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Chromatographic study of terpene derivatives on porous graphitic carbon stationary phase with β-cyclodextrin as mobile phase modifier

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Abstract

The stoichiometric coefficients and apparent formation constants (K_f) of α-terpineol, thymol, geraniol and linalool complexes with β-cyclodextrin (β-CD) were determined using HPLC with a porous graphitic carbon (PGC) chromatographic support. Measurements were performed with four different methanol–water mobile phases. All the terpene derivatives under study form 1:1 guest–CD complexes. Graphs of K_f as a function of the mobile phase composition appeared different from those classically described for RP-C_{18} and suggest that the PGC stationary phase could play an active role in the complexation process. Solute–CD inclusion and solute–stationary phase interactions may be involved in this specific behavior.

Keywords: Stationary phases, LC; Porous graphitic carbon; Mobile phase composition; Inclusion complexes; Cyclodextrins; α-Terpineol; Thymol; Geraniol; Linalool

1. Introduction

β-Cyclodextrin (β-CD) belongs to a family of torus-shaped, naturally occurring, enzymatically synthesized, cyclic oligosaccharides composed of six, seven or eight α-1,4 linked D-glucopyranose units per molecule (α-, β-, γ-CD, respectively) [1,2]. While the exterior of the molecule is hydrophilic, its relatively non-polar central cavity [3] may selectively include molecules of various species. The encapsulation of a solute inside the cyclodextrin cavity can change the physico-chemical properties of this guest molecule to a great extent. Hence cyclodextrin complexation can be used [4] to protect flavors against evaporation, atmospheric oxidation and light or heat-induced transformations. To take full advantage of the complexation potential of cyclodextrins, in depth understanding of the stoichiometry and stability of inclusion complexes is of critical importance.

Many physico-chemical methods have been successfully used to characterize inclusion complexes including UV spectroscopy [5,6], fluorescence measurements [7,8], circular dichroism [9], potentiometry [10], mass spectrometry [11,12] and nuclear magnetic resonance (NMR) spectroscopy [13]. With

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a liquid chromatographic system, it has been shown that retention characteristics of host–guest complexes will be different from those of single guest molecules [14–17]. Therefore, liquid chromatography (LC) appeared to be a satisfactory method to observe and characterize cyclodextrin–guest inclusion complexes. Modification of the retention properties of molecules, with different cyclodextrin concentrations in the mobile phase, were found to be related to the stoichiometry and the stability of the inclusion complexes thus formed, as described by Fujimura et al. [18].

Data on retention behavior, in reversed-phase high-performance liquid chromatography (RP-HPLC), with and without β-CD (the most widely used cyclodextrin) in the mobile phase have already been published for monoaromatic compounds [18], aromatic amines, polyaromatic hydrocarbons, nitrogen heterocycles, aromatic hydroxyl compounds [14,19] and with the terpene derivatives chosen for this study [20]. In the early 1980s, a comprehensive review was published by Hinze [21] which described the extent of HPLC applications using mobile phases containing cyclodextrins. Warner and co-workers have reported numerous studies of pyrene–β-CD complexes using RP18-HPLC [22–24] with methanol–water mobile phases where they described specific bindings between solutes and methanol [23]. Nowakowski et al. [25] demonstrated, in the light of thermodynamic chromatographic data and molecular modeling, that calculation of inclusion equilibrium constants was dependent on the stationary phase–mobile phase couple.

Kiselev et al. pioneered the use of porous graphitic carbon (PGC) as an adsorbent in LC [26]. Knox et al. [27] carried out systematic investigations with PGC. This stationary phase is an extremely strong adsorbent [28] due to its flat crystalline surface [29]. It has an energetically homogeneous surface [30] with minimal active sites on the edges of graphite sheets. This stationary phase is often compared to C_{18} silicas and described as a stronger hydrophobic sorbent [28]. On PGC support, the retention mechanism appears to be different compared to reversed-phase bonded silicas [31]. 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 [29] or solute–eluent interactions [30]. To study the PGC retention mechanism, comparisons with RP-HPLC are needed. On C_{18} stationary phases, linear dependence of the logarithm of the solutes capacity factors ($\log k'$) with the mobile phase composition are observed [32]. Several authors have suggested that compound retention from homologous series on RP silicas [33,34] is a function of their solubility in the mobile phase, as shown for cyclodextrins by Chatjigakis et al. [35].

