CHROMSYMP. 1521

SILANEDIOL GROUPS OF THE SILICA GEL NUCLEOSIL: ACTIVE SITES INVOLVED IN THE CHROMATOGRAPHIC BEHAVIOUR OF BASES

BETTINA PFLEIDERER and ERNST BAYER*

Institut für Organische Chemie, Auf der Morgenstelle 18, D-7400 Tübingen (F.R.G.)

SUMMARY

Fe^{3+} ions in iron(III) chloride-modified silica gels have previously been reported to be bound exclusively to silanediol groups on the surface. A silica gel (Nucleosil) was modified with various amounts of iron(III) and this masking had an influence on the retention of bases. As the iron(III) load increased, the capacity k' factors decreased and the number of plates increased. At a certain iron(III) content saturation occurred and thereafter the k' value remained constant. Only about 40% of the total silanediol groups of Nucleosil influenced retention, which can be explained by the presence of silanediols with different reactivities. Electron paramagnetic resonance measurements of the modified silica gels revealed that Fe^{3+} ions are bound to the silanediol groups as a chelate complex.

INTRODUCTION

The separation of basic compounds by high-performance liquid chromatography (HPLC) on silica gel is often accompanied by long retention times, asymmetric peaks (tailing) and a small number of plates[1,2]. The reason for this abnormal behaviour is not fully understood, but "acidic" silanol groups of the silica gel have been held responsible[1,3]. Addition of cationic modifiers, such as triethylammonium salts, to the mobile phase has been shown to improve the chromatographic behaviour, explained by masking of these active sites[4].

We have doped a silica gel, Nucleosil, with different iron(III) loads and have shown by solid-state nuclear magnetic resonance spectroscopy combined with cross-polarization (CP) and magic angle spinning (MAS) that the Fe^{3+} ions are located exclusively at silanediol groups, because the longitudinal relaxation time, T_1, of the silanediol groups, but not of silanol groups, is greatly reduced, in proportion to the iron content[5]. Moreover, the maximal iron(III) load is equal to the number of silanediol groups, assuming that one iron atom is bound to one silanediol group[5].

In order to investigate whether silanediol groups are the active sites in Nucleosil which influence the retention of bases, silica gels doped with various iron(III) loads were tested by HPLC. We chose a test developed by Daldrup and Kardel[6] for reversed-phase silicas and adapted it to native silica gels under pseudo-reversed-phase conditions.

EXPERIMENTAL

Analysis was performed on a Bruker (Karlsruhe, F.R.G.) LC 31 HPLC system with a Uvikon (Bremen, F.R.G.) 720 LC-VW detector and a Uvikon recorder 21. Electron paramagnetic resonance (EPR) experiments were carried out on a Bruker ESP 300 instrument. Stainless-steel columns (12.5 cm × 4 mm I.D.) were packed by proprietary procedures, using a slurry method.

Packing materials

The silica used was Nucleosil with a particle size of 7 μm and a surface area of 350 m^2 g^{-1} (Macherey, Nagel & Co., Düren, F.R.G.). Iron(III) chloride hexahydrate (analytical-reagent grade) dissolved in distilled water was used as the dropping solution (pH 3). At pH 3 iron(III) has been found to be completely adsorbed on silica[7]. The iron(III)-modified phases were prepared by shaking 3 g of silica for 1 h in 40 ml of aqueous solutions with different iron(III) concentrations (0.1–0.03 mol/l). The silica was filtered (G4), washed five times with 20 ml of distilled water and dried at 100°C for 12 h before being packed. The iron(III) load was determined at 500 nm photometrically with sulphosalicylic acid according to Marcenko[8].

The mobile phase was acetonitrile–buffer (5 mmol) (5:1, v/v) and the buffer was a stock solution of 6.66 g of potassium dihydrogenphosphate + 4.8 g of 85% phosphoric acid in 1 l aqueous solution, which was diluted 1:10 (pH 3.4). The flow-rate was 1 ml/min and detection was at 220 nm.

Samples

HPLC-grade acetonitrile and other reagents were obtained from Merck (Darmstadt, F.R.G.), except 5-(p-methylphenyl)-5-phenylhydantoin (MPPH) (Aldrich Europe, Beerse, Belgium). The chromatographic sample mixture contained 0.5 μg/μl of diphenhydramine hydrochloride (DPHA · HCl) and 0.5 μg/μl of MPPH.

RESULTS

The commercially available Nucleosil contains 0.14 μg/m^2 of Fe^{3+} as a natural contaminant. Doping of Nucleosil with different iron(III) concentrations resulted in silica gels with iron(III) loads of 1, 5.7, 7.4 and 17.1 μg/m^2, which were used in the HPLC test. The maximum load of 17.1 μg/m^2 of Fe^{3+} ions could not be exceeded, even with highly concentrated iron(III) doping solutions.

