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# STRUCTURE OF THE BONDED LAYER AND SELECTIVITY OF CHEMICALLY MODIFIED STATIONARY PHASES FOR CHROMATOGRAPHY

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## SUMMARY

Studies of the effects of the chemical nature and geometrical parameters of the support, the type of organosilicon anchor grouping, the length of the hydrocarbon chain, the nature of the modifier functional group and silanization of the support on stationary phase selectivity are reported.

## INTRODUCTION

The resolving power of chromatographic methods depends heavily on the selectivity factor. It is important that in high-performance liquid chromatography (HPLC), unlike gas chromatography (GC), the selectivity of separation is determined by both the mobile and stationary phases, so that the choice of stationary phases is relatively small.

In this paper we consider only one aspect of the separation problem, *viz.*, the influence of the stationary phase structure (chemically bonded to the silica surface) on selectivity. Among the factors responsible for the selectivity of stationary phases we may single out the following:

(1) The chemical nature of the support.

(2) Geometrical parameters of the support.

(3) The chemical nature of the bonded phase is based on: (a) the type of grouping responsible for fixation of the modifier on the support (anchor grouping), the size of the modifier molecules and the presence of polar functional groups; (b) the density of bonded molecules of modifier per unit area of the support surface (bonding density); (c) possible interactions of bonded groups between each other and with the support surface; (d) the type of bonded layer (monomolecular, polymolecular).

(4) Alterations of the stationary phase structure under temperature and solvent influences.

## EXPERIMENTAL

To determine the influence of the above factors on the selectivity of stationary phases we studied a set of chemically modified silicas that differed in the porous

TABLE I

CHARACTERISTICS OF SILICA SUPPORTS

| *Silica support* | *Specific surface area ($m^2/g$)* | *Effective pore diameter (nm)* |
|---|---|---|
| Silasorb Si-600 | 527 | 7 |
| Synthetic spherical | 420 | 9 |
| KCK-2 | 274 | 13 |
| C-3 | 250 | 14 |
| Silochrom C-120 | 115 | 40 |
| MCA-2 | 90 | 45 |
| Silochrom C-80 | 80 | 50 |

support structure, the nature of the bonded compound, the alkyl chain length and the type of anchor grouping. Table I shows their characteristics.

The modifiers used can be divided into three groups.

(1) Alkylsilanes of formula[1,2]. $ClR_2Si(CH_2)_nCH_3$, where R = Cl or $CH_3$ and $n$ = 0–15.

(2) Organosilicon compounds based on undecylenic acid of formula[3] $R_3Si(CH_2)_{10}X$, where with R = Cl or $OC_2H_5$ and X = CN, $CH_2N(CH_3)_2$, $COOCH_3$, $CH_2OCOCH_3$, $CH_2Cl$ or $CH_2OSi(CH_3)_3$.

(3) Functional organosilicon compounds with an alkyl chain containing not more than three methylene units: cyanopropyltrichlorosilane, aminopropyltrieth-oxysilane, chloropropyltrichlorosilane, etc.

The above compounds were fixed on the surface by a single-stage method at elevated temperatures[1,4].

For the synthesis of more complicated stationary phases a method for the further alteration of functional groups was used (surface assembly). This can be applied to groups such as carboxy, tertiary amino, alkoxy, amino acids and stable nitroxy radicals[4–6].

## RESULTS AND DISCUSSION

Let us consider in detail the influence of the above parameters of stationary phases on selectivity.

### *Chemical nature of the support*

The chemical nature of the support affects the separation selectivity with usage of modified silicas for two main reasons: (1) interaction of sample molecules with the active centres of the support surface and (2) alteration of the properties of the bonded compounds owing to their interaction with the active centres of the support surface.

With silica supports the main active centres are represented by silanol groups. As the concentration of surface silanol groups exceeds the theoretically expected concentration of bonded modifier molecules[7,8], the first factor will always act. To eliminate the influence of unreacted groups on the chromatographic properties of stationary phases, the most effective means is additional treatment of the modified supports with small-size silanizing molecules.

