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Use of the reordering/resolvation of alkyl-modified silica to characterize the microscopic heterogeneity of silica via liquid chromatography

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ABSTRACT

The microscopic heterogeneity of the surface silanols of silica was studied by liquid chromatography. By examining thermally induced reordering/resolvation of bonded alkyl chains (*i.e.*, octyl groups) in contact with water, it is possible to deduce fundamental information about differences in the microscopic surface structure of silica prepared by different manufacturing processes. In addition, deuterium exchange–mass spectrometry was used to measure the bulk silanol concentration on the same silica.

INTRODUCTION

For over two decades, chemically modified silicas have been used as liquid chromatographic stationary phases. During this time, in order to improve the performance of existing phases and to develop novel new phases, a better understanding of the bonding chemistry and the surface structure has been of paramount importance. Initially, most phases were prepared using multi-reactive silanes because of increased stability. However, present synthetic routes often employ monoreactive silanes as the reaction is more controllable and reproducible. Even so, the surfaces formed and the nature of these surfaces with respect to their chromatographic selectivities vary between manufacturers and in-house preparations among laboratories. To a large extent these differences can be attributed to the silicas used to prepare the bonded phases in terms of geometric properties such as pore structure and surface area in addition to the general nature of the surface in terms of the number and types of silanol groups present and their distribution.

The key to explaining differences in chromatographic selectivity for a given immobilized ligand, especially when using monoreactive chemistry, lies in developing a better understanding of the surface structure. Two modified silicas with nearly identical surface areas, pore structures and coverages in terms of the total amounts of bound carbon often vary in chromatographic selectivity. Hence, although the surface coverages on a macroscopic level are equivalent, on a microscopic level the distributions of the bound ligands on the surface differ. The two extremes of this variable

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bonding model are either surfaces where the chains are uniformly distributed in a homogeneous arrangement or surfaces which are made up of varying spots or patches of bound ligands distributed in a non-homogeneous fashion with tightly packed organic areas and areas of non-bonding. This latter case has been shown by many investigators to reflect more closely the bonding heterogeneity of chromatographic phases.

A large number of investigators have studied silica and derivatized silica. Because of the extensive volume of work in this area it is not possible to cite all of the references; Iler [1] and Unger [2] have written extensive texts which summarize both the physical and chemical properties of silica. Based on geometric considerations, Iler [1] suggested that amorphous silica theoretically should have a surface silanol concentration of about 8 groups/nm^2. However, experimentally determined concentrations typically vary from this predicted value. In part, this can be attributed to the technique employed to quantify the silanols, especially if they involve either a chemical derivatization or a physical sorption process. Steric effects arising from the size of the reagent, the spacing of the silanols and the pore structure of the silica (*i.e.*, higher surface area materials that may contain a number of micropores) may result in lower than expected values. A number of procedures have been reported for determining the silanol concentration, including (1) adsorbed dye methods [3], (2) chemical reactions with methyllithium and Grignard reagents [4,5], (3) infrared spectrometry [6] or (4) treatment with deuterium oxide followed by either nuclear magnetic resonance spectrometry (NMR) [7–9], mass spectrometry (MS) [10] or chromatography [4,11] to monitor the degree of isotopic exchange.

In addition to the above considerations, the processes used to synthesize and thermally treat the silica during manufacturing and drying prior to analysis influence the number, types and distribution of surface silanols. Iler [12] suggested that the hydrated surface of amorphous silica dried at 150°C in air should contain 4.5–8.0 SiOH groups/nm^2. Similarly, according to Unger [13] the maximum silanol concentration on porous silica dried at 200°C should be 4.8–5.4 groups/nm^2. Recently published experimental results are in good agreement with the latter predictions. Measured silanol concentrations for various types of chromatographic silicas have been found to range between 3 and 5 groups/nm^2 [4,5,7–11].

