Comparison of Thermoelectrically Cooled Cryocell Assisted LA-ICP-MS and Liquid ICP-MS Analysis of Metals in Kidney and Liver Samples from Beached Porpoise Carcass

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Abstract

The aim of this investigation is the determination of element concentrations and normalized elemental profiles in the inner organs of a beached harbor porpoise (*Phocaena phocaena*) carcass stranded off the coast of Cape Cod, MA, in 2003. Multiple elements (including Hg, Se, Cd, Zn, Cu, and Na) were detected and measured in liver and kidney samples. A thermoelectrically cooled sub-zero laser ablation sample cell (Hyphenated Solutions, Jonesboro, AR) was devised for the analysis of porpoise liver and kidney tissues. The temperature controlled cooling cell has a user programmable operational range between 0 and -30ºC. The cooling cell was used here to preserve the integrity of the sample during ablation and to evaluate the affect of sub-zero temperatures on reproducibility. Normalized metal ion profiles in samples were determined using LA-ICP-MS. Frozen tissue samples were ablated using a CETAC LSX-213 Laser Ablation System, operating at 213 nm. Ablated samples were carried by a Helium carrier stream into the argon plasma of a PerkinElmer Elan 9000 ICP-MS system. During laser ablation tissues were maintained at 0ºC or cooled to subzero temperatures (-10 to -20 ºC) in the laser ablation cold cell to monitor the effect of sample temperature on ablation efficiency and reproducibility.
Results from laser ablation analyses were compared to ICP-MS analyses of tissue samples digested with ultrapure nitric acid. Metals in the digested samples were measured and detected following routine ICP-MS tuning and optimization procedures built into the ELAN software. Of the metals analyzed, elevated levels of Hg (~120 and 20 ppm), Se (~132 and 56 ppm), and Cd (~7 and 65 ppm) were found in the liver and kidney respectively. Raw elemental ratios acquired by laser ablation ICP-MS analysis were - depending on the metal - similar to liquid digestion based ICP-MS values. Precision in LA-ICP-MS is known to improve for analyses of tissues cooled below sub-zero temperatures compared to those at or above room temperature. However, only slight differences in precision were identified for the three temperatures used in this study. The best precision was determined to be -10ºC for most elements.

Introduction

- Liver and Kidney tissues were extracted from a beached harbor porpoise (*Phocaena phocaena*) carcass stranded off the coast of Cape Cod, MA, in 2003
- Elevated levels of Hg, Se, and Zn may be indicative of infectious disease as opposed to physical trauma as the cause of death (Bennett et al., 2001).
- LA-ICP-MS is a powerful analytical tool for elucidation of elemental concentrations at trace levels in solid samples.
- Optimal ablation efficiency for tissues in solid phase – drying or freezing helps reduce fractionation caused in part by evaporation of water from the vicinity of the laser shot and preserves the overall integrity of the sample (Feldmann et al., 2002).
- Maintaining tissue samples at subzero temperatures within a laser ablation cyrocell improves the stability of ion currents and the precision and accuracy of analytical measurments (Becker et al., 2007).
- Cryocell designed in for this study is thermoelectrically cooled and has an operational range of 15 - -30ºC.
- Temperatures are set using a process controller which uses a solid state relay and a thermocouple to control and monitor temperature.

Instrumentation and Operating Parameters (LA-ICP-MS)

Laser ablation system - CETAC Technologies LSX-213

Wavelength	213 nm
Energy	75% (3.375 mJ)
Spot size	200 µm
Pulse Frequency	20 Hz
Ablation Method	Single Points
He flow rate	600 ml/min

213 nm is a sufficient wavelength for thorough ablation of most colored and colorless solid materials (Jackson, 2001).

ICP-MS - PerkinElmer Elan DRC II

Standard Mode	
Nebulizer gas flow	1.05 L/min
Auxillary gas flow	0.85 L/min
Plasma gas flow	15 L/min
Lens Voltage	7.50 V
ICP RF Power	1400 W
Analog Stage Voltage	-1625 V
Pulse Stage Voltage	900 V

This ICP-MS uses a dual mode 26 dynode cascading electron multiplier which provides excellent mass detection and sensitivity.

Cryocell - Hyphenated Solutions

- Operational Range 0 - -30ºC
- Equipped with a thermoelectric cooler and a copper liquid-cooled heat exchanger
- Process controller uses a thermocouple to monitor temperature and controls power applied to cell
- Device designed to accompany the CETAC Technologies laser ablation platform by modifying sample stage of the LSX-213
- Inexpensive alternative to achieve subzero temperatures without use of expensive and dangerous cryogens (e.g. liquid N_2 or liquefied propane)

Figure 1. Dissected Harbor Porpoise (*Phocaena phocaena*) on display at Arkansas State University.

Figure 2. LA-ICP-MS interface consisting of a CETAC Technologies LSX-213 laser ablation system and a PerkinElmer ELAN 9000 ICP-MS instrument.

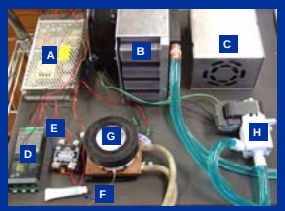

Figure 3. Components of the sub-zero cooling cell: Power supply 1 (A), Liquid chiller (B), Power supply 2 (C), Process controller (D), Solid state relay (E), Thermal grease (F), Sample Cell (G), Pump (H).

