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CHARACTERIZATION OF A NEW DEHYDROXYLATED REVERSED-PHASE MATERIAL

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SUMMARY

Reversed-phase (RP) silicas prepared by reacting silica with disilazanes or disiloxanes at temperatures of about 350°C under so-called high-temperature silylation conditions were compared with RP silicas prepared under "normal" conditions, at 139°C, and with commercially available materials, by determining the surface concentration of organic and hydroxyl groups and also their properties in liquid chromatography using both *n*-heptane and methanol-water mixtures as eluents. Relative to the conventional RP materials, the RP silicas prepared at 350°C show no marked differences in the surface concentration of the organic groups, but their hydroxyl group content is greatly reduced. This results in a different chromatographic behaviour for solutes with different polarities.

INTRODUCTION

In reversed-phase chromatography a hydrophobic packing material, produced by silylation of silica, is most often used. The degree of hydrophobicity, which influences strongly the chromatographic properties of the packing, depends on both the type and the surface concentration of the bonded organic groups and on the concentration of the unreacted silanol groups. The density of hydrophobic organic groups bonded to silica is limited by steric hindrance, and it was found that the concentration of such groups cannot be increased above 4 $\mu\text{mol m}^{-2}$, either by optimized silylation or by a post-treatment of the silylated material, so-called capping^{1,2}; this means that about half of the silanol groups present on "naked" silica remain on the surface and act during the chromatographic process.

Scott and Kucera³ employed a thermal treatment for reducing the silanol group concentration on silica. The effectiveness of this treatment for making reversed-phase materials has been proved by several authors. El Rassi and Gonnet⁴ found that a low number of silanol groups present after thermal pretreatment restricts the number of bonded organic groups, whereas Van de Venne⁵ found that a thermal pretreatment up to 400°C diminishes only the number of silanol groups determined by infrared spectroscopy but not the carbon content. According to Unger *et al.*⁶ it seems that a fully hydroxylated surface is optimal for maximum coverage with organic groups.

Following extensive work on the development of inert, high-temperature silylated glass capillaries⁷⁻¹⁰, our accumulated experience has allowed the translation of this silylation process to high-performance liquid chromatographic (HPLC) silica and the preparation of a new reversed-phase (RP) material with a greatly reduced residual silanol content. From the results of this work an explanation for the outstanding effectiveness of high-temperature silylation in the hydrophobization of silica and glass capillaries can be proposed.

EXPERIMENTAL

Materials and reactions

LiChrosorb Si 100, 10 μm (E. Merck, Darmstadt, G.F.R.) with a specific surface area, S_{BET} , of 300 $\text{m}^2 \text{g}^{-1}$ was used as starting material for the surface modification. LiChrosorb RP-8, 10 μm (Merck) and RP-18 silica, 10 μm (courtesy of Professor Engelhardt, Saarbrücken, G.F.R.) were also included in the investigations. The reagents di-*n*-hexyltetramethyldisilazane (DHTMDS), di-*n*-decyltetramethyldisilazane (DDTMDS) and di-*n*-hexyltetramethyldisiloxane (DHTMDO) were prepared from dimethyldichlorosilane by Grignard reaction with *n*-hexyl bromide and *n*-decyl bromide, respectively, and subsequent ammonolysis or hydrolysis of the purified *n*-alkyldimethylchlorosilanes¹⁰. Analytical grade solvents (VEB Laborchemie, Apolda, G.D.R.) were used in all the experiments.

Prior to the reaction, silica was dehydrated under vacuum at a temperature of 180°C for 2 h. The high-temperature silylation of silica was carried out at about 350°C in a glass ampoule. 0.01 mole reagent and 1 g of the dehydrated silica placed in an appropriate frit were put successively into the ampoule. With the ampoule in a vertical position, it was flushed with nitrogen, sealed and placed in an oven. The oven was heated at a rate of 4° min^{-1} to 350°C. After about 15 h of reaction with the vapour of the reagent the oven was cooled and the ampoule was opened. The pale brownish product was poured onto a vacuum filtering funnel, sucked dry and subsequently washed with 80 ml each of toluene, dichloromethane and acetone. Finally, the RP silica was dried under vacuum at 80°C for 4 h prior to use.

