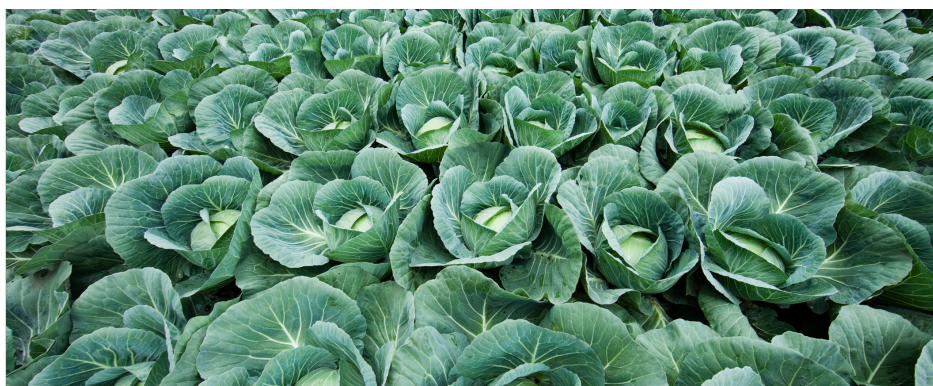**Agilent**

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Quantifying Copper Nanoparticles on Plant Leaves using Single-Particle ICP-MS

Investigating the adsorption and release of nano-pesticides on plants using the Agilent 7900 ICP-MS

Authors

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Introduction

Nano-forms of agrochemicals are potentially more efficient, reducing the quantities of chemicals needed. This development has led to increasing interest in—and use of—nano-fertilizers and nano-pesticides. Research over the past decade has improved our understanding of the characteristics and efficacy of nano-agrochemicals. But more information is needed about both their effects on crop plants and the potential for residues to remain on food products. Also, in common with other engineered nano-particles (ENPs), reliable analytical methods are needed to support research into the long-term impact of nano-agrochemicals on human health and the environment.

ICP-MS can be used to measure the NP content of environmental and food samples using a technique called single particle ICP-MS (spICP-MS) (1). spICP-MS is well-suited to nanoparticle characterization as it provides determination of the particle number and size distribution, along with the concentrations of both the particles and the dissolved element component.

Because of the high sensitivity and specificity of ICP-MS, it can provide additional—and often complementary–information compared to techniques such as dynamic light scattering (DLS) or multi-angle light scattering (MALS). ICP-MS is also fast and requires little sample preparation compared to techniques such as scanning or transmission electron microscopy (SEM, TEM), atomic force microscopy (AFM), or separation techniques such as differential centrifugal sedimentation (DCS) (2).

Copper is an essential trace element in all living organisms, but is toxic in excess (3). Cu-containing pesticides—such as copper sulfate, copper oxychloride, and copper carbonate—have been used for many years, mainly to protect against fungal infections and algae growth. Indeed, the origin of commercial fungicides can be traced to the accidental discovery of the anti-mildew properties of "Bordeaux mixture", made from copper sulfate and hydrated lime. The bright blue, bitter-tasting chemical was sprayed onto vines in the Bordeaux region of France during the 19th century to deter locals from eating the grapes. By chance, it was noticed that treated plants did not suffer from the downy mildew that was affecting vines in the area. This observation led to the discovery of the fungicidal properties of Cu^{2+}.

More recently, several nano-formulations of Cu-based pesticides have become commercially available. Although early studies indicate no obvious concerns relating to Cu-based nano-pesticides, research in this field would benefit from a routine means of characterizing and quantifying the Cu-containing nano-particles. In this study, the leaves of several edible crop plants were exposed to nano-CuO, simulating the use of a spray-applied, Cu-based nano-pesticide. After exposure to nano-CuO, the plant leaves were rinsed with de-ionized (DI) water and the Cu-containing nano-particles in the rinse water were determined using the Agilent 7900 ICP-MS in spICP-MS mode.

