

Chromatographic characterization of surfaces

Heinz Engelhardt

Angewandte Physikalische Chemie, Universität des Saarlandes,
D-6600 Saarbrücken, F.R.G.

Abstract - The influence of physical and chemical surface properties of silica on surface retention is described. The surface area within the column governs retention. The influence of sodium content at concentrations below 0.1 % on selectivity is demonstrated. For characterization of reversed phases two different approaches are described. A systematic evaluation of hydrophobic properties leads to four parameters sufficiently describing retention. A pragmatical test procedure including basic solutes permits with a single injection to evaluate stationary phases for hydrophobicity and for their suitability for separation of basic solutes.

The achievable selectivity of stationary phases for chromatography depends on their physical properties like specific surface area, pore diameter and porosity. More important than the usually available specific parameters is the knowledge of the surface area available within the column. To get these values the packing density is required, which one can get only by unpacking the column. Besides, by these commonly available parameters, chromatographic selectivity is governed additionally by the surface chemistry of the stationary phase. For pure silica, selectivity is determined by the concentration of the surface silanols (ref. 1). One can differentiate at least between two surface groups (ref. 2), the isolated silanols, and those which are hydrogen-bonded to adjacent ones. These differences can be seen by DRIFT (diffuse-reflectance infrared fourier transform spectroscopy) spectra. The sharp absorption band at 3740 cm^{-1} has been assigned to the isolated surface silanol groups, whereas hydrogen bonded groups give rise to a broad absorption band between 2500 and 3600 cm^{-1} . By thermal treatment the ratio of free to bonded silanol groups can be altered (ref. 3). Primarily at temperatures above 200°C the vicinal, hydrogen-bonded silanol groups are removed. For silicas treated at temperatures above 500°C vicinal surface silanols are no longer noticeable in the DRIFT-spectra.

In Fig. 1 these spectra are compared to the retention behaviour of solutes which can interfere with hydrogen bonds, like phenol and alcohols and of solutes, which act solely as hydrogen-bond acceptors, like acetophenone and benzoic acid esters (ref. 4). It can be seen, that the retention of solutes containing hydroxyl groups decreases with increasing temperature treatment of the stationary phase, and hence decreasing amount of hydrogen-bonded surface silanols. The decrease in retention of these solutes is largest in the temperature range between 200°C and 600°C . This indicates that these solutes are mainly adsorbed by breaking into the hydrogen bonds of the vicinal silanols. In contrast, the retention of solutes containing acceptors for hydrogen bonds is hardly influenced by thermal treatment. This indicates that these molecules with "basic" properties are adsorbed preferentially on the acidic isolated silanols, the surface concentration of which is not affected in this temperature range.

Pure silica should show in aqueous suspension an pH value around 3 to 4. It has been demonstrated (ref. 5), that commercially available silicas cover the pH range between 3.5 and 9.5. The high pH values could be correlated with the sodium content of the silicas. It is easily understandable that the retention of basic and acidic solutes is strongly affected by a sodium content below 0.15 %. In Fig. 2 a the separation of phenols and in Fig. 2 b that of anilines is demonstrated on different silica gels, where the pH had been adjusted either by an acid wash or by titration with NaOH. As eluent dry dichloromethane has been used. Besides retention also peak shapes show a distinct dependency on surface pH. Consequently, the acidic phenols are

preferably separated on an acid washed silica, whereas for separation of anilines a silica should be used whose surface has been treated with sodium ions.

So far the influence of the chemical nature of pure silica on chromatographic retention has been discussed. More than 80 % of chromatographic separations are performed with chemically bonded stationary phases, especially those of the reversed phase type. It is feasible that the silica properties discussed so far also influence solute retention with chemically modified stationary phases. Additionally, both the chemistry and the degree of surface modification have to be considered for stationary phase characterization.

