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Separation ability and stoichiometry of cyclodextrin complexes

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Abstract

Gas–liquid chromatography has been applied to search relations between selectivity towards isomers and stoichiometry of cyclodextrin complexes. The model tested compounds were: dimethylnaphthalenes and α- and β-pinenes as constitutional isomers; *cis*/*trans* decalins, anetholes and isosafroles as diastereomers and as enantiomers (+/−)-α-pinenes and (+/−)-camphenes. Experimental retention data are used to confirm a simple theoretical model that allows distinguishing formation of G·CD complexes (1:1) and $G \cdot CD_2$ complexes (1:2). Based on the experimental data, stability constants *K* were evaluated. It has been found that remarkable selectivity factor α may appear both within the range of 1:1 stoichiometry (β-CD complexes of decalins and of α- and β-pinenes) and 1:2 stoichiometry (α-CD complexes with (+/−)-α-pinenes and (+/−)-camphenes). Occasionally selectivity arises from a different composition, when one isomer forms a 1:1 stoichiometry complex while another forms a 1:2 complex (dimethylnaphthalenes, *cis*/*trans*-anetholes and *cis*/*trans*-isosafroles).

Keywords: Stability complexes; Constitutional isomers; Diastereomers; Enantiomers; Cyclodextrins; Decalins; Anetholes; Isosafroles; α-Pinene

1. Introduction

The propensity of cyclodextrins (CDs) and their derivatives to form inclusion complexes with a variety of compounds of different chemical nature has been widely taken to advantage in many chromatographic techniques, mainly for analytical purposes. Various kinds of binding forces are involved in cyclodextrin complexation and among them geometric fitting plays an important role. In consequence, these processes are very frequently endowed with a great selectivity in regard to isomers. For that reason chromatographic systems modified with CDs seem to be the most universal tool for separation of isomers of different kinds: constitutional-isomers, diastereoisomers as well as enantiomers.

Occasionally the processes of complexation between cyclodextrin–host and guest molecules themselves may be traced by chromatographic studies. The results could be helpful in understanding the mechanisms of separation and in optimization of resolution processes in which CD complexation is involved. Moreover the chromatographic studies may sometimes consist of a valuable tool indicating subjects of interest for further structural investigations.

Gas chromatographic methods modified with cyclodextrins were used for elucidation of thermodynamic parameters of complexation [1,2] as well as for evaluation of the stability constants of cyclodextrin–guest complexes [3].

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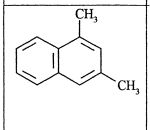
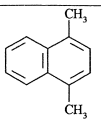
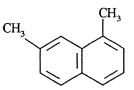
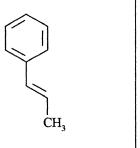
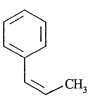
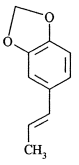
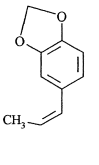
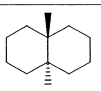
Table 1
The structural formulae of the investigated compounds.

1,3-DMN	1,4-DMN	1,7-DMN	1,8-DMN
			
Trans-anethole	Cis-anethole	Trans-isosafrole	Cis-isosafrole
			
(+)-α-pinene	(-)-α-pinene	(+)-camphene	(-)-camphene
			
(+)-β-pinene	(−)-β-pinene	Cis-decalin	Trans-decalin
			

In the present paper classical gas–liquid chromatography (GLC) has been applied to the study of stoichiometry of cyclodextrin complexes. The main goal was to answer the question when (if) the stoichiometry consists of a source of remarkable selectivity achieved in GLC. To our knowledge, no paper on this matter has been published till now although some papers referring to high-performance liquid chromatography and capillary electrophoresis studies of stoichiometry of CD complexes has already been published [4–7].

The model compounds tested exemplifying separation of various isomers were: as constitutional-dimethylnaphthalenes and α- and β-pinenes, as diastereomers — *cis*/*trans* anetholes, isosafroles and decalins and as enantiomers (+/−)-α-pinenes and (+/−)-camphenes.

The structural formulae of these compounds are shown in Table 1.

