Detection and Surface reactivity of
Engineered Nanoparticles in Water

by

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ABSTRACT

Engineered nanoparticles (NPs) pose risk potentials, if they exist in water systems at significant concentrations and if they remain reactive to cause toxicity. Three goals guided this study: (1) establishing NP detecting methods with high sensitivity to tackle low concentration and small sizes, (2) achieving assays capable of measuring NP surface reactivity and identifying surface reaction mechanisms, and (3) understanding the impact of surface adsorption of ions on surface reactivity of NPs in water.

The size detection limit of single particle inductively coupled plasma spectrometry (spICP-MS) was determined for 40 elements, demonstrating the feasibility of spICP-MS to different NP species in water. The K-means Clustering Algorithm was used to process the spICP-MS signals, and achieved precise particle-noise differentiation and quantitative particle size resolution. A dry powder assay based on NP-catalyzed methylene blue (MB) reduction was developed to rapidly and sensitively detect metallic NPs in water by measuring their catalytic reactivity.

Four different wet-chemical-based NP surface reactivity assays were demonstrated: “borohydride reducing methylene blue (BHMB)”, “ferric reducing ability of nanoparticles (FRAN)”, “electron paramagnetic resonance detection of hydroxyl radical (EPR)”, and “UV-illuminated methylene blue degradation (UVMB)”. They gave different reactivity ranking among five NP species, because they targeted for different surface reactivity types (catalytic, redox and photo reactivity) via different reaction mechanisms. Kinetic modeling frameworks on the assay outcomes revealed two surface electron transfer schemes, namely the “sacrificial reducing” and the “electrode discharging”, and separated interfering side reactions from the intended surface reaction.
The application of NPs in chemical mechanical polishing (CMP) was investigated as an industrial case to understand NP surface transformation via adsorbing ions in water. Simulation of wastewater treatment showed CMP NPs were effectively removed (>90%) by lime softening at high pH and high calcium dosage, but 20-40% of them remained in water after biomass adsorption process. III/V ions (In$^{\text{III}}$, Ga$^{\text{III}}$, and As$^{\text{III/V}}$) derived from semiconductor materials showed adsorption potentials to common CMP NPs (SiO$_2$, CeO$_2$ and Al$_2$O$_3$), and a surface complexation model was developed to determine their intrinsic complexation constants for different NP species. The adsorption of As$^{\text{III}}$ and As$^{\text{V}}$ ions onto CeO$_2$ NPs mitigated the surface reactivity of CeO$_2$ NPs suggested by the FRAN and EPR assays. The impact of the ion adsorption on the surface reactivity of CeO$_2$ NPs was related to the redox state of Ce and As on the surface, but varied with ion species and surface reaction mechanisms.
To my beloved family, who understand, accompany, and support me on my adventurous journey of pursuing dreams.

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CHAPTER 1
INTRODUCTION

In 1959, physicist Dr. Richard Feynman gave the lecture “There’s Plenty Room at the Bottom”, delivering the idea to manipulate individual atoms of matter to enable powerful and precise chemical synthesis. Rooted from Feynman’s idea, the term “nanotechnology” was formally introduced about 30 years later, opening up the door to fruitful inventions and revolutions brought about by nanotechnology in fields of material, electronics, medicine and health, and energy. Nowadays, it is commonly acknowledged that a nanomaterial is one which has at least one dimension in the range 1-100 nm. Aside from the benefits brought by nanomaterials, potential unintended risks of them to human health and the environment raised concerns among toxicology and environmental researchers, policy makers, and the public society. Investments by the U.S. Government into nanotechnology-related environmental health and safety (EHS) have been cumulated to >$ 1 billion from 2006 to 2015, and still account for a significant percentage in the National Nanotechnology Initiative (NNI)’s 2018 budget request. The goal of responsible development of nanotechnology stimulated active research in the nano-EHS fields along with pursuing the technology’s benefits.

Risk assessment of nanomaterials is the core of nano-EHS studies. A risk is established via an effective exposure to a hazard. Among broad types of nanomaterials, nanoparticles (NPs, i.e., particles between 1-100 nm in size) have particular high mobility. Engineered NPs can be discharged into industrial wastewater or released into municipal wastewater from commercial products, and eventually pose exposure to the ecological systems and humans via aqueous environment. Depending on the different phases on
the lifecycle, engineered NPs are present a variety of water systems may contain NPs. For examples, some oxide NPs (e.g., SiO$_2$, CeO$_2$ and Al$_2$O$_3$), which are used at thousands of tons per year$^{11}$ in the semiconductor chemical mechanical polishing (CMP) process, may experience a lifecycle including different water systems, e.g., the CMP slurry solution, industrial waste streams, municipal wastewater, and natural water (Figure 1.1). Scenarios where NPs are suspended in water, physically forming a colloidal system, are of importance to nano-EHS evaluation.

![Figure 1.1. Critical points in a lifecycle of oxide NPs used in the CMP process by the semiconductor industry.](Image)

Detection of NPs in water is the first need to evaluate exposures to NPs, yet current analytical techniques face challenges due to NPs’ small sizes and low concentration level in natural water. Engineered NPs are predicted to be at ng/L to µg/L levels$^{12}$ in surface water because of massive dilution during their release, challenging the detection limit of many existing techniques to detect NPs, e.g., the traditional inductively coupled plasma (ICP) with optical emission spectroscopy (OES) or mass spectroscopy (MS), and scanning or transmission microscopy (SEM and TEM). Quantifying NP size and characterizing their surface in water are also demanded. Traditional techniques sizing
particles in-situ, e.g., the dynamic light scattering (DLS), are limited by the low NP concentration, at which light is not strongly scattered to implicate particle size. DLS is also poor in quantifying a size distribution of a poly-dispersed colloidal system. Classical material surface analysis techniques, e.g., the aforementioned SEM and TEM, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), etc., require ultra high vacuum (UHV) condition for solid-sate measurements, creating a gap between analytical results obtained under UHV and the actual NP properties in water. Analytical methodologies that can directly detect and characterize engineered NPs in water media are thus greatly needed to investigate their occurrence in the aqueous environment.

Despite the definition based on small sizes, applications and implications of NPs are usually derived from their properties that may or may not be related to their sizes. Surface reactivity is a property of NPs reflecting their tendency to undergo a chemical reaction on their surface. NPs with certain high surface reactivity enabled novel technologies in antibacterials\textsuperscript{13}, catalysis\textsuperscript{14}, surface polishing\textsuperscript{15}, and medicine\textsuperscript{16}. Surface reactivity of NPs toward some reactions also controls their toxicity. For example, some NPs are reactive in reaction in which the reactive oxygen species (ROS) are formed, causing oxidative stress and thus toxicity to microorganisms and cells\textsuperscript{16,17}. Such knowledge infers a new strategy of identifying NPs in water by their surface reactivity.

Identifying NPs based on surface reactivity may lead to more accurate measures of risk-relevant exposures to NPs in natural water, because the surface reactivity and risk potential of the NPs are related. To date, the majority methods to evaluate NPs’ risks are biotic assays to assess NPs’ toxicity using living cells\textsuperscript{18} or organisms at multiple trophic levels\textsuperscript{19-21}, yet they are often expensive and/or time-consuming due to the requirement of
standardized and complicated laboratory setups. Because a NP’s toxicity is often associated with its capability of causing oxidative stress\textsuperscript{22}, abiotic and chemical-based analytical assays to assess NPs’ reactivity can be used to infer their potential toxicity. Many abiotic NP reactivity assessment assays exist, despite different names they may have, e.g., oxidative stress or potential assays\textsuperscript{22}, ROS detection assays\textsuperscript{23}, etc., yet they are commonly employed via initiating chemical reactions at the NP-water interface, with either a reactant or a product detected by an instrument to suggest the reactivity. Many studies, a good summary of which is recently reviewed\textsuperscript{22}, used one or more of these assays to analyze one or more species of NPs. While consistent and standardized assay protocols were followed to obtain results in a study, the reaction mechanisms were scarcely examined for different assays toward different NP species. Moreover, results of one NP reactivity by different assays, or, vice versa, of one assay toward different NPs, were sometimes directly combined for statistics (e.g., ranking a reactivity order)\textsuperscript{16}, but such statistics would misrepresent if the reaction mechanisms had varied across assays or NP species. Different statistics of NPs’ reactivity by different assays were also shown in some studies\textsuperscript{18, 24-27} without further mechanistic justification. As a result, a clear-cut strategy of using surface reactivity assays is needed in order to truly understand the meaning of the analysis results of one or more assays.

Studying water-suspended NPs in practical cases is a benefit to examine their real environmental impact. When the chemistry of a water solution changes, NPs in the water may transform with regard to their surface coatings\textsuperscript{18}, crystal structure\textsuperscript{18}, size\textsuperscript{28}, etc. Many existing studies on risks and toxicology of engineered NPs were based on as-synthesized materials without taking account of the transformations occurring to them,
leaving a gap between the laboratory testing results and the effect in the actual environment. A common transformation occurring to engineered NPs in water systems is the adsorption of molecules and/or ions onto their surfaces. Adsorption of natural organic matter (NOM), which is ubiquitous in natural water systems, in water could happen to metal (e.g., Ag, Au, etc)\textsuperscript{29,30} and metal oxide (e.g., TiO\textsubscript{2}, SiO\textsubscript{2}, ZnO, etc.)\textsuperscript{18} NPs, and influence their stability\textsuperscript{18} and bioavailability\textsuperscript{31}. However, adsorption-caused transformation to engineered NPs in other water systems was scarcely examined. For example, the aforementioned engineered oxide NPs used in the CMP process may sorb other chemicals during their using phase and in the industrial wastewater (Figure 1.1), where the sorbate chemicals can be derived from the semiconductor wafer materials including toxicant species such as arsenic (As). Recent technology applied novel III/V group semiconductor material (i.e., materials composed of elements in group III and V, such as GaAs, InP, etc.) due to their high electron mobility and direct band-gap structure\textsuperscript{32,33}, leading to the release of III/V ions (e.g., ionic species of In\textsuperscript{III}, Ga\textsuperscript{III}, and As\textsuperscript{III/V}) into the industrial wastewater along with NPs. Investigation of the transformation caused by III/V ions sorption to CMP NPs would be beneficial to evaluating these NPs’ risks and toxicity in the subsequent environment they exist in.

Ion adsorption caused surface transformation can change NPs’ surface reactivity. NPs with NOM adsorbed onto their surface showed decreased surface reactivity\textsuperscript{18,34} or toxicity\textsuperscript{35-37}. However, there is also evidence showing certain molecule adsorption to NP surface could enhance its surface reactivity\textsuperscript{38}. Essentially, transformation of a NP influence its surface reactivity via altering the NP’s certain intrinsic characters (Figure 1.2). Common intrinsic characters include surface groups and charge, electronic structure,
phase structure, etc. These characters can influence or control the thermodynamics or kinetics of surface reactions and thus the NP reactivity. Using the Quantitative Structure-Activity Relationship (QSAR) model, some researchers found the correlation of NP reactivity toward cytotoxicity and oxidative stress to the enthalpy of formation of the cation species constituting the NP\(^{39}\) and the NP material’s band gap\(^{18,40}\), respectively. However, QSAR models are mostly based on empirical statistics. More mechanistic justifications of the connections among surface transformation, intrinsic characters and surface reactivity of NPs (Figure 1.2) would be beneficial to understanding the variance of NP surface reactivity with the variance of environment conditions.

![Diagram of the relationship among “transformation”, “intrinsic character” and “surface reactivity” of NPs. Each arrow means a variance caused by the component at the tail to the component at the head.](image)

**Figure 1.2.** Diagram of the relationship among “transformation”, “intrinsic character” and “surface reactivity” of NPs. Each arrow means a variance caused by the component at the tail to the component at the head.

Under the themes of detection and surface reactivity of NPs in water, objectives of this study include providing effective analytical tools to detect NPs in water, exploring new
strategies of identifying NPs by surface reactivity, and examining surface reactivity variance with water conditions. NPs used in the CMP process by the semiconductor industry were particularly focused as a case study of engineered NPs used in industry, while other common engineered NPs, e.g., Au and Ag, were also used in some method development studies. A tutorial review of CMP NPs is presented in Chapter 2 to emphasize the tremendous usage, critical characteristics, and potential risks of CMP NPs in the current industry market. Covered in Chapters 3-9, the following specific research questions were addressed.

1. **Question**: How appropriate is single particle ICP-MS (spICP-MS) technology toward a wide range of engineered metallic NPs?

   **Strategy**: Sophisticated analytical and data handling methodologies were developed to evaluate the NP size detection limit of spICP-MS for 40 metal elements, deconvolute the instrument signal, and enable quantitative resolution of NP sizes. (Chapters 3 and 4)

2. **Question**: Can NPs be detected by heir surface reactivity?

   **Strategy**: An economic dry powder assay kit was developed rapidly to detect conductive material based NPs in water based on a catalysis reaction. (Chapter 5)

3. **Question**: How to assess the surface reactivity of NPs for electron transfer reactions?

   **Strategy**: The Ferric Reducing Assay for Nanoparticles (FRAN) was developed to evaluate the surface reactivity of NPs for electron transfer and the assay reaction mechanism was justified via theoretical modeling. (Chapter 6)
4. **Question**: Can typical industry onsite and municipal wastewater treatment processes efficiently remove NPs from water?

*Strategies*: Softening and activate sludge treatment processes were simulated in laboratory to investigate the removal efficiency toward CMP NPs. (Chapter 7)

5. **Question**: Do III/V ions in water adsorb to and transform engineered NPs used in CMP?

*Strategy*: Adsorption experiment was performed to investigate the adsorption of III/V ions onto CMP NPs and a theoretical sorption model was developed to examine the adsorption mechanism and thermodynamic parameters. (Chapter 8)

6. **Question**: Does ion adsorption to NPs change their reactivity?

*Strategy*: Using the case of CeO$_2$ NPs adsorb arsenic ions as an example, reactivity assays were used to investigate the reactivity difference between pristine (without ion adsorption) and transformed (with ion adsorption) CeO$_2$ NPs. (Chapter 9)

After chapter studies in response to specific research questions above, findings were synthesized at the end of dissertation (Chapter 10) with a focus on the philosophy of using NP surface reactivity assays. Different reactivity assays were compared and analyzed for functioning schemes. Recommendations on designing and using surface reactivity assays were made and used to consider potential future research needs (Chapter 11).
CHAPTER 2

REVIEW: PHYSICAL, CHEMICAL, AND IN VITRO TOXICOLOGICAL CHARACTERIZATION OF NANOPARTICLES IN CHEMICAL MECHANICAL PLANARIZATION SUSPENSIONS USED IN THE SEMICONDUCTOR INDUSTRY: TOWARDS ENVIRONMENTAL HEALTH AND SAFETY ASSESSMENTS

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- My author contribution: I participated the content design, literature review, experimental work (Figure 2.2, 2.3, 2.8, and 2.9), and a part of writing.

2.1. Abstract

This tutorial review focuses on aqueous slurries of dispersed engineered nanoparticles (ENPs) used in chemical mechanical planarization (CMP) for polishing wafers during manufacturing of semiconductors. A research consortium was assembled to procure and conduct physical, chemical, and in vitro toxicity characterization of four ENPs used in CMP. Based on input from experts in semiconductor manufacturing, slurries containing fumed silica (f-SiO$_2$), colloidal silica (c-SiO$_2$), ceria (CeO$_2$), and alumina (Al$_2$O$_3$) were selected and subsequently obtained from a commercial CMP vendor to represent realistic ENPs in simplified CMP fluids absent of proprietary stabilizers, oxidants, or other chemical additives that are known to be toxic. ENPs were stable in suspension for months, had highly positive or negative zeta potentials at their slurry working pH, and had mean diameters measured by dynamic light scattering (DLS) of 46±1 nm for c-SiO$_2$, 148±5 nm for f-SiO$_2$, 132±1 nm for CeO$_2$, and 129±2 nm for Al$_2$O$_3$, all of which were larger than the sub 100 nm diameter primary particle sizes measured by electron microscopy. The concentration of ENPs in all four slurries that caused 50% inhibition
(IC-50) was greater than 1 mg/mL based on *in vitro* assays using bioluminescence of the bacterium *Aliivibrio fischeri* and the proliferation, viability, and integrity of human cells (adenocarcinomic human alveolar basal epithelial cell line A549). In contrast to recent reports, we observed similar toxicological characteristics between c-SiO₂ and f-SiO₂, and the materials exhibited similar X-ray diffraction (XRD) spectra but different morphology observed using electron microscopy. The ENPs and CMP slurries used in this study have been made available to a number of other research groups, and it is the intention of the consortium for this paper to provide a basis for characterizing and understanding human and environmental exposures for this important class of industrial ENPs.

### 2.2. Introduction

Numerous industrial processes use large quantities of engineered nanoparticles (ENPs) in ways that do not directly end up in consumer products but instead lead to ENPs becoming discharged to sewer systems that flow to municipal wastewater treatment plants (WWTPs) and eventually to the environment as treated wastewater effluent discharged to streams and biosolids landfilled, applied to land, or incinerated. These industrially-related nanomaterials have tremendous commercial benefit but may pose workplace and/or environmental risks. For example, alumina (Al₂O₃), ceria (CeO₂), and silica (SiO₂) represent an important class of ENPs that are used in a variety of products and manufacturing applications ⁴¹. Although these materials, particularly Al₂O₃ and amorphous SiO₂, are generally believed to be relatively innocuous, it is remarkable how little can be stated about their environmental, health, and safety (EHS) characteristics. Toxicity evaluations that have been conducted on Al₂O₃, CeO₂, amorphous SiO₂, and other ENPs generally vary widely depending on the particular aspects of the particles
being assessed (e.g., particle size, physiochemical properties, and dispersion state) as well as the particular toxicity assessment method being utilized (e.g., dose, cell lines, exposure protocols, and assay end points)\textsuperscript{42,43}. Outside of carbon nanotubes (CNTs) and TiO\textsubscript{2}, there is presently little regulatory guidance and a general lack of directly actionable information or standards (e.g., particle-specific threshold limit values for occupational exposure) to guide the safe use of ENPs in occupational settings. The effectiveness of safe handling practices, efficacy of personal protective equipment, and engineering controls are predicated on knowledge of toxicity thresholds and degree of exposure, which in turn are predicated on the availability of validated procedures for their quantification in relevant media. Moreover, there is a range of alternative workplace exposure metrics such as mass concentration, particle number, particle surface area concentration, and particle size distribution whose toxicological significance and inter-relation are not well understood\textsuperscript{18,44}.

Efforts to evaluate ENP behavior in aqueous systems likewise face difficult metrology challenges\textsuperscript{19,45}. There is a need for standardized methodologies that can discriminate between dissolved and nano-sized particulates, measure particle number and size distributions, and differentiate between ENPs and naturally occurring nanoparticles. The removal of CeO\textsubscript{2}, SiO\textsubscript{2}, and Al\textsubscript{2}O\textsubscript{3} nanoparticles (NPs) during biological wastewater treatment has received some research attention\textsuperscript{46-52}, but less information exists on ENP removal during physical-chemical treatment processes often employed as on-site industrial wastewater treatment. The available literature indicates that Al\textsubscript{2}O\textsubscript{3} and CeO\textsubscript{2}, but not necessarily SiO\textsubscript{2}, are typically removed well by conventional biological wastewater treatment processes. ENPs removed from the wastewater accumulate in
biosolids and precipitated sludges, where their fate and ultimate stability are important considerations when determining environmental effects of these ENPs. Moreover, naturally occurring Al$_2$O$_3$ and SiO$_2$ NPs are believed to be common in natural water systems, and their fate is intertwined with a variety of geological and biological processes. An understanding of the background concentrations and geochemical processes that govern the occurrence and behavior of these naturally occurring NPs, and how biota respond to them, is needed to provide context for interpreting the impacts of ENP wastewater discharges and determining appropriate discharge levels. In light of these information gaps, we have pursued a multi-university collaborative research effort, the initial stages of which have centered on developing and validating basic characterization methods for Al$_2$O$_3$, CeO$_2$, and amorphous SiO$_2$.

