Elution Characteristics of Natural Cyclodextrins on Porous Graphitic Carbon

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Abstract

The retention behavior of natural α-, β-, and γ-cyclodextrins on a porous graphitic carbon (PGC) stationary phase is investigated. Unusual retention properties for reversed-phase chromatographic conditions are observed with acetonitrile–methanol and water–methanol mixtures as mobile phases. It is assumed that the retention process is governed not only by the standard solvophobic effect but also by specific interactions described as "CD-PGC" effect. The retention factor versus the volumetric methanol fraction in the mobile phase show second-order curves expressing this double mechanism hypothesis. van't Hoff plots demonstrate the contribution of these two retention processes. The retention factor of each natural cyclodextrin is shown to depend on the mobile phase property to act as a proton acceptor, according to the solvent selectivity classification described by Snyder. The "CD-PGC" effect is interpreted as an equilibrium between different interactions: cyclodextrin–PGC stationary phase, London dispersion forces, and cyclodextrin–mobile phase hydrogen bonding. The balance of these interactions may monitor the orientation of the cyclodextrin molecule facing the carbon surface, which is therefore suspected to be the major parameter of this retention mechanism.

Introduction

In high-performance liquid chromatography (HPLC), the use of cyclodextrins and their derivatives has achieved spectacular success. Cyclodextrins in chromatography have been studied for two major purposes: as chemically bonded cyclodextrin–silica stationary phases (1–5) or as mobile phase additives (6–9) in reversed-phase (RP) HPLC systems. Such processes were successful in the separation of complex mixtures (1–9). Numerous other chromatographic supports have been used for the cyclodextrin mediated separation, such as ion-exchange columns (10), silica C_{18}-bonded phases (11), and other bonded polymers (12).

The starch-derived cyclodextrins form a family of ring-like oligosaccharides containing 6, 7, 8, or 9 α(1-4)-linked D-glucopyranose units per molecule (13) (α-, β-, γ-, or δ-cyclodextrin, respectively). Selective and reversible ligand complexation by the insertion of a wide variety of organic molecules allows cyclodextrins to achieve the separation of various isomers: structural isomers (14) or stereoisomers (3,15).

In the cyclodextrin molecule, a ring of hydrogen bonds is formed intramolecularly between adjacent glucose units. The latter provokes a remarkably rigid structure of the molecule. Spectroscopic studies in aqueous solutions suggest that the conformation of cyclodextrin in solution is almost identical to its conformation in the crystalline state (16). As a consequence of these structural features, cyclodextrins possess some unique physical and chemical properties (17,18) that are used successfully in separation sciences.

Kiselev et al. (19) pioneered the use of porous graphitic carbon (PGC) as an adsorbent in liquid chromatography. Knox et al. (20) carried out systematic investigations with this type of stationary phase. PGC is an extremely strong adsorbent (21) because of its flat crystalline surface (22). PGC materials, with minimal active sites on the edges of graphite sheets, have an energetically homogeneous surface (23). This stationary phase is often compared with C_{18} silicas and described as a stronger hydrophobic sorbent (21). However, on PGC phases, the retention mechanism appears to be different in comparison with the reversed-phase bonded silicas (24). The retention mechanism on PGC support is governed by different types of interactions, such as adsorption on graphite with specific stereoselectivity due to its flat rigid surface (22) or solute–eluent interactions [23].

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It is well known that the mobile phase plays a major role in solute retention, whatever the chromatographic support used. Colin et al. (25) demonstrated that comparisons between the eluotropic strength of solvents used with classical RP18 stationary phase and carbon adsorbent couldn't be made because of the specific nature of this chromatographic support. PGC shows a specific behavior to solvent strength, which was demonstrated to be solute dependent (26,27). As a consequence, the empirical solvent strength classification described by Colin et al. (25) on graphitized carbon black stationary phase could not be applied to PGC because of the difference in the nature and particle size of these two chromatographic supports. Nevertheless, it can be said that strong solvents on carbon adsorbent, whatever the solute used (even with PGC), are big, bulky, and highly polarizable molecules such as carbon tetrachloride, chloroform, or tetrahydrofuran (25).

To understand the complex mechanism of PGC retention, comparisons with RP-HPLC are needed. RP-HPLC shows linear dependence of the logarithm of the retention factor (k) with the mobile phase volume composition (28). Several authors have suggested that compound retention from homologous series on RP silicas (29,30) was a function of their solubility in the mobile phase, as shown for cyclodextrins by Chatjigakis et al. (31).

