High-Performance Liquid Chromatography of Structural Isomers with Cyclodextrin-Poly(vinylamine)-Coated Silica Columns, Part III: Retention Mechanism Study of Nitrophenol Derivatives

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Abstract

The liquid chromatographic retention mechanism of nitrophenol isomers by isocratic elution is investigated over a range of column temperatures and mobile phase compositions using several β-cyclodextrin (β-CD) bonded stationary phases. The high-performance liquid chromatographic supports are based on silica beads coated with a polymer containing β-CD. Thermodynamic parameters for the solute transfer from the mobile to stationary phases are determined, and complex formation constant values between solute and β-CD are calculated. The thermodynamic data shows that the solute retention mechanism is dependent on the amount of bonded β-CD, and the complexation constant is dependent on temperature and mobile phase composition.

Introduction

β-cyclodextrins (β-CDs) are torus-shaped cyclic oligosaccharides made up of seven α-1,4 linked D-glycopyranose units (1,2). The inside of their cavity is apolar. They have the ability to form specific inclusion complexes with a wide variety of molecules, especially phenolic derivatives. The inclusion process is mainly influenced by size, shape, and polarity of the guest molecule (1–4). The selective inclusion property of β-CDs has been used as an advantage in many separation techniques, including classical liquid chromatography (LC) (5–9). In high-performance liquid chromatography (HPLC), two approaches have been designed for separating various compounds through β-CD complexation. The first approach is the use of β-CD as a selective component of the mobile phase in a reversed-phase system (10–13). A second approach is the use of chemically bonded β-CD silica gel stationary phases (14–17). These two procedures have been used to separate structural isomers of phenolic compounds.

In two previous publications (18,19), several chemically bonded β-CD stationary phases were prepared for HPLC based on silica beads coated with a hydrosoluble and linear polymer, poly(vinylamine), containing β-CD. These stationary phases had been characterized using nuclear magnetic resonance solid-state techniques. The retention behavior of some substituted benzene derivatives was studied by isocratic elution using ultraviolet (UV) detection. The results have demonstrated that the retention mechanism is based mainly on the formation of inclusion complexes, and additional interactions (such as acid–base and hydrophobic interactions) contribute to retention. In particularly, it was observed that the three isomers of nitrophenol were very well resolved in the sequence meta, ortho, and para, following the order of stability for binding to β-CD (the *para* isomer giving the most stable complex). Furthermore, the elution order was independent of the organic modifiers (methanol and acetonitrile) in the mobile phase. In this paper, the LC retention mechanism by isocratic elution of nitrophenol isomers was investigated over a range of column temperatures and mobile phase compositions using β-CD-bonded stationary phases containing different amounts of β-CD. Thermodynamic parameters were determined for the solute transfer from the mobile to the stationary phases, and complex formation constant values between solute and β-CD were calculated.

Experimental

Apparatus

The HPLC system consisted of a Kontron Instruments (Saint-Quentin, Yvelines, France) HPLC pump 422, an Interchim Rheodyne (Montluçon, France) model 7125 injection valve fitted with a 20-µL sample loop, and a Kontron Instruments HPLC detector 430.

Columns

The supports were prepared as reported previously (18). The columns (100 × 4.6 mm) were filled using a slurry packing technique in our laboratory. A column series with various amounts of β-CD (7, 15, 24, 36, and 53 µmol/g of silica) was used with a con-

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trolled temperature in an Interchim oven TM number 701 for temperatures higher than 20°C and an Osi Julabo (Elancourt, France) FT 200 cryoimmerser for temperatures lower than 20°C.

HPLC procedure

HPLC-grade methanol (Carlo Erba, Val de Reuil, France) was used without further purification. Water was obtained from an Elgastat (Odil, Talant, France) option I water purification system fitted with a reverse-osmosis cartridge. The mobile phase consisted of a methanol–water mixture with different methanol fractions (α) between 20 and 50%. The solutions were filtered through a 0.2-µm Millipore HA membrane and degassed by ultrasonic vibration before use. Nitrophenol (*ortho, meta,* and *para* isomers, Janssen Chimica, Beerse, Belgium) was dissolved in pure methanol at a concentration of 50 µg/mL. Volumes (20 µL) of each solute were injected. The mobile phase flow rate was fixed at 1 mL/mn, and the wavelength was 280 nm. Sodium nitrate was used as a dead time marker (Merck, Nogent-sur-Marne, France).

Temperature studies

The retention factors were determined at the temperature values of 15, 20, 25, and 30°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 compound retention time of *p*-nitrophenol 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 0.6%, making the chromatographic system sufficiently equilibrated for use after 1 h. All the solutes were injected in triplicate at each temperature and mobile phase composition.

