Journal of Chromatography, 289 (1984) 367–375
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 230

ANALYSIS OF THE ACID HYDROLYSIS PRODUCTS OF MONOFUNCTIONAL CHEMICALLY BONDED STATIONARY PHASES FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

Capillary gas chromatography of the acid hydrolysis products of monochlorosilane packing materials for high-performance liquid chromatography was used to determine the chemical composition of the bonded phases. Packing materials were acid hydrolysed, either with or without an excess of an added silica gel bonded with a different alkyl silane. The dimer products formed were extracted into hexane, and analysed by capillary gas chromatography. Determination of the identity and surface concentrations of both the primary and capping ligands was possible. This method is also valuable for the determination of more complex blended stationary phases consisting of multiple bonded ligand groups. It is suitable for quality control of both the final stationary phases and the silane reagents used in bonding

INTRODUCTION

The chromatographic properties of chemically bonded packing materials for high-performance liquid chromatography (HPLC) can be profoundly influenced by both the physical and chemical properties of the silica support[1–4]. The pore size, pore size distribution, surface area and trace metal content[5–7] will influence the surface character of the base silica material. In addition, the concentration of residual silanols[8–13] and the exact chemical composition and environment of the bonded ligand groups[14–22] will effect changes in the selectivity, even between batches of identically bonded materials[23]. In recent work in our laboratory, we have been investigating the behavior of blended stationary phases in HPLC[24,25], whereby two or more different ligands are chemically bonded to the same support material. For this type of work, as well as for comparison with and between commercially available bonded phases, it was necessary to know the exact chemical composition of the final bonded support. Not surprisingly, many facturers either will not divulge, or do not know the exact chemistry of their commercially available materials. Therefore, it was desired that simple, rapid methods be developed to analyse the bonded materials on a routine basis.

0021-9673/84/$03.00

The chemical analysis of bonded siliceous packing materials for HPLC has been accomplished by a variety of techniques. Simple tests based upon chromatographic behavior of test compounds[26–28] or elemental analyses[29] are essential in following the progress of a particular reaction or for quality control of batches, but yield little detailed structural information concerning the stationary phase composition. Infrared spectroscopy[30–34], thermal methods[21,35], photoacoustic spectroscopy[36], magic angle spinning and Fourier transform unclear magnetic resonance[37,38], direct measurements of exchange capacities[39], alkaline hydrolysis followed by derivatization and gas chromatography (GC)[40], and pyrolysis gas chromatography[41] have all been developed, and can be very powerful for the particular information desired.

GC methods seemed the most amenable to routine use and required instrumentation which would be widely available in the average laboratory. The GC–pyrolysis methods were not suitable for our purposes since, with multifunctional phases, it was necessary to determine the relative ratios of the various bonded ligands and capping agents present on the surface. The pyrograms would be too complex to analyse under these conditions without extensive use of mass spectrometry. Likewise, the dissolution of silica gel under alkaline conditions[40], followed by GC analysis, yielded somewhat complex samples which were difficult to interpret even for simple phases consisting of only one ligand type.

Therefore, it was decided that acid hydrolysis of the silane bonded ligands would be investigated, in the expectation that cleaner, more definite hydrolysis mixtures would be formed. The method was developed for the analysis of ligands bonded via monochloro bonding chemistries only, since di- and trifunctional ligands would yield multiple products and uncertain information.

EXPERIMENTAL

Production of silane dimers

All silica was bonded according to standard procedures as outlined previously[24]. Silanes used for bonding were all monofunctional and purchased either through Silar Laboratories (Scotia, NY, U.S.A.) or through Petrarch Systems (Bristol, PA, U.S.A.).

The bonded silica was exhaustively extracted with toluene, methanol and finally water to remove excess unbonded silane reagents. The material was then dried and *ca.* 0.2 g of material was placed in 60 ml of 6*N* hydrochloric acid. If specifically functionalized dimers were desired (according to Methods 2 and 3 outlined in the Results and discussion section) 2 g of functionalizing silica were added (trimethyl- or dimethylpentyl-bonded silica) to the hydrochloric acid. Finally, methanol was added to help "wet" the non-polar surface of the bonded silica. The material was stirred at 37°C overnight to assure complete cleavage of surface bonds and formation of silane dimers. Elemental carbon percentages of less than 0.5% were typical of most silica materials after the above treatment, indicating over 90% bond cleavage.

