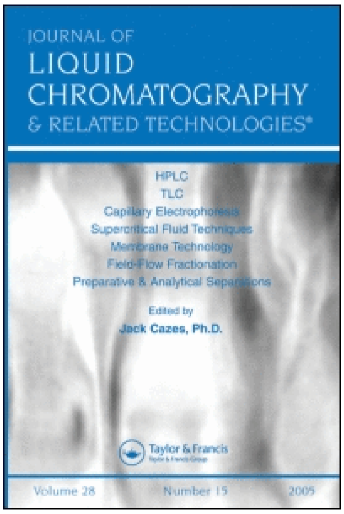

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REVERSED PHASE CHROMATOGRAPHIC STUDY OF THE INCLUSION SELECTIVITY OF TERPENE DERIVATIVES WITH β-CYCLODEXTRIN IN WATER/COSOLVENT MIXTURES

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ABSTRACT

Terpene compounds include a wide variety of molecules with very different structures and physico-chemical properties. Monoterpenes are of interest because of their volatility and aromatic properties. Inclusion complexation of these molecules with cyclodextrins will lead to chemical and volatility protection and to a possible controlled release. In this study, reversed phase liquid chromatography was used to demonstrate that some terpenes can form inclusion complexes with β-cyclodextrin. The stability of these complexes was studied for aromatic, aliphatic, or acyclic terpenes with various mobile phases.

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It is confirmed that mobile phase composition plays a major role in the complex stability. Moreover, in terms of separation, it was observed that mobile phase characteristics could modify inclusion selectivity, with retention inversions for some terpene derivatives when β-cyclodextrin was added to the mobile phase. It was also demonstrated that "rough" molecular modelling can qualitatively predict stoichiometry and relative stability of the formed inclusion complexes.

INTRODUCTION

β-Cyclodextrin belongs to a family of torus-shaped, naturally occurring, enzymatically synthesised, cyclic oligosaccharides composed of six, seven, eight, or nine α-1,4 linked D-glucopyranose units per molecule (α-, β-, γ-, and δ-cyclodextrin 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 guest molecule inside the cyclodextrin cavity can change its physico-chemical properties to a great extent. Hence cyclodextrin complexation can be used[4] as a means of protecting flavours against evaporation, atmospheric oxidation, and light or heat-induced transformations. To take full advantage of the complexation potential of cyclodextrins, deep knowledge of the stoichiometry and stability of inclusion complexes formed may be of critical importance.

Many physicochemical methods have been successfully used to characterise inclusion complexes like UV-spectroscopy,[5-6] fluorescence measurements,[7-8] circular dichroism,[9] potentiometry,[10] mass spectrometry,[11-12] or NMR spectroscopy.[13] With a liquid chromatographic system, it is obvious that retention of host-guest complexes will be different from single guest molecules. Therefore, liquid chromatography appeared to be a satisfactory method to observe and to characterise cyclodextrin-guest inclusion complexes. Modification of retention properties of molecules, with different cyclodextrin concentrations in the mobile phase, was found to be related to the stoichiometry and the stability of inclusion complexes thus formed, as described by Fujimura.[14]

Data on retention modification, in RP-HPLC, with or without β-cyclodextrin (the most widely used cyclodextrin) in the mobile phase have already been published for monoaromatic compounds,[14] aromatic amines, polyaromatic hydrocarbons, nitrogen heterocycles, and aromatic hydroxyl compounds.[15-17] In the early 80's, a considerable review was published by W. L. Hinze[18] which described the wide usefulness of HPLC applications using mobile phase containing cyclodextrins. However, recently R. Nowakowski *et al.*[19] have demonstrated, in the light of thermodynamic chromatographic data and molecular modelling, that calculation of inclusion equilibrium constants was dependent on the stationary phase-solvent couple.

While essential oils 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.[20] Therefore, using RP-HPLC, we have investigated the influence of the solvent composition on the complexation of β-cyclodextrin with the following components of essential oils: thymol (aromatic alcohol), linalool, geraniol (aliphatic alcohol), and α-terpineol (alicyclic alcohol).

EXPERIMENTAL

Apparatus

The chromatographic system consisted of a Waters model 6000 pump (Waters corporation, Milford, MA, USA), a Rheodyne injection valve model 7510 (Rheodyne incorporated, Cotati, CA, USA) fitted with a 50µL sample loop, and a Shimadzu spectrophotometric detector (220nm) model Lp6A (Shimadzu corporation, Tokyo, Japan). A Merck (Merck, Paris, France) 250mm×4.6mm i.d., packed with Lichrospher RP18, 5µm particle size, was used. The temperature of the system was monitored at 26°C by a Colora Messtechnik cryostat model WK5 (Colora Mess-technik, Wurt, Germany).

