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Advanced Analytical Techniques for the Measurement of Nanomaterials in Food and Agricultural Samples: A Review

Susmita Bandyopadhyay,[1] Jose R. Peralta-Videa,[2,3] and Jorge L. Gardea-Torresdey[1–3,*]

[1]*Environmental Science and Engineering PhD Program,* [2]*Department of Chemistry, and* [3]*University of California Center for Environmental Implications of Nanotechnology (UC CEIN), The University of Texas at El Paso, El Paso, Texas.*

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Abstract

Nanotechnology offers substantial prospects for the development of state-of-the-art products and applications for agriculture, water treatment, and food industry. Profuse use of nanoproducts will bring potential benefits to farmers, the food industry, and consumers, equally. However, after end-user applications, these products and residues will find their way into the environment. Therefore, discharged nanomaterials (NMs) need to be identified and quantified to determine their ecotoxicity and the levels of exposure. Detection and characterization of NMs and their residues in the environment, particularly in food and agricultural products, have been limited, as no single technique or method is suitable to identify and quantify NMs. In this review, we have discussed the available literature concerning detection, characterization, and measurement techniques for NMs in food and agricultural matrices, which include chromatography, flow field fractionation, electron microscopy, light scattering, and autofluorescence techniques, among others.

Key words: agriculture; food products; measurement; nanoparticles

Introduction

Nanotechnology encompasses the fabrication, characterization, and manipulation of particles <100 nm (ASTM, 2012; Nanowerk, 2012). Particles at the nanoscale have unique functional properties that are being used by many industries, including the food and agriculture sectors (Chen *et al.*, 2006; Weiss *et al.*, 2006; Klaine *et al.*, 2008; Tiede *et al.*, 2008; Guere, 2011). A considerable portion of the existing consumer products containing engineered nanoparticles/nanomaterials (ENPs/ENMs) are used in the food and agriculture fields. These ENPs/ENMs are being widely employed in the food industries to: (1) prevent microbial spoilage of packaged foods, (2) improve colors and flavors, (3) modify the texture and taste of foods, (4) detect allergens, and (5) increase the bioavailability of vitamins and minerals (Chen *et al.*, 2006; Institute of Food Science and Technology, 2006; Maynard *et al.*, 2006; Huang *et al.*, 2009). In addition, nanoclays are used as diffusion barriers and nanosilver as an antimicrobial agent in food supplements and food packaging (Day, 2005; Choy *et al.*, 2006; Sanguansri and Augustin, 2006; Chaudhry *et al.*, 2008). By March 2011, there were 1317 consumer products in the market having ENMs, including 105 food- and beverage-related products (PEN, 2012). It is expected that these numbers will increase in the near future. It has also been reported that >200 companies are conducting research and development to enhance the use of NMs in agriculture, engineering, processing, and packaging or delivery of food and nutritional supplements worldwide (Chaudhry *et al.*, 2008). Weiss *et al.* (2006) reported that nanotechnology could be beneficial for food safety with the introduction of nanobased detectors, sensors, labeling, and other applications (Table 1).

In agriculture, ENMs are mostly used to provide novel routes for pesticide delivery to plants (Scott and Chen, 2003; Chaudhry *et al.*, 2008). However, this could release an excess of ENMs in soil, ground water, and food products, with unknown consequences (Klaine *et al.*, 2008; Boxall and Molhave, 2011). ENPs like ZnO and CeO_2 ENPs, widely used in food and commercial products, are potentially toxic to humans and plants (Nel *et al.*, 2006; Lin *et al.*, 2009; Moos *et al.*, 2011; Lee *et al.*, 2012; Mihranyan *et al.*, 2012; Priester *et al.*, 2012). These, as well as silver (Ag), gold (Au), and iron oxide (Fe_3O_4) ENPs, are potentially toxic to soil microbiota (Barrena *et al.*, 2009; Ge *et al.*, 2011; Sinha *et al.*, 2011; Bandyopadhyay *et al.*, 2012a).