In the case of PGC systems, Hennion et al. (24) demonstrated that solute–stationary phase interactions (electronic interactions) are more effective than solute–solvent interactions (hydrophobic mode) in the retention mechanism of polar compounds. Koizumi et al. (32) reported cyclodextrin retention on PGC. They observed classical RP18 elution behavior with aqueous methanol mobile phases in a range of 50–70% of methanol. However, the anomalous retention behavior of peptides on PGC surface has been observed by Németh-Kiss et al. (33), where specific second-order curves were empirically explained for retention factor versus mobile phase composition.

In this report, the elution mode of cyclodextrins was studied using a PGC support. Retention data obtained with mobile phases of different natures and compositions were compared to investigate the retention process. A thermodynamic study of enthalpy–entropy compensation (34) was therefore performed in order to interpret retention factor behavior.

Experimental

Apparatus

The HPLC system consisted of an HPLC ABI Kratos (Ramsey, NJ) Spectroflow 400 pump, a Rheodyne (Cotati, CA) model 7125 valve fitted with a 5-µL sample loop, and an ICS (Launaguet, France) model M8110 differential refractometer detector. The porous graphitic carbon column used was a Shandon (100 × 4.6-mm i.d., 5.7-µm particle size) model Hypercarb S column (Eragny/Oise, France). The temperature of the system was controlled by a Mess-technik (Wurt, Germany) model WK5 cryostat.

Data were recorded with an Apple Macintosh Classic (Les Ulis, France) using a 14-bytes Keithley (Taunton, MA) model M1111 acquisition kit at a frequency of 3 Hz.

For all experiments, the mobile phase flow rate was 0.6 mL/min in order to obtain optimum signal.

Solvents

For most solutes, known strong solvents on PGC are chloroform, tetrahydrofuran, carbon tetrachloride, and dioxane (26). Under the chromatographic conditions described in this report, such solvents used as methanol cosolvents in the mobile phase, regardless of the percentage employed, led to unretained cyclodextrin peaks. As a consequence, the methanol cosolvents used were weaker ones: ethanol, isopropanol, and acetonitrile (HPLC grade used without further purification) purchased from Prolabo (Paris, France). Methanol was freshly distilled, and water was freshly bidistilled. Binary mixtures (water–methanol, ethanol–methanol, isopropanol–methanol, or acetonitrile–methanol, v/v) were filtered with a Millipore (Molsheim, France) model HVLP 0.45-µm filter before use. The range of methanol fraction (v/v) was 0.35–1.00 with a standard deviation of 0.01.

Samples

α-, β-, and γ-cyclodextrins were purchased from Sigma (St. Louis, MO) and diluted in pure fresh bidistilled water at a concentration of 6 mg/mL. These solutions were filtered with a 0.2-µm Lida filter (Kenosha, WI) prior to injection.

A randomization process (35) in the choice of solvent mixtures and cyclodextrin used was applied to avoid all linear drift. For each experiment, the column void volume was determined by injecting pure bidistilled water with a methanol mobile phase. Each injection was made in triplicate.

Data analysis

Selectivity parameters

Rohrschneider (36) and Snyder (37) have classified solvent properties. Snyder expressed the polarity index P' in three quantities (Xe, Xd, and Xn) described as "selectivity parameters". They reflect the relative ability of the solvent to function as a proton acceptor, a proton donor, or a strong dipole interactor, respectively.

Polarity P' is calculated as the sum of log K with a K polar distribution coefficient for the three reference solutes (ethanol, dioxane, and nitromethane). Xe, Xd, and Xn are calculated as the ratio log K/P' for each solute (36) with $Xe + Xd + Xn = 1$.

Using the Snyder classification process, a binary mobile phase mixture solvent polarity (P'_{mix}) can be expressed as follows (38):

$$P'_{mix} = \Phi_A(\log Ke_A + \log Kd_A + \log Kn_A) + \Phi_B(\log Ke_B + \log Kd_B + \log Kn_B) \quad \text{Eq. 1}$$

where Φ_A and Φ_B are the volume fractions of the solvents A and B.

With reference to Snyder (38),

$$\log Ke_{mix} = \Phi_A \log Ke_A + \Phi_B \log Ke_B \quad \text{Eq. 2}$$

$$\log Kd_{mix} = \Phi_A \log Kd_A + \Phi_B \log Kd_B \quad \text{Eq. 3}$$

$$\log Kn_{mix} = \Phi_A \log Kn_A + \Phi_B \log Kn_B \quad \text{Eq. 4}$$

As a consequence of Equations 1–4, selectivity parameters for a binary mixture mobile phase (Xe_{mix}, Xd_{mix}, and Xn_{mix}) can be deduced linearly in a first intention.

