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Article

Advancing Simultaneous Extraction and Sequential Single-Particle ICP-MS Analysis for Metallic Nanoparticle Mixtures in Plant Tissues

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ABSTRACT: Engineered nanoparticles (ENPs) have been increasingly used in agricultural operations, leading to an urgent need for robust methods to analyze co-occurring ENPs in plant tissues. In response, this study advanced the simultaneous extraction of coexisting silver, cerium oxide, and copper oxide ENPs in lettuce shoots and roots using macerozyme R-10 and analyzed them by single-particle inductively coupled plasma-mass spectrometry (ICP-MS). Additionally, the standard stock suspensions of the ENPs were stabilized with citrate, and the long-term stability (up to 5 months) was examined for the first time. The method performance results displayed satisfactory accuracies and precisions and achieved low particle concentration and particle size detection limits. Significantly, the oven drying process was proved not to impact the properties of the ENPs; therefore, oven-dried lettuce tissues were used in this study, which markedly expanded the applicability of this method. This robust methodology provides a timely approach to characterize and quantify multiple coexisting ENPs in plants.

KEYWORDS: *engineered nanoparticles (ENPs), citrate, simultaneous extraction of ENPs, single-particle ICP-MS, macerozyme R-10 enzyme, dried lettuce shoot and root*

1. INTRODUCTION

Nanotechnology is hailed as one of the most innovative advancements in modern science and technology and promises to revolutionize a variety of industries. In the agricultural field, nanotechnology is regarded as a sustainable approach by the United Nations Food and Agriculture Organization and the World Bank to increase crop yields and feed the growing world population.[1] Nanotechnology-enabled agrichemicals incorporating metallic engineered nanoparticles (ENPs) are particularly popular in agricultural uses. These agrichemicals are often applied to either function as pesticides and insecticides or enhance nutrient uptake. For example, silver nanoparticles (AgNPs) display strong antimicrobial properties and therefore help control pathogenic microorganisms in soil, leading to higher plant growth and yield.[2] Cerium oxide nanoparticles (CeO_2NPs) have shown potential in crop protection due to their intrinsic antioxidative capacity,[3] while copper oxide nanoparticles (CuONPs) were recently identified as an efficient fungicide for the control of *Fusarium* wilt and *Verticillium*, a major cause of yield or economic loss for crops such as almonds, tomatoes, and pistachios.[4,5] In spite of these benefits, applications of ENPs in agriculture provide a potential pathway for ENPs to enter into the human food chain by accumulating in crop tissues, which raises concerns about their possible adverse effects on animal and human health after crop consumption.[6] To maximize the benefits of nanofertilizers and nanopesticides to plant health and minimize their adverse effects, it is crucial to understand the translocation and transformation of ENPs in plant tissues, which remains unclear. Therefore, accurate monitoring of ENPs in plant tissues is critical to dissipate their safety concerns and advance the application of nanotechnology in agriculture. To date, a diverse array of methodologies has been employed to examine the accumulation of individual ENP within plant systems.[3,7,8] However, in real-world scenarios, multiple ENPs coexisting in plant tissues have been little studied. Within this complex ENP−plant matrix, the potential for mutual interference remains ambiguous. Consequently, it is imperative to develop and validate an analytical approach capable of extracting multiple intact ENPs simultaneously from plant matrices to facilitate a precise analytical investigation.

A critical challenge for ENP analysis in plant tissues is the availability of quantitative analysis techniques without changing ENP properties during sample preparation and analysis. Conventional technologies for analyzing ENPs include electron microscopy, chromatography, field flow fractionation, laser scattering, ultraviolet−visible (UV−vis) spectroscopy, etc. Difficulties generally arise for these technologies at low concentrations and in complex matrices due to poor specificity and/or sensitivity. Inductively coupled plasma-mass spectrometry (ICP-MS) is a highly sensitive and selective metal and semimetal element detection technology and has played vital roles in many research and industrial fields. ICP-MS has recently become more popular in ENP research and manufacturing, such as detecting ENP composition, impurity, and toxicity.[9−11] ICP-MS is also a very versatile technique that can be coupled with a series of other

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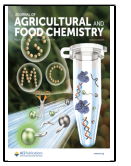

techniques, such as field flow fractionation, to meet specific analytical needs. An exciting recent development in the ICP-MS technology is its capability to operate in single-particle ICP-MS (spICP-MS) mode to directly analyze ENP size distribution and particle concentration without preacid digestion,[12,13] and it has already been used for ENP analysis in many different matrices, such as sunscreens,[14] soil,[15,16] surface water,[17] and plant tissues.[18−23]

The extraction of ENPs from plant tissues is an important step for the ENP analysis to ensure the intact ENP properties after extraction. Our previous research revealed that macerozyme R-10, a mixture of enzymes, was efficient for the extraction of CeO_2NPs from the fresh shoots of tomato, cucumber, soybean, and pumpkin.[8] A combination of enzymatic extraction and spICP-MS analysis has resulted in a precise analysis of ENP size and particle concentration in fresh plant shoots. However, this method has not been verified for the simultaneous analysis of multiple coexisting ENPs in plant tissues. In addition, the use of fresh tissues severely limited the applicability of this method to a narrow time window after the plant harvest. In many situations, analyzing ENPs in fresh plant tissue is not practical due to time and resource constraints when large numbers of fresh tissue samples must be processed within a short period of time. Moreover, when the mass of a sample is limited, analysis of multiple ENPs in a single sample through multiple extractions is not only time-consuming but also impractical. Therefore, it is critical to evaluate the feasibility of extracting coexisting ENPs simultaneously from dried plant tissues. Though simple drying can significantly expand the time window for processing plant samples, oven drying at relatively high temperatures may lead to ENP aggregation and unexpected physical and chemical reactions, and therefore, these potential effects must be evaluated by comparing the ENP extraction efficiency from both dried and fresh tissues.

