Journal of Chromatography, 218 (1981) 395–407
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,074

CHROMATOGRAPHIC CHARACTERIZATION OF SILICA SURFACES

HEINZ ENGELHARDT* and HARTMUT MÜLLER
Angewandte Physikalische Chemie, Universität des Saarlandes, Saarbrücken (G.F.R.)

SUMMARY

Columns packed with silica differ not only in their phase ratio, specific surface area per unit column volume and volume of mobile phase, but also in the pH of the silica surface. The influence of this surface pH on the separation of solutes with basic or acidic groups in dichloromethane as eluent is demonstrated. Of the commercially available silicas the irregular ones are, as expected, neutral or weakly acidic, whereas the spherical ones are either acidic (pH $\approx$ 4) or basic (pH $\approx$ 9). It is shown that the pH of the silica can easily be adjusted in order to achieve the required and optimal selectivity. These properties contribute to the selectivities of chemically bonded stationary phases based on silica.

INTRODUCTION

In chromatography, silica is the most widely used stationary phase, as a polar phase in classical liquid chromatography and as carrier for chemically bonded stationary phases, *e.g.*, for reversed-phase systems. It is commonly accepted[1–5] that its chromatographic properties depend on its specific surface area, its specific pore volume, its average pore diameter and the concentration of silanol groups per unit surface area. Because silica is a colloidal system, changes in absolute and relative retention have been traced to alterations in its chemical surface properties, sometimes called its "history"[5]. Silicas for high-performance liquid chromatography (HPLC) are sold under a variety of trade names, such as LiChrosorb, LiChrospher, Nucleosil, Partisil, Porasil, Spherisorb, Spherosil and Zorbax. They are prepared by different procedures, starting from sodium silicates, silicon tetrachloride, silicic acid esters, etc.[3,4]. The physical properties of these silicas are similar, especially if the specific surface areas per unit column volume are compared[6]. The column efficiencies obtainable have been the main aim in comparing the different brands to evaluate the relative advantages and disadvantages of spherical *versus* irregular particles[7,8]. Column efficiency is mainly affected by the average particle diameter and its distribution, and only partially by differences in physical properties such as surface area and pore diameter.

Comparing several silicas with specific surface areas between 8 and 400 m^2/g and average particle diameters between 6 and 50 μm for adequate resolution, peak capacity and time of analysis, Scott[9] demonstrated that to a first approximation the

0021-9673/81/0000–0000/$02.50

retention of test solutes increases with increasing surface area. No explanation was given for the observed deviations from this rule. From the results, it was concluded that for optimal chromatographic conditions at least four different silicas with surface areas of *ca.* 15, 75, 150 and 300–400 m^2/g should be available.

Huber and Eisenbeiss[10] demonstrated, for several silicas with specific surface areas spanning more than two orders of magnitude, that the capacity ratios (k') depend solely on the specific surface area, whereas the selectivity is fairly constant. Of course, in this comparison the k' values had been standardized for differences in the phase ratios in the different columns. In adsorption chromatography the phase ratio correlating k' and the "adsorption coefficient" is determined by the specific surface area (O_{spec}, m^2/g), the packing density (ϱ, g/cm^3) of the stationary phase and the fraction of mobile phase, ε_T, in the column. On the other hand, the selectivity, α, for the separation of adjacent pairs of the homologous series of *m*-phenylenes, *e.g.*, for *m*-sexi- and *m*-quinquephenylene, increases from 2.5 to 3.4 when the specific surface area decreases from 280 to 8 m^2/g. This may be caused by differences in the "adsorption coefficient" due to changes in the silanol concentration or in the chemical nature of the surface.

The chemical nature of the silica surface influences the retention of hydrocarbons, nitro compounds and ethers in non-polar eluents[9,10], it affects the peak shape and solute retention of medium-polar compounds in non-polar to medium-polar eluents and certainly influences the preparation and properties of chemically bonded phases. Therefore, in this work we have attempted to characterize the surface properties of some commercially available silicas by measuring the retention of non-polar to polar solutes in a non-polar eluent.

EXPERIMENTAL

Chromatographic conditions

A liquid chromatograph consisting of an M6000A pump (Waters Assoc., Milford, MA, U.S.A.), a Rheodyne 7125 sampling valve (Kontron, Munich, G.F.R.) and a 254-nm UV detector (home-built) were used. To equilibrate and to control the water content of the eluent dichloromethane, a moisture control system (MCS)[11] was used as an eluent reservoir. To maintain a water concentration of 100 ppm the funnel of the MCS was filled with 300 g of alumina (Woelm Pharma, Eschwege, G.F.R.) coated with 1% (w/w) of water. This is sufficient to equilibrate up to 500 ml of dichloromethane. The temperature of the MCS, of the eluent and of the column was maintained at 25 ± 0.1°C by a water thermostat (Haake, Karlsruhe, G.F.R.). The eluent was first equilibrated by recycling through the MCS. The columns had been equilibrated with the eluent for 14–16 h at a flow-rate of 2 cm^3/min, which was used also during chromatography. Stainless-steel columns[12] (25 × 0.41 cm I.D.) were packed as described earlier[13]. The volumes of the empty columns were determined volumetrically. The packing density was measured gravimetrically after unpacking the column and evaporation of the eluent. The samples (mono- and bifunctional benzene derivatives) were taken from the laboratory stock and, if necessary, distilled before use. Always 1 μl of each 1% solution was injected.

