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## CHROMATOGRAPHIC SELECTIVITY IN LIQUID CHROMATOGRAPHY OF POLYCONDENSED AROMATIC HYDROCARBONS ON 3-(2,4-DINITROANILINO)PROPYL SILICA\*

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### SUMMARY

The effect of the surface concentration of chemically bound 2,4-dinitroanilino groups upon the chromatographic selectivity for polycondensed aromatic hydrocarbons (PAHs) is examined. The selectivity is evaluated by means of a correlation analysis based upon a semiempirical linear free energy relationship that enables a separation of the effect of solute structure from those of other variables. The selectivity decreases with decreasing concentration of dinitroaniline groups. Sorbents with low concentrations of the bound groups yield strong peak asymmetry.

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### INTRODUCTION

Recently, several papers dealing with so-called “charge-transfer” or “donor–acceptor” liquid chromatography (DAC-LC) have been presented<sup>1–4</sup>. In this mode of LC, the formation of weak electron donor–acceptor complexes (DACs) between the solute and stationary phase is supposed to constitute the primary retention mechanism. Several sorbents based on silica modified with chemically bound electron acceptors (EAs) or electron donors (EDs) are known<sup>1–9</sup>.

It must be stressed here that the formation of DACs between solutes and stationary phases is deduced via an analogy with the complex formation taking place in homogeneous solutions. Various correlations between chromatographic retention in DAC-LC and the stability constants measured in dilute solutions or other variables depending upon the ability of solutes or stationary phases to form complexes have been used as indirect evidence of the DAC retention mechanism<sup>1,4,10</sup>.

The separation of polynuclear aromatic hydrocarbons (PAHs) on nitroaromatic stationary phases has been used as a model<sup>1,4,10,11</sup>. As in other LC modes, the retention of PAHs in DAC-LC increases with the number of carbon atoms,  $n_c$ , forming the aromatic skeleton. Despite the fact that the stability of DACs between PAHs and a strong EA also increases with  $n_c$ , no satisfactory correlation between the sta-

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bility constants measured in solutions and the retention of PAHs on bound EAs has yet been presented. For example, anthracene, forming a significantly stronger DAC than does phenanthrene has approximately the same retention as the latter<sup>6,7,10-12</sup>. The same holds for the retention of PAHs on silica impregnated with strong EAs such as tetranitrofluorenone or picric acid<sup>13,14</sup>. This can probably be explained by the lack of reliable data on the stability of DACs including large PAH molecules, and by the different situations on the sorbent surface and in dilute solution. On the sorbent surface, one PAH molecule can interact with more than one EA ligand if the surface concentration and distribution of EAs is favourable<sup>10,15</sup>.

Deviations from 1:1 stoichiometry are observed in solutions containing a large excess of one component or at higher concentrations<sup>16</sup>. This means that the relative retention of PAHs may depend upon the surface concentration of EA ligands as remarked earlier<sup>10,15</sup>. Another complication is that the ligands bound to the silica surface are not inevitably distributed in a random way as shown by Lochmüller *et al.*<sup>17</sup>.

Bearing in mind the above problems, we decided to examine the effect of the surface concentration of dinitroanilino groups upon the chromatographic selectivity for separation of PAHs. 3-(2,4-Dinitroanilino)propyl silica was chosen as a model sorbent due to its easy preparation via the reaction of bound NH<sub>2</sub> groups with 2,4-dinitrofluorobenzene<sup>4,12</sup>. The retention observed for series of PAHs was evaluated by means of a correlation analysis based upon a semiempirical linear free energy relationship (LFER).

## EXPERIMENTAL

Silica gel for high-performance liquid chromatography (HPLC), Separon SI VSK, 10–15  $\mu\text{m}$  (LP Praha, Czechoslovakia) with a specific surface area of 310 m<sup>2</sup>/g, was converted into NH<sub>2</sub>-silica using a procedure described elsewhere<sup>4</sup>. The samples thus prepared, A, B and C, contained 83, 220 and 426  $\mu\text{equivalent}$  of NH<sub>2</sub> groups per gram as determined by acid–base titration<sup>10,12</sup>. Corresponding samples of 3-(2,4-dinitroanilino)propyl silica (DNAP-silica) were prepared according to the previously described procedure<sup>4,10,12</sup>.

