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EFFECT OF THE AMOUNT OF TRIS III COATED ON SILICA GEL ON THE SEPARATION OF POLYCYCLIC AROMATIC HYDROCARBONS BY LIQUID CHROMATOGRAPHY

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SUMMARY

The effect of the amount of the stationary liquid on retention values, selectivity of separation and column efficiency were studied for 37 polycyclic aromatic hydrocarbons (PAH). The retention volumes of all PAH decrease monotonously as the extent of coating increases. The column efficiency is independent of the extent of coating until a monomolecular layer of the stationary liquid has been formed, after which it decreases rapidly at higher extents of coating. The selectivity of separation can be influenced by the amount of Tris III present. The retention of PAH on silica gel coated with Tris III is caused almost exclusively by adsorption mechanisms.

INTRODUCTION

The separation of polycyclic aromatic hydrocarbons (PAH) has always been an interesting problem from the analytical point of view and has attracted great attention[1–3] in liquid chromatography. In order to achieve an optimum separation of PAH, different combinations of stationary and mobile phases have been tested. Most of the work has dealt with the separation of only a small number of substances, and the suitability of particular systems for separating a given combination of PAH can therefore seldom be compared.

The possibility of separating PAH on silica gel coated with 1,2,3-tris(2-cyanoethoxy)propane (Tris III) by their elution with saturated hydrocarbons was reported by Randau and Schnell[4]. It follows from the work by Engelhardt and Wiedemann[5] that both the retention values and selectivity of separation can be influenced by the amount of the stationary liquid present. In this work, 37 substances were selected such that the effects of the number and configuration of aromatic rings on the retention values and the selectivity of the separation could be evaluated and the results used for estimating the chromatographic behaviour of the substances under investigation.

EXPERIMENTAL

Apparatus and materials

A chromatograph of our own construction (Fig. 1) was used. Its design ensures

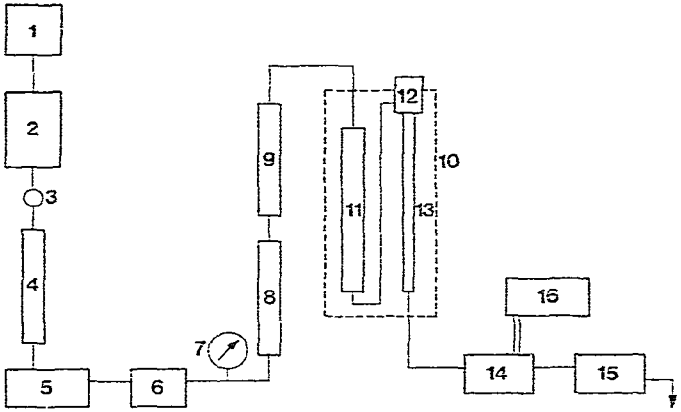


Fig. 1. Schematic diagram of the chromatograph. 1 = Reservoir; 2 = degasifier; 3 = stopcock; 4 = pre-column; 5 = pump; 6 = pressure damping system; 7 = manometer; 8 = pre-column with activated silica gel; 9 = pre-column with a coated low-surface-area support; 10 = water-bath, thermostated; 11 = pre-column with coated silica gel; 12 = injection block; 13 = column; 14 = detector; 15 = flow meter; 16 = recorder.

thorough degasification and purification of the mobile phase, its saturation with the stationary phase and precise thermostatic control of the analytical column. Pressure pulses generated by an MC 300 piston pump (Mikrotechna, Prague, Czechoslovakia) (5) are suppressed by a pressure-damping system according to Locke[6]. Pressure in the system is measured with a manometer (7). The temperature of the water-bath (10), in which a pre-column (11), an injection block (12) and an analytical column (13) are situated, is controlled by a water thermostat to a precision of better than ± 0.2°.

A capacitance detector[7] with a cell capacity of 11.7 μl and capillary inlets was used to mesaure the dead volumes. A UV analyzer (Development Workshops, Czechoslovak Academy of Sciences, Prague, Czechoslovakia) operating at a wavelength of 254 nm with a capillary cuvette of 1 mm I.D. served to detect aromatic substances. The working volume of the modified cell was about 8 μl with a mean optical path length of less than 1 mm. The inlet was packed with glass beads of diameter of 60 μm in order to decrease zone broadening[8]. The total extra column detector volumes were 66 and 71 μl for the UV and the capacitance detectors, respectively.

The flow-rate of the mobile phase was calculated from the time of the passage of the liquid through a known volume[9].

A 40–50-μm fraction was graded from 30–60-μm silica gel CH (Lachema, Brno, Czechoslovakia) with a specific surface area of 432 m^2/g. After activation for 3 h at 150°, the silica gel was coated with a chloroform solution of Tris III. The percentage of the coating is expressed as 100 times the weight of stationary phase deposited on 1 g of activated silica gel. The coated material was packed dry by the modified tap–fill method[10] into stainless-steel columns of 1.8 mm I.D. to a bed length of 490 mm; the columns were then closed at both ends with plugs of filter-paper. The column couplings and the connection of the detector and injection block were made according to Huber[11]. The packed columns were activated overnight in a stream of dry nitrogen at 150° prior to use. In measuring solubility coefficients, the analytical column (13) and the pre-column (11) were packed with a silanized support for gas chromatography, Gas-Chrom P (specific surface area 0.43 m^2/g), coated with 5.3 and 9.3% of Tris III, respectively. Columns with these packings were not activated before the use.

