Journal of Thermal Analysis, Vol. 38 (1992) 2483-2490

ANALYSIS OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY BONDED STATIONARY PHASES USING THERMOGRAVIMETRY

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(Received June 22, 1991; in revised form December 23, 1991)

A thermogravimetric method was developed for determining the C-18 bonded phase content of reversed phase high performance liquid chromatographic stationary phases. The method yielded data that were comparable to the sum of carbon and hydrogen content. Excellent agreement between the two methods was achieved by heating the stationary phase samples to 150°C in order to remove adsorbed species prior to elemental analysis.

Keywords: high-performance liquid chromatography, new thermogravimetric method

Introduction

A variety of stationary phases are employed in high performance liquid chromatography (HPLC), and the most common are based on silica gel. Silica is often given the stoichiometric composition SiO_2; however, because a non-stoichiometric amount of water is also present, silica gel can be represented by the formula $SiO_2 \cdot xH_2O$. Silica gel produced as an adsorbent for HPLC is amorphous, possesses a high surface area, and is often highly porous [1].

Knowledge of the arrangement of surface hydroxyls aids in understanding the chemical reactivity of silica. Silicon atoms present at the surface maintain their tetrahedral coordination by attachment to hydroxyls, forming silanol groups. These groups can be isolated or vicinal (located on neighboring silicon atoms). The mean surface concentration of hydroxyls varies between 7 to 10 μmole per

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square meter for most silicas. Surface hydroxyls are capable of hydrogen bonding to each other, or to water that is physically adsorbed on the surface [1, 2].

Adsorbed water and surface hydroxyls can be removed by thermal treatment. Adsorbed water is eliminated at temperatures below 150°C. At higher temperatures, surface reactions occur. Vicinal hydroxyls condense between 300° to 500°C to form siloxane groups with the elimination of water. Above 600°C sufficient thermal energy is present to permit migration and subsequent condensation of isolated hydroxyls. At 1200°C the surface is almost completely dehydroxy- lated, and contains only siloxane groups. Silica in this form is totally useless for chromatographic separations [1, 2, 3].

Due to the high polarity imparted by surface hydroxyls, silica gel itself is not a good HPLC stationary phase, hence organic reagents are used to impart nonpolar or reversed phase characteristics to the silica surface. Although organic constituents can be physically adsorbed or chemically bonded to the surface, bonded phase organic substituents are used almost exclusively because they possess the high chemical stability required in HPLC [4]. Reagents commonly employed for producing bonded stationary phases are C-18 alkoxy silanes, which provide substitution of C-18 alkyl groups for the surface hydroxyls [1, 2].

The chromatographic performance of C-18 bonded stationary phases is greatly affected by the conditions of the reaction occurring between silica and the silanizing reagents. For instance, variation in degree of substitution can have a profound effect on analyte retention times and analyte sample capacities. Therefore, strict quality control is maintained during the manufacturing processes in order to produce materials with optimum chromatographic performance characteristics [4].

A method widely used for testing C-18 bonded stationary phases is carbon and hydrogen analysis. In this method, the stationary phase sample is combusted in oxygen, and the gaseous combustion products (CO_2 and H_2O) are analyzed either spectrophotometrically, or by adsorption onto weighed traps. The percentages of carbon and hydrogen provide a direct determination of C-18 bonded phase content [4].

Determination of C-18 bonded phase content by carbon and hydrogen analysis does have certain limitations. In order to obtain an accurate measurement of hydrogen content, water not originating from the combustion of bonded phase hydrocarbon must be excluded from the measurement apparatus. It has already been demonstrated that silica, upon heating to sufficiently high temperatures, releases water by desorption and elimination [1, 2, 3]. Water originating from these sources cannot be differentiated from water produced by hydrocarbon combustion, therefore erroneously high values of total hydrogen content will be obtained.