A stable stock suspension of the ENP standard is a pivotal first step for the characterization and quantification of ENPs by spICP-MS. Currently, the spICP-MS methods for ENP analysis are increasingly used, but the preparation of a long-term stable ENP stock standard suspension remains a challenge due to the aggregation of ENPs, especially metal oxide ENPs such as CeO_2NPs and CuONPs. Without a stable ENP standard suspension, the analysis of particle size and size distribution as well as particle concentration by spICP-MS becomes unreliable. To overcome this bottleneck, different approaches have been explored. For example, Yu et al.[24] prepared zinc oxide (ZnO) nanofluids by dispersing ZnONPs in ethylene glycol and then sonicated for 3 h in the base fluid. Saeedinia et al.[25] dispersed CuONPs in oil by using an ultrasonic processor and observed that the nanofluids were uniformly dispersed for 24 h and the complete sedimentation occurred after a week. Although these studies shed new light on the stabilization of ENPs, they are not feasible for long-term storage and are not robust enough for protocol standardization. For spICP-MS method development, preparing an ENP suspension with a simplified procedure and high colloidal stability is critical.

The objectives of this study were to (1) explore new approaches to prepare stable ENP stock suspensions to optimize the spICP-MS method for AgNP, CeO_2NP, and CuONP analyses, (2) validate the effectiveness of combined macerozyme R-10 extraction with downstream spICP-MS for accurate analysis of three coexisting metallic ENPs in plants, and (3) assess the feasibility of using dried plant tissues for the spICP-MS method. Lettuce was selected as a model plant in this study because of its high consumption worldwide.[26] AgNP, CeO_2NP, and CuONP were utilized as representative metallic ENPs because of their wide applications in agriculture.[27] Both dried root and shoot samples were tested, and the impact of the drying process on the ENP fate was also evaluated. A citrate solution was applied to stabilize AgNP, CeO_2NP, and CuONP stock suspensions and examined for up to 5 months for long-term stability.

2. MATERIALS AND METHODS

2.1. Materials. All chemicals were purchased commercially and used as received. Dissolved Ag, Ce, and Cu standard solutions (10 or 1000 mg/L in 2% HNO_3) were purchased from High-Purity Standards (Charleston, SC). Ag nanopowder (30−50 nm), CeO_2NP water dispersion (30−50 nm, 20% wt), and CuONP water dispersion (25−55 nm, 20% wt) were obtained from U.S. Research Nanomaterials (Houston, TX). Sodium citrate tribasic dihydrate and 2-(*N*-morpholino) ethanesulfonic acid (MES) were purchased from Sigma-Aldrich (St. Louis, MO). Trace metal grade nitric acid (HNO_3, 67−70%) was acquired from Fisher Scientific (Pittsburgh, PA). Macerozyme R-10 was purchased from bioWORLD (Dublin, OH). Citrate-stabilized AuNPs (50 nm, 9.89×10^6 particles/mL) from Nanocomposix (San Diego, CA) were utilized to determine spICP-MS transport efficiency (TE). Ultrapure water (18.2 MΩ·cm) was produced by a Millipore water purification system (Milli-Q IQ 7000, Millipore Sigma, St., Louis, MO). All plastic wares used were precleaned by soaking in 3% HNO_3 overnight or longer prior to use.

2.2. Preparation and Characterization of Nanoparticle Suspensions and Stability Test. Ag nanopowder, CeO_2, and CuO nanosuspensions were selected and stabilized with a citrate solution. Briefly, AgNP (100 μg/L), CeO_2NP (200 μg/L), and CuONP (200 μg/L) suspensions were prepared in citrate solution (2 mM, pH 7.2) individually and then sonicated for 30 min by an ultrasonic probe (VC50, Sonics & Materials, CT) in an ice-cold water bath. Transmission electron microscopy (TEM, JEM-1400, JEOL, MA) and spICP-MS (NexION 2000P, PerkinElmer, Waltham, MA) with a single quadrupole mass spectrometer were used to characterize ENPs. TEM analysis was conducted by dropping 10 μL of AgNP, CeO_2NP, or CuONP stock suspension on the copper grid with a carbon support film (Fisher Scientific, St. Louis, MO). Images were taken with a JEM-1400 TEM operated at 120 kV. For spICP-MS analysis, AgNP, CeO_2NP, and CuONP stock suspensions were sonicated for 30 min in an ice-cold water bath sonicator (70 W, 40 kHz, FS20, Fisher Scientific, St. Louis, MO) and analyzed by spICP-MS immediately after appropriate dilution with MES buffer (20 mM, pH 5). To evaluate the storage stability of the stock suspensions, the ENP sizes and particle concentrations of these three representative metallic ENP stock suspensions were analyzed on 3, 6, 19, and 150 days after preparation, and the results were compared with the spICP-MS data obtained from the freshly prepared ENP suspensions.

2.3. Nanoparticle Extraction from Dried Lettuce Tissues. Summer Crisp lettuce seeds were purchased from Johnny's Selected Seeds (Winslow, ME) and were grown in a natural field soil collected from agricultural land in Jefferson City, MO (Carver Farm of Lincoln University of Missouri). After 76 days of growth, lettuce plants were pulled out from the soil gently, washed thoroughly with deionized water, and then separated into shoots and roots. Two groups of samples were prepared: (1) pre-oven-dried fresh samples and (2) post-oven-dried samples. For pre-oven-dried fresh samples, shoots and roots were ground to homogenates using a mortar and pestle. To ensure freshness during the grinding process, liquid nitrogen was used for cryogenic grinding. For post-oven-dried samples, the shoots and roots were oven-dried first at 60 °C overnight and then ground to powders using a mortar and pestle. Afterward, a known amount of ENP suspension mixtures was spiked into each pre-oven-dried or post-oven-dried sample for enzymatic digestion. In detail, a 50 mg

