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LIQUID CHROMATOGRAPHY ON CHEMICALLY BONDED ELECTRON DONORS AND ACCEPTORS

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CONTENTS

1. INTRODUCTION

In addition to physisorption, hydrogen bonding, solvophobic effects and other retention mechanisms, the formation of electron donor–acceptor (EDA) complexes also plays a role in liquid chromatography (LC)[1]. Complexes (substances with a defined stoichiometry and geometry) are formed by the interaction of two or more component molecules or ions. The formation is an equilibrium process and the complex formed dissociates reversibly into its components.

EDA complexes result from a weak interaction of electron donors (D) with electron acceptors (A)[2,3]:

$$A + D \overset{K_{eq}}{\rightleftarrows} AD \qquad (1)$$

The enthalpy of EDA complexation is usually of the order of a few kcal/mol and the rates of formation and dissociation of AD are very high. As the stability of EDA complexes depends not only on the structure of components but also on the polarity of the solvent, their formation has been utilized in LC for several decades[1–3].

Electron donors are defined as molecules capable of giving up an electron and their ionization potential, I_D, is a measure of the donation ability. Electron acceptors

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are able to accept an electron and this is related to their electron affinity, EA, or reduction potential. In some instances, the same molecule can act as an electron donor or acceptor, depending on the circumstances. In large molecules such as pharmaceuticals, biologically active compounds and synthetic dyestuffs, independent electron-accepting and -donating parts of the molecule can exist[2–5].

Small unsaturated or aromatic hydrocarbons are usually weak donors or very weak acceptors. Their donating or accepting capability increases with increase in the number of C=C double bonds or aromatic rings. Polynuclear aromatic hydrocarbons (PAHs) and azaarenes are therefore efficient donors of π-electrons. The replacement of a hydrogen atom in the parent molecule of PAHs with an electron-releasing substituent such as an alkyl, alkoxy or amino group increases the capability of molecule to donate π-electrons.

On the other hand, aromatic or unsaturated compounds containing several electron-withdrawing substituents such as NO_2, Cl or CN are efficient acceptors. Picryl chloride, tetracyanoethylene, *p*-phenylendiamine and other compounds are examples of strong π-donors or π-acceptors. The EDA complexation not only involves an interaction between π-electrons, but σ- or n-electrons and vacant or antibonding σ-orbitals can also play a role in the interaction.

The formation of EDA complexes is assumed to be the main interaction mechanism governing the chromatographic behaviour of solutes in so-called "charge-transfer" or EDA liquid chromatography (EDA-LC). The EDA complexation can take place in either the mobile phase or the stationary phase[1]; the latter alternative is possible using stationary phases with electron-accepting or-donating ability. Thus, the separation of PAHs on silica gel impregnated with 2,4,6-trinitrobenzene or another acceptor was reported several decades ago[6]. Nevertheless, the adsorbed stationary phases are easily washed out and this not only causes a decrease in retention but also interferes with UV detection often used in high-performance liquid chromatography (HPLC).

Various metal oxides can also have adsorption sites with acceptor or donor capability[7,8]. Pairs of these sites have been found on alumina surfaces in addition to Brönsted acid sites. As a consequence, this so-called "Lewis acidity" of alumina enhances the retention of PAHs or other electron donors compared with nearly neutral silica gel. However, the acid–base properties of many metal oxides, including alumina, are strongly dependent on the concentration of surface hydroxy groups or adsorbed water[9]. The chromatographic behaviour of thermally activated alumina is therefore easily changed by rehydration.

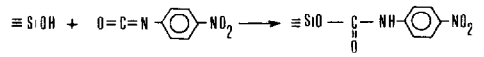
Organic electron acceptors bound to the surface of polymers have been studied in LC for over 20 years since the first such sorbent based on polystyrene gel bearing aromatic groups was reported by Ayres and Mann[10] in 1964. Hydrophilic gels such as polydextrans modified with various donors or acceptors have been prepared and tested by Porath[11]. In this instance, the separation of donors and acceptors of biological importance was achieved in aqueous mobile phases.

The advent of chemically bonded stationary phases for HPLC made it possible to prepare more selective and more efficient electron-accepting or -donating sorbents. In practice, the organic donors or acceptors are immobilized on the silica surface via a suitable silanization reaction. This review considers silica and organic polymers modified with covalently bound donors and acceptors. An attempt has been made

to review all the relevant contributions to this class of HPLC sorbents characterizing their preparation, chromatographic behaviour and practical applications.

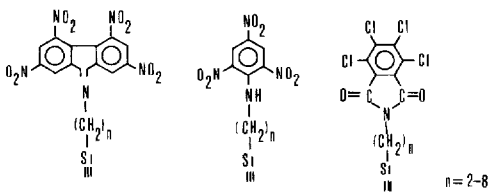
2 PREPARATION OF CHEMICALLY BONDED DONORS AND ACCEPTORS

Stationary phases for EDA-LC are mostly bonded to porous silica. The first silica modified with chemically bonded acceptor ligands was prepared via the reaction of surface silanols with *p*-nitrophenyl isocyanate by Ray and Frei[12] (Scheme 1). The resulting sorbent is unstable, being easily decomposed by moisture and light.



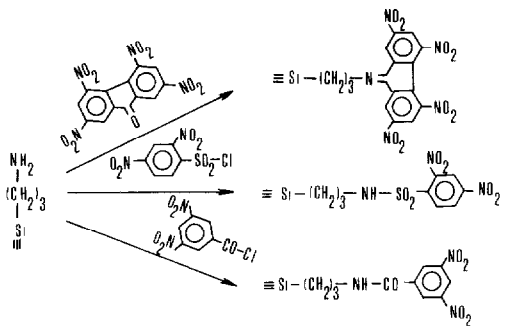
Scheme 1

Since then, several acceptor-modified silicas have been prepared and tested. Most of them are based on nitroaromatic ligands such as 2,4,5,7-tetranitrofluorenonimino, 2,4-dinitroanilino or 2,4,6-trinitroanilino groups bonded to the silica surface by means of a short aliphatic chain (see, *e.g.*, refs. 13–16) (Scheme 2). In addition to nitroaromatic ligands, tetrachlorophthalimidopropylsilica[17] has also been prepared (Scheme 2).