To give some perspective on the levels of dietary copper intake, various studies considered by the European Food Safety Authority (EFSA) indicated that total dietary intake of between ~0.7 mg/day and 1.5 mg/day was needed to maintain a healthy copper balance (4). European Union (EU) Directive 2002/46/EC on vitamins and minerals in food supplements recommends an upper limit of 4 mg/daily dose. The World Health Organization recommends a guideline value of 2 mg/L for copper in drinking water, a minimal acceptable total dietary intake of ~1.3 mg/day (from all sources) and a tolerable upper intake level of 10 mg/day (5). In the US, the Institute of Medicine (IOM) set a Recommended (minimum) Dietary Allowance (RDA) of 0.9 mg/day, and a tolerable upper intake level (UL) of 10 mg/day. The U.S. Environmental Protection Agency's Maximum Contaminant Level (MCL) for Cu in drinking water is 1.3 mg/L (ppm) (6).

Experimental

Standards

Uncoated nano-CuO with a primary particle size of 20–100 nm was bought from U.S. Research Nano-materials (Houston, TX, USA). A previous study (7) determined the hydrodynamic diameter (HDD) to be 280 ± 15 nm in DI water at pH 7 (0.5 mM phosphate buffer).

Gold NPs with a nominal diameter of 60 ± 4 nm, bought from nanoComposix (San Diego, CA), were used to determine the ICP-MS nebulization efficiency. The nebulization efficiency is the ratio of the number of particles detected divided by the number of particles known to be present in the reference solution. The nebulization efficiency value is required to allow calculation of both the particle number concentration (number of particles per mL), and to convert the measured particle signals to NP mass and therefore size (8). The 60 nm gold NP standard (99% purity) was diluted to 100 ng/L Au with DI water in a metal-free polypropylene tube.

The Cu elemental response factor—another parameter required in the equations used to convert the raw NP signal to particle size—was determined using a Cu ionic standard (1 µg/L, ppb) prepared in 1% HNO_3.

Sample preparation

Samples of the leafy vegetables kale (*Brassica oleracea*, var. Acephala Lacinato), lettuce (*Lactuca sativa* var. green leaf cultivar), and collard green (*Brassica oleracea*, var. Acephala) produced by Cal-Organic Farms (Bakersfield, CA, USA) were bought fresh on the day of analysis. Triplicate samples of leaves from each vegetable were cut into 75 × 75 mm squares. Nano-CuO, suspended in DI water and sonicated for 30 min, was then applied to the leaf surface. Ten drops (20 µL each) of nano-CuO suspension (1 mg/L particle concentration) were evenly spread onto each leaf square with an analytical pipette, ensuring that all the samples were exposed to equal amounts of nano-CuO. After air-drying for 2 h, the leaves were rinsed three times with 10 mL DI water. The three rinse solutions were collected separately in metal-free polypropylene tubes, ready for analysis. Control (unexposed) leaf samples were also prepared using the same procedure, but without exposure to the nano-CuO solution. The morphology and particle size of nano-CuO before and after application were studied via electron microscopy (FEI Tecnai G2 Sphera 200 kV TEM). For details, see the full publication cited in the More Information section.

Instrumentation

An Agilent 7900 ICP-MS was used in spICP-MS mode to determine the particle size and concentration of nano-CuO in the rinse solution samples. The instrument was equipped with a standard glass concentric nebulizer and quartz spray chamber, a quartz torch with small internal diameter injector (1.0 mm), and standard nickel cones. Samples were introduced directly into the ICP-MS with the standard peristaltic pump and tubing (i.d. 1.02 mm). The 7900 ICP-MS has a fast Time Resolved Analysis (fast TRA) mode that allows single element acquisition at a sampling rate of 100 µs (10,000 measurements per second), with no settling time between measurements.

The Single Nanoparticle Application Module of the ICP-MS MassHunter software was used for method setup, data collection, and data analysis. The Method Wizard guides the user through the entire process and automatically provides or calculates the required method parameters. The instrumental settings of the 7900 ICP-MS are given in Table 1.

Table 1. 7900 ICP-MS operating conditions.

Parameter	Value
RF power (W)	1550
Carrier gas flow rate (L/min)	0.67
Make-up gas flow rate (L/min)	0
Spray chamber temperature (°C)	2
Nebulizer pump (rps)	0.1
Sample depth (mm)	8.0
Oxide ratio (%)	1
Integration time (µs)	100
Acquisition time (s)	90
Mass monitored	^{63}Cu
ORS^4 cell mode	No gas

Before analysis, the rinse water samples were diluted with DI water to give a particle concentration between 10 and 100 ng/L. A sample inlet flow of 0.346 mL/min was used. The particle compound mass fraction (mass of the particle compound/mass of the analyte) was determined experimentally and was set to 1.33, while the CuO particle density was set to 6 g/cm^3.