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portion of the post-oven-dried shoot, 20 mg of the post-oven-dried root, 200 mg of the pre-oven-dried shoot (equivalent to approximately 50 mg of the dried shoot), or 67 mg of the pre-oven-dried root (equivalent to approximately 20 mg of the dried root) was weighted into individual 50 mL precleaned digestion tubes. The ENP suspension mixture was spiked into each sample at concentrations of 5, 0.4, and 1 μg/L of AgNP, CeO_2NP, and CuONP, respectively, in the final enzymatic digestion samples (equivalent to 1 μg of AgNP/g of dried shoot, 2.5 μg of AgNP/g of dried root, 0.08 μg of CeO_2NP/g of dried shoot, 0.2 μg of CeO_2NP/g of dried root, 0.2 μg of CuONP/g of dried shoot, and 0.5 μg of CuONP/g of dried root). The pre-oven-dried fresh samples were then oven-dried overnight at 60 °C. The rest of the experimental procedures were the same as in our previous study with fresh plant tissues.[8] Briefly, 9 mL of MES buffer (20 mM, pH 5) and 1 mL of macerozyme R-10 solution (30 mg/mL) were added to each tube. Reagent blank, reagent spike without lettuce tissue, and freshly prepared ENP standards were also processed in parallel for quality control. The samples were digested in a water bath shaker for 24 h at 37 °C and 200 rpm. The tubes were then left standing still at room temperature for 1 h to enable the separation of the tissue residue from the liquid phase. The supernatant was then collected and diluted with MES buffer as needed and sonicated for 15 min in an ice-cold water bath sonicator for the spICP-MS analysis.

2.4. Sample Analysis by spICP-MS. ENPs in the plant samples were sequentially analyzed by spICP-MS, and detailed operation and method parameters are summarized in Table 1. During the analysis, the AuNP (50 nm) standard was used to measure the TE, which was around 10% (the TE was measured daily for each experiment). Dissolved Ag, Ce, and Cu standards were utilized to establish calibration curves regularly using the matrix-match method, and then spICP-MS pulse signals were converted to ENP size according to the published method.[12] Syngistix software with the Nano Application module was used for data collection and processing.

Table 1. Optimized spICP-MS Operating Conditions and Method Parameters for AgNP, CeO_2NP, and CuONP Sequential Analyses

ICP-MS Operating Parameters			
nebulizer gas flow, L/min	0.99[a]		
auxiliary gas flow, L/min	1.2		
plasma gas flow, L/min	15		
ICP RF power	1600		
analog stage voltage	−1650		
pulse stage voltage	900		
cell entrance voltage	−9		
cell exit voltage	−9		
cell rod offset	−14		
sampler cone	nickel		
skimmer cone	nickel		
sample introduction system	cyclonic spray chamber[b]		
spICP-MS Method Parameters			
analyte[c]	Ag	Ce	Cu
mass, amu	107	140	63
dwell time, ms	0.05	0.05	0.05
scan time, s	100	100	100
density, g/cm^3	10.49	7.13	6.4
mass fraction, %	100	81.39	79.89
ionization efficiency, %	100	100	100
pump speed, rpm	−35	−35	−35

[a]Automatically optimized when each experiment was started. [b]High-sensitivity glass cyclonic spray chamber with matrix gas port for NexION 2000P. [c]These three analytes were analyzed sequentially by spICP-MS using a single quadruple ICP-MS instrument.

3. RESULTS AND DISCUSSION

3.1. Characterization and Stability of the AgNP, CeO_2NP, and CuONP Stock Suspensions. TEM images and size distribution histograms from spICP-MS of AgNP, CeO_2NP, and CuONP are shown in Figure 1. The average diameters of AgNP, CeO_2NP, and CuONP measured by TEM image were 35, 27, and 49 nm, respectively, similar to their most frequent sizes (AgNP, 42 nm; CeO_2NP, 29 nm; CuONP, 48 nm) detected by spICP-MS. To determine their stability, stock suspensions of three types of ENPs in citrate (2 mM) aqueous solution were stored in a refrigerator and tested at different times for up to 5 months. The stock suspensions were sonicated for 30 min using a water bath sonicator and then diluted with MES buffer as needed right before spICP-MS analysis at each evaluation time. MES buffer was used in sample analysis because it is a noncomplexing buffer for metal ion analysis and was also used in lettuce samples.[21,28−30] To match the matrix with lettuce sample analysis, herein, MES buffer was also used to dilute the standard ENP stock suspensions. Table 2 shows the size and particle concentration of three ENP stock suspensions on day 1 (freshly prepared), 3, 6, 19, and 150. It can be seen that the most frequent size and mean size of AgNPs are 44−47 and 74−82 nm, respectively, and the particle concentration is 28,452−33,066 particles/mL. For CeO_2NPs, the most frequent size and mean size are 28−33 and 48−53 nm, respectively, and the particle concentration is 52,751−59,206 particles/mL. For CuONPs, the most frequent size and mean size are 48−51 and 73−81 nm, respectively, and the particle concentration is 91,871−99,283 particles/mL. These results indicate that these ENPs are stable for at least 150 days as tested, and all relative standard deviations (RSDs) of the triplicated samples are ≤11.7%. In contrast, when AgNPs (100 μg/L), CeO_2NPs (200 μg/L), or CuONPs (200 μg/L) were suspended in ultrapure water, they aggregated/precipitated after 1 day, and spICP-MS was not able to quantify them (data not shown). The results demonstrated that citrate prevented AgNP, CeO_2NP, and CuONP aggregation effectively, and there were no significant changes in size or particle concentration in 5 months after the preparation of their suspensions.

3.2. Particle Size and Particle Concentration Detection Limits. The particle size detection limit can be calculated from the calibration curve of particles or dissolved metal element calibration, but the latter may be more suitable for particles with small sizes.[31,32] This study applied a dissolved calibration method to calculate the size detection limits of these three ENPs. Specifically, a threshold criterion of $\mu + 3\sigma$ (where μ and σ are the mean and standard deviation of the blank, respectively) was calculated first, and then the dissolved calibration curve (signal intensity versus dissolved concentration) was transferred to the mass per event calibration curve (signal intensity versus mass per event) using eq 1

$$W = [\eta_n q_{liq} t_{dt} C] \quad (1)$$

where W (μg/event) is the mass per event, η_n is the transport efficiency, q_{liq} (mL/ms) is the pump flow rate, t_{dt} (ms/event) is the instrument dwell time, and C (μg/mL) is the dissolved concentration.