After extraction with tetrahydrofuran in a soxhlet extractor, the samples were dried at 80°C for 4 h. Residual silanol groups were end-capped with a toluene solution of hexamethyldisilazane (HMDS) (Pierce, Rockford, IL, U.S.A.). The reaction was carried out at normal temperature with occasional shaking for 8 h. Then the samples were filtered off, washed with toluene, tetrahydrofuran and methanol and dried at 80°C for 4 h.

A liquid chromatograph PE Series 4 (Perkin-Elmer, Norwalk, CT, U.S.A.) equipped with an UV-Vis detector LC 85/Autocontrol and 3600 Datastation was used. Commercial stainless-steel columns (LP Praha), 250  $\times$  4 mm I.D., were packed by the descending technique with *n*-heptane at 600 atm. Tetrachloromethane was used as slurry liquid. Pure *n*-hexane (Winlab, Maidenhead, Berkshire, U.K.) was used as mobile phase. Columns were equilibrated overnight with the mobile phase before use. Tetrachloromethane was used as an unretained solute to determine  $t_0$ . Each measurement carried out at 20°C was repeated three times.

## RESULTS AND DISCUSSION

The experimentally measured capacity factors,  $k'$ , are summarized in Table I. The retention of PAHs on sorbent A, which has the lowest concentration of bound DNAP ligands, after end-capping is even lower than on bare silica. The end-capping with HMDS had nearly no effect in the case of sample C and only a minor effect on sample B. The retention of all solutes decreases with decreasing surface concentration of DNAP ligands. Samples A and B yielded excessive tailing and peak asymmetry; the asymmetry factor,  $A_{0.1}$ , depends upon the number of carbon atoms in the PAH molecules as shown in Fig. 1. The observed retention times of strongly skewed peaks were corrected by means of a numerical procedure proposed by Foley and Dorsey<sup>18</sup>.

*Chromatographic selectivity and structure of PAHs*

The chromatographic selectivity,  $\alpha_{2,1}$ , which is defined as  $k_2'/k_1'$ , depends not only upon the structural differences between solutes 1 and 2 but also upon the ability of the chromatographic system to respond to this difference. In DAC-LC the structure of bound EAs or EDs is expected to have the dominant influence upon  $\alpha_{2,1}$ . Nondek and Ponec<sup>4</sup> recently studied the effect of the structure of EA ligands upon the chromatographic selectivity for PAHs of several silicas modified with various EAs. Assuming that surface complexes are formed with 1:1 stoichiometry and  $\pi$ -electrons are transferred only from the highest occupied molecular orbital (HOMO) of the ED to the lowest unoccupied molecular orbital (LUMO) of the EA, they derived a simple relationship between the energies of the HOMO and LUMO and the chromatographic selectivity observed for a series of  $n$  structurally related solutes:

$$\log \alpha_{i,1} = \text{Const} \cdot \frac{(\Delta E_{\text{HOMO}})_i}{(E_{\text{HOMO}} - E_{\text{LUMO}})_1^2} \quad (i = 2, 3, \dots n) \quad (1)$$

TABLE I

CAPACITY FACTORS,  $k'$ , FOR POLYCONDENSED AROMATIC HYDROCARBONS ON DNAP-SILICA GEL

Stationary phase: DNAP-modified Separon SI VSK, 10–15  $\mu\text{m}$  (A, 83; B, 220; C, 426  $\mu\text{equivalent/g}$ ). Column: 250  $\times$  4 mm I.D. Mobile phase: *n*-hexane, flow-rate 1 ml/min. Temperature: 20°C. Sample volume: 20  $\mu\text{l}$  of ca. 0.1% solution. Detection: at 254 nm.

Solute	Column			
	A*	A	B	C
Naphthalene	0.17	0.08	0.37	0.45
Anthracene	0.24	0.14	0.97	1.23
Phenanthrene	0.25	0.11	0.90	1.31
Acenaphthene	0.37	0.07	0.39	0.52
Acenaphthylene	0.41	0.18	0.63	0.86
Fluorene	0.51	0.12	0.72	0.63
Pyrene	0.60	0.21	1.29	2.10
Fluoranthene	0.73	0.18	1.05	2.13
Picene	0.57	0.46	3.20	8.15
Benzo[a]pyrene	1.21	0.82	3.40	6.19

\* Sample A before end-capping with HMDS.