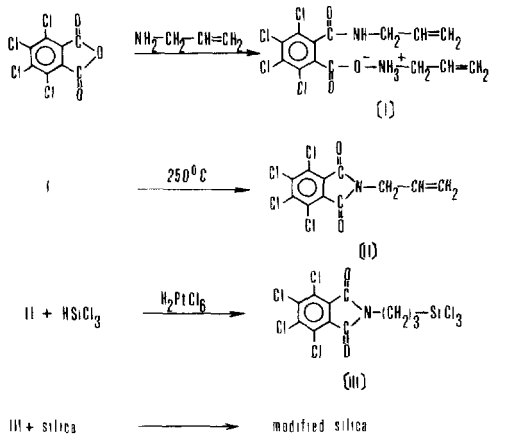
Scheme 2

In principle there are two ways to prepare sorbents with chemically bonded electron donors or acceptors. First, silica is modified with a silane having a sufficiently reactive group that is utilized in the next step of the synthesis Amino groups are often used for this purpose (see, *e.g.*, refs. 13–16 and 18–21) (Scheme 3) In the second method, the corresponding silanes are first prepared and then allowed to react with the silica surface, yielding the chemically bonded stationary phase[17,22–25]. The synthesis of the above-mentioned tetrachlorophthalimidopropylsilica reported by



Scheme 3

Holstein[17] can serve as an example (Scheme 4).



Scheme 4

With polynitroaromatic ligands, it is usually impossible to isolate the resulting silane from the reaction mixture and the crude product is therefore used in the silanization step[25].

The first method is simpler and does not require special synthetic skills, but the unreacted amino groups, being efficient n-electron donors, can interact with the bonded acceptor ligands. Hence, the surface reaction is not only to be selective but also quantitative. For example, unreacted propylamine groups have been found to form a red complex with picramidopropyl ligands. The conversion of amino groups into picramido groups can be checked by means of photoacoustic spectroscopy[26].

The low selectivity of the surface reaction, resulting in low yields of the desired ligands, is a plausible explanation for why many sorbents prepared via the surface reaction have shown poor chromatographic performance[14,19] (Fig. 1). However, the reaction of 2,4-dinitrofluorobenzene and 2,4,6-trinitrobenzenesulphonic acid with bonded primary amines can be recommended for the preparation of bonded nitroan-

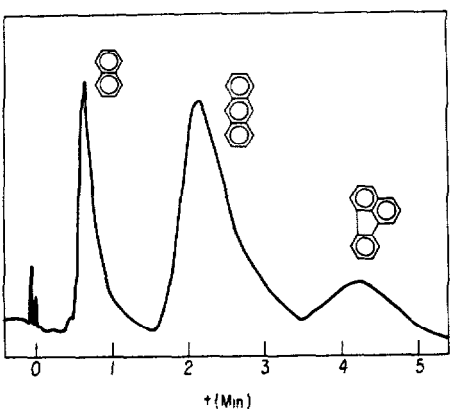
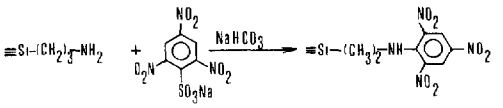


Fig. 1. Separation of naphthalene, anthracene and fluoranthene on silica modified with 3-(2,4,5,7-tetranitrofluorenimino)propyl ligands (taken from ref. 19).

ilines[27,28]. The reproducible preparation of 2,4-dinitroanilinopropylsilica has been checked by several workers[14–16,20,29,30] and the identity of the bonded ligands demonstrated by photoacoustic spectroscopy[31].

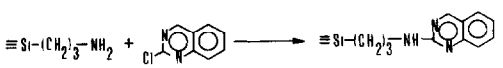
Both of the above reactions are carried out under mild conditions in aqueous media owing to the hydrolytic stability of 2,4-dinitrofluorobenzene and 2,4,6-trinitrobenzenesulphonic acid[27,28]. It has been noted that the free amino groups on γ-aminopropylsilica are largely adsorbed on silanol groups in non-polar solvents[32]. On the other hand, the use of polar aqueous media ensures better accessibility of amino groups for the surface reaction owing to their solvation. The quantitative conversion of amino groups into bonded picramide illustrates the advantageous use of polar reaction media[33] (Scheme 5).



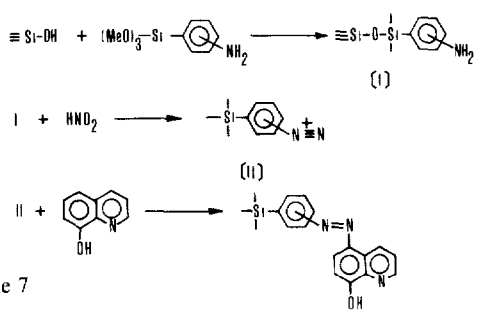
Scheme 5

If trinitrochlorobenzene is used under the above reaction conditions, free picric acid, which forms stable picrates with bonded amines, is formed. For this reason, an excess of picryl chloride was used by Eppert and Schinke[34]. The poor reproducibility of picramide-modified silica prepared from picryl chloride has been also reported by Nondek and Ponec[15].

Chemically bonded PAHs, azaaromatics and alkoxybenzenes are expected to be efficient electron donors. Phenoxy[23] or pyrene[24] ligands have been bonded to the silica surface via corresponding silanes. The reactivity of aminosilica has been utilized by Lochmüller *et al.*[31] for the preparation of a 2-quinazoline stationary phase (Scheme 6). Marshall and Mottola[35] described the preparation of silica with covalently bonded 8-quinolinol. A diazo coupling procedure was used in the last step of the synthesis (Scheme 7).



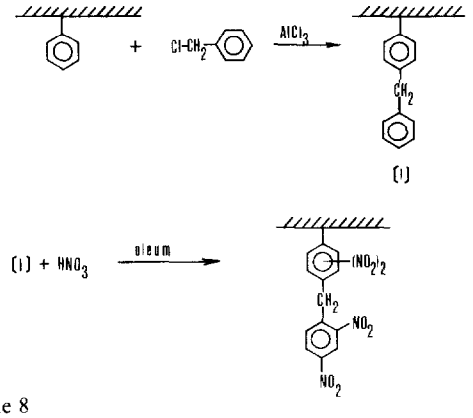
Scheme 6.



