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## ADJUSTMENT OF THE CAPACITY FACTOR BY MEANS OF THE SPECIFIC SURFACE AREA OF THE ADSORBENT FOR OPTIMAL SEPARATION IN LIQUID-SOLID CHROMATOGRAPHY

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### SUMMARY

In the optimization of a chromatographic separation with respect to resolution, speed and dilution, the capacity factor is an important parameter. Its influence on the chromatographic characteristics is discussed theoretically.

The capacity factor is determined by the distribution coefficient and the phase ratio. Only the phase ratio can be used, however, to adjust the value of the capacity factor without affecting the value of the selectivity coefficient at the same time. Micro-particulate silica and octyl silica with different specific surface areas were investigated experimentally as adsorbents for liquid-solid chromatography. It is shown that the selectivity of these materials is fairly constant for specific surface areas varying over two orders of magnitude.

The effect of the adjustment of the specific surface area is demonstrated in the separation of dyestuffs and polynuclear aromatic hydrocarbons by high-pressure liquid chromatography. The application of column switching for optimal separation using two or three columns packed with the same adsorbent but with different specific surface areas is shown.

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### INTRODUCTION

One of the process parameters that has to be adjusted in order to achieve optimal results in chromatography is the phase ratio. In general, the resolution decreases and both the separation time and the concentration of the sample increase if the phase ratio decreases. In practice, the phase ratio can be varied over two orders of magnitude.

In liquid-liquid chromatography (LLC), the phase ratio is adjusted by the degree of loading of the solid support with stationary liquid. There exist a minimal and a maximal loading<sup>1,2</sup>; the maximal loading is determined by the fraction of the pore volume of the fixed bed that is not reached by the flow, and the minimal loading is given by the minimal amount of stationary liquid required to achieve a complete

liquid coating on the surface of the solid support. In liquid–solid chromatography (LSC), the phase ratio depends on the specific surface area and to a minor extent on the specific volume of the adsorbent.

The potential of the systematic use of adsorbents with the same chemical nature but different specific surface areas has not been fully exploited so far in chromatography. In this paper, the results of a study on the adjustment of the phase ratio in LSC by using microparticulate adsorbents with different specific surface areas are presented. Both hydrophilic and hydrophobic adsorbents were used.

## THEORETICAL

In chromatography, the capacity factor appears in the expressions for the resolution, the separation time and the sample dilution. For a given type of phase system the capacity factor can be adjusted by means of the phase ratio.

### *Dependence of the resolution on the capacity factor*

The chromatographic resolution,  $R_{ji}$ , describing the degree of separation of two components  $j$  and  $i$ , is given by the expression

$$R_{ji} = \frac{t_{Rj} - t_{Ri}}{\sigma_{ii}} = (r_{ji} - 1) \frac{\kappa_i}{(1 + \kappa_i)} \left( \frac{L}{H_i} \right)^{\frac{1}{2}} \quad (1)$$

where

- $t_{Rj}, t_{Ri}$  = retention times of component  $j$  and  $i$ , respectively;
- $\sigma_{ii}$  = standard deviation of the elution peak of component  $i$ ;
- $r_{ji} = K_j/K_i$  = selectivity coefficient, that is the ratio of the distribution coefficients,  $K_j$  and  $K_i$ , of the two components  $j$  and  $i$ ;
- $\kappa_i = qK_i$  = capacity factor (distribution ratio) of component  $i$ , which is given by the distribution coefficient,  $K_i$  (determined by the chemical nature of the phase system) and the phase ratio,  $q$ .

In liquid–liquid systems, the phase ratio is the volume ratio of the two liquid phases. In liquid–solid systems, the phase ratio is the surface area to volume ratio of the adsorbent and the liquid phase;

- $L$  = length of the column;
- $H_i$  = theoretical plate height of the column for component  $i$ . It depends on, among other things, the capacity factor.

It can be seen that the expression for the chromatographic resolution contains three terms: the selectivity term  $(r_{ji} - 1)$ , the retardation term  $\kappa_i/(1 + \kappa_i)$  and the dispersion term  $(L/H_i)^{\frac{1}{2}}$ . In the discussion of the influence of the capacity factor, it has to be taken into account that the theoretical plate height also depends on this parameter<sup>3-7</sup>:

$$H_i = H_{Mi} + \underbrace{a_{fi} \cdot \frac{(1 + \kappa_i - \epsilon_f/\epsilon_m)^2}{(1 + \kappa_i)^2}}_{H_{Ef}} + \underbrace{a_{bi} \cdot \frac{\epsilon_f/\epsilon_m (1 + \kappa_i - \epsilon_f/\epsilon_m)}{(1 + \kappa_i)^2}}_{H_{Eb}} \quad (2)$$

where

$H_{Mi}$  = contribution due to the two mixing phenomena, diffusion and convective mixing;

$a_{fi}, a_{bi}$  = factors combining all parameters (flow velocity, diffusion coefficient, particle diameter, geometrical numbers) except the capacity factor. The theoretical plate height term  $H_{Ef}$ , with the factor  $a_{fi}$  describes the effect of the mass exchange process in the flowing fluid  $f$ , and the term  $H_{Eb}$  with the factor  $a_{bi}$  the effect of the mass exchange process in the fixed bed  $b$ ;

$\varepsilon_m, \varepsilon_f$  = fractions of the column cross-section that are occupied by the total mobile phase  $m$  and the flowing part  $f$  of the mobile phase (in general, a proportion  $\varepsilon_m - \varepsilon_f$  of the mobile phase is stagnant in the pores of the fixed bed).

