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SEPARATION OF ISOMERIC ALKYLBENZENES IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY THROUGH α- AND β-CYCLODEXTRIN INCLUSION COMPLEXES

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SUMMARY

The retention of various alkylbenzenes on a LiChrosorb RP-8 column was systematically studied were aqueous mobile phases containing α- or β-cyclodextrin and ethanol as an additional solvent. The model compounds tested were: ethylbenzene, *o*-, *m*- and *p*-xylenes, *o*-,*m*- and *p*-ethyltoluenes, 1,2,3-, 1,2,4- and 1,3,5-trimethylbenzenes, *n*- and iso-propylbenzenes. It was found that only β-cyclodextrin shows a distinct selectivity towards positional isomers on reversed-phase systems, enabling complete separation of both xylenes and ethyltoluenes. Two complexation stages are suggested to occur in the mobile phases containing β-cyclodextrin (solute:β-cyclodextrin = 1:1 and 1:2) in order to explain the relationship between the apparent capacity factors (k') and the β-cyclodextrin concentration.

INTRODUCTION

Two different approaches have been recently designed for the application of stereoselective processes of cyclodextrin (CD) inclusion in high-performance liquid chromatography (HPLC): the use of chemically bonded β-CD silica stationary phases[1–5] and the application of CD as the mobile phase components in reversed-phase systems[6–13].

The results obtained by the first method, especially those of recent studies performed with a commercially available, stable β-CD-bonded phase[3–5], demonstrate the great practical value of this sorbent and procedure. These studies dealt with numerous compounds, including solutes with marked hydrophobic properties, such as benzene, xylenes, anthracene and phenanthrene.

The studies dealing with the second approach concerned mainly benzene derivatives with polar functional groups, *e.g.* nitrobenzoic acids[6], nitrocinnamic acids[7], mandelic acids[8] and its derivatives[9,10], cresols and some disubstituted benzene derivatives[11], as well as some aromatic amino acids[12] and other compounds[13].

Our first attempts to adapt the second method, *i.e.* the RP systems modified by CD to the separation of hydrocarbons are reported in this paper. The tested model compounds were: ethylbenzene (EB);*o*-, *m*-, and *p*-xylenes (X); *o*-, *m*-, and *p*-ethyltoluenes (ET); 1,2,3-, 1,2,4-, and 1,3,5-trimethylbenzenes (TMB); and *n*- and *iso*-propylbenzene (PB).

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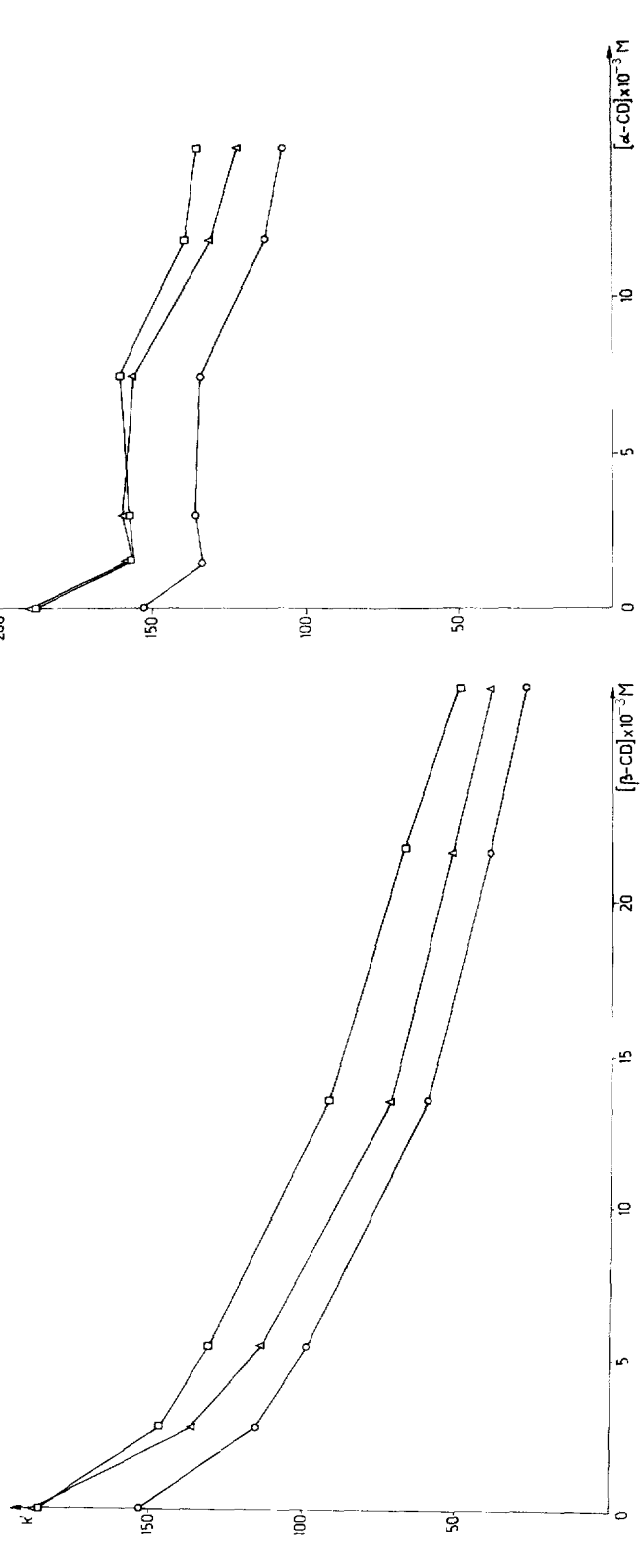


