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CHEMICALLY BONDED ELECTRON ACCEPTORS AS STATIONARY PHASES IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

On the basis of second-order perturbation theory, a relationship between chromatographic selectivity and the energy of the lowest unoccupied molecular orbital, ε_{LUMO}, is derived for chemically bonded electron acceptors. Besides 2,4-dinitroanilinopropyl silica reported earlier, other sorbents with chemically bonded polynitrobenzenes have been prepared to test the theoretical predictions. Among the new sorbents, 2,4-dinitrobenzenesulphamidopropyl silica has been found to be promising and is easy to prepare from NH_2-silica.

INTRODUCTION

Chemically bonded electron acceptors (EA) have been examined as liquid chromatographic (LC) stationary phases for nearly two decades, probably since the first LC sorbent bearing EA ligands was reported by Ayres and Mann[1] in 1964. Silica gel for high-performance liquid chromatography (HPLC), modified with EA ligands, has been prepared via reaction of surface silanols with *p*-nitrophenyl isocyanate by Ray and Frei[2] in 1972. Since that time, several EA sorbents for HPLC have been prepared and tested; most of these are based on nitroaromatic ligands bonded to the silica gel surface by means of a suitable spacer[3–8]. Other types of chemically bonded EAs have been examined, *e.g.,* tetrachlorophthalimide ligands[9].

The resulting sorbents have been used for the separation of polynuclear aromatic hydrocarbons (PAHs) or azaarenes; both groups of solutes are assumed to be electron donors. It is therefore supposed that donor–acceptor complexes are formed between the chemically bonded EAs and the above solutes[5,6,10]. The formation of such complexes, which makes possible chromatographic separation of various donors, depends however not only upon the structures of the solutes and the stationary phase but also upon other factors.

Hammers *et al.*[7] have studied the chromatographic behaviour of 2,4-dinitroanilinopropyl (DNAP), bis(3-nitrophenyl) sulphone and 2,4,7-trinitrofluoreniminopropyl ligands bonded to silica gel. If the surface of the silica gel is covered by a sufficiently dense layer of EA ligands, their self-association takes place in the presence of
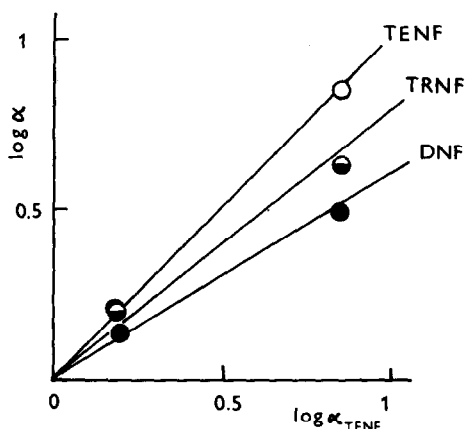
Fig. 1. Replotted selectivities of 2,4,5,7-tetranitro-(TENF), 2,4,7-trinitro- (TRNF) and 2,6-dinitrofluoren-imino (DNF) ligands in the separation of 1-, 2- and 3-methylcholanthrene. Data are taken from ref. 14. TENF-silica is used as a reference sorbent.

non-polar solvents. Thus, the penetration of solute molecules into the layer is restricted. Hammers et al.[7] assumed that slow diffusion of solute molecules into the layer of EA ligands causes the considerable asymmetry of chromatographic peaks observed on many chemically bonded EA phases.

This is the reason why the majority of reported EA phases has found only a limited use. Nevertheless, DNAP-silica, which is prepared very easily from commercially available NH_2-silica, does not result in excessive peak asymetry[7]. This sorbent was used for HPLC of PAHs and heteroaromates present in petroleum, coal liquids and shale oil[11], and found to be superior to alumina and NH_2-silica. Eppert and Schinke[8] reported the separation of PAHs in complex petrochemical samples on 2,4,6-trinitroanilinopropyl silica. This sorbent can be prepared via reaction of bonded amino groups with picryl chloride in aqueous alkaline suspensions[8,12]. A large excess of picryl chloride is required owing to its rapid solvolysis to picrate. Moreover, the conversion of amino groups into the required derivative does not proceed quantitatively and the chromatographic properties of the resulting sorbent vary from batch to batch[13]. Tetrachlorophthalimido silica, prepared by a much more complicated method by Holstein[9], has been used successfully in the analysis of coal liquids and complex PAH mixtures. It is therefore evident that the above EA silicas represent a new class of HPLC sorbents with broad application potential.