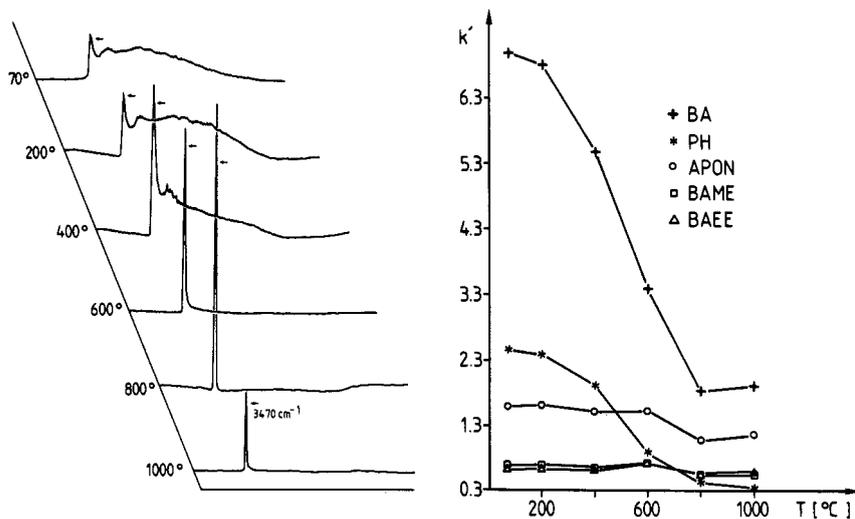


Fig. 1. DRIFT-spectra of thermally treated silica and retention of benzene derivatives on these silicas.

Eluent: dichloromethane; Solutes: (+) benzyl alcohol; (o) acetophenone; () benzoic acid methylester; () benzoic acid ethylester; (*) phenol.

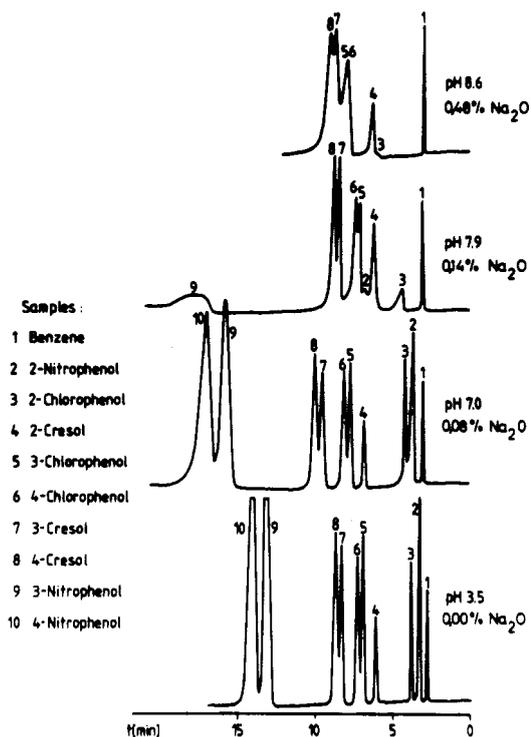


Fig. 2a. Separation of phenols at silicas differing in surface treatment with acids and bases.

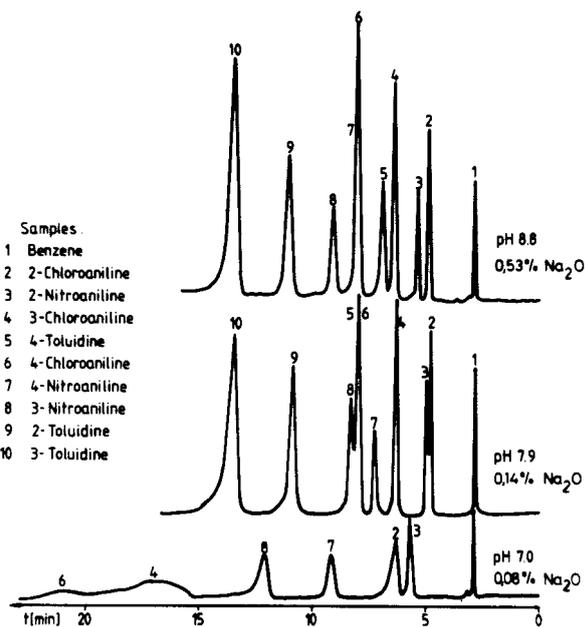


Fig. 2b. Separation of anilines at silicas differing in surface treatment with acids and bases.

