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LIQUID CHROMATOGRAPHIC RETENTION BEHAVIOUR IN THE SEPARATION OF ANTI-EPILEPTIC DRUGS ON PHENYL-, DIPHENYL-, AND TRIPHENYL-BONDED SILICAS AND GLASSES

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SUMMARY

The retention and selectivity behaviour of some anti-epileptic drugs were studied by high-performance liquid chromatography on 21 kinds of phenyl-modified porous glasses and silicas, prepared from solutions of phenyldimethylchlorosilane, diphenylmethylchlorosilane or triphenylchlorosilane in xylene, and from various kinds of glass or silica with various mean pore diameters and/or specific surface areas. From elemental analysis data for carbon, the maximum number of bonded phenyl surface groups per gram (mean pore diameter 15 nm, specific surface area 217 m^2/g, pore volume 0.85 ml/g) in phenyl-, diphenyl-, and triphenyl-bonded gels was calculated to be 0.313, 0.159, and $0.112 \cdot 10^{21}$, respectively. Using various acetonitrile–0.01 *M* potassium dihydrogen phosphate mixtures as eluents, the anti-epileptic drugs were separated on all the gels studied, but with different degrees of resolution. With increase in specific surface area on the glasses or silicas, the k' values of three anti-epileptic drugs increased. The selectivity for the separation of carbamazepin and diphenylhydantoin is discussed and explained by the π–π interaction between solutes and stationary phases. It has been shown that diphenyl and triphenyl phases are more suitable stationary phases for the selective separation of anti-epileptic drugs than monophenyl phases.

INTRODUCTION

During the past decade, high-performance liquid chromatography (HPLC), especially reversed-phase HPLC (RP-HPLC), has developed rapidly and is now accepted as a reliable and versatile analytical method for the separation and quantitation of mixtures which are soluble in liquids. Presently, chemically bonded stationary phases are used most widely as column packing materials for RP-HPLC. These materials consist of organic functional groups, such as octadecyl, octyl, phenyl, and ethyl groups, bonded to silicas. Over the past several years, much effort has been

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TABLE I

CHARACTERISTICS OF PARENT POROUS GLASSES AND SILICAS

The names and serial numbers were assigned by the authors for convenience and are not commercial names.

Sample	*Serial number*	*Mean particle size (μm)*	*Mean pore diameter (nm)*	*Specific surface area (sq.m/g)*	*Pore volume (ml/g)*
Glass	Fuji-Davison 0G	8.9	15.0	217	0.85
	Fuji-Davison 1G	8.2	33.5	69	0.57
	Fuji-Davison 2G	8.5	57.7	57	0.84
	Fuji-Davison 3G	8.7	73.1	47	0.81
Silica	Fuji-Davison 1S	8.8	50.7	78	1.05
	Fuji-Davison 2S	9.7	72.8	52	1.11
	Fuji-Davison 3S	9.6	78.7	50	1.10

directed toward the elucidation of the retention mechanism in this kind of chromatography, and at present it is generally considered that the retention of a solute in RP-HPLC is due to hydrophobic interactions involving the non-polar stationary phase, the polar mobile phase and the solute. This explanation is termed the solvophobic theory[1–6]. According to this entropically driven interaction model, it can be anticipated that physico-chemical parameters such as the surface area of the solute, its partition coefficient between two immiscible phases, and its aqueous solubility may be correlated with the retention in RP-HPLC. In practice, such correlations exist, and some physico-chemical parameters have been determined on the basis of those relationships. On the other hand, we have postulated that the important parameters of silica in HPLC determining the number of accessible alkyl amino or phenyl groups per 100 Å^2 are the pore diameter and the specific surface area[7–11]. The identification and determination of drugs in studies of bio-availability, pharmacokinetics, forensic science, etc., requires several types of column gels. Therefore, we have now investigated how the chromatographic properties of phenyl-modified supports depend on the phenyl groups bonded to the glasses. Although phenyl-modified gels are among the most important HPLC column materials[12–19], there are only few reports of physical and chemical research on phenyl-bonded columns[20]. We now report the preparation and evaluation of 12 kinds of mono-, di-, and triphenyl-modified glasses with various mean pore diameters and/or specific surface areas, and compare the results with those obtained on 9 kinds of mono-, di-, and triphenyl-modified silicas and 6 kinds of octadecyl-modified glasses or silicas.

