Trace Metals Speciation by HPLC with Plasma Source Mass Spectrometry Detection

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The analysis of environmental and biological samples often requires detection at the parts per billion (ppb) level. Plasma source mass spectrometry has potential as a method for the analysis and speciation of trace elements. This is due to the technique's highly selective nature and excellent sensitivity. In comparison to atomic emission detection, detection limits are usually two to three orders of magnitude lower for plasma MS determinations. Interfacing HPLC with plasma MS provides a means of separation that is necessary for speciation. Speciation involves the determination and quantitation of the various chemical forms of a particular element. A host of HPLC/ICP-MS techniques may be used to obtain this information. This brief report will focus on the most recent work in this area, with emphasis on the work done in our laboratory. — Environ Health Perspect 103(Suppl 1):21–23 (1996)

Key words: inductively coupled plasma, mass spectrometry, liquid chromatography, trace metal speciation

Introduction

It has been well established that trace elements in the environment can be toxic to many organisms including humans and animals. There is growing concern over their presence in the air we breathe, the water we drink, and the soil in which we grow plants and vegetables, just to name a few. Concern is well warranted, since at very low levels many trace elements may be carcinogenic, mutagenic, and teratogenic (1, 2).

Chemical speciation has become an important research area in elemental analysis, since the toxicity of an element depends on its chemical form and/or oxidation state. Is it in an inorganic, organic, or organometallic state? In what oxidation state is it present? For many environmental chemists, toxicologists, and others, the answers to these questions are extremely important (1).

Since toxicity may occur at trace and ultra-trace levels, an efficient ionization source with a highly sensitive detector is necessary. Determining the different species of an element generally is not possible with traditional atomic spectroscopy methods. By coupling the separation power of high-performance liquid chromatography (HPLC) with the low detectability of inductively coupled plasma mass spectrometry (ICP-MS), however, speciation information at the subnanogram to picogram levels is obtained. Additionally, isotope abundance information may be obtained and multielement studies may be done (1).

Plasma MS

There are two commonly used plasmas: inductively coupled and microwave induced (MIP). The former is the most common type and will be discussed in detail here. To date, there is only one use of an He-MIP as the ionization source in an HPLC-plasma MS study (3).

An ICP is formed by passing radio frequency energy (usually at 27.1 MHz) through a load coil to form an oscillating magnetic field. A plasma forms when a gas, usually argon, is "seeded" by a spark with electrons, and thus becomes conductive. Energies sufficient to ionize gaseous atoms in the magnetic field are reached. The plasma will become self-sustaining because of collisions with gaseous atoms and ohmic heating, causing further ionization. Figure 1 shows the instrumental setup for HPLC-ICP-MS. The ICP consists of a nebulizer, which creates an aerosol from solution by passage through a small orifice with high velocity gas; a spray chamber, whose purpose is to separate out the large droplets of sample that go to waste; and a torch, in which the plasma is formed and where the finer aerosol particles will travel to be ionized (4).

HPLC-ICP-MS

Solution nebulization is the type of sample introduction method used most often for plasma MS. Unfortunately, speciation information may not be obtained. Alternate sample introduction methods such as HPLC have been investigated to perform speciation studies. Interfacing an HPLC with the ICP requires little effort. All that is necessary is tubing to connect the outlet of the analytical column with the inlet of the nebulizer. In addition, commonly used flow rates in HPLC are compatible with the flow rates used in most commercial ICP instruments.

Speciation studies in our laboratory have focused primarily on elements such as arsenic, lead, and tin. These elements are of interest due to their known toxicity and widespread presence in the environment from industrial processes. As an example, occupational and environmental exposure to arsenic is of concern because of its use in herbicides and pesticides. The inorganic forms of arsenic are more toxic than the organic ones, with the relative toxicities ranging from arsenite (As(III)), which is the most toxic of the water-soluble species, to the relatively nontoxic arsenobetaine (5, 6). Arsenite is followed by arsenate (As(V)), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) in terms of toxicity (5). Human exposure is best assessed by speciation using urine, blood, and hair samples (6).

The speciation of trace metals by HPLC-plasma MS may be divided into three categories based on the separation mode: a) reversed-phase (RPLC), including ion-pairing (IPC) and micellar HPLC; b) ion-exchange (IEC); and c) size exclusion (SEC).

Reversed-phase HPLC. Reversed-phase HPLC (RPLC) is the most popular mode of HPLC separation. Accordingly, there
have been numerous reports of speciation by RPLC. Separation is achieved by partitioning the analyte between a nonpolar stationary phase and a polar mobile phase. An extension of RPLC, ion-pairing chromatography (IPC), allows the simultaneous separation of ionic and nonionic compounds. The technique involves the addition of a counter-ion or ion-pairing reagent that is of opposite charge to the analyte. The addition of surfactants as counter-ions is commonly termed micellar HPLC (7).

