

CHROM. 16,262

## BEHAVIOUR OF AROMATIC COMPOUNDS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON SIX KINDS OF SILICA GEL MODIFIED WITH FOUR TYPES OF PHENYL GROUPS

MITSUYOSHI OKAMOTO\*.\* and FUJIZO YAMADA

*Gifu Prefectural Institute of Public Health, Noishiki, 4 Chome, Gifu 500 (Japan)*

(First received August 15th, 1983; revised manuscript received September 1st, 1983)

---

### SUMMARY

The retention and selectivity behaviour of some aromatic hydrocarbons, parabens (*p*-hydroxybenzoic acid esters) and nitrochlorobenzene compounds were studied by high-performance liquid chromatography on 24 kinds of phenyl-modified silica gels, prepared with xylene solution containing phenyldimethylchlorosilane (P), diphenylmethylchlorosilane (D), triphenylchlorosilane (T) or benzyldimethylchlorosilane (B), using six kinds of silica gel with various mean pore diameters and/or specific surface areas. From elemental analysis data for carbon, the maximum number of accessible phenyl surface groups per 100 Å<sup>2</sup> of silica gel (mean pore diameter 108 Å, specific surface area 333 m<sup>2</sup>/g) in P, D, T and B gel was calculated to be 2.13, 1.90, 0.99 and 2.36, respectively. Using methanol-water mixtures as the eluent, aromatic hydrocarbons and parabens were separated on all the gels studied, but with different degrees of resolution. Nitrochlorobenzene compounds could not be separated on the P, D and T gels, but were separated on B gel and also on an octadecylsilica column.

---

### INTRODUCTION

In previous papers<sup>1-3</sup>, we postulated that the important parameters of silica gel with respect to the number of accessible alkylamino groups per 100 Å<sup>2</sup> in high-performance liquid chromatography (HPLC) are the mean pore diameter and the specific surface area. Also, we reported that the chromatographic properties of amino-modified high-performance thin-layer chromatographic plates depend largely on the length of the aminomethylene (alkylamino) chain bonded to the surface of the silica gel<sup>4</sup>. Phenyl-modified gels are important HPLC column materials but there are few reports<sup>5-7</sup> on HPLC on phenyl-bonded silicas in physical and chemical research. We have now studied the preparation and evaluation of the 24 kinds of mono-, di- and tri-phenyl- and benzyl-modified silica gels with various mean pore diameters and/or specific surface areas (Table I).

---

\* Present address: Prefectural Tajimi Hospital, Department of Pharmacy, 5-161, Maehata cho, Tajimi 507, Japan.

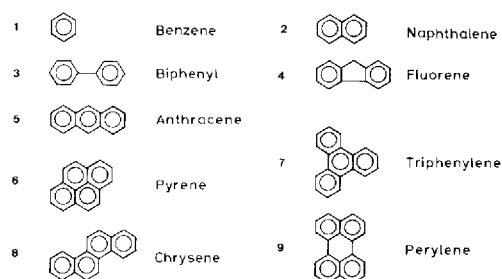


Fig. 1. Structures of PAHs used.

## EXPERIMENTAL

### Reagents

Polynuclear aromatic hydrocarbons (PAHs) (Fig. 1), ethyl, propyl and butyl paraben, *m*-nitroaniline and *o*- and *p*-nitrochlorobenzene were obtained from Wako (Osaka, Japan). Phenyltrimethylchlorosilane (P), diphenylmethylchlorosilane (D), triphenylchlorosilane (T) and benzyltrimethylchlorosilane (B) were purchased from Petrach Systems (PA, U.S.A.), and six kinds of highly microporous spherical silica gels differing in mean particle size, mean pore diameter, specific surface area and pore volume from Fuji-Davison (Nagoya, Aichi, Japan) (Table I). Octadecylsilica gel (Jasco SC-01) was purchased from Jasco (Tokyo, Japan). Methanol (Wako) was used after distillation. The other reagents and organic solvents were of analytical-reagent grade.

### Apparatus

The HPLC measurements were carried out using a Jasco Twinkle, Jasco Tri Rotar V or Jasco Tri Rotar SR-2 instrument equipped with a Jasco Uvidec-100 IV variable-wavelength detector.

### Stationary phase and elemental analysis

According to the previous method<sup>1-3</sup>, after 7 g of dried Fuji-Davison 1, 2, 3, 4, 5 or 6 had been added to 70 ml of a 3.4% solution of P in dry xylene and 3 ml of triethylamine, the silica gel suspension was refluxed for 5 h, filtered with a glass filter

TABLE I  
SILICA GELS USED

Silica gel*	Mean particle size ( $\mu\text{m}$ )	Mean pore diameter ( $\text{\AA}$ )	Specific surface area ( $\text{m}^2/\text{g}$ )	Pore volume ( $\text{ml/g}$ )
Fuji-Davison 1	5.8	108	333	0.90
Fuji-Davison 2	10.0	125	272	0.85
Fuji-Davison 3	9.0	150	224	0.84
Fuji-Davison 4	5.8	173	197	0.85
Fuji-Davison 5	9.0	187	180	0.84
Fuji-Davison 6	8.0	220	126	0.70

\* These names and serial numbers were assigned by the authors for convenience and are not commercial names.

TABLE II

SURFACE TREATMENTS AND ELEMENTAL ANALYSES FOR DIFFERENT FUJI-DAVISON SILICA GELS TREATED WITH PHENYLDIMETHYLCHLOROSILANE IN XYLENE

<i>Treated gel</i>	<i>Specific surface area (m<sup>2</sup>/g)</i>	<i>C found (%)</i>
Fuji-Davison 1-P	233	7.91
Fuji-Davison 2-P	211	7.29
Fuji-Davison 3-P	168	5.79
Fuji-Davison 4-P	147	5.17
Fuji-Davison 5-P	143	4.45
Fuji-Davison 6-P	116	3.92

(1  $\mu\text{m}$ ), washed several times with xylene, chloroform, methanol and acetone and then dried *in vacuo* at 70°C for 2 days, finally producing the silica gels for HPLC, which are listed as Fuji-Davison 1-P to 6-P, respectively, in Table II.