Mobile Phase

β-cyclodextrin was supplied by Roquette (Roquette, Ilkirch, France) and was vacuum-dried at 110°C for 48h before use. HPLC grade methanol and ethanol were purchased from Prolabo (Prolabo, Vitry, France). Water was purified by double distillation. Mobile phases consisted of aqueous solutions and were prepared according to the following procedure. After fabrication of the desired methanol/water (v/v) or ethanol/water (v/v) mixture, an accurate weight of β-cyclodextrin was added to 250mL of this binary mixture in a 500mL volumetric flask. When total dissolution at ambient temperature was observed, the remaining amount of solvent was added to bring the volume to 500mL. Maximum quantity of β-cyclodextrin that could be dissolved in such binary mixtures was preliminarily determined and has been reported elsewhere.[21] Experiments were performed at 1 and 0.7mL/min for methanol/water (v/v) and ethanol/water (v/v) respectively. The void volume of the column was determined with different mobile phase compositions by injecting 10µL samples of copper sulphate solutions (0.01mg/mL) and was found to be 2.2 ± 0.1mL. Such procedure was carried out systematically twice daily during the entire experimental program.

Samples

Terpenes used: thymol, linalool, geraniol, and α-terpineol were purchased from Sigma (Sigma, St Louis, MO, USA). They were diluted prior to use in mobile phases containing no β-cyclodextrin, at a final concentration of 0.01mg/mL. Each injection was performed at least twice.

Data Acquisition

The detector signal was acquired using a system developed in the laboratory and described elsewhere.[22-23] The ASCII data were transferred via an RS232 protocol to an IBM 486 microcomputer.

Molecular Modelling

Molecular graphic calculations were carried out on a Silicon Graphics Indigo system (Silicon Graphics, Mountain View, CA, USA) using the molecular modelling package SYBYLL 6.10 from Tripos (Tripos, St Louis, MO, USA). Initial molecular co-ordinates of β-cyclodextrin were taken from the Cambridge Crystallographic Data Bank. Terpene molecules were constructed in the "building" mode;[24] minimised energy structures were used. Manual Monte Carlo docking was used to fit the inclusion system; Van der Waals and electrostatic interaction energies were compared. Both 1:1 and 2:2 host-guest complexes were used for calculations.

RESULTS AND DISCUSSION

Effects of Methanol Percentage and β-Cyclodextrin Concentration on the Retention of Terpene Derivatives in Methanol/Water Mobile Phases

In the absence of β-cyclodextrin in the mobile phase, retention of the four different essential oils (Terpenes) increased when the water percentage increased, as shown in Figure 1A. In the solvent composition range under study, linear relationships with correlation coefficients of 0.99 between the logarithm of the retention factor k and the water percentage in the mobile phase[25] were

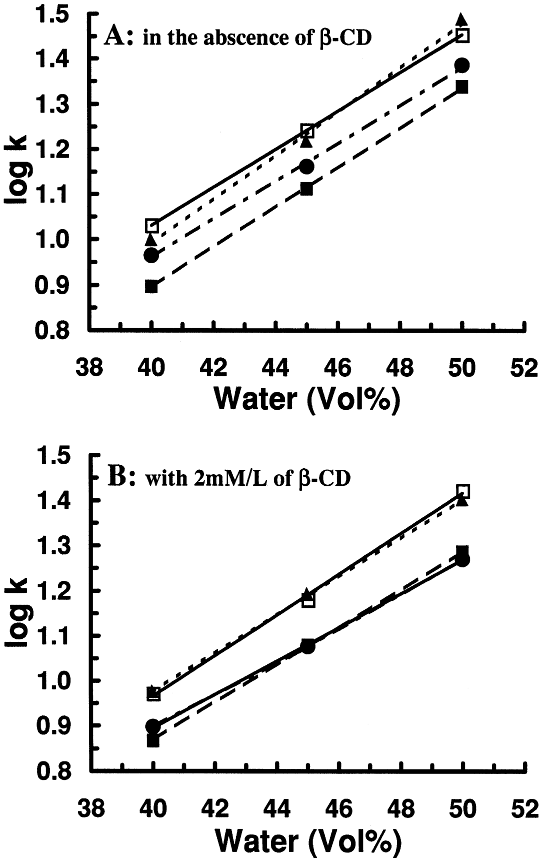


Figure 1. Correlation of the retention factor logarithm with the volumetric fraction of water in methanol/water mobile phases. Terpenes: □ linalool, ▲ geraniol, ● α-terpineol, ■ thymol. A: Without β-cyclodextrin in the mobile phase. B: With 2 mM/L β-cyclodextrin in the mobile phase.

observed and assessed the "reversed phase" elution mechanism.[26-27] β-Cyclodextrin, eluted in such a chromatographic system, was not retained indicating that negligible β-cyclodextrin-stationary phase interactions occurred. For an identical aqueous-methanol mobile phase containing β-cyclodextrin as mobile phase modifier at 2 mM/L, a linear relationship (R = 0.99) for log k of terpenes was also observed, as illustrated in Figure 1B.

