ and $\Delta S°*_{M.Ls}$ values were negative. Negative $\Delta H°_{M.Ls}$ indicated that it was energetically more favorable for the solute to be in the stationary phase. Negative $\Delta S°*_{M.Ls}$ also indicated an increase in the order of the chromatographic system as the solute was transferred from the mobile to the stationary phase. The equilibrium of complexation between the free solute and the β-CD was displaced in the direction of the free solute (noncomplexed with the β-CD). The retention mechanism of the solute involved its classical transfer from the bulk methanol–water mobile phase to the stationary phase. Retention factors decreased with increasing temperature, and $\Delta H°_{M.Ls}$ and $\Delta S°*_{M.Ls}$ were negative (Table I).

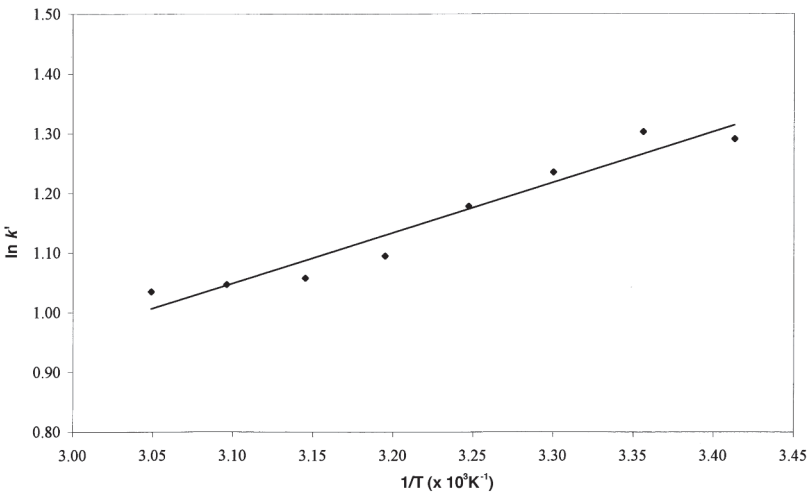


Figure 2. van't Hoff plot for 2,4-dichlorophenol at a 2.5mM β-CD concentration.

Thus, the transfer of the solute from the mobile to the stationary phase was enthalpically driven. The plots of the thermodynamic parameters ($\Delta H°_{M.Ls}$ and $\Delta S°*_{M.Ls}$) versus β-CD concentration were drawn for all dichlorophenols. Figure 3 shows, for example, $\Delta H°_{M.Ls}$ and $S°*_{M.Ls}$ values for 2,6-dichlorophenol versus β-CD concentration. When the β-CD concentration was less than a critical value *C*, $\Delta H°_{M.Ls}$ and $\Delta S°*_{M.Ls}$ became increasingly negative (Figure 3) as the β-CD concentrations increased for the six dichlorophenols. These data indicate an increase in the solute affinity for the stationary phase. This indicated that the solute had more difficulty interacting with the β-CD molecule, inducing a major displacement of the complexation equilibrium in the direction of the free solute. For concentrations greater than *C*, $\Delta H°_{M.Ls}$ and $\Delta S°*_{M.Ls}$ became decreasingly negative (Figure 3) as the β-CD concentrations increased for the six dichlorophenols. These data indicate that the complexation equilibrium tended to be progressively displaced in the direction of the complex.

Enthalpy–entropy compensation

A plot of $(\ln k')_T$ (for T = 303 K), calculated for each of the six compounds against $-\Delta H°_{M.Ls}$, was drawn at each β-CD concentration. For example, for a β-CD concentration equal to 1.5mM, the *r* value for the fit was 0.936. This can be considered adequate to verify enthalpy–entropy compensation (15). The retention mechanism can be considered independent of the molecular structure of the compound. A plot of $(\ln k')_T$ (for T = 303 K), calculated for each of the six β-CD concentration against $-\Delta H°_{M.CD}$, was drawn for each

Table I. Thermodynamic Parameters* $\Delta H°_{M.Ls}$ (kJ/mol) and $\Delta S°*_{M.Ls}$ at Different β-CD Concentrations for the Six Dichlorophenols