n-Heptane of analytical-reagent grade (Lachema, Neratovice, Czechoslovakia), dried and purified from trace amounts of benzene by passage through activated alumina prior to use, was used as the mobile phase. Water was removed from the apparatus in a pre-column (4) packed with molecular sieve 5A (Fig. 1) activated at 350°. The activated sieve was filled hot into the pre-column and *n*-heptane was poured over it. Pre-column (8) was packed with activated silica gel. Purified mobile phase was pre-saturated with the stationary phase in a pre-column (9) packed with a low-surface-area porous support of particle size about 0.1 mm, coated with up to 25% of Tris III. Equilibrium was established in a thermostated pre-column (11) between the stationary liquid coated on silica gel with which the analytical column (13) was packed and that dissolved in the mobile phase. Coarser fractions, separated by grading silica gel for the analytical column, were used to pack pre-column (11). The extent of silica gel coating for pre-column (11) and the preparation of the packing were the same as for the silica gel for column (13). Solutes (Table I) were injected with an injection syringe as solutions in the mobile phase or in benzene. All of the measurements were made at $20.0 \pm 0.2°$ and a flow-rate of the mobile phase of 0.41 ml/min, which corresponds approximately to a linear velocity of 0.6 cm/sec at a porosity of the packing of $\varepsilon = 0.4$.

The specific surface areas of the packings used (Table II) were measured on a home-made apparatus[14] by the thermal desorption method by adsorbing nitrogen at the temperature of liquid nitrogen with the use of a standard[15]. Samples of pure and coated silica gel were activated in a stream of a nitrogen–hydrogen directly in the apparatus at 150° for 25–30 min. During activation when the access of air was permitted, the stationary liquid coated on silica gel decomposed in spite of being recommended by the manufacturers[16] for use at temperatures up to 170°. The decomposition appeared as a change in the colour of the packing from white to dirty yellow. Two samples of each preparation were weighed and each was measured three times. The results differed by less than ±1% from the mean value for all extents of coating.

Saturation of the mobile phase with the stationary phase

Coating of silica gel surface with 22% of Tris III corresponds to monomolecular coverage of the surface. The surface that remains uncovered when applying smaller amounts of the stationary liquid sorbs water vapour from air and the water dissolved in the mobile phase during the preparation of the packing and the column. Alternatively, the mobile phase free from water can be saturated with water from this surface. If the mobile phase is saturated with the stationary phase, adsorption of the stationary liquid occurs on the uncovered surface of the silica gel and the amount of the stationary liquid on the surface increases. If the mobile phase is insufficiently saturated, the amount of the stationary phase in the column decreases.

As a consequence of any of the above processes, the retention data become poorly reproducible or dependent on the volume of the mobile phase used for the elution. The smaller the extent of the silica gel coating, the greater is the influence of the above factors. If good reproducibility of the retention volumes is to be obtained when using silica gel the surface of which is not completely covered with a monomolecular layer of the stationary liquid, then the mobile phase must be well purified before use, a pre-column packed with silica gel coated with the same percentage of the stationary

TABLE I
SOLUTES USED

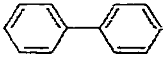
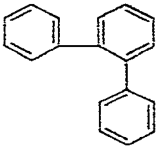
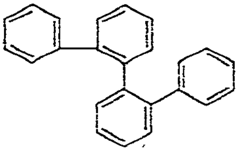
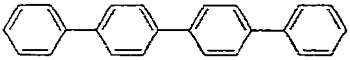
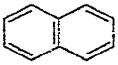
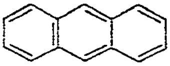
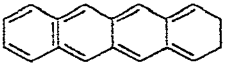
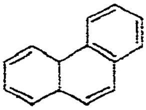
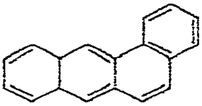
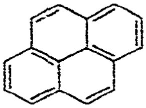
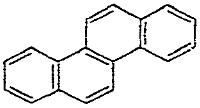
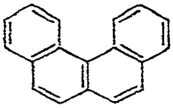
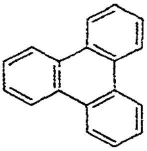
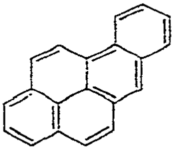
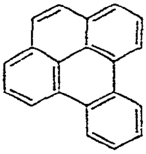
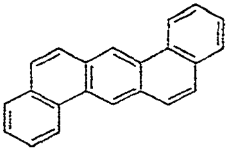
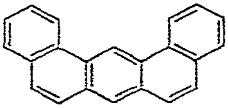
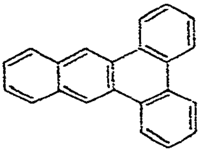
Solute No.	*Name**	*Formula*	*log* $\varepsilon_{254\ nm}$**	*Carcinogenic activity****
0	Benzene		1.9	
1	Biphenyl		4.2	
2	*o*-Terphenyl			
3	*m*-Terphenyl		4.7	
4	*p*-Terphenyl		4.0	
5	*o,o′*-Quaterphenyl			
6	*p p′*-Quaterphenyl		3.0	
7	Naphthalene		3.3	
8	Anthracene		5.0	
9	Tetracene		4.0	
10	Phenanthrene		4.8	
11	1,2-Benzanthracene		4.4	+

TABLE I *(continued)*

Solute No.	*Name**	*Formula*	*log* $\varepsilon_{254\ nm}$**	*Carcinogenic activity****
12	Pyrene		4.0	
13	Chrysene		4.5	+
14	3,4-Benzophenanthrene		4.2	
15	Triphenylene		4.9	
16	1,2-Benzopyrene (benzo[*a*]pyrene, 3,4-benzopyrene)		4.5	+++
17	4,5-Benzopyrene (benzo[*e*]pyrene, 1,2-benzopyrene)		4.3	+
18	1,2,5,6-Dibenzanthracene (dibenz[*a,h*]anthracene)		4.1	+++
19	1,2,7,8-Dibenzanthracene (dibenz[*a,j*]anthracene)			
20	1,2,3,4-Dibenzanthracene (dibenz[*a,c*]anthracene)		4.6	

