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APPLICATION OF A GRAPHITE FURNACE ATOMIC ABSORPTION DETECTOR AUTOMATICALLY COUPLED TO A HIGH-PERFORMANCE LIQUID CHROMATOGRAPH FOR SPECIATION OF METAL-CONTAINING MACROMOLECULES

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SUMMARY

High-pressure liquid chromatography (HPLC) coupled with graphite furnace atomic absorption (GFAA) is capable of sensitive, nearly non-destructive, element-specific separation and detection of a wide range of molecular species containing metals or metalloids. Applications of HPLC–GFAA techniques are discussed, including size exclusion chromatography for the analysis of experimental organometallic polymers containing chemically bonded biocidal organotin moieties, and reversed bonded phase chromatography for analysis of novel organotin silicates. In conjunction with variably sensitive optical refractive index/ultra violet absorption detection, GFAA demonstrates separation of the polymers into at least two tin-containing fractions of widely different molecular weight (MW). The relative proportions of high- and low-MW fractions have important implications with respect to performance specifications for these and similar controlled release materials. Tin-specific and silicon-specific analysis of an organotin silicate demonstrates co-elution of species containing each element. Future off/on-line ^{29}Si and ^{119}Sn Fourier transform nuclear magnetic resonance spectroscopy will demonstrate whether each element is bonded to the same molecular species.

INTRODUCTION

The automatic coupling of a flameless graphite furnace atomic absorption (GFAA) spectrophotometer with high-pressure liquid chromatography (HPLC) provides a highly sensitive detector for virtually non-destructive speciation of molecules including any of a wide range of metal- or metalloid-containing moieties[1]. Detection by GFAA of subnanogram quantities of specific elements permits potentially useful quantitative or qualitative correlations of the metal content of a species with its column retention time (t_R).

Atomic absorption coupled with HPLC has been employed in some cases where the species of interest cannot be detected directly by atomic absorption.

Interesting examples are described by Kouchiyama *et al.*[2], who used flame atomic absorption for selectively detecting potassium and magnesium ions separated as their EDTA complexes by gel chromatography; and for speciating by size various polyphosphate compounds complexed with magnesium. The complexes were eluted in the order of decreasing molecular weights[3,4]. Jones and Manahan[5,6] used copper as the AA indicator for several aminocarboxylic acids separated as their copper chelates on a weak anion exchange column. Cassidy *et al.*[7] have described the separation of various silicon-containing molecules by molecular sieve and reversed-phase chromatography, detecting silicon by flame atomic absorption. In all of these approaches the analyte was totally consumed.

Similar studies, in principle, could be applied to a wide range of soluble molecular systems containing metals or metalloids, including biopolymers[8,9], where it is often desirable to recover the isolated macromolecular organometallic components. Consequently we have employed size exclusion chromatography (SEC)–GFAA and reversed bonded phase chromatography (RBP)–GFAA, seeking to extend capabilities for speciation and recovery of such trace bioactive macromolecules. Specifically we have studied macromolecules incorporating organotin moieties on backbone chains (OMPs), and organotin silicates which are precursors of a novel type of biocidal polymer. Both of these are controlled-release biocides; *i.e.*, they are designed to release specific toxic moieties into the service environment over long periods of time. OMPs were subjected to analysis by SEC–GFAA in order to separate molecular weight (MW) fractions while continuously monitoring eluent for tin. RBP–GFAA was evaluated here to study organotin silicates, correlating the retention time of both tin-bearing and silicon-bearing molecular species. The versatility of the overall method is evident when non-specific on-line detectors of molecular properties [ultraviolet absorption (UV), refractive index (RI)] are utilized in combination with GFAA.