2. Theoretical considerations

In the GLC systems studied the stationary phases were a dilute solution of cyclodextrin (CD) in an achiral solvent (S). If a volatile substance G is eluted through the column, the process of partition in gas–liquid chromatography dealing without an addition of cyclodextrin is characterised by the process:

$$G_{(g)} \overset{K^0}{\Leftrightarrow} G_{(l)} \tag{1}$$

where K^0 is the coefficient of partition of solute G between gaseous (g) and liquid (l) phase.

$$K^0 = \frac{[G_{(l)}]}{[G_{(g)}]} \tag{2}$$

After addition of cyclodextrin to the stationary phase, we are dealing with an additional process of complexation of solute G with cyclodextrin CD. The process of complexation of solute G with *n* molecules of cyclodextrin depending on stoichiometry can be written as a set of the following reactions:

$$\begin{aligned} &G_{(l)} + CD \overset{K_1}{\Leftrightarrow} G \cdot CD \\ &G \cdot CD + CD \overset{K_2}{\Leftrightarrow} G \cdot CD_2 \\ &\vdots \\ &G \cdot CD_{n-1} + CD \overset{K_n}{\Leftrightarrow} G \cdot CD_n \\ \hline &G_{(l)} + n \cdot CD \overset{K_1 K_2 \ldots K_n}{\Leftrightarrow} G \cdot CD_n \end{aligned} \tag{3}$$

where $K_1K_2 \ldots K_n$ means the stability constant of complex of molecule G with *n* molecules of cyclodextrins.

$$K_1K_2 \ldots K_n = \prod_{i=1}^{n} K_i = \frac{[G \cdot CD_n]}{[G][CD]^n} \tag{4}$$

In this case including both processes (partition and complexation) the partition coefficient is equal to:

$$K = \frac{[G_{(l)}] + \sum_{n=1}^{N} [G \cdot CD_n]}{[G_{(g)}]} \tag{5}$$

After rearranging the final formula is:

$$K = K^0 \left(1 + \sum_{n=1}^{N} \prod_{i=1}^{n} K_i [CD]^n \right) \tag{6}$$

referring it to retention times the relation is as follows:

$$t'_r = t'^0_r \left(1 + \sum_{n=1}^{N} \prod_{i=1}^{n} K_i [CD]^n \right) \tag{7}$$

where t'^0_R and t'_R mean, respectively, the adjusted

retention times of solute G on the comparative matrix column (without cyclodextrin) and on the same twin column with addition of cyclodextrin.

Under the assumption that only complexes G·CD of 1:1 stoichiometry are formed the equations are reduced to the form [8]:

$$K = K^0(1 + K_1[\mathrm{CD}]) \quad (8)$$

where K_1 means the stability constant of complexes G·CD of 1:1 stoichiometry.

In this case the relation of retention time versus concentration of cyclodextrin is linear:

$$t_r = t_r^0(1 + K_1[CD]) \quad (9)$$

Taking into consideration the possibility of also forming complexes of 1:2 stoichiometry ($G \cdot CD_2$), the equations are as follows:

$$K = K^0(1 + K_1[\mathrm{CD}] + K_1K_2[\mathrm{CD}]^2) \quad (10)$$

where K_1 and K_1K_2 mean the stability constants of complexes G·CD and $G \cdot CD_2$ respectively.

In this case the parabolic relation of retention time versus concentration of cyclodextrin is noted:

$$t_r = t_r^0(1 + K_1[\mathrm{CD}] + K_1K_2[\mathrm{CD}]^2) \quad (11)$$

3. Experimental

3.1. Reagents

α-Cyclodextrin (α-CD), β-cyclodextrin (β-CD) were supplied by Chinoin (Budapest, Hungary). 1,3-, 1,4- 1,7- and 1,8-dimethylnaphthalenes, (+/−)α- and β-pinenes, (+/−)-camphenes, *cis*/*trans* isosafroles and *cis*/*trans* decalins were from Fluka (Buchs, Switzerland). *Cis*/*trans*-anetholes were from Roth (Karlsruhe, Germany). All other reagents and solvents were of analytical reagent grade and were used as received.

3.2. Apparatus and procedures

Gas chromatographic studies were performed using a Hewlett–Packard Model 5890 gas chromatograph equipped with a dual flame ionization detector. The peak areas and retention times were measured by means of a Hewlett–Packard 3390 A integrator.

Glass columns (2 m×4 mm I.D.) were packed with Chromosorb (60–80 mesh) coated with glycerol or formamide alone and glycerol or formamide solutions of α- or β-cyclodextrin of appropriate concentration. In the case of α-cyclodextrin, to improve its solubility, a small amount of lithium nitrate ($LiNO_3$) was added to the solution.

To simplify GC calculations — cyclodextrins concentrations have been expressed as molal solutions. The detailed information on cyclodextrins concentrations is shown in Figs. 1–8. Hold-up time t_M was measured from the methane peak.

