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REVERSED-PHASE LIQUID CHROMATOGRAPHY OF ISOMERIC ALKYLBENZENES

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SUMMARY

The separations of isomeric C_1–C_4 alkylbenzenes and polymethylbenzenes on the reversed-phase columns SAS-Hypersil, ODS-Hypersil and C_{22}-Magnusil have been compared. The correlations between log *k'* and the structural parameters carbon number, molecular connectivity index and hydrophobic surface areas were determined. The retentions were also compared with octanol–water and heptane–water partition coefficients.

INTRODUCTION

Although many studies have been carried out on the separation of the environmentally important polynuclear aromatic hydrocarbons (PAHs) by both normal- and reversed-phase liquid chromatography[1], the monocyclic aromatic hydrocarbons have not been examined in detail. In early studies, normal-phase separations on alumina[2,3] and silica[4] were carried out with pentane or fluorocarbons as eluents, but it was not easy to relate the retentions to structural features. Recently, hydroxylated silica has been used, and linear relationships have been determined for the *n*-alkylbenzenes and polymethylbenzenes between log capacity factor and number of carbon atoms[5].

Individual alkylbenzenes have been examined on reversed-phase columns by a number of authors[6–10], but the only systematic study has been of the C_1–C_9 *n*-alkylbenzenes, which were found to show a linear relationship between log capacity factor (*k'*) and carbon number[11].

The absence of polar functional groups in the alkylbenzenes suggests that they could be useful models to test the solvophobic theory of retention, which relates *k'* to the solute hydrocarbonaceous surface area[12,13]. However, other studies of hydrophobicity, with use of octanol–water distribution coefficients, have suggested that, for non-polar molecules, molecular volumes show a closer relationship than areas[14]. The concept of molecular connectivity index has similarly been proposed to relate structure to partition coefficients and hence to retention[15].

In the present work, the isomeric C_1–C_4 alkylbenzenes and mono- to tetramethylbenzenes have been examined on three reversed-phase columns. The *k'* values have been compared with the carbon numbers, reported surface areas and volumes and

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TABLE I

CAPACITY FACTORS OF ALKYLBENZENES ON REVERSED-PHASE COLUMNS AND RELATED STRUCTURAL FACTORS AND PARTITION COEFFICIENTS

Compound	*Capacity factors (solvent ratio, methanol–water)*				*Structural factors*			$\log P_{octanol-water}$ *(ref. 18)*	$\log P_{heptane-water}$ *(ref. 19)*
	SAS-Hypersil		*ODS-Hypersil (70:30)*	*C_{22}-Magnusil (50:50)*	χ*	*Surface area***	*Volume****		
	(40:60)	*(50:50)*							
Benzene	1.6	1.00	1.40	0.94	2.000	240.7	80.33	2.13	2.26
Toluene	3.3	1.67	2.60	1.76	2.410	273.9	98.81	2.69	2.85
Ethylbenzene	6.5	2.67	4.50	2.76	2.971	302.3	115.79	3.15	
o-Xylene	5.7	2.45	4.50	2.85	2.827	298.4	117.33	3.12	3.39
m-Xylene	6.5	2.67	4.96	3.15	2.821	309.1	117.33	3.20	3.54
p-Xylene	6.5	2.78	4.96	3.11	2.821	309.1	117.33	3.15	3.45
n-Propylbenzene	13.8	4.78	8.50	5.06	3.411	334.1	132.77	3.62	
Isopropylbenzene	11.1	4.00	8.10	4.23	3.354	326.0	132.77	3.66	
1,2,3-Trimethylbenzene	10.4	3.78	7.60	4.52	3.244				
1,2,4-Trimethylbenzene	11.6	4.33	8.50	5.23	3.238	331.6			
1,3,5-Trimethylbenzene	12.7	4.44	9.40	5.49	3.232		135.85	3.42	
n-Butylbenzene	–	8.89	15.7	9.47	3.971				
Isobutylbenzene	–	8.44	14.3	8.52	3.827				
sec.-Butylbenzene	–	7.33	12.7	7.47	3.892				
tert.-Butylbenzene	–	6.22	10.7	6.23	3.661		149.74	4.11	
1,2,3,4-Tetramethylbenzene	–	6.22	12.9	8.04	3.661				
1,2,3,5-Tetramethylbenzene	–	6.88	14.2	8.51	3.655				
1,2,4,5-Tetramethylbenzene	–	6.78	14.3	8.51	3.655		154.37	4.00	
p-Methylisopropylbenzene (cymene)	–	7.44	13.5	8.21	3.571				
Biphenyl	15.9	4.56	7.50	6.26	4.071			4.04	
Naphthalene[§]	6.5	2.44	4.10	3.35	3.155		122.90	3.30	

* Molecular connectivity index calculated according to ref. 15.

** Solvent cavity surface area[13].

*** Bondi volume[14].

[§] Used as internal standard.

calculated molecular connectivity indexes. Because of the possible application of liquid chromatography to the determination of Hansch distribution coefficients[16,17], the retentions were also related to octanol–water and heptane–water partition coefficients.

