

Characterization of a Range of Alkyl-Bonded Silica HPLC Stationary Phases: Chromatographic Behavior of Neutral, Acidic, and Basic Test Solutes

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Abstract

The effect of the chain length (C_1 – C_{18}) of *n*-alkyl bonded-phase silica packing materials on retention behavior is investigated using three series of substituted test solutes: biphenyls (neutral), barbiturates (weakly acidic), and pyridines (weakly basic). An excellent correlation between the capacity factor (k) and alkyl chain length is observed in the majority of cases. However, deviations from this relationship occur when the alkyl chain length is increased beyond C_8 for certain groups of solutes or when there is significant solute interaction at secondary sites on the packing material. Log k plots showed that the neutral and barbiturate solutes have similar retention mechanisms at all the alkyl chain lengths. However, the basic solutes show significant evidence of secondary interactions, which are confirmed by the peak asymmetry data and the lack of correlation between the \log_{10} octanol–water partition coefficient ($\log P$) values of the solutes and retention. There is a significant trend towards improved peak symmetry for the basic solutes when the packing materials having the highest alkyl surface density (C_1 , C_2 , and C_3 alkyl chain lengths) are used; this suggests that residual silanols are more effectively masked by the shorter alkyl chain lengths. The neutral biphenyl solutes show a progressive increase in efficiency as the chain length increases, but consistent trends are not observed for the acidic and basic solutes. The results show that the length of the bonded alkyl chain is of primary, though not sole, importance in governing the retention of acidic, basic, and neutral compounds on a series of monofunctional *n*-alkyl-bonded reversed-phase packing materials. Retention mechanisms that might explain the observed separation behavior are discussed.

Introduction

Alkyl-bonded microparticulate silica materials are used widely for reversed-phase high-performance liquid chromatographic (HPLC) separation of a broad range of compounds. Despite the improved reproducibility that can be achieved with

modern bonded phases, there are still significant differences in the chromatographic selectivity of nominally identical materials (1–3). Such fluctuations in separation performance can be attributed to the variable surface properties of the parent silicas used to prepare the bonded phases, the type and conditions of the derivatization reaction, and the nature of the bonded surface. Surface substituents that may affect the separation behavior include the amount and type of silanol groups, the presence of trace elements, or the chemical structure and surface coverage of the attached ligand (2,4,5).

Previous studies have confirmed that the chain length of the bonded alkyl ligands on reversed-phase silicas is an important variable that influences retention behavior (6–10). However, such studies have sometimes used a restricted series of alkyl chain lengths and have often used neutral compounds as model solutes. These model solutes are not representative of the wide range of pharmaceutical and chemical compounds analyzed by reversed-phase HPLC. Also, the purity of the base silica material used for bonding the alkyl groups is important, and unless carefully controlled, difficulties can arise in interpreting the resulting data, especially when the underlying silanols play a role in the separation process.

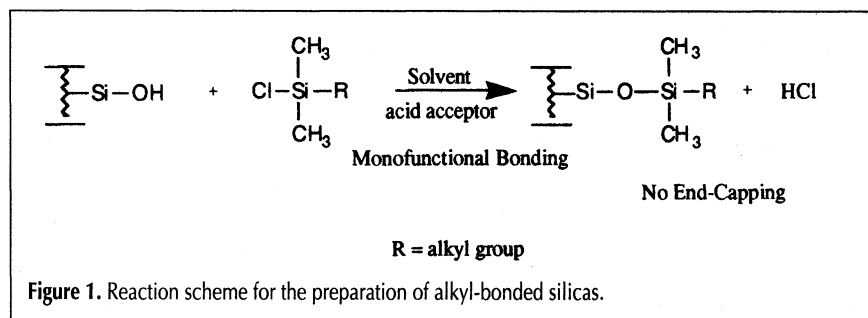
Many studies have indicated that residual silanols contribute significantly to the retention of solutes, often in a manner detrimental to separation performance (2,11,12). The use of monofunctional silanes for bonding has been reported to significantly reduce these residual silanol effects, especially when the surface coverage is near maximal (13,14), but this effect has not been investigated systematically.

The purpose of this work was to examine the effect of a range of immobilized alkyl chain lengths (C_1 to C_{18}) on the separation behavior of a series of neutral, acidic, and basic solutes. The analytes were selected to be representative of the type of small molecules typically analyzed by reversed-phase HPLC, in other words, neutral, weakly acidic, and weakly basic. Data on the surface analysis of the same range of *n*-alkyl bonded silicas have been published previously (15).

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Table I. Bulk Analysis of Percent Carbon and Surface Coverage of Base and Alkyl-Bonded Silicas

Bonded alkyl group	No. of carbon atoms	% Carbon	Surface coverage ($\mu\text{mol}/\text{m}^2$)
Base silica	—	0.06	—
Trimethylsilyl silica (C_1)	3	2.14	3.41
Dimethylethylsilyl silica (C_2)	4	2.59	3.09
Dimethylpropylsilyl silica (C_3)	5	3.00	2.87
Dimethylbutylsilyl silica (C_4)	6	3.62	2.88
Dimethyloctylsilyl silica (C_8)	10	5.86	2.80
Dimethyldodecylsilyl silica (C_{12})	14	8.14	2.78
Dimethyloctadecylsilyl silica (C_{18})	20	10.64	2.54

**Table II. Structures and Physicochemical Properties of Test Solutes**

Compound	R1	R2	log P^*	pK_a^*	% Ionized
<i>4-Substituted biphenyls (neutral)</i>					
Biphenyl			3.7		
4-Bromo biphenyl			4.6		
4-Ethyl biphenyl			4.7		
4-Acetyl biphenyl			3.4		
4-Methyl biphenyl			4.2		
4-Hydroxymethylbiphenyl			2.6		
<i>Disubstituted barbituric acids (acidic)[†]</i>					
Amobarbital	Ethyl	Isopentyl	2.01	8.07	0.001
Barbital	Ethyl	Ethyl	0.6	7.43	0.01
Butethal	Butyl	Ethyl	1.6	8.00	0.001
Phenobarbital	Ethyl	Phenyl	1.47	7.49	0.01
Secobarbital	Allyl	1-Methylbutyl	2.4	8.09	0.001
<i>3-Substituted pyridines (basic)[‡]</i>					
Pyridine			0.6	5.19	1.56
3-Amino pyridine			0.17	6.1, <3.0	11.2
3-Bromo pyridine			1.6	2.84	0.01
3-Chloro pyridine			1.43	2.84	0.01
3-Cyano pyridine			0.2	1.35	0.001
3-Ethyl pyridine			1.78	5.8	5.9
3-Acetyl pyridine			0.62	3.3	0.02

* pK_a and log P values were obtained from *CRC Handbook (21)* and *Dissociation Constants of Organic Bases in Aqueous Solution: Supplement (22)*.
[†] Ionization at pH 3.0.
[‡] Ionization at pH 7.0.

