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DETERMINATION OF HYDROXYL GROUPS AND WATER CONTENT IN SILICA BY NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY*

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SUMMARY

Deuterium exchange of the protons of hydroxyl groups in silica and the extraction of adsorbed water by deuterated trifluoroacetic acid results in a change in the amplitude of the signal due to the residual COOH groups and a chemical shift in the nuclear magnetic resonance spectrum. With the help of a calibration plot, the increase in signal amplitude can be divided into the contributions from protons of OH groups and from protons of water. The water contents determined are comparable to those obtained by thermogravimetry; the OH contents are slightly higher than values published previously. The relative decrease in OH content caused by hydrophobization and "capping" procedures is easy to follow, and so this method is useful in optimizing the production of reversed-phase bonded supports for high-performance liquid chromatography.

INTRODUCTION

The preparation of silica packing for reversed-phase chromatography involves the production of an essentially hydrophobic surface with a high degree of uniformity^{1,2}. This is usually achieved by chemical binding of different hydrophobic groups to the silica surface OH groups³. The degree of coverage of the surface has a significant effect on the adsorption behaviour of the parent support, and in order to minimize matrix interactions a dense coverage of functional groups is necessary.

Even with maximum conversion, some hydroxyl groups (*ca.* 50%) remain unreacted at the surface of the packing as they are shielded by modifying groups. The problem is complicated by the generation of new silanols from the hydrolysis of unreacted chlorosilane groups when trichlorosilanes are used as modifying agents. Attempts to eliminate these unreacted silanol groups have included their "capping" with trimethylchlorosilane (*e.g.*, ref. 3).

Although the rôle of the remaining OH groups is not yet completely clear, it has been shown that they contribute to solute retention and to peak asymmetry.

* The principle of this method was presented by M.H. at the 2nd NMR Seminar, Prague, 1974.

Moreover, they lower the chemical stability of partially covered reversed-phase packings².

To optimize the production of bonded reversed-phase supports for high-performance liquid chromatography (HPLC) it is necessary to determine the concentration of hydroxyl groups in the initial silica support and in modified and "capped" derivatives. This can be done by use of chemical or physical methods¹. Chemical methods include the treatment of silica with diborane, dimethyldichlorosilane, dimethylzinc^{4,5} or methyl lithium. The reaction with organometallic compounds yields a relatively precise measurement of the content of surface OH groups, but the reagent can only penetrate into pores that have a sufficiently large mean pore diameters and so the results obtained on silica with micropores and small mesopores are considerably lower than expected¹.

This problem is overcome by isotopic exchange with deuterium- or tritium-labelled water, which is the basis of some physical methods for the determination of OH contents¹. However, both detection methods, infrared spectroscopy for ²H₂O exchange and scintillation counting for H³HO exchange, are very complicated and time consuming and many precautions (*e.g.*, outgassing and dehydration of carefully prepared samples, separation of reactants after equilibration, hydrophobization of all glass apparatus prior to analysis) and corrections (*e.g.*, for background, light scattering, isotopic equilibrium effect)¹ have to be made.

EXPERIMENTAL

Method

Determination of OH groups and water content in silica by nuclear magnetic resonance (NMR) spectroscopy is based on two well known phenomena: the intensity of the ¹H NMR signal is proportional to the number of hydrogen atoms, and the chemical shift of the proton signal in a fast exchanging acid-water mixture depends on the relative molar concentration of each component⁶.

If deuterated trifluoroacetic acid is mixed with silica, then the increase of the residual proton signal (CF₃COOH in CF₃COO²H) corresponds to the number of H atoms exchanged from silica OH groups and from adsorbed water. Trifluoroacetic acid, being a strong acid, is believed to result in quantitative exchange of the hydrogens of the sample. Furthermore, the signal of the residual protons is shifted upfield in proportion to the amount of water extracted from the silica into the solvent.