In the case of PGC systems, Hennion et al. [31] demonstrated that solute–stationary phase interactions (electronic interactions) are more effective than solute–solvent interactions (hydrophobic mode) in the retention mechanism of polar compounds. Clarot et al. [16] reported unusual behavior of cyclodextrins retention on PGC with a methanolic aqueous mobile phase. They observed a dual retention mode depending on the mobile phase composition. In a range of 35% to 70% methanol, a classical RP-C_{18} elution behavior with aqueous–methanol mobile phases was observed [16]. Similar results were found by Koizumi et al. [17].

While terpenes are a family of compounds for which cyclodextrin complexation can be applied, few systematic studies of their inclusion complexes with cyclodextrins have been already reported [36]. Therefore, using PGC–HPLC, we investigated the influence of solvent composition on the complexation of β-CD with the following analytes: linalool, geraniol (allylic alcohols), thymol (aromatic alcohol) and α-terpineol (alicyclic alcohols). Stoichiometry and stability of the formed complexes were discussed and the PGC role in the complexation process was investigated.

2. Experimental

2.1. Chromatographic system

The HPLC system consisted of a HPLC Waters Model 590 pump (Waters, Milford, MA, USA), a Rheodyne valve Model 7125 (Rheodyne, Cotati, CA, USA), fitted with a 10-μl sample loop and a Knauer UV detector (210 nm) model variable-wavelength monitor (Knauer, Berlin, Germany). The temperature was controlled with a column oven from Dupont

(Les Ulis, France) for the column and with a Bioblock Scientific cryostat Model Polystat 22 (Bioblock, Illkirch, France) for the mobile phase. For all experiments the mobile phase flow-rate was set up and systematically controlled at 1.00±0.01 ml/min and the system temperature monitored at 25±1°C.

2.2. Columns and mobile phases

A commercially available column (100×4.6 mm I.D.) packed with Hypercarb PGC of 7 μm particle size (Shandon, Runcorn, UK) and a laboratory packed column (150×3 mm I.D.) containing LiChrosorb RP18 of 5 μm particle size (Merck, Paris, France) were used.

β-CD was supplied by Wacker (Werk Burghausen, Germany) and was used without further purification in the mobile phase. Concentrations employed were 0, 1, 2, 3 and 4 m*M*. HPLC-grade methanol (MeOH) was purchased from Prolabo (Prolabo, Paris, France) and water was freshly bidistilled. Binary mixtures of water–methanol, with water percentages from 30 to 45% (v/v), were filtered with a Millipore filter Model HVLP 0.45 μm (Molsheim, France) prior to elution.

Mobile phases were prepared according to the following procedure. After fabrication of the desired methanol–water mixture, an accurately weighed amount of β-CD was added to 250 ml of this binary mixture in a 500-ml volumetric flask. When total dissolution at ambient temperature was observed, the remaining amount of solvent was added for a final mobile phase volume of 500 ml. The maximum quantity of β-CD that can be dissolved in such binary mixtures has been reported elsewhere [37].

The void volume of the C_{18} column was determined by the elution of 10 μl of a copper sulfate solution (0.01 mg/ml) and found to be 0.84±0.01 ml. The void volume of the PGC column was determined by the elution of 10 μl of pure methanol and found to be 1.06±0.01 ml. This volume was systematically controlled through out the experimental program.

2.3. Molecular modeling

Molecular modeling calculations were carried out on a Silicon Graphics O2 system (Mountain View, CA, USA) using the Sybyl 6.3 package from Tripos (St. Louis, MO, USA). A PM3 semi-empirical calculation method was employed with a Polak–Ribiere conjugate gradient algorithm. A systematic search was used to display the terpene conformations. All the molecule torsion angles involving free rotating bonds were allowed to vary with increments of 30°. For all terpenes under study, minimum energy structures resulting from this geometrical optimization were kept.