Thermodynamic relationship

Valuable information concerning the retention mechanism in HPLC may be gained by examining the temperature dependence of retention. van't Hoff plots (39) gave absolute enthalpies and relative entropies of transfer for the solute.

$$\log k = -\Delta H°/RT + \Delta S°/R + \log \Psi \quad \text{Eq. 5}$$

with k as the solute retention factor:

$$k = (t_R - t_0)/t_0 \quad \text{Eq. 6}$$

where t_R is the retention time of the compound, t_0 is the retention time of an unretained peak, $\Delta S°$ is the entropy of transfer of the solute from the mobile phase to the stationary phase, and $\Delta H°$ is the enthalpy of transfer that measures the efficiency of the transfer of the solute from the mobile phase to the stationary phase. Negative $\Delta H°$ values mean that the solute is more effectively transferred to the stationary phase (40). T is the temperature expressed in Kelvin, R is the gas constant, and Ψ is the phase ratio (equal to 0.51) measured from the weight differences of the column when filled with solvents of different densities (methanol and chloroform) (41).

Results and Discussion

With a silica-bonded hydrocarbon stationary phase (RP-HPLC), numerous compounds are known to be eluted according to at least two different mechanisms, depending on the characteristics of the mobile phase (42,43). In these referenced cases, retention behavior can be explained by means of two different types of interactions. The first one, formalized by Horvath et al. (44), is described as the solvophobic mechanism. The second one, mainly caused by unbonded silanol sites at the surface of the silica material (42), is described as silanophilic interaction. Recently, a methodological approach was employed to elucidate intermolecular interactions in pharmacology (45) where RP-HPLC was used to demonstrate ion pair formation. When a dual elution mechanism in RP-HPLC was observed for a given solute, log k-versus-organic modifier curves showed some specific behavior associated with a second-order curve (parabolic-like) (42,43,45). Nahum and Horvath (42) observed a concave dependence of the retention factor on the composition of methanol–water mobile phase with crown ethers on an octadecyl siloxane chromatographic support. These authors explained that this retention behavior was due to silanophilic interactions between the solutes and the stationary phase as a function of the mobile phase polarity. In RP-HPLC, the cyclodextrin elution mode showed linear curves representing log k-versus-organic modifier percentages, this linearity being specific to a solvophobic mode (35). However, some discrepancies were explained with the help of molecular modeling. They involved specific complexes between stationary phase residue and cyclodextrin (46).

Theory

The elution mechanism on PGC is known to be governed by different types of interactions, including steric interactions, charge-transfer interactions, and London dispersion forces (23,24,47,48). Porous graphitic carbon is an extremely strong adsorbent, leading to positive solute–stationary phase interactions (49). The retention process is described as "hydrophobic adsorption" (24) as opposed to the "hydrophobic partitioning" (50) observed with C_{18} silicas. This adsorption mechanism on PGC is caused by the existence of large dispersion forces between the solutes and the rigid planar graphite surface, leading to the specific stereoselectivity of the PGC support. Cyclodextrins are large molecules essentially made of carbon chains with polar functions. Their rigid structure allows them to interact with the carbon stationary phase by means of dispersion forces. As already shown by Jackson (51), a PGC support does not adsorb any organic solvent molecules except tetrahydrofurane.

Cyclodextrin elution with methanol–water mobile phases

Since its development in HPLC, the PGC stationary phase is known to behave like RP-HPLC in its solvophobic mode (i.e., log k-versus-organic modifier curves are linear) (34). Surprisingly, as shown in Figure 1, when cyclodextrins were eluted on a PGC support with a binary mobile phase of methanol and water, log k-versus-methanol percentage data appeared nonlinear. Analogous with silica reversed-phase chromatography, a dual elution mode was suspected. In that case, the retention minimum was observed for a 75:25 (v/v) methanol–water mobile phase. Such a minimum allows Figure 1 to be separated into two areas. At lower methanol concentrations, for each cyclodextrin, a decrease in log k in relation to the decrease in mobile phase polarity was observed and was consistent with the solvophobic elution theory. At higher methanol concentrations (resulting in lower mobile phase polarity), the increase in log k indicated that interactions of another nature were operative and therefore played a predominant part in the retention mechanism.

