Liquid Chromatography–Laser-enhanced Ionization Spectrometry for the Speciation of Organolead Compounds

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Liquid chromatography (LC) and laser-enhanced ionization spectrometry (LEI) have been combined to provide a very sensitive method for the measurement of organolead species. Measurement is possible in environmental and biological matrices, which often contain high levels of easily ionized elements that normally interfere with LEI measurements. However, LC is able to resolve the interferents from the analytes, and LEI is sensitive enough to counterbalance the increase in detection limits that results from the use of LC. The method has been applied to the determination of organolead compounds in National Institute of Standards and Technology Standard Reference Material 1566a, Oyster Tissue. Trace levels of trimethyllead were observed in the Oyster Tissue, but concentrations were variable among the samples tested.

Keywords: *Laser-enhanced ionization; liquid chromatography; organolead; speciation*

Laser-enhanced ionization spectrometry (LEI) is a very sensitive atomic spectrometric technique for total metal analysis, with limits of detection (LODs) often in the 1–100 pg ml^{-1} range.[1–4] In LEI, one or two dye lasers are tuned to a wavelength characteristic of an electronic transition of the species of interest. The laser beam(s) are directed into an analytical flame, which serves as the atom reservoir. A potential is applied across the electrodes in the flame, and as the analyte atoms are photoexcited and collisionally ionized, the resultant current is measured across the electrodes. Laser-enhanced ionization spectrometry suffers from interferences that result from the presence of easily ionized elements (EIE) like the alkali and alkaline-earth metals. Thus, its application to the analysis of real environmental or biological samples containing high salt concentrations can be hampered. By coupling liquid chromatography (LC) to LEI, a complementary effect is observed. The LC can physically resolve analytes from interfering EIE, while the high sensitivity of LEI can counterbalance the degradation in detection limits resulting from dilution of the injected sample by the LC mobile phase. Laser-enhanced ionization spectrometry is also a good mode of detection for LC because of the compatibility of the LC flow rate with the sample aspiration rate of the burner and because of the simplicity of the interface; a short length of tubing attaches the LC column to the aspiration port of the burner. The combination of LC–LEI has been applied to the determination of organotin compounds in estuarine sediment[5] using ion-exchange separation. The use of LC–LEI for the determination of organolead compounds using a reversed-phase LC separation is described in the present paper.

There has been renewed interest in lead compounds because of recent concerns about leaded paints, lead in drinking water and lead poisoning. Organoleads were in the spotlight a few years ago because of the use of tetra-alkylleads as octane boosters in gasoline. In 1983, allowed lead concentrations in gasoline were $\leqslant 0.29$ g l^{-1} for leaded and $\leqslant 0.013$ g l^{-1} for unleaded gasoline in the United States.[6] The tetra-alkylleads decompose to form trialkylleads (which are 10–100 times more toxic than inorganic lead), dialkylleads and inorganic lead.[6]

Frequently, gas chromatography (GC) is used for separation in the analysis of organoleads. Radziuk, *et al.*[7] have compared LODs for tetra-alkyllead using five GC atomic spectrometric methods. Using 1–5 μl injections of trimethylethyllead solution, they obtained the following absolute LODs: GC–flame atomic absorption spectrometry (FAAS), 1.5 ng; GC–atomic fluorescence, 0.3 ng; GC–quartz furnace AAS, 0.1 ng; GC–quartz furnace suspended in a flame, 0.09 ng; and GC–electrothermal AAS (ETAAS), 0.03 ng.[7] Gas chromatography methods have been faulted for being unable to determine the trialkylleads due to their instability at temperatures used in GC and their reactivity with the GC column.[8,9] The dialkylleads are also thermally unstable[8] and involatile.[10] It is important to measure the ionic alkylleads since they are degradation products of the tetra-alkylleads.

An LC separation does not depend on the volatility of the analytes. Liquid chromatography has been coupled to FAAS,[8,11,12] inductively coupled plasma (ICP),[13] ICP mass spectrometry (MS)[14] and ETAAS[15–17] for the analysis of organoleads. Detection limits range over three orders of magnitude, from 500 ng ml^{-1} for the tetra-alkylleads by LC–FAAS[12] to 0.25 ng ml^{-1} for tetraethyllead by LC–ICP-MS.[14]

In the experiments described here, a reversed-phase LC column was attached to the LEI flame. Four organolead compounds and inorganic lead can be differentiated by LC, and sodium can be resolved from the lead species. Sensitive detection by LEI results in LODs comparable to those for LC–ICP-MS and GC–atomic spectrometric techniques. The method was applied to the measurement of organolead compounds in a National Institute of Science and Technology Standard Reference Material (SRM) 1566a Oyster Tissue; trimethyllead was found in a few samples that were taken from one bottle.

