ABSTRACT: Zinkicide is a systemic bactericidal formulation containing protein-size fluorescent zinc oxide-based nanoparticles (nano-ZnO). Previous studies have shown that Zinkicide is effective in controlling citrus diseases. Its field performance as an antimicrobial agent has been linked to the bioavailability of zinc ions (Zn²⁺) at the target site. It is therefore important to monitor Zn²⁺ release from Zinkicide so that application rates and frequency can be estimated. In this study, we present a simplistic approach designed to monitor Zinkicide nanoparticle dissolution rates in water and acidic buffer solutions using traditional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The evolution of nano-ZnO in the polyacrylamide gel scaffolds was studied by exciting the sample with UV light and detecting the fluorescence of nano-ZnO. Fluorescence intensities measured with this assay allowed for quantitative analysis of molecular weight changes of nano-ZnO in citrate buffer, a surrogate of citrus juice. Our results demonstrated that citrate buffer induced the greatest degradation of Zinkicide. Fluorescence intensity fluctuations were observed over time, indicating interactions of citrate with the surface of nano-ZnO. These findings provide a new approach to quantify the dissolution of nanoparticles in simulated environments, even when other analytical methods lack sensitivity because of the small size of the system (≈4 nm).

1. INTRODUCTION

Engineered nanomaterials have greatly improved a plethora of applications including the manufacturing of biomedical, industrial, and consumer products.¹⁻³ Specifically, nanoparticles (NPs) have gained traction in the agriculture sector because of their unique physicochemical and multifunctional potential,⁴ which provides new avenues to induce higher efficacies for crop protection and crop yields.⁴⁻⁶ Zinc oxide NPs (nano-ZnO) in particular are beneficial for a variety of agriculture practices such as pest management, fertilizer treatment, and drug delivery.⁵⁻⁸ Several groups have reported on the development of ZnO materials as an alternative management tactic for conventional copper (Cu)-based bactericidal spray applications.⁶⁻⁹ Among them, Zinkicide, a nano-ZnO-based bactericide, has demonstrated outstanding antimicrobial activity against citrus canker, citrus melanose, and citrus scab.¹⁰ Ultrasmall ZnO NPs (<10 nm) exhibit special traits for sustainable crop treatment because of their small packaging, providing the ability to penetrate the vascular system of the plant.¹⁰ These site-targeted treatments are important for antagonizing bacteria inside the crop which is where a variety of systemic diseases take homage.⁹⁻¹¹ Furthermore, ZnO NPs exhibit bespoke multifunctional characteristics such as antimicrobial, bactericidal, and micronutrient efficacies.¹²

A problematic crop disease is Huanglongbing, also known as citrus greening, which is a systemic citrus disease.⁸ This disease is bacterial-borne and has decimated the citrus industry worldwide. The current status of the citrus industry urges the development of treatments such as ZnO NPs to suppress pathogen progression.⁸ An agriculture-grade variation of Zinkicide has been developed in view of offering growers with an effective systemic bactericide engineered for the treatment of citrus greening. Zinkicide contains ultrasmall (4.0 nm average size) spherical ZnO NPs highly dispersible in solution. By design, nano-ZnO is expected to be fully degraded in planta and produce micronutrient Zn²⁺ ions via dissolution kinetics.³⁻⁵,¹²⁻¹⁷ However, evaluating the changes in particle size of nano-ZnO in aqueous solution and quantifying the change of NP characteristics over time are challenging given the small size of the NPs (D < 4.0 nm).

