

CHROM. 21 298

CHROMATOGRAPHIC EVALUATION OF OLIGOMERIC C₈ REVERSED PHASES FOR USE IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

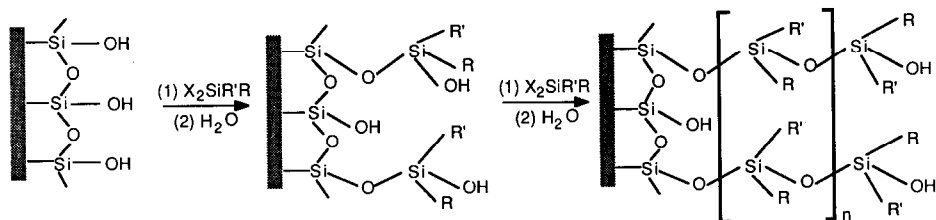
Stepwise silanisation of porous silica gel with *n*-octyldichlorosilane and subsequent hydrolysis of the unreacted chlorine atoms have been shown to produce a dense-layered C₈ chemically bonded stationary phase. Increase in carbon load results in decrease in specific surface area and pore volume of the starting silica material. Chromatographic properties obtained for aromatic hydrocarbons and alkyl benzoates under isocratic conditions were correlated with carbon content of the stationary phases. It was found that at high carbon content (> 11%) both capacity factor and separation factor are independent of carbon load.

INTRODUCTION

Over the past decade, reversed-phase high-performance liquid chromatography (RP-HPLC) has become a popular analytical technique. The use of the technique has led to intensive studies both on column design and synthesis of the stationary phases employed. These non-polar stationary phases are produced by the reaction of organochlorosilanes (or alkoxy-silanes) with the silanol groups on the surface of the porous silica. Generally, organochlorosilanes are preferred as the silanising agent, and depending on the functional group of the reactive moiety (mono-, di- or trihalides), monomeric or polymeric phases are produced.

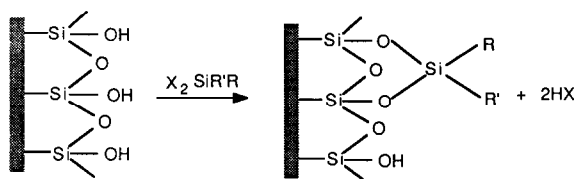
Treatment of the porous silica with di- or trichlorosilanes, for example, octylmethyl-dichlorosilane, results in the formation of polymeric phases in which the alkyl groups are attached to the silica surface via siloxane bonds. Unreacted halogen atoms are hydrolysed by traces of water present in the reaction medium and the newly formed silanols can either undergo condensation reaction with surface hydroxyl groups and/or react with more halogenated silane molecules to produce cross-linked polymeric phase.

Apart from the above reaction mechanism, a new, dense, polymer layer can be built up on the silica surface by exhaustive silanisation under controlled conditions resulting in a multilayer of the desired thickness. In this case, after the initial silanisation and subsequent hydrolysis of the unreacted functional group of the



Scheme 1.

organic moiety, the modified silica is again silanised with another portion of the starting silanising agent to form a second layer and so on. The structure of the final product is that of an oligomer made up of repeating units of the silanising agent as shown in Scheme 1, where $R' = CH_3$, $R = CH_3(CH_2)_7$ and $n = 0, 1, 2$, etc., depending on the number of reaction steps. Another possible reaction mechanism between silica and bifunctional silanes under anhydrous conditions is that of a silane molecule with two adjacent surface silanol groups to form a closed ring of siloxane bonds as shown in Scheme 2.



Scheme 2.

Whilst both reaction mechanisms are possible, the reaction shown in Scheme 2 is sterically unlikely to occur and if it actually takes place, it can be viewed as a terminating step since all the reactive functional groups of the silanising agent are covalently bonded during the first reaction. Consequently, subsequent treatment of the bonded silica with more silane molecules will result in little or no change in the carbon loading.