EXPERIMENTAL

Reagents

Phenobarbital (PB), carbamazepine (CBZ), diphenylhydantoin (DPHT), and sodium valproate (SV) were obtained from Kanebo Yakuhin (Osaka, Japan). Phenyldimethylchlorosilane (P), diphenylmethylchlorosilane (D), triphenylchlorosilane (T), and octadecyldimethylchlorosilane (ODS) were purchased from Petrach Systems (PA, U.S.A.); various kinds of highly porous glasses (G) or silicas (S) differing in mean particle size, mean pore diameter, specific surface area, and pore volume from Fuji-Davison (Nagoya, Aichi, Japan) (Table I); and acetonitrile (HPLC grade) from Wako (Osaka, Japan). The other reagents and organic solvents were of analytical reagent grade.

Apparatus

The micro-HPLC measurements were carried out using a microfeeder MF-2 (Azuma Electric, Tokyo, Japan) with a Toyosoda (Tokyo, Japan) 8000 UV detector and a fused-silica capillary column of 300 × 0.53 mm I.D., packed with mono-, di-, triphenyl, or ODS-bonded glass or silica. Other HPLC measurements were carried out using a Twincle instrument (Jasco, Tokyo, Japan) equipped with a Uvidec-100 IV variable-wavelength detector (Jasco) and 150 × 4.6 mm I.D. column.

Stationary phases and elemental analysis

Following to a previously described method[7–10], 3.5 g of dried Fuji-Davison 0G, 1G, 2G, 3G, 1S, 2S, or 3S and 1.5 ml of triethylamine, were added to 35 ml of

TABLE II

CHARACTERISTICS OF POROUS GLASSES TREATED WITH P, D, T, OR ODS

P = phenyldimethylchlorosilane; D = diphenylmethylchlorosilane; T = triphenylchlorosilane; ODS = octadecyldimethylchlorosilane.

Column glass	*Specific surface area (m^2/g)*	*C found (%)*	*Average pore diameter (nm)*	*Pore volume (ml/g)*	*No. of surface groups per gram ($\times 10^{21}$)*	*No. of surface groups per 100 Å²*
Fuji-Davison 0G-P	131.0	4.99	13.4	0.77	0.313	2.39
Fuji-Davison 1G-P	50.8	1.93	33.0	0.58	0.121	2.38
Fuji-Davison 2G-P	46.5	1.71	51.9	0.64	0.107	2.31
Fuji-Davison 3G-P	37.9	1.27	72.0	0.63	0.080	2.10
Fuji-Davison 0G-D	138.0	4.13	13.1	0.84	0.159	1.25
Fuji-Davison 1G-D	59.2	2.43	32.5	0.58	0.094	1.58
Fuji-Davison 2G-D	54.4	2.23	52.1	0.68	0.086	1.58
Fuji-Davison 3G-D	40.9	1.47	71.4	0.75	0.057	1.39
Fuji-Davison 0G-T	135.0	4.11	13.3	0.73	0.112	1.21
Fuji-Davison 1G-T	57.8	1.57	32.5	0.56	0.044	0.76
Fuji-Davison 2G-T	52.1	1.35	51.7	0.75	0.038	0.72
Fuji-Davison 3G-T	41.6	1.10	69.0	0.74	0.031	0.74
Fuji-Davison 0G-ODS	107.0	5.73	13.1	0.80	0.131	1.22
Fuji-Davison 1G-ODS	46.4	2.92	32.8	0.57	0.073	1.58
Fuji-Davison 2G-ODS	43.9	2.71	53.0	0.70	0.068	1.55
Fuji-Davison 3G-ODS	33.7	2.20	69.9	0.62	0.055	1.63

TABLE III

CHARACTERISTICS OF POROUS SILICAS TREATED WITH P, D, T, OR ODS

P, D, T, and ODS refer to compounds in Table II.