The significance of this mode is enhanced especially when it is applied to the separation of organic bases, and also to the compounds that can form strong hydrogen bonds. As an example, let us consider the sorption isotherms of benzene and nitrobenzene from water on silica modified by alkyl groups. Fig. 1 shows that additional treatment with trimethylchlorosilane decreases the sorption of the more polar nitrobenzene to a larger extent then benzene.

The sorption changes can be followed more easily for aniline–benzene with water–methanol (9:1) as the eluent[9]. On additionally silanized ODS stationary phase (obtained by treatment of silica with octadecyltrichlorosilane) the selectivity is to 0.4–0.5, whereas on the non-silanized stationary phase it is 33. Hence interaction with the support is of major importance.

In the separation of methyl-substituted phenols, whose hydroxy groups are capable of forming hydrogen bonds with the surface silanols, we can observe the same effect: additional silanization with a mixture of trimethylchlorosilane (TMS) and hexamethyldisilazane (HMDS) results in a considerable decrease in the retention of the samples and an alteration in selectivity (cyanopropyl stationary phase; mobile phase, *n*-hexane–isopropyl alcohol, 100:1)[10]. Other instances of the influence of the support will be considered when discussing the other stationary phase properties.

*Geometric characteristics of the support*

The porous structure of silica is one of the main factors affecting the properties of stationary phases, including selectivity. For instance, in the separation of large molecules in the gel chromatography of polymers, the correct choice of a porous structure proves to be the main factor determining selectivity. But does the porous structure affect the separation selectivity of smaller molecules? This question has been given almost no consideration, although in our opinion it should be taken into account. For instance, the difference in the geometric characteristics of the supports is one of the important parameters responsible for the lack of reproducibility of widely used ODS phases. Even modifiers with shorter chain lengths (10–16 carbon atoms) defy attempts to achieve the greatest possible surface density of bonded groups when using silica with an average pore diameter of 7–8 nm. Table II shows the density of cyanodecyl and hexadecyl groups bonded to support with different porous characteristics.

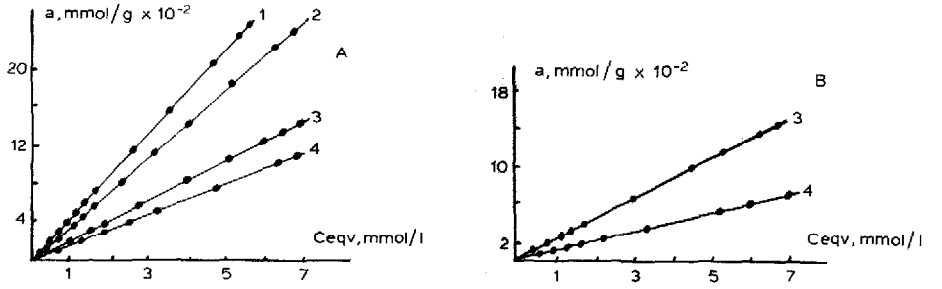



Fig. 1. Sorption isotherms of (A) benzene and (B) nitrobenzene from water on Silochrom C-80 modified by alkyltrichlorosilanes at 20°C: (1) Silochrom C-80 treated with $C_{16}H_{33}SiCl_3$; (2) as (1), additionally silanized with TMS; (3) Silochrom C-80 treated with $C_8H_{17}SiCl_3$; (4) as (3), additionally silanized with TMS.

TABLE II

EFFECT OF POROUS STRUCTURE OF SUPPORT ON BONDING DENSITY OF TRICHLOROSILANES WITH LONG HYDROCARBON CHAINS

| *Modifier* | *Silica characteristics* | | *C (%)* | *Bonding density (groups/nm²)* |
|---|---|---|---|---|
| | *Specific surface area ($m^2/g$)* | *Effective pore diameter (nm)* | | |
| $C_{16}H_{33}SiCl_3$ | 90 | 45 | 5.8 | 2.2 |
| | 115 | 40 | 8.0 | 2.2 |
| | 250 | 14 | 13.0 | 2.1 |
| | 274 | 13 | 14.5 | 2.1 |
| | 420 | 9 | 19.9 | 1.8 |
| | 527 | 7 | 18.7 | 1.4 |
| $Cl_3Si(CH_2)_{10}CN$ | 115 | 40 | 5.3 | 2.3 |
| | 229 | 14 | 10.0 | 2.3 |
| | 274 | 13 | 10.6 | 2.2 |
| | 527 | 7 | 13.4 | 1.5 |

It follows that for silica with an average pore diameter of 7 nm the bonding density does not exeed 65% of the maximum value, calculated on the basis of the dimensions of the organosilicon anchor group. However, supports with the same porous structure are frequently used to obtain ODS phases where steric hindrance is much greater.