One principal use of Al$_2$O$_3$, CeO$_2$, and amorphous SiO$_2$ nanoparticles is chemical mechanical planarization (CMP) where particles in the form of abrasive slurries are used to polish wafers when fabricating integrated circuits in the semiconductor industry. In this application, the ENPs are used to manufacture the product (semiconductor chips), but are not incorporated into the product. CMP nanoparticles constituted nearly 60% of the total $1$ billion worldwide market for nano-powders in 2005.

This paper aims to characterize ENPs in model CMP suspensions, to understand the ramifications of ENP properties on potential exposure and toxicity to environmental systems and humans, and to discuss research needs associated with the next generation of semiconductor manufacturing. Four model CMP fluids, each being the simplest formulation to generate a stable suspension of ENPs, were obtained from a major commercial slurry manufacturer. These slurries were thoroughly characterized via
physical-chemical means, including intra-laboratory comparisons of nano-measurements. Large volumes of these model CMP suspensions were procured and have been made available to several investigators that study workplace exposure, human and ecosystem toxicity, industrial treatment efficiencies, sewer discharge and wastewater treatment removal, fate and transport, and fundamental aspects of the nano-bio interface. In vitro assays were conducted using different cell lines to compare the relative toxicity of ENPs used in CMP. Finally, a discussion identifies potential scenarios for workplace exposure and flux of ENPs from CMP processes into the sewer system.

2.2.1 Nanoparticle Use in Semiconductor Production

Al₂O₃, CeO₂, and amorphous SiO₂ ENPs are used in a variety of applications beyond CMP. For example, Al₂O₃ is used in the production of tires, paper, catalyst, polymers, and personal care products; CeO₂ is used in fuel additives, catalyst, and biomedical applications; and SiO₂ is used in the manufacture of tires, paper, paints, coatings, catalyst, cement, polymers, and personal care products. Annual production volume of Al₂O₃, CeO₂, and SiO₂ has been estimated and presented in a number of recent publications but varies widely, as indicated in Table 2.1.

Table 2.1. Estimates of the annual production (metric tons/year) of Al₂O₃, CeO₂, and SiO₂.

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<tr>
<td>Al₂O₃</td>
<td>&gt; 200,000</td>
<td>18,500 - 35,000</td>
<td>200,000</td>
<td>55 - 5,500</td>
</tr>
<tr>
<td>CeO₂</td>
<td>&lt; 10,000</td>
<td>7,500 - 10,000</td>
<td>10,000</td>
<td>5.5 - 550</td>
</tr>
<tr>
<td>SiO₂</td>
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CMP fluids remove materials by a combination of chemical and mechanical (or abrasive) actions to achieve highly smooth and planar material surfaces. CMP, which can be used to planarize a variety of materials including dielectrics, semiconductors, metals, polymers, and composites, is one of the most important semiconductor processes for achieving the performance goals of modern microprocessor and memory chips.\textsuperscript{62,63} Fig. 2.1 shows a typical CMP operation scheme where a robotic arm loads a semiconductor wafer onto a rotating plate, a quantity of CMP slurry is dispensed, and a pad is engaged in a polishing action that uses the slurry to planarize the wafer surface. The polishing step is followed by additional automated rinse and pad cleaning steps. Following the CMP operation, the height of the wafer surface is typically uniform to within $\pm 1$ nm, or less. Because even the smallest scratch or surface imperfection can ruin the geometry of the integrated circuit being fabricated into a wafer, CMP slurries are typically crafted with highly controlled particle size distributions, along with additives, as described below.
The inorganic oxide abrasive particles are an important constituent for CMP slurries, with the three most commonly used abrasive particles being $\text{Al}_2\text{O}_3$, amorphous $\text{SiO}_2$, and $\text{CeO}_2^{55,64}$. Depending on the particular application, particle size in CMP slurries can vary from 20 to 200 nm, and trends are toward CMP particles $< 100$ nm in size to achieve highly polished surfaces $^{64,65}$. In a given slurry formulation, these particles usually have a uniform shape and size.

Amorphous $\text{SiO}_2$ can be distinguished as fumed silica ($f$-$\text{SiO}_2$) and colloidal $\text{SiO}_2$ ($c$-$\text{SiO}_2$) based on the synthesis methods. $f$-$\text{SiO}_2$ is formed in a pyrogenic process by oxidizing chlorosilane ($\text{SiCl}_4$) at high temperature $^{66}$. $c$-$\text{SiO}_2$ is formed in liquid phase by precipitating a Si precursor (e.g., $\text{Na}_2\text{SiO}_3$) $^{67}$. A widely referenced method of synthesizing $c$-$\text{SiO}_2$ that uses a tetraalkyl silicate as the Si precursor was presented by Stöber $^{68}$. $\text{CeO}_2$ NPs used in CMP slurries have a crystalline structure, thus often yielding sharp edges, corners, and apexes $^{69}$. $\text{Al}_2\text{O}_3$ NPs used in CMP slurries can be $\alpha$-$\text{Al}_2\text{O}_3$, $\beta$-$\text{Al}_2\text{O}_3$, $\delta$-$\text{Al}_2\text{O}_3$, or fumed $\text{Al}_2\text{O}_3^{70}$. $\text{Al}_2\text{O}_3$ is softer than $\text{SiO}_2$ or $\text{CeO}_2$ and sometimes can be coated with a hard surface such as $\text{SiO}_2$ to enhance semi-conductor polishing $^{64}$.

In addition to the metal oxide NPs, CMP slurries may also contain a number of additives, such as pH adjustment agents, oxidizers, dispersants, complexing agents, surfactants, biocides, and corrosion inhibitors, as summarized in Table 2.2. For instance, one additive may serve to aid in the selective dissolution and solubilization of a material that is present at the wafer surface, and a second additive may serve to protect or inhibit
the removal of a different material that is present on the exposed wafer surface during the CMP process. Likewise, surface active agents may be added to influence particle surface charge and help maintain a stable particle dispersion.

**Table 2.2. Typical CMP slurry additives**

<table>
<thead>
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<th>Component</th>
<th>Function</th>
<th>Examples</th>
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<tbody>
<tr>
<td>Abrasive particles</td>
<td>Polish surface</td>
<td>Al₂O₃, CeO₂, amorphous SiO₂</td>
</tr>
<tr>
<td>pH adjust</td>
<td>Adjust and buffer pH</td>
<td>HCl, KOH, HNO₃, NH₄OH, H₃PO₄, TMAH, NH₄OH, buffers</td>
</tr>
<tr>
<td>Complexing agents</td>
<td>Solubilize dissolved metals</td>
<td>Amino acids (glycine, etc), carboxylic acids (citric acid, etc)</td>
</tr>
<tr>
<td>Oxidizers</td>
<td>Promote metal removal via oxidative dissolution</td>
<td>H₂O₂, Ferric nitrate, KIO₄, KMnO₄, etc.</td>
</tr>
<tr>
<td>Corrosion inhibitors</td>
<td>Selectivity against removal of certain surfaces,</td>
<td>Benzotriazole (BTA), 3-amino-triazole</td>
</tr>
<tr>
<td></td>
<td>corrosion inhibition</td>
<td></td>
</tr>
<tr>
<td>Surface active</td>
<td>Maintain metal oxide particles in a dispersed state</td>
<td>Polyacrylic acid, polyethylene glycol polymer, cetyl trimethyl ammonium bromide, polyethylene cetyl ether</td>
</tr>
<tr>
<td>organics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High MW polymers</td>
<td>Flocculant and/or coat abrasives to &quot;cushion&quot; their</td>
<td>High MW Polyethylene oxide</td>
</tr>
<tr>
<td></td>
<td>abrasiveness</td>
<td></td>
</tr>
<tr>
<td>Biocides</td>
<td>Prevent biological growth</td>
<td>Hydrogen peroxide and others</td>
</tr>
</tbody>
</table>

In a manufacturing line, a combination of CMP slurries is fed to a fleet of CMP tools. In each tool, the wafers undergo the CMP process and are rinsed with deionized (DI) water, and further chemicals may be added to clean and/or recondition the wafer.
polishing pads. A typical wafer production step might involve applying between 0.2 and 0.8 L of CMP slurry, 1 to 2 L of rinse water, and another 5 or more liters of pad cleaner and rinse water. The total quantity of wastewater generated per wafer undergoing CMP polishing may be on the order of 10 or more liters. The effluent wastewater contains the original slurries, associated rinse waters, and dissolved and particulate material that is removed from the wafer during the CMP operation. The thickness of material removed from the wafer surface may vary from a few nanometers to 100 or more nanometers. If, for instance, a 100 nm blanket layer of Cu is removed from a 300 mm diameter wafer surface, 64 mg Cu/wafer would be added to the wastewater.

Detailed characterization of the compositional change that a CMP slurry undergoes throughout a given CMP process has not been reported in the literature. However, there are reports that the particle size distribution in CMP waste is typically broader than the particle size distribution of the virgin slurry \cite{71, 72}, which is probably due to the release of small particles from the wafer surface, the formation of aggregates, or both.

### 2.3. Analytical & Experimental Methods

#### 2.3.1. CMP Fluid Procurement

Through an industry-university collaboration supported by the Semiconductor Research Corporation (SRC) and the SRC Engineering Research Center for Environmentally Benign Semiconductor Manufacturing, our consortium was able to work with a CMP slurry provider (Cabot Inc.) to design and procure large volumes of four fairly simple, industrially relevant, and well characterized CMP slurries. Because the slurries were custom synthesized, there were no intellectual property challenges to
overcome nor any proprietary chemical additives. Table 2.3 summarizes the physiochemical properties of the four CMP slurries, including information provided by the manufacturer (shown in the top row). Acidic $c$-SiO$_2$ was prepared in acetic acid while $f$-SiO$_2$ was in a basic solution of KOH. The CeO$_2$ slurry was provided without any additives. The Al$_2$O$_3$ slurry was provided in dilute nitric acid. CMP NPs were dispersed using a common industry method involving a high-energy dispersion machine. 

Table 2.3. Summary of key characteristics for the model CMP slurry composition.

<table>
<thead>
<tr>
<th>Name</th>
<th>$c$-SiO$_2$</th>
<th>$f$-SiO$_2$</th>
<th>CeO$_2$</th>
<th>Al$_2$O$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer Reported</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Material</td>
<td>Colloidal SiO$_2$</td>
<td>Fumed SiO$_2$</td>
<td>CeO$_2$</td>
<td>Al$_2$O$_3$</td>
</tr>
<tr>
<td>- Composition</td>
<td>3% SiO$_2$</td>
<td>5% SiO$_2$</td>
<td>1% CeO$_2$</td>
<td>3% Al$_2$O$_3$</td>
</tr>
<tr>
<td>- Additive</td>
<td>&lt; 1% acetic acid</td>
<td>&lt; 1% KOH</td>
<td>none</td>
<td>&lt;1% nitric acid</td>
</tr>
<tr>
<td>- pH</td>
<td>2.5 – 4.5</td>
<td>10</td>
<td>3-4</td>
<td>4.5-5.0</td>
</tr>
<tr>
<td>- Particle size (nm)</td>
<td>50-60</td>
<td>120-140</td>
<td>60-100</td>
<td>80-100</td>
</tr>
<tr>
<td>Primary metal concentration</td>
<td>27 g Si/L</td>
<td>50 g Si/L</td>
<td>9.6 g Ce/L</td>
<td>29 g Al/L</td>
</tr>
<tr>
<td>Dissolved organic carbon (DOC; mg/L)</td>
<td>320.5 ± 0.5</td>
<td>4.84 ± 0.03</td>
<td>1.90 ± 0.03</td>
<td>6.77 ± 0.18</td>
</tr>
<tr>
<td>Other additives</td>
<td>801.9 ± 1.3 mg/L acetic acid</td>
<td>--</td>
<td>--</td>
<td>134.7 ± 0.8 mg NO$_3$/L BDL* for nitrite</td>
</tr>
<tr>
<td>Diameter by SEM (nm)</td>
<td>37 ± 7</td>
<td>38 ± 14</td>
<td>43 ± 16</td>
<td>85± 21</td>
</tr>
<tr>
<td>Diameter by TEM (nm)</td>
<td>36 ± 9</td>
<td>ND#</td>
<td>39 ± 19</td>
<td>38 ± 16</td>
</tr>
<tr>
<td>Mean diameter by DLS</td>
<td>46 ± 0.2</td>
<td>148 ± 5.1</td>
<td>132 ± 0.1</td>
<td>129± 1.6</td>
</tr>
</tbody>
</table>
2.3.2. Particle Sizing and Zeta Potential Analysis

Particle sizing was conducted using Brookhaven ZetaPALS or Bl-200SM and Malvern ZS ZEN3600 instruments, different laser wavelengths (659, 488, 633 nm), and different scattering angles (90°, 90°, 173°). Refractive indexes were 1.765 for Al₂O₃, 2.200 for CeO₂, and 1.542 for SiO₂. The electrophoretic mobilities (EPMs) of the slurries were measured using either the Malvern Zetasizer Nano ZS ZEN3600 or the Brookhaven ZetaPALS Analyzer. The EPMs of the slurries were then converted into zeta (ζ) potentials using the Smoluchowski equation. Slurries were prepared for both measurements using either DI water or 10 mM ionic strength solutions (adjusted with either NaCl or NaHCO₃), and the EPM measurements were conducted over a broad range of pH conditions (3–11).

Nanoparticle Tracking Analysis (NTA) was performed using a NanoSight LM10 instrument (NanoSight Ltd., Amesbury, United Kingdom) equipped with a 405 nm (blue) laser source, a temperature-controlled chamber, and a scientific CMOS camera (Hamamatsu). A video (30 s) of each sample was collected and analyzed using NTA 2.3
Build 011 software (NanoSight Ltd.). The concentration (particles per mL) was calculated as the average of three replicates.

Single particle ICP-MS (spICP-MS) is an emerging nano-analysis to size and quantify NPs in liquid matrices. An ICP-MS instrument (Perkin-Elmer NexION 300q) was placed in time-resolved analysis mode in which the signal was recorded every dwell time (integration time of one reading by the detector) of 10 ms. Thus, the detection of a particle gave a pulse signal. The sample flow rate was 0.6–0.7 mL/min. Si$^{29}$, Ce$^{140}$, and Al$^{27}$ were used as the analyte isotopes for SiO$_2$, CeO$_2$, and Al$_2$O$_3$ NPs.

### 2.3.3. Chemical Digestion and Analysis of CMP Nanoparticles

All NPs were digested using a microwave-assisted system and a suitable digestion mixture. Tetramethylammonium hydroxide (4 mL of TMAH, 25%) was used to digest SiO$_2$ NPs samples (11 mL). For CeO$_2$ and Al$_2$O$_3$, 2 mL HF (50%), 2 mL HCl (30%, J. T. Baker), and 6 mL HNO$_3$ (70%) were added to the sample, and the total volume was adjusted to 15-20 mL. The microwave was operated as follows: ramping to 150°C in 15 min; next ramping to 180°C in 15 min; and holding at 180°C for 30 min. Metals were analyzed using an ICP-MS (Thermo X series II ICP-MS).

### 2.3.4. Separation of Nanoparticles from Dissolved Ions

Two methods were employed and compared to separate NPs from dissolved ions. First, a centrifugal ultrafiltration device (Millipore, Darmstadt, Germany), which combines an ultrafiltration (UF, 30 kDa nominal molecular weight limit) membrane and a centrifugation tube, was adopted as a tool to separate NPs and the ionic species (liquid phase). Samples in the centrifugal UF devices (10 mL) were centrifuged at 5000 ×g for 30 min. To
demonstrate the effectiveness of the centrifugal UF device to separate NPs and dissolved species, a commercial SiO$_2$ nanoparticle (PM1040, Nissan Chemical, Houston) and an ionic SiO$_2$ standard solution (HACH, Loveland) were used. Three samples containing: 1) 1000 µg/L (as SiO$_2$) ionic standard and 1000 µg/L (as SiO$_2$) NPs; 2) 1000 µg/L NPs; and 3) 1000 µg/L ionic standard were tested in triplicate. The recoveries of filtrate and concentrate for all cases were $\geq 94\%$. In the second method, slurries were centrifuged in a two-step procedure to remove NPs and provide supernatants for analysis. Slurry aliquots (1.5 mL) were centrifuged at 20,000 ×g for 60 min, and 1.2 mL of the supernatant was collected. Subsequently, the supernatant was centrifuged at 100,000 ×g for 60 min.

2.3.5. Anions and Dissolved Organic Carbon

Acetate was monitored using an Agilent 7890A gas chromatography system (Agilent Technologies, Santa Clara, CA, USA) fitted with a Restek Stabilwax-DA column (30 m x 0.35 mm, ID 0.25 µm) and a flame ionization detector. Nitrate was analyzed by suppressed conductivity–ion chromatography using a Dionex IC-3000 system (Sunnyvale, CA, USA) fitted with a Dionex IonPac AS11 analytical column (4 mm x 250 mm) and an AG11 guard column (4 mm x 40 mm). The flow rate was 1 mL/min, and run time was 10 min per sample. An isocratic mobile phase containing 30 mM KOH was employed. The dissolved organic carbon (DOC) was determined using a Shimadzu TOC-500A total organic carbon analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA).
2.3.6. Solid State Characterization

Scanning electron microscopy systems equipped with an energy dispersive X-ray microanalysis system (SEM/EDX) (FEG ESEM Philips XL30 with EDAX system) and high-resolution transmission electron microscopy systems (HR-TEM) coupled with energy dispersive X-ray spectroscopy (EDX) (Philips CM200 FEG HR-TEM/STEM) were used. X-ray diffraction was performed using an Agilent-Gemini X-Ray diffractometer with a molybdenum source in a Bragg–Brentano arrangement. All slurries were dried to a constant mass at 125ºC prior to analysis. The Fourier transform infrared spectroscopy (FTIR) analysis was performed in an attenuated total reflectance (ATR) spectrophotometer (Varian 600 FT-IR) in the range of 400-4000 cm\(^{-1}\) at a resolution of 1 cm\(^{-1}\).

2.3.7. CMPs Catalytic Activity Analysis

The catalytic activity of CMPs (c-SiO\(_2\) and f-SiO\(_2\)) using our Colorimetric Assay to Detect Engineered nanoparticles (CADE) technique described elsewhere in detail. Briefly, CADE employs a dye, methylene blue (MB), and a reducing agent, sodium borohydride (BH\(_4\)), to colorimetrically assess the catalytic activity of nanoparticles in an aqueous media (see SI for more information).

2.3.8. In Vitro Assays

*In vitro* assays were conducted using marine bacterium *Aliivibrio fischeri* (MicroTox\textsuperscript{®} Bioasssay) and adenocarcinomic human alveolar basal epithelial cells (A549 cell viability, ATCC\textsuperscript{®} CCL-185™). The dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit (Sigma Aldrich) was used to quantitatively
evaluate the cell viability of A549 cells after exposure to the CMP slurries, and the Lactate dehydrogenase (LDH) kit (Sigma Aldrich) was used to evaluate the membrane integrity of A549 cells. Proliferation of A549 cells was measured by two methods: determination of cell numbers by staining nucleic acids with crystal violet dye (CV), or direct counting of cell numbers with a Coulter counter. Details of these methods are provided in Supplemental Information.

2.4. Results

2.4.1. Chemical Composition of Nanoparticles and CMP Slurries

The “model slurries" employed in the initial phase of this work represent the simplest possible stable dispersion of particles in water. As such, they contrast with the complexity of commercial CMP slurries that are formulated with a wide variety of ingredients, including a number of chemicals that are known to be toxic on their own and surface active and redox active chemicals (Table 2.2) that are intended to influence particle behavior. Bulk primary metal concentrations in the as-received slurries ranged from 9.6 to 50 g/L and agreed with the manufacturer reported data of 1 to 5% (Table 2.3). Digested slurries were analyzed for additional elements (Fig. 2.2) to quantify the presence of impurities, especially elements potentially toxic to cells. Each slurry contained different ratios of trace elements relative to the primary CMP NP. Fig. 2.2 presents the concentrations of elements in the slurry that were detected at concentrations above laboratory blanks. Calcium and zinc were detected as impurities in all the samples at levels of 10 to 100 mg/L, which is roughly 1000 times lower than the primary metal elements (Si, Ce, or Al) that were present in the slurry at 9.6 to 50 g/L. The SiO2-based
slurries contained aluminum at 1 to 20 mg/L and titanium, iron, and small amounts of either gold, magnesium, and/or copper at concentrations below 5 mg/L. The high concentration of potassium in the f-SiO₂ slurry is associated with addition of KOH (Table 2.1). The Al₂O₃ slurry contained less than 1 mg/L iron, magnesium, titanium, zirconium, copper, and chromium. The CeO₂ slurry contained the largest number of detectable elements, including hafnium, palladium, silver, and gold, which may have been co-occurring elements residual from the mining and separation process. Likewise, impurities in the feedstocks for SiO₂ and Al₂O₃ probably cause impurities in the slurries and could possibly be used as unique markers for tracing the fate of CMP NPs in the environment as has been attempted for other types of NPs (e.g., TiO₂ NPs from sunscreens into rivers 79). Analysis of metals was performed at two different universities, and comparable results were obtained.

