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A rapid and precise procedure for Pb isotopes in whole blood by Fe co-precipitation and MC-ICPMS analysis

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Abstract

Elevated Pb levels in humans through environmental exposure are a significant health concern requiring scientific study of the sources of, and physiological response to this toxin. This requires a simple and precise method for measuring radiogenic Pb isotopes and Pb levels in blood. Presented here is a combination of methods for separation and analysis of Pb previously used predominantly for geologic samples. This includes separation of Pb from the complex matrix of blood samples using an Fe co-precipitation method, followed by isotopic analysis by multi-collector inductively coupled plasma mass spectrometry. Evaluation of the efficacy of this procedure shows that the precision of sample preparations as measured by % difference between the ²⁰⁷Pb/²⁰⁶Pb of duplicate analyses averages 0.064% ($n = 48$). Using the same preparation and analysis techniques to measure Pb concentrations by isotope dilution resulted in a reproducibility of better than 6%. The method was successfully used to measure uptake of ingested soil Pb in a study of the bioavailability of Pb in contaminated soils.

1. Introduction

Stable Pb isotopes have been used in a wide array of environmental studies due to the ability to measure isotopic ratios very precisely in comparison to the range of values found in nature. This paper focuses on the analysis of Pb isotopes in a complex biological matrix, specifically blood. Uses of Pb isotopic data in blood and other tissues have included tracing sources of environmental Pb to an organism (Manton, 1977; Rabinowitz, 1987; Rabinowitz, 1994; Gulson et al., 1994); tracing remobilization and transport of Pb within an organism (Gulson et al., 1995; Smith et al., 1996; Manton et al., 2003); and estimating the bioavailable fraction of Pb in environmental materials, typically soils (Graziano et al., 1996; Maddaloni et al., 1998). These studies are all based on a small number of analyses, due in part to the laborious sample preparation required by thermal ionization mass spectrometry (TIMS).

Multi-collector inductively coupled plasma mass spectrometry (MC-ICPMS) offers improvements in throughput without loss of precision over TIMS. TIMS requires near complete matrix removal and thus demands a total digest and column separation (Manton,

1977) before analysis. The complex matrix of blood makes it difficult to quantitatively separate Pb, and TIMS analysis is particularly sensitive to incomplete matrix removal, resulting in reduced Pb ionization and the potential for inaccurate mass fractionation correction as the sample matrix is not identical to the standards used. The greater tolerance of ICPMS for matrix can allow for simpler preparation. Investigators have used ICPMS to determine blood Pb levels by aspirating whole blood samples that were first diluted 10:1 with solutions of Triton-X 100, ammonia and EDTA (Schutz et al., 1996), and have prepared serum for Pb determination by diluting with weak HNO₃ (Vanhoe et al., 1994).

A distinct advantage of MC-ICPMS over TIMS is the ability to monitor and correct for mass fractionation during the ICPMS analysis by the addition of Tl to samples and standards. Although mass fractionation is much larger for MC-ICPMS analysis than it is for TIMS (about 1% vs. 0.05%, respectively), the ²⁰⁵Tl/²⁰³Tl ratio can be used to correct the Pb ratios, since this bias is predominantly mass dependent and not significantly element dependent. For TIMS analysis, mass fractionation can be corrected in-run by double- and triple-spiking techniques, but this requires analyzing each sample twice, necessitating larger samples and more time (Hamelin et al., 1985). For most routine TIMS analyses, mass fractionation is not corrected in run but is estimated by calculating the mass bias/AMU based on separate analyses of standard solutions (Rehkamper and Halliday, 1998; White et al., 2000).

Single collector ICP-MS also enjoys the potential advantages of increased throughput in comparison to TIMS and has been used for Pb isotopes in blood. However, the precision of the isotopic measurements is not as good as MC-ICPMS (Gwiazda et al., 1998).

The authors have combined previously established procedures for Pb isolation and isotopic analysis in order to increase sample throughput, and assess the strengths and weaknesses of the procedure. A simple extraction method for isolating Pb from small (2 mL) samples of human blood followed by multiple collector ICPMS isotope analysis is presented. Whole blood is treated with 1 N HNO₃, and Pb is co-precipitated from the supernate with Fe. Data are presented from a study on the uptake rate of Pb from soil ingested by human subjects (a primary pathway for childhood exposure).