As can be seen from eqn. 2, the contribution of the mass exchange in the fluid stream increases monotonously with the capacity factor, approaching a constant value. The contribution of the mass exchange in the fixed bed has a maximum at a certain value of the capacity factor. The overall dependence of the theoretical plate height on the capacity factor is determined by the relative magnitude of the factors  $a_{fi}$  and  $a_{bi}$ . In Fig. 1 it can be seen that the overall curve rises monotonously to a limiting value if the term for mass exchange in the flowing fluid dominates. Otherwise, a maximum appears at a certain value of the capacity factor. This behaviour has been verified experimentally<sup>6</sup>.

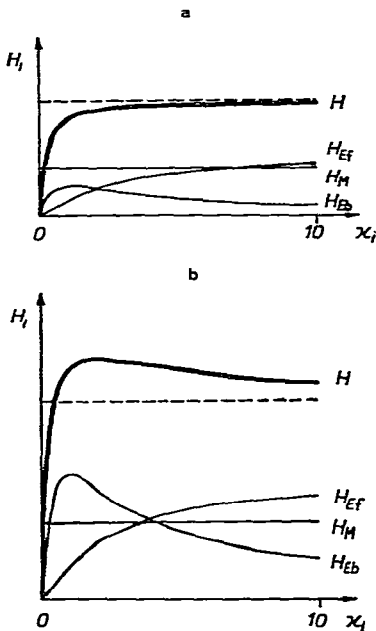


Fig. 1. Plot of the theoretical plate height as function of the capacity factor. The curves are calculated according to eqn. 2 using sets of values for the constants which are found in practice: (a)  $H_M = 20 \mu\text{m}$ ,  $a_f = 25 \mu\text{m}$ ,  $a_b = 50 \mu\text{m}$ ,  $\varepsilon_f/\varepsilon_m = 0.5$ ; (b)  $H_M = 30 \mu\text{m}$ ,  $a_f = 50 \mu\text{m}$ ,  $a_b = 200 \mu\text{m}$ ,  $\varepsilon_f/\varepsilon_m = 0.5$ .

By combining eqns. 1 and 2, an expression describing the dependence of the resolution on the capacity factor is derived:

$$R_{ji} = \frac{(r_{ji} - 1) L^{\pm}}{\left[ H_{Mi} \left( \frac{1 + \kappa_i}{\kappa_i} \right)^2 + a_{fi} \cdot \frac{(1 + \kappa_i - \epsilon_f/\epsilon_m)^2}{\kappa_i^2} + a_{bi} \cdot \frac{\epsilon_f/\epsilon_m (1 + \kappa_i - \epsilon_f/\epsilon_m)}{\kappa_i^2} \right]^{\pm}} \quad (3)$$

The phase ratio,  $q$ , can be adjusted by means of the liquid loading of the solid support in LLC or the specific surface area of the adsorbent in LSC. A change in the phase ratio also affects in principle the factor  $a_{bi}$ , but this is a minor effect in comparison with the influence on the capacity factor.

From eqn. 3, it can be seen that the resolution increases with increasing capacity factor, approaching a limiting value  $(r_{ji} - 1) L^{\pm} / [H_{Mi} + a_{fi}]^{\pm}$ . This is illustrated in Fig. 2, where the term  $\kappa_i / (1 + \kappa_i) H_i^{\pm}$ , which is equal to the reciprocal of the nominator in eqn. 3, is plotted as function of  $\kappa_i$ .

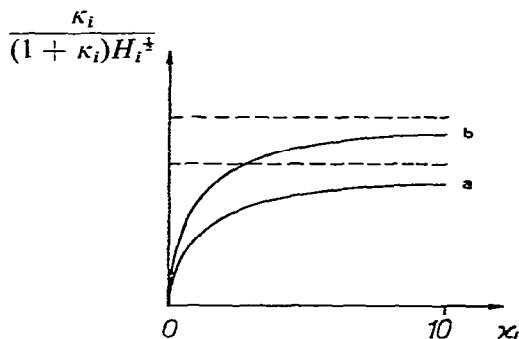


Fig. 2. Plot of the reciprocal of the term in the equation for the resolution against the capacity factor. The curves are calculated according to eqn. 3 and correspond to the theoretical plate height curves in Fig. 1.

#### *Dependence of the separation time on the capacity factor*

In the optimization of a chromatographic process, the resolution is considered together with other process characteristics such as the separation time. The time necessary to achieve a given minimal resolution for all components in the mixture is given by the equation<sup>8</sup>

$$t_{Rl} = \left( \frac{R_{(n+1)n}}{r_{(n+1)n} - 1} \right)^2 \left( \frac{1 + \kappa_n}{\kappa_n} \right)^2 \frac{H_n}{u} (1 + \kappa_l) \quad (4)$$

where

- $t_{Rl}$  = retention time of the last eluting component,  $l$ , of the mixture;
- $u$  = mobile phase velocity;
- $n, n + 1$  = the two most difficult to separate components of the mixture.

