Detection of nanoparticles on plant tissues using sp-ICP-MS

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Where do nanomaterials come from?

Particles with at least one dimension in the nano-scale (1~100 nm)

**Natural Sources**
Produced by redox reactions, weathering, mining, volcanos, dust storms

**Unintentionally produced NPs**
Emitted to air, water and soil from combustion, wear, metal polishing and metal working, electric motors etc.

**Engineered NPs**
Synthesized for a specific purpose. Usually embedded in other products
Engineered Nano-Materials (ENMs)

Application of ENMs in Agriculture

- Nano-pesticide/fungicide/bactericide
- Nano-fertilizer
ENM concentrations expected to be at ng/L to ug/L levels at point of release.

Lazareva and Keller, ACS Sus Chem, 2014
ENMs in Environment

Environmental Implications of Cu Based ENMs

Theme 1
Nanoparticle characterization & synthesis of Fe-doped Cu NPs

Theme 2
% hatching
HTS with zebrafish

Theme 3
LCA of Cu ENMs

Theme 4
Effects on crop plants & soil bacteria

Theme 5
Effects on fish & invertebrates

Theme 6
Transformation of Cu ENMs in septic systems
F&T modeling of Cu concentrations

Theme 7
Alternatives Analysis case study & workshop (Cu in marine paints)

How can we “see” ENMs?

Analytical Challenge

• Can we detect ENMs in water and other environmental matrices?
• Composition?
• Size?
• Quantity?
• Other characteristics?

No EPA methods available to date…
Methods for ENMs Characterization

- **Imaging** methods (TEM, SEM, AFM) are often definitive for detection, shape and size determination. Not quantitative or representative. Labor-intensive.

- **Spectroscopic/optical** methods (UV-Vis, dynamic light scattering) simple, but subject to interferences. No elemental information. DLS needs sharp size distribution.

- **Hyphenated techniques** (Chromatographic (or other online) separation coupled with ICP-MS detection). Allow representative samples, provide good particle size resolution, high elemental sensitivity but no information on individual particles

  - FFF-ICP-MS
  - CE-ICP-MS
  - HPLC-ICP-MS

- **Single particle ICP-MS**
Methods for ENMs Characterization

**Single Particle ICP-MS (spICP-MS)**

- Each nanoparticle gives a transient signal (a plume of ions generated from the particle)
- Use time resolved data acquisition and analysis
- Measure particle concentration, particle effective diameter and composition
Single particle ICP-MS

Reference Materials

60 nm Gold Nanospheres

- Unagglomerated and monodisperse
- Mean diameter: 60 nm ± 4 nm
spICP-MS workflow

ICP-MS TRA data

Response vs frequency

- Analyte response factor > Mass of analyte in particle
- Nebulization efficiency (calculated from reference material)
- Analyte density
- Analyte mass fraction in sample particle

Size distribution

Tabulate and Report

<table>
<thead>
<tr>
<th>Sample</th>
<th>Date/Time</th>
<th>Type</th>
<th>Sample Name</th>
<th>Nebulization Efficiency</th>
<th>Particles</th>
<th>Conc (particles)</th>
<th>Conc (ppb)</th>
<th>Conc (ppm)</th>
<th>Conc (ppb)</th>
<th>Size (nm)</th>
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</table>
## Precision and Accuracy

<table>
<thead>
<tr>
<th>Sample (Prepared concentration)</th>
<th>Observed Concentration (particles/L)</th>
<th>Observed Concentration (ng/L)</th>
<th>Observed Particle Size (nm)</th>
<th>Reference Particle Size obtained by TEM (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST 8013 Nominal 60nm (100 ng/L)</td>
<td>$5.59 \times 10^7$</td>
<td>103</td>
<td>55</td>
<td>$56.0 \pm 0.5$</td>
</tr>
<tr>
<td>NIST 8012 Nominal 30nm (10 ng/L)</td>
<td>$4.27 \times 10^7$</td>
<td>10.5</td>
<td>28</td>
<td>$27.6 \pm 2.1$</td>
</tr>
</tbody>
</table>
Applications?