Experimental

Chemicals

Sodium phosphate, di-sodium hydrogen phosphate, potassium dihydrogen phosphate, and methanol (all HPLC grade) were obtained from Fisons (Loughborough, UK). Deionized, purified water was produced using an Elgastat water purification system (Elga Ltd; Cheltenham, UK). Chromatographic-grade microporous silica was provided by Shandon HPLC (Runcorn, UK), and *n*-dimethylalkylchlorosilanes were obtained from Fluka Chemicals (Dorset, UK). All other chemicals were of analytical grade or better. All solvents required for moisture-sensitive reactions were dried before use. Chemical reactions were performed in inert conditions under 1 atmosphere of dry nitrogen.

Preparation of the spherical base silica

A single batch (1.0 kg) of microparticulate base silica was prepared by the gelation of a colloidal silica under controlled conditions. After emulsification, washing, and sintering, the base silica was classified, and the particle size distribution was measured on an Elzone 120 instrument (Particle Data Laboratories; Elmhurst, IL). The average particle size was determined to be $4.57 \pm 0.63 \mu\text{m}$ (mean \pm standard deviation). The base silica was then modified via a hydration process to produce the finished product used in the production of *n*-alkyl-bonded silicas. This process involved boiling the silica in 5mM HCl for 13 h, resulting in an adjustment of the apparent pH of the silica in aqueous solution and a conversion of surface siloxanes to silanols. The pore size and pore volume of the silica particles were determined using mercury porosimetry (Micrometrics 9300 Analyzer), and the surface area was measured by BET single point analysis (Micrometrics Flowsorb 2300). The base silica material had a pore volume of $0.72 \text{ cm}^3/\text{g}$, a pore size of 130 \AA , and a surface area of $174 \text{ m}^2/\text{g}$. The chromatographic selectivity parameters were characteristic of commercial grade, 5- μm particle size Hypersil. This batch of parent silica was used to prepare the bonded silicas used in this study.

Synthesis of the alkyl-bonded silica materials

A series of 5- μm alkyl-bonded silicas was prepared from the single batch of the hydrated Hypersil base silica using the appropriate *n*-dimethylalkylsilyl chlorides with

alkyl chain lengths of between C₁ and C₁₈. Monofunctional chlorosilanes were used in the reaction, and the subsequent *n*-alkyl bonded silica materials were not endcapped. The alkyl silane (40% v/w in toluene) was reacted with 10.0 g of the base silica with 10% w/w of base and refluxed for 6 h. The reaction product was dried in a vacuum oven for 12 h and allowed to cool. It was then washed in hexane (100 mL), acetone (100 mL), acetone–water (10 mL; 50:50), and acetone (100 mL). The final product was dried in an oven at 80°C prior to use. A series of *n*-alkyl-bonded silicas were synthesized using this method; their structures are shown in Table I. The reaction pathway was similar to that outlined in Figure 1.

The batches of bonded silica materials were prepared specially for this project and were not made available commercially by Shandon HPLC. All the columns were subjected to rigorous quality control testing to ensure that the chromatographic performance was within the criteria accepted for a high-efficiency reversed-phase packing material. The columns were slurry-packed into 10-cm × 4.6-mm i.d. stainless steel columns at a pressure of 8000 psi using acetone as a packing and slurry solvent. The packing was retained using compression fittings and 2-μm metal frits. Prior to testing, the columns were conditioned with methanol–water (70:30).

HPLC equipment

The liquid chromatographic system consisted of two Gilson Model 303 HPLC pumps (Gilson Medical Instruments; Villiers-

le-Bel, France), a Gilson 116 ultraviolet detector, a Gilson 231/401 autoinjector, and Gilson 715 integration and data handling software. The column temperature was controlled using a column oven set at 30°C (Jones Chromatography; Mid Glamorgan, UK). All mobile phases were degassed by helium sparge before use.

Chromatographic measurements

Three series of test solutes were chosen, comprising non-polar, weakly acidic, and weakly basic compounds of pharmaceutical relevance: 4-substituted biphenyls, disubstituted barbiturates, and 3-substituted pyridines, respectively. The substituents on each of the test series were selected to include a wide range of lipophilicities. The log₁₀ octanol–water partition coefficient (log *P*) values, *pK_a* values, and percentage ionization at mobile phase pH of all the test compounds are shown in Table II. The test solutes, including acids and bases, were chosen to be predominantly in their un-ionized form at the pH of analysis. The stability of the test solutes under the conditions of storage and analysis was established to ensure that degradation products did not interfere with the chromatographic analysis.

For the barbiturate standards, the mobile phase was pH 3.0 aqueous potassium phosphate buffer (0.005M)–methanol (68:32), and the detection wavelength was 210 nm. For the biphenyl standards, the mobile phase was water–methanol (39:61), and the detection wavelength was 260 nm. For the pyridine standards, the mobile phase was pH 7.0 aqueous sodium phosphate buffer (0.005M)–methanol (87:13), and the detection wavelength was 205 nm. The standard solutions of all the test compounds were made up in the appropriate HPLC mobile phase at a concentration of 50 or 100 μg/mL. An injection volume of 20 μL was used for analysis of all the test solutions, and the flow rate was 1.0 mL/min. Under these conditions, there was no evidence of column overload for any of the test solutes.