This review embraces different measurement techniques for ENPs/ENMs in food and agricultural products, and the challenges associated with them. The available literatures on food additives, food processing and packaging, along with agricultural products containing NMs are discussed. The reported benefits of these products have been discussed in Tables 1 and 2, along with specific examples. Accordingly, the review provides the list for the separation and characterization techniques for NMs present in food- and agriculture-related products (Figs. 1 and 2).

**Corresponding author:* Environmental Science and Engineering PhD Program, Department of Chemistry, The University of Texas at El Paso, 500 West University Ave., El Paso, TX 79968. *Phone:* 915-737-5359; *Fax:* 915-747-5748; *E-mail:* jgardea@utep.edu

Table 1. Examples of the Current Use of Nanomaterials in Foods and Food Packaging

Type of product	*Purpose*	*Nanocontent*	*References*
Food processing	Increase potency and bioavailability. Increased stability of foods during processing and storage.	Molecular cages (1–5 nm diameter) made from silica mineral hydride complex: Nanoscale silicone complex. Nanosized mycrohydrin.	Canham (2007) Chen *et al.* (2006) Dickinson *et al.* (2004)
Nutritional drink	Increase reactivity and bioavailability. Increase solubility of certain vitamins and minerals.	Iron NPs of 300 nm: Micelles and nanocapsules.	Magnuson *et al.* (2011) McClements *et al.* (2009) Huang *et al.* (2009) Pegg and Shahidi (2007)
Food contact material (cooking equipment, crockery, and other kitchenware)	Provide antibacterial properties.	Ag NPs of different size.	Chen *et al.* (2010)
Food packaging; adhesive for food packages/containers	Provide strong adhesion. When used as an adhesive they require less water and, thus, less time and energy to dry.	Starch nanospheres (50–150 nm). These NPs have 400× the surface area of natural starch particles.	PEN (2012)
Food packaging	Prevent penetration of oxygen and gas of the wrapping (plastics), extending the product's shelf life.	Si NPs in a polymer-based nanocomposite.	LeGood and Clarke (2006)
Food additive	Increases absorption within the body (including individual cells).	Nanoscale micelle (nanocapsule) of lipophilic or water-insoluble substances	Shi *et al.* (2006)

Ag, silver; Si, silicon; NP, nanoparticle.

Table 2. Examples of the Current Use of Nanomaterials in Agriculture and Related Processes

Type of product	*Purpose*	*Nanocontent*	*References*
Plant growth treatment	Increase the potency of active ingredients, potentially reducing the quantity to be applied.	Nanoemulsions (~100 nm).	Barati (2010) Pandey *et al.* (2010)
Nanotech delivery systems	Delivering pesticides, fertilizers, and other agrochemicals.	Nanocapsules.	Perea-de-Lugue and Rubilaes (2009) Mukal *et al.* (2009) Maysinger *et al.* (2007)
Nanosensors for soil health	Monitoring of soil conditions and crop growth.	NPs and quantum dots.	Guere (2011) Scott (2002)
Delivering agents	Delivering DNA to plants.	Mesoporous Si NPs (3 nm).	Torney *et al.* (2007)
Agri-waste management products	Recycling of agricultural wastes using newly developed solvents and a technique called electrospinning. Fertilizer or pesticide absorbents.	Nanofibers (~100 nm) produced from cotton fiber.	Lang (2003)
Biofuel production and processes	Catalyst to provide simple and cost-effective conversion of cellulose from waste plant parts into ethanol.	NPs (metal or metal oxide) of different size as catalyst.	Liou and Wu (2010)
Nanobarcodes and nanoprocessing	Monitoring the quality of agricultural procedures. Tagging pathogens in farmlands.	Microscopic probes (nanobarcodes) that could tag multiple pathogens in a farm, which can be detected using any fluorescent-based equipment.	Li *et al.* (2005)

