

Note

Retention of pyridine and 2,6-dimethylpyridine on silanized silica

A simple test on residual silanols?

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The residual silanol groups present on the surface of silanized silica can influence the chromatographic behaviour of polar solutes in reversed-phase liquid chromatography (RPLC). In particular, basic solutes such as amines, amino acids or aza-aromatics interact with the silanols via an ion-exchange or hydrogen-bonding retention mechanism^{1,2}. The accessibility of the silanol groups depends on the structure of the layer of bound alkyl groups³; "monomeric" or "polymeric" layers can be formed according to the reaction conditions of silanization (see, *e.g.*, refs. 4 and 5).

The concentration and chemical character can be observed directly by means of spectroscopic⁶⁻¹⁰ or chemical^{4,11} methods, which are not feasible for the testing of RPLC columns in the laboratory. For this purpose, several empirical procedures have been proposed employing normal- or reversed-phase separations of test solutes¹²⁻¹⁵. Also the adsorption of polar dyes, *e.g.*, methyl red, serves as a simple qualitative test for the extent of surface polarity^{12,16,17}.

The aim of this work was to study the retention of various polar solutes on silanized silica in a normal-phase system as a function of the surface concentration of residual silanol groups.

EXPERIMENTAL

A spherically shaped silica gel, $d_p = 10 \mu\text{m}$, $S_{\text{BET}} = 360 \text{ m}^2/\text{g}$, was used for the preparation of monomeric C_{18} -silica via a reaction with octadecyldimethylchlorosilane. Samples of octadecylsilica with various concentrations of the bound phase were prepared under altered reaction conditions as described in detail elsewhere¹⁸. An irregularly shaped silica, $d_p = 10 \mu\text{m}$, $S_{\text{BET}} = 190 \text{ m}^2/\text{g}$, was dried at 180°C overnight and modified with octadecyltrichlorosilane to prepare polymeric C_{18} -silica.

The silanization of 10 g of silica was carried out with 5 g of silane in 100 ml

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of dry, boiling toluene for 6 h. The product was filtered off and washed with dry toluene, acetone and methanol. The product was shaken with water-methanol (1:1) saturated with sodium carbonate in order to hydrolyse the residual Si-Cl bonds. After 30 min, the silica was filtered off, washed with distilled water and methanol and dried at 100°C for 3 h. Equal portions of the silanized silica were treated with hexamethyldisilazane (HMDS) to change the concentration of residual silanols; a suspension of 2 g of silica in 10 ml of dry toluene was shaken with 0.5 ml of HMDS for 5 min, 30 min, 2 h, 8 h and 24 h. The resulting samples were carefully washed with methanol to remove unreacted HMDS and dried for 2 h at 120°C.

Methyl red (50 mg), purified according to ref. 17 was dissolved in 100 ml of dry toluene; 0.1 g of silanized silica was shaken with 5 ml of this solution for 1 h. The colour of the sample was observed after sedimentation. The determination of surface silanol groups by reaction with a gaseous complex of dimethylzinc with tetrahydrofuran has been described previously⁴.

The samples were packed into stainless-steel columns (100 × 4 mm I.D.) using a slurry packing procedure; methanol was used as the slurry liquid. A laboratory-constructed liquid chromatograph consisting of a reciprocating pump (Altex 110 A), a sampling valve (Rheodyne 70-10) with a 20- μ l loop and a UVM-4 UV-VIS detector (Development Workshops, ČSAV, Prague, Czechoslovakia) operated at 254 nm was used. All measurements were carried out at ambient temperature using *n*-heptane-chloroform (75:25) as the mobile phase. Solvents of analytical-reagent grade were dried over molecular sieves before use. The tested columns were flushed with acetone, chloroform and the mobile phase for 1 h. Test solutes were diluted with mobile phase to *ca.* 0.05% (v/v) and injected individually. The capacity factors were calculated assuming toluene to be a non-retained solute.

RESULTS AND DISCUSSION

In Table I, capacity factors are given for the retention of pyridine (P), 2,6-dimethylpyridine (DMP), acetone (A), nitrobenzene (N) and benzo[*a*]pyrene (BP) on a series of silanized silicas with a monomeric alkyl layer. The sorbents were prepared by silanization of bare silica with various amounts of octadecyldimethylchlorosilane. The surface concentration of residual silanol groups was determined by means of their reaction with dimethylzinc tetrahydrofuranate⁴. The methyl red adsorbed on the silica surface from dilute benzene solution changed colour from yellow to violet with increasing concentration of silanol groups.

The methyl red adsorption seems to be a sensitive test for residual silanol groups¹². This test is based on the fact that methyl red, 2-[[4-(dimethylamino)phenyl]azo]benzoic acid, turns red when adsorbed on acidic sites such as silanol groups. However, the dyestuff could also be adsorbed on other polar adsorption sites and the amount of methyl red adsorbed from non-polar solutions cannot be taken, *a priori*, as a measure of the silanol concentration. It was stressed by Shapiro and Kolthoff¹⁹ and Benesi²⁰ that the amount of methyl red adsorbed depends on surface area only.