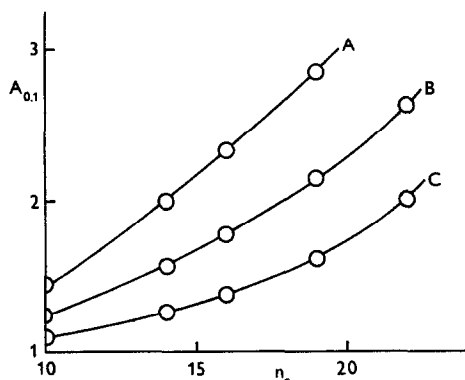


Fig. 1. The dependence of peak asymmetry,  $A_{0.1}$ , upon the number of carbon atoms,  $n_c$ , in the PAH molecule (naphthalene, anthracene, fluoranthene, chrysene and coronene). Values of  $A_{0.1}$  were calculated at 10% of peak height. Curves: A, 83; B, 220; C, 426  $\mu$ equivalent DNAP groups per gram. Mobile phase: pure *n*-hexane, flow-rate 1 ml/min. Temperature: 20°C.

$(\Delta E_{\text{HOMO}})_i$  is the difference between the energies of the HOMOs of solutes  $l$  and  $i$ . Solute  $l$  serves as a standard.

Due to the crudeness of the quantum chemical model, eqn. 1 has only a limited value for numerical calculations, nevertheless it indicates that the chromatographic selectivity is enhanced by increasing the electron-accepting ability of stationary phase, which depends upon the structure of the bound ligands.

This relationship also shows that two independent factors influence the selectivity. The first, expressed as  $\Delta E_{\text{HOMO}}$ , includes the difference in structure of the solutes which affects the ability to donate  $\pi$ -electrons. The rest of eqn. 1 reflects the ability of EA ligands to form complexes within the series of structurally related solutes.

Thus, the chromatographic selectivity can be expressed as a linear free energy relationship (LFER) in accordance with the general theory of LFERs<sup>4</sup>:

$$\log \alpha_{i,1} = \kappa \delta_i \quad (2)$$

In our quantum chemical approximation,  $\delta_i = (\Delta E_{\text{HOMO}})_i$  and  $\kappa$  represents the rest of eqn. 1. As a semiempirical LFER, eqn. 2 is more general than eqn. 1, since it may include several independent factors.

The retention of PAHs on DNAP-silica has therefore been recalculated as  $\log \alpha_{i,1}$  and correlated with the data given by Hammers *et al.*<sup>11</sup> for the same chromatographic system. These data served as a base for calculation of the standard set of  $\delta$  constants given in Table II.

#### Interpretation of $\delta$ constants

If the formation of DACs is responsible for the retention of PAHs on DNAP-silica, the  $\delta$  constants should be correlated with  $E_{\text{HOMO}}$ , ionization potentials,  $I_d$ , or stability constants,  $K_{\text{eq}}$ , measured in solution. These parameters are given in Table II together with other structural constants of PAHs.

The main problem in the correlation analysis is that all these parameters are

TABLE II

COMPARISON OF RETENTION CONSTANTS,  $\delta$ , WITH VARIOUS STRUCTURAL PARAMETERS OF PAHs

$I_d^0$  = Vertical ionization potential taken from ref. 23.  $E_{\text{HOMO}}$  = energy of HOMO taken from ref. 24;  $\alpha_{\text{exp}}$  = average molecular polarizability taken from ref. 25;  $DE_{\text{HMO}}$  = delocalization energy calculated by Hückel method and taken from ref. 24;  $\chi$  = molecular connectivity calculated according to ref. 22;  $K_{\text{eq}}$  = equilibrium constant of DAC between PAH and pyrromelic dianhydride taken from ref. 26.