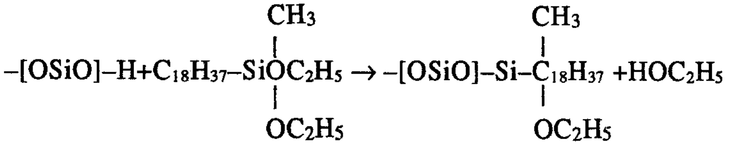
Because of the limitations of carbon and hydrogen analysis, other methods have been sought for analyzing and evaluating C-18 bonded stationary phases. For example, differential scanning calorimetry (DSC) has been used to measure the energy released upon combustion, which can be related to total bonded phase content. However, this method has enjoyed limited success due to the difficulties in determining the appropriate conversion factor between energy and bonded phase content [5].

In this study, thermogravimetry was used for the analysis of C-18 bonded stationary phases. Water and organic species adsorbed onto the stationary phase samples was removed by heating to relatively low temperatures, and thus excluded from the calculation of bonded phase content. This resulted in better agreement with elemental analysis data.

Experimental

A number of HPLC bonded stationary phases was analyzed by thermogravimetry. Several materials were supplied by Streng and Gutnikov that had been used for HPLC determination of fatty acid profiles of lipids [6]. These samples were prepared in the following manner.

LiChrosorb Si 100 (E. Merck, Art. 9309) was reacted with methyloctadecyldiethoxysilane (Petrarch Systems Inc), according to the reaction



$$-[OSiO]-H+C_{18}H_{37}-\underset{\displaystyle OC_2H_5}{\overset{\displaystyle CH_3}{\overset{|}{\underset{|}{Si}}}}OC_2H_5 \rightarrow -[OSiO]-Si-\underset{\displaystyle OC_2H_5}{\overset{\displaystyle CH_3}{\overset{|}{\underset{|}{C}}}}_{18}H_{37} + HOC_2H_5 \qquad (1)$$

where –[OSiO]–H represents the silica matrix.

The product was end-capped with hexamethyldisilazane (Petrarch Systems Inc., H7300EG), according to the reaction:

$$\begin{gathered}-[OSiO]-H+(CH_3)_3C-Si-NH-Si-C(CH_3)_3 \rightarrow \\ -[OSiO]-Si-C(CH_3)_3 + H_2N-Si-C(CH_3)_3\end{gathered} \qquad (2)$$

In order to remove traces of iron and other chromatographic interferents from the silica, the product was treated three times with dilute HCl and methanol, then dried at 70°C. A final treatment with methyloctadecyldiethoxysilane was performed in order to ensure that no active sites remained on the silica.

An HPLC C-18 bonded stationary phase employed by Gutnikov and Hung in earlier studies, previously analyzed at the University of Saarbrücken for carbon content by gas chromatography of the combustion products, was also obtained for analysis [7]. The base silica in this sample, LiChrosorb Si 60, was treated with C-18 silanizing reagent and end-capped twice.

In addition to these samples, two untreated silica samples from Merck were analyzed for comparative purposes. They were: LiChrosorb Si 60 (E. Merck Reagents, cat 9387, batch NoYF 325); and LiChrosphere Si 100 (E. Merck Reagents, cat 9312, batch VV 2122).

Thermogravimetric analyses were performed on a Mettler TA3000 system, consisting of the TG50 thermobalance and TC10A controller. TG data were recorded between 25° and 1000°C, using a heating rate of 10 deg/min. TG blank measurements were made using uncovered, empty 150 μl alumina crucibles. The thermobalance was calibrated against the Curie temperatures of standard Mettler Alumel, Mumetal, and Trafoperm. Data evaluation was performed using a Lotus 123 database program.

A suitable purge gas was found through preliminary testing. Oxygen and air proved to be unsatisfactory purge gases because bonded stationary phase sample was expelled from the crucible during analysis due to the violence of the oxidation reactions. In contrast, no irregularities were exhibited in the TG data when nitrogen was used as purge gas. Subsequent TG analyses were conducted using ultra high purity nitrogen as purge gas with a flow rate of 100 ml/min.

Initial sample weights were obtained on the external balance pan located outside of the purged thermobalance furnace, because rapid volatile desorption from the samples in the dry nitrogen gas stream resulted in a steady weight decrease. After the initial weight was recorded on the external pan, the sample crucibles were placed on the balance support, and the furnace raised into place for analysis.