In order to maintain the same homogeneity of the magnetic field in all measurements, *p*-xylene is added as an internal standard. The signal from its methyl groups is used for field-frequency lock, and the signal intensity due to aromatic hydrogens serves for checking and, if necessary, for correction of the intensity of the COOH signal. Namely, the intensity of this signal corresponds to the sum of residual protons in deuterated trifluoroacetic acid, protons from OH groups of silica and protons from water adsorbed on silica only when the intensity of the signal due to aromatic protons of xylene is the same in a blank as in the experiment with the sample. Generally this is not true, and a correction, *f*, must be used (for the meaning of symbols see Table I):

$$A_{\text{H}}^{\circ} + A_{\text{OH}} + A_{\text{H}_2\text{O}} = A_{\text{H}}f; \quad f = A_x^{\circ}/A_x \quad (1)$$

TABLE I
SYMBOLS USED IN CALCULATIONS

Experiment	Signal amplitude		Chemical shift of CF_3COOH	Weight	
	CF_3COOH	Xylene		Solvent	Sample
Blank	A_H^0	A_x^0	ν_H^0	W^0	—
Calibration	A_H'	A_x'	ν_H'	W'	W_{H_2O}
Determination	A_H	A_x	ν_H	W	W_{H_2O}

The same procedure is used for the calibration experiments in which an increasing amount of water is added to a known amount of solvent ($CF_3COO^2H +$ xylene):

$$A_H^0 + A'_{H_2O} = A'_H f; \quad f = A_x^0 / A'_x \quad (2)$$

For the calibration plot two dependences are used: the increase of the COOH signal amplitude and the change of its chemical shift with increasing amount of water added. The plot in Fig. 1 was constructed using the values of Table II.

To be able to compare the results of calibration with those from experiments

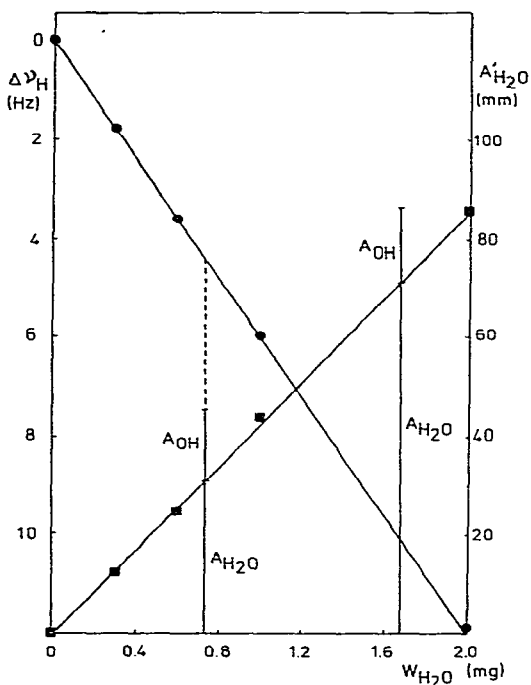


Fig. 1. Calibration plots of $\Delta\nu_H$ versus W_{H_2O} (●) and of A'_{H_2O} versus W_{H_2O} (■) with slope $S = 43$. Examples: left, $\Delta\nu_H = 4.4$ Hz, $A = 45$ mm; $A_{OH} = 14$ mm, $A_{H_2O} = 31$ mm, $W_{H_2O} = 0.73$ mg, $W_{OH} = 0.61$ mg; right, $\Delta\nu_H = 10.1$ Hz, $A = 86$ mm, $A_{OH} = 15$ mm, $A_{H_2O} = 71$ mm, $W_{H_2O} = 1.68$ mg, $W_{OH} = 0.66$ mg.

TABLE II
VALUES USED FOR THE CONSTRUCTION OF CALIBRATION PLOT

Units: W_{H_2O} , mg; A' , mm; ν_H , Hz. $\Delta\nu_H = \nu_H - \nu_H^0$; $W' = 701.15$ mg.

No.	W_{H_2O}	A'_x	A'_H	$A'_H \cdot f$	A'_{H_2O}	ν_H	$\Delta\nu_H$
1	0	97.0*	95.0**	95.0	0	518.9***	0
2	0.3	95.5	105.0	107.2	12.2	517.1	1.8
3	0.6	95.0	117.0	119.5	24.5	515.3	3.6
4	1.0	96.0	137.0	138.4	43.4	512.9	6.0
5	2.0	99.0	184.0	180.3	85.3	507.0	11.9

* A'_x .

** A'_H .

*** ν_H .

with silica, it is necessary to use exactly the same amount of solvent mixture in each case, or to correct the experimental results by use of a factor g for different weights, after subtraction of a blank:

$$A = A_{OH} + A_{H_2O} = (A_{Hf} - A_H^0) \quad g; g = W/W' \quad (3)$$

Using the calibration plot, the results are evaluated as follows. The difference between the COOH chemical shift for the mixture of acid and silica and the COOH chemical shift in the blank, *i.e.*, $\nu_H - \nu_H^0$, is found on the calibration plot of $\Delta\nu_H$ versus W_{H_2O} . At this point, a line parallel to the y -axis is drawn and the abscissa corresponding to the amplitude A from eqn. 3 is defined on it using the scale for A'_{H_2O} . The part of this abscissa under the calibration plot of A'_{H_2O} versus W_{H_2O} then describes the signal enhancement due to the protons of water extracted from silica, and the part above it corresponds to the protons exchanged from OH groups of silica by deuterium. Two examples of this procedure are given in Fig. 1.

