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DEPT OF TOXIC SUBSTANCES CONTROL
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Career and
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Jeffrey Wong, Ph.D.
Department of Toxic Substances Control
101 "I" Street
P.O. Box 806
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Re: DTSC Chemical Information Call-In – Nanometals, Nanometal Oxides, and Quantum Dots

Dear Dr. Wong,

In response to the January 4, 2011 call-in letter from the Department of Toxic Substance Control (DTSC), the University of Southern California submits the following chemical information and analytical test methods regarding the specified nanomaterials: Nano Silver, Nano Zero Valent Iron, Nano Cerium Oxide, Nano Titanium dioxide, Nano Zinc Oxide, and Quantum dots {California Health and Safety Code §57018-57020}.

The University of Southern California (USC), a leading academic research institution with affiliated hospitals, encompasses over one thousand research laboratories. To obtain the requested information from USC researchers and faculty advisors who may produce, use or oversee the study of nanoparticles, several approaches were employed. Users self-identified in response to a memo distributed to the USC research community; additionally, research protocols submitted to institutional safety committees and annual safety audit reports were reviewed. Information was verified by interview using the DTSC questionnaire as a guide.

Following is the compilation of chemical information and analytical test methods used at USC for the specified nanomaterials.

Nanomaterials at USC; their Chemical and Physical Properties

USC both produces, and purchases from commercial sources (e.g., Invitrogen and e-Biosciences), Nano Silver, Nano Titanium Dioxide, Nano Zinc Oxide and Quantum Dots (but not Nano Zero Valent Iron or Nano Cerium Oxide,) for research and development purposes within USC facilities. The USC materials are not employed for commercial applications.

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Maximum annual quantities produced in USC labs or procured from commercial vendors range from two micrograms to 80 grams, stored in volumes from five micro-milliliters to 100 milliliters. Quantities used in any one laboratory procedure are very minute.

Physical shapes of these materials are most commonly spherical, but also include rod and tripod configurations. Known densities range from one to seven grams per cubic centimeter; surface areas vary from 10 to 300 square nanometers. Nanoparticles are created or purchased only in liquid or solid form. All nanoparticle manipulations are conducted in liquid phase, submerged in a liquid or contained within a controlled atmosphere, minimizing the possibility of airborne emission.

Particle surfaces are modified and subsequently functionalized with amines, carboxylates, polyethylene glycol, mercaptopropionic acid, or dodecanethiol. The materials handled at USC pose little risk of flammability, oxidation or explosion due to the minimal quantities and low concentrations used. While high solvent use present a flammability hazard that may be exacerbated by the reactivity of nanoparticles, no current protocols use a high volume of solvent matrices, and USC Environmental Health and Safety closely monitors the chemical inventory. Currently used forms of the nanoparticles and nano-solutions are considered stable for the laboratory processes employed and for storage in existing glass containers.

All waste involving nanoparticles and nano-contaminated materials is treated as toxic chemical waste, and incinerated through an approved waste treatment facility.

Analytical Test Methods

Paragraphs 1) through 7) below describe current USC applications of the specified nanoparticles, and the associated quantitative and qualitative test methods in use. Referenced publications, included with this memo, provide additional detail regarding analytical test methods.

1) Nano silver and quantum dots are used for in-vitro and in-vivo cancer treatment studies to evaluate biodistribution and molecular behavior. These nanomaterials are purchased from Invitrogen in micromolar liquid concentrations in water, buffer or solvent matrices. Nano silver, and quantum dots created from cadmium selenide or zinc sulfide, are functionalized with a peptide coating. Pre- and post-processing nanomaterial concentration is determined using UV/Vis optical absorption measurements. At peak absorbance wavelengths, the cross-sectional absorption of the nanomaterial in various matrices is compared to a blank sample to infer concentration using Beer-Lambert law, reported in moles/liter. Cross-section absorption values for different nanomaterials are available in scientific literature (Attachment A, "Experimental Determination of the Extinction Coefficient of CdTe, CdSe, and CdS Nanocrystals").

   Other nanomaterial detection methods in liquid matrices include size exclusion chromatography analysis and dynamic light scattering. Nanomaterial presence in a non-liquid matrix (e.g., in cells or tissue) is primarily evaluated using transmission electron microscopy (TEM). These characterization methods are used for qualitative assessment, and no concentrations are inferred.