Figure 2.2. Concentrations of elements other than the primary metal (Si, Ce or Al) in the four CMP slurries. Open bars represent the element concentration analyzed after a
full sample digestion by ICP-MS; shaded bars represent the detectable concentration of these elements in the digestion blank control sample. A significant concentration difference between the slurry sample and blank control sample demonstrates the existence of the corresponding element in the slurry sample.

In order to differentiate elements associated with the CMP particles from elements dissolved in solution, supernatants after high-speed centrifugation or permeates of 30kDA ultrafilters were analyzed (Fig. 2.3). Both preparation methods yielded very similar concentrations, thus validating their use in laboratories that may only have access to one method (Fig. 2.3). In general, the same elements detected in the digested, as-received slurry were also detectable in the NP-free solutions. In all cases, the concentrations of all detectable elements in the aqueous aliquots of the slurries were far below levels detected in the as-received digested slurry, and most trace elements were below 1000 µg/L. This suggests that the NPs partially dissolved and released ionic forms of these elements. Whereas levels of zinc were high in some CMP slurry NPs (Fig. 2.2), they were much lower in free solution (Fig. 2.3). The as-received slurries were diluted many fold prior to toxicity testing, and the predicted effect of dissolved zinc was below levels of concern for toxicity. Elevated levels of potassium in the f-SiO$_2$ slurry and supernatant were notable but expected because the slurry was adjusted to basic pH with KOH (Table 2.3).
Figure 2.3. Concentration of elements other than the major metals (Si, Ce or Al) in the liquid phase of four CMP slurries. Open bars represent liquid sample prepared by centrifugal ultrafiltration; solid bar represent liquid sample prepared by high speed centrifugation.

Three of the slurries had low levels of dissolved organic carbon (1.9 to 6.7 mg/L), but the c-SiO$_2$ dispersion had much higher DOC (320 mg/L) because high levels of acetic acid were present (~800 mg/L) (Table 2.3). Nitrate was detected (135 mg NO$_3^-$/L) in the Al$_2$O$_3$ slurry that contained nitric acid. Both the acetic acid and nitrate were associated with pH control agents added to adjust pH to levels where the NPs should be stable in suspension.

2.4.2. Solid-State Analysis of Nanoparticles

XRD spectra of the NPs in the CMP slurries were obtained to characterize their crystalline nature and purity (Fig. 2.4). The two SiO$_2$ samples gave similar spectra, showing a broad halo in XRD pattern, clearly indicating an amorphous SiO$_2$ structure.
The CeO$_2$ slurry shows strong peaks at (111), (200), and (220) for CeO$_2$ that are consistent with literature$^{80}$. The Al$_2$O$_3$ slurry shows a strong peak at (111) and weaker peaks at (311) and (400), as observed elsewhere$^{81}$.

Figure 2.4. X-ray diffraction pattern of SiO$_2$, CeO$_2$, and Al$_2$O$_3$ slurries after drying.

Figure 2.5 shows FTIR analysis of the slurries. Broad stretching around 3000–3500 cm$^{-1}$ is attributed to OH stretch from water, and the peak around 1650 cm$^{-1}$ is attributed to C=C stretching and indicates the presence of organic contaminants in the slurries. Colloidal and fumed SiO$_2$ showed a band around 1120 cm$^{-1}$ corresponding to asymmetric stretching vibration of Si-O-Si band$^{82}$ in which the bridging oxygen atom moves parallel to the Si-Si lines in the opposite direction to their Si neighbors and a second band around ~470 cm$^{-1}$ corresponding to Si-O rocking vibration where the oxygen atom moves perpendicular to the Si-O-Si plane. FTIR spectra for the other two slurries show Ce-O and Al-O stretching in the region of 500–750 cm$^{-1}$$^{80,81}$. 
Differentiating forms of silica is important to the semiconductor industry which uses both fumed and colloidal silica for CMP operations. Fumed silica has been used since CMP processes were first developed in the 1980s and provides a comparatively inexpensive and rapid means of planarizing oxide surfaces. However, it is stable only at alkaline pH and generally provides a lower quality surface than colloidal silica, which became available in the 1990s. Fumed silica particles are normally multi-fractal, irregularly shaped with sharp edges and surfaces and is produced via high temperature combustion of SiCl₄ with oxygen, whereas colloidal silica, made via a sol-gel process using either water glass (Na or K silicates) or tetramethoxysilane (TMOS) and tetraethoxysilane (TEOS), is available in the form of uniform spherical particles over a wide range of pH and size distributions and is generally used where a highly smooth surface is required. Despite very different uses by industry, it can be difficult to differentiate f-SiO₂ from c-SiO₂ using common solid-state characterization methods.
However, literature incorporating nuclear magnetic resonance analysis suggests that the surface hydroxyl concentration and formation of bi-nuclear surface complexes with metals in solution reacting with the SiO$_2$ surfaces (i.e., proximity of surface group) are perhaps more important than the morphological structure.$^{83, 84}$

### 2.4.3. Shape and Size Characterization

Table 2.3 summarizes CMP NP sizing information and shows sizing of primary particles via electron microscopy differs from measurements of the NPs in dispersions where aggregates are present.

Fig. 2.6 shows imaging and sizing results using electron microscopy. The c-SiO$_2$ NPs are nearly spherical, compared with more angular and rectangular shaped CeO$_2$ NPs. The $f$-SiO$_2$ NPs appeared fused together to an extent not apparent with SiO$_2$. The angular shape and non-spherical morphology of three of the four NPs was initially unexpected because CMP NPs are routinely described as nearly spherical polishing agents. However, the non-spherical nature of some CMP NPs influences their ability to scratch surfaces being polished.$^{85}$ The Al$_2$O$_3$ NPs appeared to be aggregates of smaller primary particles having a broad range of diameters. Using both TEM and SEM images, the particle size distributions of the shortest dimension of the NPs were made (Fig. 2.6 and Table 2.3). The primary particles in the two SiO$_2$ slurries were similar (30 to 40 nm), and were also similar to the CeO$_2$ NPs. The broader range of primary particle sizes for the Al$_2$O$_3$ resulted in a larger mean diameter and larger size distribution than the other NPs. Differences between SEM and TEM analysis was low (Table 2.3), except for the Al$_2$O$_3$ NPs, which may have been associated with the modest number of primary particles counted given the large observed distribution in diameters.
Figure 2.6. TEM images and TEM based particle size distributions for CMP nanoparticles. The size distribution histogram for colloidal silica, ceria, or alumina is obtained by sizing > 50 particles under the corresponding TEM images. Fumed silica particles were not sized because of their coalesced state.

The particle size distribution of slurries diluted approximately 10:1 with DI water was also analyzed by DLS in six separate laboratories, resulting in the following mean diameters: 46±10 nm for c-SiO2, 137±10 nm for CeO2, 141±28 nm for Al2O3, and 158±16 nm for f-SiO2. Statistically, there is little difference between the three larger NPs, but in all cases the order of mean sizes was consistent with c-SiO2 having the smallest diameter and Al2O3, CeO2, and f-SiO2 the largest and relatively similar diameter. Differences in absolute diameters may be attributed to six different operators and three instrument models that used different laser wavelengths (659, 488, 633 nm) and different scattering angles (90°, 90°, 173°). The hydrodynamic diameter of the CMP NPs was larger compared to the size determined for the primary particles by electron microscopy.
c-SiO$_2$ was an exception, and the particle size determined with these two techniques was relatively similar. In contrast, the DLS sizing of the f-SiO$_2$ resulted in an average size nearly 2-3 fold higher than determined for the primary NPs, reflective of the aggregated nature of the primary NPs into a dendritic morphology, and the hydrodynamic size of the CeO$_2$ NPs was approximately two-fold higher compared to the primary particle size determined by electron microscopy (Table 2.3, Fig. 2.6).

The NTA trends in NP size from smallest to largest were consistent with DLS measurements, with exception of CeO$_2$, which was sized smaller by NTA (79 nm) than by DLS (132 nm). NTA analysis also determines particle number concentrations, which were (#particles/ x10$^{12}$ per mL): 4.7 ± 0.2 for c-SiO$_2$, 13.0 ± 0.3 for f-SiO$_2$, 3.9 ± 0.2 for CeO$_2$, and 22.0 ± 1.1 for Al$_2$O$_3$.

An additional sizing method, spICP-MS, was also employed to evaluate the particle size distribution in the CMP slurries (Fig. 2.7). In this method, the cloud of ions generated from the ablation of a single particle is detected as a pulse above the background by utilizing short dwell times. Calculated mean diameters from spICP-MS analysis were 144 ± 26, 60 ± 28, and 66 ± 23 nm for f-SiO$_2$, CeO$_2$, and Al$_2$O$_3$, respectively. Limitations brought on by molecular interfering ions (e.g., dinitrogen) hindered the sizing of the SiO$_2$ NPs below 100 nm and biased the diameter toward a larger mean size than actually present in the sample. The average particle sizes determined for f-SiO$_2$ using spICP-MS, DLS, and NTA are very similar (Table 2.3). The size of c-SiO$_2$ was below current spICP-MS detection limits for silica, indicating it has smaller diameter than f-SiO$_2$, which is consistent with DLS, NTA, and SEM/TEM.

Advances in micro-second dwell time ICP-MS technology and analysis may be capable
of improving size resolution for $c$-SiO$_2$ or other NPs with high background noise or poor detection resolution $^{86,87}$. Mean diameters for Al$_2$O$_3$ and CeO$_2$ by spICP-MS were similar to each other and between electron microscopy methods and DLS or NTA results. The size distributions of NPs based upon spICP-MS lose some resolution relative to background below ~25 nm for Al$_2$O$_3$ and CeO$_2$, which biases the mean to slightly larger sizes. The size ranges near the peak of the Gaussian distributions (Fig. 2.7) are in closer agreement with SEM/TEM results.
Figure 2.7. Size distributions of Al₂O₃ (A), CeO₂ (B), and f-SiO₂ (C) CMP slurries by single particle ICP-MS.

Few studies compare size measurements across as many techniques on the same number of different, well-dispersed NPs as present in these CMP slurries. Figure 2.8 compares mean diameters reported by the manufacturer to those measured by the various techniques reported herein. Within any single evaluation technique, the size trends from smallest to largest are generally consistent, but significant differences in absolute size...
vary dramatically. This points to both the bias and assumptions of each technique (e.g., hydrated radius, mineral structure, density). Whereas DLS and NTA detect the hydrodynamic size, spICP-MS and SEM/TEM are not impacted by the hydrated nature of NPs and thus hydrodynamic state partially accounts for observed differences in mean diameters reported in Table 2.3 and shown in Figure 2.8.

![Comparison of the average particle size values determined in this study for the various CMP slurries using different techniques with values reported by the slurry manufacturer.](image)

**Figure 2.8.** Comparison of the average particle size values determined in this study for the various CMP slurries using different techniques with values reported by the slurry manufacturer.

### 2.4.4. Stability of Nanoparticles in Different Matrices

Surface charge is a critical factor influencing the stability (i.e., aggregation potential) of NP dispersions. Zeta potential measurements of the CMP slurries (Table 2.3), diluted with ultrapure water to concentrations suitable for zeta potential analysis, resulted in highly positively charged NPs (> +40 mV) for CeO$_2$ and Al$_2$O$_3$ or very negatively charged (< -20 mV) for the two SiO$_2$ NPs. Zeta potentials this far from zero indicate very
stable NP suspensions. The manufacturer claims that the NPs in the CMP slurries would remain stable for at least two years if stored in the dark at room temperature. Fig. 2.9 shows both the zeta potentials and DLS measurements obtained at the same time. Separate measurements performed six months later showed no discernible differences in zeta potential or DLS.

**Figure 2.9.** Dynamic light scattering (bars) and zeta potential analysis (squares) of CMP slurries (ambient slurry pH). Polydispersity index (Pdi) values are shown for DLS data.

Figure 2.10 shows the electrophoretic mobilities (EPMs) of four CMP NPs as a function of pH. At pH higher than 2, the c-SiO₂ and f-SiO₂ were negatively charged, and the magnitude of surface charge generally increased with increasing pH. The f-SiO₂ was almost neutral at pH 2, which is consistent with the reported pH of zero point of charge (pH_{ZPC}) of 2.0 for SiO₂. The pH_{ZPC} of c-SiO₂ was lower than 2. CeO₂ and Al₂O₃ colloids were both positively charged at pH lower than 7.0, and their surface charges reversed when pH was elevated to 11. By extrapolation, the pH_{ZPC} for CeO₂ and Al₂O₃ colloids were determined to be approximately 8 and 10, respectively, which are consistent with the reported pH_{ZPC} for CeO₂ (8.1) and for Al₂O₃ (8.2–10).
Figure 2.10. EPMs of four CMP nanoparticles in 1 mM NaCl solutions prepared at different pH conditions. The error bars represent the standard deviation of triplicates.

2.4.5. Surface Reactivity of Silica Nanomaterials

The CADE uses the reduction rate of methylene blue by borohydride, which depends directly on the catalytic activity of nanoparticles in CMP. Results in SI showed a statistical difference at the 95% confidence interval between the catalytic reactivity in a control from the catalytic activity induced by \( f \)- or \( c \)-SiO\(_2\) present at 100 ppm. The \( f \)-SiO\(_2\) nanoparticles were also more catalytically active than the \( c \)-SiO\(_2\) nanoparticles from CMP slurries. At fixed CMP mass concentration, the surface charge of nanoparticles may have an influence on the catalytic reactivity of CMPs. We believe negatively charged particles, \( c \)-SiO\(_2\) and \( f \)-SiO\(_2\), with a surface charge of -21 and -50 mV, may electrostatically repel BH\(_4\) molecules to the surface of the particle, which then inhibit the reduction of MB, resulting in high \( \beta \) values. According to Azad et al. when BH\(_4\) absorbs to the surface of nanoparticles, it creates a negatively charged layer that attracts cationic organic dyes,
such as CADE\textsuperscript{91}. This electrostatic attraction or repulsion between particle surfaces and the reducing agents increase or decrease the reduction rate of MB.

2.4.6. In Vitro Toxicity

The potential toxicity of model CMP slurries to bacteria \textit{A. fischeri} was assessed using the Microtox\textsuperscript{®} assay. Microtox\textsuperscript{®} assay is a highly sensitive test that is widely used to monitor the toxicity of effluents and evaluate the toxic effects of chemical compounds\textsuperscript{92}. The results of the test have been shown to correlate well with toxicity values for fish, crustaceans, and algae for a wide range of organic and inorganic chemicals. The results in Table 4 indicate that the CMP NPs were not or only mildly inhibitory to the metabolic activity of \textit{A. fischeri} at high concentrations ranging from 0.7 to 1.3 mg/mL, depending on the assay. No effect was observed when cells were exposed to \textit{f-SiO}\textsubscript{2} and CeO\textsubscript{2} NPs. This observation is similar to the CADE analysis shows that \textit{f-SiO}\textsubscript{2} posses higher catalytic reactivity than \textit{c-SiO}\textsubscript{2}, where surface redox reactivity in nanoparticles is a key emerging property related to potential cellular toxicity. Exposure to a concentration of 1.3 mg/mL of the \textit{c-SiO}\textsubscript{2} and Al\textsubscript{2}O\textsubscript{3} suspensions resulted in 37.6 and 28.4\% inhibition, respectively.

For the eukaryote toxicity tests with A549 cells, the IC-50 values for proliferation and plasma membrane integrity were in the range 1 to 4 mg/mL for both \textit{c-SiO}\textsubscript{2} and \textit{f-SiO}\textsubscript{2}. The viability tests using MTT resulted in IC-50 values for both forms of SiO\textsubscript{2} in the range of 1 to 2 mg/mL. The CeO\textsubscript{2} and Al\textsubscript{2}O\textsubscript{3} had negligible effect in any of the A549 cell assays at the highest tested slurry concentrations. In both prokaryotic and eukaryotic cell assays, the CMP NPs were unstable and aggregated when in biological medium. This was especially pronounced for CeO\textsubscript{2} and Al\textsubscript{2}O\textsubscript{3}. 

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With the prokaryote *A. fischeri*, none of the CMP metal oxides NPs resulted in as much as a 50% decrease in bioluminescence in the Microtox® assay after a 30 min exposure. Thus, CMP NPs do not appear to be very toxic to *A. fischeri*. However, there was a statistically significant reduction in bioluminescence from bacteria exposed to *c*-SiO$_2$ and Al$_2$O$_3$ at 1.3 mg/mL for 30 min (37.6 and 28.4% inhibition, respectively). It is therefore likely that there could be more significant adverse effects at higher doses and longer exposure times. Literature results confirm the low toxicity of silica, ceria, and alumina NPs towards *A. fischeri*. No appreciable effects were observed in Microtox® assays supplemented with nominal concentrations of CeO$_2$ and Al$_2$O$_3$ up to 0.1 mg/mL. Similarly, amorphous SiO$_2$ NPs of different diameters (50 and 100 nm) were not toxic to *A. fischeri* at a concentration as high as 1 mg/mL.

There are many studies in the literature on the toxicity of SiO$_2$ NPs towards various cultured mammalian cells with a wide range of toxicity values reported. Factors that influence silica NP toxicity include cell type, differences in the physical and surface properties of the NPs, and the type of toxicity assay. The IC-50 values reported here for the various assays with A549 cells (in the range of 1 to 4 mg/mL for a 24 hour exposure) are consistent with data in the literature. For example, Lin et al. (2006) observed at most a 17% reduction in viability after exposing A549 cells to 15 nm SiO$_2$ NPs for 24 hours, and at 72 h exposure the viability was reduced by about half. Yu et al. (2011) reported no effect of *c*-SiO$_2$ colloidal silica on A549 cells in a 24 h exposure up to the highest concentration tested of 0.5 mg/mL. Yu et al. (2011) also reported that a murine macrophage cell line (RAW 264.7) responded to *c*-SiO$_2$ with an IC-50 of ~200 µg/mL, emphasizing that different cell types may respond differently to SiO$_2$. Using the
MTT assay and an impedance-based assay, Otero-Gonzalez et al. (2012) found IC-50 values in the range of 0.17-0.23 mg/mL with human bronchial epithelial cells (16HBE14o-) exposed to amorphous SiO$_2$ for 48 h. Zhang et al. (2012) reported that f-SiO$_2$ prepared by a high temperature process was significantly more toxic than c-SiO$_2$ prepared by a low temperature process. They attributed the result to different surface chemistries generated by the different synthesis methods. Nevertheless, we observed little difference in the toxicity of colloidal and fumed SiO$_2$ in a variety of assays with A549 cells.

The biological effects of CeO$_2$ have been enigmatic because of reports that it is both an oxidant capable of generating reactive oxygen species (ROS) and an anti-oxidant capable of protecting cells from oxidants by consuming ROS. The different oxidation properties are attributed to the presence of both Ce(III) and Ce(IV) in NPs. Interpretation of the literature has been confusing because the same type of NP could seemingly be oxidizing or anti-oxidizing, toxic or non-toxic. Recent work from the Baer group has brought insight to the problem. CeO$_2$ is usually made by one of three methods: high temperature heating (> 300°C), heated in a solvent (< 100°C), or prepared at room temperature. Karakoti et al. (2012) grouped biological responses to CeO$_2$ in the literature according to synthesis method and noticed that most (but not all) of the CeO$_2$ made by the high temperature or high temperature in solvent methods were either pro-oxidative or had both oxidative and anti-oxidative properties as reported in the various assays used in papers. CeO$_2$ made at room temperature was, with one exception, anti-oxidative. This analysis is another example demonstrating that the method of nanoparticle synthesis can have a large influence on its properties and biological effects. However, the CMP
CeO$_2$ NPs we examined had no measureable toxicity in our A549 assays up to the highest concentrations tested.