EXPERIMENTAL

Instrumental methods

The HPLC–GFAA system is outlined schematically in Fig. 1. An Altex Model 100, dual-piston high-pressure pump (Altex Scientific, Berkeley, Calif., U.S.A.) provided stable flow of mobile phases, incremented in 0.01 $ml \cdot min^{-1}$ steps. Isocratic conditions were employed. Organometallic compounds in solution were injected (typically 50 or 100 μl) into the HPLC system via an on-line high-pressure syringe loading sample injector (Model 7120, Rheodyne, Berkeley, Calif., U.S.A.). The columns employed for SEC were packed with porous, highly cross-linked styrene–divinyl benzene copolymer with a particle size of 10 μm. Two such columns were used in series, having nominal pore sizes of 10^3 and 10^2 Å, with column dimensions 300 × 7.8 mm I.D. (Waters Assoc., Milford, Mass., U.S.A.). For reversed-phase chromatography, a column was employed of dimensions 250 × 3.2 mm I.D., packed with LiChrosorb C_{18}, with a particle size of 10 μm (Altex Scientific). Columns were connected in series by 1.6 mm O.D., 0.2 mm I.D. stainless steel tubing to either an adjustable wavelength ($\lambda = 254$ nm in this work) UV detector (Altex Model 153, Altex Scientific) or to a Knauer RI/UV dual detector (Utopia Instrument, Joliet, Ill., U.S.A.) maintained at 25° by a thermostated water bath (Lauda K4R circulating

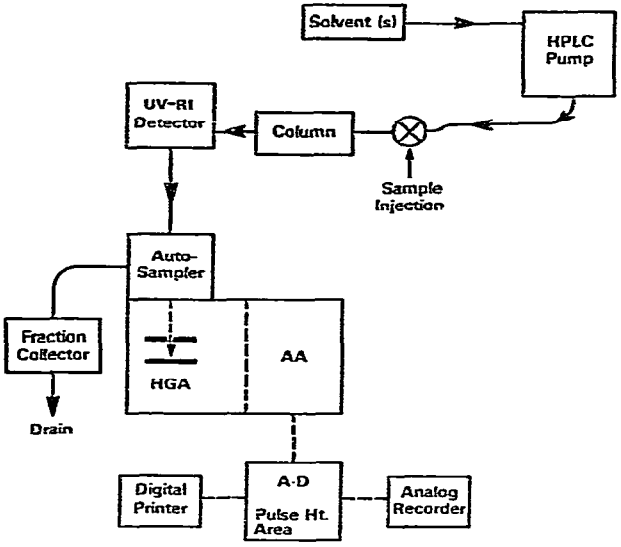


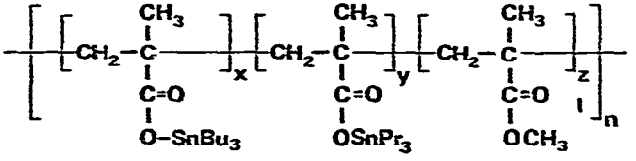
Fig. 1. The overall HPLC–GFAA system is summarized in this block diagram, including certain accessory devices (an optical detector, digitization equipment). The fraction collector may be used for a segmental stream analysis[1]; sample may be collected for off-line analysis; or excess sample may simply be drained off.

constant temperature bath, Brinkman, Westbury, N.Y., U.S.A.). Columns were operated at room temperature (22°). Medium walled PTFE tubing (1.6 mm O.D., 0.7 mm I.D.) transported effluent from the detector cell outlet to a specially constructed PTFE "well-sampler", described previously in greater detail[1]. Effluent from the HPLC column continuously entering the well sampler from the bottom flowed into a 50-μl sample well and excess liquid was withdrawn at the top by gentle suction. An AS-1 Auto Sampler automatic pipette (Perkin-Elmer, Norwalk, Conn., U.S.A.) withdrew, in the present experiments, 20-μl aliquots from the sample well at approximately 50 sec intervals. Thus, at a HPLC flow-rate of 1 ml min^{-1} only 2.4% of eluent was consumed. Either of two Perkin-Elmer atomic absorption spectrophotometers (indicated in captions) was used for specific detection: a Model 460 dual beam instrument with deuterium lamp background corrector and an HGA 2200 graphite furnace atomizer or a Model 360 dual beam instrument with deuterium lamp background corrector and HGA 2100 graphite furnace atomizer. In GFAA chromatograms, the observed peak heights represent measured absorbances due to the quantities of an element contained in a 20-μl aliquot introduced into the furnace and volatilized at a high temperature (for tin and silicon, about 2700°). At concentrations much greater than 20 ng per 20 μl, deviations from linearity were observed in a plot of absorbance versus concentration[10]. For this reason, quantitation of tin in OMPs was not extensively examined in the present study.