Materials

The following silica stationary phases were studied: Hypersil (Shandon, Frankfurt/Main, G.F.R.), LiChrosorb, LiChrospher (Merck, Darmstadt, G.F.R.), Nucleosil (Machery, Nagel & Co., Düren, G.F.R.), Porasil (Waters), Spherisorb (Phase Separations, Queensferry, Great Britain) and Zorbax (DuPont, Bad Nauheim, G.F.R.). A home-made silica (H 80-10) was also used. If available, materials with a particle diameters of 10 μm were used.

Physical characterization

The specific surface areas were determined by nitrogen adsorption using an Areameter II (Fa. Ströhlein Labortechnik, Stuttgart, G.F.R.) applying the "single-point differential method" according to Haul and Dümbgen[14,15] (DIN 66132). The silicas were dried in stream of nitrogen at 130°C for 24 h.

Pore volumes and average pore diameters were determined by exclusion chromatography[16] using polystyrene standards between $2.6 \cdot 10^6$ and 600 daltons. The volume of the eluent within the column (V_M) was defined by the elution volume of benzene, and the interstitial volume (V_Z) from the elution volume of the $2.6 \cdot 10^6$ dalton polystyrene standard. From these volumes and that of the empty column (V_K) the total porosity ε_T (V_M/V_K) and the interstitial porosity ε_Z (V_Z/V_K) were calculated. In Table I the physical properties and columns parameters of some of the silicas are summarized.

The pH values of the silicas were determined in 1% (w/w) suspensions in doubly distilled water with a glass electrode (EA 121) and a Metrohm E 536 potentiograph (Metrohm, Herisau, Switzerland)[17].

RESULTS AND DISCUSSION

Normalization of capacity ratios

The k' values depend on the polarity of the sample and of the eluent and, of course, on the properties of the stationary phase. The k' values are proportional to the adsorption coefficient and to the phase ratio. If identical samples and eluents are used and the k' values are standardized for differences in the phase ratio, the "adsorption coefficient", K^*, thus obtained should depend only on the surface properties of

TABLE I

PHYSICAL PROPERTIES OF THE SILICA USED

Silica	*Specific surface area* (m^2/g)	*Average pore diameter* (*nm*)	*Specific pore volume* (cm^3/g)	*Column porosities*		*Packing density* (g/cm^3)
				ε_T	ε_z	
Hypersil	170	11.5	0.7	0.78	0.43	0.53
LiChrosorb Si 100	320	11.1	1.2	0.83	0.41	0.34
Porasil	350	10.0	1.1	0.88	0.46	0.37
Spherisorb	190	8.1	0.6	0.77	0.43	0.61
Zorbax BPSil	300	5.6	0.5	0.71	0.42	0.60
H 80-10	450	7.5	1.1	0.84	0.45	0.37

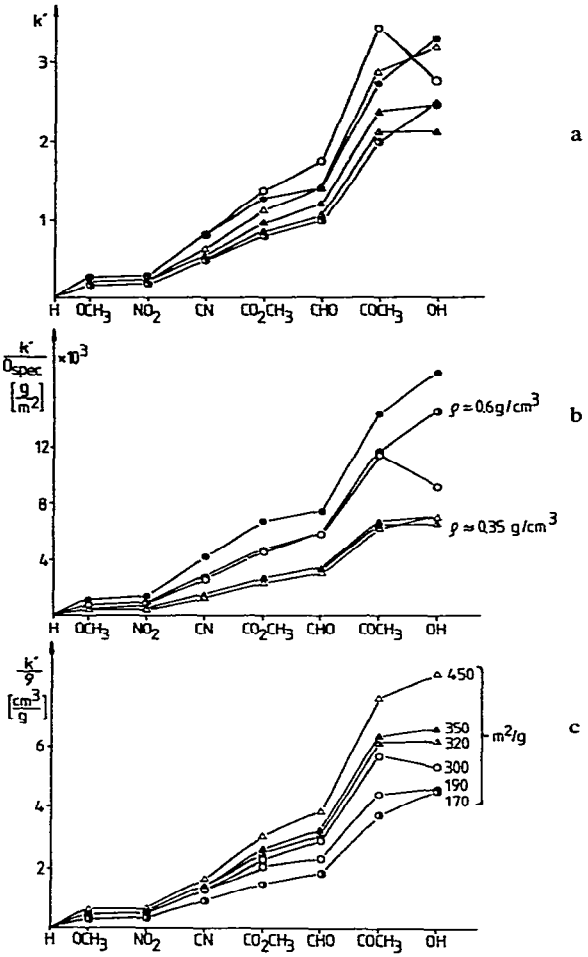

Fig. 1. Normalization of retention parameter. (a) Measured k' values; (b) k' normalized for differences in specific surface areas; (c) k' normalized for packing density differences. Stationary phases: Hypersil (◑); LiChrosorb Si 100 (▲); Porasil (▲); Spherisorb (●); Zorbax (○); H 80-10 (△). Columns: 25 cm × 4.1 mm I.D. Eluent: Dichloromethane containing 100 ppm of water. Flow-rate: 2 cm^3/min. Samples: Benzene, anisole, nitrobenzene, benzonitrile, methyl benzoate, benzaldehyde, acetophenone, phenol.

the stationary phases. In adsorption chromatography usually the specific surface area is taken as the volume of the stationary phase.