Also, after 7 g of dried Fuji-Davison 1, 2, 3, 4, 5 or 6 had been added to 70 ml of a 3.4% dry xylene solution of D, T or B, the same procedure as with the Fuji-Davison 1-P to 6-P series in Table II was carried out, producing Fuji-Davison 1-D to

TABLE III

SURFACE TREATMENTS AND ELEMENTAL ANALYSES FOR DIFFERENT FUJI-DAVISON SILICA GELS TREATED WITH DIPHENYLMETHYLCHLOROSILANE IN XYLENE

<i>Treated gel</i>	<i>Specific surface area (m<sup>2</sup>/g)</i>	<i>C found (%)</i>
Fuji-Davison 1-D	196	9.67
Fuji-Davison 2-D	187	9.05
Fuji-Davison 3-D	123	8.43
Fuji-Davison 4-D	107	6.32
Fuji-Davison 5-D	104	5.92
Fuji-Davison 6-D	87	4.89

6-D, 1-T to 6-T and 1-B to 6-B, which are shown in Tables III, IV and V, respectively. Hereafter, Fuji-Davison 1-P to 6-P, 1-D to 6-D, 1-T to 6-T and 1-B to 6-B will be referred to as "column gels".

The carbon contents of each column gel were determined by elemental analysis using an MT-3 Yanagimoto CHN elemental analyser, giving the data indicated as "Found" in Tables II-V. The specific surface areas of the column gels were determined

TABLE IV

SURFACE TREATMENTS AND ELEMENTAL ANALYSES FOR DIFFERENT FUJI-DAVISON SILICA GELS TREATED WITH TRIPHENYLCHLOROSILANE IN XYLENE

<i>Treated gel</i>	<i>Specific surface area (m<sup>2</sup>/g)</i>	<i>C found (%)</i>
Fuji-Davison 1-T	244	8.62
Fuji-Davison 2-T	236	7.73
Fuji-Davison 3-T	178	6.37
Fuji-Davison 4-T	167	5.57
Fuji-Davison 5-T	160	5.18
Fuji-Davison 6-T	124	4.61

TABLE V

SURFACE TREATMENTS AND ELEMENTAL ANALYSES FOR DIFFERENT FUJI-DAVISON SILICA GELS TREATED WITH BENZYL DIMETHYLCHLOROSILANE IN XYLENE

<i>Treated gel</i>	<i>Specific surface area (m<sup>2</sup>/g)</i>	<i>C found (%)</i>
Fuji-Davison 1-B	208	8.82
Fuji-Davison 2-B	194	7.85
Fuji-Davison 3-B	161	6.44
Fuji-Davison 4-B	148	5.65
Fuji-Davison 5-B	137	5.34
Fuji-Davison 6-B	114	4.25

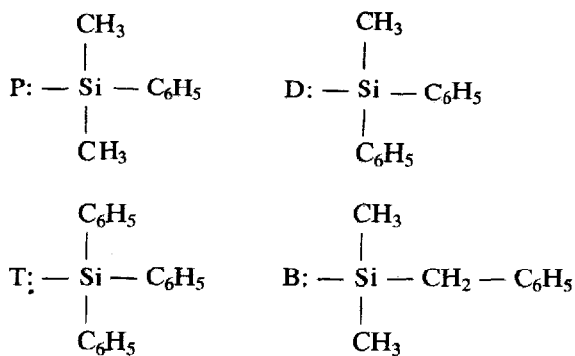
with an SA-1000 Shibata surface area pore volume analyser, giving the data in Tables II-V.

### Column preparation

The column gels were packed into stainless-steel columns (250 × 4.6 mm I.D.) using a balanced density method through a 30-ml stainless-steel packer at a rate of 450 kg/cm<sup>2</sup> with a Kyowa Seimitsu KHW-20 ultra-high-pressure pump.

### RESULTS AND DISCUSSION

From the elemental analysis of silica gel treated with P, D, T or B, the number of accessible phenyl, diphenyl, triphenyl or benzyl surface groups per gram or per 100 Å<sup>2</sup> of silica gel can be calculated by the following procedure. If P, D, T or B is substituted monofunctionally on silica gel, the surface structure of silica gel can be written as follows:



According to the previous methods<sup>1,2</sup>, the number of accessible phenyl surface groups per gram or per 100 Å<sup>2</sup> of silica gel surface is then given by

$$[(C/100) / 12.011 \cdot 8] \cdot 6.022 \cdot 10^{23} \quad (1)$$

or

$$[(C/100) / 12.011 \cdot 8] \cdot 6.022 \cdot 10^{23} / S \cdot 10^{18} \quad (2)$$

The number of accessible diphenyl surface groups per gram or per 100 Å<sup>2</sup> of silica gel surface is given by

$$[(C/100) / 12.011 \cdot 13] \cdot 6.022 \cdot 10^{23} \quad (3)$$

or

$$[(C/100) / 12.011 \cdot 13] \cdot 6.022 \cdot 10^{23} / S \cdot 10^{18} \quad (4)$$

The number of accessible triphenyl surface groups per gram or per 100 Å<sup>2</sup> of silica gel surface is given by

$$[(C/100) / 12.011 \cdot 18] \cdot 6.022 \cdot 10^{23} \quad (5)$$

or

$$[(C/100) / 12.011 \cdot 18] \cdot 6.022 \cdot 10^{23} / S \cdot 10^{18} \quad (6)$$

The number of accessible benzyl surface groups per gram or per 100 Å<sup>2</sup> of silica gel surface is then given by

$$[(C/100) / 12.011 \cdot 9] \cdot 6.022 \cdot 10^{23} \quad (7)$$

or

$$[(C/100) / 12.011 \cdot 9] \cdot 6.022 \cdot 10^{23} / S \cdot 10^{18} \quad (8)$$

where  $C$  = weight percentage of carbon,  $6.022 \cdot 10^{23}$  = Avogadro's number and  $S$  = specific surface area (m<sup>2</sup>/g) of treated silica gel.

Substitution of the values of  $C$  found by elemental analysis into eqns. 1–8 gives the number of accessible phenyl, diphenyl, triphenyl and benzyl surface groups per gram or per 100 Å<sup>2</sup> of silica gel surface, indicated in Tables VI, VII, VIII and IX, respectively.

