

Synergistic Effect of Heptakis(2,3,6-tri-*o*-pentyl)- β -Cyclodextrin and *o*-Methyl-*p*-Phenylene-bis-(*p*-Heptoxy Benzoate) Mixed Stationary Phase in Capillary Gas Chromatography

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Abstract

The separations for benzene–cyclohexane, cyclohexane–hexane, halogen hydrocarbons, aromatics, phenol, and alcohol on heptakis(2,3,6-tri-*o*-pentyl)- β -cyclodextrin and *o*-methyl-*p*-phenylene-bis-(*p*-heptoxy benzoate) liquid crystalline mixed stationary phase in capillary gas chromatography are carried out. It is observed that the chromatographic retention properties for most of the pairs tested on the mixed stationary phase are not the simple addition of 2 pure phases. These facts show that there exists a synergistic effect in the mixed stationary phase. This effect depends on the mixing ratio of mixed stationary phases and the linear velocity of the carrier gas.

Introduction

Cyclodextrin derivatives have been extensively used as gas chromatographic (GC) stationary phases. There are a large number of reports about cyclodextrin for the separation of chiral compounds and position isomers. Cyclodextrin is a cyclic molecule with a cavity structure and is able to form inclusion complexes with solutes. It has already become the focal point of supramolecular chemistry.

Liquid crystalline stationary phases have temperature-dependent ordered structures, and solutes interact with this ordered structure. Although most of compounds on nonpolar stationary phases elute according to their increasing boiling points, they elute by solute geometry on liquid crystalline. The major factor affecting this behavior was the length-to-breadth ratio (L/B). The

solute's molecular shape, although of less importance, was also a significant retention-affecting factor, having an effect contrary to L/B values on the elution order (1). Liquid crystals can demonstrate nematic, cholesteric, or smectic properties within a certain temperature range. It has been generally accepted that nematic phases produced a better chromatographic resolution than smectic phases (2).

The investigation in our recent work has shown the existence of a synergistic effect in some mixed GC stationary phases, each having a special selectivity (3–5). The synergistic effect was discovered in 1957 by Blake (6) in the study of the extraction of uranium. It has been widely studied in the extraction of inorganic or organic compounds. It is also reported in capillary electrophoresis (7). Therefore, it is interesting to see whether there is also a synergistic effect in the cyclodextrin liquid crystalline system.

Experimental

Reagents

The synthesis, purification, and characterization of heptakis(2,3,6-tri-*o*-pentyl)- β -cyclodextrin were achieved by using a method reported by Laufer et al (8). Liquid crystalline *o*-methyl-*p*-phenylene-bis-(*p*-heptoxy benzoate) (MPBH_pB) and SE-54 were chromatographic-grade reagents (Shanghai First Reagents Factory, China). All tested compounds were analytical reagent-grade (Beijing Chemical Factory, Beijing China).

Chromatographic column preparation

Fused-silica capillary columns manufactured by the Yongnian Optical Fiber Factory (Hebei Province, China) were prepared by

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Table I. Characteristics of the 15 Capillary Columns

Column	Column dimension (m × mm)	Film thickness (mm)	<i>k</i>	Temperature (°C)	Optimal linear velocity (cm/s)	Column efficiency (plates/m)	Compound tested
M ₁	10 × 0.25	0.28	3.86	115	12.4	4347	dodecane
M ₂	8.5 × 0.25	0.28	3.78	115	12.3	4257	dodecane
M ₃	9.5 × 0.25	0.28	3.85	115	12.7	3470	dodecane
CD ₁	10 × 0.25	0.28	3.05	125	14.7	1422	dodecane
CD ₂	8.5 × 0.25	0.28	3.04	125	14.2	1488	dodecane
CD ₃	9.5 × 0.25	0.28	3.11	125	14.3	1631	dodecane
[CD-M _{1,1}] ₁	10 × 0.25	0.28	3.66	115	12.8	2725	dodecane
[CD-M _{1,1}] ₂	8.5 × 0.25	0.28	3.65	115	12.9	2518	dodecane
[CD-M _{1,1}] ₃	9.5 × 0.25	0.28	3.67	115	12.7	2907	dodecane
[CD-M _{2,1}] ₁	10 × 0.25	0.28	3.48	120	13.5	1290	dodecane
[CD-M _{2,1}] ₂	8.5 × 0.25	0.28	3.53	120	13.2	1210	dodecane
[CD-M _{2,1}] ₃	9.5 × 0.25	0.28	3.53	120	13.1	1443	dodecane
[CD-M _{1,2}] ₁	10 × 0.25	0.28	3.83	110	12.2	4078	dodecane
[CD-M _{1,2}] ₂	8.5 × 0.25	0.28	3.75	110	12.6	3364	dodecane
[CD-M _{1,2}] ₃	9.5 × 0.25	0.28	3.78	110	12.3	3516	dodecane

