

CHROM. 10,404

## REVERSED-PHASE LIQUID-LIQUID CHROMATOGRAPHY OF AROMATICS ON MACROPOROUS POLYSTYRENE GEL

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### SUMMARY

Retention data of polar substituted benzenes and naphthalenes were measured using reversed-phase liquid-liquid chromatography on macroporous polystyrene gel with methanol-water and acetonitrile-water as the eluents. The results of the chromatographic measurements are expressed in terms of retention indices ( $I$ ). Considerable differences in the behaviour of the individual solutes were found in the two eluents. A method is suggested for predicting the retention indices of polysubstituted solutes as the sum of increments ( $\Delta \log I$ ) from the monosubstituted compounds. To verify the liquid-liquid partition mechanism controlling this chromatographic separation, the partition coefficients of several compounds were measured for the model system toluene-aqueous methanol.

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### INTRODUCTION

In previous work<sup>1</sup>, the behaviour of aromatic hydrocarbons on a macroporous polystyrene gel was studied using a mixture of methanol, diethyl ether and water as the eluent. This paper extends the gel characteristics to aromatic solutes with polar functional groups and shows how the elution behaviour of these substances is affected by the choice of the eluent. The reversed-phase partition chromatographic mechanism has been proposed<sup>1</sup> as the governing principle for separations on the materials studied. Nakae and Muto<sup>2</sup> have interpreted the chromatographic properties of a styrene-divinylbenzene copolymer and the temperature dependence of retention volumes in terms of the same mechanism. In an attempt to verify this mechanism, the extraction equilibria of selected compounds were measured in a model system consisting of toluene-aqueous methanol.

### EXPERIMENTAL

A glass column (30 cm × 4 mm I.D.) packed with a crosslinked polystyrene gel in the form of spherical particles 25-32 μm in diameter and with an exclusion limit of molecular weight 40,000 (obtained using benzene as the eluent and a Waters polystyrene standard) was used. The dead volume of the column was measured as the elution volume of water. The column was packed with a gel suspension in metha-

not; the pressure of eluent was maintained at 2.5–3.0 MPa and the packing was gradually added. Methanol–water and acetonitrile–water were employed as eluents, each at volume ratios of 7:3 and 3:2, the composition depending on the retention volumes of the solutes measured.

The eluent was delivered by a ISCO Dialagrad, Model 384, pulseless pump. The pressure was *ca.* 3.0 MPa at a flow-rate of 40 ml/h. The detector was a Waters Assoc. differential UV monitor with a cell of dead volume 8  $\mu$ l and an optical path length of 1 cm, operating at a constant wavelength of 254 nm.

The extraction equilibria were studied in a separating funnel provided with a thermostating jacket. The sample was dissolved in methanol–water (4:1) to give a total volume of 30 ml, the mixture was transferred to the funnel and then 30 ml of toluene were added. To approximate infinite dilution conditions, a concentration of 1 g of sample per litre of methanol was used. The mixture was shaken vigorously and allowed to equilibrate for 2 h; samples of both phases were then removed and measured on a Unicam SP 800B UV spectrophotometer. The phase samples and the original methanolic solution were diluted so that the solvent composition was identical. In addition to methanol, the pure phases corresponding to the equilibrium composition of the two layers in the absence of the sample were used for the dilution. In this way, even substances whose UV spectra depend strongly on the solvent composition could be evaluated accurately.

## RESULTS AND DISCUSSION

The results of the chromatographic experiments are expressed in terms of retention indices (*I*) (ref. 3). For benzene, naphthalene and phenanthrene, the log *I* values were defined as 1, 2 and 3, respectively. The retention index of a solute with log *I* > 1 is calculated according to the equation

$$\log I = \log I_n + \frac{\log R_x - \log R_n}{\log R_{n+1} - \log R_n} \quad (1)$$

where *R* is the corrected elution volume and subscripts *n*, *n* + 1 and *x* correspond to the lower and higher standards and to the solute measured, respectively.

For solutes with log *I* < 1 the benzene–naphthalene interval must be extrapolated:

$$\log I = 1 - \frac{\log R_B - \log R_x}{\log R_N - \log R_B} \quad (2)$$

where subscripts B and N refer to benzene and naphthalene, respectively.