In order to verify the TG results, samples of the bonded stationary phases were submitted to Galbraith labs for carbon and hydrogen analysis. In this method, a Leco 800 analyzer was used to heat the samples to 950°C in an oxygen stream, and the gaseous combustion products swept into an infrared gas cell for analysis. Carbon content was determined by CO_2 absorption bands, and hydrogen content by H_2O absorption bands [8].

To provide additional information on the relationship between adsorbed species and bonded phase content, several samples were heated to 150°C, and subsequently analyzed for carbon and hydrogen content.

Results

The untreated silicas displayed similar weight loss curves (Fig. 1). All three samples exhibited a 3% weight loss between 150° and 995°C attributed to the reactions involving elimination of water and dehydroxylation. The temperature range over which these reactions occur is in good agreement with the literature reports [1, 2].

The TG data for the bonded stationary phase samples appear in Fig. 2. The large weight loss step in the curves between 150° and 995°C was attributed to pyrolysis of the C-18 groups. Bonded phase content was calculated as the weight difference between 150° and 995°C, relative to sample weight at 150°C. Percent volatiles was defined as the difference in sample weight between 25° and 150°C, relative to the sample weight at 25°C.

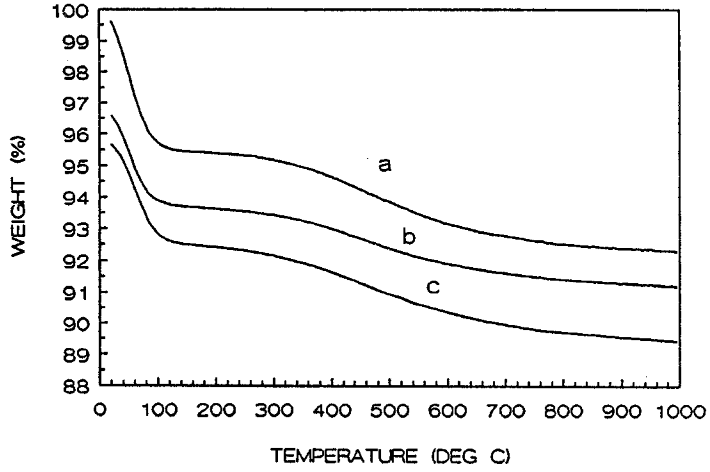


Fig. 1 Thermogravimetry curves for untreated silicas, offset for overlay: *a*) LiChrosphere Si 100; *b*) LiChrosorb Si 100; *c*) LiChrosorb Si 60

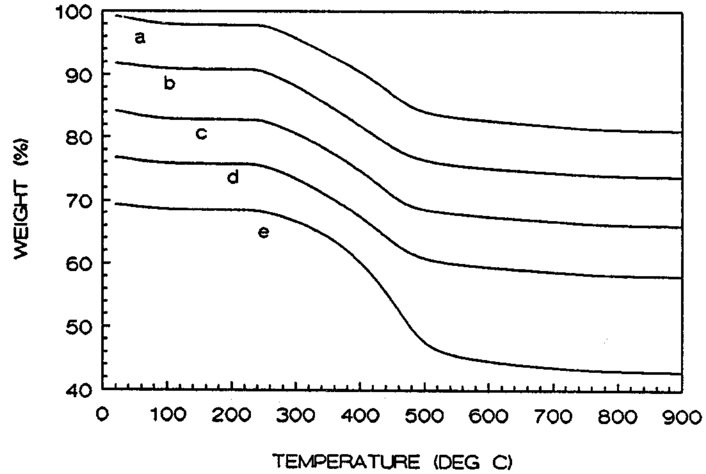


Fig. 2 Thermogravimetry curves for bonded stationary phases, offset for overlay: *a*) silanized Si 100; *b*) silanized and end-capped Si 100; *c*) acid-washed sample *b*; *d*) re-silanized sample *c*; *e*) silanized and endcapped Si 60

Table 1 summarizes the results of thermogravimetry and carbon and hydrogen analysis. The total weight loss between 25° and 995°C is in good agreement with the sum of carbon and hydrogen content for the entire set of bonded stationary phases (an average relative difference of 4.3%).

