# Use of Cold Cell Assisted LA-ICP-MS to Detect Semiconductor Nanocrystals Metals Accumulated in Tissues of Exposed Animals

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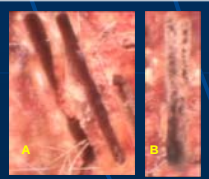
## Abstract

Mice were exposed to a water aerosol containing of 2-20 ppb semiconductor nanocrystals. The nanoparticles contained a cadmium-selenium core coated with zinc sulfide and an organic polymer. After exsanguination, tissue samples (lung, blood, brain, liver) were collected from exposed and unexposed mice. Immediately after collection samples were frozen in liquid nitrogen and stored at -80 awaiting analysis. Metal ion concentrations in samples were determined using LA-ICP-MS. Frozen tissue samples were ablated using a Cetac LSX-213, operating at 213 nm. Ablated samples were carried by both an argon and helium carrier stream into the argon plasma of a PerkinElmer DRC II ICP-MS. During laser ablation tissues were maintained at subzero temperatures ( -1 to -10 oC) in a laser ablation cold cell (Hyphenated Solutions, Jonesboro, AR). ELAN software was used to analyze spectra. Cd and Se were used as markers of nanocrystals in tissues. Na, Fe and K concentrations were constant among tissues and were used to normalize counts of all metals. Comparisons of solid standards using both the sub-zero and room temperature sample cells revealed no difference in total counts for each metal. Cd counts from brain tissue samples of exposed animals were 20% higher than those measured in tissues from unexposed animals while the liver tissues revealed a reverse trend. ICP-MS nebulizers gas (Ar) flow rate was 1 L/min. Laser ablation operating parameters used for both sample lines and spots were %100 energy (6 mJ), 20 Hz pulse repetition rate, 20 um/sec scan rate, and a 200 µm diameter sampling area.

## Introduction

- Mice were exposed by breathing an aerosol that contained 2 ppb semiconductor nanocrystals.
- The semiconductor nanocrystals used contained Cd, Zn, and Se. Cd and Se are highly toxic metals.
- Tissues (brain and liver) from exposed and unexposed animals were analyzed by laser ablation ICP-MS to detect and measure metals from nanocrystals.
- By maintaining tissue samples at subzero temperatures within a laser ablation cyrocell the efficiency and reproducibility of both the ablation and analyses may be improved.
- Sub-zero temperatures reduce redistribution of soluble analytes within samples during analyses and help preserve tissues for further analyses.
- Cold cells have been used successfully in experiments requiring ablation of *Xenopus* oocytes, tissue samples and frozen biological fluids.

Cryocell design and components



Pictures of ablated liver in cold cell (A) and warm cell (B)

## Instrumentation and Operating Parameters (LA-ICP-MS)

Laser ablation system - CETAC Technologies LSX-213

| | |
|---|---|
| Wavelength | 213 nm |
| Energy | 100% (6 mJ) |
| Spot size | 200 µm |
| Pulse Frequency | 20 Hz |
| Scan rate | 20 µm/sec |
| He flow rate | 500 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 | 0.95 L/min |
| Auxillary gas flow | 0.85 L/min |
| Plasma gas flow | 15 L/min |
| Lens Voltage | 7.00 V |
| ICP RF Power | 1400 W |
| Analog Stage Voltage | -1900 V |
| Pulse Stage Voltage | 1100 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 - -20ºC
- Equipped with a thermoelectric cooler and a copper liquid-cooled heat exchanger
- Process controller monitors and controls temperature and power applied to cell
- Device designed to accompany the Cetac Technologies laser ablation platform by modifying sample stage of the LSX-213
- Provides subzero surface and sampling cell without use of expensive and dangerous cryogens (e.g. liquid $N_2$ or liquefied propane)

## Materials and Methods

Mice were exposed to a water aerosol containing 2 ppb nanocrystals while confined within a 2.5 L Plexiglas™ exposure chamber. The mouse was placed in the chamber and an aerosol was generated by a nebulizer for 15 minutes. The mice remained in the box for an additional 15 minutes before tissue samples were taken. Controls were treated identically except that there were no nanocrystals in the water used to generate the aerosol.

Following the exposure the mice were exsanguinated and perfused with PBS. Blood and tissue samples from the brain and liver were collected, frozen in liquid nitrogen and stored in a -80°C freezer.

Brain and liver tissues were removed from the -80ºC freezer and allowed to equilibrate in the cell for 5 minutes. Using the Cetac LSX-213 software a line scan was programmed and ablated using the chosen parameters.