2.4. Samples

The terpenes used were thymol, linalool, geraniol and α-terpineol, purchased from Sigma (St. Louis, MO, USA) whose structures are shown in Fig. 1. They were diluted in mobile phases containing no β-CD at 0.3 mg/ml. Both anhydrous D-glucose and

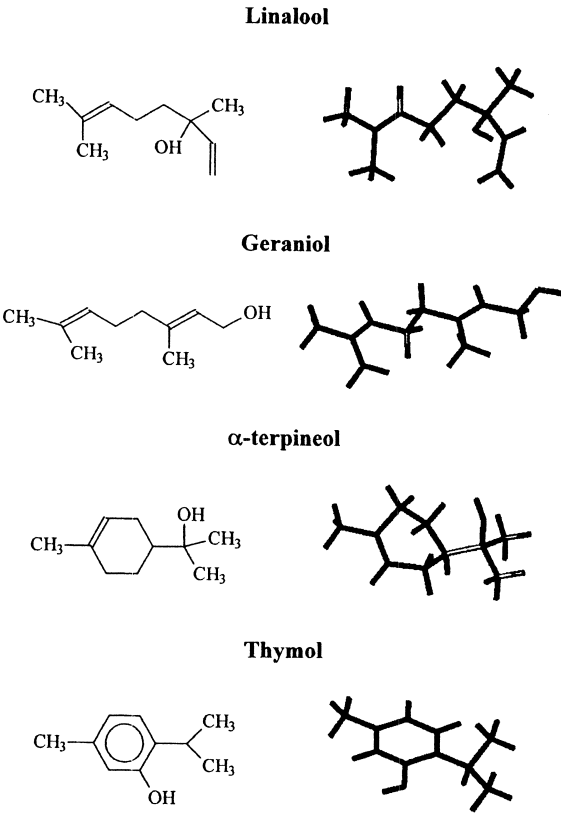

Fig. 1. Terpene structures: left side: planar geometry. right side: calculated molecular modeling minimum energy structure.

sulfanilic acid were purchased from Prolabo (Fontenay sous Bois, France), and were used as control probes.

2.5. Data acquisition and peak profile analyses

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

For all experiments, peak characteristic probes were calculated with algorithms and methodologies previously described [38].

2.6. Statistical treatment

Each compound was eluted in triplicate and the corresponding capacity factors calculated. Capacity factor behavior studies, under different experimental conditions, were systematically tested in terms of linearity and nonlinearity. Tolerance curves leading to uncertainty scores on slopes and intercepts are described in the Results section.

3. Results and discussion

3.1. Experimental methodology

In RP18-HPLC, encapsulation of solutes with β-CD is a relatively well known mechanism [14,18,22,39]. PGC is often compared to C_{18} silicas and described as a stronger hydrophobic sorbent [28].

In the absence of data in the literature concerning cyclodextrin complexation with PGC, specific information was needed to elucidate the inclusion process. Solute retention modifications can be attributed to the presence of cyclodextrins in the mobile phase at different concentrations.

To examine the possible effect of β-CD on solvent strength of the mobile phase, various amounts of D-glucose, corresponding to 1 to 4 m*M* of β-CD [18] in the number of glucose units, were added to the methanol–water (40:60, v/v) mobile phase and the retention of the four terpenes checked, as shown in Table 1. As no retention modifications were observed, and with the hypothesis that no glucose–terpenes complexes exist, possible elution modifications observed in the presence of β-CD cannot therefore be attributed to solvent strength modifications.

In order to investigate the possible surface modification of PGC in the presence of cyclodextrin, a system control using sulfoanilic acid was injected in the system. This solute cannot be complexed by cyclodextrins [40]. In a first step, runs of 0.1 mg/ml sulfoanilic acid with 0, 2 and 4 m*M* β-CD in water–methanol (40:60, v/v) as mobile phases with a RP18 column led to the conclusion that no host–guest complexation occurred. As shown in Table 2, no retention modifications of sulfoanilic acid, regardless of β-CD concentration, were observed, leading to the conclusion that this compound is a good candidate for a PGC surface study. Similar experiments with PGC were then done under identical chromatographic conditions. Results, illustrated in Table 2, demonstrated that no retention modifications of sulfoanilic acid occurred, indicating that no changes on the PGC surface with any β-CD concentration happened.

