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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMNS OF SMALL DIAMETER

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SUMMARY

By using a specially designed column system, wall effects can be minimized. An approximately linear relationship between retention volume (V_R) and peak width ($2\Delta V_{1/2}$) (in volume units) for various solutes on columns with different diameters was obtained:

$$2\Delta V_{1/2} = a' + b'V_R$$

Systematic experiments on the influence of the dead volume of the detector system on the values of a' and b' were carried out, which indicated that there is a large influence on a' but little on b'. By using the improved column system and home-made UV detector, a column of I.D. 2 mm and length 10 cm with an efficiency of more than 6000 theoretical plates was obtained.

INTRODUCTION

Currently used commercially available high-performance chromatographic columns with efficiencies of more than 4000 theoretical plates and packed with microparticulate silica have an I.D. of at least 4 mm and a length of 10 cm[1–3]. It has been found that the column efficiency decreases with decrease in column diameter[4–7]. The concept of the "infinite diameter effect" was proposed to explain these results[8,9]. However, small inner diameters with the same column efficiency will require less solvent to achieve the same separation, as the flow-rate of the eluent is proportional to the square of the column diameter. Improvements in the injection system can minimize the wall effect. By using a home-made, specially designed injection system we have carried out a systematic investigation of the influence of column diameter on efficiency. A linear relationship between retention volume and peak width (in volume units) for different solutes for columns with different diameters was obtained. A systematic investigation of the influence of extra-column effects produced by connectors and detectors on the two constants of the above-mentioned linear relationship was carried out and indicated a great influence of the volume of the detector system

on the column efficiency. By using a home-made UV detector we obtained a column of I.D. 2 mm and length 10 cm with an efficiency of more than 6000 theoretical plates[10].

THEORETICAL

HETP is preferably used to evaluate the column efficiency. Generally the plate equation is very complicated. For example, the equation given by Horváth and Lin[12] for liquid chromatography is

$$H = H_{disp} + H_{e.diff} + H_{l.diff} + H_{kin} \tag{1}$$

where

$$H_{disp.} = \frac{2\gamma D_m}{u_e} + \frac{2\lambda d_p u_e^{1/3}}{u_e^{1/3} + \omega(D_m/d_p)^{1/3}} \tag{2}$$

$$H_{e.diff} = \frac{\kappa(k_0 + k' + k_0 k')^2 \, d_p^{5/3} u_e^{2/3}}{(1 + k_0)^2 \, (1 + k')^2 \, D_m^{2/3}} \tag{3}$$

$$H_{l.diff} = \frac{\theta(k_0 + k' + k_0 k')^2 \, d_p^2 u_e}{30 D_m k_0 \, (1 + k_0)^2 \, (1 + k')^2} \tag{4}$$

$$H_{kin} = \frac{2k' u_e}{(1 + k_0) \, (1 + k')^2 \, k_d} \tag{5}$$

and H is the conventional plate height, and u_e, D_m and d_p are the interstitial mobile phase velocity, the diffusivity of the solute in the bulk mobile phase and the particle diameter respectively. The value of k_0 is given by $\varepsilon_i \, (1 - \varepsilon_e)/\varepsilon_e$, where ε_i and ε_e are the appropriate intraparticular and interstitial porosities, respectively. γ, λ, ω and κ are structural parameters of the column packing and θ is the tortuosity factor for the porous particles. k' is the column capacity ratio and k_d is the desorption rate constant.