NP sample preparation and dilution were performed on the day of the analysis to avoid sample degradation and to minimize nano-CuO dissolution. Before initial dilution of the samples, and again before analysis, all solutions were placed in an ultrasonic bath for 10 min. Sonication ensures that the suspended particles are distributed homogeneously.

Results and Discussion

Control samples – leaves not exposed to CuO NPs

The control samples (rinse water obtained from leaves not exposed to nano-CuO) were analyzed by spICP-MS for Cu-containing NPs. It was assumed that any Cu-containing particles detected would have the same physicochemical properties (density and Cu content) as nano-CuO. Any NPs found on the control samples may have arisen from the growing environment or have been introduced along the normal produce distribution chain.

Primary (first) rinse water samples from the non-exposed leaves were analyzed by spICP-MS. Cu-containing NPs in the 20–50 nm size range were detected in the rinse water from all three leaf types, as shown in Figure 1. The concentrations of the NPs were, however, at very low levels, from 30 to 90 ng/L in the first rinse water samples (Figure 2). The water collected from the two additional rinse cycles was also analyzed and the results are also shown in Figure 2.
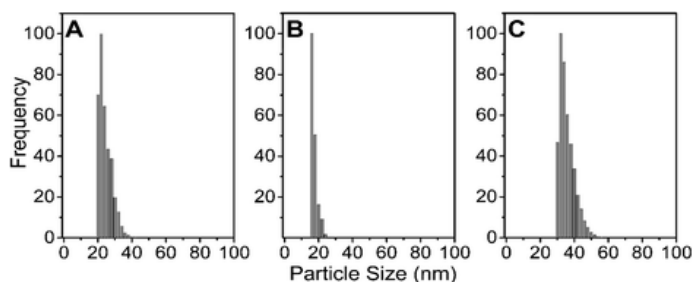

Figure 1. Particle size distribution of Cu-based nano-particles from the initial rinse of unexposed (no CuO NP addition) samples of **A** green leaf lettuce, **B** collard green, and **C** kale. Data was obtained using spICP-MS.*
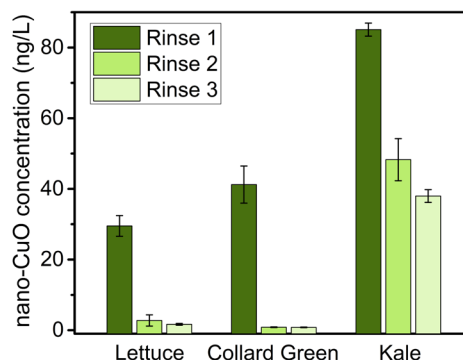

Figure 2. Cu-based NP concentration in rinse waters from unexposed (no CuO NP addition) samples of three types of leaf tissues. Error bars show standard deviation calculated from the triplicate analysis. Data was obtained using spICP-MS*.

Most Cu-containing NPs were removed from lettuce and collard green by the first rinse (only 5% remained in the third rinse, compared to the level found in the first rinse). Kale produced much higher total particle concentrations (sum of Cu NP concentration measured in all three rinse steps), at around 4 or 5 times the total nano-Cu levels found on the other two plants. Kale also appeared to retain more of the NPs after the initial rinse step, likely due to the roughness of its leaves. The concentration of NPs in the third rinse water sample from the non-exposed kale leaves was equivalent to 43% of the concentration of the first rinse water sample.

Nano-CuO exposed leaf tissue rinse water samples

The rinse water samples collected from leaf samples of each plant exposed to nano-CuO were analyzed using the same spICP-MS method. The median and mean NP size data is given in Table 2. In the first rinse water samples, most of the detected nano-CuO particles were between 30 and 78 nm diameter. The removal of most of the small particles in the first rinse meant that the median and mean particle sizes detected were larger in the second rinse cycle for lettuce and in the third rinse cycle for all three leaf types. The results suggest that small particles are more easily removed from the surface of all three leaf crops (particularly the smooth surface of lettuce), while larger NPs may remain after the first (and second) rinse, perhaps indicating that they get trapped in surface crevices or stomata.