(Continued on p. 52)

TABLE I *(continued)*

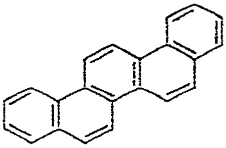
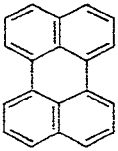
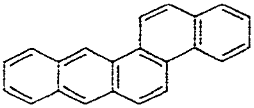
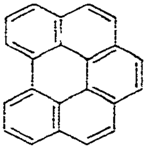
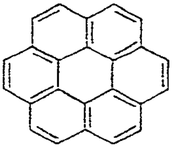
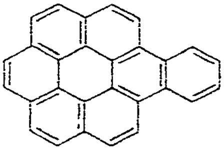
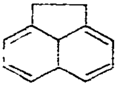
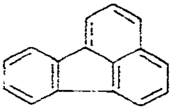
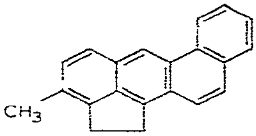
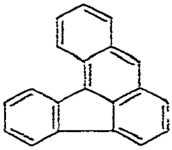
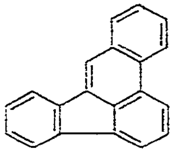
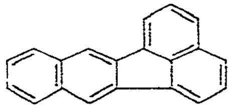
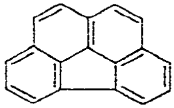
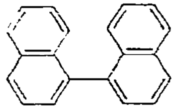
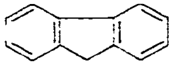
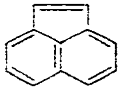
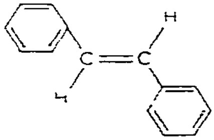
Solute No.	*Name*[*]	*Formula*	*log* $\varepsilon_{254\ nm}$[**]	*Carcinogenic activity*[***]
21	Picene		4.9	
22	Perylene		4.6	
23	3,4-Benzotetraphene		4.5	
24	1,12-Benzoperylene		4.2	
25	Coronene		3.7	
26	1,2-Benzocoronene			
27	Acenaphthene		3.2	
28	Fluoranthene		4.1	
29	20-Methylcholanthrene		4.4	+++

TABLE I *(continued)*

Solute No.	*Name**	*Formula*	*$\log \varepsilon_{254\,nm}$***	*Carcinogenic activity****
30	1,2-Benzofluoranthene (benzo[*a*]fluoranthene, 2,3-benzofluoranthene)		4.8	
31	2,3-Benzofluoranthene (benzo[*b*]fluoranthene, 3,4-benzofluoranthene)		4.5	+
32	8,9-Benzofluoranthene (benzo[*k*]fluoranthene, 11,12-benzofluoranthene)		4.7	
33	1,10-Benzofluoranthene (benzo[*m,n,o*]fluoranthene. 2,13-benzofluoranthene)		4.7	
34	1,1′-Binaphthyl		3.7	
35	Fluorene		4.1	
36	Acenaphthylene		3.2	
37	*trans*-Stilbene		3.8	

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* Names of the compounds are used in agreement with the "Definitive Rules for the Nomenclature of Organic Chemistry" accepted at the IUPAC Conference, Paris, 1957. Other names commonly found in the literature are given in brackets.

** Logarithms of the extinction coefficients at 254 nm were taken from various sources[12,13] and serve as a rough guide to the sensitivity of detection. The solvents used in their measurements are therefore not given.

*** Carcinogenic activity: +, mild; +++, strong.

TABLE II

DEAD VOLUMES AND COLUMN EFFICIENCIES MEASURED BY INJECTING NONANE AND SQUALANE

Coating (%)	*Weight (g)*	*Specific surface area of packing (m^2/g)*	*Nonane*		*Squalane*	
			Dead volume (μl)	*HETP (mm)*	*Dead volume (μl)*	*HETP (mm)*
60.2	1.086	64.3	0.692	0.65	0.702	1.3
40.7	0.922	136	0.766	0.76	0.765	1.4
20.2	0.835	264	0.883	0.46	0.883	0.88
9.8	0.701	337	0.893	0.49	0.853	0.78
5.1	0.646	382	0.935	0.48	0.922	1.03
1.0	0.587	412	0.916	0.64	0.891	1.60
0.0	0.588	432	0.982	0.61	0.866	1.33

phase as the silica gel in the column must be provided and thermostated together with the column in addition to the mobile phase being saturated with the stationary liquid, and the columns and pre-columns for the preparation of which silica gel was used must be reactivated prior to use on each occasion. When these requirements were observed, the measured retention data for all 37 solutes differed from the mean value by more than ±1% only exceptionally for all of the coatings used.

Measurements of the dead volumes of the columns and flow-rate of the mobile phase

Nonane and squalane were used to measure the dead volume. Both substances are so chemically similar to the liquid phase that there are no reasons for the origin of any measurable retention[17] in the chromatographic system that was used. As follows from Table II, the dead volumes, corrected for the volume of the couplings, are in good agreement (better than 2%) for both pure and coated silica gel.

However, the values of the height equivalent to a theoretical plate (HETP) that were calculated for the two solutes differ considerably. The HETP values found by injecting squalane are roughly twice as great as those for nonane .Using the data of Reid and Sherwood[18], it follows from the Wilke–Chang equation[19] that the diffusion coefficients of nonane and squalane in *n*-heptane are $2.2 \cdot 10^{-5}$ and $1.08 \cdot 10^{-5}$ cm^2/sec, respectively. The value of the HETP contribution depending on the flow-rate is inversely proportional to the value of the diffusion coefficient of the solute in the mobile phase for the substances that are not retained[20]. The difference in the calculated efficiencies can therefore be ascribed to the different values of the diffusion coefficients of nonane and squalane in *n*-heptane.