To evaluate the hydrophobic properties of bonded phases the retention of molecules of a homologous series at varying solvent composition can be used. Simple linear relationships for the dependence of $\ln k'$ on solute chain length and on eluent composition are obtained which can be combined, as shown in Fig. 3. It is, therefore, possible to describe solute retention in a given solvent system and hence characterize the properties of a stationary phase (ref. 6). The retention depends on

$$\ln k' = A + B \cdot n + C_x + D \cdot n \cdot x$$

where x is the weight fraction of water in the eluent ($x = 0$ for the pure organic eluent, hence retention increases with increasing x) and n is the number of carbon atoms of the homologous series.

The four parameters can be identified as:

- A = $\ln k'$ for the basis of the homologous series ($n = 0$) in pure strong eluent ($x = 0$);
- B = logarithm of the methylene group selectivity in pure strong eluent ($x = 0$);
- C = the change of $\ln k'$ with increasing water content for the basis of the homologous series ($n = 0$);
- D = logarithmic change of methylene group selectivity with increasing water content.

As can be seen from Fig. 3, all lines intersect for a common value n^* of the chain length, and a hypothetical eluent composition x^* at which all solutes would be eluted at the same time. Consequently, the solutes do not distinguish between both phases. With increasing chain length a higher proportion of organic solvent is required to offset stationary phase hydrophobicity. In the case of methanol the pure eluent is not strong enough, more than 100 % would be necessary, thus corresponding to negative water content values of x . For octyl reversed phases the values of x^* are close to -0.14 ("114 % methanol"), whereas for octadecyl phases 126 % methanol is needed ($x^* = -0.26$) to suppress selectivity. So x^* can be used to distinguish between the different types of reversed phases, as can be seen in Fig. 4, where x^* values are plotted vs chain length of different solutes.

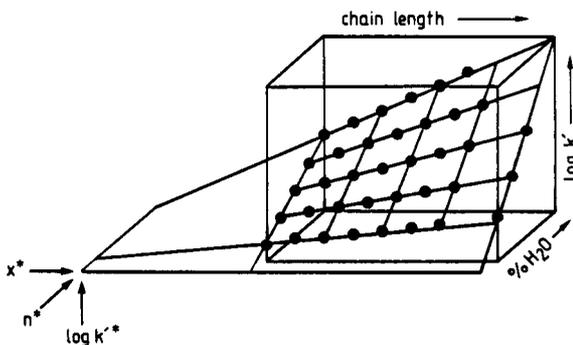


Fig. 3. Dependence of k' on solute chain length. Eluent: methanol-water; Solutes: phenylalkanes.

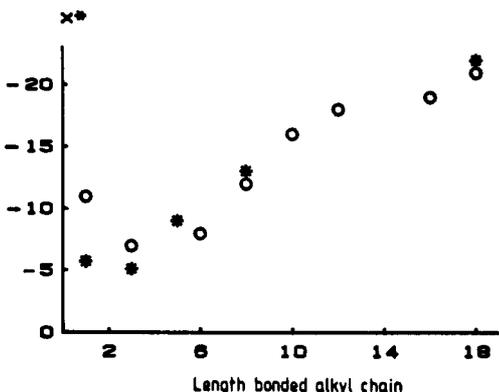


Fig. 4. Hypothetical eluent composition for reversed phase characterization.

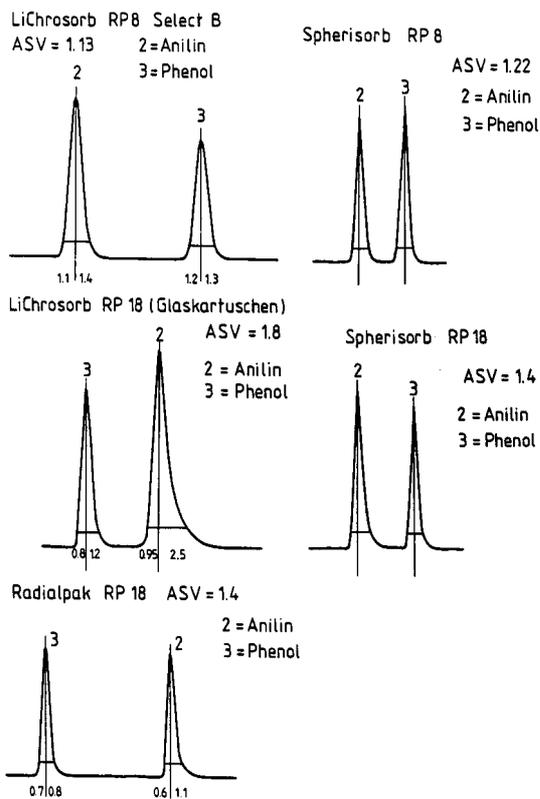


Fig. 5. Characterization of reversed phases by elution sequence and peak asymmetry.