PAH	$\delta$	$n_c$	$I_d^0$	$E_{\text{HOMO}}$	$\alpha_{\text{exp}}$	$DE_{\text{HMO}}$	$\chi$	$K_{\text{eq}}$
Naphthalene	0.00	10	8.14	0.000	17.5	3.683	3.405	1.3
Phenanthrene	0.66	14	8.02	0.013	24.7	5.448	4.892	2.6
Anthracene	0.64	14	7.42	0.204	25.9	5.314	4.809	3.7
Fluoranthene	1.01	16	7.76	0.123	28.4	—	5.565	7.9
Pyrene	1.04	16	7.56	0.173	29.3	6.506	5.559	9.0
Chrysene	1.32	18	7.74	0.098	33.1	7.190	6.226	14.1
Benzo[a]pyrene	1.69	20	7.17	0.247	—	—	6.970	—
Perylene	1.79	20	7.03	0.244	—	—	6.975	19.4
Picene	1.95	22	—	0.117	—	—	7.637	—

more or less proportional to the molecular size given, *e.g.*, in terms of the number of carbon atoms,  $n_c$ . It could be erroneously concluded from the correlation between  $\delta$  and  $n_c$  or molecular area,  $A_s$ , calculated according to Snyder<sup>20</sup> (Table III) that the retention of PAHs depends only upon the area occupied by the PAH molecule adsorbed in a "flat position". However, the best correlation (Table III) has been obtained between  $\delta$  and the delocalization energies,  $DE_{\text{HMO}}$ . Being a measure of the stability of the PAH molecules, these energies depend not only upon electronic factors but also on the molecular size. Molecular connectivity or branching index,  $\chi$ , a topological index proposed by Randic<sup>21,22</sup>, also correlates well with  $\delta$ . The molecular connectivity reflects the size as well as the shape of PAH molecules.

On the other hand, the unsatisfactory correlation between  $\delta$  and  $E_{\text{HOMO}}$  or  $I_d^0$  indicates the complicated nature of the solute-sorbent interaction. Thus, the correlation analysis reveals that DACs present on the surface of DNAP-silica differ from the "charge-transfer" complexes studied in dilute solutions.

TABLE III

CORRELATION BETWEEN  $\delta$  AND VARIOUS STRUCTURAL PARAMETERS

$n_{\text{exp}}$  = Number of points; numerical values of the structural parameters are given in Table II,  $A_s$  calculated according to ref. 20.

Relationship	Correlation coefficient	$n_{\text{exp}}$	Eqn.
$\delta = -1.36 + 0.373 DE_{\text{HMO}}$	0.9994	5	3
$\delta = -1.65 + 0.479 \chi$	0.9976	5	4
$\delta = -1.69 + 0.169 n_c$	0.9970	9	5
$\delta = -1.51 + 0.086 \alpha_{\text{exp}}$	0.9930	6	6
$\delta = -0.03 + 1.245 \log K_{\text{eq}}$	0.9710	7	7
$\delta = -2.02 + 0.271 A_s$	0.9581	9	8
$\delta = 10.52 - 1.25 I_d^0$	0.8215	9	9
$\delta = 0.53 + 4.38 E_{\text{HOMO}}$	0.6248	9	10

### Effect of surface concentration of DNAP groups

In Table IV, the values of  $\kappa$  are summarized for varying experimental conditions, e.g., concentration of DNAP groups, mobile phase composition or temperature. According to eqn. 1, the selectivity of the stationary phase in DAC-LC depends only upon the molecular structure of the bound EAs. From Table IV, one can see that  $\kappa$  (reflecting the ability of the stationary phase to form complexes) decreases with decreasing concentration of DNAP groups. One can explain this on the basis of the formation of the above mentioned non-stoichiometric complexes. If the silica surface is covered by a dense layer of EA ligands, the molecules of PAHs can interact, according to their molecular area, with a more or less constant number of EA groups. On the other hand, at low surface concentrations of these groups, even relatively large PAH molecules can interact with only one EA ligand. Thus the retention of PAHs on sorbent A, where the average distance between the neighbouring DNAP groups is about 24 Å as calculated according to ref. 17, should parallel the stabilities of the charge-transfer complexes in dilute solutions.

