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COMPARATIVE STUDIES OF THE CHROMATOGRAPHIC PROPERTIES OF α - AND β -CYCLODEXTRIN IN GAS-SOLID AND GAS-LIQUID CHROMATOGRAPHY

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SUMMARY

A systematic study was carried out of the properties of α - and β -cyclodextrins as selective agents for the separation of various compounds, mainly isomers, in gas-liquid and gas-solid chromatographic systems. The test compounds included aliphatic and aromatic hydrocarbons (xylenes, trimethylbenzenes, diethylbenzenes) and alcohols (propanols, butanols). The possible interaction mechanism is discussed in terms of molecular inclusion, the effect of the nature of the matrix solvent on the chromatographic properties of the cyclodextrin solutions and the selectivity due to the inclusion process. Examples of chromatographic separations are given.

INTRODUCTION

The selective properties of cyclodextrins (CDs), based on inclusion processes, have been widely used in separation methods. Interesting results have also been obtained in gas chromatography¹⁻⁶. Three different stationary phases have been employed, namely, solid CDs^{1-3,6}, polyurethane resins containing CDs⁴ and β -CD solutions in formamide⁵.

The present work compares the chromatographic properties of α - and β -CDs as selective components of gas chromatographic stationary phases, in the form of the solids or dissolved in a suitable solvent matrix. The test substances comprised *n*-alkanes, cyclohexane, benzene, propanols, butanols, *o*-, *m*- and *p*-xylenes, ethylbenzene, *o*-, *m*- and *p*-diethylbenzenes and trimethylbenzenes used individually or as mixtures.

EXPERIMENTAL

α - and β -CDs were obtained from Chinoin (Budapest, Hungary). The stationary phases for the gas-solid chromatographic (GSC) measurements were prepared by coating Chromosorb W (60-80 mesh) with 10% (w/w) α -CD or β -CD, from a

dimethylformamide (DMF) solution. The DMF was removed at 95°C *in vacuo* and the phase was packed into a glass column (120 cm × 3 mm I.D.).

For the gas-liquid chromatographic (GLC) measurements, Celite (80-120 mesh) was coated with solutions of α - and β -CD in formamide (F) or ethylene glycol (EG), using 4.54 g of the solvent and 20 g of Celite. The amounts of α - and β -CD were 0.3, 0.6, 0.9 and 1.2 g. The detailed preparation procedure is described elsewhere⁵. Glass columns (200 cm × 4 mm I.D.) were used.

All the substances used were of p.a. purity or reagent grade and were not further purified.

The GLC measurements were performed on a Hewlett-Packard 7620 A chromatograph equipped with a flame ionization detector. The GSC measurements were carried out on a Hewlett-Packard 5700 A instrument and on a Chrom 41 instrument (Laboratorní Přístroje, Prague, Czechoslovakia), both equipped with a flame ionization detector. The samples were injected with Hamilton microsyringes. The GLC measurements were performed at temperatures from 50 to 70°C and the GSC measurements at 90°C and at the temperatures used in previous work¹.

RESULTS AND DISCUSSION

Samples of various structural types and geometries were selected for the measurements, falling into three groups.

Aliphatic hydrocarbons

The retention data obtained on α - and β -CD varied greatly both in GSC and GLC. As an example, the behaviour of *n*-hexane, cyclohexane and benzene can be given. In the GSC system the elution order on α -CD corresponds to the concept of inclusion, *i.e.*, cyclohexane (b.p. 80.7°C), benzene (b.p. 80.1°C) and *n*-hexane (b.p. 68.7°C), while the order on β -CD, hexane, cyclohexane, benzene, corresponds to an interaction with a polar stationary phase².

