

CHROM. 12,930

REVERSED-PHASE LIQUID CHROMATOGRAPHY OF AROMATIC NITRO COMPOUNDS ON A MACROPOROUS POLYSTYRENE GEL

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(Received May 6th, 1980)

SUMMARY

The retention behaviour of some nitrobenzene derivatives was studied by reversed-phase chromatography on the macroporous polystyrene gel Separon SE with methanol–water, acetonitrile–water, tetrahydrofuran–water and methanol–water with adjusted pH as the eluents. The effect of the mobile phase and the presence of additional polar and non-polar substituents in the molecule of the solute on the retention were investigated.

Using methanol–water (80:20) the contribution of the nitro group to the retention of the molecule is positive for substances containing both polar and non-polar functional groups. The contribution of the nitro group did not change if a mobile phase with adjusted pH was used. With acetonitrile–water and tetrahydrofuran–water the situation was more complicated.

INTRODUCTION

In reversed-phase liquid chromatography (RP-LC) separation of substances can be generally characterized by several fundamental features¹: (i) hydrocarbons are eluted in order of increasing number of carbon atoms in the molecule; (ii) the retention volume decreases if the molecule is substituted with polar functional groups, except nitro and halogens; (iii) steric shielding of polar functional groups increases the retention volume; (iv) the chromatographic system does not retard ions and compounds in the ionic form.

In RP-LC the contribution of a halogen substituent to the retention of the whole molecule is positive, irrespective of the sorbent used and the composition of the mobile phase^{2–4}. Halogen exert a positive mesomeric (+M) and a negative inductive (–I) effect. As a consequence of the competition of these two effects operating in opposite directions, halogens are among the weakest first class substituents⁵. Halobenzenes behave in a similar manner to alkylbenzenes in RP-LC, *i.e.*, the retention increases with the bulk (atomic weight) of the halogens.

The situation is more complicated with the nitro group. This group, exerting

—M and —I effects on the aromatic system, can be classed as a medium polar substituent⁵. Substances containing a nitro group exhibit selective interactions associated with charge transfer between the substance and the mobile phase. When the substance involves additional polar or non-polar substituents, the electron configuration of the molecule alters appreciably, and so does its chromatographic behaviour.

The relationships between the chromatographic behaviour of nitro compounds and their structure have been studied extensively in thin-layer chromatography (TLC) by adsorption on silica gel⁶. In addition to the effect of the position and type of the substituent⁷, the dependences of the R_M values on the mobile phase composition were examined⁸ and simple molecular models of adsorption of nitro compounds on silica gel were devised^{9,10}.

The objective of the present work was to study the retention behaviour of aromatic nitro compounds in RP-LC on Separon SE. The effect of the mobile phase composition and of the presence of additional polar and non-polar substituents in the molecule on the retention were investigated.

EXPERIMENTAL

The chromatographic measurements were carried out on a liquid chromatograph with a stainless-steel column (300 × 6 mm I.D.) packed with Separon SE macroporous gel in the form of spherical particles, 25–32 μm in diameter, with an exclusion limit of molecular weight 300,000 daltons. The sorbent was obtained from Laboratorní přístroje (Prague, Czechoslovakia). The eluent was delivered by means of a VLD 30 high-pressure linear dosing apparatus, manufactured at the Development Workshop, Czechoslovak Academy of Sciences (Prague, Czechoslovakia), and a UV (254 nm) detector with a 10- μl cell and an optical path length of 1 cm was obtained from the same manufacturer.

The mobile phases were methanol–water (MeOH–H₂O) (80:20), acetonitrile–water (AcN–H₂O) (63:37), tetrahydrofuran–water (THF–H₂O) (50:50) and MeOH–H₂O (80:20) of pH = 3.3, 7 and 11.9, and the flow-rate was 60 ml/h.

The standards for chromatography and the solvents for the preparation of the mobile phases were of reagent-grade purity.

