CHROMSYMP. 745

DETERMINATION OF SILANOL GROUPS ON PACKINGS FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY BY MEANS OF ISOTOPIC EXCHANGE

J. GOWOREK*, F. NOOITGEDACHT, M. RIJKHOF and H. POPPE*

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam (The Netherlands)

SUMMARY

A fast, easy, and non-destructive method has been developed for the determination of silanol groups in liquid chromatography columns by using common high-performance liquid chromatographic instrumentation. The method is based on the complete exchange of the silanol protons with deuterium.

INTRODUCTION

The presence of residual silanol groups in chemically modified packings is assumed by many workers to be responsible for a number of undesirable chromatographic effects, such as peak tailing and non-reproducible peak positions. Also, it is assumed that the variations in relative retention observed with different brands of packing material, or even different batches of the same brand, can be attributed to a varying influence of the silanol groups. Therefore, it would be useful to have available a method for the determination of those groups that is fast, feasible with common high-performance liquid chromatographic (HPLC) instrumentation, and involves no destruction of (expensive) HPLC columns.

We have previously observed[1] rapid deuteron–proton exchange under HPLC conditions between hydroxyl groups in solvent components and the silanol groups on the packing, and we speculated about the use of this effect for the determination of the silanol content. In this paper we report some preliminary results obtained with this method.

THEORETICAL

The experiment is performed as follows. First, the column is equilibrated with an "inert" solvent, A. By "inert" we mean that the solvent molecules do not contain protons that exchange at a measurable rate under the prevailing conditions. On the

---

* Present address: Department of Physical Chemistry, Marie Curie-Sklodowska University, Nowotki 12, 20-031 Lublin, Poland.

basis of a wealth of experimental results[2], it can be safely assumed that all aryl and alkyl hydrogens, with the possible exception of those activated by carbonyl groups, do not exchange and that all oxygen-bound hydrogens do. Thus acetonitrile, benzene, and dichloromethane are inert, but methanol is not. Solvent A must be dried carefully, and the equilibration time must be long enough for all physically adsorbed water to be removed from the column. After equilibration, the eluent is switched to one containing a given [preferably *ca.* 1% (v/v)] concentration of a proton-exchange solvent, B; methanol and water are the most convenient. The breakthrough volume, $V_{R,B}$, of B is then measured.

Next, the solvent-delivery system is switched to a solvent having the same composition, except that now its B component is isotopically tagged with deuterium or tritium (B*). The breakthrough of the tag can then be measured by refractometry, radioassay, mass spectrometry, or atomic emission spectrometry, and the retention volume (V_{R,B^*}) can be determined. The amount of silanols in the column (in moles) is then calculated from

$$n_{SiOH} = (V_{R,B^*} - V_{R,B})c_B \tag{1}$$

where c_B is the molar concentration of B and B* in the two solvents applied.

Refinements

When molecule B is doubly tagged, as is the case with water, one should use, instead,

$$n_{SiOH} = 2(V_{R,B^*} - V_{R,B})c_B \tag{1a}$$

When breakthrough curves show significant broadening, *e.g.* due to a low plate number, and/or the absence of a self-sharpening effect[3,4], one should determine the breakthrough volume as described in Fig. 1.

Derivation

Following Riedo and Kováts[5] we make use of the "molar component capacity", *i.e.* the total amount of component i (not compound, as used by Riedo and Kováts, as this would preclude the treatment of isotopic exchange reactions!) in the column in equilibrium, M_i, which is an unambigious function of the composition of the mobile phase. The retention of step change in the concentration from $c_{n,i}$ to $c_{n+1,i}$, from equilibrium state n to equilibrium state $n+1$, can be derived by considering the
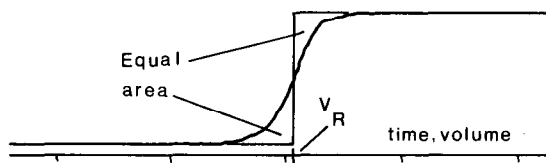

Fig. 1. Determination of the breakthrough retention volume with broadened steps. The perpendicular line is drawn to make the two indicated areas equal. With digital integration, the position of the line can be calculated directly from the integration of the step.

