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SELECTION OF THE SOLID SUPPORT — A CRUCIAL STEP FOR THE REALIZATION OF SOLVENT GENERATED LIQUID-LIQUID CHROMATOGRAPHY

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SUMMARY

Solvent-generated liquid-liquid chromatography (SGLLC) is a LLC technique in which the stationary liquid is generated dynamically by the mobile phase. This approach allows the advantages of liquid-liquid distribution as a retention mechanism to be used without the disadvantage of "column bleeding". The selection of suitable "inert" solid supports for LLC based on the correlation between chromatographic retention and static liquid distribution data is described. The retention mechanism is verified and the magnitude of residual adsorption contributions to retention in the LLC mode is estimated. The similarity of the retention behaviour of columns operated in either the LLC or the liquid-solid chromatographic (LSC) mode was investigated by two classification methods: by comparing the correlation coefficients of capacity factors for pairs of columns and by cluster analysis. The results demonstrate that a number of solid supports show only insignificant adsorption effects in the LLC mode and that on these columns operated in the LLC mode retention data are more similar than on columns operated in the LSC mode.

INTRODUCTION

Two retention mechanisms can be applied in chromatography: adsorption and partition. In adsorption chromatography the sample distributes between the bulk mobile phase (gas or liquid) and the surface layer on a stationary solid. In partition chromatography the stationary phase is a bulk liquid phase coating a solid support. Liquid chromatography can therefore be realized in two different forms: liquid-solid chromatography (LSC) and liquid-liquid chromatography (LLC).

The partition mode offers two advantages: versatility and reproducibility. In its

"classical" form, where a porous solid support is loaded with a given amount of stationary liquid which wets its surface better than the mobile phase, it suffers from column instability. If the difference in the wettability of the solid support between the mobile and the stationary liquid is too small, erosion of the stationary liquid phase occurs ("column bleeding").

This disadvantage can be overcome by "solvent-generated" LLC, where the stationary liquid phase is generated dynamically by the mobile phase¹⁻⁸. In this approach, one of the phases of a liquid-liquid system is applied as a mobile phase and a solid support is used which is better wetted by the other phase of the liquid-liquid system. Under these conditions a multimolecular layer is formed on the surface of the solid support which has the properties of the liquid phase in equilibrium with the mobile liquid phase. This type of LLC offers high column stability in addition to the other advantages of LLC. The technique is very versatile as a wide range of column selectivities can be obtained with the same column packing using the corresponding phases of different liquid-liquid systems as the mobile phase. If an inert solid support is used, *i.e.*, adsorption of solutes is negligible, the columns can be prepared with high reproducibility depending only on the reproducibility of the composition of the liquid mixtures used as mobile phases.

The aim of the work reported here was to determine the magnitude of residual adsorption effects and to demonstrate that LLC with negligible adsorption of solutes can be realized.

EXPERIMENTAL

Apparatus and procedures

Liquid-liquid partition coefficients and chromatographic data were determined according to the methods and with the instruments described previously^{4,5}.

Test compounds

Analytical-reagent grade test compounds listed in Table I were used.

Column packings

Tables II and III summarize the specifications of the chromatographic columns investigated, listing the column dimensions, packing mode and the characteristics of the support materials. Two types of columns, packed with hydrophobic and hydrophilic porous solids, were studied.

Eluents

Chromatographic experiments were performed in either the liquid-liquid or the liquid-solid mode. Tables IV and V give the compositions of the liquid phases used as eluents. The water-rich phases are used for columns with hydrophobic packings and the water-poor phases for hydrophilic packings. LLC was carried out using as eluent one of the phases of the ternary liquid-liquid phase system formed by water-ethanol-2,2,4-trimethylpentane described previously^{4,5}. Table IV also includes the mixing ratio of the solvents used for the preparation of the liquid-liquid system and the composition of the resulting coexisting liquid phases. Stationary liquid phases were generated dynamically via the mobile phase by pumping the more polar liquid phase

TABLE I
TEST COMPOUNDS

<i>Type of column packing</i>	<i>Test compound</i>	<i>Supplier</i>	
Hydrophobic	α -Naphthol	E. Merck (Darmstadt, F.R.G.)	
	Naphthalene		
	Anthracene		
	Phenanthrene		
	Benzene		
	Diphenyl		
	Triphenylene	Aldrich (Milwaukee, WI, U.S.A.)	
	Pyrene		
	9-Phenylanthracene		
	9,10-Diphenylanthracene		
	Benzo[ghi]perylene		
	Benzo[a]anthracene		
	Acenaphthylene		
	Dibenz[a,c]anthracene		
	Dibenz[a,h]anthracene		
	Benzo[a]pyrene		Sigma (St. Louis, MO, U.S.A.)
	Benzo[e]pyrene		
	Triphenylmethane		
	Chrysene	EGA Chemie (Steinheim, F.R.G.)	
	Fluorene		
	Acenaphthene		
	Indeno[1,2,3-cd]pyrene		
	Fluoranthene	Schuchardt (Munich, F.R.G.)	
	Perylene		
	<i>o</i> -Terphenyl	Koch-Light Labs. (Colnbrook, Bucks., U.K.)	
	<i>p</i> -Terphenyl		
	Indene		
Hydrophilic	Decylbenzene	E. Merck	
	Testosterone		
	Phenol		
	<i>o</i> -Cresol		
	<i>m</i> -Cresol		
	<i>p</i> -Cresol		
	2,4-Dimethylphenol		
	2,5-Dimethylphenol		
	3,4-Dimethylphenol		
	Progesterone		Sigma
	Andrenosterone		
	Androstene-1,7-dione		
	Methyltestosterone		
	Barban	EPA (Washington, DC, U.S.A.)	
	Baygon	Bayer (Leverkusen, F.R.G.)	
Carbaryl	Union Carbide (New York, NY, U.S.A.)		

TABLE II
SPECIFICATIONS OF HYDROPHOBIC ALKYL-SILICA COLUMNS

Column No. ^a	Dimensions (mm)	Packing material						Trade name
		Packing mode	Carbon number of bonded alkyl	Carbon content (% m/m)	Pore diameter (nm)	Particle size (μm)	Particle shape	
1	250 × 4.0	Prepacked	8	—	10	5	Spherical	LiChrospher 100 RP-8
2	250 × 4.6	Custom-made	18	10.5	10	10	Irregular	LiChrosorb Si-100, C ₁₈
3	250 × 4.6	Custom-made	8	11.0	6	10	Irregular	LiChrosorb RP-8
4	250 × 4.6	Custom-made	18	10.6	10	5	Spherical	ODS-Hypersil
5	250 × 4.0	Prepacked	8	—	6	5	Irregular	LiChrosorb RP-select B
6	250 × 4.6	Custom-made	18	17.6	10	10	Irregular	Experimental
7	250 × 4.0	Prepacked	18	—	10	7	Spherical	Nucleosil 10 C ₁₈
8	250 × 4.6	Custom-made	18	17.3	6	10	Irregular	Experimental
9	250 × 4.6	Custom-made	18	13.9	10	10	Spherical	Nucleosil 10 C ₁₈
10	250 × 4.6	Custom-made	18	17.2	6	10	Irregular	Experimental
11	250 × 4.0	Prepacked	18	19.4	6	7	Irregular	Experimental
12	250 × 4.6	Custom-made	18	6.8	—	10	Spherical	Partisil-10, ODS

^a Suppliers: E. Merck (Darmstadt, F.R.G.) (1,3,5,9,10,11); Shandon Southern (Runcorn, U.K.) (4); Macherey, Nagel & Co. (Düren, F.R.G.) (7,9); Polish Chemical Reagents (Gliwice, Poland) (2); Z. Suprynowicz (University of Lublin, Poland) (6); Whatman (Maidstone, U.K.) (12).

TABLE III
SPECIFICATIONS OF HYDROPHILIC COLUMNS (SILICA AND ALUMINA)

Supplier: E. Merck.

