

CHROM. 14,256

TEMPERATURE AND ELUENT EFFECTS ON THE SELECTIVITY OF SOME NITROAROMATIC BONDED PHASES IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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(Received July 13th, 1981)

SUMMARY

Nucleosil 10 NH₂ has been converted into 2,4-dinitroanilino, bis(3-nitrophenyl) sulphone and 2,4,7-trinitrofluorenimine silicas. Net retention volumes per gram of sorbent have been measured for a number of monosubstituted benzenes and unsubstituted polycyclic aromatic hydrocarbons, using *n*-hexane and dichloromethane-hexane (35:65) as eluent at 10, 25 and 40°C. The influences of temperature and of eluent strength on the bonded phase properties are described and related to the structure of the phases. At equilibrium, net retention data can be described in terms of the adsorption model, developed by Snyder for bare adsorbents.

INTRODUCTION

Tetranitrofluorenone (TENF), either physically adsorbed¹ or chemically bound to porous silica²⁻⁵, and 3-(2,4-dinitroanilino)propylsilyl silica^{6,7} have been the subject of several chromatographic investigations. The complexing ability of nitrated aromatic compounds towards potential electron donors is well-known⁸, as is the fact that such complexing sites on a silica carrier can give rise to an improved selectivity. Hemetsberger *et al.*⁵ and Nondek *et al.*⁷ related retention data on this type of bonded phases to complex association constants determined by means of suitable spectroscopic methods. Although a correlation was found, it is not evident whether high-performance liquid chromatography (HPLC) can yield well-defined complexation constants. In this respect, the relatively large concentration of ligands on the silica surface seems to hamper a straightforward interpretation of the results.

In this work, attention is focused on the effects of temperature and eluent strength on solute retention, and an attempt is made to relate these effects to the structure of the bonded phase. Although the proposed approach has a limited scope, the obtained results may form a basis for further investigations in this field. This work is part of a project, the aim of which is to examine the applicability of Snyder's adsorption model⁹ (for bare adsorbents) to bonded phases with a moderate surface coverage of monomers. Previously reported retention data on octadecylsilyl-^{10,11}, N-

2-cyanoethyl-N-methylamino¹² and aminobutylsilica¹³ could be described reasonably well in terms of the parameters of this model. Provided that the chromatographic conditions obey the underlying assumptions of the model, it has proved to be a valuable tool for understanding the retention behaviour of simple solutes on these phases. Encouraged by these results, we proceeded to complexing phases with a relatively high amount of bound organic material.

THEORETICAL

In this work, only the most simple and basic concepts of Snyder's adsorption model will be considered. For a more extended account the reader is referred to Snyder's book⁹ and previous papers on this subject¹¹⁻¹³.

The net retention volume per gram of adsorbent in the column is given by

$$\log (V_N/W) = \log V_A + \alpha (S^0 - \varepsilon^0 A_S) \quad (1)$$

where α is the adsorbent activity, S^0 a measure of the Lewis basicity (or acidity) of the solute, ε^0 the (mean) eluent strength and A_S the (effective) surface area of the adsorbate molecule (expressed in units of 8.5 \AA^2 , the surface area of a $-\text{CH}=\text{}$ group in benzene). V_A is related to the specific surface area of the adsorbent. The eluent strength scale was arbitrarily fixed with $\varepsilon^0 = 0$ for *n*-hexane. If S^0 values on the bonded phase are equal (or proportional) to those on bare silica, the adsorbent parameters α and $\log V_A$ are readily obtained from plots of $\log (V_N/W)$ data of monosubstituted benzenes in hexane *versus* their S^0 values on silica. Of course the magnitude of α is related to $\alpha = 0.83$ of a (wide-pore) silica⁹. In order to obtain A_S values from eqn. 1, it is useful to apply ε^0 values which hold on bare silica. This holds true even when complex formation contributes to solute retention¹³. It was shown that, on silica and on bonded phases, A_S values of monosubstituted benzenes are given by

$$A_S = A_S (\text{calc.}) + \gamma_i \Delta a_i (\text{SiO}_2) \quad (2)$$

where $A_S (\text{calc.}) = A_S$ when no solute and eluent localization (due to adsorbent heterogeneity) occurs. $\Delta a_i (\text{SiO}_2)$ is the difference between A_S and $A_S (\text{calc.})$ on a deactivated silica. As these cases are rather rare, a correction term is usually required. The localization parameter, γ_i can readily be evaluated with $A_S (\text{calc.})$ and $\Delta a_i (\text{SiO}_2)$ values given by Snyder⁹. On bare silica and on the previously examined bonded phases, γ_i did not significantly depend on temperature, nor on the substituent *i*.

The S^0 values of unsubstituted polycyclic aromatic hydrocarbons are readily obtained by applying the estimated α and $\log V_A$ in eqn. 1. They can be described by

$$S^0 = n Q_{-\text{C}=\text{}}^0 - \zeta (n - 6) \quad (3)$$

where n is the number of aromatic carbon atoms of the arene and $Q_{-\text{C}=\text{}}^0$ the contribution of a $-\text{CH}=\text{}$ moiety in benzene (a non-delocalized solute) to its S^0 value. The second term accounts for delocalization effects. On a wide-pore bare silica, $\zeta = 0.14$ (ref. 14), but owing to complex formation of the arenes with bonded phases ζ can decrease and even become negative¹³.

Finally, the A_s values of the arenes can be compared to those on bare silica given by

$$A_s = 6 + 0.8(h - 6) + 0.25(c - h) \quad (4)$$

where c and h are the numbers of carbon atoms and protons in the arenes, respectively.

If both the solute and eluent molecules can form a complex with bonded phase sites, the effective eluent strength will be larger than that on silica. Hence, the term $\varepsilon^0 A_s$ in eqn. 1 (and thus the obtained A_s values) will be anomalously large¹³. If $\alpha\zeta$ is small or even negative (which points to complex formation by the solute), but the A_s values are similar to those on silica, the eluent molecules apparently have a similar affinity to bonded phase sites and silanol groups. These rules of thumb are consistent with the adsorption model and will be applied in the following.