As can be seen from these tables, an increase in the specific surface area of silica

TABLE VI  
NUMBER OF ACCESSIBLE PHENYL SURFACE GROUPS PER GRAM OR PER 100 Å<sup>2</sup>

<i>Column Gel</i>	<i>No. of accessible phenyl surface groups per gram</i> ( $\times 10^{21}$ )	<i>No. of accessible phenyl surface groups per 100 Å<sup>2</sup></i>
Fuji-Davison 1-P	0.496	2.13
Fuji-Davison 2-P	0.457	2.17
Fuji-Davison 3-P	0.363	2.16
Fuji-Davison 4-P	0.324	2.20
Fuji-Davison 5-P	0.282	1.97
Fuji-Davison 6-P	0.246	2.11

TABLE VII

NUMBER OF ACCESSIBLE DIPHENYL SURFACE GROUPS PER GRAM OR PER 100 Å<sup>2</sup>

<i>Column gel</i>	<i>No. of accessible diphenyl surface groups per gram (<math>\times 10^{21}</math>)</i>	<i>No. of accessible diphenyl surface groups per 100 Å<sup>2</sup></i>
Fuji-Davison 1-D	0.373	1.90
Fuji-Davison 2-D	0.349	1.87
Fuji-Davison 3-D	0.325	2.64
Fuji-Davison 4-D	0.244	2.28
Fuji-Davison 5-D	0.228	2.20
Fuji-Davison 6-D	0.189	2.17

TABLE VIII

NUMBER OF ACCESSIBLE TRIPHENYL SURFACE GROUPS PER GRAM OR PER 100 Å<sup>2</sup>

<i>Column gel</i>	<i>No. of accessible triphenyl surface groups per gram (<math>\times 10^{21}</math>)</i>	<i>No. of accessible triphenyl surface groups per 100 Å<sup>2</sup></i>
Fuji-Davison 1-T	0.240	0.99
Fuji-Davison 2-T	0.215	0.91
Fuji-Davison 3-T	0.177	1.00
Fuji-Davison 4-T	0.155	0.93
Fuji-Davison 5-T	0.144	0.90
Fuji-Davison 6-T	0.128	1.04

TABLE IX

NUMBER OF ACCESSIBLE BENZYL SURFACE GROUPS PER GRAM OR PER 100 Å<sup>2</sup>

<i>Column gel</i>	<i>No. of accessible benzyl surface groups per gram (<math>\times 10^{21}</math>)</i>	<i>No. of accessible benzyl surface groups per 100 Å<sup>2</sup></i>
Fuji-Davison 1-B	0.491	2.36
Fuji-Davison 2-B	0.437	2.25
Fuji-Davison 3-B	0.357	2.23
Fuji-Davison 4-B	0.315	2.13
Fuji-Davison 5-B	0.297	2.17
Fuji-Davison 6-B	0.237	2.08

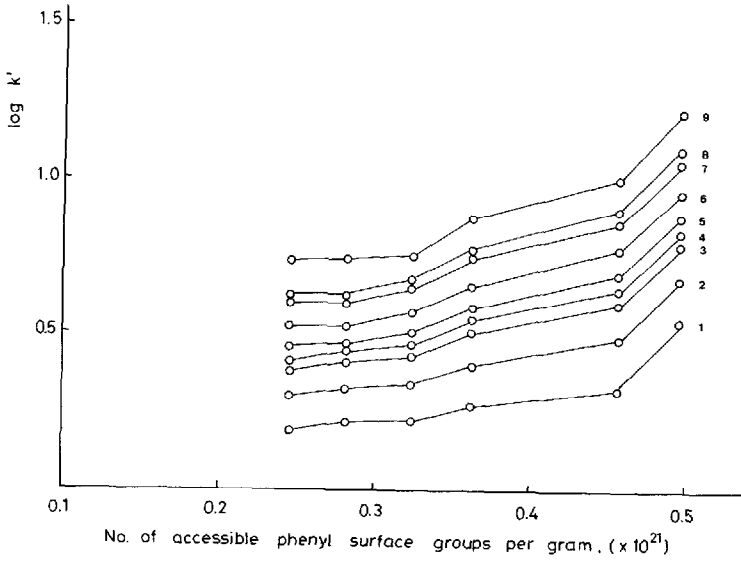


Fig. 2. Correlations between the capacity factors ( $k'$ ) of PAHs and the number of accessible phenyl surface groups per gram of silica gel. Columns: Fuji-Davison 1-P, 2-P, 3-P, 4-P, 5-P and 6-P. Mobile phase: methanol-water (70:30). Wavelength of UV detection: 260 nm. Flow-rate: 1.0 ml/min. Sample: the numbers refer to compounds in Fig. 1.

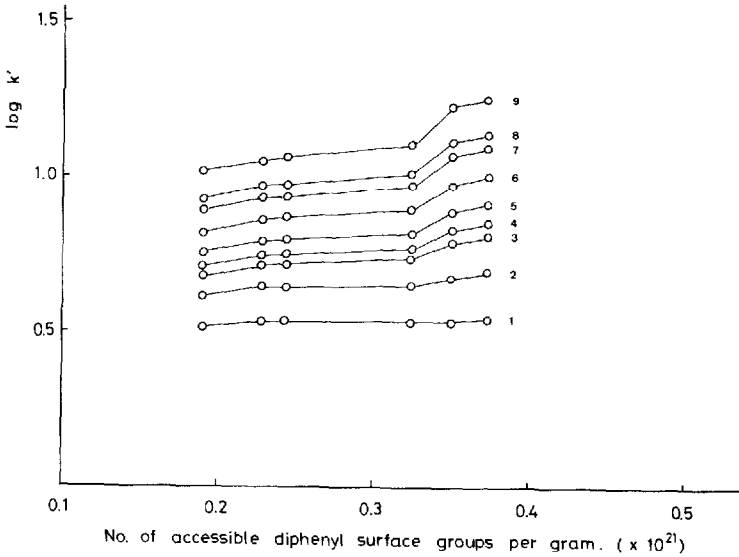


Fig. 3. Correlations between the capacity factors ( $k'$ ) of PAHs and the number of accessible diphenyl surface groups per gram of silica gel. Columns: Fuji-Davison 1-D, 2-D, 3-D, 4-D, 5-D and 6-D. Conditions as in Fig. 2.