RESULTS AND DISCUSSION

Some papers dealing with the effect of the mobile phase composition on the retention behaviour of substances containing polar functional groups have reported in some instances different effects of some functional groups on the retention behaviour of the entire molecule^{2,4,11}. The differences found for sorbents with chemically bonded phases are probably due to the presence of unreacted and unshielded silanol groups. Silanol groups exert unfavourable effects on the retention of the substances separated and on the peak symmetry, and their influence must therefore be minimized. Sorbents with chemically bonded phases are usually characterized in terms of the amount of the organic phase on the support surface in $\mu\text{mol}/\text{m}^2$, and the blocking of the silanol groups is expressed through the capacity factor values of some polar substances measured in a non-polar eluent. For instance,

Tanaka *et al.*⁴ employed anisole, methyl benzoate and acetophenone, using dry *n*-heptane as the mobile phase.

Separon SE is prepared by copolymerization of styrene with ethylene dimethacrylate. This sorbent does not contain hydroxy groups and behaves as a purely non-polar stationary phase¹². Comparison of the k' values of anisole, methyl benzoate and acetophenone on Separon SE with the results obtained by Tanaka *et al.*⁴ for sorbents with chemically bonded phase indicates that Separon SE acts as a sorbent with a completely covered surface (Table I). The capacity factors were measured by using the procedure reported by Tanaka *et al.*⁴.

TABLE I
CHARACTERISTICS OF SORBENTS

Values for Hypersil are taken from ref. 4.

Sorbent	Size of particles (μm)	Column dimensions (mm)	Surface coverage ($\mu\text{mol}/\text{m}^2$)	k' with <i>n</i> -heptane mobile phase		
				Anisole	Methyl benzoate	Acetophenone
Hypersil C ₈	5	150 × 4.6	3.4	0.2	1.6	5.3
Hypersil C ₈ + TMS	5	150 × 4.6	3.4	0.1	0.2	0.55
Separon SE	25-32	300 × 6	—	0.1	0.2	0.19

The results of the chromatographic measurements are given in terms of retention indices (I). By Popl *et al.*'s definition², $\log I = 1, 2$ and 3 for benzene, naphthalene and phenanthrene, respectively, and the retention index of any substance is calculated as

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

where R is the reduced retention volume and the subscripts n , $n + 1$ and x refer to the nearest lower and higher standards and the substance in question, respectively.

The retention volumes and capacity factors of benzene, naphthalene phenanthrene are given in Table II.

TABLE II
CORRECTED RETENTION VOLUMES, V' , AND CAPACITY FACTORS, k' , OF BENZENE, NAPHTHALENE AND PHENANTHRENE

Compound	$\log I$	MeOH-H ₂ O (80:20)		AcN-H ₂ O (63:37)		THF-H ₂ O (50:50)	
		V' (ml)	k'	V' (ml)	k'	V' (ml)	k'
Benzene	1	13.19	3.07	13.54	4.35	13.23	3.05
Naphthalene	2	49.33	11.49	35.71	11.48	21.81	5.03
Phenanthrene	3	184.49	42.99	97.99	31.51	30.55	7.05

Table III list the retention indices ($\log I_{\text{meas}}$) of the aromatic nitro compounds examined, together with the differences, $D = \log I_{\text{meas}} - \log I_{\text{theor}}$. The $\log I_{\text{theor}}$ values were calculated from the contributions of the functional groups involved

TABLE III
RETENTION INDICES FOR SUBSTITUTED NITROBENZENES AND DEVIATIONS (*D*)