This paper describes the preparation of *n*-octylsilyl reversed phases by repeated silanisation of the silica support with *n*-octylmethyldichlorosilane and hydrolysing the unreacted chloro group of the silane as shown in Scheme 1. The effect of such exhaustive silanisation on the physical characteristics of the oligomeric bonded phases produced are discussed. Data on the chromatographic properties of each oligomeric phase for aromatic hydrocarbons, alkyl benzoates and strongly polar solutes are reported and correlated with carbon load.

EXPERIMENTAL

Materials

The spherical silica gel [average particle diameter = $20 \mu m$, specific surface area (S_{BET}) = $250 m^2 g^{-1}$, specific pore volume (V_p) = $1.08 ml g^{-1}$] used in this work was

a research sample from Professor K. K. Unger's laboratory (Johannes Gutenberg Universität, Mainz, F.R.G.).

Octylmethylchlorosilane was prepared by a hydrosilation reaction¹ of distilled octene (Aldrich) with methylchlorosilane (Fluka). The reaction was catalysed by chloroplatinic acid (Johnson Mathey Chemicals, London, U.K.). The chemical composition of the compound was confirmed by elemental analysis [Anal. calc. for C₉H₂₀SiCl₂ (227.25): C (47.56%), H (8.81%), Cl (31.26%); found: C (47.50%), H (8.78%), Cl (31.20%)].

HPLC-grade solvents were used for all the chromatographic measurements. Mobile phases were prepared on a volume basis and degassed before use. Solutes were analytical grade and were made up in the mobile phase.

Elemental analyses were performed on the phases at the Microanalysis Laboratory (University College, London, U.K.).

The S_{BET} [molecular cross-sectional area of nitrogen ($a_{\text{m}(\text{N}_2)}$) = 16.2 Å² molecule⁻¹] and $V_{\text{p}(\text{N}_2)}$ values of the untreated and treated silica supports were determined according to the BET nitrogen adsorption isotherm using a Micromeritics Model 2100E physical adsorption analyzer (Micromeritics, Norcross, GA, U.S.A.).

Column packing

The bonded phases were packed into 250 × 4.6 mm I.D. stainless-steel columns fitted with stainless-steel frits of 2 μm porosity (Thames Chromatography). The columns were slurry packed using a high-pressure Haskel pump, Model DSTV-122. The slurry mixture consisted of isopropyl alcohol–cyclohexanol–chloroform (2:1:1, v/v). Methanol was used as the displacement solvent and columns were packed at a pressure of about 8000 p.s.i.

Chromatography

The chromatographic equipment used consisted of a Shimadzu LC-5A pump and an SPD 2AM variable-wavelength UV detector operated at 254 nm. Solutes were injected into the column using a Rheodyne 7143 valve fitted with a 5-μl internal loop. The void volume, t_0 , was obtained by including potassium nitrate in the test mixtures and was used to calculate the capacity ratio, k' , defined as $k' = (t_{\text{R}} - t_0)/t_0$, where t_{R} is retention time of the test solute.

Preparation of bonded phases

The bonded phases were prepared using the fluidised bed technique in the apparatus previously described^{2,3}. About 50.0 g of silica gel was placed in the fluidising tower and hydrothermally treated at 200°C for 6 h using nitrogen as the fluidising gas. This was undertaken to remove any physically adsorbed water and to ensure a reproducible level of surface silanols. The treated silica was silanised under anhydrous conditions with octylmethylchlorosilane at 200°C for 4 h using dry nitrogen as the fluidising gas. The bonded material was again hydrolysed with steam as in the hydrothermal treatment and the above silanisation reaction was repeated. This procedure was repeated nine times. Samples were taken at the end of each reaction step and packed for evaluation.

TABLE I
CHARACTERISTICS OF THE BONDED PHASES

Reaction step number	% Carbon (g/g)	Surface area S_{BET} ($\text{m}^2 \text{g}^{-1}$)	Pore volume V_p (ml g^{-1})
0	0.00	250.00	1.00
1	7.58	153.04	0.82
2	9.69	144.99	0.71
3	11.14	132.69	0.60
4	12.18	126.59	0.63
5	12.69	118.64	0.59
6	14.43	115.97	0.59
7	14.90	107.11	0.54
8	15.80	106.50	0.49
9	16.79	105.21	0.48
10	17.45	105.14	0.45

RESULTS AND DISCUSSION

Characteristics of the bonded phases

The surface of the silica is only reacted once since subsequent silanisation occurred on the newly formed silanols produced by hydrolysis of the residual halogen atoms. Hence the stationary phases are characterised only by the percentage carbon load as shown in Table I. Included in Table I are values of the specific surface area and specific pore volume of the bonded phases.