The method of Gerding and Hagel[9], based on the passage of the air bubbles, introduced into a stream of liquid, by a calibrated known volume (*e.g.*, with a pipette with a volume of 1 ml), was used to measure the flow-rate of the mobile phase. Air bubbles that are injected behind the detector outlet into a stream of the degasified liquid dissolve in it, which causes an error of up to 30%. The injected air was therefore replaced with an ammoniacal solution of copper(II) sulphate, which does not mix with the mobile phase.

RESULTS

Selectivity of the separation

All of the investigated solutes and benzene were eluted from uncoated silica gel in asymmetric zones. A slight asymmetry of zones occurred even with solutes that have a high retention capacity, *e.g.*, isomeric dibenzanthracenes, even at a content of 5.1% of the stationary liquid which covers 23.2% of the surface of the silica gel used. Very low extents of coating (less than 5.1% of Tris III) are therefore not interesting from the analytical point of view. As the extent of coating increased, the specific retention volumes decreased and, a Tris III monolayer having been formed on the surface of silica gel, the column efficiency was rapidly impaired (Table V). The dependence of the specific retention volumes on the extent of coating was therefore measured within the range 5–60% only. The results are summarized in Table III.

The specific retention volumes of all of the solutes tested decreased monotonously as the extent of coating increased over the whole range investigated. The most distinct changes in the retention volumes, selectivity of separation and retention order occurred at extents of coating that were not sufficient to create a monomolecular layer of the stationary phase on the surface (less than 22% of Tris III). The specific retention volumes decreased substantially more slowly at higher extents of coating and the retention changed approximately in inverse proportion to the extent of coating. The retention order changed only rarely. For instance, for 4,5-benzopyrene and 1,2-benzopyrene, the selectivity factor ($K_1/K_2 - 1$) increased from 0.120 for 60.2% coating to 0.175 for 20.2% coating; it decreased to 0.070 for 9.8% coating and both substances were eluted in the same volumes at a 5.1% coating of the stationary liquid.

Silica gel coated with Tris III showed good selectivity towards the separation of substances with various numbers of rings that belong to the same conformational type, *e.g.*, acenes, *p*-polyphenyls or aromatics with five-membered rings (Figs. 2 and 3), at all extents of coating studied. *p*-Polyphenyls always have higher retentions than acenes with an identical number of the rings. The differences increase both with an increasing number of rings at a given extent of coating and with a decreasing extent of coating. The selectivity of the coated silica gel is considerably worse for the separation of isomeric compounds that have the same number of rings (Fig. 3). It follows from a comparison of the retention values with the structures of aromatics being separated that only an isomer with a high degree of fusion, the rings of which are strongly clustered, can be separated from isomers the rings of which are arranged so that they can create chains of approximately equal lengths.

The influence of the extent of ring fusion on the selectivity of separation can also be illustrated for compounds with five benzene rings. The solutes tested form two groups that can be well separated from one another: 1,2-benzopyrene, 3,4-benzopyrene and perylene, and dibenzanthracenes, picene and 3,4-benzotetraphene. The separation of the compounds in each group requires the extent of silica gel coating to be carefully selected and highly efficient columns to be used. However, for some combinations of these compounds, separation is not possible even under these conditions. The situation is similar with compounds with four benzene rings in their molecules.