4. Results and discussion

We have selected for this study a number of compounds for which very high selectivity has been observed on cyclodextrin phases. The structural formulae of the listed compounds are quoted in Table 1. At the first attempt, it has been assumed that just in the case of high selectivities, their relations with stoichiometry (strong or none) or with structural differences will be well pronounced and more easy to rationalize than those of low selectivities.

The examples of chromatograms presenting high selectivities of α- and β-cyclodextrins towards constitutional isomers, diastereomers and enantiomers are shown in Fig. 1.

5. Separation of isomers within 1:1 stoichiometry

5.1. Cis and trans decalins

Fig. 2 presents the values of retention times and separation factors of *cis* and *trans* decalins as the function of the β-CD concentration. It is seen that the course of relation t'_R vs. [β-CD] is approximately linear. The behaviour of separation factor $\alpha_{cis/trans}$ initially increasing with increasing β-CD concentration, but then asymptotically reaching a constant value above a certain [CD] concentration confirms this statement. Such relations indicate the formation of 1:1 stoichiometry complexes between both decalins and β-CD. Evaluated from these data [according to Eq. (9)] stability constants values are presented in Table 2.

In summary, one may conclude that β-CD in-

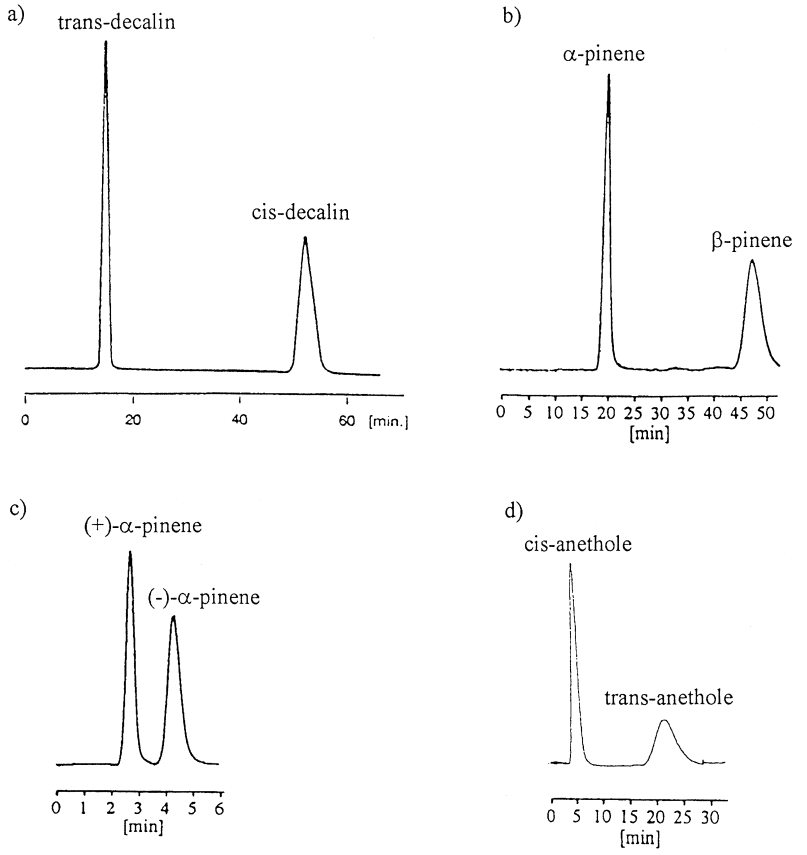


Fig. 1. Chromatograms of *cis* and *trans* decalins (a), α- and β-pinenes (b), (+)- and (−)-α-pinenes (c) and *cis* and *trans* anetholes (d). Chromatographic conditions: columns 2 m×4 mm I.D. filled with Chromosorb W 60/80 mesh coated with: 0.16 m solution of β-cyclodextrin in formamide, temp. 70°C, flow-rate 40 ml/min (a); 0.08 molal solution of β-cyclodextrin in formamide; temp. 40°C, flow-rate 40 ml/min (b), 0.09 molal solution of α-cyclodextrin in formamide, temp. 40°C, flow-rate 40 ml/min (c); 0.09 molal solution of α-cyclodextrin in glycerol; temp. 80°C, flow-rate 40 ml/min (d).

cludes weakly one molecule of *trans*-decalin while very strongly that of *cis* isomer. The difference in inclusion of isomeric decalins in β-CD cavity must be considerable. However till now gas–liquid chromatography investigations did not bring more information concerning structural differences between inclusion complexes.