Fig. 1 shows the effect of the exhaustive silanisation on carbon loading of the bonded phases. It can be seen that there is a rapid increase in the percentage carbon load between step 1 and 3 after which further silanisation steps result in a linear increase in carbon load over the range of steps investigated. This observation suggests that the reaction according to Scheme 1 dominates at all stages of the process and more newly formed hydroxyls are available for bonding on hydrolysing the unreacted chlorine

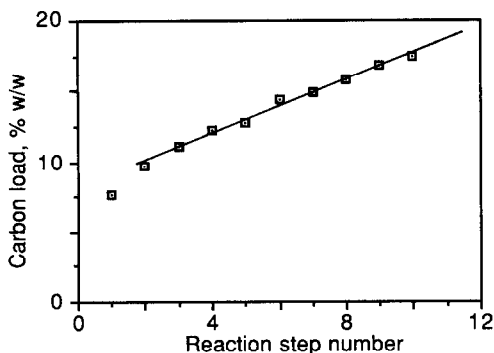


Fig. 1. Plot of percentage carbon load against reaction step number.

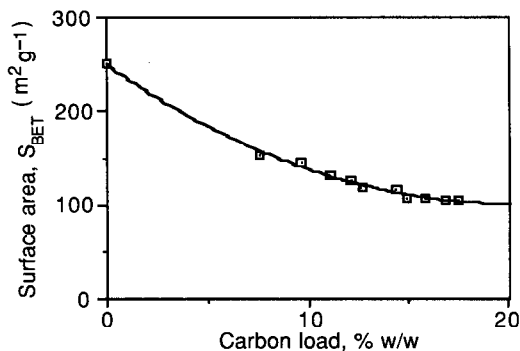


Fig. 2. Effect of carbon load on specific surface area of the silica support.

atoms. However the extent of further reaction may reach a limiting value due to steric hindrance and/or non-availability of sufficient hydroxyl groups for further bonding. A similar investigation⁴ of such a silanisation process with octadecyltrichlorosilane showed a minimal increase in carbon loading after the first three steps. While this might be true considering the molecular cross-sectional area of C₁₈ as compared to that of C₈, Verzele and Mussche⁵ have shown that their bonding procedure depends on the amount of water added to the reaction mixture and this directly affects the carbon load. However, their procedures were carried out in solvents; thus the procedure is different to the present one.

The effect of surface modification on surface area and pore volume of the phases produced is shown in Figs. 2 and 3 respectively. Generally, the surface area and pore volume decreases with increase in carbon load as measured by BET nitrogen adsorption isotherm up to step 7 after which a limiting value is reached for the surface area but pore volume continues to decrease in a linear manner. As shown in Table I both the surface area and the pore volume have decreased by about 60%. This is thought to be due to the increase in hydrocarbonaceous layer of the alkyl ligand on the porous silica matrix. The effect becomes minimal when further reactions with the silanols are sterically hindered indicating a dense surface coverage. As noted by Erdel

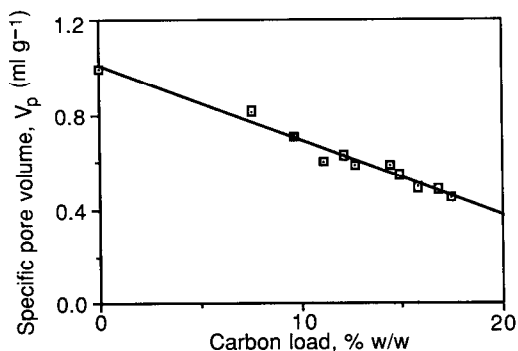


Fig. 3. Effect of carbon load on specific pore volume of the silica support.