In light of the above observations, it can be concluded that all terpene retention modifications

Table 1
Capacity factors (k') of terpenes with a methanol–water (60:40, v/v) mobile phase containing 0 to 28 m*M* glucose as mobile phase modifier (flow-rate, 1.00±0.01 ml/min; temperature, 25±1°C)

	k'				
	0 m*M* β-CD, 0 m*M* glucose	0 m*M* β-CD, 7 m*M* glucose	0 m*M* β-CD, 14 m*M* glucose	0 m*M* β-CD, 21 m*M* glucose	0 m*M* β-CD, 28 m*M* glucose
α-Terpineol	14.68	14.66	14.68	14.65	14.65
Linalool	10.21	10.22	10.21	10.24	10.22
Thymol	11.11	11.11	11.09	11.08	11.10
Geraniol	12.25	12.27	12.26	12.27	12.27

Table 2
Capacity factors (k') of sulfanilic acid with a methanol–water (60:40, v/v) mobile phase containing 0 to 4 m*M* of β-CD as mobile phase modifier, on two different stationary phases: C_{18} and PGC (flow-rate, 1.00±0.01 ml/min; temperature, 25±1°C)

	k'				
	0 m*M* $(\beta\text{-CD})_T$	1 m*M* $(\beta\text{-CD})_T$	2 m*M* $(\beta\text{-CD})_T$	3 m*M* $(\beta\text{-CD})_T$	4 m*M* $(\beta\text{-CD})_T$
C_{18}	0.39	0.34	0.37	0.40	0.37
PGC	1.28	1.25	1.28	1.31	1.26

with PGC using the chromatographic conditions described herein can be attributed to a complexation process with β-CD.

3.2. Effect of methanol percentage on complexation

Mobile phase composition plays a major role in solute retention, regardless of the chromatographic support used. Colin and Guiochon [41] demonstrated that comparisons between the eluotropic strength of solvents used with a classical RP18 stationary phase and carbon adsorbent are not accurate because of the specific nature of this last chromatographic support. When samples were eluted in a PGC/mobile phase couple, the apparent solvent strength of the carrier phase was solute dependent [42,43]. Nevertheless, we have shown in a previous report [16] that, with cyclodextrins as solutes on a PGC stationary phase, methanol was a stronger solvent than water.

Fig. 2 represents the logarithm of the capacity factor (k') of terpenes as a function of water percentage in the mobile phase, with 0 m*M* and 4 m*M* of β-CD in Fig. 2A and in Fig. 2B, respectively. When no β-CD is added to the mobile phase, retention of all four terpene derivatives increased with increasing water percentage, as shown in Fig. 2A. In this solvent composition range (30 to 45% of water in water–methanol mixtures), linear relationships (correlation coefficient>0.99) between the retention factor (k') logarithm of terpenes and the water percentage in the mobile phase [44] were observed illustrating a classical ''reversed-phase'' elution mechanism [45,46]. Similar linear relationships of log k' versus water percentage (R>0.99) were also obtained with 4 m*M* of β-CD, as observed in Fig. 2B. These log k' patterns in the presence or absence of β-CD indicated that retention was essentially driven by a single mechanism involving hydrophobic interactions [47]. This retention characteristic was observed regardless of the β-CD concentration added to the mobile phase. The entire range of β-CD