Column silica	*Specific surface area (m^2/g)*	*C found (%)*	*Average pore diameter (nm)*	*Pore volume (ml/g)*	*No. of surface groups per gram ($\times 10^{21}$)*	*No. of surface groups per 100 Å^2*
Fuji-Davison 1S-P	56.2	2.15	47.2	0.94	0.135	2.40
Fuji-Davison 2S-P	47.0	1.58	71.1	1.08	0.099	2.11
Fuji-Davison 3S-P	38.8	1.46	73.5	1.09	0.092	2.39
Fuji-Davison 1S-D	63.5	2.71	47.5	0.98	0.105	1.65
Fuji-Davison 2S-D	50.0	2.26	69.9	1.01	0.087	1.74
Fuji-Davison 3S-D	38.7	1.90	75.4	1.01	0.073	1.89
Fuji-Davison 1S-T	64.7	1.65	47.0	0.92	0.046	0.71
Fuji-Davison 2S-T	49.8	1.36	68.5	1.05	0.038	0.76
Fuji-Davison 3S-T	44.7	1.22	72.1	1.05	0.034	0.76
Fuji-Davison 1S-ODS	59.7	1.71	48.5	0.97	0.043	0.72
Fuji-Davison 2S-ODS	42.7	1.44	68.8	0.96	0.036	0.85
Fuji-Davison 3S-ODS	39.8	1.27	73.3	1.00	0.032	0.80

a 3.4% solution of P in dry xylene. The glass or silica suspension was refluxed for 5 h, filtered through a glass filter (1 μm porosity), washed several times with xylene, chloroform, methanol, and acetone in succession, and then dried *in vacuo* at 70°C for 2 days, finally producing the glasses or silicas for HPLC, which are listed as Fuji-Davison 0G-P to 3G-P, 1S-P to 3S-P, respectively, in Tables II and III.

Similarly, after 3.5 g of dried Fuji-Davison 0G, 1G, 2G, 3G, 1S, 2S, or 3S had been added to 35 ml of a 3.4% dry xylene solution of D, T, or ODS, the same procedure as with the Fuji-Davison 0G-P to 3G-P or 1S-P to 3S-P series in Tables II and III was carried out, producing the Fuji-Davison 0G-D to 3G-D, 0G-T to 3G-T, 1S-D to 3S-D, 1S-T to 3S-T, 0G-ODS to 3G-ODS, and 1S-ODS to 3S-ODS, shown in Tables II and III. Hereafter, Fuji-Davison 0G-P to 3G-P, 0G-D to 3G-D, 0G-T to 3G-T, and 0G-ODS to 3G-ODS will be referred to as "column glasses", and Fuji-Davison 1S-P to 3S-P, 1S-D to 3S-D, 1S-T to 3S-T, and 1S-ODS to 3S-ODS will be referred to as "column silicas", respectively.

The carbon contents of column glasses and silicas were determined by elemental analysis, using an MT-3 CHN elemental analyser (Yanagimoto, Kyoto, Japan). These data are indicated as "Found" in Tables II and III. The specific surface areas, mean pore diameters, and pore volumes of the column glasses or column silicas were determined with an MOD-220 porosimeter (Carlo Erba, Milan, Italy) and an SA-1000 surface-area pore-volume analyser (Shibata, Tokyo, Japan), and the data are shown in Tables II and III.

Column preparation

The column glasses or column silicas were packed into the fused-silica capillary columns (300 × 0.53 mm I.D.) or into the stainless-steel columns (150 × 4.6 mm I.D.) by the slurry technique.