Scheme 7

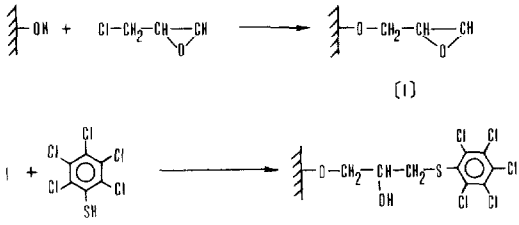
Organic gels modified with donors or acceptors are the second group of sorbents for EDA-LC. Divinylbenzene–styrene copolymer was modified by a conventional Friedel–Crafts benzylation with benzyl chloride (Scheme 8). The reaction prod-

uct was nitrated with a mixture of oleum and fuming nitric acid. The resulting polynitrobenzylpolystyrene resin permitted the complete separation of anthracene and pyrene despite the very low efficiency of the column used. In the early work of Ayres and Mann[10], charge-transfer spectral bands of complexes formed by the resin and PAHs were observed in the region of 350–500 nm Polymers modified by electron acceptors were also reported by Smets *et al.*[36].



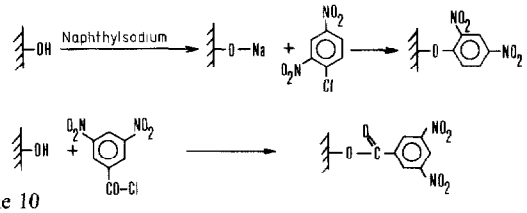
Scheme 8

Porath and co-workers[11,37,38] modified Sephadex G-25 polydextran gel with several organic electron donors and acceptors. In the first step, the gel was treated with 1-chloro-2,3-epoxypropane and the reactive epoxide groups bonded to the polymer surface permitted the immobilization of various ligands, *e.g.*, the pentachlorothiophenyl group[11] (Scheme 9).



Scheme 9

Šmídl and Pecka[39,40] prepared methacrylate copolymers modified with 2,4-dinitrophenoxy and 3,5-dinitrobenzoyl ligands. They utilized the reactivity of hydroxy groups present on the surface of the methacrylate gel used. The copolymer was arylated with dinitrochlorobenzene or esterified with dinitrobenzoyl chloride (Scheme 10).



Scheme 10

Natural polymers have also been chemically modified with electron donors and acceptors. Glucose esterified with 3-(pentamethylphenyl)propionic or 3-(9-phenanthryl)propionic acid has been used for the thin-layer chromatography (TLC) of nitrotoluenes and quinones[41]. Riboflavin has been covalently bonded to cellulose for the separation of biologically active electron donors[42].

3 RETENTION MECHANISM IN EDA-LC

In this type of LC, the chromatographic retention depends on the stability of EDA complexes formed between the stationary phase and the solute. The formation of these complexes, which is assumed to be the governing retention mechanism, is influenced by factors identical with those in a homogeneous phase.

The ideas about the formation of EDA complexes in the gas phase or dilute solutions are based on Mulliken's theory of charge-transfer complexes[43] In this theory, an electron donor (D) and acceptor (A) usually form a weak complex stabilized by charge transfer Two electronic states are assumed· non-bonding (DA) and dative (D^+A^-). The resulting wave function is

$$\psi_N(DA) = a\psi_0(DA) + b\psi_1(D^+A^-) \tag{2}$$

The formation of the dative state contributes to the stabilization of the ground state ($a \gg b$) Hence, the main factor influencing the stability of an EDA complex is the electronic structure of D and A.

However, the main difference between the complexation in homogeneous dilute solutions and on a sorbent surface, which may play a decisive role in EDA-LC, is given by two additional factors (1) a large surface concentration of bonded ligands permits the formation of non-stechiometric complexes; and (2) the limited motion of immobilized ligands causes steric hindrance of EDA complexation For these reasons, the equilibrium constants, K_{eq}, measured in dilute solutions may not correlate well with log k'.

As stated above, the stability of EDA complexes is influenced by the structure of both participants, the solute and the bonded ligands To separate the contribution of other experimental factors such as temperature and solvent effects or varying concentration of "active" ligands, the structure–retention relationships will be discussed in terms of chromatographic selectivity. This permits the comparison of different experiments, the evaluation of various sorbents and a better discussion of the retention mechanism.

3 1 Chromatographic selectivity in EDA-LC

The chromatographic selectivity, $\alpha_{i,1}$ is defined as the ratio of capacity factors, k'_i/k'_1, for a given pair of solutes. As the individual capacity factors are proportional to an equilibrium constant K_{eq}

$$\log k' \approx \log K_{eq} = -\Delta G^\circ/RT \tag{3}$$

log $\alpha_{i,1}$ can be approximated as

$$\log \alpha_{i,1} \doteq (\Delta H^\circ)_i - (\Delta H^\circ)_1 \tag{4}$$

$$(i = 1, 2, 3, \ldots, n)$$

in a series of n structurally related solutes (ΔS° = constant). In EDA-LC, it is possible to substitute the enthalpic terms in eqn. 4 with an interaction energy, ΔE, which is a measure of the stability of the complex.

Nondek and Ponec[15] attempted to predict log $\alpha_{i,1}$ using a simple quantum-chemical model, in which only π-electrons are transferred between frontier orbitals of A and D. Thus, ΔE may be approximated by the equation of Klopman and Salem[44–46]:

$$-\Delta E = \frac{2\Sigma c^2_{\mathrm{HOMO}}\, c^2_{\mathrm{LUMO}}\, \beta^2}{E_{\mathrm{HOMO}} - E_{\mathrm{LUMO}}} \tag{5}$$

where c_{HOMO} and c_{LUMO} are frontier orbital coefficients, E_{HOMO} and E_{LUMO} are energies of these orbitals and β is the corresponding resonance integral. Assuming that the numerator in eqn. 5 is a constant in a series of structurally related solutes, the following relationship between the energies of frontier orbitals and the chromatographic selectivity or relative stability of EDA complexes has been found[15]:

$$\log \alpha_{i,1} = \text{Constant} \cdot \frac{(\Delta E_{\mathrm{HOMO}})_i}{(E_{\mathrm{HOMO}} - E_{\mathrm{LUMO}})_1}$$
$$(\Delta E_{\mathrm{HOMO}})_i = (E_{\mathrm{HOMO}})_i - (E_{\mathrm{HOMO}})_1 \tag{6}$$

Nondek and Ponec[15] tried to verify this relationship by studying the retention of several PAHs on four electron acceptor-modified silicas. They observed a qualitative agreement despite the fact that the selectivity is influenced by many experimental factors such as the surface concentration of the acceptor ligands[47].