The retention behaviors of the compounds in the neutral, acidic, and basic test mixtures were characterized as follows. The retention factors (*k*), peak asymmetry factors, and plate numbers (*N*) were measured from replicate (*n* = 6) injections of each individual test compound on each column evaluated. The retention factors, plate numbers, and asymmetry factors were calculated using the following formula:

$$k = (t_R - t_M) / t_M \quad \text{Eq 1}$$

where *t_M* is the mobile phase hold-up time, and *t_R* is the total retention time of the solute. The hold-up time was taken as the time from injection to the moment when the trace solvent disturbance crossed the baseline. The solvent disturbance was generated by the sol-

Table III. Retention Factors, Asymmetry Factors, and Plate Numbers for Biphenyls

Alkyl chain length	Biphenyl	4-Bromo	4-Ethyl	4-Acetyl	4-Methyl	4-Hydroxymethyl
<i>Retention factors</i>						
1	2.55	4.00	4.91	2.30	3.64	1.66
2	3.31	5.61	7.09	2.85	4.96	1.98
3	3.81	6.48	8.29	2.96	5.69	2.14
4	6.10	8.03	10.31	3.20	6.86	2.33
8	10.21	20.54	26.70	6.23	16.78	3.76
12	14.93	33.81	45.06	8.58	27.16	4.61
18	19.33	49.00	65.20	10.16	38.29	4.93
<i>Asymmetry factors</i>						
1	1.61	1.35	1.23	1.83	1.43	1.77
2	1.59	1.16	1.08	1.59	1.24	1.75
3	1.29	1.00	0.91	1.48	1.08	1.26
4	1.18	0.92	0.87	1.41	1.01	1.67
8	1.09	0.91	0.94	1.30	0.97	1.66
12	1.09	1.07	1.10	1.16	1.08	1.43
18	1.42	1.11	1.13	1.21	1.13	1.42
<i>Plate numbers</i>						
1	1412	1808	2213	953	1732	750
2	1263	2374	2795	1263	2195	883
3	1922	2617	3201	1474	2497	1018
4	2181	3032	3314	1418	2683	1157
8	4719	5011	6245	2874	5689	1695
12	5085	5463	5235	3739	5426	2238
18	5500	5893	5670	3992	5836	2040

vent in which the sample was dissolved. In all the mobile phases used, t_M was found to be 0.8 min at a flow rate of 1.0 mL/min using a 100- \times 4.6-mm column.

$$N = 5.54 (t_R/w_h)^2 \quad \text{Eq 2}$$

where w_h is the peak width at half height.

The peak asymmetry factor was calculated according to Kirkland and co-workers (16).

Elemental analysis and surface coverage

Elemental analysis data of percent carbon was obtained for each of the bonded-phase materials using an EC-12 Carbon Determinator (Leco; St. Joseph, MI). A known amount of silica was placed in the furnace and combusted in pure oxygen. The carbon in the sample was oxidized to yield CO₂ and CO (which was converted to CO₂ with a platinum catalyst). The amount of CO₂ formed was determined using infrared radiation, and from that, the percentage of carbon present in the sample was calculated to an accuracy of $\pm 0.04\%$. The carbon percent was used to calculate the ligand surface density according to the following equation:

$$\text{surface coverage } (\mu\text{mol}/\text{m}^2) = \frac{\%C \times 10^6}{1200 nA} \quad \text{Eq 3}$$

where %C is expressed as a ratio of the weights, n is the number of carbons in the bonded phase, and A is the surface area of the substrate (in meters squared per gram).

The percentage carbon values and the surface coverage data are reported in Table I.

Results and Discussion

The chromatographic experiments were performed with a series of alkyl bonded materials derived from a single batch of microporous spherical base silica. The use of the same support material for all of the bonded materials has enabled this investigation to focus on the role of the immobilized n -alkyl chains in the chromatographic separation of the test solutes without the variability introduced by the use of different batches of base silica. Care was taken when the bonded silicas were prepared and when the columns were packed to ensure that the only variable was the chain length of the alkyl group attached to the base silica. It should be noted that surface coverage decreased slightly with increasing chain length (Table I), but this should not invalidate any conclusions. The single batch of silica base material, on which the bonded alkyl groups were immobilized, was shown to have passed all the appropriate quality assurance checks for a commercial material. The quality assurance checks also confirmed that the manufacture and column

packing of the n -alkyl-bonded materials was performed to a high degree of reproducibility and was within the standards of normal commercial materials according to both the standard physical and chromatographic tests.

Thus a comprehensive set of data has been gathered on the effect of alkyl chain length on the chromatographic behavior of test solutes. The data are representative only of this particular batch of silica and the type of bonding process used. However, because this type of manufacturing process is similar to that used for several other commercially available materials, the results are likely to have more general significance to the separation behavior of other monofunctional n -alkyl bonded phases.

The retention behavior of test solutes was not determined by measuring adsorption isotherms but by a more practical approach using precise chromatographic studies to compare the retention behavior of a series of specially chosen test compounds. The test solutes were selected to cover a range of lipophilicities for each series of neutral, basic, and acidic compounds, and care was taken to ensure that the compounds eluted from the columns under reversed-phase conditions without mobile phase additives, apart from dilute buffers. The neutral biphenyls were used to assess the hydrophobic or partitioning interactions with the stationary phase, and it

Table IV. Retention Factors, Asymmetry Factors, and Plate Numbers for Barbiturates

Alkyl chain length	Ethylisopentyl	Diethyl	Ethylbutyl	Ethylphenyl	Allyl 1-methylbutyl
<i>Retention factors</i>					
1	9.25	1.96	5.48	3.48	12.18
2	14.25	2.38	7.86	4.73	19.40
3	17.13	2.59	9.08	5.25	23.46
4	20.50	2.76	10.54	5.98	28.24
8	34.50	3.19	16.18	8.88	51.09
12	35.75	3.08	16.41	8.96	54.06
18	37.88	3.13	17.24	9.54	58.26
<i>Asymmetry factors</i>					
1	0.80	1.52	0.94	1.12	0.72
2	0.76	1.39	0.84	0.98	0.69
3	0.70	1.29	0.78	0.90	0.65
4	0.65	1.27	0.73	0.85	0.63
8	0.85	1.33	0.86	0.92	0.85
12	1.12	1.50	1.14	1.15	1.13
18	1.09	1.49	1.12	1.13	1.09
<i>Plate numbers</i>					
1	4995	2706	4442	3581	5210
2	5581	2866	5091	4107	5641
3	5213	3266	4970	4338	5211
4	5697	3533	5401	4691	5540
8	5466	3217	5363	4729	5344
12	4746	2540	4682	4067	4518
18	5740	3000	5596	4938	5578

has been assumed that these test compounds do not interact significantly with surface silanols or other ionic surface species in the predominantly aqueous-based mobile phases used. The barbiturates were chosen as acidic probes and the substituted pyridines as basic probes to assess the potential nonpartitioning (i.e., secondary) interactions of these classes of solutes with the packing material surface.