An internal standard (such as pentyl silane dimer) was prepared and added to the reaction mixture. Equilibration times as well as choice of internal standard is critical to achieve quantitative results. Hexane (30 ml) was then added to the acid methanol mixture and mixed for 1.0 h. The hexane layer was subsequently analysed by capillary GC.

Instrumental conditions

GC analyses were performed using an HP 3750 gas chromatograph modified with an SGE (Scientific Glass Engineering, Austin, TX, U.S.A.) capillary injection system. The capillary column used for all analysis was an SGE 25-m SE-30 column. Peak identification was confirmed using an HP 5840 gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) coupled to an HP 5985B mass spectrometer. Kovats retention index are reported for several symmetric silane dimers in Table I.

TABLE I

KOVATS RETENTION INDICES FOR SYMMETRIC DIMER SILANES

Column: SE-30 (SGE Gloss); other conditions as described in Experimental section.

*Dimers**	*Indices*
C_5Si–O–SiC_5	1379
C_8Si–O–SiC_8	1941
ClC_3Si–O–SiC_3Cl	1499
PhC_3Si–O–SiC_3Ph	2274

* C_5Si = dimethylpentylsilane; C_8Si = dimethyloctylsilane; ClC_3Si = 3-chloropropyldimethylsilane; PhC_3Si = 3-phenylpropyldimethylsilane.

Data were reported and integrated using a HP 3390A electronic integrator. All analyses were temperature programmed from 100 to 250°C at 8°C/min, after an initial hold time of 3 min. Throughout, 1-μl injections were split 10:1 to reduce column load. The routine analysis of silica materials was performed for a year without column failure or significant loss of system resolution.

All elemental analyses used for comparison of ligand coverages were performed by Robertson Laboratory (Florham Park, NJ, U.S.A.).

RESULTS AND DISCUSSION

Acid hydrolysis of bonded phases

One of the problems encountered with acid hydrolysis of silane-bonded phases is that dimerization of the silane ligands is favored[42], as illustrated in Method 1 of Fig. 1. Such dimerization can be helpful for the analysis of simple monoligand phases, or a source of added complexity in the case of multiple ligands, *e.g.*, capping reagents or blnded stationary phases. Symmetric dimers are formed when uncapped monofunctional support materials are subjected to acid hydrolysis. If the support material is end-capped, typically with trimethylchlorosilane (TMCS), both symmetric and asymmetric dimers form in proportions dependent both upon the kinetics of hydrolysis, and upon the rate of dimer formation. If multiple ligands are bonded to the support, numerous unique dimers can be formed, greatly complicating the analysis.

Three basic approaches have been developed in this study to eliminate these complications, each one being suited for a particular type of stationary phase. First, if only the surface concentration of the primary ligand is of interest, simple acid hydrolysis with dimerization is used. This approach works best for determination of the primary ligand, *e.g.* C_8, and for the estimation of impurities present. The for-