The value obtained by $\mu + 3\sigma$ was inputted into this new calibration curve to determine the mass per event (particle mass). We assumed that AgNP, CeO_2NP, and CuONP had

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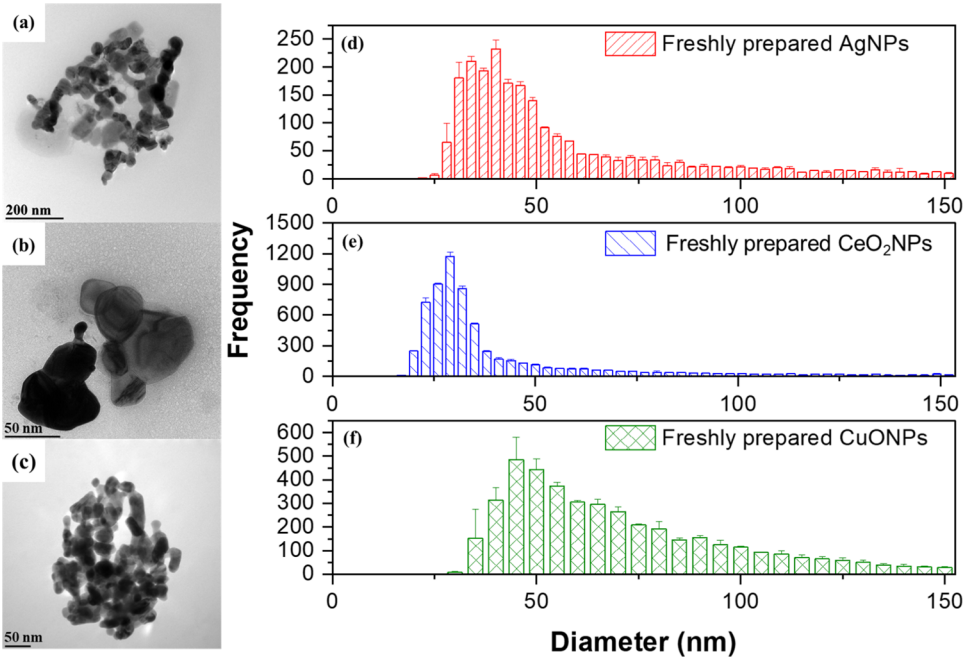

Figure 1. Characterization of AgNPs, CeO_2NPs, and CuONPs was performed by TEM and spICP-MS. (a–c) TEM images of AgNPs, CeO_2NPs, and CuONPs, respectively. (d–f) Size distribution histograms of AgNPs, CeO_2NPs, and CuONPs, respectively, detected by spICP-MS. The error bars in the histograms represent standard deviation (n = 3).

Table 2. Stability Test of AgNP, CeO_2NP, and CuONP Stock Suspensions[a]

	most frequent size (nm)			mean size (nm)			particle concentration (particles/mL)		
time	AgNP	CeO_2NP	CuONP	AgNP	CeO_2NP	CuONP	AgNP	CeO_2NP	CuONP
1st day[b]	44.3 (1.8%)	30.4 (5.6%)	50.1 (1.0%)	83.1 (1.3%)	55.3 (0.5%)	76.7 (2.6%)	29,859 (6.5%)	52,131 (4.8%)	99,283 (3.9%)
3rd day	47.4 (1.9%)	29.2 (2.4%)	47.8 (1.7%)	72.3 (2.6%)	56.1 (3.2%)	79.6 (1.6%)	30,784 (7.8%)	53,515 (5.5%)	96,983 (3.0%)
6th day	45.2 (2.7%)	31.2 (4.2%)	49.4 (1.8%)	74.9 (2.8%)	56.3 (2.1%)	73.4 (2.0%)	32,946 (7.0%)	58,541 (5.5%)	93,180 (2.7%)
19th day	44.9 (2.7%)	28.4 (7.4%)	51.1 (2.7%)	74.0 (2.4%)	52.8 (2.7%)	73.3 (0.8%)	29,322 (9.3%)	58,607 (4.8%)	93,625 (2.0%)
150th day	47.4 (3.4%)	32.5 (7.7%)	49.6 (3.4%)	84.2 (1.5%)	54.7 (1.5%)	81.2 (2.7%)	27,731 (11.7%)	56,387 (4.8%)	91,871 (4.0%)

[a]Reported values are the average of three replicates (%RSD). [b]1st day means the test was performed as soon as freshly prepared stock suspensions were prepared.

spherical geometry and then applied eq 2 to calculate the particle diameter.

$$d = \sqrt[3]{\left[\frac{6m}{\pi\rho}\right]} \quad (2)$$

Here, d is the particle diameter, m is the particle mass, and ρ is the particle density.[33,34]

The calculated size detection limits of AgNP, CeO_2NP, and CuONP were 19, 16, and 24 nm, respectively, smaller than previous studies, which is maybe due to the improved sensitivity and stability of the new model of ICP-MS instrument.[8,35]

To determine the particle concentration detection limits, a series of ENP stock suspensions with different mass concentrations were diluted in MES buffer (AgNP: 20, 50, 125, 250, and 500 ng/L; CeO_2NP: 2, 4, 10, 20, and 40 ng/L; CuONP: 5, 10, 25, 50, and 100 ng/L) and analyzed by spICP-MS. Table 3 depicts the most frequent size, mean size, particle concentration, and precision of particle concentration, and the graph shows the correlation between mass concentration and particle concentration for these three ENPs. For AgNPs ranging from 50 to 500 ng/L, both precision (0.7–8.5%) and linearity were satisfactory. Further decreasing concentration resulted in poor linearity and precision. Therefore, the particle concentration detection limit of AgNPs was around 2631 particles/mL (50 ng/L). The same approach was utilized for CeO_2NP and CuONP concentration detection limit determination, and they were around 4,586 particles/mL (4 ng/L) and 4110 particles/mL (25 ng/L), respectively.