integral mass balance[3,4,6,7]. Neglecting compression and contraction effects, one arrives at:

$$V_{R,n,n+1}(c_{n+1,i} - c_{n,i}) = M_{n+1,i} - M_{n,i} \qquad (2)$$

Applying this relation to the two subsequent steps in the experiment, the first time for compound B, and the second time for the isotopic atom, leads to the following:

(1) The retention volume $V_{R,B}$ measures the amount, M_B, of compound B in the column, regardless of whether B is adsorbed or present in the mobile phase (whatever the definition of the latter).

(2) The retention volume, V_{R,B^*}, measures the amount of exchangeable protons, M_H, in the column.

These two quantities are equal, except for the exchangeable protons in the stationary phase, *i.e.* the silanols (and the hydroxyls of possible diol-groups in the packing).

Precision

The amount of i in the column, M_i, can be defined as[5]

$$M_i = V_{m/CX}C_i + S\Gamma_{i/CX} \qquad (3)$$

where $V_{m/CX}$ and $\Gamma_{i/CX}$ are the mobile phase volume and surface excess of i, respectively, using some convention, CX, to fix the position of the Gibbs dividing plane, and S is the surface area of the adsorbent. The retention volume in the first step is therefore equal to

$$V_{R,B} = V_{m/CX} + S\Gamma_{B/CX}/c_B \qquad (4)$$

That of the step in the second experiment is

$$\begin{aligned} V_{R,B^*} &= V_{m/CX} + S\Gamma_{H/CX}/c_H \\ &= V_{m/CX} + S\Gamma_{B/CX}/c_B + S\Gamma_{SiOH}/c_B \qquad (5) \end{aligned}$$

In order to preserve a reasonable precision when determining the difference between these two quantities, it is imperative to work under conditions where the SiOH term in eqn. 5 is not too small in comparison with the other terms. This means that c_B should be low. On the other hand, depending on the detection system used, it might be necessary to have a reasonably high value for c_B in order to allow the detection of the isotope step. With the refractive index system used in our work, it was impossible to use c_B values much below 1% (v/v). This probably limits the precision of the method at present.

Accuracy

The derivation of the preceding equations is straightforward and does not leave much room for systematic errors. At the low concentrations of B used, and with reasonably low flow-rates, the contraction and compression effects can indeed be neglected. The assumption that equilibrium is reached after every step is more dif-

ficult to verify. In particular, the completeness of the "drying" in the pre-equilibration and the completeness of isotopic exchange in the second step of the experiment may be questioned. In the section on Results and Discussion we shall show that a study of temperature and flow-rate dependencies, together with a study of the shape of the steps, indicate that a large proportion of the silanols is indeed accessible with this method.

It is important to note that the isotope effect on distribution equilibria does not lead to a systematic error when the proposed method is used. This is due to the fact that "carrier-free" materials are used (in the case of isotopic labelling, this is always possible), and a full stoichiometric exchange of the hydrogens is accomplished during the second step.