Let us consider the simplest model of silica, modified by ODS groups. We can consider two possible cases: (a) bonded groups are stretched to the maximum length and are perpendicular to the support surface ("brush" structure); and (b) bonded groups are in an appropriate liquid condition.

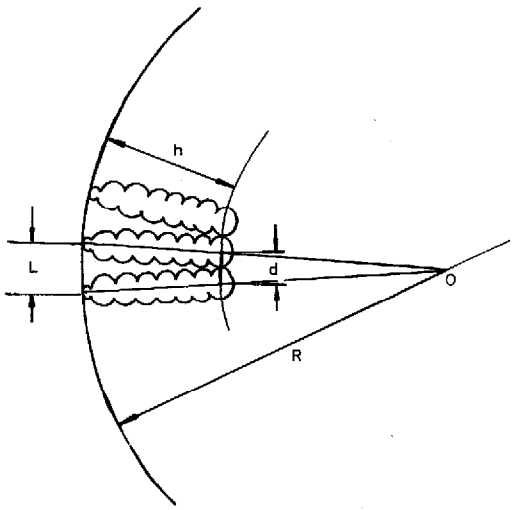



Fig. 2. Model of bonded groups within small-diameter pores.

Usually transitional conditions are used[8,11]. It is clear that irrespective of the bonded layer structure, touching of the bonded chain ends appears to be the limiting factor with regard to the increase in surface density when the pore diameter decreases. Therefore, the following equation applies (see Fig. 2):

$$L/d = R/(R-h)$$

or solving the equation for $R$ we obtain

$$R = h(1-d/L)$$

Knowing the analytical expression for the distribution of pores according to their dimensions in the initial support, one can obtain the maximum bonding density for different modifiers.

Using the above equations it is possible to solve another problem, *viz.*, to find the minimum pore diameter at which the maximum bonding density can still be obtained. In the special case of alkyldimethylsilyl phases we can introduce an experimental value $L = 0.73$[8] and $D$ equal to double the Van der Waals radius of methyl group (0.40 nm):

$$R = h/0.45$$

Table III gives the smallest pore diameter at which the maximum surface density of alkyldimethylsilyl groups can be achieved, *i.e.*, the support should not contain pores with $D$ smaller than the critical value.

Table III indicates that the maximum bonding density can be obtained only when using silica with a pore diameter larger than 10–11 nm. The application of such a support nowadays is more the exception than the rule. Most commercial ODS stationary phases are based on supports with an average pore diameter of 8–10 nm, *i.e.*, pores with diameters less than 10–11 nm predominate. This leads to a considerable increase in the amount of unmodified silanol groups, which remain partly accessible to the sample molecules. We have already demonstrated the influence that silanol groups have on the selectivity of separation. The situation is also aggravated by the fact that different support batches vary in pore diameter, thus distorting the contribution of the accessible silanol groups. We should add that experimental measurements of the thickness of the bonded layer of ODS phases showed that it does not differ much from the length of the molecule of the modifier[8], so that the 8 nm

TABLE III

SMALLEST PORE DIAMETERS AT WHICH THE MAXIMUM BONDING DENSITY OF ALKYLDIMETHYLSILYL GROUPS CAN BE ACHIEVED

| *Number of carbon atoms in n-hydrocarbon chain* | *Length of bonding chain (nm)* | *Smallest pore diameter (nm)* |
|---|---|---|
| 8 | 1.19 | 5.2 |
| 16 | 2.20 | 9.8 |
| 18 | 2.45 | 10.8 |
| 22 | 2.95 | 13.2 |