Because of the physical and chemical properties of commercial-grade Zinkicide, a variety of conventional characterization tools have failed to provide reliable size measurements. Dynamic light scattering (DLS)¹⁸⁻²¹ is the most commonly used technique for the characterization of NPs in aqueous solution. DLS is predominantly used for classifying NP size and size distributions. However, the ultrasmall size of this material falls under the limit of detection of DLS (<10 nm). Ultraviolet–visible (UV–vis) absorption spectroscopy²²,²³ is considered a valuable technique for NP–derived materials; however, in the case of Zinkicide, absorption
interferences from other chemicals present in the stock hinder the analysis of NP traits. Inductively coupled plasma mass spectrometry (ICP-MS) has been used for metal NP characterization quite extensively but was deemed unreliable for Zinkicide particle size characterization in the separate investigations. High-resolution transmission electron microscopy (HRTEM) is a reliable tool to measure electron-dense metals and metal oxide particles in the vacuum state. A HRTEM study has revealed the size of the Zinkicide particles. A synchrotron X-ray absorption/fluorescence-based technique is suitable for detecting metal NPs in plant tissue and has been successfully used to detect Zinkicide residues in planta. However, this technique has several limitations including detection reliability for the 4.0 nm sized ZnO particles. Because of increased dissolution rates of ultrasmall ZnO NPs in solution, the use of synchrotron X-ray absorption/fluorescence measurements is not suitable for NPs dispersed in solution. Lastly, small-angle neutron scattering (SANS) was found to be suitable to measure the particle size in aqueous suspension but requires extensive preparation and suffers from limited access to the sophisticated characterization machinery. However, this technique is time-consuming and determining the size of the NPs is arduous in materials prepared with highly saturated solutions. Because of the high concentration needed for SANS characterization, mimicking the application rate for in-field concentration conditions and analyzing dissolution of the particles will be challenging. On the other hand, the determination of NP sizes using gel electrophoresis has been minimally investigated. Traditionally, agarose gel electrophoresis has been used for separation and purification of NPs, yet measurements similar to protein studies have rarely been conducted. Krizkova et al. reported that they qualitatively measured NP sizes using agarose gel electrophoresis and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). They used SDS-PAGE to separate different sized NPs using similar processes as traditional NP purification with gel electrophoresis tools.

In this study, we have developed a new protocol using SDS-PAGE combined with fluorescence imaging for the detection and quantification of Zinkicide dissolution in aqueous solutions. The protocol was developed on the premise that Zinkicide particle sizes are comparable to protein dimensions and that they exhibit fluorescent properties when exposed to UV light, which is compatible with conventional gel imaging stations. Taking advantage of the inherent emission property of Zinkicide, the fluorescence intensity of Zinkicide dispersed in aqueous solution was monitored over time. Quantitative analysis of Zinkicide in polyacrylamide gels allowed for tracking its relative molecular weight changes in water and citric acid buffer over time. The present study provides a useful tool for detecting and monitoring the dissolution of ZnO bactericidal NPs in aqueous solutions.

2. RESULTS AND DISCUSSION

2.1. Zinkicide Detection and Concentration Measurements. Figure S1 is a representative HRTEM image showing particle size, crystallinity, and morphology of Zinkicide NPs in the vacuum state. The image reveals the spherical shape of the particles with average diameters of ~4 nm. First, the fluorescence of Zinkicide upon UV excitation was assessed (Figure S2). Agriculture-grade Zinkicide stock (57,000 ppm (v/v) of metallic Zn) was supplied by the TradeMark Nitrogen Inc. (Tampa, FL). Zinkicide was diluted to 1000 ppm from the original stock solution of 57,000 ppm in two separate sample fractions with water and citric acid buffer and was deposited on ethanol-cleaned glass slides. The fluorescence emission of the two samples was captured using ChemiDoc XR+ with a UV preset for excitation (Figure S2a). Complementary images were acquired using the UV-transilluminator for UV excitation and obtained with a camera phone (Figure S2b). These measurements confirmed sufficient fluorescence intensities of Zinkicide in aqueous solutions upon UV light excitation.

The detection of Zinkicide using polyacrylamide gel (15%) electrophoresis was performed using UV imaging of SDS-PAGE gels loaded with Zinkicide NPs diluted in water with varying concentrations (Figure 1 and Table S1). Figure 1a,b exhibits the two-dimensional (2D) gel image of Zinkicide and the three-dimensional (3D) rendering of the gel captured with the gel-imaging device. The 3D image was oriented into a 3D landscape utilizing the Bio-Rad Image Lab software (see Methods for details) to make the intensity profile changes easier to observe (Figure 1b). Analysis of the UV images of the gels allowed us to quantify Zinkicide concentration-dependent fluorescence intensities (Figure 1c) ranging from 445 to 28,500 ppm (Figure 1). Zinkicide concentrations were determined to have linear proportionality to fluorescence intensity emissions of Zinkicide. Similar measurements were carried out to measure fluorescence intensity emissions of lower stock concentrations of Zinkicide (Figure S3 and Table S2). To achieve this, Zinkicide was diluted from a stock

![Figure 1](https://dx.doi.org/10.1021/acsomega.9b02893)  
ACS Omega 2020, 5, 1402–1407
concentration of 1000 ppm to lower concentrations using twofold serial dilutions in water. Next, the particles were loaded in the 15% polyacrylamide gels, imaged, and quantified using the Bio-Rad ChemiDoc XRS+ and Bio-Rad Image Lab software. The results show that using SDS-PAGE UV gel imaging and analysis, Zinkicide concentrations ranging from 16 to 28,500 ppm could be measured. The intensities coincided with the designed concentration dilutions and were found to exhibit high degrees of proportionality (Figure S3 and Table S2).