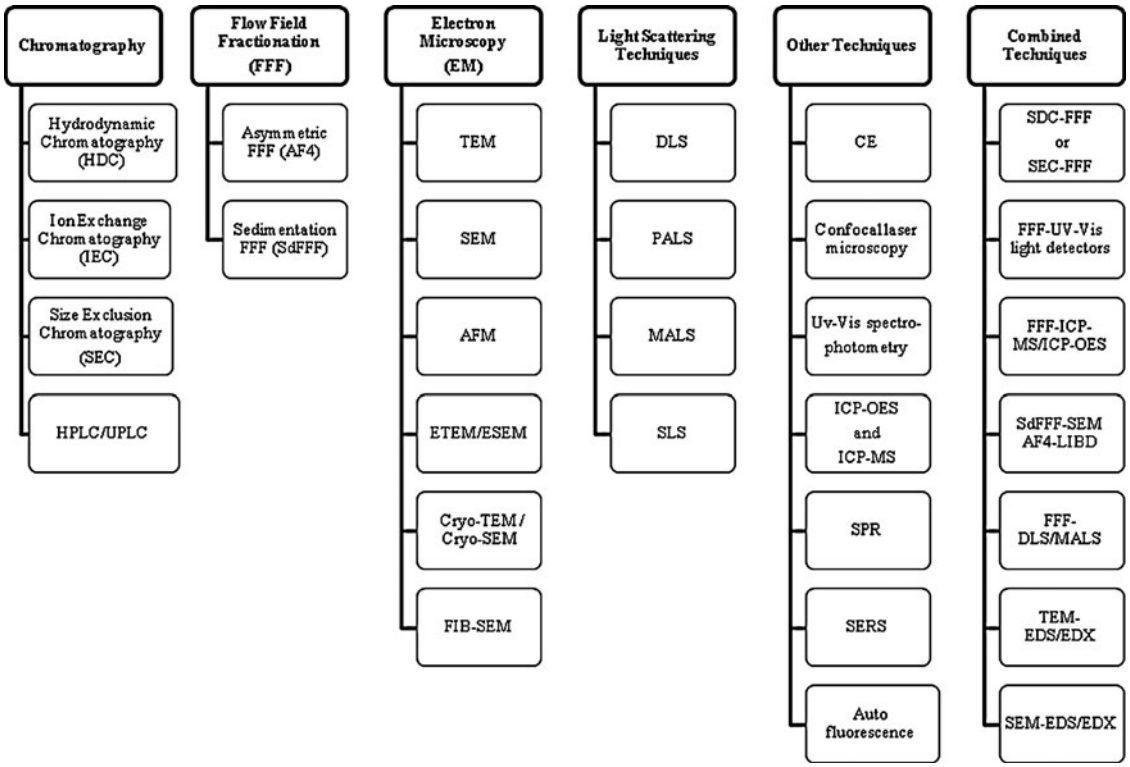

FIG. 1. List of available separation and detection techniques for the measurement of nanomaterials (NMs)/nanoparticles (NPs) in food and agriculture products. AFM, atomic force microscopy; CE, capillary electrophoresis; DLS, dynamic light scattering; EDS/EDX, energy dispersive X-ray spectroscopy; EM, electron microscopy; ETEM/ESEM, environmental TEM or SEM; FIB, focused ion beam; HPLC/UPLC, high- or ultra-performance liquid chromatography; ICP, inductively coupled plasma; LIBD, laser-induced breakdown detection; MALS, multiangle light scattering; MS, mass spectrometry; OES, optical emission spectrometry; PALS, phase analysis light scattering; SDC, sample displacement chromatography; SEM, scanning EM; SERS, surface enhanced Raman scattering; SLS, static light scattering; SPR, surface plasmon resonance; TEM, transmission EM; UV-vis, ultraviolet-visible.