Case study: ENMs pathway into plants

- Foliar application
- Delivery to soil surface as slow-release NPs
- Delivery below ground in fertigation suspensions
- Application with biosolids

- Pathway matters in terms of delivered [ENM] and bioavailability

Can we detect ENMs in Edible Plant Tissues with spICP-MS?
Copper Oxide Nanoparticles (nano-CuO)

<table>
<thead>
<tr>
<th>Property</th>
<th>nano-CuO</th>
</tr>
</thead>
<tbody>
<tr>
<td>primary particle size (nm)</td>
<td>50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>hydrodynamic diameter&lt;sup&gt;b&lt;/sup&gt; (nm)</td>
<td>280 ± 15</td>
</tr>
<tr>
<td>copper content (wt %)</td>
<td>74.3 ± 1.2</td>
</tr>
<tr>
<td>main copper phase</td>
<td>monoclinic CuO</td>
</tr>
<tr>
<td>density (g/cm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>6.349</td>
</tr>
<tr>
<td>BET surface area (m&lt;sup&gt;2&lt;/sup&gt;/g)</td>
<td>12.31 ± 0.05</td>
</tr>
<tr>
<td>isoelectric point (IEP)</td>
<td>6.3</td>
</tr>
<tr>
<td>CCC at pH 7 (mM NaCl)</td>
<td>40</td>
</tr>
<tr>
<td>water content (wt %)</td>
<td>0.23</td>
</tr>
</tbody>
</table>

<sup>a</sup>As provided by the manufacturer.
<sup>b</sup>Measurement was done in DI water at pH 7.

TEM Image of nano-CuO

Size distribution of nano-CuO in DI at pH 7 (via DLS)
Analyze nano-CuO with spICP-MS


![Graph A](image1)

**Graph A**: Measured nano-CuO Concentration (ng/L) vs. Nominal nano-CuO Concentration (ng/L).

![Graph B](image2)

**Graph B**: Frequency vs. Particle Size (nm).

<table>
<thead>
<tr>
<th>Nominal nano-CuO concentration (ng/L)</th>
<th>Ionic Cu concentration (ng/L)</th>
<th>Median size (nm)</th>
<th>Mean size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avg.</td>
<td>Std.</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>1</td>
<td>0.11</td>
<td>0.01</td>
<td>9.3</td>
</tr>
<tr>
<td>10</td>
<td>0.39</td>
<td>0.04</td>
<td>10.8</td>
</tr>
<tr>
<td>50</td>
<td>2.33</td>
<td>0.06</td>
<td>2.5</td>
</tr>
<tr>
<td>100</td>
<td>2.78</td>
<td>0.20</td>
<td>7.3</td>
</tr>
<tr>
<td>250</td>
<td>32.12</td>
<td>3.36</td>
<td>10.4</td>
</tr>
<tr>
<td>500</td>
<td>35.10</td>
<td>2.47</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Detect ENMs in leaf tissues via spICP-MS

Organic Vegetables

Kale
(\textit{Brassica oleracea}, var. \textit{Acephala Lacinato})

Lettuce
(\textit{Lactuca sativa} var. green leaf cultivar)

Collard Green
(\textit{Lactuca sativa} var. green leaf cultivar)
Leaf surface roughness

**ESEM**

Rinse Leaf with DI water

Detect with spICP-MS

A
Lettuce

B
Collard Green

C
Kale

A

B

C

Frequency
0 20 40 60 80 100
0 20 40 60 80 100
0 20 40 60 80 100
Particle Size (nm)

nano-CuO concentration (ng/L)
0 20 40 60 80

Lettuce
Collard Green
Kale

Rinse 1  Rinse 2  Rinse 3
Expose Leaf to nano-CuO

Lettuce
Collard Green
Kale

2-hour air dry
Rinse Leaf with DI after nano-CuO exposure

Detect with spICP-MS
Rinse Leaf with DI after nano-CuO exposure

Detect with spICP-MS

- Concentrations in first rinse around 500-750 µg/L
- Residual washable concentration after 2 rinses is less than 10 µg/L
- Leaf surface roughness may influence residual
Any nano-CuO within the leaf tissues?

Enzymatic digestion

Macerozyme R-10 enzyme

- Mixed with plant tissue samples to digest tissues and release nano-CuO
- 24 hr digestion
- Neutral pH to avoid digesting nano-CuO
- Filtration
- Analyze with spICP-MS

Lettuce

Collard Green

Kale
Yes! Leaf tissues retain ENMs
Conclusions

✓ spICP-MS offers a great approach for quantitative analysis of nanoparticles

✓ Provides concentration, size distribution, composition, dissolved ion concentration

✓ Can be applied to water and some biological tissues

✓ NPs were found in all rinse water samples, as individual nanoparticles as well as aggregates

✓ The concentration of nano-CuO in rinse water was highly related to leaf surface characteristics

✓ Substantial fraction of the nano-CuO can remain on the leaf surface or perhaps even enter via the stomata.

✓ After three cycles of rinse, the residual Cu concentration were below any toxicity concern for humans
Acknowledgments