Despite its crudeness, the above model can serve as a rational basis for the synthesis of more selective electron acceptor-modified sorbents bearing aromatic ligands[15]. The selectivity depends not only on the number of electron-accepting substituents (NO_2, Cl, CN, etc.) attached to the aromatic skeleton, but also on the nature of the spacer connecting the skeleton with the silica surface. The dependence of $\alpha_{i,1}$ on the structure of bonded ligands is also shown in the separation of methylcholanthrenes on nitrofluorenone ligands studied by Lochmüller *et al.*[13]. The selectivity increases with increase in the number of nitro groups attached to the fluorenoniminopropyl ligands (Fig. 2).

The experimental results of Šmídl[39] are also in qualitative agreement with the quantum-chemical model, as 3,5-dinitrobenzoyl ligands attached to a gel matrix show greater selectivity than 2,4-dinitrophenoxy ligands. In this instance, the selectivity $\alpha_{i,1}$ does not depend on the preparation procedure or the surface concentration of EA ligands and clearly reflects the influence of substituent position and the nature of the spacer (Table 1).

As mentioned above, the quantum-chemical model is of only limited value for numerical calculations of $\alpha_{i,1}$. However, it shows that log $\alpha_{i,1}$ is a combination of two independent factors: the structural difference of the solutes and the ability of bonded ligands to form EDA complexes. Thus, the selectivity can be expressed as[47]

$$\log \alpha_{i,1} = \kappa\delta_i \tag{7}$$

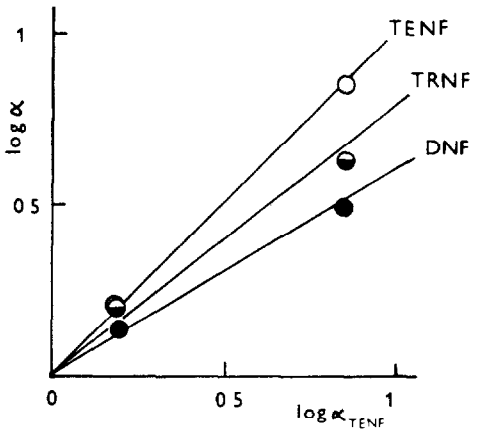


Fig 2 Replotted selectivities of 2,4,5,7-tetranitro- (TENF), 2,4,7-trinitro-(TRNF) and 2,6-dinitrofluorenimino (DNF) ligands in the separation of 1-, 2- and 3-methylcholanthene Data taken from ref 13; TENF-silica used as a reference sorbent (taken from ref 15)

This linear free energy relationship (LFER) is more general than eqn. 6 because it can be used for the quantification of several independent interaction mechanisms.

As PAHs are frequently used in testing acceptor-modified silicas, the set of δ constants has been calculated (Table 2). They can serve for the calculation of the κ parameter characterizing the stationary phase. In Table 3, various acceptor-modified silicas are surveyed; it is evident that the most efficient sorbent is 2,4,7,9-tetranitrofluorenoneoximesilica prepared by Hemetsberger *et al* [22] and the weakest is Nucleosil-NO_2, which is assumed to be *p*-nitropropylbenzenesilica[21,48].

Nevertheless, it is evident from Table 3 that the chromatographic selectivity of the sorbent expressed by κ is influenced by the density of bonded acceptor ligands, the temperature and the polarity of the mobile phase. Sorbents with low coverages

TABLE 1

CHROMATOGRAPHIC SELECTIVITY IN EDA-LC OF PAHs OVER MODIFIED HYDROXYETHYLMETHACRYLATE GELS*

PAH	*Chromatographic selectivity,* $\alpha_{i,1}$			
	*Acylation (%)***		*Arylation (%)****	
	18	*63*	*20*	*60*
Naphthalene§	0 00	0 00	0.00	0 00
Anthracene	0 52	0 61	0 49	0 45
Phenanthrene	0 59	0 67	0 47	0 51
Fluoranthene	0 87	1.01	0 72	0 64
Chrysene	1 11	1.18	0 91	0.92

* Recalculated from ref 39, *n*-hexane as mobile phase
** Surface OH groups acylated with 3,5-dinitrobenzoyl chloride (% conversion)
*** Surface OH groups arylated with 2,4-dinitrochlorobenzene (% conversion).
§ Taken as a reference solute

TABLE 2

RETENTION CONSTANTS (δ) OF PAHs*

PAH	δ	*PAH*	δ
Naphthalene**	0 00	Chrysene	1 32
Phenanthrene	0 66	Benzo[*a*]pyrene	1 69
Anthracene	0 64	Perylene	1 79
Fluoranthene	1 01	Picene	1 95
Pyrene	1 04		

* Taken from ref. 47
** Taken as a reference solute

show poor chromatographic performance, probably owing to non-homogeneity of the surface layer. This non-homogeneity is increased by unreacted amino groups, provided that aminosilica is used as a starting material.