Cyclodextrins elution order

As shown in Figure 1, the elution order of the three cyclodextrins was the same, regardless of the percentage of methanol in the mobile phase. The α-cyclodextrin eluted first, the β-cyclodextrin eluted in an intermediate position, and the γ-cyclodextrin was found to be the most retained. The elution order chromatogram is shown in Figure 2. This elution order was observed for all mobile phase compositions where the solvophobic effect was prominent (left part of Figure 1) but also when other interactions occurred (right part of Figure 1). In RP-HPLC, the elution order was determined by cyclodextrin solubility or related to its hydrophobic surface. In contrast, with the PGC stationary phase, the elution order appeared to be size dependent, the smallest molecule (α-cyclodextrin) being the least retained.

Retention mechanism

Discussions proposed by Bij et al. (43) for RP-HPLC were not appropriate to explain the second-order curves in Figure 1 (obtained using PGC stationary phase) because of the different natures of the chromatographic support. PGC does not have any silanol groups, its flat surface being devoid of any functional sites. Nevertheless, second-order curves in Figure 1 showed a strong similarity with those described as a dual mechanisms in RP-HPLC with a silica support. Therefore, a dual mechanism using a PGC stationary phase (in the case of methanol–water mobile phase) was highly suspected. When the solvophobic elution mode was no longer observed (right part of Figure 1), the predominant retention mechanism was related to a possible change in solute–solvent interactions. The intensity of these interactions was linked to the modification of the mobile phase polarity.

To assess this mechanism, different binary mixtures of methanol–cosolvent were used as mobile phases, and their polarity roles in the retention process were investigated. In a first series of experiments, the use of acetonitrile as methanol cosolvent led to a second-order curve, as shown in Figure 3. At first glance, such results are similar to those in Figure 1 involving water as a cosolvent. When using ethanol or isopropanol as methanol cosolvents, concave curves were absent, as illustrated in Figures 4 and 5. Ethanol and isopropanol are less polar solvents than methanol. An increase in volume fraction of these two cosolvents led to a decrease in mobile phase polarity. In Figures 4 and 5, when the methanol percentage in the mobile phase increased, log k for each cyclodextrin increased as in a classical reversed-phase elution mechanism.

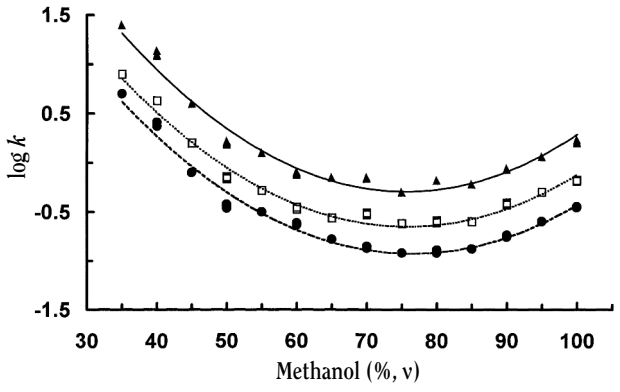

Figure 1. Correlation of retention factor logarithm with the volumetric fraction of methanol in methanol–water (v/v) mobile phases (temperature, 296 ± 1 K). Cyclodextrins: ●, α-CD; □, β-CD; ▲, γ-CD.

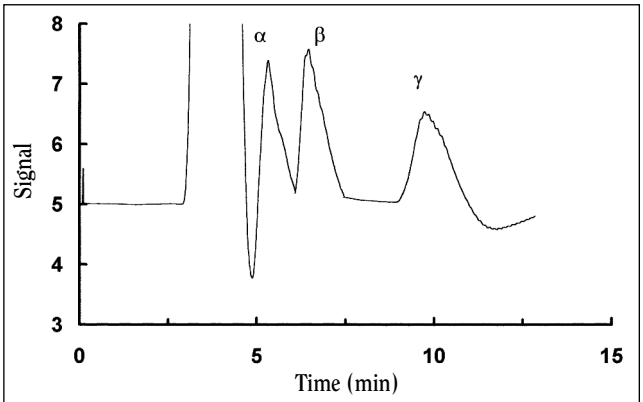

Figure 2. Elution order chromatogram of α-, β-, and γ-cyclodextrin with a 100% methanol mobile phase (temperature, 296 ± 1 K).