Table II. McReynolds Constants of Columns M, CD, and CD-M_{1,1} at 120°C*

Column		X'	Y'	Z'	U'	S'	Average
M	<i>I</i>	714	714	738	800	822	757
	ΔI	61	124	111	148	123	113
CD	<i>I</i>	775	831	775	878	918	835
	ΔI	122	241	148	226	219	191
CD-M _{1,1}	<i>I</i>	750	817	772	881	979	839
	ΔI	97	227	145	229	280	195

* X', benzene; Y', 1-butanol; Z', 2-pentanone; U', nitropropane; S', pyridine. ΔI , $I_{\text{polar}} - I_{\text{squalane}}$.

static coating them with 0.45% (w/v) dichloromethane solutions of stationary phase. After the capillary column was purged with nitrogen at 240°C for 4 h, it was filled with dichloromethane solution. Then, one end of the capillary column was sealed, and the other end was connected to a vacuum line. Dichloromethane was gently evaporated for approximately 12–15 h at a constant temperature of 35°C. The column was flushed with nitrogen for 2 h and conditioned between 80°C and 140°C (increasing its temperature in 20°C increments, remaining at each temperature for 2 h, and finishing at 160°C for 6 h). Column CD was heptakis(2,3,6-tri-*o*-pentyl)- β -cyclodextrin. Column M was MPBHpB diluted in SE-54, which was used as a solvent for ease of coating MPBHpB (mass ratio MPBHpB–SE-54, 1:2). Column CD-M_{2,1} was the mixed stationary phases heptakis(2,3,6-tri-*o*-pentyl)- β -cyclodextrin and MPBHpB–SE-54 (6:1:2). Column CD-M_{1,1} was the mixed stationary phases heptakis(2,3,6-tri-*o*-pentyl)- β -cyclodextrin and MPBHpB–SE-54 (3:1:2). Column CD-M_{1,2} was the mixed stationary phases heptakis(2,3,6-tri-*o*-pentyl)- β -cyclodextrin and MPBHpB–SE-54 (3:2:4).

Apparatus

A Shimadzu (Tokyo, Japan) model GC-9A GC fitted with a cap-

illary control unit, a splitter injection port, and a flame ionization detector was used. The carrier gas was nitrogen at a linear velocity of 11.2–11.6 cm/s. The injection split ratio was 80:1.

Results and Discussion

Table I summarizes the chromatographic characteristics of 15 columns. Three capillary columns were made for the columns CD, M, CD-M_{1,1}, CD-M_{2,1}, and CD-M_{1,2}. Dodecane was used to evaluate column efficiency. All

columns possessed fine reproducibility and no poor efficiency. The polarity of CD, M, and CD-M_{1,1} stationary phases are expressed by the McReynolds constants in Table II. The column temperature was 120°C. All data in Table II are an average of the retention indexes of the same 3 columns with which each compound was determined 3 times. The relative standard deviation of (*I*) is less than 1.2%. The U', S', and average values of the mixed stationary phase are greater than with either pure stationary phase; it is different than general experimental results. This fact indicates that the interaction mechanism of the mixed stationary phase with some guest molecules has been changed in comparison with either column CD or M. There might be synergistic effect on some compounds in the mixed stationary phase.