Substitution of eqn. 2 into eqn. 4 and replacement of  $\kappa_l$  by  $(\kappa_l/\kappa_n)\kappa_n = r_{ln}\kappa_n$  results in an expression which allows one to discuss the effect of the capacity factor on the separation time:

$$t_{RI} = \left( \frac{R_{(n+1)n}}{r_{(n+1)n} - 1} \right)^2 \left[ \frac{H_{Mn}}{u} \left( \frac{1 + \kappa_n}{\kappa_n} \right)^2 + \frac{a_{fn}}{u} \cdot \frac{(1 + \kappa_n - \varepsilon_f/\varepsilon_m)^2}{\kappa_n^2} + \frac{a_{bn}}{u} \cdot \frac{\varepsilon_f/\varepsilon_m (1 + \kappa_n - \varepsilon_f/\varepsilon_m)}{\kappa_n^2} \right] (1 + r_{In} \kappa_n) \quad (5)$$

At increasing mobile phase velocity, the terms  $H_{Mn}/u$  and  $a_{fn}/u$  decrease to zero and the term  $a_{bn}/u$  decreases to a constant value  $(a_{bn}/u)_{lim}$  (refs. 3-7). Therefore, the separation time  $t_{RI}$  decreases with increasing mobile phase velocity and approaches a limiting value, which decreases with increasing capacity factor to an ultimate limit of  $[R_{(n+1)n}/(r_{(n+1)n} - 1)]^2 (a_{bn}/u)_{lim} (\varepsilon_f/\varepsilon_m) r_{In}$ .

The capacity factor can be increased by increasing the phase ratio. In practice, the phase ratio can only be increased up to a given value and the limiting value of  $t_{RI}$  is approached only if the distribution coefficient is large enough to fulfil the condition  $\kappa_n \gg 1 - \varepsilon_f/\varepsilon_m$  and  $r_{In} \kappa_n \gg 1$  at the maximal attainable phase ratio.

#### *Dependence of the peak height on the capacity factor*

The broadening of the concentration peak of a component in the chromatographic process results in a decrease of its height. For a given amount  $Q_i$  of component  $i$ , the maximal concentration (peak height),  $(c_i^m)_{L \max.}$ , in the mobile phase  $m$  at the end  $L$  of the column, *i.e.* in the column effluent, is described by the expression<sup>3-7</sup>

$$(c_i^m)_{L \max.} = \frac{Q_i}{(2\pi)^{\frac{1}{2}} \varepsilon_m A (H_i L)^{\frac{1}{2}} (1 + \kappa_i)} \quad (6)$$

where  $A$  is the cross-sectional area of the column. Substitution of eqn. 2 into eqn. 6 gives an expression for the dependence of the height of the elution peak on the capacity factor:

$$(c_i^m)_{L \max.} = \frac{Q_i}{(2\pi)^{\frac{1}{2}} \varepsilon_m A L^{\frac{1}{2}} [H_{Mi}(1 + \kappa_i)^2 + a_{fi}(1 + \kappa_i - \varepsilon_f/\varepsilon_m)^2 + a_{bi}(\varepsilon_f/\varepsilon_m)(1 + \kappa_i - \varepsilon_f/\varepsilon_m)]^{\frac{1}{2}}} \quad (7)$$

This equation describes the dilution of the sample due to the chromatographic process, assuming that the width of the input peak of the sample is negligible in comparison with the width of the output peak. It can be seen from eqn. 7 that the peak height increases up to a limiting value with decreasing capacity factor.

From eqns. 3, 5 and 7, it can be concluded that a large value of the capacity factor is favourable with respect to the resolution and the separation time (provided that a sufficiently high flow velocity is attainable) but unfavourable with respect to the peak height. If the flow velocity is not high enough, the separation time will have a minimum at a certain value of the capacity factor, depending on the relative magnitudes of  $H_{Mi}$ ,  $a_{fi}$  and  $a_{bi}$ .

It should be emphasized that in liquid chromatography the theory has been experimentally verified only for a range of about two orders of magnitude with respect to the mobile phase velocity (0.1-10 mm·sec<sup>-1</sup>) and the capacity factor (0.5-50).

Extrapolations far beyond these ranges can cause serious errors as effects neglected in the present theory can become significant. However, the theoretical conclusions are useful for predicting trends for conditions that are not too far from the experimentally verified range.

## EXPERIMENTAL

### Apparatus

In the chromatographic experiments, three types of high-pressure liquid chromatographs with variable-wavelength UV detectors were used (Model S 200 with a PM 4 detector and Model S 100 with a PM 2 detector from Siemens, Karlsruhe, G.F.R., and Model 8500 from Varian Instruments Div., Walnut Creek, Calif., U.S.A., with a Model SF-770 detector from Schoeffel Instrument Corp., Westwood, N.J., U.S.A.).

For column switching, a high-pressure three-way switching valve (Siemens) was installed in all three chromatographs. The flow scheme of the column switching arrangements is shown in Fig. 3.

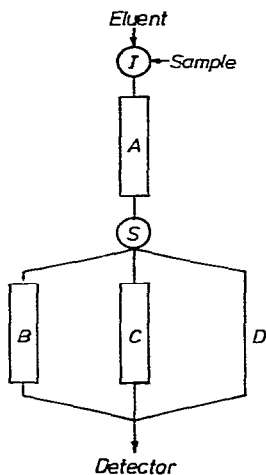


Fig. 3. Flow scheme of the column switching arrangement. I = Sample injection device; S = switching valve; A,B,C = chromatographic columns; D = capillary tubing.