2.2. Intensity Tracking of Zinkicide. The evolution of Zinkicide fluorescence intensity changes in solution was evaluated using polyacrylamide gel (15%) electrophoresis and imaging of SDS-PAGE gels (Figure 2 and Table S3). Zinkicide was diluted in deionized water to 1000 ppm and incubated at increasing times.

Figure 2. Intensity tracking of Zinkicide. (a) Representative 2D SDS-PAGE image of Zinkicide diluted at 1000 ppm in double deionized water (ddH2O) at varying time incubations. (b) 3D rendering of SDS-PAGE gel of Zinkicide NPs. (c) Average fluorescence intensity of Zinkicide over 16 days. Uncertainty bars represent the standard deviation (SD) (d) Corresponding UV-transilluminator image of the gel. The loading of Zinkicide was 1000 ppm at the following time points: lane 1: 0 h control, lane 2: 10 day, lane 3: 13 day, lane 4: 14 day, lane 5: 15 day, and lane 6: 16 day.

The time selected ranged from 10 to 16 days to determine the fate of the NPs as the foliar treatments on citrus trees at recommended spray frequencies of the bactericides for in-field applications.8,9 The aged Zinkicide was loaded into the SDS-PAGE gels under constant loading conditions and run with our electrophoresis protocol. The 2D and 3D gel images were captured and analyzed. Fluorescence intensity changes of aged Zinkicide in aqueous solution were quantified (Figure 2 and Table S3).

Fluctuations in fluorescence intensities were observed in the 2D and 3D images where the low point of intensity was recorded in lane 3 and the highest intensity was measured in lane 6 (Figure 2a,b). The plot in Figure 2c indicates that time-dependent imaging and tracking of Zinkicide in tandem with SDS-PAGE were applicable. To provide further evidence of the validity of tracking fluorescence intensity variation of Zinkicide with gel electrophoresis, we used UV-transilluminator imaging (Figure 2d) to characterize the same gel previously studied with the Bio-Rad device. In the UV-transilluminator image, the low point of intensity was observed at lane 3 and the high point of intensity was recorded at lane 6 (Figure 2d). This result was consistent with the high precision measurements obtained using the Bio-Rad ChemiDoc XRS+. The samples incubated for longer time intervals became more fluorescent.

The fluctuation of fluorescence intensities could be linked to increase in surface defects because of aging of Zinkicide in solution. In addition, ZnO NPs exposed to solutions over time are known to release zinc ions via the dissolution process.4,14,21,31,32 The dissolution kinetics have been shown to produce increased fluorescence in time-dependent photoluminescence studies with ZnO NPs.33 There are three general factors that could contribute to dissolution and/or the generation of surface defects for NPs: (i) surface chemistry effects, (ii) external factors, and (iii) size and surface area effects.34 These physiochemical factors could independently or collectively contribute to dissolution of Zinkicide and promote increased fluorescence.

2.3. Tracking Molecular Weight Changes of Zinkicide in Different Media. Zinkicide molecular weight changes were tracked using polyacrylamide gel electrophoresis and UV imaging of gels loaded with Zinkicide NPs dispersed in water and citric acid buffer over time. The results reveal that the overall molecular weight decrease of Zinkicide is a function of the solution pH (Figures 3 and S4, and Table S4). The molecular weight of Zinkicide in citric acid buffer (pH = 3.0) decreased faster than in ddH2O (pH = 7.0). After 7 days, Zinkicide NPs incubated in citric acid buffer resulted in a molecular weight decrease of 12.23% while those incubated in ddH2O caused a molecular weight decrease of 4.18%. We tracked these molecular weight changes as a percentage decrease because the native Zinkicide molecular weights were found to exhibit molecular weights less than 10 kDa.