This standardization procedure is demonstrated in Fig. 1. The k' values of benzene derivatives as obtained with the different silica columns are shown in Fig. 1a. The k' values increase with increasing sample polarity, as expected, and no comparison of the different silicas is possible, because their order changes from solute to solute. The normalization for the differences in specific surface area is demonstrated in Fig. 1b. The retention values are much higher with the spherical than with the irregular silicas, owing to the larger packing densities of the spherical materials (larger surface area per unit column volume). If the k' values are normalized for

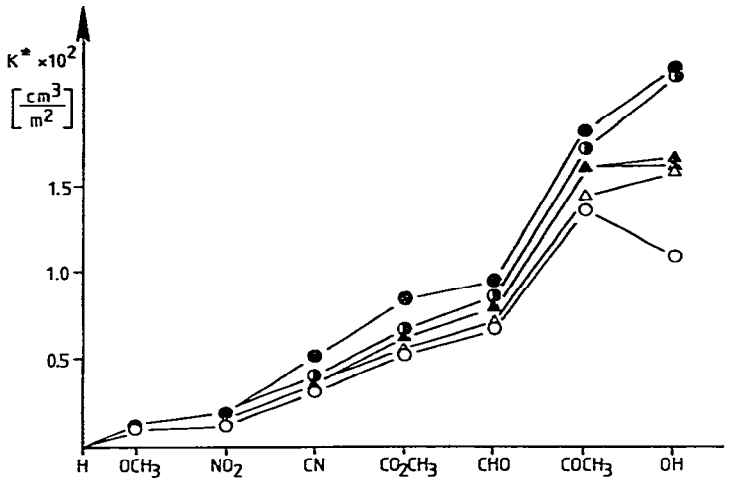

Fig. 2. Adsorption coefficient, K^*, for neutral and acidic solutes on different silicas. Conditions as in Fig. 1.

differences in packing density, as shown in Fig. 1c, they increase with increasing surface area. This is another indication that specific values (usually given per gram) are less important in chromatography because retention depends only on the values per unit column volume. Therefore, the k' values have to be normalized for the specific surface areas and for the packing densities. Table I shows that the packed columns differ also in their total porosities, ε_T, *i.e.*, the volume of mobile phase per unit column volume. For these reasons in this paper the "adsorption coefficient", K^* (cm^3/m^2), was calculated according to

$$K^* = k' \cdot \frac{\varepsilon_T}{O_{spec} \cdot \varrho}$$

to compare the retention behaviour of the solutes on the different silica columns[10].

In Fig. 2 these K^* values are plotted for different samples on the different silicas. If the surface properties of the silicas were to be identical, an identical sorption mechanism should result in a single line. For slightly retained solutes such as anisole and nitrobenzene this may be partly true, but for more polar solutes large variations in K^* reflect the differences in the surface nature of the silicas. The K^* values are very divergent with the most polar component in this series, *e.g.*, phenol.

Surprisingly large changes in K^* are observed if basic or other nitrogen-containing solutes are separated on different silicas, as shown in Fig. 3. The order of elution of the anilines on the silicas is not a function of their basicity or their hydrogen bonding capabilities. From Zorbax columns these components could not eluted in a reasonable time ($k' < 20$) with the standard conditions applied. This different behaviour of Zorbax to all the other silicas cannot be explained solely by differences in the surface concentration of the weakly acidic silanol groups[4].

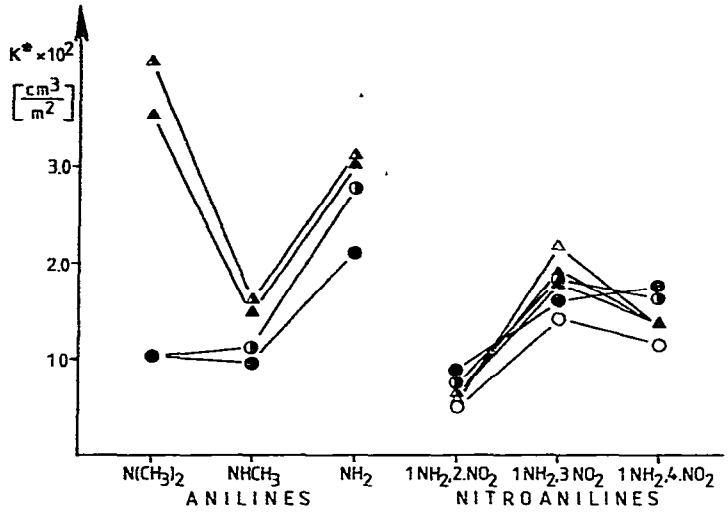

Fig. 3. Adsorption coefficient, K^*, for basic solutes. Conditions as in Fig. 1. Solutes: N,N-dimethylaniline, N-methylaniline, aniline and isomeric nitroanilines.