Discussion

Separation and characterization of NMs in food and agricultural samples

Food and agricultural samples are heterogeneous systems, which may contain a mixture of natural NPs (NNPs) and ENPs of different composition (Tiede *et al.*, 2008; Kammer *et al.*, 2011). The mixing of ENPs with NNPs will impact their agglomeration and reactivity in any medium (Tiede *et al.*, 2008; Farre *et al.*, 2011). Hence, sometimes the samples require separation or pretreatment before characterization (Tiede *et al.*, 2008). Sample preparation and prefractionation can be done in different stages or analytical processes to reduce complexity of the sample matrices with minimum alteration (Tiede *et al.*, 2008; Bandyopadhyay *et al.*, 2012b). In addition, physicochemical parameters, such as size, type, surface charge, and reactivity, might influence the fate, transport, and ecotoxicology of NMs (Magnuson *et al.*, 2011; Pycke *et al.*, 2011; Bandyopadhyay *et al.*, 2012b). Available separation and characterization techniques are discussed in detail.

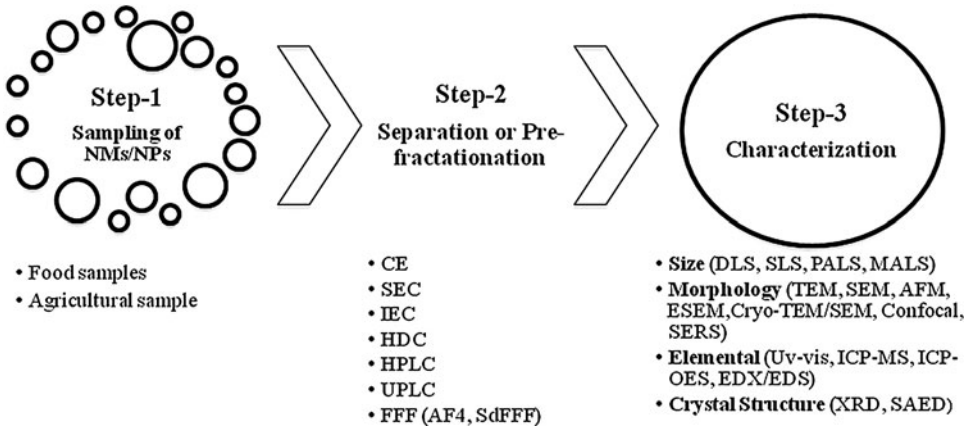

FIG. 2. Different steps for measuring NMs/NPs present in food and agricultural samples along with their corresponding analytical tools/techniques. SAED, selected area electron diffraction; XRD, X-ray diffraction.

Separation techniques. Several separation techniques can be used for the detection of NMs in food/agricultural samples (da Silva *et al.*, 2011; Farre *et al.*, 2011; Pycke *et al.*, 2011; Bandyopadhyay *et al.*, 2012b). Separation or prefractionation can be achieved by a variety of techniques, including capillary electrophoresis (CE), chromatography, and flow field fractionation (FFF), among others (Magnuson *et al.,* 2011). Recently, Magnuson *et al.* (2011) reported the use of high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography, and CE with FFF as efficient separation techniques for NMs in food products.

In chromatography, compounds can be separated based on their charge (weak/strong cation or anion exchange chromatography; [IEC]), molecular mass (size exclusion chromatography [SEC]), hydrophobicity/polarity (reversed-phase HPLC, hydrophobic interaction chromatography), and specific characteristics (affinity chromatography), depending on the type of materials in the stationary phase (Williams *et al.*, 2002; Lead and Wilkinson, 2006; Tiede *et al.*, 2008). Luykx *et al.* (2008) reported the use of SEC and IEC to measure NMs in different food matrices. For example, SEC, coupled to refractive index and multiangle light scattering (MALS) detectors, has been used to characterize polysaccharides present in food samples (Fee *et al.*, 2003; Hokputsa *et al.*, 2004). SEC can also be coupled with a range of detection techniques to characterize NPs, along with the monitoring of the size fractionation (Song *et al.*, 2004; Helfrich *et al.*, 2006).