Experimental

Laser-enhanced ionization spectrometry

Two dye lasers were pumped by a frequency-doubled Nd : YAG laser. The first dye laser contained Rhodamine 6G; the beam was frequency-doubled to 283.31 nm, which excited the lead atom from the 6p level of the ground state to the 7s level at 35287 cm^{-1} (Fig. 1). The second dye laser, containing Rhodamine 610, was tuned to 600.19 nm. This beam excited the lead atom from 35287 cm^{-1} to the 8p level at 51944 cm^{-1}, 7876 cm^{-1} from the lead ionization limit of 59820 cm^{-1}. From here, the lead atom was collisionally ionized. The water-cooled cathode was operated at −1000 V and an air–acetylene flame was used. The LEI measurements were performed using a

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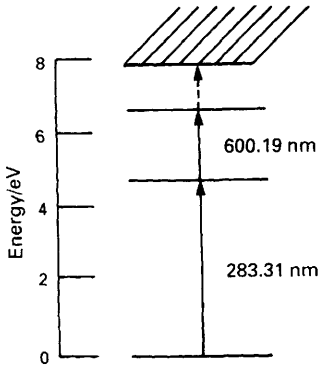

Fig. 1 Two-step photoexcitation and collisional ionization of lead

Model OGS-1 LEI Burner System (Sopra, Bois-Colombes, France), which consists of the burner, cathode, resistor (10 kΩ), capacitor (100 pF), pre-amplifier and high-voltage source in a radiofrequency shielded box. Optics were aligned to superimpose the laser beams a few millimeters below the cathode. Data were acquired using a Stanford Research Systems Model SR250 gated integrator and SR245 computer interface. A schematic diagram of the set-up is shown in Fig. 2.

Liquid chromatography

The organolead compounds were separated on a Zorbax ODS reversed-phase column using an LKB 2150 pump with a Rheodyne 7125 injector and a 20 μl sample loop. The method is an adaptation of that of MacCrehan and Durst,[18] using an isocratic mixture of methanol, water and ammonium acetate. Various solvent strengths were studied. A mixture of methanol–water (9+1) containing 0.01 mol l^{-1} ammonium acetate was determined to give the best separation of triethyl-, trimethyl-, tetramethyl- and tetraethyl-lead. The eluent flow rate was 1 ml min^{-1} and the aspiration rate of the flame without connection to the LC was 8.6 ml min^{-1}.

Sample Preparation

Two sample preparation procedures were studied for the analysis of Oyster Tissue: an extraction and a digestion. The extraction method was similar to the one that had previously been successful for rapidly extracting organotin compounds from sediment.[5] A 0.5 g portion of freeze-dried, powdered Oyster Tissue (SRM 1566a), 3 ml of a mixture of hexanes and 3 ml of a 0.5 mol l^{-1} solution of chelating agent were placed in centrifuge tubes in an ultrasonicating bath for 60 min. The use of chelating agents is frequently reported in the literature on organolead.[19–21] Sodium diethyldithiocarbamate (NaDDC) and ammonium pyrrolidin-1-yldithioformate [ammonium pyrrolidine dithiocarbanate (APDC)] were investigated in the present work. After ultrasonication, samples were centrifuged and the supernatants were removed and filtered through 50 μm syringe filters. Using each chelating agent, both spiked and unspiked samples were extracted. A spiked sample was prepared by adding 100 μg ml^{-1} of lead as each of tetramethyllead, trimethyllead, tetraethyllead and triethyllead in methanol–water (7+3) to an Oyster Tissue sample prior to extraction. Both spiked and unspiked pairs of samples were placed in the refrigerator (40 °C) overnight so that the Oyster Tissue could potentially absorb the spikes.

The digestion method was an adaptation of the procedure of Chau *et al.*[19] Oyster Tissue samples (0.5 g) were digested in 5 ml of 20% m/v tetramethylammonium hydroxide (TMAH) overnight at room temperature. The pH was then adjusted to 8 by the addition of 50% v/v 12 mol l^{-1} hydrochloric acid. A 3 ml aliquot of 0.5 mol l^{-1} NaDDC solution, 3 ml of toluene and 2 g of sodium chloride (to increase the solubility of the trialkylleads in the toluene) were added, and the mixture was shaken by hand for 20 min. The mixture was then centrifuged to separate the phases. The toluene phase was removed and was filtered through a 0.50 μm syringe filter. Spiked and unspiked samples were digested. Approximately 100 μg ml^{-1} of each of the four organolead compounds were spiked into the Oyster Tissue prior to digestion.