In addition, the asymmetry factor and plate number of each of the solutes on each of the packing materials were examined. Peak efficiency and asymmetry properties are partly due to the quality of the packed bed of the bonded silica particles, but they also reflect the kinetics of secondary interaction between the packing material and solute.

Effect of alkyl chain length on retention

The retention behavior of all test solutes on the series of bonded silicas, expressed as retention factors, are given in Tables III–V. The dependence of k on alkyl chain length is shown in Figure 2. The retention values for each set of probes are assumed to be solely dependent on the interaction of the probes with the surface of the packing material because all the chromatographic conditions were maintained constant throughout the experiments.

For the series of neutral, acidic, and basic probes, there was

an approximately linear relationship between k and alkyl chain length. This relationship held from C_1 to C_{18} for the neutral biphenyls, but for certain pyridines and for all of the barbiturates, there was a break point in the linear correlation indicated by a reduced slope to the relationship. Almost identical plots (not shown) were obtained when k was plotted against the bulk carbon content (i.e., carbon loading) of the packing materials. The chromatographic behavior of the pyridines was not as consistent as the biphenyls or the barbiturates, with significant deviations in linearity for several of the solutes, in particular the 3-acetyl pyridine and 3-amino pyridine. This anomalous behavior was attributed to secondary interactions with the packing materials.

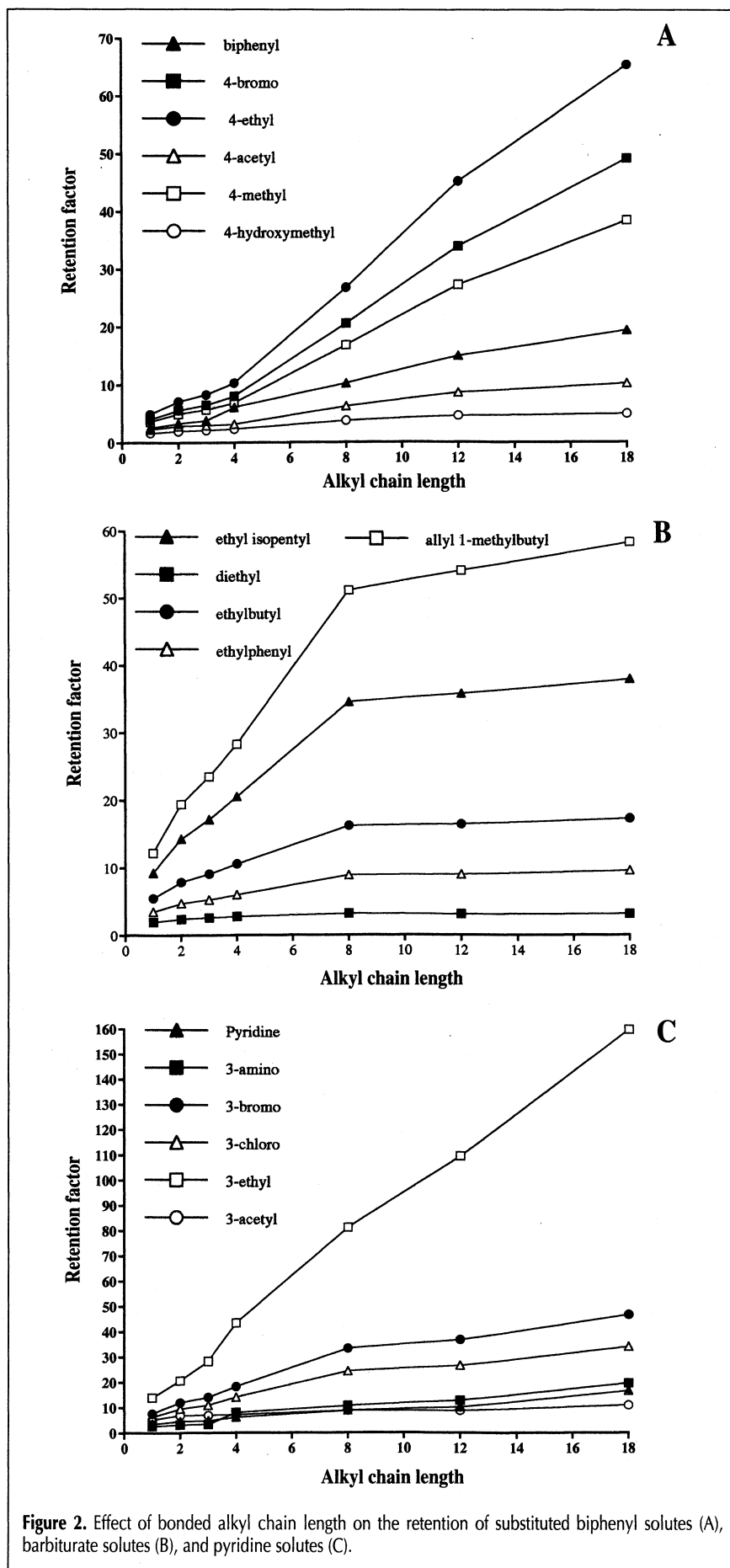
A continuous increase in retention with increasing alkyl chain length has been observed by other investigators (6,8,17,18), although this has usually been reported as a correlation with $\log k$ rather than k . The linear correlation between alkyl chain length and k can be predicted from hydrophobic or partition type interactions because as the chain length is doubled, the amount of stationary phase is doubled, and it would be expected that the retention volume would also double. The data from our investigation is consistent with these findings, with the exception of the deviation from linearity at higher alkyl chain lengths (C_8 or greater), which was particularly noticeable for the barbiturate (acidic) solutes and certain of the pyridine (basic) solutes.

These solutes demonstrated a definite break point at a critical alkyl chain length of C_8 for all of the test solutes, above which there was little or no increase in retention. Berendsen and de Galen (14) also found a similar effect in a series of n -alkyl bonded phases (C_1 to C_{22}) with a variety of different solutes. These effects cannot adequately be explained by traditional solvophobic interaction mechanisms but fit more closely with a mechanism of retention where the solute can partition into the alkyl chains of the stationary phase. One explanation for the observation is that, for the longer alkyl chains, only the exterior part of the chain participates in the retention process due to the interactions between the hydrocarbon chains themselves under certain solvent conditions. A reversed-phase retention mechanism has been postulated in which the interaction of the solute was with only the outermost part of the alkyl chain and for which a larger solute requires a longer alkyl chain to become fully retained (14). According to this explanation, there is only limited penetration of the solute into the bonded phase, and hence, above a certain chain length, there will be no further increase in retention once full penetration of the solute has occurred.