Compound		[β-CD] = 0mM	[β-CD] = 0.5mM	[β-CD] = 1mM	[β-CD] = 1.5mM	[β-CD] = 2mM	[β-CD] = 2.5mM
1	$\Delta H°_{M.Ls}$	–4.6 (0.1)	–4.2 (0.1)	–3.9 (0.1)	–10.9 (0.3)	–10.6 (0.2)	–5.6 (0.1)
	$\Delta S°*_{M.Ls}$	–0.9 (0.0)	–0.9 (0.1)	–0.8 (0.1)	–3.6 (0.1)	–3.6 (0.1)	–1.4 (0.1)
2	$\Delta H°_{M.Ls}$	–4.9 (0.1)	–4.5 (0.1)	–4.2 (0.1)	–11.6 (0.4)	–11.6 (0.2)	–7.1 (0.1)
	$\Delta S°*_{M.Ls}$	–0.7 (0.1)	–0.7 (0.1)	–0.7 (0.1)	–3.6 (0.1)	–3.6 (0.1)	–1.7 (0.1)
3	$\Delta H°_{M.Ls}$	–4.6 (0.2)	–4.1 (0.1)	–3.7 (0.1)	–11.2 (0.4)	–8.2 (0.1)	–6.7 (0.1)
	$\Delta S°*_{M.Ls}$	–0.2 (0.0)	–0.3 (0.0)	–0.6 (0.0)	–3.5 (0.1)	–2.3 (0.1)	–1.6 (0.1)
4	$\Delta H°_{M.Ls}$	–2.7 (0.0)	–2.8 (0.1)	–3.9 (0.1)	–10.9 (0.3)	–12.2 (0.1)	–5.5 (0.1)
	$\Delta S°*_{M.Ls}$	–0.3 (0.0)	–0.5 (0.0)	–0.7 (0.1)	–3.8 (0.1)	–4.3 (0.1)	–1.5 (0.1)
5	$\Delta H°_{M.Ls}$	–5.9 (0.2)	–4.7 (0.1)	–3.8 (0.2)	–11.3 (0.2)	–12.7 (0.1)	–6.9 (0.1)
	$\Delta S°*_{M.Ls}$	–1.1 (0.1)	–0.8 (0.0)	–0.5 (0.1)	–3.4 (0.1)	–4.0 (0.1)	–1.6 (0.1)
6	$\Delta H°_{M.Ls}$	–6.6 (0.2)	–5.3 (0.1)	–4.2 (0.2)	–13.0 (0.1)	–12.6 (0.1)	–11.1 (0.1)
	$\Delta S°*_{M.Ls}$	–0.9 (0.1)	–0.7 (0.1)	–0.2 (0.0)	–3.7 (0.1)	–3.5 (0.1)	–2.8 (0.1)

* Transfer from the mobile to the stationary phase.
† Standard deviations in parentheses.
‡ See the corresponding compound in Figure 1.

dichlorophenol. For example, for 2,4-dichlorophenol, the *r* value for the fit was 0.963. This can be considered adequate to verify enthalpy–entropy compensation (15). The retention mechanism can be thought to be independent of the quantity of β-CD dissolved in the mobile phase.

Association constant values between solute and β-CD

In accordance with Equation 13, linear plots were obtained for all solutes at y/x = 1/3, inducing a M.$(\beta\text{-CD})_3$ association. The *r* value for the linear fit was greater than 0.985. The association constants were calculated for different temperatures. Their values decreased with increasing temperatures. For example, the *K* values are given for each compound at T = 35 and 55°C in Table II. As predicted by the model explained in the section on elution order, 3,4- and 3,5-dichlorophenols that had no chloro groups in the ortho position of the hydroxy groups have a greater possibility of solvation by methanol–water clusters, inducing a decreasing possibility of complexation by β-CD and thus a lower association constant value. It can be noted that the stoichiometry 1:3 (solute:β-CD) of the complex and the largest possible diameter of a substituted chlorophenol (approximately 7.05 Å) (9) were close to the size of the β-CD cavity (7.8 Å). These data indicate that the complex would probably not be a real inclusion complex with a solute molecule inside the β-CD cavity but rather an association of solute and β-CD, suggesting the exclusion of dichlorophenol isomers from the cavity. In this case, these compounds are likely to interact with the hydroxyl groups on the β-CD molecule rim (9) through strong hydrogen bonding or steric interactions in relation to the compound structure.