### Chemicals

All experiments were carried out with pre-packed Hibar® columns (E. Merck, Darmstadt, G.F.R.) of dimensions  $250 \times 4$  or  $250 \times 3$  mm. The columns were packed with adsorbents of mean particle diameter  $10 \mu\text{m}$ . The following types of adsorbents were used: LiChrosorb Si 60, LiChrospher Si 100, Si 500, Si 1000 and Si 4000 silica (Merck) and LiChrospher 100 CH-8, 500 CH-8, 1000 CH-8 and 4000 CH-8 (Merck) octyl silica.

The eluents were prepared from chromatographic-grade solvents (LiChrosolv, Merck). For the hydrophilic silica adsorbents, *n*-heptane or toluene partially saturated with water was used as the eluent, and for the hydrophobic octyl silica adsorbents a mixture of methanol and water (doubly distilled).

## RESULTS AND DISCUSSION

In the optimization of a chromatographic separation, it is essential that the various process parameters can be adjusted independently. Consequently, in LSC the selectivity of the adsorbent should be independent of its specific surface area. In practice, the useful range of the capacity factor is about two orders of magnitude (0.5–50). On the one hand, the resolution vanishes if the capacity factor becomes too small, and on the other, both the separation time and the sample dilution (which affects the precision and the lower limit of detection) become too large if the capacity factor is too high. The capacity factors of the components of a sample mixture can be adjusted to the appropriate range by means of the phase ratio of the column.

A problem arises however, when the components of the sample are chemically very different. In such a case it is not possible to find a phase system that allows one to place all sample components in the useful capacity factor range by the appropriate choice of a fixed phase ratio. It is then necessary to adjust the phase ratio gradually during the separation process by column switching. Even when the sample components can be shifted into the useful capacity factor range by the choice of a fixed phase ratio, its gradual adjustment allows a better approach to the optimal separation characteristics.

*Interdependence of the selectivity and the specific surface area of adsorbents*

In order to examine the interdependence of the selectivity and the specific surface area of a given chemical type of adsorbent, the capacity factors of different compounds were determined for adsorbents with different specific surface areas but with the same chemical nature of the surface, using the same liquid phase. The capacity factor was determined from the retention time of the test component and the retention time of an unretarded tracer.

For the verification of the assumed independence of the adsorption coefficient of the specific surface area, a linear regression of the capacity factor and the specific surface area can be made. In adsorption chromatography, the phase ratio is determined by the specific surface area,  $\alpha_g$  ( $\text{m}^2 \cdot \text{g}^{-1}$ ), and the packing density,  $\rho$  ( $\text{g} \cdot \text{cm}^{-3}$ ), of the adsorbent:  $q = \alpha_g \rho / \varepsilon_m$  ( $\text{cm}^{-1}$ ). Therefore, a linear relationship exists between the capacity factor and the specific surface area provided that the adsorption coefficient, the packing density and the specific pore volume remain constant. The specific surface areas of the adsorbents were determined according to the B.E.T. nitrogen adsorption method. The results of the linear regression of a number of test compounds for silica are presented in Table I. It can be seen that there is a good linear relationship, which proves that the adsorption per unit surface area is independent of the specific surface area of the adsorbent.

In Table II, the results of another test are given for two octyl silica adsorbents with different specific surface areas. For a number of test compounds, the capacity factors and the selectivity coefficients of consecutive eluting components are given. The small variations in the values of the selectivity coefficients, which vary on average by only 2.9%, indicate that the selectivity of the two adsorbents is independent of their specific surface area, within the limits of experimental error. These results prove that the chemical properties of both surfaces are the same. The absolute values of the capacity factors have a ratio of 10.6:1, which reflects the ratio of the specific surface

TABLE I

## LINEAR REGRESSION OF THE CAPACITY FACTOR AND THE SPECIFIC SURFACE AREA

Column, Hibar,  $250 \times 4$  mm; stationary phase, silica,  $10 \mu\text{m}$ , LiChrospher Si 100, Si 500, Si 1000, Si 4000; mobile phase, *n*-heptane partially saturated to 30% with water; temperature,  $22^\circ$ .

Linear regression:  $\kappa_1 = (a_0 \pm s_0) + (a_1 \pm s_1)\alpha_g$

The specific surface area,  $\alpha_g$ , was measured by the BET method. The linearity is characterized by the correlation coefficient,  $r$ .

Compound	$a_0 \pm s_0$	$a_1 \pm s_1$	$r$	Adsorbent					
				Si 4000	Si 1000	Si 500	Si 100		
				$\alpha_g$ ( $\text{m}^2 \cdot \text{g}^{-1}$ )	$\kappa_1$	$\alpha_g$ ( $\text{m}^2 \cdot \text{g}^{-1}$ )	$\kappa_1$	$\alpha_g$ ( $\text{m}^2 \cdot \text{g}^{-1}$ )	$\kappa_1$
<i>m</i> -Diphenylene	$0.10 \pm 0.05$	$0.008 \pm 0.0004$	0.9962	8	0.16	25	0.32	280	2.40
<i>m</i> -Triphenylene	$0.25 \pm 0.16$	$0.019 \pm 0.0001$	0.9938		0.40		0.63		5.60
<i>m</i> -Tetraphenylene	$0.55 \pm 0.47$	$0.044 \pm 0.003$	0.9930		0.69		1.55		12.6
<i>m</i> -Pentaphenylene	$1.23 \pm 0.70$	$0.097 \pm 0.005$	0.9964		2.32		3.63		31.6



TABLE II

## CAPACITY FACTORS AND SELECTIVITY COEFFICIENTS ON ADSORBENTS WITH THE SAME CHEMICAL NATURE BUT DIFFERENT SPECIFIC SURFACE AREAS

Columns, octyl silica, 10  $\mu\text{m}$ , LiChrospher 1000 CH-8 or 100 CH-8; liquid phase, water-methanol (3:7); temperature, 20°. The capacity factor,  $\bar{\kappa}_n$ , is the mean value of four replicate measurements;  $s_{\bar{\kappa}_n}$  is its standard deviation.