2) A comparative study of two analytical techniques uses nano silver clusters. The first technique produces particles in a cryogenic helium superfluid matrix (nanodroplets). Clusters are deposited on a TEM grid using a continuous beam of helium droplets for the assembly and surface

deposition of nano silver; the surface is imaged for study and quantification. Images of clusters on the amorphous carbon substrates are obtained at short deposition times to determine the size distribution of the metal clusters.

The second helium droplet technique, under development, is an in-Situ nano silver structure determination employing laser scattering of clusters in helium. Supporting journal articles are included with this memo (Attachment B, "Surface Deposition and Imaging of Large Ag Clusters Formed in HE Droplets" and C, "Photosoabsorption of Ag_N(N ~ 6-6000) Nanoclusters Formed in Helium Droplets: Transition from Compact to Multicenter Aggregation").

3) Nano titanium dioxide is viewed under TEM in a study of particle sizes and shapes.

4) Zinc oxide nanoparticles are involved in a study of translocation mechanisms in biological cells, tissues and organs. Primary cultured rat alveolar epithelial cell monolayers (RAECMs) are exposed to ZnO nanoparticles at several μg/ml concentrations. Cellular effects of interest, measured as functions of concentration and time, include morphologic changes, lactate dehydrogenase release, cell membrane integrity, intracellular reactive oxygen species, and mitochondrial activity. A Millicell-ERS quantifies transepithelial electrical resistance, and potential difference in the presence or absence of varying ZnO concentrations in apical fluid, as a function of exposure time. ZnO nanoparticle suspensions in water are sonicated, vortexed, then evaluated by Zetasizer to determine the zeta potential on the surface charge of ZnO nanoparticles. The ICP-MS method is also utilized for ZnO nanoparticle quantitation (Attachment D, "Alveolar Epithelial Cell Injury Due to Zinc Oxide Nanoparticle Exposure").

5) Quantum dots used in a kidney filtration study involves amine functionalization on a modified nanoparticle surface (neutral charge; surface area: 20 nm). 100 microliters of a micro-molar nanocrystal solution in a DMSO matrix are conjugated with a fluorescent dye, and injected into small animals. Quantum dot concentration in the animals' circulatory system is determined by fluorescent intensity during in-vivo animal imaging. Upon completion of the imaging, the animals are sacrificed, their circulatory system flushed with saline, and the perfusate collected as a chemical waste. Nanoparticle waste materials are shipped to a waste treatment site for incineration.

6) An optical behavior study uses lead sulfide quantum dots produced in liquid form through chemical synthesis (3 gram per year). These spherical nanoparticles (size: 6 nm; surface area: 12-300 nm^2; density: 7.6 gr/cm^3) are functionalized with carboxylate coating. The particles are oxidized by thermal exposure and are soluble in organic solvent. Estimates of both particle size and size distribution are obtained using TEM. Visible light absorption optical spectroscopy is used to estimate quantum dot concentration in toluene. The amount of light absorbed by quantum dots at a given wavelength is determined by the material's properties and concentrations, from which quantity can be rendered.

7) An analytical study produces approximately 80 grams per year of cadmium selenide quantum dots dissolved in solvent, e.g., 150 mg/ml toluene, functionalized with fatty acids or a fatty amine coating. Solid crystals are obtained by drying a measured volume of solution, weighing the residue, and performing Thermo Gravimetric Analysis (TGA). The high temperature TGA mass represents the pure inorganic, and its concentration in a given solvent can be calculated.

This innovative analytical test method is soon to be published in scientific journals, and will be provided to DTSC upon request.

Additional References

USC Nano Researchers have been extremely cooperative in responding to the DTSC's appeal for information, and expressed their willingness to furnish additional information upon request.

Respectfully submitted,

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Attachment A

Chem. Mater. **2003,** *15,* 2854–2860

Experimental Determination of the Extinction Coefficient of CdTe, CdSe, and CdS Nanocrystals

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Received February 20, 2003. Revised Manuscript Received April 26, 2003

The extinction coefficient per mole of nanocrystals at the first exitonic absorption peak, ϵ, for high-quality CdTe, CdSe, and CdS nanocrystals was found to be strongly dependent on the size of the nanocrystals, between a square and a cubic dependence. The measurements were carried out using either nanocrystals purified with monitored purification procedures or nanocrystals prepared through controlled etching methods. The nature of the surface ligands, the refractive index of the solvents, the PL quantum yield of the nanocrystals, the methods used for the synthesis of the nanocrystals, and the temperature for the measurements all did not show detectable influence on the extinction coefficient for a given sized nanocrystal within experimental error.