However, it has been known for almost 20 years that in practice there are some simple and approximately linear relationships between peak widths and retention values, as given in the following equations[13,14]:

$$2\Delta t_{1/2} = a_2 + b_2 t_R' \tag{6}$$

$$2\Delta t_{1/2} = a + b t_R \tag{7}$$

$$2\Delta V_{1/2} = a' + b' V_R \tag{8}$$

where $2\Delta t_{1/2}$ and $2\Delta V_{1/2}$ are the peak width at half-height in time and volume units respectively. V_R, t_R, t_R' t_R^0 and F are the retention volume, retention time, adjusted retention time of the solute, retention time of non-sorbed substance and mobile phase

flow-rate, respectively. The relationships between these parameters and constants are as follows:

$$2\Delta V_{1/2} = 2\Delta t_{1/2} F \tag{9}$$

$$a_2 = a + b_{tR}^0 \tag{10}$$

$$b' = b_2 = b \tag{11}$$

$$a' = aF \tag{12}$$

From these empirical relationships we can calculate the plate height, H, of the solute with a capacity factor k', and also H^1 and H^∞, which are the plate heights for the solute with a capacity factor, k', equal to unity or approaching infinity, respectively.

$$H = \frac{L}{n} = \frac{L}{5.54\left(\frac{t_R}{2\Delta t_{1/2}}\right)^2} = \frac{L}{5.54\left(\frac{V_R}{2\Delta V_{1/2}}\right)^2} = \frac{Lb^2}{5.54}\left(\frac{k' + 1 + \frac{au}{bL}}{1 + k'}\right)^2$$

$$= \frac{Lb_2^2}{5.54}\left(\frac{\frac{a_2 u}{b_2 L} + k'}{1 + k'}\right) = \frac{Lb'^2}{5.54}\left(\frac{1 + \frac{a'u}{b'LF} + k'}{1 + k'}\right) = H^\infty\left(\frac{\beta + k'}{1 + k'}\right)^2 \tag{13}$$

The three constants in eqn. 13 can be calculated from the following equations:

$$H^\infty = \frac{Lb^2}{5.54} = \frac{Lb'^2}{5.54} = \frac{Lb_2^2}{5.54} \tag{14}$$

$$H^1 = H^\infty\left(\frac{\beta + 1}{2}\right)^2 \tag{15}$$

$$\beta = 1 + \frac{au}{bL} = 1 + \frac{a'u}{Fb'L} = 2\sqrt{\frac{H^1}{H^\infty}} - 1 \tag{16}$$

We can obtain the equations for H^∞ and H^1 when infinity and unity, respectively, are substituted for k' in eqn. 1:

$$H^\infty = \frac{2\gamma D_m}{u_e} + \frac{2\lambda d_p u_e^{1/3}}{u_e^{1/3} + \omega(D_m/d_p)^{1/3}} + \frac{\kappa d_p^{5/3} u_e^{2/3}}{(1 + k_0)^2 D_m^{2/3}} + \frac{\theta d_p^2 u_e}{30 D_m k_0 (1 + k_0)^2} \tag{17}$$

$$H^1 = \frac{2\gamma D_m}{u_e} + \frac{2\lambda d_p u_e^{1/3}}{u_e^{1/3} + \omega(D_m/d_p)^{1/3}} + \frac{\kappa (1 + 2k_0)^2 d_p^{5/3} u_e^{2/3}}{4(1 + k_0)^2 D_m}$$

$$+ \frac{\theta (1 + 2k_0)^2 d_p^2 u_e}{120 D_m k_0 (1 + k_0)^2} + \frac{2u_e}{4(1 + k_0) k_d} + H_{ext.} \tag{18}$$

In developing the high-performance liquid chromatographic column system, the extra-column effects must be taken into consideration. Obviously, the dead volume in the detector and connector must be minimized, and even so the extra-column effects must still be added to the plate height equation. Because for the solute with k' approaching infinity the extra-column effect is comparatively small and can be neglected, we only added this term to the equation for H^1.

Eqn. 14 shows that the constants b, b_2 and b' are determined by H^∞ and, according to eqn. 17, they will not depend seriously on the extra-column effects. Nevertheless, it may have more serious influence on H^1 and also on constants a, a_2 and a'. Further, in these equations no wall effects have been taken into consideration. Only when the wall effect is not the major controlling factor in band broadening will the approximately linear relationship in eqn. 8 between peak width (in volume units) and retention volume be preserved for different solutes on columns with different inner diameters. Especially in such instances it will be very interesting to carry out some systematic experiments on the influence of the dead volume of the detector and connector on a'. It must be remembered that these are only approximate empirical relationships and it is possible that the wall effect would have some influence on b'.