Table 1

Retention Factor of Terpenes for Various Cyclodextrin Concentrations for Three Different Methanol /Water (V/V) Mobile Phases

A					
β-Cyclodextrin (mM):	**0**	**1**	**2**		**0**
Glucose (mM):	**0**	**0**	**0**		**21**
Thymol	7.89	7.72	7.36		7.91
α-Terpineol	9.22	8.46	7.89		9.22
Geraniol	10.72	9.88	9.45		10.73
Linalool	9.96	9.67	9.36		9.96

B					
β-Cyclodextrin (mM):	**0**	**1**	**2**	**3**	**0**
Glucose (mM):	**0**	**0**	**0**	**0**	**21**
Thymol	12.95	12.34	12.03	11.50	12.95
α-Terpineol	14.52	13.21	11.94	11.14	14.52
Geraniol	17.45	16.50	15.54	15.01	17.45
Linalool	16.50	15.89	15.14	14.88	16.50

C					
β-Cyclodextrin (mM):	**0**	**1**	**2**	**3**	**0**
Glucose (mM):	**0**	**0**	**0**	**0**	**21**
Thymol	21.77	20.25	19.31	18.10	21.75
α-Terpineol	24.30	22.78	18.30	16.90	24.32
Geraniol	28.37	26.79	24.75	23.63	28.37
Linalool	30.69	28.05	26.39	24.24	30.70

A: 60/40, B: 55/45, C: 50/50.

When Figures 1A and 1B were compared, different retention characteristics were evidenced. Both curves showed linear relationships between retention and water percentage whose origins have been already explained.[25,14] Such behaviour indicated, in both cases, that retention was essentially driven by a single mechanism involving hydrophobic interactions.[28] When comparing Figure 1A and Figure 1B, the systematic decrease in retention of terpenes in the presence of 2 mM/L β-cyclodextrin, suggested that β-cyclodextrin-terpene complexes formed were more hydrophilic than pure solutes, thereby the complexes were eluted faster. These results were analogous to those observed for polycyclic aromatic hydrocarbons (PAH).[29]

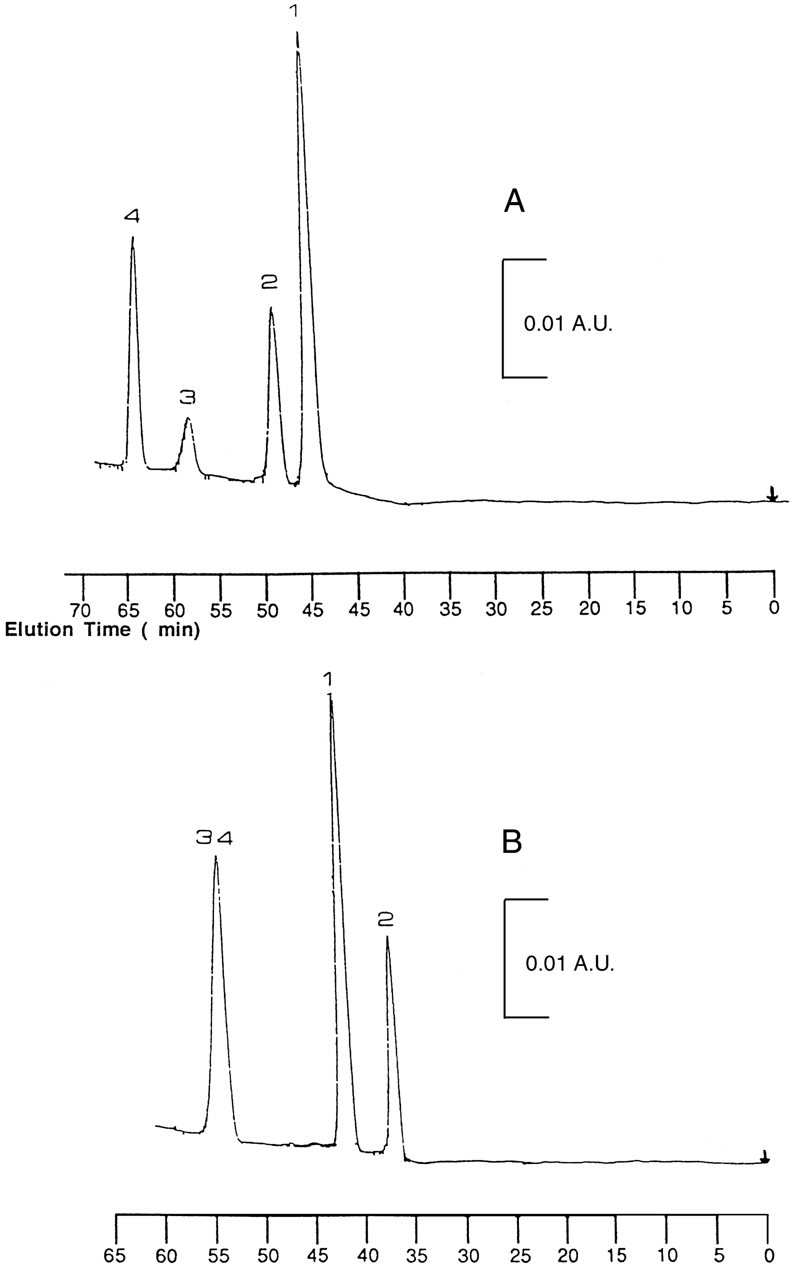
Retention factors of the four terpenes were therefore determined for different concentrations of β-cyclodextrin $[(CD)_T]$ in three different methanol/water mobile phases : 60/40, 55/45, 50/50 (v/v). Results are reported in Tables IA, IB and IC respectively. To eliminate the possible effect of β-cyclodextrin on the elution strength of the mobile phase, 21 mM/L of glucose were added to the water/methanol mobile phase and retention of the essential oils checked, as shown in Table 1. Since no retention modification of terpenes in the presence of glucose was observed, no glucose-terpene complexes were assumed to be formed. Therefore, with β-cyclodextrin, retention modifications can be attributed to the complex of the terpene trapped in the hydrophobic β-cyclodextrin cavity. This inclusion association will interact differently with the hydrocarbon moieties (C_{18}) of the stationary phase than with the terpene molecule alone. Thus, the inclusion complex formed reduces the hydrophobic area of the terpene considered, as illustrated in Table 1 by the decrease in retention of all terpenes for an increase of β-cyclodextrin concentration in the mobile phase.