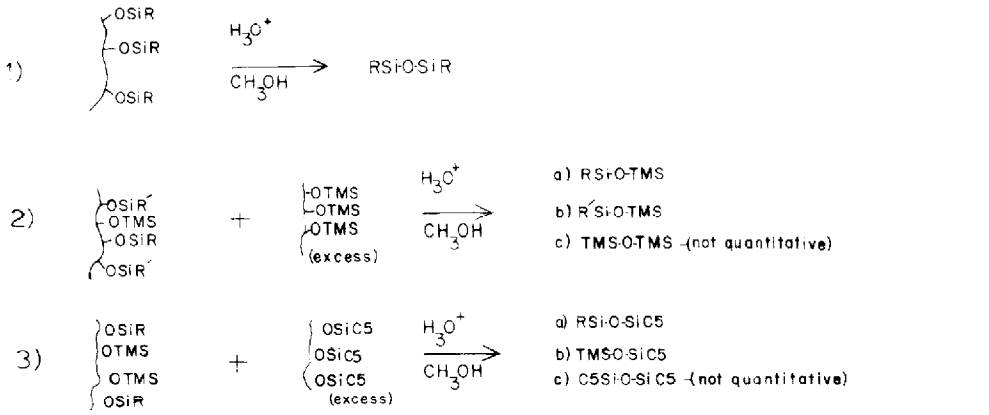

Fig. 1. The bonded silica materials are acid cleaved and dimers analysed using capillary GC (see Experimental). According to three methods, the bonded material can be (1) acid hydrolyzed and the dimer products extracted and directly analysed by capillary GC, or (2) the packing material of interest may be hydrolyzed along with an excess of trimethylsilane bonded silica, forming TMS derivatives of the original bonded ligand or ligands, or (3) the packing material is hydrolyzed with excess C_x bonded silica, where C_x is a silane of unique chain length. The C_x derivatives can then be separated and analysed for the surface concentrations of both capping agent and primary ligands present.

mation of trimethylsilyl (TMS)–C_8 dimers will be minimal, provided the relative surface concentration of capping agent is low.

The second approach involves determination of the relative concentrations of primary ligand and capping agent. In this case, excess large diameter (inexpensive) silica bonded with TMS is hydrolysed along with the bonded silica of interest. Under these conditions, the formation of a trimethylsilyl-ether derivative of the ligand is favored, due to the locally high concentrations of TMS. TMS derivatives of the silanes were produced in excess of 96% yield. For studies concerning multiple ligand bonding and mixed phases, this approach proved to be simple, producing excellent qualitative and quantitative results. However, using TMS as the derivatizing agent obviously precluded obtaining information concerning the concentrations of TMS capping agent on the silica.

If information concerning the degree of capping was needed, a third method was developed in which an excess of large particle silica bonded with a ligand of unique chain length (*e.g.*, C_5 silane) was added to the packing during the hydrolysis step. Although this technique is more expensive than using TMS silica, all ligands of interest and the degree of capping can be determined in a single GC run by analysis of the C_x derivatives. The experimental procedures for each of these three methods are detailed in the Experimental section.

Analysis of reversed-phase materials

An example of a simple dimethyloctylmonochlorosilane bonded phase analysed using the first dimer method is presented in Fig. 2. The average ligand concentration was found to be $3.88 \cdot 10^{-4}$ moles g^{-1}, compared with a value of $5.60 \cdot 10^{-4}$ moles g^{-1} calculated by elemental analysis. The lower values for the GC method were consistently observed, and were attributed to several sources. The elemental

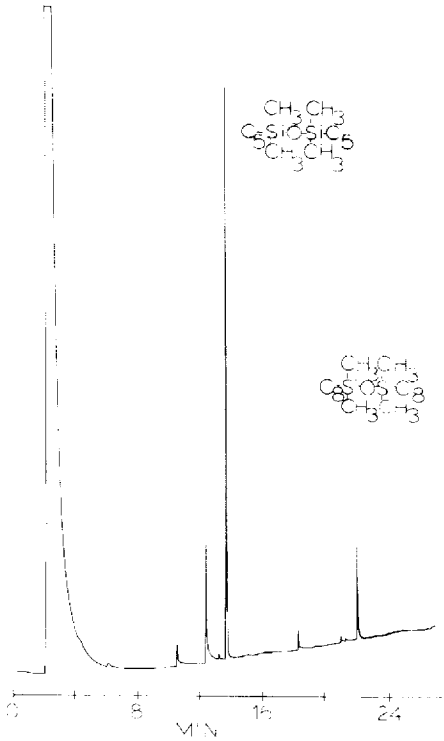

Fig. 2. Direct analysis by capillary GC of the acid hydrolysis products of a dimethyloctyl 10-μm silica phase, using C_5 dimer as an internal standard. The smaller unidentified peaks were from the internal standard and from slight impurities in the hexane extractant. GC conditions: column, SGE BP-1; split injection; temperature, from 100 to 250°C at 8°C/min. Other instrumental and reaction conditions are described in the Experimental section.