EXPERIMENTAL

Equipment

The K-1 column system (adsorption type) made in our Institute is shown in
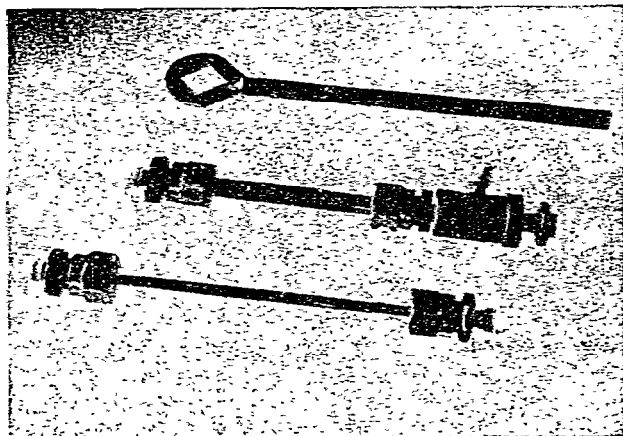

Fig. 1. K-1 (adsorption type) column system made in our Institute.

TABLE I

VOLUMES OF THREE UV DETECTOR SYSTEMS

No. of system	Cell volume of UV detector (μl)	Dead volume of connector (μl)	Total volume of the system (μl)
1	8*	13	21
2	8*	3	11
3	1.2**	0.8	2

* Supplied by Analytical Instruments Factory, Peking, China.
** Home-made in our Institute.

Fig. 1. The microparticulate irregular silica YWG (particle size 3–5 μm) produced by Tsing Tao Ocean Chemical Plant (Tsing Tao, Shandong, China) was used in all experiments. A specially designed central injecting sample injector was used, as shown in Fig. 1. Precision-bore stainless-steel tubing of length 10 cm and with inner diameters of 2, 3, 4, 5 and 6 mm were supplied by the Yan An Steel Tubing Factory, Shanghai, China. The unbalanced high-pressure slurry technique was used to pack these columns; the composition of the slurry was carbon tetrachloride–dioxan–silica (10:5:1, by weight)[11].

A home-made 250-ml syringe pump was used. Three kinds of UV detector–connector systems were used, as shown in Table I.

Mobile phase and samples

n-Hexane without further purification was used as the mobile phase at a linear velocity of 0.9–1 mm/sec.

Sample solutes were ordinary chemical reagents and were dissolved in *n*-hexane. Ethylene tetrachloride was used as the unretained solute. The concentrations of ethylene tetrachloride, benzene, naphthalene, biphenyl and phenanthrene in *n*-hexane were 24, 53, 5, 1.3 and 0.5 μg/μl, respectively. The sample volume should be less than 0.2 μl for the column of 2 mm I.D. in order to prevent overloading.