EXPERIMENTAL

Equipment

Fig. 2a is a schematic diagram of the equipment used. A high-pressure membrane pump (Model 1515, Orlita, Giessen, F.R.G.) was used to supply the mobile phase. A large loop in the injection valve was applied to switch to the new mobile phase composition. This loop (12.5 ml), consisting of 3.95 m of stainless-steel tubing, 0.5 mm I.D., was usually filled by using a high-pressure pump (Model b-100-S-2, Eldex Labs., Menlo Park, CA, U.S.A.). Alternatively, a larger-bore loop was used, but this had the disadvantage that more deuterated water is wasted because of the larger dispersion in such a tube. Valve V_1 permits pre-pressurization of the loop contents. Without this, the compression effect after the "injection" of 12.5 ml by means of valve V_2 would lead (at 100 bar) to a "loss" in the mobile phase flow of some 100 μl, which would lead to drastic systematic errors[6]. Fig. 2b illustrates the operation of the injection system.
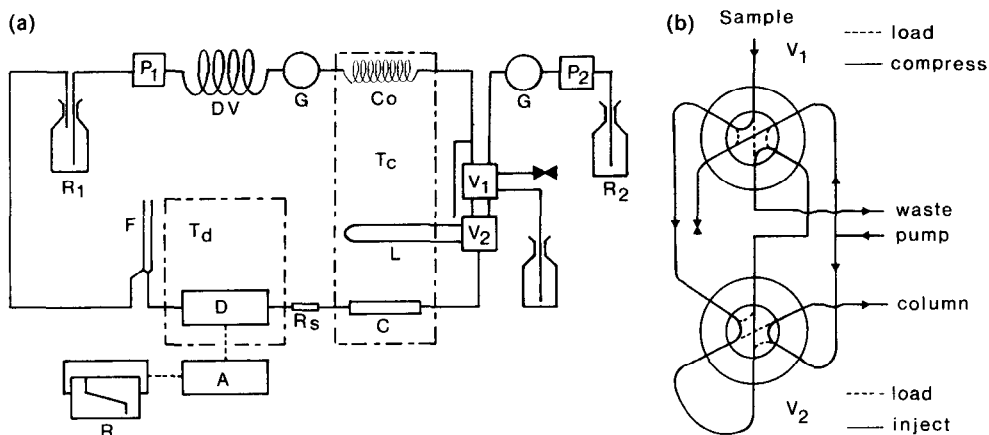

Fig. 2 (a). The experimental set-up: R_1 = solution reservoir; R_2 = sample reservoir; P_1 = pump for elution; P_2 = pump for filling the loop (not essential); DV = damping valve; F = pressure gauge; V_1 and V_2 = sampling valves; L = loop with 12.5-ml volume; C = chromatographic column under study; Rs = restrictor; D = refractometer; A = amplifier; R = recorder; F = liquid flow-meter; T_d = thermostat for the detector; T_c = thermostat for the column; Co = coil for pre-thermostatting the mobile phase. (b) Injection system.

TABLE I

PACKING MATERIALS USED AND RESULTS FOR SILANOL CONTENT

Material	n_{SiOH} found	
	mmol/g	mmol/g base silica
LiChrosorb Si-60 (10 μm)	3.91	
LiChrosorb RP-8 (10 μm)	0.96	
LiChrosorb RP-2 (10 μm)	1.19	
Zorbax-Sil (5–6 μm)	1.86	
Zorbax-ODS (5–6 μm)		0.41–0.57*
Zorbax-C$_8$ (5–6 μm)		0.18
Zorbax-phenyl (5–6 μm)		0.35

* See Discussion and Fig. 5.

The step elution functions were observed by means of a refractometer (Model LC1, Siemens, Karlsruhe, F.R.G.). The whole system was temperature-controlled by two circulating-liquid thermostats, one for the detector (T_d, needed for observing the isotope breakthrough at the highest sensitivity) and one for the column (T_c). The latter was filled with mineral oil in order to allow measurements up to 373 K. The mobile phase was pre-thermostatted in coil Co. A restrictor, Rs, was included to prevent the mobile phase boiling at the end of the column.

Flow-rates were measured frequently during experiments by means of a simple buret-type flow-meter, F, and a stopwatch.

Column and chemicals

The columns (15 cm × 4.6 mm I.D.) were slurry-packed, with tetrachloromethane as the slurry liquid and methanol as the driving liquid. The packings investigated are listed in Table I.

Doubly distilled water and p.a.-quality acetonitrile and deuterium oxide were used. Acetonitrile was carefully dried over activated silica gel prior to use.

Procedures

The columns were equilibrated with the starting solvent, usually for 15 min, after it had been established that longer equilibration would not lead to different results for a particular packing. The value of the retention time was found from the recorder tracing, as indicated under Theoretical. The flow-rate was ca. 2 ml/min. For very fine particles it might be necessary to use a lower flow-rate in order to avoid compression and thermal effects. In cases were the carbon content is known, we report the silanol content as millimoles per gram of bare silica, in other cases as millimoles per gram of packing. The amount of packing was determined by emptying the columns after the experiment. However, in most cases the amount can be estimated from the column volume and known packing characteristics.