Compound	MeOH-H ₂ O (80:20)		AcN-H ₂ O (63:37)		THF-H ₂ O (50:50)	
	log <i>I</i> _{meas}	<i>D</i>	log <i>I</i> _{meas}	<i>D</i>	log <i>I</i> _{meas}	<i>D</i>
Nitrobenzene	1.31	—	0.84	—	0.97	—
<i>m</i> -Dinitrobenzene	1.78	+0.16	0.86	+0.18	1.87	+0.93
Aniline	0.50	—	0.02	—	-0.83	—
<i>o</i> -Nitroaniline	1.31	+0.50	0.75	+0.89	0.15	+1.01
<i>m</i> -Nitroaniline	1.29	+0.48	0.53	+0.67	0.22	+1.08
<i>p</i> -Nitroaniline	1.18	+0.37	0.37	+0.51	-0.66	+0.20
2,4-Dinitroaniline	1.89	+0.77	0.60	+0.90	0.42	+1.31
Toluene	1.23	—	1.22	—	1.52	—
<i>o</i> -Nitrotoluene	1.52	-0.02	1.06	0.00	1.33	-0.16
<i>p</i> -Nitrotoluene	1.55	+0.01	1.10	+0.04	1.36	-0.13
2,4-Dinitrotoluene	2.03	+0.18	1.10	+0.20	2.52	+1.06
2,4,6-Trinitrotoluene	2.58	+0.42	1.34	+0.60	4.99	+3.56
Phenol	0.42	—	0.05	—	-1.06	—
<i>o</i> -Nitrophenol	1.23	+0.50	0.53	+0.64	0.73	+1.82
<i>m</i> -Nitrophenol	1.15	+0.42	0.18	+0.29	-0.07	+1.02
<i>p</i> -Nitrophenol	1.18	+0.45	-0.42	-0.31	-0.32	-0.77
2-Nitro-4-methylaniline	1.59	+0.55	0.91	+0.83	1.49	+1.83
Chlorobenzene	1.36	—	1.33	—	1.69	—
<i>o</i> -Nitrochlorobenzene	1.56	-0.11	1.10	-0.07	1.12	-0.54
<i>p</i> -Nitrochlorobenzene	1.76	+0.09	1.29	+0.12	2.32	+0.66
2,4-Dinitrochlorobenzene	2.06	+0.08	1.25	+0.24	2.91	+1.28
2,5-Dichloronitrobenzene	2.01	-0.02	1.65	+0.15	2.76	+0.41
Acetophenone	0.79	—	0.40	—	0.23	—
<i>p</i> -Nitroacetophenone	1.32	+0.22	0.52	+0.28	1.06	+0.86

(Table IV), which were obtained for a given mobile phase as the differences between the values for the corresponding monosubstituted benzene derivative and benzene itself. In this manner the effect of the various substituents on the retention behaviour of the entire molecule can be conveniently characterized; this approach, however, is not satisfactory when the various substituents in the molecule interact with each other.

Phenol and nitrophenols exhibited lower log *I*_{meas} values in the AcN-H₂O and THF-H₂O systems than in MeOH-H₂O because of a strong interaction with the

TABLE IV
Δ log *I* CONTRIBUTIONS FOR VARIOUS FUNCTIONAL GROUPS IN SUBSTITUTED BENZENES

Functional group	Mobile phase		
	MeOH-H ₂ O (80:20)	AcN-H ₂ O (63:37)	THF-H ₂ O (50:50)
NO ₂	+0.31	-0.16	-0.03
OH	-0.58	-0.95	-2.06
NH ₂	-0.50	-0.98	-1.83
CH ₃	+0.23	+0.22	+0.52
Cl	+0.36	+0.33	+0.69
COCH ₃	-0.21	-0.60	-0.77

mobile phase, involving dipole-dipole interactions and hydrogen bond formation¹¹. As the hydrophobic selectivity of MeOH-H₂O and THF-H₂O is normalized (benzene possesses the same retention volume), it is possible to assess the contribution of the hydroxy group to the retention behaviour of the molecule in the given eluent⁴. The lowest log I_{mcs} value for phenol on Separon SE, and therefore also the lowest $\Delta \log I$ contribution of the hydroxy group, was obtained in the THF-H₂O system. This disagrees with the findings of Bakalyar³, who observed, using LiChrosorb RP-8, a decrease in the k' values of phenols in AcN-H₂O compared with MeOH-H₂O and, on the other hand, an approximately 50% increase in these values in THF-H₂O.