TABLE I

CORRECTED RETENTION VOLUMES, *V'*, AND CAPACITY FACTORS, *k'*, OF BENZENE AND NAPHTHALENE

Compound	Log <i>I</i>	CH <sub>3</sub> OH–H <sub>2</sub> O				CH <sub>3</sub> CN–H <sub>2</sub> O			
		3:2		7:3		3:2		7:3	
		<i>V'</i> (ml)	<i>k'</i>	<i>V'</i> (ml)	<i>k'</i>	<i>V'</i> (ml)	<i>k'</i>	<i>V'</i> (ml)	<i>k'</i>
Benzene	1	9.8	4.8	6.4	3.1	9.2	4.5	4.5	2.2
Naphthalene	2	31.2	15.2	20.2	9.9	21.4	10.4	9.9	4.8

The elution volumes and capacity factors for the standards are given in Table I. The capacity factors of all of the substances vary considerably according to the content of water in the mobile phase, whereas the retention indices are virtually independent of this ratio. The use of a mobile phase with a higher water content permitted more precise measurements of the retention indices for solutes with low

TABLE II

RETENTION INDICES (LOG *I*) FOR SUBSTITUTED BENZENES AND ESTABLISHED DEVIATIONS (*D*)

$D = \log I_{\text{meas}} - \log I_{\text{calc}}$ , where  $\log I_{\text{calc}}$  has been calculated as the sum of substituent contributions given in Table IV.

Compound	Mobile phase			
	$\text{CH}_3\text{OH}-\text{H}_2\text{O}$		$\text{CH}_3\text{CN}-\text{H}_2\text{O}$	
	$\log I_{\text{meas}}$	<i>D</i>	$\log I_{\text{meas}}$	<i>D</i>
Benzene	1.00	—	1.00	—
Phenol	0.51	-0.12	0.07	-0.09
Thiophenol	0.52	—	0.29	—
Nitrobenzene	1.29	0.01	0.87	-0.03
Chlorobenzene	1.32	—	1.42	—
Bromobenzene	1.46	-0.03	1.60	-0.05
Iodobenzene	1.73	—	1.89	—
Benzoic acid	0.57	0.42	-0.26	-0.31
Benzaldehyde	0.75	—	0.43	—
Methyl benzoate	0.60	—	0.74	—
Benzonitrile	0.82	—	0.46	—
Aniline	0.55	—	0.37	—
N,N-Dimethylaniline	1.46	—	-0.69	—
<i>p</i> -Dibromobenzene	1.93	-0.05	2.35	0.05
1,3,5-Tribromobenzene	2.56	0.09	2.92	-0.03
<i>o</i> -Cresol	0.79	-0.06	0.38	-0.11
2,6-Xylenol	1.03	-0.05	0.72	-0.10
<i>o</i> -Nitrotoluene	1.48	-0.02	1.23	0.00
<i>p</i> -Nitrotoluene	1.47	-0.03	1.19	-0.04
1-Fluoro-2,4-dinitrobenzene	1.79	—	0.92	—
1-Chloro-2,4-dinitrobenzene	1.93	0.05	1.40	0.18
<i>m</i> -Nitrophenol	1.33	0.42	0.32	0.26
2-Chloro-4-nitrophenol	1.16	-0.44	0.52	-0.80
Picric acid	1.54	0.07	0.71	0.85
Resorcinol	0.12	-0.14	-0.71	-0.03
Hydroquinone	0.01	-0.25	-1.10	-0.42
Pyrogallol	-0.04	0.15	-0.84	0.68
<i>p</i> -Toluenesulphonic acid	0.60	0.08	-0.88	0.08
<i>p</i> -Xylenesulphonic acid	0.66	-0.08	-0.72	-0.09
<i>p</i> -Hydroxybenzoic acid	-0.07	0.15	-0.73	0.06
3,5-Dinitrobenzoic acid	0.13	-0.58	0.09	0.24
<i>o</i> -Aminobenzoic acid	0.67	0.97	-0.09	0.67
<i>o</i> -Aminophenol	0.23	0.05	-1.33	-0.86
<i>p</i> -Aminophenol	-0.13	-0.31	-1.29	-0.82
<i>o</i> -Nitroaniline	1.33	0.50	0.80	0.53
<i>m</i> -Nitroaniline	1.25	0.42	0.66	0.39
<i>p</i> -Nitroaniline	1.07	0.24	0.49	0.22
2,4-Toluediamine	0.09	-0.23	-1.66	-1.73
<i>p</i> -toluidine	0.53	-0.24	-1.46	-2.16