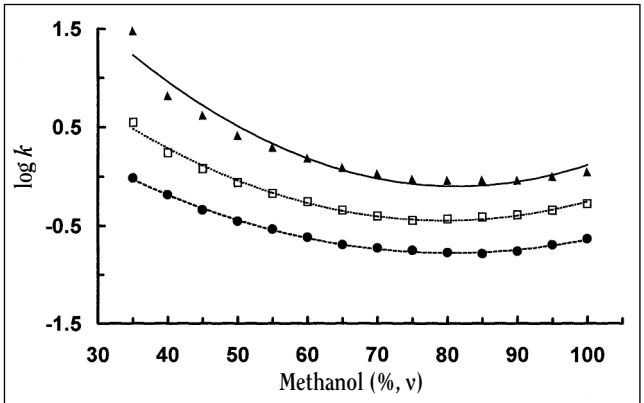

Figure 3. Correlation of retention factor logarithm with the volumetric fraction of methanol in methanol–acetonitrile (v/v) mobile phases (temperature, 296 ± 1 K). Cyclodextrins: ●, α-CD; □, β-CD; ▲, γ-CD.

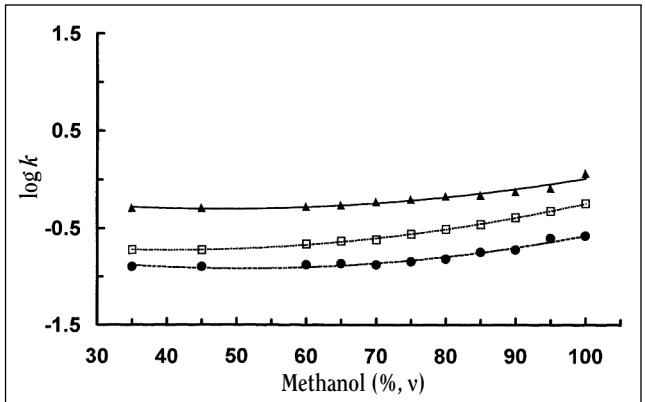

Figure 4. Correlation of retention factor logarithm with volumetric fraction of methanol in methanol–isopropanol (v/v) mobile phases (temperature, 296 ± 1 K). Cyclodextrins: ●, α-CD; □, β-CD; ▲, γ-CD.

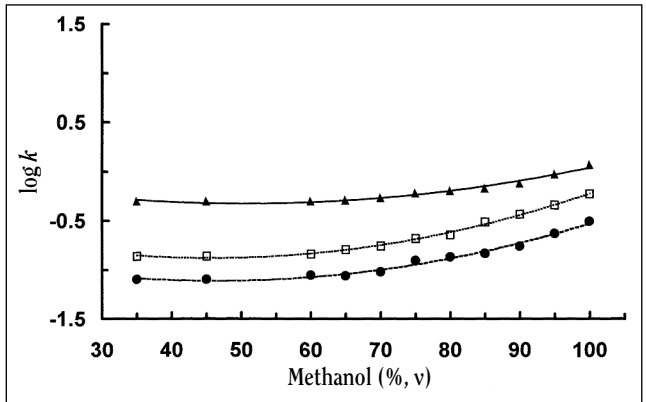

Figure 5. Correlation of retention factor logarithm with volumetric fraction of methanol in methanol–ethanol (v/v) mobile phases (temperature, 296 ± 1 K). Cyclodextrins: ●, α-CD; □, β-CD; ▲, γ-CD.

Results obtained with the four different mobile phases for natural cyclodextrins on PGC demonstrated that the possible retention mechanisms observed on the right part of Figures 1 and 3 may be mainly controlled by solute–solvent interactions, which can be roughly described as the "CD-PGC" effect.

Temperature effects on retention

To describe cyclodextrin retention on a PGC stationary phase, a thermodynamic approach was employed similar to thermodynamic studies developed for C_{18} reversed phase. With a C_{18} RP-HPLC column, van't Hoff plots (39) show a linear regression for a single elution mechanism, which is described as the "solvophobic" elution mode. With the hypothesis that retention of α-, β-, and γ-cyclodextrins on a PGC support is driven by a dual mechanism under conditions analogous to those of Figures 1 and 3, the retention factor *k* obtained can be described as a complex combination of at least two factors that represent the solvophobic and "CD-PGC" elution modes, respectively.

van't Hoff plots were drawn from 293 to 333 ± 1 K under different eluent conditions; pure methanol and three different methanol–water mixtures were used as mobile phases. $\Delta H°$ and $\Delta S°$ are reported in Table I. For each cyclodextrin studied, the magnitude of $\Delta H°$ was greater than that of $\Delta S°$, indicating that enthalpy played a much greater role in the transfer of the solute between the mobile and the stationary phase and therefore in the retention process. Analogous with what is known of C_{18} RP-HPLC, the enthalpy of transfer can be assumed to depend on both types of elution modes (43):

$$\Delta H° = f(\Delta H°_1; \Delta H°_2) \qquad \text{Eq. 7}$$

where $\Delta H°_1$ is the enthalpy probe for solvophobic interactions, and $\Delta H°_2$ is an image of the "CD-PGC" retention.