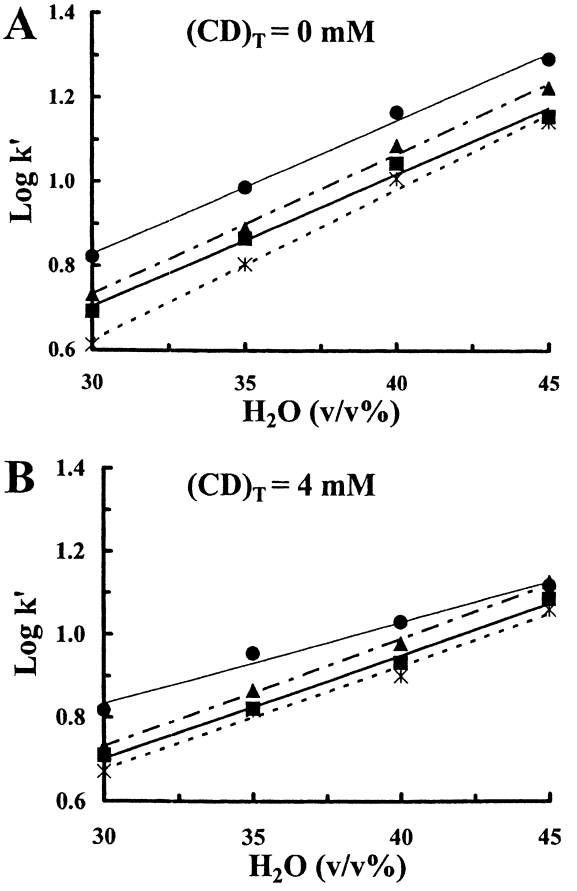

Fig. 2. Correlation of the log of the capacity factor (log k') with volumetric fraction of water in methanol–water (v/v) mobile phases (flow-rate, 1.00±0.01 ml/min; temperature, 25±1°C). Terpenes: ○ α-terpineol, ■ thymol, ▲ geraniol, ★ linalool. (A) Without β-CD in the mobile phase, (B) with 4 m*M* β-CD in the mobile phase.

Table 3
Capacity factors of terpenes for four different methanol–water (v/v) mobile phases containing 0 to 4 m*M* of β-CD (flow-rate, 1.00±0.01 ml/min; temperature, 25±1°C)

	Mobile phase, MeOH–water (v/v)	*k′*				
		0 m*M* β-CD	1 m*M* β-CD	2 m*M* β-CD	3 m*M* β-CD	4 m*M* β-CD
α-Terpineol	55:45	19.68	18.91	14.06	11.59	11.40
	60:40	14.68	13.15	11.54	11.74	11.17
	65:35	9.70	8.82	8.06	7.96	7.51
	70:30	6.63	6.33	5.82	5.51	5.20
Linalool	55:45	13.99	14.02	11.02	10.90	9.84
	60:40	10.21	9.30	8.75	8.54	8.31
	65:35	6.38	6.13	5.70	5.60	5.23
	70:30	4.09	3.94	3.89	3.64	3.42
Thymol	55:45	14.39	14.10	12.24	12.14	10.50
	60:40	11.11	10.08	9.17	9.28	8.94
	65:35	7.35	6.48	6.01	5.96	5.35
	70:30	4.92	4.64	4.40	4.17	3.83
Geraniol	55:45	16.82	16.55	13.18	13.10	11.63
	60:40	12.25	11.16	10.08	10.35	9.92
	65:35	7.76	7.06	6.60	6.61	5.93
	70:30	5.39	4.90	4.57	4.53	4.06

concentrations led to retention characteristics summarized in Table 3.

3.3. Elution order

On PGC, as observed in Fig. 2A, the elution order of all four terpene derivatives, without cyclodextrin modifier and with all mobile phases used, is constant: linalool, thymol, geraniol and finally α-terpineol. On PGC, retention of such family of compounds is related to size, polarity and deformability of the molecules [14,30]. The four terpenes studied can be classified in two groups, the first one containing the two aliphatic compounds: geraniol and linalool, the second one including the cyclic molecules: thymol (aromatic) and α-terpineol (alicyclic).

Considering the two aliphatic compounds, it is observed in Fig. 2A that linalool was eluted first, demonstrating that the hydroxyl position in the molecule plays a major role in the retention process [48]. In the case of geraniol, the OH functional group positioned in a terminal position allows the molecule to have a best alignment on the PGC surface, in comparison to linalool where the OH group is in the middle, as observed in the structures shown in Fig. 1. This effect is particularly intense when the stereochemistry of the analyte molecule forces the polar group to be close to the graphite surface. This specific effect, called ''polar retention effect on graphite'' [48], appears to be additive to the normal hydrophobic and dispersive effects found with conventional reversed-phase materials. For both cyclic compounds, it can be observed in Fig. 2A that thymol was eluted first showing the influence of the solute molecule flexibility in the PGC retention mechanism. Thymol is rigid because of the aromatic ring and has a non planar geometry due to methyl substituents, as observed in Fig. 1. When cyclodextrin was added to the mobile phase, the observed elution order between the four terpene derivatives was found to be: linalool, thymol, geraniol and α-terpineol. This classification was observed for all mobile phases used and whatever the CD concentration added, as shown on Table 3. As an exception, an elution inversion between geraniol and α-terpineol was observed for the mobile phase methanol–water (55:45, v/v), with 3 and 4 m*M* β-CD. Such observations suggested possibilities of selectivity

modifications when complex formation was achieved.