Continuous wave proton nuclear magnetic resonance (NMR) spectra were obtained at 60 mHz on a Varian NMR Spectrometer (Varian Assoc., Palo Alto, Calif., U.S.A.). Samples were run in 5-mm NMR tubes as neat liquids, employing tetramethylsilane (TMS) as an external standard.

Samples and reagents

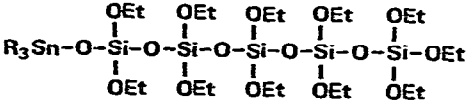
Samples of organometallic polymers were provided by the U.S. Naval Ship R & D Center, Annapolis, Md., U.S.A. Dyckman and Montemarano[11,12] have described the preparation, structures and testing of the materials designated OMP-1, OMP-2 and OMP-4. They and their associates have selected these polymers for formulation and testing as experimental antifouling coatings. OMP-1 is described as poly(tri-*n*-butyltin methacrylate–tri-*n*-propyltin methacrylate–methyl methacryiate) (Fig. 2). OMP-2 is poly(tri-*n*-butyltin methacrylate–methyl methacrylate), and OMP-4 is the tri-*n*-butyltin ester of poly(methyl vinyl ether–maleic anhydride).



n, x, y, z = repeating monomeric units

Fig. 2. Suggested chemical formula for OMP-1. The subscripts *x*, *y*, *z*, and *n* represent numbers of monomers. Actual numeric values cannot be assigned.

Several organotin silicates were studied which were organostannoxy derivatives of ethyl orthopentasilicate, described by Gysegem *et al.*[13]. Of these, we discuss here only the tricyclohexylstannoxy derivative (see Fig. 3).



R = S, Alkyl, Phenyl

Fig. 3. Suggested chemical formula for the tri-organostannoxy derivative of ethyl orthopentasilicate.

Polystyrene compounds serving as molecular weight standards were obtained from Arro Labs., Joliet, Ill., U.S.A.

The solvents used were tetrahydrofuran (THF, ACS reagent grade), absolute ethanol (USP grade), and acetonitrile (spectrograde). These solvents were purified by filtration, using organic solvent clarification kits of 0.5 μm pore size. Insoluble materials were removed from sample solutions by filtration, using organic sample clarification kits of 0.45 μm pore size (Waters Assoc.).

RESULTS AND DISCUSSION

Size exclusion chromatography

All three of the polymers (OMP-1, OMP-2, and OMP-4) were provided in a solution of Stoddard solvent (mineral spirits) and appeared to be completely soluble in THF. The chromatograms shown in Fig. 4 were obtained using THF as both solvent and eluent.