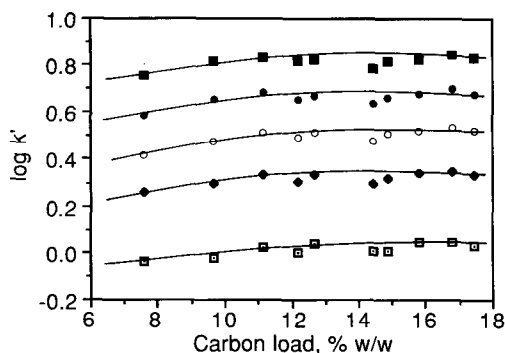


Fig. 4. Plot of $\log k'$ for aromatic hydrocarbons against carbon load. Symbol representation: (\square) benzene, (\blacklozenge) naphthalene, (\circ) biphenyl, (\bullet) anthracene, (\blacksquare) pyrene.

and Unger⁶ surface modification of porous silica leads to: (i) a specific increase in weight of the starting material, (ii) a decrease in the adsorption potential of the bonded phase since the pore structures are determined from adsorption and desorption measurements, and (iii) an increase in the thickness of the surface layer which in turn decreases the mean pore diameter of the support. The above effects becomes more significant with purely microporous materials and can lead to total blockage of the pores when exhaustively silanised thus resulting in effectively a non-porous packing.

Chromatographic properties

Retention times and capacity ratio. The retention properties of the stationary phases were determined at 20°C using methanol–water (70:30, v/v) as mobile phase. The dependence of the logarithm of capacity factor on carbon content of the bonded phases are shown in Figs. 4 and 5 for the test solutes. As shown in Figs. 4 and 5, the capacity factor increased slightly up to about 11% carbon after which it remains almost constant with further increase in carbon load. Statistical analysis of the results at carbon load greater than 11% gave no significant difference in $\ln k'$ with increasing

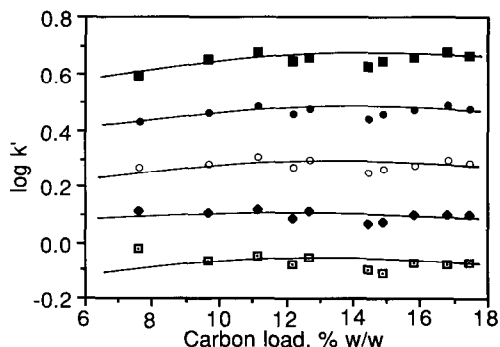


Fig. 5. Plot of $\log k'$ for *n*-alkyl benzoates against carbon load. Symbol representation: (\square) methyl benzoate, (\blacklozenge) ethyl benzoate, (\circ) propyl benzoate, (\bullet) butyl benzoate, (\blacksquare) pentyl benzoate.

carbon load. Evidence for the absence of significant differences in the means and variances of the results was obtained when the calculated t and F values are less than the true or statistics table values ($P = 0.05$). Thus while the carbon load increased with the reaction step, the retention properties of the solutes are independent of the carbon content of more than 11%. However there is a slight difference in the shape of the graph when compared to that obtained with increasing carbon content or alkyl chain length⁷⁻⁹ of the bonded ligand. This observation confirms the importance of bonded ligand structure and conformation on solute retention in reversed-phase chromatography.

While several mechanisms¹⁰⁻¹² have been proposed for solute retention in reversed-phase chromatography, the experimental results given in this paper indicate that the logarithm of the capacity factor depends on the molecular contact area between the test solutes and the hydrocarbonaceous layer of the stationary phase. Therefore, for an oligomeric phase solute molecules are unable to interact completely with the ligands and further increase in the extent of the bonded alkyl layer reduces the effective interactive surface between the solute molecules and the stationary phase.

Fig. 6 clearly illustrates the effect of non-polar interaction on retention times for two types of solute with increasing carbon content (columns 1, 3, 5 and 10).

It was observed that the retention of aromatic hydrocarbons slightly increased with increasing carbon content. A possible explanation for this is that the aromatic molecules are capable of penetrating into the hydrocarbon layer as compared to polar compounds where the retention generally decreases with carbon load simply because the molecules either interact with the surface layer or a small proportion of the ligands. Also the decrease in solute retention times can be attributed to the decrease in the specific pore volume of the silica matrix resulting in some solutes being partially excluded from the pores of the silica support.