Some test sample pairs (benzene–cyclohexane, cyclohexane–hexane, halogen hydrocarbons, aromatics, phenol, alcohol) were separated on columns CD, M, and CD-M_{1,1}. The results of the separation expressed as the separation factor (α) are listed in Table III. All experimental data are an average of α measured on 3 columns of each stationary phase with 3 analyses on each column. The relative standard deviation is less than 1.0%. Comparing the α values on column CD-M_{1,1} with those on column CD and M exhibits the obvious positive synergistic

effects for ethylbenzene–toluene, toluene–benzene, *o*-xylene–*p*-xylene, styrene–ethylbenzene, *p*-chlorotoluene–*o*-chlorotoluene, *p*-cresol–*o*-cresol, *n*-octanol–*n*-heptanol, cyclohexanol–hexanol, and *p*-nitrotoluene–*o*-nitrotoluene, and the obvious negative synergistic effect for dichloromethane–*tert*-butylchloride also occurred. All the separations of aromatic derivatives on CD-M_{1:1} have a positive synergistic effect in Table III.

Table IV lists the values of α for some solute pairs on column CD-M_{2:1}, CD-M_{1:1}, and CD-M_{1:2}. The ratio of the stationary phases is 2:1 in column CD-M_{2:1}, 1:1 in column CD-M_{1:1}, and 1:2 in column CD-M_{1:2}. All experimental data are also an average of 3 columns of each stationary phase and 3 analyses with each column. The relative standard deviation of α is also below 1.0%. It can be seen that the synergistic effect depends on the relative amounts of the individual phases in the mixed stationary phase. For instance, the separation factor of the styrene–ethylbenzene pair measured on the column CD-M_{1:1} ($\alpha = 1.51$) is between those obtained on columns coated solely with the pure derivatized β -cyclodextrin ($\alpha = 1.52$) and those with the pure liquid crystalline ($\alpha = 1.35$), but the separation on column CD-M_{2:1} ($\alpha = 1.55$) is better than on column CD ($\alpha = 1.52$) and column M ($\alpha = 1.35$), which exhibits a positive synergistic effect.

Figures 1–3 summarize the results for separations of 1,2-dichloroethane–dichloromethane, toluene–benzene, and *p*-cresol–*o*-cresol pairs on 5 columns (CD₁, M₁, [CD-M_{1:1}]₁, [CD-M_{2:1}]₁, and [CD-M_{1:2}]₁) at different temperatures. The separation factors α were measured 3 times with each column. They are expressed by plots of α against the stationary phase ratio. Figures 1–3 reveal that α became greater at lower column temperature, but the synergistic effect is not approximately affected.

The effect of the carrier gas linear velocity was also examined. It is generally known that the separation factor α is not changed with carrier gas linear velocity, but here it is more pronounced at lower carrier gas linear velocity on column [CD-M_{2:1}]₁. Every α is an average of 5 determinations with column [CD-M_{2:1}]₁. The relative standard deviation of α is below 0.3%. Table V shows the changes in a values of toluene–benzene and *p*-cresol–*o*-cresol at different carrier gas linear velocities.