3.4. Equilibria analyses

Several authors have already described the different equilibria occurring in a HPLC system when cyclodextrins were added as organic modifiers in the mobile phase [14,18,22,39]. As previously demonstrated [16], free β-CDs are not retained in the solvent range studied (55 to 70% of methanol). As a consequence, interactions between cyclodextrin molecules and complexed solutes could be neglected in the theoretical treatment of the retention mechanism.

Since PGC behaves as a classical hydrophobic chromatographic support (i.e., linear relationship between log k' with water mobile phase percentage and negligible cyclodextrin–stationary phase interactions), equilibria written for RP-HPLC can be extended to the PGC stationary phase.

Mosheni and Hurtubise [14] proposed an equation taking into account equilibria involving formation of 1:1 host–guest complexes:

$$\frac{1}{k'} = \frac{1}{k'_0} + K_f \cdot \frac{([CD]_m)}{k'_0} \quad (1)$$

where k' is the capacity factor of the solute, k'_0 the solute capacity factor in the absence of β-CD, K_f is the apparent formation constant of the inclusion complex and $([CD]_m)$ is the equilibrium concentration of β-CD.

The cyclodextrin concentration $([CD]_m)$ used in Eq. (1) is not the total analytical one $([CD]_T)$ because methanol can form weak complexes with β-CD in competition with solutes. The relationship between $([CD]_m)$ and $([CD]_T)$ is given in the following equation:

$$([CD]_m) = \frac{([CD]_T)}{1 + K_m[M]} \quad (2)$$

with [M] the mobile phase methanol concentration, K_m describes the affinity of the organic modifier for the CD cavity and has been determined to be 0.32 M^{-1} for methanol at 25°C [49].

Moeder et al. [39] added an equilibrium including the formation of a 1:2 guest–CD complex via a precursor 1:1 complex which leads to the following equation:

$$\frac{1}{k'} = \frac{1}{k'_0} + K_f \cdot \frac{([CD]_m^2)}{k'_0} \quad (3)$$

Stoichiometry and stability constants of terpene–CD inclusion complexes under study have been already published for a RP18 stationary phase [20]. 1:1 guest–CD complexes were observed for many other terpenes as well [22,39]. Because of PGC specificity concerning the retention mechanism, both 1:1 and 1:2 complexes were considered.

3.5. Effect of β-CD concentration on complexation

When β-CD was added to the mobile phase (1 to 4 mM), capacity factors of all terpenes under study decreased whatever the methanol percentage used, as illustrated in Table 3. Such results were analogous to those observed with the same terpenes on a C_{18} column [20]. As observed in Table 3, the k' decrease in each terpene derivative, with increasing amounts of β-CD, was always most pronounced for the smallest mobile phase methanol concentration (55%, v/v). To explain such behavior, different major interactions should be taken into account: cyclodextrin–PGC interactions, solvent strength and terpene–CD inclusion.

In a previous study [16], it was shown that, in the mobile phase range studied, the distribution equilibrium of β-CD between the PGC support and aqueous–methanol mobile phases was negligible. For a methanol–water (55:45, v/v) mobile phase, the large decrease in the terpene capacity factor, observed in Table 3, could be explained by cyclodextrin complexation: the higher the β-CD concentration, the faster the elution. For an increasing eluent methanol concentration (55–70%, v/v), the decrease in the mobile phase polarity provokes a decrease in complexation. Furthermore, the existing competition between methanol and terpene for access to the cyclodextrin hydrophobic cavity must be considered, since the association constant of methanol with β-CD is 0.32 M^{-1} [49]. At high methanol percentage (70%, v/v), a substantial amount of methanol can interact with β-CD leading to competition with terpene complexation.

3.6. Stoichiometry and stability constant of the formed complexes

The determination of stoichiometric ratios for different CD–terpene complexes formed was achieved with the help of both Eqs. (1) and (3). As a result, the reciprocal of k' for each terpene was plotted as a function of ($[CD]_m$) and ($[CD]_m^2$). Correlation coefficients arising from these two plots were determined and shown in Table 4. Whatever the terpene derivatives and the mobile phase under study, correlation coefficients corresponding to 1:1 complexes were always greater than those calculated for 1:2 complexes. Consequently, complexes in 1:1 terpene–CD ratio were favored. Such observations were in good agreement with those previously obtained with a C_{18} column [20].