Selectivity. Selectivity of two solutes, 1 and 2 is defined as

$$\alpha = \frac{k'_2}{k'_1} \quad (k'_2 > k'_1) \quad (1)$$

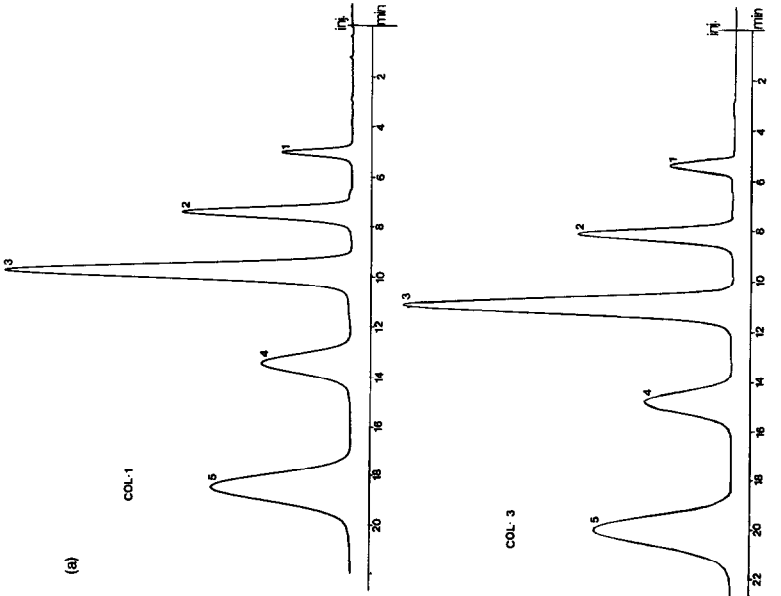
As shown in Figs. 7 and 8 the selectivity for various solute pairs generally increases up to about 10% carbon load after which it remains substantially constant with increasing carbon content. This stresses the importance of retention time being independent of carbon content at high carbon load

Resolution. Resolution, R_s , for a given pair of solutes is expressed as

$$R_s = \frac{t_{R_2} - t_{R_1}}{0.5(w_1 + w_2)} \quad (2)$$

where t_{R_i} and w_i are the retention time and baseline peak width for solute i . As shown in Fig. 6, the solutes are completely resolved ($R_s > 1$) except on columns 5 and 10 where acetone and 2-acetylpyridine peaks overlapped which will partly be due to slightly different column efficiencies.

Peak asymmetry. In order to qualitatively determine the presence of residual silanols on the bonded phases, peak asymmetry, A_s , was calculated for a variety of solutes. This parameter is defined as