Fig. 1. Capacity factors (k') as a function of concentration of β-CD for xylenes. Data points: ○ = *ortho*; □ = *meta*; △ = *para*. Solvent composition, ethanol–water (20:80, v/v). Column, LiChrosorb RP-8.

Fig. 2. Capacity factors (k') as a function of concentration of α-CD for xylenes. Data points: ○ = *ortho*; □ = *meta*; △ = *para*). Solvent composition, ethanol–water (20:80, v/v). Column, LiChrosorb RP-8.

EXPERIMENTAL

Reagents

α-CD was supplied by Janssen Chimica (Beerse, Belgium) and β-CD by Chinoin (Budapest, Hungary). All other materials were of analytical or reagent grade and were used without further purification.

Apparatus and procedures

Chromatographic measurements were performed with a Model 302 apparatus (Institute of Physical Chemistry, Polish Academy of Sciences, Warsaw, Poland) equipped with a UV detector (254 nm) with Z-shaped passage (volume 8 μl). Samples were injected with a Rheodyne Model 7120 injection valve with a 5-μl sample loop. Stainless-steel columns (50 × 4.0 mm I.D.) were packed with 10-μm LiChrosorb RP-8 (Merck, Darmstadt, F.R.G.) by the slurry method.

Chromatographic measurements of k' values *versus* CD concentration were carried out at a constant flow-rate of 3.6 ml/min and constant temperature of 25 ± 0.1°C (maintained by a water jacket). The mobile phases consisted of aqueous solutions, containing 20 vol. % of ethanol (96%) and appropriate concentrations of α- or β-CD. The ethanolic solutions of solutes ($1.0 \cdot 10^{-3}$–$3.0 \cdot 10^{-3}$ M) were injected into the column both separately and as mixtures.

The stability constants of CD complexes, K, and the capacity factors, $k'_{G\cdot CD}$, of the compounds being investigated were evaluated by the least-squares method, using eqn. 1, which was derived earlier[7] on the assumption that CD complexes with a 1:1 stoichiometry are formed exclusively.

$$k' = \frac{k'_G - k'}{K[CD]_m} + k'_{G\cdot CD} \tag{1}$$

where G stands for guest molecule (solute), K is the stability constant of the (G · CD) complex, and k'_G and $k'_{G\cdot CD}$ are the capacity factors of the free guest molecule and its CD complex, respectively; $[CD]_m$ is the concentration of cyclodextrin in mobile phase solution.

RESULTS AND DISCUSSION

The main problem in the application of reversed-phase systems modified with CD to the separation of hydrophobic compounds was to find an appropriate water-miscible solvent, which would enhance the solubility of hydrocarbons in the aqueous mobile phases and diminish their adsorption on the stationary phase and would, at the same time, dissolve the CD, thus preserving the selective effects of CD complexation. After consideration of the results of preliminary studies and our experience in this field, we selected 20 vol. % ethanolic solutions. This composition of the solvent corresponds to the maximum for β-CD solubility, while it preserves comparatively well the selectivity due to β-CD complexation.

The influence of the α- and β-CD concentrations on the apparent capacity factors (k') is exemplified in Figs. 1 and 2 by the behaviour of xylenes. Similar effects were observed for the other compounds investigated. The addition of α- and β-CD

TABLE I

CAPACITY FACTORS (k') OF TESTED COMPOUNDS

Determined on an LiChrosorb RP-8 column at different β-CD concentrations in water–ethanol (80:20, v/v) and calculated from eqn. 2 $k'_{G\cdot CD}$, K and r values.