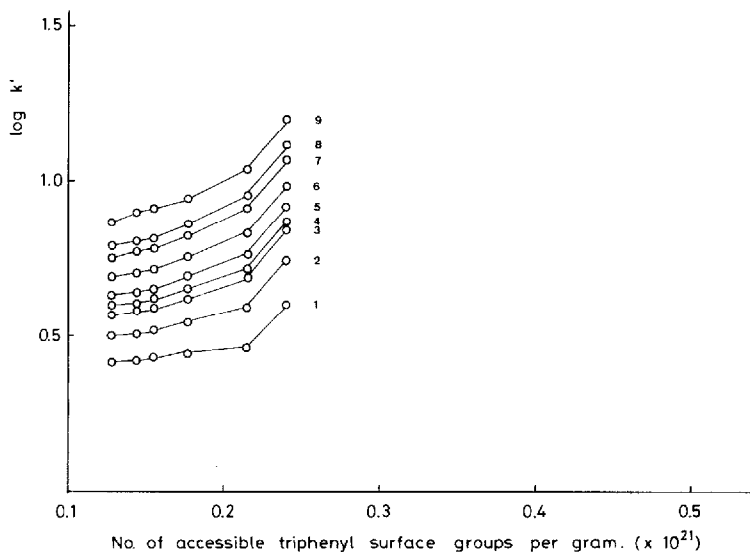


Fig. 4. Correlations between the capacity factors ( $k'$ ) of PAHs and the number of accessible triphenyl surface groups per gram of silica gel. Columns: Fuji-Davison 1-T, 2-T, 3-T, 4-T, 5-T and 6-T. Conditions as in Fig. 2.

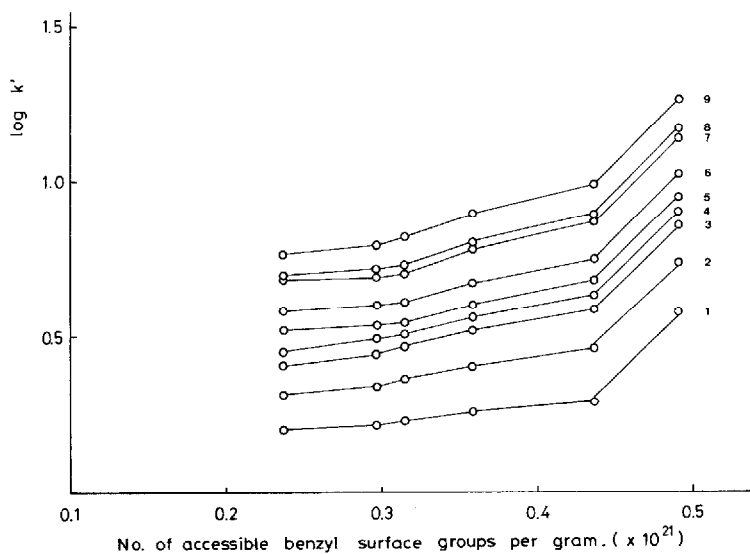


Fig. 5. Correlations between the capacity factors ( $k'$ ) of PAHs and the numbers of accessible benzyl surface groups per gram of silica gel. Columns: Fuji-Davison 1-B, 2-B, 3-B, 4-B, 5-B and 6-B. Conditions as in Fig. 2.



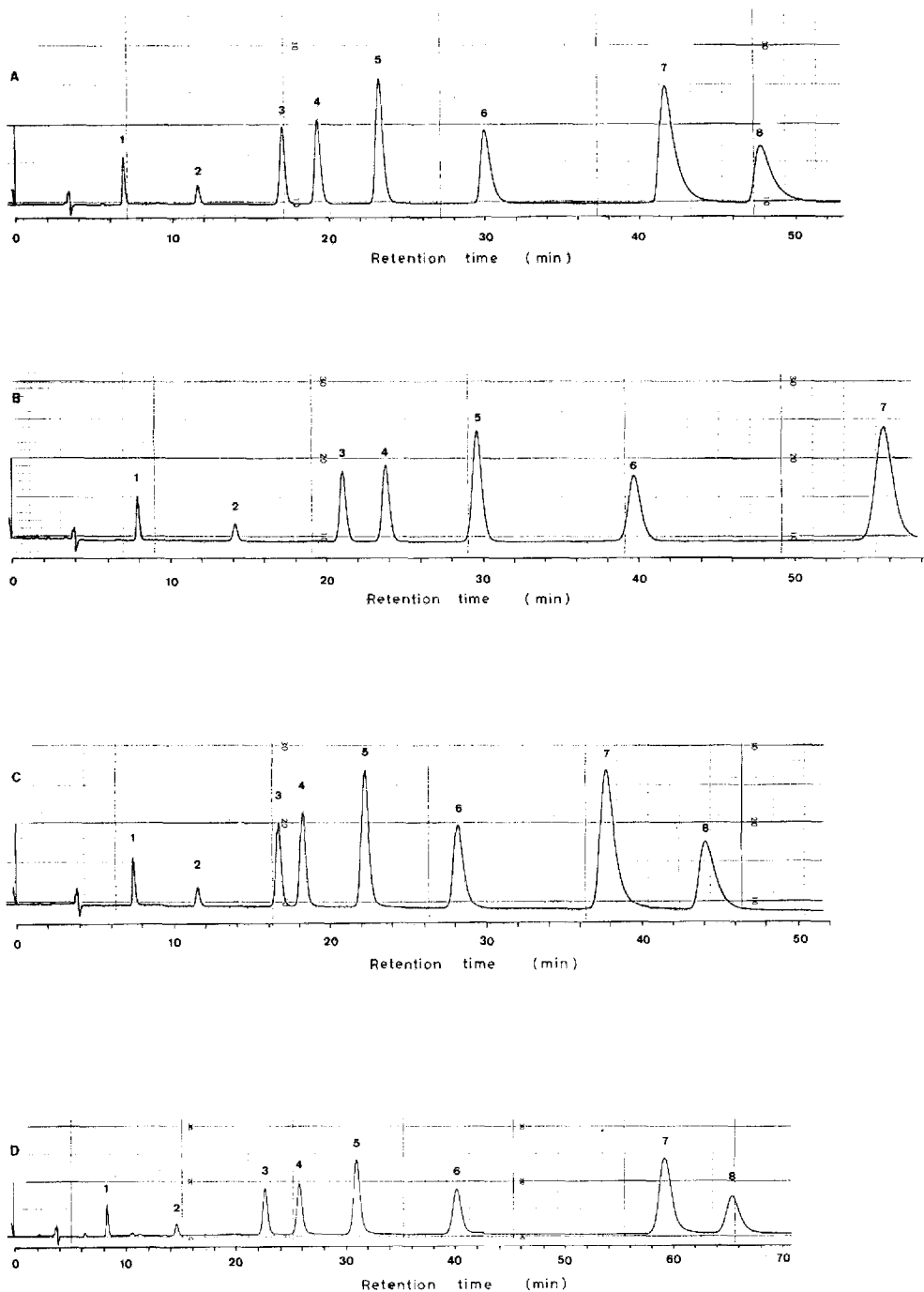


Fig. 6. Separation of PAHs on Fuji-Davison 1-P, 1-D, 1-T and 1-B. Columns: (A) 1-P; (B) 1-D; (C) 1-T; (D) 1-B (each  $250 \times 4.6$  mm I.D.). Mobile phase: methanol-water (60:40). Conditions as in Fig. 2. Sample: the numbers refer to compounds in Fig. 1.