Table III. Separation Factor (α) on Columns CD, CD-M_{1:1}, and M

Temperature	Compounds tested	α_{CD}	$\alpha_{CD-M_{1:1}}$	α_M
60°C	benzene–cyclohexane	1.49	1.45	1.36
	cyclohexane–hexane	2.58	2.13	1.41
	1,2-dichloroethane–chloroform	1.10	1.31	1.84
	chloroform–dichloromethane	2.65	2.43	1.59
	dichloromethane– <i>tert</i> butyl chloride	1.92	1.75	2.44
80°C	cyclohexanone–methyl isobutyl ketone	4.47	4.16	3.73
	methyl isobutyl ketone–2-pentanone	1.46	1.43	1.40
	2-pentanone–1-butanone	2.02	1.88	1.84
	styrene–ethylbenzene	1.52	1.51	1.36
	ethylbenzene–toluene	1.99	2.02	2.00
	toluene–benzene	2.20	2.32	2.27
90°C	<i>o</i> -xylene– <i>p</i> -xylene	1.23	1.26	1.19
	styrene–ethylbenzene	1.48	1.50	1.34
	ethylbenzene–toluene	1.90	1.95	1.74
	toluene–benzene	2.10	2.17	2.14
100°C	<i>o</i> -xylene– <i>p</i> -xylene	1.22	1.24	1.18
	<i>p</i> -chlorotoluene– <i>o</i> -chlorotoluene	1.05	1.08	1.06
	<i>p</i> -cresol– <i>o</i> -cresol	1.30	1.32	1.21
140°C	<i>o</i> -cresol–phenol	1.23	1.30	1.47
	<i>n</i> -octanol– <i>n</i> -heptanol	1.73	1.75	1.62
150°C	<i>n</i> -heptanol–cyclohexanol	1.27	1.38	1.55
	cyclohexanol–hexanol	1.25	1.33	1.24
	<i>p</i> -nitrotoluene– <i>o</i> -nitrotoluene	1.43	1.47	1.37

Table IV. Separation Factor (α) on Columns CD-M_{2:1}, CD-M_{1:1}, and CD-M_{1:2}

Temperature	Compounds tested	$\alpha_{CD-M_{2:1}}$	$\alpha_{CD-M_{1:1}}$	$\alpha_{CD-M_{1:2}}$
60°C	benzene–cyclohexane	1.65	1.45	1.39
	cyclohexane–hexane	2.14	2.13	2.11
	1,2-dichloroethane–chloroform	1.25	1.31	1.58
	chloroform–dichloromethane	2.53	2.43	2.10
	dichloromethane– <i>tert</i> butyl chloride	1.87	1.75	1.33
80°C	styrene–ethylbenzene	1.55	1.51	1.48
	ethylbenzene–toluene	2.01	2.02	2.01
	toluene–benzene	2.26	2.32	2.35
	<i>o</i> -xylene– <i>p</i> -xylene	1.25	1.26	1.23
90°C	styrene–ethylbenzene	1.52	1.50	1.47
	ethylbenzene–toluene	1.92	1.95	1.96
	toluene–benzene	2.15	2.17	2.18
	<i>o</i> -xylene– <i>p</i> -xylene	1.24	1.24	1.21
100°C	<i>p</i> -chlorotoluene– <i>o</i> -chlorotoluene	1.05	1.08	1.08
	<i>p</i> -cresol– <i>o</i> -cresol	1.32	1.32	1.30
140°C	<i>o</i> -cresol–phenol	1.28	1.30	1.38
	<i>n</i> -octanol– <i>n</i> -heptanol	1.76	1.75	1.73
150°C	<i>n</i> -heptanol–cyclohexanol	1.35	1.38	1.40
	cyclohexanol–hexanol	1.34	1.33	1.26
	<i>p</i> -nitrotoluene– <i>o</i> -nitrotoluene	1.45	1.47	1.44

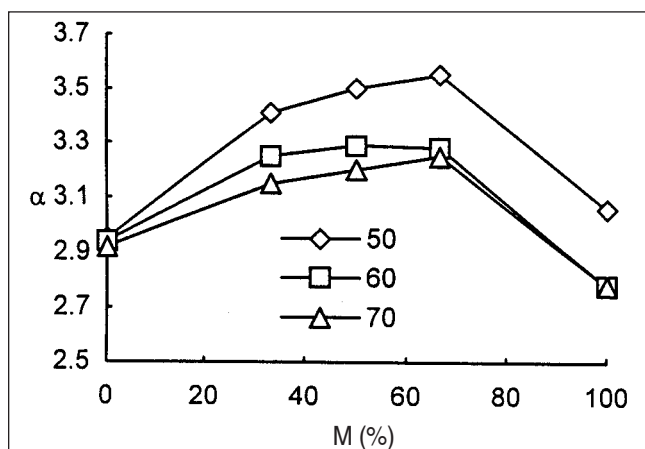


Figure 1. Plot of $\alpha_{1,2\text{-dichloroethane-dichloromethane}}$ versus M% for mixed stationary phases on columns CD_1 , M_1 , $[CD-M_{2:1}]_1$, $[CD-M_{1:1}]_1$, and $[CD-M_{1:2}]_1$ at 50, 60, and 70°C.