Interesting results have been obtained in the GLC system where the liquid matrix was varied in order to study the effect of the solvent. The solubility of CD is sufficiently high in both formamide and ethylene glycol, while the permittivities (dielectric constants) of the two solvents differ significantly. Fig. 1 illustrates comparative measurements with the two CDs in a formamide solution and in pure formamide, where it is seen that the separations on α - and β -CD are different. For example, α -CD forms no inclusion complexes with hexane, cyclohexane and only weakly includes benzene, while the retention of cyclohexane and benzene is pronounced on β -CD.

It follows from the separation factor and the adjusted retention times ($t_M = 35$ sec) which can be calculated from the examples given in Figs. 1 and 2 that the matrix affects both the capacity factor and the separation factor. Therefore, the stability of sorbates with CD constants must be dependent on the matrix; *e.g.*, β -CD in an EG solution seems to be more suitable for selective separations of benzene, cyclohexane and hexane than β -CD in a formamide solution.

In contrast to the GSC measurements, the elution order in GLC is always hexane, cyclohexane, benzene. It seems that the polarity of the solvent plays the predominant rôle, but the cavity dimensions also exert an effect with respect to the solvent used (see Fig. 2), where competitive sorption may occur.

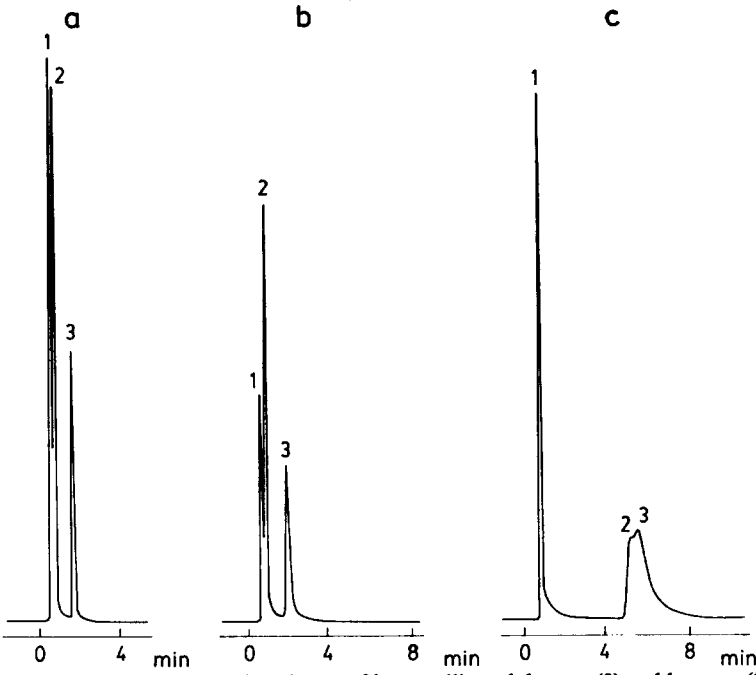


Fig. 1. Chromatograms of a mixture of hexane (1), cyclohexane (2) and benzene (3), obtained at 50°C on columns packed with 0.0 mole % (a), 1.24 mole % (b) of α -CD and 1.18 mole % (c) of β -CD in formamide.