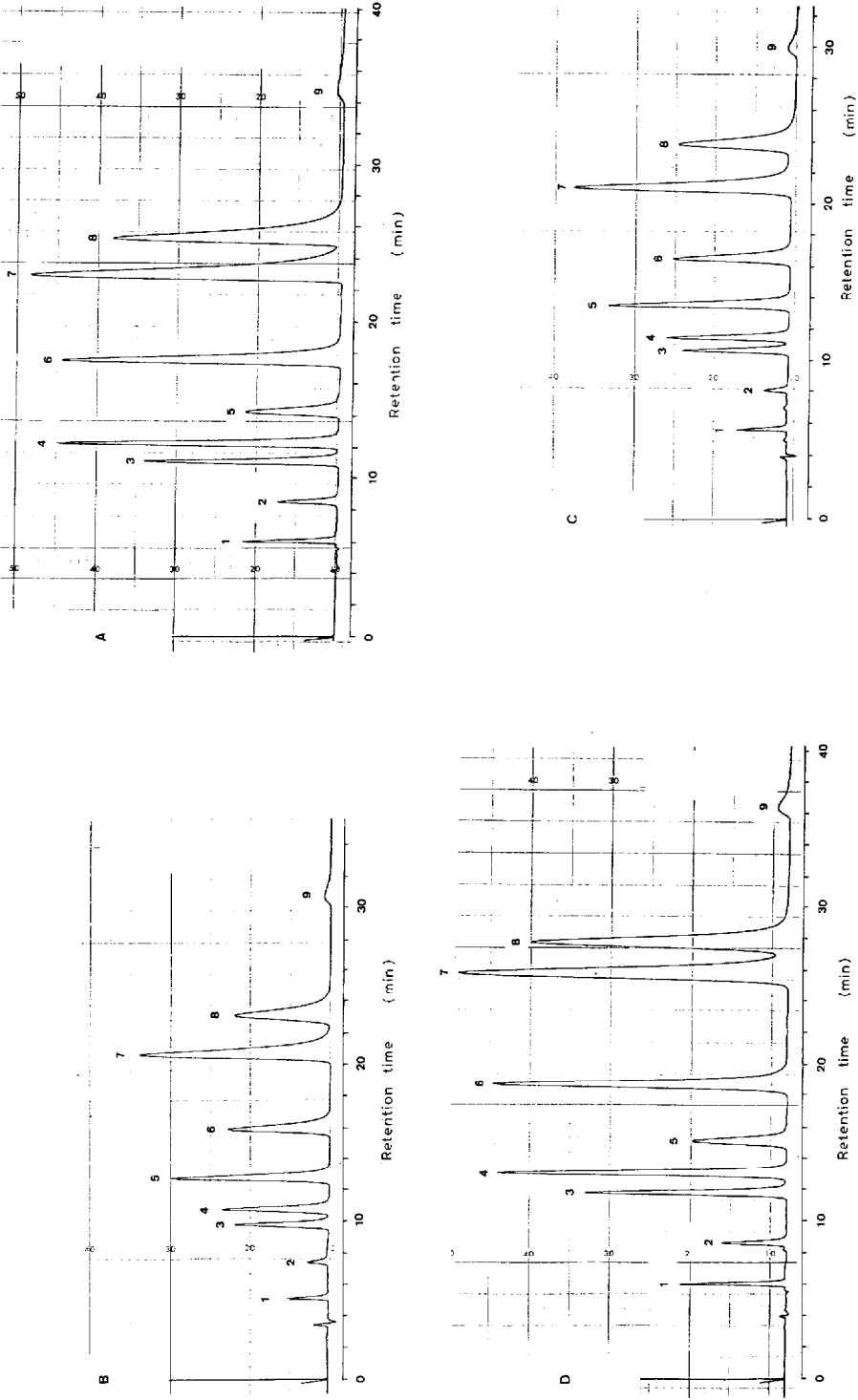


Fig. 7. Separation of PAHs on Fuji-Davison 6-P, 6-D, 6-T and 6-B. Columns: (A) 6-P; (B) 6-D; (C) 6-T; (D) 6-B (each 250 × 4.6 mm I.D.). Conditions as in Fig. 6.

gel increases the number of accessible surface groups per gram, but does not change the number of accessible surface groups per  $100 \text{ \AA}^2$ . Figs. 2, 3, 4 and 5 show the correlations between the capacity factors ( $k'$ ) of PAHs and the number of accessible P, D, T and B surface groups, respectively, per gram of silica gel surface.

It can be seen that, an increase in the number of accessible surface groups per gram of silica gel surface increases  $\log k'$ . The  $\log k'$  curves obtained with the P and B column gel series provide similar resolution and retention to the D or T column gel series. With increase in specific surface area, the  $\log k'$  values of PAHs increase to high value.

Figs. 6 and 7 show typical liquid chromatograms obtained with PAHs on Fuji-Davison 1-P, 1-D, 1-T, 1-B, 6-P, 6-D, 6-T and 6-B. It can be seen that the Fuji-Davison 1 series provide longer retention time than the 6 series under the same conditions.

Figs. 8–11 show the correlations between the  $\log k'$  values of PAHs or parabens and the bulky effect of the bonded phenyl groups. As the bulkiness of the bonded phenyl groups increases from phenyl ( $1\phi$ ) to diphenyl ( $2\phi$ ) to triphenyl ( $3\phi$ ), steric exclusion of the solute from the support surface can be expected to increase dramatically. This provides a tool for investigating the elution mechanism in reversed-phase chromatography. As can be seen from Figs. 8–11, the maximum retention and resolution occur near diphenyl. Also, it is interesting that the  $\log k'$  curves showed opposite trends with mobile phases consisting of 70:30 and 60:40 methanol–water.

