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# CHARGE-TRANSFER LIQUID CHROMATOGRAPHY OF AROMATIC HYDROCARBONS AND POLYARYL ALKANES

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## SUMMARY

The chromatographic properties of LiChrosorb Si 60 silica gel, with chemically bonded 3-(2,4-dinitranilino)propyl (DNAP) groups (0.89 mequiv./g), were examined in the high-performance liquid chromatography of a series of polynuclear aromatic hydrocarbons (PAHs), alkyl aromatic hydrocarbons and polyaryl alkanes. The capacity factors, $k'_{CTC}$, of solutes decreased rapidly with increasing chloroform content in the *n*-heptane–chloroform mobile phase. A linear correlation was found between the terms $1/(\log k'_{CTC} + 1.28)$ and the vertical ionization potentials, $I_D^v$, of medium-sized PAHs, providing strong evidence for charge-transfer interactions of the solutes with the DNAP groups. Data are presented reflecting significant differences in the behaviour of PAHs in charge-transfer, adsorption elution and reversed-phase chromatography.

## INTRODUCTION

The formation of charge-transfer (CT) complexes of a proper electron donor with aromatic hydrocarbons has been utilized in gas and liquid chromatography since the early 1950s[1,2]. The first chemically bonded stationary phase for high-performance liquid chromatography (HPLC), which was based on anchored 3-(2,4,5,7-tetranitrofluorenimino)propyldiethoxysiloxane groups, was reported by Lochmüller and Amoss[3]. Porath and co-workers[4–6] prepared and examined Sepharose and Sephadex gels bearing bonded dinitrophenyl or *p*-benzoquinone groups. The use of these gels has been recommended for the water-mediated chromatography of polar aromatic compounds[4–6]. The strong retention of the solutes on modified Sephadex or Sepharose gels has been attributed to the formation of immobilised CT complexes and this type of liquid chromatography was termed charge-transfer chromatography (CTC)[4]; however, several additional effects may be responsible for the observed increase in retention. Recently, the separation and determination of a number of polycyclic aromatic hydrocarbons in environmental dust samples was described using HPLC with LiChrosorb RP-15, LiChrosorb $NH_2$ and Nucleosil $5NO_2$ as the column supports and acetonitrile–water or isooctane–dichloroethane as the mobile phase[7].

In a previous paper[8], we reported the preparation and chromatographic properties of silica gel modified by chemically bonded 3-(2,4-dinitranilino)propyl (DNAP) groups, which were formed by reaction of initially bonded amino groups with 2,4-dinitrofluorobenzene[9]. In comparison with unmodified silica, the silica with bonded DNAP groups exhibited a higher affinity to condensed aromatic hydrocarbons. Taking into account the heats of adsorption ($\Delta H_{ads}$) of the solutes on the DNAP-modified gel, we concluded that the observed increase in retention of the aromatic hydrocarbons was due mainly to the formation of weak CT complexes[8]. This proposal was in accordance with the weak electron-acceptor characteristic of a DNAP group in which the alkylamino function attached to the $\pi$-electron system decreases the electron-withdrawing effect of both nitro groups.

The purpose of this work was to gain further insight into the separation mechanism of aromatic hydrocarbons on a DNAP stationary phase. The examination of a series of polynuclear aromatic hydrocarbons (PAHs) and alkylaromatic hydrocarbons as well as polyaryl alkanes has made it possible to study the relationship between the structure and the chromatographic behaviour of the solutes. Benzo[*a*]-pyrene and 20-methylcholanthrane, both strongly carcinogenic compounds, were also included. The fact that chloroform interacts via hydrogen bonds with aromatic $\pi$-donors is well known[10]. For this reason, a mixture of *n*-heptane and chloroform was used as the mobile phase. Variation of the composition of the mobile phase over a wide range of concentrations allowed us to test the versatility of the DNAP stationary phase.

