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β-CYCLODEXTRIN AS A SELECTIVE AGENT FOR THE SEPARATION OF *o*-, *m*- AND *p*-XYLENE AND ETHYLBENZENE MIXTURES IN GAS–LIQUID CHROMATOGRAPHY

DANUTA SYBILSKA* and TOMASZ KOŚCIELSKI
Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44, Warsaw 01-224 (Poland)
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SUMMARY

The dependence of the elution patterns of *o*-, *m*- and *p*-xylenes and ethylbenzene on the β-cyclodextrin concentration in the formamide stationary phase deposited on Celite has been studied in the temperature range 50–70°C. It has been found that stereoselective molecular inclusion processes take place in the formamide medium, thus, modifying its chromatographic properties. Such systems result in a gain in stereoselectivity while at the same time retaining all the advantages of partition gas chromatography. The conditions for complete separation of the investigated compounds have been elaborated.

INTRODUCTION

The most remarkable property of cyclodextrins (CDs) is their great ability to form inclusion compounds with various molecules and ions. Attempts to make use of this phenomenon for the separation of various mixtures have mainly been by use of liquid chromatography[1,2]. There is little information concerning the behaviour of CDs in gas chromatography (GC). The retention data of various compounds were studied using stationary phases containing crystalline α- and β-CDs[3] and polyurethane incorporating CDs[4]. Under these conditions, molecular inclusion plays a significant rôle in the sorption process. The Japanese authors[4] proposed that CD-polyurethane resins be applied in analytical practice for the concentration of some aromatic compounds.

The aim of this work was to answer the question: can CDs be used to modify the resolution properties of liquid stationary phases in gas chromatography? In other words: can CDs be applied to the analytical separation of various compounds by partition GC?

The studies were performed using β-CD solutions in formamide; *o*-, *m*- and *p*-xylenes and ethylbenzene were chosen as the model compounds.

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EXPERIMENTAL

Reagents

β-CD was supplied by Chinoin (Budapest, Hungary). Celite (80–120 mesh) for gas chromatography was from BDH (Poole, Great Britain). Pure formamide was supplied by POCh (Gliwice, Poland). All materials were of analytical or reagent grade and were used without further purification.

Apparatus and procedures

Chromatographic studies were performed using a Hewlett-Packard 7620 A gas chromatograph equipped with dual flame ionization detectors. Glass columns (2 m × 4 mm I.D.) were used. In the experiments special care was taken to maintain constant the inlet pressure (2.75 atm) and helium flow velocity (50 ml/min).

The compounds were injected (0.2 μl) separately or as mixtures using Hamilton microsyringes. The retention time of each compound was determined as the mean value (relative error $<0.5\%$) from each of series of six injections.

The stationary phase was prepared as follows. An aqueous solution containing β-CD and formamide was deposited on Celite. The resulting slurry was shaken for about 10 min and then the excess of water was slowly evaporated at low pressure (20 mmHg) and at a temperature of 50°C. In all the experiments the quantities of formamide (4.54 g) and Celite (20 g) were constant. The amount of β-CD was varied: 0.3, 0.6, 0.9 or 1.2 g. The columns were weighed before and after packing to give the mass of coated support in the column; the mean value for all the columns was 12 ± 0.5 g.

In each case two columns were prepared: one with a given solution of β-CD in formamide and the second (reference) containing formamide alone. These two columns when placed in the chromatograph oven enabled performance comparisons under the same conditions, thus excluding many sources of error. The two flame ionization detectors were connected to two electrometers and could be operated with two columns at the same time. Each injection on the β-CD containing column was followed almost at once by the injection on the reference column.

The studies were carried out in the temperature range 50–70°C.

RESULTS AND DISCUSSION

The chromatograms presented in Fig. 1 show the dependence of the separation of a mixture of *o*-, *m*- and *p*-xylenes and ethylbenzene on the concentration of β-CD in the formamide solution used to coat the Celite column. Fig. 2 demonstrates the influence of the temperature on the separation process for the same mixtures. In these experiments the stationary phase comprised a 1.18 mol % solution of β-CD in formamide.

A comparison of the elution patterns in Fig. 1 leads to the conclusion that inclusion of the solutes in the β-CD cavity takes place in the formamide medium. For each species investigated, the adjusted retention times followed the relationship:

$$t'_R \text{ (β-CD, formamide)} > t'_R \text{ (formamide)}$$

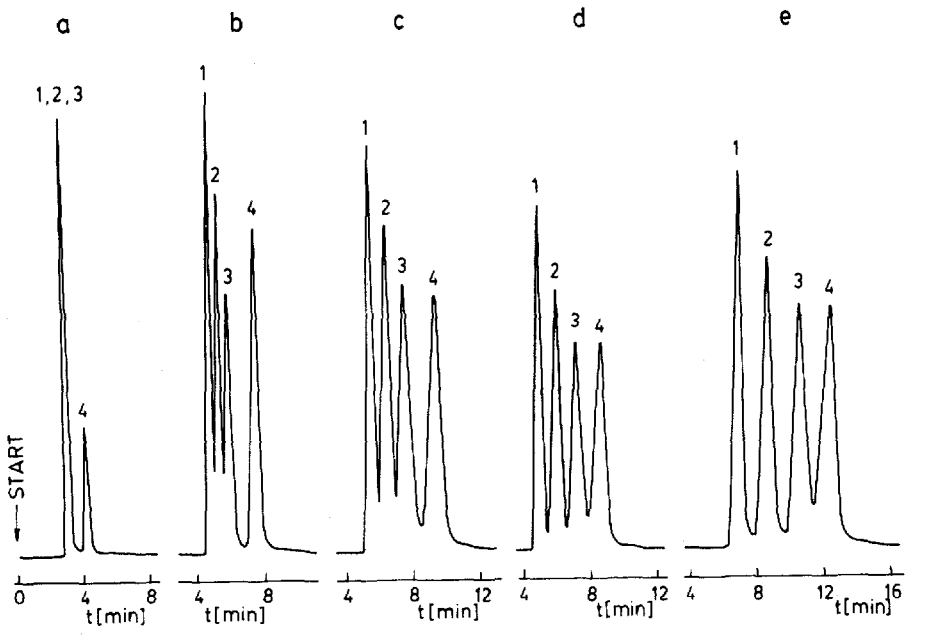

Fig. 1. Chromatograms of a mixture of *o*-(4), *m*-(1) and *p*-(2)xylenes and ethylbenzene (3) at 60°C on columns (2 m × 4 mm I.D.) packed with 0.0 (a), 0.296 (b), 0.59 (c), 0.88 (d) and 1.18 mol % (e) of β-CD in formamide solution, deposited on Celite (4.54 g per 20 g). Sample size: 0.3 μl; composition; *o*-xylene (0.7974 g), *m*-xylene (0.7546 g), *p*-xylene (0.7262 g) and ethylbenzene (0.7080 g).

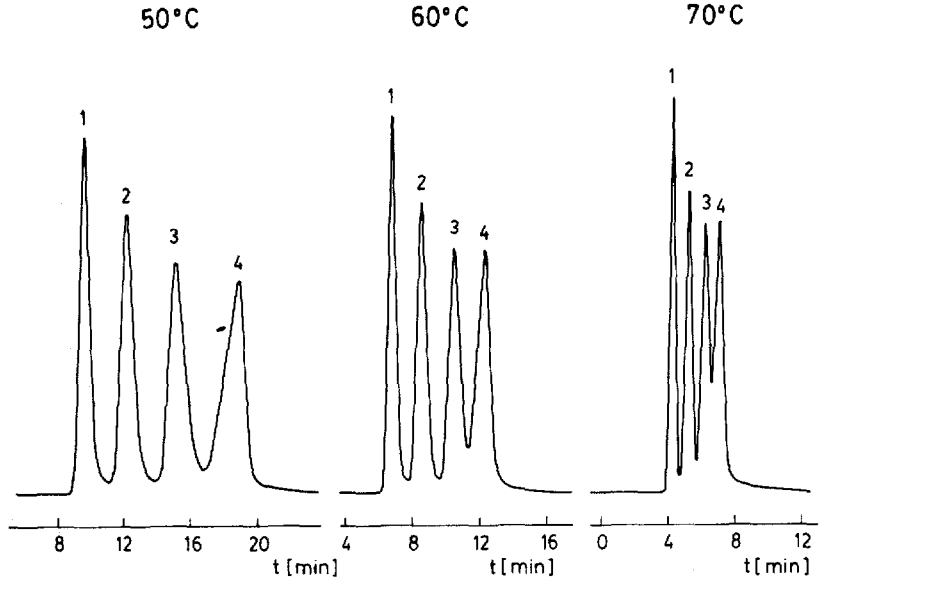

Fig. 2. Chromatograms of the same mixture as in Fig. 1e at 50°C, 60°C and 70°C. Sample size: 0.3 μl. Helium flow: 50 ml/min.

TABLE I

SEPARATION FACTORS AT 70°C FOR COLUMNS WITH DIFFERENT CLNCENTRATIONS OF β-CD

o-, *m*-, *p*-X = *o*-, *m*-, *p*-Xylenes; etb = ethylbenzene.

Concentration of β-CD in formamide (mol %)	$\alpha_{p\text{-}X/m\text{-}X}$	$\alpha_{etb/p\text{-}X}$	$\alpha_{o\text{-}X/etb}$	$\alpha_{etb/m\text{-}X}$	$\alpha_{o\text{-}X/m\text{-}X}$
0	0.965	0.974	1.485	0.940	1.396
0.30	1.114	1.107	1.277	1.233	1.574
0.59	1.145	1.116	1.222	1.278	1.562
0.88	1.221	1.135	1.244	1.386	1.724
1.18	1.223	1.364	1.057	1.668	1.763

A characteristic feature of this inclusion process is its stereoselectivity. The separation factors, α, of pairs of the compounds investigated, calculated as the ratios of their adjusted retention times, are given in Table I.