No.	Dimensions (mm)	Packing mode	Packing material				Trade name
			Base material	Particle size (μm)	Particle shape		
1	250 × 4	Prepacked	Silica	10	Spherical	LiChrospher Si 100	
2	250 × 4	Prepacked	Silica	10	Irregular	LiChrosorb Si 100	
3	250 × 4	Custom-made	Silica	7	Irregular	Experimental	
4	250 × 4	Prepacked	Alumina	10	Irregular	LiChrosorb Alox T	

TABLE IV
LIQUID PHASES USED FOR LIQUID-LIQUID CHROMATOGRAPHY AND PARTITION

Liquid-phase component	Composition (% m/m)					
	Liquid-liquid phase system I			Liquid-liquid phase system II		
	Overall liquid-liquid system	More polar liquid phase	Less polar liquid phase	Overall liquid-liquid system	More polar liquid phase	Less polar liquid phase
Water	36.04	39.51	0.09	3.15	18.50	0.10
Ethanol	54.62	59.80	1.11	17.01	75.00	5.50
2,2,4-Trimethylpentane	9.34	0.69	98.80	79.84	6.50	94.40

TABLE V
LIQUID PHASES USED AS ELUENTS FOR LIQUID-SOLID CHROMATOGRAPHY

<i>Liquid-phase component</i>	<i>Composition (% , m/m)</i>	
	<i>Eluents for hydrophobic adsorbents</i>	<i>Eluents for hydrophilic adsorbents</i>
Water	38.18	0.10
Ethanol	61.16	7.68
2,2,4-Trimethylpentane	0.66	92.22

of system I through columns packed with hydrophobic support materials or the less polar liquid phase of system II through columns packed with hydrophilic support materials.

RESULTS AND DISCUSSION

Retention data

In order to characterize the chromatographic retention of different porous materials either as a solid support for the stationary liquid in solvent-generated liquid-liquid partition chromatography or as an adsorbent in liquid-solid adsorption chromatography, the retentions of a set of test substances were measured on each column packing operated in either the partition or adsorption mode. As the columns investigated had different diameters the results are given as normalized retention, V_R/V_C , where V_R is the retention volume and V_C the volume of the column.

Tables VI and VII give the retention data for 27 test compounds on 12 columns packed with the hydrophobic porous solids operated in either the liquid-liquid or the liquid-solid mode. The water-rich phase of the liquid-liquid phase system I described in Table IV is used in the liquid-liquid mode and the water-rich liquid phase described in Table V as the eluent in the liquid-solid mode. Column 12, packed with Partisil-10 ODS, was excluded from further tests as the retention data showed that it was not possible to obtain a significant retention on this column in the liquid-liquid mode applying the dynamic method for the generation of the stationary liquid phase via the mobile phase. As this support material contains only 6.75% carbon, this is probably due to the relatively high residual silanol density on its surface.

Table VIII and IX give the retention data for sixteen test compounds on four columns packed with hydrophilic porous solid supports operated in either the liquid-liquid or the liquid-solid mode. The water-poor phase of the liquid-liquid system II described in Table IV is used as the eluent in the liquid-liquid mode and the water-poor phase described in Table V in the liquid-solid mode. Tables VI and VIII also include the partition coefficients of the sixteen test compounds for the liquid-liquid systems I and II described in Table IV.

TABLE VI
RETENTION DATA FOR 27 TEST COMPOUNDS ON 12 COLUMNS PACKED WITH HYDROPHOBIC POROUS SOLIDS OBTAINED IN THE LIQUID-LIQUID MODE TOGETHER WITH CORRESPONDING LIQUID-LIQUID PARTITION COEFFICIENTS OF 16 TEST COMPOUNDS

Solute Index <i>i</i>	Name	Partition coefficient, $K_1^{(u,l)}$ (<i>s</i> , <i>n</i> = 4)	Normalized retention, V_{Ri}/V_C											
			1	2	3	4	5	6	7	8	9	10	11	12
1	α -Naphthol	0.0845 (0.001)	0.38	0.45	0.43	0.44	0.58	0.39	0.50	0.44	0.53	0.44	0.39	0.80
2	Benzene	6.15 (0.30)	2.57	2.39	2.59	1.99	1.88	2.53	2.16	2.15	2.41	2.15	2.12	0.88
3	Acenaphthylene	—	2.99	2.87	3.00	2.29	2.25	3.11	2.64	2.75	3.34	2.71	2.89	1.11
4	Naphthalene	7.78 (0.03)	3.17	3.08	3.15	2.55	2.34	3.30	2.69	2.88	3.40	2.83	2.99	1.02
5	Indene	—	3.47	3.29	3.48	2.96	2.45	3.57	2.91	2.96	3.43	2.87	3.01	1.02
6	Phenanthrene	8.79 (0.04)	3.73	3.67	3.74	3.15	2.82	4.15	3.47	3.75	4.77	3.79	4.27	1.25
7	Fluoranthene	9.46 (0.17)	3.91	4.27	3.98	3.28	2.95	4.95	3.84	4.26	5.57	4.32	4.69	1.33
8	Anthracene	9.39 (0.10)	4.06	3.99	4.11	3.26	3.01	4.69	3.75	4.16	5.13	4.20	4.50	1.27
9	Pyrene	9.17 (0.20)	4.14	3.93	4.70	3.49	3.16	4.82	4.09	4.58	5.99	4.68	5.00	1.43
10	Triphenylene	9.48 (0.32)	4.30	4.07	4.28	3.68	3.24	4.94	4.32	4.94	6.82	5.11	5.72	1.57
11	Chrysene	10.05 (0.06)	4.49	4.38	4.50	3.83	3.44	5.47	4.61	5.54	7.37	6.07	6.61	1.65
12	Fluorene	10.53 (0.00)	4.56	4.41	4.54	3.74	3.29	5.11	4.00	4.30	5.25	4.31	4.68	1.18
13	Perylene	10.72 (0.31)	4.43	4.46	5.11	3.99	3.53	5.81	5.63	6.29	9.10	6.87	7.78	1.81
14	Diphenyl	—	4.53	4.39	4.55	3.56	3.11	4.72	3.88	3.86	4.60	3.85	3.98	1.15
15	Benzol[<i>a</i>]pyrene	—	4.55	4.54	4.71	4.02	3.67	5.84	5.75	6.39	9.11	6.93	7.77	1.82
16	Benzol[<i>a</i>]anthracene	11.70 (0.12)	4.82	4.74	4.87	4.20	3.62	5.84	4.88	5.85	7.76	6.40	6.82	1.63
17	Benzol[<i>a</i>]pyrene	—	4.86	5.00	5.12	4.47	3.96	6.75	6.43	7.39	9.80	8.60	9.35	1.92
18	Benzol[<i>g</i>]perylene	—	5.13	5.11	5.30	4.72	4.26	7.29	7.26	8.82	12.29	10.63	11.59	2.25
19	Indeno[1,2,3- <i>cd</i>]pyrene	—	5.24	5.42	5.49	4.88	4.31	9.78	7.40	9.33	12.46	11.39	12.24	2.31
20	Acenaphthene	13.05 (0.05)	5.26	5.30	5.30	4.28	3.70	5.93	4.53	4.88	5.86	4.82	5.23	1.23
21	Dibenz[<i>a,c</i>]anthracene	—	5.54	5.42	5.62	4.98	4.29	7.28	6.94	8.04	11.93	9.40	10.55	2.14
22	Dibenz[<i>a,h</i>]anthracene	—	5.85	6.05	6.05	5.43	4.68	8.33	7.79	9.73	12.81	11.73	12.60	2.28
23	<i>o</i> -Terphenyl	—	7.85	7.52	7.81	5.69	5.11	7.92	5.61	6.27	7.73	6.14	6.65	1.37
24	<i>p</i> -Terphenyl	—	8.81	8.53	8.87	6.74	5.80	9.70	7.07	8.84	10.12	8.55	9.18	1.64
25	9-Phenylanthracene	23.30 (0.52)	9.13	8.87	9.27	7.38	5.92	9.78	7.95	8.05	10.44	7.76	8.67	1.79
26	Triphenylmethane	27.12 (0.83)	10.80	9.58	9.94	7.71	6.56	10.41	7.46	7.71	9.58	7.53	8.10	1.49
27	9,10-Diphenylanthracene	53.97 (0.95)	20.30	19.76	20.40	15.97	12.93	21.81	16.27	17.57	20.96	15.86	17.11	2.50