## EXPERIMENTAL

LiChrosorb Si 60 silica gel (E. Merck, Darmstadt, G.F.R.) had a particle diameter of 8.3–11.5 $\mu$m. 2,4-Dinitrofluorobenzene (Fluka, Buchs, Switzerland) and 3-aminopropyltriethoxysilane (Pierce, Rockford, Ill., U.S.A.), both reagent-grade chemicals, were used without further purification. PAHs (Institute for Organic Syntheses, Rybitví, Czechoslovakia; Fluka; Aldrich-Europe, Beerse, Belgium; Koch-Light Labs., Colnbrook, Great Britain) were used as obtained. Pyrene of technical purity was repeatedly crystallized from benzene. The preparation of diphenylalkanes, triphenylalkanes and tetraphenylalkanes and of 9,9′-bifluorene, 9,9-dimethylfluorene[11,12] and 9,10-dihydroanthracene[13] has been described elsewhere. *n*-Heptane (Loba-Chemie, Vienna, Austria) and chloroform (Lachema, Brno, Czechoslovakia), both reagent-grade chemicals, were used as purchased; 2-ethylhexanol (Chemical Works, Záluží, Czechoslovakia) was purified by rectification prior to use.

LiChrosorb Si 60 gel with bonded DNAP groups was prepared according to a procedure described previously[8,9]; the assay of the DNAP groups was 0.89 mequiv./g. Three stainless-steel columns (30 cm × 6.0 mm O.D. × 4.2 mm I.D.) were packed using a slurry technique; modified LiChrosorb Si 60 gel (2 g) was suspended in 40 ml of 2-ethylhexanol and fed into the columns under a pressure of 350 atm. 2-Ethylhexanol was washed out with acetone and the columns were flushed with pure *n*-heptane (0.5 ml/min) for 24 h. All of the columns were tested by benzene sampling and the one with the greatest efficiency (2400 plates at a flow-rate of 0.5 ml/min) was used for the measurements.

The apparatus used for HPLC was the same as described previously[8], except

that a differential refractometer R401 (Waters Assoc.) was additionally attached and the column was carefully thermostated. The solute samples were injected on to the column by means of a six-port sampling valve equipped with a 20-$\mu$l loop or a septumless injector (Varian 8500) using the stop-flow technique. The capacity factors, $k'_{CTC}$, were related to the retention volume of pure *n*-pentane considered to be a non-retained solute.

## RESULTS AND DISCUSSION

The capacity factors determined for a number of PAHs are given in Table I. If the CT interactions are operative in a chromatographic system, the values of $k'_{CTC}$ would be proportional to those of the equilibrium association constants, $K_a$, of CT complexes. In such a case, the following simplified equation derived from the perturbation theory of quantum chemistry[14] should hold:

$$\log k'_{CTC} = A/(I_D^v + EA_A) + B \quad (1)$$

Eqn. 1, where $I_D^v$ is the vertical ionization potential of the donor, $EA_A$ is the electron affinity of the acceptor and $A$ and $B$ are empirical constants, is valid for a series of

TABLE I

CAPACITY FACTORS, $k'_{CTC}$, FOR POLYNUCLEAR AROMATIC HYDROCARBONS

Stationary phase, DNAP-modified LiChrosorb Si 60 gel; mobile phase, *n*-heptane–chloroform; flow-rate, 1 ml/min; sample volume, 20 $\mu$l of *ca.* 0.1% solution of the solute; temperature, 22°.