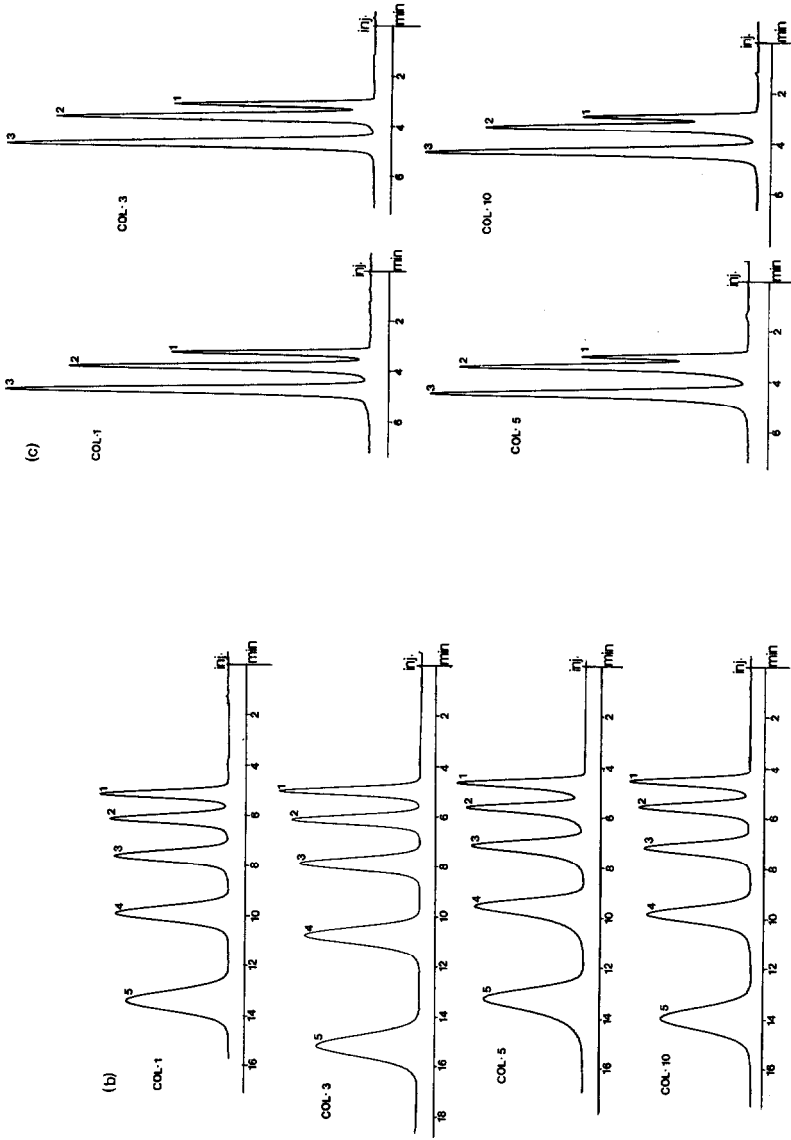


Fig. 6. Chromatograms of the test solutes. Column: 250 × 4.6 mm I.D. Mobile phase: methanol-water (70:30, v/v). Flow-rate: 1 ml/min. Temperature: 20°C. Detector: UV photometer, wavelength 254 nm. Solutes: (a) 1 = benzene; 2 = naphthalene; 3 = biphenyl; 4 = anthracene; 5 = pyrene. (b) 1 = Methyl benzoate; 2 = ethyl benzoate; 3 = propyl benzoate; 4 = butyl benzoate; 5 = pentyl benzoate. (c) 1 = Acetone; 2 = 2-acetyl pyridine; 3 = nitrobenzene.

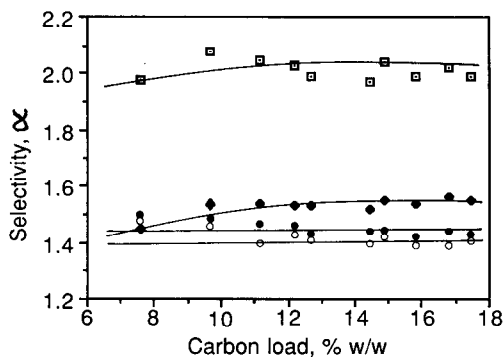


Fig. 7. Dependence of selectivity for aromatic hydrocarbons on carbon load. Symbol representation: (□) naphthalene-benzene, (◆) biphenyl-naphthalene, (●) anthracene-biphenyl, (○) pyrene-anthracene.

$$A_s = \frac{w_t}{w_l} \quad (3)$$

where w_t and w_l are the trailing and the leading half widths at 10% peak height respectively. An examination of Fig. 6 shows that the peaks both for polar and non-polar solutes were symmetrical and values of A_s as obtained from similar chromatograms under identical conditions are shown in Table II for columns 1, 3, 5, 7, 9 and 10. Surprisingly, A_s is about unity for all solutes indicating the absence of an appreciable number of residual silanols considering the fact that the phases were not endcapped. Alternatively, the acidic surface silanols, held to be the cause of peak tailing, are shielded by the oligomeric surface layer and hence are unable to interact. Amongst the six representative columns, column 3 produced the best symmetrical peaks with $A_s \approx 1.00$.