RESULTS

The peak widths and retention times of different solutes determined on columns with different inner diameters are given in Table II. Fig. 2 shows the linear
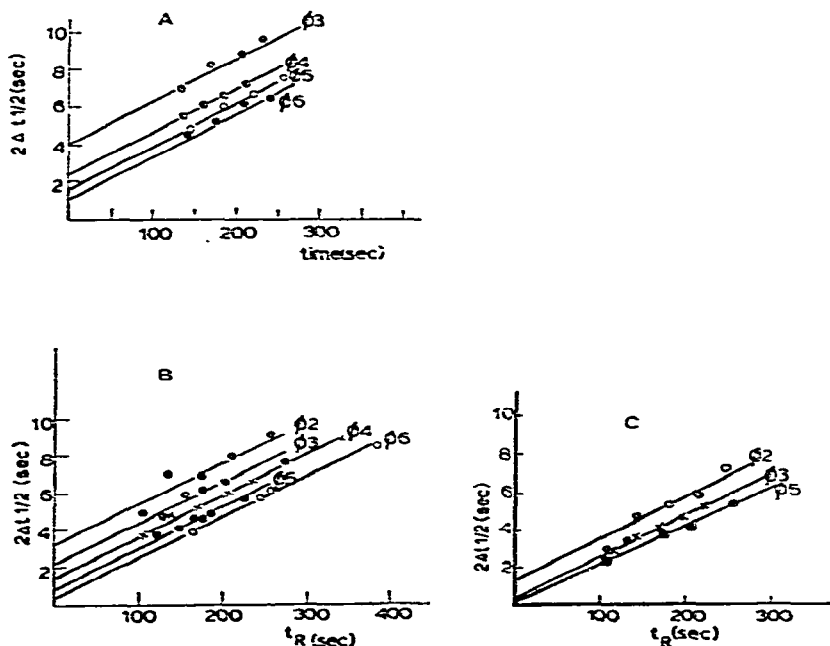

Fig. 2. Peak width *versus* retention time on columns with different inner diameters. Dead volume of detector system: A, 21 μl; B, 11 μl; C, 2 μl.

TABLE II

MEASURED PEAK WIDTHS (IN TIME UNITS) AND RETENTION TIMES OF DIFFERENT SOLUTES ON COLUMNS WITH DIFFERENT INNER DIAMETERS

Average values of more than 3 experiments. Column length, 10 cm.

Dead volume of detector system (µl)	Column I.D. (mm)	Flow-rate of mobile phase (µl/sec)	Ethylene tetrachloride		Benzene		Naphthalene		Biphenyl		Phenanthrene	
			t_R (sec)	$2\Delta t_{1/2}$ (sec)	t_R (sec)	$2\Delta t_{1/2}$ (sec)	t_R (sec)	$2\Delta t_{1/2}$ (sec)	t_R (sec)	$2\Delta t_{1/2}$ (sec)	t_R (sec)	$2\Delta t_{1/2}$ (sec)
21	3	5.5			134	6.6	169	7.8	205	8.6	231	9.1
	4	9.7			137	5.5	162	6.1	186	6.5	208	7.2
	5	15.0			145	4.8	183	6.0	220	6.7	257	7.4
	6	22.0			141	4.5	175	5.2	210	6.0	242	6.5
11	2	2.5	106	4.8	135	6.0	174	6.9	210	7.9	256	9.5
	3	5.9			125	4.7	151	5.9	175	6.2	198	6.6
	4	10.3	104	3.7	136	4.8	170	5.2	207	6.1	233	6.5
	5	16.3			121	3.7	145	4.0	167	4.4	183	4.7
	6	24.8			135	3.7	175	4.5	222	5.5	251	6.0
2	2	2.5	109	2.9	144	4.6	179	5.2	217	5.8	249	7.3
	3	5.4	112	2.6	139	3.6	172	4.1	193	4.5	224	5.3
	5	16.0	103	2.3	133	3.3	173	3.8	208	4.2	256	5.4

TABLE III

CALCULATED PEAK WIDTHS (IN VOLUME UNITS) AND RETENTION VOLUMES OF DIFFERENT SOLUTES ON COLUMNS WITH DIFFERENT INNER DIAMETERS

Column length, 10 cm.