Results and Discussion

An eluent of methanol–water, (9+1) containing 0.01 mol l^{-1} of ammonium acetate, resulted in good separation of the organolead species (Fig. 3). Liquid-chromatography–LEI has an LOD for tetraethyllead of 0.9 ng ml^{-1} or 20 pg of lead for a 20 μl injection using a 1 s time constant. The LOD is calculated from the data shown in Fig. 4, the chromatogram of a 20 ng ml^{-1} solution of lead as tetraethyllead in hexanes. This LOD is the concentration that would give a signal equal to three times the root mean square chromatographic baseline noise and is comparable to LODs of 0.05 ng ml^{-1} obtained by GC–ETAAS[21,22] (25 pg absolute)[21] and 0.25 ng ml^{-1} (25 pg absolute) by LC–ICP-MS.[14] The LODs for LC–ICP-MS and GC–ETAAS obtained in refs. 14 and 21, respectively, were also calculated as three times the standard deviation of the baseline noise divided by the slope of the calibration curve; the method used for calculating the LOD for GC–ETAAS in ref. 22 was not stated. The lowest LOD reported in the literature is 0.18 pg ml^{-1} for a unique method in which tri- and di-methyllead are ethylated by sodium tetraethylborate, the ethylated compounds trapped, and then thermally desorbed and flushed into a quartz furnace where atomic absorption is measured.[10] This detection limit is orders of magnitude lower

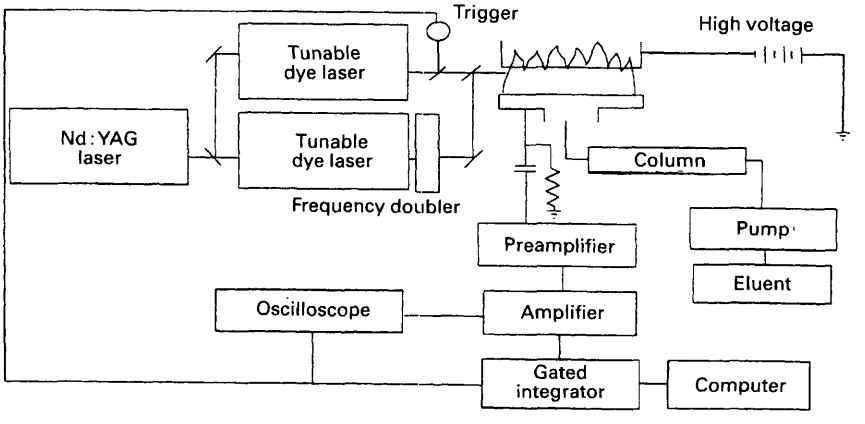

Fig. 2 Schematic diagram of the instrument

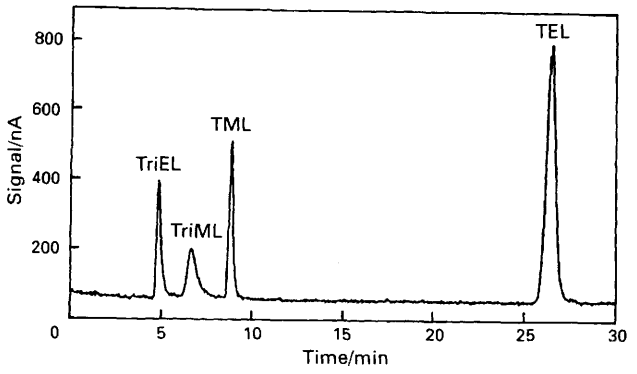

Fig. 3 Chromatogram of LC separation of four organolead compounds, equivalent to 500 ng ml^{-1} of lead each, in 70% methanol–30% water. TriEL = triethyllead; TriML = trimethyllead; TML = tetramethyllead; TEL = tetraethyllead. The eluent is 9 + 1 methanol–water containing 0.01 mol l^{-1} ammonium acetate and is pumped at 1 ml min^{-1}. Sample size = 20 µl

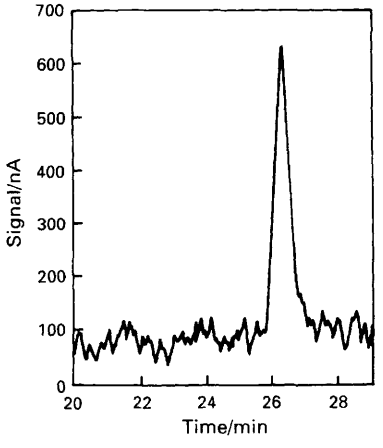

Fig. 4 Chromatogram showing 20 ng ml^{-1} of lead as TEL in hexanes using chromatographic conditions described in Fig. 3. Detection limit is 0.9 ng ml^{-1} of lead, or 20 pg absolute for a 20 µl injection

than anything else reported; unfortunately, it is applicable only to the measurement of tri- and di-methyllead.