RESULTS AND DISCUSSION

From the elemental analysis of glasses or silicas treated with P, D, T, or ODS, the number of bonded phenyl, diphenyl, triphenyl or octadecyl surface groups per gram or per 100 Å^2 of glass or silica were calculated by the previous described procedure[7–10]. The results are given in Tables II and III. As can be seen from Tables II and III, an increase in the specific surface area of glass or silica increases the number of bonded surface groups per gram, but does not change the number of bonded surface groups per 100 Å^2.

Fig. 1 shows typical liquid chromatograms, obtained with three anti-epileptic drugs on Fuji-Davison 1G-P, 2G-P, 1S-P, and 2S-P. It can be seen that the Fuji-Davison 1G and 1S series provide longer retention times than the 2G or 2S series under the same conditions. These results show that three of the anti-epileptic drugs can be separated on the column glasses as well as on the column silicas, with acetonitrile–0.01 *M* potassium dihydrogen phosphate mixtures as eluents.

Fig. 2 shows a typical liquid chromatogram on the Fuji-Davison 3G-T and 3S-T. As can be seen from Fig. 2, CBZ and DPHT were separated on the column glass (3G-T) but not on the column silica (3S-T).

Fig. 3 shows the correlations between the capacity factor of three anti-epileptic drugs and the number of bonded P, D, T, and ODS surface groups on the column glasses and column silicas.

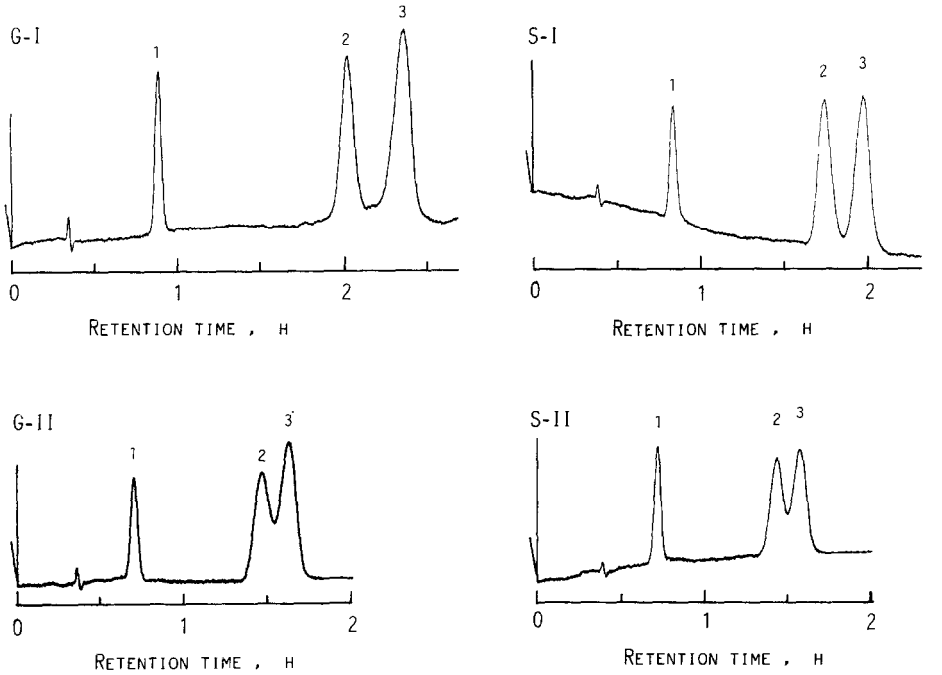

Fig. 1. Micro-HPLC chromatograms of three anti-epileptic drugs on column glasses and column silicas. Columns: 300 × 0.53 mm I.D.; G-I = Fuji-Davison 1G-P, G-II = Fuji-Davison 2G-P, S-I = Fuji-Davison 1S-P; S-II = Fuji-Davison 2S-P. Mobile phase: acetonitrile–0.01 *M* potassium dihydrogen phosphate (1.4, v/v). UV detection: 220 nm Flow-rate: 2.7 μl/min Samples. 1 = PB, 2 = CBZ, 3 = DPHT

Fig. 4 shows the capacity factors of three anti-epileptic drugs and the bulk effect of the bonded phenyl groups on the column glasses and column silicas. As can be seen from Fig. 4, the maximum retention and resolution on the column glasses as well as on the column silicas is obtained with diphenyl.