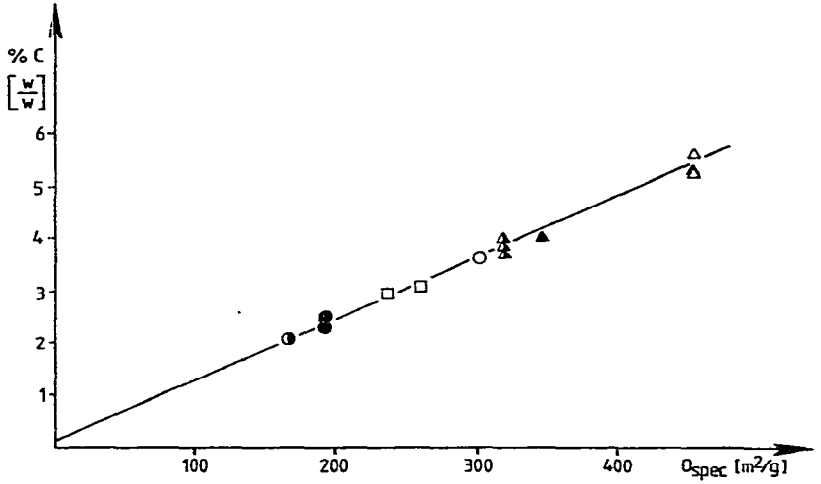

Fig. 4. Specific surface area and carbon content after silanization. Silanizing agent: trimethylchlorosilane. Silicas as in Fig. 1.

Surface properties

Retention on silica should depend on the surface concentration of the silanol groups. Because the silicas used here are prepared in different ways[3,4], we first tried to trace the selectivities demonstrated in Figs. 2 and 3 to differences in surface silanol concentrations. The total amount of surface silanols can be calculated from the total amount of water lost at 1000°C[18] minus the amount of physisorbed water, determined from the same silica at the same time by Karl-Fischer titration[19]. Despite the large differences in their specific surface areas for LiChrosorb Si 100 and Spherisorb, values between 8.9 and 8.6 μmol/m^2 were obtained by this method. On average, 8.7 $\pm$

TABLE II

pH VALUES OF DIFFERENT SILICAS MEASURED IN A 1% (w/w) AQUEOUS SUSPENSION

Silica	*pH*	*Regular (R) or irregular (I)*
Zorbax BPSil	3.9	R
LiChrospher Si 100	5.3	R
Nucleosil 100-7	5.7	R
H 80-10 (home-made)	6.5	I
LiChrosorb Si 100	7.0	I
Porasil	7.2	I
Partisil 10	7.5	I
LiChrosorb Si 60	7.8	I
Polygosil 60-5	8.0	I
Spherosil XOA 400	8.1	R
Hypersil	9.0	R
LiChrospher Si 1000	9.2	R
Spherisorb S 10 W	9.5	R
LiChrospher Si 500	9.9	R

0.2 μmol/m^2 had been determined for all the silicas with widely differing specific surface areas. These values are in good agreement with those given in the literature[4,20].

When a silica surface is modified with chlorosilanes, only the accessible silanol groups should react. Differences in this silanol concentration should result in different carbon contents per unit surface area of the chemically modified phases[21]. To prevent problems with reactivity and accessibility, trimethylchlorosilane was used as a silanizing agent. Fig. 4 shows that the relationship between carbon content and specific surface area is linear. The achievable surface concentration of the trimethylsilyl group was independent of the silica at 3.7 $\pm$ 0.2 μmol/m^2. These values cannot explain the differences in the chromatographic properties of the silicas.

The large differences in the elution behaviour of the anilines led us to compare the pH values of the different silicas in aqueous suspensions[17]. It is expected that the surface of pure silica should be weakly acidic owing to the pK_a value of 9.8 for orthosilicic acid[4]. A suspension of silica in neutral de-salted water should give a pH value of about 5 (ref. 3). Surprisingly, the pH values of the silicas measured in a 1% (w/w) suspension in neutral doubly distilled water deviated greatly from this value. In Table II some of the commercially available silicas are listed in order of increasing pH value. Irregular silicas seem to have the expected pH values between 6 and 7, whereas the specially prepared spherical silicas for HPLC are either acidic or basic.

