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STUDY OF THE STEREOSPECIFIC PROPERTIES OF CYCLODEXTRINS AS GAS–SOLID CHROMATOGRAPHIC STATIONARY PHASES

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SUMMARY

A comparative study was carried out of the stereospecific properties of α-, β- and γ-cyclodextrins, using a set of monoalkyl-, dialkyl- and trialkylbenzenes. The relationship between the relative retention and the boiling points of the test substances were studied and, on this basis, the retention mechanism was discussed. The elution order of variously substituted aromatic hydrocarbons, affected by the steric arrangement of the substances and by the size of the cyclodextrin cavity, permits separations of complex mixtures, as demonstrated in the chromatograms given.

INTRODUCTION

Inclusion is a specific spatial interaction in which the molecule of a "guest", or at least part of it, is included in the cavity of the "host", so as to attain a state of minimal energy. In analytical chemistry, inclusion phenomena have found the widest use in separation methods, especially chromatography[1,2]. Increased attention has recently been focused on the formation of inclusion compounds of cyclodextrins (CDs)[3,4]. CDs and their derivatives have some advantages over other host substances, *e.g.* the ability to form complexes both in the solid state and in aqueous solutions[5]. Thus, CDs have been used both in liquid and gas chromatography. In the former method they have been used not only as polymer or chemically bonded stationary phases[6–11], but also as selective components of the mobile phase[12–15]. In the latter method they have been used as solid stationary phases in gas–solid chromatography (GSC)[16–20] and as components of the stationary liquids in gas–liquid chromatography (GLC)[20–22].

An increased interest in the use of CDs in modern chromatographic methods appeared in the eighties. In addition to important separations based on the selectivity of the inclusion process, chromatographic data can also be used to elucidate the mechanism and type of interaction underlying the inclusion process. GSC is suitable for the latter purpose, as the inclusion of the guest from the gaseous phase into a solid CD is unaffected by any solvent.

Our previous works[16–20] dealt with the formation of inclusion complexes of CDs with substances present in the gaseous phase and verified the existence of this process, which was previously known to exist primarily in solution. We pointed out

the possibilities of using the selective properties of CDs in chromatographic separations, in the preparation of well-defined gaseous mixtures with low concentrations of the components, and in preadsorption of traces of some pollutants in water[23].

At present, we are interested in comparing the stereoselective properties of α-, β- and γ-CDs with respect to various structural and positional isomers of some aromatic hydrocarbons and in separations of these substances, which are otherwise difficult to resolve.

EXPERIMENTAL

The α-, β- and γ-CDs used were obtained from Chinoin (Budapest, Hungary). The stationary phases were prepared by coating Chromosorb W (60–80 mesh) with a CD from a dimethylformamide solution. The detailed procedure can be found elsewhere[16,17]. The coverages used were 7–10%, ensuring complete coverage of the support with the CD.

The measurements were carried out on a CHROM 4 gas chromatograph with a flame ionization detector (Laboratorní Přístroje, Prague, Czechoslovakia), using 120-cm long glass columns, 2–3 mm I.D. Saturated vapours of the substances were injected with a Hamilton microsyringe. The mono-, di- and trisubstituted benzenes were of the highest purity available.

RESULTS AND DISCUSSION

In continuation of our previous research, the extent to which alkyl groups of various size and shape, substituted on the benzene ring, may suppress or enhance inclusion of the guest molecule in the β- and γ-CD cavities, were studied. The results were compared with the data obtained for α-CD. The experimental data are summarized in Table I.

With β-CD, all the monoalkylbenzenes are eluted in the order of their boiling points, except for *sec.*- and *tert.*-butylbenzene. The greater volume of the molecule of *sec.*-butylbenzene, caused by free rotation of the longer side-chain around C-1, seems to be the decisive factor, causing a weaker interaction with β-CD. The two isomers, differing by 4°C in their boiling points, can be separated on the basis of the different volumes of their substituents.

The somewhat increased retention of toluene and benzene can be explained by the inclusion of the whole molecules in the β-CD cavity, in agreement with the dimensions of the β-CD cavity (7.8–8.0 Å) and the length of these molecules (≈ 6.5 Å). The data obtained for monoalkyl derivatives suggest that the length of the chain, and to a certain extent also the degree of its branching, do not affect the inclusion process and that the same parts of the molecules participate in the interaction with the β-CD cavity.