As 1:1 complexation occurred, linear graphs of $1/k'$ versus $(CD)_m$ were plotted with the four water–methanol mobile phases containing 30, 35, 40 and 45% (v/v) water. Fig. 3 represents this plot for a methanol–water (70:30, v/v) mobile phase. Apparent formation constant (K_f) values, were obtained using linear regression slopes and intercepts from a method previously described. Finally, K_f values were plotted as a function of the mobile phase water percentage in Fig. 4A–D for α-terpineol, thymol, geraniol and linalool, respectively. The standard deviations were determined with the statistical procedure described in the Experimental section. All K_f values obtained were of the same magnitude as those described in the literature [20].

Table 4
Correlation coefficients (R) arising from Eqs. (1) and (3) (for 1:1 and 1:2 terpene–CD complexes, respectively), with four different methanol–water (v/v) mobile phases

	MeOH (%, v/v)	Correlation coefficient	
		1:1 using Eq. (1)	1:2 using Eq. (3)
α-Terpineol	55	0.964	0.908
	60	0.952	0.812
	65	0.973	0.891
	70	0.991	0.958
Linalool	55	0.953	0.909
	60	0.972	0.864
	65	0.981	0.949
	70	0.990	0.951
Thymol	55	0.964	0.958
	60	0.952	0.803
	65	0.972	0.909
	70	0.994	0.980
Geraniol	55	0.961	0.921
	60	0.949	0.772
	65	0.962	0.918
	70	0.973	0.943

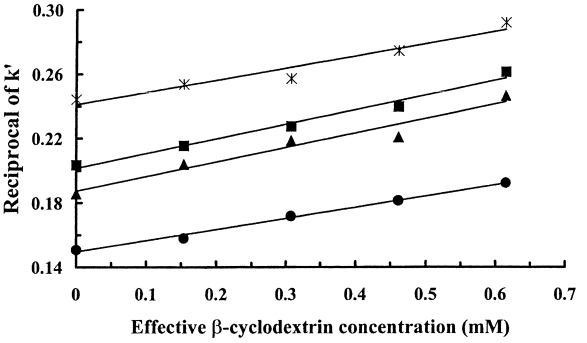

Fig. 3. Reciprocal of retention factor k' vs. effective β-CD concentration ($[CD]_m$) with a methanol–water (70:30, v/v) mobile phase (flow-rate, 1.00±0.01 ml/min; temperature, 25±1°C). Terpenes: ○ α-terpineol, ■ thymol, ▲ geraniol, ★ linalool.

By comparing Fig. 4A–D, a similar calculated K_f between 30 and 40% water in the mobile phase is observed for all the solutes tested (≈400). A slight increase of the K_f values appeared from 40 to 45% for the two aliphatic derivatives (Fig. 4C and D). A larger increase is observed for α-terpineol (Fig. 4A), K_f remained stable for thymol (Fig. 4B).

Several authors explained the increase in K_f as a function of the mobile phase water content with an enhanced competition of methanol and solute for the cyclodextrin cavity [14,39]. As a matter of fact, the classical equation used for K_f calculation (Eq. (1)) employed the analytical cyclodextrin concentration ($[CD]_m$) and not the total one ($[CD]_T$). Nevertheless, the increase in K_f values, principally observed for α-terpineol in Fig. 4A, is interpreted using hydrophobic interactions which are known to play a key role in the inclusion process. The transfer of a solute containing a hydrophobic moiety, like terpenes, from a polar solvent to the hydrophobic cyclodextrin cavity, produces a large decrease in the solute free energy leading to a favored complexation. As the mobile phase increases in polarity, the polarity difference between the CD cavity and the eluent will