Table 1 Comparison between weight loss and sum of carbon and hydrogen content for high performance liquid chromatography bonded stationary phases

	Unheated sample		Sample heated to 150°C	
ID.	Weight loss/% 25° to 995°C	Sum of %C and %H	Weight loss/% 150° to 995°C	Sum of %C and %H
a	18.40	17.80	17.12	16.94
b	18.22	1854	17.30	17.58
c	18.59	16.79	17.23	17.06
d	18.96	19.24	17.94	18.26
e	26.79	25.73	26.04	25.66

Note: a) LiChrosorb Si 100 silanized with C-18 hydrocarbon; b) sample (*a*) endcapped with C1 hydrocarbon; c) sample (*b*) washed with dilute HCl in methanol; d) sample (*c*) resilanized with C-18 hydrocarbon; e) silanized LiChrosorb Si 60. ID's refer to designations appearing in Fig. 2.

Excellent agreement between the two methods was obtained by first heating samples to 150°C to remove adsorbed species (such as water, solvent and unreacted silanizing reagents) prior to carbon and hydrogen analysis. An average relative difference of 1.4% was observed between the two methods for these samples.

Discussion

As shown in Table 2, the volatile content was approximately equal to the change in carbon and hydrogen content after heating (except for the acid-washed sample). This indicates that most adsorbed species contained only carbon and hydrogen. However, the acid-washed sample had a volatile content of 1.6%, but exhibited a negligible change in carbon and hydrogen content after heating. This suggests that the adsorbed species may be water and/or HCl introduced by the acid treatment.

The primary difficulty of using thermogravimetry to determine bonded phase content is that gaseous pyrolysis products are not identified. Evolved gas analysis could be used for this purpose, but was unavailable for this study.

In the previous section, the weight loss step between 150° and 995°C was attributed solely to pyrolysis of C-18 groups. This attribution is correct if all surface hydroxyls underwent reaction with the C-18 silanizing reagent. Otherwise, a small weight loss due to dehydroxylation would occur simultaneously with pyrolysis. Presumably, this assumption introduces only a slight inaccuracy in the final calculation.

Table 2 Effect of heating on carbon and hydrogen content of high performance liquid chromatography bonded stationary phases

	%C + %H		Change in	Weight loss/%
ID.	Unheated	Heated	%C + %H	25°–150°C
a	17.80	16.68	1.12	1.54
b	18.54	17.38	1.16	1.12
c	16.79	16.78	0.01	1.64
d	19.24	18.03	1.21	1.24
e	25.73	25.40	0.33	1.02

Note: a) LiChrosorb Si 100 silanized with C-18 hydrocarbon; b) sample (*a*) endcapped with C1 hydrocarbon; c) sample (*b*) washed with dilute HCl in methanol; d) sample (*c*) resilanized with C-18 hydrocarbon; e) silanized LiChrosorb Si 60. ID's refer to designations appearing in Fig. 2. All percentages based on weight of unheated samples at 25°C.

Conclusions

Thermogravimetry appears to be a useful method for studying high-performance liquid chromatography stationary phases. The results obtained by this method can also be valuable for clarifying discrepancies that are encountered from more traditional methods, such as elemental analysis. The method outlined in this paper could also be applied to the study of more complex bonded phases, such as those containing cyano or amine groups. Carbon, hydrogen and nitrogen analysis could be used to verify the TG results from these materials.

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This research stemmed from the author's Master's degree research undertaken at California State Polytechnic University Pomona, and conducted at the Getty Conservation Institute (GCI).

The author is greatly indebted to the following colleagues at the GCI for their advice during the course of this project: Neville Agnew, Charles Selwitz, Dusan Stulik and David Scott. The HPLC bonded stationary phase samples were prepared by John Streng, California State Polytechnic University, Pomona.

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Zusammenfassung — Es wurde eine thermogravimetrische Methode entwickelt, um den C-18 gebundenen Phasengehalt von stationären Phasen bei Hochleistungsflüssigkeitsumkehrchromatographie zu bestimmen. Die Methode ergab Daten, die mit der Summe von Kohlenstoff- und Wasserstoffgehalt übereinstimmen. Eine ausgezeichnete Übereinstimmung der beiden Methoden ergibt sich, wenn vor der Elementaranalyse die Proben der stationären Phasen auf 150°C erhitzt werden, um adsorbierte Stoffe zu entfernen.