| *Hydrocarbon* | *Chloroform content in mobile phase (%, v/v)* | | | |
|---|---|---|---|---|
| | *5* | *20* | *50* | *100* |
| Benzene* | 0.22 | 0.10 | <0.10 | <0.10 |
| Naphthalene | 0.80 | 0.25 | <0.10 | <0.10 |
| Biphenyl | 0.81 | 0.24 | <0.10 | <0.10 |
| *p*-Terphenyl | 1.89 | 0.43 | <0.10 | <0.10 |
| Fluorene | 1.41 | 0.47 | <0.10 | <0.10 |
| 9,9'-Bifluorene | 7.25 | 1.00 | 0.23 | <0.10 |
| Acenaphthene | 1.04 | 0.39 | <0.10 | <0.10 |
| Acenaphthylene | 1.81 | 0.61 | 0.12 | <0.10 |
| Phenanthrene | 1.41 | 0.44 | <0.10 | <0.10 |
| Anthracene | 2.99 | 0.89 | 0.20 | <0.10 |
| 9,10-Dihydroanthracene | 0.99 | 0.32 | <0.10 | <0.10 |
| Benzo[*b*]fluorene | 4.81 | 0.38 | <0.10 | <0.10 |
| Fluoranthene | 5.78 | 1.87 | 0.64 | 0.15 |
| Pyrene | 6.45 | 2.08 | 0.70 | 0.20 |
| Tetracene | 6.23 | 2.11 | 0.66 | 0.18 |
| Picene | >10.0 | 6.32 | 1.56 | 0.38 |
| Chrysene | 11.17 | 2.78 | 0.77 | 0.31 |
| Truxene | >10.0 | 6.10 | 1.15 | 0.29 |
| 20-Methylcholanthrene | >10.0 | 5.03 | 1.28 | 0.35 |
| Benzo[*a*]pyrene | >10.0 | 6.46 | 1.87 | 0.43 |
| Perylene | >10.0 | 7.80 | 3.25 | 0.75 |
| *o*-Phenylenepyrene | >10.0 | 10.35 | 4.22 | 0.86 |
| Coronene | >10.0 | >10.0 | >10.0 | 2.50 |
| Decacyclene | >10.0 | >10.0 | >10.0 | 4.03 |

* Data given for comparison.

complexes of one acceptor with several donors, provided that the structure of the complexes and solvation, steric and other effects are nearly constant. In accordance with eqn. 1, the plot of $\log k'_{CTC}$ *versus* $I_D^v$ is non-linear (Fig. 1). However, when $1/(\log k'_{CTC} + 1.28)$, resulting from the linearized regression of eqn. 1, was plotted against $I_D^v$ (refs. 15–19) of the medium-sized PAHs, a linear correlation was obtained, which could be expressed by the equation

$$1/(\log k'_{CTC} + 1.28) = 1.45\, I_D^v + 7.05 \quad (2)$$

The correlation coefficient, $r$, was 0.9985 and the critical value of $r$ for a 99.9% confidence level was 0.9507. We consider this correlation to be strong evidence for CT interactions governing the chromatographic behaviour of PAHs on DNAP-modified LiChrosorb Si 60. The correlation failed for some PAHs with larger molecules; hydrocarbons, such as picene and chrysene, exhibited retentions greater than those calculated according to eqn. 2. This result may be explained either by non-constancy of $A$ or $B$ in eqn. 1 or by the interaction of a relatively large hydrocarbon molecule with two DNAP groups.

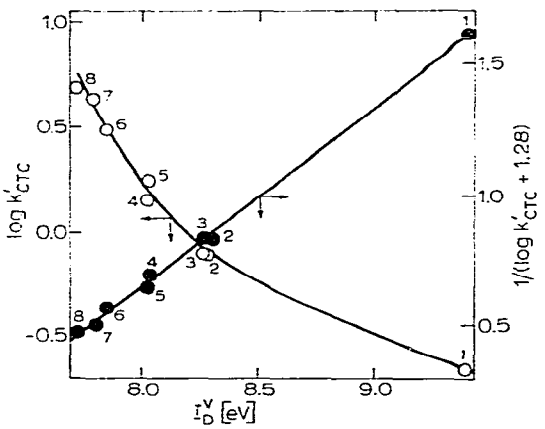



Fig. 1. Correlation between the capacity factors, $k'_{C1C}$ (Table I; *n*-heptane–chloroform, 95:5) and the vertical ionization potentials, $I_D^v$, for PAHs. 1 = Benzene; 2 = naphthalene; 3 = biphenyl; 4 = phenanthrene; 5 = acenaphthylene; 6 = anthracene; 7 = fluoranthene; 8 = pyrene.