Thomson and Reynolds[16] prepared four chemically bonded nitroaniline stationary phases 2,4-dinitroanilinopropyl-, 2,4,6-trinitroanilinopropyl-, 2,4-dinitroanilinooctyl- and 2,4,6-trinitroanilinooctylsilica. The results of elemental analysis showed excellent correlation between the carbon and nitrogen percentages for surface coverage of the 2,4-dinitroanilinopropylsilica prepared according to Nondek and Málek[20] in aqueous solution. The other three sorbents show higher carbon contents, which indicates incomplete conversion of bonded amine to nitroaniline ligands, probably owing to unsuitable experimental conditions being used. The trinitroanilinopro-

TABLE 3

COMPARISON OF VARIOUS ACCEPTORS BOUND ON SILICA IN EDA-LC OF PAHs

Acceptor ligand A	κ	*[A] (μmol/m^2)*	*Mobile phase composition*	r_{correl}	n_{exp}*	*Ref.*
2,4-Dinitroaniline	1 03	2 5	*n*-Hexane (25°C)	0 9953	12	14
	0 94	2 5	*n*-Hexane (40°C)	0 9969	12	14
	0 54	0 7	*n*-Hexane	0 9585	9	47
2,4,6-Trinitroaniline	0 75	–	Cyclohexane–10% EtOAc	0 9928	5	25
3,5-Dinitrobenzamide	0 71	–	*n*-Hexane–5% CH_2Cl_2	0 9907	7	21
	1 03	–	*n*-Hexane–5% CH_2Cl_2	0 9876	5	75
2,4,6-Trinitrophenyl propyl ether	0 88	2 5	*n*-Hexane–25% CH_2Cl_2	0 9975	5	75
2,4,7,9-Tetranitro-fluorenoneoxime	1 75	3 0	*n*-Hexane–20% CH_2Cl_2	0 9741	6	22
	1 44	3 0	*n*-Hexane–40% CH_2Cl_2	0 9573	7	22
	1 15	3 0	CH_2Cl_2	0 9760	7	22
Tetrachlorophthalimide	0 82	3 3	*n*-Hexane–20% CH_2Cl_2	0 9813	11	17
Pentafluorobenzamide	0 52	4 1	*n*-Hexane	0 9482	7	64
Caffeine	0 98	2 5	*n*-Hexane–25% CH_2Cl_2	0 9974	5	62
	0 88	2 3	*n*-Hexane–25% CH_2Cl_2	0 9958	5	63
Nucleosil-NO_2	0 57	–	*n*-Hexane–10% $CHCl_3$	0 9924	10	48
	0 45	–	Isooctane–10% CH_2Cl_2	0 9937	5	60
	0 68	–	*n*-Hexane–5% CH_2Cl_2	0 9990	5	75

* Number of experimental points used in correlation to give r_{correl}

pyl- and octyl-substituted sorbents show lower selectivity than 2,4-dinitroanilinopropylsilica[16].

Šmídl[39] studied the retention of several PAHs on Separon H-1000 arylated or acylated with dinitrochlorobenzene or dinitrobenzoyl chloride. Recalculating the reported k' values to selectivities relative to naphthalene, one can see the independence of selectivity from the surface concentration of acceptor ligands (Table 1). As the surface concentration of bonded ligands is unknown in the above gels, it is impossible to compare Šmídl's results with the data given in Table 3.

3.2. Temperature and solvent effects in EDA-LC

Obviously the retention of PAHs on bonded acceptors decreases with increasing temperature and solvent polarity[14,20,22,29]. The same holds for the chromatographic selectivity, $\alpha_{i,1}$, as is evident from κ values given in Table 3. As the enthalpy of complexation, $-\Delta H$, decreases with increasing complex stability, the retention of stronger donors will be decreased more with increase in temperature[20,22]. Consequently, a lower selectivity is observed at higher temperatures[48].

As for the composition of mobile phase, both the retention and the selectivity decrease with increasing polarity. It is interesting that similar trends in the relative stability of EDA complexes have been observed in solutions, *e g.*, the relative K_{eq} are decreased in a series of several donors with fluoranil or 1,4-dicyano-2,3,5,6-tetrafluorobenzene as acceptors if tetrachloromethane is replaced with the more polar chloroform[49]

It must be pointed out that the solvent effects on EDA complexation can be characterized as either non-specific or specific solvation of A or D. The latter can be described as competing equilibria[2]:

$$D + A \overset{K_{eq}}{\rightleftarrows} DA \tag{8}$$

$$D + S \overset{K_{solv}}{\rightleftarrows} DS \tag{9}$$

Assuming that [S] $\gg$ [DS] and [A°] $\gg$ [DA], which is perfectly fulfilled under LC conditions, one can derive

$$k'_n = k'_s (1 + K^D_{solv} [S]) \tag{10}$$

where

$$k'_n = K_{corr} [A°] \tag{11}$$

$$k'_{solv} = K_{eq} [A°] \tag{12}$$

The concentration of bound acceptor ligands is [A°], K_{eq} is the equilibrium constant given by eqns. 8 and 9 and K_{corr} is a corrected equilibrium constant derived by Drago *et al.*[50]. K_{corr} does not involve the specific solvation competing with the solute–stationary phase complexation. Eqn. 10 enables K_{solv} and k'_n to be determined. A similar equation can be derived for the specific solvation of A.

If the solvent S competes for the both A and D, an analogous equation has been derived by Bishop and Sutton[51]

$$K_{eq} = K_{eq}^{solv} (1 + K_{solv}^{D} [S]) (1 + K_{solv}^{A} [S]) \quad (13)$$

The specific solvation effects characterized by the equilibrium constants K_A^{solv} and K_D^{solv} proceed via EDA interaction and/or hydrogen bonding. Such competition is assumed to be the major solvent effect of dioxane and ethers, which are known to be effective n-donors; chloroform and dichloromethane have been shown to hydrogen bond to aromatic π-donors[2].

Recalculating the retention data taken from previous papers[22,29], one can plot reciprocal capacity factors, $1/k'_s$, against the concentration [S] of chloroform or dichloromethane in *n*-hexane and *n*-heptane (Fig. 3). The slope of the lines is K_{solv}^{D} according to eqn. 10; for the retention of phenanthrene and naphthalene on 2,4-dinitroanilino- and 2,4,5,7-tetranitrofluorenoneoximesilica, it is evident that k'_n agrees well with the capacity factors measured in pure *n*-alkanes ([S] = 0). The estimated value of K_{solv}^{D} is 4.6 l/mol for phenanthrene–chloroform, 1.7 l/mol for naphthalene–chloroform and 0.6 l/mol for naphthalene–dichloromethane complexes.