Compounds	k' *[β-CD](M)*						$k'_{G\cdot CD}$	K	r
	0.0	*0.0027*	*0.0054*	*0.0135*	*0.0216*	*0.0270*			
Ethylbenzene	179	125	102	61	43	31	1.8	155	0.988
o-Xylene	153	115	99	60	40	28	−7.7	107	0.971
m-Xylene	186	147	131	92	68	50	14.0	100	0.952
p-Xylene	187	136	114	72	53	40	7.8	140	0.985
o-Ethyltoluene	385	276	203	83	43	22	−111.0	106	0.996
m-Ethyltoluene	445	334	264	160	103	68	−51.0	106	0.997
p-Ethyltoluene	456	301	218	125	85	53	−17.7	182	0.996
1,2,3-Trimethylbenzene	348	266	172	55	21	12	−143.0	90	0.897
1,2,4-Trimethylbenzene	415	320	243	126	76	52	−113.0	85	0.991
1,3,5-Trimethylbenzene	485	472	451	385	348	280			
n-Propylbenzene	554	298	193	91	57	40	−23.3	299	0.999
*Iso*propylbenzene	459	221	137	62	39	27	−15.2	378	0.999

was followed by a decrease in the observed values of the capacity factors of the solutes. This suggests that the adsorption of CD complexes is less than that of corresponding free guest molecules

$$k'_G > k'_{G\cdot GD}$$

These effects are more marked for β-CD than α-CD, and the k' *versus* [β-CD] relation is more monotonous.

The values of apparent capacity factors of the compounds investigated, deter-

TABLE II

CAPACITY FACTORS (k') OF TESTED COMPOUNDS

Determined on LiChrosorb RP-8 column at different α-CD concentrations in water–ethanol (80:20, v/v)

Compounds	*[α-CD]M*					
	0.0	*0.003*	*0.006*	*0.015*	*0.024*	*0.030*
Ethylbenzene	179	152	152	146	121	114
o-Xylene	153	134	136	135	113	108
m-Xylene	186	156	158	161	140	136
p-Xylene	187	158	160	157	131	122
o-Ethyltoluene	385	353	370	330	325	269
m-Ethyltoluene	445	404	423	359	331	284
p-Ethyltoluene	456	412	426	320	247	209
1,2,3-Trimethylbenzene	348	331	325	325	288	258
1,2,4-Trimethylbenzene	415	398	404	393	353	322
1,3,5-Trimethylbenzene	485	472	455	438	387	344
n-Propylbenzene	554	522	466	370	299	263
*Iso*propylbenzene	459	427	393	376	329	311

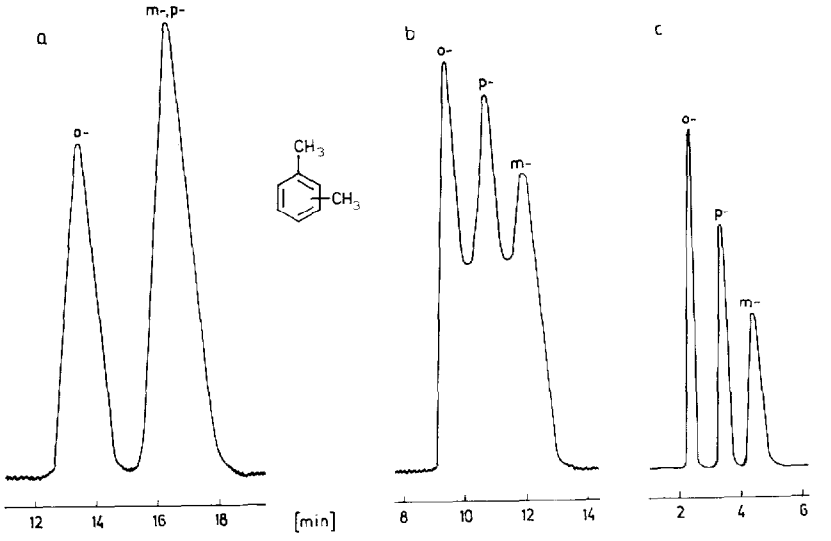


Fig. 3. Separation of xylenes (a) without CD, (b) with $3 \cdot 10^{-2}$ *M* α-CD, (c) with $2.7 \cdot 10^{-2}$ *M* β-CD. Conditions: column, LiChrosorb RP-8 10 μm, 50 × 4.0 mm I.D.; solvent composition, ethanol–water (20:80, v/v); flow-rate, 3.6 ml/min; temperature, 25 ± 1°C.

mined for α- and β-CD solutions of various concentrations, are collected in the Tables I and II. The last three columns in Table I give the values for the stability constants of β-CD complexes (K), their capacity factors ($k'_{G\text{-}\beta\text{-}CD}$) and regression coefficients (r), evaluated according eqn. 1.