As Fig. 2 shows, the retention of PAHs decreases sharply with increasing chloroform content in the *n*-heptane–chloroform mixture; this concentration dependence is probably due to competitive interactions of chloroform with DNAP groups.

An attempt was made to obtain spectral evidence for the CT complexes. The UV spectra of anthracene, pyrene and perylene solutions were measured by means of a known technique[20] in the presence of 2,4-dinitro-N-ethylaniline (DNE) as a model compound. The concentrations of each of the hydrocarbons and DNE in isooctane as solvent varied between $10^{-6}$ and $10^{-4}$ mol/l. Weak changes in the anthracene spectra were observed in the range from $35 \cdot 10^3$ to $38 \cdot 10^3$ $cm^{-1}$.

Another group of solutes examined was alkyl aromatic hydrocarbons. As shown in Table II, the values of $k'_{CTC}$ for methylbenzenes increase with increasing number of methyl groups and values of the association constants, $K_a$, for the hydro-

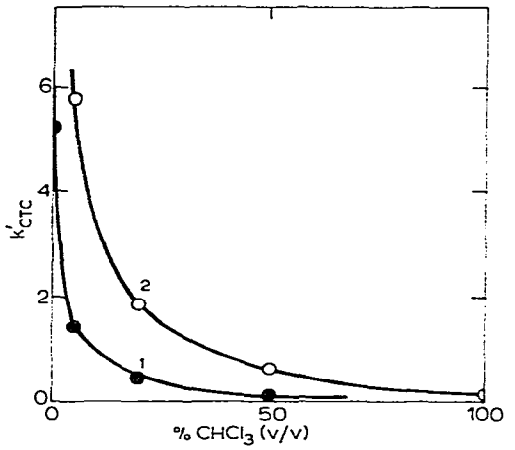



Fig. 2. Dependence of the capacity factors, $k'_{CTC}$, for phenanthrene (1) and fluoranthene (2) on the chloroform content in *n*-heptane–chloroform as the mobile phase.

TABLE II

CAPACITY FACTORS, $k'_{CTC}$, AND EQUILIBRIUM ASSOCIATION CONSTANTS, $K_a$, FOR ALKYL AROMATIC HYDROCARBONS AND POLYARYL ALKANES

Conditions as in Table I.

| *Hydrocarbon* | $k'_{CTC}$ | | $K_a$* |
|---|---|---|---|
| | *0% (v/v) $CHCl_3$ in mobile phase* | *5% (v/v) $CHCl_3$ in mobile phase* | |
| Benzene** | 0.35 | 0.22 | 0.50 |
| *p*-Xylene | 0.32 | 0.20 | 0.92 |
| 1,3,5-Trimethylbenzene | 0.34 | 0.20 | 1.27 |
| 1,2,4,5-Tetramethylbenzene | 0.43 | 0.26 | 2.11 |
| Pentamethylbenzene | 0.58 | 0.32 | 3.09 |
| Hexamethylbenzene | 0.76 | 0.42 | 5.11 |
| Ethylbenzene | 0.29 | 0.17 | 0.71 |
| 1,4-Diisopropylbenzene | 0.15 | <0.10 | — |
| Naphthalene** | 1.32 | 0.80 | — |
| 1-Methylnaphthalene | 1.45 | 0.86 | — |
| 2-Ethylnaphthalene | 1.20 | 0.66 | — |
| 1,2,3,4-Tetrahydronaphthalene | 0.42 | 0.25 | — |
| 1,1-Diphenylpropane | 0.97 | 0.44 | — |
| 2,2-Diphenylpropane | 0.65 | 0.38 | — |
| 2,2-Diphenylbutane | 0.57 | 0.35 | — |
| Triphenylmethane | 1.72 | 0.80 | 0.92 |
| 1,1,1-Triphenylethane | 1.42 | 0.71 | — |
| 1,2,2-Triphenylpropane | 1.27 | 0.67 | — |
| 1,1,2,2-Tetraphenylethane | 8.08 | 2.54 | — |
| 1,1,4,4-Tetraphenylbutane | 6.57 | 1.99 | — |
| 2,2,4,4-Tetraphenylhexane | 3.10 | 0.88 | — |

* Equilibrium association constants for the hydrocarbon–1,3,5-trinitrobenzene CT complexes[21].

