Note

Retention parameters and eluent composition in thin-layer and high-performance liquid chromatography of aromatic nitro compounds in reversed-phase systems

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It has been emphasized by several authors[1–5] that the systematic use of gradient elution chromatography depends on the formulation of quantitative relationships between the solute retention and the composition of the eluent. For the so-called reversed-phase liquid–solid systems (aquoorganic eluent–silanized silica) this relationship is given by the simple semiempirical formula

$$\log k' = R_M = a + bc$$

where a and b are constants and c is the concentration of the modifier (methanol, acetonitrile, etc.) in the eluent, expressed usually in volume fractions (or volume per cent). This relationship, reported first for analogous liquid–liquid partition systems[6–8], has been confirmed by a large number of studies (*e.g.*, refs. 7–34 in ref. 1) although for some solute–solvent systems deviations from linearity have been reported (*e.g.*, refs. 2, 9). Melander, and co-workers[9,10] utilized the formula to investigate the relationship between the hydrophobic properties of alkylbenzenes and their chromatographic parameters, and discussed the application of the method in quantitative structure–activity relationships (QSAR) of biologically active solutes[11].

On the other hand, the comparison of retention data from thin-layer (TLC) and high-performance liquid chromatography (HPLC), has been investigated mainly for normal phase systems[12]; the comparison is of practical interest owing to the potential use of TLC as a pilot technique for the optimization of HPLC, including preparative separations.

In the present work the retention behaviour of aromatic nitro compounds on thin layers of methyl- and octadecylsilica (RP-2 and RP-18) was compared with column HPLC data obtained for octadecylsilica. Mixtures of water and methanol were used as eluents of good separation selectivity[3,13,14].

EXPERIMENTAL

Thin-layer chromatography of nitro compounds (Table I) was carried out using 10 × 10 cm glass plates with a 0.25-mm layer of silanized silica (RP-2) and HPTLC plates of RP-18 silica (E. Merck, Darmstadt, G.F.R.). One-microlitre samples

of 0.1 % solutions of solutes in methanol were spotted 1 cm from the edge and the plates immersed in a 0.4-cm layer of the eluent (60% methanol for RP-2 plates and 80% methanol for RP-18 plates). The chromatograms were eluted to a distance of 9 cm. The nitro compounds were visible as yellow spots or were localized under UV light.

Column HPLC was carried out using a liquid chromatograph* with 200-ml syringe pump, 5-μl sample injection valve, steel column (250 × 4 mm I.D.) packed with 10-μm LiChrosorb RP-18 (E. Merck) and 254-nm UV detector. The flow-rate was 0.6–1.2 cm^3 min^{-1} depending on the hydrophobicity of the compounds. The column dead volume was determined using an aqueous solution of potassium bichromate.

RESULTS AND DISCUSSION

The chromatographic results (HPLC) are represented as plots of log k' vs. percentage methanol (Fig. 1), which are linear for almost the whole concentration range investigated and spread fanwise owing to increased selectivity at higher water contents in the eluent[15], especially for less polar solutes. The selectivity, Δ log k', can be directly estimated from the plots (log α = log k'_2 — log k'_1).

Comparison of the retention of nitro-, 1,4-dinitro- and 1,3,5-trinitrobenzene shows that the Δ log k' (NO$_2$) value is not constant, being smaller for the third nitro group. This is presumably due to the fact that the mesomeric effect (—M) decreases with the number of nitro groups already present in the benzene molecule so that these compounds cannot be considered as a homologous series[16].

For nitro derivatives of naphthalene and biphenyl the Δ log k'(NO$_2$) values are also differentiated. As usual, the larger size of the non-polar part of naphthalene and biphenyl in comparison to benzene derivatives causes stronger retention, expecially at higher water contents.

Weaker retention of 2-chloronitrobenzene in comparison to the *para* isomer (Fig. 1b) is presumably due to the smaller conjugation effect in the former caused by steric repulsion between the two dipoles and increased polarity (dipole moment). The introduction of a second nitro group into the *para* position of 2-chloronitrobenzene further weakens the retention, and this effect is augmented when chlorine is substituted by fluorine. On the other hand, introduction of one or two methyl groups into nitrobenzene increases the hydrophobic properties and thus prolongs the retention times.