For comparison purposes, other RP silicas were also prepared with the same silylating reagents in boiling *m*-xylene. One gram of LiChrosorb Si 100 was allowed to react with 0.01 mole reagent dissolved in 40 ml dried *m*-xylene at 139°C for 8 h. The reaction mixture was then treated as described above.

Physical and chemical characterization of the RP silicas

Elemental analysis of the RP silicas prepared was followed by the determination of their residual hydroxyl group content, the pH value of their suspension and their reflection IR spectrum.

A simple method, based on the reaction of OH groups with methylolithium, was developed for the determination of the silanol content of both the "naked" and modified silicas. The method has been described in detail¹¹. Essentially, the dehydrated silica or RP silica sample (about 50 mg) was allowed to react with a solution of methylolithium in dry di-*n*-butyl ether using a 3-ml glass vessel connected to a glass capillary (ca. 10 m \times ca. 0.4 mm I.D.). The amount of methane formed was estimated by measuring the displacement of the di-*n*-butyl ether plug in the capillary. The

surface concentration of silanol groups, α_{OH} ($\mu\text{mole m}^{-2}$), can be calculated from the amount of methane

$$\alpha_{\text{OH}} = \frac{N \cdot 1000}{E \cdot S \cdot \left[1 - \frac{P_c (M - 1)}{n_c \cdot 1200} \right]}$$

where N is the amount (μmole) of methane formed, E the weight (mg) of the RP or silica sample, S the specific surface area ($\text{m}^2 \text{g}^{-1}$) prior to the modification, P_c the carbon content (% w/w) of the RP sample, n_c the number of carbon atoms in the bonded organosilyl group and M the molecular mass of this group. The relative standard deviation of the method is about 6%.

The pH value of 1% (w/w) suspensions of the silicas in isopropanol-0.05 M aqueous potassium nitrate solution (1:1, w/w) was determined by a glass electrode and a digital pH meter (Radelkis, Hungary), calibrated with aqueous buffer standards.

Chromatographic characterization of the RP silicas

For the liquid chromatographic characterization of the small silica samples available, a micro-HPLC apparatus was built using vitreous silica capillaries as column material (courtesy of Scientific Glass Engineering, Australia). The packed capillaries were connected via home-made T-pieces to the injection valve (home-made) fitted with a 2- μl sample loop and to the UV detector Model UV-50 (Varian, U.S.A.). The T-pieces serve for splitting the sample before it enters the column, and for the addition of a make-up flow at the exit of the column (prior to the detector cell). The eluent delivery lines, injection valve and column were placed in a cylindrical, thermally-isolated box maintained at $23 \pm 0.3^\circ\text{C}$. A Model FR-30 pump (Knauer, G.F.R.) was used to deliver the eluent. The vitreous silica capillaries ($300 \times 0.33 \text{ mm}$ I.D.) were slurry packed, using freshly purified tetrachloromethane as suspension liquid and n -heptane as pumping liquid.

The packing density of the columns was calculated from the weight of the dried material measured after unpacking the columns and from the volume of the capillary.

For the HPLC separations on 4 mm I.D. columns a chromatograph Model Liquochrom OE 307 (Labor-MIM, Hungary) was used.