EXPERIMENTAL

Liquid chromatography was carried out with the use of a Pye-Unicam XPS pump with pulse damper, a Shandon syringe injector and columns (10 cm × 5 mm) and an Altex Model 153 detector at 254 nm; solvent flow-rate was 1.0 or 1.3 ml min^{-1}.

The columns were packed with 5-μm SAS-Hypersil, ODS-Hypersil (Shandon Southern, Runcorn, Great Britain) or C_{22}-Magnusil (Magnus Scientific, Sandbach, Great Britain). Solvents were HPLC-grade methanol (Fisons, Loughborough, Great Britain) and distilled water. The alkylbenzenes were laboratory grade, obtained from a number of sources, and samples were injected, by using a stop-flow technique, as dilute solutions in methanol–water (7:3). Naphthalene was used as internal standard.

RESULTS AND DISCUSSION

Retention parameters

In an early study of a range of alkylbenzenes with differing substitution patterns and various alkyl groups, Sleight reported a correlation between log capacity factor and total number of carbon atoms[7]. Close correlations have subsequently been established for *n*-alkylbezenes and other homologous series of solutes, and the general equation $\log k'_n = pn + q$ has been derived, in which p and q are characteristic constants for a particular homologous series and column and solvent combination[11]. These values can be used to express the ability of the column to separate adjacent homologues, as the column selectivity $\alpha = k'_{n+1}/k'_n = 10^p$, the value of α varying as the polarity (ε) of the solvent.

In the present work, the k' values of 19 alkyl- and polymethylbenzenes have been determined on three reversed-phase columns (Table I), the solvents being chosen to give reasonable retention times in each instance. The ODS-Hypersil column, as expected, showed a greater retention than the Short Alkyl Silica (SAS-Hypersil) column, but, unexpectedly, the C_{22}-alkyl bonded column, rather than having greater retention, was similar to the SAS column. The relative order of elution of the monocyclic compounds was effectively the same on all three columns, and, on plotting k' for the ODS and C_{22} columns, a single line was produced (correlation 0.9971), (ODS and SAS correlation, 0.9799).

As in the earlier study, there was reasonable overall correlation between log k' of the alkylbenzenes on the ODS-column and their carbon numbers (Table II). If just the homologous *n*-alkylbenzenes were considered, as expected, a much closer correlation was found for all three columns. The values of p, q and α are similar to those reported for the C_1–C_9 alkylbenzenes on LiChrosorb RP-8 and RP-18 (ref. 11) and are in agreement with the reported trend to higher values of p and α with increased eluotropic strength of the solvent.

Although not a true homologous series, polymethylbenzenes with adjacent substituents again correspond to a systematic structural change. They also showed a

TABLE II
RETENTION-PARAMETER RELATIONSHIPS BETWEEN CAPACITY FACTORS AND CARBON NUMBERS FOR ALKYLBENZENE ON REVERSE-PHASE CHROMATOGRAPHY

Compounds	*Column*	*Mobile phase ratio (methanol–water)*	*Correlation*	*p*	*q*	*α*
Alkylbenzenes	ODS Hypersil	70:30	0.9903	0.240	−1.256	1.74
n-Alkylbenzenes	C_{22} Magnusil	50:50	0.9986	0.247	−1.510	1.77
	ODS Hypersil	70:30	0.9998	0.262	−1.424	1.83
	SAS Hypersil	50:50	0.9984	0.236	−1.658	1.72
		40:60	0.9998	0.311	−1.432	2.04
	RP-8*	100:00	–	0.066	−0.925	1.16
		90:10	–	0.101	−0.586	1.26
		80:20	–	0.154	−0.309	1.43
	RP-18*	100:00	–	0.100	−0.73	1.26
		90:10	–	0.151	−0.420	1.42
		80:20	–	0.183	−0.080	1.52
Methylbenzenes**	C_{22}-Magnusil	50:50	0.9983	0.232	−1.406	1.71
	ODS-Hypersil	70:30	0.9994	0.239	−1.274	1.73
	SAS Hypersil	50:50	0.9989	0.194	−1.157	1.56

* From ref. 11.

** Benzene, toluene, 1,2-dimethylbenzene, 1,2,3-trimethylbenzene and 1,2,3,4-tetramethylbenzene.

good linear relationship between log k' and carbon number (Table II), but with a flatter slope than for the *n*-alkylbenzenes. The different slope could be due to the increasing electron density on the benzene ring because of the polyalkyl substitution. This effect should be relatively small for a non-specific absorbent, but had a marked effect in the study on hydroxylated silica. Although retention decreased with increasing chain length of monoalkylbenzenes, polymethylation resulted in an increase in retention time^5.

As with many gas chromatography columns, it was not possible to resolve the three xylenes, although the *ortho*-isomer was well separated from *meta*- and *para*-xylene.

Two bicyclic compounds (biphenyl and naphthalene) were also examined; both were eluted much earlier than would be expected from their carbon numbers. On the ODS column, naphthalene (C_{10}) would be predicted to have a k' value of 13.8 (experimental 4.1) and biphenyl (C_{12}) a value of 42 (experimental 7.5). The additional aromatic ring in each compound apparently makes it more polar than a corresponding alkyl chain.