As with many nanomaterials, there is a diversity of literature and opinions on whether Al$_2$O$_3$ NPs are toxic. For example, nano-Al$_2$O$_3$ at 100 to 1000 mg/L was toxic to cultured human brain microvascular endothelial cells and also reduced tight junctions in brain endothelial cells in cerebral vasculature after infusion into rats$^{102}$. Al$_2$O$_3$ NPs were at least mildly toxic to osteoblast-like UMR 106 cells using assays of mitochondrial and lysosome function$^{103}$, and they were cytotoxic and genotoxic with CHO-K1 cells$^{104}$. Otero-Gonzalez et al. (2012) observed that exposing human bronchial epithelial cells (16HBE14o-) to 1 mg/mL of nano-Al$_2$O$_3$ (< 50 nm) for 48 h resulted in 50% inhibition in the MTT assay$^{99}$. In the same study, the IC-50 value determined for nano-Al$_2$O$_3$ using an impedance-based real-time cell analyzer was 0.3 mg/mL. In recent work, Al$_2$O$_3$ NPs were reported toxic to plant cells in culture and toxic to fresh water algae$^{105,106}$. In contrast, Al$_2$O$_3$ NPs had no measurable toxicity with mouse L929 cells and normal human fibroblasts$^{107}$. Moreover, even at high concentrations, nano-Al$_2$O$_3$ did not affect the phagocytic activity of rat alveolar macrophages$^{108}$. Our results with Al$_2$O$_3$ CMP slurry failed to find any toxic response with A549 cells in three different types of assays.

Toxicity data developed herein for $f$- and $c$-SiO$_2$, Al$_2$O$_3$, and CeO$_2$ were integrated with human and other organisms. These data are summarized along with corresponding IC-50 and the half maximal effective concentration (EC-50) data reported in the literature in Fig. 2.11. The IC/EC-50 concentrations for silica are higher than for alumina, which in turn is higher than for ceria. This is good because it is in reverse order of their prevalence and use in most semiconductor fabrication facilities (fabs). Silica,
which is used in most abundance, has the highest IC/EC-50 values and is therefore least toxic. It is evident that for a given material type, there is considerable variation among the IC/EC-50 data, depending upon the particular test type, cell line or test organism, test duration, and test endpoint.
Figure 2.11. Integration of human- and eco-toxicity findings for three major classes of metal-based nanoparticles used in CMP slurries. Green bars show data from our work, and blue bars are based upon other literature reports using similar ENPs of similar composition.

2.4.7. Impact of Findings on Semiconductor Industry

Another key aspect of this work has been a collaborative effort between universities and the semiconductor industry to determine the conditions and concentration ranges necessary for the ENP analytical methods. Using a materials balance from one fab and drawing from reported concentration data in the literature (see Supplemental Information), SiO$_2$ concentrations in the effluent wastewater that comes directly from CMP operations might typically be on the order of 1,000 mg/L, alumina concentrations on the order of 10 to 100 mg/L, and cerium concentrations on the order of 1 mg/L or less. Cerium is less prevalently used than either silica or alumina in CMP operations, and none of the referenced literature reports listed cerium concentration in wastewater. According to materials usage records at one fab, silica, alumina, and ceria may be used in
proportions of roughly 90:9:1. However, slurry formulations are both proprietary and dynamic.

There are also significant differences between fabs in the manner that CMP wastewater is routed through the fab and treated. Some, like the fab described in Supplemental Information, employ a physical-chemical wastewater treatment system for the composite CMP water, followed by dilution and equalization with other on-site wastewater flows before treatment by an on-site biological wastewater system. For this particular fab, the waste streams that represent potential gateways for releasing Al$_2$O$_3$, CeO$_2$, and SiO$_2$ ENPs to the environment are the solids concentrate produced by the CMP wastewater treatment process and/or discharges from municipal wastewater treatment plants that receive sewer discharges from the fab. In this fab, the on-site industrial CMP wastewater treatment process produces a filter cake with a 52% water content and measured SiO$_2$ and Al$_2$O$_3$ concentrations of 77 and 8 wt%, respectively. Although this particular filter cake was recycled for the production of cement, it demonstrates the importance of evaluating the fate and long term stability of the solids concentrate waste streams from on-site CMP wastewater treatment processes as the ENP composition of waste sludges may range from less than 1 wt % to greater than 75 wt %. The treated effluent from municipal biological wastewater treatments is typically discharged to surface waters, and the waste sewage sludges or biosolds disposed as land soil amendments (~60%), landfills (~20%), or incinerated with ash being landfilled (~20%)

109. SEM analysis of ENPs at the influent and effluent of on-site chemical wastewater treatment processes at a fab (see SI) indicate the presence of SiO$_2$ ENPs. While both locations have ENPs approximately 70 nm in size, the effluent SiO$_2$ NPs appear to have
slightly different surface morphologies. Overall, the results and analytical methods herein with the four CMP slurries can be applied to effluent streams in fabs to determine ENP behavior and fate. Ideally, speciated and size fractionated ENP concentration data are available for the influent, effluent, and waste biosolids, such that a materials balance account can be made across wastewater treatment facilities.

Analytical method development is relevant not only for assessing the fate of ENPs, but also for determining their impact on biological processes (industrial on-site or off-site municipal facilities). For instance, Zheng et al. (2012) reported 35% inhibition of N removal efficiency at 50 mg/L of 80 nm SiO$_2$. Others observed a 37% inhibition of O$_2$ uptake rate for 50 mg/L of 50 nm SiO$_2$. Details for a particular fab with on-site chemical and biological treatment is described in SI. The mass balance indicates 2 mg/L of SiO$_2$ influent to the on-site treatment facility and 0.2 mg/L in the effluent from the biological wastewater treatment step. Thus, the biological treatment is important in reducing ENP levels. If a fab doesn't pre-treat wastestreams in the fab or have extensive dilution with other on-site wastewater flows, its potential ENP influent concentrations to the biological treatment process could be several tens of mg/l or greater, which may inhibit the performance of the biological wastewater treatment process.

2.5. Conclusions

A principal objective of this work was to develop a common set of ENP samples that could be shared between different laboratories and used to develop validated analytical methods for characterizing CMP slurries and their associated waste streams. The ENPs in the “model slurries” are representative of those used in commercial CMP slurries, but they lack the additives that are commonly employed in commercial slurries and thus are
intended as only a first step in analytical method development. Moreover, the four test slurries are models of the raw unused slurries prior to contact with wafers in CMP operations, and so likewise these model slurries serve as only a first step towards our ultimate goal of using validated methods to characterize the behavior and fate of alumina, ceria, and silica ENPs in real fab wastewaters and effluent discharges. Towards these goals we have developed metal oxide digestion methods that are appropriate for determining $f$- and $c$-SiO$_2$, Al$_2$O$_3$, and CeO$_2$ concentrations. We have demonstrated two alternative methods, a centrifuge and an ultrafiltration method, for distinguishing between dissolved and ENP concentrations. We have demonstrated the applicability of four different particle size distribution methods and highlighted their relative differences.

Cytotoxicity using prokaryotic and eukaryotic toxicity assays showed a low inhibitory potential of the four ENPs in the CMP slurries. The concentrations of ENPs in all four slurries causing 50% inhibition (IC-50) were greater than 1 mg/mL based upon in vitro assays using bioluminescence of the bacterium Aliivibrio fischeri and proliferation and viability or integrity of human cells (adenocarcinomic human alveolar basal epithelial cell line A549). In contrast with some previous reports, $f$-SiO$_2$ was not significantly more toxic than $c$-SiO$_2$ in the CMP slurries, despite having different sizes and morphologies but similar characterization by FTIR and XRD. Additional characterization techniques that probe surface reactivity or number and proximity of surface hydroxyl groups are needed to improve our understanding of discrepancies in the literature. Otherwise, the levels of toxicity of the ENPs towards human cells or model aquatic organisms were similar to literature reports and suggest monitoring at mg/L levels would be adequate to meet IC-50 levels. IC-50 values (> 1000 mg/L) are much higher than ENP
concentrations expected in semiconductor effluents, which may be on the order 0.2 mg/L CeO₂, 4 mg/L Al₂O₃, and 40 mg/L of SiO₂ when on-site chemical treatment is employed. Among the most interesting observations was the ability of the CMP slurry manufacturer to produce 1 to > 5 wt% ENPs that have remained dispersed in solution for many months (i.e., stable; no aggregation). The special-order slurries did not contain organic surfactants, and we demonstrated through comprehensive analysis of the solution that there were no added stabilizers other than pH adjustment. The slurry manufacturers were able to disperse the ENPs using mechanical, sound, or other non-chemical means and then maintain a very highly negative (less than -20 mV for c- and f-SiO₂) or very highly positive (greater than +40 mV for Al₂O₃ and CeO₂) zeta potential through pH adjustment.

The size, morphology, and composition of the ENPs in the CMP slurries differed. Size measurements by TEM, SEM, and spICP-MS agreed well and were smaller than measurements by DLS and NTA, which accounted for the hydrodynamic influence of the nanoparticles. There was excellent agreement among multiple laboratories performing DLS measurements on the well-dispersed ENPs. f-SiO₂ ENPs contained small primary particles agglomerated together into dendritic structures whereas the c-SiO₂ ENPs were present as small and usually singular (non-agglomerated) particles, indicating that the synthesis method impacts the morphology more than structural properties measured by FTIR, XRD, or XPS. CeO₂ ENPs were cubic shaped and generally not-agglomerated whereas Al₂O₃ nanoparticles contained a wide range of primary particle sizes and were agglomerated together. Elemental analysis of the ENPs (Fig. 2.2) revealed the presence of trace constituents that, while representing low weight percentages of the nanoparticles,
might influence their reactivity and or ability to be traced in the environment. Such elemental data has not been reported for other nanoparticles, and the presence of some metals may be related to the purity of silica, ceria, or alumina used in bulk by the CMP slurry manufacturer, compared against high grade purity levels typically used in laboratory studies that synthesize nanoparticles for specific research applications. Unrelated observations, yet similar conclusions, have been reported when yttrium and other trace metals were reported in carbon nanotubes 112. Additional research is needed to understand the implications of differences in stock reagent purity on nanoparticle properties as production of ENPs scales up.

2.6. Acknowledgements
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2.7. Supporting Information

2.7.1. CMPs Catalytic Activity Analysis

Method Description. The catalytic activity of CMPs (c-SiO2 and f-SiO2) using our Colorimetric Assay to Detect Engineered nanoparticles (CADE) technique 1. CADE employs a dye, methylene blue (MB), and a reducing agent, sodium borohydride (BH4),
to colorimetrically assess the catalytic activity of nanoparticles in an aqueous media. CCADE leverages the surface catalytic redox properties of nanoparticles to provide a simple colorimetric detection assay for CMPs. Figure S2.1A inset shows a schematic representation of the catalytic electron transfer mechanism between MB and BH4 in presence of nanoparticles. When CMPs are introduced to MB-BH4 solution, nanoparticles serve as a catalyst for reducing the dye-reductant agent pair by promoting the electron transfer between the dye and reductant. Multiple studies report that nanoscale metals (but not their bulk counterparts) are catalytically active due to the reduction of their redox potential 2. Thus, to act as an effective catalyst, the redox potential of CMPs needs to be found between the redox potential of MB and BH4 since these reduction reactions are thermodynamically favorable but not kinetically 3,4. CCADE consists of final concentrations of 40 μM Methylene blue (CAS: 7220-79-3, Sigma–Aldrich, St. Louis, MO), 10 mM Sodium Borohydrate (CAS: 16940-66-2, Sigma–Aldrich, St. Louis, MO), and 10 mM N-(2-hydroxy- ethyl) piperazine-N’-(2-ethanesulfonic acid) (HEPES, CAS: 7365–45-9 Sigma–Aldrich, St. Louis, MO) at pH = 7. We chose 10 mM HEPES as a buffering agent to maintain the pH constant during our experimental procedures and the buffer concentration has sufficient buffering capacity without inducing particles instability, i.e. aggregation due to reduction in electric double layer thickness or surface charge as a result of high ionic strength. We prepared all our working solution by dispersing CMPs in ultrapure water (18.3 MΩ-cm, Milli-Q Advantage A10® system, Millipore Corp., Billerica, MA). The resulting solution was
placed on a rotary shaker (50 rpm) for 1 h at room temperature (23 °C) prior to each experiment. We begin each experiment by mixing with a micro magnetic stirring bar, driven by an external magnetic mixer (Cat: H370170, Scienceware, Wayne, NJ) for ~2 min. Our CMPs working solution is mixed with a small aliquot of concentrated BH₄ and HEPES solution to reach a final concentration of 10 mM for both reagents. Then, we add a small volume of concentrated dye that provides a final concentration of 40 μM in a 2.5 mL working volume. We continuously monitoring CADE’s absorbance with a portable UV-Vis spectrometer (USB2000+XR1-ES Ocean Optics, Dundin, FL) at the maximum dye’s absorbance peak of λₘₐₓ= 665 nm. CMP detection studies were performed in 3 mL disposable methacrylate cuvette (Cat: 9014, Perfector Scientific, Atascadero, CA) with an optical path length of 1 cm.

Results. Figure S2.1A shows the optical density of dye-reductant solution at λₘₐₓ=665 nm as a function of time. CADE solution in the absence of nanoparticles has a stable OD of ~2.8, showing that the color of the dye remains unaltered for ~25 s. This indicates that the dye reduction is insignificant when nanoparticles are not in solution. We measured the optical density of CADE for long experimental times, ~1 h, in absence of nanoparticles and show that OD decreases less than 0.09 over the duration of the experiment (data not shown here). To demonstrate the effectiveness of our catalytic detection assay, we use a commercial available Au nanoparticle with a nominal diameter of 100 nm (DAC1001, NanoCompsix, San Diego, CA). After 25 seconds, Au nanoparticles are added to the solution with a final concentration of 300 ppb which results in an exponentially decrease of OD from 2.8 to 2.2 over ~75 s. Then, the OD
plateaus and remains steady for the duration of the experiment. We fit the absorbance as a function of time with an exponential curve, given as,

$$OD = OD_0 \exp\left(-\left(\frac{t}{\tau}\right)\right) + \beta,$$

(s2.1)

where $OD$ is the optical density of CADE solution, $OD_0$ is the optical density at $t=0$, $\tau$ is the exponential decay rate constant, $t$ is the time transpired from the addition of the nanoparticles, and $\beta$ is a constant that represents the asymptotic $OD$ value. $OD$ data processing and analysis was performed by a custom code written in MATLAB (MathWorks Inc., Natick, MA). We fit $\tau$ and $\beta$ values (41.8 s and 2.2 for the data shown in Figure S2.1A), which respectively represent the decay time at which the dye absorbance is reduced to $1/e$ of its initial value and the steady state $OD$ that is reached at long times. We explored the use of decay rate constant for quantifying the reduction reactions, but ultimately chose $\beta$ to quantify the catalytic activity of CMPs since this asymptotic constant is unbiased from experimental artifacts (e.g., particle diffusion in the solution, formation of bubbles, etc). $\beta$ values have a dynamic range of 0.01 to 2.8 and potentially serve as a nanoparticle reactivity indicator.
Figure S2.1. Optical Density of CADE assay at pH 7.0 (40 µM of MB, 10 mM NaBH₄ and 10mM HEPES) in the presence of gold nanoparticles at a fixed mass concentration of 300 ppb recorded at 665 nm. Dash line indicates a fit to an exponential decay model 

\[ OD = OD_0 \exp\left(-\frac{t}{\tau}\right) + \beta \quad (R^2 = 0.978) \]

\( \beta \) is the asymptotic value of the optical density.

Nanoparticles are added at \( t = 28 \) s causing CADE optical density to decrease (A) and schematic diagram of dye reduction electron transfer mechanism in presence of nanoparticles, inset. \( \beta \)-value as a function of CMPs type at a fixed mass concentration of 100 ppm. Control experiments correlate to absence of CMPs in the assay. \( \beta \)-values are an average over 5 experiments with error bars that denote 95% confidence intervals (B). ANOVA test and subsequent comparison of means test showed that all three samples differed significantly from each other (\( \alpha = 0.05, \) p-value < 0.0005). This test shows that the difference in the \( \beta \)-value means are likely due to the presence of the different CMPs and not due to random error.
The reduction of MB by borohydride depends directly on the catalytic activity of nanoparticles in CMPs. Figure S2.1B shows $\beta$ values as a function of CMPs composition ($f$-SiO$_2$ and $c$-SiO$_2$) at a constant concentration of 100 ppb. All $\beta$ values are reported herein as the mean of at least five experimental measurements with error bars that denote the 95% confident intervals ($\alpha = 0.05$). An analysis of variance (ANOVA) and subsequent comparison of means test showed that all three samples differed significantly from each other at an $\alpha$=0.05 level (p-value < 0.0005 for all comparisons). This test shows that the difference in the $\beta$ value means are likely due to the presence of the different nanoparticles and not due to random error. In the absence of CMPs in the CADE solution, the $\beta$ value is ~2.8, representing the highest absorbance of dye solution. The $\beta$ values range from 2.6 for $c$-SiO$_2$ to 2.4 for $f$-SiO$_2$. Lower $\beta$ values correspond to higher CMP catalytic activity. At fixed CMP mass concentration, the surface charge of nanoparticles may have an influence on the catalytic reactivity of CMPs. We believe negatively charged particles, $c$-SiO$_2$ and $f$-SiO$_2$, with a surface charge of ~21 and -50 mV, may electrostatically repel BH$_4$ molecules to the surface of the particle, which then inhibit the reduction of MB, resulting in high $\beta$ values. According to Azad et al. when BH$_4$ absorbs to the surface of nanoparticles, it creates a negatively charged layer that attracts cationic organic dyes, such as CADE. This electrostatic attraction or repulsion between particle surfaces and the reducing agents increase or decrease the reduction rate of MB. Other factors that we also believe may play a roll in the reduction of CADE are
hydrophobicity of the dye, complex formation of the dye with anionic surfactants, repulsion between the dye and charged surfactant—these factors are ongoing work in our labs.

2.7.2. Detailed Experimental Methods for In Vitro Assays

Assays were conducted using CMP slurries with marine bacterium *Aliivibrio fischeri* (MicroTox Bioasssay), adenocarcinomic human alveolar basal epithelial cells (A549 cell viability, ATCC® CCL-185™) with the dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay kit (Sigma Aldrich) to quantitatively evaluate the cell viability of A549 cells after exposure to the slurries and Lactate dehydrogenase (LDH) kit (Sigma Aldrich) to evaluate the membrane integrity of A549 cells, proliferation of A549 cells was measured by two methods: determination of cell numbers by staining nucleic acids with crystal violet dye (CV)⁶, or direct counting of cell numbers with a Coulter counter. Details of these methods are provided in Supplemental Information.

*Microtox Bioassay.* The Microtox® Model 500 analyzer (Strategic Diagnostics, Inc. SDIX, Newark, DE, USA) was used to measure changes in the bioluminescence produced by the marine bacterium *Aliivibrio fischeri* (lyophilized culture of *A. fischeri* NRRL-B-11177, AZUR Environmental, Carlsbad, CA, USA). Bioluminescence inhibition was measured at a 30-min exposure time to a dilution series of concentrations with three replicates per test concentration ⁷. All assays were performed at 25°C. The acute toxicity data were obtained and analyzed using the MicrotoxOmni software (Strategic Diagnostics, Inc. SDIX, Newark, DE, USA).
Viability Assay with A549 cells. A549 cells (adenocarcinomic human alveolar basal epithelial cells, ATCC® CCL-185™) were obtained from the American Type Culture Collection (Manassas, VA, USA) and were cultured in F-12K media supplemented with 10% (v/v) FBS in a 37°C incubator with 95% air and 5% CO₂. The dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was used to quantitatively evaluate the cell viability of A549 cells after exposure to the slurries. MTT is converted to purple formazan crystals by mitochondrial reductase enzymes that are present in viable cells. The amount of formazan produced is proportional to the number of cells and can be measured using a spectrophotometer.