HPLC allows the separation of pigments, carbohydrates, vitamins, additives, mycotoxins, amino acids, proteins, lipids, chiral compounds, and triglycerides in fats and oils (Luykx *et al.*, 2008; Magnuson *et al.*, 2011). Hydrodynamic chromatography (HDC) is also a very efficient technique to separate NPs in food and agricultural samples based on their hydrodynamic radius (Tiede *et al.*, 2008). HDC coupled with an ultraviolet–visible (UV-vis) detector has been used for the size characterization of colloidal suspensions and biomolecules in food and biological samples (Williams *et al.*, 2002; Blom *et al.*, 2003; Luykx *et al.*, 2008).

FFF, a technique similar to HPLC, can be used to separate NMs based on thermal or hydraulic gradients, electrical forces, and sedimentation (Hassellov *et al.*, 2008; Luykx *et al.*, 2008; Bolea *et al.*, 2010). The general principles of the FFF technique are described in detail elsewhere (Giddings, 1993; Schimpf *et al.*, 2000). It is a flexible elution technique where simultaneous separation and measurement can be done across a broad macromolecular colloidal particulate, ranging from about 1 nm to more than 100 μm (Giddings, 1993). A major advantage of this method is the lack of a stationary phase, thus restraining the interaction between the sample and the equipment surfaces (Giddings, 1993; Schimpf *et al.*, 2000). FFF can be used by an online or offline detection mode for the analysis of complex food samples (Kammer *et al.*, 2011). For instance, FFF coupled with online detectors, such as inductively coupled plasma-mass spectrometry (ICP-MS)/optical emission spectrometry, has been used for elemental analysis of metallic NPs (Kammer *et al.*, 2011; Baalousha *et al.*, 2006). FFF can be coupled with fluorescence, MS, and light scattering techniques for the quantitative detection of NMs in complex systems (Hassellov *et al.*, 2008). Kammer *et al.* (2011) reported that FFF combined with HPLC and UV-vis light detectors is an efficient tool for the detection of NMs in food samples. Soil and sediments can be analyzed by using FFF in combination with other online/offline detectors. For example, ZnO NPs have been separated from soil particles through FFF (Gimbert *et al.*, 2007).

Sedimentation FFF (SdFFF) has been used for characterizing micrometer size (You *et al.*, 2002) and submicrometer size particles in food materials (Jussila *et al.*, 1997; Udabage *et al.*, 1997; Udabage *et al.*, 2003). SdFFF is suitable for separation and characterization of emulsions in food samples. It is an elution-based analytical technique, which provides high-resolution separation of NMs in gentle, low shear conditions. Saeseaw *et al.* (2005) reported the use of SdFFF for the measurement of small food particles present in different types of flour and milk samples. SdFFF, coupled with ICP-MS, has been used for the characterization of particle size and elemental distribution in soil colloids (Ranville *et al.*, 2005).

Asymmetric flow FFF (AF4) is another technique for NM characterization. Bouby *et al.* (2004) reported the characterization of Fe_3O_4/hydroxide colloids by using a combined AF4 and laser-induced breakdown technique with trace detection limit of 1 mg/L. This combination can be ideal for measuring NMs in agricultural soil, aquatic samples, and/or humic substances containing Fe_3O_4/hydroxide. AF4, in combination with MALS, has gained importance in the field of food science to detect submicron size particles (Hupfeld *et al.*, 2009).

Detection or characterization of NMs in food and agriculture. The most widely used detection techniques for NMs in food and agricultural samples include: microscopic and spectroscopic techniques, dynamic light scattering (DLS), surface plasmon resonance (SPR), and autofluorescence, among others (Caldwell *et al.*, 1992; Contado *et al.*, 1999; You *et al.*, 2002; Durand *et al.*, 2003; Regnault *et al.*, 2004; Arfvidsson *et al.*, 2004; González-Melendi *et al.*, 2008; Rebe Raz *et al.*, 2012).