2. Material and methods

2.1. Sample preparation

An effective and reproducible Pb isolation method is desirable in order to remove organic material, which may cause C deposits on the ICPMS sample uptake system, and to remove elements that may cause matrix interferences in the ICPMS.

Whole blood samples are treated with heparin at the time blood is collected in an effort to prevent coagulation. Further sample preparation is performed later in the laboratory. For isotopic analysis of blood Pb, 8 mL 1 N HNO₃ is added to 2 mL of blood to rupture red cell membranes and leach Pb. Insoluble material is removed by centrifugation. The clear supernatant is treated with 1000 µL concentrated NH₄OH to raise the solution pH to ~10, followed by 50 µL of a 1000 mg/L/Fe solution. This causes Fe oxy-hydroxides ("FeOH") to precipitate quantitatively, co-precipitating Pb with it (Bruninx and Vanmeyl, 1975). Dissolved organic material and salts are largely left in solution. The solution is centrifuged to separate the FeOH precipitate, which is then rinsed with distilled water several times. The precipitate is then taken up in 1 mL 0.14 N HNO₃ and 900 µL of it are added to 100 µL of 250 µg/L Tl. At this point, the sample is ready for introduction into the MC-ICPMS sampling system. The run solutions range from approximately 5–50 µg/L Pb with a constant 25 µg/L Tl. Since mass 203 and 205 are monitored for mass bias corrections via Tl (see

below), enriched Pb-205 spikes cannot be used to obtain isotope composition and concentration data by isotope dilution in a single run.

Blood Pb concentration measurements were done using 3 ways: (1) by isotope dilution of blood spiked with enriched ^{206}Pb solution (NIST 983) and prepared as above; (2) by isotope dilution of blood spiked with enriched ^{206}Pb solution and totally digested (described below); and (3) by measuring signal intensity of samples prepared as above and compared to a known Pb concentration standard. For the isotope dilution method, the primary enriched ^{206}Pb solution was diluted to approximately 600 ng/L, and 50 μL of this dilution were added to blood prior to adding HNO_3 in order to get an appropriate $^{206}\text{Pb}/^{208}\text{Pb}$ ratio to minimize errors.

For total digests, 1 mL of whole blood was mixed with the enriched spike in 7 mL Teflon vials. Two milliliters of concentrated HNO_3 was added, and the vials were closed and heated on a hot plate until the solutions became clear (about 2 h). They were evaporated to dryness, taking care to avoid heating the samples too rapidly in order to avoid sample loss. The remaining solids were redissolved in 4 mL 1 N HNO_3 and transferred to centrifuge tubes with 4 mL water. From this point on, samples were treated as above – pH was raised with NH_4OH , Fe solution added, and the resulting precipitate collected and redissolved in 0.014 N HNO_3 .

2.2. In-house reference material

To the best of our knowledge, there is no available blood reference material with certified values for radiogenic Pb isotopes. Therefore, we prepared an in house human blood reference material, HBRM-1. Out-of-date packed red blood cells were mixed with saline solution and homogenized. From this solution over 170 seven mL aliquots were taken and stored in a freezer in acid cleaned plastic vials. This reference material is not identical to whole blood, in that it contains none of the species found in plasma (clotting agents, etc.), allowing it to be more stable for long-term frozen storage. An aliquot of HBRM-1 is routinely digested and analyzed when running whole blood samples. To date, HBRM-1 has not been analyzed by other laboratories. The authors are willing to offer aliquots to other researchers interested in inter-laboratory comparison.

2.3. Analysis by MC-ICPMS

Analyses were completed on a Thermo-Elemental Axiom multicollector ICPMS equipped with 10 Faraday cups and a single electron multiplier. The RF output is 1250 W, and the nebulizer gas flow rate is 1–1.2 L/min. Sample solutions are introduced to the plasma using a PFA nebulizer (20–100 $\mu\text{L}/\text{min}$) in an Aridus desolvating sample introduction system. This system removes H_2O from the sample solutions by passing the sample past a desolvating membrane, thus providing a dry sample to the plasma, increasing ionization efficiency by a factor of 5–10 over other sample introduction systems.