To examine this hypothesis, we have correlated  $\log \alpha_{i,1}$  on sorbents A, B and C with the logarithms of the equilibrium constants,  $K_{eq}$ , quoted by Ilmet and Rashba<sup>26</sup>. These stability constants for complexes of pyrromelitic dianhydride with several PAHs represent a consistent set of data. In the case of sorbent A, a satisfactory correlation coefficient,  $r = 0.9935$  ( $r_{crit} = 0.9912$  for the 99.9% significance level,  $n_{exp} = 5$ ), has been obtained. Significantly lower correlation coefficients,  $r = 0.8862$  and  $0.9419$ , were obtained for sorbents B and C, respectively. Thus, the complexation of PAHs on sorbent A seems to be closer to 1:1 stoichiometry than is the case on sorbents B and C.

The influence of ligand distribution can be modelled by means of a Monte Carlo procedure<sup>27</sup>. A series of surfaces with different coverages and extents of ligand association have been generated to simulate uniform and non-uniform distributions of DNAP ligands. The random adsorption of solutes having different molecular areas shows that an effect of the ligand "clustering" upon selectivity can be expected only

TABLE IV

COMPLEXATION ABILITY OF VARIOUS DNAP-SILICA SAMPLES IN SEPARATION OF PAHs

Mobile phase	$\kappa$	Concentration of DNAP groups ( $\mu\text{mol/m}$ )	Temperature ( $^{\circ}\text{C}$ )	$r$	$n_{exp}$	Ref.
Hexane	1.05	2.5	10	0.9978	12	11
Hexane	1.03	2.5	25	0.9953	12	11
Hexane	0.94	2.5	40	0.9969	12	11
Heptane (1% chloroform)	0.88	2.5	20	0.9392	7	12
Heptane (5% chloroform)	0.86	—	20	0.9982	13	10
Heptane (20% chloroform)	0.82	—	20	0.9986	11	10
Hexane	0.72	1.4	20	0.9964	9	Sample C
Hexane	0.62	0.7	20	0.8265	8	Sample B
Hexane	0.54	0.3	20	0.9585	9	Sample A
Hexane	0.65	0.3	20	0.6027	9	Sample A*
Hexane (1% isopropanol)	0.63	1.4	20	0.9870	10	28

\* Sample A before treatment with HMDS.

at low coverages, up to 20–30%. On the other hand, a broader spectrum of adsorption energies has been found for large solutes at lower coverages regardless of the ligand distribution. This could also be a plausible explanation of the peak asymmetry observed on sorbents A and B (Fig. 1).

Another explanation has been presented by Hammers *et al.*<sup>11</sup>. These authors suppose that the slow diffusion of solute molecules into the layer of bound EA ligands, which should be preferred at higher coverages, causes the peak asymmetry and increases the reduced plate height. Our results are however in contradiction with this idea.

In Table IV the experimental results from Table I are compared with retention data published previously<sup>10–12</sup> by means of eqn. 2. Very good correlation between  $\delta$  and  $\log \alpha_{i,1}$  is observed in all cases. The ability of DNAP-silica to interact with PAHs expressed in terms of  $\kappa$  decreases with increasing temperature and the mobile phase polarity. This is in an agreement with the general theory of DAC<sup>16</sup>. If a polar modifier such as isopropanol is added to the mobile phase, the poor peak asymmetry exhibited by DNAP-silica at low surface coverages (samples A and B) is not improved<sup>28</sup>.

## CONCLUSIONS

(1) In DAC-LC of a series of structurally related solutes, the chromatographic selectivity,  $\log \alpha_{i,1}$ , can be expressed by means of two separable terms. The first of these characterizes the structural differences between solutes  $l$  and  $i$ , and the second term reflects the ability of bound ligands to form complexes within the series of solutes.

(2) The retention of PAHs on DNAP-silica does not depend only upon the donation ability of their  $\pi$ -electron system but it is also influenced by molecular size.

(3) The formation of surface complexes between several adjacent bound ligands and one molecule of PAH is supposed. This non-stoichiometric complexation could be enhanced by non-uniform distributions even at low concentrations of bound ligands ("clusters" or "islands").

(4) The selectivity of DNAP-silica in DAC-LC of PAHs is decreased with decreasing surface coverage. Sorbents with low coverages yield strong peak asymmetry, probably by a broad spectrum of interactions between the solute molecule and the group of adjacent ligands.

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