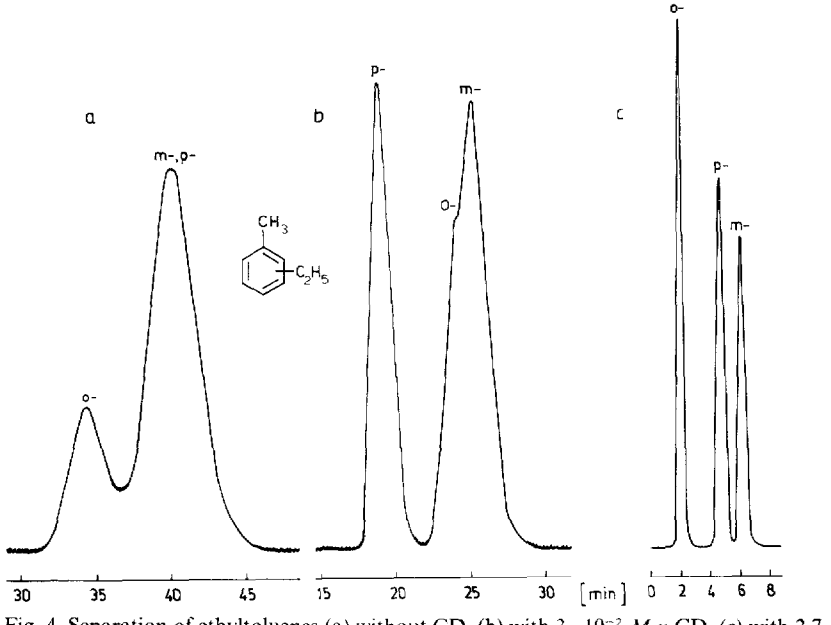
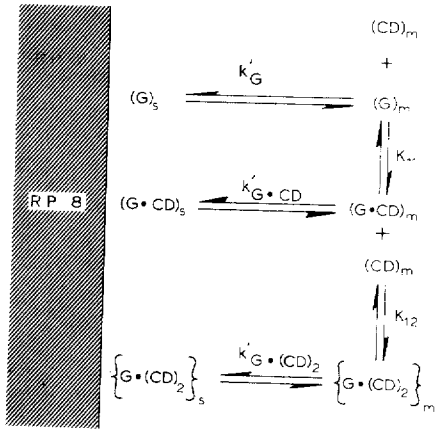


Fig. 4. Separation of ethyltoluenes (a) without CD, (b) with $3 \cdot 10^{-2}$ *M* α-CD, (c) with $2.7 \cdot 10^{-2}$ *M* β-CD. Conditions as in Fig. 3.

Chromatograms illustrating the separation of xylenes, ethyltoluenes, and trimethylbenzenes are shown in Figs. 3–5. They enable a comparison of the chromatographic properties and selectivities due to α- and β-CD complexation between these isomers. It should be stressed that only β-CD shows the remarkable selectivity towards positional isomers of alkylbenzenes on the reversed-phase system. The observed order of elution of isomers is identical for both xylenes and ethyltoluenes: *ortho*, *para* and then *meta*. The improvement in the resolution of positional isomers due to α-CD complexation is not so obvious.

Our results for the separation of xylenes in the RP system modified with β-CD are consistent with those obtained by Armstrong *et al.*[4], who used β-CD in the stationary phase; the sequence of stability constants of β-CD complexes, evaluated by both methods, is the same: $K_m < K_o < K_p$.

The calculated negative values for the capacity factors of β-CD complexes shown in Table I require some discussion. They may suggest that the first assumption of eqn. 1 (complex stoichiometry 1:1) is not entirely true. In the case of hydrophobic compounds such as alkylbenzenes, it is quite plausible that two complexation stages (1:1 and 1:2) occur in the polar mobile phase solutions. By introducing this additional complexation step, we obtain the following new scheme of equilibria in a reversed-phase system containing CD



where

$$K_{11} = \frac{[G \cdot CD]_m}{[CD]_m[G]_m}$$

and

$$K_{12} = \frac{[G \cdot (CD)_2]_m}{[G \cdot CD]_m[CD]_m}$$

and the subscripts m and s denote mobile and stationary phase, respectively.For this