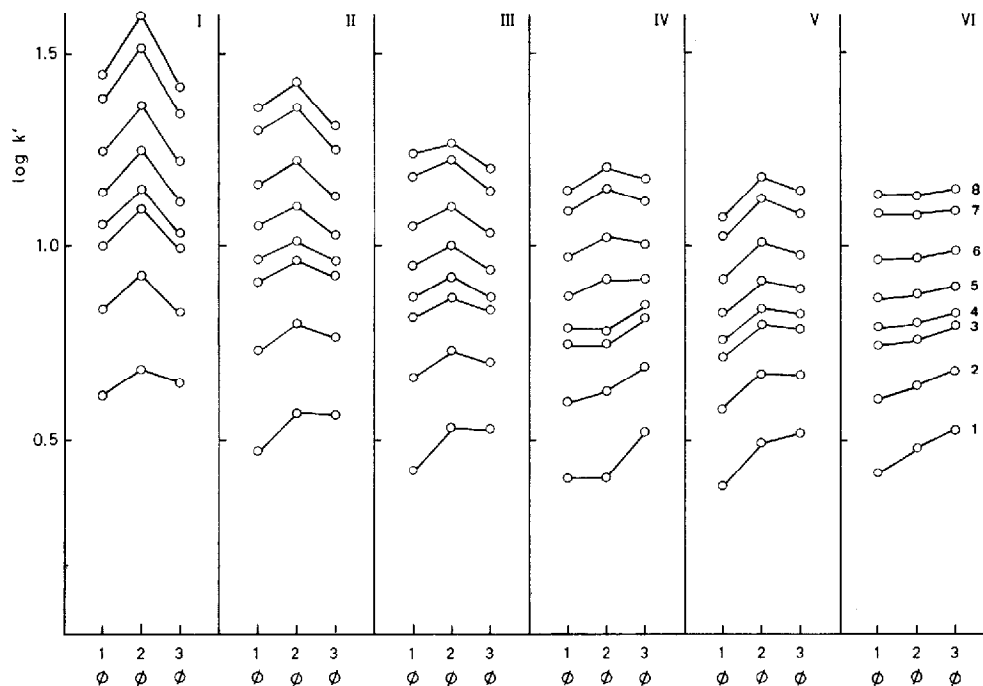


Fig. 8. Correlations between the  $\log k'$  of PAHs and the bulky effect of bonded phenyl groups. I, Fuji-Davison 1; II, Fuji-Davison 2; III, Fuji-Davison 3; IV Fuji-Davison 4; V, Fuji-Davison 5; VI, Fuji-Davison 6.  $1\phi$ : Monophenyl-treated silica gel;  $2\phi$ : diphenyl-treated silica gel;  $3\phi$ : triphenyl-treated silica gel. Mobile phase: methanol–water (60 : 40). Conditions as in Fig. 2.

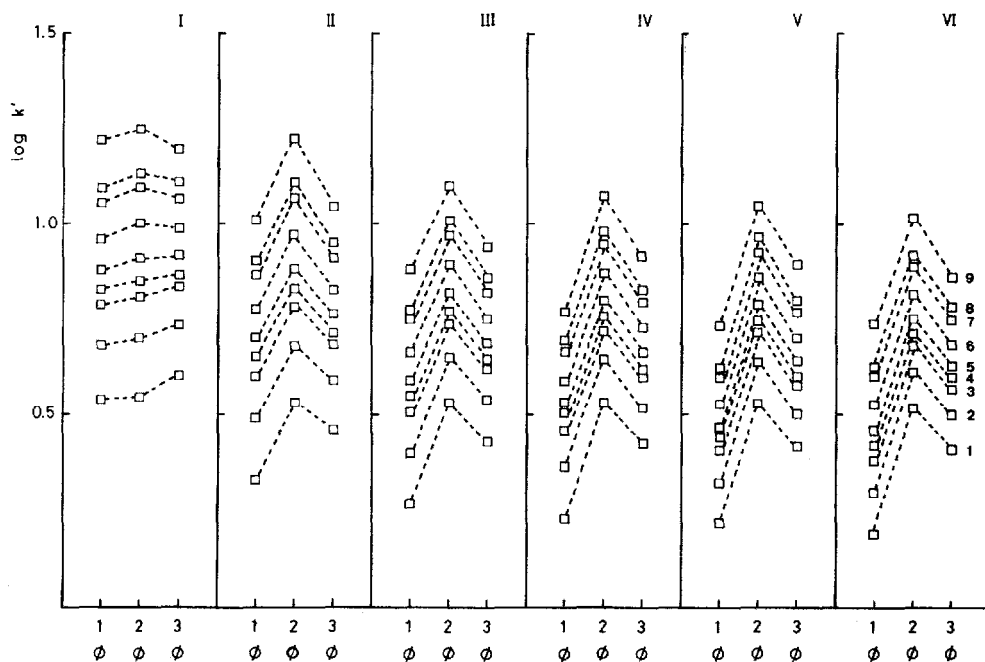


Fig. 9. Correlations between the  $\log k'$  of PAHs and the bulky effect of bonded phenyl groups. Mobile phase: methanol-water (70:30). Conditions as in Fig. 8.

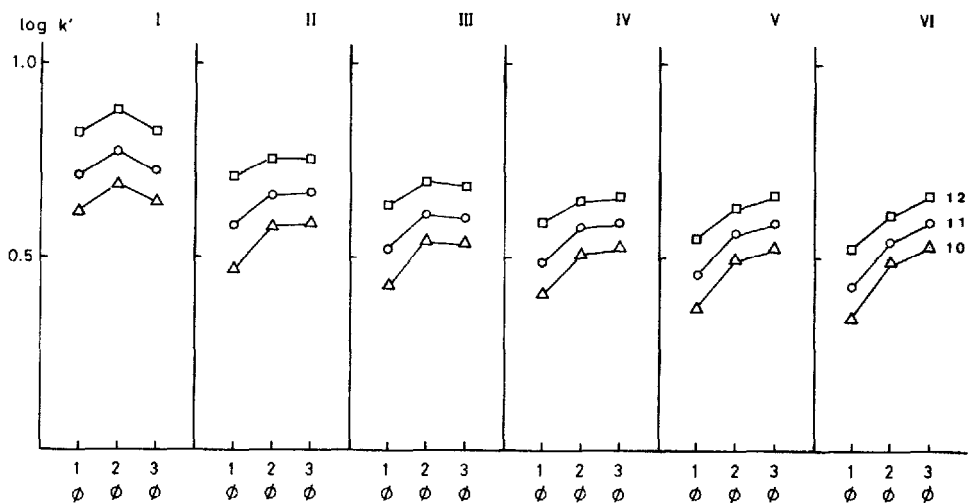


Fig. 10. Correlations between the  $\log k'$  of parabens and the bulky effect of bonded phenyl groups. 10, Ethyl paraben; 11, propyl paraben; 12, butyl paraben. Mobile phase: methanol-water (60:40). Conditions as in Fig. 8.