o-Nitrophenol, in which a strong intramolecular bond forms and the -OH group is sterically shielded, exhibits the highest log I_{mcs} value of the three isomeric nitrophenols. In AcN-H₂O and THF-H₂O, the log I values of nitrophenols increase in the order *p*-, *m*-, *o*-; in MeOH-H₂O the order is *m*-, *p*-, *o*-. In AcN and THF [$\delta_b = 15.91 \cdot 10^3$ and $15.49 \cdot 10^3$ (J/l)^{1/2}, respectively], their ability to act as proton acceptors obviously plays a part, the probability of formation of the substance-mobile phase hydrogen bond decreasing in the order *p*-, *m*-, *o*-. With MeOH-H₂O, the ability of MeOH to act both as a proton donor and a proton acceptor shows up here [$\delta_a = \delta_b = 34.75 \cdot 10^3$ (J/l)^{1/2}]¹⁵. The probability of the two types of interactions is virtually the same for the *p*- and *m*-isomers; for *o*-nitrophenol it is substantially lower because of the intramolecular bonding and steric shielding. This view is supported by the log I_{mcs} values of the *p*- and *m*-isomers, which do not differ appreciably. With these substances, dissociation in the system in question plays a role, the elution order in the various eluents being in accordance with the dissociation constants¹³.

For nitroanilines, the log I_{mcs} values increase in the MeOH-H₂O and AcN-H₂O systems in the order *p*-, *m*-, *o*-, and in THF-H₂O in the order *p*-, *o*-, *m*-. The dissociation has no effect in any of the eluents, owing to the very low dissociation constants¹⁴ (see the measurements in MeOH-H₂O of pH = 3.3 in Table V). With nitroanilines, acceptor-donor and donor-acceptor intermolecular bonds can form with the mobile phase, with nitrophenols. No satisfactory explanation could be found for the behaviour of nitroanilines in THF-H₂O.

For *o*-nitrochlorobenzene, the log I_{mcs} value was found to exceed log I_{theor} , owing to the strong intramolecular bonding between the two functional groups. On the other hand, *p*-nitrochlorobenzene, in which the substituents have little effect on each other and where, moreover, the -M and -I effects of the nitro group appear to the full extent, exhibits a longer retention time than that which corresponds to the theoretical value. Thus the difference D is negative for *o*-nitrochlorobenzene and positive for *p*-nitrochlorobenzene in all of the mobile phases used.

The greatest differences between the experimental and theoretical log I values were found in THF-H₂O for substances containing two or more nitro groups in a molecule. Their retention behaviour is probably governed by their dipole-dipole interaction. THF, with a rather bulky molecule, for steric reasons is not capable of giving rise to such strong dipole-dipole interactions as, for instance, AcN. This may account for the high log I_{mcs} values observed for those substances in THF, the difference D being positive in all instances.

The effect of pH on the retention behaviour of aromatic nitro compounds was investigated in the MeOH-H₂O (80:20) system of pH 3.3, 7.0 and 11.9 (Table V).