Table II. K (M-1) Values at Two Temperatures (35 and 55°C) for the Six Dichlorophenols

	T = 35°C	T = 55°C
2,3-Dichlorophenol (1)	369	179
2,4-Dichlorophenol (2)	340	164
2,5-Dichlorophenol (3)	368	172
2,6-Dichlorophenol (4)	452	269
3,4-Dichlorophenol (5)	121	98
3,6-Dichlorophenol (6)	111	83

Table III. Thermodynamic Parameters* (Solute Association with β-CD) for the Six Dichlorophenols

Compound‡	$\Delta H°_{M.CD\,y/x}$	$\Delta S°_{M.CD\,y/x}$	$T\Delta S°_{M.CD\,y/x}$	$\Delta G°_{M.CD\,y/x}$
1	–31.17 (0.05)	–45.56 (0.09)	–13.58 (0.07)	–17.59 (0.06)
2	–30.63 (0.06)	–52.21 (0.09)	–15.56 (0.08)	–15.07 (0.05)
3	–32.91 (0.01)	–56.29 (0.08)	–16.78 (0.08)	–16.13 (0.04)
4	–24.56 (0.02)	–16.21 (0.06)	–4.83 (0.02)	–19.73 (0.04)
5	–9.12 (0.05)	–0.08 (0.01)	–0.02 (0.01)	–9.10 (0.01)
6	–11.45 (0.04)	–0.38 (0.01)	–0.11 (0.01)	–11.34 (0.02)

* $\Delta H°_{M.CDy/x}$ (kJ/mol), $\Delta S°_{M.CDy/x}$ (J/mol.K), $T\Delta S°_{M.CDy/x}$ (kJ/mol), and $\Delta G°_{M.CDy/x}$ (kJ/mol) at 25°C.
† Standard deviations in parentheses.
‡ See the corresponding compound in Figure 1.

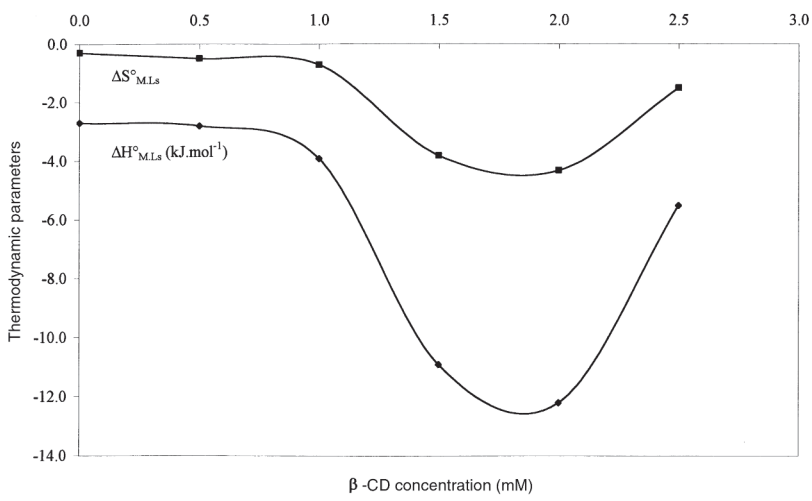


Figure 3. Thermodynamic parameters (transfer from the mobile to the stationary phase) $\Delta H°_{M.Ls}$ (kJ/mol) and $\Delta S°*_{M.Ls}$ values versus β-CD concentration for 2,6-dichlorophenol.

Enthalpy, entropy, and Gibbs free energy changes for the solute association with cyclodextrin

The van't Hoff plots of Equation 15 (ln *K* versus 1/*T*) for all samples were linear. The correlation coefficient *r* for all the fits was greater than 0.990. $\Delta H°_{M.b\text{-}CDy/x}$, $\Delta S°_{M.b\text{-}CDy/x}$, and $\Delta G°_{M.b\text{-}CDy/x}$ (calculated using Equation 17 at 25°C) values are listed in Table III. A comparison of these values with those reported in the literature (8,9) show that the orders of magnitude are in good agreement. $\Delta H°_{M.b\text{-}CDy/x}$ was negative, and this indicates that it is energetically favorable for the solute to interact with the β-CD molecules. Obviously, the largest changes in enthalpy would be for the dichlorophenol isomers with the highest association constant. For the entropy change, the values obtained were negative, proving the apparent lower degrees of freedom of the solute associated with the β-CD molecule. It can be noted that for every solute evaluated, when $\Delta H°_{M.b\text{-}CDy/x}$ was compared with $T\Delta S°_{M.b\text{-}CDy/x}$ (Table III) over the temperature range, the magnitude of $\Delta H°_{M.b\text{-}CDy/x}$ was always greater than that of $T\Delta S°_{M.b\text{-}CDy/x}$. This indicates that enthalpy played a greater role in the association process, and therefore in the retention process, than entropy.