RESULTS AND DISCUSSION

LiChrosorb Si 100 samples were modified by silylation with DHTMDS, DDTMDS and DHTMDO, respectively, under "normal" conditions at 139°C , called here "low-temperature silylation" (LTS), and at 350°C , called here "high-temperature silylation" (HTS), yielding RP-6 and RP-10 chemically bonded phases. In order to describe the true physicochemical properties of the RP silicas, it is necessary that, along with the number of organic groups, the residual hydroxyl groups which contribute to the retention of even weakly polar solutes¹²⁻¹⁵ should also be determined. The silanol content was determined by the methyl lithium method¹¹ (*cf.*, Experimental). The surface concentration of the hydroxyl groups of the "naked" silica, Li-

Chrosorb Si 100, $\alpha_{\text{OH}_\sigma}$ was $7.3 \pm 0.5 \mu\text{mole m}^{-2}$. This value is in reasonable agreement with the generally accepted average value for silica: $8 \mu\text{mole m}^{-2}$ (refs. 5 and 16–19). The somewhat lower value found might be explained by considering that some OH groups in the micro-pores are inaccessible to the solvated methyllithium molecules¹⁷.

The results of elemental analysis and OH group determination for our LTS and HTS samples, as well as for the RP-8 (Merck) and the RP-18 (Engelhardt) material, are summarized in Table I. A comparison of both the carbon content and the concentration of organosilyl groups, α_C , calculated by the formula given by Berendsen *et al.*² for the RP-6 and RP-10 materials shows that the values obtained for LTS phases 1 and 5 are in the expected range^{2,20,21}. Their α_C values are on average 0.7% (absolute) smaller than those of the reference RP silicas 8 and 9. It is interesting that the α_C values of the HTS materials are not drastically different from those of the LTS materials which suggests the presence of a bonded monolayer. This conclusion is supported by the comparison of the IR spectra of LTS and HTS RP silicas. The IR bands in the 2900–3000 cm^{-1} range, which are typical for the stretching vibration of C–H, are nearly identical in shape and intensity for the LTS and HTS materials.

The effect of HTS on α_C changes with the reagent used. HTS, using DHTMDS as reagent, produces increased surface concentrations (2 and 3) close to the highest possible value^{2,22}. Compared to the corresponding LTS product, smaller surface concentrations are obtained with DDTMDS (6 and 7). The RP-6 material prepared by HTS using DHTMDO (4) also has lower values. All phases prepared by HTS

TABLE I
SURFACE CONCENTRATIONS, α , ON RP SILICAS

For the calculation see text.

Silica	Reaction procedure	Reagent	Further treatment	No.
RP-6	LTS	DHTMDS	—	1
	HTS	DHTMDS	—	2
	HTS	DHTMDS	2 h in boiling water	2a
	HTS	DHTMDS	—	3
	HTS	DHTMDS	2 h in boiling methanol	3a
	HTS	DHTMDO	—	4
RP-10	LTS	DDTMDS	—	5
	HTS	DDTMDS	—	6
	HTS	DDTMDS	—	7
RP-8	Merck**			8
RP-18	Engelhardt**			9
Si 100	Thermal treatment at 350°C for 15 h		—	10
RP-6	LTS	DHTMDS	—	10a

* $\alpha_{\text{OH}_0} = 7.3 \pm 0.5 \mu\text{mol m}^{-2}$.

** For the calculations a specific surface area of 300 $\text{m}^2 \text{g}^{-1}$ and the α_{OH_0} value given above for the starting material were used.

with disilazanes contain an average of 0.3–0.5% (w/w) nitrogen. This value can be reduced almost by a factor of two by refluxing the material with water or methanol for 2 h (2a and 3a). A similar finding was reported by Aue and Wickramanayake²³ using silazanes as silylating agents. The reduction of the nitrogen content does not simultaneously decrease the carbon content, which means that the organosilyl groups are not bonded to the silica surface via nitrogen atoms. The nitrogen content found may result from sorbed ammonia or ammonium ions evolved during HTS.

It is interesting to consider the silanol group concentration, α_{OH} , along with α_C and the original value, α_{OH_0} , of the parent silica. The difference between α_{OH_0} and the sum of α_{OH} and α_C gives $\Delta\alpha$ shown in the last column of Table I. As expected, the silanol concentration is about $4 \mu\text{mole m}^{-2}$ for the LTS RP materials 1 and 5, and for the materials 8 and 9. The $\Delta\alpha$ values are scattered around zero, well within the range of the experimental error. This means that all silanol groups detected in the starting material are still present after modification, either as organosilyl groups or as unreacted silanols.