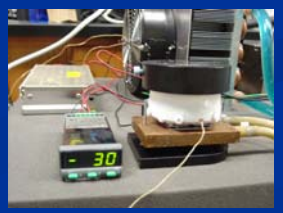

Figure 4. Sub-zero cooling cell (Hyphenated Solutions, Jonesboro, AR) cooled to -30ºC. The cell was specifically designed for LA-ICP-MS using CETAC laser ablation systems.

Materials and Methods

The harbor porpoise (*Phocaena phocaena*) was dissected following standard necropsy procedures. The organs (including kidney and liver tissues) were removed and small subsamples (~ 2 g wet weight) were sectioned and transferred to a -80ºC freezer. After allowing the cold cell to cool to the desired temperature (beginning with -20ºC), the kidney and liver tissues were removed from the -80ºC freezer, placed in the cell, and allowed to equilibrate for 5 minutes. Using the CETAC LSX-213 software single points were programmed, defocused, and ablated using the chosen ablation parameters. Experiments were conducted at 0ºC, -10ºC, and -20ºC to monitor changes in signal at different sub-zero temperatures.

Liver	0 C	%RSD	-10 C	%RSD	-20 C	%RSD
Zn	129618	13	117546	4	76279	13
Cu	130512	2	113805	8	40260	16
Se	70146	4	68660	7	46365	11
Hg	277508	8	300181	7	176948	27
Cd	17573	13	14523	12	9301	3
Pb	284	21	171	14	316	49
Kidney						
Zn	115616	3	137191	10	36187	3
Cu	45741	1	52946	8	13708	5
Se	17555	4	24866	0	5817	23
Hg	42107	9	41112	8	11121	20
Cd	79121	7	84268	5	34832	8
Pb	520	6	571	5	364	29

Figure 5. Average elemental CPS and %RSD measured by LA-ICP-MS at 0ºC, -10ºC, and -20ºC.

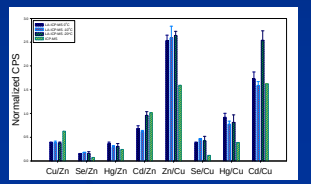

Figure 6. Normalized CPS in kidney tissue. Ratios were similar for all LA-ICP-MS experiments (p-value >0.05) with the exception of Cd/Cu (p-value <0.05 for -20ºC analyses). Agreement with solution based ICP-MS was poor (p-value <0.05) for all ratios.

	Liver	Kidney
Cu	118.93	40.45
Zn	326.29	214.24
Se	132.09	56.63
Cd	7.22	65.64
Hg	118.39	16.50
Pb	0.16	0.14

Figure 7. Elemental concentration in ppm in porpoise liver and kidney tissues measured by digestion based ICP-MS.

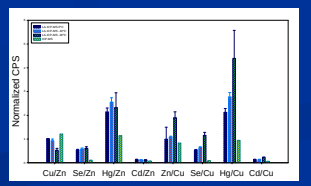

Figure 8. Normalized CPS in liver tissue. Ratios were similar (p-value >0.05) at 0 and -10ºC. With the exception of Cu/Zn and Zn/Cu, agreement was poor (p-value <0.05) for solution based ICP-MS comparisons. Agreement was also poor for LA-ICP-MS comparisons at -20ºC with the exception of Se/Zn, Hg/Zn, and Cd/Zn.

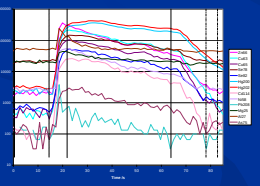

Figure 9. LA-ICP-MS spectra obtained for liver tissue maintained at -10ºC using the cryocell. Vertical designate the blank, signal, and matrix during data reduction.

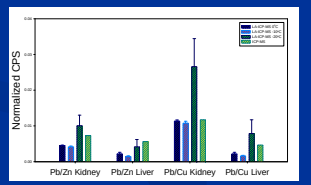

Figure 10. Normalized CPS for Pb in liver and kidney tissues. Ratios were similar (p-value >0.05) at 0 and -10ºC. Agreement was poor (p-value < 0.05) for comparisons to -20ºC and solution based ICP-MS with the exception of the ICP-MS Pb/Cu ratio in the kidney.

Results & Discussion

- Average CPS decreases with lower temperature (lowest average counts at -20ºC). Optimal balance between CPS and precision at -10ºC. Ion signal stability in LA-ICP-MS spectra highest at -10ºC.
- Ratios obtained by LA-ICP-MS were generally much higher than digestion based ICP-MS. The differences could be affected by many factors such as wet vs. dry plasma, plasma mass load and temperature, homogeneity of the aerosol, surface sampling (LA) vs. complete digestion, and surface and sub-sample differences.
- High amounts of Zn, Hg, and Se indicate the porpoise may have suffered from infectious disease or possibly methyl mercury poisoning (presence of MeHg was determined in separate study).
- Cryo cell successfully used to control and maintain sub-zero temperature for analysis of tissues by LA-ICP-MS.