TABLE IV

EFFECT OF HYDROCARBON CHAIN LENGTH AND NATURE OF FUNCTIONAL GROUPS ON CHARACTERISTICS OF CHEMICALLY MODIFIED SILICA

| *Modifier* | *Support* | *C (%)* | *Bonding density (groups/nm²)* |
|---|---|---|---|
| $Cl_3SiC_4H_9$ | Silochrom | 2.1 | 2.3 |
| $Cl(CH_3)_2SiC_4H_9$ | C-80 | 3.0 | 2.2 |
| $Cl_3SiC_8H_{17}$ | | 3.7 | 2.2 |
| $Cl_3SiC_{16}H_{33}$ | | 6.9 | 2.1 |
| $Cl_3Si(CH_2)_{10}CN$ | Silochrom | 5.3 | 2.3 |
| $Cl_3Si(CH_2)_{10}COOCH_3$ | C-120 | 5.6 | 2.3 |
| $Cl_3Si(CH_2)_{11}OCOCH_3$ | | 5.3 | 2.0 |
| $(C_2H_5O)_3Si(CH_2)_{11}OSi(CH_3)_3$ | Silochrom | 3.8 | 1.6 |
| $(C_2H_5O)_3Si(CH_2)_3OCH_2CH{-}CH_2$ (O) | C-120 | 1.6 | 1.4 |
| $(C_2H_5O)_3Si(CH_2)_{11}N(CH_3)_2$ | | 3.1 | 1.1 |

diameter pores become halved in size under modification. Bearing in mind the solvation, it seems possible that we may anticipate the effects such as cooperative sorption even for relatively small sample molecules in pores of such a diameter. Therefore, the porosity of the support is one of main factors that should be taken into account to achieve reproducible results for the selectivity of stationary phases. In particular, to obtain an ODS phase the minimal critical diameter should be not less than 10–11 nm.

*Chemical nature of the bonded layer*

The above data show that in order to obtain reproducible properties of stationary phases we have to achieve the highest density of bonded molecules and maximal screening of silanol groups. Now, let us consider the dependence of bonding density on the molecular structure of the modifier. Table IV gives the bonding density of the modifiers with different structures on the same wide-pore support, Silochrom, whose geometric structure does not affect the bonding density.

The data in Table IV show that for wide-pore silica the chain length and the presence of functional groups have little effect on the bonding density of modifiers with chlorosilicon anchor groups. Passing to triethoxysilicons considerably reduces this value, especially for modifiers with amino groups. The latter effect is due to adsorption of the amino groups on the silica surface, which prevents the modification reaction.

It is simpler to analyse the role of anchor groups (trichlorosilyl or dimethylchlorosilyl) in the case of alkyl-bonded stationary phases. Because the sizes of these anchor groups are the same, the maximum bonding density should be the same. This is why the selectivity of the stationary phases must be identical, but only when we eliminate the influence of the support, *i.e.*, the influence of silanol groups. The experimental data confirm this. For a support with a sufficient pore diameter ($D_{pore}$

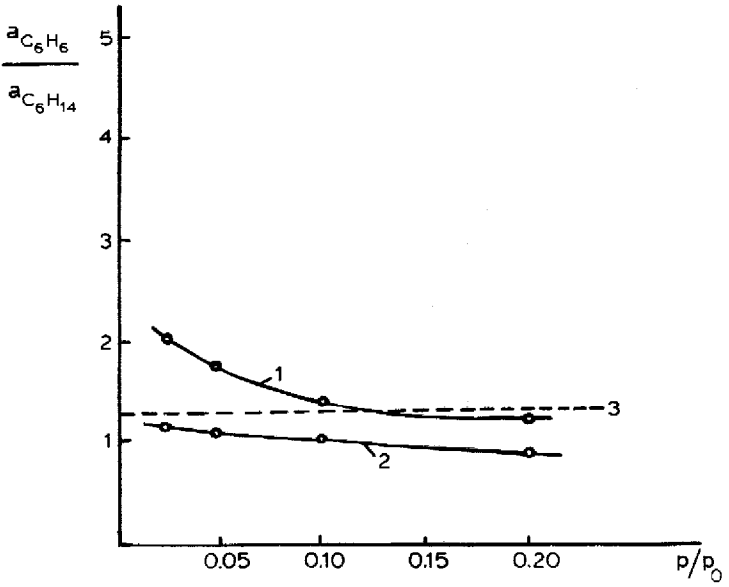



Fig. 3. Dependence of ratio of benzene to hexane sorption values on relative pressure: (1) Silichrom C-80 treated with $C_{16}H_{33}SiCl_3$; (2) as (1), additionally silanized with TMS; (3) graphitized carbon black.