EXPERIMENTAL

Chemicals and sorbent characterization

All solutes and reagents (Fluka, Buchs, Switzerland) were of the highest available purity and were used as received. Dichloromethane and *n*-hexane were supplied by Baker (Deventer, The Netherlands) and were dried with molecular sieve 5A.

Nucleosil 10 NH₂, particle diameter 10 μm (Macherey, Nagel & Co., Düren, G.F.R.), from the same batch as used in a previous investigation¹³ was the starting material for the first two modifications described below. From elemental analysis (4.25% C and 1.24% N) and its BET surface area ($388 \pm 2 \text{ m}^2/\text{g}$) it was concluded that the monomers are aminobutyl groups, that the amounts of C, N and aminobutylsilyl (ABS) per gram of bare silica are 0.0473, 0.0138 and 0.1133 g, respectively, that the surface concentration of ABS groups is $2.54 \pm 0.02 \mu\text{mole}/\text{m}^2$. It was assumed that the silane had reacted bifunctionally and that the remaining reactive group was hydrolysed.

Preparation of 2,4-dinitroanilino (DNA)-silica

A suspension of 3 g Nucleosil 10 NH₂ in 25 ml of 0.7 M NaHCO₃ was degassed and homogenized by sonication. To this mixture a solution of 1.6 g 2,4-dinitrofluorobenzene in 20 ml ethanol was added dropwise while the contents of the flask were gently swirled. The mixture was allowed to react for 20 h at room temperature. Thereafter, the yellow product was neutralized, rinsed with distilled water, methanol and acetone and dried at 60°C and 1 mmHg for 2 h. Elemental analysis showed the presence of 9.14% C. Combination of this result with those for the starting ABS-silica yields an amount of 0.1586 g of bound 2,4-dinitrophenyl (DNP) per gram of bare silica, which corresponds to a surface coverage of 2.45 μmole dinitrophenyl per m² and 0.09 μmole ABS per m². A similar large yield of DNP groups was reported by Nondek and Málek⁶.

Preparation of bis(3-nitrophenyl) sulphone (DNPS)-silica

A 3-g amount of Nucleosil 10 NH₂ was suspended in 130 ml of 0.025 M

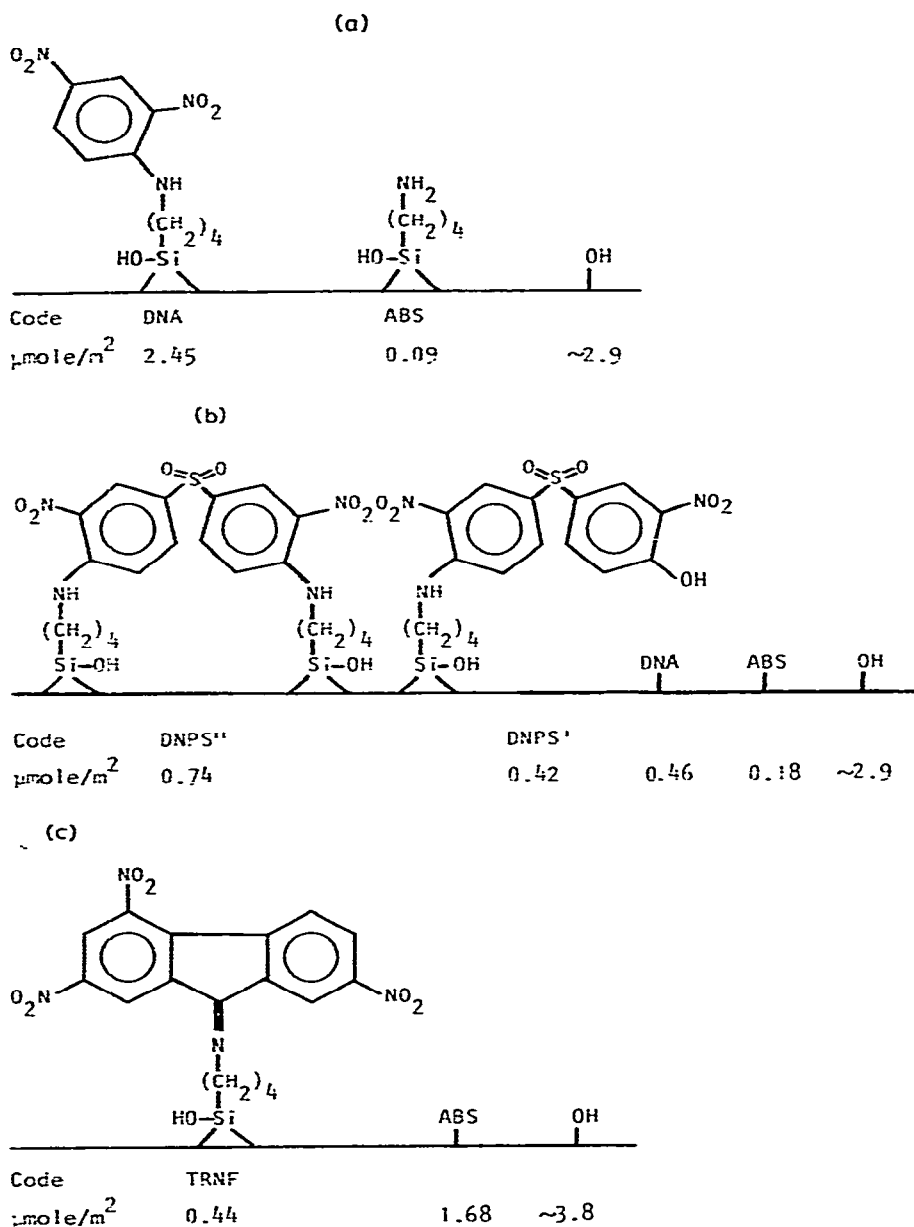


Fig. 1. Structure, code and surface concentration of bound groups on DNA-silica (a), DNPS-silica (b) and TRNF-silica (c).