Dead volume of detector system (μl)	Column I.D. (mm)	Flow-rate of mobile phase ($\mu l/sec$)	Ethylene tetrachloride		Benzene		Naphthalene		Biphenyl		Phenanthrene	
			V_R (μl)	$2\Delta V_{1/2}$ (μl)	V_R (μl)	$2\Delta V_{1/2}$ (μl)	V_R (μl)	$2\Delta V_{1/2}$ (μl)	V_R (μl)	$2\Delta V_{1/2}$ (μl)	V_R (μl)	$2\Delta V_{1/2}$ (μl)
21	3	5.5			737	36	919	43	1128	47	1270	50
	4	9.7			1319	53	1571	59	1795	63	2008	70
	5	15.0			2179	72	2747	90	3300	101	3860	111
	6	22.0			3109	100	3859	114	4631	132	5336	143
11	2	2.5	267	12	340	15	438	17	529	20	645	24
	3	5.9			737	28	890	35	1032	37	1174	39
	4	10.3	1068	38	1397	49	1746	53	2132	63	2393	67
	5	16.3			1970	60	2360	66	2720	69	2980	77
	6	24.8			3344	92	4335	111	5500	136	6219	149
2	2	2.5	266	7	353	11	438	13	531	14	610	18
	3	5.4	600	14	745	19	922	22	1035	24	1200	28
	5	16.0	1668	38	2138	54	2800	62	3372	68	4146	88

TABLE IV

CALCULATED VALUES OF a' AND b' IN EQN. 8 USING DIFFERENT DETECTOR SYSTEMS

No. of detector system	Dead volume of detector system (μl)	a' (μl)	b'^*	Correlation coefficient
1	21	20.5 ± 1.5	0.024	0.996
2	11	11.0 ± 1.3	0.023	0.996
3	2	1.7 ± 1.2	0.022	0.995

* Average value: $b' = 0.023$.

relationships between $2\Delta t_{1/2}$ and t_R on columns with different inner diameters and detector systems.

Table III gives the peak widths ($2\Delta V_{1/2}$) (in volume units) and retention volumes (V_R) calculated from $2\Delta t_{1/2}$ and t_R multiplied by the flow-rate of the mobile phase. Fig. 3 shows $2\Delta V_{1/2}$ *versus* V_R for different solutes on columns with different inner diameters but with the same detector system. There are three curves for three detector systems. The values of the constants a' and b' in eqn. 8 calculated by regression analysis on these three detector systems are given in Table IV. It can be clearly seen that the dead volume of the detector system has a great influence on a', but b' remains almost constant for all three detector systems.

The value of a' decreases linearly with decrease in the dead volume of the detector system. Whereas a solute has the same capacity factor, k', using columns with different inner diameters, it will have a smaller retention volume for a smaller

TABLE V

COMPARISON OF CALCULATED (H_{calc}) AND EXPERIMENTAL (H_{exp}) VALUES OF HETP FOR DIFFERENT SUBSTANCES ON THE COLUMNS WITH DIFFERENT INNER DIAMETERS
Column length, 10 cm.

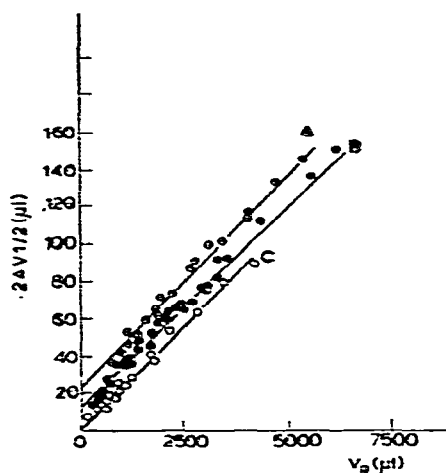| Dead volume of detector system (μl)	Column I.D. (mm)	Flow-rate of mobile phase ($\mu l/sec$)	Constants in eqn. 8 a' (μl)	b'	Constants in eqns. 14 and 16 H^{∞} (μm)	β^*	Ethylene tetrachloride k'	H_{calc} (μm)	H_{exp} (μm)
21	3	5.5	20.5	0.024	9.6	2.52			
	4	9.7				1.85			
	5	15.0				1.54			
	6	22.0				1.37			
11	2	2.5	11.0	0.023		2.37	0	53	37
	3	5.9				1.81			
	4	10.3				1.45	0	20	23
	5	16.3				1.29			
	6	24.8				1.20			
2	2	2.5	1.7	0.022		1.21	0	14	13
	3	5.4				1.13	0	12	10
	5	16.0				1.05	0	10	9

* By using the average value $b' = 0.023$.