The amount in mg of water in the sample is read directly from the x -axis scale for W_{H_2O} and knowing the weight of silica, W_s , the percentage of water can be calculated. For OH protons, the length in mm of the A_{OH} abscissa (A'_{H_2O} scale) is divided by the slope, S , of the calibration plot of A'_{H_2O} versus W_{H_2O} and multiplied by 1.888, *i.e.*, (mol. wt. 2OH)/(mol. wt. H₂O). The percentage of OH groups in the silica sample is then calculated from:

$$\% H_2O = 100 \cdot W_{OH}/W_s; \quad W_{OH} = 1.888 \cdot A_{OH}/S \quad (4)$$

Chemicals and instruments

Deuterated trifluoroacetic acid was prepared from trifluoroacetic anhydride⁷ and the equivalent amount of deuterium oxide (99.8% ²H; E. Merck, Darmstadt, G.F.R.). *p*-Xylene (Lachema, Brno, Czechoslovakia) was redistilled before use and checked for impurities by NMR spectrometry. A solution for measurement was prepared by mixing CF₃COO²H with *p*-xylene in a volume ratio of 100:3.

Silasorb 300 (10 μ m) was obtained from Lachema. Modified Silasorbs were

prepared in our Institute. Porasil 60/A, 75–125 μm (Waters Assoc., Milford, MA, U.S.A.), and Merckogel SI 500A were commercial materials.

All measurements were performed on a Tesla BS 467A 60-MHz NMR spectrometer (Tesla, Brno, Czechoslovakia), equipped with a temperature probe and operated in the CW mode. A derivatograph (MOM, Budapest, Hungary) was used for the thermogravimetric determinations.

Working procedure

Blank. To a 5-mm NMR tube were added 500 μl of the $\text{CF}_3\text{COO}^2\text{H}$ -xylene solution using a micropipette. The tube was tightly capped, weighed, W' , and equilibrated in the temperature probe of the spectrometer at 30°C. The methyl signal of xylene was used as field-frequency lock and signals of the residual COOH protons and xylene aromatic protons were recorded (Table II, No. 1) seven times, each with a small offset to avoid overlapping of signals. The strongest and the weakest signal in each group were omitted and the mean amplitude of the remaining five signals was calculated for COOH, A_{H}° , and for xylene, A_{X}° . The chemical shift of the COOH signal was measured by stationary method with the help of an external audio frequency generator (by the same procedure as the INDOR technique). The frequency was read from the frequency counter with an accuracy of ± 0.1 Hz; the mean of five measurements was used as ν_{H}° .

Calibration. To the $\text{CF}_3\text{COO}^2\text{H}$ -xylene solution in an NMR tube, a known amount of water, $W_{\text{H}_2\text{O}}$ (Table II), was added by Hamilton microsyringe. The mixture was thoroughly shaken and the measuring procedure was repeated as for the blank. In this way at least five points per calibration plot should be obtained.

Determination. About 20 mg of silica were weighed into an NMR tube, W_s , then 500 μl of the $\text{CF}_3\text{COO}^2\text{H}$ -xylene solution were added and the tube was reweighed (difference, W). The mixture was thoroughly shaken and centrifuged for ca. 15 min to settle the silica at the bottom of the NMR tube. Allowance for the "new bottom" of the tube, caused by settled silica, has to be made when positioning the tube in the temperature probe. The subsequent procedure was the same as for the blank.

It is recommended to use a fresh blank each day before measurement of samples since trifluoroacetic acid is hygroscopic and the amplitude and the chemical shift of the COOH signal changes with time. The calibration is not affected by small changes of water content.

RESULTS AND DISCUSSION

The results of the determinations of OH groups and water contents in different silicas and modified silicas are given in Table III.

First it is necessary to comment on the possible sources of errors. One is the temperature dependence of the COOH chemical shift. All our measurements were done in the temperature probe at 30°C, since at room temperature the thermal stability during the day was insufficient and reproducible results were difficult to obtain.

