Fast multi-element quantification of nanoparticles in water supplies and wastewater treatment using single particle ICP-MS

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This material is in part supported by the National Science Foundation and Environmental Protection Agency under Cooperative Agreement # NSF-EF0830117. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the US Environmental Protection Agency.
Outline

• What are nanoparticles and where can we find them?
• Nanoparticle (NP) characterization
• NP analysis by ICP-MS:
  • Important concepts
  • Instrumentation
  • NP workflow
• Application:
  • Analysis in water samples
  • Analysis in biological tissues
What is a nanomaterial?

Transistors of 20-30 Years ago

1 mm 100 µm 10 µm 1 µm 100 nm 10 nm 1 nm 100 pm

Micro-scale

Nano-scale

Drug molecule

Quantum dot

Human cell

Bacterium cell

Virus

Protein

DNA

Individual atom

© 2009 Created by Sean Nash
Where do they come from?

Particles with at least one dimension in the nano-scale (1~100 nm); 1 nm = 10^{-9} m

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
Unique Properties of ENMs

- Huge surface area, with most atoms exposed to surrounding environment
- High surface energy
- Quantum effects
Why does it matter?

- Enhanced reactivity
- Enhanced interaction with photons
- Easily interact with biomolecules
- Can enter cell membranes
(all flows in metric tons/yr, 2010 estimates from Future Markets, Inc.)

Keller et al., JNR 2013
Keller and Lazareva, ES&TL, 2014
Personal Care Products

• Large application for many ENMs
• Direct human contact
• ENMs go into wastewater
• Small amount to landfill
Predicted Initial [ENM] in SF Bay

ENM concentrations expected to be at ng/L to ug/L levels at point of release

Lazareva and Keller, ACS Sus Chem, 2014
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 NP 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.

- **Single particle ICP-MS**
Methods for NP characterization

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

Transmission Electron Microscopy (TEM)

<table>
<thead>
<tr>
<th>Benefits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent size and shape characterization for individual particles</td>
<td>Time consuming preparation</td>
</tr>
<tr>
<td>Capability to detect very small particles</td>
<td>Subject to preparation artifacts</td>
</tr>
<tr>
<td></td>
<td>Limited elemental information (hard to be quantitative)</td>
</tr>
</tbody>
</table>
## Methods for NP characterization

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

### UV-Vis spectroscopy

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>C/C₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-400</td>
<td></td>
</tr>
</tbody>
</table>

- Mesocosm freshwater
- Stormwater
- Mesocosm effluent
- Treated Effluent
- Lagoon
- Groundwater
- Santa Clara River
- Seawater

Keller et al., ES&T 2010

### Dynamic Light Scattering

- Statistics Graph (18 measurements)

Adeyemi, Keller et al., ES&T 2015

### Benefits and Limitations

<table>
<thead>
<tr>
<th>Benefits</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low cost</td>
<td>Suspension stability</td>
</tr>
<tr>
<td>Capability to detect very small particles</td>
<td>Subject to preparation artifacts &amp; interferences</td>
</tr>
<tr>
<td>Several water matrices</td>
<td>No elemental information</td>
</tr>
<tr>
<td></td>
<td>&gt; 1-10 ppm in sample</td>
</tr>
</tbody>
</table>
Methods for NP 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 size & size distribution, and composition

![Signal from one nanoparticle event](Image)

Nanoparticle sample
Important points for single nanoparticle analysis

- Need fast acquisition (short dwell time) to capture each NP
- Just shortening the dwell time alone does not solve problem
- Need to integrate signal over multiple scans to:
  - Accurately quantify signal from single particle
  - Help identify overlapping peaks

Duration for one NP event (0.5-1 msec)
Microsecond Scanning with no Settling Time

- Short Dwell Time (0.1ms)

• Peak Integration Mode

  - “Peak Integration Mode” is automatically enabled for SNP
  - Data Analysis software employed when dwell time is <1 ms

![Single nanoparticle (SNP) event](image)

Duration for one NP event (0.5-1msec)
Mass Spectrometer used in our studies

- 7900 quadrupole ICP-MS – ultra-sensitive, ultra-fast 0.1 ms dwell
- Fully automated acquisition and data analysis configuration within MassHunter 4.3 software guided by Method Wizard
- Supports single particle mode
What is sNP analysis?

- **Sample Types for single nanoparticle analysis**

  - **Ionic Blank**
    - Blank sample

  - **Ionic Standard**
    - Dissolved metal solution

  - **Reference Material**
    - Metal suspension liquid.
    - Known particle size
    - Known conc.
    - Used to calibrate nebulizer efficiency ($\eta_n$).

  - **Unknown Sample**
    - Metal suspension liquid
    - To be analyzed
    - Unknown particle size and conc.
What is Nebulizer Efficiency?