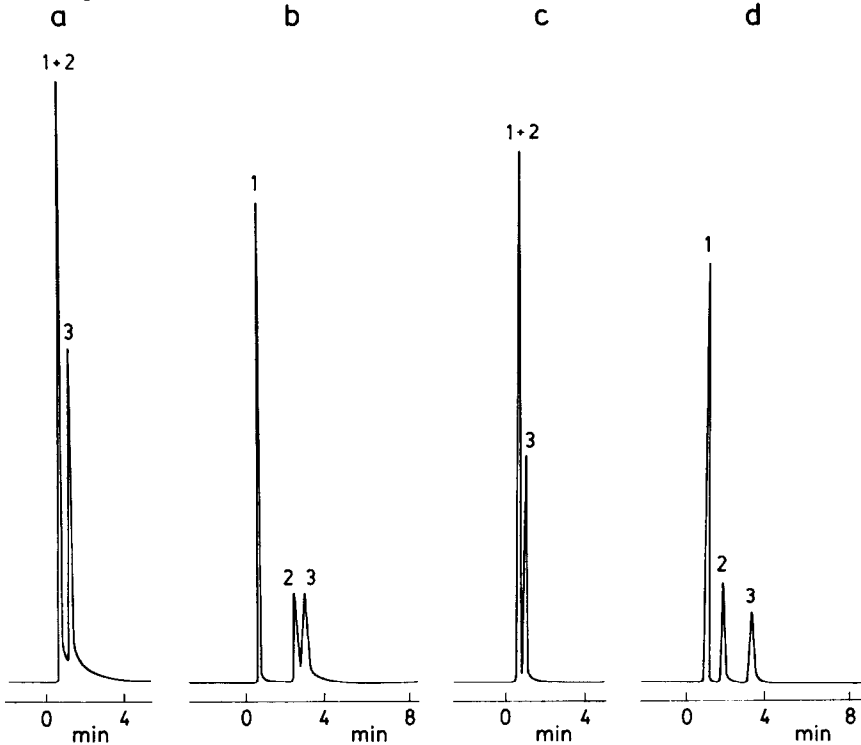


Fig. 2. Chromatograms of a mixture of hexane (1), cyclohexane (2) and benzene (3), obtained at 60°C on columns packed with 0.0 (a) and 0.30 mole % (b) of β -CD in formamide and 0.0 (c) and 0.36 mole % (d) in β -CD in ethylene glycol.

Alcohols

The elution order of isomeric propanols and butanols in GSC and GLC systems is determined by the boiling points of the substances, *i.e.*, isopropanol, *n*-propanol and *tert.*-butanol, *sec.*-butanol, isobutanol, *n*-butanol.

In GSC, stereoselectivity is not pronounced, in contrast to that observed for alkanes, and the retention is primarily determined by the interaction of the hydroxyl groups of the host and guest. The somewhat higher retentions obtained on α -CD can be explained by the interaction with the smaller α -CD cavity, leading to a closer contact and a partial effect of inclusion.

The GLC behaviour of α - and β -CD is different, in spite of yielding the same elution order. Different behaviours of the pure solvent and of CD solutions were again observed. Whereas isopropanol and *n*-propanol are eluted within 8 min in pure ethylene glycol, addition of β -CD increases the retention about four times and that of α -CD about three times. A similar situation is encountered with isomeric butanols (Fig. 3a, b), separated either in ethylene glycol or formamide solutions of β -CD. It can be seen that the inclusion process of butanols is more important in a solvent of a lower permittivity, *i.e.*, ethylene glycol.

Liquid stationary phases containing CDs are always more selective than the solvent itself. This effect can be explained in terms of a combination of processes involving the CD cavity, the solvent and the test substance. It seems that the most important of these is the replacement of solvent molecules included in the CD cavity by molecules of the chromatographed substances capable of forming inclusion complexes with CD, and their elution back into the mobile phase. From our experiments it is difficult to explain why this effect is more pronounced with β -CD than with α -CD.

Aromatic compounds

It is known that aromatic compounds readily form inclusion complexes with CD. Therefore, variously substituted benzenes were studied in the GSC and GLC systems.

The results of GSC measurement with monoalkylbenzenes, given in Table I, indicate strong retention of *n*-alkylbenzenes on α -CD. Branching of the alkyl chain substantially decreases the retention, similar to branching of alkanes. *tert.*-Butylbenzene has a substantially weaker retention on α -CD than benzene.

Methyl groups in the positions 1,2-, 1,3-, 1,2,3-, 1,2,4- and 1,3,5- on the benzene ring increase the molecular volume and thus cause a pronounced decrease in retention (see Table I); 1,3,5- and 1,2,3-trimethylbenzenes are eluted much more rapidly than benzene. This indicates that inclusion is the most important factors in the retention of benzene. The retention data obtained on β -CD again reflect the effect of the cavity size and are generally lower than on α -CD. With xylenes, even the retention order is different, *i.e.*, the retention order on α -CD is *p*-xylene > *m*-xylene > *o*-xylene, whereas *o*-xylene is the most strongly retained on β -CD.