Methods

Secondary chemical equilibria

In an HPLC system containing β-CD in the stationary phase, the following equilibria will be established: the adsorption of the free solute (*S*) to β-CD molecules in the stationary phases

$$S_m \rightleftharpoons S_s \qquad \text{Eq 1}$$

and the complexation of the free solute with β-CD molecules

$$S_s + (\beta\text{-CD})_s \rightleftharpoons (S.\,\beta\text{-CD})_s \qquad \text{Eq 2}$$

where the subscripts s and m denote the stationary and mobile phases, respectively.

It is also supposed that only 1:1 complexes are formed between nitrophenol and β-CD molecules (20). The complexation constant between the solute and the β-CD molecules, K_f, is expressed by the following equation:

$$K_f = [(S.\,\beta\text{-CD})]_s/[S]_s[\beta\text{-CD}]_s \qquad \text{Eq 3}$$

where [(*S*. β-CD)], [*S*], and [β-CD] correspond to complex, solute, and β-CD concentrations, respectively.

The solute capacity factor k' is related to the partage constant K by:

$$k' = \phi K = \phi([S]_s + [S.\,\beta\text{-CD}]_s)/[S]_m \qquad \text{Eq 4}$$

where ϕ is the phase ratio of the column (volume of stationary phase divided by volume of mobile phase).
Combining Equations 3 and 4 gives:

$$k' = \phi([S]_s/[S]_m) + K_f\phi[\beta\text{-CD}]_s \qquad \text{Eq 5}$$

The value of K_f is obtained from the slope-to-intercept ratio of the capacity factor plot of each eluting solute versus the concentration of cyclodextrin incorporated in the stationary phase.

Thermodynamic relationships

Solute retention is usually expressed in terms of k' by the well-known equation:

$$\ln k' = -\Delta H°/RT + \Delta S°* \qquad \text{Eq 6}$$

$$\Delta S°* = \Delta S°/R + \ln \phi \qquad \text{Eq 7}$$

where $\Delta H°$ and $\Delta S°$ are the enthalpy and entropy, respectively, of the transfer of the solute from the mobile to the stationary phases, T is the temperature, and R is the gas constant. ln k' versus $1/T$ is called a van't Hoff plot. For a linear plot, the slope and intercept are $-\Delta H°/R$ and $\Delta S°*$, respectively.

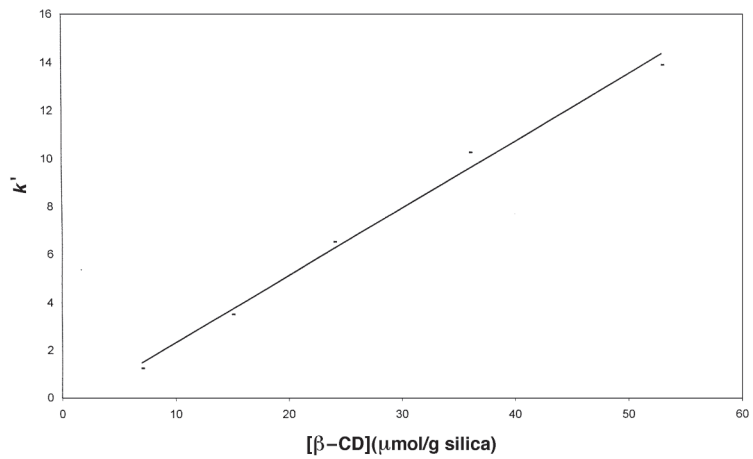

Figure 1. Plot of k' versus β-CD concentration in the stationary phase for *o*-nitrophenol (α = 30% and T = 30°C).

Results and Discussion

Complexation constant values between solute and β-CD

According to Equation 5, linear plots were obtained for all solutes (Figure 1). The correla-

tion coefficient *r* for the linear fit always exceeded 0.994. The complexation constants were calculated for different mobile phase compositions and temperatures.

Effect of methanol content

Nitrophenol retention times decreased with increasing methanol fraction in the mobile phase. Furthermore, the complexation constants were smaller for the mobile phase with the highest methanol content. For example, the K_f values are given for *p*-nitrophenol at 30°C in Table I. These results first demonstrate that the stability of the complex between β-CD and nitrophenol is reduced as the solute solubility increases in less polar bulk phases. For the mobile phase with the highest methanol fraction, the solute had a lower affinity for β-CD-bonded stationary phase than for the mobile phase with the lowest methanol fraction. Moreover, at high methanol fractions, the methanol molecules tend to be included in the β-CD cavities of the stationary phase: the methanol competes with the solute for inclusion in the β-CD cavities (21,22). Thus, under these conditions, K_f values decreased with increasing methanol content in the mobile phase.