Despite the complex nature of the chromatographic process, the structure of chemically bonded EAs is one of the principal factors influencing the performance of the corresponding sorbent, as demonstrated by Lochmüller et al.[14]. If the capacity factors expressed in terms of chromatographic selectivities are replotted (Fig. 1), it is evident that sorbent selectivity increases with increasing number of nitro groups attached to the fluoreniminopropyl ligands.

Bearing in mind the application potential of chemically bonded EA stationary phases, we have attempted to prepare other sorbents with enhanced complexing abilities. According to the theory of electron donor–acceptor complexes[15], the stability of such complexes depends upon the electron affinity, A_G, of the acceptor and

the ionization potential, I_p, of the donor. These quantities can be approximated via Koopmans theorem[16] by the quantum chemically calculated energies of the lowest unoccupied (LUMO) and highest occupied (HOMO) molecular orbitals of the acceptor and donor respectively.

A relationship between chromatographic selectivity and the above parameters has been derived on the basis of second-order perturbation theory. The energies, ε_{LUMO}, calculated for a series of di- and trinitrobenzenes can be used for prediction of the selectivities of the corresponding EA ligands. Besides DNAP-silica reported earlier[5], another three chemically bonded EA silicas have been prepared and tested to confirm the validity of our predictions (Scheme 1).

DNAP   DNSP   DNBP   TNAP
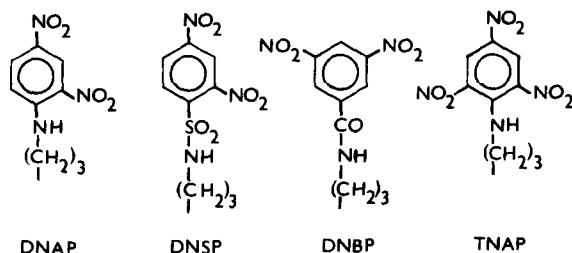
Scheme 1.

THEORETICAL

Chromatographic selectivity, $\alpha_{1,2}$, is defined as a ratio of capacity factors, k'_1/k'_2, for a given pair of solutes. The individual capacity factors are assumed to be proportional to an equilibrium complexing constant, K_{eq}:

$$\log k' \approx \log K_{eq} = -\Delta G^0/RT \tag{1}$$

Assuming negligible changes of ΔS^0 throughout a given series of n solutes, the selectivity, $\alpha_{i,1}$ can be approximated as

$$\log \alpha_{i,1} \approx (\Delta H^0)_i - (\Delta H^0)_1 \tag{2}$$

where $i = 2,3,...\ n$.

Within the scope of this simplified approach, it is possible to substitute the enthalpic terms in eqn. 2 with an interaction energy, ΔE, which is taken as a measure of the complex stability. Under the simplifying assumption that the complex formation is caused by the transfer of an electron only from the HOMO of the donor to the LUMO of the acceptor, as shown in Scheme 2, the corresponding stabilization energy, ΔE, is given by

$$\Delta E = \frac{(c_{v,\text{HOMO}}\ c_{\mu,\text{LUMO}})^2}{\varepsilon_{\text{HOMO}} - \varepsilon_{\text{LUMO}}} \cdot \beta^2_{v,\mu} \tag{3}$$

where $c_{v,\text{HOMO}}$ and $c_{\mu,\text{LUMO}}$ are expansion coefficients at centres μ and v in the HOMO and LUMO, respectively, and $\beta_{v,\mu}$ is a resonance integral characterizing the inter-

action between the centres μ and v[17]. In a series of structurally related complexes, the numerator of eqn. 3 is assumed to be constant.

Combining eqns, 2 and 3, the selectivity $\alpha_{i,1}$ can be expressed for a pair of solutes with $(\varepsilon_{HOMO})_1$ and $(\varepsilon_{HOMO})_i$, both being greater than ε_{LUMO}:

$$\log \alpha_{i,1} = \text{constant} \cdot \left[\frac{1}{(\varepsilon_{HOMO} - \varepsilon_{LUMO})_i} - \frac{1}{(\varepsilon_{HOMO} - \varepsilon_{LUMO})_1} \right] \tag{4}$$