> 10 nm), the selectivities of silica modified by octadecyltrichlorosilane and additionally silanized with TMS compared with a stationary phase with bonded dimethyloctadecylmonochlorosilane are almost the same[9].

A similar conclusion can be derived from isotherms of benzene and hexane sorption on these modified silicas. Fig. 3 shows the dependence of the benzene-to-hexane sorption value on the relative pressure. According to these data, the additional treatment of silica modified by $C_{16}H_{33}SiCl_3$ with TMS results in a non-specific surface resembling the surface of graphitized carbon black[2].

Of course, we refer only to stationary phases obtained under conditions that prevent polycondensation of trichlorosilanes. Polycondensed bonded phases are less reproducible and are the subject of special considerations.

Hence the process of modification by alkylchlorosilanes when one takes into account the influence of all the above factors must result in stationary phases with similar properties, irrespective of the anchor group. However, applying modern spectral methods, if needed, we may obtain data on the origin of the stationary phase. For instance, using the integral curve of $^{13}C$ NMR spectra we can calculate the ratio between the amount of bonded modifier and that of the silanizing agent. The tests carried out show aproximately 0.8 bonded TMS groups for each $C_{16}H_{33}$ group bonded to silica, which agrees with the carbon elemental analysis. Extra data can be derived with the help of the impulse NMR method (spin echo method). The natural width of the NMR line shows the mobility of the groups responsible for that width. A sample modified by $C_{16}H_{33}SiCl_3$ is characterized by a one-component falling curve of spin echo, whereas the overall falling curve for the same sample additionally silanized by TMS has two components reflecting protons of groups of different mobility. Times $T_2$ (time of spin–spin relaxation, inverse to the resonance width) for fast

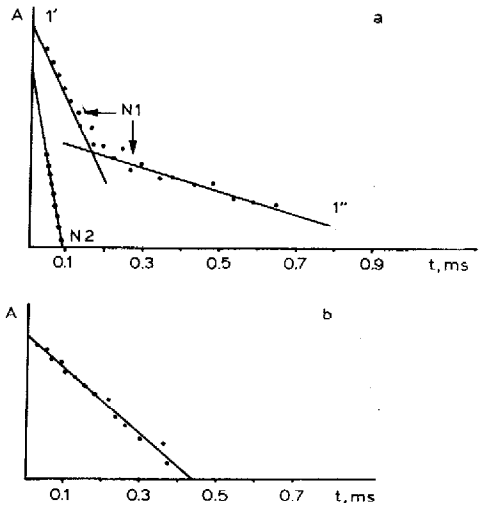



Fig. 4. Falling curves of spin-echo resonance for modified silicas. (a) KCK-2 with bonded $Cl_3SiC_{16}H_{33}$, additionally silanized with TMS; (b) KCK-2 with bonded $Cl_3SiC_{16}H_{33}$. (a) The right curve (N1) illustrates the presence of two components reflecting protons of groups of different mobility (experimental data). In order to obtain the relaxation time of the fast falling component, the left curve (N2) is calculated by subtraction of curve 1'' from curve 1'