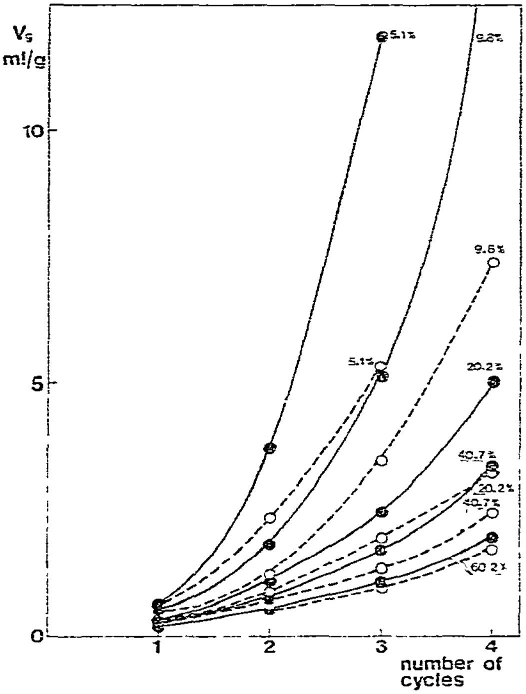
TABLE III

EFFECT OF THE EXTENT OF THE SILICA GEL COATING ON THE SPECIFIC RETENTION VOLUME RELATIVE TO 1 g OF THE PACKING

Elution order	*5.1%*		*9.8%*		*20.2%*		*40.7%*		*60.2%*	
	Sample No.	V_g *(ml/g)*	*Sample No.*	V_g *(ml/g)*	*Sample No.*	V_g *(ml/g)*	*Sample No.*	V_g *(ml/g)*	*Sample No.*	V_g *(ml/g)*
1	7	2.33	7	1.45	7	0.865	27	0.605	27	0.436
2	27	2.33	27	1.58	27	0.968	7	0.654	1	0.523
3	1	3.72	1	1.82	1	1.01	1	0.768	5	0.549
4	36	3.73	36	2.40	2	1.34	2	0.877	7	0.552
5	35	4.66	35	2.54	36	1.47	35	0.948	2	0.560
6	8	5.31	2	2.69	35	1.49	5	1.02	35	0.706
7	10	5.61	37	3.39	5	1.70	36	1.10	34	0.809
8	37	5.61	8	3.45	8	1.87	37	1.28	36	0.868
9	12	6.78	10	3.60	37	2.02	8	1.33	37	0.893
10	34	7.73	5	3.80	10	2.02	34	1.37	8	0.963
11	28	9.00	34	4.20	3	2.30	10	1.48	3	1.04
12	2	9.48	12	4.63	34	2.32	3	1.59	4	1.06
13	33	9.73	3	4.70	4	2.39	4	1.69	10	1.10
14	3	9.75	4	5.11	12	2.56	12	1.76	12	1.29
15	4	12.4	28	5.27	28	2.68	28	1.94	28	1.48
16	14	14.5	33	6.52	33	3.29	33	2.31	29	1.48
17	11	14.6	9	7.43	9	3.44	9	2.41	33	1.60
18	15	15.1	14	7.57	14	3.92	29	2.56	9	1.70
19	13	16.0	11	7.95	11	3.94	14	2.77	13	1.94
20	32	18.5	15	8.57	15	4.42	11	2.80	6	1.95
21	22	18.6	13	8.78	13	4.43	13	2.96	14	1.98
22	16	18.8	29	9.76	29	4.54	15	3.05	11	2.00
23	17	18.8	16	10.6	16	4.90	16	3.33	15	2.24
24	29	18.9	17	11.3	6	5.02	6	3.34	16	2.35
25	31	19.9	32	11.5	32	5.60	32	3.62	32	2.45
26	30	19.9	31	11.8	17	5.76	17	3.72	30	2.52
27	24	23.7	30	11.9	31	5.79	31	3.74	31	2.53
28	25	26.1	22	12.2	30	5.83	30	3.74	17	2.63
29	23	31.5	6	14.5	22	6.12	22	4.00	22	2.84
30	19	21.5	24	14.7	24	7.05	24	4.36	24	2.95
31	21	32.3	19	16.9	25	7.19	25	4.69	25	2.96
32	20	33.6	25	17.0	21	7.95	23	5.49	23	3.75
33	18	35.6	23	18.3	18	8.26	21	5.50	21	3.78
34	5	—	18	18.6	19	8.53	19	5.61	19	3.80
35	6	—	20	18.8	23	8.78	18	5.76	18	4.04
36	9	—	21	19.0	20	9.37	20	6.29	20	4.41
37	26	—	26	—	26	14.9	26	9.89	26	6.01

Mechanism of the retention of PAH

The monotonous decrease in the retention values of all substances tested with the extent of coating does not correspond to the course common in the separation systems. Also, the solubility coefficients of some solutes that were determined experimentally suggest that dissolution is not the main separation mechanism in the chromatographic system studied. The retention of PAH on Tris III-coated silica gel is caused to a large extent by adsorption mechanisms. Adsorption on the surface of



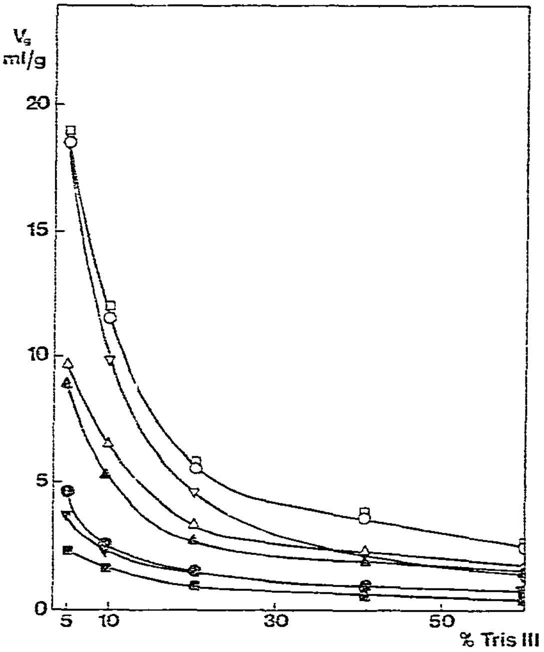


Fig. 2. Dependence of the specific retention volume relative to 1 g of packing on the number of rings for acenes and *p*-polyphenyls at various extents of coating (indicated on the curves). ●, Acenes (benzene, naphthalene, anthracene, tetracene); ○, *p*-polyphenyls (benzene, biphenyl, *p*-terphenyl, *p*-quaterphenyl).

Fig. 3. Dependence of the specific retention volumes relative to 1 g of packing on the extent of coating for substances with a five-membered ring in the molecule. ■, Acenaphthene; ▼, acenaphthylene; ●, fluorene; ▲, fluoranthene; △, 1,10-benzofluoranthene; ○, 8,9-benzofluoranthene; □, 1,2-benzofluoranthene and 2,3-benzofluoranthene; ▽, 20-methylcholanthrene.

silica gel that is not coated with the stationary liquid, on the coated surface and on the surface of the stationary liquid should be taken into consideration.

According to Kiselev's classification[21], silica gel belongs to the group of adsorbents of the second type, which have surface acidic hydroxy groups, and Tris III to the molecules of group B, which have a locally concentrated density of electrons. The deposition of Tris III molecules on the surface of silica gel is therefore accompanied by the formation of hydrogen bonds between the surface hydroxy groups of the silica gel and ether oxygen atoms or cyano end-groups of the Tris III molecules. From the conformational viewpoint, it is necessary for Tris III molecules to be orientated parallel with the surface. With this type of deposition, the thickness of the layer adsorbed, determined by the size of the most bulky methyl groups, is 4.6 Å. On the silica gel used, with a specific area of 432 m^2/g, the volume of the layer adsorbed is 0.199 ml. Complete coverage of this layer with Tris III with a specific gravity of 1.109 (at 20°) is achieved with a coating of 22% (w/w).

