

CHROM. 17,463

CARBON ANALYSIS FOR THE STUDY OF BONDED PHASES IN LIQUID CHROMATOGRAPHY

BARRY I. DIAMONDSTONE, STEPHEN A. WISE and LANE C. SANDER*

Center for Analytical Chemistry, National Bureau of Standards, Gaithersburg, MD 20899 (U.S.A.)

(Received December 3rd, 1984)

SUMMARY

The procedure outlined permits carbon analyses to be performed on a variety of samples using a carbon analyzer originally intended for use with metal samples. By mixing small quantities of a relatively high-carbon sample with low-carbon steel, the problems of explosive combustion and inductive coupling are overcome. The technique results in highly reproducible measurements which might not be possible using other methods. These measurements are extremely useful in assessing substrate modification, endcapping, and stability studies.

INTRODUCTION

The study of bonded stationary phases for use in liquid chromatography (LC) requires careful measurement of the physical properties of the sorbent materials. Such measurements are often correlated with chromatographic properties of the phase in an attempt to better understand solute retention mechanisms and to gain insight on how to tailor bonded phases to solve a given separation problem. Unfortunately, spectral techniques (*e.g.* ultraviolet and infrared spectroscopy) have only limited applicability to the study of bonded phases. In previous work alkyl chain conformation of bonded phases was studied using Fourier transform infrared spectrometry¹. The bonded phase environment has been studied using fluorescence spectroscopy²⁻⁴ and nuclear magnetic resonance spectroscopy⁵⁻⁸. In each instance qualitative, rather than quantitative measurements, were performed on the phases. Because of interferences from the silica matrix and low absorptivity of most bonded-phase ligands, spectral techniques generally lack sufficient sensitivity for bonded-phase quantification.

By far the most prevalent method for measuring phase loading of LC bonded phases is elemental carbon analysis. Usually the chemically modified silica is burned in an oxygen environment and the carbon dioxide that is produced is quantified infrared spectroscopy. Alternatively, a measure of phase loading can be made gravimetrically by determining the weight loss upon burning. The precision of phase loading measurements is of considerable importance. The precision of gravimetric determinations is inherently limited by sample size and balance precision. Carbon

analysis by quantification of combustion gases is less dependent on weight measurements and may have better precision.

In this work the precision of carbon analyses is measured for high- and low-percent carbon bonded-phase substrates. The need for high precision is demonstrated by examples in which small changes in overall carbon loading result from changes in the conditions of bonded-phase synthesis. Implications and applications of the technique to other materials are discussed.

EXPERIMENTAL

Bonded phases

Polymeric and oligomeric phases were prepared by the reaction of octadecyltrichlorosilane with Vydac TPB-10 silica*. In a typical polymeric phase synthesis 3.5 g of silica, dried at 150°C under reduced pressure, were added to 100 ml of carbon tetrachloride. To this slurry, 10 ml of octadecyltrichlorosilane were added followed by the addition of 0.5 ml water. The reaction slurry was refluxed for 4 h and then filtered, washed and dried. A series of oligomeric phases were synthesized in a step-wise fashion by anhydrous reaction of the silica with octadecyltrichlorosilane followed by bonded-phase hydrolysis and further reaction with octadecyltrichlorosilane. Each "cycle" adds carbon to the silica and builds the oligomeric phase one C_{18} unit at a time. For phases that were endcapped, hexamethyldisilazane was used as the silanizing reagent. The details of these reaction schemes were reported in an earlier paper⁹.

Carbon analyses

The equipment used to determine carbon in these experiments is commonly used to determine carbon in steels and other metal alloys. Typical sample weights for metal analyses range from 0.5 to 1.0 g. The carbon values normally range from a few ppm to about 7%. The metallic character of the samples serves an important function in the analysis since this allows the sample to couple with the radio frequency (RF) furnace to produce proper burning conditions for volatilizing the carbon as carbon dioxide.

The chromatographic bonded phases used in this study are made up of extremely fine particles with carbon concentrations as high as 20–25%. If samples of this material weighing 0.5–1.0 g are mixed with the flux normally used for metal analyses and burned directly in the furnace, explosive combustion may occur. This would create inconsistent results and may even damage the instrumentation. Therefore, a procedure was developed that permits smaller samples of the bonded phases to be used for analysis. By using the technique described below, the carbon levels measured by the infrared detection system were similar to those for metal samples. In addition, by using a metallic catalyst along with the flux, samples readily absorbed energy by coupling with the induction furnace and burned efficiently.

* Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Prior to analysis, bonded phases were dried at 150°C under reduced pressure for 2 h. Samples were weighed to four significant figures in preburned ceramic crucibles, with weights typically ranging from 10 to 25 mg. To the weighed samples were added 1 g of a low-carbon steel standard (10 ± 4 ppm carbon) and an accelerator flux. The flux used in this experiment is a commercially available, proprietary tungsten-based mixture that couples with the RF power and permits efficient sample combustion. In the absence of this flux, nearly all samples burn slowly and incompletely. The carbon analyses were performed by combustion of the bonded phases in a gas-fusion carbon-sulfur analyzer. Standard reference materials (SRM) 361, 362, and 365 (low-carbon steel standards, National Bureau of Standards, Gaithersburg, MD, U.S.A.) were used as calibration standards and control samples.