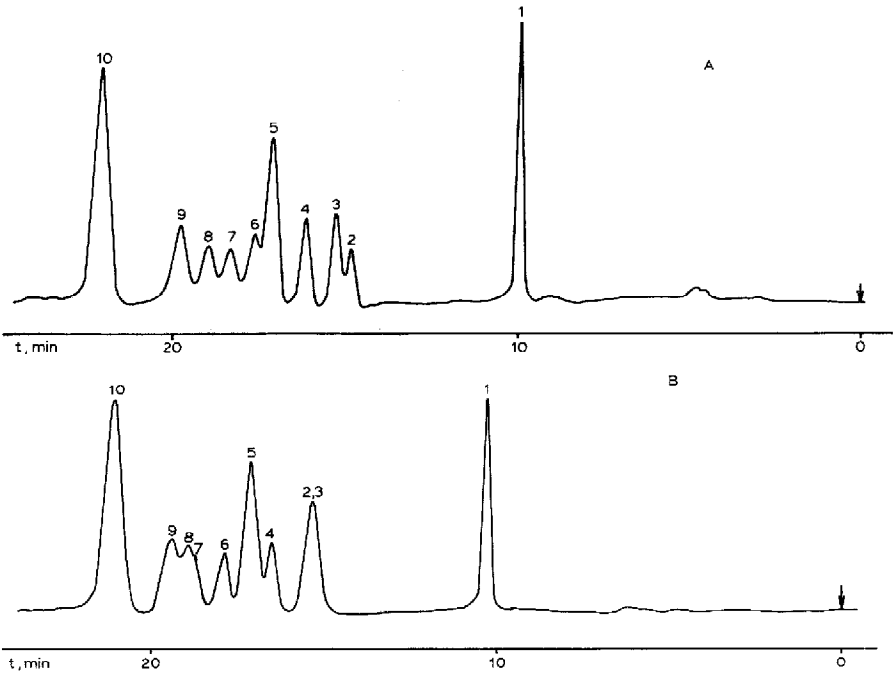
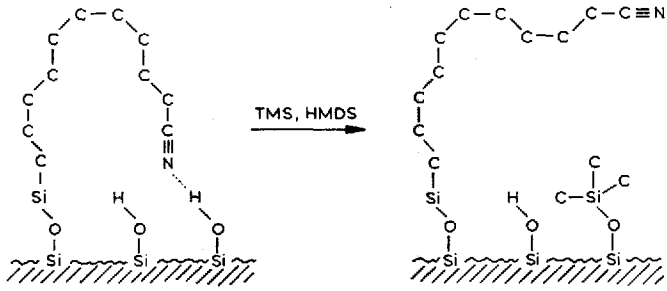



Fig. 5. Chromatograms of methyl-substituted phenols. Eluent, *n*-hexane–isopropyl alcohol (100:1); flow-rate, 1 $cm^3$/min; temperature, 20°C; column, 250 × 4.6 mm I.D.; particle size, 7.5 μm. Stationary phase: (B) laboratory-made Si-600 treated with $Cl_3Si(CH_2)_{10}CN$; (A) the same, additionally silanized with TMS–HMDS. 1 = 2,6-xylenol; 2 = 2,5-xylenol; 3 = 2,3-xylenol; 4 = 2,4-xylenol; 5 = *o*-cresol; 6 = 3,5-xylenol; 7 = 3,4-xylenol; 8 = *m*-cresol; 9 = *p*-cresol; 10 = phenol.

and slow falling components were about 0.1 and 0.44 ms, respectively (see Fig. 4).

The insertion of functional groups into the bonded layer has a drastic effect on the selectivity of the stationary phase. Unfortunately, the contribution of the functional groups is difficult to predict because in most instances they interact to some extent with silanol groups of the support. This condition of bonded groups in the bonded layer is called an "arch" structure[11,12]. For instance, it is common knowledge that the basicity of primary amines when bonded decreases 10–100-fold[13]. The alcohol groups are capable of reacting with silanol surface groups at elevated temperatures[12].

As an example of how this is manifested in chromatography we may use the chromatograms of methyl-substituted phenols on silica modified by cyanodecyl groups (Fig. 5). As shown earlier, the additional silanization of cyanopropyl phase considerably diminishes the retention. In our case the retention remains almost unchanged. This phenomenon can be easily explained if we assume that part of the most active silanol groups formed hydrogen bonds with nitrile groups, so that additional silanization has almost no effect on the concentration of sorption centres:




Consideration of the interaction of functional groups with the support surface can be profitably used in the creation of reversed stationary phases for amine separations. It is logical to assume that in the surface layer containing long-chain aminoalkyl groups the active silanols would form hydrogen bonds with the bonded amines. The virtual absence of strong adsorption centres in amines ought to have a positive effect on the peak shapes and selectivity of separation. The efficiency of stationary phases with bonded long-chain amines can be illustrated by the separation of benzene derivatives (Fig. 6A).

The application of modifiers with relatively long chains (10–12 carbon atoms) and terminal functional groups is advantageous because the separation is then determined not only by specific interactions of molecules of the sample with functional groups, but also by dispersion interactions with the hydrocarbon chains. For example, silica with carboxy groups bonded with long hydrocarbon radicals can be used in both reversed and normal-phase methods and also in ion chromatography[14].