It is assumed that inclusion complexes formation between guest solutes and the CD cavity is dependent upon the geometry, size, and physico-chemical properties of the included solute.[15] In Table 1, it can be observed that the extend of the decrease in the essential oil-β-cyclodextrin complex retention is highly dependent on the solute structure. Modifications of the retention for α-terpineol are more significant than for geraniol, linalool, or thymol. Moreover, these retention modifications, when β-cyclodextrin was added to the mobile phase, led to an inversion of the retention order, as observed by H. Lamparczyck *et al.*[29] for PAH. Such inversion indicates that β-cyclodextrin-inclusion complex formation modifies mobile phase selectivity of terpenes.

As shown in Figures 2A and 2B, without β-cyclodextrin as mobile phase modifier, thymol was eluted before α-terpineol; the elution order was inverted when using 3mM of β-cyclodextrin in the mobile phase. When retention of linalool and geraniol were compared, it was observed that they were not separated in the presence of β-cyclodextrin whereas linalool was eluted before geraniol in a simple 50/50 (v/v) methanol/water mixture. When inclusion complex formation with β-cyclodextrin is possible, improvements in the separation process may be achieved in two ways : a decrease in the analysis time and a favourable change in the selectivity.

Stability and Stoichiometry of the Inclusion Compounds in Water/Methanol Mobile Phases

When inclusion complex is possible, modifications of the retention factor, with different concentrations of β-cyclodextrin in a given water/methanol


A
1
2
3
4
0.01 A.U.
70 65 60 55 50 45 45 40 35 30 25 20 15 10 5 0
Elution Time (min)
B
1
2
34
0.01 A.U.
65 60 55 50 45 40 35 30 25 20 15 10 5 0

mobile phase, can be related to the modification of the stability constant of the complex[14-15]. Such equations in reversed phase chromatography have been already described by Sybilska *et al*.[16-17] However, it should be remembered that the organic modifier can also form inclusion complexes with β-cyclodextrin.[30] Competition between solutes and solvent for inclusion reduces the concentration of cyclodextrin free to associate with terpenes $(CD)_M$.

$$(CD)_T = (CD)_M + (CD\text{-}O) \qquad (1)$$

where $(CD)_T$ and $(CD)_M$ are the total and the mobile phase β-cyclodextrin concentration, respectively , and (CD-O) the concentration of complex formed between CD and the organic component of the mobile phase.[15] The effective concentration $(CD)_M$ should therefore be used to determine the stoichiometry and the stability of all the CD-terpene complexes formed. For all the molecules, and mobile phase compositions under study, a linear relationship was observed between 1/k and $(CD)_m$, as shown in Figure 3A. Correlation coefficients are in a 99% range, which confirmed that the stoichiometry of the complexes formed between cyclodextrin and the terpene derivatives were of 1:1 type. Using equations already described,[14-17,30] stability constants of the inclusion complexes were determined and illustrated in Figure 3B. An increase in the stability constants of all the molecules under study was observed when the percentage of water increased. Such results have been previously observed in spectrophotometric[31] and chromatographic[15-17,29] reports on the inclusion of aromatic and polyaromatic compounds in cyclodextrins.