NaHCO_3 in water-dimethylformamide (DMF) (18:42) in a three-necked flask. After degassing and homogenizing, a solution of 0.45 g bis(4-fluoro-3-nitrophenyl) sulphone in 10 ml ethanol was added dropwise while the contents of the flask were swirled. The number of moles of reagent is half the number of moles of ABS groups in order to promote a bifunctional reaction. The reaction mixture was kept at 60°C under

gentle reflux. During the reaction the pH was monitored and maintained below a pH meter reading of $\text{pH} = 10$ (at which silica is stable in this medium) by bubbling CO_2 through the suspension. The reaction was stopped after 120 h when its rate had become very low (as followed by spectrophotometric analyses of the supernatant at 420 nm). The yellow product was neutralized, rinsed and dried as described above. Elemental analysis gave 9.03% C and 2.08% N. From these results and those for ABS-silica, it can be estimated that the weight of bound sulphone per gram of bare silica and the surface coverage of sulphone are 0.135 ± 0.003 g and 1.14 ± 0.01 $\mu\text{mole}/\text{m}^2$ (bifunctional reaction), or 0.145 ± 0.003 g and 1.16 ± 0.01 $\mu\text{mole}/\text{m}^2$ respectively (monofunctional reaction). This indicates a 83% conversion of the sulphone. In order to estimate the extent of bifunctional reaction, 200 mg of the product were suspended in 10 ml of 0.01 N HCl and degassed. After 12 h the hydrochloric acid was washed out and titrated with a CO_2 -free borax solution using methyl red-bromocresol green as indicator. The surface coverage of free ABS groups appeared to be 0.64 $\mu\text{mole}/\text{m}^2$. It can now easily be estimated that 64% of the bound sulphone reacted bifunctionally.

Finally, the product was treated with 2,4-dinitrofluorobenzene (as described above) in order to reduce the number of ABS groups. Elemental analysis showed 9.86% C. From this result it can be estimated that the final product contains 0.46 μmole 2,4-dinitrophenyl per m^2 and that 0.18 μmole ABS per m^2 are left on the silica surface.

Preparation of 2,4,7-trinitrofluorenimine (TRNF)-silica

A 3-g amount of Nucleosil 10 NH_2 (surface coverage 2.12 μmole ABS per m^2) was suspended in a solution of 1.2 g 2,4,7-trinitrofluorenone in 60 ml dry tetrahydrofuran (THF). The mixture was degassed and homogenized and was allowed to react for 4 days in the dark at room temperature. The brown product was thoroughly washed with THF and dichloromethane and dried at 50°C and 1 mmHg. Elemental analysis showed 5.81% C and 1.75% N. It was concluded that the surface concentration of TRNF groups is about 0.44 $\mu\text{mole}/\text{m}^2$. A similar low yield was obtained for TENF-silica, prepared with 2,4,5,7-tetranitrofluorenone. These surface concentrations are roughly in accord with those reported by Lochmüller *et al.*⁴

The stability of the DNA- and the DNPS-phase was good. The fluorenimine phases, however, showed a slow decomposition in dichloromethane, as judged from an increased background of the UV-detector after storage of the columns for several months. In particular, the TENF-phase was troublesome and only plate height measurements will be reported for this bonded phase. TRNF-silica was examined more closely, and apart from its surface concentration, the results seem reliable enough to characterize its sorbent properties.

Apparatus and procedure

The columns (precision-bore stainless steel, 25 cm \times 2.1 mm I.D.) were packed by means of a slurry technique. The slurries (10 wt. % sorbent in dry tetrachloromethane) were degassed and homogenized by sonication and forced into the columns with 200 ml *n*-hexane at 350 atm. Finally, 300 ml dry dichloromethane were flushed through the columns. The weights of the sorbents in the columns were 0.46 g (DNA-), 0.43 g (DNPS-) and 0.40 g (TRNF-silica).

The apparatus and the measuring technique have been described previously¹¹. The sample size was less than 25 μg . The reproducibility of triplicate V_N measurements was about 10 μl or 2% for strongly sorbed solutes. The column temperature was regulated by means of a water-jacket around the column and a heat exchanger. By means of some valves, a rapid switch to a second water thermostat could be performed. This arrangement made possible a 15°-change of the column temperature within a few minutes. These temperature jumps were applied in order to measure desorption entropies and enthalpies at a fixed sorbent state, as will be outlined below.

The following eluents were used: *n*-hexane (H, $\varepsilon^0 = 0$) and *n*-hexane-dichloromethane (65:35) (B, $\varepsilon^0 = 0.22$ according to Snyder¹⁵). Measurements were made at 10, 15 and 40°C.

RESULTS AND DISCUSSION

Effects of sorbent state on solute retention and dispersion

In a preliminary investigation on the solute retention, some interesting phenomena were observed.

(1) *Temperature effects.* An abrupt jump of the eluent temperature from 25 to 40°C caused an instantaneous decrease of V_N , as expected. Thereafter, however, V_N appeared to increase steadily to a constant value. A reversed jump from 40 to 25°C had an analogous, but opposite, effect on the retention behaviour. These experiments were performed at a small pressure drop across the columns (50 atm) and low flow-rates (< 0.5 ml/min). The solute sorption-desorption equilibrium is fast (as usual), as a three-fold increase of the flow-rate did not affect the magnitude of V_N . After a temperature jump, constant V_N data were obtained after about 5.5 h (DNA-silica), 3 h (DNPS-silica) and 2 h (TRNF-silica) of pumping. These equilibration times were roughly the same for both 15°-jumps (positive or negative) and did not clearly depend on the original temperature (10, 25 or 40°C). In the binary eluent B the equilibration times were shorter (1.5 h).