Effect of temperature

As for reversed-phase systems, K_f values decreased with increasing temperatures. For example, the K_f values for *o*-, *m*- and *p*-nitrophenol at 15 and 30°C for a mobile phase composed of 20% methanol are given in Table II. *p*-Nitrophenol had the highest formation constant values in comparison with *o*- and *m*-nitrophenol. This result demonstrates that the β-CD size is more appropriate for *p*-nitrophenol than for *o*- and *m*-nitrophenol, which had the highest steric hindrance.

Enthalpy, entropy changes for the solute transfer from the mobile to the stationary phases

The van't Hoff plots of Equation 6 (ln *k*' versus 1/*T*) for all samples were linear at all β-CD concentrations in the stationary phase and at all methanol contents in the mobile phase (Figure 2). The *r* value for all the fits was greater than 0.997. $\Delta H°$ and $\Delta S°$* values for *o*-, *m*-, and *p*-nitrophenol at α = 20% for various β-CD concentrations in the stationary phase are listed in Table III. The results demonstrate that the largest changes in enthalpy (from –39.98 to –8.71 kJ/mol) and entropy (from –14.23 to 0.54) would be for *p*-nitrophenol, the nitrophenol isomer which has the highest formation constant.

Table I. $K_f \times 10^{-6}/_{mol}$ Values at 30° C for *p*-Nitrophenol as a Function of Mobile Phase Composition

α*	20%	30%	40%	50%
K_f	1.28	0.98	0.67	0.45

*Methanol fraction in the mobile phase.

Table II. $K_f \times 10^{-6}/_{mol}$ Values at Two Temperatures for *o*-, *m*-, and *p*-Nitrophenol (α = 20%)

	K_f	
	T **= 15° C**	***T*** **= 30° C**
o-nitrophenol	0.48	0.46
m-nitrophenol	0.10	0.04
p-nitrophenol	1.38	1.28

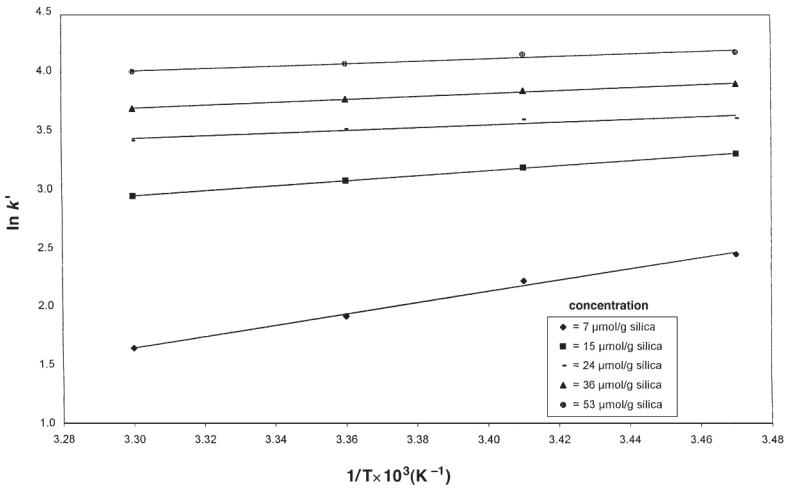

Figure 2. Plot of ln *k*' versus 1/*T* for *p*-nitrophenol at various β-CD concentrations in the stationary phase (α = 20%).

Table III. Thermodynamic Parameters (with Standards Deviations) at Different β–CD Concentrations in the Stationary Phase (α = 20%)

		[β–CD] (µmol/g silica)				
		7	**15**	**24**	**36**	**53**
$\Delta H°$	*o*–nitrophenol	–28.17 (0.4)	–22.77 (0.3)	–18.06 (0.2)	–8.18 (0.2)	–5.93 (0.1)
	m–nitrophenol	–12.15 (0.2)	–10.09 (0.1)	–9.11 (0.1)	–8.17 (0.2)	–4.00 (0.3)
	p–nitrophenol	–39.98 (0.5)	–17.66 (0.1)	–9.61 (0.2)	–10.38 (.02)	–8.71 (0.2)
$\Delta S°$*	*o*–nitrophenol	–10.73 (0.4)	–7.58 (0.2)	–5.08 (0.06)	–0.62 (0.02)	0.55 (0.02)
	m–nitrophenol	–7.70 (0.3)	–3.12 (0.2)	–0.13 (0.01)	–0.08 (0.01)	0.53 (0.02)
	p–nitrophenol	–14.23 (0.3)	–4.07 (0.2)	–0.39 (0.02)	–0.44 (0.01)	0.54 (0.01)