The idea of characterizing the microscopic heterogeneity of silica by a method which requires no assumptions to be made about surface area, pore geometry and silanol distribution is appealing and should be potentially useful in explaining differences in chromatographic selectivity for similar bonded phases prepared on different silica substrates. In an effort to develop such a method a series of silicas produced by different manufactures were chemically modified by derivatization with *n*-octyltrichlorosilane. Subsequently, the reordering/resolvation of these materials in contact with water were studied by liquid chromatography [14]. Under such conditions the surface immobilized chains can be made thermally to undergo changes in their conformation. These changes have been described in terms of a characteristic reordering/resolvation temperature which is dependent on chain length and functionality of the bound ligand, but is independent of bonding chemistry and surface coverage within a certain critical range [15–18]. To date all previous investigations of reordering/resolvation have been carried out on a single type of silica, LiChrosorb Si-60. This work represents an extension of previous work to examine the influence of the substrate on

reordering/resolvation and the use of these data to characterize the microscopic nature of the surface.

EXPERIMENTAL

Materials

n-Octyltrichlorosilane and deuterium oxide (99.8 atom% of D) were purchased from Huls America (Levittown, PA, USA) and Aldrich (Milwaukee, WI, USA), respectively. Analytical-reagent grade toluene and high performance liquid chromatographic grade acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA, USA). Deionized water was produced in-house with a Milli-Q reagent water purification system (Millipore, El Paso, TX, USA).

Chromatographic-grade silicas were purchased from various manufactures: LiChrosorb Si-100 and Si-60 from E. Merck (Darmstadt, Germany), Partisil-10 from Whatman (Clifton, NJ, USA), Zorbax SIL from Rockland Technologies (Newport, DE, USA) and ICN 7–12 was from ICN Biochemicals (Cleveland, OH, USA). The physical properties of these materials are listed in Table I.

Water-saturated toluene was prepared by vigorously mixing toluene with water and allowing it to separate into two layers. Dry toluene was made by refluxing toluene with calcium hydride overnight.

Synthesis

About 1.7 g of a given silica were added to 30 ml of deionized water, the mixture was shaken, allowed to stand for 2–3 h and the excess water was decanted off. The resulting silica was dried at 120°C for 3 h then it was transferred to a special reaction vessel with a fritted-glass bottom [15] and 170 ml of water-saturated toluene were added. After allowing the silica to equilibrate overnight the water-saturated toluene was removed and the silica was reacted under reflux conditions with 30 ml of a 15% solution of *n*-octyltrichlorosilane in dry toluene for 8 h. During the modification, dry nitrogen was bubbled through the bottom of the reaction vessel to stir the mixture and to expel the HCl generated. Subsequently, the silica was washed four times with 50-ml portions of both dry and water-saturated toluene and then twice with 50 ml portions of diethyl ether. The material was dried at 120°C.

TABLE I

PHYSICAL PROPERTIES OF THE SILICA STUDIED

Data are from manufacturers' literature.

Silica	Particle size (μm)	Surface area (m^2/g)	Pore size (Å)
LiChrosorb Si-60	10	550	60
LiChrosorb Si-100	10	420	100
Zorbax SIL	7	350	70–80
Partisil-10	10	>400	80
ICN 7–12	7–12	500–600	60

Column packing

A slurry was prepared by combining about 0.8 g of a given modified silica with *ca.* 30 ml of 2-propanol in a dynamic packing apparatus. The mixture was stirred for about 15 min and the apparatus sealed. The modified silica was packed by the ascending technique into 25 cm × 2.4 mm I.D. stainless-steel columns using a Haskel (Burbank, CA, USA) Model DSTV-52-C air-driven fluid pump and a packing pressure of 6000 p.s.i. [19]. Methanol was used as the delivery solvent.

Equipment

A Hewlett-Packard (Palo Alto, CA, USA) Model HP5995 gas chromatograph–mass spectrometer equipped with a Model 9825A desktop computer was used to make the deuterium exchange measurements. In doing this, the gas chromatograph was not employed. Rather, the samples were introduced directly into the quadrupole mass analyzer by slowly bleeding the vapors developed above small L-shaped glass tubes (1/4 in. O.D.) which were attached through the auxiliary mass calibration inlet port. To maximize sensitivity and minimize interferences, the analyzer was set to scan over a mass range from 16.0 to 22.0 u. The mass analyzer temperature was 200°C and the electron impact (70 eV) ionization source was set at 150°C.