The MTT assay kit was purchased from Sigma Aldrich. A549 cells were seeded in 96 well plates at a density 10,000 cells/cm². After seeding, the cells were incubated for 24 h to allow time for attachment. The media were changed after 24 h of incubation and the slurries at respective concentrations (10, 1, 0.1 µL per well each of which had a final volume of 150 µL) were added. The final concentrations in media for each of the slurries in mg/mL were: l-c-SiO₂, 2.03, 0.203, and 0.0203; f-SiO₂, 3.34, 0.334, and 0.0334; CeO₂, 0.52, 0.052, and 0.0052; Al₂O₃, 2.01, 0.201, and 0.0201. After 48 hours of incubation followed by addition of slurries, the media was changed and then the cells were incubated for an additional 24 hours after which the cells were treated with MTT reagent and incubated for four hours. After 4 hours, purple formazan crystals were formed. The crystals were dissolved using the MTT solvent and the absorbance was measured at 570 nm. The absorbance was compared to the controls (positive-lysis buffer and negative-untreated cells) and one-way Anova test was performed to analyze the significance of the results with a confidence interval of 95%.
**Membrane Integrity Assay with A549 cells.** Cell culture conditions were as in the previous section. Lactate dehydrogenase (LDH) was used to evaluate the membrane integrity of A549 cells exposed to slurries. LDH is a stable cytoplasmic enzyme present in most cell types and leaks out of cells into the media if there is damage to the plasma membrane. LDH catalyses the oxidation of lactate to pyruvate through the reduction of nicotinamide adenine dinucleotide (NAD) to NADH.

The LDH assay kit was purchased from Sigma Aldrich. A549 cells were seeded in 96 well plates at a density 10,000 cells/cm². After seeding, the cells were incubated for 24 hours to allow time for attachment. The media was changed after 24 hours of incubation and slurries at respective concentrations were added as in the MTT assay. After 48 hours of incubation followed by addition of slurries to the cells, the assay was performed and the absorbance was collected at a wavelength of 490 nm. The absorbance was compared to the controls (positive-lysis buffer and negative-untreated cells) and one-way Anova test was performed to analyze the significance of the results with a confidence interval of 95%.

**Cell Proliferation Assays with A549 cells.** The effect of exposure to different concentrations of slurry on the proliferation of A549 cells was measured by two methods: determination of cell numbers by staining nucleic acids with crystal violet dye (CV)⁶, or direct counting of cell numbers with a Coulter counter. These assays are sensitive to both cytotoxic materials that may kill cells and to cytostatic materials that may reduce cell growth but not be acutely toxic. A549 cells were cultured in F-12K media supplemented with 10 mM HEPES, pH 7.4 and 10% (v/v) FBS in a 37°C incubator with 90% air and 10% CO₂. For the assay, A549 cells were plated at a density of 3x10⁴ cells/well in a 48-
well plate. The next day, just prior to use, the pH of the slurries were adjusted to pH 7.4 by diluting in 20 mM HEPES buffer at pH 7.4 and further addition of small volumes of 1 M NaOH or 1 M HCl, for c-SiO₂ or the f-SiO₂, respectively. The slurries were diluted to 2x the final concentration in H₂O from the pH adjusted stock solutions and mixed with an equal volume of 2x concentrated media containing 20% FBS plus antibiotics penicillin (200 U/mL) and streptomycin (0.2 mg/mL). After 24 hours of incubation, the medium was removed and the cells were extensively washed. For experiments with c-SiO₂ and f-SiO₂, the cell number was obtained either by CV staining or after trypsinization to detach the cells by direct cell counting and the cumulative data from 4 assays was averaged. For CeO₂ and Al₂O₃, due to the large extent of aggregation of the slurries, only data from cell counting was used. The number of cells in samples that were not treated with slurries was set at 100% proliferation for comparison with cells exposed to slurries.

2.7.3. Life Cycle & Toxicity Implications

**Tool Design & Occupational Exposure Evaluations.** CMP tools are typically designed to conform to Semiconductor Equipment and Materials International (SEMI) guidelines for health and safety. Conformance to these guidelines involves occupational exposure performance testing to demonstrate that the tool provides adequate isolation of the operator and maintenance personnel from the chemicals used in the tools. For operators, the guidelines require that the measured chemical concentration in the worst case personal breathing zone during normal operation of the tool must be less than 1% of the relevant occupational exposure limit. For maintenance operations, the guidelines require that the maximum chemical concentrations must be less than 25% of the relevant occupational exposure limit.
Although these well established and highly protective guidelines are widely employed for chemicals, their application to ENP is impeded by both metrology challenges and the absence of occupational exposure threshold limits for ENP. Shepard and Brenner (2013) conducted occupational exposure monitoring in CMP areas and associated support areas in an operating development fab. Their work reported that airborne particle levels in an operating fab are generally low, and often below detectable levels; but also highlighted the need for more sensitive sample collection and analysis methods for occupational exposure monitoring of ENP. With this in mind, one of the principal goals of the present work has been to develop and distribute a set of characterized “model slurries” with the intent of facilitating method development for occupational exposure monitoring, and the developing toxicity data that applies to relevant occupational exposures.

2.7.4. Wafer Production & CMP Usage

The quantity CMP slurries and of the individual types of particles that are used by a fab differ according to the design and the physical dimension of the integrated circuits being fabricated into a wafer, as well as the wafer size and production capacity of the fab. Many advanced semiconductor manufacturing facilities (fabs) are currently fabricating integrated circuits at the 32, 22 and 14 (nm) technology nodes, on wafers that are either 200 or 300 mm in diameter.

In 2011, the worldwide production capacity for semiconductor wafers was estimated to be on the order of 260,000 wafers per day, with roughly 68% produced as 300-mm wafers, 25% produced as 200-mm wafers, and 7% as wafers that are less than 200-mm in diameter. The CMP process is used repetitively throughout the production
of a single wafer, and typically involves the use of several different slurry formulations, as appropriate to the particular material being planarized. The number of CMP steps required to fabricate a wafer has trended upward with more advanced technology nodes. Whereas fewer than 20 CMP steps may typically have been involved in the production of wafers at the 250 (nm) technology node, it has been projected that a typical 16 (nm) technology node wafer may require more than 40 CMP individual CMP steps, involving 10 or more different slurry types\textsuperscript{10}.

**Example Facility: Characterizing CMP Wastewater Effluents.** The composition and quantity of CMP wastewater produced by a given semiconductor fab varies according to the type and design of the integrated circuits that are produced, the particular slurries that are used, the size of the wafers and the production capacity of the fab, as well as facility dependent infrastructure. Characterization of the ENP life cycle and fate in a facility requires a consideration of the compositional changes along the particle flow path through the facility specific wastewater system, and how those changes influence particle behavior and fate.

Figure S2.2 illustrates the flow path of CMP slurry particles through a particular Fab. Slurry, rinse water, wafers and brush cleaner are fed into CMP tools (Node A). The polished wafer exits the tool and the effluent wastewater consists of the feed streams to the tool, and a combination of dissolved and particulate material removed from the wafer, and possibly residual pad material that may be dislodged (Node B). Materials balance estimates for this particular fab indicate that the average total SiO\textsubscript{2}, Al\textsubscript{2}O\textsubscript{3}, and CeO\textsubscript{2} concentrations in the composite wastewater exiting the CMP tools would be on the order of 1,000 mg/l, 100 mg/l and 5 mg/l, respectively. These estimates are consistent with
CMP effluent concentrations for silica and aluminum, as reported in the literature, as summarized here:

<table>
<thead>
<tr>
<th>Total [Si] (mg/l)</th>
<th>tot [Al] (mg/l)</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>400 - 800</td>
<td>NA</td>
<td>“Oxide” CMP WW effluent from Hsinchu Park (Taiwan)</td>
<td>Den et al (2006)</td>
</tr>
<tr>
<td>810 tot, 362 after 0.45 um</td>
<td>NA</td>
<td>CMP WW effluent from Hsinchu Park (Taiwan).</td>
<td>Huang et al (2004)</td>
</tr>
<tr>
<td>1580 tot, 398 passing 0.2 um</td>
<td>NA</td>
<td>CMP WW effluent from a 300 mm fab in southern Taiwan.</td>
<td>Kuan and Hu (2009)</td>
</tr>
<tr>
<td>98 - 224</td>
<td>.01 - 11.8</td>
<td>Oxide and metal CMP waste from semiconductor fab in Taiwan</td>
<td>Lo and Lo (2004)</td>
</tr>
<tr>
<td>4000</td>
<td>NA</td>
<td>Downstream of ultrafilter at DRAM manufacturer in Hsinchu Park, Taiwan</td>
<td>Tsai et al (2007)</td>
</tr>
<tr>
<td>609 as Si</td>
<td>4.8</td>
<td>“Oxide-CMP” WW from wafer fab in southern Taiwan</td>
<td>Yang et al (2003,2004)</td>
</tr>
</tbody>
</table>

CeO$_2$ concentrations were not reported in the CMP wastewater effluent concentrations that were available in the literatures sources that were reviewed. The concentrations of Al$_2$O$_3$, CeO$_2$, and SiO$_2$ that would be in downstream processes vary considerably, depending on fab specific wastewater system configuration, and the removal efficiency for the treatment processes that are in place. In the absence of an upstream treatment process that removes CMP particles, the influent concentrations to biotreatment at this particular fab would be on the order of 40, 4, and 0.2 (mg/l) for silica, alumina, and ceria. With the CMP wastewater treatment process, the alumina and silica concentrations are estimated to be on the order of 0.6 mg/L Al$_2$O$_3$ and 2 mg/L SiO$_2$. The solids concentrate from a CMP wastewater treatment process may produce a sludge that is 77 % SiO$_2$, 8 % Al$_2$O$_3$ and less than 0.5 % CeO$_2$.

The wastewater effluent from the individual CMP tools flows to a collection and equalization tank which in turn is fed to physicochemical treatment process that uses lime (Node C) primarily to precipitate and coagulate dissolved metals and solids at a high pH. The precipitated and coagulated solids are removed via an overflow clarifier (Node D). The treated aqueous effluent exits the CMP wastewater treatment process (Node E), and
mixes with treated and untreated effluents from other semiconductor manufacturing processes within the facility (Node F). Following equalization and the addition of acid or base (Node G) for neutralization the combined wastewater is routed to an activated sludge type process, where with the addition of sanitary waste (Node I) biological wastewater treatment occurs. The waste biosolids are collected as a sludge (Node K), and disposed as hazardous waste. The treated effluent from the biotreatment process (Node L) undergoes disinfection and is discharged to a stream (Node F). In some cases where local discharge permits can be met, following Node F or G composite fluids containing CMP NPs may be directly discharged to sewers without additional on-site treatment. This situation is more common where the flows from the fabs are small relative to regional sewer flow rates.
Although the ENP from CMP processes in other fabs will follow facility specific flow paths, this particular system serves as a useful point of departure for a consideration of the metrology challenges in characterizing the lifecycle of CMP ENP from their point of use, through to the node points by which they leave the facility.

Conventional mass concentration based and particle count based analytical methods do not provide a convenient tool for tracing the behavior and fate of ENP in aqueous system. In addition to the individual types of ENPs (Al₂O₃, CeO₂, and SiO₂) that are used in the CMP processes, other particles are likely created and destroyed depending on
the governing physicochemical processes at a particular point in the flow path. The lime precipitation process, for instance is based upon the addition of lime slurry, which both contains particles, and initiates the formation of metal hydroxide and other new particles via precipitation.

Particle count and particle size distribution provide measures of particle concentration, but they do not differentiate the composition of the particles. The total elemental composition of digested wastewater samples are likewise indiscriminate measures unless coupled with specialized fractionation methods that can differentiate between particles and dissolved matter. For cerium, which is uncommon in source waters or acids/bases, and likely predominately attributable to the CeO$_2$ used in CMP, the total digested mass concentration is likely a good indicator of the total mass of CeO$_2$. However, both aluminum and silicon are common constituents of wastewater treatment and other chemical feeds. Further, they are often the very materials being removed from wafer surfaces via CMP and other Fab manufacturing processes. The total aluminum and silicon concentrations do however provide bounding information regarding the maximum quantity of Al$_2$O$_3$ or SiO$_2$ particles that could be present. For this fab, for instance, the final effluent concentration of aluminum is 0.2 mg/L and thus represents an upper bound on the total mass of Al$_2$O$_3$ particles that could be present.

SEM and TEM imaging of the particles in aqueous samples provides useful qualitative information regarding the presence and form of particles. Figure S2.3 for instance was prepared by evaporating a drop of influent wastewater from Node B, and shows copious quantities of uniform round particles with an approximate diameter of 70 nm. Using EDX, these particles were identified to be SiO$_2$. Figure S2.3 shows a SEM/EDX image of
a similarly prepared effluent sample from Node E. These particles are likewise 70 nm SiO$_2$ particles, but shown to be weathered. Figure S2.4 shows a corresponding SEM/EDX image of a sample collected from within the gravity clarifier, and clearly shows an agglomerate of SiO$_2$ particles. The agglomerate is seen to be composed largely of the rounded 70-nm SiO$_2$ particles in the influent, and therefore establish that the CMP SiO$_2$ particles are undergoing removal via agglomeration and settling.

A compositional analysis of the lime sludge collected at Node D shows indicates that the sludge is approximately 77 % SiO$_2$, 8 % Al$_2$O$_3$, and 10% CaO. The sludge CeO$_2$ concentrations were not reported, and may have been below detectable limits. Correcting for the moisture content of the sludge and comparing to slurry usage records indicates that on the order of 95% of the SiO$_2$ and 85% of the Al$_2$O$_3$ NPs from the CMP slurries is removed in the lime precipitation process.

A compositional analysis of the lime sludge collected at Node D shows indicates that the sludge is approximately 77 % SiO$_2$, 8 % Al$_2$O$_3$, and 10% CaO. The sludge CeO$_2$ concentrations were not reported, and may have been below detectable limits. Correcting for the moisture content of the sludge and comparing to slurry usage records indicates that on the order of 95% of the SiO$_2$ and 85% of the Al$_2$O$_3$ NPs from the CMP slurries is removed in the lime precipitation process.

**Figure S2.3** SEM image of ENP in the influent (Node B) and effluent (Node E) to the CMP wastewater treatment process.

Operative NP Removal Processes. The concentrations of ENPs across this flow system are indicated to be affected by dilution, dissolution, and agglomeration, coagulation and sedimentation. It is likely, but unconfirmed that they also undergo
additional removal by partitioning onto biosolids in the biological wastewater treatment process (WWTP).

Based upon materials balance considerations in this system, the slurry particles undergo on the order of 25x dilution from the point of effluent discharge from the CMP tools (Node B) to their feed point into the biological WWTP (Node H). Absent removal in the lime precipitation process, the estimated feed concentrations into the biological WWTP would be on the order of 0.2 mg/L CeO₂, 4 mg/L Al₂O₃, and 40 mg/L of SiO₂. With the removal in the lime treatment process inferred from the sludge to particle balance, it is estimated that the actual ENPs feed concentrations into the biological WWTP are on the order of 0.6 mg/L Al₂O₃ and 20 mg/L SiO₂. Measured concentrations of SiO₂ and CeO₂ are not available for the final effluent discharge point from the facility, but the total measured aluminum concentration averages about 0.2 mg/L. This concentration is comprised of the total dissolved and particulate aluminum, from all sources, and thus only serves as an upper bound on what the maximum Al₂O₃ concentration could be. Repeat efforts to use SEM/EDX to image evaporated samples of wastewater from the treated final effluent (Node L) and the points upstream (Node M) and downstream (Node N) of the final effluent discharge point did not show any NPs. Future efforts to characterize ENP in these samples will employ a centrifugal filtration method as means of concentrating the particles and distinguishing the particulate and dissolved fractions.
Figure S2.4. SEM image of coagulated particles removed in the clarifier.
CHAPTER 3

NANOPARTICLE SIZE DETECTION LIMITS BY SINGLE PARTICLE ICP-MS FOR 40 ELEMENTS

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3.1. Abstract

The quantification and characterization of natural, engineered, and incidental nano- to micro-size particles are beneficial to assessing a nanomaterial’s performance in manufacturing, their fate and transport in the environment, and their potential risk to human health. Single particle inductively coupled plasma mass spectrometry (spICP-MS) can sensitively quantify the amount and size distribution of metallic nanoparticles suspended in aqueous matrices. To accurately obtain the nanoparticle size distribution, it is critical to have knowledge of the size detection limit (denoted as $D_{\text{min}}$) using spICP-MS for a wide range of elements other than a few available assessed ones, that have been or will be synthesized into engineered nanoparticles. Herein is described a method to estimate the size detection limit using spICP-MS and then apply it to nanoparticles composed of 40 different elements. The calculated $D_{\text{min}}$ values correspond well for a few of the elements with their detectable sizes that are available in the literature. Assuming each nanoparticle sample is composed of one element, $D_{\text{min}}$ values vary substantially among the 40 elements: Ta, U, Ir, Rh, Th, Ce, and Hf showed the lowest $D_{\text{min}}$ values $\leq$ 10 nm; Bi, W, In, Pb, Pt, Ag, Au, Tl, Pd, Y, Ru, Cd, and Sb had $D_{\text{min}}$ in the range of 11-20 nm; $D_{\text{min}}$ values of Co, Sr, Sn, Zr, Ba, Te, Mo, Ni, V, Cu, Cr, Mg, Zn, Fe, Al, Li, and Ti were located in 21-80 nm; and Se, Ca and Si showed high $D_{\text{min}}$ values greater than 200 nm. A range of parameters that
influence the \( D_{\text{min}} \), such as instrument sensitivity, nanoparticle density, and background noise are demonstrated. It is observed that when the background noise is low, the instrument sensitivity and nanoparticle density dominate the \( D_{\text{min}} \) significantly. Approaches for reducing the \( D_{\text{min}} \), e.g., collision cell technology (CCT) and analyte isotope selection are also discussed. To validate the \( D_{\text{min}} \) estimation approach, size distributions for three engineered nanoparticle samples were obtained using spICP-MS. The use of this methodology confirms that the observed minimum detectable sizes are consistent with the calculated \( D_{\text{min}} \) values. Overall, this work identifies the elements and nanoparticles to which current spICP-MS approaches can be applied, in order to enable quantification of very small nanoparticles at low concentrations in aqueous media.

3.2. Introduction

A wide variety of engineered nanomaterials are used today in many consumer products, such as textiles (e.g., Ag), food additives (e.g., SiO\(_2\) and TiO\(_2\)), cosmetics (e.g., TiO\(_2\) and ZnO), and polishing slurries (e.g., SiO\(_2\), Al\(_2\)O\(_3\) and CeO\(_2\)) in electronics manufacturing\(^{64, 113-115}\). The implications of nanomaterials released into the environment are of increasing concern to researchers\(^4, 5, 116\) and regulatory agencies\(^6\) due to their potential risks to humans and the health of the ecosystem. Nanomaterials are commonly defined as materials having one dimension in the range 1-100 nm\(^3, 117\). They exhibit different properties from bulk materials in terms of their environmental effects, including bioavailability, toxicity, and ending points\(^{116, 118}\). Specific size dependencies of toxicity and reactivity for different nanoparticles have also been demonstrated\(^{119-121}\). In order to evaluate the potential environmental risk of nanoparticles, it is critical to quantify and characterize their exposures in relevant environmental media. Material flow analysis
modeling\textsuperscript{12} and production quantity based estimation\textsuperscript{122, 123} have been used in the past to predict the concentration of nanoparticles in the environmental media. However, there are very few robust analytical approaches that actually detect nanoparticles in the environment, a significant challenge herein being that nanoparticles have been predicted to occur at very low environmentally relevant concentrations, in the range of ng/L to µg/L\textsuperscript{12}. Hence, in support of environmental nano studies, analytical techniques are needed to detect nanoparticles at extremely low concentration levels and provide more information about the nanoparticle size.