We interpreted that the greater effect of molecular weight changes in citric acid buffer could be attributed to the acidic environments of the buffer solution which in turn induced greater degradation of the NPs over extended time intervals.4,12,14 This may be directly related to increased dissolution of the bactericidal NPs.16 It has been shown that the dissolution rates of ZnO NPs are inversely proportional to the particles size and surface area.13 Hence, smaller NPs or NPs which have been degraded can increase the rate of ion dissolution.7 The in vivo acidic environment in citrus is within the range of the citric acid buffer environment (pH 3.0) of our in vitro studies.14,15 This result is crucial for future applications of ZnO for bactericidal crop management tactics. The
molecular weight results identified the notion that if the ZnO NPs are indeed systemically delivered to the interior compartment of crops, the material would have the magnitude to degrade readily at the fruit level of the crops and in fruit juices.9,14,35,36 Tracking molecular weight changes of Zinkicide is important for predicting its stability in planta. The design of Zinkicide aging studies in buffer-controlled solutions is important for providing evidence that the material should be able to safely degrade over time.

3. CONCLUSIONS

In this study, using SDS-PAGE, we have developed a facile and cost-effective tool to monitor the dissolution of Zinkicide, a bactericidal NP treatment designed to manage citrus diseases. We established the linear dependency between Zinkicide fluorescence intensity and its concentration in the gel. Next, we established that the dissolution of Zinkicide dispersed in aqueous solutions is accompanied by a molecular weight change and a change in the fluorescence intensity. The acidity of the aqueous solvents used to mimic the conditions of Zinkicide in the field was found to have a greater impact on the kinetics of dissolution. The developed protocol for quantification of NPs in aqueous solution can be utilized for other fluorescent NP characterization as a unique and cost-effective methodology. Several traditional NP characterization tools are expensive, time-consuming, and nonreliable, especially for ultra-small NPs (<10 nm) because of high dispersibility and dissolution of smaller NPs at low concentrations. These findings are important for stability assessments of commercially available nanomaterials in solution.

4. METHODS

4.1. NP Materials. Agriculture-grade Zinkicide (4.5% metallic Zn) was obtained from our collaborator TradeMark Nitrogen Inc. (TMN, Tampa, FL).27 The material was studied as received without further purification. Figure S1 shows the representative HRTEM image of Zinkicide.

4.2. NP Preparation and Loading in SDS-PAGE Gels. Bio-Rad Mini-PROTEAN Tetrad glass/short plates were used for hand-casting 8.3 cm × 7.3 cm SDS-PAGE gels (Bio-Rad, USA). Stacking gel concentration of 6% and resolving gel acrylamide concentration of 15% were determined to be the optimal gel conditions for resolving changes in Zinkicide. Twenty microliters of the NP samples was aliquoted into labeled 0.6 mL test tubes and vortexed. An amount of 2.5 μL of 4× Laemmli sample buffer dye was added to the samples. For gel orientation, only (not used for molecular weight quantification) 10 μL of the Bio-Rad Precision Plus Protein All Blue Standard was loaded into the first lane of the 15-well gels. The device was run at 150 V, 500 mA, for 50 min in 500 mL of Tris-glycine electrode buffer. Following electrophoresis, the gels were excised from the glass plates and stored in Rainin gel box containers with ddH2O to prevent drying. No stain or destain protocols were used in these experiments.

4.3. UV SDS-PAGE Gel Imaging and Analysis. Gel samples were imaged with the Bio-Rad Chemidoc XRS+, which uses a mercury UVB lamp with a wavelength of 302 nm. The device was computer-controlled using Bio-Rad Image Lab software for image acquisition. Once gels were placed into the device, the Image Lab software was loaded and the preset parameter (UV) was selected for imaging. The gels were aligned manually and using the digital alignment grid in the software. The images were captured using the imaging device and saved as .scn files for analysis with the same software. Secondary image files were saved as .tiff for image documentation.

Gel analysis was performed using the same software. The software enables precise measurements of molecular weight, fluorescence intensity, relative mobility (Rf), and quantity tools for relative and absolute volumes. For molecular weight studies, a custom molecular weight ladder was developed. The custom digital marker was designed to measure the Zinkicide molecular weight from 0 to 100% in the gels with the Image Lab software. This setting was used to track the molecular weight changes of the samples over time when incubated in pH-dependent media. The selected marker bands were adjusted using the digital band manipulation tools in the software to maximize the coverage of the bottom and top bands in the custom marker system. These markers were placed in three positions across the gel to sustain detection integrity and precision. The values of relative molecular weights were recorded using the software, and the percent decrease was calculated by using the molecular weight of the control (t = 0 h) in relation to the other samples.