Attachment 3

THE JOURNAL OF
PHYSICAL CHEMISTRY A

ARTICLE

pubs.acs.org/JPCA

Surface Deposition and Imaging of Large Ag Clusters Formed in He Droplets

Evgeny Loginov, Luis F. Gomez, and Andrey F. Vilesov*

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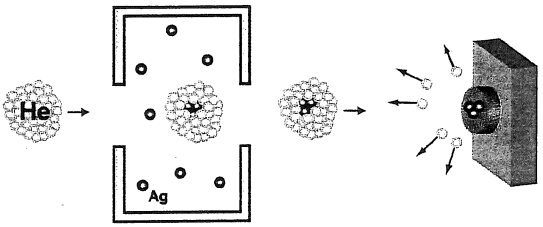
ABSTRACT: The utility of a continuous beam of He droplets for the assembly and surface deposition of Ag_N clusters, $\langle N \rangle \sim 300-6000$, is studied with transmission electron microscopy. Images of the clusters on amorphous carbon substrates obtained at short deposition times have provided for a measure of the size distribution of the metal clusters. The average sizes of the deposited clusters are in good agreement with an energy balance based estimate of Ag_N cluster growth in He droplets. Measurements of the deposition rate indicate that upon impact with the surface the He-embedded cluster is attached with high probability. The stability of the deposited clusters on the substrate is discussed.



Photoabsorption of $Ag_N(N \sim 6\text{–}6000)$ Nanoclusters Formed in Helium Droplets: Transition from Compact to Multicenter Aggregation

Evgeny Loginov,[1] Luis F. Gomez,[1] Naihao Chiang,[1] Avik Halder,[2] Nicholas Guggemos,[2] Vitaly V. Kresin,[2,*] and Andrey F. Vilesov[1,2,†]

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(Received 16 September 2010; published 10 June 2011)

Ag_N clusters with up to thousands of atoms were grown in large He droplets and studied by optical spectroscopy. For $N \lesssim 10^3$ the spectra are dominated by a surface plasmon resonance near 3.8 eV and a broad feature in the UV, consistent with absorption by individual metallic particles. Larger clusters reveal unexpectedly strong broad absorption at low frequencies, extending down to ≈ 0.5 eV. This suggests a transition from single-center to multicenter formation, in agreement with estimates of cluster growth kinetics in He droplets. Moreover, the spectra of large clusters develop a characteristic dispersion profile at 3.5–4.5 eV, indicative of the coexistence of localized and delocalized electronic excitations in composite clusters, as predicted theoretically.

DOI: 10.1103/PhysRevLett.106.233401 PACS numbers: 36.40.Mr, 36.40.Vz, 61.46.Bc, 78.67.Sc

Attachment D

PubMed Search

Display Settings: Abstract

Am J Respir Crit Care Med. 2010 Dec 1;182(11):1398-409. Epub 2010 Jul 16.

Final Version ajrccm.atsjournals.org

Alveolar epithelial cell injury due to zinc oxide nanoparticle exposure.

Kim YH, Fazlollahi F, Kennedy IM, Yacobi NR, Hamm-Alvarez SF, Borok Z, Kim KJ, Crandall ED.

Will Rogers Institute Pulmonary Research Center, University of Southern California, Los Angeles, California, USA.

Abstract

RATIONALE: Although inhalation of zinc oxide (ZnO) nanoparticles (NPs) is known to cause systemic disease (i. metal fume fever), little is known about mechanisms underlying injury to alveolar epithelium.

OBJECTIVES: Investigate ZnO NP-induced injury to alveolar epithelium by exposing primary cultured rat alveola epithelial cell monolayers (RAECMs) to ZnO NPs.

METHODS: RAECMs were exposed apically to ZnO NPs or, in some experiments, to culture fluid containing ZnC free Zn released from ZnO NPs. Transepithelial electrical resistance (R(T)) and equivalent short-circuit current (I(were assessed as functions of concentration and time. Morphologic changes, lactate dehydrogenase release, cel membrane integrity, intracellular reactive oxygen species (ROS), and mitochondrial activity were measured.