An alternative explanation for the change in slope of the k versus chain length above C_8 may be the altered bonded alkyl chain orien-

Table V. Retention Factors, Asymmetry Factors, and Plate Numbers for Pyridine

Alkyl chain length	Pyridine	3-Amino	3-Bromo	3-Chloro	3-Ethyl	3-Acetyl
<i>Retention factors</i>						
1	3.56	2.73	7.63	6.34	13.96	5.29
2	4.51	3.21	11.99	9.50	20.65	6.86
3	4.81	3.40	14.05	10.96	28.29	6.94
4	6.16	8.03	18.28	14.10	43.39	7.13
8	8.84	10.73	33.30	24.36	81.09	8.75
12	10.21	12.85	36.81	26.63	109.41	8.78
18	16.63	19.60	46.68	34.05	159.69	10.95
<i>Asymmetry factors</i>						
1	6.24	7.26	2.11	2.24	4.42	1.72
2	7.55	13.60	1.75	1.93	3.13	1.61
3	6.07	12.39	1.26	1.41	4.61	1.39
4	16.90	22.60	1.65	1.80	12.43	1.87
8	14.07	18.04	2.05	2.46	16.01	3.48
12	5.52	13.21	1.95	3.00	10.65	3.43
18	15.56	15.88	2.41	3.05	4.27	6.13
<i>Plate numbers</i>						
1	905	824	3030	3053	1156	3382
2	1186	467	4886	4689	884	4831
3	1229	385	5058	4879	1845	4363
4	513	42	5623	5368	580	4556
8	365	34	4576	4303	448	3699
12	121	22	2510	3705	137	2002
18	65	12	3982	3427	51	1075



tation in relation to the silica support surface under certain mobile phase conditions. An increase in the solvent polarity of the mobile phase (e.g., at high percentages of water) has been reported to affect the orientation and fluidity of the longer alkyl chains; the chains are in a more "bristle-like" topography in the presence of a high percentage of organic solvent, and they are in a flatter "blanket-like" orientation at high water concentrations (17,19). Thus, it is argued that the longer alkyl chains (C_8 , C_{12} , and C_{18}) will interact preferentially with each other forming overlapping chains that lie flat on the surface, which provide a coating on the surface of the silica of a similar hydrophobic character despite the increase in chain length. Conversely, the shorter alkyl bonded ligands of C_1 to C_4 do not have the fluidity and mobility of the longer chains, and hence there is likely to be only a small change in orientation between high and low methanol concentrations, with little or no flattening of the chains. Thus the relationship between retention and chain length still applies for these shorter alkyl ligands. The actual retention mechanism may change according to the experimental conditions used, and may involve either one of the above effects or a combination of both effects.

Differences in the mobile phases used for each of the series of solutes may partly explain the observed anomalies in retention behavior between C_8 to C_{18} , since the biphenyls were analyzed in a high percentage of methanol (61%), whereas the barbiturates (32% methanol) and the pyridines (10% methanol) were analyzed in a predominantly aqueous environment. However, it seems likely that the effect of alkyl chain length on retention behavior observed in this study is not anomalous but can be explained in terms of the orientation and topography of the bonded-alkyl groups on the surface of the silica. The exact mechanism for the observed effects of alkyl chain length on retention remain uncertain, since there is insufficient data available in this study to confirm one of several possible hypotheses.

The chromatographic characteristics of 3-amino pyridine are of interest because it is the only solute, out of all those tested, that changed its relative retention with respect to the other solutes as the alkyl chain length increased. This particular solute had an extremely strong secondary interaction with the packing material, as indicated by peak tailing, which was more apparent at lengths

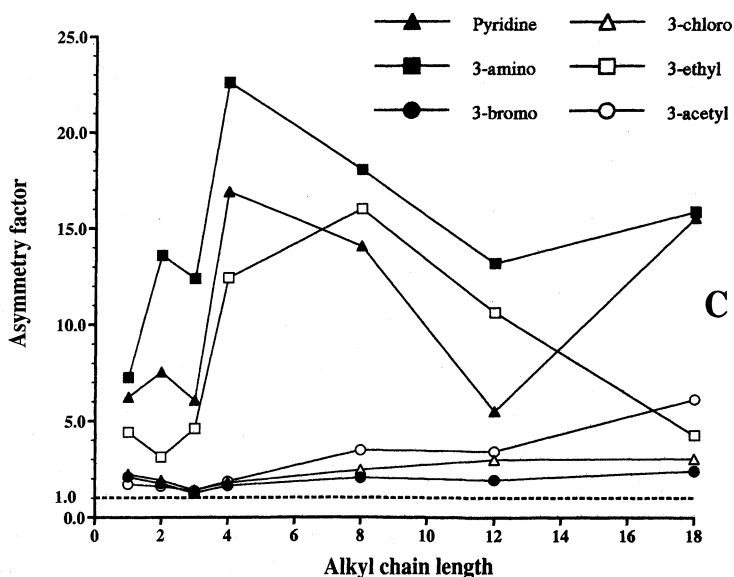
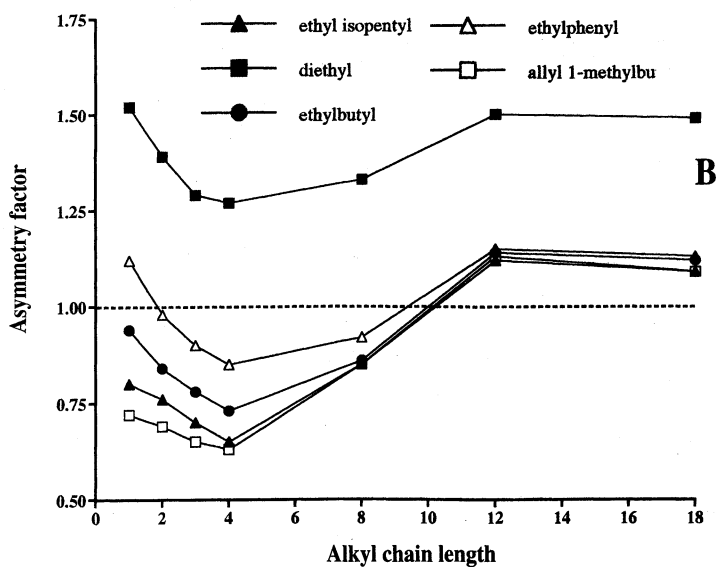
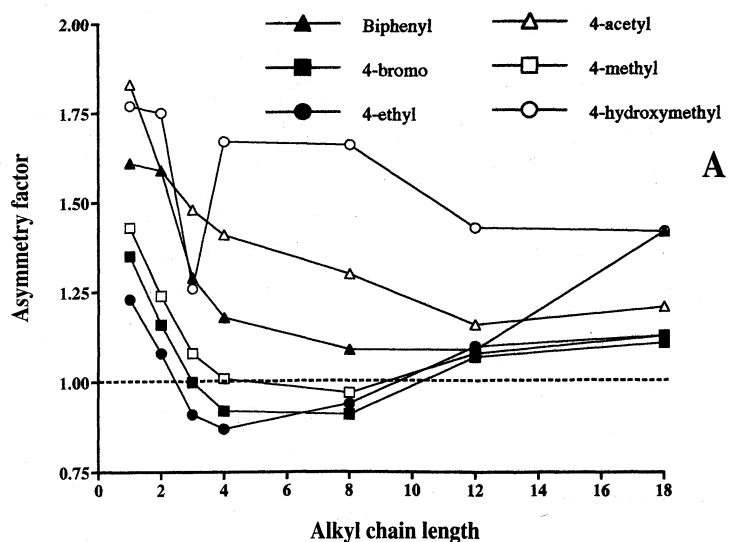