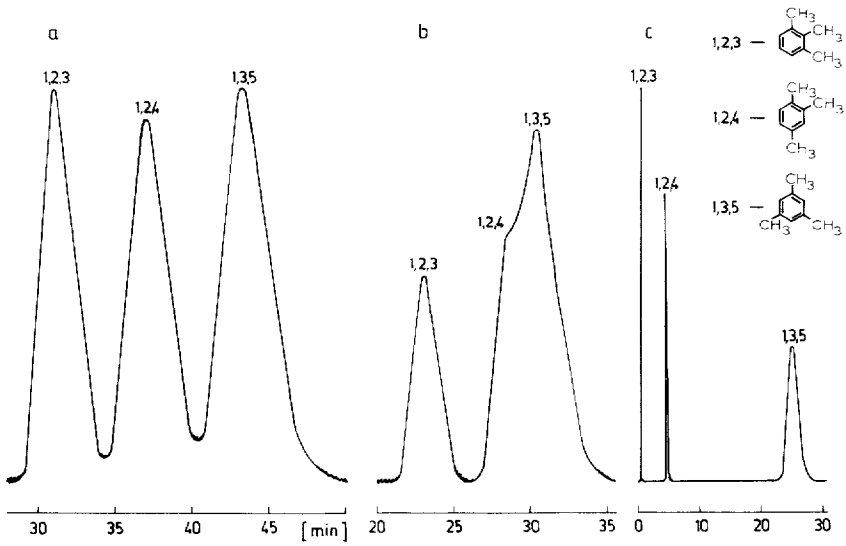


Fig. 5. Separations of trimethylbenzenes (a) without CD, (b) with $3 \cdot 10^{-2}$ *M* α-CD, (c) with $2.7 \cdot 10^{-2}$ *M* β-CD. Conditions as in Fig. 3.

scheme the apparent capacity factor, k', can be expressed as follows:

$$k = \frac{k'_G + k'_{G\cdot CD} K_{11}[CD]_m + k'_{G\cdot(CD)_2} K_{11} K_{12}[CD]_m^2}{1 + K_{11}[CD]_m + K_{11}K_{12}[CD]_m^2} \quad (2)$$

We have performed some preliminary calculations with assumed values of k'_G, $k'_{G\cdot CD}$, $k'_{G\cdot(CD)_2}$, K_{11} and K_{12} using eqn. 2 and compared the results with those from eqn. 1. This showed that negative values of k'_G can be obtained on the basis of eqn. 1 only if the second complexation step really occurs. Moreover, stability constant values calculated with eqn. 1 differ from K_{11} values obtained from eqn. 2 by no more than 10%. Therefore the simpler eqn. 1 can be considered adequate for approximating K_{11} values. Unfortunately, neither of these equations describes the observed k' *versus* [α-CD] relation. Further work on an accurate computation of all stability constants, K_{11} and K_{12}, and on a model of the chromatographic process with α-CD as a mobile phase component is in progress.

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REFERENCES

1 K. Fujimura, T. Ueda and T. Ando, *Anal. Chem.*, 55 (1983) 446.
2 Y. Kawaguchi, M. Tanaka, M. Nakae, Y. Mizobuchi and T. Shono, *Anal. Chem.*, 55 (1983) 1852.

3 D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.*, 22 (1984) 411.
4 D. W. Armstrong, W. DeMond, A. Alak, W. L. Hinze, T. E. Riehl and K. H. Bui, *Anal. Chem.*, 57 (1985) 234.
5 W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. DeMond, A. Alak and T. Ward, *Anal. Chem.*, 57 (1985) 237.
6 D. Sybilska, J. Lipkowski and J. Wóycikowski, *J. Chromatogr.*, 253 (1982) 95.
7 D. Sybilska, J. Dębowski, J. Jurczak and J. Żukowski, *J. Chromatogr.*, 286 (1984) 163.
8 J. Dębowski, D. Sybilska and J. Jurczak, *J. Chromatogr.*, 237 (1982) 303.
9 J. Dębowski, D. Sybilska and J. Jurczak, *Chromatographia*, 16 (1982) 198.
10 J. Dębowski, D. Sybilska and J. Jurczak, *J. Chromatogr.*, 282 (1983) 83.
11 J. Żukowski, D. Sybilska and J. Jurczak, *Anal. Chem.*, 57 (1985) 2215.
12 J. Dębowski, J. Jurczak, D. Sybilska and J. Żukowski, *J. Chromatogr.*, 329 (1985) 206.
13 Y. Nobuhara, S. Hirano and Y. Nakanishi, *J. Chromatogr.*, 258 (1983) 276.