Compound	n	$\bar{\kappa}_n \pm s_{\bar{\kappa}_n}$		$r_{(n+1)n}$	
		1000 CH-8	100 CH-8	1000 CH-8	100 CH-8
Benzene	1	0.13 $\pm$ 0.01	1.19 $\pm$ 0.01	1.85	2.18
Naphthalene	2	0.24 $\pm$ 0.01	2.60 $\pm$ 0.03	1.29	1.25
Acenaphthylene	3	0.31 $\pm$ 0.01	3.24 $\pm$ 0.06	1.52	1.61
Acenaphthene	4	0.47 $\pm$ 0.01	5.21 $\pm$ 0.05	1.06	1.05
Phenanthrene	5	0.50 $\pm$ 0.01	5.46 $\pm$ 0.02	1.10	1.09
Anthracene	6	0.55 $\pm$ 0.01	5.93 $\pm$ 0.02	1.35	1.27
Fluoranthene	7	0.74 $\pm$ 0.01	7.54 $\pm$ 0.08	1.09	1.11
Pyrene	8	0.81 $\pm$ 0.01	8.39 $\pm$ 0.12	1.42	1.43
2,3-Benzofluorene	9	1.15 $\pm$ 0.02	12.02 $\pm$ 0.02	1.02	1.01
Chrysene	10	1.17 $\pm$ 0.02	12.20 $\pm$ 0.02	1.06	1.12
1,2-Benzanthracene	11	1.24 $\pm$ 0.01	13.6 $\pm$ 0.2	1.49	1.41
Perylene	12	1.85 $\pm$ 0.03	19.3 $\pm$ 0.3	1.17	1.15
3,4-Benzopyrene	13	2.18 $\pm$ 0.03	22.1 $\pm$ 0.6	1.29	1.34
1,2,5,6-Dibenzanthracene	14	2.81 $\pm$ 0.03	29.6 $\pm$ 1.1	1.05	1.04
Picene	15	2.94 $\pm$ 0.05	30.8 $\pm$ 0.6	1.18	1.15
Indenopyrene	16	3.46 $\pm$ 0.03	35.3 $\pm$ 0.2		

areas of the two adsorbents. It seems feasible to develop a convenient method for the determination of specific surface areas based on these observations.

*Adjustment of a fixed phase ratio for optimal separation*

In solving the problem of a chromatographic separation, first a phase system has to be found that offers sufficient selectivity for all components of the mixture.

TABLE III

## LIPOHILIC DYESTUFFS SEPARATED

The numbers correspond to the sequence in the chromatograms. Several of the products consist of two major components, which are indicated by Roman numerals.

No.	Name	Manufacturer
1	Ceresviolett BRN I	Bayer
2	Ceresviolett BRN II	Bayer
3	Ceresschwarz G	Bayer
4	Ceresbraun B/I	Bayer
5	Fettgelb 3 G	Hoechst
6	Bleu VIF organol.J.N.	Francolor
7	Ceresrot G	Bayer
8	Ceresbraun RRN I	Bayer
9	Ceresbraun RRN II	Bayer
10	Ceresbraun B II	Bayer
11	Fettgelb 3 GL I	Sandoz
12	Fettgelb 3 GL II	Sandoz