Conclusion

In this paper, the retention mechanism in HPLC and the inclusion complex formation with β-CD were assessed for six dichlorophenol isomers. The stoichiometries were determined, and the association constants were measured in relation to temperature. The thermodynamic parameter trends were determined over a wide range of column temperatures. Most of the evidence would indicate that in the chromatographic conditions exam-

ined, the dichlorophenol isomers did not form genuine inclusion complexes with β-CD. The interactions between these compounds and β-CD seemed to be an association where the dichlorophenols interacted with hydroxyl groups on the β-CD rim. Enthalpy–entropy compensation revealed that the dichlorophenol isomer retention was independent of both the chloro group position on the phenol molecule and the β-CD quantity dissolved in the mobile phase.

References

1. J. Szejtli. *Cyclodextrins and their inclusion complexes.* Akadémiai Kiadó, Budapest, Hungary, 1982.
2. I. Ciucanu and W.A. König. Immobilization of peralkylated β-cyclodextrin on silica gel for high-performance liquid chromatography. *J. Chromatogr. A* **685:** 166–71 (1994).
3. W. Stammel and H. Thomas. A simple and rapid method for the separation of the (*R*)- and (*S*)-enantiomers of the tetrahydroisoquinoline alkaloid sasolinol by high-performance liquid chromatography. *Anal. Lett.* **26:** 2513–24 (1993).
4. E. Ameyibor and J.T. Stewart. Enantiomeric HPLC separation of selected chiral drugs using native and derivatized β-cyclodextrins as chiral mobile phase additives. *J. Liq. Chrom. Rel. Technol.* **6:** 855–69 (1997).
5. H. Lamparczyk and P.K. Zarzycki. Effect of temperature on separation of estradiol stereoisomers and equilin by liquid chromatography using mobile phases modified with β-cyclodextrin. *J. Pharm. Biomed. Anal.* **13:** 543–49 (1995).
6. M. Sueyasu, T. Ikeda, K. Otsubo, T. Taniyama, T. Aoyama, and R. Oishi. Enantioselective determination of thiamylal in human serum by high-performance liquid chromatography. *J. Chromatogr. B* **665:** 133–37 (1995).
7. W. Stammel, B. Woesle, and H. Thomas. Enantiomeric separation of tetrahydroisoquinoline alkaloids by high-performance liquid chromatography with β-cyclodextrin as chiral selector. *Chiralit.* **7:** 10–19 (1995).
8. Y.C. Guillaume and C. Guinchard. New statistical approach to a gas chromatography retention model: application to dichlorophenol isomers. *J. Phys. Chem. B* **101:** 8390–94 (1997).
9. M. Paleologou, S. Li, and W.C. Purdy. Liquid chromatographic retention behavior and separation of chlorophenols on a β-cyclodextrin bonded-phase column, part I. Monoaromatic chlorophenols: retention behavior. *J. Chromatogr. A* **28:** 311–18 (1990).
10. L.A. Cole and J.G. Dorsey. Temperature dependence of retention in reversed-phase liquid chromatography. 1. Stationary-phase considerations. *Anal. Chem.* **64:** 1317–23 (1992).
11. S. Landau and E. Grushka. High performance liquid chromatographic studies of some cyclodextrin complexes. *Isr. J. Chem.* **30:** 271–79 (1990).
12. L.C. Sander and L.R. Field. Effect of eluent composition on thermodynamic properties in high performance liquid chromatography. *Anal. Chem.* **52:** 2009–2013 (1980).
13. N. Sadlej-Sosnowska. Molecular complexation: β-cyclodextrin and steroid hormones inclusion complexes studied by high-performance liquid chromatography. *Eur. J. Pharm. Sci.* **3:** 1–5 (1995).
14. Y.C. Guillaume and C. Guinchard. Participation of cluster species in the solvation mechanism of a weak polar solute in a methanol/water mixture over a 0.2–0.7 water fraction range: high performance liquid chromatography study. *Anal. Chem.* **70:** 608–615 (1998).
15. L.A. Cole, J.G. Dorsey, and K.A. Dill. Temperature dependence of retention in reversed-phase liquid chromatography. 2. Mobile-phase considerations. *Anal. Chem.* **64:** 1324–27 (1992).

Manuscript accepted September 24, 1999.