4.4. Zinkicide Fluorescence Intensity Measurements on Glass Slides. Zinkicide was diluted to 1000 ppm in water and citric acid buffer in separate microcentrifuge tubes. Two glass slides were rinsed with 70% ethanol and water and dried with KimWipes. Twenty microliters of the Zinkicide sample diluted with water was loaded onto the first slide and 20 μL of the Zinkicide sample diluted with citric acid buffer was loaded onto the second slide. The slides were placed in the Bio-Rad Chemidoc XRS+ and captured using the UV preset in Bio-Rad Image Lab Software. The identical slides were placed on the UV-transilluminator and the images were captured with a smart phone camera.

4.5. Zinkicide Detection and Quantification. The NPs were vortexed for 1 min and shaken vigorously in the stock 50 mL conical tubes to disperse the nanoparticle solutions. The samples for the concentration measurement using SDS-PAGE were serially diluted from the stock concentration of 28,500 ppm using twofold serial dilutions for the following six sample conditions.

For intensity measurement, NPs were diluted to 1000 ppm in 15 mL conical tubes with ddH2O for all sample conditions. The diluted samples (1000 ppm) were aged at selected time intervals which included 0 h control, 10 day, 10 day, 14 day, 15 day, and 16 day. The diluted/aged samples were loaded into 15% SDS-PAGE gels and run under the prior-listed SDS-PAGE protocol. The 2D and 3D images were captured with the Bio-Rad Chemidoc XRS+ and the secondary images were obtained using a UV-transilluminator device and captured with a camera phone. Image analysis was conducted with Bio-Rad Image Lab software.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b02893. HRTEM image of Zinkicide NPs; fluorescence intensity controls; Zinkicide detection and concentration measurements using SDS-PAGE; tracking of time-dependent molecular weight changes; fluorescence intensity at varying ranges of concentrations; time-dependent
intensity tracking; and molecular weight changes in water and citric acid buffer over time.

AUTHOR INFORMATION

Corresponding Author
Ellen H. Kang – University of Central Florida, Orlando, Florida; orcid.org/0000-0003-2785-3479; Email: ellen.kang@ucf.edu

Other Authors
Zachary T. Untracht – University of Central Florida, Orlando, Florida
Ali Ozcan – University of Central Florida, Orlando, Florida; orcid.org/0000-0001-6781-535X
Swadeshmukul Santra – University of Central Florida, Orlando, Florida; orcid.org/0000-0001-5929-5323

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b02893

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This study was supported by funds provided by the University of Central Florida (UCF) (to E.H.K.). The authors acknowledge the UCF Materials Innovation and Sustainable Agriculture (MISA) Center, UCF Biomolecular Research Annex, and UCF Advanced Materials Procession and Analysis Center (MCF) for facilities and technical support. We acknowledge partial support obtained from the Citrus Research and Development Foundation (CRDF project # 907) and USDA-NIFA-SCRI (grant # 2015-70016-23010). The authors would like to thank Dr. Laurene Tetard for critical reading and helpful suggestions for the manuscript and the Kang Lab members for their continued support and assistance.