3.3. AgNP, CeO_2NP, and CuONP Analyses in Dried Lettuce Shoots and Roots. Based on the previous studies, macerozyme R-10 was able to extract some ENPs from plant tissues without causing ENP dissolution or aggregation.[8,18,36,37] The current study validated this approach for simultaneous extraction and analysis of coexisting ENPs in both dried lettuce shoots and roots. To evaluate if oven drying has any impact on ENPs, we spiked a known amount of the ENP suspension mixture into lettuce samples pre- and post-oven-drying processes and then oven-dried the pre-oven-dried samples for simultaneous extraction of these coexisting ENPs. Reagent blank, spiked reagent, and freshly prepared ENP standards were also all processed in parallel (n = 3) for quality control. Table 4 displays the size, particle concentration, and spike recovery data in spiked pre- and post-oven-dried samples. The reagent spike results indicate that the macerozyme R-10 digestion has no impact on the sizes and particle concentrations of the spiked coexisting AgNP, CeO_2NP, and CuONP.

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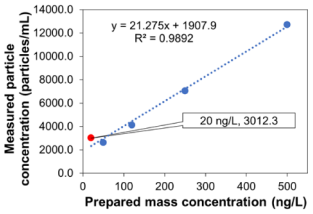
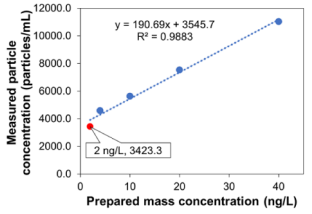
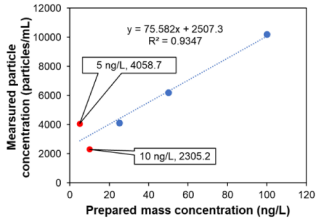
Table 3. Determined Particle Concentration Detection Limit of AgNP, CeO_2NP, and CuONP by spICP-MS[a]

	Mass concentration (ng/L)	Most frequent size (nm)	Mean size (nm)	Measured particle concentration (particles/mL)	Graph
AgNP	500	42.1 (1.4%)	79.7 (0.8%)	12,724 (3.3%)	
	250	45.2 (0.7%)	79.3 (3.2%)	7,042 (4.9%)	
	125	42.3 (2.8%)	67.0 (6.9%)	4,130 (7.6%)	
	50	38.6 (4.4%)	58.7 (9.4%)	2,631 (8.5%)	
	20	33.1 (3.3%)	39.7 (6.3%)	3,012 (12.8%)	
CeO_2NP	40	29.2 (2.7%)	66.3 (3.8%)	11,034 (9.8%)	
	20	27.9 (5.4%)	60.3 (2.5%)	7,542 (3.9%)	
	10	27.3 (5.1%)	54.7 (7.3%)	5,636 (7.6%)	
	4	26.8 (3.4%)	46.0 (10.9%)	4,586 (5.4%)	
	2	23.5 (8.1%)	42.0 (4.8%)	3,423 (15.3%)	
CuONP	100	53.3 (0.6%)	76.5 (0.5%)	10,212 (5.3%)	
	50	53.7 (0.4%)	79.1 (1.9%)	6,211 (5.3%)	
	25	51.4 (1.0%)	77.0 (5.6%)	4,110 (13.1%)	
	10	47.0 (4.0%)	68.2 (9.2%)	2,305 (24.8%)	
	5	38.5 (3.9%)	57.1 (8.9%)	4,059 (17.3%)	

[a]Reported values are the average of three replicates (%RSD).

Figure 2 displays the similarity of the detected ENP size distributions between the spiked reagent and spiked shoot and root samples. Compared with freshly prepared ENP suspensions, the size distributions in the spiked reagent and spiked samples are comparable. The reproducibility is also very good, with %RSDs ≤ 8.2% for all spiked samples. The RSDs of CeO_2NP in a few unspiked samples were slightly higher due to near-quantification detection limits. Particle concentration spike recoveries of AgNP, CeO_2NP, and CuONP were 84.4−107.3, 89.7−94.3, and 96.6−106.2%, respectively. These results demonstrate that the oven drying process has no impact on particle size and concentration, and this method is feasible for coexisting metallic ENPs analyses in both dried plant shoot and root samples. It is significant because it is a common practice to oven-dry fresh plant tissues to obtain the dry biomass of plants to extend storage time and simplify storage conditions such as at room temperature and limited space. Other advantages include the need for a low sample quantity and more efficient extraction when multi-ENPs can be extracted in a single extraction process. In addition, monitoring ENPs in both shoot and root makes it possible to understand the uptake, transportation, and interaction of ENPs in the plant−soil system. Figures S1−S3 show representative spICP-MS raw data of AgNP (^{107}Ag), CeO_2NP (^{140}Ce), and CuONP (^{63}Cu) in the reagent blank, reagent spike, control lettuce shoots and roots (no ENP treatment), spiked lettuce shoots and roots, and freshly prepared ENP suspensions. It should be noted that sporadic pulse signals of ^{107}Ag, ^{140}Ce, and ^{63}Cu were observed in lettuce shoots and roots without spiking ENPs. Ag, Ce, and Cu are elements commonly present in natural soils in different states, including different particulate species, such as silver sulfide (Ag_2S), cerium phosphate ($CePO_4$), and copper sulfide (Cu_2S) NPs,[7,38] which can be taken up by lettuce as well. Though they were treated as AgNP, CeO_2NP, and CuONP pulse signals, the specific species were not clear because spICP-MS cannot distinguish different species of particles. Nevertheless, the concentrations of the naturally occurring particles in the plant samples are insignificant, if present (see the raw data in Figures S1−S3). They do not show any significant impact on the results of spiked ENP analyses in the lettuce samples.