5.2. α and β-Pinenes

Fig. 3 presents retention times and separation factor of constitutional isomers: α- and β-pinenes depending on β-CD concentration in formamide. Linear relation t'_R vs. [β-CD] has been observed and in consequence the separation factor α remains constant above a certain CD concentration. Alike to decalins this behaviour suggests the formation of 1:1 stoichiometry complexes. Stability constant values evaluated at 40°C in formamide medium using Eq. (9) are summarized in the Table 2.

A very high separation factor $\alpha_{\beta/\alpha}$ amounting to a value of 2 gives evidence of a great difference in the structures of β- and α-pinene β-CD complexes of 1:1 stoichiometry. It is worth mentioning that in contrast to α-CD, no enantioselectivity has been observed. It may exist but it is too small to be discovered by the not very efficient classical gas–liquid chromatography.

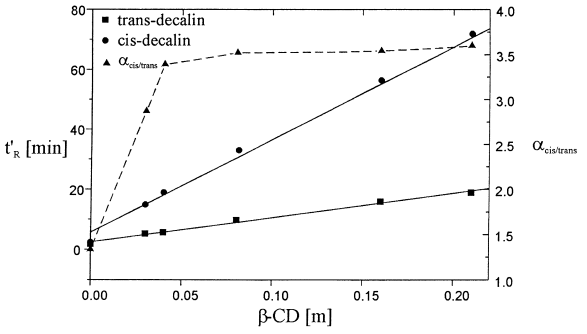


Fig. 2. Relation between adjusted retention times t'_R of *cis* and *trans* decalins (left axis) and separation factor $\alpha_{cis/trans}$ (right axis, dashed lined) versus β-CD concentration. Chromatographic conditions: columns 2 m×4 mm I.D. filled with Chromosorb W 60/80 mesh coated with β-CD solution in formamide; temp. 70°C, flow-rate 40 ml/min.

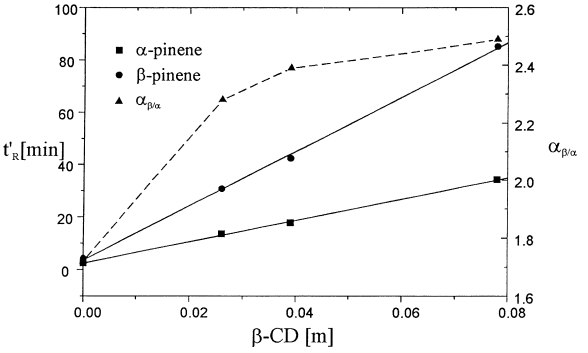


Fig. 3. Relation between adjusted retention times t'_R of α- and β-pinenes (left axis) and separation factor $\alpha_{\beta/\alpha}$ (right axis, dashed lined) versus β-CD concentration. Chromatographic conditions: columns as in Fig. 2, temp. 40°C, flow-rate 40 ml/min.

6. Separation of enantiomers within 1:2 stoichiometry

Figs. 4 and 5 present retention times and enantioseparation factors α of chiral α-pinenes and camphenes as the function of the α-CD concentration. All the presented relations t'_R vs. [α-CD] are parabolic and the enantioseparation factor does not remain constant. These findings suggest inclusion of one molecule of guest by two molecules of α-CD. As to our knowledge this is the first information giving evidence of $G \cdot CD_2$ complexes erasing from gas chromatographic data.

Evaluated stability constants of complexes of α-pinenes and camphenes with α-CD according to Eq. (11) are summarized in the Table 2. Enantioseparation factors reach considerably high values, very rarely in practice. Commonly observed enantioseparation factors are close to 1.0 and for this reason separation requires columns of high efficiency.

Under the conditions described in this paper

Table 2
The stability constants of complexes formed between cyclodextrins and studied compounds