Table I. Thermodynamic Results and Correlation Coefficients *r* of van't Hoff Plots for Each Cyclodextrin with Different Methanol–Water Mobile Phases

Methanol–water (v/v)	CD	ΔH° (cal/mol)	ΔS° (cal/mol)	*r*
100/0	α	n.d.*	n.d.*	n.d.*
100/0	β	576 ± 94	2.70 ± 1.32	0.81
100/0	γ	545 ± 81	3.33 ± 1.28	0.84
80/20	α	n.d.*	n.d.*	n.d.*
80/20	β	410 ± 106	1.35 ± 1.36	0.75
80/20	γ	334 ± 90	1.65 ± 1.35	0.74
60/40	α	–403 ± 82	–2.01 ± 2.00	0.79
60/40	β	–245 ± 56	–0.56 ± 0.09	0.79
60/40	γ	–152 ± 76	–0.22 ± 0.58	0.80
40/60	α	–1170 ± 112	–3.66 ± 1.37	0.97
40/60	β	–852 ± 77	–1.83 ± 1.26	0.98
40/60	γ	–1226 ± 68	–2.25 ± 1.23	0.99

* n.d., not determined.

As illustrated in Table I, with 40% methanol in the mobile phase, satisfactory correlation coefficients were obtained (≈ 1), demonstrating that retention was governed only by the solvophobic retention mode. Between 40 and 75% methanol, the loss of linearity can be explained by an increase in the "CD-PGC" effect. With more than 75% methanol, the "CD-PGC" effect became predominant, with correlation values reaching 0.84 for a 100%-methanol mobile phase. Such results were in agreement with the dual sorption model according to Equation 7. The more negative the $\Delta H°$ value, the more effectively the solute is transferred to the stationary phase by solvophobic effect (34). The more positive the $\Delta H°$ value, the more effectively the solute binds to the stationary phase by the "CD-PGC" effect. A continuous increase in $\Delta H°$ values was observed as the methanol percentage in the mobile phase increased, as shown in Figure 6. For $\Delta H° = 0$, cyclodextrins were energetically attracted neither by the PGC stationary phase nor the mobile phase. The corresponding methanol percentage was smaller for γ- and β-cyclodextrin (64 and 67%, respectively) than for α-cyclodextrin (80%). Therefore, the "CD-PGC" effect may be less involved in the retention mechanism of α-cyclodextrin than in that of β- and γ-cyclodextrin.

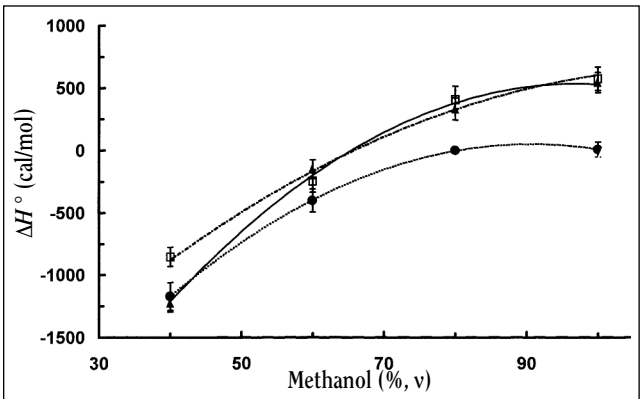

Figure 6. Enthalpy of transfer between mobile and stationary phases as a function of the volumetric fraction of methanol in different methanol–water (v/v) mobile phases. Cyclodextrins: ●, α-CD; □, β-CD; ▲, γ-CD.

Solvent effects on retention

Another way to understand processes involved in the "CD-PGC" mechanism could be a study of selectivity parameters described by the Snyder solvent selectivity classification (37). Snyder's solvent classification is based on the different and selected intermolecular interactions occurring between solute and solvent, such as hydrogen bonding (*Xe* and *Xd*) or dipolar interactions (*Xn*). Methanol cosolvents for which the "CD-PGC" effect was observed were water and acetonitrile, as illustrated in Figures 1 and 3, respectively. According to Snyder,