Since $(\varepsilon_{HOMO})_i = (\varepsilon_{HOMO})_1 + (\Delta\varepsilon_{HOMO})_i$, eqn. 4 can be rearranged for small values of $\Delta\varepsilon_{HOMO}$ to give a semiempirical relationship between $\log \alpha_{i,1}$ and the complexing ability of solutes and stationary phase, expressed by $(\varepsilon_{HOMO})_1$, $(\varepsilon_{HOMO})_i$ and ε_{LUMO}:

$$\log \alpha_{i,1} \approx \text{constant} \cdot \frac{(\Delta\varepsilon_{HOMO})_i}{(\varepsilon_{HOMO} - \varepsilon_{LUMO})_1^2} \tag{5}$$

It is evident on the basis of eqn. 5 that one can compare the complexing abilities of two different EA ligands I and II by plotting the logarithmic values of the selectivities measured for a given series of solutes, as shown in Fig. 1. The slopes of the correlation lines are proportional to the ratio $(\varepsilon_{HOMO} - \varepsilon_{LUMO}^I)^2/(\varepsilon_{HOMO} - \varepsilon_{LUMO}^{II})^2$. As a consequence, the complexing ability in a series of chemically bonded EAs will therefore increase with decreasing ε_{LUMO} (Scheme II).
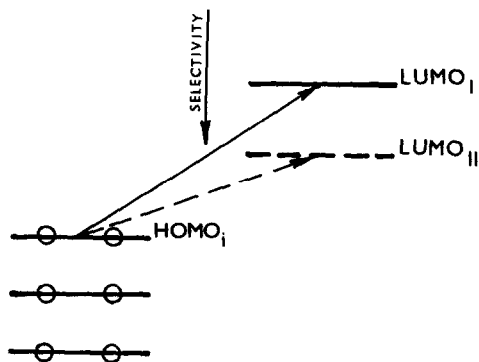
Scheme 2.

EXPERIMENTAL

Chemicals and solvents
3,5-Dinitrobenzoyl chloride and 2,4-dinitrobenzenesulphonyl chloride, both of purum grade, were supplied by Fluka (Buchs, Switzerland). Picryl chloride was prepared from dry picric acid and phosphorus pentachloride. 3-Aminopropyltriethoxysilane (Merck-Schuchardt, Hohenbrunn, F.R.G.) of reagent grade was used as obtained.

Tetrahydrofuran and toluene were dried over sodium and purified by distillation. Methanol, tetrachloromethane and dichloromethane (Lachema, Brno,

Czechoslovakia) were of analytical purity grade, as was n-heptane (Loba Chemie, Wien, Austria).

Preparation of sorbents

Twenty grams of silica gel for HPLC, Separon SI VSK, 10–15 μm (LP Praha, Czechoslovakia), were dried at 100°C for 6 h and then suspended in 100 ml of dry toluene and 4 ml of 3-aminopropyltriethoxysilane. The mixture was refluxed gently with mixing for 2 h. The product was filtered off, washed with dry toluene and methanol and dried at 60°C for 4 h; 0.92 mmol/g of amino groups were found by titration[5,10]. The product was used for the preparation of all resulting EA sorbents.

DNAP-silica was prepared according to the previously described procedure[5,10,12]. The same procedure was used for the preparation of 2,4-dinitrobenzene-sulphamidopropyl (DNSP)-silica via reaction of NH_2 groups with 2,4-dinitroben-zenesulphonyl chloride. 3,5-Dinitrobenzamido (DNBP)-silica was prepared by shaking 3 g of NH_2-silica suspended in 20 ml of dry tetrahydrofuran with 5 mmol of 3,5-dinitrobenzoyl chloride and 1 ml of dry trimethylamine for 1 h. The crude product was filtered off, washed with dry tetrahydrofuran and methanol. The sorbent was then treated with 10 ml of 1% HCl in methanol to remove the excess of trimethyl-amine. The same procedure was used for the preparation of 2,4,6-trinitroanilinopro-pyl (TNAP)-silica.

After washing with methanol and drying at 60°C, the sorbents were placed into a Soxhlet extractor and extracted with dry tetrahydrofuran for 8 h. If a protic solvent such as methanol is used for the extraction, solvolysis of Si–O–Si bonds takes place, decreasing the content of bonded ligands. Finally, the sorbents were dried at 80°C for 4 h, sieved through a mesh (30μm) and used for the preparation of columns. Elemental analysis indicated that *ca.* 85% of the NH_2 groups were converted to TNAP groups. The conversion is more than 90% for DNBP-silica and almost quan-titative for DNSP- and DNAP-silica. The high conversion into DNAP groups has also been reported by Hammers *et al.*[7].