MEASUREMENTS AND MAIN RESULTS: Apical exposure to 176 μg/ml ZnO NPs decreased R(T) and I(EQ) of RAECMs by 100% over 24 hours, whereas exposure to 11 μg/ml ZnO NPs had little effect. Changes in R(T) and caused by 176 μg/ml ZnO NPs were irreversible. ZnO NP effects on R(T) yielded half-maximal concentrations of approximately 20 μg/ml. Apical exposure for 24 hours to 176 μg/ml ZnO NPs induced decreases in mitochondrial and increases in lactate dehydrogenase release, permeability to fluorescein sulfonic acid, increased intracellular I and translocation of ZnO NPs from apical to basolateral fluid (most likely across injured cells and/or damaged paracellular pathways).

CONCLUSIONS: ZnO NPs cause severe injury to RAECMs in a dose- and time-dependent manner, mediated, a part, by free Zn released from ZnO NPs, mitochondrial dysfunction, and increased intracellular ROS.

PMID:20639441[PubMed - indexed for MEDLINE] PMCID: PMC3029930[Available on 2011/12/1]

Publication Types, MeSH Terms, Substances, Grant Support

LinkOut - more resources

Alveolar Epithelial Cell Injury Due to Zinc Oxide Nanoparticle Exposure

Yong Ho Kim[1,2], Farnoosh Fazlollahi[1,8], Ian M. Kennedy[9], Nazanin R. Yacobi[1,8], Sarah F. Hamm-Alvarez[6], Zea Borok[1,2,3], Kwang-Jin Kim[1,2,5,6,7], and Edward D. Crandall[1,2,4,8]

[1]Will Rogers Institute Pulmonary Research Center, [2]Departments of Medicine, [3]Biochemistry and Molecular Biology, [4]Pathology, [5]Physiology and Biophysics, [6]Pharmacology and Pharmaceutical Sciences, [7]Biomedical Engineering, and [8]Chemical Engineering and Materials Science, University of Southern California, Los Angeles, California; and [9]Department of Mechanical and Aeronautical Engineering, University of California, Davis, California

Rationale: Although inhalation of zinc oxide (ZnO) nanoparticles (NPs) is known to cause systemic disease (i.e., metal fume fever), little is known about mechanisms underlying injury to alveolar epithelium.

Objectives: Investigate ZnO NP–induced injury to alveolar epithelium by exposing primary cultured rat alveolar epithelial cell monolayers (RAECMs) to ZnO NPs.

Methods: RAECMs were exposed apically to ZnO NPs or, in some experiments, to culture fluid containing $ZnCl_2$ or free Zn released from ZnO NPs. Transepithelial electrical resistance (R_T) and equivalent short-circuit current (I_{EQ}) were assessed as functions of concentration and time. Morphologic changes, lactate dehydrogenase release, cell membrane integrity, intracellular reactive oxygen species (ROS), and mitochondrial activity were measured.

Measurements and Main Results: Apical exposure to 176 μg/ml ZnO NPs decreased R_T and I_{EQ} of RAECMs by 100% over 24 hours, whereas exposure to 11 μg/ml ZnO NPs had little effect. Changes in R_T and I_{EQ} caused by 176 μg/ml ZnO NPs were irreversible. ZnO NP effects on R_T yielded half-maximal concentrations of approximately 20 μg/ml. Apical exposure for 24 hours to 176 μg/ml ZnO NPs induced decreases in mitochondrial activity and increases in lactate dehydrogenase release, permeability to fluorescein sulfonic acid, increased intracellular ROS, and translocation of ZnO NPs from apical to basolateral fluid (most likely across injured cells and/or damaged paracellular pathways).

Conclusions: ZnO NPs cause severe injury to RAECMs in a dose- and time-dependent manner, mediated, at least in part, by free Zn released from ZnO NPs, mitochondrial dysfunction, and increased intracellular ROS.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

It is well documented that inhaled zinc oxide (ZnO) nanoparticles (NPs) can be associated with systemic adverse effects (e.g., metal fume fever). However, the mechanisms underlying interactions between ZnO NPs and alveolar epithelium, and their adverse effects on alveolar epithelium, are not fully understood.

What This Study Adds to the Field

ZnO NP–induced injury to alveolar epithelium appears to be mediated at least in part by Zn^{2+} derived from ZnO NPs, increased intracellular reactive oxygen species, and mitochondrial dysfunction.

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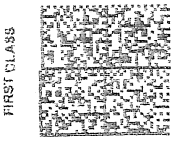

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