The retention of the above solutes was measured with a non-polar mobile phase, *n*-heptane-chloroform (3:1, v/v). As is evident from Fig. 1, a fairly linear dependence between retention of polar solutes and silanol concentration is observed

TABLE I

BASIC CHARACTERISTICS OF MONOMERIC OCTADECYLSILICA AND THE RETENTION OF TEST SOLUTES UNDER NORMAL-PHASE CONDITIONS

| Sample | S_{BET} (m^2/g) | C (%) | C_{SiOH} ($mmol/g$) | Methyl red test | Capacity factors (k') [*] | | | | |
|--------|--------------------------|----------|----------------------------|-----------------|--|------|-----|-----|------|
| | | | | | P | DMP | A | N | BP |
| I | 338 | 5.1 | 1.7 | Violet-red | 19.0 | 15.8 | 3.7 | 2.2 | 0.68 |
| II | 262 | 11.4 | 1.1 | Violet-red | 13.0 | 11.2 | 2.7 | 1.9 | 0.66 |
| III | 246 | 13.5 | 1.2 | Red | 13.0 | 10.1 | 2.6 | 1.4 | 0.62 |
| IV | 225 | 16.1 | 0.9 | Pink | 7.7 | 5.3 | 1.8 | 1.3 | 0.57 |
| V | 175 | 24.5 | 0.3 | Yellow | 3.4 | 2.3 | 1.1 | 0.6 | 0.54 |

* Solutes are abbreviated as follows: P = pyridine; DMP = 2,6-dimethylpyridine; A = acetone; N = nitrobenzene; BP = benzo[a]pyrene.

under the normal-phase conditions. In contrast, the retention of non-polar BP does not show a similar linear trend (Table I).

It was stated by Snyder²¹ that surface hydroxyl groups are the only important sites for the adsorption of unsaturated or polar molecules and that the adsorption energy increases with increasing basicity of the sorbate. It is evident that the retention

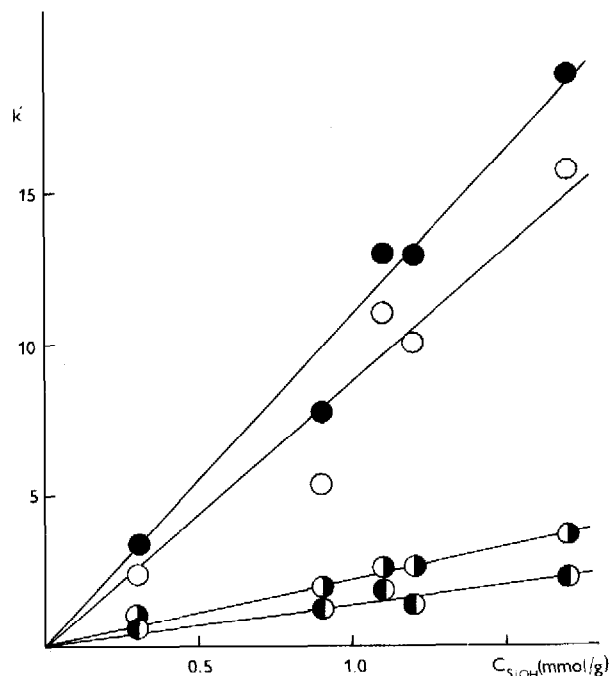


Fig. 1. Linear dependence of capacity factor, k' , on concentration of silanol groups, C_{SiOH} , for "monomeric" ODS-silica: (●) pyridine; (○) 2,6-dimethylpyridine; (◐) acetone; (◑) nitrobenzene. Mobile phase, *n*-heptane-chloroform (3:1, v/v); flow-rate, 1.0 ml/min; temperature, 20°C.

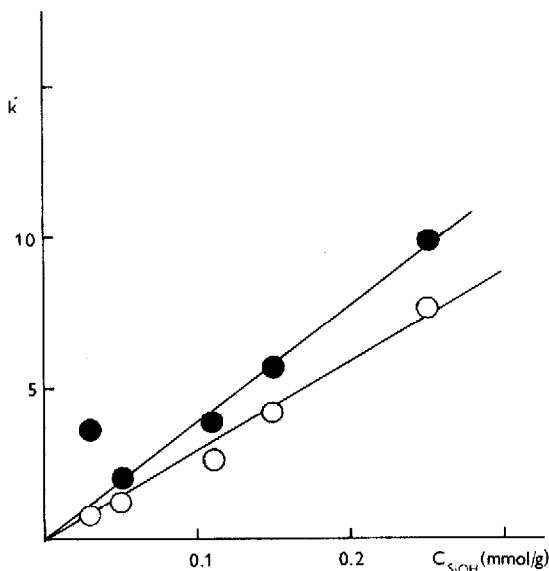
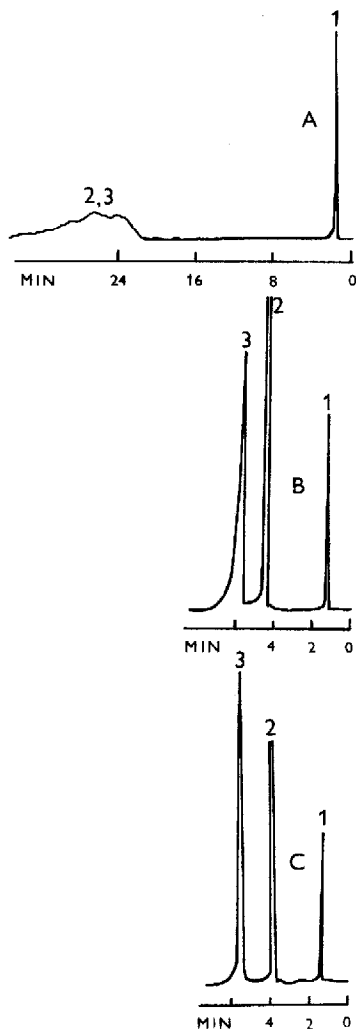