In interactions between the molecules of Tris III, the formation of hydrogen bonds cannot occur. Intramolecular interactions of Tris III are therefore infrequent compared with intermolecular interactions of the stationary liquid with the silica gel surface. As a consequence, a second layer of molecules of the stationary phase can be formed only after a complete coverage of the silica gel with Tris III molecules. Hence adsorption on an uncoated surface of silica gel can take place at coverages less than 22% (w/w) only.

During the chromatographic process, PAH can be dissolved in both the mobile and stationary phases. From the viewpoint of the establishment of adsorption equilibria, both liquids act as solvents and there is no substantial difference in their functions. On dissolving PAH in either of the two liquid phases, strong specific interactions between the solute and solvent do not occur. The same applies to the solvation of the Tris III molecule in the mobile phase and approximately also to solvation in the stationary liquid. The contributions of solvation can therefore be neglected according to Snyder[22]. The possibility of PAH adsorption on the silica gel surface coated with Tris III from either of the two liquids then determines adsorption interactions only. Only relatively weak specific interactions with the surface groups of silica gel[21] originate in the adsorption of the PAH that have free electron pairs of π-bonds in their molecules. In contrast, very strong specific interactions (hydrogen bond formation) occur in the adsorption of Tris III. The standard free energy of adsorption of PAH on the silica gel surface is therefore always less than the standard free energy of adsorption of Tris III and their difference, $-\Delta G_a^0$, must be negative. It follows from the relationship[22]

$$\log K_{\mathrm{th}} = -\Delta G_a^0/2.3\ RT$$

where K_{th} is the thermodynamic equilibrium constant, R is the gas constant and T the absolute temperature, that the adsorption of PAH on the silica gel surface covered with Tris III cannot occur.

Adsorption on the surface of the stationary liquid is therefore the only mechanism that evokes the retention if the silica gel is covered with a monomolecular layer of the stationary liquid. It follows, however, from a comparison of the retentions at an extent of coating greater than 22% with the contribution of dissolution (Table IV) that adsorption on the surface of the stationary liquid plays a significant role even at other extents of coating. It follows further from the solubility coefficients of some PAH

TABLE IV

CONTRIBUTION OF DISSOLUTION TO THE TOTAL RETENTION ON SILICA GEL COATED WITH 40.7% AND 60.2% OF TRIS III

It was assumed in the calculation that the whole volume of the stationary phase that was coated on silica gel participates in the dissolution.

Compound	*Retention volume (ml/g of packing)*		*K_g (ml/g)*	*Contribution of dissolution to total retention (%)*	
	40.7% coating	*60.2% coating*		*40.7% coating*	*60.2% coating*
1,2-Benzopyrene	3.33	2.35	2.2	18	35
1,2,3,4-Dibenzanthracene	5.76	4.04	2.6	13	24

that were measured directly and from Table IV that dissolution contributes to the total retention at very high extents of coating only.

Efficiency

Changes in column efficiency owing to the coating were studied at a flow-rate of 0.41 ml/min. It follows from the results in Table V that the efficiencies of the columns packed with silica gel coated with 5.1, 9.8 and 20.2% of Tris III are virtually identical for a given solute. The slight differences are caused by experimental errors. The column efficiency decreases to approximately half for the silica gel coated with 40.7%

TABLE V

INFLUENCE OF THE EXTENT OF SILICA GEL COATING ON COLUMN EFFICIENCY

The results given are HETP values (in mm).

Sample No.	*Extent of coating*				
	5.1%	*9.8%*	*20.2%*	*40.7%*	*60.2%*
1	0.73	0.68	0.59	1.4	2.8
2	0.85	0.83	0.82	1.6	2.8
3	0.84	0.86	0.88	1.8	4.3
4	0.82	0.76	0.87	1.3	3.5
5	—	0.85	1.01	1.7	—
6	—	—	3.28	4.0	—
7	0.55	0.52	0.53	1.2	3.1
8	0.72	0.59	0.60	1.5	4.3
9	—	0.73	—	1.6	—
10	0.72	0.69	0.97	2.3	4.6
11	0.79	0.86	0.84	1.9	—
12	0.88	0.69	0.82	1.8	5.3
13	0.92	0.75	1.02	1.8	5.4
14	0.84	0.81	0.78	2.0	—
15	0.84	0.85	0.89	1.7	5.5
16	0.80	0.73	0.84	1.8	4.8
17	0.92	0.79	0.70	1.9	5.8
18	1.0	0.70	0.87	1.6	4.8
19	0.97	0.85	0.80	1.8	5.6
20	0.90	0.85	0.90	1.9	5.5
21	—	—	0.77	1.5	—
22	0.95	0.94	0.93	2.1	6.0
23	0.91	0.79	0.79	1.7	4.2
24	1.04	0.88	0.94	2.0	6.7
25	1.9	1.7	1.9	35	8.5
26	—	—	—	2.4	—
27	0.72	0.65	0.99	1.3	2.8
28	0.68	0.68	0.80	1.70	6.5
29	0.86	0.81	0.82	1.4	5.2
30	0.90	0.76	0.82	1.5	6.0
31	0.90	0.72	0.78	1.5	5.5
32	0.90	0.75	0.81	1.5	5.6
33	0.82	0.78	0.78	1.6	6.4
34	1.45	0.84	1.1	1.9	4.9
35	0.75	0.57	0.92	1.2	4.6
36	0.81	0.64	0.68	1.6	4.2
37	1.33	0.74	0.76	1.3	4.5

of Tris III. The HETP values vary between 1 and 2 mm (Fig. 4) for most of the solutes. As the content of the stationary liquid increases to 60.2%, the HETP increases to 3–7 mm for most of the substances. Having compared the results with those in Table II, it can be concluded that the impaired efficiency obtained for volumes of the stationary liquid that exceed monolayer coverage (over 22%) should be ascribed to the properties of the packing and not to the manner of its deposition in the chromatographic column.