Analyses were in static mode with mass 206 in the axial Faraday cup (Table 1). This collector configuration allows the simultaneous measurement of all masses of interest, including the Tl masses that are used for the mass bias correction. Mass bias corrections were done by normal methods (i.e., the exponential law approach), assuming natural abundances. In addition to the Tl and Pb masses, ^{202}Hg is monitored in order to correct for the potential ^{204}Hg interference on ^{204}Pb . Although Hg interference is rarely seen, it can be present in the Ar carrier gas used in the ICPMS. Automation was achieved using a Gilson 201 autosampler, with sample introduced to the nebulizer using a peristaltic pump integrated with the Axiom MC-ICPMS. An analysis consists of 3 blocks of 10 ratios, at 5 s per ratio, and takes approximately 10 min including uptake and washout. Typically the residual signal

after 5 min of washout is less than 0.1% of the sample signal intensity, and thus has no detectable effect on the following sample. Background measurements are made at mass 205.5 (the half-mass offset). The on-line data acquisition program incorporates corrections for mass bias and for potential isobaric interference of ^{204}Hg on ^{204}Pb .

2.4. Analysis by TIMS

For comparison, a limited number of analyses were completed by thermal ionization mass spectrometry (TIMS). Blood samples were processed as described above up to the point of taking up the washed FeOH precipitate in 1 mL 0.14 N HNO_3 , after which Pb was isolated by anion exchange column chemistry, concentrated by evaporation, loaded on Re filaments with silica gel, and analyzed on a VG sector 54-30 mass spectrometer at Lamont-Doherty Earth Observatory in static mode, following standard procedures (Cameron et al., 1969). Ratios were corrected for cup bias and mass fractionation as determined with reference material from the National Bureau of Standards (NBS 981). Signal strength for the blood samples was much lower than expected, probably due to suppression of ionization due to impurities not removed by the chemical separation procedure (further support for the MC-ICPMS technique, which appears to be less sensitive to matrix effects).

3. Results and discussion

3.1. Instrumental sensitivity and blanks

Sensitivity ranged from 30–70 mV/ppb Pb (measured on mass 208) over different run dates. Sample intensities ranged from about 180–3500 mV. Procedural blanks were generally between 2 and 5 mV, and were generally at least 80–100 times lower than the lowest sample. However, there were a few blanks, early in the use of this method, that were considerably higher and probably indicated a contamination problem.

3.2. Concentrations and recovery

Three methods of measuring Pb concentration in blood were compared on selected samples: (1) isotope dilution plus the 1 N HNO_3 leach and Fe co-precipitation, (2) isotope dilution plus total digest, and (3) simply measuring signal intensity of samples prepared for isotopic analysis by 1 N HNO_3 leach and Fe co-precipitation. The second method is more time-consuming, but the authors view it as the most reliable, since it is the only method in which a clear solution (without blood cell material) is obtained. The third approach has the advantage of requiring only one preparation for both concentration and isotopic ratios.

Comparison data (Fig. 1) shows that the two isotope dilution measurements are very similar, and are very reproducible by this method. Therefore, it is concluded that the first method (i.e., isotope dilution plus the 1 N HNO_3 leach and Fe co-precipitation) is the preferred way to measure Pb concentrations in blood.

Concentrations by signal intensity are 40–68% of the isotope dilution values. These lower measured levels are evidence that Pb recovery of the leach/co-precipitation is incomplete. This may be explained by a loss of Pb either in the separation of the 1 N HNO_3 supernate from the insoluble blood material (i.e., some Pb may be discarded with the red blood cells that are removed by centrifuging), or in co-precipitation (some Pb may not come out of solution). However, the good agreement of the two isotope dilution methods is evidence that, if Pb is lost with the removed red blood cells, the Pb in the cells is easily exchangeable with Pb in solution.