A dependence on the steric properties is especially marked with disubstituted aromatics. It can be seen from Fig. 1, depicting the logarithmic dependence of the relative retentions on the boiling points of the test substances, that all the substances, except for the xylenes, are eluted in the order, *o-*, *m-* and *p-*isomer. With xylenes, the arrangement of the methyl groups in the *ortho* position does not prevent the benzene ring from at least partially penetrating into the β-CD cavity (Fig. 2, left). This fact

TABLE I

RETENTION DATA ON β- AND γ-CD STATIONARY PHASES AT 90°C

Guest compound	b.p. (°C)	β-CD		γ-CD	
		t'_R (s)	$\log r_{12}$	t'_R (s)	$\log r_{12}$
Benzene	80.1	14.9	0	5.1	0
Toluene	110.6	14.3	−0.016	15.1	0.470
Ethylbenzene	136.2	22.2	0.173	30.3	0.770
n-Propylbenzene	159.2	55.1	0.568	57.7	1.053
n-Butylbenzene	183.0	150.8	1.006	110.3	1.334
p-Xylene	138.4	22.0	0.169	24.0	0.672
m-Xylene	139.1	19.7	0.121	30.9	0.781
o-Xylene	144.4	24.1	0.209	79.5	1.192
Isopropylbenzene	152.4	41.9	0.450	52.1	1.009
Vinylbenzene	145.8	39.2	0.421	44.9	0.945
Allylbenzene	156.0	68.5	0.663	70.4	1.140
1,3,5-Trimethylbenzene	164.7	19.1	0.109	39.9	0.890
1,2,4-Trimethylbenzene	169.4	54.7	0.565	61.3	1.080
1,2,3-Trimethylbenzene	176.1	35.5	0.377	147.0	1.460
tert.-Butylbenzene	169.0	103.4	0.842	103.7	1.308
sec.-Butylbenzene	173.0	62.7	0.625	100.4	1.294
m-Ethyltoluene	161.3	30.9	0.317	45.7	0.952
p-Ethyltoluene	162.0	43.8	0.469	41.4	0.909
o-Ethyltoluene	165.2	29.8	0.301	91.7	1.255
m-Diethylbenzene	181.5	57.5	0.587	81.4	1.203
p-Diethylbenzene	183.8	142.8	0.982	79.9	1.195
o-Diethylbenzene	184.2	38.2	0.410	153.7	1.479
m-Cymene	176.0	76.1	0.709	−	−
p-Cymene	177.1	110.3	0.870	77.2	1.180
o-Cymene	178.2	37.5	0.401	139.5	1.438
m-Propyltoluene	182.0	81.6	0.739	89.0	1.242
p-Propyltoluene	183.0	119.6	0.905	95.2	1.271
o-Propyltoluene	185.0	66.5	0.650	155.8	1.485
Neopentylbenzene	186.0	180.9	1.085	315.9	1.792
m-Diisopropylbenzene	203.2	264.6	1.250	222.1	1.639
p-Diisopropylbenzene	210.4	642.1	1.635	277.0	1.735

has been verified by others[24], as well as by comparing a model of o-xylene with the β-CD cavity. More voluminous substituents cause steric hindrance and thus the o-isomers of higher homologues are eluted first. The highest stability of all the test p-dialkyl derivatives is caused by close contact of their molecules with β-CD (Fig. 2, right). The separation of o- and m-isomers depends considerably on the sizes of the two substituents; their retention behaviour is shown in Fig. 3. The differences in the relative retentions are rather small for ethyl- and propyltoluenes, but those for the isomers of cymene and diethylbenzene are large, caused mainly by weak interaction of the *ortho* isomers with the β-CD cavity. In all cases, the *para* isomers are retained the most.