Obviously, a temperature change causes a reversible alteration of the sorptive properties of the bound layers. As the mean distance between adjacent ABS groups is about 9.5 Å and the conversion of these groups into DNA and DNPS groups is nearly complete, the mutual distance of the latter groups must also be small. Further, the total area of these groups is about equal to the silica surface area underneath. Therefore, it seems justified to assume that they are arranged in a rather dense layer on top of more or less upright-standing spacer groups. With this picture in mind, the following equilibria in the bound layers can be expected



where S and A denote the solute and the DNA or DNPS groups, respectively. As the TRNF content is rather low, but ABS-TRNF (donor-acceptor) interaction is feasible, the following equilibria will be of prime importance



where D denotes a "free" ABS group. It is noted that the amino groups on ABS-silica are largely adsorbed to silanol groups in the weak eluents used¹². Finally, it has been shown that (free) ABS groups can form complexes with dichloromethane¹³:



As a result of a positive temperature jump all equilibria will shift to the left, but due to the restricted mobility of the anchored groups, the equilibria 7 and 11 will shift much more slowly than the equilibria 5,6,8,9 and 10. Hence, after a sharp drop, V_N will increase slowly to a constant value. This explanation is based on the plausible assumption that AS complexes are far more stronger than AA \cdots S (or AD \cdots S) interactions.

From the foregoing it is evident that the influence of the temperature on solute desorption can only be evaluated unambiguously at a fixed sorbent state. This can be achieved by extrapolation of net retention volumes after abrupt jumps from 25 to 10°C and 25 to 40°C to zero equilibration time, denoted by (A25, E10) and (A25, E40). Of course, the (A25, E25) datum can also be used. Analogously, the data (A10, E25) and (A40, E25) can be combined with (A10, E40) and (A40, E40), respectively. The change of the chemical potential of the solute on desorption is given by

$$\Delta\mu_{d,E}^0 = RT \ln (V_N/W\beta_E) \quad (13)$$

where β_E is the effective volume of the sorbing phase per gram sorbent. From $\ln (V_N/W)$ data at different temperatures (but at the same sorbent state), $\Delta S_d^0 - R \ln \beta$ and $\Delta \bar{H}_d^0$ values can be estimated in 15°-intervals. Results for acetophenone and benzonitrile on DNA- and DNPS-silica in both eluents are given in Table I. In Fig. 2, $\Delta \bar{H}_{d,E}^0$ is plotted versus $\Delta S_{d,E}^0 - R \ln \beta_E$. A pair of parallel lines (slope 360°K) is obtained for the eluents. Hence, apart from a temperature effect on both thermodynamic quantities which gives rise to a displacement of the data points alongside the lines, there is at least one mechanism which occurs only in one of the eluents. As the slopes of the lines which connect corresponding data points on the two lines ($450 \pm 20^\circ\text{K}$ and $520 \pm 30^\circ\text{K}$ at 290.7°K and 305.7°K , respectively) only depend on temperature, and the estimated errors in the $\Delta \bar{H}_d^0$ and ΔS_d^0 values are rather small (see Table I), it seems justified to conclude that the occurrence of the two lines in Fig. 2 be attributed to a single mechanism¹⁶ and a small second-order temperature effect (on this mechanism, probably). The occurrence of two lines cannot merely be due to the trival term $-R \ln (\beta_B/\beta_H)$ because by sorption of dichloromethane in the bond layers, $\beta_B/\beta_H > 1$. Hence, at equal values of $\Delta \bar{H}_{d,B}^0$ and $\Delta \bar{H}_{d,H}^0$, $\Delta S_{d,B}^0 - \Delta S_{d,H}^0 > 2.8 \text{ cal/mole} \cdot ^\circ\text{K}$. However, it is conceivable that the solute molecules can to some extent

TABLE I

$$A\beta_1^0 + RT \ln \beta_1, \Delta S_0^0 - R \ln \beta_1 \text{ AND } \Delta H_0^0 \text{ VALUES OF BENZONITRILE AND ACETOPHENONE ON DNA- AND DNPS-SILICA}$$

Data are given for the sorbate at 10, 25 and 40 C in hexane (E = I) and in the binary eluent (E = B). Errors in the three quantities are about 7 cal/mole, 0.7 cal/mole · K and 0.2 kcal/mole, respectively.

	DNA-silica						DNPS-silica					
	A10		A25		A40		A10		A25		A40	
	β_1	ΔS_0^0	β_1	ΔS_0^0	β_1	ΔS_0^0	β_1	ΔS_0^0	β_1	ΔS_0^0	β_1	ΔS_0^0
$A\beta_1^0 + RT \ln \beta_1$ (cal/mole)												
	Benzonitrile											
	11,10	1814	2009	B	246	337	11,10	1802	2087	B	324	428
	25	1577	1747	1897	259	205	25	1597	1842	2018	246	286
	40		1562	1691	143	115	40	1605	1777		158	215
Acetophenone	11,10	2087	2359	B	505	622	11,10	2139	2540	B	674	804
	25	1801	2033	368	478	546	25	1856	2238	546	628	737
	40		1820	1992	358	416	40	1935	2193		473	559
$\Delta S_0^0 - R \ln \beta_1$ (cal/mole · K)												
	Benzonitrile											
	11,17.5	15.8	17.5	B	6.4	8.8	11,17.5	13.7	20.2	B	5.2	9.5
	32.5	12.3	13.7	6.0	7.7	32.5	15.8	16.1		8.5	9.3	
	11,17.5	19.1	21.7	B	9.1	9.6	11,17.5	18.9	20.1	B	8.5	11.7
	32.5	17.5	17.5	18.2	8.0	8.7	32.5	16.3	23.0		10.3	11.9
ΔH_0^0 (kcal/mole)												
	Benzonitrile											
	11,17.5	6.3	7.0	B	2.1	2.8	11,17.5	5.7	8.0	B	1.8	3.1
	32.5	5.4	6.0	2.6	2.0	32.5	6.6	6.8		2.8	3.1	
	11,17.5	7.5	8.5	B	3.1	3.3	11,17.5	7.5	8.2	B	3.1	4.1
	32.5	7.3	7.3	7.7	2.9	3.1	32.5	7.0	9.4		3.7	4.3

enter the solvated ligand layers in the binary solvent, whereas in hexane, adsorption on top of the bound layers prevails. As a result of the solvation of the ligands (and solute molecules), the complex stability (and hence $\Delta\bar{H}_d^0$) will be diminished. But due to the constraint of the motions of the solute molecules within the dense ligand layers, $\Delta\bar{S}_d^0$ will not decrease proportionally (*i.e.*, according to the slope 360°K), but less. Unfortunately, it is not possible to establish whether the observed solvent effect on $\Delta\bar{H}_d^0$ and $\Delta\bar{S}_d^0$ is typical for dense ligand layers, because corresponding data in dilute solutions are scarce and generally not very reliable⁸.