Analysis of Oyster Tissue

Because oysters live in waters where organolead pollution from gasoline-powered boat engines is possible, SRM 1566a seemed a likely matrix in which to find organolead compounds. When the oyster tissue was extracted using hexane, chromatograms using both NaDDC and APDC as the chelating agents showed barely detectable tetraethyllead peaks. The NaDDC extract also appeared to contain a small amount of triethyllead. Both chromatograms showed large concentrations of sodium and inorganic lead. Using the hexane extraction method, 100 µg ml^{-1} spikes of the organoleads were recovered at a level of only about 0.5%.

When the Oyster Tissue was digested in TMAH, inorganic lead and trimethyllead peaks were clearly observed in several unspiked samples (Fig. 5), but a high degree of variation between samples was observed, as is shown in Table 1. Recoveries of organolead from one spiked sample were as follows: triethyllead, 48%; trimethyllead, 45%; tetramethyllead, 24%; and tetraethyllead, 48%. Although these recovery data result from the digestion of only one spiked sample, a 45% recovery implies that the actual trimethyllead concentration in some of the Oyster Tissue samples may have been closer to 3 ng g^{-1} of lead as trimethyllead. No organoleads were detected in a second bottle of Oyster Tissue. The variability in

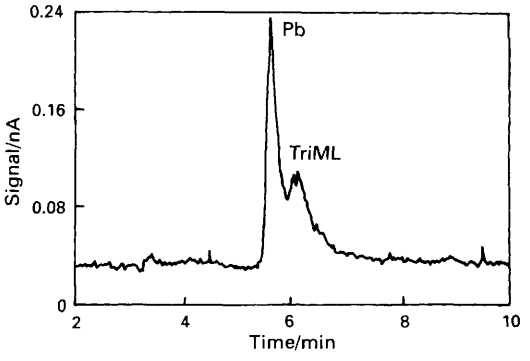

Fig. 5 Chromatogram for the separation of inorganic lead and TriML in oyster tissue by LC. Chromatographic conditions are described in Fig. 3

Table 1 Organolead concentrations in five oyster tissue samples

Sample No.	Concentration/ng g^{-1}
1	1.34
2	1.23
3	3.04
4	1.49
5	0.49

organolead concentration in different bottles of Oyster Tissue might have resulted from inhomogeneity or from differences in storage, *i.e.*, whether one bottle was exposed to more light or higher temperatures than another. Variability within a bottle could arise from the particular location of the sample in the bottle, *i.e.*, a sample at the top of the bottle could be exposed to more light and air than a sample from the centre of the bottle where it would be surrounded by Oyster Tissue. The bottles were not mixed before the samples were removed. This SRM is certified only for the total concentration of lead (0.371 mg kg^{-1}) and there is no information regarding the speciation of lead.

The absence of tetra-alkylleads in these samples is not entirely unexpected; the tetra-alkylleads are not only fairly volatile, but also decompose rather rapidly when exposed to sunlight. The half-life of tetramethyllead in the atmosphere is 10 h in summer and 34 h in winter; the half-life of tetraethyllead is 2 h in summer and 8 h in winter.[6] The trialkylleads that are formed are converted into dialkylleads, which are then de-alkylated to form inorganic lead. The use of tetraalkylleads as anti-knock agents relies on the ease with which they can be oxidized to form lead oxide. All samples tested showed large inorganic lead peaks and, in many cases, little organic lead.

It is obvious that both sample preparation procedures used in this study were inadequate. Further work is necessary to determine whether irreproducible levels of trimethyllead in the Oyster Tissue were a result of inhomogeneity or the fault of the sample preparation method or of the analyst. At the very least, the method of sample preparation needs to be improved to increase spike recoveries. However, while the Oyster Tissue experiments were not entirely successful, they do demonstrate the applicability of LC–LEI to the analysis of very low levels of organolead species in a real sample.

This work was included in the dissertation of K.S.E., which was submitted in partial fulfillment of the requirements for the Ph.D. degree from the University of Maryland. 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 Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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