By adding the steel and flux on top of the modified silica, the possibility of losing sample by "blow-out" during ignition was minimized. The carbon dioxide evolved during sample combustion passes through an infrared detector cell and is quantified. A catalyst within the instrument is used to assure that all of the carbon is converted to carbon dioxide rather than carbon monoxide. Blank values for the sample crucible and flux are determined and appropriate corrections to the total carbon calibration curve are made. Finally, the known carbon value of the low-carbon steel is subtracted from the displayed percent carbon and this corrected value along with the weight of modified silica is used to determine total carbon in the bonded phase.

RESULTS AND DISCUSSION

Because small differences in phase loading can give rise to large changes in chromatographic properties, precise measurements of bonded phase carbon loading are essential for meaningful phase characterization. To evaluate the precision of bonded-phase carbon determinations, eight measurements were performed on a polymeric C_{18} bonded phase. Sample weights ranged from 12 to 18 mg for six of the samples. Considerably larger samples were used for the remaining samples (122 and 190 mg) to investigate any influence of sample size on carbon determination. Percent carbon values for the eight analyses are given in Table I. For the low-weight samples the

TABLE I
PERCENT CARBON ON A POLYMERIC C_{18} BONDED PHASE

Sample	Sample weight (mg)	Carbon (% w/w)
1	17.5	8.35
2	12.7	8.16
3	15.0	8.30
4	17.7	8.24
5	17.3	8.25
6	17.9	8.25
7	121.9	8.24
8	190.3	8.40

$$\bar{x} = 8.26$$

$$\text{S.D.} = 0.06$$

95% confidence interval ranges from 8.10 to 8.42% carbon. The two results for the higher-weight samples fall within this range (8.24 and 8.40% for 122 and 190 mg samples, respectively) indicating that no apparent differences exist within the uncertainty of the measurement. The average and standard deviation are 8.26 and 0.06% carbon, respectively [relative standard deviation (R.S.D.) = 0.8%].

Similar measurements were made on samples of the unbonded silica substrate. Silica consists of silicon, oxygen, and trace levels of other elements. Recently we reported physical and chemical properties for a series of 22 silicas including measurement of the percentage of carbon in the unbonded substrate¹⁰. This value, referred to as "background carbon" varied among the silicas from trace levels to nearly 1% (w/w) carbon. The substrate used in this study was determined to contain 0.43% carbon (S.D. = 0.015%, R.S.D. = 3.5%).

One example of a need for precision in carbon analyses is in comparing the efficacy of endcapping procedures. Endcapping is a technique that is used to reduce the number of unreacted silanol groups at the bonded silica surface. Even under rigorous conditions, only about half of the surface silanol sites are modified with long-chain alkyl silane reagents. This is a consequence of steric hindrance between adjacent alkyl chains bonded to the surface. Unmodified silanols are shielded from reaction with free alkyl silane molecules. Very small silane molecules are less sterically hindered and are able to react at a greater fraction of the surface silanols. Thus, the reaction of trimethylchlorosilane or hexamethyldisilazane with bonded-phase sorbents should give rise to small increases in carbon loading. However, in a study by Tanaka *et al.*¹¹ it was concluded that evaluation of endcapping reactions by carbon analysis was "invalid", due to the imprecision of the measurement. Berendsen *et al.*¹² disagreed with this finding and suggested that increases in carbon loading should be readily observed if the measurements are made with a precision of 0.1% carbon absolute. Such increases were not observed in their work and they concluded that endcapping reactions were ineffective for their phases.

In our study of oligomeric phases, selected phases were endcapped by treatment with hexamethyldisilazane (see below). In each instance, small increases in carbon loading were observed after silanization with hexamethyldisilazane (see Fig. 1). This indicates that additional silanols were modified by hexamethyldisilazane during endcapping that were inaccessible to reaction with C₁₈ silane molecules. In a similar study⁹, half of a lot of a C₁₈ polymeric phase was endcapped with hexamethyldisilazane and carbon loading measurements were made on the capped and uncapped phases. The carbon loadings for these phases were 8.93% and 8.77%, respectively. In general, differences among the effects of endcapping procedures reported in the literature can probably be attributed to differences in the bonded-phase materials prior to endcapping. Certainly variations in surface coverage values and silanizing reagents will be affected the most by endcapping reactions. The question of silanol accessibility is an important topic in liquid chromatography and deserves further study.