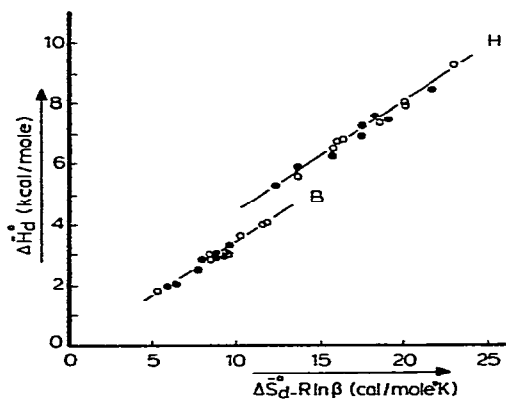


Fig. 2. Plots of $\Delta\bar{H}_d^0$ versus $\Delta\bar{S}_d^0 - R \ln \beta$ for benzonitrile and acetophenone on DNA-silica (O) and DNPS-silica (●) at 17.5 and 32.5°C in *n*-hexane (H) and in the binary eluent (B).

(2) *Effects of eluent change.* After a change from H to B, equilibrium is attained by flushing about 50 ml of B through the columns (*i.e.*, 3 h of pumping at 50 atm) (Fig. 3a). The same time is required to reach apparent equilibrium on DNPS-silica after a change from B to H. On DNA- and TRNF-silica this time is about 16 h. In all instances the column temperature was 40°C. The thermostat kept on overnight, but the pump was stopped. Next day, retention measurements were made at small time intervals, starting immediately after actuation of the pump. During a period of about 20–30 min the retention volumes decreased by about 40%, but then increased steadily to their final values on the day before (Fig. 3c and d). In the following days the retention “dents” grew smaller and smaller and after 42 h of pumping (700 ml) *true* equilibrium is achieved on DNPS-silica; on DNA- and TRNF-silica about 940 ml hexane are required (*i.e.*, 56 h of pumping). On DNA-silica, the pump was stopped, before apparent equilibrium was reached. Next day, the V_N values seemed to increase from the level reached the day before (Fig. 3b).

The following explanation is proposed. Before the change from B to H the bonded phases are in the solvated (“swollen”) state. When apparent equilibrium is attained in hexane, most but not all the dichloromethane is removed from the “shrunken” sorbing phases. The remaining dichloromethane diffuses very slowly into the mobile phase. When the flow is stopped for a sufficiently long period, the dichloromethane concentration in the hexane can increase to such an extent that partial reswelling occurs. When the hexane starts flowing again two phenomena occur with opposite effects on solute retention. On the one hand (part of) the dichloromethane diffuses rapidly out of the “reswollen” sorbing phase into the hexane. This solvates the

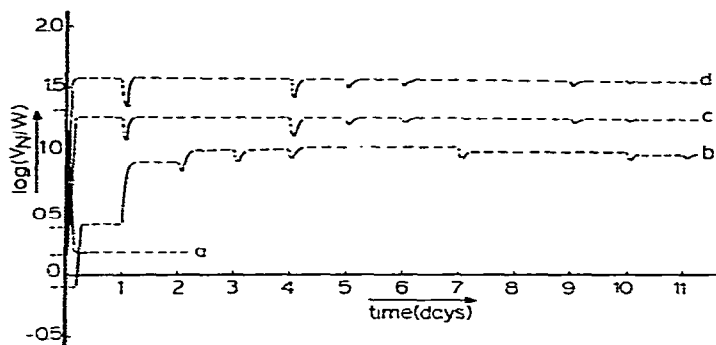


Fig. 3. Plots of $\log(V_N/W)$ data at 40°C versus time after the change from eluent H to B for chrysene on DNPS-silica (a), and after the change from B to H on DNA-silica for methyl benzoate (b), and on DNPS-silica for benzonitrile (c) and acetophenone (d).

sorbing phase as it moves through the column and causes a decrease in retention. On the other hand, an increasing amount of the sorbing phase comes into contact with hexane. This causes an increase in retention, which is slowed down by the slow self-association of the ligand groups. The two effects counterbalance after 20–30 min. The retention volumes become constant when equilibrium 7 or 11 has been re-established and the sorbent is again in its stable "shrunken" state.

This explanation is supported by gas chromatographic analyses of the eluate during the first retention "dent" observed on the DNPS column. During the first 4 min of pumping the dichloromethane concentration increased sharply. Thereafter it decreased steadily until it became constant (about 0.4%, v/v) after 2 h (*i.e.*, when the sorbent has reached its "shrunken" state).

The above mentioned equilibration phenomena are also apparent on a silica with a moderate coverage of bound pyridylethylsilyl groups. Obviously they are commonly encountered on this type of aromatic polar bonded phases.

(3) *Peak dispersion.* Strongly retained solutes (capacity ratio, $k' > 12$) showed skewed peaks, in accord with the results of Lochmüller and Amoss³. As the net retention volumes are independent of the flow-rate, peak skewness cannot be attributed to the slow rate of complex dissociation. In the foregoing it is proposed that di- and monomeric complexing sites with different sorptive strengths are present in the bound layer. Thus, apart from the possibility that residual silanol groups contribute to the retention, non-linear isotherms can be expected which explains peak skewing on this type of bonded phases. In the following, only symmetrical peaks are examined.

According to Done and Knox¹⁷, reduced plate heights ($h \equiv H/d_p$) are related to reduced linear flow-rates ($v \equiv v d_p/D_M$) by

$$h \approx h_{\text{extra}} + 1.4v + v^{0.33} + Cv \quad (14)$$

where h_{extra} is the trivial contribution of peak broadening outside the column, d_p is the particle diameter, v the linear flow-rate and D_M the solute diffusion coefficient in the mobile phase. D_M values can be estimated with the Wilke–Chang equation¹⁸.