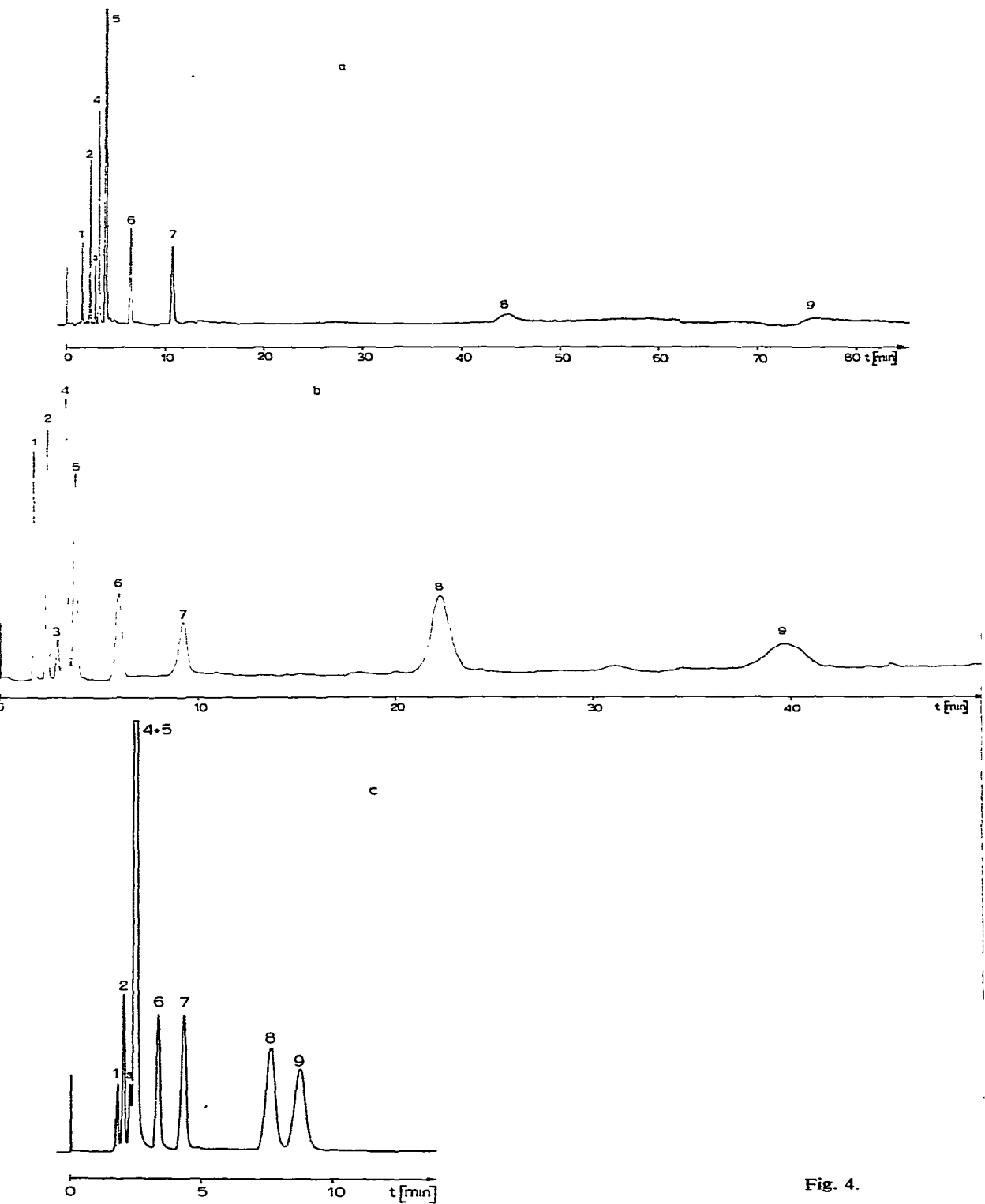


Fig. 4.

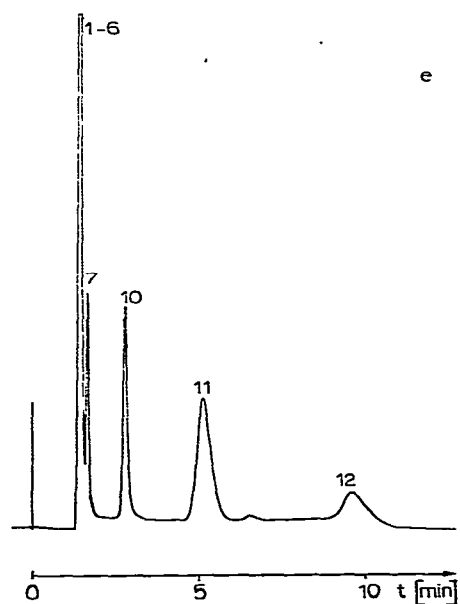
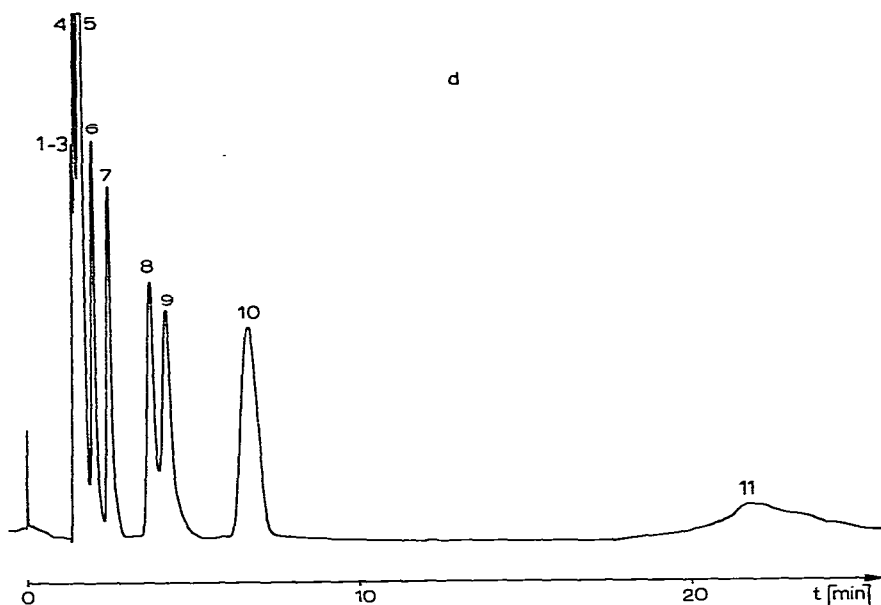


Fig. 4. Fixed adjustment of the phase ratio for silica columns. Sample, lipophilic dyestuffs (for identification, see Table III); injection volume,  $10 \mu\text{l}$ . Column, Hibar,  $250 \times 4 \text{ mm}$ ; stationary phase, silica,  $10 \mu\text{m}$ , (a) LiChrosorb Si 60, LiChrospher (b) Si 100, (c) Si 500, (d) Si 1000, (e) Si 4000; mobile phase, toluene saturated partially with water by means of a precolumn of LiChrosorb Si 60 with 6% (w/w) water; flow-rate,  $2 \text{ ml} \cdot \text{min}^{-1}$ ; pressure, 70 bar; temperature,  $22^\circ$ . Detector, UV absorbance at  $365 \text{ nm}$ .