Single particle inductively coupled plasma spectrometry (spICP-MS) is promising in that it provides the capability to quantify and size nanoparticles in the environmental media. spICP-MS is a technique that operates based on traditional ICP-MS, but in a “single particle” mode, i.e., by introducing metal-based nanoparticles individually into the instrument, and then recording the time-resolved analysis (TRA) intensity within each short dwell time. Deguedre et al. systematically described the concept of using single particle mode on ICP-MS to quantify and size metal-based colloids in a series of studies\textsuperscript{75, 124, 125}. As a technique for measuring nanoparticles in aqueous matrices, spICP-MS holds two substantial advantages that directly meet the requirement for nanoparticle analysis in environmental media. First, spICP-MS has very low concentration detection limits. Individual nanoparticles can only be detected in sufficiently diluted samples, normally at part per trillion levels, which meet the environmentally relevant concentrations of nanoparticles\textsuperscript{12}. Second, other than detecting a nanoparticle composition, spICP-MS can simultaneously reflect the nanoparticle size information from the particle signal intensity\textsuperscript{75}. Quantified size distribution can then be obtained by using a reference
nanoparticle standard with a known size\textsuperscript{76}. Nano-analysis techniques, in general, lack the ability to quantify size and concentration simultaneously. For example, ICP-MS hyphenation techniques, such as flow field fractionation (FFF)\textsuperscript{126-128} and hydrodynamic chromatography\textsuperscript{129, 130} can effectively resolve and characterize the nanoparticle size distribution; however, they are unable to deal with very low nanoparticle concentrations at ng/L level\textsuperscript{128, 131}. Transmission electron microscopy (TEM) can capture nanoparticle images, from which a user can read the particle size; however, it is difficult to apply TEM to quantify nanoparticles in environmental media at low concentrations (ng/L to µg/L). TEM also does not directly work with liquid samples, and the sample preparation, may lead to artifactual errors\textsuperscript{132}.

Applications of spICP-MS to real environmentally or biologically relevant samples have been demonstrated on algal growth medium\textsuperscript{77}, matrices containing humic acids\textsuperscript{77}, wastewater\textsuperscript{133, 134}, immunoassay (antibody-nanoparticle complex)\textsuperscript{135}, and biological tissues\textsuperscript{136}. Based on the decision to apply spICP-MS to environmental analysis, we asserted that the size detection limit (or the minimum detectable size, as denoted as D\textsubscript{min} throughout this article) was a critical issue for spICP-MS operation. Most current studies on spICP-MS still test with commercial standard nanoparticle samples that carry known information about their properties; however, for an unknown sample, sizes below the low end of the measured size distribution will not be apparent \textit{without} the knowledge of the D\textsubscript{min} for a specific nanoparticle. D\textsubscript{min} values have not been assessed for most nanoparticles other than a few species (e.g., Ag and Au\textsuperscript{137-139}). On the other hand, there are dozens of other elements being developed for the engineering of nanoparticles, and currently there is no guidance on the applicability of spICP-MS to detect their sizes. D\textsubscript{min}
depends on the ability of the instrument to distinguish nanoparticle pulse signals from background noise, and therefore, changes with the analyte elements due to parameters including isotopic abundance and background noise derived from interference ions produced in the plasma. A common way to identify particle pulse signals apart from background noise is by establishing a threshold of $\mu+3\sigma$ ($\mu$ and $\sigma$ correspond to the mean and standard deviation of the background data set)\textsuperscript{76}, although other data processing techniques have also been recently developed\textsuperscript{140}. Laborda et al. recommended that size detection limit be related to the mean and variance of the Poisson distribution describing the background signals\textsuperscript{141}. In their study, however, the theoretically recommended size detection limit was experimentally tested only for silver nanoparticles\textsuperscript{141}. A robust methodology to assess $D_{\text{min}}$ for a wide range of elements using spICP-MS is highly desirable, particularly since spICP-MS holds great promise for applicability to a wide variety of nanoparticle species.

The aim of this study is to estimate the $D_{\text{min}}$ values using spICP-MS for a wide range of elements (40 metallic elements). Based on the nanoparticle’s intrinsic properties (e.g., density and isotope composition), instrument sensitivity, and statistical data analysis, $D_{\text{min}}$ values specific to nanoparticle elemental compositions are calculated. This paper consists of four parts: (1) methodology development for calculating $D_{\text{min}}$ using spICP-MS and determining parameterizing factors that influence $D_{\text{min}}$; (2) element specific mass spectra data collection for 40 elements and calculation of their $D_{\text{min}}$ values equivalent to diameters; (3) comparison of the calculated $D_{\text{min}}$ values against the literature reported on nanoparticles size detected through spICP-MS and against spICP-MS measurements of three representative metallic nanoparticles (Ag, Au, and TiO$_2$) in our lab; and (4)
discussion of the environmental relevance of $D_{\text{min}}$ and use of spICP-MS for emerging nanoparticle applications.

### 3.3. Theory

#### 3.3.1. Computational Strategy for Converting spICP-MS Data into Nanoparticle Size

The methodology by Pace et al.\textsuperscript{76} for raw data conversion of spICP-MS, namely, output as intensity (in counts per second, cps) versus time, is adopted here to determine particle size distribution. As a mass spectrometry, the instrument directly reads the mass of a specific element. Based on the assumption that nanoparticle density is constant and that all nanoparticles are spherical, the nanoparticle sizes can be calculated using the following relationships:

$$
D = \left( \frac{6M_{\text{NP}}}{\rho \cdot \pi} \right)^{\frac{1}{3}} = \left( \frac{6I_{\text{NP}}}{R \cdot f_a \cdot \rho \cdot \pi} \right)^{\frac{1}{3}} = \left( \frac{6\eta \cdot I_{\text{NP}}}{K \cdot f_a \cdot \rho \cdot \pi} \right)^{\frac{1}{3}},
$$

(3.1)

where $M_{\text{NP}}$ (µg) is the mass of the analyte element in a nanoparticle; $I_{\text{NP}}$ (cps) is the corresponding intensity of the interested analyte element in the nanoparticle; $R$ (cps/µg) is sensitivity of the detector for the element of the analyte; $f_a$ is the mass fraction of analyzed metallic element in the nanoparticle; $K$ (cps/µg) is the slope of the calibration curve of ionic standards solution; $\eta$ (%) is the transport efficiency; and $\rho$ and $D$ are the density and diameters of the nanoparticle. Details on derivation of Eq. (3.1) can be found in the Supporting Information section.
3.3.2. Determination of the $D_{\text{min}}$ specific to nanoparticle composition

During the spICP-MS analysis, particles are atomized and ionized in the plasma and each one forms a pack of atoms that will be detected, thereby yielding an intensity reading reflected as a pulse signal. If the particle is too small, the signal of this particle will be masked in the background noise with no pulse signal identified, which eventually determines the $D_{\text{min}}$. Based on the principle of interpreting spICP-MS signals to particle sizes, a calculation for the minimum size can be constructed. Laborda et al. originally suggested a $D_{\text{min}}$ calculation method based on the quantile of the standard normal distribution that describes the signals\textsuperscript{141, 142}. Herein, we adopt a similar idea by using the $3\sigma$ threshold method to identify the particle signal from the background noise. The basic principle lies in calculating the threshold of $\mu + 3\sigma$ repeatedly until no additional particle signals are differentiated. Thus, $3\sigma$ indicates the magnitude of the background noise, which depends on the background signals. Assuming the background noise of a nanoparticle suspension sample is comparable to that of its matrix blank, the $3\sigma$ of which can be experimentally determined and thus the $D_{\text{min}}$ can be calculated. To estimate the most conservative $D_{\text{min}}$, we choose the simplest sample matrix, the deionized (DI) water, based on which background signals by spICP-MS for 40 elements are measured. In this case, the particle signal is defined as a signal 3 times above the standard deviation of DI background ($3\sigma_{\text{DI}}$); $D_{\text{min}}$ can be then calculated as follows:

$$D_{\text{min}} = \left( \frac{6 \times 3\sigma_{\text{DI}}}{R \cdot f \cdot \rho \cdot \pi} \right)^{\frac{1}{3}}. \quad (3.2)$$

$D_{\text{min}}$ is a function of nanoparticle composition. If the different compositions give different analyte elements, different magnitudes of background noise will be obtained for
each unique element because of different interference ions specific to the analyte
element. If different nanoparticle compositions share the same analyte element, e.g.,
 hematite (Fe₂O₃) and goethite (FeOOH), the same background noise magnitude will still
result in different size detection limits because the analyte fraction and nanoparticle
density are different based on Eq. (3.2). However, in this case, the Dₘᵢᵢ values of the
two-nanoparticle species can be interconverted based on Eq. (3.2), which leads to:

\[
D_{\text{min,2}} = D_{\text{min,1}} \times \left[ \left( \frac{f_{a,2}}{f_{a,1}} \right) \cdot \left( \frac{\rho_2}{\rho_1} \right) \right]^{\frac{1}{3}},
\]

where 1 and 2 correspond to two nanoparticle species sharing the same analyte element.

3.4. Experimental Methods

3.4.1. Selected metal elements and their measurement

Among the 40 elements selected to evaluate the Dₘᵢᵢ values, some elements have
already been incorporated into commercialized nanoparticles based on the literature¹⁴³
and vendor website¹⁴⁴, while others may see future use. Table 3.1 summarizes the
selected metal elements that were studied, along with the tested isotopes, ICP-MS
operating modes, and instrument sensitivities. Most of the elements were measured in
the normal mode using Argon as the carrier gas. Some elements were also measured in
the collision cell technology (CCT) mode. Sensitivity (Rᵢ) of the mass detector for each
element was determined based on Eq. (s7) in the Supporting Information section. Rᵢ
describes the detection efficiencies of ICP-MS from the plasma to the detector and is not
influenced by the nebulization efficiency. Transport efficiency was measured for each
analysis using 100 nm Au NP and ionic Au standard based on Eq. (s6) in the supporting
information. The measured value of η varied from 1.5% to 2.1% during the investigation
period (~ 4 months). Multiple isotopes were analyzed for some elements, with different sensitivities determined. All sensitivity values lie in the range of \(1.14 \times 10^{11} \sim 6.87 \times 10^{13}\) cps/\(\mu\)g (Table 3.1), with Uranium having the highest sensitivity and Silicon the lowest.

### 3.4.2. Reagents and sample preparation

The ionic standard for Ag, Al, Au, Ba, Bi, Ca, Cd, Ce, Co, Cu, Fe, Mo, Mg, Ni, Pb, Se, Si, Ta, Th, Ti, Tl, U, V, and Zn were purchased from VHG Labs (Manchester, UK) and Cr, Hf, In, Ir, Li, Pd, Pt, Ru, Rh, Sb, Sn, Sr, Te, W, Y, and Zr were purchased from UltraScientific (N. Kingstown, RI). Standard solutions with a concentration range of 100 ng/L to 2000 ng/L were prepared in 2% HCl (Ultrex, JT Baker Inc., Philipsburg, NJ) for Au, Hf, Ir, Pd, Pt, Rh, Ru, Te, Sn, and 2% HNO\(_3\) (OmniTrace, EMD Chemicals, Gibbstown, NJ) for the other elements. Four sizes of Au nanoparticle samples (20, 50, 70 nm, and 100 nm) with citrate coating were obtained from British Biocell International (BBI, Cardiff, UK) and a silver nanoparticle sample (80 nm) was purchased from NanoComposix, Inc. (San Diego, USA). A food grade titanium dioxide powder sample (E171) was purchased from a commercial supplier (Fiorio Colori Spa, Italy). The E171 TiO\(_2\) sample was suspended into DI water with bath ultrasonication for 30 min followed by centrifugation at 1,500 G for 30 min. The supernatant was collected and used for this study. Deionized water (18.3 M\(\Omega\) cm, NANOpure Infinity, LA, USA) was used for dilution of samples.
Table 3.1. Selected elements for $D_{\text{min}}$ evaluation and their isotopes, ICP-MS operation modes and instrument sensitivities in the test

<table>
<thead>
<tr>
<th>Element</th>
<th>Isotope</th>
<th>ICP-MS operation mode</th>
<th>Sensitivity $R$ (cps/ug)</th>
<th>Element</th>
<th>Isotope</th>
<th>ICP-MS operation mode</th>
<th>Sensitivity $R$ (cps/ug)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag</td>
<td>107</td>
<td>normal</td>
<td>1.92E+13</td>
<td>Pd</td>
<td>105</td>
<td>normal</td>
<td>4.68E+12</td>
</tr>
<tr>
<td>Al</td>
<td>27</td>
<td>normal</td>
<td>1.18E+13</td>
<td>Pt</td>
<td>195</td>
<td>normal</td>
<td>8.70E+12</td>
</tr>
<tr>
<td>Au</td>
<td>197</td>
<td>normal</td>
<td>1.32E+13</td>
<td>Rh</td>
<td>103</td>
<td>normal</td>
<td>4.23E+13</td>
</tr>
<tr>
<td>Ba</td>
<td>137</td>
<td>normal</td>
<td>4.46E+12</td>
<td>Ru</td>
<td>101</td>
<td>normal</td>
<td>3.83E+12</td>
</tr>
<tr>
<td>Bi</td>
<td>209</td>
<td>normal</td>
<td>3.64E+13</td>
<td>Sb</td>
<td>121</td>
<td>normal</td>
<td>4.39E+12</td>
</tr>
<tr>
<td>Ca</td>
<td>44</td>
<td>normal</td>
<td>5.54E+11</td>
<td>Si</td>
<td>29</td>
<td>normal</td>
<td>1.14E+11</td>
</tr>
<tr>
<td>Cd</td>
<td>111</td>
<td>normal</td>
<td>5.71E+12</td>
<td>Sn</td>
<td>118</td>
<td>normal</td>
<td>7.75E+12</td>
</tr>
<tr>
<td>Ce</td>
<td>140</td>
<td>normal</td>
<td>6.31E+13</td>
<td>Se</td>
<td>76</td>
<td>normal</td>
<td>2.48E+11</td>
</tr>
<tr>
<td>Co</td>
<td>59</td>
<td>normal</td>
<td>9.43E+12</td>
<td>Sr</td>
<td>88</td>
<td>normal</td>
<td>1.94E+13</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>CCT</td>
<td>1.54E+12</td>
<td>Ta</td>
<td>181</td>
<td>normal</td>
<td>6.12E+13</td>
</tr>
<tr>
<td>Cr</td>
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<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
3.4.3. Instruments

X-Series II ICP-MS (Thermo Fisher Scientific Inc., Waltham, MA) was used for spICP-MS analysis. The sample introduction system consists of a glass concentric nebulizer and a conical spray chamber with impact bead. Normal mode and CCT mode were applied to investigate the effect of these different modes on $D_{\text{min}}$ evaluation. A mixed gas of 7% H$_2$ and 93% He was used as the reaction gas for CCT mode. The instrument was tuned using a solution containing Li, Ce, In, and U at a concentration of 10 µg/L for high sensitivity of Li, In, and U and minimum oxide levels (CeO/Ce < 2%). The peristaltic pump speed was maintained at 30 rpm, and the flow rate was measured before each analysis and found to be in the range of 0.67~0.71 ml/min. For spICP-MS analysis, the instrument was operated in TRA mode to output signal of cps versus time. The duration time for each run was set at 1 min with dwell time of 10 ms per reading. A single isotope was monitored for each measurement, and the investigated isotopes are summarized in Table 3.1.

3.5. Results and Discussion

3.5.1. Evaluation of $D_{\text{min}}$ values for nanoparticles composed of different elements

Figure 3.1 shows the calculated $D_{\text{min}}$ values for nanoparticles corresponding to 40 elements based on Eq. (3.2) by assuming that the nanoparticle composition is a single element (i.e., $f_a$=1). Figure 3.1 also includes data (triangles) on the sizes of nanoparticles measured by spICP-MS in previous literature reports$^{75, 112, 124, 125, 128, 135, 137, 138, 145-150}$. Table S3.1 in the Supporting Information section summarizes values obtained from the literature. For nanoparticles composed of multiple elements, $D_{\text{min}}$ can be converted from
$D_{\text{min}}$ of the corresponding single element nanoparticle based on Eq. (3.3). $D_{\text{min}}$ values of some nanoparticles composed of multiple elements, e.g., the typical oxide compound nanoparticles for each element, are calculated and shown in Table S3.2 in the Supporting Information section.

**Figure 3.1.** Evaluated $D_{\text{min}}$ of spICP-MS for 40 elements and reported detectable sizes of some nanoparticles from previous literature. For some elements, different isotopes and/or different instrument operation modes are investigated all corresponding $D_{\text{min}}$ values are shown. All the evaluated $D_{\text{min}}$ are performed by assuming single element nanoparticles (i.e., $f_{\alpha} = 1$).

Figure 3.1 demonstrates that the calculated $D_{\text{min}}$ values varies significantly with different elements in a wide range, i.e., < 10 nm to 463 nm. Low $D_{\text{min}}$ values ($\leq 10$ nm) can be achieved for elements, including Ta, U, Ir, Rh, Th, and Ce, whereas high $D_{\text{min}}$ values (> 200 nm) exist for Se, Ca, and Si. For most elements (except for Se, Ca, and Si), $D_{\text{min}}$ can be achieved below 100 nm (all are < 80 nm, as seen in Figure 3.1) using spICP-
MS, indicating the viability of spICP-MS as a nano-analysis technique, when the 1-100 nm size range is adopted for defining nanomaterials. Virtually, all previously reported detectable nanoparticle sizes from spICP-MS are above our calculated $D_{\text{min}}$ values, indicating the calculated $D_{\text{min}}$ is probably a best-case size detection limit. For example, Degueldre et al. reported the analysis of gold colloids with spICP-MS using Au particles from 80 nm to 250 nm and Hu et al. reported the smallest detectable size of 15 nm for Au nanoparticle, which agrees well with the calculated $D_{\text{min}}$ of 13 nm for Au nanoparticles in our study. Ag nanoparticle samples with nominal size at 20 nm (the lower end of their size distributions are even less than 20 nm) at minimum has been detected by spICP-MS, which fairly agree with our calculated $D_{\text{min}}$ of 13 nm. An exception to calculated $D_{\text{min}}$ being less than observed detectable size was for SiO$_2$. Olesik and Gray have reported SiO$_2$ particles with a minimum diameter of 403 nm detected using spICP-MS. This result is below our calculated $D_{\text{min}}$, which is 463 nm as Si and equivalent to 572 nm as SiO$_2$ particles. A possible explanation for the disparity may be the use of NH$_3$ as a reaction gas for the reaction/collision cell technology in the study by Olesik and Gray, which can then effectively diminish the interfere ion production (e.g., $^{14}$N$^{15}$N, $^{14}$N$_2$H, $^{13}$C$^{16}$O, $^{12}$C$^{17}$O, or $^{12}$C$^{16}$O$^{1}$H interfere with $^{29}$Si) in plasma. In the present study, use of NH$_3$ as a reaction gas for the CCT mode was not set up. Few studies have reported $D_{\text{min}}$ values for nanoparticles composed of elements other than these examples. Thus, Figure 3.1 effectively demonstrates the instructional value for future applications of spICP-MS to evaluate which nanoparticle elemental compositions the method may, or may not, be appropriately applied to.
To demonstrate factors that influence $D_{\text{min}}$, Figure 3.2 shows the calculated $D_{\text{min}}$ values of the selected elements on a coordinate of $R_i \times \rho_i$ (the product of instrument sensitivity and nanoparticle density) versus $3\sigma_{\text{DI}}$ (an indication of the background noise). The equi-diameter curves (dashed line) are determined based on Eq. (3.2). From the contour map of the equi-diameter curves, the smaller $D_{\text{min}}$ occurs for elements with higher $R_i \times \rho_i$ and lower $3\sigma_{\text{DI}}$. The equi-diameter curves become intense as the $R_i \times \rho_i$ and $3\sigma_{\text{DI}}$ diminish, indicating that the $D_{\text{min}}$ fluctuates greatly with varied sensitivity, nanoparticles density, and background noise when these parameters become small. Most of the 40 elements have low background noise in the range of 100-1000 cps, where $R_i \times \rho_i$ is greater than $\sim 10^{14}$ cps$\cdot$g$\cdot$cm$^{-3}$ and yields $D_{\text{min}}$ values less than 20 nm, indicating that $D_{\text{min}}$ is more dominated by the products of nanoparticle density and sensitivity when a relatively low background noise is achieved. Some elements (V, Te, Sb, Cd, Ru, Pd, Hf, Ta, and U (not shown because of the graph scope)) have $3\sigma_{\text{DI}}$ values equal to 100 cps, which is the minimum signal magnitude the instrument can read, and their $D_{\text{min}}$ values are greatly influenced by $R_i \times \rho_i$. Accordingly, the $D_{\text{min}}$ values of some elements (e.g., Li, Ti, Fe, and Mg) are more limited by the instrument sensitivity and nanoparticle density; whereas the poor $D_{\text{min}}$ values of elements like Al, Se, Ca, and Si (data points of the last two is beyond the scope of Figure 3.2) are also attributable to the high background noise. Figure 3.2 indicates decreasing background noise and/or increasing the sensitivity, fulfilled by, e.g., the CCT operation mode and the selection of analyte isotopes can lower the $D_{\text{min}}$ for a specific nanoparticle.
**Figure 3.2.** $D_{\text{min}}$ map as a function of background noise ($3\sigma_{DI}$) and the product of sensitivity and nanoparticle density ($R_i \times \rho_i$). Dashed lines are equi-diameter curves determined based on eq 2.