Care must be taken when other components in addition only to trifluoroacetic acid and water form the fast exchanging system; e.g., traces of HCl can shift the position of signals in such a way that the calibration is no longer valid for the division

TABLE III
CONTENTS OF HYDROXYL GROUPS AND WATER

Samples: 1, starting material for modifications (Lachema); 2, same as 1, but heated to 520°C before analysis; 3, modified by phenylethyl groups; 4, same as 3, but capped with $(\text{CH}_3)_3\text{SiCl}$; 5, modified by $\text{C}_{18}\text{H}_{37}\text{SiCl}_3$ and hexamethyldisilazane; 6, modified by $\text{C}_{18}\text{H}_{37}\text{SiCl}_3$ and, after hydrolysis, capped with Me_3SiCl ; 7, same as 6, but different conditions of capping; 8, modified by $\text{C}_{18}\text{H}_{37}\text{SiCl}_3$ and, after hydrolysis, capped with hexamethyldisilazane; 9 and 10, commercial materials (see-Experimental section).

No.	Sample	Surface ($\text{m}^2 \text{g}^{-1}$)	Hydroxyl content			Water content	
			%	mM g^{-1}	$\mu\text{M m}^{-2}$	%	TG %
1	Silasorb 300—0	248	4.2	2.4	9.9	2.0	—
2	Silasorb 300—1	248	3.1	1.8	7.4	1.0	—
3	Silasorb Ph 6—0	248	4.4	2.6	10.5	1.6	1.4
4	Silasorb Ph 6—1	248	2.9	1.7	6.8	1.0	1.1
5	Silasorb C_{18} —a	288	4.3	2.5	8.8	1.3	1.0
6	Silasorb C_{18} —b	288	4.2	2.5	8.6	1.2	1.0
7	Silasorb C_{18} —c	248	3.3	1.9	7.7	0.9	0.9
8	Silasorb C_{18} —d	288	3.2	1.9	6.5	1.1	0.8
9	Porasil 60/A	513	3.7	2.1	4.2	5.7	5.8
10	Merckogel SI 500A	67	1.6	0.9	14.0	1.3	1.7

of A into A_{OH} and $A_{\text{H}_2\text{O}}$. In this case the water content has to be determined by another method, *e.g.*, by thermogravimetry. Our results in Table III are in good agreement with those obtained by thermogravimetric determinations (TG % H_2O).

Another possible source of error can be in the alteration of the signal amplitude by "noise". Our procedure of omitting the two extremes from seven measurements is designed to minimize this effect. We repeated the measurement of the COOH signal amplitude 49 times and found that the coefficient of variation was 0.76. These measurements were then divided into seven groups each of seven values and the coefficients of variation were determined, the maximum value being 1.06. However, when the maximum and minimum value in each group were omitted, the highest coefficient of variation was only 0.68, *i.e.*, comparable to that from a large number of measurements.

The reproducibility of the method was tested on Merckogel SI 500A (Table IV) and found to be the same or better than that of other physical methods⁸⁻¹⁰.

The results of the determination of OH content show that the effect of different reaction conditions for modification and "capping" of silicas can be studied by this method. The highest content of hydroxyl groups on the surface of porous silica was

TABLE IV

STATISTICAL EVALUATION OF RESULTS OBTAINED FOR MERCKOGEL SI 500A

\bar{x} = Mean value; $R = x_{\max} - x_{\min}$; $\pm k_n R$ = reliability; s_R = standard deviation; C.V. = $100 \cdot s_R / \bar{x}$ = coefficient of variation (relative standard deviation).

No.	% OH	% H ₂ O
1	1.60	1.42
2	1.69	1.30
3	1.49	1.33
4	1.59	1.18
\bar{x}	1.59	1.31
R	0.20	2.24
$\pm k_n R$	0.14	0.17
s_R	0.097	0.116
C.V.	6.1	8.9

reported^{1,10,11} to be 7.0–9.5 $\mu\text{M}/\text{m}^2$. Our result for Silasorb 300 was slightly higher, and surprisingly high for Merckogel SI 500A. Such a high value is probably caused by the presence of micropores in the silica which are not detected by surface measurements but are accessible to the proton–deuterium exchange. Our method may therefore permit new insight into the structure of porous silica.

In conclusion, the NMR spectrometric method presented here gives comparable results with other physical methods for the determination of OH groups and water contents in porous silica and modified silicas. Moreover, it is faster and much easier to perform, since both determinations are made together without the need for precautions in the preparation of samples.

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