TABLE IV

## CAPACITY FACTOR RANGE OF SILICA COLUMNS WITH DIFFERENT SPECIFIC SURFACE AREAS FOR LIPOPHILIC DYESTUFFS

Eluent, toluene partially saturated with water (same as in Fig. 4); temperature, 22°. The maximal and minimal values of the capacity factor within the range 0.5–50 are given.

Adsorbent	Dyestuff No.*	Capacity factor
LiChrospher Si 60	2	0.98
	8	38
LiChrospher Si 100	3	1.19
	9	30
LiChrospher Si 500	4	0.58
	11	33
LiChrospher Si 1000	6	0.50
	11	15
LiChrospher Si 4000	7	0.42
	12	6.7

\* Dyestuffs are numbered as in Table III.

Then the phase ratio has to be adjusted so as to bring the total mixture within a favourable range of the capacity factor.

For the separation of lipophilic dyestuffs (see Table III), it was found that silica offers sufficient selectivity for all compounds to be separated. Chromatographic experiments were carried out with silicas with different specific surface areas. The chromatograms are shown in Fig. 4. In Table II, the smallest and highest values of the

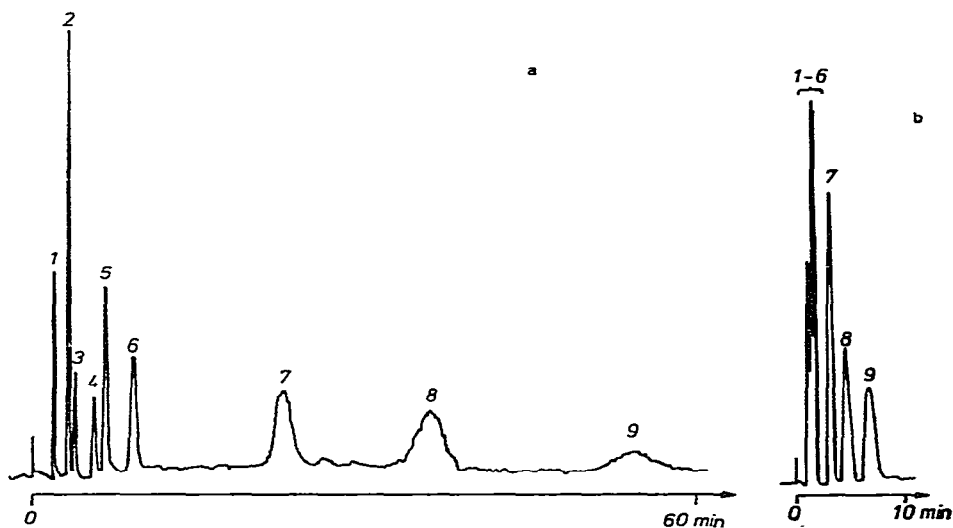


Fig. 5. Fixed adjustment of the phase ratio for octyl silica columns. Sample: (1) benzene; (2) naphthalene; (3) acenaphthylene; (4) acenaphthene; (5) anthracene; (6) pyrene; (7) 3,4-benzopyrene; (8) indenopyrene; (9) 2,3,5,6-dibenzofluoranthene. Injection volume, 50  $\mu$ l. Column, Hibar, 250  $\times$  3 mm; stationary phase, octyl silica, 10  $\mu$ m, LiChrospher (a) 100 CH-8, (b) 1000 CH-8; mobile phase, methanol-water (7:3); flow-rate, 1.5 ml  $\cdot$  min<sup>-1</sup>; pressure, 120 bar; temperature, 20°. Detector, UV absorbance at 254 nm.