An analogous study was carried out in the GLC system for diethylbenzenes (Fig. 4) and xylenes with ethylbenzene (Fig. 5), using α - and β -CD dissolved in formamide. With formamide alone, *p*-diethylbenzene is separated from the *o*- and *m*-isomers. On addition of α -CD, the separation properties of formamide completely disappear, while in the presence of β -CD all the three isomers are separated in the

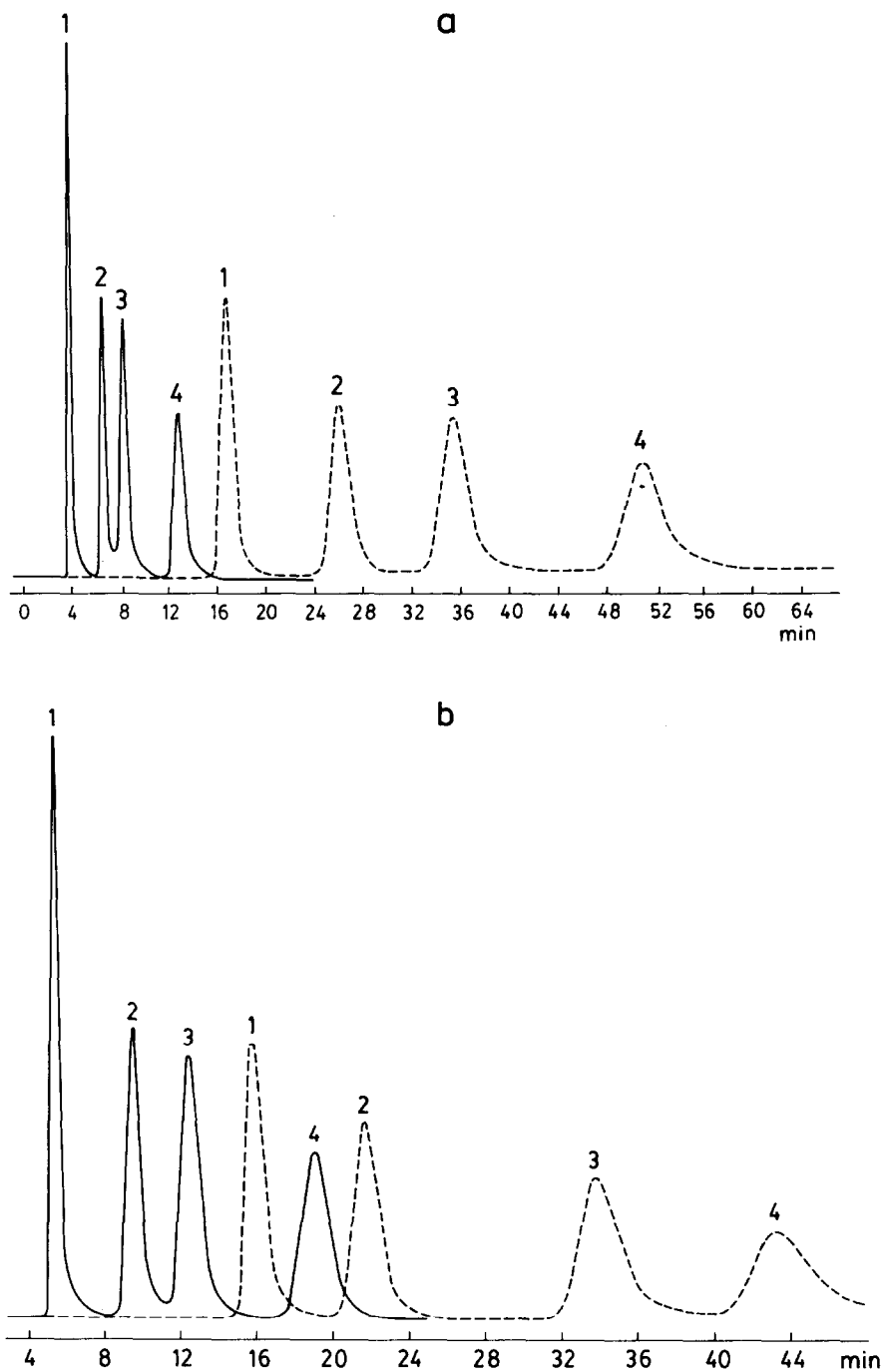


Fig. 3. Chromatograms of a mixture of C₄ alcohols, *tert.*-butanol (1), *sec.*-butanol (2), isobutanol (3) and *n*-butanol (4), obtained at 70°C. a, 0.0 (—) and 0.36 mole % (---) of β -CD in ethylene glycol; b, 0.0 (—) and 0.36 mole % (---) of β -CD in formamide.

TABLE I
RELATIVE RETENTION ($r_{1,2}$) OR AROMATIC HYDROCARBONS ON α - AND β -CD

Hydrocarbon	Boiling point (°C)	$r_{1,2}$	
		α -CD	β -CD
Benzene	80.1	1.00	1.00
Toluene	110.6	2.25	0.963
Ethylbenzene	136.2	8.64	1.50
Propylbenzene	159.2	18.5	3.11
Isopropylbenzene	152.4	1.03	2.54
Butylbenzene	183	—	6.11
<i>sec.</i> -Butylbenzene	173	0.942	4.00
<i>tert.</i> -Butylbenzene	169	0.122	5.56
<i>p</i> -Xylene	138.4	13.5	1.16
<i>m</i> -Xylene	139.1	1.52	0.935
<i>o</i> -Xylene	144.4	0.882	1.47
1,3,5-Trimethylbenzene	164.7	0.120	0.778
1,2,4-Trimethylbenzene	169.4	1.37	2.63
1,2,3-Trimethylbenzene	176.1	0.211	1.60

order m - > o - > p -. Addition of α -CD does not affect the separation of xylene isomers and ethylbenzene in formamide. On the other hand, addition of β -CD leads to a good separation of all the components, in the order m -, p -xylene, ethylbenzene, o -xylene.

As with alcohols, β -CD exhibits better separation properties in the GLC system, whereas in GSC the order of positional isomers follows the relationship of the