In the case of HTS materials, a much lower α_{OH} value is obtained resulting in high $\Delta\alpha$ values between $2.5 \mu\text{mole m}^{-2}$ (2) and $3.4 \mu\text{mole m}^{-2}$ (7). This can be explained, for example, by postulating the formation of a new surface structural element. As discussed in ref. 9 this element could be a siloxane bridge formed by a reaction of a bonded organosilyl group with a neighbouring silanol group in an elimination-type reaction. Only bonded OH groups should have a tendency to enter into such reactions. There is indeed a correlation between the value of 46% bonded OH groups

Content (% , w/w)			Surface concentration ($\mu\text{mole m}^{-2}$)			
Carbon	Hydrogen	Nitrogen	Organic groups, α_C	Hydroxyl groups, α_{OH}	$\Sigma\alpha = \alpha_C + \alpha_{OH}$	$\Delta\alpha = \alpha_{OH_0} - (\alpha_C + \alpha_{OH})^*$
7.81	1.87	0.00	3.1	4.3	7.4	-0.1
9.00	2.00	0.52	3.6	1.2	4.8	2.5
8.98	2.00	0.21	3.6	1.0	4.6	2.7
9.26	2.03	0.43	3.7	1.2	4.9	2.4
9.35	2.13	0.28	3.8	0.7	4.5	2.8
5.97	1.40	0.04	2.3	1.7	4.0	3.3
10.43	2.25	0.02	2.8	4.5	7.3	0.0
7.87	1.76	0.30	2.1	2.2	4.3	3.0
7.20	1.67	0.31	1.9	2.0	3.9	3.4
11.12	2.30	0.00	3.8	3.4	7.2	0.1
19.41	3.88	0.00	3.6	3.7	7.3	0.0
0.00	0.00	0.00	0.0	5.5	5.5	1.8
7.06	1.54	0.00	2.7	2.4	5.1	2.2

TABLE II

k' VALUES OF TEST COMPOUNDS USING DIFFERENT RP MATERIALS AND COLUMN PACKING DENSITIES

Eluent: *n*-heptane, 10 ppm water.

Solute	No. of column			
	I	II	III	IV
	Phase			
	LiChrosorb RP-8	RP-6, LTS (1)	RP-6, HTS (3)	RP-6, HTS (4)
	Packing density (g cm^{-3})			
	0.42	0.37	0.36	0.38
Benzene	0.03	0.01	0.01	0.00
Nitrobenzene	0.18	0.08	0.07	0.13
Benzaldehyde	0.96	0.63	0.14	0.45
<i>o</i> -Nitroaniline	2.95	1.95	0.46	0.56
Phenol	3.97	3.31	0.57	0.96
Benzyl alcohol	7.04	3.98	0.74	1.34

reported by Davydov *et al.*²⁴ which corresponds to $3.4 \mu\text{mole m}^{-2}$ for the LiChrosorb Si 100 used, and the average $\Delta\alpha$ value of $3.0 \mu\text{mole m}^{-2}$ for the HTS RP materials.

The siloxane bridges formed, however, should be strained. Therefore it is surprising that they do not undergo hydrolysis or methanolysis during post-reaction with water or methanol (2a and 3a). It may be that this new surface site is more complicated in nature than envisioned here.

The hydroxyl group concentration decreases after treatment with water or methanol in correspondence with the decreased nitrogen content. This might imply that the true hydroxyl group concentration in the HTS materials is still smaller (and those of the new surface sites higher) because nitrogen—if present as ammonia—may also react with the methyl lithium reagent, leading to erroneous results. The information available at this point does not lead to an unequivocal explanation of the observed data.