analysis cannot take into account the presence of multiple ligands on the surface with any degree of accuracy. Impurities in the bonded phase, consisting of silanes of different chain length or other unknown organics, will yield elemental percentages not representative of the true surface ligand concentration. Also, if any residual solvents remain adsorbed within the silica pores, high elemental values will result. The efficiency of cleavage of the ligands under acidic conditions is another variable; however, extensive testing of the silica after hydrolysis using elemental analyses indicated the lack of significant carbon remaining on the silica.

Analysis of multifunctional phases

Figs. 3A and B show an application in which the ligand concentrations for a stationary phase consisting of multiple bonded ligands (in this case a C_8/phenyl-SO_3^- mixed-mode reversed-phase/cation-exchange phase) is monitored. In the synthesis of these multifunctional phases, it was necessary to optimizate the sulfonation reaction of the phenyl ligand with chlorosulfonic acid, and to monitor the extent of hydrolysis occuring during this step. The degree of sulfonation was determined by following the decrease in peak area of the propylphenylsilane–TMS derivative over

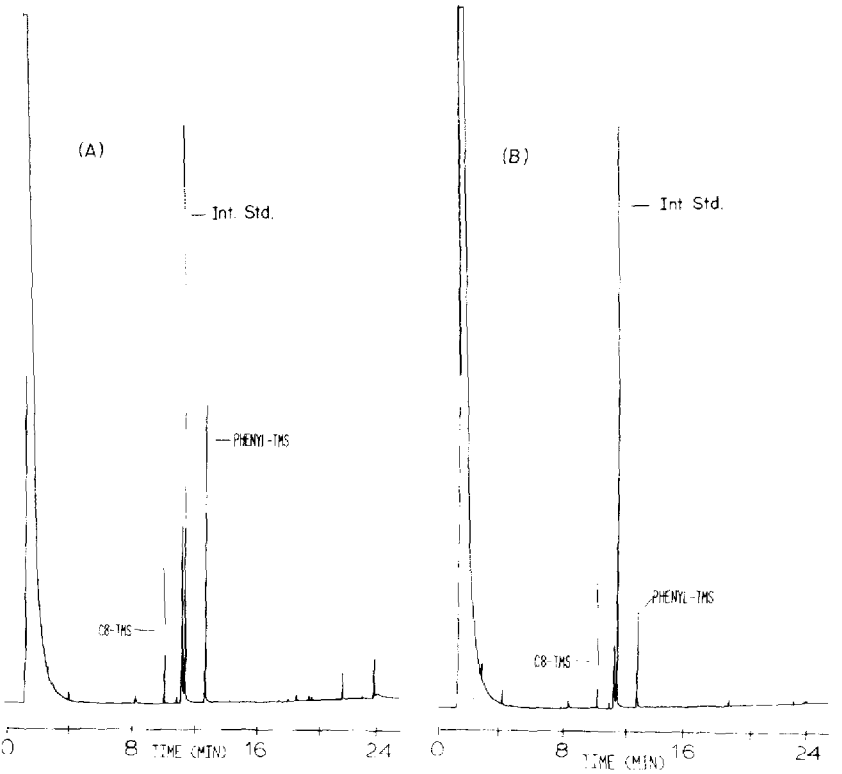

Fig. 3. Analysis of chemically bonded mixed phase (phenyl-SO_3/C_8) (A) before and (B) after sulfonation of the phase with chlorosulfonic acid. The efficiency of the sulfonation reaction was reflected by the loss of dimethylpropylphenyl peak area, since the sulfonated products were not volatile. The hydrolysis of all ligands was reflected in the loss of dimethyloctyl silane peak area.

the course of the sulfonation. Decrease in dimethyloctylsilane–TMS derivative reflected loss of ligands due to hydrolysis.