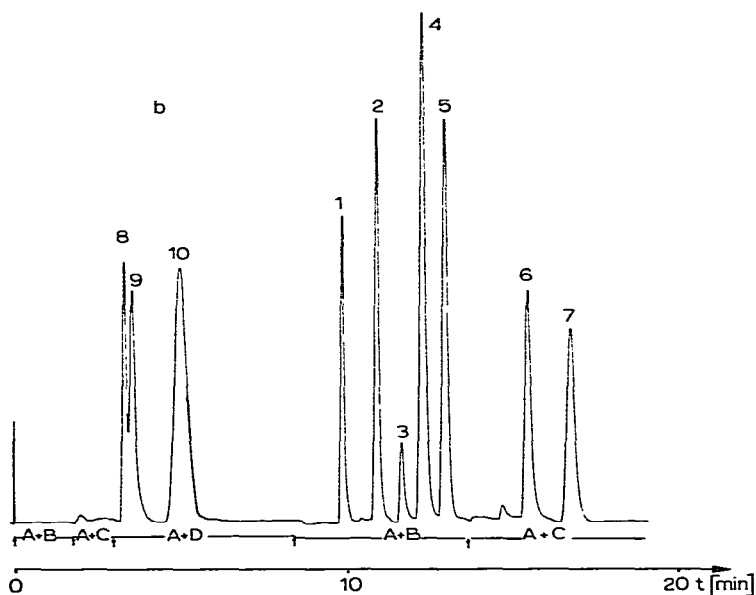
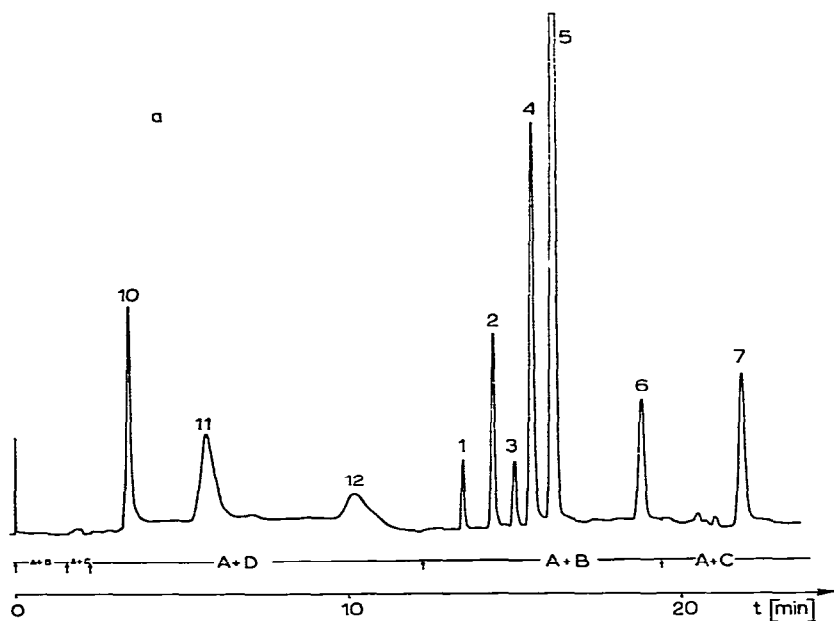


Fig. 6. Gradual adjustment of the phase ratio for silica columns by column switching. Sample components, as in Fig. 4. Columns, Hibar,  $250 \times 4$  mm; silica,  $10 \mu\text{m}$ , (A) LiChrospher (a) Si 4000, (b) Si 1000; (B) LiChrosorb Si 60; (C) LiChrospher Si 500; (D) capillary tubing; mobile phase, temperature and detector as in Fig. 4.

capacity factor measured on a certain adsorbent are given. From the data in Table IV and the chromatograms in Fig. 4, it can be concluded that at times only a fraction of the total number of compounds can be placed within the useful range of the capacity

factor. From the chromatograms it can be seen that components with too small values of the capacity factor are no longer resolved. On the other hand, components with too large values of the capacity factor can no longer be detected. In addition, the separation time becomes rather long.

For the separation of a number of polycyclic aromatic hydrocarbons, octyl silica has a satisfactory selectivity. The effect of the specific surface area for this type of adsorbent is demonstrated by the chromatograms in Fig. 5. It can be seen that with a suitable choice of the phase ratio, a given mixture can be separated within the useful capacity factor range (Fig. 5a). Beyond component 6, however, the distance between consecutive components is long, resulting in a long separation time and a reduced precision in the detection of the last component.

#### *Adjustment of a gradually changing phase ratio for optimal separation*

The best results in the optimization of a chromatographic separation by means of the phase ratio may be expected if the phase ratio is adjusted gradually during the separation. This can be performed in practice by column switching<sup>9</sup>. In LSC, this approach requires adsorbents with different specific surface areas.

The improvement resulting from the application of column switching in the separation of the dyestuffs can be seen from the comparison of the chromatograms in Figs. 6a and 6b with those in Figs. 4a–4e. It becomes possible in this manner to place more components within the useful capacity factor range. As a result of the gradual adjustment of the phase ratio, more components of the dyestuff mixture can be separated and detected and a shorter separation time is achieved.

The ideal solution of a separation problem in chromatography would be the elution of the components one immediately after the other with only a short distance

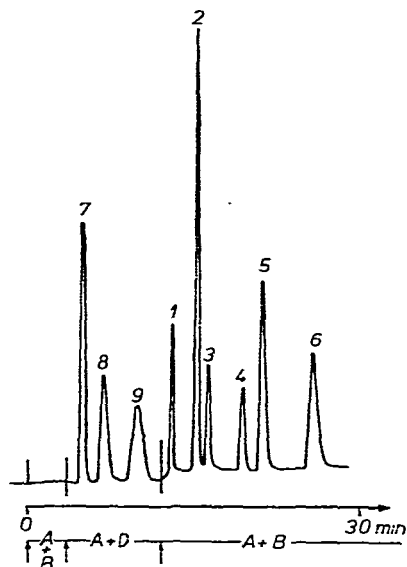


Fig. 7. Gradual adjustment of the phase ratio for octyl silica columns by column switching. Sample, as in Fig. 5. Columns, Hibar, 250 × 3 mm; stationary phase, octyl silica, 10 μm, LiChrospher (A) 1000 CH-8. (B) 100 CH-8. (D) capillary tubing; mobile phase, temperature and detector as in Fig. 5.

between them. The required distance is determined by the desired resolution. The exploitation of adsorbents with different specific surface areas by column switching offers a means of approaching this aim to a certain extent. The chromatogram in Fig. 7 shows how far the separation of polycyclic aromatic hydrocarbons can be improved in this manner compared with the results shown in Fig. 5. The distances between the peaks have been drastically reduced. As a result, the separation time is about half and the precision of detection of the last component is significantly increased.

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