The chromatographic results of this report show that this is also observed for alicyclic (α-terpineol) and even aliphatic (geraniol) molecules. The decrease in stability of inclusion complexes with increasing concentration of methanol in the mobile phase cannot be attributed to an enhanced competition of methanol and solute for cyclodextrin cavity, since the equation employed[30] uses the effective concentration, and not the total one. Therefore, these experimental results are interpreted using hydrophobic interactions, which are thought to play a key role in the inclusion process. The transfer of the molecule with a hydrophobic moiety, like terpenes, from the polar solvent to the hydrophobic β-cyclodextrin cavity produces a decrease in the free energy of solute-stationary phase association. It is all the more intense as the difference, in terms of polarity, is greater between the two environments (cyclodextrin cavity and solvent).

Figure 2 (left). Elution order with a 50/50 (v/v) methanol/water mobile phase. (1) Thymol, (2) α-Terpineol, (3) Linalool, (4) Geraniol. A: Without β-cyclodextrin in the mobile phase. B: With 3 mM/L β-cyclodextrin in the mobile phase.

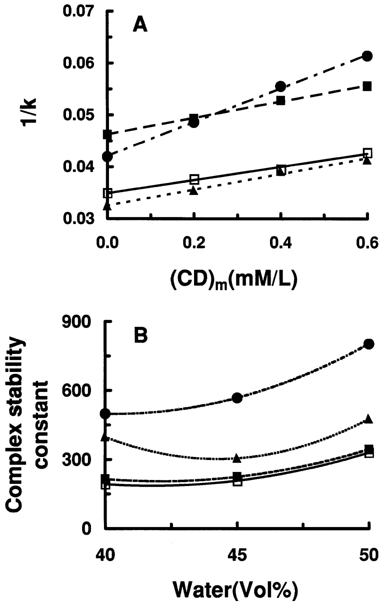


Figure 3. A: Reciprocal of retention factor k vs. effective β-cyclodextrin concentration $(CD)_m$ with a 50/50 (v/v) methanol/water mobile phase. B: Relationship between the complex stability constant and the volumetric fraction of water in methanol/water mobile phases. For key to symbols refer to Figure 1.

Stability and Stoichiometry of the Inclusion Compounds in Water/Ethanol Mobile Phases

Water/ethanol mobile phases were investigated because ethanol forms a stronger complex with β-cyclodextrin than methanol does. The formation constant of ethanol-β-cyclodextrin inclusion complex is three times as great (0.93 M^{-1} for ethanol and 0.32 M^{-1} for methanol) as for methanolic complex.[30] Therefore, it was necessary to solubilise higher concentrations of cyclodextrin.[21] Retention properties of the different terpenes were characterised with three different mobile phases of 50/50, 60/40, 70/30 water/ethanol (v/v) mixtures. With ethanol/water mobile phases, an increase in the organic modifier percentage caused a decrease in the retention factor of all terpene derivatives studied. As observed on Figure 4A, the decrease in retention of all terpene solutes with the increase of total β-cyclodextrin concentration $(CD)_T$ implicates the formation of soluble inclusion complexes, which were more hydrophilic than pure terpenes. A linear relationship between 1/k and the effective concentration of β-cyclodextrin $(CD)_M$ was observed for each solute ($R > 0.94$), as shown in

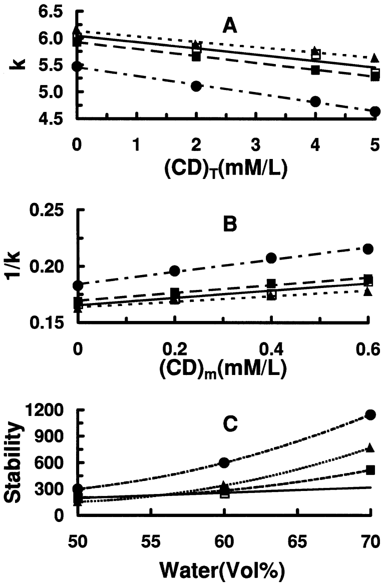


Figure 4. A: Effect of total cyclodextrin concentration $(CD)_T$ on terpenes retention with a 50/50 (v/v) ethanol/water mobile phase. B: Reciprocal of retention factor k vs. effective β-cyclodextrin concentration $(CD)_m$ with a 50/50 (v/v) ethanol/water mobile phase. C: Relationship between the complex stability constant and the volumetric fraction of water in ethanol/water mobile phases. For key to symbols refer to Figure 1.