3.3. Isotope ratios

Internal precision of isotope ratios was excellent with precision on $^{206}\text{Pb}/^{207}\text{Pb}$ averaging 0.018% (2σ , $n = 3$ runs per sample, 152 samples) over 5 run dates. Total precision, based on 18 replicate digests from 12 different vials of HBRM-1, the in-house reference material, is presented in Table 2. Note that environmental studies typically use the $^{206}\text{Pb}/^{207}\text{Pb}$, rather than the more typical $^{207}\text{Pb}/^{206}\text{Pb}$ used in geological studies. Ratios to ^{204}Pb are significantly less precise due to the relatively low ion current of this least abundant isotope. However, it is possible to increase sample size or dwell time on mass 204 in order to improve the statistics on ^{204}Pb if improved precision for that isotope is necessary. A limited number of samples were run by TIMS as a simple estimate of accuracy of the MC-ICPMS results. Results for $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{206}\text{Pb}/^{204}\text{Pb}$ agreed within errors of the measurements. Results for $^{208}\text{Pb}/^{206}\text{Pb}$ agreed within 2 standard deviations of the measurements.

Reproducibility of actual blood samples is evaluated by total procedural and analytical duplicates from duplicate aliquots of blood taken from the same sample vacutainer done by the Fe hydroxide co-precipitation method. Fig. 2 shows that these duplicates typically have reproducibility in line with the internal standard, although outliers do occur. The % difference for $^{206}\text{Pb}/^{207}\text{Pb}$ in duplicate digests ranged from 0.0002% to 0.58%, and averaged 0.064% ($n = 48$ pairs). The only possible explanation for the few poor replicates is contamination. However, the procedural blanks do not support that. Regardless, the data in the next section shows that this reproducibility is more than adequate to discern the changes in isotope ratio due to ingestion of soil Pb.

3.4. Application to human toxicity

The authors have applied the Fe co-precipitation/ MC-ICPMS technique for precise determinations of Pb isotopes in blood to a study of the uptake of Pb into blood after ingestion of soil, a primary pathway for childhood exposure. Using the more laborious sample preparation that is necessary for TIMS analyses on blood samples, this isotope dilution approach was previously used by Maddaloni et al. (1998) to study bioavailability of Pb in soil at a mixed mining-smelter site. This prior study found that the bioavailability fraction was an order of magnitude higher when the subjects had fasted, with fasted subjects having an average bio-available fraction of the soil of 26% (Maddaloni et al., 1998). The clinical procedures of the previous study were followed: each subject, after giving informed consent, received a screening physical examination and a 3-day hospital admission during which they had standardized meals and fasting times and ingested a gelatin pill containing 250 lg of Pb per 70-kg body weight. Blood samples were collected prior to ingestion and over a 30-h time period following ingestion.

The primary objectives of this on-going study is to understand human bioavailability of Pb of soils contaminated with Pb from different sources (smelting vs mining vs diffuse urban sources) and to test the effectiveness of a simple soil amendment for reducing bioavailability of soil Pb. The full protocols and results of this study will be reported elsewhere. Here the authors focus on consistency of results obtained by the method by showing examples of time series of Pb isotope ratios in whole blood after ingestion of soil. Uptake rates were determined for untreated and remediated Pb contaminated soil with known Pb isotope composition. A time series of blood samples was taken prior to and at intervals after ingestion (Fig. 3). As can be seen in this plot, there is a progressive increase in $^{206}\text{Pb}/^{207}\text{Pb}$ as the Pb is processed by the body. The background value of ca. 1.19 is clearly altered by the soil Pb (ca. 1.38), despite the fact that total Pb blood levels only increased by about 20%. This is an important point as small amounts of contaminants can be studied without detriment to the test subject. Further, the potential exists to identify the provenance of the contamination based on the Pb isotope signature. The systematic change in the $^{206}\text{Pb}/^{207}\text{Pb}$

towards the value of the ingested soil (Fig. 4) is a strong indication that the procedure is robust.