The liquid chromatographic system consisted of a Laboratory Data Control (Riviera Beach, FL, USA) Constametric III pump, an Altex Model 152 UV detector set at 254 nm and a Rheodyne (Berkeley, CA, USA) Model 7010 injection valve with a Model 7012 loop filler port. The column temperature was controlled in a water-bath equipped with a Fisher Scientific Model 730 isotemp immersion circulator and a Neslab Instrument (Portsmouth, NH, USA) Model EN-350 flow-through liquid cooler. The flow-rate of the mobile phase was measured by a Phase Separation (Queensberry, Clwyd, UK) Model FLOSOA electronic flow meter which was connected to the outlet of the UV detector. The detector output signal was recorded and analyzed using an IBM Instruments (Danbury, CT, USA) Model 9000 computer system and chromatographic applications program (CAP).

Deuterium exchange and mass spectrometric measurements

A set of standard solutions were prepared by adding from 1–5% of water to D_2O. The different silica samples were dried at 150°C under vacuum for 24 h to remove adsorbed water. After cooling to room temperature in a desiccator, 60–80 mg of silica were weighed into L-shaped glass sample tubes, 200 μl of deuterium oxide which contained 1% of water were added and the tubes were sealed. To facilitate complete wetting of the material (*i.e.*, to release the gas from the pores) the sealed sample tubes were placed in an ultrasonic bath and sonicated for at least 30 min. Subsequently, the samples were allowed to stand for at least 4 h before analysis.

Before each standard and sample measurement, a pure deuterium oxide background was run to insure that no exchange peaks were present in the mass spectrum. Samples were introduced directly into the analyser section via the auxiliary calibration inlet port at a pressure of $2 \cdot 10^{-5}$ Torr (1 Torr = 133.3 Pa) (monitored by a vacuum ionization gauge). Spectra were recorded for a minimum of 10 min. Plateau values were reached after about 0.5 min of sample introduction time. At least ten values were averaged from the reported ion tables over the last 5 min of acquired spectra. Separate sample introductions and mass spectra were collected a minimum of

three times on each tube. Each silica was studied a minimum of at least three complete times on separate days.

Liquid chromatographic measurements

Each column was first conditioned with 100 ml of acetonitrile followed by an equal amount of water at the lowest temperature studied, 10°C. Acetonitrile was used to minimize entrapment problems [20]. After conditioning, the retention times of three test solutes (resorcinol, phenol and *p*-cresol) were measured every 5°C up to 80°C (initial evaluation). This was carried out using deionized water as the mobile phase at a flow-rate of 1.0 ml/min. At least two injections of each solute were made at all temperatures. After completing the initial evaluation, the column was cooled to 10°C and the retention times of the test solutes were measured again over the same temperature range (re-evaluation). Between all temperature changes the pump was switched off until the column had reached thermal equilibrium. All columns were studied at least twice by the above procedure (*i.e.*, conditioning, initial evaluation and re-evaluation).

The mean capacity factors, k', were calculated from multiple injections of the test solutes using deuterium oxide to determine the void volume. Void volume measurements were made at each temperature studied.

RESULTS AND DISCUSSION

Although there are a number of methods for determining the number and types of silanol groups on the surface of silica, the results obtained typically give macroscopic information about the system. In order to reduce these macroscopic data to a microscopic level, assumptions must first be made in terms of the surface area and pore geometry of the support and the distribution of the silanols on its surface. In the current study the surface silanol concentrations for several different silicas were measured using a conventional bulk characterization procedure, deuterium exchange followed by mass spectrometry. Subsequently, these same silicas were initially modified via chemical derivatization with *n*-octyltrichlorosilane and then the reordering/resolvation of the materials in contact with water measured by liquid chromatography. The resulting data appear to reflect differences in microscopic heterogeneity of the surfaces as discussed below.