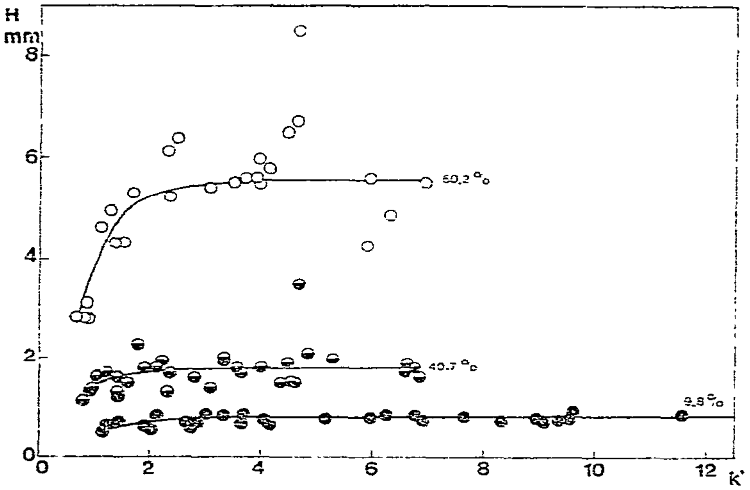


Fig. 4. HETP values (*H*) for various extents of coating (indicated on the curves) at a linear mobile-phase flow-rate of 0.6 cm/sec.

Measurement of solubility coefficients

For the direct measurement of the solubility coefficients, benzene, acenaphthylene, anthracene, 1,2-benzopyrene and 1,2,3,4-dibenzanthracene were selected as their retentions cover the whole range of the retentions of the set of samples being used. A low-surface-area support coated with the greatest possible amounts of the stationary liquid was selected in order to suppress the adsorption contributions. The correction for adsorption on the stationary liquid surface is considerably less than the error of the measurement.

The retention volumes of the samples injected differed only slightly from the retention volume of nonane. The measurements provided acceptable results for 1,2-benzopyrene and 1,2,3,4-dibenzanthracene only. Values of the solubility coefficient of 2.2 ± 0.4 and 2.6 ± 0.3 ml/g were found for 1,2-benzopyrene and 1,2,3,4-dibenzanthracene, respectively.

DISCUSSION

It follows from the mechanism of the retention of PAH that the measured values must be correlated with surface areas and the properties of the surface in order to interpret the results.

The extent of coating affects firstly the total surface area of the packing, which is the sum of the surface areas of the film of the stationary liquid and the uncoated silica gel surface (Fig. 5). The effect of the surface area on the retention values can be eliminated by relating the surface area to 1 m^2. However, even then the retention values of all of the PAH decrease as the amount of the stationary phase increases (Fig. 6). This decrease can be explained by the differences in the adsorption activities of the uncoated silica gel surface and the Tris III-coated surface and by the changes in their relative proportions of the total surface area (Fig. 5). The increase in the relative specific retention volumes relative to 1 m^2 with an increase in the extent of coating over 22% can be explained by the contribution of dissolution. It follows from the measurements of the partition coefficients and their values for 1,2-benzopyrene and 1,2,3,4-dibenzanthracene that the values for the other solutes tested also will be very low compared with the values common in the dissolution systems. The value of the contribution of dissolution increases as the extent of coating increases, while the surface area of the stationary phase, and hence also of the contribution of adsorption to the total retention, decreases[22]. Hence the relative contribution of dissolution to the total retention increases. It is equal to the difference between the value of the specific retention volume relative to 1 m^2 for the coating of 22% which lies in the minimum of the dependence (Fig. 6) and the value calculated for a given higher extent of coating.

It is known[23] that in suitable adsorption systems the establishment of partition

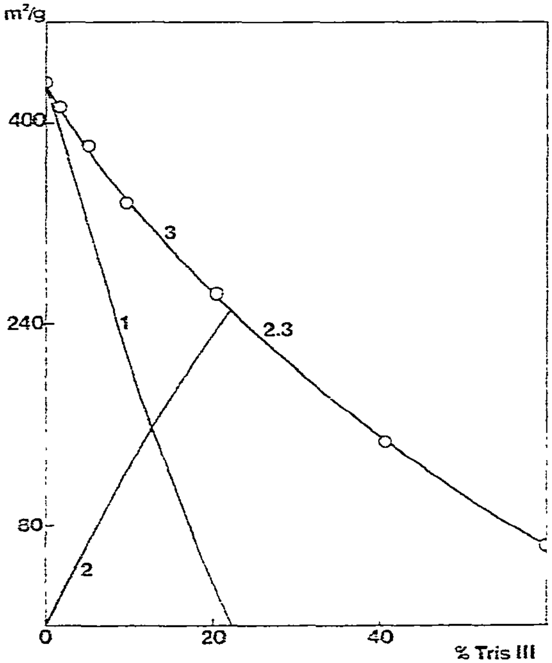


Fig. 5. Change in the specific surface area of silica gel at various extents of coating with Tris III. 1, Fraction of surface area of silica gel that was not coated with Tris III; 2, surface area of the surface created by coating with Tris III; 3, total surface area of the coated silica gel. ○, Experimental values.