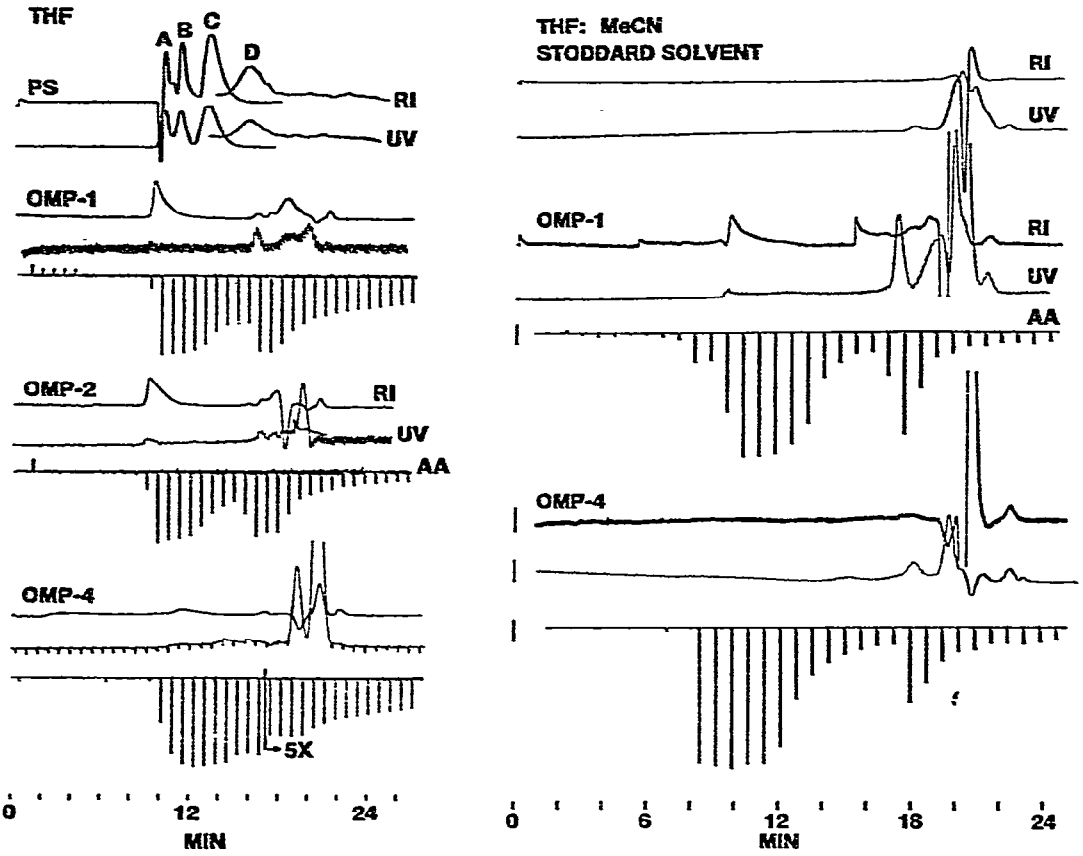


Fig. 4. SEC–GFAA of OMPs in THF. Perkin-Elmer 460 detector. Columns: Waters 10^3 and 10^2 Å size exclusion columns in series. Flow = 1 ml min^{-1}. Pressure = 300–500 p.s.i. Sensitivity of RI detector: $1 \times (6 \cdot 10^{-5})\Delta RI$ = full scale deflection (FSD). Sensitivity of UV detector. 1 absorption unit (a.u.) = FSD (OMPs 1, 2); 0.5 a.u. = FSD (OMP 4). Concentration of PS standards, 500 μg/100 μl; molecular weights: A = 111,000; B = 19,000; C = 4000; D = 600. Typical injected concentrations: OMP-1, 1000 μg/100 μl; OMP-2, 1350 μg/100 μl; OMP-4, 2300 μg/100 μl.

Fig. 5. SEC–GFAA of OMPs in THF–acetonitrile. Perkin-Elmer 460 detector. Waters 10^3 and 10^2 Å size exclusion columns in series. Solvent, THF–CH_3CN (19:1). Injection 100 μl. Flow = 1 ml min^{-1}. Pressure = 500 p.s.i. RI sensitivity, FSD = $(6 \cdot 10^{-5})\Delta RI$. UV sensitivity: OMP-1, FSD = 0.25 a.u., OMP-4, FSD = 0.5 a.u. Typical injected concentration: OMP-1, 1220 μg/100 μl; OMP-4, 2360 μg/100 μl. Specific measured relative tin peak areas:

	A (≈ 8–12 min)	*%*	*B (≈ 18 min)*	*%*
OMP-1	29.86	72	11.62	28
OMP-4	35.72	73	13.37	27

In the case of both OMP-1 and OMP-2, two fractions, at least, are clearly apparent in the RI chromatograms. By comparison with MW standards of polystyrene (PS), the fraction that is first eluted has a MW of about 111,000; the second has a MW probably less than 600. Differences in the composition of the lower-MW fraction are quite evident, as could be expected from the batch compositions (Experimental). The RI chromatogram of OMP-4 indicates the presence

of a fraction having a MW of about 19,000, but none similar to the high-MW fraction of OMP-1 and OMP-2.

In each case there are wide separations of high- and low-MW fractions. However, looking at AA chromatograms, the apparent separation of peaks is quite evidently contraindicated. One does see the onset of an early peak in the chromatograms of OMP-1 and OMP-2 which corresponds very closely to the t_R of the RI peaks. Also at a t_R corresponding to that of standards of lower MW, a second peak is seen. The AA data show that column overloading with severe tailing as a consequence is necessary in order to attain threshold concentrations for optical detection of these polymers. To obtain adequate peak resolution will require using polymer concentrations diminished by one or two orders of magnitude, with sacrifice of conventional optical detection.