TABLE III  
RETENTION INDICES (LOG *I*) FOR SUBSTITUTED NAPHTHALENES

Compound	Mobile phase	
	CH <sub>3</sub> OH-H <sub>2</sub> O	CH <sub>3</sub> CN-H <sub>2</sub> O
α-Naphthol	1.65	1.06
β-Naphthol	1.65	0.88
α-Naphthylamine	1.80	0.68
β-Naphthylamine	1.65	—
α-Chloronaphthalene	2.32	2.47
α-Bromonaphthalene	2.51	2.67
α-Nitronaphthalene	2.34	1.85
α-Naphthoic acid	0.10	—

retentions. The reproducibility of log *I* with change in the composition of the mobile phase is approximately  $\pm 0.05$ ; for solutes with small values of log *I* (below 0.5), the reproducibility is lower (about  $\pm 0.2$ ). This error is caused mainly by the inaccuracy in the determination of very short retention times.

The measured retention indices are given in Tables II and III. Considerable differences in the behaviour of the individual solutes in the aqueous methanol and aqueous acetonitrile mobile phases can be seen. The halobenzenes shows similar affinities towards both eluents and the individual members can easily be separated (Fig. 1). Other substances exhibit larger or smaller differences, so that the selectivity can be affected to a considerable extent by the choice of the eluent. For example, benzoic acid, benzaldehyde and methyl benzoate cannot be separated in aqueous methanol, whereas aqueous acetonitrile gives good results (Fig. 2). Similar conclusions were drawn by Locke<sup>4</sup> for reversed-phase partition chromatography when interactions in the non-polar stationary phase are weak and the separation properties of the system depend exclusively on the eluent composition or the solubility of the solute in the mobile phase.

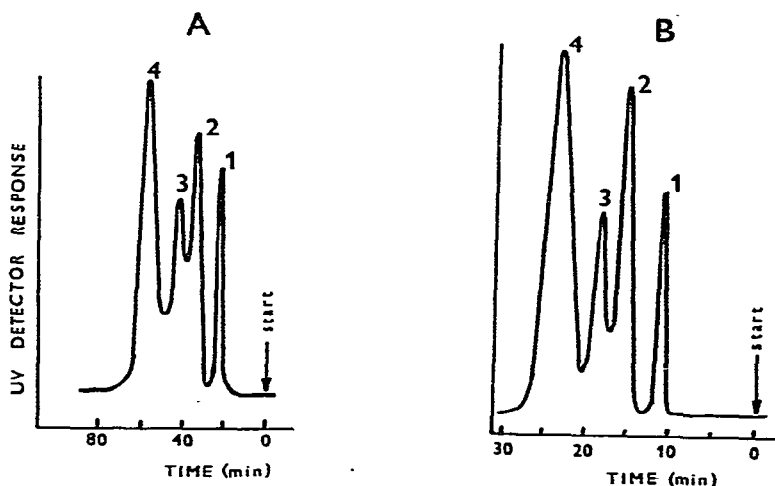


Fig. 1. Separation of halobenzenes: 1 = benzene; 2 = chlorobenzene; 3 = bromobenzene; 4 = iodobenzene. Eluent: (a) methanol-water (7:3), 20 ml/h; (b) acetonitrile-water (3:2) 40 ml/h.

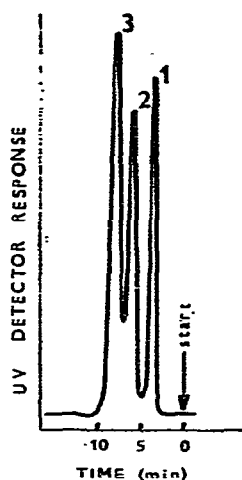


Fig. 2. Separation of benzenes with carboxylic substituents: 1 = benzoic acid; 2 = benzaldehyde; 3 = methyl benzoate. Eluent: acetonitrile-water (3:2), 40 ml/h.