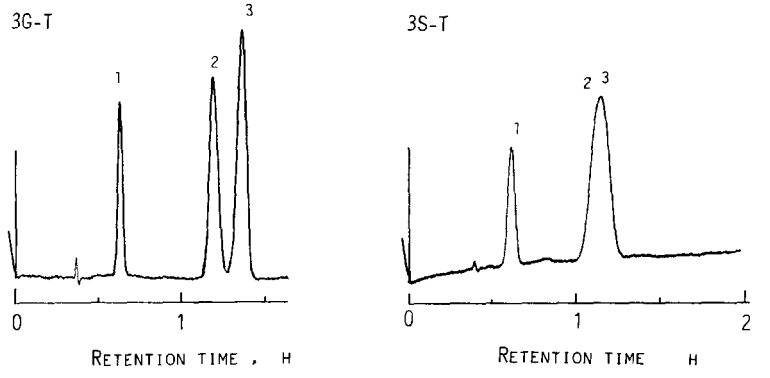

Fig. 2. Typical micro liquid chromatogram on Fuji-Davison 3G-T and 3S-T. Columns: 3G-T = Fuji-Davison 3G-T, 3S-T = Fuji-Davison 3S-T. Other conditions and solutes as in Fig. 1.

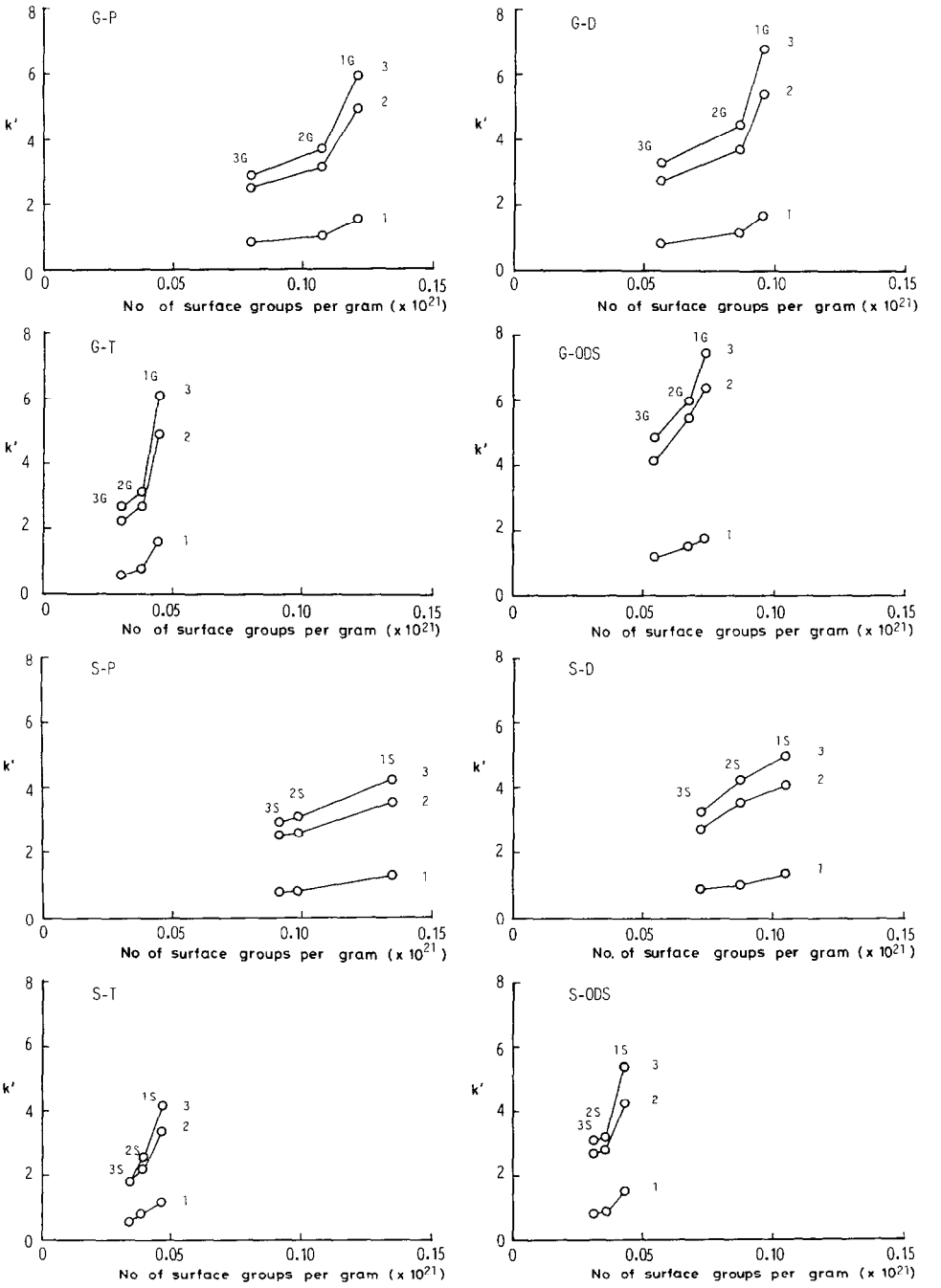

Fig. 3. Correlation between the capacity factors (k') of three anti-epileptic drugs and the number of P, D, T, and ODS groups on the surface of the column glasses and the column silicas. Columns: G-P = Fuji-Davison 1G-P, 2G-P, 3G-P; G-D = Fuji-Davison 1G-D, 2G-D, 3G-D; G-T = Fuji-Davison 1G-T, 2G-T, 3G-T; G-ODS = Fuji-Davison 1G-ODS, 2G-ODS, 3G-ODS; S-P = Fuji-Davison 1S-P, 2S-P, 3S-P; S-D = Fuji-Davison 1S-D, 2S-D, 3S-D; S-T = Fuji-Davison 1S-T, 2S-T, 3S-T; S-ODS = Fuji-Davison 1S-ODS, 2S-ODS, 3S-ODS. P, D, T, and ODS refer to compounds in Tables II and III. Other conditions and solutes as in Fig. 1.