REFERENCES

(1) Mukhopadhyay, S. S. Nanotechnology in agriculture: prospects and constraints. Nanotechnol., Sci. Appl. 2014, 7, 63−71.
(2) Cho, E. J.; Holback, H.; Liu, K. C.; Abouelmagd, S. A.; Park, J.; Yeo, Y. Nanoparticle Characterization: State of the Art, Challenges, and Emerging Technologies. Mol. Pharm. 2013, 10, 2093−2110.
(3) López-Serrano, A.; et al. Nanoparticles: a global vision. Characterization, separation, and quantification methods. Potential environmental and health impact. Anal. Methods 2014, 6, 38−56.
(4) Vogelsberger, W.; Schmidt, J.; Roeolfs, F. Dissolution kinetics of oxide nanoparticles: The observation of an unusual behaviour. Colloids Surf., A 2008, 324, 51−57.
(5) Sabir, S.; Arshad, M.; Chaudhari, S. K. Zinc oxide nanoparticles for revolutionizing agriculture: synthesis and applications. Sci. World J. 2014, 2014, 925494.
(6) Rajput, V. D.; Minkina, T. M.; Behal, A.; Sushkova, S. N.; Mandzhieva, S.; Singh, R.; Gorovtsov, A.; Tsitsilashvili, V. S.; Purvis, W. O.; Ghazaryan, K. A.; Movsesyan, H. S. Effects of zinc-oxide nanoparticles on soil, plants, animals and soil organisms: A review. Environ. Nanotechnol. Monit. Manag. 2018, 9, 76−84.
(7) Reddy Pullagurala, V. L.; Adisa, I. O.; Rawat, S.; Kim, B.; Barrios, A. C.; Medina-Velo, I. A.; Hernandez-Viecas, J. A.; Peralta-Videz, J. R.; Gardea-Torresdey, J. L. Finding the conditions for the beneficial use of ZnO nanoparticles towards plants-A review. Environ. Pollut. 2018, 241, 1175−1181.
(8) Graham, J. H.; Johnson, E. G.; Myers, M. E.; Young, M.; Rajasekaran, P.; Das, S.; Santra, S. Potential of Nano-Formulated Zinc Oxide for Control of Citrus Canker on Grapefruit Trees. Plant Dis. 2016, 100, 2442−2447.
(9) Young, M.; Ozcan, A.; Myers, M. E.; Johnson, E. G.; Graham, J. H.; Santra, S. Multimodal Generally Recognized as Safe ZnO/Nanocopper Composite: A Novel Antimicrobial Material for the Management of Citrus Phytopathogens. J. Agric. Food Chem. 2018, 66, 6604.
(10) Bagchi, D.; Rathnam, V. S. S.; Lemmens, P.; Banerjee, I.; Pal, S. K. NIR-Light-Active ZnO-Based Nanohybrids for Bacterial Biofilm Treatment. ACS Omega 2018, 3, 10877−10885.
(11) Servin, A.; Elmer, W.; Mukherjee, A.; De la Torre-Roche, R.; Hamdi, H.; White, J. C.; Bindraban, P.; Dimoka, C. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. J. Nanopart. Res. 2015, 17, 92.
(12) Dias, S. S.; Ismail, A. B.; Mohamad, A. A. Effect of pH on ZnO nanoparticle properties synthesized by sol−gel centrifugation. J. Alloys Compd. 2010, 499, 231−237.
(13) David, C. A.; Galceran, J.; Rey-Castro, C.; Puy, J.; Companies, E.; Salvador, J.; Monné, J.; Wallace, R.; Vakourov, A. Dissolution Kinetics and Solubility of ZnO Nanoparticles Followed by AGNES. J. Phys. Chem. C 2012, 116, 11758−11767.
(14) Mudunkotuwang, J.; Rupasinghe, T.; Wu, C.-M.; Grassian, V. H. Dissolution of ZnO nanoparticles at circumneutral pH: a study of size effects in the presence and absence of citric acid. Langmuir 2012, 28, 396−403.
(15) Pauel, I.; Bar-Tal, A.; Robart, N.; Ephrath, J.; Cohen, S. Water quality changes seasonal variations in root respiration, xylem CO 2 and sap pH in citrus orchards. Agric. Water Manag. 2018, 197, 147−157.
(16) Hale, P. S.; Maddox, L. M.; Shapter, J. G.; Voelcker, N. H.; Ford, M. J.; Waclawik, E. R. Growth Kinetics and Modeling of ZnO Nanoparticles. J. Chem. Educ. 2005, 82, 775.
(17) Dutta, T.; Bagchi, D.; Bera, A.; Das, S.; Adhikari, T.; Pal, S. K. Surface Engineered ZnO-Humic/Citrate Interfaces: Photoinduced Charge Carrier Dynamics and Potential Application for Smart and Sustained Delivery of Zn Micronutrient. ACS Sustainable Chem. Eng. 2019, 7, 10920−10930.
(18) Lim, J.; Yeap, S. P.; Che, H. X.; Low, S. C. Characterization of magnetic nanoparticles by dynamic light scattering. Nanoscale Res. Lett. 2013, 8, 381.
(19) Murdoch, R. C.; Braydich-Stolle, L.; Schrand, A. M.; Schlager, J. I.; Hussain, S. M. Characterization of nanomaterial dispersion in solution prior to in vitro exposure using dynamic light scattering technique. Toxicol. Sci. 2008, 101, 239−253.
(20) Lu, X.-Y.; Wu, D.-C.; Li, Z.-J.; Chen, G.-Q. Polymer nanoprop. Prog. Mol. Biol. Transl. Sci. 2011, 104, 299−323.
(21) Odzak, N.; Kistler, D.; Sigg, L. Influence of daylight on the fate of silver and zinc oxide nanoparticles in natural aquatic environments. Environ. Pollut. 2017, 226, 1−11.
(22) Desai, R.; Mankad, V.; Gupta, S.; Jha, P. Size Distribution of Silver Nanoparticles: UV-Visible Spectroscopic Assessment. Nanosci. Nanotechnol. Lett. 2012, 4, 30−34.
(23) Bindhu, M. R.; Sethy, V.; Umadevi, M. Synthesis, characterization and SERS activity of biosynthesized silver nanoparticles. Spectrochim. Acta, Part A 2013, 115, 409−415.
(24) Helfrich, A.; Bruichert, W.; Bettmer, J. Size characterisation of Au nanoparticles by ICP-MS coupling techniques. J. Anal. At. Spectrom. 2006, 21, 431.
(25) Soldati, T.; Schiwa, M. Powering membrane traffic in endocytosis and recycling. Nat. Rev. Mol. Cell Biol. 2006, 7, 897−908.
(26) Avellan, A.; Simonin, M.; McGivney, E.; Bossa, N.; Spielman-Sun, E.; Rocca, J. D.; Bernhardt, E. S.; Geitner, N. K.; Unrine, J. M.; Wiesner, M. R.; Lowry, G. V. Gold nanoparticle biodissolution by a freshwater macrophyte and its associated microbiome. Nat. Nano-technol. 2018, 13, 1072−1077.
(27) Rawal, T. B.; Ozcan, A.; Liu, S.-H.; Pingali, S. V.; Akbilgic, O.; Tetard, L.; O'Neill, H.; Santra, S.; Petridis, L. Interaction of Zinc Oxide Nanoparticles with Water: Implications for Catalytic Activity. ACS Appl. Nano Mater. 2019, 2, 4257.
(28) Krizkova, S.; Dostalova, S.; Michalek, P.; Nejdl, L.; Kominkova, M.; Milosavljevic, V.; Moulick, A.; Vuculovicova, M.; Kopel, P.; Adam, V.; Kizek, R. SDS-PAGE as a Tool for Hydrodynamic Diameter-Dependent Separation of Quantum Dots. Chromatographia 2015, 78, 785−793.