In this way, the decrease in the κ factor with increasing concentration of the polar component S complexing with solutes can be explained. If only the bonded acceptor ligands were solvated specifically ($K_{solv}^{D} = 0$), all the experimental points obtained for different solutes should fit only one correlation line having a slope of K_{solv}^{A}. Non-linear correlations are expected, provided A and D form complexes with S (K_{solv}^{A}, $K_{solv}^{B} \neq 0$). Using a similar approach, Hemetsberger *et al.*[22] found that the bonded tetranitrofluorenoneoxime ligands form A_2S complexes with several polar solvents. They assumed that D competes with *n* molecules of S for A. This way, a "sandwich" structure of swollen ligand layers is formed. The complexing strengths of the solvents increase in the order isopropyl chloride < dichloromethane < tet-

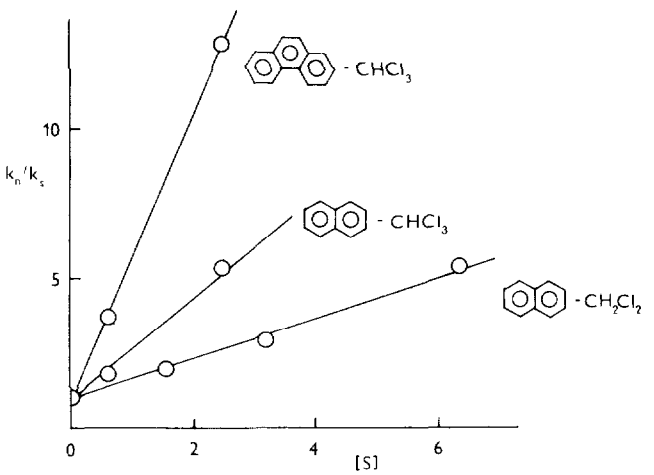


Fig. 3. Plot of reciprocal capacity factors, k'_s, of naphthalene and phenanthrene against the concentration, [S], of polar solvent ($CHCl_3$, CH_2Cl_2) in a non-polar mobile phase (*n*-hexane, *n*-heptane) according to eqn. 13. Data taken from refs. 22 and 29.

rahydrofuran < acetone < ethyl acetate. The specific solvation of bonded ligands with n-donors, *e.g.*, alcohols, esters or ethers, can be assumed. However, chlorinated hydrocarbons such as dichloromethane or chloroform are not known to interact specifically with organic electron acceptors.

The effect of mobile phase polarity on the retention of PAHs over tetranitrofluorenoneoximesilica has been thoroughly discussed by Hemetsberger *et al* [22]. They used a model derived by Filakov and Borovikov[52] under the assumption that the stability of EDA complexes is determined by dipole dipole electrostatic actions. Assuming that the dipole moments of D and A are unchanged by the complexation, a linear relationship (eqn. 14) has been derived[52].

$$\log k' = a + b/\varepsilon \tag{14}$$

The plots of log k' versus $1/\varepsilon$ are not exactly linear, however, for the experimental data of Hemetsberger *et al.*[22].

As has been mentioned elsewhere[2], there is no simple correlation between K_{eq} and bulk polarity parameters of solvents such as dielectric constant, ε, although a general trend to lower K_{eq} with increasing ε is observed. For strong complexes, the reverse trend occurs, however[2] These complexes with significantly larger dipoles gain extra stabilization from the inductive interactions with polar solvents.

In very polar solvents, the ionic states of both components are stabilized The stabilization energy will depend on the dielectric constant of the solvent according to Born's equation[3,11].

$$-\Delta H_{solv} \approx \left(1 - \frac{1}{\varepsilon}\right)\left(\frac{1}{R_{A^-}} + \frac{1}{R_{D^+}}\right) \tag{15}$$

where R_{A^-} and R_{D^+} are the ionic radii of ions A^- and D^+, respectively Thus, the solvation energy calculated with eqn. 14 may amount to about 100 kcal/mol in water[3]. EDA-LC in reversed-phase systems therefore seems to be an interesting alternative to the normal-phase separations discussed above.

The first attempt in this direction was made by Hunt *et al.*[53], who used bonded phthalimide for the separation of PAHs. The retention order of these solutes is very

TABLE 4

RETENTION OF NITROBENZENES IN REVERSED-PHASE LC ON BONDED ARYL ETHER AND C-18 STATIONARY PHASES

Solute	*Capacity factor**	
	Aryl ether	*C-18*
Benzene	2 98	8 18
Nitrobenzene	3 76	6 95
1,2-Dinitrobenzene	4 40	5 54
1,3-Dinitrobenzene	4.88	4 58
1,3,5-Trinitrobenzene	6.94	3 13

* Taken from ref 23

TABLE 5

SELECTIVITY IN EDA REVERSED-PHASE LC OF ALKYLAROMATICS ON 2,4,5,7-TETRANITROFLUORENONEOXIME (TNF) IN COMPARISON WITH C-18 STATIONARY PHASE*

Solute	*Chromatographic selectivity,* $\alpha_{i,1}$	
	TNF	*C-18*
1-Methylnaphthalene	1.00	1.00
2-Methylnaphthalene	1.22	1.00
Acenaphthene	1.58	1.25
Acenaphthylene	2.41	1.12

* Taken from ref. 54, mobile phase, methanol–water (95:5)

close to that for the C-18 phase used for the comparison. Porath[11] also pointed out that EDA complexation might be enhanced owing to solvent effects in water-mediated EDA-LC over modified Sephadex gels. It was clearly shown by Mourey and Siggia[23] that EDA complexation can operate along with the solvatophobic effect obvious in reversed-phase LC. They studied the retention of nitrobenzenes on bonded phenoxy groups acting as an acceptor. The elution order of nitrobenzenes is completely changed by EDA complexation compared with a conventional C-18 phase (Table 4).

Hemetsberger and Ricken[54] studied systematically the chromatographic behaviour of bonded tetranitrofluorenoneoxime ligands in the reversed-phase LC of PAHs. They found that the EDA complexation acted together with the solvophobic effect. The heats of adsorption, $-\Delta H_{ads}$, are much higher for the nitro than for C-18 phase as a result of EDA complexation, the $-\Delta H_{ads}$ values vary to a greater extent with the structure of the solutes, indicating that more specific solute–ligand interactions are involved. In Table 5, the chromatographic selectivities of four structurally related solutes with the same number of carbon atoms are given. Under conventional reversed-phase LC conditions, the selectivity on a C-18 phase is lower than that on the nitro phase; *e.g.*, acenaphthylene, forming relatively stronger EDA complexes with acceptors than naphthalene or acenaphthene, possesses a considerably enhanced retention in EDA reversed-phase LC.