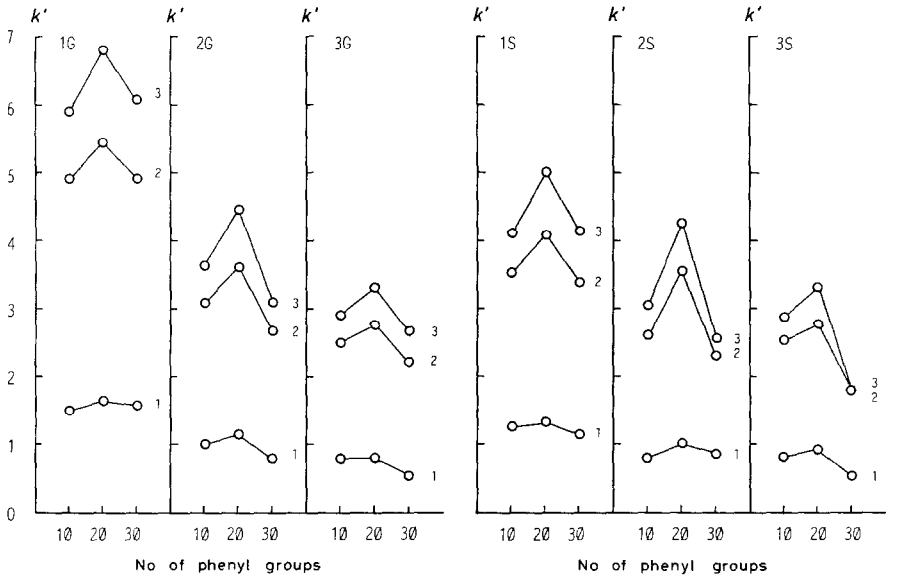

Fig. 4. Capacity factors (k') of three anti-epileptic drugs and the bulk effect of the bonded phenyl groups on the column glasses and the column silicas. 1G = Fuji-Davison 1G series; 2G = Fuji-Davison 2G series; 3G = Fuji-Davison 3G series. 1S = Fuji-Davison 1S series; 2S = Fuji-Davison 2S series; 3S = Fuji-Davison 3S series, 10 = Monophenyl, 20 = di-phenyl, 30 = triphenyl. Conditions and solutes as in Fig. 1.

TABLE IV

CAPACITY FACTORS (k') OF THREE ANTI-EPILEPTIC DRUGS ON COLUMN GLASS TREATED WITH P, D, T, OR ODS

P, D, T, and ODS refer to compounds in Table II. Conditions as in Fig. 1.

Column glass	k'			α*
	PB	*CBZ*	*DPHT*	
Fuji-Davison 0G-P**	2.07	6.08	7.95	1.31
Fuji-Davison 0G-D**	2.32	7.33	8.97	1.22
Fuji-Davison 0G-T**	2.30	6.89	8.91	1.29
Fuji-Davison 0G-ODS**	3.18	10.94	12.84	1.17
Fuji-Davison 1G-P	1.55	4.91	5.89	1.20
Fuji-Davison 1G-D	1.74	5.62	7.04	1.25
Fuji-Davison 1G-T	1.66	5.13	6.37	1.24
Fuji-Davison 1G-ODS	1.96	6.73	7.92	1.18
Fuji-Davison 2G-P	1.00	3.18	3 64	1.15
Fuji-Davison 2G-D	1.14	3.71	4.46	1.20
Fuji-Davison 2G-T	0.78	2.68	3.07	1 15
Fuji-Davison 2G-ODS	1.50	5.46	6.00	1.10
Fuji-Davison 3G-P	0.74	2.42	2.77	1 14
Fuji-Davison 3G-D	0.80	2.57	3.08	1.20
Fuji-Davison 3G-T	0 72	2.19	2 66	1.21
Fuji-Davison 3G-ODS	1.13	4.20	4.53	1.08

* Separation factor (α) between CBZ and DPHT = k'_{DPHT}/k'_{CBZ}

** Column dimensions: 110 × 0.53 mm I.D.

Tables IV and V show the capacity factors (k') of three anti-epileptic drugs on the various column gels with P, D, T, and ODS surface groups. It appears that the separation between CBZ and DPHT strongly depends on the characteristics of the stationary phases. D and T column gels offer better selectivity for the separation of CBZ and DPHT than the P and ODS column gels, although the capacity factors on the ODS column gels were larger than those obtained with all other column gels. This unique behaviour has also been observed in a study of the elution characteristics of large polycyclic aromatic hydrocarbons (PAHs) with D and T column gels in our previous work[21].

In order to explain this experimental observation, we have tried to model the chemical conformation of CBZ and DPHT by a computer graphics techniquc. From this experiment, it was clear that the two phenyl rings of DPHT have more freedom to interact with the phenyl groups of the bonded stationary phases than those of CBZ. The two phenyl rings of CBZ are relatively inflexible and cannot easily be positioned such as to interact with the phenyl groups of the phase. Therefore, the characteristic retention behaviour of CBZ and DPHT on the D and T column gels can be explained by the fact that dominant π–π interactions between the solutes and phenyl groups of the bonded stationary phases causes large retention for molecules with freely movable aromatic rings in their structures. A similar explanation has been proposed by Tanaka *et al.* in 1982 for the retention behaviour of PAHs on a pyrenylethyl bonded gel[22].