We attempted to produce a RP-6 material with a low content of silanol groups by a thermal pretreatment according to El Rassi and Gonnet⁴ and van de Venne⁵ (10 and 10a of Table I). This treatment reduces the silanol content to a value of $5.5 \mu\text{mole m}^{-2}$ (10). The following low-temperature silylation leads to an α_c value of $2.7 \mu\text{mole m}^{-2}$ and an α_{OH} value of $2.4 \mu\text{mole m}^{-2}$ (10a), which means that the good results of HTS are not obtained.

For a preliminary chromatographic characterization of the residual silanol groups, several aromatic compounds of different polarities were chromatographed using *n*-heptane (adjusted to 10 ppm water) as eluent because the retention in such a system is mainly caused by silanophilic interactions¹⁵. In Table II the resulting capacity factors (k') for the RP-6 materials and LiChrosorb RP-8 are summarized. Note that the standard deviation of the k' values at about 0.5 is lower than 10%.

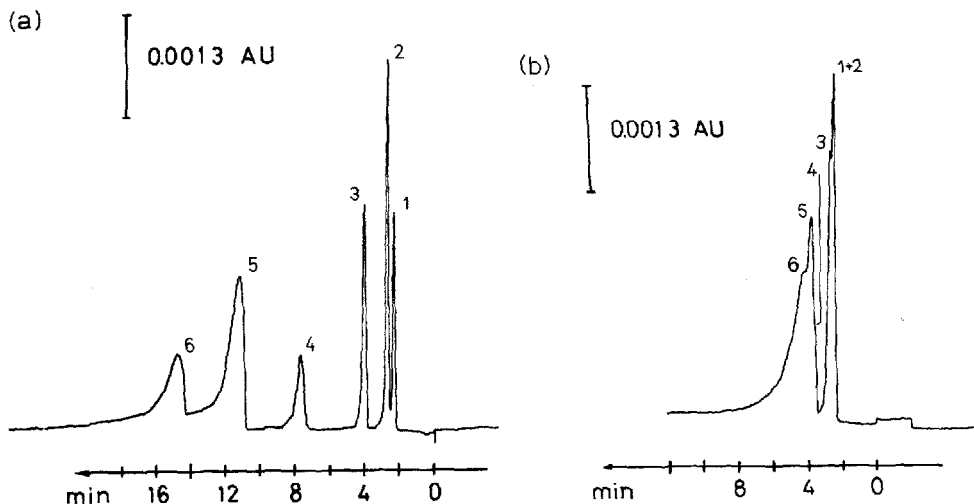


Fig. 1. The effects of residual hydroxyl groups using *n*-heptane as eluent (10 ppm water). Column: 300 × 0.33 I.D., vitreous silica capillary packed with LTS RP-6 (1), chromatogram a, and HTS RP-6 (3), chromatogram b. Flow-rate: 20 μl min⁻¹. Solutes: 1 = benzene; 2 = nitrobenzene; 3 = benzaldehyde; 4 = *o*-nitroaniline; 5 = phenol; 6 = benzyl alcohol.

The packing densities of the columns are also included in Table II. It can be seen that at nearly identical packing densities the *k'* values of the more polar test compounds are very small on the RP materials 3 and 4, indicating a low residual silanol group content in direct correspondence with the small α_{OH} values given in Table I. The strong effect of the reduced silanol group content produced by HTS is also illustrated by the two chromatograms in Fig. 1.