On the basis of this concept of the formation of inclusion complexes, it is possible to explain why the elution order of the isomers studied is different from that on common stationary phases, on which the separation of the *para* isomer might be
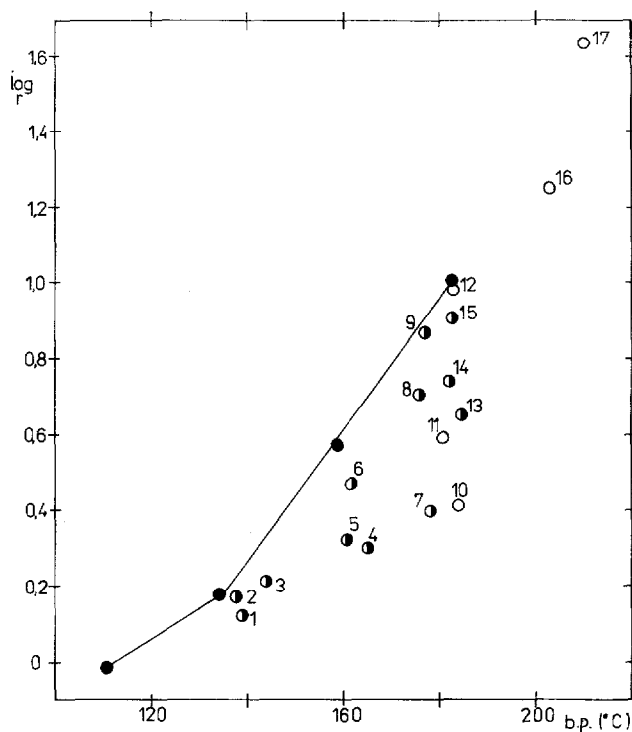
Fig. 1. Relation between log r_{12} on β-CD and the boiling point for alkyl- and dialkylbenzenes. (●) n-Alkylbenzenes: toluene, ethylbenzene, propylbenzene, butylbenzene. (1) m-Xylene, (2) p-xylene, (3) o-xylene, (4) o-ethyltoluene, (5) m-ethyltoluene, (6) p-ethyltoluene, (7) o-cymene, (8) m-cymene, (9) p-cymene, (10) o-diethyltoluene, (11) m-diethylbenzene, (12) p-diethylbenzene, (13) o-propyltoluene, (14) m-propyltoluene, (15) p-propyltoluene, (16) m-diisopropylbenzene, (17) p-diisopropylbenzene.

difficult and the *ortho* isomer is always eluted last. The selectivity of the inclusion process permits a very good separation of all three isomers, as demonstrated below in practical chromatograms.

The results of measurements with γ-CD and the same set of substances are different, owing to the larger dimensions of the γ-CD cavity. All alkylbenzenes can freely enter the γ-CD cavity and are not strongly retained, as demonstrated by the linear dependence of the logarithm of the relative retention on the boiling points for the homologous series of n-alkylbenzenes (Fig. 4). The separation of these substances is unaffected by inclusion and is controlled by interaction between the polar groups of the CD and the n-alkyl derivatives of benzene. An increased retention has been
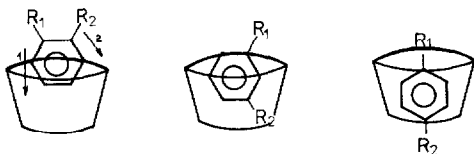

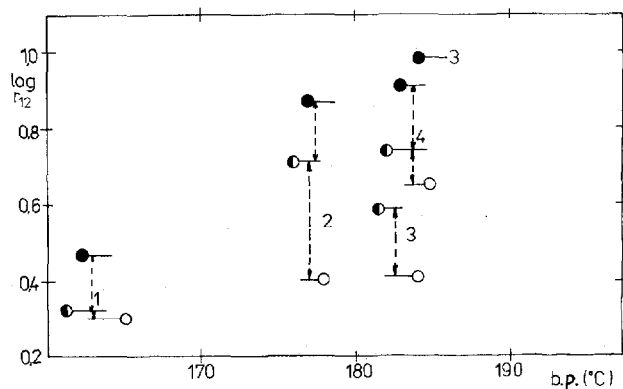Fig. 2. Orientation of dialkylbenzenes in the β-CD cavity.