Owing to the formation of an intramolecular hydrogen bond, 2-nitrophenol (Fig. 1c) is slightly more hydrophobic than its isomers and is thus more strongly retained; a similar effect is observed for 2-nitroaniline (Fig. 1b) in comparison to its *meta* and *para* isomers (Fig. 1c). Generally, for polar compounds having donor–acceptor properties (nitrophenols, nitroanilines, nitrobenzaldehydes) the interactions with the mobile phase, expecially hydrogen bonding, largely compensate the hydrophobic interactions with the RP adsorbent so that their retention is weak.

To determine quantitative relations between HPLC and TLC data, log k' values (HPLC) were plotted against R_M values (TLC). Owing to the limited wettability of RP adsorbents by aqueous solutions, 80% methanol was used as eluent for RP-18

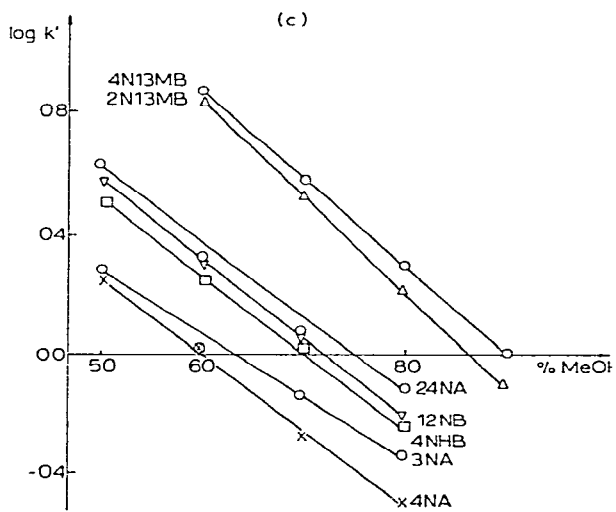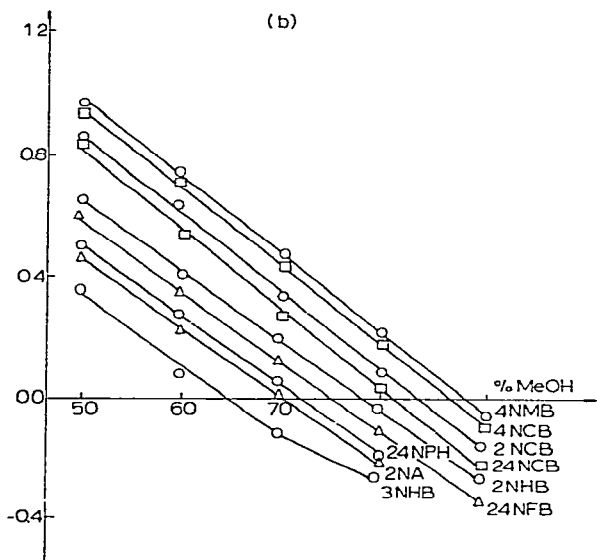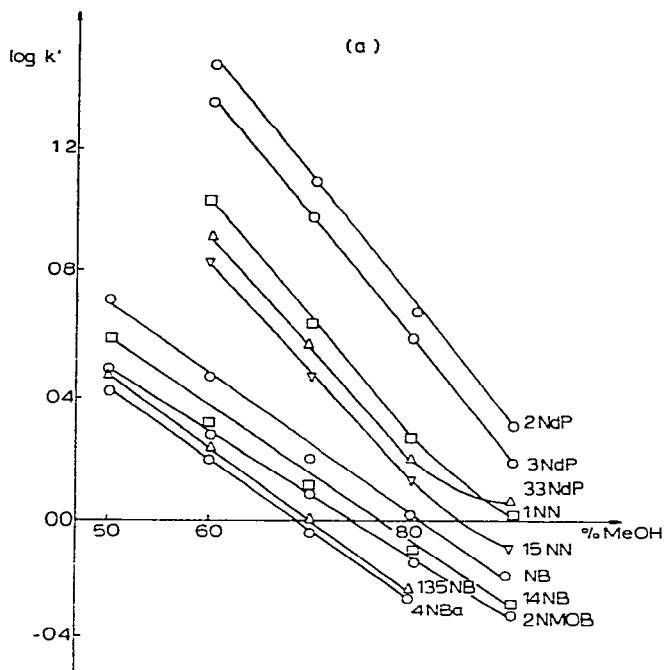* Produced by Institute of Physical Chemistry of the Polish Academy of Sciences, Warsaw, Poland.