RESULTS AND DISCUSSION

Fig. 3 shows a sample chromatogram of the two steps, as observed when the
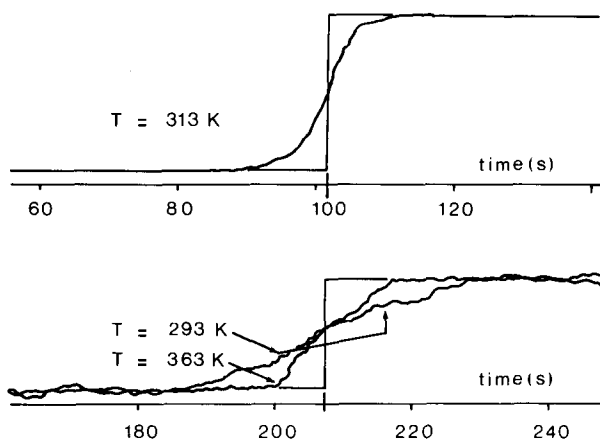
Fig. 3. Example of the curves obtained in the first (attenuation, 256) and second step (attenuation, 1) for an experiment with a Zorbax-ODS column.

packing was Zorbax-ODS. As can be seen, it is difficult to observe the step to deuterated water solution, owing to the small difference in the refractive index and the low concentration (0.7% v/v) used. However, replicate determinations indicated reasonable precision, *ca.* 1%, in the retention volume.

Sometimes the tracing in the second part of the experiment shows a second step, which usually comes earlier. This is the result of a non-ideal matching of the concentrations, c_B and c_{B^*}, and the position of this step corresponds to that of a minor disturbance[1,4,6,7] experiment. When water isotherms are linear, the silanols can be determined in one experiment. However, because of uncertainties with respect to the isotherm shape on various packings, this is not preferable to the proposed procedure.

The same figure indicates the difference between experiments at high and low temperature: the step is much sharper at higher temperature. With better signal-to-noise ratio it would be possible to extract kinetic information on the exchange rate of the isotopes. As the chemical kinetics[2] are fast, and the rate of transport in the interstices and pores can be estimated from the behaviour of normal solutes, this approach would yield information on the rate at which silanols are accessible.

In these experiments, we focused on the equilibrium phenomena. Fig. 4 shows the retention volumes and the obtained estimated of n_{SiOH} as a function of temperature for the Zorbax Sil material. As can be seen, the estimate is virtually constant, as is expected in the given temperature range, despite the significant variation of the two retention volumes. This indicates that the amount of accessible silanols does not depend on temperature, and the most likely explanation is that all silanols participate in the exchange reaction.

In experiments in which the flow-rate through the column was varied (not shown), a constant value for n_{SiOH} was found. This again suggests that no silanols, that exchanging at an extremely low rate, are present. Similar results were obtained with most of the packing materials used, *i.e.* the n_{SiOH} values found were virtually independent of temperature.

