CASE STUDY

Nanomaterials in Food

Robert Thomas
Scientific Solutions Inc.

Prof. Jorge Gardea-Torresdey with some of his research group members

Customer Story: University of Texas at El Paso Pushing the Boundaries to Understand the Impact of Manufactured Nanomaterials on our Food Supply

It is well-recognized that engineered or manufactured nanomaterials (ENM/MNM) can exhibit unique physical, chemical, and biological characteristics, which are not exhibited by the bulk materials with the same composition. This is particularly true of metal-containing nanomaterials which are used for a variety of consumer, healthcare and industrial applications. Although many of them are incorporated into the product matrix such as cadmium crystal-based quantum dots used in the production of solar panels, a significant number are used in applications where they are either intentionally released or somehow find their way into the environment. Some of these applications include:

- The use of fabrics containing silver (Ag) nanoparticles to kill bacteria, which when washed, release silver at varying rates during the washing cycle
- Many cosmetics and personal care products such as mascara, lipstick and sunscreen contain pigments manufactured with nano zinc oxide (ZnO) or titanium oxide (TiO_2) particles for their UV-resistant properties
- Some commercial diesel fuels contain cerium oxide (CeO_2) nanoparticles, which are used as a catalyst to increase surface area to improve the fuel's combustion properties
- The use of silica (SiO_2) nanoparticle slurries to polish silicon wafers using the semiconductor chemical-mechanical planarization (CMP) polishing process

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Characterization of Nanomaterials

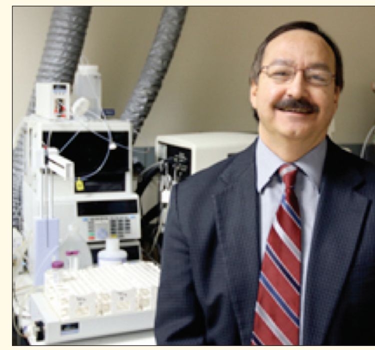
Even though the properties of these manufactured nanomaterials are ideally-suited for the applications they are intended for, they may eventually end up being a serious ecological concern when they are either emitted directly into the atmosphere (in the case of diesel fumes) or into residential and industrial wastewater treatment plants as with the other three application areas. So in order to ensure the continued development of nanotechnology products, there is clearly a need to evaluate the risks posed by these manufactured nanoparticles when they are discharged into the environment at potentially harmful concentrations. Much of the early studies have focused on understanding how they enter and impact watershed and hydrologic systems. Some of these investigations have involved the use of nanometrology tools such as electron microscopy, chromatography, centrifugation, laser light scattering, ultrafiltration and more recently, inductively coupled plasma mass spectroscopic methods to assess the size, size distribution, surface characteristics, shape, and chemical composition of the particles under investigation. Different manufactured nanoparticles (MNP) will have different properties and will therefore behave very differently when they enter the environment, so it is very clear, the analytical techniques must be able to detect the nanoparticles with high specificity and extreme sensitivity.[1]

Impact of Nanomaterials on Soil Fertility

Understanding all these metrics for hydrologic systems is very important, but now researchers are going a step further and beginning to investigate the impact of nanoparticles on soil fertility and their uptake by crops grown for human consumption. Some of the many questions they are trying to answer are whether the nanoparticles remain as the metal or metallic oxide or whether are they converted into a biomolecular form as they are absorbed through the root system into the vegetable, leaf or fruit, which eventually is consumed by the human population. These are critical questions that need to be answered; which will require a different set of tools to not only quantitate the concentration of the metallic nanomaterial, but also to know whether it's been bio-transformed as the plant goes through its growing cycle.

Research at the University of Texas, El Paso

One of the leading research groups in the study of nanomaterials is the "Department of Chemistry and Environmental Science and Engineering Ph.D. Program" at The University of Texas at El Paso (UTEP), under the leadership of Professor Jorge Gardea-Torresdey. He has worked in the field of environmental nanometrology for almost 20 years and some of his many research interests include the applications of spectroscopic techniques to environmental chemistry; phytoremediation (treatment of environmental pollution through the use of plants), novel methods for the bio-production of nanoparticles and study of the fate of nanoparticles in the environment. He is also a co-investigator at the "University of California Center for Environmental Implications of Nanotechnology (UCCEIN)", a joint collaboration with other institutions including the University of California in Los Angeles and Santa Barbara, and funded by the National Science Foundation (NSF) and the Environmental Protection Agency (EPA). Professor Gardea-Torresdey is very excited about this project:



Professor Jorge Gardea-Torresdey

"This was fantastic news for the University, as the Center was one of the recipients of a $24M multi-institutional grant headquartered at UCLA to investigate the fate of nanomaterials in the environment and particularly how they impact the growth rate of crops and vegetables. It means we will be able to dedicate a team of scientists, Ph.D. students and undergraduates to understand this very complex issue."