** Data given for comparison.

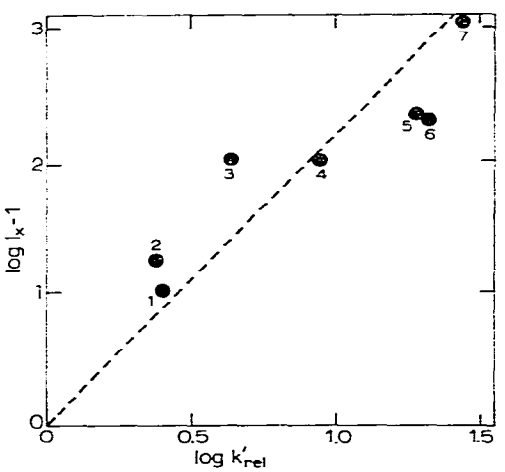
carbon–trinitrobenzene CT complexes[21]. This relationship does not hold, however, for higher alkylbenzenes and naphthalenes.

At this stage it appeared interesting to compare the behaviour of PAHs in CTC with that in adsorption elution chromatography (AEC)[22–25] and reversed-phase chromatography (RPC)[26–28]. The separation of PAHs by AEC has been studied by Popl *et al.*[25] and the adsorption properties of the solutes were expressed by means of the retention indices, $I_x$. In order to obtain comparable data, we modified eqn. 4 in ref. 25 by inserting the capacity factors, $k'_{CTC}$; this modification resulted in a linear relationship between $I_x$ and $k'_{CTC}$ expressed by the equation

$$\log I_x - 1 = C \log k'_{rel} \tag{3}$$

where $k'_{rel}$ are the capacity factors, $k'_{CTC}$, of PAHs relative to benzene and $C$ is a constant. The values of $k'_{rel}$ were calculated from the capacity factors, $k'_{CTC}$, given in Table I (mobile phase: 20%, v/v, chloroform in *n*-heptane). When $\log I_x - 1$ was plotted against $\log k'_{rel}$ (Fig. 3), a satisfactory correlation was obtained only for naphthalene, anthracene and chrysene; the points for other PAHs were scattered along the regression line. This poor correlation (correlation coefficient $r = 0.8889$) clearly reflects the contrasting interaction mechanisms operative in the AEC and CTC of PAHs. Similar differences occurred between the RPC and CTC of these solutes (Fig. 4). Whereas RPC shows a linear increase in $\log k'_{RPC}$ (refs. 26–28) with increasing number of carbon atoms ($N_c$) in the PAH molecule, an analogous plot of $\log k'_{CTC}$ *versus* $N_c$ is widely scattered, indicating the importance of structural effects in the CTC of this group of hydrocarbons.

From the practical point of view, CTC on a DNAP bonded stationary phase



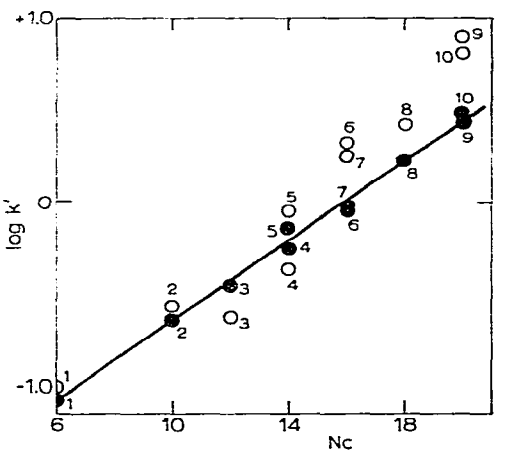




Fig. 3. Relationship between the relative capacity factors, $k'_{rel}$, in charge-transfer chromatography (DNAP-modified LiChrosorb Si 60 gel; *n*-heptane–chloroform, 80:20) and the retention indices, $I_x$, in adsorption elution chromatography (alumina; *n*-pentane) of PAHs. 1 = Naphthalene; 2 = biphenyl; 3 = phenanthrene; 4 = anthracene; 5 = fluoranthene; 6 = pyrene; 7 = chrysene.