Optimization of dispersion and electrostatic interaction contributions in the case of bonded phases with long-chain amino groups made it possible to obtain a stationary phase efficient and selective in the separation of oligonucleotides (Fig. 6B)[15].

The concentration of functional groups on the surface often has a major influence on the selectivity of separation. As an example we may consider the fact that a decrease in the concentration of chiral functional groups for by 10–100-fold alters

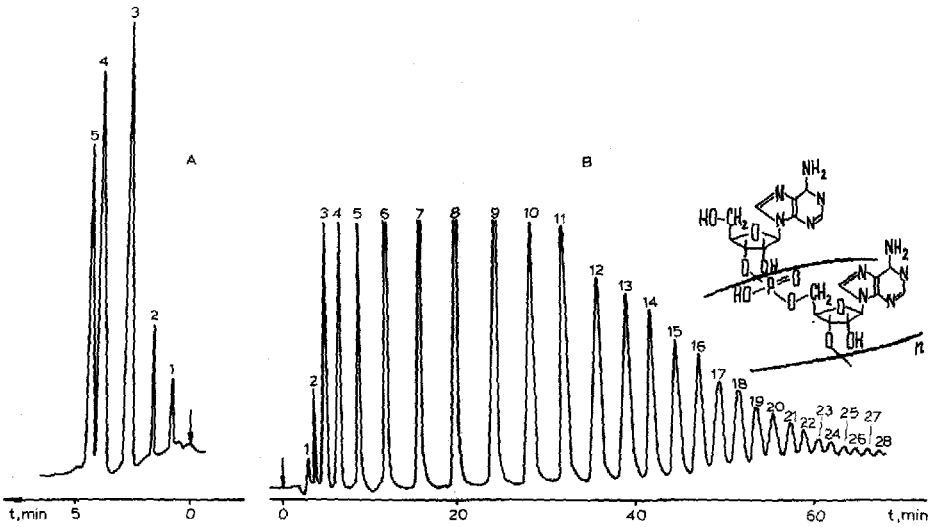



Fig. 6. Chromatograms of (A) benzene derivatives and (B) oligoriboadenilates with common formula r-A(pA)$_n$ on laboratory-made stationary phase with long-chain amine bonded to silica (KCK-2) (particle size 6 $\mu$m). (A) Column, 80 × 6 mm I.D.; eluent, acetonitrile–water (20:80); flow-rate, 1.8 cm$^3$/min; temperature, 25°C. 1 = benzoic acid; 2 = aniline; 3 = acetophenone; 4 = nitrobenzene; 5 = benzene. (B) Column, 250 × 4.6 mm I.D.; eluent, (a) 50% methanol, 0.2 *M* ammonium acetate, and (b) 70% methanol, 1.5 *M* ammonium acetate (pH 7.2), programmed at 0.5% (b) increase/min; flow-rate, 50 cm$^3$/h; temperature, 60°C.

the mechanism of bonding of the separated molecules to the stationary phase, resulting in an increase in the selectivity coefficient in the separation of enantiomers of amino acid derivatives of more than two[16].

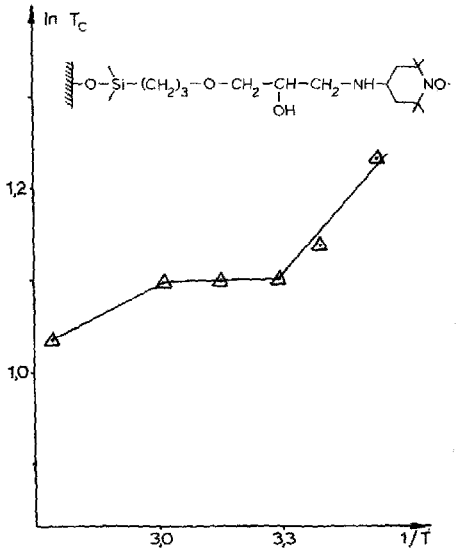



Fig. 7. Dependence of correlation time on temperature for bonded nitroxide radicals.