In Fig. 4, $h - (1.4v + v^{0.33})$ data for nitrobenzene and benzonitrile are plotted versus v . The slope of the plots, although dependent on v on DNPS-silica, represents

the mass transfer parameter C . On DNA-silica (and the fluorenone phases) $C \approx 0.2$, *i.e.*, rather close to the values obtained on wide-pore silicas where $C \lesssim 0.06$ (ref. 19) (wall effects²⁰ probably contribute to C in our narrow-bore columns). In the binary eluent B similar plate height data were obtained. On DNPS-silica however, $C \approx 2.5$ and in addition C depends strongly on the eluent strength.

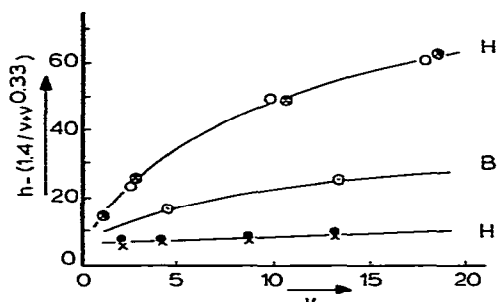


Fig. 4. The influence of slow mass transfer at 25°C in the bound layers of DNA-silica [nitrobenzene, $k' = 3.97$ (●); benzonitrile, $k' = 9.96$ (×)] and of DNPS-silica [nitrobenzene, $k' = 5.08$ (○) and benzonitrile, $k' = 10.46$ (⊗)] in *n*-hexane (H) and of DNPS-silica [nitrobenzene, $k' = 0.43$ (○)] in the binary eluent (B).

In view of the slow diffusion of dichloromethane through the "shrunken" bound layers, it is concluded from the rapid mass transfer on DNA-silica (and the fluorenone phases) that the solutes are mainly adsorbed on top of the bound layer of these phases. On DNPS-silica the solutes are probably partly sorbed within the polar part of the bound layer. In the binary eluent "swelling" occurs and molecular diffusion is less severely restricted.

Snyder's adsorption model

In view of the proposed picture of the bonded phases derived from the rather intricate solute retention behaviour, an attempt to apply a monolayer adsorption model may seem a precarious undertaking. However, it will be shown below that this model describes the retention data surprisingly well, although caution should be exercised in attributing a physical meaning to the obtained parameters. In the following only true equilibrium retention data will be considered.

(1) *Adsorbent activity*. Experimental $\log(V_N/W)$ data for some monosubstituted benzenes on DNA-, DNPS- and TRNF-silica in *n*-hexane are given in Table II. Linear regression analysis of these data on S^0 values, given by Snyder⁹ for bare silica, yields the α and $\log V_A$ values presented in Table II (see eqn. 1). The data for anisole, methyl benzoate and acetophenone were excluded because they appear to deviate significantly from a plot of $\log(V_N/W)$ data on the present sorbents *versus* those on ODS-silica. Similar deviations were observed on ABS-silica and can be ascribed to steric effects on complexation¹³. The α values on DNA- and DNPS-silica appear to be about equal to those on ODS-silica¹¹, where $\alpha = 0.51 \pm 0.01$ (25°C) and 0.48 ± 0.01 (43.5°C). Obviously, these bonded phases have approximately equal effective strengths towards monosubstituted benzenes as free silanol groups. The deviations from the regression line are largely systematic and indicate that the S^0 values on bare silica deviate slightly from the effective values on the bonded phases. An interpretation of the $\log V_A$ data in terms of a monolayer adsorption model is not feasible.

TABLE II

log (V_N^*/V) DATA FOR MONOSUBSTITUTED BENZENES ON DNA-, DNPS- AND TRNF-SILICA IN HEXANE, AND α log V_A VALUES, THEIR ERRORS AND THE STANDARD ERROR OF THE FIT (s_f)

<i>i</i>	S^0	<i>DNA-silica</i>			<i>DNPS-silica</i>			<i>TRNF-silica</i>	
		10°C	25°C	40°C	10°C	25°C	40°C	25°C	40°C
H	1.50	-0.49	-0.52	-0.55	-0.43	-0.47	-0.55	-0.35	-0.40
F	1.35	-0.40	-0.43	-0.46	-0.29	-0.42	-0.51	-0.31	-0.37
Cl	1.30	-0.34	-0.36	-0.38	-0.26	-0.39	-0.47	-0.25	-0.33
Br	1.33	-0.28	-0.30	-0.34	-0.22	-0.30	-0.38	-0.17	-0.25
CH ₃	1.61	-0.49	-0.52	-0.55	-0.37	-0.48	-0.58	-0.33	-0.42
SCH ₃	2.79	0.19	0.14	0.06	0.20	0.17	0.08	0.27	0.20
OCH ₃ *	3.33	0.25	0.19	0.12	0.28	0.26	0.22	0.35	0.31
NO ₂	4.27	0.99	0.88	0.79	0.97	0.92	0.83	0.86	0.78
CO ₂ CH ₃ *	4.95	1.15	1.05	0.96	1.17	1.18	1.09	1.00	0.92
CN	4.83	1.40	1.28	1.18	1.39	1.35	1.24	1.15	1.06
COCH ₃ *	6.19	1.61	1.49	1.39	1.65	1.64	1.53	1.46	1.36
α		0.50	0.48	0.45	0.47	0.49	0.49	0.41	0.40
s		0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.02
log V_A		-1.12	-1.11	-1.11	-0.99	-1.11	-1.19	-0.86	-0.92
s		0.14	0.10	0.10	0.10	0.09	0.09	0.07	0.06
s_f		0.14	0.14	0.14	0.13	0.12	0.12	0.10	0.09

* Excluded from regression analyses (see text).