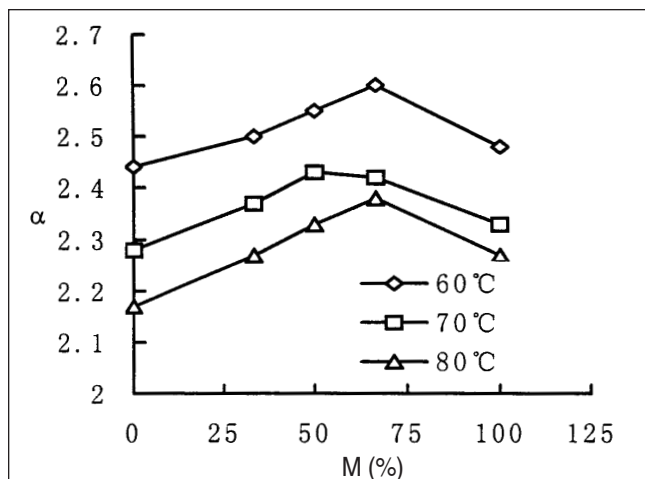


Figure 2. Plot of $\alpha_{\text{toluene-benzene}}$ versus M% for mixed stationary phases on columns CD_1 , M_1 , $[CD-M_{2:1}]_1$, $[CD-M_{1:1}]_1$, and $[CD-M_{1:2}]_1$ at 60, 70, and 80°C.

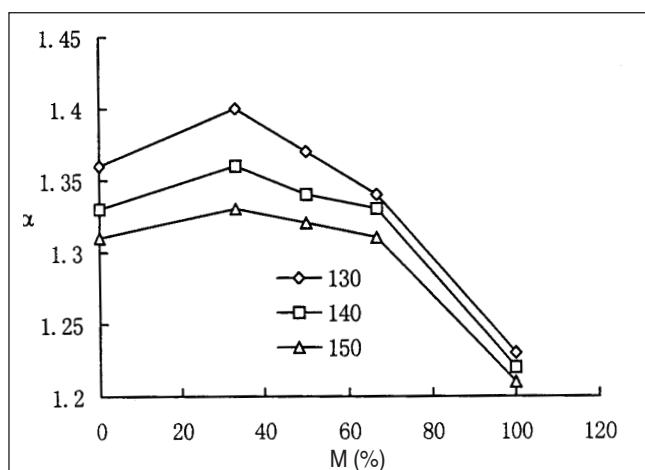


Figure 3. Plot of $\alpha_{p\text{-cresol-o-cresol}}$ versus M% for mixed stationary phases on columns CD_1 , M_1 , $[CD-M_{2:1}]_1$, $[CD-M_{1:1}]_1$, and $[CD-M_{1:2}]_1$ at 130, 140, and 150°C.

Table V. Separation Factor (α) on Column $CD-M_{2:1}$ at Different Carrier Gas Linear Velocity

Temperature	Compounds tested	Carrier gas linear velocity (cm/s)	$[\alpha_{CD-M_{2:1}}]_1$
60°C	toluene-benzene	6.6	2.50
		11.8	2.48
		19.5	2.46
150°C	<i>p</i> -cresol- <i>o</i> -cresol	37.5	2.41
		6.7	1.34
		10.5	1.33
		17.4	1.32
		39.3	1.29

Conclusion

A synergistic effect of the mixed stationary phases (each having special selectivity) may exist. The mixture's chromatographic property is not the simple addition of the chromatographic properties of the two pure phases. It is assumed that the synergistic effect is based on the supramolecular actions of mixed GC stationary phases on the solute molecules (9). This effect depends on the mixing ratio and the linear velocity of the carrier gas. The positive synergistic effect is very useful in the separation of some substance pairs. Further studies of the mechanism of the synergistic effect are underway.

Acknowledgments

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