These data further demonstrate that the macerozyme R-10 digestion method can simultaneously extract AgNPs, CeO_2NPs, and CuONPs from both dried lettuce shoots and roots without causing aggregation or dissolution and has satisfactory precisions and accuracies. Overall, our study showed that enzymatic extraction of three coexisting ENPs from dried plant tissues followed by spICP-MS measurement is a robust technique for routine applications of plant sample analyses. With the increasing detections of multiple ENPs in the same agricultural system, a method that can effectively quantify and characterize ENP mixtures in a single plant sample will greatly contribute to understanding the fate and translocation of ENPs and to assessing food safety risks caused by the use of agricultural nanotechnology. This method can also benefit other fields interested in understanding the occurrence, fate, and transport of ENPs in the environment. It should be noted that this study used a single quadruple ICP-MS instrument. Though the coexisting ENPs were extracted simultaneously, they were detected sequentially due to the limitation of this instrument. If a time-of-flight mass spectrometer or a magnetic sector mass spectrometer is

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Table 4. AgNP, CeO_2NP, and CuONP Analyses Result in Dried Lettuce Samples[a]

sample	most frequent size (nm)		mean size (nm)		particle concentration (particles/mL)		particle concentration spike recovery	
	pre-oven-dried	post-oven-dried	pre-oven-dried	post-oven-dried	pre-oven-dried	post-oven-dried	pre-oven-dried	post-oven-dried
				AgNPs				
reagent blank	ND	ND	ND	ND	ND	ND		
spiked reagent	48.8 (5.3%)	50.5 (4.4%)	77.1 (0.6%)	72.3 (1.7%)	101,352 (1.9%)	113,632 (1.2%)	84.4%	97.3%
shoot sample	ND	ND	ND	ND	ND	ND		
spiked shoot sample	47.2 (4.7%)	49.1 (1.0%)	72.3 (1.8%)	66.3 (1.8%)	115,797 (1.1%)	102,497 (1.6%)	96.4%	87.8%
root sample	ND	ND	ND	ND	ND	ND		
spiked root sample	48.1 (3.1%)	49.6 (3.4%)	74.5 (2.6%)	65.7 (3.0%)	119,874 (1.6%)	125,316 (1.7%)	99.8%	107.3%
fresh AgNPs	44.7 (2.7%)	47.8 (1.7%)	78.2 (0.8%)	78.8 (0.4%)	120,124 (1.9%)	116,763 (2.8%)		
				CeO_2NPs				
reagent blank	ND	ND	ND	ND	ND	ND		
spiked reagent	28.9 (4.2%)	29.2 (3.1%)	55.3 (4.5%)	60.1 (2.5%)	113,534 (1.1%)	114,792 (1.1%)	90.7%	92.5%
shoot sample	25.1 (17.5%)	30.3 (7.9%)	40.1 (9.5%)	35.3 (6.5%)	5144 (4.4%)	5781 (3.5%)		
spiked shoot sample	31.2 (8.3%)	34.5 (4.9%)	56.8 (3.7%)	54.8 (5.5%)	123,215 (1.2%)	121,479 (2.1%)	94.3%	93.2%
root sample	29.6 (4.4%)	32.5 (6.8%)	42.2 (10.7%)	41.7 (6.0%)	9893 (8.2%)	10,112 (3.6%)		
spiked root sample	35.4 (2.5%)	35.2 (4.8%)	60.3 (2.7%)	56.3 (0.7%)	125,138 (1.8%)	121,528 (4.5%)	92.0%	89.7%
fresh CeO2NPs	31.0 (2.3%)	31.9 (1.6%)	59.6 (4.0%)	58.2 (2.9%)	125,216 (2.5%)	124,155 (3.7%)		
				CuONPs				
reagent blank	ND	ND	ND	ND	ND	ND		
spiked reagent	50.3 (1.4%)	53.4 (2.4%)	78.2 (4.2%)	70.1 (3.4%)	89,558 (2.4%)	94,639 (3.4%)	96.8%	101.1%
shoot sample	ND	ND	ND	ND	ND	ND		
spiked shoot sample	51.8 (2.5%)	53.3 (4.3%)	77.6 (1.9%)	75.3 (2.8%)	93,738 (3.9%)	99,325 (4.4%)	101.4%	106.2%
root sample	ND	ND	ND	ND	ND	ND		
spiked root sample	53.4 (2.1%)	55.3 (2.2%)	76.8 (0.7%)	74.3 (2.0%)	95,118 (4.3%)	90,384 (2.7%)	102.8%	96.6%
fresh CuONPs	51.3 (1.2%)	53.2 (0.9%)	76.1 (1.2%)	75.3 (0.8%)	92,487 (3.6%)	93,568 (2.3%)		

[a]Reported values are the averages (%RSD). ND means not quantitatively detected due to the size or particle concentration being lower than the detection limit.

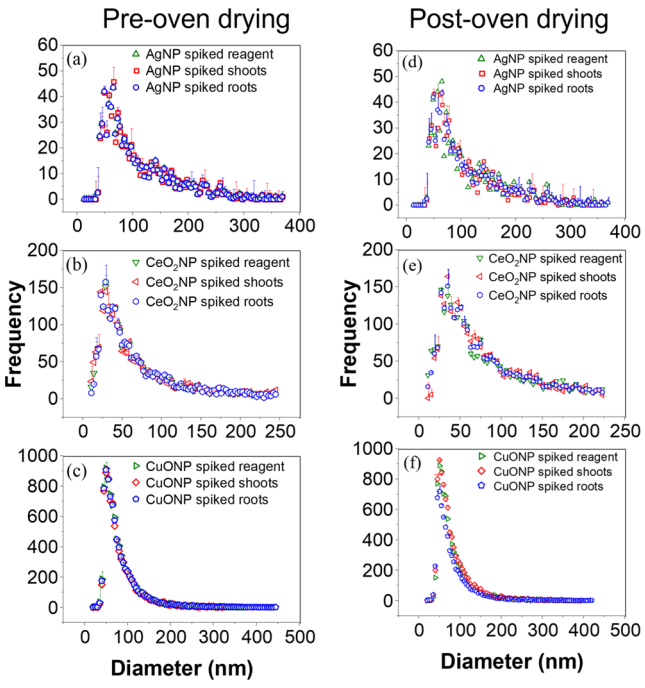

Figure 2. AgNP (a, d), CeO_2NP (b, e), and CuONP (c, f) size distribution histograms in spiked reagent and pre- and post-oven-dried spiked lettuce shoot and root samples. Error bars represent standard deviation (n = 3).

available, these ENPs are expected can be detected simultaneously to further save time and sample amount.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.3c09783.

spICP-MS raw data of AgNPs (Figure S1); spICP-MS raw data of CeO_2NPs (Figure S2), and spICP-MS raw data of CuONPs (Figure S3) (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ENP, engineered nanoparticle; ICP-MS, inductively coupled plasma-mass spectrometry; spICP-MS, single-particle inductively coupled plasma-mass spectrometry; AgNP, silver nanoparticle; CeO_2NP, cerium dioxide nanoparticle; CuONP, copper oxide nanoparticle; MES, 2-(*N*-morpholino)-ethanesulfonic acid; HNO_3, nitric acid; TE, transport efficiency; TEM, transmission electron microscopy; RSD, relative standard deviation

■ REFERENCES

(1) Asadishad, B.; Chahal, S.; Akbari, A.; Cianciarelli, V.; Azodi, M.; Ghoshal, S.; Tufenkji, N. Amendment of agricultural soil with metal nanoparticles: effects on soil enzyme activity and microbial community composition. *Environ. Sci. Technol.* **2018**, *52* (4), 1908−1918.