TABLE III

log (V_N^*/V) DATA FOR SOME ARENES ON DNA-, DNPS- AND TRNF-SILICA IN HEXANE, AND ζ VALUES FOR FUSED ARENES AND POLYPHENYLS, THEIR STANDARD ERRORS AND THE STANDARD ERROR OF THE FIT (s_f)

<i>Solute</i>	<i>n</i>	<i>DNA-silica</i>			<i>DNPS-silica</i>			<i>TRNF-silica</i>	
		10°C	25°C	40°C	10°C	25°C	40°C	25°C	40°C
Benzene	6	-0.49	-0.52	-0.55	-0.43	-0.47	-0.55	-0.35	-0.40
Naphthalene	10	0.22	0.15	0.06	0.20	0.13	0.04	0.23	0.15
Acenaphthene*	10	0.38	0.29	0.21	0.35	0.28	0.18	0.34	0.29
Fluorene	12	0.55	0.45	0.36	0.52	0.46	0.35	0.56	0.49
Anthracene	14	0.90	0.78	0.66	0.87	0.79	0.66	0.85	0.76
Phenanthrene	14	0.91	0.79	0.68	0.88	0.80	0.67	0.86	0.76
Fluoranthene	16	1.28	1.13	1.01	1.24	1.14	1.00	1.21	1.09
Pyrene	16	1.28	1.15	1.01	1.25	1.14	1.00	1.20	1.12
Chrysene	18	1.62	1.46	1.31	1.59	1.47	1.32	1.56	1.43
Benz[a]pyrene	20	2.00	1.85	1.66	1.97	1.85	1.66	1.98	1.88
Perylene	20	2.11	1.92	1.76	2.04	1.92	1.74	2.02	1.92
Bibenzyl*	12	0.06	-0.01	-0.09	0.07	0.00	-0.08	0.23	0.16
Biphenyl	12	0.22	0.13	0.08	0.18	0.14	0.03	0.27	0.21
<i>p</i> -Terphenyl	18	0.80	0.67	0.61	0.75	0.70	0.57	0.83	0.81
<i>p,p'</i> -Quaterphenyl	24	1.38	1.22	1.18	1.32	1.22	1.07	1.35	1.23
<i>Fused arenes</i> ζ		-0.158	-0.151	-0.154	-0.166	-0.143	-0.128	-0.206	-0.209
s		0.007	0.007	0.008	0.009	0.007	0.006	0.012	0.013
<i>Polyphenyls</i> ζ		-0.002	0.006	-0.007	0.001	0.010	0.018	-0.025	-0.027
s		0.009	0.006	0.005	0.004	0.009	0.008	0.007	0.014
s_f		0.09	0.08	0.09	0.10	0.08	0.07	0.12	0.18

* Outlier, excluded from regression analyses (see text).

Both α and $\log V_A$ values on TRNF-silica are close to those on ABS-silica¹³ where $\alpha = 0.39 \pm 0.01$ and $\log V_A = -0.79 \pm 0.04$ at 25°C. This result is expected since the surface concentration of the ABS groups is rather large on this mixed-phase sorbent. The low activity of ABS-silica in weak eluents is due to the adsorption of the amino groups to free silanol groups left on the silica surface¹². It is interesting that α increases when the adsorption of the bulky DNP and DNPS groups is hampered.

(2) *Solute-sorbent interactions.* Experimental $\log (V_N/W)$ data on polycyclic aromatic hydrocarbons in hexane are given in Table III. Apart from acenaphthene and bibenzyl*, the S^0 values (obtained from eqn. 1) can be described accurately by means of eqn. 3 with different ζ values for the fused arenes and the polyphenyls. These ζ values are given in Table III. According to eqn. 3, the selectivity of the bonded phase j towards arenes can be evaluated from

$$\log (V_{N,n+1}/V_{N,n})_j = \alpha_j (Q_{-C=}^0 - \zeta_j) \quad (15)$$

where $Q_{-C=}^0 = 0.204 \pm 0.004$. At 25°C the following data (for fused arenes and polyphenyls, respectively) are obtained: DNPS- \approx DNA- (0.170; 0.095) \geq TRNF- (0.168; 0.092) $>$ ABS- (0.112; 0.089) $>$ bare silica (0.091; -). We conclude:

(i) Despite their different structures, TRNF-, DNA- and DNPS-silica form complexes with effectively equal stability

(ii) Obviously, arenes can form complexes with electron acceptor and electron donor ligands, but with the nitroaromatic phases the interactions are stronger than with ABS-silica

(iii) On all complexing phases, a polyphenyl shows weaker interaction than is expected for a fused arene with the same number of aromatic carbon atoms. This is due to the small extent of π -conjugation in the slightly twisted polyphenyl molecules, which limits the stability of the charge-transfer complex¹³

(iv) The sharp increase of the selectivity caused by only a small amount of TRNF groups points to a strong complexing ability of these electron acceptor sites. Recently, Lochmüller *et al.*⁴ showed that the retention of arenes substantially increases with the degree of nitration of the fluorenone nucleus.

(3) *Primary eluent effects.* Experimental $\log (V_N/W)$ data for some monosubstituted benzenes and arenes in the binary eluent B are given in Tables IV and V, respectively. Combination of these data with those in hexane gives A_S values (eqn. 1), from which γ_i values for the benzenes can be calculated by means of eqn. 2. An analysis of the variance²¹ of the γ_i values revealed that they depend on the substituent i and on the bonded phase, but the influence of temperature appeared to be insignificant. The A_S values of the arenes are only slightly temperature-dependent. Therefore it suffices to consider the mean γ_i and A_S values presented in Tables IV and V, respectively.

The A_S values of the monosubstituted benzenes and arenes on DNA- and DNPS-silica are about 15% smaller than those on ODS-silica¹¹. The similarity of the relative deviations for both solute classes points to a change of the sorbent properties in the binary eluent: the small A_S (and γ_i) values on DNA- and DNPS-silica can be attributed to a small but significant increase of the sorbent activity α_B

* The rather small S^0 value of bibenzyl may be related to the poor stabilization of the π,π -complex. Acenaphthene shows rather large S^0 values, presumably due to inductive effects by its methylene groups.