Figure 3. Effect of bonded alkyl chain length on the asymmetry factor of substituted biphenyl solutes (A), barbiturate solutes (B), and pyridine solutes (C).

of C_4 and longer. The reversal of retention order between C_3 and C_4 may have been linked to a change in the type of interaction between the solute and the packing material due to the change in alkyl chain length. There is good evidence (presented later) that several of the substituted pyridines undergo strong secondary interactions with residual surface substituents of the silica. Hence, the retention mechanism will be a mixture of partitioning and secondary interactions. This more complex mechanism of retention may explain the erratic behavior of these basic probes and the poorer correlation of their retention behavior with alkyl chain length compared with the acidic and neutral compounds.

Effect of alkyl chain length on the asymmetry factor

Care was taken to eliminate potential causes of peak asymmetry that were not due to solute–packing material interactions, such as the quality of the packed bed of the bonded silica particles, sample overload, extra column effects, and inadequate buffering. Instances of peak tailing were deemed to be a measure of secondary interactions between the solute and the packing material. It was not possible to determine the specific nature of these secondary interactions, but it has been assumed that they were primarily due to unreacted silanols on the silica surface with a possible smaller influence as a result of surface bound trace metals.

The peak asymmetry factor for the biphenyl and barbiturate probes was within acceptable limits on all the columns, ranging between 0.7 and 1.8 (Figures 3A and 3B). Asymmetry factor values of less than 1.0 indicate peak fronting, which is normally attributed to the quality of the packed bed rather than an interaction with the bonded phase. Peak fronting was noted for four out of the five barbiturates and three out of the six biphenyls. The reason for this effect is not clear, but it may be due to changes in the chain length of the bonded materials affecting the quality of the column packing achieved. The results for the biphenyl and barbiturate probes show little evidence of secondary interactions with the packing material, which indicates that both of these sets of compounds are retained on the test columns primarily by a reversed-phase type interaction with the bonded phase.

All the basic substituted pyridine probes demonstrated a notably worse peak symmetry than either the biphenyls or the bar-