The classical light scattering technique can provide the structural information and, in combination with DLS or FFF, the shape of the particles (Brar and Verma, 2011). DLS (also known as photon correlation spectroscopy) uses the scattered light to measure the rate of diffusion of NPs and provides a size distribution in terms of hydrodynamic diameter. This is suitable for sensing small aggregated proteins ($<0.01\%$ weight) in various food samples (Brar and Verma, 2011). Yegin and Lamprecht (2006) reported the use of DLS for size characterization of lipid nanocapsules. Durand *et al.* (2003) described the use of DLS (along with optical microscopy) for the size measurement of natural particles ($\sim$1–3 μm) present in milk. The surface structure of the casein micelle NPs was also achieved in simple and rapid experimentation using DLS (Griffin *et al.*, 1983; Griffin *et al.*, 1988; Alexander and Dalgleish, 2006). However, it is hard to quantify accurately the presence of any aggregates with DLS. This problem can be overcome by using the phase analysis light scattering (PALS) technique. PALS has been used to determine the isoelectric point and electrophoretic mobility of the whey protein isolate solution (Vanapalli and Coupland, 2000). Static light scattering is also considered as another rapid and reproducible light scattering technique for food samples varying from 0.05 to 2000 μm. This technique has already been used for the particle size measurement of dairy products (Michalski *at al.*, 2001), casein micelles (Huppertz and deKruif, 2007), lactose crystals (Mimouni *et al.*, 2005), skimmed milk (Gaucher *et al.*, 2007), and whole milk (Saveyn *et al.*, 2006; Ahmad *et al.*, 2008).

Electron microscopy (EM) techniques are widely used to determine the size, shape, and other elemental properties of NPs/NMs in food matrices. Standard EM instruments

facilitate size and shape determination of NMs with a better resolution. Transmission EM (TEM) is one of the indispensable nanoscale imaging techniques for the characterization of NMs <200 nm in food and agricultural samples. In TEM, electrons are transmitted through the sample to acquire an image (Peters *et al.*, 2011). This technique is suitable for imaging NPs with a resolution of 0.5 nm (Tiede *et al.*, 2008). The NPs appear as dark dots on a lighter background, as the density of the inorganic NPs is higher compared with the background in the food matrix. TEM has been employed to measure milk-protein-based nanotubes, the shape of serum albumin NPs, and enzyme-functionalized peptide nanotubes (Luykx *et al.*, 2008). TEM, coupled with an energy dispersive X-ray spectroscopy (EDS or EDX) detector is used to get the elemental compositions of NMs, while at the same time, TEM images can provide the size, morphology, and size distribution of NMs with accuracy of $\pm 5\%$ (Burleson *et al.*, 2004). However, this technique is mostly used to localize and identify inorganic particles. This technique is not helpful in organic NPs, as carbon is the major element in the NPs and the food matrix (Peters *et al.*, 2011). Recently, by using TEM, the scanning electron microscope (SEM), EDS, along with Zetasizer (DLS), Zhang *et al.* (2012) determined the size, shape, and elemental characteristics of AgNPs present in Ag-contaminated pears. The authors mentioned that the combination of two or more techniques is suitable for characterizing the NPs in the food samples (Zhang *et al.*, 2012). SEM can be employed for detecting larger particles (achieving a spatial resolution of ~ 500 nm) (Burleson *et al.*, 2004). SEM provides a high-resolution image of a sample surface in a distinctive three-dimensional appearance. Additionally, the SEM has been used to observe the morphology of polysaccharide NPs, protein NPs, and the liposomal NPs (Luykx *et al.*, 2008).

The sample preparation for the EM study can be very tedious, as it requires thin sections for imaging (Dudkiewicz *et al.*, 2011). Additionally, standard EM instruments (TEM and SEM) operate under high-vacuum conditions; therefore, samples containing water cannot be imaged before sample preparation. In addition, chemical fixation and dehydration of the samples is required before imaging, which can produce artifacts (Burleson *et al.*, 2004). Liquid samples and emulsions (e.g., milk, yogurt, or salad dressings) can be encapsulated in agar and/or chemically fixed followed by dehydration before the TEM or SEM analysis (Kalab and Larocque, 1996; Egelandsdal *et al.*, 1999). An environmental TEM or SEM (ETEM/ESEM) can be employed to characterize samples in wet conditions or without chemical fixation. The food and agricultural samples can be imaged in a controlled atmosphere in ETEM, whereas, in ESEM, hydrated samples can be imaged as the samples remain under high vapor pressure. Reports indicate that it is possible to image samples with 100% relative humidity by controlling the vapor pressure (Burleson *et al.*, 2004; Dudkiewicz *et al.*, 2011). ESEM has been employed to investigate the presence of inorganic microsized and nanosized contaminants in food products (Gatti *et al.*, 2009).