TABLE V

RETENTION INDICES FOR SUBSTITUTED NITROBENZENES IN THE MeOH-H₂O (80:20) MOBILE PHASE WITH ADJUSTED pH

Compound	pH of mobile phase		
	3.3	7.0	11.9
Nitrobenzene	1.30	1.31	1.32
<i>m</i> -Dinitrobenzene	1.65	1.78	1.60
Aniline	-0.13	0.50	0.40
<i>o</i> -Nitroaniline	1.26	1.31	1.18
<i>m</i> -Nitroaniline	1.19	1.29	1.26
2,4-Dinitroaniline	1.69	1.89	1.72
Toluene	1.23	1.23	1.25
<i>o</i> -Nitrotoluene	1.41	1.52	1.52
<i>p</i> -Nitrotoluene	1.44	1.55	1.55
2,4-Dinitrotoluene	1.91	2.03	1.86
2,4,6-Trinitrotoluene	2.27	2.58	2.36
Phenol	0.44	0.42	-1.58
<i>o</i> -Nitrophenol	1.19	1.23	-3.88
<i>m</i> -Nitrophenol	1.09	1.15	-1.91
<i>p</i> -Nitrophenol	1.08	1.18	-1.75
2-Nitro-4-methylaniline	1.39	1.59	1.66
Chlorobenzene	1.31	1.36	1.31
<i>o</i> -Nitrochlorobenzene	1.54	1.56	1.54
<i>p</i> -Nitrochlorobenzene	1.62	1.76	1.75
2,4-Dinitrochlorobenzene	1.92	2.06	1.74
2,5-Dichloronitrobenzene	1.90	2.01	1.91
Acetophenone	0.64	0.79	0.67
<i>p</i> -Nitroacetophenone	1.33	1.32	1.34

No changes in the retention indices were observed for substances not capable of dissociation, the differences being within the limits of the measurement precision.

Substances containing an amine or a hydroxy group displayed different $\log I_{\text{meas}}$ values at pH 3.3 and 11.9 in MeOH-H₂O, in accordance with their dissociation constants.

Based on the data obtained, some general conclusions can be drawn concerning the retention behaviour of aromatic nitro compounds on Separon SE.

In the MeOH-H₂O (80:20) mobile phase the nitro group contribution to the retention of the molecule is positive for substances containing both polar and non-polar functional groups. The contribution of the nitro group is higher (D is positive) for polar substances with basic or acidic functional groups. When assessing the retention behaviour of a substance from the individual contributions of the substituents involved, the highest differences are encountered if the nitro group is in an *ortho* position with respect to a polar functional group; if only non-polar substituents are present in the substance in addition to the nitro group, the retention behaviour can be forecast fairly accurately based on the increments for individual substituents.

The contribution of the nitro group did not change if a mobile phase with adjusted pH was used; the differences found (Table V) lay within the error of measurement.

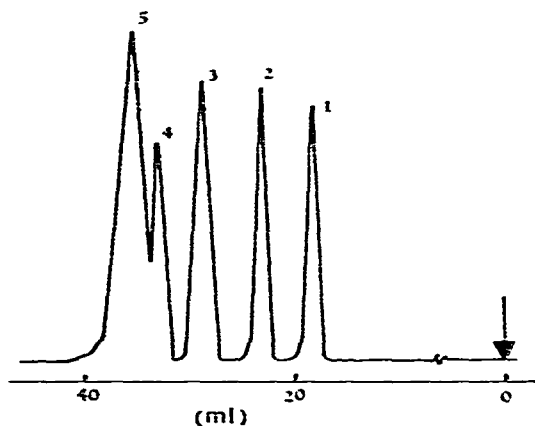


Fig. 1. Separation of chloronitrobenzenes. Column: 300 × 6 mm I.D., Separon SE. Eluent: tetrahydrofuran-water (50:50), 60 ml/h. UV detector at 254 nm. Peaks: 1 = *o*-nitrochlorobenzene; 2 = chlorobenzene; 3 = *p*-nitrochlorobenzene; 4 = 2,5-dichloronitrobenzene; 5 = 2,4-dinitrochlorobenzene.

With AcN-H₂O (63:37) and THF-H₂O (50:50) the situation is more complicated and conclusions cannot be drawn so clearly as in the preceding case. In AcN-H₂O and THF-H₂O the nitro group contributes negatively to the retention of the molecule if the substance contains non-polar groups (halonitrobenzenes, alkyl-nitrobenzenes), whereas if the substance contains medium polar or polar functional groups, basic or acidic, the contribution of the nitro group is positive. The AcN-H₂O and particularly the THF-H₂O systems give rise to specific interactions between the mobile phase and the substance, and are suitable for the separation of aromatic nitro compounds (Fig. 1).

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