With the column containing formamide alone, only *o*-xylene forms a separate peak while all the other C_8H_{10} aromatics are eluted together; thus is due to the relatively great difference (about 4°C) between boiling point of *o*-xylene and those of the other compounds. However, in the presence of β-CD in formamide solution, the shape of the molecules is distinguished and each compound is eluted separately. The polarizability, which changes only slightly in the case of xylenes, plays a minor rôle.

The processes involved in the formation of β-CD molecular inclusion complexes which take place in formamide medium, except stereoselectivity and suitable capacity, meet the other requirements of the dynamic chromatographic method, primarily those of reversibility and fast equilibration. The elution curves are symmetrical and the retention of each compound is independent of its concentration and of the presence of other substances in the injected sample. Table II gives the number of theoretical plates, *N*, of the columns and Table III lists the resolution, R_s, established graphically for neighbouring peaks. It is evident that the loss of efficiency caused by complexation processes is relatively small, not exceeding 12–24 % of the reference value for the column containing formamide alone. Because of the gain in

TABLE II

NUMBER OF THEORETICAL PLATES, *N*, OF THE COLUMNS AT 60°C FOR DIFFERENT CONCENTRATIONS OF β-CD IN FORMAMIDE SOLUTION

Concentration of β-CD in formamide (mol %)	*N**
0	1250
0.59	950
0.88	1050
1.18	1100

* Calculated on the basis of the peak of *o*-xylene.

TABLE III

RESOLUTION, R_s, FOR *o*-, *m*-, *p*-XYLENES AND ETHYLBENZENE AT DIFFERENT CONCENTRATIONS OF β-CD AND AT DIFFERENT TEMPERATURES

Concentration of β-CD in formamide (mol %)	R_s	*Temperature*		
		50°C	*60°C*	*70°C*
0.0	$R_{etb,p\text{-}X}$ / $R_{p\text{-}X,m\text{-}X}$		<0.40	
	$R_{m\text{-}X,o\text{-}X}$	1.81	1.92	1.69
0.30	$R_{m\text{-}X,p\text{-}X}$	1.25	1.17	0.80
	$R_{p\text{-}X,etb}$	1.10	1.14	0.67
	$R_{etb,o\text{-}X}$	2.22	2.00	1.86
0.59	$R_{m\text{-}X,p\text{-}X}$	1.26	1.20	1.20
	$R_{p\text{-}X,etb}$	1.13	1.16	1.09
	$R_{etb,o\text{-}X}$	1.53	1.42	1.00
0.88	$R_{m\text{-}X,p\text{-}X}$	1.56	1.33	1.40
	$R_{p\text{-}X,etb}$	1.25	1.25	1.45
	$R_{etb,o\text{-}X}$	1.29	1.29	1.00
1.18	$R_{m\text{-}X,p\text{-}X}$	1.88	1.52	1.38
	$R_{p\text{-}X,etb}$	1.60	1.47	1.44
	$R_{etb,o\text{-}X}$	1.57	1.33	1.05

selectivity with molecular inclusion, these systems exhibit better resolution towards the compounds investigated.

Taking into account the time of separation and the values of R_s as a function of β-CD concentration and of temperature, it may be concluded that the following conditions are suitable for the analysis of *o*-, *m*- and *p*-xylene and ethylbenzene mixtures: β-CD concentration, 1.18 ml %; temperature, 60°C. Under these conditions the chromatographic columns are stable and may be used for several weeks.

Therefore we have obtained an affirmative answer for the question posed at the outset of this work. β-CD can modify the resolution properties of partition GC systems under suitably chosen conditions. The observed stereoselectivity seems to be a very promising feature for various chromatographic separations.

The opinion generally accepted until quite recently, that CDs form inclusion complexes exclusively in aqueous solutions, does not therefore seem to be correct. Formamide itself constitutes a suitable liquid matrix for β-CD inclusion processes.

In the course of this work special attention was paid to maintaining constant values of the inlet pressure and helium carrier gas flow velocity. Under these conditions it was possible to compare the relative stabilities of β-CD complexes while having no means of establishing their exact values[5–8]. This evaluation was performed on the assumption that only complexes of 1:1 stoichiometry are formed and hence

$$t'_{\beta\text{-CD}} = t'_0 (1 + K [\beta\text{-CD}])$$

where $t'_{\beta\text{-CD}}$ and t'_0 are respectively the adjusted retention times of a solute on the column containing β-CD in formamide solution and on the reference column contain-

ing pure formamide; K is the stability constant of a 1:1 β-CD complex, *e.g.*, for *o*-xylene;

$$K_{ortho} = \frac{[o\text{-X} \cdot \beta\text{-CD}]}{[o\text{-X}]\,[\beta\text{-CD}]}$$

For dilute β-CD solutions ($[\beta\text{-CD}] < 0.296$ mol %) the following sequence has been etablished at 60°C (etb = ethylbenzene):

$$K_{ortho} < K_{meta} < K_{para} < K_{\text{etb}}$$

This sequence changes in more concentrated β-CD solutions, where the above straight line depencence (for 1:1 stoichiometry) is no longer valid, and may suggest that complexes of other than 1:1 stoichiometry are formed. Further studies on the application of CDs in partition GC are in progress.

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