Deuterium exchange and mass spectrometric measurements

The surface silanol concentrations for the five different chromatographic-grade porous silicas were measured by deuterium exchange–mass spectrometry. Prior to making these measurements each material was treated as described under Experimental. During the development stages of this work, it was found to be necessary to add 1% of water to the deuterium oxide in order to reach a total level of exchanged water which gave reproducible mass spectrometric readings and which fit in the linear region of calibration graphs generated by adding known amounts of water (*i.e.*, from 1 to 5%) to deuterium oxide.

Fig. 1 shows a representative calibration graph obtained by plotting the exchange ratio for the 19–20 peaks *vs.* the logarithm of the percentage of water added. At water levels between 1 and 5% the graphs were linear with correlation coefficients

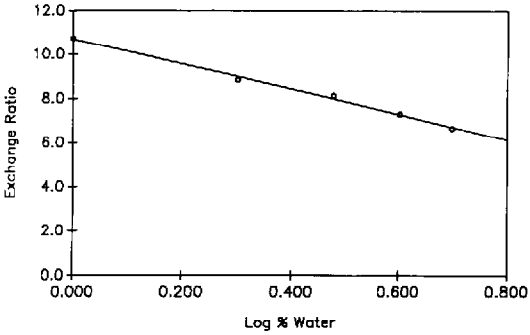

Fig. 1. Representative calibration graph: exchange ratio of the 19–20 u peaks *vs.* log (% water added).

of 0.99 or better. Before each calibration and sample measurement, a pure deuterium oxide background was run to insure the absence of a 19 u peak in the mass spectrum. Readings were taken by alternating samples and standards.

The concentration of surface silanols in moles per gram of material are given in Table II for the five silicas studied. The surface concentrations are also reported in terms of the number of silanol groups per nm^2 based on the manufacturers' listed surface area data summarized in Table I. The measured values ranged between 3.4 and 4.9 silanols/nm^2. These data are in good agreement with values reported by Iler [12] and other recently published data [4,5,7–11]. For LiChrosorb-100 where a direct comparison can be made, the current value of 4.42 groups/nm^2 and an earlier reported value by Welsch and Frank [4] of 4.40 groups/nm^2 are identical. However, both the current value and that of Welsch and Frank are slightly lower than that reported by an alternative deuterium exchange method [11] (*i.e.*, 5.1 groups/nm^2). For Zorbax SIL, the current value of 4.41 silanols/nm^2 is slightly higher than that previously obtained by solid-state NMR methods [8,9] of 3.0–3.6 groups/nm^2. It seems reasonable that some of this difference might be attributed to variations in surface area between manufactured batches of Zorbax or treatment and drying steps in the manufacturing and preanalysis handling of the silica. However, it is unclear whether these factors would account for all of the difference.

TABLE II

MEASURED SILANOL CONCENTRATIONS FOR THE SILICAS STUDIED

Silica	Silanol concentration (mmol/g)	Groups/nm^2 [a]
LiChrosorb Si-60	3.14	3.44 ± 0.24
LiChrosorb Si-100	3.09	4.42 ± 0.09
Zorbax SIL	2.56	4.41 ± 0.82
Partisil-10	3.22	4.86 ± 0.13
ICN 7–12	3.07	3.37 ± 0.76

[a] Data were measured by deuterium exchange–mass spectrometry and are based on the manufacturers' reported surface areas listed in Table I.

The value of 3.44 groups/nm^2 for LiChrosorb Si-60 seems low compared with that obtained for LiChrosorb Si-100 and may reflect differences in pore structure and wetting. Similar numbers were obtained for another higher surface area material, *i.e.*, 3.37 groups/nm^2 for ICN 7–12. The current results are consistent with data reported for other materials with smaller pores such as Porasil 60 silica [7]. In all instances the above calculated values (column 2 in Table II) assume a homogeneous distribution of the silanol groups on the surface.