3.3. Surface EDA complexes

It must be pointed out that the existence of well defined EDA complexes between solutes and surface-bound ligands is deduced mainly by analogy with the complexation taking place in homogeneous solutions. Visual or spectral observations of these complexes have not been reported, the only exception being the early work of Ayres and Mann[10]. Various correlations between the retention of solutes and their stability constants for EDA complexation observed in dilute solutions, ionization potentials, I_D, electron affinities, EA, etc., have been used as indirect evidence of the existence of surface EDA complexes[15,22,29].

PAHs have mainly been used as model solutes in these studies and their retention is increased with increasing molecular size, as in other known LC systems such as reversed-phase or adsorption LC. Nondek and Minárik[47] discussed various cor-

relations between the retention of PAHs on 2,4-dinitranilino bonded ligands and various structural parameters. The correlation analysis reveals that the retention depends on molecular size more than on I_D or E_{HOMO} The same holds for all the systems included in Table 3 as they correlate well with the δ constants.

The dependence of the retention of PAHs retention on their molecular size may be explained by the formation of surface complexes between one molecule of a PAH and several adjacent ligands[29,47,55]. This non-stoichiometric complexation could be enhanced by a non-uniform distribution of bound ligands even at very low concentrations of ligands forming "clusters" or "islands" on silica surface. Nondek and Minárik[47] observed that the retention of PAHs expressed as log $\alpha_{i,1}$ correlates better with K_{eq} at low concentrations of 2,4-dinitroanilino ligands. On the other hand, the results of Šmídl[39] obtained for organic gels bearing nitroaromatic ligands show that the selectivity is independent of the surface concentration of ligands.

Aromatics with polar substituents such as OH, NO_2 and $N(CH_3)_2$ seem to interact with bonded nitroaromatic ligands not only via simple π–π complexation. Their chromatographic behaviour therefore does not correspond with the expected retention order and the nitroaromatic ligands probably act as a strongly polar stationary phase[56].

In connection with the discussion of surface EDA complexes, the structure and properties of the ligand layer are important. Individual polar ligands interact either mutually or with the silica surface. The aggregation depends on the temperature and solvation, as shown by Hammers *et al.*[32]. The self-association of ligands occurs to some extent in the presence of weakly polar solvents. If the association equilibrium is perturbed by a temperature change, a new state of the ligand layer is established after a few hours. In non-polar solvents, adsorption of solute molecules on top of the ligand layer prevails, whereas in more polar solvents the solute molecules are assumed to penetrate into the swollen layer. The "swelling" and "shrinking" of the layer following a change in solvent composition is a relatively slow process. As has been shown by Nondek and Chvalovský[57], the sorptive properties of 2,4-dinitranilino- and 2,4-dinitrobenzenesulphonamidosilica depend on the extent of swelling.

The structure of the surface layer of 2,4-dinitranilino ligands has also been studied by Lochmüller *et al.*[31] by means of photoacoustic spectroscopy It was found that the bonded ligands behave as a solid in non-polar solvents. This is a consequence of the inability of these solvents to overcome the attraction of adjacent ligands for each other and for the silica surface. The attraction mimics the precipitation of ligands and could yield solid-like spectra. A decreased motional freedom of bound dinitranilino ligands has been proposed by Van Miltenburg and Hammers[58] on the basis of specific heat measurements.

4 PRACTICAL APPLICATIONS

Chemically bonded electron acceptors have been used by various workers for the analysis of complex mixtures of PAHs such as crude oil, petrochemical fractions and products and coal hydrogenates. Holstein[17] demonstrated the practical utility of EDA-LC in the analysis of the above samples over tetrachlorophthalimidopropylsilica. Eppert and Schinke[34] achieved a class separation of higher boiling PAH

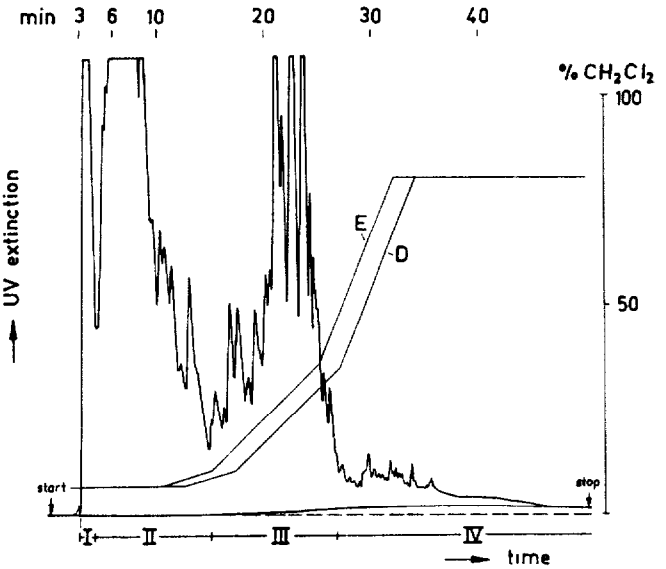


Fig 4 Gradient separation of PAHs present in diesel fuel on chemically bonded 2,4,6-trinitroanilinopropylsilica I–IV, Number of benzene rings, *n*-hexane–dichloromethane used as mobile phase (taken from ref 34)

mixtures over 2,4,6-trinitroanilinopropylsilica (Fig. 4). Thomson and co-workers[16,30] used 2,4-dinitroanilino- and 2,4,6-trinitroanilinopropylsilica for the analysis of liquid fossil fuels. From their comparative study, it is evident that this type of chemically modified silica is superior to alumina and aminopropylsilica, which are often used for this purpose.

Matsunaga[59] found Nucleosil-NO_2 to have greater selectivity than -NH_2, -CN or bare Nucleosil in separations of PAHs. The same was observed earlier by Lankmayr and Müller[60]. Holstein and Severin[61] separated a recycle oil into 23 fractions by means of tetrachlorophthalimidopropylsilica. Felix and co-workers[62,63] tested chemically bonded caffeine in the separation of PAHs and petroleum asphaltenes. The performance of the caffeine-modified silica is comparable to that of nitroaromatic stationary phases (Table 3). Another novel sorbent for HPLC[64], pentafluorobenzimidopropylsilica, seems to be a weak electron-accepting phase with a similar selectivity to that of Nucleosil-NO_2 (Table 3).