Determination of capping agents

An excess of a C_5 bonded material was used to determine the primary bonded ligand plus capping concentrations on the silica support for several commercial and laboratory bonded phases (Method 3). A series of bonded phases were prepared from silane bonding mixtures varying in their TMCS concentrations from 0% to 89%. The data concerning bonding mixture percentages *versus* the actual surface concentrations of the trimethyl- and dimethyloctyl-silanes are presented in Table II.

A commercial C_8 material is included in the list in Table II. The gas chromatogram of the commercial phase is shown in Fig. 4. It is interesting to note the rather large concentration of TMS relative to dimethyloctyl groups for this dimethyloctyl phase. Using direct analytical methods, such as the one presented here, should yield interesting data concerning various commercial phases, and the relationships between their particular chemistries and their chromatographic properties. A more unified understanding of differences between phases may result as this type of data becomes available.

TABLE II

DETERMINATION OF C_8/TMS CAPPING RATIOS USING CAPILLARY GC

Analysis of a series of test phases, plus one commercial phase, for the surface concentrations of dimethyloctylsilane and trimethylchlorosilane groups, using dimethylpentylsilane as the derivatizing agent. The test silicas were prepared using the indicated mole ratios of monochlorosilane reagents (last column), producing the indicating actual coverages (column 2). It is interesting to note that the commercial material labeled as C_8 in fact has a surface comprised of nearly 70% trimethylsilane groups. Little information concerning the degree of capping agent can be discerned from the elemental data alone.

Bonded material	*C_8 (%)*	*TMS %*	*Elemental carbon (%)*	*Mole percent of reactants during bonding*	
				C_8 (%)	*TMCS (%)*
Silica 100:0	100	0	9.2	100	0
Silica 49:51	3.4	96.6	5.4	49	51
Silica 11:89	0	100	4.8	11	89
Commercial C_8 silica	31	69	8.5	–	–

This method was also found to be very useful for checking the purity of chlorosilane reagents used in the various bonding procedures. Commercial dimethyloctyl-silanes were generally found to be fairly pure, while several dimethyloctadecyl- and dimethyldodecyl-monochlorosilanes had gross impurities of numerous other chain lengths.

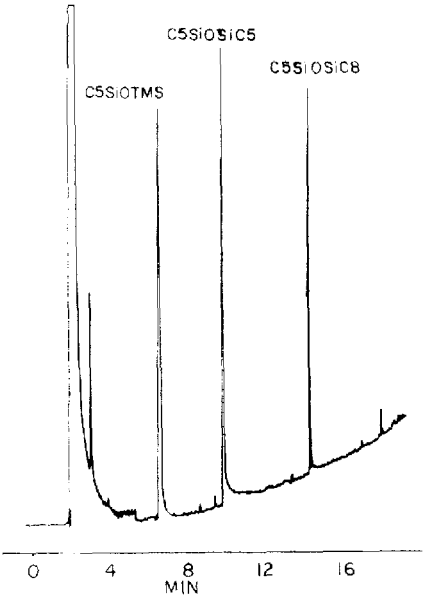

Fig. 4. End-capping a analysis of a commercial C_8 material using Method 3. An excess of C_5 bonded silica was added to the reversed-phase material during hydrolysis. Under these conditions, the C_5 derivatives of the reversed-phase ligands are preferentially formed, allowing for the direct monitoring of both TMS capping agent and primary ligand identification and quantification. Other conditions as in Fig. 2.

Accuracy and precision

In general, analysis by any of the three GC methods described produced ligand concentrations significantly lower than obtained using elemental analysis, even with carefully prepared monofunctional phases. As previously discussed, there are several possible sources for this discrepancy.

For single batches of bonded phases, relative standard deviations of 3–11% were observed. The real usefulness of the methods was in the qualitative information obtained. Details concerning surface coverages of multiple ligand groups, relative ratios of capping reagents, and of overall silica coverage were obtainable with very simple instrumentation available in most analytical laboratories. Much of this information could otherwise only be obtained by using other very sophisticated methods, if at all.

ACKNOWLEDGEMENT

We acknowledge the support of the National Science Foundation, grant CHE 8100224, for support of this research.

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