(29) Hanauer, M.; Pierrat, S.; Zins, I.; Lotz, A.; Sönnichsen, C. Inga Zins, Alexander Lotz, and; So1nnichsen*, C., Separation of Nanoparticles by Gel Electrophoresis According to Size and Shape. Nano Lett. 2007, 7, 2881−2885.

(30) Robertson, J. D.; Rizzello, L.; Avila-Olias, M.; Gaitzsch, J.; Contini, C.; Magoñ, M. S.; Renshaw, S. A.; Battaglia, G. Purification of Nanoparticles by Size and Shape. Sci. Rep. 2016, 6, 27494.

(31) Zhang, H.; Chen, B.; Banfield, J. F. Particle Size and pH Effects on Nanoparticle Dissolution. J. Phys. Chem. C 2010, 114, 14876−14884.

(32) Milani, N.; McLaughlin, M. J.; Stacey, S. P.; Kirby, J. K.; Hettiarachchi, G. M.; Beak, D. G.; Cornelis, G. Dissolution kinetics of macronutrient fertilizers coated with manufactured zinc oxide nanoparticles. J. Agric. Food Chem. 2012, 60, 3991−3998.

(33) Monticone, S.; Tufeu, R.; Kanaev, A. V. Complex Nature of the UV and Visible Fluorescence of Colloidal ZnO Nanoparticles. J. Phys. Chem. B 1998, 102, 2854−2862.

(34) Misra, S. K.; Dybowska, A.; Berhanu, D.; Luoma, S. N.; Valsami-Jones, E. The complexity of nanoparticle dissolution and its importance in nanotoxicological studies. Sci. Total Environ. 2012, 438, 225−232.

(35) Casida, J. E. Pesticide Interactions: Mechanisms, Benefits, and Risks. J. Agric. Food Chem. 2017, 65, 4553−4561.

(36) Zuverza-Mena, N.; Martínez-Fernández, D.; Du, W.; Hernandez-Viezcas, J. A.; Bonilla-Bird, N.; López-Moreno, M. L.; Komárek, M.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L. Exposure of engineered nanomaterials to plants: Insights into the physiological and biochemical responses-A review. Plant Physiol. Biochem. 2017, 110, 236−264.