Fig. 3. The steric effect of substituents on the retention of *ortho* and *meta* isomers of dialkylbenzenes on β-CD. (○) *Ortho* isomers, (◐) *meta* isomers, (●) *para* isomers. (1) Ethyltoluenes, (2) cymenes, (3) diethylbenzenes, (4) propyltoluenes.
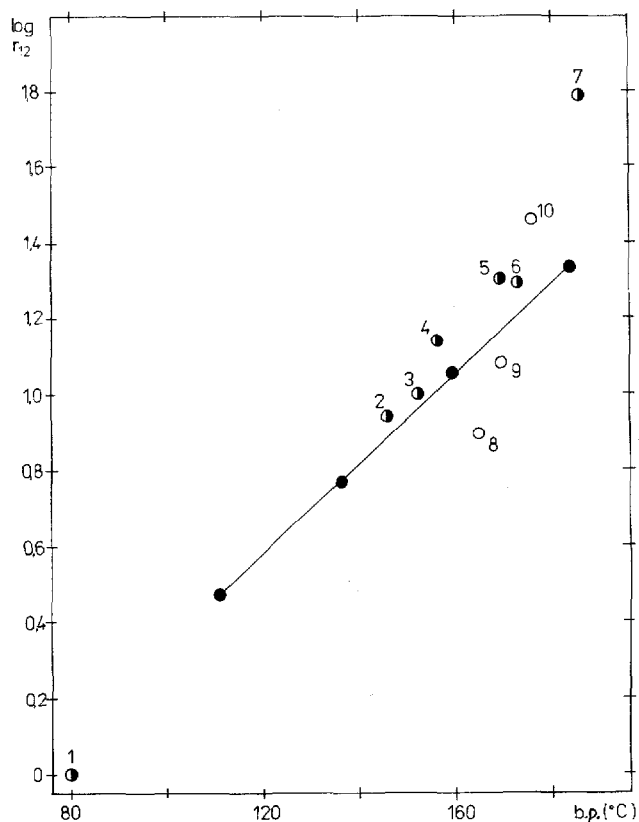

Fig. 4. Relation between log r_{12} on γ-CD and the boiling point. (●) Toluene, ethylbenzene, propylbenzene, butylbenzene. (1) Benzene, (2) vinylbenzene, (3) isopropylbenzene, (4) allylbenzene, (5) *tert.*-butylbenzene, (6) *sec.*-butylbenzene, (7) neopentylbenzene, (8) 1,3,5-trimethylbenzene, (9) 1,2,4-trimethylbenzene, (10) 1,2,3-trimethylbenzene.

found for *tert.*- and *sec.*-butylbenzene and also for neopentylbenzene. The stabiliz-
ation of the latter substance in the γ-CD cavity may partly be caused by hindrance
to free rotation of its voluminous side-chain. Rotation of the voluminous *tert.*-butyl
group may give rise to a spatial cloud with dimensions corresponding to the size of
the γ-CD cavity. This may explain the fact that neopentylbenzene is retained longer
by γ-CD than the approximately equally long molecule of *p*-diisopropylbenzene (the
b.p. of which is almost 25°C higher). The latter is more rigid because of the more
limited spatial arrangement of its substituents.

The different behaviour of *n*-alkylbenzenes and the corresponding *p*-disubsti-
tuted benzene derivatives can be explained analogously. It follows from Fig. 5 that
the relative retentions of all the *para* isomers are lower than those for the correspond-
ing *n*-alkylbenzenes, even if some boiling points are higher than those for *n*-alkyl-
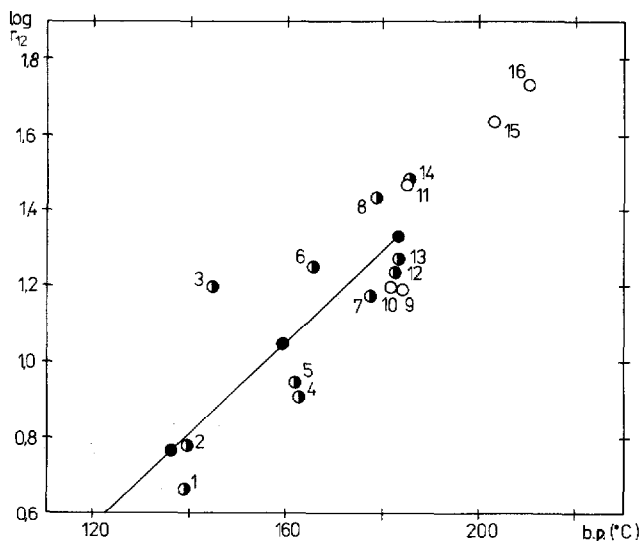benzenes with the same number of carbon atoms.