TABLE VII
AS TABLE VI, DATA OBTAINED IN THE LIQUID-SOLID MODE

Solute	Normalized retention, V_{Ri}/V_C											
	1	2	3	4	5	6	7	8	9	10	11	12
1 α -Naphthol	0.89	0.87	0.89	0.92	0.71	0.60	0.81	0.92	0.88	0.77	0.66	0.84
2 Benzene	1.49	1.53	1.37	1.30	1.76	1.86	1.35	1.51	1.81	1.39	1.57	0.84
3 Acenaphthylene	1.77	2.00	1.69	1.58	1.98	2.49	1.83	2.15	2.75	1.98	2.29	1.02
4 Naphthalene	1.87	1.83	1.73	1.54	2.09	2.40	1.72	2.08	2.51	1.90	2.23	0.97
5 Indene	1.94	1.98	1.64	2.11	2.11	2.53	1.74	2.11	2.48	1.81	2.19	1.01
6 Phenanthrene	2.26	2.40	2.12	1.99	2.54	3.13	2.36	3.03	3.87	2.76	3.27	1.16
7 Fluoranthene	2.43	2.48	2.31	2.22	2.68	3.63	2.74	3.61	4.67	3.33	3.89	1.26
8 Anthracene	2.38	2.43	2.27	2.12	2.68	3.52	2.49	3.28	4.13	3.04	3.78	1.19
9 Pyrene	2.96	2.67	2.42	2.34	2.81	3.90	2.90	3.88	5.02	3.60	4.38	1.31
10 Triphenylene	2.70	2.84	2.47	2.48	2.96	3.62	3.47	4.55	6.02	4.15	5.12	1.42
11 Chrysene	2.78	2.99	2.51	2.64	3.10	4.00	3.73	4.87	6.53	4.93	6.09	1.65
12 Fluorene	2.41	2.52	2.29	2.12	2.84	3.55	2.47	3.15	3.93	2.77	3.41	1.14
13 Perylene	2.55	3.37	2.89	3.07	3.32	5.24	4.71	6.10	8.26	6.10	8.49	1.70
14 Diphenyl	2.32	2.53	1.93	1.98	2.69	3.37	2.20	2.77	3.36	2.35	2.94	1.07
15 Benzol[e]pyrene	2.46	3.55	3.04	3.05	2.82	4.67	4.34	5.95	8.37	6.02	7.64	1.64
16 Benz[<i>a</i>]anthracene	3.53	3.06	2.61	2.80	3.58	4.22	3.86	5.05	6.84	4.95	6.14	1.47
17 Benzol[<i>a</i>]pyrene	3.04	3.90	3.19	3.37	3.61	5.39	4.84	6.88	9.19	7.42	9.08	1.73
18 Benzol[<i>ghi</i>]perylene	3.58	4.32	3.57	3.85	3.98	6.14	6.23	8.55	12.13	9.29	11.93	2.01
19 Indeno[1,2,3- <i>cd</i>]pyrene	7.18	4.41	3.60	3.98	4.00	6.80	6.39	8.87	11.95	10.04	12.63	2.05
20 Acenaphthene	2.63	2.69	2.41	2.29	3.16	3.90	2.56	3.30	4.10	2.85	3.64	1.15
21 Dibenz[<i>a,c</i>]anthracene	3.57	4.19	3.53	3.79	3.98	6.07	5.82	7.73	11.55	7.89	10.29	1.88
22 Dibenz[<i>a,h</i>]anthracene	3.50	4.81	3.57	4.08	4.09	6.69	6.31	8.87	12.10	9.91	12.53	1.99
23 <i>o</i> -Terphenyl	3.49	4.02	3.50	3.65	4.32	4.34	2.99	3.88	5.78	3.30	3.92	1.22
24 <i>p</i> -Terphenyl	4.26	4.76	3.75	3.65	4.79	5.81	4.03	5.81	7.86	5.16	6.45	1.47
25 9-Phenylanthracene	3.21	4.82	3.27	3.80	5.13	5.20	4.14	5.55	7.57	4.63	5.78	1.48
26 Triphenylmethane	4.53	4.06	3.08	3.33	5.33	5.40	3.86	4.36	6.24	3.64	5.11	1.26
27 9,10-Diphenylanthracene	3.82	9.40	5.45	7.12	10.40	10.13	7.20	9.76	14.14	7.83	9.86	1.89

TABLE VIII

NORMALIZED RETENTION DATA OF SIXTEEN TEST COMPOUNDS ON FOUR COLUMNS PACKED WITH HYDROPHILIC POROUS SOLIDS OBTAINED IN THE LIQUID-LIQUID MODE TOGETHER WITH THE CORRESPONDING LIQUID-LIQUID PARTITION COEFFICIENTS

Solute		Partition coefficient, $K_i^{L/L}$ ($s, n = 4$)		Normalized retention, V_{Ri}/V_C			
Index i	Name			1	2	3	4
1	Decylbenzene	0.005	(0.001)	0.72	0.71	0.62	0.73
2	2,4-Dimethylphenol	7.11	(0.23)	2.29	2.61	3.01	1.35
3	2,5-Dimethylphenol	7.23	(0.10)	2.31	2.69	3.07	1.35
4	Progesterone	7.87	(0.18)	2.73	3.20	3.97	1.35
5	<i>o</i> -Cresol	8.84	(0.04)	2.62	3.14	3.61	1.49
6	3,4-Dimethylphenol	9.33	(0.15)	2.72	3.20	3.66	1.57
7	<i>m</i> -Cresol	10.86	(0.02)	3.03	3.61	4.16	1.68
8	<i>p</i> -Cresol	11.42	(0.13)	3.03	3.66	4.16	1.72
9	Barban	13.82	(0.14)	3.70	4.53	5.85	1.78
10	Phenol	15.49	(0.25)	3.52	4.30	5.05	1.91
11	Androstene-1,7-dione	15.88	(0.04)	4.29	5.15	6.94	1.73
12	Methyltestosterone	21.51	(0.78)	5.77	7.04	9.45	2.24
13	Baygon	22.31	(0.81)	5.10	6.28	15.42	2.01
14	Carbaryl	26.00	(0.16)	6.01	7.53	10.07	1.73
15	Testosterone	31.38	(0.34)	7.85	9.69	13.32	2.90
16	Andrenosterone	59.20	(1.45)	12.67	15.35	22.57	3.35

TABLE IX

AS TABLE X, DATA OBTAINED IN THE LIQUID-SOLID MODE

Solute		Normalized retention, V_{Ri}/V_C			
Index i	Name	1	2	3	4
1	Decylbenzene	0.82	0.83	0.83	0.76
2	2,4-Dimethylphenol	1.32	1.53	1.55	1.11
3	2,5-Dimethylphenol	1.34	1.54	1.54	1.10
4	Progesterone	1.67	1.87	2.04	0.97
5	<i>o</i> -Cresol	1.37	1.64	1.66	1.18
6	3,4-Dimethylphenol	1.43	1.63	1.66	1.29
7	<i>m</i> -Cresol	1.44	1.73	1.74	1.39
8	<i>p</i> -Cresol	1.46	1.72	1.72	1.42
9	Barban	1.65	2.02	2.09	1.09
10	Phenol	1.54	1.84	1.82	1.57
11	Androstene-1,7-dione	2.05	2.10	2.67	1.02
12	Methyltestosterone	2.19	2.65	2.95	1.15
13	Baygon	2.07	2.49	2.65	1.10
14	Carbaryl	2.37	2.98	3.08	1.47
15	Testosterone	2.54	3.13	3.46	1.29
16	Andrenosterone	4.37	5.11	5.77	1.29

TABLE X

CORRELATION OF RETENTION VOLUMES WITH LIQUID-LIQUID PARTITION COEFFICIENTS ACCORDING TO EQN. 1

Correlation coefficients and test of the significance of the difference of the correlation coefficient Δr of a column and the column giving the highest correlation coefficient. $t_{\Delta r} > 2.02$ indicates a difference which is significant at the 95% confidence level.