The different properties of LTS and HTS materials also became evident using methanol-water mixtures as eluents. According to Tanaka *et al.*¹⁵ the change in group contribution to the retention caused by different numbers of silanol groups is

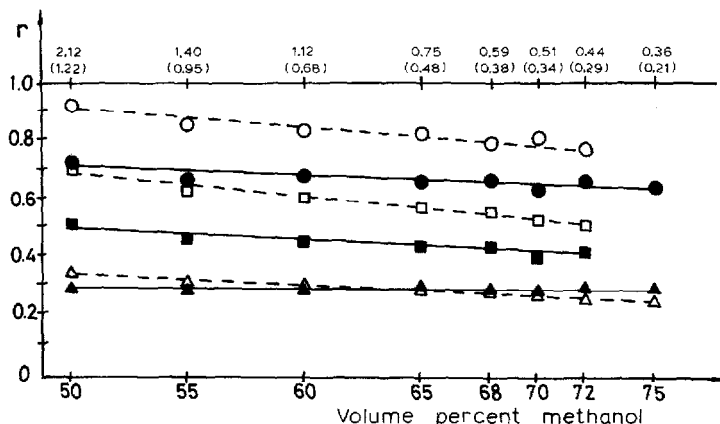


Fig. 2. *r* values ($r = k'_x/k'_{benzene}$) as a function of the methanol content of the aqueous eluent for nitrobenzene (○, ●), *o*-nitroaniline (□, ■) and phenol (Δ, ▲); open symbols for LTS RP-6 (1), full symbols for HTS RP-6 (3). The *k'* values of benzene are shown on the line at *r* = 1.0 (LTS values in brackets).

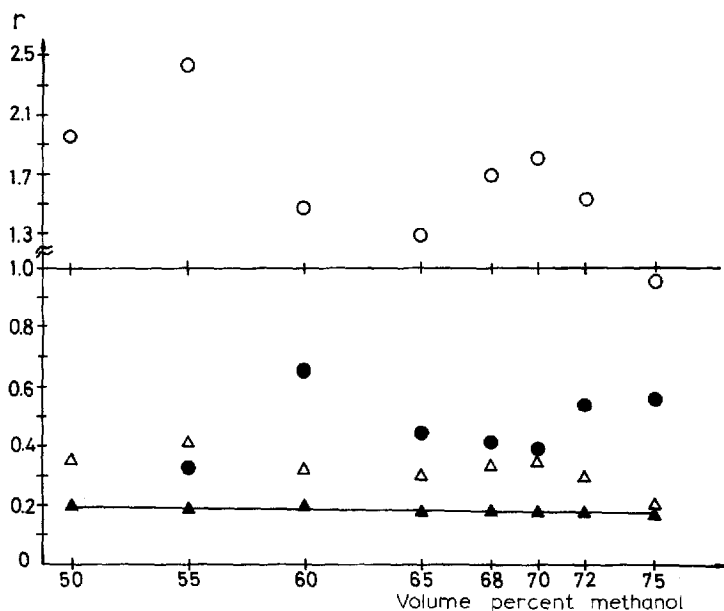


Fig. 3. r values as a function of the methanol content of the aqueous eluent for pyridine (○, ●) and aniline (△, ▲). Symbols as in Fig. 2.

relatively high in methanol-water mixtures. The k' values, for example, of benzene (*cf.*, Fig. 2) measured on the HTS RP-6 column III are about 1.5 times higher than those on the LTS RP-6 column II. It is not clear whether this is caused only by the different α_C and α_{OH} values of the RP materials 3 and 1, or partly by an additional hydrophobic interaction of the new surface site (siloxane bridges) with the solutes. Young²⁵ reported that siloxane bridges on the silica surface are essentially hydrophobic.

The effect of these different materials on the retention of polar and weakly basic solutes, x , is illustrated in Figs. 2 and 3 where the relative retentions, $r = k'_x/k'_{\text{benzene}}$, are plotted against the solvent composition. Note that, in order to stress

TABLE III

pH VALUES OF A 10-mg SAMPLE SUSPENDED IN A MIXTURE OF 0.5 g ISOPROPRANOL AND 0.5 g OF 0.2 M AQUEOUS KNO_3

Sample	No. in Table I	pH
Solvent mixture	—	6.25 ± 0.02
LiChrosorb Si 100	—	5.67
RP-6 LTS	1	6.44
RP-6 HTS	3	6.83
RP-6 HTS	4	6.25
RP-10 LTS	5	6.75
RP-10 HTS	6	6.42
RP-8	8	4.53
RP-18	9	6.82