Fig. 2. Linear dependence of capacity factor, k' , on concentration of silanol groups, C_{SiOH} , for "polymeric" ODS-silica after end-capping with HMDS: (●) pyridine; (○) 2,6-dimethylpyridine. Other conditions as in Fig. 1.

Fig. 3. Separation of (1) toluene, (2) 2,6-dimethylpyridine and (3) pyridine on (A) bare silica, (B) silica treated with octadecyldimethylchlorosilane and (C) ODS-silica end-capped. Mobile phase, *n*-heptane-chloroform (3:1, v/v); flow-rate, 1 ml/min; temperature, 20°C.

of P and DMP as basic solutes is more sensitive to silanol concentration than the retention of acetone or nitrobenzene (Table I). Benesi²² expected an increased specificity of sterically hindered pyridines, *e.g.*, DMP, to interact with surface silanol groups owing to the relative flexibility of the hydroxyl group in the hydrogen-bonding interaction with the hindered free electron pair of nitrogen. Under our experimental conditions the linearity found for the both basic solutes is virtually the same, however (Fig. 1).

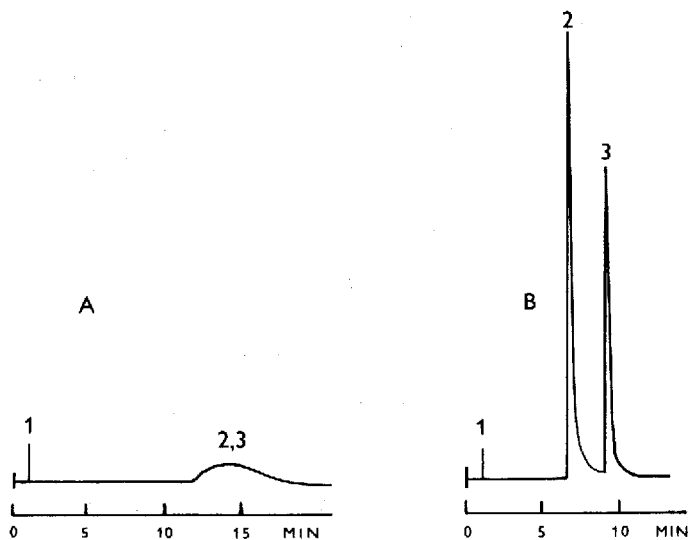


Fig. 4. Separation of (1) toluene, (2) 2,6-dimethylpyridine and (3) pyridine on (B) a column packed with Spherisorb ODS (5 μm) and (A) the same column after 2 months of use at pH > 7.

The linear dependence of the capacity factors of the basic solutes, P and DMP, on silanol concentration was also tested with polymeric ODS-silica, as shown in Fig. 2. The second series was prepared via the reaction of bare silica with octadecyltrichlorosilane. The residual silanol groups were end-capped to varying extents with HMDS. It is interesting that the slopes of the linear dependences differ markedly for monomeric and polymeric surfaces. This may indicate relatively greater acidity of the silanol groups in the polymeric layer. Further, the markedly different sorption properties of polymeric and monomeric alkyl layers can also depend on the different accessibilities of the residual silanol groups and on the varying amounts of adsorbed water^{4,5}. Consequently, the quantitative determination of residual silanol groups, e.g., given as millimoles of hydroxyl groups per gram of sorbent, if a constant density of the packing is assumed, is impossible using the capacity factors (k') of P and DMP.

In addition, we used the separation of P and DMP for the testing of columns packed with RPLC sorbents. Fig. 3 shows the separation of the above bases on a bare silica, silica treated with octadecyldimethylchlorosilane and end-capped ODS-silica. The improved peak symmetry achieved with the end-capped ODS-silica indicates an enhanced homogeneity of the alkyl layer. Also, deterioration of a commercial column was observed by means of the above basic solutes (Fig. 4).

In conclusion, the normal-phase separation of basic solutes such as P and DMP can serve as a simple, semi-quantitative and non-destructive test for residual silanol groups present on the surface of RPLC sorbents.

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