The concentrations of nano-CuO in the first rinse water samples from the exposed leaves ranged from 500 to 750 µg/L, as shown in Figure 3 (note the log scale).
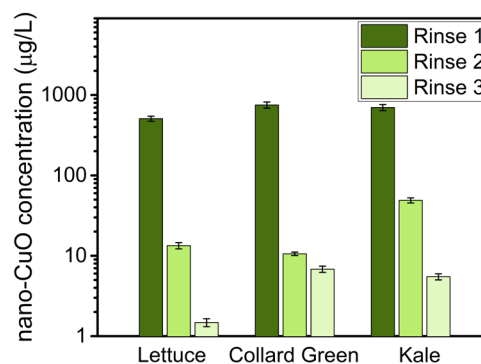

Figure 3. CuO particle concentration (log scale) in rinse water from leaf tissues exposed to nano-CuO. Error bars show standard deviation calculated from the triplicate analysis. Data was obtained using spICP-MS*.

The highest concentration was detected in collard green leaf rinse water (752.5 µg/L), followed closely by kale, and lettuce leaves (508.6 µg/L). The results show that the concentration of Cu-based NPs in the first rinse water is affected by leaf surface characteristics.

Table 2. Median and mean size of nano-CuO in rinse water from exposed leaf tissues. Data was obtained using spICP-MS*.

Parameter	Rinse cycle	Median size (nm)			Mean size (nm)		
		Average	Std. Dev.	RSD (%)	Average	Std. Dev.	RSD (%)
Lettuce	1	34.80	1.88	5.4	45.61	5.21	11.4
Lettuce	2	233.68	10.51	4.5	229.29	14.46	6.3
Lettuce	3	236.17	12.79	5.4	221.87	17.88	8.1
Collard green	1	30.36	2.53	8.3	78.32	6.09	7.8
Collard green	2	22.92	1.68	7.3	23.76	1.96	8.2
Collard green	3	135.48	13.01	9.6	153.04	11.77	7.7
Kale	1	35.14	2.63	7.5	76.00	3.40	4.5
Kale	2	32.63	0.74	2.3	36.37	3.16	8.7
Kale	3	135.48	13.01	9.6	163.91	11.98	7.3

Kale leaf has the highest surface hydrophobicity and surface roughness, which likely channels the applied NP suspension and rinse water through a limited region of the leaf. In contrast, the smoother and more hydrophilic leaves of lettuce plants are likely to allow nano-pesticides to spread more widely over the surface.

Compared to the nano-CuO concentrations detected in the first rinse water samples, the levels found in rinse water samples 2 and 3 were significantly lower for all three leaf types, especially for lettuce leaves. As found for the unexposed leaves, kale appeared to retain more of the nano-Cu beyond the first rinse step, as compared to the other leaf types. In all cases, the residual Cu concentrations were below the 1.3 ppm MCL stated by the EPA for Cu in drinking water.

Conclusions

Nano-particles present in food and environmental samples can be determined routinely using an Agilent 7900 ICP-MS in spICP-MS mode. Method development is simplified using the ICP-MS MassHunter single particle application software module. The ICP-MS MassHunter Method Wizard automatically generates all acquisition and data analysis parameters, and even recommends a sample list.

Using nano-CuO to simulate a Cu-based nano-pesticide, the spICP-MS method was used to characterize the NPs in rinse water from three varieties of exposed crop plants. spICP-MS provides a combination of particle size distribution, particle concentration, and dissolved element concentration in a single rapid analysis. This information is generally not available with other techniques. NPs were detected in the rinse water samples in the ppb range. This range is below the U.S. EPA's MCL for Cu in drinking water of 1.3 mg/L (ppm). The study showed that washing the leaves of food crops is a simple and efficient way to remove NPs.

spICP-MS can be used to research the interactions between ENPs, such as nano-agrochemicals, and plants. It would also be a useful technique in the monitoring of nano-agrochemicals in runoff waters, plant tissues, and wash water. Greater understanding of these issues could influence nano-pesticide development and reduce the risk of human and environmental exposure to ENPs.

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More Information

A.A. Keller, Y. Huang, and J. Nelson, Detection of nanoparticles in edible plant tissues exposed to nano-copper using single-particle ICP-MS, *J Nanopart Res*, **2018**, 20: 101. https://doi.org/10.1007/s11051-018-4192-8

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