Fig. 5. Relation between log r_{12} on γ-CD and the boiling point of alkyl- and dialkylbenzenes. (●) Ethyl-
benzene, propylbenzene, butylbenzene. (1) *p*-Xylene, (2) *m*-xylene, (3) *o*-xylene, (4) *p*-ethyltoluene, (5)
m-ethyltoluene, (6) *o*-methyltoluene, (7) *p*-cymene, (8) *o*-cymene, (9) *p*-diethylbenzene, (10) *m*-diethylben-
zene, (11) *o*-diethylbenzene, (12) *m*-propyltoluene, (13) *p*-propyltoluene, (14) *o*-propyltoluene, (15) *m*-di-
isopropylbenzene, (16) *p*-diisopropylbenzene.

Among disubstituted alkylbenzenes, the *ortho* isomers are retained the most.
This is in agreement with their larger molecules coming into closer contact with the
γ-CD cavity. Weak interactions of the *meta* and *para* isomers are reflected in small
differences in their retention, so that these isomers cannot be separated, except for
xylenes and diisopropylbenzenes.

Among trimethylbenzenes (see Fig. 4), the 1,3,5-isomer is retained the least.
Similar to β-CD, a symmetrical distribution of the substituents prevents penetration,
even into the larger γ-CD cavity. In contrast to β-CD, the 1,2,3-arrangement of
methyl groups causes an increased retention on γ-CD, similar to *o*-dialkylbenzene.

Hence, the elution order is different from that with β-CD; the 1,3,5-isomer is retained the least and the 1,2,3-isomer is retained the most.

To form a general hypothesis of spatial interactions of aromatic compounds with α-, β- and γ-CD, the above results can be supported by other published data. Thermodynamic quantities for inclusion interactions of CDs with benzene[25] indicate that only part of the benzene molecule is included in the α-CD cavity and that the molecule is located close to the polar hydroxyl groups of α-CD. The close contact of the benzene molecule with the β-CD cavity is reflected in the values of the equilibrium constants for the complexes formed, which follow the order: $\beta \gg \alpha > \gamma$.

Using these data and the results obtained on application of Corey-Pauling-Koltun models[26], it can be concluded that the stability of the α-CD complexes with n-alkylbenzenes is given by the placement of the alkyl group inside the CD cavity.
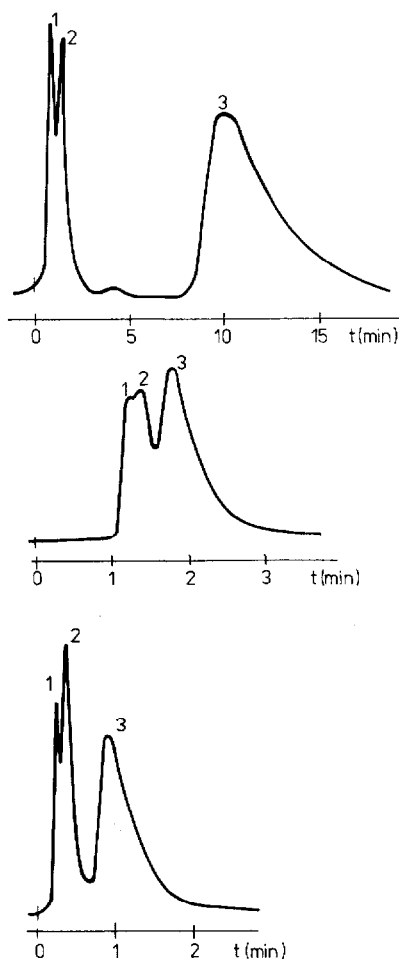

Fig. 6. Separation of xylenes on (from top to bottom) α-, β- and γ-CD. Carrier gas flow-rate, 35 ml/min; column temperature, 90°C. Elution order on α-CD: o-, m-, p-xylene; on β-CD: m-, p-, o-xylene; on γ-CD: p-, m-, o-xylene.