### 3.5.2. Approaches to lower the $D_{\text{min}}$ of spICP-MS

The unique application of spICP-MS is the conversion of the detectable mass concentration of the atom packet corresponding to a particle within a transient time (dwell time) to the particle size. CCT is often adopted to diminish the interference ions so as to decrease the background noise. Basically, CCT places a reaction/collision cell before the analyzer quadruple, in which a reaction gas (e.g. H$_2$ or NH$_3$) is injected to collide and react with polyatomic interfering ions to remove their interference to the analyte or to transform the analyte ions to other species that are not interfered with. To illustrate this, we measured $D_{\text{min}}$ values of 6 elements in the CCT mode, including Co, Ni,
V, Cu, Zn, and Fe (circles in Figure 3.1). CCT can decrease the background noise, quantitatively reflected as the decreased $3\sigma_{DI}$. However, this effectiveness varied with different elements. Among the 6 tested elements, CCT decreased the $D_{\text{min}}$ values for V, Cu, and Fe, but even increased the $D_{\text{min}}$ values for Co, Ni and Zn. This is because CCT often also decreases the instrument sensitivity (Table 3.1), along with the background noise. Figure 3.2 shows that when the background noise stays generally in the lower range, instrument sensitivity will remarkably dominate the $D_{\text{min}}$. Thus, we expect that only a sufficiently massive (in order to compensate the decrease of instrument sensitivity) drop of background can yield an appreciably lowered $D_{\text{min}}$. This is virtually effective for elements with significant isobaric/polyatomic interferences in plasma. Evidently, Fe yields a more than 100 times drop in $3\sigma_{DI}$ from the normal mode (75800 cps) to CCT mode (600 cps), which, in turn, decreases the calculated $D_{\text{min}}$ of Fe from 119 nm in normal mode to 55 nm in CCT mode (Figure 3.1). However, a decrease of $3\sigma_{DI}$ for the other 5 elements is small (<10 times, data not shown), leading to inappreciable lowered $D_{\text{min}}$ values (V and Cu), or even increased $D_{\text{min}}$ values (Co, Ni, and Zn). It is noteworthy that background noise is also influenced by the selected dwell time. In the current work the dwell time was set at 10 ms, which is adopted from a reported optimization$^{128}$. However, recent work shows that an extremely short dwell time at microsecond level can effectively lower the background noise for detecting silica nanoparticles, yielding decreased $D_{\text{min}}$$^{152}$.

For a specific instrument setup, another way to achieve higher detection sensitivity for an element is to manipulate the selection of isotopes to be measured, since $R_i$ is isotope specific for a given element. Currently, spICP-MS is operated with only one isotope
measured in each analysis. For a given analyte, the selection of isotope depends on the sensitivity ($R_i$), background noise ($3\sigma_{\text{DI}}$), and the relative abundance of the isotope. In our experiment, different isotopes were measured and compared for selected elements, including magnesium ($^{24}\text{Mg}$ and $^{26}\text{Mg}$), nickel ($^{58}\text{Ni}$ and $^{60}\text{Ni}$), titanium ($^{47}\text{Ti}$ and $^{49}\text{Ti}$), zirconium ($^{90}\text{Zr}$ and $^{94}\text{Zr}$), and zinc ($^{64}\text{Zn}$ and $^{66}\text{Zn}$) were measured using two different isotopes (Figure 3.1). The more abundant isotope of an element gives higher sensitivity except for Ni (the abundance of $^{58}\text{Ni}$ and $^{60}\text{Ni}$ differ slightly in the range of ~2.1%, and thus may show less difference between their sensitivities.). However, higher background noise can compromise the higher sensitivity advantage for more abundant isotopes. For example, $^{47}\text{Ti}$ (abundance of 7.75%) possesses higher $R_i$ (Table 3.1) than $^{49}\text{Ti}$ (abundance of 5.51%), but $^{49}\text{Ti}$ shows a lower $D_{\text{min}}$ (75 nm) than $^{47}\text{Ti}$ (93 nm) because of its lower $3\sigma_{\text{DI}}$, indicating the importance of the factor of background noise when selecting an analyte isotope. Using $^{49}\text{Ti}$ as the analyte, relative standard deviation (RSD) of 36%, was obtained for $R_i$ based on 5 cycles of analysis, thereby indicating that tuning and optimizing the instrument mass detector can also alter $R_i$ slightly. Notably, $R_i$ is dependent on the ICP-MS instrument itself. Instrument from different manufactures may varied in the sensitivity. Thus different instruments possibly yield $D_{\text{min}}$ differences, the magnitude of which is not evaluated in this work.

Other than the above mentioned instrument operation approaches, $D_{\text{min}}$ can also be potentially lowered through improvements in the signal processing methodology used. The $D_{\text{min}}$ estimation method described in this study is based on the traditional $3\sigma$ signal processing approach. Cornelis and Hassellöv have proposed using a Polygaussian probability mass function to describe the background signal and discriminate the particle
signal. This approach is feasible in instances where the ionic metal species exists or when the particle size is small and an overlap occurs between background signals and particle signals\textsuperscript{140}. We expect that this methodology can possibly decrease the size detection limits for spICP-MS than \( D_{\text{min}} \) calculated in this study.

3.5.3. Application of spICP-MS for three representative engineered nanoparticles.

To further validate our calculated \( D_{\text{min}} \) values for spICP-MS, spICP-MS was used to analyze three representative engineered nanoparticles, including Au nanoparticles, Ag nanoparticles, and food grade TiO\textsubscript{2} nanopowders. Figure 3.1 and Table S3.2 show the calculated \( D_{\text{min}} \) values for these three elements, which are 13 nm for Au nanoparticles and Ag nanoparticles, and 75 nm for Ti nanoparticles (equivalent to 91 nm as TiO\textsubscript{2} nanoparticles). Figure 3.3 shows the time-resolved spICP-MS response signals and the calculated size distribution histograms for the 3 samples. The histogram charts in Figure 3.3 illustrate the limit lines corresponding to different coefficients of \( \sigma \) (i.e., 3\( \sigma \), 4\( \sigma \) or 5\( \sigma \)), as bigger \( \sigma \)'s coefficients can practically lower the probability that a background signal is misidentified as a particle signal, and thus increase the confidence of obtained particle size distribution\textsuperscript{134, 141}. For the Au nanoparticles (Figure 3.3a), samples with three different nominal sizes (20, 50, and 70 nm) were analyzed. Using 3\( \sigma \) as the particle detection threshold, the complete size distributions of 50 nm and 70 nm Au nanoparticles are captured by spICP-MS. From this result, we see the nominal 70 nm sample contain more nanoparticles sized greater than 70 nm (e.g., 80-90 nm). We trust this result as spICP-MS has been demonstrated to well characterize Au nanoparticles in this size range with different samples in our lab. It is possibly aggregation has occurred with the 70 nm sample as it ages. However, for the 20 nm Au nanoparticle sample, the detectable size
was cut off at 14 nm by the $3\sigma$ threshold and 17 nm by the $5\sigma$ threshold, both of which are close to our calculated $D_{\text{min}}$ of 13 nm for Au nanoparticles. The observed minimum detectable sizes for Ag nanoparticles (Figure 3.3b) and TiO$_2$ (Figure 3.3c) are 17 nm and 95 nm based on $3\sigma$ threshold respectively; therefore, both reach the calculated $D_{\text{min}}$ values in this study. Increasing the coefficient of $\sigma$ raises the minimum detectable size as expected from the different cutoff thresholds in Figure 3.3 for all three samples: from $3\sigma$ to $5\sigma$, it increases from 14 nm to 17 nm for Au nanoparticles, from 17 nm to 20 nm for Ag nanoparticles, and from 95 to 129 nm for TiO$_2$ nanoparticles. Using higher $\sigma$ coefficient can increase the confidence of the determined particle events$^{134}$ but meanwhile lead to higher $D_{\text{min}}$. The selection of $\sigma$ coefficient remains arbitrary so far and new particle event discrimination approach has been recommended, e.g., the K-means clustering algorithm$^{153}$. The size distribution of the same TiO$_2$ sample was previously determined to be in the range of 40 to 220 nm based on a TEM analysis$^{114}$. The distribution is also fairly comparable to the spICP-MS result for the range above 90 nm (Figure 3.3c). This comparison suggests spICP-MS can be used to determine the size of TiO$_2$ above $\sim$90 nm, which agrees with our calculated $D_{\text{min}}$ at 91 nm (obtained without running a real TiO$_2$ nanoparticle sample). An analysis of nanoparticles with these three elements supports the validity of the $D_{\text{min}}$ database for 40 elements calculated in this study, and can serve as guidelines for future spICP-MS applications.
Figure 3.3. Raw signal spectra of spICP-MS and the corresponding size distribution histograms: (a) Au nanoparticles (NP), (b) Ag nanoparticles, and (c) E171 TiO$_2$ nanopowders. Dashed lines in the size distribution histograms describe particle thresholds determined by different multiple of standard deviation.
3.5.4. Implications for analysis of environmental samples.

spICP-MS, as an emerging technique, has been applied to very limited fields when compared to existing lab-scale studies. To the best of our knowledge, few studies\textsuperscript{77,133,134,136} have analyzed environmentally relevant samples using spICP-MS. We propose that the unique advantages of spICP-MS makes it suitable for a variety of environmental nano-analysis applications, such as: (1) for prospecting the natural, engineered, or incidental metal-based nanoparticles in various aqueous environmental media, e.g., drinking water, river water, groundwater, storm water, etc.; (2) for analyzing nanoparticles in non-aqueous media after extracting the nanoparticles into aqueous phases, thus to prospect airborne nanoparticles and nanomaterials in solid media (e.g., soil and biomass). For example, spICP-MS can be used to monitor the potential Pb-based particles released from pipes to drinking water, and the fate of engineered nanoparticles through the wastewater treatment processes. For all potential application of spICP-MS to the environmentally relevant analysis, foreknowing the $D_{\text{min}}$ is imperative to evaluate the reliability of analysis results. A predicted $D_{\text{min}}$ will evidence whether the absence of sizes below a minimum detected value is attributed to the actual nonexistence or to the analysis limitation. For example, a variety of other uncommon metal-based nanoparticles can also be measured, using spICP-MS such as a group of rare earth elements (REEs, specifically Scandium, Yttrium and Lanthanides elements), the nanoparticles of which are of concern for their known as well as unknown environmental impacts\textsuperscript{154}. Our study has predicted the $D_{\text{min}}$ values of some REE based nanoparticles, e.g., 16 nm for Y and 10 nm for Ce. $D_{\text{min}}$ for other REEs can also be estimated based on the method described here. To enable potential applications of spICP-MS for more nanoparticle compositions, the
current work has estimated the minimum sizes that can be detected by using this technique for 40 elements (which covers most metal species). This small database thus offers initial guidelines for adopting spICP-MS instrumentation for nanoparticles of interest. It also demonstrates that spICP-MS can detect at < 20 nm diameter sizes for some elements based particles, e.g., Rh, Ce, Pb, Pt, Ag, and Au; for other elements, however, spICP-MS is unable to detect when the size becomes too small. For example, TiO$_2$ nanoparticles are predicted to exist in the environment at significant concentrations (0.7-16 µg/L in water)\textsuperscript{155}. Our study shows that the current spICP-MS strategy cannot detect TiO$_2$ nanoparticles with sizes < ~90 nm.

To apply spICP-MS directly to environmental nano-analysis, we ran environmental media samples, including river water, tap water and wastewater effluent from three local wastewater treatment plants (WWTP) in phoenix area of Arizona, to detect particulate Ti, Ce and Ag. Table 3.2 lists the minimum detected particle sizes for the three elements. For each element we assume the most particle composition, i.e., TiO$_2$, CeO$_2$ or Ag, based on which the particle size was calculated. The estimated D$_{\text{min}}$ values are: 91 nm for TiO$_2$, 10 nm for CeO$_2$ and 13 nm for Ag in Figure 3.1. Values in Table 3.2 give all minimum detected particle size greater than our estimated D$_{\text{min}}$, except for CeO$_2$ in tap water, which has comparable minimum size (9 nm) to the estimated D$_{\text{min}}$ (10 nm). Taking account of all the sample from 6 environmental matrices, the minimum detected particle size values are fairly close, with the mean values of 130 nm for TiO$_2$, 19 nm for CeO$_2$ and 21 nm for Ag. This result indicates that complex aqueous environmental matrices contain inorganic ions, which can potentially be the same elements to the nanoparticles of interest, and organic matter that can influence the ability to achieve calculated D$_{\text{min}}$ values. Such
interferences may increase the baseline signal or otherwise increase the magnitude of $3\sigma$ in the environmental sample relative to the $3\sigma$ value in ultrapure water containing nanoparticles. Fortunately, strategies to “clean up” sample matrices for food media and ionic metal interferences have recently been proposed\textsuperscript{156,157}. Again, the calculated $D_{\text{min}}$ values in ultrapure water represent the lowest minimum size predicted for detected using spICP-MS.

**Table 3.2.** The minimum particle size detected in river water, tap water and wastewater effluent

<table>
<thead>
<tr>
<th>Assumed nanoparticle composition</th>
<th>Verde River</th>
<th>Salt River</th>
<th>Tap water</th>
<th>WWT P #1</th>
<th>WWT P #2</th>
<th>WWT TP #3</th>
<th>Mean</th>
<th>Standard deviation</th>
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<td>TiO$_2$</td>
<td>186</td>
<td>120</td>
<td>119</td>
<td>112</td>
<td>123</td>
<td>122</td>
<td>130</td>
<td>28</td>
</tr>
<tr>
<td>CeO$_2$</td>
<td>33</td>
<td>21</td>
<td>9</td>
<td>15</td>
<td>20</td>
<td>17</td>
<td>19</td>
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</tr>
<tr>
<td>Ag</td>
<td>18</td>
<td>16</td>
<td>26</td>
<td>22</td>
<td>18</td>
<td>23</td>
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<td>4</td>
</tr>
</tbody>
</table>

All size values are determined base on $3\sigma$ principle. All units are in nm.

This work summarizes the factors that can influence $D_{\text{min}}$ and also suggests possible methods to lower $D_{\text{min}}$. For a specific environmental sample, obtaining the best knowledge of the sample matrix for desired nanoparticles can be helpful to increase the instrument sensitivity and to deconvolute the background noise. For example, some appropriate sample pretreatments (e.g., a mild digestion to remove complicated constituents other than nanoparticles) can help remove the interference from the sample matrix. Seeking more effective signal processing methodology to more accurately remove the background signal, manipulating the measurement modes (normal and CCT), and selecting the best analyte isotope, all can potentially help lower the $D_{\text{min}}$. In
conclusion, this paper demonstrates $D_{\text{min}}$ values for a wide variety of elements using spICP-MS, which is based on current signal processing methods (i.e., the $3\sigma$ principle). It provides guidance for future applications of spICP-MS for environmentally relevant nano-analysis.

3.6. Acknowledgements

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3.7. Supporting Information

Computational strategy for converting spICP-MS data into nanoparticle size.

We adopted the basic methodology presented by Pace et al.\textsuperscript{76} to convert the raw data of spICP-MS, which is output as intensity (in counts per second, cps) versus time, to the particle size distribution.

The signal intensity ($I_{\text{MS}}$, cps) of ICP-MS is the sum of analyte intensity ($I_a$, cps) and background intensity ($I_{BK}$, cps), which is given by:

$$I_{\text{MS}} = I_a + I_{BK} \quad (\text{s3.1})$$

Analyte intensity ($I_a$) is proportional to the mass of analyte delivered to the detector, which gives:

$$I_{\text{MS}} = M_a \cdot R + I_{BK} \quad (\text{s3.2})$$

where $R$ (cps/µg) is sensitivity of the detector for the element of the analyte, and $M_a$ (µg) is the mass of the analyte detected within one dwell time. The sensitivity of detector $R$ can be obtained using intensity of a reference nanoparticle with known size (based on
which \( M_a \) can be evaluated) and blank intensity. If the reference particle composed of interested element is not available, \( R \) can be calculated using available reference nanoparticles with assumption that the transport efficiency is constant regardless of the particle composition as described by Pace et al.\(^7\). The transport efficiency represents the percentage of analyte transporting through the sample introduction system to the mass detector of the instrument. One transport efficiency determination method is based upon the different transport behavior of ionic sample and nanoparticle sample. Briefly, in case of nanoparticle sample, the signal intensity (corresponding to the mass of a nanoparticle delivered to the mass detector) is independent of the sample amount entering the plasma but dependent on the size of the nanoparticle, i.e., bigger size nanoparticle gives higher intensity because of its bigger mass, if the density is constant. Whereby the signal intensity of the ionic sample (corresponding to the mass of ions delivered to the mass detector) is affected by the transport efficiency of the sample introduction system because only a certain fraction of the aspirated sample volume introduced to the spray chamber enters the plasma to be detected eventually. The intensity of the ionic sample can be expressed by:

\[
I_{MS} = M_{in} \cdot \eta \cdot R + I_{BK},
\]

where \( M_{in} \) (µg) is the mass of the analyte ion entering the spray chamber within one dwell time and \( \eta \) (%) is the transport efficiency. With a known sample flow rate, analyte concentration, and dwell time, \( M_{in} \) can be determined by:

\[
M_{in} = q \cdot C \cdot t_{dwell},
\]
where \( q \) (ml/min) is sample flow rate controlled by the peristaltic pump, \( C \) (µg/mL) is concentration of the dissolved metal, \( t_{\text{dwell}} \) (min) is dwell time, and \( \eta \) is the transport efficiency.

Practically, we determine the sensitivity \( R \) using the reference nanoparticle sample (100nm Au nanoparticle standard with known size are used in the present study) based on eq. (2). By running the ionic sample corresponding the reference nanoparticle sample (i.e., the ionic Au standard solution in the present study), the calibration curve can be obtained with the relationship as:

\[
I_{\text{MS}} = M_{\text{in}} \cdot K + I_{\text{BK}},
\]

where \( K \) is the slope of the calibration curve of ionic standards solution. By comparing eq. (3.3) and (3.4), the transport efficiency can be calculated as:

\[
\eta = \frac{K}{R}.
\]

So far the transport efficiency can be determined by running Au nanoparticle standard with known homogeneous size and ionic Au standard solution. With assumption that \( \eta \) is constant regardless of the particle composition, sensitivity corresponding to all other desired elements can be obtained using ionic standard solution of different elements based on:

\[
R_i = \frac{K_i}{\eta},
\]

where \( R_i \) and \( K_i \) are the sensitivity and ionic standard calibration curve slope for desired element \( i \), respectively.