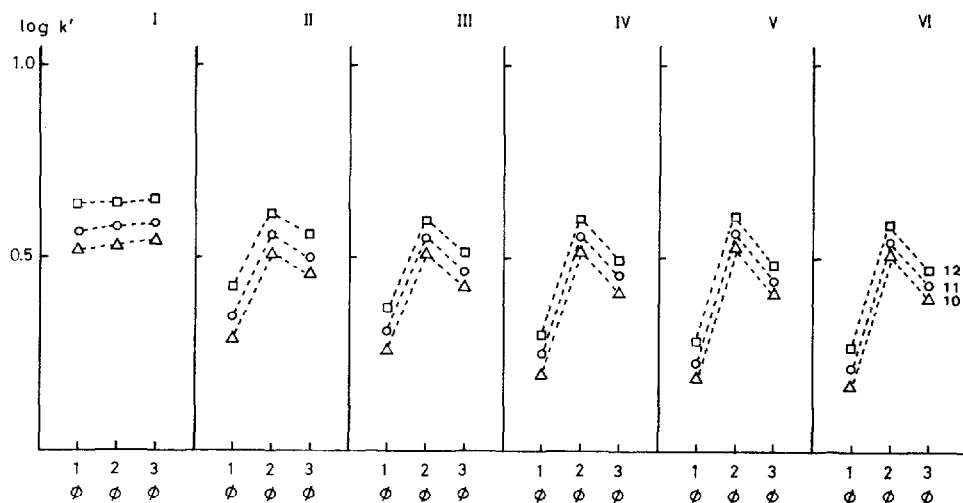


Fig. 11. Correlations between the  $\log k'$  of parabens and the bulky effect of bonded phenyl groups. Mobile phase: methanol-water (70 : 30). Conditions as in Fig. 10.

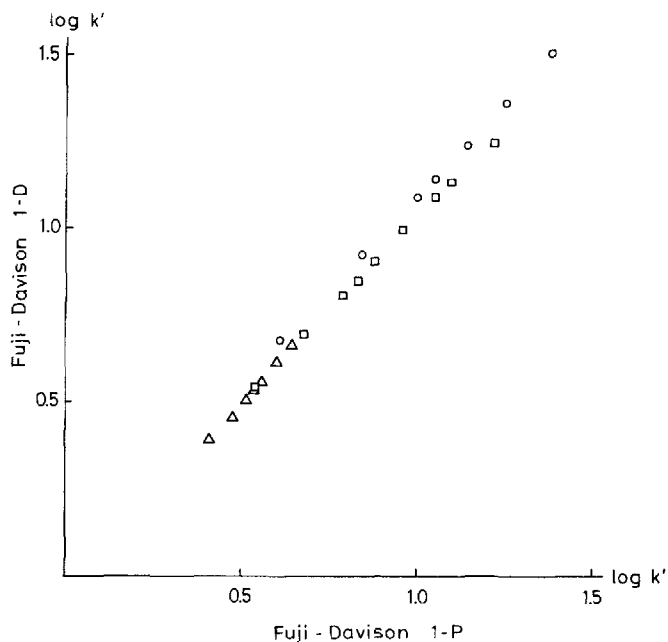


Fig. 12.  $\log k'$  on a P column gel versus  $\log k'$  on a D column gel. Mobile phase:  $\Delta$ , methanol-water (80 : 20);  $\square$ , methanol-water (70 : 30);  $\circ$ , methanol-water (60 : 40). Operating conditions as in Fig. 2.

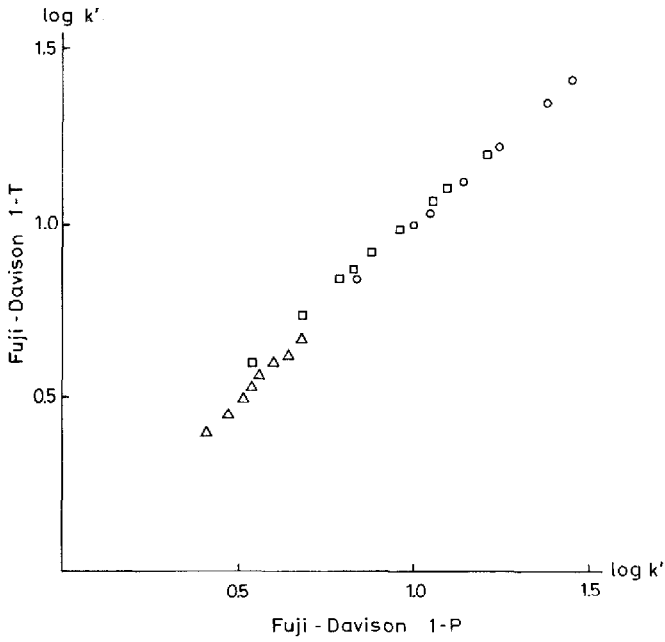


Fig. 13.  $\log k'$  on a P column gel *versus*  $\log k'$  on a T column gel. Operating conditions as in Fig. 12.

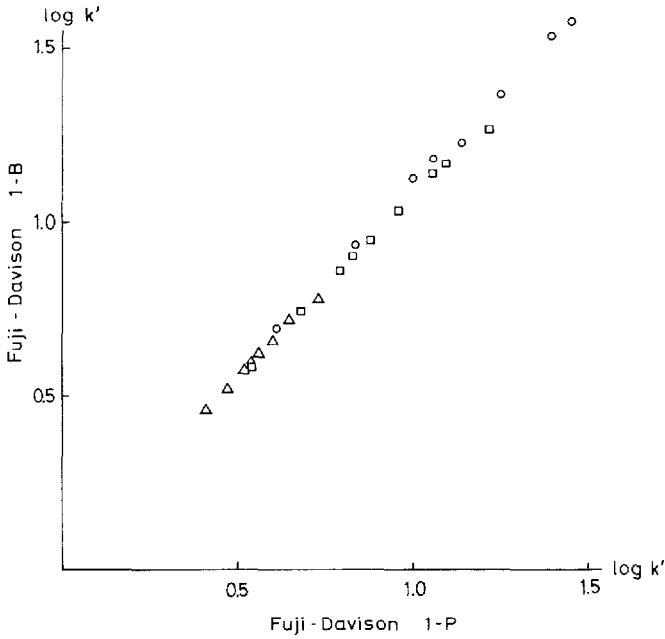


Fig. 14.  $\log k'$  on a P column gel *versus*  $\log k'$  on a B column gel. Operating conditions as in Fig. 12.