Line scans were ablated 5, 30, and 60 minutes after samples were placed in the cold cell so that changes in signal could be correlated with tissue temperature. Tissue and ablation pit integrity were also monitored. The cold cell was maintained at an average temperature range of -5 to -10 ºC.

| Element | MACS-1 standard cold vs warm: P value | Statistical Difference ? | NIST 612 glass standard cold vs warm: P value | Statistical Difference ? |
|---|---|---|---|---|
| Cd | 0.1019 | n | 0.1392 | n |
| Mg | 0.1076 | n | 0.1139 | n |
| Cr | 0.0657 | n | 0.0679 | n |
| Pb | 0.0544 | n | 0.1029 | n |
| Fe | 0.1305 | n | 0.1545 | n |
| Zn | 0.0875 | n | 0.1657 | n |
| Na | 0.1163 | n | 0.2650 | n |
| Se | 0.0596 | n | 0.4076 | n |
| P | 0.0600 | n | 0.2743 | n |
| B | 0.2299 | n | 0.2623 | n |

**Table 1.** Elemental differences for select metals in the MACS-1 (USGS) and NIST glass standards measured using LA-ICP-MS with both cold and warm sample cells.

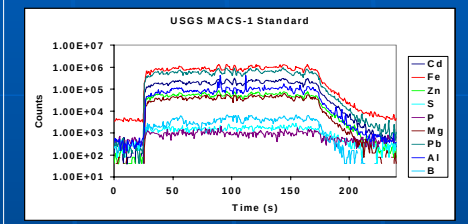


**Figure 5.** Counts obtained using LA-ICP-MS for the USGS MACS standard placed in the cold sample cell (T = -5ºC).

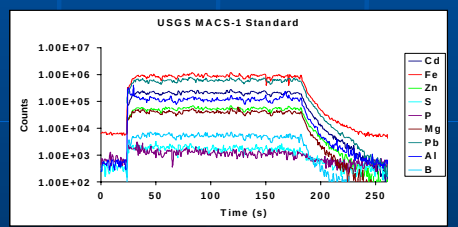


**Figure 6.** Counts obtained using LA-ICP-MS for the USGS MACS standard placed in the warm sample cell (T = 23ºC).

## Results & Discussion

- No statistical difference in counts for NIST 612 glass and USGS MACS-1 standards in the cold or warm sample cells. This is important because it verified that the cold cell does not contribute to loss of analyte signal. Changes in signal are purely sample dependent.
- Although on average counts were higher for warmer tissues, visual inspection of ablation lines of samples in the cold cell revealed deeper, more reproducible ablation with minimal damage to surrounding tissue. This is especially important when a limited amount of sample is available and for tissues that must be maintained at subzero temperatures.
- Average temperature for tissues in the cold and warm cells were -17ºC and 13ºC respectively.

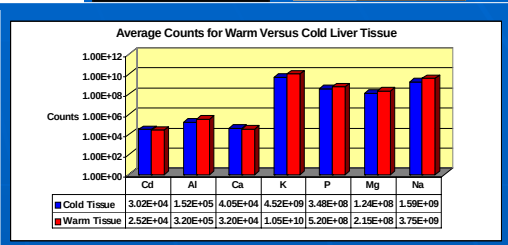


**Figure 1.** Average counts detected by ICP-MS from liver tissues sampled with laser ablation at various temperatures. Average cold temperature (blue bar) recorded over one hour was -13ºC and average warm temperature (red bar) was 17ºC.

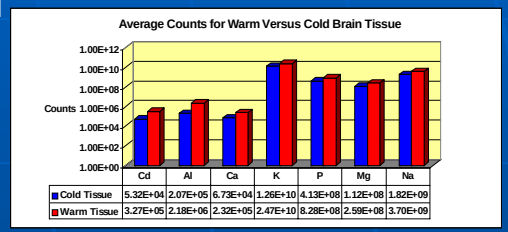


**Figure 2.** Average counts detected by ICP-MS from brain tissues sampled with laser ablation at various temperatures. Average cold temperature (blue bar) recorded over one hour was -13ºC and average warm temperature (red bar) was 17ºC.

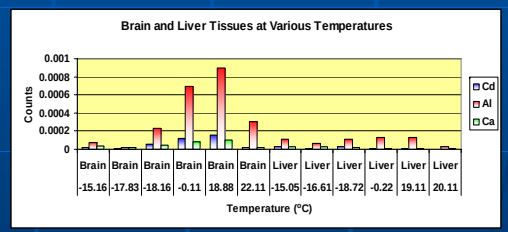


**Figure 3.** Counts normalized to Na for brain and liver tissues for various temperatures in the cold and warm sample cells.

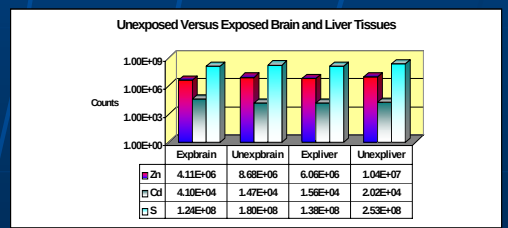


**Figure 4.** Counts for rat brain and liver tissues exposed and unexposed to semiconductor nanocrystals composed of Zn, Cd, and S.

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