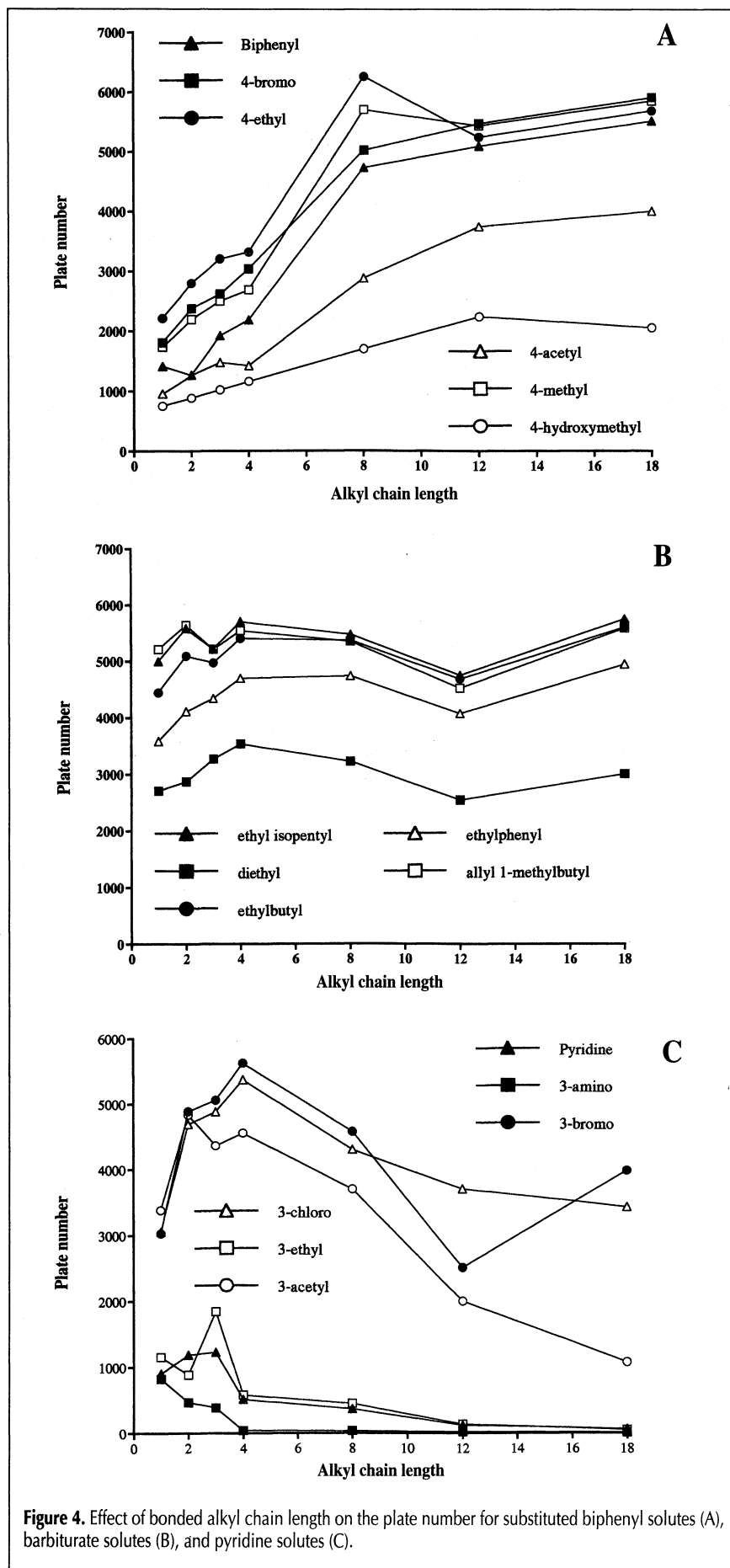


Figure 4. Effect of bonded alkyl chain length on the plate number for substituted biphenyl solutes (A), barbiturate solutes (B), and pyridine solutes (C).

biturates, with the values of asymmetry factor ranging between 1.5 to 23 (Figure 3C). Three of the pyridines (acetyl, chloro, and bromo) gave consistently better asymmetry values between 1.5 and 5.0. The remaining three substituted pyridines (pyridine, 3-amino, 3-ethyl) gave extremely tailed peaks with asymmetry factors ranging between 5.0 and 23. These data demonstrate that, to a greater or lesser extent, all the pyridines have a significant secondary interaction with the packing material on all the columns analyzed. This kind of strong secondary interaction between basic compounds and bonded silicas is typical of many commercial materials and appears to result from the presence of residual acidic silanol groups or trace metals (2). The pK_a values for the substituted pyridines indicate that all the compounds are predominantly un-ionized at the mobile phase pH, although the three pyridines with the highest asymmetry values were also partly ionized (Table II). Hence a small degree of ionization of the pyridines appears to markedly affect the secondary interaction of these solutes with the packing material, suggesting the involvement of an electrostatic or ionic force.

There was a significant trend towards lower asymmetry values (i.e., less secondary interaction) for the basic compounds with the packing materials having the C_1 , C_2 , and C_3 alkyl bonded phases. This suggests that, for the basic probes used, the residual silanols were more effectively masked by the shorter alkyl chains rather than the longer ones. The surface density data (Table I) indicates that the C_1 , C_2 , and C_3 ligands have the highest surface density (in terms of micromoles per squared meter) of the silica surface and hence may provide a more effective masking of the surface silanols than the longer alkyl chains, presumably due to steric effects. In the 90% aqueous mobile phase used for the analysis of the basic solutes, it is expected that the longer alkyl chains would be in a nonextended, flattened conformation and hence would not provide sufficient depth to mask solute interactions with underlying silanols. An alternative explanation for this observation is that the longer alkyl chains are not evenly distributed over the silica surface, leaving uncoated areas where secondary interactions may take place. The shorter alkyl chains, with their higher surface ligand density, may provide more effective masking of the underlying silanols.

Effect of alkyl chain length on plate number

For the biphenyls, there was a consistent trend towards improved efficiency as chain length increased (Figure 4A). The most retained solutes showed the best efficiencies, suggesting that extra-column effects may have reduced the efficiency of the least retained compounds and that these effects played a less important role as the chain length increased. This argument is supported by the observation that k values for certain biphenyl solutes on the C₁–C₄ columns were in the range of 1.9 to 3.0. For the barbiturates, there was no consistent trend for efficiency in relation to the alkyl chain length (Figure 4B). As with the biphenyls, the lowest efficiencies were observed for the least retained solutes, which again suggests that extra-column effects may be involved. The pyridines could be divided into two groups according to the degree of secondary interactions with the packing material (Figure 4C). The pyridines with the worst asymmetry values also gave the lowest efficiencies on all the columns, and those with better asymmetry values gave the highest efficiencies. The first group had a noticeably improved efficiency for alkyl chain lengths of C₁–C₃ with poorer efficiencies at C₈ to C₁₈, which correlates with the observations on peak asymmetry of these three pyridines.

The column plate numbers for the un-ionized biphenyl and barbiturate solutes were consistently less than 6000 in this study, whereas the expected values for small neutral molecules such as these are normally greater than 6500 for Hypersil type packing materials (Shandon HPLC technical data). The source of this minor discrepancy is not clear but might be related to either extra-column effects or to packing quality. In any case, the slightly lower column efficiency does not affect the conclusions derived from this investigation.

Intra column comparisons of secondary retention effects

Plots of $\log k_A$ vs $\log k_B$ derived from the retention data of a single set of solutes and the same mobile phase can be used to examine the extent of secondary (or silanophilic) retention effects on a series of packing materials (20). A linear correlation, with unit slope, between the $\log k_A$ versus $\log k_B$ plot for columns A and B with different bonded-alkyl chain lengths, indicates that the two columns have an identical separation behavior. The case of linear correlation with a slope different from unity suggests a similar, though not identical, physico-chemical basis of retention on the two columns. Poor correla-

tion between the $\log k_A$ versus $\log k_B$ plots is taken as proof that there is a significant secondary interaction taking place, presumably with residual surface silanol or metal groups. Hence the $\log k_A$ versus $\log k_B$ plots provide a powerful method to compare the retention behavior of a set of reversed-phase materials when a series of test compounds are evaluated with the same mobile phase, as in this study.

The retention data from all the solutes and columns were used to construct a series of $\log k$ plots. The results are shown in Tables VI–VIII. Linear correlations ($r^2 > 0.95$) were obtained for all the columns for both the neutral (biphenyls) and acidic (barbiturate) solutes. The C₈, C₁₂, and C₁₈ columns with the barbiturate and biphenyl solutes all had slopes close to unity, indicating that the additional chain length had little effect on the retention mechanism with this group of solutes and confirming the data from the retention versus alkyl chain length plots. The pyridine solutes deviated considerably from a linear correlation with the C₁₈ packing material; the C₁, C₂, and C₃ columns having a correlation coefficient ($r^2 > 0.75$) indicating that these short alkyl chain columns have a significant secondary retention mechanism present. These data indicate that the relative importance of reversed-phase and secondary interactions may vary with the solute type and the length of the bonded alkyl chain.