Correlation with physical parameters

Although the relationship between k' and number of carbon atoms has a useful general predictive power for homologues, it cannot propose the relative order of elution of isomeric compounds such as the four butylbenzenes. Attempts have been made, therefore, to devise relationships that will more closely relate structure and retention. These have invariably been based on models of the nature of the interaction between the sample and the stationary phase.

The solvophobic hypothesis proposes that the retention of a solute is dependent on the decrease in repulsion between the hydrocarbonaceous surface area of the sample and the solvent, which occurs when the sample interacts with the non-polar stationary phase[12]. Similar considerations have been used to relate the solvent cavity surface areas of hydrocarbons with their solubilities in water[13] and the molecular areas and volumes of non-polar molecules with their partition coefficients[14].

For the alkylbenzenes examined in the present study, values have been reported for the surface area[13] of 9 compounds and for the Bondi volume[14] of 11 compounds (Table I). Comparison of these values with *k′* on SAS- and ODS-Hypersil columns showed a good correlation (Table III), and if the homologous *n*-alkylbenzenes only were considered, the relationship was closer. From the Bondi-volume correlation, the expected *k′* of naphthalene can be predicted as 5.6, in closer agreement with the experimental value of 4.1 than the earlier prediction on the basis of carbon number.

However, surface areas and volumes are not easy to determine. The readily calculated molecular connectivity index χ, which is derived from the topology of the molecule, has been shown to relate closely to water solubility and partition coefficient[15], and it has been suggested that this parameter can be related to *k′* values in chromatography[6,16]. The calculated values for the compounds in the present study (Table I) showed a reasonable correlation with log *k′* (Table III), although SAS was better than ODS. Again, if the *n*-alkylbenzenes only were considered, the correlations were improved. Because of the similarity of the relative retention on ODS and C_{22} columns, separate correlations for the latter were not determined.

Close examination suggested that, despite its relationship to the topology of the molecule, the connectivity index could not predict the order of elution of the isomeric isobutyl- and *sec.*-butylbenzenes, or the large difference in retention between *tert.*-butylbenzene ($k' = 6.23$) and 1,2,3,4-tetramethylbenzene ($k' = 8.04$), each of which had the same connectivity index, χ (3.661).

By using the connectivity index of the bicyclo compounds, the expected *k′* values were calculated to be naphthalene 6.65 (found 4.10) and biphenyl 21.1 (found 7.50), results similar to those found from the carbon-number relationships.

Despite claims to the contrary, it therefore appears that the connectivity index is little better than carbon number in relating *k′* to structure for these compounds and

TABLE III

RELATIONSHIP BETWEEN LOG CAPACITY FACTORS AND PHYSICAL PARAMETERS FOR ALKYLBENZENES ON REVERSE-PHASE COLUMNS

*Physical property**	*Correlation coefficients** (number of compounds)*			
	*SAS-Hypersil****		*ODS-Hypersil*	
	Alkylbenzenes	*n-Alkylbenzenes*	*Alkylbenzenes*	*n-Alkylbenzenes*
Solvent cavity surface area	0.9943 (9)	0.9992 (4)	0.9951 (9)	0.9994 (4)
Bondi volume	0.9931 (11)	0.9985 (4)	0.9913 (11)	0.9995 (4)
Molecular connectivity index	0.9901 (19)	0.9995 (5)	0.9756 (19)	0.9985 (5)
Parition coefficients				
Log $P_{octanol-water}$	0.9835 (11)	0.9974 (4)	0.9707 (11)	0.9980 (4)
Log $P_{heptane-water}$	0.9948 (5)	—[§]	0.9982 (5)	—[§]

* Values from table I.

** Excludes naphthalene and biphenyl.

*** Solvent: methanol–water (50:50).

[§] Insufficient samples.

generally fails to correlate with the relative retentions of isomeric compounds. Its only close correlation, that *n*-alkylbenzenes, is explicable by the incremental change of index with members of a homologous series. Overall, it shows a poorer correlation than does surface area or volume with capacity factor. Similar problems were noted by Colin and Guiochon[11], who compared k' values for isomeric C_4 and C_5 alcohols with surface area and connectivity index and found an inability to predict the relative elution of isomers.

Because of the relationship between k' values and partition coefficients, it has been proposed that liquid chromatography could be used to determine Hansch partition coefficients ($\log P_{\text{octanol-water}}$), which are important in studies of drug structure–activity relationships[16–18]. For the accurate comparison of compounds with different functional groups, it has been found necessary to impregnate hydrocarbon bonded phases with octanol[16,17], but this should not be necessary for the relative comparison of compounds of similar type.

As with physical parameters, there was a good correlation between $\log P_{\text{octanol-water}}$ and $\log k'$ for the *n*-alkylbenzenes, but, for a wider group of compounds, correlation was poorer. With a limited number of examples, there was good correlation with $\log P_{\text{heptane-water}}$.

CONCLUSION

The *n*-alkylbenzenes show good correlation between $\log k'$ and carbon number and structural parameters, but, for isomeric alkylbenzenes, the correlation is much weaker and the physical parameters are often little better than carbon number in predicting k' values.

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