Figure 4B for a 50/50 (v/v) ethanol/water mobile phase. Such correlation coefficients indicated that inclusion complexes of 1:1 stoichiometry were formed. These results confirm previous chromatographic study[15] of polyaromatic inclusion compounds. Using the methodology described above, the stability constants of the inclusion complexes formed for ethanol/water mobile phases with β-cyclodextrin concentration equal to 3 mM were determined. Results are given in Figure 4C. In this Figure, the inclusion selectivity was modulated by the percentage of ethanol contained in the mobile mixture. This effect could be observed, especially, with thymol and geraniol where the change in the solvent mixture composition triggered in a complete inversion of the inclusion selectivity. In a 50/50 (v/v) mixture, the terpene-cyclodextrin complex appeared to be more stable with thymol than with geraniol, whereas in a 70/30 (v/v) water/ethanol mixture, the opposite was observed.

This variance of behaviour indicated the major role played by the mobile phase organic modifier in the inclusion process. By comparing the stability constants of the inclusion complexes for methanol/water and ethanol/water with a same organic modifier percentage (50%), as observed in Figures 3B and 4C, it can be concluded that inclusion complexes formed in methanol/water mixtures were more stable than those in ethanol/water. It must be emphasised, that, in the light of a recent review in the field of RP-HPLC retention mechanism, restrictions must be expressed concerning the relative effect of organic co-solvents in the mobile phase. Tchapla *et al.*[32] described, qualitatively, the complicated effect of the solvent mixture on the stationary phase structure, which suggested a highly complex host-guest stationary phase structure. Furthermore, solute-stationary phase interactions are involved, as described by R. Tijssen.[33]

Molecular Modelling

The possibility of using molecular modelling to characterise the mechanism of the inclusion process is intriguing. However, complexity of processes involving: solvent-host, solvent-column, host-column, substrate-column, and substrate-host, make quantitative estimation models difficult to achieve. The probability of inclusion between cyclodextrins and the apolar stationary phase has been therefore suggested.[19] Such results strongly support the idea that, when cyclodextrin was added to the mobile phase, solutes and apolar stationary phases were in competition for inclusion. As of now, no satisfactory model exists for the structural modification of water in alcohol/water systems:[34] it renders a total theoretical analysis of the chromatographic system difficult,[35] due to huge calculation procedures.

Not many experimental works on chromatography and inclusion processes take into account molecular modelling to date,[36] few in HPLC[19]. However, some relevant information may be deduced from the treatment of closely analogous molecules within cavity, assuming that the driving factor in the inclusion process is the difference between host-guest interaction energies. In particular, Van der Waals and host-guest hydrogen bonding energies are involved, as previously suggested on the sole basis of chromatographic data by H. Lamparczyck *et al.*[29] Such molecular graphic procedures have already been applied successfully in the case of enantioselective complexation in gas chromatography.[36]

Two approaches can be set up for the analysis of CD inclusion compounds via molecular modelling. First, solvent molecules will be induced in a molecular dynamics calculation which should take into account solvent/co-solvent and solvent-guest reorganisation effects. As relevant models are not established so far, even for those studied in this report (water/methanol or water/ethanol),[37] to describe how terpenes may modulate the water structure, results obtained from molecular dynamic calculations should be treated with great caution. A second methodological approach is based on the comparison of

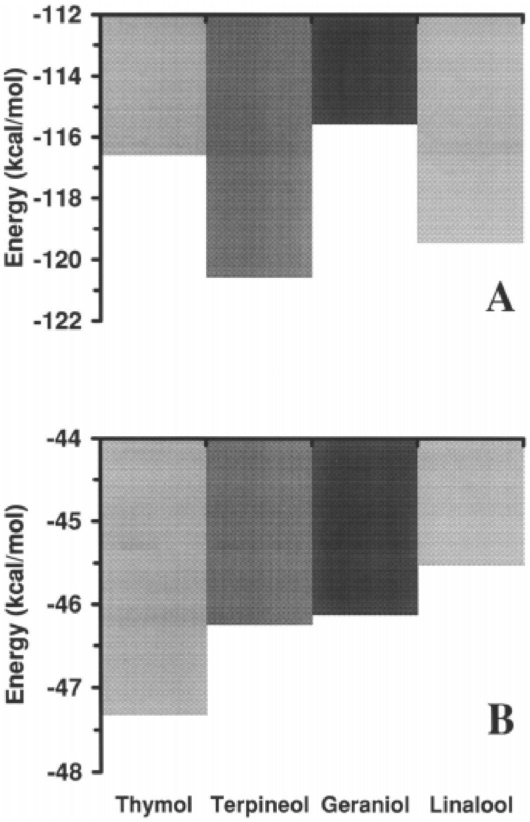


Figure 5. Van der Waals interaction energies for β-cyclodextrin-terpenes systems derived from modelling studies. A: For 2:2 systems. B: For 1:1 systems.