Fig. 1. log *k'* values of nitro compounds plotted against concentration (volume per cent) of methanol in the eluent. Active solid: octadecylsilica. Notation of solutes as in Table I.

plates; 60% methanol for RP-2 plates gave an appropriate range of R_F values (0.2–0.8) for most solutes.

The sequence of solutes on RP-18 HPTLC plates and for HPLC using LiChrosorb RP-18 was found to be identical (Fig. 2); however, for RP-2 plates there were some changes in the sequence (Fig. 3). The regression equations for the correlations are:

$$\log k_{18}\quad = 0.93\ R_{M(18)} + 0.13\qquad (r = 0.992)$$
$$\log k_{18}\quad + 1.35\ R_{M(2)}\ + 0.64\qquad (r = 0.930)$$
$$R_{M(18)} = 0.94\ R_{M(2)}\ + 0.09\qquad (r = 0.900)$$

Thus for identical adsorbents the correlation of TLC and HPLC retention data of nitro compounds is very good in spite of differences in the properties of the solutes and the limited accuracy of the TLC technique; a minor shift of the straight line relative to the origin of the coordinates is presumably due to differences in the phase ratios ($m_{ads}:V_0$). The spread of the points is considerably greater for comparison of TLC data for the system RP-2–60% methanol and HPLC data (RP-18–80% methanol), although for homologues the correlation was quite good[18]. Moreover, the higher slope of the plot (Fig. 3) shows that the RP-18 system is generally more selective, although for individual pairs of solutes the RP-2 system may be more selective[19]. The effect of brush length on selectivity is also seen from the third equation which shows the only partial analogy of TLC data for RP-2 and RP-18 systems.
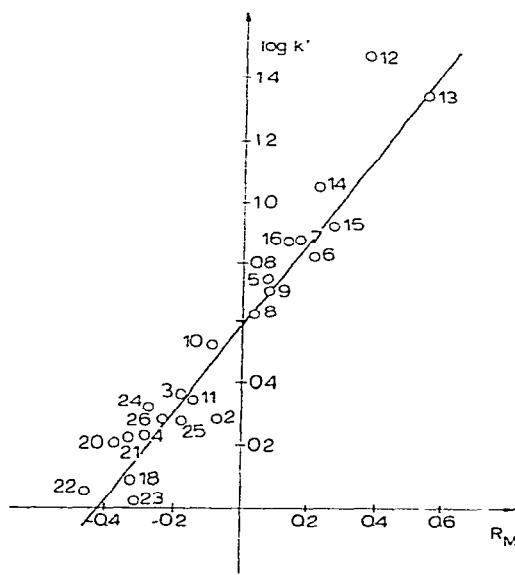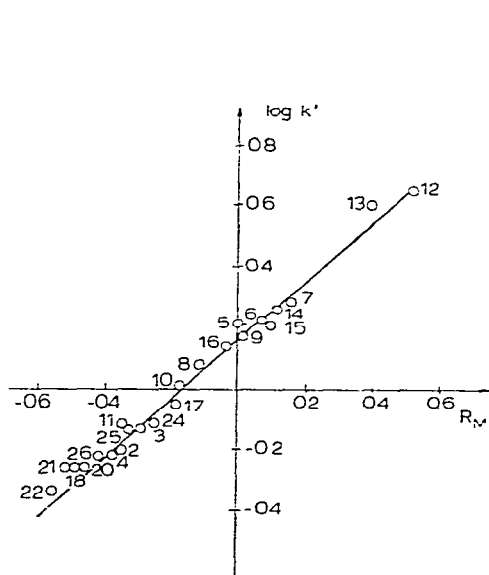

Fig. 2. log k' values of nitro compounds (HPLC) plotted against TLC data. Chromatographic system: octadecylsilica–80% methanol. Identification of solutes as in Table I.

Fig. 3. log k' values of nitro compounds obtained for the system octadecylsilica–60% methanol plotted against TLC R_M values for the system silanized silica (RP-2)–60% methanol.