Fig. 4. Dependences of the logarithms of capacity factors, $\log k'_{RPC}$ (●), in reversed-phase chromatography and $\log k'_{CTC}$ (○) in charge-transfer chromatography on the number of carbon atoms ($N_c$) in PAHs. 1 = Benzene, 2 = naphthalene; 3 = biphenyl; 4 = phenanthrene; 5 = anthracene; 6 = pyrene; 7 = fluoranthene; 8 = chrysene; 9 = perylene; 10 = benzo[*a*]pyrene.

has several advantages over the widely used RPC and AEC of aromatic hydrocarbons. The enhanced interaction between the stationary phase and the solutes may be advantageous for gradient elution, particularly for the separation of complex mixtures. Moreover, the high-molecular-weight condensed aromatic hydrocarbons are more soluble in chloroform or *n*-heptane–chloroform mixtures than in water–methanol or in pure alkanes. This feature may be useful in preparative HPLC. The versality of the CTC on DNAP-modified LiChrosorb Si 60 gel is illustrated by the separation of three hydrocarbon mixtures (Fig. 5). An even greater selectivity is expected for 3-(2,4,6-trinitranilino)propyl stationary phase[9], which is at present under investigation.

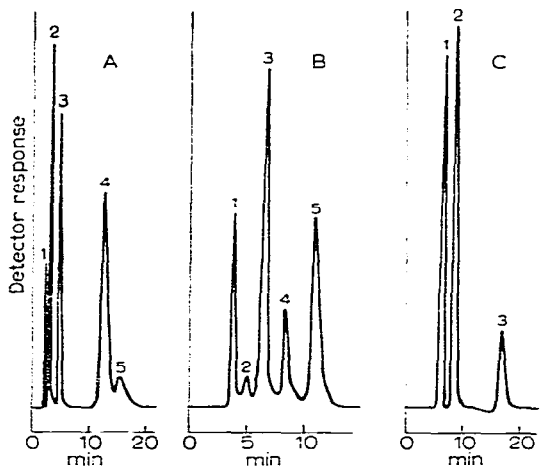



Fig. 5. Separation of aromatic hydrocarbon mixtures on DNAP-modified LiChrosorb Si 60 gel with detection at 254 nm. (A) Peaks: 1 = benzene; 2 = 9,9-dimethylfluorene; 3 = fluorene; 4 = benzo[*b*]fluorene; 5 = 9,9-bifluorene (flow-rate, 1.0 ml/min; sample volume, 5 μl of *ca.* 1% solution; *n*-heptane–chloroform, 95:5). (B) Peaks: 1 = naphthalene; 2 = anthracene; 3 = pyrene; 4 = 20-methylcholanthrene; 5 = benzo[*a*]pyrene (flow-rate, 1.0 ml/min; sample volume, 5 μl of *ca.* 1% solution; *n*-heptane–chloroform, 50:50). (C) Peaks: 1 = chrysene; 2 = perylene; 3 = coronene (flow-rate, 0.6 ml/min; sample volume, 5 μl of *ca.* 1% solution; pure chloroform).

## CONCLUSIONS

The study of the chromatographic behaviour of PAHs, alkyl aromatic hydrocarbons and polyaryl alkanes has enabled us to examine the nature of the interactions between the DNAP-bonded stationary phase and the solutes. The linear correlation between the vertical ionization potentials and a modified term for the capacity factors of medium-sized PAHs strongly supports the involvement of CT interactions of the DNAP groups with the aromatic hydrocarbons. Structural effects play an important role in the CTC of these solutes. Considering the chromatographic behaviour of PAHs, the characteristic of the separation process in CTC is significantly different from that in AEC and RPC.

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