Column preparation and chromatography

A 2-g amount of sorbent was slurried in 10 ml of tetrachloromethane under sonication for 5 min. The stainless-steel columns (LP Praha, Czechoslovakia), 250 × 4 mm I.D., were packed by the descending technique, with n-heptane at 600 atm using an air-driven pump DSF-100 (Haskel, Burbank, CA, U.S.A.).

Chromatographic measurements were performed on an apparatus comprising an Altex 110 A pump (Altex, Berkeley, CA, U.S.A.), Rheodyne 70-10 sampling valve (Rheodyne, Berkeley, CA, U.S.A.) with 20-μl loop and an UVM-4 detector (Devel-opment Workshops, Czechoslovakia Academy of Sciences, Prague, Czechoslovakia) operated at 254 nm.

n-Heptane–dichloromethane (3:1 or 10:1) was used as the mobile phase at flow-rate of 1 ml/min. Each column was equilibrated overnight with the mobile phase before measurement. Solutes were dissolved in mobile phase ($\approx$ 1 mg per 10 ml). Tetrachloromethane was used as an unretained solute to determine t_0. Each mea-surement carried out at ambient temperature was repeated three times.

TABLE I

ENERGIES OF THE LOWEST UNOCCUPIED MOLECULAR ORBITALS ε_{LUMO} (IN β UNITS) FOR SUBSTITUTED DI- AND TRINITROBENZENES

Substituent	2,4-	3,5-	2,4,6-
-O-CH$_3$	-0.3549	-0.3505	-0.3698
-NH$_2$	-0.3549	-0.3525	-0.3700
-SO$_2$ NH$_2$	-0.3549	-0.3528	-0.3700
-CH$_3$	-0.3549	-0.3532	-0.3728
-CO-NH$_2$	-0.3593	-0.3563	-0.3903

RESULTS AND DISCUSSION

The quantum chemical calculations of ε_{LUMO} for several potentially interesting acceptors have been performed by a simple Hückel method (HMO) with standard parameters proposed by Streitwieser[18] and Gerdil and Lucken[19]. The resulting values, are given in Table I. In accordance with chemical intuition, the negative values of ε_{LUMO} demonstrate an enormous ability of these EAs to accept electrons, e.g., ε_{LUMO} of the parent compound, benzene, is $+1.0$.

The complexing ability of all the EAs studied is influenced first by the number of nitro groups attached to the aromatic skeleton. The position of such groups also plays an importat rôle, as does the nature of the substituent that acts as a spacer. In this work, the short aliphatic chain $-(CH_2)_3-$ in spacers was approximated by a hydrogen atom. Thus, the $-NH_2$ group was taken instead of $-NH-(CH_2)_3-$, etc., as the corresponding HMO parameters are available only for simple substituents[18,19]. Despite the relative crudeness of the HMO model, it is evident on the basis of the calculated ε_{LUMO} values that electron donating spacers such as $-NH-(CH_2)_n-$ or $-O-(CH_2)_n-$ decrease the complexing ability of EA ligands. On the other hand, electron accepting spacers such as $-CO-NH-(CH_2)_n-$ enhance the complexing ability.
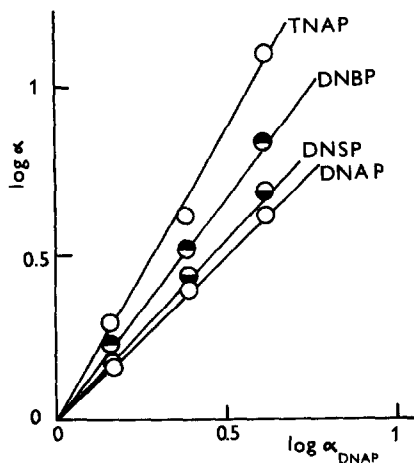

Fig. 2. Plot of log α for naphthalene (standard), fluorene, anthracene and fluoranthene. Mobile phase: n-heptane-dichloromethane (3:1). DNAP-silica is taken as a reference sorbent.

   In order to verify these conclusions we have prepared and tested three EA sorbents: DNSP-silica; DNBP-silica and TNAP-silica. Their complexing ability was compared with that of DNAP-silica reported earlier[5,7].