TABLE V

DEPENDENCE OF CORRELATION TIME OF SAMPLES MODIFIED BY NITROXIDE RADICALS ON TYPE OF SOLVENT

Sample as in Fig. 7.

| *Solvent* | *Correlation time* ($10^{-11}$ *s*) |
|---|---|
| Benzene | 12 |
| Dichloromethane | 6.8 |
| *sec.*-Butanol | 2.4 |
| Ethanol | 0.95 |

*Influence of temperature and solvent on the stationary phase structure*

To determine the influence of temperature and solvent on the stationary phase structure it is most convenient to use ESR spectroscopy. For this purpose we obtained a set of silicas modified by nitroxide radicals. Fig. 7 shows an example of the dependence of the correlation time, $\tau_c$, which characterizes the mobility of radicals, on the temperature for one of the modified silicas. It is clear that an increase in temperature from ambient to 60°C considerably changes the state of bonded groups. At ambient temperature the functional groups are strongly bonded to silanol groups and move slowly and intermittently, whereas at elevated temperatures the contribution of free motion increases, approaching the molecular state in solution. Such a rearrangement usually takes place at temperatures of 30–40°C. This may explain the characteristics of selectivity changes when the temperature is varied during the chromatographic run. Similar phenomena can also be observed on changing the eluent polarity. A transition from a non-polar solvent (benzene) to polar solvents (dichloromethane, alcohols) also leads to a greater contribution of free motion of bonded groups (Table V). In our opinion, one should not disregard this when interpreting the results of chromatographic runs in relation to the selectivity of separation.

Thus, according to the above consideration of different factors affecting the selectivity of separation, it is clear that the selectivity is determined by the totality of parameters that are negligible at first sight, which significantly change the retention values and sometimes the elution sequence of different compounds.

REFERENCES

1 S. M. Staroverov, A. A. Serdan, V. A. Malinovskii and G. V. Lisichkin, *Zh. Vses. Khim. Ova.*, 24 (1979) 296.
2 A. V. Kiselev, G. V. Lisichkin, Yu. S. Nikitin, A. A. Serdan, S. M. Staroverov and N. K. Shonia, *Zh. Fiz. Khim.*, 57 (1983) 1829.
3 S. M. Staroverov, P. N. Nesterenko and G. V. Lisichkin, *Zh. Obshch. Khim.*, 49 (1979) 2487.
4 S. M. Staroverov, P. N. Nesterenko and G. V. Lisichkin, *Vestn. Mosk. Univ., Ser. II, Khim.*, 21 (1980) 370.
5 G. V. Lisichkin and S. M. Staroverov, *Vestn. Mosk. Univ., Ser. II, Khim.*, 21 (1980) 307.
6 V. A. Malinovskii, S. M. Staroverov and G. V. Lisichkin, *Vestn. Mosk. Univ., Ser. II, Khim.*, 25 (1984) 80.
7 I. Yu. Babkin and A. V. Kiselev, *Zh. Fiz. Khim.*, 36 (1962) 1326.
8 G. E. Berendsen, K. A. Pikaart and L. de Galan, *J. Liq. Chromatogr.*, 3 (1980) 1437.

9 H. Engelgardt, B. Dreyer and H. Schmidt, *Chromatographia*, 16 (1982) 11.
10 S. M. Staroverov, G. V. Lisichkin and E. L. Styskin, *Chromatographia*, 19 (1986) in press.
11 S. M. Staroverov, Yu. S. Nikitin and G. V. Lisichkin, *Zh. Fiz. Khim.*, 56 (1982) 2813.
12 N. K. Shonia, S. M. Staroverov, Yu. S. Nikitin and G. V. Lisichkin, *Zh. Fiz. Khim.*, 58 (1984) 702.
13 G. V. Kudryavtsev and G. V. Lisichkin, *Zh. Fiz. Khim.*, 55 (1981) 1352.
14 G. V. Lisichkin, S. M. Staroverov, A. A. Serdan, A. I. Ageev and Ya. I. Yashin, *Neftekhimiya*, 23 (1983) 712.
15 G. V. Lisichkin and S. M. Staroverov, *Zh. Vses. Khim. Ova.*, 28 (1983) 47.
16 B. Feibush, M. J. Cohan and B. L. Karger, *J. Chromatogr.*, 282 (1983) 3.