(2) Khot, L. R.; Sankaran, S.; Maja, J. M.; Ehsani, R.; Schuster, E. W. Applications of nanomaterials in agricultural production and crop protection: a review. *Crop Prot.* **2012**, *35*, 64−70.

(3) Dai, Y.; Chen, F.; Yue, L.; Li, T.; Jiang, Z.; Xu, Z.; Wang, Z.; Xing, B. Uptake, transport, and transformation of CeO2 nanoparticles by strawberry and their impact on the rhizosphere bacterial community. *ACS Sustainable Chem. Eng.* **2020**, *8* (12), 4792−4800.

(4) Ashraf, H.; Anjum, T.; Riaz, S.; Ahmad, I. S.; Irudayaraj, J.; Javed, S.; Qaiser, U.; Naseem, S. Inhibition mechanism of green-synthesized copper oxide nanoparticles from Cassia fistula towards Fusarium oxysporum by boosting growth and defense response in tomatoes. *Environ. Sci.: Nano* **2021**, *8* (6), 1729−1748.

(5) Elmer, W. H.; White, J. C. The use of metallic oxide nanoparticles to enhance growth of tomatoes and eggplants in disease infested soil or soilless medium. *Environ. Sci.: Nano* **2016**, *3* (5), 1072−1079.

(6) Zhang, P.; Wu, X.; Guo, Z.; Yang, X.; Hu, X.; Lynch, I. Stress response and nutrient homeostasis in lettuce (Lactuca sativa) exposed to graphene quantum dots are modulated by particle surface functionalization. *Adv. Biol.* **2021**, *5* (4), No. 2000778.

(7) Huang, D.; Dang, F.; Huang, Y.; Chen, N.; Zhou, D. Uptake, translocation, and transformation of silver nanoparticles in plants. *Environ. Sci.: Nano* **2022**, *9* (1), 12−39.

(8) Dan, Y.; Ma, X.; Zhang, W.; Liu, K.; Stephan, C.; Shi, H. Single particle ICP-MS method development for the determination of plant uptake and accumulation of CeO2 nanoparticles. *Anal. Bioanal. Chem.* **2016**, *408* (19), 5157−5167.

(9) Schwab, F.; Zhai, G.; Kern, M.; Turner, A.; Schnoor, J. L.; Wiesner, M. R. Barriers, pathways and processes for uptake, translocation and accumulation of nanomaterials in plants−Critical review. *Nanotoxicology* **2016**, *10* (3), 257−278.

(10) Schwabe, F.; Tanner, S.; Schulin, R.; Rotzetter, A.; Stark, W.; von Quadt, A.; Nowack, B. Dissolved cerium contributes to uptake of Ce in the presence of differently sized CeO2-nanoparticles by three crop plants. *Metallomics* **2015**, *7* (3), 466−477.

(11) Ulrich, A.; Losert, S.; Bendixen, N.; Al-Kattan, A.; Hagendorfer, H.; Nowack, B.; Adlhart, C.; Ebert, J.; Lattuada, M.; Hungerbühler, K. Critical aspects of sample handling for direct nanoparticle analysis and analytical challenges using asymmetric field flow fractionation in a multi-detector approach. *J. Anal. At. Spectrom.* **2012**, *27* (7), 1120−1130.

(12) Pace, H. E.; Rogers, N. J.; Jarolimek, C.; Coleman, V. A.; Higgins, C. P.; Ranville, J. F. Determining transport efficiency for the purpose of counting and sizing nanoparticles via single particle inductively coupled plasma mass spectrometry. *Anal. Chem.* **2011**, *83* (24), 9361−9369.

(13) Peters, R. J. B.; Rivera, Z. H.; van Bemmel, G.; Marvin, H. J. P.; Weigel, S.; Bouwmeester, H. Development and validation of single particle ICP-MS for sizing and quantitative determination of nano-silver in chicken meat. *Anal. Bioanal. Chem.* **2014**, *406* (16), 3875−3885.

(14) Dan, Y.; Shi, H.; Stephan, C.; Liang, X. Rapid analysis of titanium dioxide nanoparticles in sunscreens using single particle inductively coupled plasma−mass spectrometry. *Microchem. J.* **2015**, *122*, 119−126.

(15) Navratilova, J.; Praetorius, A.; Gondikas, A.; Fabienke, W.; Von der Kammer, F.; Hofmann, T. Detection of engineered copper nanoparticles in soil using single particle ICP-MS. *Int. J. Environ. Res. Public Health* **2015**, *12* (12), 15756−15768.

(16) Schwertfeger, D. M.; Velicogna, J. R.; Jesmer, A. H.; Saatcioglu, S.; McShane, H.; Scroggins, R. P.; Princz, J. I. Extracting metallic nanoparticles from soils for quantitative analysis: method development using engineered silver nanoparticles and SP-ICP-MS. *Anal. Chem.* **2017**, *89* (4), 2505−2513.

(17) Fréchette-Viens, L.; Hadioui, M.; Wilkinson, K. J. Quantification of ZnO nanoparticles and other Zn containing colloids in natural waters using a high sensitivity single particle ICP-MS. *Talanta* **2019**, *200*, 156−162.

(18) Dan, Y.; Zhang, W.; Xue, R.; Ma, X.; Stephan, C.; Shi, H. Characterization of gold nanoparticle uptake by tomato plants using enzymatic extraction followed by single-particle inductively coupled plasma−mass spectrometry analysis. *Environ. Sci. Technol.* **2015**, *49* (5), 3007−3014.