Fig. 3. Peak width (in volume units) *versus* retention volume on columns with different inner diameters by using different dead volumes of the detector system. Dead volume of detector system: A, 21 μl; B, 11 μl; C, 2 μl.

column inner diameter. The efficiency of the column with a smaller inner diameter is greatly improved by decreasing the dead volume of the detector system.

We used average values of b' and different values of a' for three different detector systems to calculate the values of β and H^{∞} from eqns. 14 and 16, respectively. Then, by using these values of β and H^{∞}, we calculated the HETP values for different

Benzene			Naphthalene			Biphenyl			Phenanthrene		
k'	H_{calc} (μm)	H_{exp} (μm)	k'	H_{calc} (μm)	H_{exp} (μm)	k'	H_{calc} (μm)	H_{exp} (μm)	k'	H_{calc} (μm)	H_{exp} (μm)
0.25	47	43	0.57	37	40	0.91	31	31	1.15	28	28
0.25	27	29	0.47	24	26	0.69	22	22	0.88	20	22
0.28	19	20	0.61	17	19	0.94	16	17	1.24	15	15
0.29	16	19	0.61	15	16	0.93	14	15	1.22	13	13
0.27	42	35	0.64	32	29	0.98	27	26	1.42	23	25
0.23	26	26	0.48	23	28	0.72	21	23	0.95	19	20
0.31	17	22	0.63	16	17	0.99	14	16	1.24	14	14
0.21	15	17	0.45	14	14	0.67	13	13	0.83	13	12
0.39	13	14	0.80	12	12	1.29	11	11	1.59	11	10
0.32	14	19	0.64	13	15	0.99	12	13	1.28	12	15
0.24	12	12	0.54	11	10	0.72	11	10	1.00	11	10
0.29	10	12	0.68	10	9	1.02	10	8	1.49	10	8

TABLE VI

INFLUENCE OF THE DEAD VOLUME OF DETECTOR SYSTEMS ON THE PEAK SYMMETRY

Column I.D. (mm)	Solute									
	Ethylene tetrachloride		Benzene		Naphthalene		Biphenyl		Phenanthrene	
	Dead volume (µl)									
	11	2	11	2	11	2	11	2	11	2
2	5.0	—	3.7	1.7	3.0	1.9	2.4	1.9	2.2	2.2
3	2.6	2.0	2.4	1.8	2.0	1.9	2.0	1.6	1.9	1.5
4	1.6	1.6	2.0	1.5	1.7	1.6	1.7	1.5	1.7	1.6
5	—	—	1.6	—	1.6	—	1.4	—	1.4	—
6	1.8	1.6	1.8	1.4	1.4	1.3	1.3	1.3	1.2	1.1

solutes on columns with different inner diameters for three different detector systems according to eqn. 13 and compared the results with the values determined experimentally, as shown in Table V. The calculated and experimental values are in good agreement.

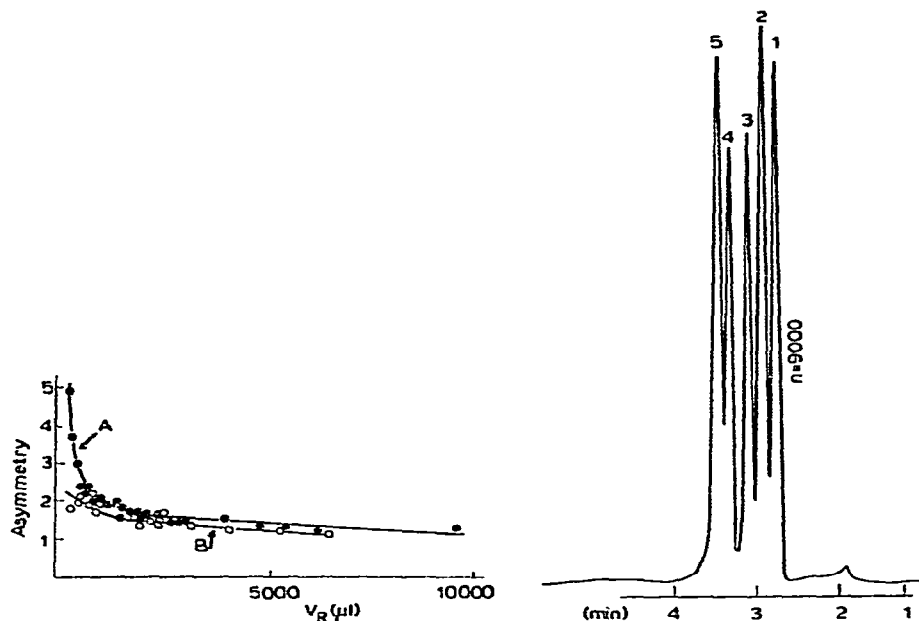It must be pointed out that a decrease in the dead volume of the detector