Branching of the side-chain gives rise to a substantial decrease in the retention. Similar results were obtained earlier in a chromatogrpahic study of the interaction of the corresponding alkanes with α-CD[16,17]. With disubstituted benzene derivatives, the typical order of the stabilities of the complexes is *ortho < meta < para* isomer. The highest stability of the complex of the *para* isomer is given by the fact that this molecule can closely fill the α-CD cavity with one of its substituents, probably that which is more hydrophobic. The steric hindrance caused by the arrangement of the substituents in the *meta* position partially prevents penetration into the α-CD cavity. The arrangement in the *ortho* position represents an even greater steric hindrance.

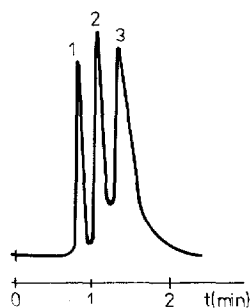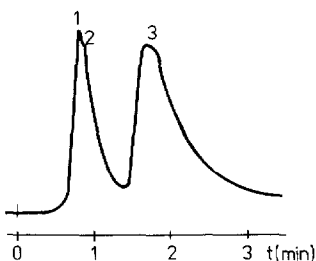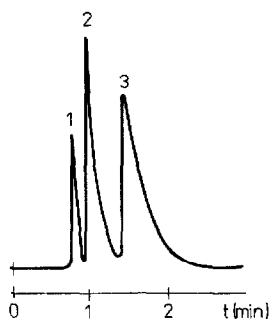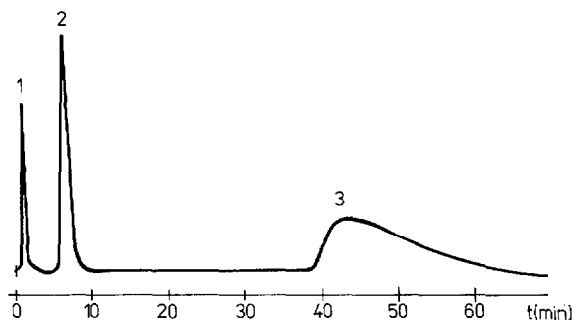The same elution order for dialkylbenzenes is mostly preserved with β-CD, but

Fig. 7. Separation of diethylbenzenes on (from top to bottom) α-, β- and γ-CD. Carrier gas flow-rate, 35 ml/min; column temperature, 90°C. Elution order on α- and β-CD: *o-*, *m-*, *p-*diethylbenzene; on γ-CD: *p-*, *m-*, *o-*diethylbenzene.

Fig. 8. Separation of cymenes on β-CD. Carrier gas flow-rate, 35 ml/min; column temperature, 90°C. Elution order: *o-*, *m-*, *p-*cymene.

the steric hindrances involved are smaller. Therefore, xylenes (as shown above) may be eluted in the order: *meta* < *para* < *ortho* isomer (see Fig. 6). In the group of dialkylbenzenes, the steric arrangement of the guest molecule affects the interaction with β-CD to a much greater extent, and branching or lengthening of the chain of one of the substituents by a single homologous increment leads to substantially greater differences in the retention of the isomers. The improved selectivity of β-CD toward more voluminous dialkylbenzenes permits a very good separation of all three isomers of diethylbenzene (*cf.* Fig. 7) or cymene (see Fig. 8).

The large cavity of γ-CD causes a deterioration in the separation of most dialkylbenzenes. Only xylenes are exceptional (see Fig. 6). On the other hand, trimethylbenzenes were resolved relatively well (Fig. 9).
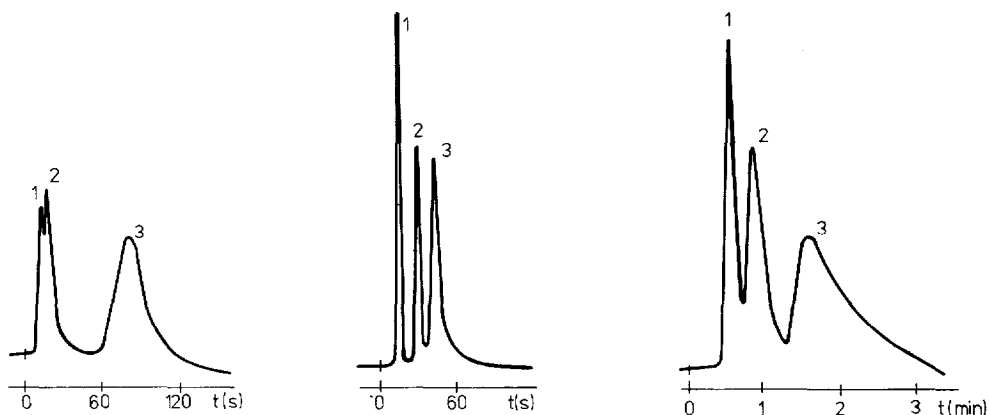