Retention behaviour of homologous series. Earlier work¹³⁻¹⁵ on the retention behaviour of homologous series in reversed-phase chromatography has shown that the

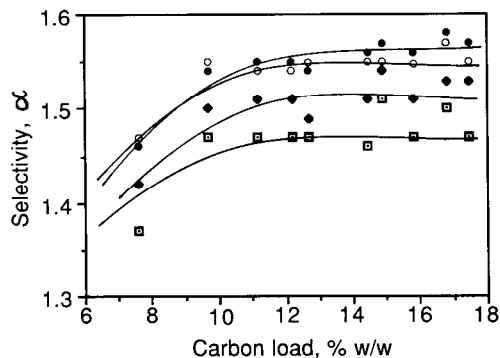


Fig. 8. Dependence of selectivity for *n*-alkyl benzoates on carbon load. Symbol representation: (□) ethyl-methyl benzoate, (◆) propyl-ethyl benzoate, (○) butyl-propyl benzoate, (●) pentyl-butyl benzoate.

TABLE II
PEAK ASYMMETRY

Step or column number	Coverage (weight%)	Peak asymmetry		
		Pyrene	Pentyl benzoate	Nitrobenzene
1	7.58	1.01	1.06	1.00
3	11.14	1.02	1.01	1.04
5	12.69	1.13	1.15	1.03
7	14.90	1.04	1.04	1.04
9	16.79	1.05	1.05	1.04
10	17.45	1.08	1.05	1.03

logarithm of capacity factor depends linearly on the carbon number or number of methylene groups in a homologous series of bonded ligands. A similar trend was observed in this work as shown in Figs. 9 and 10 for aromatic hydrocarbons and *n*-alkyl benzoates, respectively. Each plot shows an increase in retention time with increasing molecular surface area of the eluite. This set of lines can be expressed as

$$\log k' = an_c + b \quad (4)$$

where n_c is the number of carbon atoms or CH₂ groups and a and b are constants determined by the mobile phase, stationary phase and the nature of the eluite. The slopes and intercepts for each series obtained on each column are given in Table III. From the plots and the correlation coefficient values which are near unity in all cases it can be concluded that the experimental data gave excellent fit. The slopes are represented by $a = 0.18 \pm 0.0095$ and 0.081 ± 0.018 and the intercepts by $b = -0.077 \pm 0.022$ and -0.48 ± 0.034 for alkyl benzoates and aromatic hydrocarbons, respectively. Substituting the mean values of a and b into eqn. 4 for each series gives two linear equations, that is, for alkyl benzoates, eqn. 4 becomes

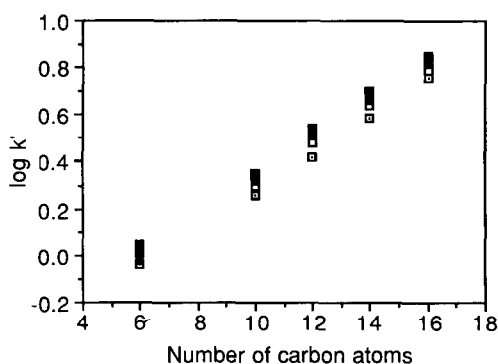


Fig. 9. Plot of $\log k'$ for aromatic hydrocarbons versus carbon number. Symbol representation: (□) column 1, (◆) column 2, (●) column 3, (○) column 4, (■) column 5, (◻) column 6, (▲) column 7, (▼) column 8, (△) column 9, (▽) column 10.

TABLE III

PARAMETERS OF $\log k' = an_c + b$ RELATIONSHIPS FOR HOMOLOGOUS *n*-ALKYL BENZOATES AND AROMATIC HYDROCARBONS

Correlation coefficient, $r \approx 1$ for all the plots.