these cosolvents differ mainly by their dipolar interactions (*Xn* values), 0.25 and 0.42, respectively. Therefore, when the "CD-PGC" effect was observed (from 75 to 100% methanol with water as a cosolvent, from 78 to 100% methanol with acetonitrile as a cosolvent), it may be related to solute–solvent hydrogen bonding and thus the ability of the mobile phase to be a proton acceptor (*Xe*) or proton donor (*Xd*). To support this hypothesis, the systematic effect of Snyder's *Xe* parameter on cyclodextrin retention in different solvent systems was studied. Figure 7 shows the cyclodextrin retention factor as a function of mobile phase *Xe* values derived from Figures 1, 3, 4, and 5. Xe values for methanol–cosolvent mixtures were calculated by a combination of Equations 1–4. Curves A and B in Figure 7 were obtained with mobile phases composed of binary mixtures of methanol with ethanol and isopropanol cosolvents, respectively, whereas curves C and D resulted from mobile phases composed of binary mixtures of methanol with water and acetonitrile cosolvents, respectively. Figure 7 can be divided into three areas: two for curves C and D (zones 1 and 2) and the third one pertaining to curves A and B (zone 3). Curves A and B show a continuous decrease in the retention factor versus *Xe* values of the mobile phase. These two curves represent the measured retention factor of cyclodextrins in the integral experiment range of mobile phase composition. The continuous decrease in log *k* observed in zone 3 suggests a single solvophobic elution mode, as previously shown in Figures 4 and 5. The other two areas of Figure 7 involve curves C and D (zone 1 and 2). For each cyclodextrin studied, second-order polynomial fitting curves behave similarly with a calculated minimum *Xe* = 0.44, as described in Table II. These second-order curves define two domains: one associated with *Xe* < 0.44 (zone 1), the second with *Xe* > 0.44 (zone 2). In zone 1, a decrease in log *k* values as the mobile phase methanol percentage increases is consistent with the solvophobic elution mode already described for curves A and B. In zone 2 (0.44 < *Xe* < 0.48), an increase in each log *k* was systematically observed and could be considered specific to the "CD-PGC" effect.

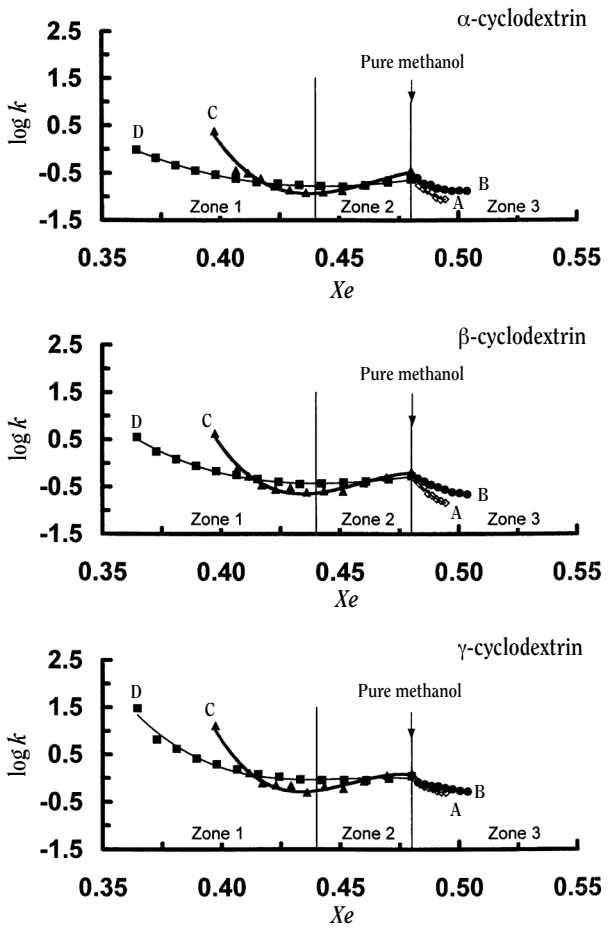

Figure 7. Retention factor logarithm as a function of proton acceptor capacity (*Xe*) for different ethanol–methanol (A), isopropanol–methanol (B), water–methanol (C), and acetonitrile–methanol (D) mobile phases (temperature, 296 ± 1 K). Zone 1, *Xe* < 0.44; zone 2, 0.44 < *Xe* < 0.48; zone 3, *Xe* > 0.48.