<i>Hydrophobic solid support</i>			<i>Hydrophilic solid support</i>		
<i>Column No.</i>	<i>Correlation coefficient, r</i>	<i>Student factor, t_{Δr}</i>	<i>Column No.</i>	<i>Correlation coefficient, r</i>	<i>Student factor, t_{Δr}</i>
1	0.9993		1	0.9950	
2	0.9985	0.98	2	0.9940	3.24
3	0.9974	1.70	3	0.954	23.7
4	0.9968	2.00	4	0.930	42.3
5	0.9966	2.07			
6	0.9942	2.76			
7	0.982	4.27			
8	0.971	4.93			
9	0.947	5.73			
10	0.947	5.75			
11	0.904	5.91			

Verification of liquid-liquid partition as retention mode

In order to verify liquid-liquid partition as the retention mechanism for columns operated in the liquid-liquid mode, retention volumes, V_{Ri} , were correlated with static liquid-liquid partition coefficients $K_i^{(L/L)}$ according to the linear regression equation

$$V_{Ri} = V^{(m)} + V^{(s)}K_i^{(L/L)} \quad (1)$$

where $V^{(m)}$ and $V^{(s)}$ are the volumes of the mobile and stationary liquid phases, respectively.

Retention by a pure liquid-liquid partition mechanism leads to a linear correlation coefficient $r = 1.000$. Table X lists the correlation coefficients, r , and the Student's factors, $t_{\Delta r}$, which allow the assessment of the statistical significance of the difference between the correlation coefficient for the best column (column 1) and those for other columns. The deviation of the correlation coefficient from 1.0000 is a measure of adsorption contributions to solute retention. The correlation coefficients were rounded using the following conventions: (1) the maximum rounding interval is defined by half of the standard deviation of the data; and (2) the significant number of digits is defined such that the lowest digit is found within the maximum rounding interval. These conventions were used in spite of the unsymmetrical error distribution of the correlation coefficient, as this asymmetry has only a negligible consequence for the rounding of correlation coefficients by the method used. A value of $t_{\Delta r} > 2.02$ indicates that the differences between correlation coefficients is significant at the 95% probability level.

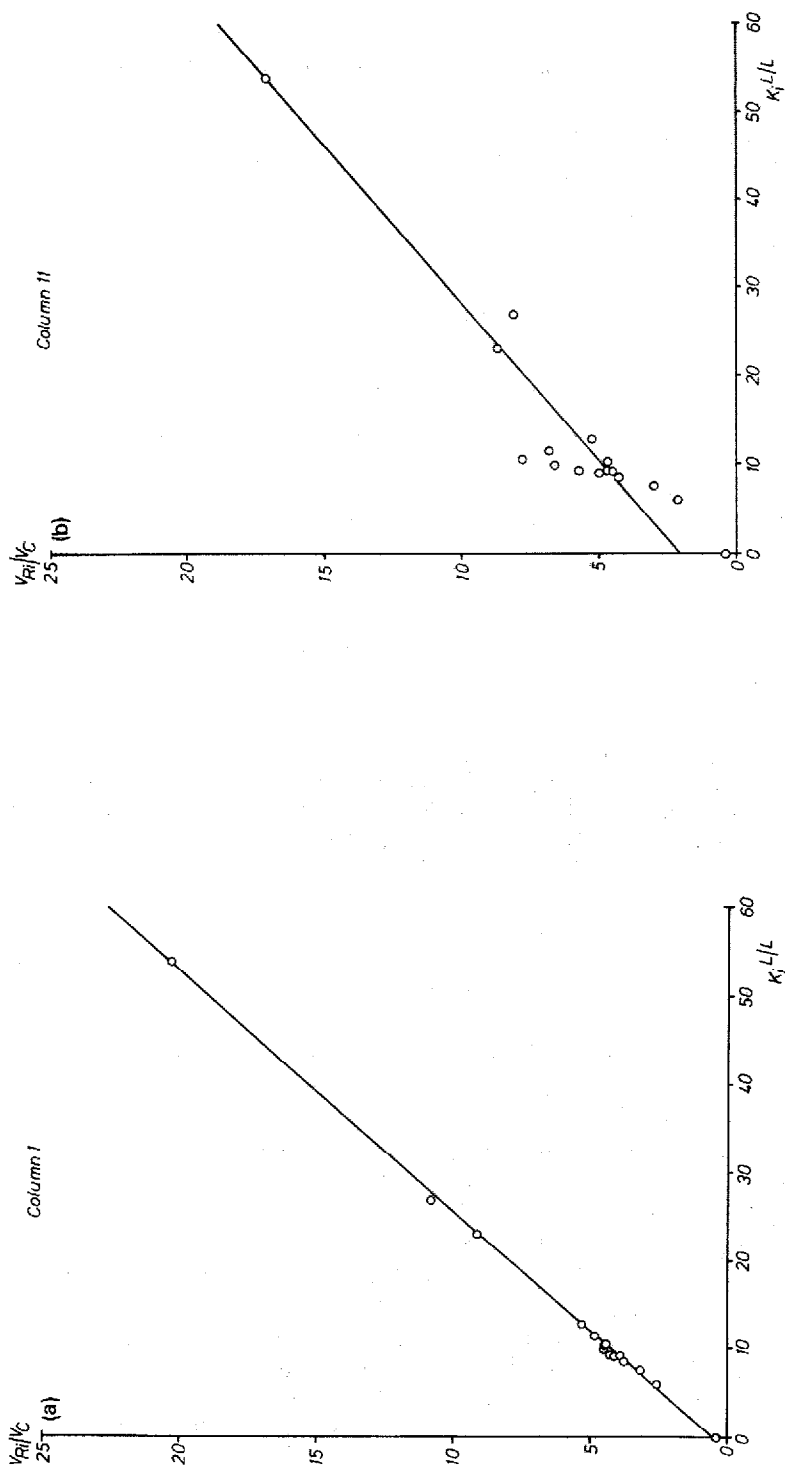


Fig. 1. Interdependence of retention volumes, V_{RL} , and liquid-liquid partition coefficients, $K_1^{L/L}$, for two typical columns packed with hydrophobic solid supports. The results are representative of columns packings with negligible or significant adsorption effects. Column specifications are given in Table II.

From Table X for columns with hydrophobic solid supports it can be seen that the columns packed with this material can be classified into two groups. The first group (columns 1–5) consists of columns with a retention completely determined by liquid–liquid partition. They have correlation coefficients in the range 0.9993–0.9966, which differ insignificantly from $r = 1.0000$ at the 95% probability level, indicating that adsorption effects are negligible (column 5 is on the limit of significance at this probability level). The second group (columns 6–11) consists of columns with correlation coefficients in the range 0.9942–0.9398, which are significantly different from $r = 1.0000$, indicating adsorption contributions to solute retention increasing from column 6 to column 11. Column 6 is still very similar to the group of columns 1–5 which shows that the adsorption effects, although significant, are only small in this instance. The retention mechanism with columns 1–5 is pure liquid–liquid partition, whereas columns 6–11 have a mixed mechanism retarding solutes by partition and adsorption with a predominance of partition. For hydrophobic columns Fig. 1 clearly illustrates the difference between a pure partition mechanism and a distinct mixed mechanism with significant adsorption effects. The different slopes of the regression lines can be attributed to different specific surface areas of the solid supports resulting in different volumes of the stationary liquid and to differences in residual adsorption effects (see Table XV).