A method has been developed for the characterization of the individual substituents, permitting the calculation of  $\log I$  as the sum of  $\Delta \log I$  increments from the individual substituents (Table IV). These contributions predict the retention index with deviations ( $D$ ) given in Table II. In this way, substances substituted with a methyl group, halogens, some phenols and nitro compounds can readily be characterized. The application of the contributions is limited in the case of intramolecular substituents interaction. Rather large deviations were found for nitrophenols, polyphenols and solutes with carboxylic groups using aqueous acetonitrile as the eluent. The poorest results were obtained with amino compounds. There is no explanation

TABLE IV

CONTRIBUTIONS ( $\Delta \log I$ ) FOR VARIOUS FUNCTIONAL GROUPS IN SUBSTITUTED BENZENES

Functional group	Mobile phase	
	CH <sub>3</sub> OH-H <sub>2</sub> O	CH <sub>3</sub> CN-H <sub>2</sub> O
CH <sub>3</sub>	0.22	0.33
F	0.23	0.12
Cl	0.32	0.42
Br	0.49	0.65
I	0.73	0.89
NO <sub>2</sub>	0.28	-0.10
OH	-0.37	-0.84
SH	-0.48	-0.71
CHO	-0.25	-0.57
COOCH <sub>3</sub>	-0.40	-0.26
C≡N	-0.18	-0.54
SO <sub>3</sub> H	-0.70	-2.29
COOH	-0.85	-0.95
N(CH <sub>3</sub> ) <sub>2</sub>	0.46	-1.70
NH <sub>2</sub>	-0.45	-0.63

TABLE V

VALUES OF SOLUTE PARTITION COEFFICIENT,  $k^{25}$ , FOR TOLUENE/METHANOL-WATER (4:1) AT 25°

Compound	Log $k^{25}$	Compound	Log $k^{25}$
Phenol	-0.47	1-Fluoro-2,4-dinitrobenzene	0.76
Nitrobenzene	0.72	1-Chloro-2,4-dinitrobenzene	0.83
Benzoic acid	-0.30	<i>p</i> -Hydroxybenzoic acid	-1.78
Aniline	-0.28	2,4-Toluenediamine	-0.90
N,N-Dimethylaniline	0.87	Naphthalene	1.06

for the large difference between the retention indices of aminobenzene and *p*-toluidine with the aqueous acetonitrile as the eluent.

The chromatographic behaviour of substituted naphthalenes (Table III) is similar to that of benzenes. For halo-, nitro- and hydroxynaphthalenes and methanol-water as the eluent, the contributions found for the substituted benzenes can be employed with good precision. It seems that there are differences in the elution volumes depending on the position of substitution, which were not observed for alkyl substituents<sup>1</sup>.

The results of the extraction equilibrium experiments are summarized in Table V, in which the partition coefficients ( $\log k^{25}$ ), i.e., equilibrium concentration of the solute in the toluene phase to that in the methanolic phase at 25° are given. In Fig. 3, the linear dependence of  $\log I$  on  $\log k^{25}$  is shown for aqueous methanol as the eluent:

$$\log I = 0.780 \log k^{25} + 0.972$$

(correlation coefficient  $r = 0.951$ ). Although the correlation coefficient is not high, this dependence demonstrates a relationship between the elution behaviour and the distribution coefficient in model liquid phases. As the extraction system approximates

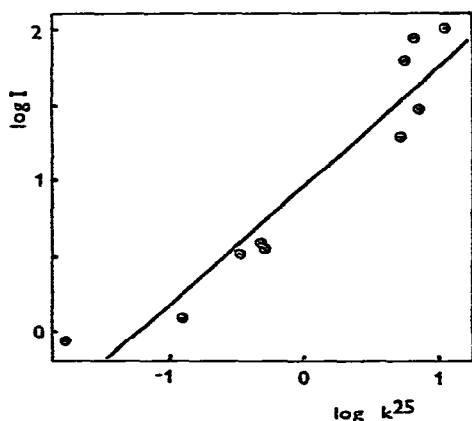


Fig. 3. Dependence of retention index,  $I$ , on solute partition coefficient,  $k^{25}$ , for toluene/methanol-water (4:1) at 25°.

the character of the gel matrix only roughly, this correlation can be considered to indicate the probability of the partition mechanism controlling the chromatographic system studied.

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