Column number	<i>n</i> -Alkyl benzoates		Aromatic hydrocarbons	
	Slope <i>a</i>	Intercept <i>b</i>	Slope <i>a</i>	Intercept <i>b</i>
1	0.1559	-0.0340	0.0796	-0.5254
2	0.1803	-0.0738	0.0846	-0.5357
3	0.1835	-0.0614	0.0821	-0.4739
4	0.1827	-0.0900	0.0822	-0.4987
5	0.1794	-0.0626	0.0795	-0.4436
6	0.1822	-0.0974	0.0789	-0.4745
7	0.1891	-0.1074	0.0819	-0.4867
8	0.1832	-0.0810	0.0793	-0.4365
9	0.1900	-0.0842	0.0814	-0.4454
10	0.1849	-0.0810	0.0807	-0.4565
Mean	0.1811	-0.0773	0.0810	-0.4777
S.D.	0.0095	-0.0218	0.0177	-0.0342

$$\log k' = 0.18n_c - 0.077 \quad (5a)$$

and for aromatic hydrocarbons

$$\log k' = 0.081n_c - 0.48 \quad (5b)$$

Therefore eqns. 5a and 5b can be used to estimate the retention for any member of the series under the same chromatographic conditions. Furthermore, an examination of Table III shows that *b* values for benzoates are generally less than that of aromatic hydrocarbons which may be due to the increase in polarity of the test solutes.

Column efficiency. As shown in Table IV, column performance in terms of reduced plate height, *h* ($h = H/d_p$) and reduced velocity, *v* ($v = ud_p/D_m$) where *H* is

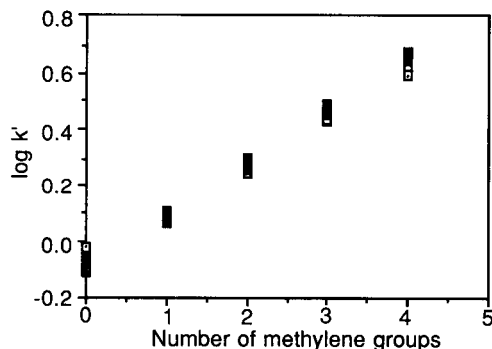


Fig. 10. Plot of $\log k'$ for *n*-alkyl benzoates versus number of methylene groups. Symbol representation as for Fig. 9.

TABLE IV

EFFECT OF CARBON LOAD ON REDUCED PARAMETERS FOR ANTHRACENE ($k' = 4$)

Column number	Carbon load (% w/w)	h	v
1	7.58	9.19	5.43
3	11.14	8.34	5.39
5	12.59	10.91	5.67
7	14.90	10.57	5.49
9	16.79	9.84	5.41
10	17.45	10.15	5.64

plate height and D_m the diffusion coefficient of the solute, were obtained for columns 1, 3, 5, 7, 9 and 10 using the elution peak of anthracene ($k' = 4$) and mobile phase flow-rate of 1 ml min^{-1} . While there is no clear correlation between carbon load and reduced plate height, h , column 3 has the minimum h value and hence appears to be the best packing material. Little appears to be gained on increasing the number of the reactions steps above $n = 3$ on the performance of the stationary phases produced.

CONCLUSION

This work has demonstrated that both the pore volume and surface area of the silica support decreases with increasing carbon load for an oligomeric series of reversed phases. Furthermore while the surface area approached a limiting value with increasing carbon load, the pore volume continued to decrease in a linear manner over the range of bonding reactions investigated.

The results have shown that after the third reaction all the columns exhibit very similar chromatographic properties, indicating that the type of interaction between the solutes and the bonded phases are the same for all the columns. Linear correlations were obtained between $\log k'$ and carbon number for members of a homologous series and the slope of the plots increases up to 11% carbon load after which it remained almost constant. Amongst the two classes of test solutes, the slope of $\log k'$ versus carbon number for alkyl benzoates is greater than that of aromatic hydrocarbons indicating that the benzoates are more retained than aromatic hydrocarbons.

While selectivity for all the columns appeared to be affected by the carbon load up to 10%, all the columns produced symmetrical peaks. Hence with fluidised bed method good oligomeric packings can be produced.

Based on these results, further investigation on stability and the influence of temperature and mobile phase composition on the chromatographic properties of the phases are in progress in the authors laboratory and the results will be the subject of a subsequent paper.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. K. K. Unger and his research students for the supply of the silica and their assistance during the study visit of one of us (S.O.A.) to the F.R.G. We would also like to thank Prof. R. P. W. Scott for his helpful comments.

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