With the columns in Table X packed with hydrophilic supports, the highest correlation coefficient found is significantly lower than the value $r = 1.0000$ for a pure liquid–liquid retention mechanism. This result is not unexpected as the set of test compounds is less uniform and highly polar groups on the solid support surface have strong interactions not only with mobile phase components such as water or ethanol, but also with polar solutes. Adsorption contributions to solute retention are therefore likely to play a more prominent role. The data show that silica (columns 1–3) is to be preferred to (basic) alumina (column 4) as a support material for LLC with the liquid–liquid system investigated. However, even within the group of silica columns, significant differences are found. Column 1 offers the best conditions to approach solvent-generated LLC with a polar stationary phase. Fig. 2 shows the correlations for the hydrophilic columns with the smallest and the largest adsorption effects.

In the preceding discussion it was demonstrated that liquid–liquid partition can be proved to be the retention mechanism by correlating retention data and liquid–liquid partition coefficients. The suitability of a support material for LLC can therefore be tested by determining the retention of a number of test substances and correlating these data with the static partition coefficients measured in equilibrium experiments. In order to optimize the expenditure of this test method, a minimum number of test compounds should be selected.

Starting with the sixteen substances used initially, one test compound at a time is omitted from the full set and the calculation of the linear regression is repeated with the remainder. The effect of the omission of a compound on the slope of the regression line was chosen as a criterion for the significance of the contribution of this compound to the regression. The following compounds were found to contribute most to the non-linearity of the regression: α -naphthol, benzene, perylene, triphenylmethane and 9,10-diphenylanthracene. As triphenylmethane is unfavourable with respect to its detection sensitivity it was replaced with 9-phenylanthracene without significant changes in the slope and correlation coefficient. For column 11, for exam-

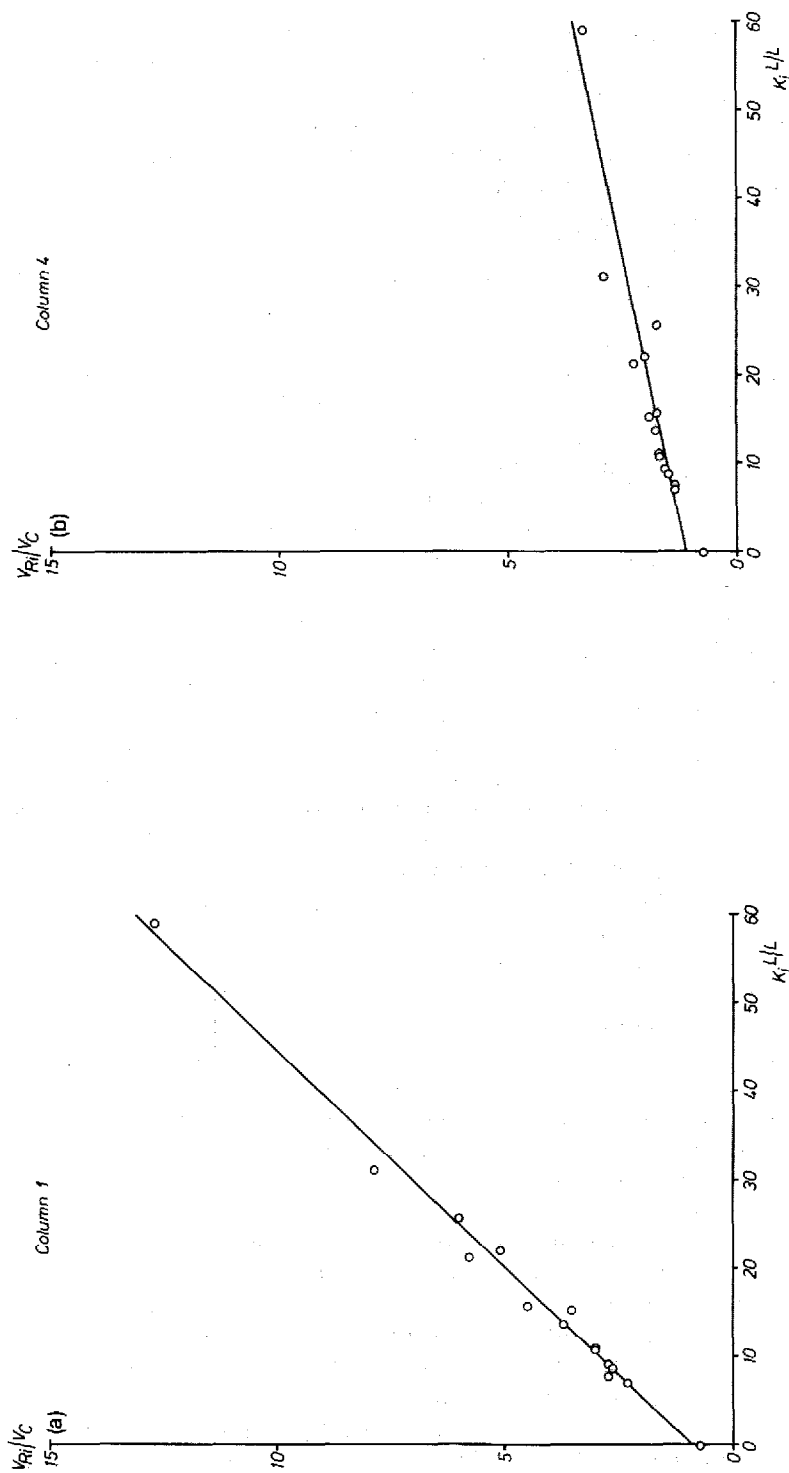


Fig. 2. Interdependence of retention volumes, V_R , and liquid-liquid partition coefficients, K_L^L , for typical columns packed with hydrophilic solid supports. The results are representative of column packings with slight or strong adsorption effects. Column specifications are given in Table III.

TABLE XI

SELECTED LINEAR REGRESSION DATA OF RETENTION VOLUMES AND LIQUID-LIQUID PARTITION COEFFICIENTS ACCORDING TO EQN. 1 FOR HYDROPHOBIC COLUMNS

Column No.	Compounds	Slope \pm S.D.	Intercept \pm S.D.	Correlation coefficient
1	Total set of 16 test compounds	0.369 \pm 0.004	0.54 \pm 0.07	0.9993
	Reduced set of 5 test compounds:			
	With triphenylmethane	0.371 \pm 0.005	0.41 \pm 0.13	0.9998
	With 9-phenylanthracene	0.370 \pm 0.002	0.39 \pm 0.07	0.9999
5	Total set of 16 test compounds	0.222 \pm 0.005	0.87 \pm 0.09	0.9965
	Reduced set of 5 test compounds:			
	With triphenylmethane	0.226 \pm 0.007	0.65 \pm 0.20	0.9984
	With 9-phenylanthracene	0.229 \pm 0.006	0.68 \pm 0.17	0.9988
6	Total set of 16 test compounds	0.383 \pm 0.01	0.95 \pm 0.20	0.9942
	Reduced set of 5 test compounds:			
	With triphenylmethane	0.391 \pm 0.02	0.52 \pm 0.52	0.9966
	With 9-phenylanthracene	0.399 \pm 0.02	0.63 \pm 0.41	0.9987
7	Total set of 16 test compounds	0.277 \pm 0.01	1.19 \pm 0.26	0.9823
	Reduced set of 5 test compounds:			
	With triphenylmethane	0.280 \pm 0.03	0.91 \pm 0.79	0.9849
	With 9-phenylanthracene	0.284 \pm 0.02	1.11 \pm 0.68	0.9897
11	Total set of 16 test compounds	0.280 \pm 0.03	2.0 \pm 0.5	0.9399
	Reduced set of 5 test compounds:			
	With triphenylmethane	0.289 \pm 0.05	1.4 \pm 1.4	0.9573
	With 9-phenylanthracene	0.311 \pm 0.04	1.6 \pm 1.3	0.9644

ple, which has the lowest correlation coefficient, the values found for the regression parameters of the full test set and the reduced subsets selected are shown in Table XI. It can be seen that a reduced test set of five compounds, including 9-phenylanthracene, does not lead to a regression significantly different from the total test set of sixteen compounds. In practice, this set of only five compounds can therefore be used to test the liquid-liquid retention behaviour of a column for polynuclear aromatic hydrocarbons.