➢ Calibration - Nebulizer Efficiency ($\eta_n$)

Reference Material

85 particles (Detected)

915 particles (Went to drain)

1000 particles (Introduced)

$\eta_n = \frac{85}{1000} = 0.085 \text{ or } 8.5\%$
Workflow for NP characterization

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

Workflow:
1. Convert ICP-MS TRA data
2. Tabulate data
3. Calculate size distribution
4. Report results
Reference Material

60 nm Gold Nanospheres

- Unagglomerated and monodisperse
- Mean diameter: 60 nm ± 4 nm
## Precision and Accuracy (NIST 8012 and 8013)

<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 ± 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 ± 2.1</td>
</tr>
</tbody>
</table>
Size distribution of Reference Materials

Graph A shows the size distribution of 60 nm Ag NPs RM, while Graph B shows the size distribution of 60 nm Au NPs RM.
Collecting NP samples in WWTP process

Samples were collected at:
- Raw water before primary clarifier
- Effluent of secondary clarifier
- Reclaimed water after UF
- Waste sludge from the activated sludge basin
- Anaerobic sludge from the sludge digester

Cervantes et al., Water Research 2019
Calibration curves of silver (Ag) nanoparticles in:
- 1 mM citrate buffer solution
- synthetic waste water (SWW)
- DI water

Cervantes et al., Water Research 2019
Ag NPs in WWTP process: Concentrations

- Found Ag NPs and Ag\(^+\) in all steps
- Concentrations within range predicted by Material Flow Analysis (Keller et al., 2014)
- Majority of mass as Ag\(^+\)
- Backwash water concentrates NPs that recirculate

Cervantes et al., Water Research 2019
Ag NPs in WWTP process: Size distributions

Cervantes et al., Water Research 2019
Ag NPs in WWTP process: Transformation

- Spiked 300 µg/L as NPs
- After 15 days, Ag NPs are still present in synthetic wastewater mostly as NPs
- More transformation to Ag\(^+\) as the NPs pass through the various processes

Cervantes et al., Water Research 2019
Ag NPs in WWTP process: Conclusions

- Can detect Ag NPs in WWTP process
- Concentrations as Ag NPs in low ng/L
- Around 96% of influent load removed through WWTP (including UF step)
- Most NPs are below 100 nm within the process
- Considerable dissolution of Ag NPs, releasing Ag^+
- sp-ICP-MS is effective for monitoring NP concentrations in WWTP effluent

Cervantes et al., Water Research 2019
Advanced operation – Multi-Element Screening

➢ Sample prep: In case number of elements is 2+

1. Ionic Blank
2. Ionic Standard
3. Reference Material
4. Unknown Sample
5. Ionic Standard for Reference Material

Contains both of A and B
Such as: 1ppb A + 1ppb B

- Element A
- Element B
- Mixture of A and B
Multi-element sp-ICP-MS analysis

• Very similar operating parameters as single element
• Agilent software able to acquire information for up to 16 elements
• Considered both aqueous phase and sludge
• Sludge was centrifuged at 5,000 RPM for 20 min
• Recovery of spiked Au NPs from sludge was
  ▪ 64% spiked pre-centrifugation
  ▪ 107% spiked post-centrifugation
Advanced operation – Multi-Element Screening

➢ Operation

Run Method wizard and Select “Rapid - Multi Element Screening”.

![MassHunter Method Wizard](image)
Multi-element sp-ICP-MS analysis

Water Samples

Nanoparticle concentration (ng/L)

- Mg
- Fe
- Ti
- Mn
- Ce
- Zn
- Cu
- Ni
- Al
- Ag
- Au
- Co
- Cd

- Raw wastewater
- Secondary effluent
- UF effluent

August 11, 2019
Removal of NPs in WWTP

Nanoparticle Removal Efficiency

Effluent  UF

Cd  Co  Au  Ag  Al  Ni  Cu  Zn  Ce  Mn  Ti  Fe  Mg
NP size in Water Streams

![Graph showing size distribution of nanoparticles in influent and effluent](image)

- **Influent**
  - Size distribution for nanoparticles such as Ag, Au, Co, Cd, Al, Ce, Ni, Zn, Cu, Mn, Ti, Fe, and Mg.
  - The size distribution is shown in nm (nanometers).

- **Effluent**
  - Size distribution for the same nanoparticles as the influent.
  - The size distribution is shown in nm (nanometers).
Multi-element sp-ICP-MS analysis

![Bar chart showing nanoparticle concentration (ng/kg) for various elements in Sludge Samples. Elements include Mg, Fe, Ti, Mn, Ce, Zn, Cu, Ni, Al, Ag, Au, Co, and Cd.](image-url)
NP size in Sludge

Secondary sludge

Anaerobic sludge
Size distribution of detected Ag NPs in (A) Raw; (B) Effluent; (C) waste sludge; (D) anaerobic sludge.

Pre-centrifugation spiked Ag NPs reference materials (60 nm) in (E) Raw; (F) Effluent; (G) waste sludge; (H) anaerobic sludge.
Size Distribution TiO$_2$ NPs

TiO$_2$ NPs in (A) Raw; (B) Effluent; (C) waste sludge and (D) anaerobic sludge samples
Conclusions

• splICP-MS offers the best approach for quantitative analysis of nanoparticles
• Provides concentration, size distribution, composition, dissolved ion concentration
• Can be applied to water and wastewater samples
• Most likely approach for commercial analysis
• Challenges remain with regards to samples that contain high levels of natural NPs
Acknowledgements

Yuxiong Huang, now Prof. at TBSI @ Shenzhen
Pabel Cervantes, postdoc at UCSB
Jenny Nelson, Agilent Technologies
Craig Marvin, Agilent Technologies
Tarun Anumol, Agilent Technologies