Understanding Nanoparticles on the Growth of Soybeans

A recent investigation carried out by the Center, was to study how nanomaterials affected the growth rate and development of soybeans. This study took over 12 months to complete and was just published in the August, 2012 on-line issue of the Proceedings of the National Academy of Sciences (PNAS).[2] Soybeans are the fifth-largest crop in global agricultural production and the second-largest crop in the United States. Moreover in 2009, the U.S. exported $30 billion worth of soybean, making it the ideal candidate for this kind of study.

Soil samples with nanoparticles in one of the growth chambers.

The study focused on two MNMs, cerium oxide (nano-CeO_2) and zinc oxide (nano-ZnO) powders which can enter the ecosystem relatively easily via the use of biosolids from waste water treatment plants and exhaust fumes from diesel-powered farm equipment. The researchers found that the two nanomaterials in the study could profoundly alter soil-based food crop quality and yield. They came to this conclusion by measuring stem length, leaf count and other related properties which are well-recognized metrics for studying the health of the soybean plant. In particular, for plants that were exposed to ZnO nanoparticles, high levels of zinc were distributed throughout edible plant tissues, even into the soybean itself, whereas in the case of exposure to CeO_2, the plant's nitrogen fixation capability – a process by which nitrogen in the atmosphere is converted into ammonia – was severely compromised. These results indicated that we are not only affecting the biochemistry of the plant, but it also represents a broader risk to the food chain because it would result in an increased demand for synthetic fertilizer to offset the reduced nitrogen fixation.

Professor Jorge Gardea-Torresdey.

Solution to Meet the Challenge

Professor Gardea-Torresdey has played an integral role in this study, by carrying out much of the trace element measurements using an Optima 4300 DV ICP-OES for the higher level elemental concentrations such as Ca, Na, P, Zn, Cu, Fe in the soil and the plant material and an ELAN DRC II ICP-MS instrument for determining the ultratrace levels of elements like Ce. Over the years Professor Gardea-Torresdey has come to rely exclusively on PerkinElmer for all his atomic spectroscopy needs:

> *"I have used PerkinElmer equipment for the past 35 years. In my opinion, the quality and reliability of your instrumentation is second to none. And the rare times we have needed assistance, your technical and service support organization has always been there to guide us through the problem."*

We are very pleased that Professor Gardea-Torresdey holds us in such high esteem, but he is very realistic about the role of atomic spectroscopic techniques and challenges he faces in the study of the uptake of nanoparticles. He explained that ICP-MS is an excellent technique for carrying out the ultratrace analysis of environmental samples and when used in the single particle mode,[3] or coupled with separation techniques such as field flow fractionation (FFF),[4] can generate unbelievably high quality data on the size and distribution of nanoparticles suspended in aqueous-type samples. However, he emphasized that the technique has its limitations when it comes to studying how the nanomaterial is distributed throughout the root, stem, leaf or fruit/nut/bean of the plant or in what form the metallic particle exists.

Other Analytical Techniques

He further explained how other techniques such as scanning electron microscopy (SEM) combined with energy dispersive X-ray fluorescence (EDXRF), in addition to X-ray microscopy (XRM) and Synchrotron X-ray Absorption (XAS), are all playing an important role in not only assessing the visual distribution and localized analysis of the metallic nanoparticles in various parts of the plant tissue, but they are also telling us whether it is present as an inorganic or organic species. This is extremely important when trying to understand whether the metallic nanoparticles are remaining intact, or whether they are having a deleterious effect on the genetic make-up at the plant's cellular level.