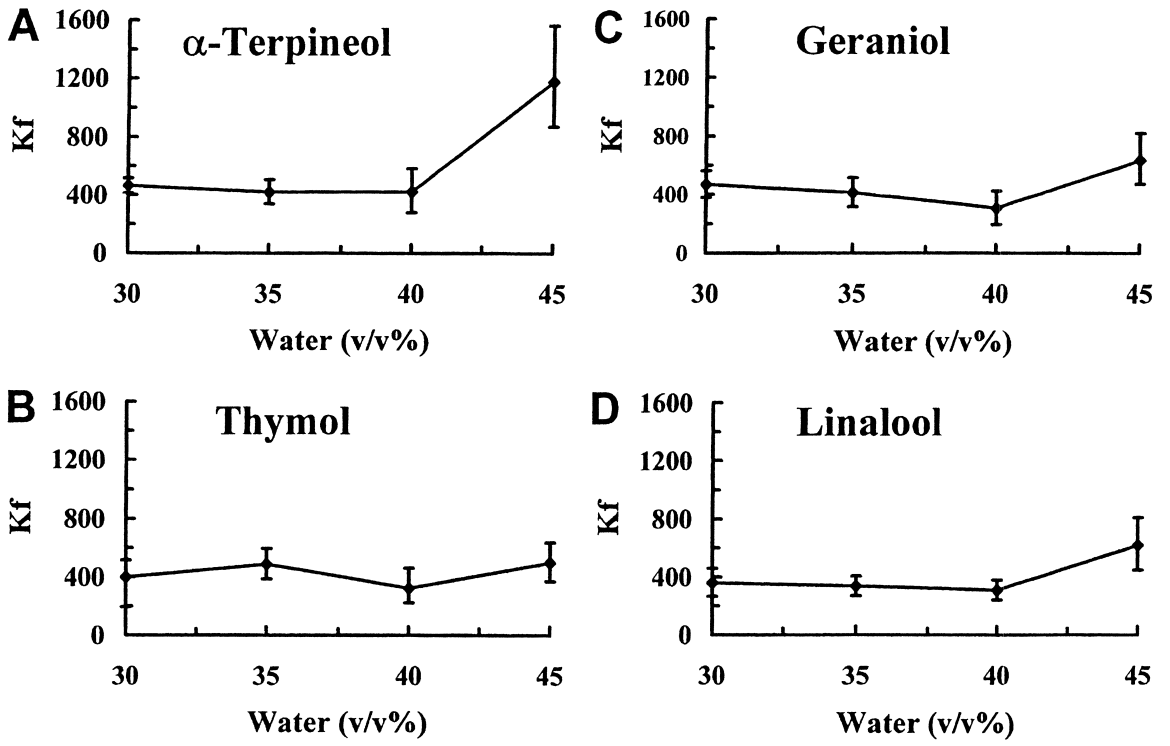

Fig. 4. Apparent formation constant (K_f) of terpene–β-CD complexes as a function of water percentage in methanol–water (v/v) mobile phases (flow-rate, 1.00±0.01 ml/min; temperature, 25±1°C). Terpenes: A, α-terpineol; B, thymol; C, geraniol; D, linalool.

be more intense. Consequently, complex formation will be favored [20]. This hypothesis can explain the increase in K_f values observed between 40 and 45% (v/v) water in Fig. 4A, C and D, as expected in classical RP-HPLC but is in contradiction with the first part of these curves. In the RP18-HPLC system, this K_f behavior was interpreted by Muñoz de la Peña et al. [23] as cooperative binding between the terpenes and methanol in the CD cavity. With PGC adsorbents, other interactions must be operative in the complex formation process that takes into account the carbon stationary phase. It is well known that the retention mechanism on graphite is different from that observed with C_{18} [16,50]. Hydrophobic interactions controlled CD complexation (CD cavity/mobile phase polarity difference) and retention (analyte expelled from the mobile phase). These interactions led to opposing effects involving competition of terpene solutes for the PGC surface and the cyclodextrin cavity. In the first zone of Fig. 4A, C and D, where K_f has a constant value, the graphite support can play the role of complexation restrictor. When the mobile phase polarity becomes greater (water content = 40–45%, v/v) the PGC hydrophobic role becomes negligible compared to the complexation process, leading to an increase in the apparent formation constant K_f.

4. Conclusion

Stoichiometric ratios and apparent formation constants K_f of terpene derivative–β-CD complexes with a graphite carbon stationary phase have been determined. Stoichiometric coefficients obtained were found to be similar to those described with a RP18 column [20]. K_f values were in the same magnitude as those reported in the literature but their patterns as a function of the mobile phase composition gave different results. A proposed mechanism involving both interactions intervening in the encapsulation process and in the adsorption mecha-

nism suggests an active role for graphite in the complex formation. Such observations led to complex equilibria description when PGC was used for cyclodextrin inclusion observations. Additionally, PGC behaves uniquely when β-CD is added to the mobile phase, leading to new possibilities in complex driven separation processes.

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