The sample mixture used contained DPHA · HCl and MPPH. DPHA · HCl is a basic drug that is extremely sensitive to the polar silanol groups[6], whereas the retention of MPPH has been shown to be independent of the polarity of different silica gels[6]. In preliminary experiments, we investigated the influence of the Fe^{3+} modification on the retention of MPPH. The capacity factors (k') were not influenced by the iron(III) load and were therefore used as a reference. The iron(III) load of the Nucleosil has a great influence on the retention time and the number of plates for DPHA · HCl. Increasing the iron(III) concentration results in a decrease in k' values and an increase in the plate number (N) (Table I). These effects are not reversible. Prolonged washing with the eluent for 1 day reduces the iron(III) load by 30% to a stable value, and further washing has no great effect. The k' values increase slightly,

TABLE I

DEPENDENCE OF k' VALUES AND NUMBER OF PLATES, N, OF DPHA · HCl ON THE IRON(III) CONTENT OF IRON(III)-MODIFIED NUCLEOSIL

$[Fe^{3+}]$ $(\mu g/m^2)$	k'	N/m
0.14	1.68	2000
1.00	1.56	3060
5.7	1.22	3650
7.4	1.09	4430
17.1	1.05	7240

but never reach the unmodified level. A plot of ln k' *versus* iron(III) content (Fig. 1) shows saturation kinetics, and this allows the determination of the amount of iron necessary for saturation of the active sites of Nucleosil. Extrapolation of the steepline in Fig. 1 to the abscissa gives a value of 8.85 $\mu g/m^2$ of Fe^{3+} ions, corresponding to 0.159 $\mu mol/m^2$. Even higher iron(III) loads have no greater influence on the retention time. Although Fe^{3+} ions can mask all silanediol groups in Nucleosil[5], only a proportion of the total silanediol groups are actually active sites. Whereas the total silanediol group concentration has been estimated to be 0.37 $\mu mol/m^2$ (ref. 5), the concentration of active sites is 0.159 $\mu mol/m^2$, and therefore only *ca.* 40% of the silandiol groups in Nucleosil influence the retention times of bases, such as those used in the HPLC test.

Native and iron(III)-modified silica gels were examined by EPR in order to determine the configuration of iron(III) bound to the surface. Fig. 2 shows the derivative spectra at 77 K for a native silica gel and an iron(III)-modified phase (5.7 $\mu g/m^2$ of Fe^{3+} ions). The native silica gel shows a weak $g = 4.29$ resonance and a signal at $g = 2.014$, whereas the modified phases show a shoulder resonance at $g = 8.6$,
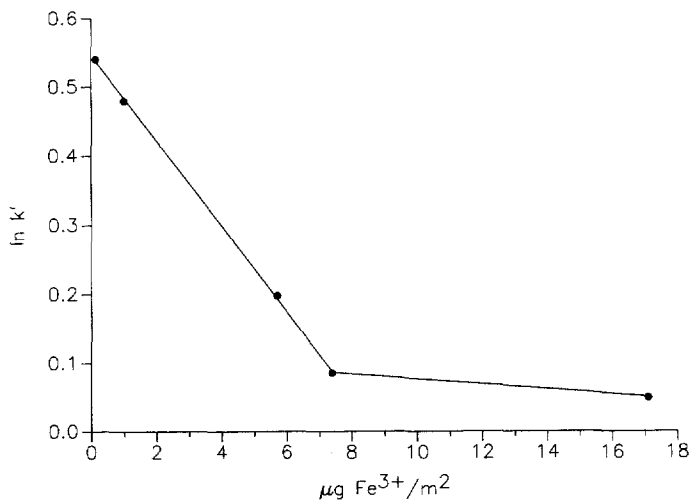

Fig. 1. Plot of the logarithm of k' values of DPHA · HCl *versus* iron(III) content of iron(III) chloride-modified Nucleosil.
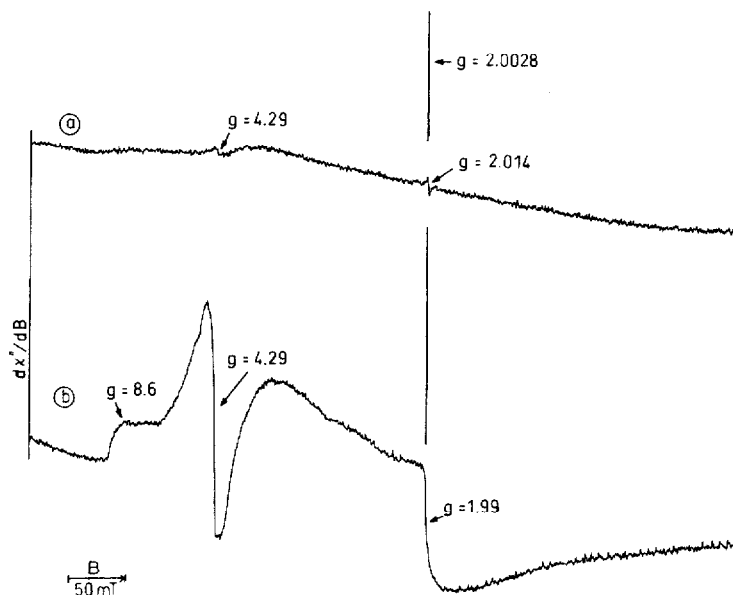
Fig. 2. Derivative of the absorption, $d\chi''/dB$, at 77 K for (a) native Nucleosil and (b) iron(III)-modified Nucleosil (5.7 $\mu g/m^2$ of Fe^{3+} ions).