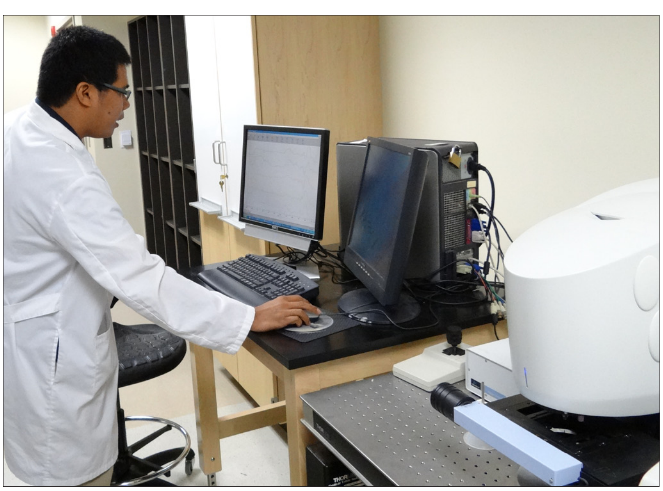
However, when a traditional bulk analysis technique like XRF, with a typical spatial resolution of 100-1000 microns (µm), is used in conjunction with SEM, it is limited in its applicability to the analysis of nanoparticles, which are all less than 100 nanometers (nm) in diameter. This means that micro-XRF techniques are far better suited to the problem, because they have the ability to sample significantly smaller areas. This is achieved by using additional optical components to reduce the diameter of the excitation beam, in order that nanoscale features on the sample can be analyzed. Unfortunately, this approach has the effect of blocking the majority of the emitted photons, which adversely affects the technique's sensitivity and trace element detection capability. As a result the micro-XRF technique is mainly used for the determination of higher concentrations analytes in very small features of a bulk material.

Synchrotron X-Ray Techniques

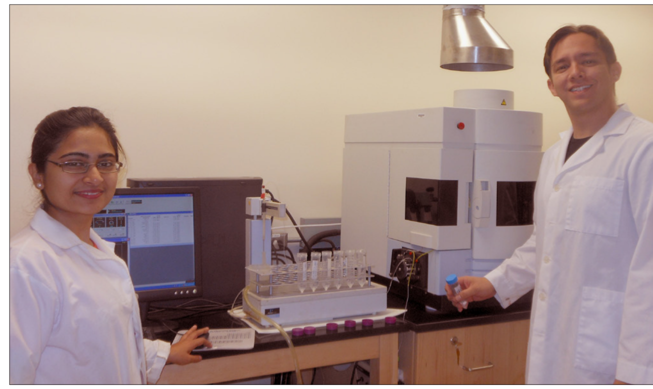
So the only way these X-ray techniques can be applied to the analysis of nanoparticles, is to utilize X-rays generated by a synchrotron light source. For those of you who are not familiar with this technique, a synchrotron generates X-rays by accelerating a beam of electrons around a circular vacuum tube at approximately the speed of light. As the electrons circle the tube, they emit energy in the form of photons of light, with wavelengths in the range of 0.1 nm – 100 nm. However, in order to produce light with enough intensity to generate high analyte signals with low background noise, the size of the vacuum tube needs to be almost the size of a football field. These are such large, specialized pieces of equipment that only 40 of these synchrotron light sources exist throughout the world and only 6 in the U.S. This fact was brought to our attention by Professor Gardea-Torresdey when he said:

"This poses a real problem for our investigations, because we need the use of a synchrotron light source coupled with these micro-X-ray techniques to complete our studies. We take all our samples either to Stanford University Synchrotron Light Source (SSRL), Lawrence Berkeley Labs, or the European Synchrotron Radiation Facility (ESRF), in Grenoble, France. This capability is critical for any group that is carrying out research in this area."

These techniques were in fact used in a recent publication by Professor Gardea-Torresdey and his co-workers to study the impact of TiO_2 on hydroponically-grown cucumber plants, and specifically to track the presence and chemical speciation of Ti within the plant tissues.[5] Results showed that at all concentrations, TiO_2 significantly increased root length. By using micro-XRF it was found that Ti was transported from the roots to the leaf trichomes (hairs), suggesting that this might be a possible secretion mechanism for the Ti. The micro-XAS spectra showed that the absorbed Ti was present as TiO_2 within the cucumber tissues, showing that the TiO_2 nanoparticles were not bio-transformed into a different species, as the plant went through its growing cycle.



Cyren Rico, one of Dr. Gardea's Ph.D. students, in the lab.



Dr. Gardea's Ph.D. students Sanghamitra Majumdar and Jose Hernandez use the ICP-OES to analyze plant samples.