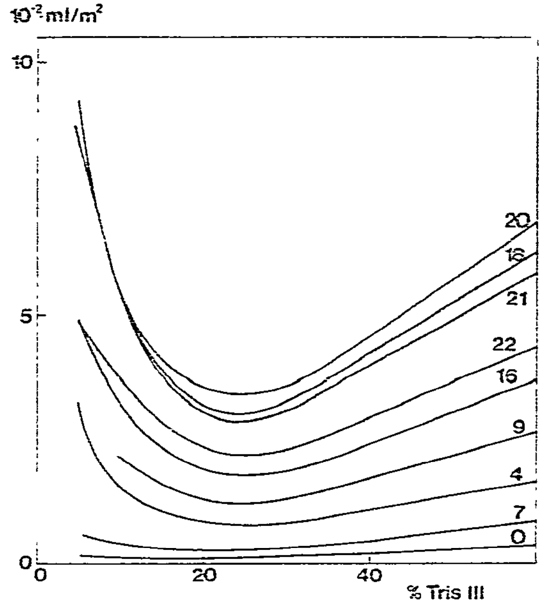


Fig. 6. Dependence of the specific retention volume relative to 1 m^2 on the extent of coating. The numbers on the curves identify solutes listed in Table I.

equilibria is controlled only by diffusion, so that the HETP values for various solutes at a given flow-rate are merely a function of the values of the diffusion coefficients in the mobile phase. They do not depend on the capacity factors.

It is obvious from Table VI that the correlation of the HETP values with the diffusion coefficients is much more justified than the correlation with the capacity factors. An apparent dependence of the HETP values on *k'*, for substances with small molecules, results from the fact that the increase in the size of the molecule (connected with the increased retention) is accompanied by a significant change in the diffusion coefficient, *D*. The difference between the effects of *D* and *k'* on the HETP values can be seen for substances with a larger number of rings in their molecules, the diffusion coefficients of which differ only slightly with the size of the molecule. Evidence against the dependence of the HETP values on *k'* is also provided by the fact that significant differences can be found in the retention values of isomeric solutes with the same number of rings whereas the diffusion coefficients calculated according to the Wilke–Chang equation[19] either change only slightly or not at all.

CONCLUSIONS

Tris III-coated silica gel distinguishes well structural differences in PAH that have small molecules. The resolving power of the column decreases as the size of the solute molecule increases and only a group separation can be obtained. The retention value is determined mainly by the adsorption on the surface of the stationary liquid and on the uncoated silica gel surface. As a consequence of the differences in the quality of the surfaces of silica gel and Tris III and of their relative contents at different ex-

TABLE VI

RELATIONSHIP BETWEEN THE DIFFUSION COEFFICIENTS (*D*) AND HETP

Number of rings	*Compound*	$V_b^{0.6}$ *(ml/mole)**	$D \cdot 10^5$ *(cm²/sec)*	*9.8% coating*		*40% coating*	
				HETP (mm)	*k'*	*HETP (mm)*	*k'*
1	Benzene	15.52	3.34	0.4	0.37	0.9	0.39
2	Naphthalene	20.02	2.56	0.5	1.14	1.2	0.787
2	Biphenyl	22.06	2.35	0.8	1.43	1.4	0.924
3	*o*-Terphenyl	28.98	1.79	0.8	2.12	1.8	1.23
3	*m*-Terphenyl	28.98	1.79	0.9	3.70	1.3	1.92
3	*p*-Terphenyl	28.98	1.79	0.8	4.07	1.7	2.26
3	Anthracene	23.78	2.18	0.6	2.72	1.5	1.60
4	*o,o'*-Quaterphenyl	31.20	1.66	0.9	3.00	1.7	1.92
4	*p,p'*-Quaterphenyl	31.20	1.66	—	9.7	4.0	4.02
4	Tetracene	27.52	1.88	0.73	5.9	1.6	—
4	Pyrene	25.00	2.08	0.7	3.65	1.8	2.12
4	1,2-Benzanthracene	27.52	1.88	0.9	6.27	1.9	3.37
4	Chrysene	27.52	1.88	0.8	6.92	1.8	3.56
4	Triphenylene	27.52	1.88	0.9	6.77	1.7	3.67
4	Fluoranthene	24.18	2.14	0.7	4.16	1.7	2.34
5	1,2-Benzopyrene	28.47	1.88	0.7	8.33	1.8	4.00
5	Benzofluoranthene	27.74	1.87	0.8	9.30	1.5	4.51
5	1,2,5,6-Dibenzanthracene	30.80	1.69	0.7	14.6	1.6	6.85
5	1,2,3,4-Dibenzanthracene	30.80	1.69	0.9	14.9	1.7	7.57
5	3,4-Benzotetraphene	30.80	1.69	0.8	14.4	1.7	6.61
5	Perylene	28.46	1.82	0.9	9.63	2.1	4.82
6	1,12-Benzoperylene	29.39	1.76	0.9	11.56	2.0	5.24
7	Coronene	33.88	1.45	1.7	13.4	3.5	5.65

* V_b is the molar volume of the compound at its boiling point. According to Wilke–Chang's equation[19] D is inversely proportional to the quantity $V_b^{0.6}$.

tents of coating, the selectivity of the separation can be influenced by the amount of the stationary liquid present.

From the point of view of the speed of separation (time of analysis), silica gel with the surface almost completely coated with the stationary liquid is the most suitable. It has a sufficient selectivity at relatively low retentions for most of the solutes. It is the least sensitive of all the materials with high efficiencies to the precision of the preparation and changes that can be caused by the passage of the mobile phase. The column efficiency does not depend on *k'*, which, in comparison with the dissolution systems, is a practical advantage, particularly in separating solutes with high retentions.

The chromatographic system used belongs to the systems that are often used in the liquid chromatography: a non-polar mobile phase is combined with a highly polar stationary phase coated on a polar adsorbent that has a very strong adsorption activity. It follows from the results of the measurements that a merely formal analogy of the chromatographic systems based on the deposition of the stationary liquid on a solid phase is not sufficient to be able to draw the conclusion that the partition mechanism of dissolution is the controlling mechanism in both instances. On the contrary, it suggests that in liquid chromatography, where adsorbents with large surface areas are often used as supports for the stationary phases, the adsorption mechanism may occur much more frequently than is generally supposed.

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