Classification of columns

Comparison of column retention in pairs. Linear correlation between the capacity factors of a set of test compounds measured on two columns yields information on the similarity of retention on this pair of columns. The differences in the correlation coefficients obtained with a given column operated in the liquid-liquid and the liquid-solid modes indicates which of the two modes is likely to produce more reproducible results when changing to another batch of the same support material or to another material of the same type.

Table XII summarizes the correlation coefficients of the capacity factors for all possible pairs of the alkylsilica columns listed in Table II for operation of the columns in the LLC and LSC modes. Table XIII shows the corresponding data for columns packed with hydrophilic support materials. Correlation coefficients are given together with their confidence intervals at the 95% probability level. The correlation coeffi-

TABLE XII

CHARACTERIZATION OF THE SIMILARITY OF RETENTION FOR PAIRS OF HYDROPHOBIC (ALKYL-SILICA) COLUMNS BY MEANS OF THE CORRELATION COEFFICIENTS OF THE CAPACITY FACTORS OF 27 COMPOUNDS

The data are rounded using the 95% confidence limits given in the second line.

Column No.	Correlation coefficients with confidence limits				
	2	3	4	5	6
<i>Liquid-liquid mode</i>					
1	0.9978 0.9990-0.9948	0.9977 0.9990-0.9946	0.992 0.997-0.982	0.992 0.996-0.981	0.980 0.991-0.953
2		0.9985 0.9994-0.9966	0.9961 0.9983-0.9910	0.9952 0.9979-0.9890	0.987 0.994-0.970
3			0.9955 0.9981-0.9895	0.9946 0.9977-0.9875	0.985 0.994-0.966
4				0.9987 0.9994-0.9969	0.995 0.998-0.988
5					0.994 0.998-0.987
6					
7					
8					
9					
10					
<i>Liquid-solid mode</i>					
1	0.98 0.99-0.94	0.95 0.98-0.89	0.96 0.98-0.90	0.98 0.99-0.96	0.94 0.97-0.86
2		0.96 0.98-0.92	0.987 0.994-0.969	0.97 0.99-0.93	0.96 0.98-0.90
3			0.97 0.99-0.93	0.91 0.96-0.80	0.98 0.99-0.95
4				0.94 0.97-0.86	0.98 0.99-0.95
5					0.90 0.95-0.77
6					
7					
8					
9					
10					

7	8	9	10	11
0.92	0.87	0.81	0.74	0.74
0.96-0.82	0.94-0.73	0.91-0.60	0.88-0.49	0.88-0.48
0.93	0.89	0.82	0.76	0.76
0.97-0.85	0.95-0.76	0.92-0.64	0.89-0.53	0.89-0.52
0.93	0.89	0.82	0.76	0.76
0.97-0.85	0.95-0.76	0.92-0.63	0.89-0.52	0.89-0.51
0.957	0.92	0.86	0.81	0.80
0.981-0.903	0.96-0.82	0.94-0.71	0.91-0.60	0.91-0.59
0.960	0.93	0.87	0.82	0.81
0.983-0.910	0.97-0.84	0.94-0.73	0.92-0.62	0.91-0.62
0.977	0.951	0.90	0.85	0.85
0.990-0.948	0.978-0.889	0.96-0.78	0.94-0.70	0.93-0.69
	0.990	0.970	0.94	0.936
	0.996-0.976	0.987-0.931	0.97-0.86	0.972-0.858
		0.988	0.973	0.971
		0.995-0.973	0.988-0.937	0.987-0.933
			0.988	0.990
			0.995-0.973	0.996-0.978
				0.9992
				0.9997-0.9981
0.80	0.7	0.8	0.6	0.6
0.91-0.58	0.9-0.5	0.9-0.6	0.8-0.3	0.8-0.3
0.84	0.83	0.85	0.7	0.7
0.93-0.67	0.92-0.65	0.93-0.68	0.9-0.4	0.9-0.4
0.91	0.90	0.92	0.8	0.8
0.96-0.80	0.96-0.79	0.96-0.82	0.9-0.6	0.9-0.6
0.90	0.90	0.91	0.8	0.8
0.96-0.79	0.95-0.77	0.96-0.80	0.9-0.6	0.9-0.6
0.7	0.7	0.7	0.54	0.5
0.9-0.5	0.9-0.4	0.9-0.5	0.77-0.20	0.8-0.2
0.94	0.93	0.94	0.84	0.85
0.97-0.87	0.97-0.85	0.97-0.86	0.93-0.67	0.93-0.68
	0.996	0.997	0.97	0.97
	0.998-0.991	0.999-0.992	0.99-0.92	0.99-0.93
		0.997	0.98	0.98
		0.999-0.992	0.99-0.94	0.99-0.94
			0.97	0.97
			0.98-0.92	0.99-0.92
				0.998
				0.999-0.995

TABLE XIII

CHARACTERIZATION OF THE SIMILARITY OF RETENTION OF HYDROPHILIC (SILICA AND ALUMINA) COLUMNS IN PAIRS BY MEANS OF THE CORRELATION COEFFICIENT OF THE CAPACITY FACTORS FOR 16 TEST COMPOUNDS

The data are rounded using the 95% confidence interval given in the second line.

Mode	Column No.	Correlation coefficients with confidence limits		
		1	2	3
Liquid-liquid	2	0.998		
		0.999-0.994		
	3	0.95	0.95	
		0.98-0.84	0.98-0.84	
4	0.94		0.89	
	0.98-0.82	0.98-0.83	0.97-0.68	
Liquid-solid	2	0.997		
		0.998-0.989		
	3	0.998	0.998	
		0.999-0.993	0.999-0.993	
4	0.3	0.3	0.3	
	0.7-0	0.7-0	0.7-0	

coefficients were rounded according to the convention given above. The columns in Tables XII and XIII are arranged in such a way that the correlation coefficients for both the LLC and the LSC modes increase from left to right and from top to bottom. The sequence of columns obtained is the same as that found with the correlation coefficients of retention volumes and liquid-liquid partition coefficients.

The data for hydrophobic columns in Table XII also show that the correlation coefficients are higher in the LLC mode considerably more often than in the LSC mode. In the LLC mode correlation coefficients $r \geq 0.992$ were found for the comparison of the packings used in columns 1-5. In order to allow a correct interpretation of this observation one has to consider the significance of the difference in the correlation coefficients in both modes. This significance can be evaluated by calculating the Student factor, $t_{\Delta r}$, for the difference. A value above 2.02 indicates that the difference is significant at the 95% probability level. It was found that the correlation coefficients are significantly better for fourteen pairs of columns when they are operated in the LLC mode. Only two pairs of columns show significantly higher correlation coefficients in the LSC mode. With the exception of one case (columns 1 and 5) all column pairs formed from the first five columns have higher correlation coefficients in the LLC than in the LSC mode. These results are shown in Fig. 3. Operating hydrophobic columns in the LLC mode should therefore lead to significant improvements in the reproducibility of retention data on different supports of the same type but different batches or origin. This is an important conclusion as especially changes in retention characteristics from batch to batch are troublesome in the practice.