Infrared Microspectroscopy

Even though Professor Gardea-Torresdey relies on PerkinElmer atomic spectroscopic techniques for the characterization of metallic nanoparticles in plant material, he also utilizes Fourier Transform-Infrared Spectroscopy (FT-IR) coupled with microspectroscopy (MS) to supplement the data. FT-IR-IMS is a rapid technique for component analysis of plants exposed to biotic stress (damage done by other living organisms) and abiotic stressors (damage caused by non-living factors like nanomaterials). Its chemical imaging capability can determine the structural changes and spatial distribution of macromolecules in plants and plant material. Within his research group, the PerkinElmer Spotlight FT-IR system combined with an IR microscope has been employed to elucidate macromolecular modifications to various parts of plant tissue including the cell wall, epidermis (skin), cortex (outer layer), and xylem (vessels that transport water), which have been treated with heavy metals and nanoparticles. In fact his research group recently published a paper using data generated by the Spotlight FT-IR-IMS system and the Optima 4300 ICP-OES to get a better understanding of the impact of chromium species on the *Parkinsonia Aculeata* plant, a spiny shrub belonging to the pea family, which is native to the deserts of West Texas.[6]

Importance of Teaching

Clearly, Professor Gardea-Torresdey is on the leading edge of this very active area of research, particularly in his role as a co-investigator in NSF/EPA funded "UC Center for Environmental Implications of Nanotechnology". But even though he has authored over 300 publications and issued 5 U.S. patents for environmental remediation, Professor Gardea-Torresdey has not forgotten about his teaching responsibilities. Over the past 20 years, he has graduated 25 Ph.D. students in Environmental Science and Engineering and Chemistry, and 29 students have received their Masters degrees under his mentorship. In fact, the State of Texas recently honored him with one of the most prestigious awards that can be bestowed on a higher education professor. The Minnie Stevens Piper Foundation announced in May of this year that he was being named one of 10 Piper Professors for excellence in teaching at the college level in Texas.

If you have ever met Professor Jorge Gardea-Torresdey, you know that this award is thoroughly deserved. He takes his chemistry and environmental science teaching responsibilities very seriously and as a result has a wonderful relationship with his students. But he is also passionate about his research into the fate of nanomaterials on the environment. We are very proud to be associated with such an outstanding researcher and teacher and also very proud to call him a PerkinElmer customer. We'll leave the last words to Jorge:

"This is a very exciting time to be a researcher in this dynamic field of environmental chemistry. There is no question that PerkinElmer instrumentation has been critical to our nanometrology studies. I can categorically say that my reputation and success in this field has been very much linked to PerkinElmer and their outstanding application, technical and service support team."

Thank you Jorge for those kind words. We very much appreciate your support also.

Further Reading

1. Nanotechnology and Engineered Nanomaterials – A Primer; A.W. Salamon, P. Courtney, I. Shuttler; PerkinElmer, Inc., www.perkinelmer.com/nano, 2010.
2. Soybean Susceptibility to Manufactured Nanomaterials with Evidence for Food Quality and Soil Fertility Interruption: J.H. Priester, Y. Gea, R.E. Mielkea, A.M. Horsta, S. Cole Moritz, K. Espinosae, J. Gelbf, S.L. Walkerg, R.M. Nisbet, Youn-Joo Ani, J.P. Schimel, R.G. Palmere, J.A. Hernandez-Viezcasc, L. Zhao, J.L. Gardea-Torresdey, and P.A. Holden; Proceedings of the National Academy of Sciences (PNAS), August, 2012; www.pnas.org/cgi/doi/10.1073/pnas.1205431109.
3. Detection of Nanoparticulate Silver Using Single Particle Inductively Coupled Plasma Mass Spectrometry: D.M. Mitrano, E.K. Leshner, A. Bednar, J. Monserud, C.P. Higgins, and J.F. Ranville; Environmental Toxicology and Chemistry, Vol. 31, No. 1, pp. 115–121, (2012).
4. An Overview of the Capabilities of Field-Flow-Fractionation Coupled with ICP-MS to Separate, Detect and Quantitate Engineered Nanoparticles: J. Ranville, K. Neubauer, R. Thomas; Spectroscopy (27 (9), pp 36-44, (2012).
5. Synchrotron Micro-XRF and Micro-XANES Confirmation of the Uptake and Translocation of TiO_2 Nanoparticles in Cucumber Plants; A.D. Servin, H. Castillo-Michel, J.A. Hernandez-Viezcas, B. Corral Diaz, J.R. Peralta-Videa, J.L. Gardea-Torresdey; Environmental Science and Technology, 46, 7637-7643, 2012.
6. Use of Plasma-based Spectroscopy and Infrared Microspectroscopy Techniques to Determine the Uptake and Effects of Chromium (III) and Chromium (VI) on *Parkinsonia Aculeate*; Y. Zhao, J.R. Peralta-Videa, M.L. Lopez-Moreno, G.B. Saupe, J.L. Gardea-Torresdey; International Journal of Phytoremediation 13(S1):17–33, 2011.

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