   The capacity factors of the tested solutes, naphthalene (ε_{HOMO} = -0.618), fluorene (ε_{HOMO} = -0.67), anthracene (ε_{HOMO} = -0.414) and fluoranthene (ε_{HOMO} = -0.618), have been measured under identical conditions; the calculated selectivities are referenced to naphthalene. DNAP-silica serves as a reference sorbent in the logarithmic plot of $\alpha_{i,1}$ shown in Fig. 2. From the slopes of the correlation lines, it is evident that the complexing ability increases in the order:

DNAP $\approx$ DNSP < DNBP < TNAP

Comparing this order with the ε_{LUMO} values given in Table I, one can see that the experimental results are in agreement with our simple model (eqn. 5) that can therefore serve as a rational basis for the synthesis of more selective and efficient EA silicas for HPLC.

   It must be pointed out that the conversion of NH_2-silica into these EA sorbents cannot be taken as an universal procedure despite the fact that the reactivity of NH_2-silica has been utilized by several authors[3,5,7]. In our case, the reaction of 3,5-dinitrobenzoyl chloride and picryl chloride with NH_2-silica does not proceed quantitatively. The unreacted NH_2 groups may block the adjacent EA ligands, decreasing the capacity and selectivity of the sorbent. The preparation of chemically bonded EAs via the corresponding silanes is therefore superior to this approach.

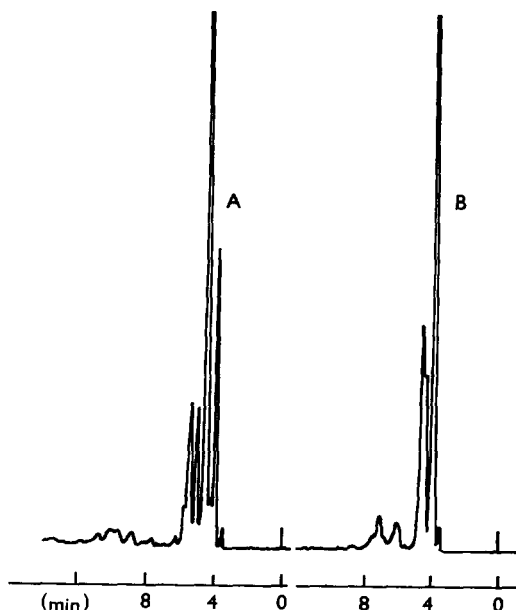   On the other hand, the reaction of NH_2-silica with 2,4-dinitrobenzenesul-

Fig. 3. Comparative separations of a mixture of chlorinated phenylbutanes on DNSP-silica (A) and DNAP-silica (B). Mobile phase: n-heptane–dichloromethane (10:1); flow-rate 1.5 ml/min.
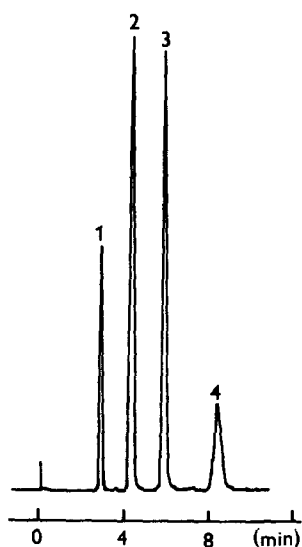
Fig. 4. Separation of benzene (1), naphthalene (2), phenanthrene (3) and anthracene (4) on DNSP-silica. Mobile phase as in Fig. 3.

phonyl chloride proceeds quantitatively according to the results of elemental analysis. The DNSP-silica prepared easily from NH_2-silica seems to be an useful sorbent for the separation of PAHs. The comparison of DNAP- and DNSP-silica in the separation of a mixture of phenylbutanes polychlorinated at the alkyl chain is shown in Fig. 3. DNSP-silica has yielded symmetrical peaks (Fig. 4); reduced plate heights, $h \approx 4$, have been obtained for several PAHs over a broad range of flow-rates. Because of the promising chromatographic properties of the above sorbent, a thorough examination of DNSP-silica is now being carried out.

CONCLUSIONS

(1) The chromatographic selectivity of chemically bonded EA phases increases with increasing electron affinity of the ligands. The experimental values of electron affinity can be approximated theoretically by a simple HMO method.

(2) The complexing ability and selectivity depends not only upon the number of electron-accepting substituents ($-NO_2$, $-Cl$, $-CN$, etc.) attached to the aromatic skeleton, but also upon their positions and the nature of the spacer.

(3) 2,4-Dinitrobenzenesulphamidopropyl silica appears to be a promising sorbent for HPLC.

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