Both points (n^* and x^*) of intersection occur at the same k' . This value k^* is very small and is at the point of intersection, where the partition coefficient for all solutes equals one, a measure of the phase ratio. However, due to the uncertainty of dead time measurements and the extremely small values (for RP 18 k^* is around 0.1) it is dangerous to use this value also for stationary phase characterization.

For exact evaluation measurements for various solutes at different eluent compositions are necessary. Only one facet of stationary phase properties, the hydrophobic character, can be evaluated by this way. Because many important solutes to be separated by HPLC contain basic nitrogen, also basic solutes have to be included in stationary phase characterization. The most distinct differences in selectivity are observed for basic solutes. Consequently, a pragmatic test procedure has been developed (ref. 7) to evaluate stationary phases for both major properties: their hydrophobic (retentive) character and their behaviour against basic nitrogen containing solutes. As eluent a water methanol mixture (49/51 w/w) is used, where the retention of simple benzene derivatives is in the optimal range and the stationary phase is still wetted. The hydrophobic selectivity is recognized via retention and selectivity of toluene and ethylbenzene. As basic solutes the isomeric toluidines are used, together with aniline and NN-dimethylaniline. Phenol is eluted closely to aniline early in the chromatograms. It is used to determine the influence of the equipment and column quality on peak shape. If phenol is eluted with a symmetrical peak, whereas aniline with asymmetry this can be attributed to silanophilic interaction. Additionally, benzoic acid ethylester has been included in the test mixture. It serves as a fast marker whether RP 8 or RP 18 has been packed into the column. With the latter stationary phases it is always eluted in front of toluene, whereas with octyl phases it is eluted after toluene.

The elution order and the peak asymmetry relationship of aniline and phenol are good measures to describe the properties of stationary phases for the separation of basic solutes. This is demonstrated in Fig. 5 for different stationary phases. When aniline is eluted in front of phenol symmetric peaks are obtained and the relationship of peak asymmetry of aniline and phenol is usually below 1.3. With such stationary phases all other basic solutes are eluted with symmetrical peaks and the isomeric toluidines (identical hydrophobicity but different basicity) are not separated: silanophilic interaction does not contribute significantly to solute retention. With these "good" stationary phases the retention of basic solutes is also independent of sample size, if this does not exceed 10^{-3} g sample/g stationary phase.

In comparing more than 50 different reversed phases it has been found that retention increases linearly with carbon content, whereas relative retention approaches a limiting value when the carbon content increases above 10 % (w/w).

It should be mentioned that with all these measurements eluent composition and column temperature have to be monitored and maintained exactly. The retention time varies in the range of 3 % (at $k' = 1$) and 13 % (at $k' = 10$) if the eluent composition varies for 1 %. The influence of temperature variations on retention is also noticeable and can reach values up to 3 % per °C temperature change.

This simple test procedure with one injection at one eluent composition gives a good approximation of the suitability of a stationary phase for a given separation problem and permits to determine stationary phase stability. Hydrolytic instability of stationary phases can easily be recognized by the elution behaviour of the basic solutes. Their retention and peak shape is much earlier and more significantly affected than the hydrophobic selectivity determined with non polar hydrocarbons.

REFERENCES

1. L.R. Snyder, Principles of Adsorption Chromatography, Marcel Dekker, New York (1968)
2. V.Y. Davidov, L.T. Zhuravlev, A.V. Kiselev, J. Phys. Chem. **38**, 1108 (1964)
3. L.D. Belyakova, A.V. Kiselev, N.V. Kovaleva, Anal. Chem. **36**, 1517 (1964)
4. M. Mauss, H. Engelhardt, J. Chromatogr. **371**, 235 (1986)
5. H. Engelhardt, H. Müller, J. Chromatogr. **218**, 395 (1981)
6. M. Czok, H. Engelhardt, submitted to Chromatographia
7. M. Jungheim, Ph.D. Thesis, Saarbrücken (1986)