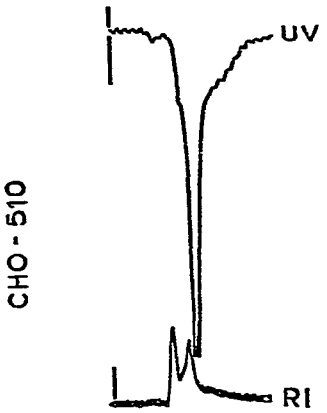
The discussion of OMP-1 and -2 also applies to OMP-4, with one exception. Strong AA signals for high-MW fractions are evident even though the RI signal is very weak. Again, optimal resolution will require a decrease in polymer concentration. As shown in Fig. 5, adding 5% of CH_3CN to THF provides somewhat more rapid elution of both high- and low-MW fractions, with superior resolution of AA peaks. The RI difference here is smaller than that observed when THF is the solvent, presumably because CH_3CN has a lower RI than THF. Again, resolution would be further improved by dilution of sample, with a diminished capacity, however, for tandem optical detection, or for off-line analysis. The relatively high ratio of low-MW polymer indicated in Fig. 5, as in Fig. 4, may result from a high proportion of oligomers, to unreacted monomers of organotin, or possibly from dissociated organotin moieties resulting from the hydrolysis of esters.

In order to better correlate molecular properties with a performance specification, it is desirable to incorporate most of the tin in organometallic polymers within a high polymer fraction having a narrow MW range[14]. Hence, this type of direct analysis, associating metal content with molecular-weight fraction, will play a future role both in developing the polymerization method and in standardizing physical properties of the polymer product.

Reversed bonded phase chromatography

Several organotin silicates were examined. These have been synthesized for use as monomer precursors of novel controlled release organotin "inorganic" polymer systems[13]. Stannoxy derivatives of ethyl orthopentasilicates are subjected to acid or base hydrolysis. The products, on condensation *in situ*, form biocidal organotin polysiloxanes[13]. Chromatographic speciation of the tricyclohexylstannoxy derivative of ethyl orthopentasilicate (CHO-510) was undertaken with a solvent/eluent of absolute ethanol to examine the relationship of elution times for Si- and Sn-containing species.

Fig. 6 shows RI/UV spectra of CHO-510. The component absorbing UV light at 254 nm is eluted as one intensely absorbing peak, with some tailing. However, there are two much less pronounced RI peaks. These might be due to the presence of isomers or to dissociated materials. Since loss from the parent compound of R_3Sn sites providing controlled release could adversely affect performance criteria, the GFAA spectrum (Fig. 7) assumes great significance. Here we can see that tin is eluted at the same time as silicon, a necessary consequence if both elements exist, as desired, in the same molecular species.



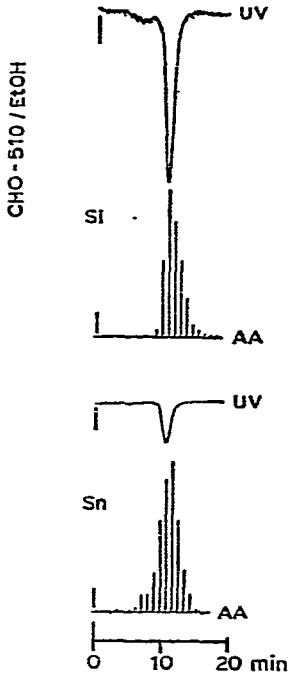


Fig. 6. Tri-cyclohexylstannoxy derivative of orthopentasilicate, prepared with a Si:Sn stoichiometric ratio of 5:1 (CHO-510). Column: C_{18} reversed bonded phase (RBP); mobile phase, absolute ethanol. Flow = 0.25 ml min^{-1}, pressure = 280 p.s.i. Injection 50 μl, 100 μg of analyte. Solvent, absolute ethanol. Detector sensitivities: UV, FSD = 0.25 a.u.; RI, $(6 \cdot 10^{-8})\Delta RI$ = FSD.

Fig. 7. Sn- and Si-specific chromatograms of CHO-510. Perkin-Elmer 360 detector. Column: C_{18} RBP. Mobile phase, absolute ethanol. Flow = 0.25 ml min^{-1}. Pressure = 280 p.s.i. Injection 50 μl, 7.15 μg of analyte. Solvent absolute ethanol. Detector sensitivities: GFAA, for Si, 10 mV = FSD; for Sn, 2 mV = FSD, operating at 251.6 and 286.3 nm, respectively. UV for Si, FSD = 0.005 a.u.; for Sn FSD = 0.02 a.u., operating at 254 nm. Retention volumes: (UV) 12.8 ml; Si (AA) 12.8 ml; Sn (AA) 12.8 ml.