Liquid chromatographic studies of reordering/resolvation

The orientation of silica-immobilized alkyl chains in contact with water are influenced by cohesive, hydrophobic and specific interactions between the chain, solvent and surface. [16]. A two-state model has been proposed where the bonded chains may be in either an aggregated/collapsed conformation or in a more extended/solvated conformation, depending on the thermal conditons. These conformational differences have been studied as a function of various properties of the bonded groups, including their size, shape and polarity (*i.e.*, the presence of functional groups) as well as the bonding chemistry [15–18,19–27]. To date, all previous investigations have been carried out using a single type of silica substrate, LiChrosorb Si-60.

In the current study, columns were packed with different octyl-modified silicas, conditioned with acetonitrile, and the retentions of three solutes were measured as a function of temperature using water as the mobile phase. These experiments were conducted in a similar fashion to those reported by Gilpin's group [15–18, 27].

Representative plots of ln k' *vs.* $1/T$, where T (K) is the column temperature, are shown in Fig. 2 for phenol chromatographed on the Zorbax- and Partisil-modified silicas. Plots were also constructed for the other test solutes on these same modified

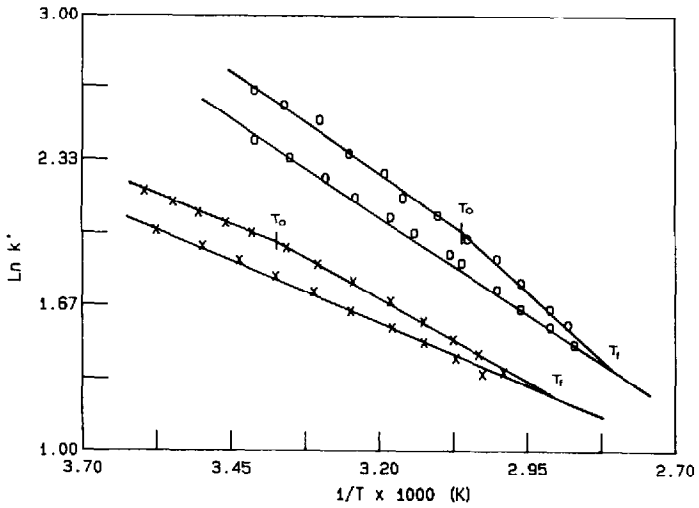

Fig. 2. Representative plots of ln k' *vs.* $1/T$ which illustrate the differences in thermally induced reordering/resolvation for octyl groups chemically bonded to two different silica substrates. T_o and T_f represent the initial and final surface reordening/resolvation temperatures, respectively. (○) Zorbax silica; (×) Partisil silica.

silicas and on the other modified silicas. The general shapes of all the curves were similar to each other and to curves reported previously [15] for octyl-modified LiChrosorb Si-60. However, the curves differed in the onset temperature (T_o) for reordering/resolvation and the temperature range (ΔT) over which thermal reordering/resolvation occurred.

Summarized in the Table III are T_o values for the various octyl-modified silicas. These data are averages from at least duplicate injections for the three different solutes, resorcinol, phenol and *p*-cresol. In all instances at least two different reaction batches for a given silica were studied. For comparative purposes the data for LiChrosorb Si-60 reported earlier [15] are also included in Table III. The data in Table III show that the reordering/resolvation temperature is independent of surface coverage (*i.e.*, at least for the range studied) for a given silica. This trend is also in agreement with results from earlier studies [15,17]. For example, although the surface coverages of the different reaction batches of octyl-modified Partisil range from 8.8 to 11.0% bound carbon, the average reordering/resolvation temperatures are statistically not significantly different. This is also true of the other materials listed in Table III. These results imply that within a critical range of chain density bonding occurs in subunits or patches on the surface. Increasing the extent of the reaction may increase the size of a subunit or the formation of more subunits but it does not increase the bonding density within a subunit. Thus, the reordering/resolvation temperature is independent of the number of subunits but depends on the spacing (*i.e.*, distribution/heterogeneity of the surface silanols) of the bonded groups within a subunit.