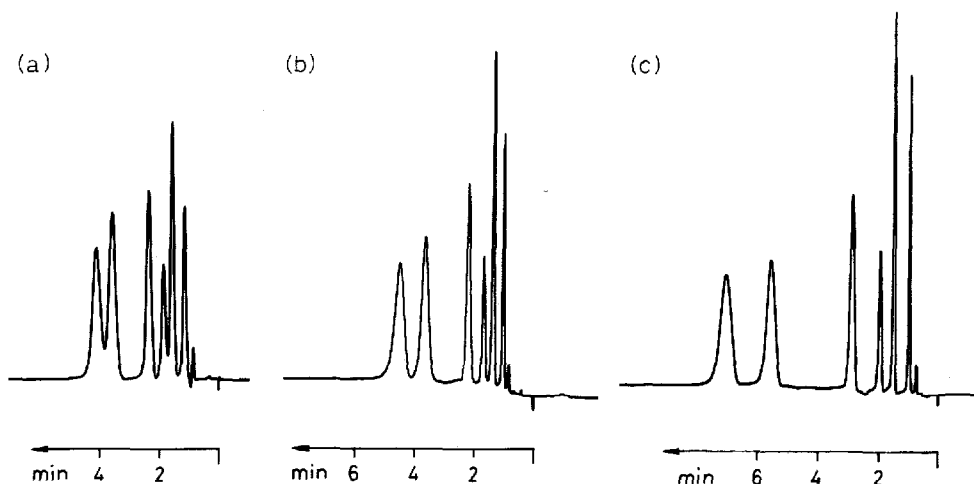


Fig. 4. Separation of phenol, anisole, phenetole, naphthalene, fluorene and anthracene (in order of elution) on LTS RP-6 (1) (a), HTS RP-6 (3) (b) and LiChrosorb RP-8 (c). Column: 100×4 mm I.D. Eluent: methanol-water (60:40, v/v). Flow-rate: 1.2 ml min^{-1} . Detection: 254 nm.

the silanophilic interactions, the solvents were not buffered. The relative retentions obtained on the OH group-rich LTS RP-6 material are generally higher (except for some values of phenol) than those obtained on the HTS RP-6 material due to the higher contribution of silanophilic interactions to the retention. This contribution is especially large in the case of pyridine, where the retention order is reversed. That the increased r values determined on LTS material are to be attributed only to the higher surface hydroxyl group concentration and not to the different acidities of LTS and HTS RP silicas can be seen from the pH values of their suspensions which differ only slightly (Table III). In the r versus methanol concentration plot, straight lines are obtained for nitrobenzene, *o*-nitroaniline and phenol (Fig. 2), but curves for pyridine and aniline (Fig. 3). This is not surprising because several factors^{14,22,26-29} contribute to the retention of basic substances which are influenced in very different ways by the methanol concentration of the eluent. The straight lines obtained for nitrobenzene, *o*-nitroaniline and phenol in case of LTS RP-6 (column II) show a negative slope. This means that the selectivity changes with changing eluent composition owing to the relatively high amount of surface silanol groups. These negative slopes become smaller or approach zero in the case of HTS RP-6 (column III), indicating that on such, more hydrophobic materials different methanol contents change only the elution power of the solvent, not the selectivity.

These characteristics of the HTS materials are especially obvious in the case of basic solutes. The so-called irregularities in retention behaviour¹² become smaller for pyridine and negligible for aniline, resulting in a straight line. This means that the contribution of the OH groups to the retention is largely suppressed using HTS material.

Beside the special selectivity discussed above, the new material should also have a high efficiency for use in HPLC. As illustrated by the chromatograms of Fig. 4, the HTS RP-6 material exhibits practically the same efficiency as the comparable LTS RP-6 material or LiChrosorb RP-8.

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