Table I lists the results obtained at 295 K. The effect of the chemical modifi-
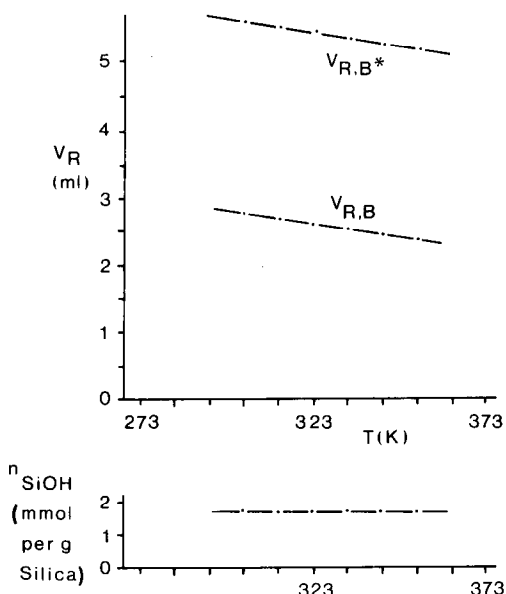
Fig. 4. Retention volumes, $V_{R,B}$ and $V_{R,B}^*$, and estimate of silanol content, n_{SiOH}, as a function of temperature. Column packing, Zorbax Sil.
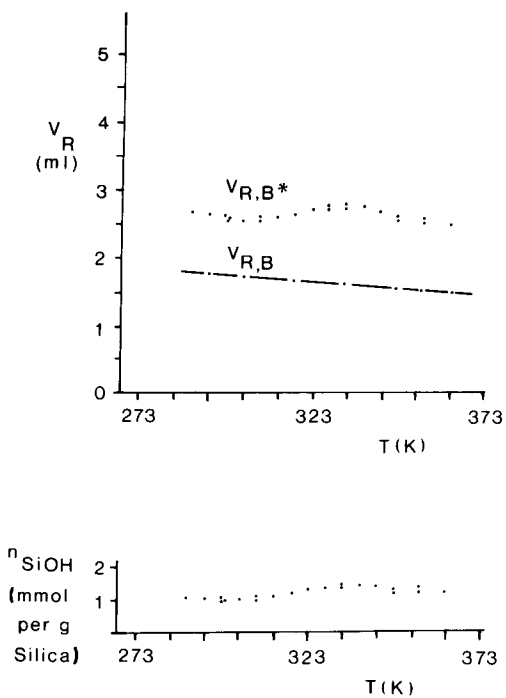
Fig. 5. Retention volumes, $V_{R,B}$ and $V_{R,B}^*$, and estimate of silanol content, n_{SiOH}, as a function of temperature. Column packing, Zorbax ODS.

cation on the amount of accessible silanols is clearly visible. However, interpretation in quantitative terms is not yet possible, as all the necessary data on surface area, percent carbon, and on the base silica are unavailable.

For Zorbax-ODS the observed amount of silanols depends significantly on the temperature (Fig. 5). In the plot of the estimated n_{SiOH} values against temperature a sigmoidal shape can be observed. The increase of the n_{SiOH} values at *ca.* 320 K might be the result of the fact that at that temperature source of the silanols become accessible. However, the graph, as it is, suggests that superimposed on such an increase at a given temperature, there is an overall negative slope. No explanation for this observation is available. Such a slope would be obtained if the column were not completely dried at the beginning of an experiment, because any remaining physically adsorbed water would be indistinguishable from silanols in the subsequent procedure. The resulting positive bias would also decrease at higher temperature because of the increased water content of the mobile phase. However, the precautions we have taken against water contamination, and the fact that we did not find a systematic effect of pre-equilibration time, make this explanation rather inplausible.

CONCLUSION

It has been shown that isotopic exchange of hydrogen atoms of silanol groups can be used under HPLC conditions to measure the amount of accessible silanols on the surface of bonded and non-bonded silicas. A substantial fraction (if not all) of the silanols participate in the stoichiometric exchange. Further work should be devoted to the comparison of our results with those from other methods, the interpretation in terms of the original silanol content of the base silica, and the extent of bonding. The possibility of studying the rate of exchange should also be explored.

ACKNOWLEDGEMENT

REFERENCES

1 E. H. Slaats, W. Markovski, J. Fekete and H. Poppe, *J. Chromatogr.*, 207 (1981) 299.
2 A. I. Shatenshtein, *Exchange and the Replacement of Hydrogen in Organic Compounds*, authorized translation from the Russian, C. N. Turton and T. I. Turton, Consultant Bureau, New York, 1962.
3 D. DeVault, *J. Am. Chem. Soc.*, 65 (1943) 532.
4 J. F. K. Huber and R. G. Gerritse, *J. Chromatogr.*, 58 (1971) 137.
5 F. Riedo and E. sz. Kováts, *J. Chromatogr.*, 239 (1982) 1.
6 A. W. J. de Jong, J. C. Kraak, H. Poppe and F. Nooitgedacht, *J. Chromatogr.*, 193 (1980) 181.
7 J. Jacobson, J. Frenz and Cs. Horváth, *J. Chromatogr.*, 316 (1984) 53.