It should be added that the pH values depend on the concentration of neutral salts in the suspension. Generally, addition of a neutral salt leads to a decrease in the pH of the suspension, owing to an exchange of hydrogen ions of the silanols against the cations of the added salt forced by the law of mass action. On adding sodium chloride to obtain a 30% (w/w) solution, the pH of the LiChrosorb Si 100 suspension decreased from 7.0 to 4.5 and that of Spherisorb from 9.4 to 6.0, whereas that of Zorbax decreased only from 3.9 to 3.3.

pH and chromatographic retention

These pH values, of course, cannot be related to the pH of the silica used in a chromatographic column with a non-polar eluent. However, it can be demonstrated that the pH value measured in aqueous suspension can be correlated with the retention behaviour of polar and non-polar solutes. Fig. 5 shows the separation of anilines and *m*-nitroanilines on a "basic" (Hypersil) (Fig. 5a) and on a "neutral" (Porasil) (Fig. 5b) silica. With the "neutral" silica the strongest base, N,N-dimethylaniline, is eluted last, whereas on the "basic" Hypersil it is eluted as the first of the anilines. The

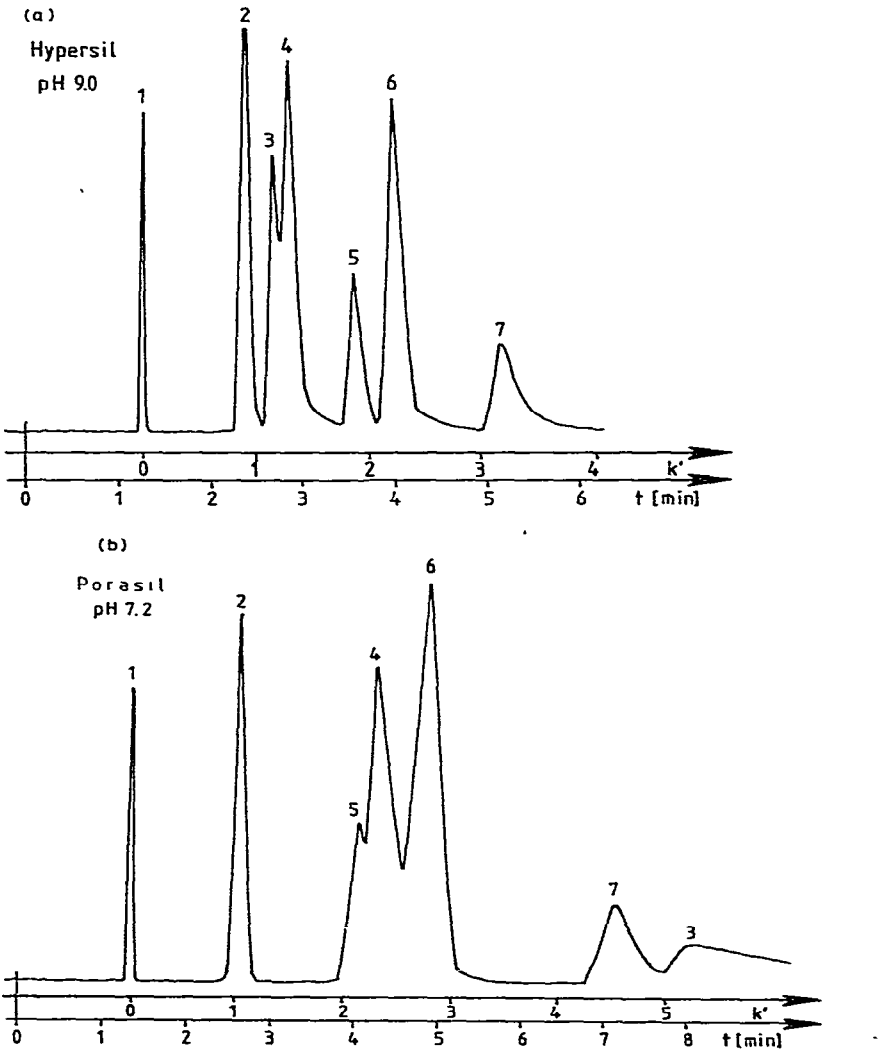

Fig. 5. Separation of basic compounds on "basic" and "neutral" silica. Chromatographic conditions as in Fig. 1. Stationary phases: (a) Hypersil and (b) Porasil. Solutes: 1 = benzene; 2 = *o*-nitroaniline; 3 = N,N-dimethylaniline; 4 = N-methylaniline; 5 = *p*-nitroaniline; 6 = *m*-nitroaniline; 7 = aniline.

efficiency and the symmetry of the peaks also increase with increasing pH of the silica surface. The relative retentions of the nitroanilines also change as a function of the "neutral" or "basic" silica surface. From "acidic" silicas (pH < 6.5) the anilines cannot be eluted as measurable peaks within a reasonable time.

The influence of the pH of the silicas on the separation of acidic compounds is demonstrated in Fig. 6. Phenol is eluted as a very symmetrical peak from the "acidic" Zorbax column (Fig. 6b), whereas it is eluted with severe tailing from the "basic" Spherisorb column (Fig. 6a). The absolute and relative retentions of the neutral phenyl alcohols are also influenced by the pH of the silicas.