$\Delta H°$ values

$\Delta H°$* values

When the solute is removed from the mobile phase to the β-CD-bonded stationary phase, the relatively weak solute–solvent interactions are replaced by strong solute–cyclodextrin-specific interactions (attractive hydrophobic, van der Waals, hydrogen bonding, and dipole–dipole interactions between the interior of the cyclodextrin cavity and the portion of the solute inside the cavity), inducing negative $\Delta H°$ values (Table III) in the entire range of β-CD concentrations. This indicates that it is energetically more favorable for the solute to be included in the β-CD cavity. The transfer of the solute from the mobile phase to the stationary phase is enthalpically driven. When the β-CD concentration increases in the column, the β-CD molecules are closer to each other, increasing steric hindrance and decreasing the β-CD cavity access for the solutes. Thus, the complete strong-specific interactions between the solute and the cyclodextrin cavity interior are progressively replaced by only hydrogen bonding between the functional groups on the rim of cyclodextrin (primary and secondary hydroxyl groups) and the hydrophilic portion of the solute, inducing increased $\Delta H°$ values with increasing β-CD concentrations.

$\Delta S°$* values

The strong-specific interactions between the interior of the β-CD cavity and the solute implies a loss of freedom for the compound in the inclusion complex, inducing negative $\Delta S°$* values when the solute is removed from the mobile phase to the β-CD-bonded stationary phase for β-CD concentrations lower than 53 µmol/g silica. When the β-CD concentration increases in the column, the steric hindrance of β-CD molecules between themselves decreases the possibility for the solute to be included in the cyclodextrin cavity. Thus, the solute is retained by hydrogen bonds on the cyclodextrin rim, inducing a gain of freedom and explaining the increasing $\Delta S°$* values with increasing β-CD concentration until a positive value at the β-CD concentration of 53 µmol/g silica.

From an analytical point of view, it was already observed (19) that the separation efficiency varies with the concentration of β-CD in the stationary phase. More precisely, the height of the theoretical plate passes through an optimum (minimum) value for a β-CD concentration of 20–25 µmol/g of silica and increases beyond this value. This is in accordance with the aforementioned observations concerning the variations of $\Delta H°$ and $\Delta S°$*.

Enthalpy–entropy compensation

The investigation of the enthalpy–entropy compensation temperature is a thermodynamic approach to the analysis of physicochemical data (23). Mathematically, the enthalpy–entropy compensation can be expressed by the following formula:

$$\Delta H° = \beta \Delta S° + \Delta G°_b \qquad \text{Eq 8}$$

where $\Delta G°_b$ is the Gibbs free energy of a physicochemical interaction at a compensation temperature β. According to Equation 8, 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 ($\Delta G°_b$) at temperature β. Therefore, if enthalpy–entropy compensation is observed for *o*-, *m*-, and *p*-nitrophenols, these compounds will have the same retention time at the compensation temperature β, although the temperature dependencies of their retention times may differ. By combining Equations 6, 7, and 8, the following equations are obtained (24,25):

$$\ln k'_T = \ln k'_b - (\Delta H°/R)(1/T - 1/\beta) \qquad \text{Eq 9}$$

$$\ln k'_b = (-\Delta G°_b/R\beta) + \ln \phi \qquad \text{Eq 10}$$

Equation 9 shows that if a plot of $\ln k'_T$ against $-\Delta H°$ is linear, then the *o*-, *m*-, and *p*-nitrophenols are retained by an essentially identical interaction mechanism. A plot of $\ln k'_T$ (for T = 293 K) against $-\Delta H°$ calculated for each of the three solutes at all β-CD concentrations was drawn. The correlation coefficient r for the linear fit was greater than 0.970. This high degree of correlation can be considered adequate to verify enthalpy–entropy compensation for this chromatographic system (26). Thus, it can be concluded that the retention mechanism can be thought to be independent of the isomeric configuration of the *o*-, *m*-, and *p*-nitrophenol compounds. This suggests that all of the isomers bind in the same way on the β-CD-bonded stationary phase with different interaction strengths that are dependent of the isomer.

Conclusion

The retention mechanism in HPLC was studied, and the inclusion complex formations with β-CD were measured in relation to temperature for nitrophenol isomers. The thermodynamic parameter trends were determined over a range of β-CD-bonded stationary phases. The *p*-nitrophenol, which had a linear geometry, exhibited the highest K_f values, whereas *o*- and *m*-nitrophenols, for which the proximity of the OH and NO_2 substituents resulted in steric hindrance, had the lowest K_f values. Enthalpy–entropy compensation revealed that the retention mechanism of nitrophenol isomers was independent of the relative positions of the OH and NO_2 substituents.

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