Fig. 4. Influence of the dead volume of the detector system on the peak symmetry. Peak asymmetry is calculated at 1/10 peak height. Dead volume of the detector system: A, 11 µl; B, 2 µl.

Fig. 5. Chromatogram of some benzoates. Column: 15 cm × 2 mm I.D. Packing: YWG-5 (5 µm). Eluent: CH$_2$Cl$_2$, 0.17 ml/min. Inlet pressure: 31 atm. Detector: UV, 254 nm (dead volume of detector system = 2 µl). Peaks: (1) isopentyl benzoate; (2) n-butyl benzoate; (3) n-propyl benzoate; (4) ethyl benzoate; (5) methyl benzoate.

systems also improved the peak asymmetry of columns with a small internal diameter, as shown in Table VI and Fig. 4.

By using the improved home-made injection and detector systems we obtained a column of I.D. 2 mm and length 10 cm with an efficiency of more than 6000 theoretical plates. Some typical chromatograms are shown in Figs. 5–7.
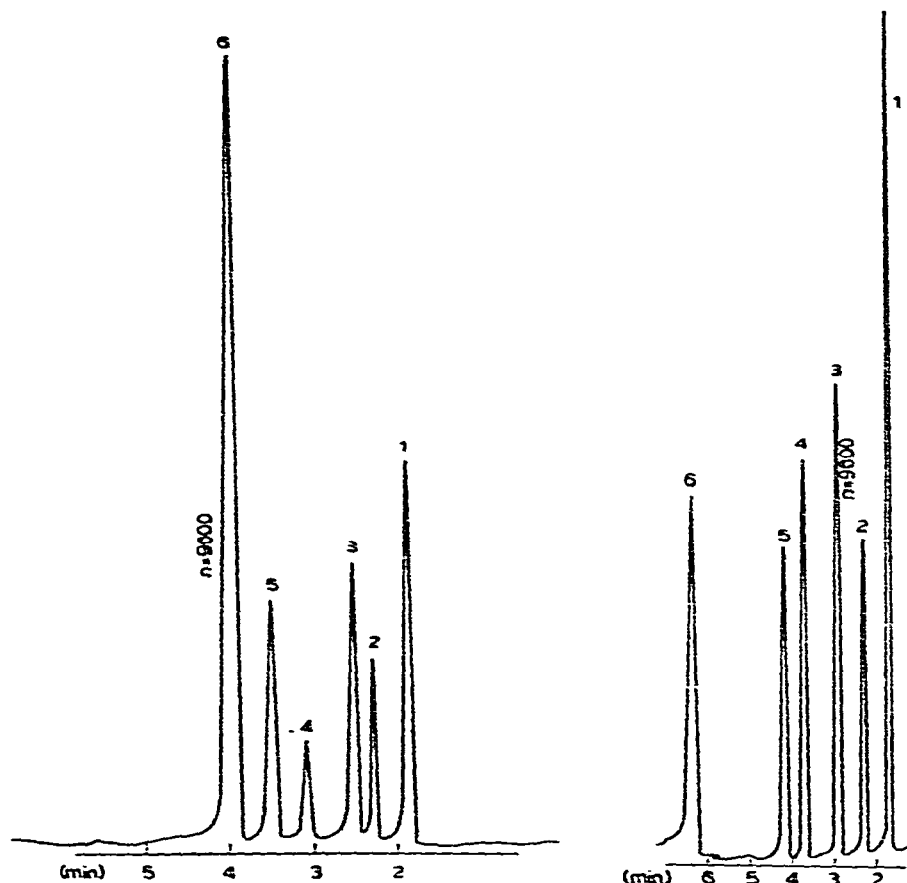