A second example illustrating a need for precise carbon measurements is given by the study of oligomeric phases. Oligomeric phases have properties intermediate to monomeric and polymeric phases, and are synthesized sequentially. A series of nine oligomeric phases were synthesized using octadecyltrichlorosilane⁹. In the first step of the oligomeric synthesis, silica was modified with the silane under anhydrous

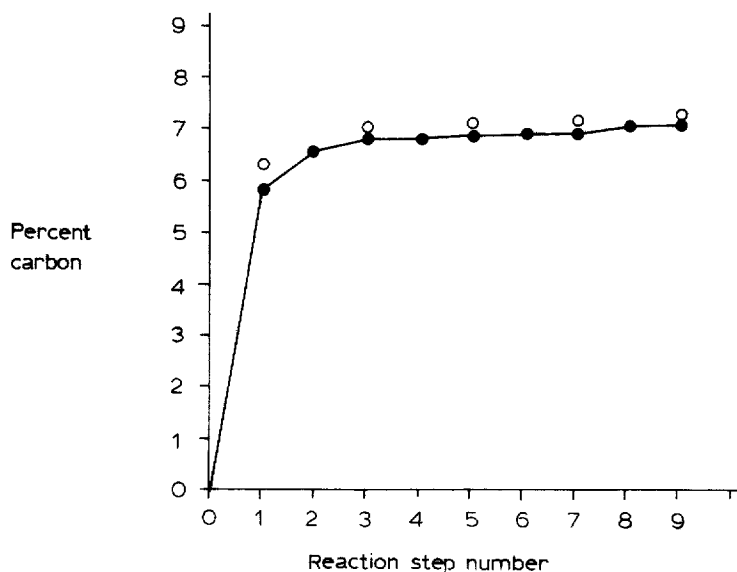


Fig. 1. Carbon loading plotted as a function of reaction step number for oligomeric phase synthesis. Circles represent carbon loading after endcapping with hexamethyldisilazane.

conditions, resulting in essentially a C_{18} monomeric phase. After filtering and washing, the phase was partially hydrolyzed. This converts unreacted chloro groups to new silane silanols, but does not remove any of the bonded phase itself. These silanols will react with additional octadecyltrichlorosilane when added, and thus the oligomer is synthesized one C_{18} unit at a time. Carbon analysis can be readily utilized to follow the extent of the reaction. Percent carbon *versus* reaction step number is plotted in Fig. 1 for an oligomeric synthesis⁹. Phase loading can be seen to increase significantly during the first three reaction steps. Smaller increases are observed for each of the remaining six steps. In fact, it is evident that greater increases in phase loading result from endcapping with hexamethyldisilazane than from reaction with octadecyltrichlorosilane. Steric considerations effectively limit the C_{18} oligomeric synthesis even though silanols are available for reaction.

The need for precise carbon measurements is further evident when phase stability is studied. To test the stability of a C_{18} polymeric phase, the sorbent was extracted for 24 h by refluxing in carbon tetrachloride. After filtering and washing, the extracted bonded phase was dried and carbon determinations were made. Three measurements were made on the untreated phase (6.77, 6.82 and 6.85% carbon; average 6.81%; S.D. = 0.04%) and the extracted phase (6.78, 6.87 and 6.85% carbon; average 6.83%; S.D. = 0.04%). Since no apparent difference in carbon was measured after extraction, it is clear that the bonded phase remained fixed to the silica surface throughout the extraction procedure.

REFERENCES

- 1 L. C. Sander, J. B. Callis and L. R. Field, *Anal. Chem.*, 55 (1983) 1068.
- 2 C. H. Lochmüller, D. B. Marshall and D. R. Wilder, *Anal. Chim. Acta*, 130 (1981) 31.

- 3 C. H. Lochmüller, D. B. Marshall and J. M. Harris, *Anal. Chim. Acta*, 131 (1981) 263.
- 4 C. H. Lochmüller, A. S. Colborn, M. L. Hunnicutt and J. M. Harris, *Anal. Chem.*, 55 (1983) 1344.
- 5 G. R. Hays, A. D. H. Clague, R. Haus and G. van der Velden, *Appl. Surf. Sci.*, 10 (1982) 247.
- 6 E. Bayer, K. Albert, J. Reiners, M. Nieder and D. Müller, *J. Chromatogr.*, 264 (1983) 197.
- 7 R. K. Gilpin and M. E. Gangoda, *J. Chromatogr. Sci.*, 21 (1983) 352.
- 8 H. A. Claessens, L. J. M. van de Ven, J. W. de Haan, C. A. Cramers and N. Vonk, *J. High Resolut. Chromatogr. Chromatogr. Comm.*, 6 (1983) 433.
- 9 L. C. Sander and S. A. Wise, *Anal. Chem.*, 56 (1984) 504.
- 10 L. C. Sander and S. A. Wise, *J. Chromatogr.*, 316 (1984) 163.
- 11 N. Tanaka, H. Goodell and B. L. Karger, *J. Chromatogr.*, 158 (1978) 233.
- 12 G. E. Berendsen, K. A. Pikaart and L. de Galan, *J. Liquid Chromatogr.*, 3 (1980) 1437.