GFAA analysis of CHO-510 in ethanol solution showed an absorbance at 251.6 nm using a Si-specific lamp (Fig. 8), which is numerically equal to that observed at 286.3 nm, using a Sn-specific lamp. If the injected compound was eluted as a single species, one should expect signals of equal strength when measurements are taken at the respective wavelengths. The total signal (peak area) for Si in the chromatographic eluent, measured on a digital printer, however, exceeds that for Sn by about 3.6/1 (Si = 68,500 μV/sec; Sn = 19,100 μV/sec). Thus, it appears that the amount of tin which passes through the column is appreciably less than that present in the injected quantity of CHO-510.

A dissociation of tin-containing bonds (Sn–O–Si) either on the column or in storage must be inferred from this retention of tin-containing moieties on the column, possibly as a result of alcoholysis with the ethanol mobile phase. The loss of tin signal indicates a need to compare the behavior of compounds bearing R_3Sn substituents in different column/eluent systems, but also illustrates the means to eventually determine slow release rates for this biocidal moiety.

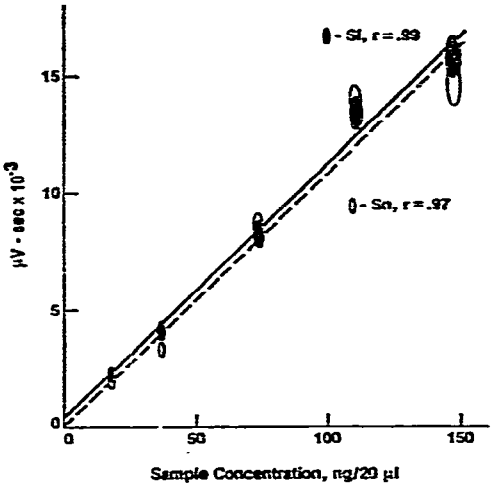


Fig. 8. GFAA analysis of CHO-510 using lamps element-specific for Si operating at 251.6 nm and Sn operating at 286.3 nm. Perkin-Elmer 360 detector. These represent calibration lines for total element analysis before injection.

Figs. 9 and 10 show that continuous wave–NMR is capable of detecting the presence of the cyclohexyl moieties in CHO-510. These are proton continuous wave–NMR spectra of ethyl orthopentasilicate and CHO-510, respectively. The complex peaks downfield in Fig. 9 result from $-CH_2O-$ groups having slightly different orientations in the molecule. The sharp upfield triplet arises from CH_3- groups, all having essentially the same molecular environment. The difference in Fig. 10 consists of a low-intensity complex peak due to tricyclohexyltin protons, that is absent from Fig. 9. These data are useful, but do not demonstrate whether the Sn-