Cryo-TEM/Cryo-SEM can be used to acquire high-resolution images of biological samples under high vacuum and below ambient temperature (between -100°C and -175°C). The lower temperature (typically the vitrified state) allows the life-like appearance of the sample and helps to obtain the micrograph of hydrated and chemically unmodified state of the sample (Dudkiewicz *et al.*, 2011). This is ideal for samples that cannot be fixed chemically (e.g., fat- and polysaccharide-based food samples). Cryo-SEM can be applied for imaging NMs in suspensions, solid lipid NPs, or micelles. Dudkiewicz *et al.* (2011) reported that Cryo-SEM has a higher resolution compared to ESEM. Nonetheless, ESEM can be used to observe the dynamic changes associated to the sample morphology.

Atomic force microscopy (AFM) is also considered a powerful tool to investigate the fine structural information of food materials. AFM can detect irregularities in the polymer structure that usually hindered the detection in whole sample-based analyses (Round *et al.*, 1996). Thus, AFM imaging provides the potential to characterize the integral heterogeneous assemblies of food macromolecules (Yang *et al.*, 2006, 2007).

Soil and sediment samples containing NMs with unique properties, such as light absorption, fluorescence, or containing a rare metal, can be effectively analyzed by TEM or SEM techniques (von der Kammer *et al.*, 2006). These properties are also sensitive for UV-vis spectrophotometry, where colored species (such as ferrihydrite and humic colloids) in soil samples can be detected (Bouby *el al.*, 2004).

In addition to EM, confocal laser microscopy can also be used to detect NMs in agricultural samples, specifically, in plant and microbial systems. This technique was used to detect CeO_2 and ZnO NPs inside corn plant tissue. Confocal microscope images showed NP aggregates in root epidermis, cortex, and some NP aggregates in the xylem vessels (Zhao *et al.*, 2012a, 2012b).

SPR or surface-enhanced Raman spectrometry-based approaches are also being employed to measure NPs (Tiede *et al.*, 2008; Rebe Raz *et al.*, 2012). A recent report described the use of human metallothionein-based SPR sensors to detect and measure AgNPs in food and environmental samples (Rebe Raz *et al.*, 2012). The authors acknowledged SPR as a rapid screening tool that can provide real-time automated measurements. The sensitivity of the sensor increased as the size of the AgNPs increased, probably due to the enhancement of the SPR signal, which is proportional to the mass of the binding analyte (Rebe Raz *et al.*, 2012).

Autofluorescence can also be used to analyze agricultural samples. NMs can be detected with this technique: glutaraldehyde-fixed plant samples. González-Melendi *et al.* (2008) reported the use of autofluorescence to detect core shell magnetic NPs in *Cucurbita pepo* plants. The plant samples showed the presence of NPs inside the cell wall of the xylem vessels. Actually, the presence of lignin in the cell wall of the plant tissue helps to autofluoresce the samples. Therefore, the method can visualize some particles associated with the cell wall of xylem vessels, which are highly autofluorescent due to their major component—lignin (González-Melendi *et al.*, 2008).

Conclusion

In summary, there are many analytical methods/tools for the separation and detection of the NMs in food and agricultural samples. These include chromatography, light scattering techniques (classical and advanced), and electron microscopy, among others. However, to the best of the authors' knowledge, a limited number of studies have been reported for the detection of NMs in food and agricultural samples. Therefore, extended future studies are needed to

understand the environmental fate, transport, and ecotoxicity of the released ENMs/ENPs.

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Author Disclosure Statement

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