Fig. 9. Separation of trimethylbenzenes on (from left to right) α-, β- and γ-CD. Carrier gas flow-rates, 60 ml/min (α-CD), 35 ml/min (β- and γ-CD); column temperature, 90°C. Elution oreder on α- and β-CD: 1,3,5-, 1,2,3- and 1,2,4-trimethylbenzene; on γ-CD: 1,3,5-, 1,2,4- and 1,2,3-trimethylbenzene.
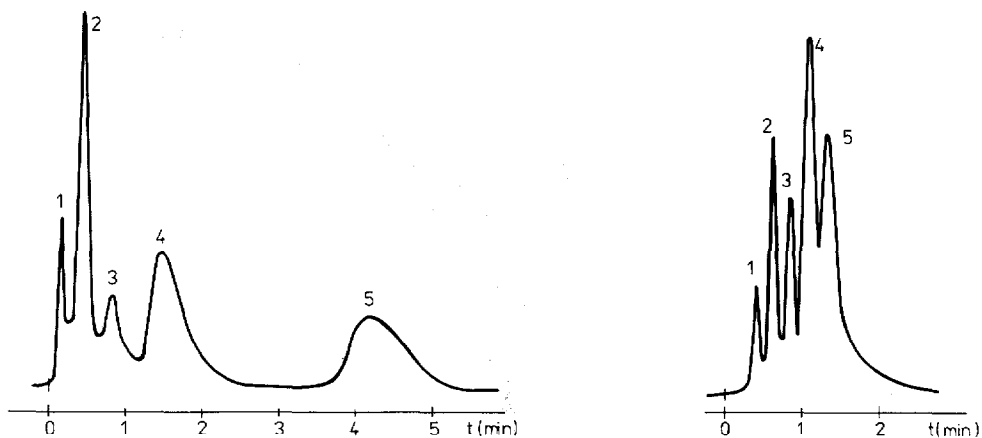

Fig. 10. Separation of monoalkylbenzenes on β-CD. Carrier gas flow-rate, 35 ml/min, column temperature, 90°C. (1) Ethylbenzene, (2) isopropylbenzene, (3) *sec.*-butylbenzene, (4) *tert.*-butylbenzene, (5) neopentylbenzene.

Fig. 11. Separation of monoalkylbenzenes on γ-CD. Carrier gas flow-rate, 35 ml/min, column temperature, 90°C. (1) Toluene, (2) ethylbenzene, (3) propylbenzene, (4) butylbenzene, (5) neopentylbenzene.

Both β- and γ-CD can be used as stationary phases for the separation of monoalkylbenzenes. β-CD permits the rapid separation of many alkylbenzenes, including *sec.-*, and *tert.*-butylbenzene, *sec.*-butylbenzene being eluted before the tertiary isomer, which has a lower boiling point (see Fig. 10). Fig. 11 depicts the separation of some monoalkylbenzenes on a γ-CD stationary phase, which also takes a relatively short time. The chromatogram shows increased selectivity toward the large molecules of neopentylbenzene and the complete separation of this substance from *n*-butylbenzene.

Therefore, chromatographic measurements allowed not only the formulation of certain conclusions on interactions operative in the GSC system with α-, β- and γ-CD, but also optimization of the conditions for analytical use of cyclodextrins as selective stationary phases. Another important step in this direction will be the preparation of reproducible capillary columns with CD stationary phases, which should yield good separation efficiency and selectivity.

ACKNOWLEDGEMENT

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