closely related systems. It may be assumed that the stoichiometry of inclusion compounds and the general structural geometry of the systems will be highly similar. Therefore, we may suppose that the inclusion complex solvent, solvent/co-solvent and guest/solvent interaction energies will not differ substantially and, in a first approximation, may be neglected. From this second approach, a comparison of Van der Waals interaction energies should give information on the host/guest configuration. Moreover, comparison of Van der Waals energies of the two possible host/guest complexes 1:1 or 2:2 with the experimentally derived empirical stoichiometry will suggest which of these is present in the chromatographic system. The second system 2:2 is the one found in the dimeric solid state inclusion complexes usual for β-cyclodextrin.[37] In Figure 5A and 5B were schematised calculated Van der Waals interaction energies for 2:2 and 1:1complexes, respectively.

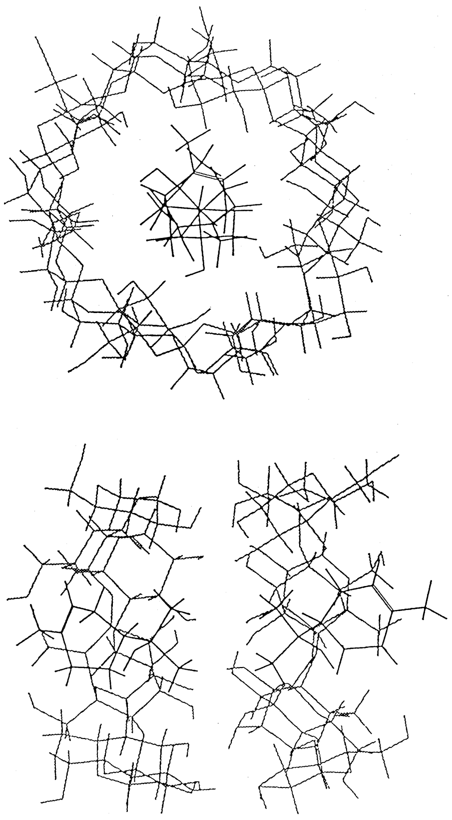


Figure 6. Molecular modelling derived structure for the 2:2 β-cyclodextrin α-terpineol system.

At first glance, it could be observed, for all solutes studied, that the energy of 1:1 complex was always greater than for the 2:2 one. Such results indicated that the four terpene/cyclodextrin complexes formed were in the shape of dimers in the solution. This concords also with information on aggregation of cyclodextrin systems.[38] Figure 6 shows possible molecular geometry of α-terpineol/β-cyclodextrin complex. As observed in Figure 5B, Van der Waals energies of complexes formed with thymol (-116 Kcal/mol), linalool (-119 Kcal/mol), geraniol (-116 Kcal/mol), and α-terpineol (-120 Kcal/mol) were similar and no clear differences can be made. Nevertheless, it was suggested that the molecular shape of solute molecules should be taken into account in the presence of the solvent.[29]

Given also the change in relative position of association constants with solvent composition, it would probably appear difficult to use the simplistic theoretical procedure previously described to get appropriate correlation with experimental chromatographic results. This approach can be valid one for large Van der Waals energy differences (>10Kcal/mol), but when slight differences are observed, the more subtle factor of solvent organisation must be taken into account.

CONCLUSION

Food or drug flavour analysis management is a complex situation. In the food industry, long term product storage may reduce product taste. As aromatization is performed using terpenes, the opportunity, demonstrated in this report, to protect terpenes by encapsulation in β-cyclodextrin internal cavity may be of some practical importance. The use of liquid chromatography to study inclusion complexation, when compared to other techniques, appears more versatile, particularly when the inclusion process does not modify spectrophotometric or electrochemical properties of the molecules studied. Given the wide variety of mobile or stationary phase selectivity and strength, it is possible to adapt elution conditions to gain interesting retention.

From a chromatographic point of view, selectivity and retention modifications by inclusion complexation are suitable tools for separation optimisation, in particular with the development of modified cyclodextrins. However, the complicated competing role of stationary phase with molecules able to be included in cyclodextrin cavity is a restricting factor for quantitative study. Molecular modelling interpretations are complicated by the presence of at least four components: cyclodextrin, the inclusion host, the stationary phase, and the solvent.