How to interpret "CD-PGC" effect

According to the preceding paragraph, the retention behavior of cyclodextrins on PGC appeared different from that observed with RP-HPLC (31). As already observed in Figures 1 and 3, the solvophobic model is not adequate for the prediction of cyclodextrin retention with a stationary carbon phase. PGC stationary phase is much more hydrophobic than any other usual reversed-phase chromatographic support (51). The thorough solute adsorption mechanism on a PGC support is not clearly understood at present, but many authors described the strong interactions between the PGC stationary phase and solutes as London dispersion forces (24,47). It has been proposed that polar solutes would interact with the graphite surface in a nonplanar manner with a specific orientation (23). Such an orientation may be related to the polar surface of the α-, β, and γ-cyclodextrin, leading to a different adsorptive strength due to the cyclodextrin size. This hypothesis matches the size dependence elution order seen in Figures 1 and 3. As a preliminary conclusion, the "CD-PGC" effect involves London dispersion forces between the solute and the stationary phase. However, as shown in Figures 1, 3, 4, and 5, the form of the curve depends on the cosolvent used. A single interpretation with London dispersion forces is not sufficient to explain the

Table II. Minimum Values and Regression Coefficients r^2 of Polynomial Fittings to Second-Order Curves of log *k* versus *Xe*, According to Cyclodextrin Type and Methanol Cosolvent*

Cosolvent	CD	Methanol (%volume ± 1%)	Xe	r^2
Water	α	75	0.44	0.96
Water	β	75	0.44	0.97
Water	γ	75	0.44	0.96
Acetonitrile	α	78	0.44	0.99
Acetonitrile	β	78	0.44	0.99
Acetonitrile	γ	78	0.44	0.98

* Temperature is 296 ± 1 K.

nonlinearity of log *k*-versus-mobile phase methanol percentage observed in Figures 1 and 3.

When the mobile phase methanol percentage increases, the London interaction forces of the solute versus the stationary phase are kept constant. Therefore, as observed in Figures 1, 3, 4, and 5, additional criteria involving the mobile phase composition must be taken into account. Hydrogen bonding is suspected to be responsible for solute–solvent interactions (51). With *Xe* mobile phase values being a probe of the solvent proton acceptor ability, it can be assumed that primary and secondary hydroxyl groups of cyclodextrin molecules are involved in hydrogen bonding interactions with the solvent system. Hydrogen bonding between cyclodextrin solutes and solvent, acting as a single interaction, would also lead to a decreased or constant retention factor value as the mobile phase methanol percentage increases. As observed for curves C and D of Figure 7 (zone 2), retention factors of each cyclodextrin increase as the hydrogen bond acceptor capacity of the mobile phase increases ($0.44 < Xe < 0.48$).

The two single interpretations previously described (i.e., London dispersion forces and hydrogen bonding) cannot explain the concavity of the curves observed in Figures 1 and 3 and would even lead to an opposite result. The "CD-PGC" effect might be more complex, and interdependence between interactions should be taken into account. Wan et al. (23) have proposed that interactions between the polar substituent and the solvent may influence the orientation of the solute molecule facing the carbon adsorbent surface. Therefore, hydrogen bonding between the cyclodextrin and mobile phase may influence the molecule alignment facing the PGC surface. When water or acetonitrile were used as methanol cosolvents (curves C and D of Figure 7), the increase in the mobile phase proton acceptor ability (zone 1, $Xe < 0.44$) led to an increase in the retention factor of each cyclodextrin. Such results indicate less effective solute–solvent interactions. Therefore, cyclodextrins (considered hydrogen bond acceptors) may interact less with solvents when the mobile phase methanol percentage increases. The "CD-PGC" effect may be described as a balance between mobile phase–cyclodextrin solute interactions via hydrogen bonding and London dispersion forces involved in PGC support–cyclodextrin interactions, leading the cyclodextrin angle in relation to the PGC surface to be modified (23).

Conclusion

The elution mode of cyclodextrins on a porous graphitic carbon as chromatographic support was experimentally shown to be different from that observed with C_{18} stationary phases. The "CD-PGC" effect was evidenced for a specific range and nature of mobile phase. It can be interpreted as a balance between mobile phase–cyclodextrin solute interactions and London dispersion forces involved in PGC support–cyclodextrin interactions. Solute–solvent–stationary phase interactions are difficult to interpret, and Wan's hypothesis (23) of the solute angle with the stationary phase is consistent with all the experimental data of this report.

For highly aqueous concentrations, natural cyclodextrins are almost irreversibly retained on the carbon surface. Therefore, a new stationary phase composed of adsorbed cyclodextrins will be able to complex different solutes in their internal cavities. Such a support may offer new separation capacities in the solvophobic domain. In that case, solute–stationary phase interactions will depend on the equilibrium constant of complexation between solutes and fixed cyclodextrins.

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