a signal at $g = 4.296$ and a resonance at $g = 1.99$. Castner et al.[9] explained the resonance at $g = 4.29$ and 2.014 occurring in native silica gels as a high spin of the $^6S_{5/2}$ Fe^{3+} ion. These Fe^{3+} ions are isolated and not coupled, occupying sites similar to silicon in the network. After doping, the intensity of the $g = 4.29$ peak increased strongly, and a resonance at $g = 1.99$ appeared. Here, the Fe^{3+} ions are isolated and also not coupled. The resonance at $g = 1.99$ is due to a strongly asymmetric rhombohedral surrounding for Fe^{3+} ons on the surface[10]. For Fe^{3+} ions occupying silicon sites within the network in a more symmetrical surrounding, the resonance at $g = 1.99$ should be weak[9]. These measurements and the fact that the effects of masking active sites with Fe^{3+} ions are not reversible indicate a strong specific interaction between the Fe^{3+} ions and the silanediol groups. The Fe^{3+} ions could form a chelate complex with the oxygen atoms of silanediols.

DISCUSSION

The retention characteristics of bases in native silica gels under pseudo-reversed-phase condtions are complex functions of the organic solvent and buffer concentrations, and also pH[1]. In the chromatographic test system used, the mobile phase contains a high concentration of organic solvent and the silanophilic interactions are strengthened. DPHA · HCl is protonated in the mobile phase (pH 3.4)[6]. The interaction with active sites can be explained by an ion-exchange mechanism, represented by

$$NR_3 + H^+ \rightleftharpoons NR_3H^+ \tag{1}$$

$$SiOH \rightleftharpoons SiO^- + H^+ \tag{2}$$

$$SiO^- + NR_3H^+ \rightleftharpoons SiO^-NR_3H^+ \tag{3}$$

The masking of acidic SiO^- groups (eqn. 2) by Fe^{3+} influences the ion-exchange strength, because the number of available SiO^- groups is reduced and the ion-exchange equilibrium constant changes. With this model, the curves in Fig. 1 can be readily explained. In iron(III)-modified silica gels with an iron(III) content below the saturation level, the k' values are influenced by the remaining active silanediol groups. This process can be correlated with the steep branch in Fig. 1. After saturation, all active sites are masked.

Acidic silanediol groups could be among the active sites in both native silica gels and in reversed-phase materials: CP–MAS solid-state nuclear magnetic resonance spectra of chemically modified silica gels revealed that silanediol groups remain present on the surface[12]. Silanol groups could also be active sites contributing to the retention of bases. However, the interaction between active silanol groups and basic compounds cannot be investigated with the test system used, as Fe^{3+} ions bind exclusively to silanediol groups. To determine the contribution of active silanol groups, a reagent specially affecting silanol groups must be found.

REFERENCES

1 C. B. Cox and R. W. Stout, *J. Chromatogr.*, 384 (1987) 315.
2 R. Schwarzenbach, *J. Chromatogr.*, 334 (1985) 35.
3 H. A. Claessens, L. J. N. van den Ven, J. W. de Haan and C. A. Cramers, *Chromatographia*, 20 (1985) 582.
4 J. E. Rivier, *J. Liq. Chromatogr.*, 1 (1978) 343.
5 B. Pfleiderer, *Master Thesis*, University of Tübingen, Tübingen, 1987.
6 T. Daldrup and B. Kardel, *Chromatographia*, 18 (1984) 315.
7 F. Vydra and J. Galba, *Fresenius Z. Anal. Chem.*, 235 (1968) 166.
8 Z. Marcenko, *Spectrometric Determination of Elements*, Wiley, New York, 1979.
9 J. R. Castner, G. S. Newell, W. C. Holton and C. P. Slichter, *J. Chem. Phys.*, 32 (1960) 668.
10 H. H. Wickmann, M. P. Klein and D. A. Shirley, *J. Chem. Phys.*, 42 (1965) 2113.
11 B. Pfleiderer, K. Albert, E. Bayer, L. van de Ven, J. de Haan and C. A. Cramers, *J. Phys. Chem.*, submitted for publication.