Compound	CD, solvent, temperature	K_1 (M^{-1})	K_1K_2 (M^{-2})	K_2 (M^{-1})
cis-Decalin	β-CD, formamide, 70°C	128.0	–	–
trans-Decalin	β-CD, formamide, 70°C	45.0	–	–
α-Pinene	β-CD, formamide, 40°C	162.0	–	–
β-Pinene	β-CD, formamide, 40°C	241.0	–	–
(+)-α-Pinene	α-CD, formamide, 40°C	1.4	132.0	94.0
(−)-α-Pinene	α-CD, formamide, 40°C	2.8	320.0	114.0
(+)-Camphene	α-CD, formamide, 40°C	1.7	84.0	49.0
(−)-Camphene	α-CD, formamide, 40°C	4.3	256.0	60.0
1,3-DMIN	β-CD, glycerol, 80°C	10.0	–	–
1,4-DMIN	β-CD, glycerol, 80°C	10.0	–	–
1,7-DMIN	β-CD, glycerol, 80°C	8.0	–	–
1,8-DMN	β-CD, glycerol, 80°C	5.5	1832.0	333.0
cis-Anethole	α-CD, glycerol, 80°C	6.0	–	–
trans-Anethole	α-CD, glycerol, 80°C	1.3	255.0	196.0
cis-Isosafrole	α-CD, glycerol, 80°C	11.0	–	–
trans-Isosafrole	α-CD, glycerol, 80°C	2.0	232.0	116.0

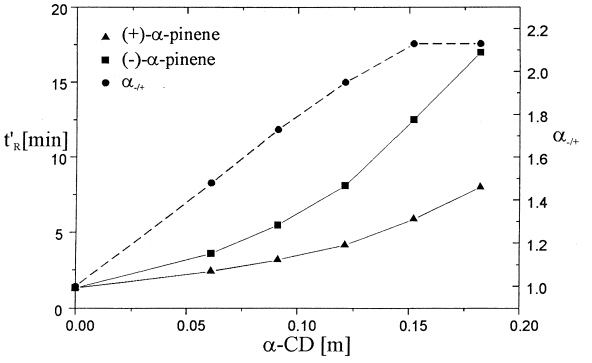


Fig. 4. Relation between adjusted retention times t'_R of (+)- and (−)-α-pinenes (left axis) and separation factor $\alpha_{-/+}$ (right axis, dashed lined) versus α-CD concentration. Chromatographic conditions: columns 2 m×4 mm I.D. filled with Chromosorb W 60/80 mesh coated with α-CD solution in formamide temp. 40°C, flow-rate 40 ml/min.

enantioseparation factor reaches the values amounting to 2÷3. Thus the baseline resolution could be achieved on classic short-packed gas–liquid columns of great capacity and moderate efficiency. In consequence the large sample amounts introduced on such columns enable micropreparative resolutions.

Numerous isomeric terpenoids behave similarly to α-pinene and camphene. They form with α-CD complexes of 1:2 stoichiometry and these processes are endowed with remarkable enantioselectivity.

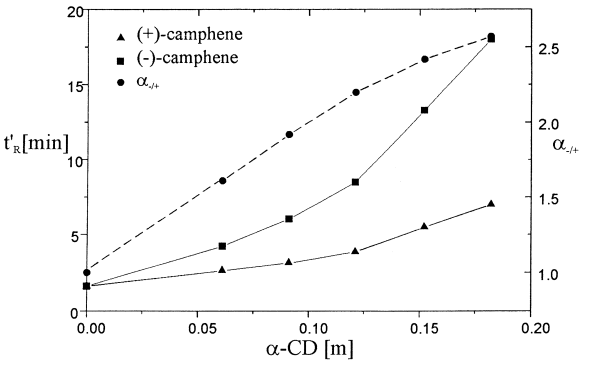


Fig. 5. Relation between adjusted retention times t'_R of (+)- and (−)-camphenes (left axis) and separation factor $\alpha_{-/+}$ (right axis, dashed lined) versus α-CD concentration. Chromatographic conditions as in Fig. 4.

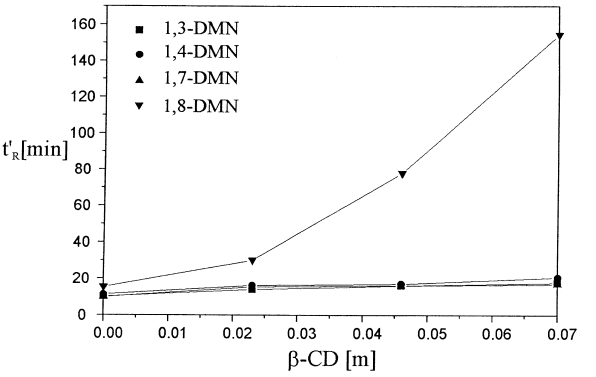


Fig. 6. Relation between adjusted retention times t'_R of 1,3-, 1,4-, 1,7- and 1,8-dimethylnaphthalenes versus β-CD concentration. Chromatographic conditions: columns 2 m×4 mm I.D. filled with Chromosorb W 60/80 mesh coated with β-CD solution in glycerol temp. 80°C, flow-rate 40 ml/min.