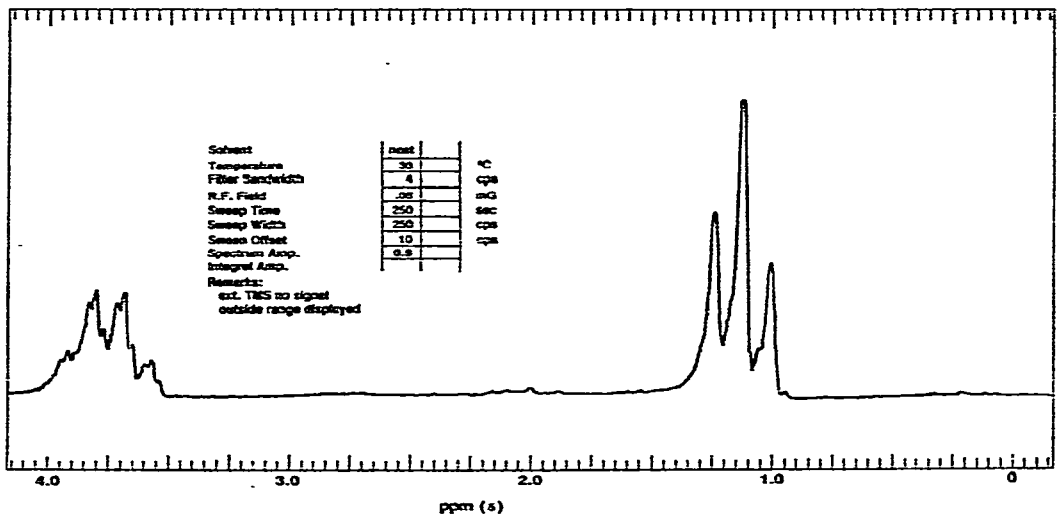


Fig. 9. Proton continuous wave–NMR spectrum of ethyl orthopentasilicate, neat. Formula given in Fig. 3. The downfield multiplet is assigned to $-CH_2-O-$ protons; the triplet arises from CH_3-protons.

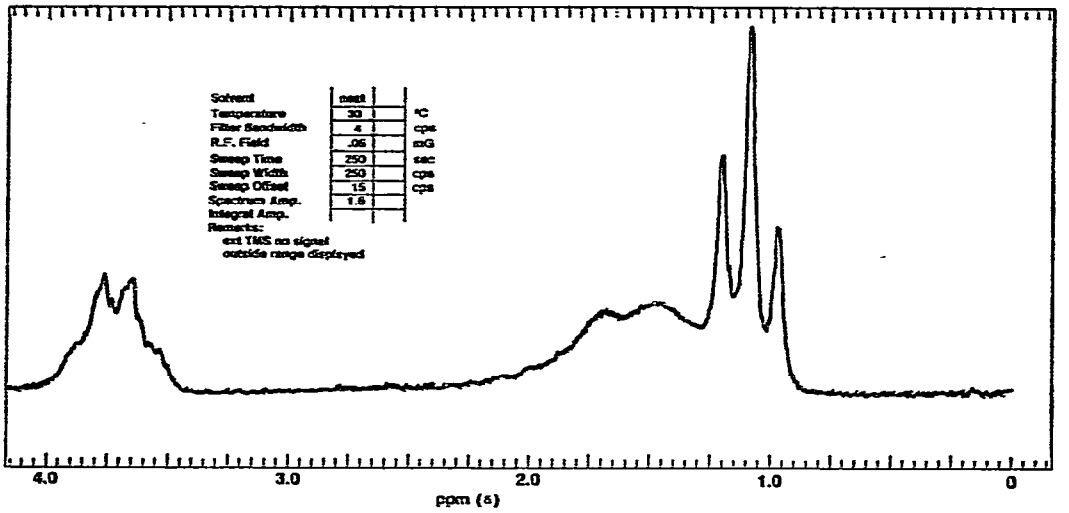


Fig. 10. Proton continuous wave–NMR spectrum of tricyclohexylstannoxy derivative of ethyl orthopentasilicate (CHO-510) (Fig. 3). In addition to the peaks observed in Fig. 9, the broad doublet occurring at $\Delta = 3.2$ ppm results from tricyclohexyl stannyl protons.

and Si-containing species are identical. Conclusive evidence on this point is anticipated by means of direct ^{29}Si and ^{119}Sn Fourier transform–NMR examination, with the installation of new National Bureau of Standards facilities[15]. Moreover NMR can offer a highly significant potential on-line characterization technique[16] for future studies.

CONCLUSION

HPLC–GFAA techniques (*e.g.* SEC and RBP) are capable of providing sensitive detection criteria for characterizing molecules bearing specific elements, such as controlled release biocidal organometal macromolecules. Supplementary methods of detection and analysis such as on-line optical detectors and off- or on-line NMR, complement and extend the usefulness of HPLC–GFAA, but disparity in the various detector sensitivities, in some cases by orders of magnitude, must be noted. The opportunity to utilize additional characterization techniques down stream of the GFAA detector is provided by the consumption of only a small amount of HPLC eluent by the GFAA, typically 2–5% depending on HPLC flow-rate. Commercial interfaces for coupling a mass spectrometer to HPLC are on the market[16] and direct coupling of HPLC to Fourier transform–NMR has recently been reported[17]. Such systems would further amplify the usefulness of HPLC–GFAA for element-specific characterization of metal- or metalloid-containing macromolecular species.

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Certain commercial materials and equipment are identified in this paper in order to specify the experimental procedures. In no case does such identification imply recommendation or endorsement by the National Bureau of Standards nor does it imply that the material or equipment identified is necessarily the best available for the purpose.

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