TABLE IV
 $\log (V_N/W)$ DATA FOR SOME MONOSUBSTITUTED BENZENES ON DNA-, DNPS- AND TRNF-SILICA IN THE BINARY ELUENT B, Σa_i (calc.), Aa_i (SiO_2) AND MEAN \bar{v}_i VALUES

<i>i</i>	$\log (V_N/W)$				Σa_i (calc.) ^a				Aa_i (SiO_2) ^b		$\bar{v}_i \pm s$		DNPS-	TRNF-
	DNA-silica	DNPS-silica	TRNF-silica	TRNF-silica	40 C	25 C	40 C	25 C	40 C	25 C	40 C	25 C		
NO_2	-0.02	-0.07	-0.15	0.01	-0.04	-0.11	-0.20	-0.27	7.3	6.2	0.31 ± 0.03	0.27 ± 0.04	0.68 ± 0.02	
CO_2CH_3	0.01	-0.03	-0.09	0.14	0.10	0.02	-0.08	-0.14	8.3	5.8	0.36 ± 0.03	0.29 ± 0.01	0.64 ± 0.02	
CN	0.19	0.15	0.10	0.25	0.21	0.15	-0.01	-0.07	6.6	7.8	0.55 ± 0.01	0.51 ± 0.04	0.81 ± 0.01	
COCH_3	0.39	0.35	0.29	0.52	0.46	0.39	0.27	0.20	7.5	7.7	0.45 ± 0.02	0.43 ± 0.02	0.73 ± 0.01	

TABLE V
 $\log (V_N/W)$ DATA FOR SOME ARENES ON DNA-, DNPS- AND TRNF-SILICA IN THE BINARY ELUENT B, AND CALCULATED AND (MEAN) EXPERIMENTAL A_3 VALUES

Solute	$\log (V_N/W)$				A_3 (calc.)				DNA- $\bar{v}_3 \pm s$	DNPS-	TRNF-	
	DNA-silica	DNPS-silica	TRNF-silica	TRNF-silica	40 C	25 C	40 C	25 C				
Anthracene	-0.12	-0.19	-0.26	-0.11	-0.16	-0.16	-0.28	-0.14	10.2	9.3 ± 0.1	9.0 ± 0.3	11.2 ± 0.2
Phenanthrene	-0.08	-0.16	-0.23	-0.05	-0.16	-0.26	-0.26	-0.12	10.2	9.1 ± 0.1	8.8 ± 0.2	11.0 ± 0.1
Fluoranthene	0.21	0.13	0.04	0.21	0.11	0.02	0.14	0.08	10.7	9.7 ± 0.1	9.5 ± 0.4	10.7 ± 0.2
Pyrene	0.31	0.21	0.10	0.29	0.19	0.07	0.20	0.20	10.7	9.0 ± 0.2	8.9 ± 0.3	11.3 ± 0.1
Chrysene	0.40	0.30	0.21	0.39	0.28	0.17	0.42	0.33	12.3	11.1 ± 0.1	11.1 ± 0.4	12.6 ± 0.1
Benz[a]pyrene	0.81	0.68	0.55	0.77	0.63	0.51	0.86	0.74	12.8	11.0 ± 0.2	11.2 ± 0.4	12.7 ± 0.3
Perylene	0.92	0.78	0.66	0.88	0.73	0.59	0.93	0.80	12.8	10.9 ± 0.1	11.0 ± 0.2	12.4 ± 0.3
<i>p</i> -Terphenyl				-0.39	-0.46	-0.58	-0.47	-0.47	13.4		10.8 ± 0.2	14.1 ± 0.2
<i>p,p'</i> -Quaterphenyl	-0.22	-0.27	-0.34	-0.27	-0.30	-0.42	-0.14	-0.21	17.1	14.7 ± 0.5	14.4 ± 0.7	16.4 ± 0.1

(0.04 ± 0.01) and the sorbent volume ($12 \pm 7\%$) in the binary eluent due to "swelling" of the sorbing phase. The γ_i and A_s values on TRNF-silica are larger than those on the DNA-phase. Obviously, residual ABS groups in this phase are involved which can cause a substantial increase of A_s values¹³.

CONCLUSIONS

In bonded phases with a sufficiently large coverage of (polar) ligands on flexible spacer chains, self-association can occur to some extent in the presence of apolar or weakly polar solvents. After a perturbation of the association equilibrium by a sudden change of the temperature, the new sorbent state is established only after a few hours. The slow rate of this type of relaxation process facilitates the determination of solute desorption enthalpies and entropies at a fixed sorbent state.

An analysis of the desorption enthalpies and entropies for benzonitrile and acetophenone on DNA- and DNPS-silica shows that the sorption mechanisms in a hexane and in a dichloromethane-hexane eluent are different. In the former, adsorption on top of the bound layer prevails, whereas in the latter the solute molecules can to some extent enter the solvated ligand layers.

An eluent change from 35% dichloromethane in hexane to pure hexane requires a 40-60 h flush of hexane before reproducible retention data are obtained. The slow transfer of the dichloromethane to the mobile phase is due to the difficulty in permeating the associated layer of ligand groups, which is formed in the hexane eluent. A reversed eluent change requires only a few hours flush of the binary eluent through the columns, because of the solvation and "swelling" of the bound layers in this eluent.

On DNPS-silica an enormous contribution to the plate height occurs as a result of partial penetration of the solutes into the bound layer and of restricted solute diffusion in this layer.

Equilibrium net retention data on these bonded phases can be described very well with the basic equations of Snyder's adsorption model. The sorbent activity of DNA- and of DNPS-silica is close to that of free silanol groups. TRNF-silica has an activity close to ABS-silica due to the large amount of ABS groups in this mixed bonded phase. The adsorption model is a useful tool for comparing the complexing abilities of bonded phases. The complex stability appears to depend on the extent of π -conjugation in the solute molecules.

Due to diminished self-association of the ligands and "swelling" of the bound layer in the binary eluent, the eluent effect is slightly smaller than predicted by Snyder's model.

From a practical point of view, the fluorenimine phases are not recommended because of their poor chemical stability. Under isothermal and isocratic conditions, DNA-silica is quite useful.

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