On the other hand, as can be expected, an increase in the number of bonded surface groups per gram of glass or silica surface increases the capacity factor. With increases in specific surface area, the capacity factor values of three anti-epileptic drugs also increase. This chromatographic behaviour has also been observed in a

TABLE V

CAPACITY FACTORS (k') OF THREE ANTI-EPILEPTIC DRUGS ON COLUMN SILICA TREATED WITH P, D, T, OR ODS

P, D, T, and ODS refer to compounds in Table II. Conditions as in Fig. 1.

Column silica	k'			α^*
	PB	*CBZ*	*DPHT*	
Fuji-Davison 1S-P	1.25	3.52	4.12	1.17
Fuji-Davison 1S-D	1.32	4.07	4.98	1.22
Fuji-Davison 1S-T	1.13	3.38	4.13	1.22
Fuji-Davison 1S-ODS	1.47	4.25	5.35	1.26
Fuji-Davison 2S-P	0 80	2.60	3.05	1.17
Fuji-Davison 2S-D	1.02	3.55	4.24	1.19
Fuji-Davison 2S-T	0.74	2.29	2.53	1.10
Fuji-Davison 2S-ODS	0.85	2.78	3.21	1.15
Fuji-Davison 3S-P	0.78	2.52	2.86	1.13
Fuji-Davison 3S-D	0.87	2.76	3.30	1.20
Fuji-Davison 3S-T	0.53	1.79	1.79	1.00
Fuji-Davison 3S-ODS	0.78	2.72	3.11	1.14

* See Table IV.

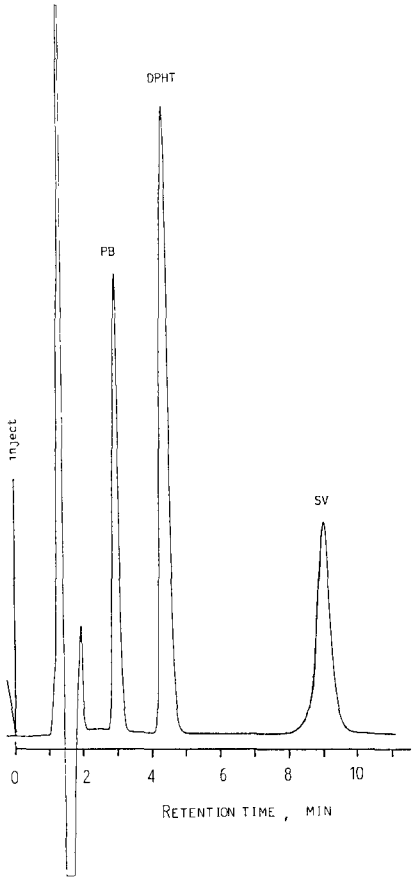

Fig. 5. Typical HPLC chromatogram of anti-epileptic drugs on the ODS-modified glass. Column: Fuji-Davison 1G-ODS, 150 × 4.6 mm I.D. Mobile phase: acetonitrile–0.01 *N* citric acid–0.01 *N* disodium hydrogen phosphate (35:43:22, v/v). UV detection: 205 nm. Flow-rate: 1.0 ml/min. Samples: PB = phenobarbital, DPHT = diphenylhydantoin, SV = sodium valproate

previous study[11] on silicas with smaller pore diameters (10 to 20 nm) and larger specific surface areas (100 to 300 sq. m/g) compared to the silicas in the present work.

Fig. 5 shows a typical liquid chromatogram of antiepileptic drugs on the ODS-modified glass (Fuji-Davison 1G-ODS).

It is concluded from the present investigation that it is not sufficient to evaluate column gels solely on the basis of the carbon content of chemically bonded reversed-phase materials. The pore size distribution of glass or silica supports, the bulkiness of the ligands bonded to the glass or silica, and the molecular size of the solute must also be considered.

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