All the above studies show that chemically bonded acceptors permit the group analysis of PAHs according to the number of aromatic rings as the retention of alkylaromatics is insensitive to the degree of alkylation to a great extent[16,17,30,34].

As azaarenes and other nitrogen bases are efficient donors of π- and n-electrons, the use of chemically bonded acceptors for their HPLC analysis seems to be advantageous. The first separation of azaarenes by EDA-LC was attempted by Ray and Frei[12]. Tetrachlorophthalimidopropylsilica[17] and the commercial sorbent Nucleosil-NO_2[48] have been also used for this purpose. Nondek and Chvalovský[57] measured the retention of 22 azaarenes, anilines and alkylaromatic amines on 2,4-dinitrobenzensulphonamidosilica and 2,4-dinitroanilinosilica. As PAHs with three or four rings and weak nitrogen bases such as pyrroles and anilines are less retained

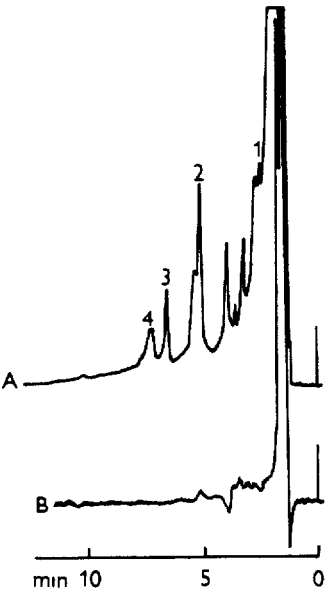


Fig 5. On-line pre-concentration of various impurities present in kerosene· peak 1 is identical with aniline, 2 with quinoline, 3 with pyridine and 4 with isoquinoline All the impurities are present in parts per million amounts The retention of PAHs was checked by means of fluorimetric detection (B), the upper chromatogram was obtained with a UV detector (A) (taken from ref 57).

than pyridines and other azaaromatics, the above sorbents have been tested for the on-line pre-concentration and LC analysis of azaarenes in gasoline, kerosene and diesel fuel (Fig. 5).

Many workers have used gradient elution for the analysis of complex mixtures of PAHs. Hammers *et al.*[32] pointed out that a layer of nitroaromatic ligands is in a

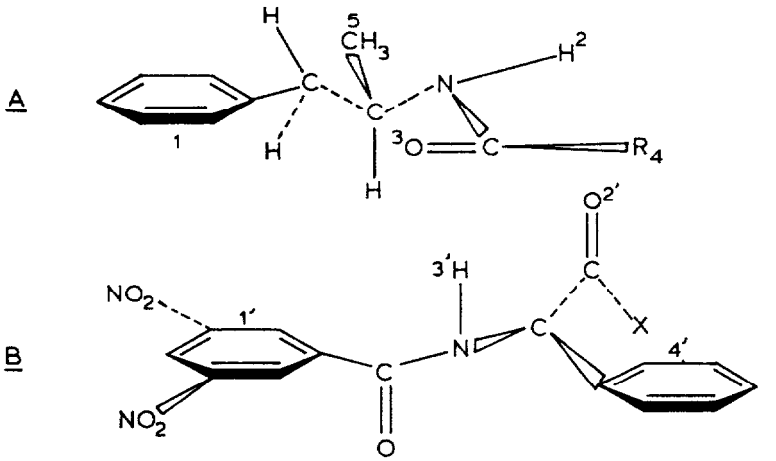


Fig 6 Bonding interaction between solute [(*S*)-1-phenyl-2-aminopropanamide] and chiral nitroaromatic stationary phase [(*R*)-N-(3,5-dinitrobenzyl)phenylglycine] (taken from ref 74)

swollen state depending on the polarity of the mobile phase. In dichloromethane, the layer is fully swollen and the subsequent shrinking in pure *n*-heptane is a relatively slow process. Nondek and Chvalovský[57] showed that the adsorption capacity of nitroaromatic phases depends on the extent of swelling. Hence these sorbents act under non-equilibrium conditions if a gradient regime is used.

The practical use of pyrene ligands bonded to silica has been reported by Lochmüller *et al.*[24]. The sorbent shows a good selectivity in the separation of nitroaromatics present in the particulate matter emitted by diesel engines. The formation of EDA complexes may play an important role in the separation of enantiomers on optically active nitroaromatic stationary phases[65–68]. This type of chiral stationary phase was developed by Pirkle and co-workers[69–72] using the concept of three-centre interaction between the solute and chiral ligands[73]. EDA interaction takes place simultaneously with other types of interaction mechanisms, ensuring better complexation of one enantiomer[74,75] (Fig. 6).

5 CONCLUSIONS

The rational utilization of EDA complexation in LC offers not only a better physico-chemical understanding of the separation process, but also improved chromatographic separations in many instances Both normal-phase separations and the use of reversed-phase LC systems are possible with the broad range of chemically bonded electron acceptors and donors available. EDA complexation used in combination with other specific interactions such as hydrogen bonding leads to efficient separations of racemic mixtures.

EDA-LC has been used frequently in separations of PAH mixtures. However, other classes of organic compounds such as azaaromatics, amines and chlorinated and nitrated aromatics could be separated efficiently by means of EDA-LC. The specific sorption properties of sorbents modified with electron donors or acceptors make it possible to use on-line pre-concentration techniques in connection with EDA-LC.

Finally, the study of complexation processes taking place in solutions may be supported by the use of HPLC, as has been demonstrated here. Relative stability constants, specific solvation effects and enthalpies of complexation found by means of HPLC agree well with the results of spectroscopic measurements

The main aim of this review has been to draw the attention of users of LC to this promising field. The potential of the application of EDA-LC, however, must be demonstrated by its ability to solve more real-life problems.

6 SUMMARY

The preparation and chromatographic properties of chemically bonded electron donors and acceptors are reviewed. The retention mechanism based upon the formation of donor–acceptor complexes is critically examined. Chromatographic selectivity expressed as a linear free energy relationship is used to compare various electron acceptors chemically bonded on silica in the separation of polynuclear aromatic hydrocarbons. The practical applications of donor–acceptor interactions in liquid chromatography are discussed.

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