7. Separation of isomers due to difference in stoichiometry of CD complexes

7.1. Dimethylnaphthalenes (DMNS)

Fig. 6 shows the retention times of 1,8-, 1,4-, 1,3- and 1,7-dimethylnaphthalenes as the function of β-CD concentration. It is seen that the relations between retention times of 1,3-, 1,4- and 1,7-dimethylnaphthalenes and [β-CD] are linear and they grow very slowly. In contrast, retention times of 1,8-DMN grow fast and according to square function giving parabola. This indicates the formation of 1:2 stoichiometry complexes: 1,8-DMN·$(\beta\text{-CD})_2$. In consequence the separation factors of 1,3/1,4 and 1,3/1,7 are very close to 1, while separation factors 1,8/1,3, 1,8/1,4 and 1,8/1,7 are great and for this great value, the different stoichiometry of β-CD complexes with 1,8-DMN and other DMNs are responsible.

Evaluated stability constants of complexes formed between β-CD and dimethylnaphthalenes according to Eqs. (9) and (11) are presented in Table 2.

7.2. Cis and trans anetholes and isosafroles

Figs. 7 and 8 present the retention times and separation factor $\alpha_{trans/cis}$ of anetholes and isosafroles versus α-CD concentration. It is seen that

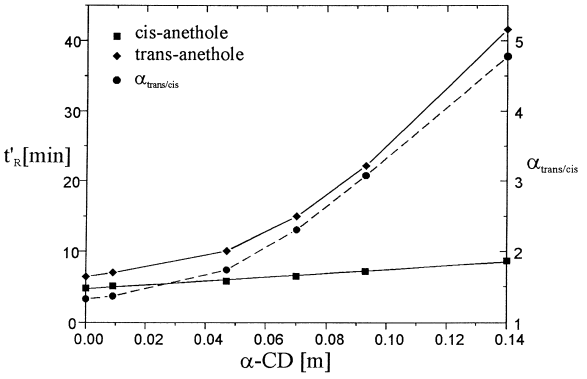


Fig. 7. Relation between adjusted retention times t'_R of *cis* and *trans* anetholes (left axis) and separation factor $\alpha_{trans/cis}$ (right axis, dashed lined) versus α-CD concentration. Chromatographic conditions: columns 2 m×4 mm I.D. filled with Chromosorb W 60/80 mesh coated with α-CD solution in glycerol, temp. 80°C, flow-rate 40 ml/min.

retention times of *cis* isomers are an approximately linear function of [α-CD] while the retention of *trans* gives parabola.

This time α grows with CD concentration according to a parabolic function. Evaluated from the data given in Figs. 6 and 7, stability constants values according to Eqs. (9) and (11) are presented in Table 2.

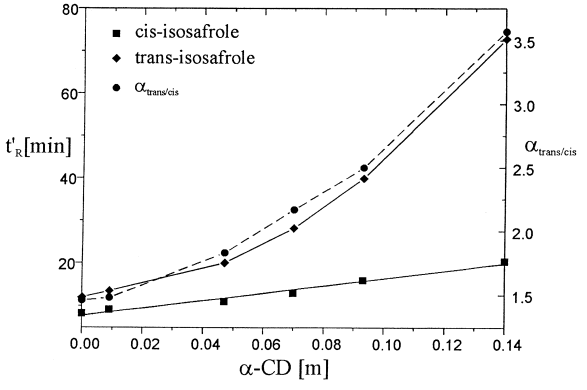


Fig. 8. Relation between adjusted retention times t'_R of *cis* and *trans* isosafroles (left axis) and separation factor $\alpha_{trans/cis}$ (right axis, dashed lined) versus α-CD concentration. Chromatographic conditions as in Fig. 7.

It is seen that the separation of DMNs, anetholes and isosafroles are due not only to structural differences but mainly to different compositions. The results presented above seem to make evident that not only liquid chromatography methods but also gas–liquid chromatography may be used to study the stoichiometry of cyclodextrin complexation. In column liquid chromatography, the most reliable data could be determined from the relation: $1/k$ versus concentration of cyclodextrin used as additive to the mobile phase solution [3].

In gas–liquid chromatography the relation between retention times and cyclodextrin concentration in stationary phase should be followed. Thus a very important problem is to obtain a series of columns filled with solid support covered with cyclodextrins solutions of various well-known concentrations. Under conditions of classical gas–liquid chromatography, this requirement could be relatively easy accomplished especially when involatile native cyclodextrins and essentially involatile solvent under appropriate temperature are used.

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