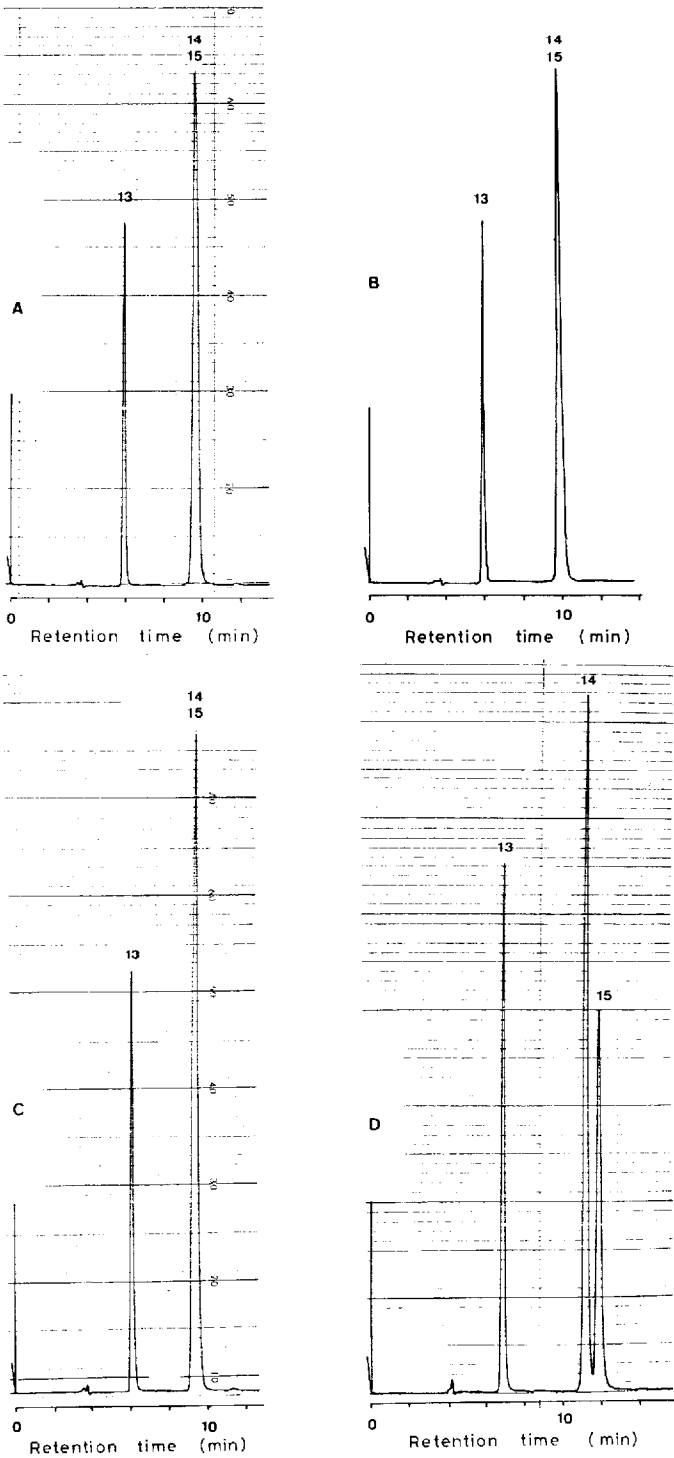


Fig. 15.

(Continued on p. 76)

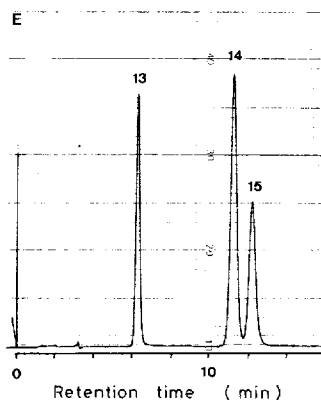


Fig. 15. Separation of *m*-nitroaniline and *o*- and *p*-nitrochlorobenzene on P, D, T, B and octadecyl column gels. Mobile phase: methanol water (60:40). 13, *m*-Nitroaniline; 14, *o*-nitrochlorobenzene; 15, *p*-nitrochlorobenzene. Column: (A) Fuji-Davison 1-P; (B) 1-D; (C) 1-T; (D) 1-B; (E) Jascosil SC-01 (ODS). Operating conditions as in Fig. 2.

Figs. 12, 13 and 14 show plots of  $\log k'$  on a P column gel versus  $\log k'$  on a D column gel,  $\log k'$  on a P column gel versus  $\log k'$  on a T column gel and  $\log k'$  on a P column gel versus  $\log k'$  on a B column gel, respectively.

Fig. 15 shows the correlations between the five gels of the P, D, T, B and octadecyl series using *m*-nitroaniline and *o*- and *p*-nitrochlorobenzene. The nitrochlorobenzenes could not be separated on the P, D or T column gels with methanol-water mixtures, but were separated on the B and octadecyl column gels. It is interesting that nitrochlorobenzene compounds can be separated with interaction of benzyl functional groups on the silica surface.

#### ACKNOWLEDGEMENT

The authors are indebted to a research subsidy from the Toyota Foundation.

#### REFERENCES

- 1 M. Okamoto, *J. Chromatogr.*, 202 (1980) 55.
- 2 M. Okamoto and H. Kishimoto, *J. Chromatogr.*, 212 (1981) 251.
- 3 M. Okamoto and F. Yamada, *J. Chromatogr.*, 247 (1982) 167.
- 4 M. Okamoto, F. Yamada and T. Omori, *Chromatographia*, 16 (1982) 152.
- 5 C. J. Little, A.D. Dale and M.B. Evans, *J. Chromatogr.*, 153 (1978) 381.
- 6 K.K. Unger, N. Becker and P. Roumeliotis, *J. Chromatogr.*, 125 (1976) 115.
- 7 J.J. Kirkland, *Chromatographia*, 8 (1975) 661.