The correlation data of the hydrophilic columns shows that silica columns have high correlation coefficients and are similar in both modes of chromatography. The alumina column is not too different from the silica columns in the liquid-liquid mode but very different in the liquid-solid mode. This again demonstrates that column

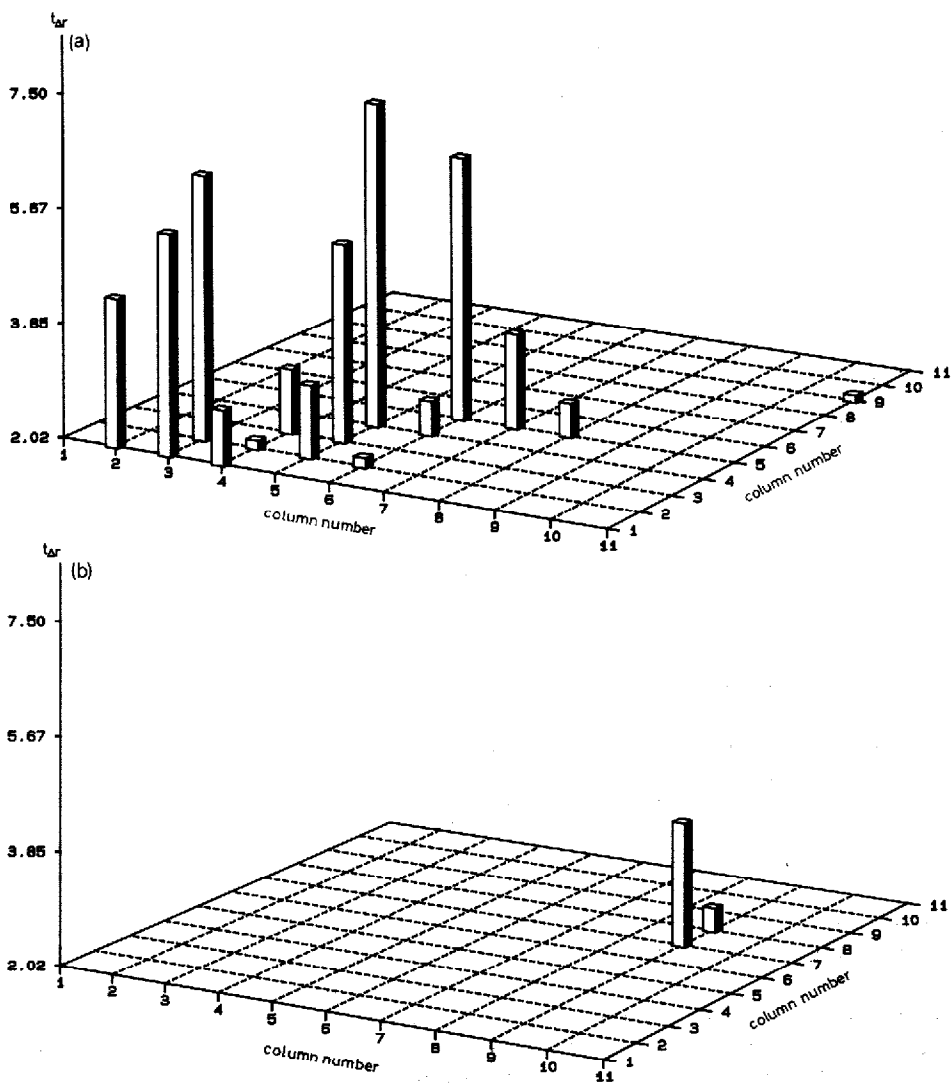


Fig. 3. Bar graph showing the Student factor t_{Ar} as a measure of the significance of the difference between the correlation coefficients of the capacity factors on pairs of columns. (a) LLC mode; (b) LSC mode.

retention is more reproducible in LLC than in LSC, because in LLC solutes are dissolved in the bulk liquid and not adsorbed at the surface.

Comparison of column retention by pattern recognition. The similarity of the retention characteristics of columns can also be described by applying pattern recognition techniques to the capacity factors of a number of analytes on these columns. In this approach a column is described as a point in the multi-dimensional space defined by the capacity factors. The capacity factor data were investigated by cluster analysis

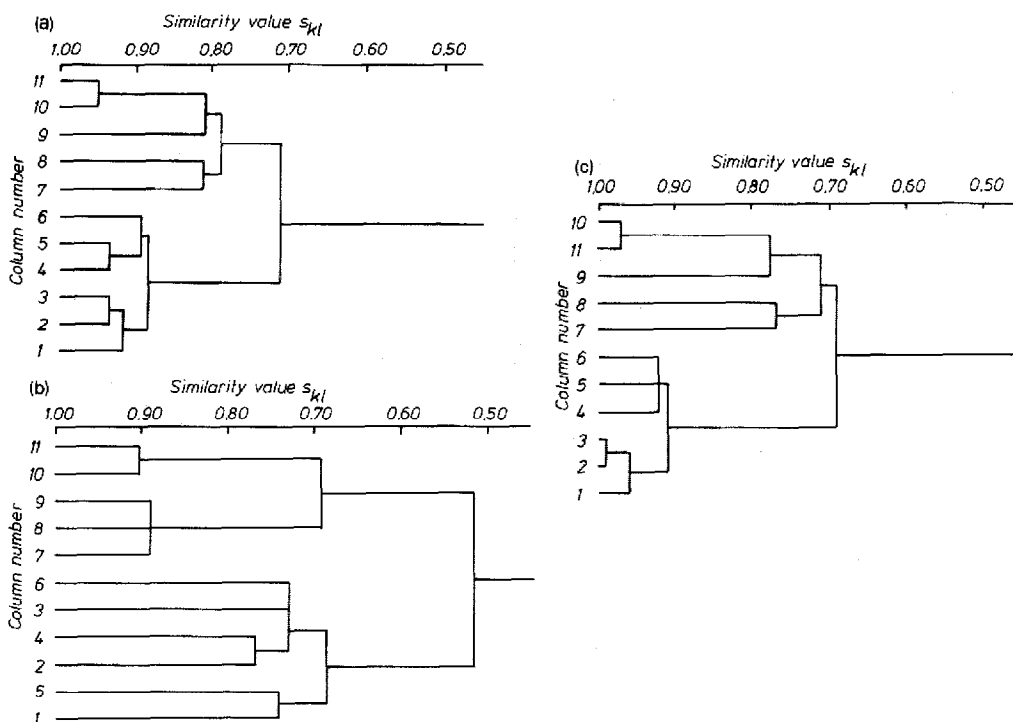


Fig. 4. Hierarchical dendrograms for capacity factors of a number of test compounds on eleven alkylsilica columns (test compounds and column specifications are given in Tables I and II). Computation by single link method of clustering. (Programm Arthur, Version 1-9-77). (a) LLC mode, 27 test compounds; (b) LSC mode, 27 test compounds; (c) LLC mode, 5 test compounds.

using the Euclidian distance to define the similarity values, s_{kl} , for the columns, k and l (for more details, see ref. 9).

The results of cluster analysis applied to the complete data set for 27 compounds and 11 alkylsilica columns are shown graphically in Fig. 4. The columns operated in the LLC mode (Fig. 4a) behave significantly more similarly than those operated in the LSC mode (Fig. 4b). At a similarity level of 0.71 the set of eleven columns splits into two clusters containing columns 1-6 and 7-11. Each of these two clusters is again divided into two clusters consisting of columns 1-3 and 4-6 on the one hand and columns 7-8 and 9-11 on the other. Next the triple clusters split up into double and single clusters and finally each column forms a particular cluster at a similarity level above 0.94. The results obtained in the classification of eleven alkylsilica columns by cluster analysis lead to the same sequence of columns as obtained by a pairwise comparison of columns. The cluster containing columns 1-6 corresponds to the group of columns giving the best linear regression of retention data with liquid-liquid partition coefficients.

It is important to optimize and validate the choice of the set of test compounds and, for practical reasons, to reduce their number to a minimum. For this purpose the magnitude of the effect of a test compound on the cluster formation was investigated

by determining step by step the test compounds which are least significant for the results of cluster analysis. In order of increasing significance, the following series was obtained: fluoranthene, chrysene, fluorene, phenanthrene, pyrene, triphenylene, perylene, phenanthracene, naphthalene, benzanthracene, anthracene, 9-phenylanthracene. The remaining set of the five most significant test compounds consists of α -naphthol, benzene, acenaphthylene, triphenylmethane and 9,10-diphenylphenanthracene. Triphenylmethane was replaced by 9-phenylanthracene for the same practical reasons as in the selection of a reduced set of test compounds for the verification of the liquid-liquid retention mechanism. The result of cluster analysis in the five-dimensional space defined by this reduced set of five test compounds is shown in Fig. 4c. A comparison with Fig. 4a shows that the clustering of columns determined with 27 and 5 test compounds is nearly identical. Especially the splitting into clusters follows the same path in both instances. Only the similarity levels of the splitting points are different. The splitting starts at a slightly lower similarity level of 0.69 and is completed at a higher level of 0.99. These results show that columns can be classified fairly well with only five test compounds, one of them being unretarded.