(19) Wojcieszek, J.; Jiménez-Lamana, J.; Bierła, K.; Ruzik, L.; Asztemborska, M.; Jarosz, M.; Szpunar, J. Uptake, translocation, size characterization and localization of cerium oxide nanoparticles in radish (Raphanus sativus L.). *Sci. Total Environ.* **2019**, *683*, 284−292.

(20) Dang, F.; Wang, Q.; Cai, W.; Zhou, D.; Xing, B. Uptake kinetics of silver nanoparticles by plant: relative importance of particles and dissolved ions. *Nanotoxicology* **2020**, *14* (5), 654−666.

(21) Xu, L.; Wang, X.; Shi, H.; Hua, B.; Burken, J. G.; Ma, X.; Yang, H.; Yang, J. J. Uptake of Engineered Metallic Nanoparticles in Soil by Lettuce in Single and Binary Nanoparticle Systems. *ACS Sustainable Chem. Eng.* **2022**, *10* (50), 16692−16700.

(22) Zhang, W.-Y.; Wang, Q.; Li, M.; Dang, F.; Zhou, D.-M. Nonselective uptake of silver and gold nanoparticles by wheat. *Nanotoxicology* **2019**, *13* (8), 1073−1086.

(23) Dang, F.; Chen, Y.-Z.; Huang, Y.-N.; Hintelmann, H.; Si, Y.-B.; Zhou, D.-M. Discerning the sources of silver nanoparticle in a terrestrial food chain by stable isotope tracer technique. *Environ. Sci. Technol.* **2019**, *53* (7), 3802−3810.

(24) Yu, W.; Xie, H.; Chen, L.; Li, Y. Investigation of thermal conductivity and viscosity of ethylene glycol based ZnO nanofluid. *Thermochim. Acta* **2009**, *491* (1−2), 92−96.

(25) Saeedinia, M.; Akhavan-Behabadi, M. A.; Nasr, M. Experimental study on heat transfer and pressure drop of nanofluid

flow in a horizontal coiled wire inserted tube under constant heat flux. *Exp. Therm. Fluid Sci.* **2012**, *36*, 158−168.
(26) Shatilov, M.; Razin, A.; Ivanova, M. Analysis of the World Lettuce Market. *IOP Conf. Ser.: Earth Environ Sci* **2019**, No. 012053, DOI: 10.1088/1755-1315/395/1/012053.
(27) Adisa, I. O.; Pullagurala, V. L. R.; Peralta-Videa, J. R.; Dimkpa, C. O.; Elmer, W. H.; Gardea-Torresdey, J. L.; White, J. C. Recent advances in nano-enabled fertilizers and pesticides: a critical review of mechanisms of action. *Environ. Sci.: Nano* **2019**, *6* (7), 2002−2030.
(28) Good, N. E.; Winget, G. D.; Winter, W.; Connolly, T. N.; Izawa, S.; Singh, R. M. Hydrogen ion buffers for biological research. *Biochemistry* **1966**, *5* (2), 467−477.
(29) Kandegedara, A.; Rorabacher, D. B. Noncomplexing tertiary amines as "better" buffers covering the range of pH 3− 11. Temperature dependence of their acid dissociation constants. *Anal. Chem.* **1999**, *71* (15), 3140−3144.
(30) Ferreira, C. M. H.; Pinto, I. S. S.; Soares, E. V.; Soares, H. M. V. M. (Un) suitability of the use of pH buffers in biological, biochemical and environmental studies and their interaction with metal ions−a review. *RSC Adv.* **2015**, *5* (39), 30989−31003.
(31) Witzler, M.; Küllmer, F.; Hirtz, A.; Günther, K. Validation of gold and silver nanoparticle analysis in fruit juices by single-particle ICP-MS without sample pretreatment. *J. Agric. Food Chem.* **2016**, *64* (20), 4165−4170.
(32) Witzler, M.; Kuellmer, F.; Guenther, K. Validating a single-particle ICP-MS method to measure nanoparticles in human whole blood for nanotoxicology. *Anal. Lett.* **2018**, *51* (4), 587−599.
(33) Pace, H. E.; Rogers, N. J.; Jarolimek, C.; Coleman, V. A.; Gray, E. P.; Higgins, C. P.; Ranville, J. F. Single particle inductively coupled plasma-mass spectrometry: a performance evaluation and method comparison in the determination of nanoparticle size. *Environ. Sci. Technol.* **2012**, *46* (22), 12272−12280.
(34) Lee, S.; Bi, X.; Reed, R. B.; Ranville, J. F.; Herckes, P.; Westerhoff, P. Nanoparticle size detection limits by single particle ICP-MS for 40 elements. *Environ. Sci. Technol.* **2014**, *48* (17), 10291−10300.
(35) Weigel, S.; Peters, R.; Loeschner, K.; Grombe, R.; Linsinger, T. P. Results of an interlaboratory method performance study for the size determination and quantification of silver nanoparticles in chicken meat by single-particle inductively coupled plasma mass spectrometry (sp-ICP-MS). *Anal. Bioanal. Chem.* **2017**, *409*, 4839−4848.
(36) Wei, W.-J.; Li, L.; Gao, Y.-P.; Wang, Q.; Zhou, Y.-Y.; Liu, X.; Yang, Y. Enzyme digestion combined with SP-ICP-MS analysis to characterize the bioaccumulation of gold nanoparticles by mustard and lettuce plants. *Sci. Total Environ.* **2021**, *777*, No. 146038.
(37) Keller, A. A.; Huang, Y.; Nelson, J. Detection of nanoparticles in edible plant tissues exposed to nano-copper using single-particle ICP-MS. *J. Nanopart. Res.* **2018**, *20*, No. 101, DOI: 10.1007/s11051-018-4192-8.
(38) Szakas, S. E.; Lancaster, R.; Kaegi, R.; Gundlach-Graham, A. Quantification and classification of engineered, incidental, and natural cerium-containing particles by spICP-TOFMS. *Environ. Sci.: Nano* **2022**, *9* (5), 1627−1638.

https://doi.org/10.1021/acs.jafc.3c09783
J. Agric. Food Chem. 2024, 72, 11251−11258