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REFERENCES

1. J. Szejtli, in **Cyclodextrin Technology**, Kluwer Academic Publishers, Dordrecht, 1990.

2. F. Schardinger, Zentrebl. Bakteriol. Parasitenkd., **129**, 188-214 (1911).

3. K. W. Jr. Street, J. Liq. Chromatogr., **10**, 655-662 (1987).

4. J. Sejtli, L. Szente, E. Banky-Elod, Acta Chim. Acad. Sci. Hung., **101**, 27-35 (1979).

5. S. Hamai, J. Phys. Chem., **93**, 2074-2078 (1989).

6. A. Bavari, J. Szejtli, J. Inclusion Phenom., **1**, 151-157 (1983).

7. J. W. Carr, J. M. Harris, Anal. Chem., **58**, 626-631 (1986).

8. G. Nelson, I. M. Warner, J. Phys. Chem., **94**, 576-581 (1990).

9. K. Uekama, F. Hirayama, S. Yamasaki, M. Otagiri, K. Ikeda, Chem. Lett., 1389-1395, (1977).

10. K. A. Connors, in **Binding Constants: a Measurement of Molecular Complex Stability,** J. Wiley and Sons, New York, 1987.

11. P. Cescutti, D. Garozzo, R. Rizzo, Carbohyd. Res., **290**, 105-115 (1996).

12. P. Cescutti, D. Garozzo, R. Rizzo, Carbohyd. Res., **302**, 1-6 (1997).

13. A. F. Casy, A. D. Mercer, Magn. Res. Chem., **26**, 765-774 (1988).

14. K. Fujimura, T. Ueda, M. Kitagawa, H. Takayanagi, T. Ando, Anal. Chem., **58**, 2668-2674 (1986).

15. R. M. Mohseni, R. J. Hurtubise, J. Chromatogr., **499**, 395-410 (1990).

16. D. Sybilska, J. Lipkowski, J. Woycikowski, J. Chomatogr., **253**, 95-100 (1982).

17. D. Sybilska, J. Debowski, J. Jurczak, J. Zukowski, J. Chromatogr., **286**, 163-170 (1984).

18. W. L. Hinze, Separ. Purif. Method, **10**,159-237 (1981).

19. R. Nowakowski, P. Cardot, A. W. Coleman, E. Villard, G. Guiochon, Anal. Chem., **67** 259-266, (1995).

20. Y. Ikeda, K. Matsumoto, T. Fuwa, K. Uekama, Yakug. Zasshi, **102**, 83-91 (1982).

21. A. K. Chatjikagis, C. Donze, A. W. Coleman, Ph. Cardot, Anal. Chem., **64**, 1632-1634 (1992).

22. J. P. Olivo, Ph. Cardot, I. Igniatiadis, C. Vidal-Madjar, J. Chromatogr., **395**, 383-393 (1987).

23. Ph. Cardot, Y. Trolliard, S. Tembely, E. Guernet-Nivaud, Chromatographia, **33**, 361-368 (1992).

24. U. R. Dharmawardana, S. D. Christian, E. E. Tucker, R. W. Taylor, J. F. Scamehorn, Langmuir, **9**, 2258-2263 (1993).

25. L. R. Snyder, J. W. Dolan, J. R. Gant, J. Chromatogr., **165**, 3-30 (1979).

26. C. Horvath, W. Melander, I. Molnar, J.Chromatogr., **125**, 129-156 (1976).

27. C. Horvath, W. Melander, J. Chromatogr. Sci., **15**, 393-404 (1977).

28. X. Pepin, L. Attali, C. Domrault, S. Gallet, J. M. Metreau, Y. Reault, P. J. P. Cardot, M. Imalalen, C. Dubernet, E. Soma, P. Couvreur, J. Chromatogr. B, **702**, 181-191 (1997).

29. H. Lamparczyk, P. Zazycki, R. S. Ochocka, D. Sibilska, Chromatographia, **30**, 91-97 (1990).

30. Y. Matsui, K. Mochida, Bull. Chem. Soc. Jpn, **52**, 2808-2814 (1979).

31. K. A. Connors, M. J. Mulski, A. Paulson, J. Org. Chem., **57**, 1794-1802 (1992).

32. A. Tchapla, S. Heron, E. Lesellier, H. Colin, J. Chromatogr., **656**, 81-112 (1993).

33. R. Tijssen, P. J. Shoenmakers, M. R. Bohmer, L. K. Koopal, H. A. Billiet, J. Chromatogr., **656**, 135-196 (1993).

34. F. Franks in **Water Science Reviews 1**, F. Franks ed., Cambridge University Press, Cambridge, 1987.

35. M. R. Schure, 19th International Symposium on Chromatography, Abstract 4.L.4., Sept 13-18, 1992.

36. F. Kobor, K. Angermund, G. Shomburg, J. High Resolut., **16**, 299-311 (1993).

37. G. Hebas in **Cyclodextrins and the Industrial Uses**, D. Duchène, ed., Editions de Santé, Paris, 1987.

38. A. W. Coleman, I. Nicolis, J. Incl. Phenom., **13**, 139-143 (1992).

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