The reduced set of test compounds selected for the classification of columns is the same as the set selected for the verification of the liquid-liquid retention mechanism except for one compound. Acenaphthylene was selected in the first instance and perylene in the second. This difference is not unexpected, as the aims of the selection were different: the similarity of retention characteristics of the columns was consid-

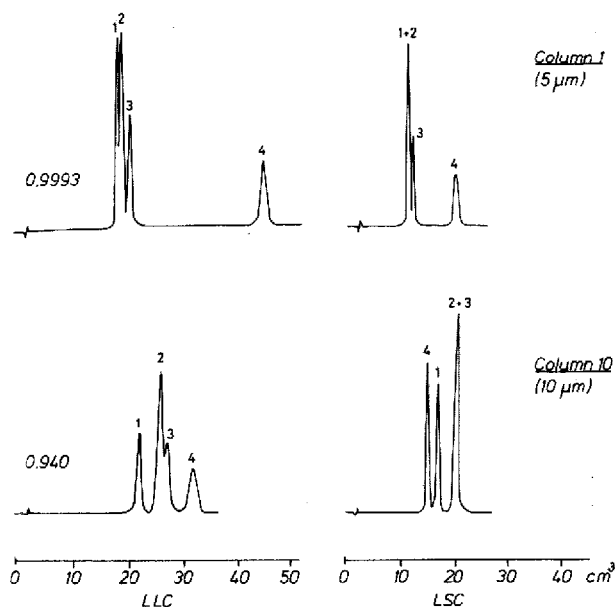


Fig. 5. Chromatograms of a test mixture on different hydrophobic support material operated in the LSC and LLC modes. The results are typical of solid supports with negligible or significant adsorption effects. The column packings are characterized by the correlation coefficients given in Table X. Test mixture: 1 = triphenylene; 2 = chrysene; 3 = benz[a]anthracene; 4 = triphenylmethane. For column specifications, see Table II.

ered in the first instance and the similarity of the correlation of chromatographic and liquid-liquid partition data in the second.

Illustration of column similarity by chromatograms. A test mixtures of four polynuclear aromatic hydrocarbons was chosen to illustrate column retention behaviour. The four test compounds were selected with regard to their sensitivity to adsorption effects. The test mixture was separated in both the LLC and LSC modes. Typical chromatograms obtained in these experiments are shown in Fig. 5. They represent the behaviour of columns with negligible and with significant solid support adsorption effects in the LLC mode.

In comparing the chromatograms, column efficiency has to be left out of consideration as packings of different particle size were used. The patterns of the chromatograms are significantly more similar in the LLC mode than in the LSC mode, where even changes of the peak sequence occur. Further, the resolution of the critical pairs of compounds 1-2, 2-3 and 1-4 is satisfactory for all columns in the LLC mode but not in the LSC mode. It was found that the similarity of the chromatograms obtained in the LLC mode decreases from column 1 to column 10, which agrees with the results of the column classification methods described above.

TABLE XIV

ESTIMATION OF THE CONTRIBUTION OF ADSORPTION TO SOLUTE RETENTION IN LLC

Residual adsorption effects are estimated from the difference in the regression (reg) and experimental data (exp) of the mobile phase and stationary liquid volumes of the columns ($\pm \Delta V$ = confidence limit of data at 95% probability level).

Column No.	Regression coefficient, r	$[V^{(m)} \pm \Delta V^{(m)}]_{cal}$ (cm^3)	$[V^{(m)} \pm \Delta V^{(m)}]_{exp}$ (cm^3)	$[V^{(s)} \pm \Delta V^{(s)}]_{cal}$ (cm^3)	$[V^{(s)} \pm \Delta V^{(s)}]_{exp}$ (cm^3)
<i>Alkylsilica solid supports</i>					
1	0.999	1.7 \pm 0.5	1.20	1.16 \pm 0.03	0.54
2	0.998	1.9 \pm 0.6	1.40	1.10 \pm 0.05	0.49
3	0.997	2.1 \pm 0.9	1.34	1.14 \pm 0.05	0.50
4	0.997	2.1 \pm 0.7	1.39	0.88 \pm 0.04	0.49
5	0.997	2.7 \pm 0.6	1.83	0.70 \pm 0.03	0.37
6	0.994	3.0 \pm 1.4	1.21	1.20 \pm 0.07	0.54
7	0.982	3.7 \pm 1.7	1.56	0.87 \pm 0.09	0.44
8	0.971	4.4 \pm 2.4	1.39	0.92 \pm 0.13	0.49
9	0.947	6.7 \pm 3.8	1.65	1.09 \pm 0.21	0.42
10	0.947	5.0 \pm 3.0	1.38	0.82 \pm 1.16	0.49
11	0.940	6.5 \pm 3.3	1.21	0.88 \pm 0.18	0.54
<i>Silica and alumina solid supports</i>					
1	0.995	2.7 \pm 0.8	2.25	0.64 \pm 0.04	0.25
2	0.994	3.0 \pm 1.0	2.22	0.79 \pm 0.05	0.26
3	0.954	1.8 \pm 4.8	1.95	1.24 \pm 0.22	0.33
4	0.928	3.5 \pm 0.6	2.28	0.13 \pm 0.03	0.24

Quantitative determination of residual adsorption effects in solvent generated LLC

Table XIV shows the results of another attempt to quantify adsorption contributions to the retention volume in LLC by comparing linear regression data for $V^{(m)}$ and $V^{(s)}$ with experimental data. Assuming a pure liquid-liquid partition mechanism, eqn. 1 can be used to calculate the mobile and stationary phase volumes by linear regression. These data, $V_{\text{cal}}^{(m)}$ and $V_{\text{cal}}^{(s)}$, are then compared with the experimental data, $V_{\text{exp}}^{(m)}$ and $V_{\text{exp}}^{(s)}$. The volume of the mobile phase, $V_{\text{exp}}^{(m)}$, is determined by measuring the elution volume of an unretarded compound or by evaluating the refractive index signal caused by the sample injection. An experimental estimate for the volume of the stationary liquid phase, $V_{\text{exp}}^{(s)}$, is obtained from the column volume and the mobile phase volume assuming a particle porosity of 0.28. The difference between the calculated and the experimental data indicates the magnitude of the adsorption contributions.

From Table XIV it can be concluded that for the hydrophobic solid supports the difference $V_{\text{cal}}^{(m)} - V_{\text{exp}}^{(m)}$ increases from column 1 to 11. For the first four columns the increase is not significant, however. As already found in the previous tests, these columns do not differ significantly in their retention characteristics. Column 5 is a limiting case as the difference with columns 1-4 is not distinct. Columns 6-11 show a significant difference between the calculated and experimental volumes of the mobile phase. The difference $V_{\text{cal}}^{(s)} - V_{\text{exp}}^{(s)}$ does not show a clear trend with the column number but scatters by up to a factor 2. This behaviour suggests a compound-specific deviation from the liquid-liquid partition behaviour. This is not unexpected as theory does not suggest a high correlation of the contribution to solute retention caused by adsorption on the solid support surface with solution in the bulk stationary liquid phase. Differences in the absolute value of the stationary liquid volume may be attributed to differences in the specific surface area of the solid supports.

For the hydrophilic solid supports it can be seen that there is a significant difference between both mobile phase volumes and therefore a significant residual adsorption only with the alumina support. For the silica supports no significant difference is found between the calculated and experimental volumes of the mobile phase. On the other hand, the difference between the calculated and the experimental values of the volume of the stationary phase is found to be significant and increases from column 1 to 3. This result does not only depend on the adsorption strength but also reflects differences in the specific surface area of the solid supports.

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