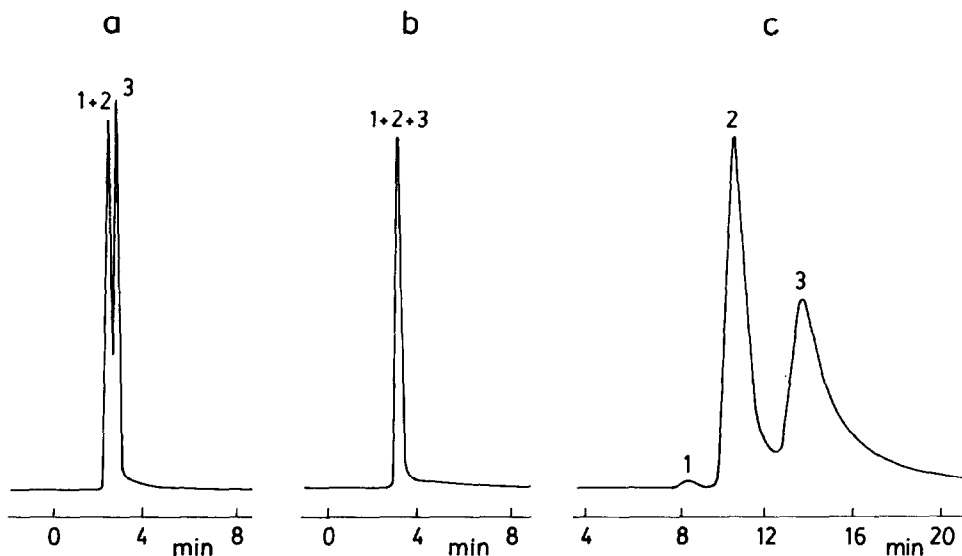


Fig. 4. Chromatograms of a mixture of o -(2), m -(1) and p -(3)-diethylbenzenes at 70°C on columns packed with 0.0 mole % (a), 1.24 mole % (b) of α -CD and 1.18 mole % (c) of β -CD in formamide.

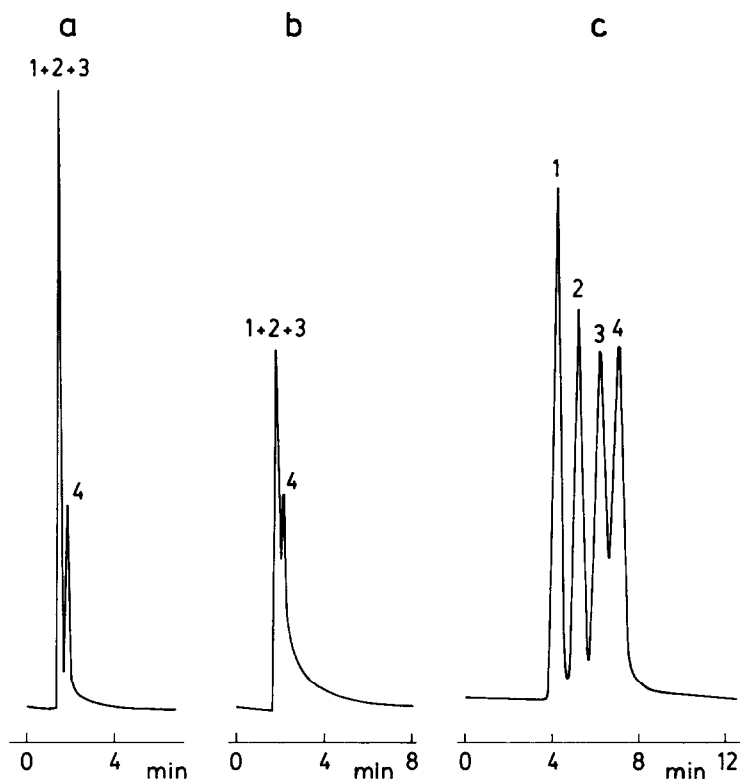


Fig. 5. Chromatograms of a mixture of *o*-(4), *m*-(1), *p*-(2)-xylenes and ethylbenzene (3) at 70°C on columns packed with 0.0 mole % (a), 1.24 mole % (b) of α -CD and 1.18 mole % (c) of β -CD in formamide.

molecular geometry to the cavity shape. For example, xylenes are more strongly retained on α -CD and are eluted in the order *o*-, *m*-, *p*-xylene. On β -CD, the elution order is changed to *m*-, *p*-, *o*-xylene (as in GLC), which can be explained by a larger β -CD cavity and differences in the vapour pressures. Again it is seen that in the three-component GLC system it is more difficult to explain the interaction mechanism and further experimental measurements are needed; this work is in progress.

CONCLUSIONS

The results obtained confirmed the occurrence of inclusion of molecules from the gaseous phase, both for the interaction of gaseous sorbates with solid CD and with a solution of CD. The GSC measurements are easier to interpret from the point of view of the interactions leading to separation, but are subject to well-known drawbacks, such as the non-linearity of the separation isotherm leading to variable retention data and sometimes also slow kinetics. On the other hand, the three-component system used in GLC is difficult to describe, but the advantages of a liquid stationary phase are retained and addition of CD significantly improves the selectivity. This system is thus more advantageous, especially in analytical applications.

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