TABLE I

RETENTION DATA OF AROMATIC NITRO COMPOUNDS FOR HPLC (RP-18) AND TLC (RP-18 AND RP-2)

No.	Solute	Abbreviation	HPLC 80% methanol k'	HPLC 80% methanol $\log k'$	HPLC 60% methanol k'	HPLC 60% methanol $\log k'$	TLC RP-18–80% methanol R_F	TLC RP-18–80% methanol R_M	TLC RP-2–60% methanol R_F	TLC RP-2–60% methanol R_M
1	Nitrobenzene	NB	1.08	0.03	1.91	0.28	0.69	−0.35	0.54	−0.02
2	1,2-Dinitrobenzene	12NB	0.63	−0.20	2.29	0.36	0.65	−0.27	0.60	−0.18
3	1,4-Dinitrobenzene	14NB	0.72	−0.14	1.74	0.24	0.70	−0.37	0.66	−0.29
4	1,3,5-Trinitrobenzene	135NB	0.59	−0.23	5.63	0.75	0.50	0	0.46	0.07
5	4-Nitrotoluene	4NMB	1.59	0.20	6.76	0.83	0.45	0.07	0.38	0.21
6	2-Nitro-1,3-methylbenzene	2N13MB	1.70	0.23	7.24	0.87	0.40	0.18	0.40	0.18
7	4-Nitro-1,3-methylbenzene	4N13MB	1.91	0.28	4.27	0.63	0.57	−0.12	0.48	0.03
8	2-Chloronitrobenzene	2NCB	1.17	0.07	5.13	0.71	0.48	0.03	0.46	0.07
9	4-Chloronitrobenzene	4NCB	1.48	0.17	3.39	0.53	0.59	−0.16	0.54	−0.07
10	1-Chloro-2,4-dinitrobenzene	24NCB	1.05	0.02	2.19	0.34	0.68	−0.33	0.58	−0.14
11	1-Fluoro-2,4-dinitrobenzene	24NFB	0.77	−0.11						
12	2-Nitrobiphenyl	2NdP	4.50	0.65	30.2	1.48	0.23	0.53	0.30	0.37
13	3-Nitrobiphenyl	3NdP	3.98	0.60	22.4	1.35	0.28	0.41	0.22	0.55
14	3,3'-Dinitrobiphenyl	33'NdP	1.80	0.26	11.5	1.06	0.43	0.12	0.38	0.21
15	1-Nitronaphthalene	1NN	1.58	0.20	8.32	0.92	0.44	0.1	0.35	0.27
16	1,5-Dinitronaphthalene	15NN	1.35	0.13	7.41	0.87	0.52	−0.03	0.42	0.14
17	2-Nitrophenol	2NHB	0.89	−0.05	2.51	0.40	0.60	−0.18	0.77	−0.52
18	3-Nitrophenol	3NHB	0.55	−0.26	1.23	0.09	0.75	−0.48	0.68	−0.33
19	4-Nitrophenol	4NHB	0.57	−0.24	1.57	0.24	0.90		0.85	
20	4-Nitrobenzaldehyde	4NBa	0.55	−0.26	1.62	0.21	0.74	−0.45	0.70	−0.37
21	2-Nitroaniline	2NA	0.55	−0.26	1.70	0.23	0.76	−0.50	0.68	−0.33
22	3-Nitroaniline	3NA	0.47	−0.33	1.13	0.05	0.78	−0.55	0.73	−0.43
23	4-Nitroaniline	4NA	0.33	−0.48	1.05	0.02	0.90		0.68	−0.33
24	2,4-Dinitroaniline	24NA	0.77	−0.11	2.09	0.32	0.64	−0.25	0.65	−0.27
25	2-Nitroanisole	2NMOB	0.72	−0.14	1.86	0.27	0.67	−0.31	0.60	−0.18
26	2,4-Dinitrophenylhydrazine	24NPH	0.62	−0.21	1.91	0.28	0.72	−0.41	0.63	−0.23

The correlations indicate that approximate optimization of eluents for HPLC can be carried out even using the less expensive and more wettable RP-2 sorbent, at least as far as the range of k' values is concerned. The use of the same adsorbent in pilot TLC experiments and HPLC secures much better correlation, permitting also preliminary determination of programs for gradient elution.

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