While MIP is not generally accepted as an HPLC detector due to difficulties with sample handling, coupling RPLC to a helium MIP-MS system has been reported (3). This report provides an example of RPLC for the speciation of several halogenated organic compounds of bromine, iodine, and chlorine. Additionally, the feasibility of analyzing nonmetallic compounds by HPLC-ICP-MS is indicated.

**Ion-pairing Chromatography.** The first published paper using ion-pair RPLC with ICP-MS detection was also the first reported use of plasma-MS as an HPLC detector (8). Since then, several papers have been published employing IPC. The major advantage of ion-pairing lies in its capability to analyze samples containing both ions and molecular species (9).

The effect of inorganic tin chloride on the separation of trimethyl, tributyl, and triphenyltin chlorides has been investigated by Kumar and co-workers (10). The use of silica-based columns was previously reported for the separation of organotin compounds (11). Kumar and co-workers have found that inorganic tin was retained strongly on both a C18 silica column and a polymeric-based column, with the effect being more pronounced on the former column. The method was evaluated with extracted material from tuna fish tissue. Tributyltin chloride and triphenyltin were separated and the results reported for both a certified reference material and tuna purchased locally.

Gradient HPLC coupled with ICP-MS was used by Al-Rashdan et al. (12) to separate triethyllead chloride, tetraethyllead, triphenyllead chloride, and inorganic lead (Pb(II)). The viability of this method was verified using water and fuel reference materials. In earlier work, Al-Rashdan et al. (13) investigated lead speciation by both HPLC-ICP-AES (atomic emission spectroscopy) and HPLC-ICP-MS techniques employing reversed-phase, ion-pairing, and ion-exchange HPLC modes.

**Micellar HPLC.** The speciation of alkyltin compounds has been performed by Suyani et al. (14) using a sodium dodecyl sulfate (SDS) micellar mobile phase. Trimeethyltin chloride, triethyltin bromide, and tripropyltin chloride were separated and an additional separation scheme was used on monoethyltin trichloride, diethyltin dichloride, and trimethyltin chloride. While chromatographic efficiencies are comparable to hydroorganic mobile phase methods, unique retention mechanisms are possible using micellar mobile phases. Through the minimization of organic solvent usage, other advantages such as low cost, low toxicity, and greater plasma compatibility are obtained. Additionally, when micellar gradient elution studies are performed, no equilibration time is required, thereby saving time over commonly used reversed-phase gradient studies that do require column reequilibration (9).

**Ion-exchange Chromatography.** The majority of elemental speciation separations have been performed by IEC. A reason for its popularity as an elemental speciation method stems from its potential to separate ions and some polar molecules, including complex ions and neutral compounds. Selectivity, sample retention, and the extent of ionization all depend upon the pH of the mobile phase, with the resolution of nonpolar compounds being possible by ligand-exchange reactions or ionic complex formation (15).

The speciation of arsenic has been extensively studied by our group using anion-exchange HPLC (5,6,16). The first of these reports was presented by Heitkemper et al. (5), who used a weak anion-exchange column to separate four arsenic compounds commonly found in urine: arsenite, arsenate, DMA, and MMA. Due to the formation of an ArCl⁺ interference in the plasma, which corresponds with a mass-to-charge ratio (m/z) of 75, the detection of arsenite was complicated. This problem was addressed in a paper by Sheppard et al. (16) by resolving the chloride-containing species chromatographically from the arsenic peaks. Later work by Sheppard et al. (6) involved the determination of four arsenic species in freeze-dried urine, club soda, and wine.

**Size-exclusion Chromatography.** Only a few speciation studies have used SEC with ICP-MS detection. SEC employs a column with packing material containing pores capable of distinguishing between analytes; the separation depends upon the molecular size of the compound of interest (9). Investigations have included the determination of cadmium in metalloprotein species (metallothionein and ferritin) (17). Cadmium speciation in pig kidney after gastrointestinal digestion has also been accomplished (18).

**Future Directions**

There are some problems associated with sample introduction by HPLC. First, the inefficiency of the nebulizer-spray chamber apparatus requires further study. Second,
mobile phases containing high total dissolved solids of greater than 0.2% may cause clogging of the nebulizer, sampler, and skimmer orifices. Finally, organic mobile phases at high concentrations may cause instability in the plasma. All these difficulties lead to a decrease in sensitivity. These problems need to be addressed. While some studies have been done, further attention is warranted in the future. Additionally, a wider range of real samples needs to be made amenable to separation by these techniques while at the same time maintaining the integrity of the individual species.

Speciation studies in our laboratory focusing on tin, arsenic, and lead have led us into current work on chromium and vanadium using both ICP-AES and ICP-MS detection. Chromium and vanadium, along with a host of other trace metals, are of toxicological interest due to their presence in the environment. It is clear that the development of techniques to analyze such species at sub-parts per billion levels will continue to be a major area of research.

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