4. Conclusions

1. A simple sample preparation for whole blood, consisting of a HNO_3 leach combined with Fe co-precipitation, was developed and tested. Stable Pb isotope ratios were analyzed by MC-ICPMS.
2. Isotope ratios were measured precisely.
3. Concentrations were also measured precisely by isotope dilution.
4. This method makes possible analyses of large numbers of samples in a reasonable time. Therefore, studies that require large amounts of data are now more feasible.

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Fig. 1.

Comparison of blood Pb concentrations by 3 methods. A1 and A2 are replicate preparations of a whole blood sample. HBRM-1 35, 75, and 125 are preparations of the in-house reference material HBRM-1 (with aliquots from vial 35, vial 75 and vial 125).

Fig. 2. Reproducibility of duplicate sample preparations and analyses (as % difference of $^{206}\text{Pb}/^{207}\text{Pb}$ ratio). Total number of sample pairs = 48.

Fig. 3.

Pb isotope time series determined from blood samples taken from two subjects after ingestion of a gel capsule filled with either the untreated soil or the treated soil. Duplicate analyses of all time points are displayed on the graph.

Fig. 4. Mixing plot showing evolution of blood Pb isotope ratios toward composition of ingested soil. Best fit line is based on blood values only and is extrapolated to overlap with value of ingested soil.

Table 1
Masses analyzed

Mass	Faraday	Comments
202	L4	For $^{202}\text{Hg}/^{204}\text{Hg}$ to correct for isobaric interference on ^{204}Pb
203	L3	For $^{203}\text{Tl}/^{205}\text{Tl}$ ratio to correct for mass fractionation
204	L2	
205	L1	For $^{203}\text{Tl}/^{205}\text{Tl}$ ratio to correct for mass fractionation
206	Axial	
207	H1	
208	H2	

Table 2
Precision by MC-ICPMS and TIMS on in-house reference material and NIST 981

		$^{207}\text{Pb}/^{206}\text{Pb}$	$^{206}\text{Pb}/^{204}\text{Pb}$	$^{208}\text{Pb}/^{206}\text{Pb}$	$^{206}\text{Pb}/^{207}\text{Pb}$	Total Pb ($\mu\text{g/dL}$)
HBRM-1: MC-ICPMS	Average	0.84037	18.631	2.0574	1.1899	1.52
	$2 \times \text{rsd}$	0.049%	0.21%	0.23%	0.049%	5.0%
	$2 \times \text{rse}$	0.011%	0.05%	0.05%	0.01%	
	<i>n</i>	18	18	18	18	9
HBRM-1: TIMS	Average	0.84074	18.578	2.0583	1.1894	
	% Diff.	0.061%	0.35%	0.010%	0.06%	
	<i>n</i>	2	2	2	2	
NIST 981: MC-ICPMS	Average	0.91464	16.937	2.1681	1.0933	
	$2 \times \text{rsd}$	0.015%	0.067%	0.029%	0.015%	
	$2 \times \text{rse}$	0.005%	0.024%	0.010%	0.005%	
	<i>n</i>	8	8	8	8	
NIST 981: TIMS	Average	0.91464	16.937	2.1681	1.0933	
	$2 \times \text{rsd}$	0.027%	0.060%	0.058%	0.027%	
	$2 \times \text{rse}$	0.014%	0.030%	0.029%	0.014%	
	<i>n</i>	4	4	4	4	
Subject A after 5 h: MC-ICPMS	Average	0.76788	20.646	1.9420	1.3023	
	<i>n</i>	1	1	1	1	
Subject A after 5 h: TIMS	Average	0.76748	20.541	1.9394	1.3030	
	<i>n</i>	1	1	1	1	
	% Diff.	0.052%	0.51%	0.14%	0.051%	
Subject B after 5 h: MC-ICPMS	Average	0.82278	19.092	2.0343	1.2154	
	<i>n</i>	1	1	1	1	
Subject B after 5 h: TIMS	Average	0.82241	19.033	2.0322	1.2159	
	<i>n</i>	1	1	1	1	
	% Diff.	0.046%	0.31%	0.11%	0.046%	

For HBRM-1, MC-ICPMS data from 12 different vials, 4 analysis dates; TIMS data from 2 vials, one analysis date.