It seems that the selectivity for the separation of aromatic hydrocarbons with

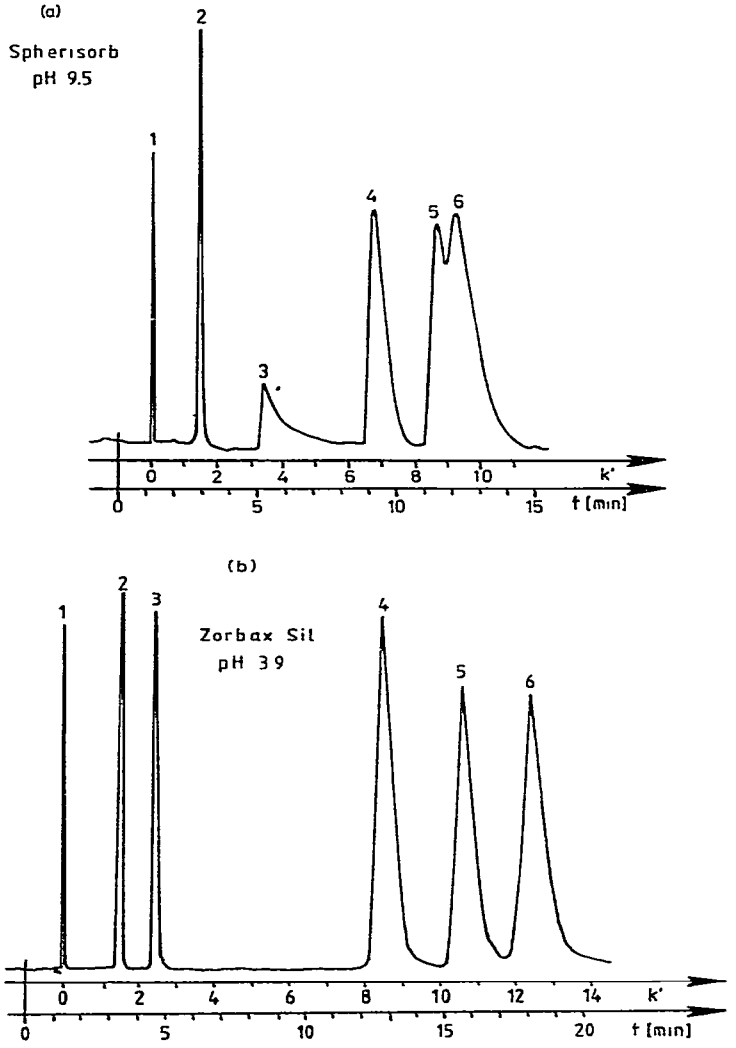

Fig. 6. Separation of neutral and acidic compounds on "basic" and "acidic" silica. Chromatographic conditions as in Fig. 1. Stationary phases: (a) Spherisorb and (b) Zorbax. Solutes: 1 = benzene; 2 = benzaldehyde; 3 = phenol; 4 = benzyl alcohol; 5 = 2-phenylethanol; 6 = 3-phenylpropanol-1.

n-heptane as eluent is also affected by the surface pH. This may explain the increase in the selectivity when changing from a large surface area silica (LiChrospher Si 100) to one with a smaller surface area (LiChrospher Si 4000)[10].

Acid- and base-treated silicas

To confirm these experimental results the pHs of different silicas were adjusted to high values by titration with 0.1 *N* sodium hydroxide in an aqueous suspension containing 20% (w/w) sodium chloride[22]. To obtain acidic surfaces, 10 g of silica were treated three or four times with 200 ml of 1 *N* hydrochloric acid. These materials were washed with distilled water until the pH of the washings was neutral. After drying they were packed as usual and their chromatographic properties determined. The basic treatment leads to a smaller specific surface area and to a larger average pore diameter if a pH of 8.5 is exceeded. By this treatment the surface area of LiChrosorb Si 100 decreased from 320 to 240 m^2/g and its pore diameter increased from 11 to 13 nm if treated at a pH of *ca.* 9.

The alteration of the chromatographic selectivity for the separation of nitrogen compounds is demonstrated in Fig. 7 for basic-treated silicas. The higher the pH of the initially neutral silica was increased, the smaller the k' values of the anilines became and the more symmetrical their elution curves. It should be noted that the elution order approaches that on the Hypersil column (Fig. 5a) if the pH of the treated silica exceeds 9.

The initially basic Spherisorb was adjusted to a pH of 6.5. The separation of phenols and phenyl alcohols with this material is shown in Fig. 8. By comparing the elution profile of phenol here with that on the starting material (Fig. 6a), the improvement obtained is remarkable. As mentioned before, the selectivity for the separation of the phenyl alcohols is also improved. After the acid treatment of this initially basic material the anilines can no longer be eluted.

Similar changes in the selectivity of silica on treating silicas with acidic or basic buffer solutions were described by Schwarzenbach[23,24]. It would not be surprising if the selectivity achieved with such systems were to be different from those obtained with titrated silicas. By coating silicas with buffers the surface is also covered with the anionic counter ions, which also may contribute to stationary phase selectivity. Of course, adding acids or bases to the eluent can also help in adjusting the selectivity.