Fig. 6. Chromatogram of some aromatic compounds. Conditions as in Fig. 5. Peaks: (1) naphthalene; (2) benzyl methyl ether; (3) m-dinitrobenzene; (4) 2,6-dimethylphenol; (5) methyl benzoate; (6) benzyl ethyl ketone.

Fig. 7. Chromatograms of some aromatic hydrocarbons. Conditions as in Fig. 5, except eluent, n-heptane (0.17 ml/min). Peaks: (1) ethylene tetrachloride; (2) benzene; (3) naphthalene; (4) biphenyl; (5) phenanthrene.

CONCLUSIONS

Using the home-made column system, the influence of wall effects on column efficiency can be minimized. An approximately linear relationship between retention volume (V_R) and peak width $(2\Delta V_{1/2})$ (in volume units) for various solutes on columns with different inner diameters was obtained (eqn. 8).

For the three detector systems with dead volumes of 21 μl, 11 μl and 2 μl we obtained values of the constants in eqn. 8 of $b' = 0.024, 0.023, 0.022$ (average 0.023) and $a' = 20.5 \pm 1.5$, 11.0 ± 1.3 and 1.7 ± 1.2 μl, respectively. The volume of the detector system has a great influence on a' but only a slight influence on b'.

Using the average value of b' (0.023) and the above three values of a', we calculated β and H^{∞} from eqns. 14 and 16 and the HETP values according to eqn. 13 for various solutes on columns with different inner diameters for three different detector systems. The calculated values were in good agreement with the experimental values.

By decreasing the volume of the detector system the efficiency of columns with smaller inner diameter and peak asymmetry can be greatly improved.

ACKNOWLEDGEMENTS

REFERENCES

1 *J. Chromatogr. Sci.*, 16 (1978) 13A.
2 *J. Chromatogr. Sci.*, 16 (1978) 8A.
3 *Anal. Chem.*, 49 (1977) 515A.
4 J. P. Wolf, *Anal. Chem.*, 45 (1973) 1248.
5 T. J. N. Webber and E. H. McKerrell, *J. Chromatogr.*, 122 (1976) 243.
6 J. J. DeStefano and H. C. Beachell, *J. Chromatogr., Sci.*, 8 (1970) 434.
7 J. J. DeStefano and H. C. Beachell, *J. Chromatogr. Sci.*, 10 (1972) 654.
8 J. H. Knox and J. F. Parcher, *Anal. Chem.*, 41 (1969) 1599.
9 J. H. Knox, *J. Chromatogr. Sci.*, 15 (1977) 352.
10 Lu Peichang, *Some Theoretical Considerations of Column Chromatography*, Paper presented at *Third Chinese National Symposium on Chromatography, Dalian, March 23–28, 1979*.
11 Group of Chromatography Institute of Chemical Physics, Academica Sinica (Editors), *Microparticulate Packings for HPLC*, Science Press, Peking, 1980.
12 Cs. Horváth and H. J. Lin, *J. Chromatogr.*, 149 (1978) 43.
13 Lu Peichang, Lü Zufang and Li Haochon, *Kexue Tongbao*, 22 (1957) 699.
14 Lu Peichang, Zhou Liangmo and Ma Tsoulan, *Sci. Sinica*, 14 (1965) 705.