Table VII. Comparison of the Retention Behavior of Substituted Biphenyl Solute Using Data from $\log k_A$ versus $\log k_B$ Plots

Stationary phase		Slope	Correlation coefficient (r^2)
Column A	Column B		
C ₁₈	C ₁₂	1.140	0.999
	C ₈	1.322	0.996
	C ₄	1.745	0.993
	C ₃	1.936	0.988
	C ₂	2.078	0.981
	C ₁	2.447	0.977
	C ₁₂	C ₈	1.160
C ₄		1.532	0.995
C ₃		1.701	0.992
C ₂		1.825	0.984
C ₁		2.150	0.980
C ₈	C ₄	1.319	0.994
	C ₃	1.463	0.991
	C ₂	1.570	0.982
	C ₁	1.849	0.977
C ₄	C ₃	1.110	0.997
	C ₂	1.191	0.988
	C ₁	1.402	0.983
C ₃	C ₂	1.076	0.996
	C ₁	1.268	0.994
C ₂	C ₁	1.180	0.999

Table VI. Correlation Between $\log P$ and $\log k$

Column	Linear correlations (r^2)		
	Biphenyls (neutral)	Barbiturates (acidic)	Pyridines (basic)
C ₁	0.93	0.87	0.66
C ₂	0.92	0.86	0.73
C ₃	0.91	0.86	0.69
C ₄	0.91	0.85	0.58
C ₈	0.91	0.84	0.61
C ₁₂	0.90	0.83	0.54
C ₁₈	0.90	0.83	0.49

Correlation of log *P* with retention factor

A range of log *P* values was chosen for each series of solutes by selecting appropriate functional groups as substituents. The wide range of lipophilicities resulting from this approach (Table II) ensures that the test compounds are typical of many pharmaceutical and chemical chromatographic separations. In an ideal reversed-phase stationary phase, the retention should correlate with log *P* values for each series of compounds. Any deviations from this relationship indicate that other types of nonreversed-phase interactions are taking place. The log *P* values were correlated with the retention factor by plotting log *P* versus log *k* for each set of test solutes on each bonded column. The correlation coefficients of these plots are shown in Table IX. There was a strong linear relationship between log *k* and log *P* for the biphenyl compounds ($r^2 > 0.90$) and the barbiturates ($r^2 > 0.83$) over the range of bonded-alkyl stationary phases. An unexpected trend of improved correlation between log *P* and retention as the bonded alkyl chain length decreased was apparent for both these sets of solutes. The pyridine compounds showed a poor correlation between log *P* and retention ($r^2 = 0.49$ to 0.73). These data support the conclusion that the biphenyl (neutral) and barbiturate (acidic) test compounds are retained on these columns by a predominantly reversed-phase mechanism, whereas the pyridines (basic) deviate significantly from this relationship.

Conclusion

The results show that the length of the bonded alkyl chain is of primary, though not sole, importance in governing the retention of acidic, basic, and neutral compounds on the series of monofunctional *n*-alkyl-bonded reversed-phase packing materials used in this study. An excellent correlation between *k* and alkyl chain length was obtained in the majority of cases. Deviations from this relationship occurred when the alkyl chain length was increased beyond C₈ for certain groups of solutes or when there was significant solute interaction at secondary sites on the packing material. In practical terms, this means that the use of C₁₈ columns would offer little advantage in performance compared with a C₈ column for many commonly analyzed weak acids and bases. For the basic compounds, peak tailing was notably worse on the columns with the longer alkyl chains (C₈, C₁₂, C₁₈), but much improved at C₁ to C₄ chain length. Thus it appears that the shorter alkyl chain lengths, with their greater surface coverage, provide a superior masking of the unwanted interactions with the silica surface on these nonendcapped materials. Although these conclusions apply strictly only to the series of packing materials synthesized for this study, it is reasonable to assume that these findings would find a more general application to a wider range of commercial monofunctional *n*-alkyl-bonded silicas used for the separation of pharmaceutical and chemical compounds.

Table VIII. Comparison of the Retention Behavior of Substituted Barbiturate Solute Using Data from Log *k_A* versus Log *k_B* Plots

Stationary phase		Slope	Correlation coefficient (r^2)
Column A	Column B		
C ₁₈	C ₁₂	1.015	0.999
	C ₈	1.047	0.999
	C ₄	1.230	0.997
	C ₃	1.297	0.996
	C ₂	1.366	0.997
	C ₁	1.561	0.995
C ₁₂	C ₈	1.031	0.999
	C ₄	1.212	0.998
	C ₃	1.278	0.997
	C ₂	1.345	0.998
	C ₁	1.538	0.996
C ₈	C ₄	1.175	0.998
	C ₃	1.239	0.998
	C ₂	1.305	0.998
	C ₁	1.493	0.998
C ₄	C ₃	1.055	0.999
	C ₂	1.110	0.999
	C ₁	1.270	0.999
C ₃	C ₂	1.053	0.999
	C ₁	1.205	0.999
C ₂	C ₁	1.144	0.999

Table IX. Comparison of the Retention Behavior of Substituted Pyridine Solute Using Data from Log *k_A* versus Log *k_B* Plots

Stationary phase		Slope	Correlation coefficient (r^2)
Column A	Column B		
C ₁₈	C ₁₂	0.988	0.986
	C ₈	1.046	0.962
	C ₄	1.263	0.955
	C ₃	1.078	0.756
	C ₂	1.175	0.690
	C ₁	1.382	0.701
C ₁₂	C ₈	1.069	0.993
	C ₄	1.291	0.987
	C ₃	1.133	0.825
	C ₂	1.247	0.769
	C ₁	1.457	0.771
C ₈	C ₄	1.205	0.990
	C ₃	1.083	0.868
	C ₂	1.199	0.819
	C ₁	1.395	0.814
C ₄	C ₃	0.893	0.866
	C ₂	0.819	0.819
	C ₁	1.162	0.829
C ₃	C ₂	1.137	0.995
	C ₁	1.323	0.989
C ₂	C ₁	1.162	0.991

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