Characterization of packed columns

From the above it is evident that one should know which kind of silica has been used in preparing a column, if acidic or basic solutes have to be separated. When anilines and other basic nitrogen compounds cannot be eluted from a column, it follows that it was packed with acidic silica. If phenol is eluted with more tailing than for neutral compounds, the surface of the silica may be neutral or basic. In this case, acid washing of the packed column may improve the elution profile of phenol and other acidic compounds. An analogous basic treatment of a packed column, however, is impossible and in this case one should use another type of silica.

The relative retentions of *p*- and *o*-nitroaniline are hardly affected by the pH of the stationary phase, and in our work were between 2.0 and 2.2, whereas the α values for *m*- and *p*- and also for *m*- and *o*-nitroaniline show a more pronounced dependence on the pH of the silicas. It seems possible to use these selectivities for surface charac-

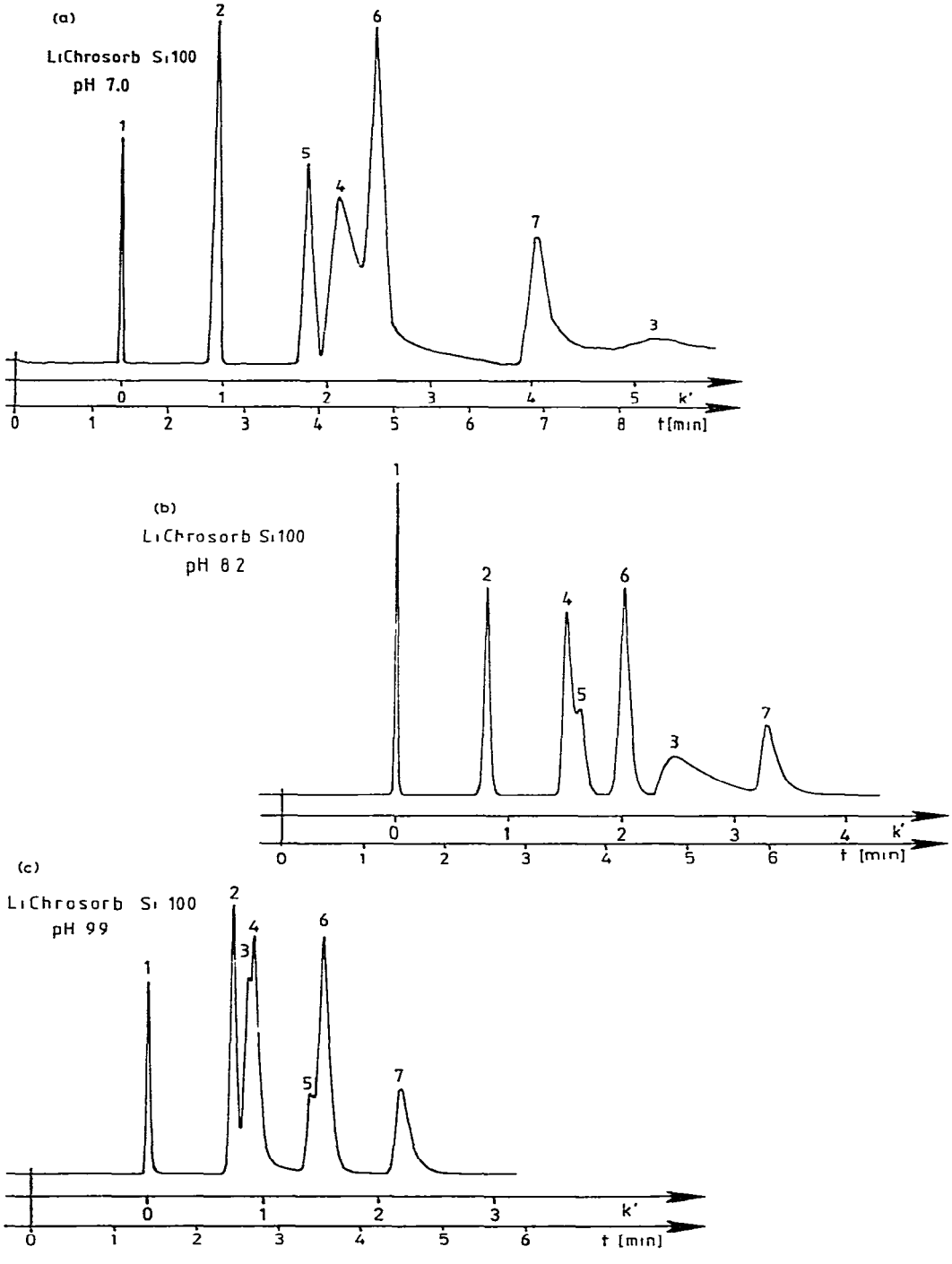

Fig. 7. Separation of basic solutes on basic-treated silica. Conditions and solutes as in Fig. 5. Stationary phases: LiChrosorb Si 100, (a) untreated, (b) pH adjusted to 8.2, (c) pH adjusted to 9.9.