Different silicas produced via different processes should vary in the number and

TABLE III

SURFACE COVERAGE AND REORDERING/RESOLVATION TEMPERATURE FOR THE OCTYL-MODIFIED SILICAS

Silica	Column	Carbon (%)	Onset temperature[a] (°C)
LiChrosorb Si-60		9.5[b]	Av. 44.7 ± 0.7[b]
LiChrosorb Si-100	a	7.4	44.1
	b	7.2	45.5
	c	6.8	44.1
			Av. 44.6 ± 0.7
Zorbax SIL	a	10.0	54.5
	b	7.2	56.2
	c	7.2	52.5
			Av. 54.5 ± 1.5
Partisil-10	a	11.0	22.4
	b	8.8	24.9
			Av. 23.7 ± 1.3
ICN 7–12	a	8.9	35.4
	b	9.3	35.7
			Av. 35.6 ± 0.2

[a] Determined from linear fits of ln k' *vs.* $1/T$ data labeled point T_o in Fig. 2.
[b] Data from ref. 15.

average spacing of silanol groups. As shown in Table III, the reordering/resolvation temperatures for the octyl-modified surfaces are significantly different for silicas produced by different manufacturers. On the other hand, for LiChrosorb Si-60 and Si-100, which are manufactured by a similar process, the reordering/resolvation temperatures are similar. For Zorbax and Partisil, reordering/resolvation start at about 54 and 24°C, respectively. Their physical properties such as pore size and surface area (Table I) are similar, but their reordering/resolvation temperatures differ by more than 30°C. These observations further support the idea that resolvation is controlled by the microscopic distribution of the bound chains (*i.e.*, the silanol distribution), not gross physical properties of the silica.

Table IV gives mean data for T_o and T_f, the initial and final surface reordering/resolvation temperatures, for the various octyl-modified silicas. The total temperature ranges (ΔT) over which thermally induced reordering/resolvation occurred are also listed in Table IV. As cohesive and hydrophobic interactions are dependent on chain length, for a given chain length, T_o, T_f and ΔT should be related to the distribution and spacing of the bonded groups on the surface and thus should reflect differences in the heterogeneity and concentration of the silanols on the silica substrate. Again, comparing Zorbax and Partisil, the range for Partisil, $\Delta T = 44.5$°C, is about twice that for Zorbax, $\Delta T = 25.3$°C. These results imply that there is a greater degree of heterogeneity in terms of the silanol distribution of Partisil compared with Zorbax.

CONCLUSION

Deuterium exchange mass spectrometry was used to measure the macroscopic silanol concentrations of silica produced by different manufacturers, which ranged from 3 to 5 groups/nm^2. These data show neither a significant difference between the materials nor a direct relationship with reordering/resolvation of the materials in contact with water. As macroscopic measurements such as surface coverage and silanol concentration determined by gas chromatography–mass spectrometry give only average properties of the surface, they do not satisfactorily characterize the heterogeneity of the surface of silica. It is this latter aspect that the chromatographic method described in the current work has addressed.

Based on the reordering/resolvation data obtained in this or in similar studies applied to other substrates, it may be possible to explain either similarities of differences in the chromatographic performance of bonded phases prepared on various types of silica. As reordering/resolvation of the surface are dependent on chain length

TABLE IV

TEMPERATURE RANGE OVER WHICH SURFACE REORDERING/RESOLVATION OCCURS

Silica	T_o(°C)	T_f(°C)[a]	$\Delta T = T_f - T_o$ (°C)
LiChrosorb Si-100	44.6	71.8	27.2
Zorbax SIL	54.4	79.7	25.3
Partisil-10	23.7	68.2	44.5
ICN 7–12	35.6	76.8	41.2

[a] T_f is the final temperature where all the surface is solvated.

[16], for a given chain length, T_o and ΔT should be related to the distribution and spacing of the bonded groups on the surface and should reflect differences in the heterogeneity and concentration of silanols on the silica substrate which arise during manufacture.

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