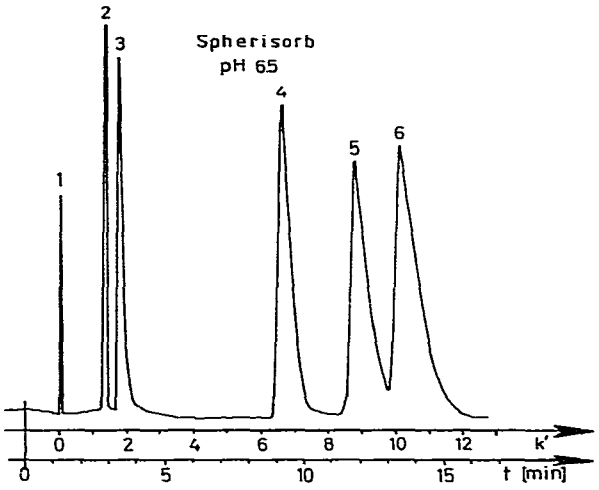

Fig. 8. Separation of neutral and acidic compounds on an acid-treated "basic" silica. Conditions and solutes as in Fig. 6. Stationary phase: Spherisorb, pH adjusted to 6.5.

terization; however, this needs further investigation with more and different silica samples.

CONCLUSION

The different commercially available silicas differ in their physical properties such as specific surface area, specific pore volume and average pore diameter, and the columns contain different amounts of stationary and mobile phase per unit column volume. If the retention parameters are normalized for this, distinct selectivities of the different silicas are still noticeable. Not only silicas with the expected neutral surface reaction but also acidic and basic silicas are commercially available, but this information is usually not stated on the labels as has been the custom for alumina preparations[25,26].

For the separation of neutral substances the efficiencies and selectivities obtainable with these different silicas may be optimal, reproducible and sufficient. However, if polar samples have to be separated the surface characteristics may influence peak retention, peak shape and efficiency. Hence "acidic silicas" may prove to be more efficient with neutral and acidic solutes than with basic, nitrogen-containing samples. The opposite, of course, may be true for "basic silicas" and the separation of basic or neutral samples. The classical and usually neutral silicas are in between these extremes. Owing to slight changes in their apparent pH around 7, these silicas may prove better for acidic or for basic solutes. Because of these problems there is no ideal silica for the separation of both acidic and basic samples. It is difficult to determine these characteristics with pre-packed columns. Therefore, it is recommended that the manufacturers should state at least the pH of their silicas.

It seems evident that these properties of the silicas also influence the properties of bonded phases prepared from them. In these cases, where aqueous eluents have to

be used, the acidity or basicity of the starting carrier material is also a contributory determinant.

ACKNOWLEDGEMENT

Financial support of this work by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, is gratefully appreciated.

REFERENCES

1 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
2 H. Engelhardt, *High Performance Liquid Chromatography*, Springer, New York, Heidelberg, Berlin, 1979.
3 K. K. Unger, *Porous Silica*, Elsevier, Amsterdam, 1979.
4 R. K. Iler, *The Chemistry of Silica*, Wiley-Interscience, New York, 1979.
5 H. Engelhardt, in Cs. Horváth (Editor), *High Performance Liquid Chromatography, Advances and Perspectives*, Vol. 2, Academic Press, New York, 1980, pp. 57–111.
6 R. Ohmacht and I. Halász, *Chromatographia*, 14 (1981) 155.
7 K. K. Unger, W. Messer and K. F. Krebs, *J. Chromatogr.*, 149 (1978) 1.
8 R. Ohmacht and I. Halász, *Chromatographia*, 14 (1981) 216.
9 R. P. W. Scott, *J. Chromatogr. Sci.*, 12 (1974) 473.
10 J. F. K. Huber and F. Eisenbeiss, *J. Chromatogr.*, 149 (1978) 127.
11 W. Böhme and H. Engelhardt, *J. Chromatogr.*, 133 (1977) 67.
12 J. Asshauer and I. Halász, *J. Chromatogr. Sci.*, 12 (1974) 139.
13 H. Elgass, H. Engelhardt and I. Halász, *Z. Anal. Chem.*, 294 (1979) 97.
14 R. Haul and G. Dümbgen, *Chem.-Ing. Techn.*, 32 (1960) 349.
15 R. Haul and G. Dümbgen, *Chem.-Ing. Techn.*, 35 (1963) 586.
16 I. Halász and K. Martin, *Angew. Chem.*, 90 (1978) 954.
17 DIN 53200, Beuth Verlag, Berlin, 1978.
18 DIN 55921, Beuth Verlag, Berlin, 1980.
19 W. Noll, K. Damm and R. Fauss, *Kolloid Z.*, 169 (1960) 18.
20 H. P. Boehm, *Advan. Catal.*, 16 (1966) 226.
21 H. Schmidt, *Ph.D. Thesis*, Saarbrücken, 1978.
22 G. W. Sears, Jr., *Anal. Chem.*, 28 (1956) 1981.
23 R. Schwarzenbach, *J. Liq. Chromatogr.*, 2 (1979) 205.
24 R. Schwarzenbach, *J. Chromatogr.*, 202 (1980) 397.
25 G. Hesse, I. Daniel and G. Wohlleben, *Angew. Chem.*, 64 (1952) 103.
26 H. Böhme, H. J. Bohn and J. Roehr, *Arch. Pharm.*, 299 (1966) 282.