By assuming the density of nanoparticle is constant and all nanoparticles are spherical, the size of nanoparticles can be calculated using the following relationships:
$M_{NP} = \frac{(I_{MS} - I_{BK})}{R \cdot f_a} = \frac{\eta \cdot I_{NP}}{K \cdot f_a}$, \hspace{1cm} (s3.8)

\[D = \left(\frac{6M_{NP}}{\rho \cdot \pi}\right)^\frac{1}{3} = \left(\frac{6I_{NP}}{R \cdot f_a \cdot \rho \cdot \pi}\right)^\frac{1}{3} = \left(\frac{6\eta \cdot I_{NP}}{K \cdot f_a \cdot \rho \cdot \pi}\right)^\frac{1}{3}, \hspace{1cm} (s3.9)\]

where $M_{NP}$ (µg) is the mass of the analyte element in a nanoparticle, $I_{NP}$ (cps) is the corresponding intensity of the interested analyte element in the nanoparticle, $\rho$ and $D$ are density and diameter of the nanoparticle, and $f_a$ is the mass fraction of analyzed metallic element in the nanoparticle.

Table S3.1. Detectable particle sizes reported by literatures

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<tr>
<th>Particle</th>
<th>Isotope</th>
<th>Detection limit (nm)</th>
<th>Studied particle (nm)</th>
<th>Note</th>
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<td>$^{107}$Ag</td>
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Dynamic reaction cell, distribution, 41 fg, 324 nm as Si,
ICP-OES, 50 fg, 345 nm as Si
Milled powder
100-10,000 nm mixture
2.6 fg, 64 nm as U
0.76 fg, 60 nm as Zr
1.3 fg, 84 nm as Ti
0.03 fg, 28 nm as Al
11 fg, 139 nm as Fe

*calculated using mass of detection limit in the reference

**Table S3.2.** Evaluated $D_{\text{min}}$ for investigated elements of different particle compositions.

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*Information is adopted from reference 14.¹⁶⁸*
CHAPTER 4

QUANTITATIVE RESOLUTION OF NANOPARTICLE SIZES USING SINGLE PARTICLE INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY WITH THE K-MEANS CLUSTERING ALGORITHM


4.1. Abstract

Sensitive and accurate characterization of nanoparticle size in aqueous matrices at environmentally relevant concentrations is still challenging for current nano-analysis techniques. Single particle inductively coupled plasma mass spectrometry (spICP-MS) is an emerging method to characterize the size distribution of nanoparticles and determine their concentrations. Herein for the first time the K-means clustering algorithm is applied to signal processing of spICP-MS raw data. Compared with currently used data processing approaches, the K-means algorithm improved discrimination of particle signals from background signals and provides a sophisticated, statistically based method to quantitatively resolve different size groups contained within a nanoparticle suspension. In tests with commercial Au nanoparticles (AuNPs), spICP-MS with the K-means clustering algorithm can quantitatively discriminate secondary “impurity-size nanoparticles,” present at fractions of less than 2% by mass, from primary-size nanoparticles with the minimum resolvable size difference between the primary and secondary nanoparticles at ~20 nm. AuNP mixtures in which 80-nm particles act as the “primary size group” and 20-nm, 50-nm, or 100-nm particles act as the “impurity size group” were analyzed by spICP-MS, which reliably measured percentages of secondary...
impurity-size nanoparticles that are consistent with the expected experimentally
determined values. Compared with dynamic light scattering (DLS), spICP-MS has
remarkably better particle size resolution capability. We also demonstrated the size
measurement advantage of spICP-MS over DLS for commercial CeO₂ nanoparticles that
are commonly used in the semiconductor industry, where quality control of the
nanoparticle size distribution is critical for the wafer polishing process.

4.2. **Introduction**

Advances in nanotechnology, which employs materials at the nanometer scale, have
led to wide incorporation of nanomaterials in industrial processes and commercial
products. Quality assurance and control (QA/QC) of nanoparticle size is
often crucial when employing nanomaterials in industrial processes. For instance, in the
semiconductor industry, the chemical mechanical polishing (CMP) process uses
monodisperse nanoparticles, e.g., CeO₂, Al₂O₃, and SiO₂, as abrasives in slurries to
planarize the wafer surface. The nanoparticle size distribution significantly influences
CMP efficiency and the resulting substrate surface properties. Both the average and
the standard deviation of the size of abrasive particles in CMP slurries can influence the
material removal rate (MRR) of the CMP process. A small amount of large particles
generated by slurry agglomeration and aggregation can lead to an increase in surface
roughness and defects. Quantitatively characterizing the particle size distribution and
detecting any small percentage of “impurity-size” particles outside the size range of the
primary monodisperse particles is critical for QA/QC of CMP operation. Techniques
including acoustic spectroscopy and static light scattering have been investigated for
the detection of large particles in a monodisperse particle slurry. Although these
techniques were reported to be capable of detecting a small percentage of large, coarse particles, the size differences they can resolve are often no less than several hundreds of nanometers\textsuperscript{15,173}. Transmission electron microscopy (TEM) produces images of nanoparticles with high resolution at the nanometer level. However, TEM is time consuming and artifactual because it requires dried particle samples that are imaged under vacuum. The agglomeration that occurs during the drying step makes it difficult, if not impossible, to quantify the agglomeration in the original liquid suspension. Also, the small sample size (number of particles counted in the TEM images) limits the statistical accuracy of particle sizing with TEM images\textsuperscript{132}. Dynamic light scattering (DLS) is another common technique used to measure the particle size of a CMP slurry\textsuperscript{174,175}. However, DLS has limitations when dealing with polydisperse systems. For example, Fiella et al. found that DLS cannot resolve two sizes of particles mixed together when the difference between them is less than \textasciitilde 30 nm; furthermore, the measured size distributions are significantly biased, generally toward large particles, when the difference between the sizes is great (e.g., \textasciitilde 900 nm)\textsuperscript{176}. Methods for resolving different sizes of particles in polydisperse dispersions still need improved sensitivity and accuracy to facilitate better analysis and control of CMP slurry quality. For many nanoparticle applications, it is a considerable challenge to sensitively resolve different nanoparticle sizes and thus accurately distinguish impurity-size nanoparticles from primary monodisperse nanoparticles.

Single particle inductively coupled plasma-mass spectrometry (spICP-MS) is an emerging technique in which traditional ICP-MS instrumentation is operated in a time-resolved mode. Degueldre et al. originally reported the use of a single-particle counting
mode with ICP-MS to determine the particle size distributions of several colloids in aqueous solutions, some of which were at the nano-scale\textsuperscript{75, 124, 125}. Basically, as a dilute nanoparticle suspension is introduced into the ICP-MS, each particle is individually atomized and ionized in the plasma. The instrument detects the resulting ion cloud as a packet of ions so as to generate a signal pulse, which is integrated over a specific dwell time. The signal readings of the instrument are documented in terms of counts per second versus time as the direct output of spICP-MS. In recent years researchers have applied spICP-MS to nanoparticles including Ag, TiO\textsubscript{2}, and ZnO\textsuperscript{112, 128, 137}. Furthermore, Pace et al.\textsuperscript{76} presented improved calibration and data-processing approaches in which the transport efficiency of the instrument plays a critical role in both quantifying and sizing nanoparticles. Later the transport efficiency was increased remarkably by employing monodisperse microdroplet generators (MDGs)\textsuperscript{138, 177, 178}. However, spICP-MS still faces some challenges, in particular how to accurately discriminate particle signals, especially those arising from the smallest particles in the distribution, from background signals. An approach that uses three times the standard deviation (denoted by $\sigma$) of the entire signal data set as a threshold is often adopted to differentiate particle signals from background\textsuperscript{76, 134}; in this technique, the threshold of $\mu + 3\sigma$ is calculated, and signals above this threshold are removed from the signal data set and clustered into particle signal group. The process is repeated until no additional particle signals are differentiated. In some cases this method incorrectly treats background signals as particle signals and thus overestimates the number of small particles. Tuoriniemi et al.\textsuperscript{134} tried to overcome this issue by increasing the $3\sigma$ threshold to $5\sigma$ on the basis of experimental optimization. However, this method can arbitrarily classify particle signals as background signals in
cases in which <5σ works better as the threshold. Thus, the multiplier (n) for σ is subjective and difficult to determine, making the differentiation of background signals from signals arising from small nanoparticles ambiguous. Another challenge is accurately drawing a line between background and particle signals, which requires a more rational signal processing approach to assist spICP-MS in quantifying and sizing nanoparticles.

The present work addresses these two challenges in order to improve the technical robustness of spICP-MS. First, we apply the K-means algorithm to the signal processing of the spICP-MS technique for the first time to assist the differentiation of particle signals from background signals. We compare this new method with the current standard deviation (i.e., μ + n × σ) threshold method to demonstrate the advantage of its clustering ability. Second, we test the ability of the spICP-MS technique (with the K-means algorithm) to quantitatively differentiate a very small number of secondary impurity-size nanoparticles mixed with primary monodisperse nanoparticles to demonstrate its potential applicability in QA/QC for nanoparticle size homogeneity, which is required for industrial nanomaterials such as CMP slurries. We also compared the size resolution ability of the spICP-MS and DLS techniques to show the higher accuracy and other advantages of spICP-MS. Overall, this work demonstrates the viability of a statistical signal processing algorithm to process spICP-MS data and the size resolution ability of spICP-MS with the K-means algorithm.
4.3. Theory

4.3.1. The K-means clustering algorithm for spICP-MS data processing.

Clustering algorithms are widely used in problems in which a set of data instances need to be defined into multiple groups based on the similarity among them. Among the clustering algorithms that have been developed, the K-means algorithm is a robust one commonly used to partition a data set into \( k \) groups with \( k \) centers, i.e. the mean value, determined for each group. The optimization principle is to find the optimal \( k \) centers so as to minimize the mean squared distance from each data point to its nearest center\(^\text{179}\). Additional details on the K-means algorithm are found elsewhere\(^\text{180-182}\). Researchers have modified the K-means algorithm to make it applicable to processing many types of data including GPS\(^\text{183}\), color quantization, and image segmentation\(^\text{179}\).

The raw data generated by spICP-MS (Figure 4.1) is a data set of signal readings in counts per second (cps) versus time (ms) for a sample. Each data point represents either a particle signal (a pulse) or background signals (baseline). The magnitude of the data point (the height of the pulse) of the particle signal is related to the particle size and the number of pulses is related to the particle concentration, given assumptions about the elemental composition, particle geometry, and density\(^\text{75, 76}\). However, to manipulate these two conversions so as to quantify and size nanoparticles simultaneously, separation of the particle and background signals is a critical first step. This is essentially a data clustering process. In some cases, we also need to discriminate between different dominant sizes of particles contained in a single sample, as shown by the CMP example mentioned above. Discriminating among the particle size groups is essentially another
data clustering problem within the particle signal data set. Therefore, the K-means algorithm is appropriate for spICP-MS signal processing.

The statistical algorithms necessary to apply K-means clustering theory to spICP-MS data processing are as follows:

1. Let \( \{C_1, C_2, \ldots, C_k\} \) be the initial \( k \) cluster centers (corresponding to \( k \) groups) for \( M \) data points of one sample data set \( D \).
2. Assign the \( i^{th} \) data point \( d_i \) in \( D \) to its closest cluster center \( C_j \), where \( j \) is the \( j^{th} \) cluster center (group).
3. Update \( \{C_1, C_2, \ldots, C_k\} \) to be the averages of points contained in each group, i.e.,

\[
C_j = \frac{\sum_{d_j \in G_j} d_j}{\text{Data Number in } G_j} \quad (j = 1, 2, \ldots, k),
\]

where \( G_j \) is the \( j^{th} \) group.
4. Iterate 2 and 3 until the convergence of \( \{C_1, C_2, \ldots, C_k\} \).
5. Return \( \{C_1, C_2, \ldots, C_k\} \) and \( k \) groups determined by the cluster centers.

When a sample contains monodisperse nanoparticles, manipulating the K-means algorithm with \( k = 2 \) leads to a boundary between background signals and particle signals (Figure 4.1a). In Figure 4.1b, above the background-particle boundary, another boundary is obtained via the K-means algorithm that differentiates two groups of nanoparticles corresponding to nominally sized 50-nm and 80-nm AuNPs. In this case, the resolution of different dominant sizes of particles could be realized either by increasing the \( k \) value (i.e., group number) or by iteratively applying the K-means algorithm with appropriate \( k \) values. For example, for samples containing two sizes of nanoparticles, setting \( k = 3 \) clusters the whole data set into three groups; the minimum center group corresponds to the background signals, and the other two groups correspond to the two sizes of nanoparticles. Alternatively, one can first set \( k = 2 \) to differentiate the background and
particle groups and then use $k = 2$ on the particle group to differentiate the two sizes. The boundaries drawn in Figure 4.1b were obtained via the second approach. Practically, the K-means algorithm can be used in multiple procedures with different $k$ values to determine which one yields the most reasonable results. Future work will seek approaches to optimize $k$ values as well to achieve best clustering results.

**Figure 4.1.** spICP-MS raw data for BBI AuNPs: (a) sample of 80-nm particles in 113 ppt concentration showing the thresholds of the $n \times \sigma$ method and the K-means algorithm, (b) sample of 50-nm particles (8 ppt) mixed with 80-nm particles (92 ppt); the boundary lines determined by the K-means algorithm cluster all signals into three groups: from bottom to top, background signal, 50-nm group, and 80-nm group.
4.4. Experimental Section

4.4.1. Chemicals.

Monodisperse gold nanoparticles with nominal diameters of 20 nm, 50 nm, 80 nm, and 100 nm as reported by the manufacturer were purchased from British Biocell International (BBI, Cardiff, UK) and used without further purification. Reference gold nanoparticles with a nominal diameter of 60 nm were obtained from the U.S. National Institute of Standards and Technology (NIST). A nominal 30-nm cerium oxide nanoparticle suspension was purchased from Alfa Aesar (Ward Hill, USA). Ionic gold and cerium standards for ICP-MS calibration were purchased from VHGs Labs (Manchester, USA) and diluted to different concentrations using 2% HCl (for gold) or 2% HNO₃ (for cerium) prepared with ultrapure concentrated hydrochloric acid (ULTREX II Ultrapure Reagent, J.T. Baker) or nitric acid (ULTREX II Ultrapure Reagent, J.T. Baker). The same concentrated hydrochloric acid and nitric acid were used for gold nanoparticle digestion to determine the stock concentration. In addition, 18.3 MΩ nanopure water (Barnstead Thermolyne) was used for dilution in the experiment.

4.4.2. Characterization of gold nanoparticles

The particle size was characterized by spICP-MS, dynamic light scattering (DLS), and transmission electron microscopy (TEM). Details about these analyses can be found in the Supplementary Information. The concentration and mean diameter of each AuNP product are reported in Table 4.1. Some of the size distribution characterization results are shown in Figure S4.1 in the Supplementary Information.

Table 4.1. Mean size and initial concentration of the AuNPs used in this study
### Nominal size

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<th>80 nm</th>
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<td>96 ± 6</td>
<td>56 ± 0</td>
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</table>

<sup>a</sup>Mean size determined by sizing 100–200 particles from TEM images.  
<sup>b</sup>Data not analyzed due to insufficient sample.  
<sup>c</sup>Uncertainty determined from duplicate analysis.

### 4.4.3. ICP-MS analysis

A Thermo X series II ICP-MS was used for all ICP-MS analyses. For spICP-MS analysis, the instrument was placed in time-resolved analysis (TRA) mode, and 60 s was set as the measurement duration for each sample. The dwell time (integration time of one reading by the detector) was set at 10 ms. The instrument was tuned before each analysis using a multielement tuning solution containing Li, Ce, In, and U at a concentration of 10 µg•L<sup>-1</sup>. The forward power of the ICP-MS was maintained at 1300–1400 W. The sample introduction system is composed of a glass nebulizer and a conical spray chamber. The sample flow rate was set in the 0.6–0.7 ml•min<sup>-1</sup> range, and the specific value was measured before each run and used for further data processing. The nebulizer transport efficiency was determined for each analysis using the method published by Pace et al.<sup>76</sup>, and normally a value at ~1.7% was obtained. The gold isotope of 197Au and cerium isotope of 140Ce were used for the measurement. All spICP-MS signal data (CPS versus time) were exported into a spreadsheet and further processed with MATLAB (2011b, version 7.13.0.564).
4.4.4. Sample preparation and analysis.

All samples for spICP-MS analysis were diluted to appropriate concentrations at part per trillion levels. The actual mass concentrations of different nanoparticles varied with size to yield suitable number concentrations for effective analysis. For the size resolution experiments, gold nanoparticles of two different diameters were mixed together by spiking a secondary size (20-nm, 50-nm, or 100-nm) nanoparticle suspension into a primary size (80-nm) nanoparticle suspension at volume ratios of 1:99, 3:97, 5:95, 8:92, and 10:90. The volume ratios were later converted to the mass percentage of each size after measuring the stock concentrations for all AuNPs. All prepared samples are analyzed by spICP-MS (see the Supplementary Information for more details about the instrument operation).

4.5. Results and discussion

4.5.1. Comparison of particle signal discrimination with the K-means algorithm versus the \( n \times \sigma \) threshold method

Figure 4.1a, corresponding to 80-nm AuNPs, shows the thresholds of \( \mu + 3\sigma \), \( \mu + 4\sigma \), \( \mu + 5\sigma \) and the K-means with \( k = 2 \). Assuming each particle has a diameter of 80 nm and based on measured ICP-MS instrument parameters (i.e., flow rate and transport efficiency), ~243 particles min\(^{-1}\) were predicted to enter the plasma and be detected (i.e., \( f_p \) in Eq. 1). The standard deviation threshold method gave the particle number as 328 min\(^{-1}\) for 3\( \sigma \), 286 min\(^{-1}\) for 4\( \sigma \), and 275 min\(^{-1}\) for 5\( \sigma \). In contrast, the K-means algorithm method calculates the particle number to be 263 min\(^{-1}\), which is closer to the theoretically calculated number concentration than any of the \( n \times \sigma \) threshold methods. It can be
statistically estimated there is always a fraction of background signals cannot be removed by $n \times \sigma$ method\textsuperscript{41}. As seen in Figure 4.1a, choosing $3\sigma$ or $4\sigma$ as the threshold resulted in some small-magnitude signals being assigned to the particle group. These small-magnitude signals can be either background signals or derived from sample contaminant or partially detected particles, and taking them as particle signals may overestimate the nanoparticle concentration and bias the size distribution. The $5\sigma$ threshold better filtered the background compared with $3\sigma$ and $4\sigma$. Generally, however, the selection of a $\sigma$ coefficient is ambiguous and arbitrary. As the K-means algorithm draws a line higher than 3, 4, or 5 times the standard deviation, it is much more likely to filter (into the background signal group) all the small-magnitude signals that bias the effective size distribution. The drawback of the $n \times \sigma$ threshold method is that the selection process for particle signals is directed only against the background signals because the method calculates the standard deviation only of the background signal as the threshold. This can lead to the misidentification as particle signal pulses of some large and highly fluctuating background signals, which can be caused by, for example, instrument instability or sample impurity that causes interference with the analyte. The K-means clustering algorithm statistically minimizes the total within-cluster variation, for both background group and particle group, by minimizing the squared distance of each point to its cluster center. Thus, the K-means algorithm takes into account the patterns of not only the background signal group but also the particle signal group when performing the clustering, which increases the accuracy of the boundary obtained compared with the unidirectional $n \times \sigma$ method. Compared to the traditional $n \times \sigma$ method, the K-means algorithm eliminates the ambiguity of the multiplier for $\sigma$. 

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According to the study of Pace et al., particle size distribution can be obtained from the raw signal of spICP-MS (more details can be found in the Supplementary Information). The raw data in Figure 4.1a was converted to particle size to produce size distribution histograms for 80-nm AuNPs with different signal processing methods, as shown in Figure 4.2. Both 4σ (Figure 4.2a) and 5σ (Figure 4.2b) give rise to a few particles binned to the 20 nm to 70 nm range, which are highly likely attributable to the background signals, whereas the K-means algorithm eliminates these signals, yielding a more accurate size characterization of the sample. Another possibility is these small particles might be a few particles that were detected on the boundary of two dwell times, which can lead to the division of one pulse into two smaller pulses that appear as smaller sizes. The dwell time applied in the present study is 10 ms, which is a conservative selection, which avoids capturing partial particle events but limits the upper concentration limit of the method due to possible coincidence events.
Figure 4.2. (a–e) Particle size distribution histograms of BBI 80-nm AuNPs determined by different methods: (a) spICP-MS with 4σ threshold signal processing, (b) spICP-MS with 5σ threshold signal processing, (c) spICP-MS with K-means algorithm signal processing, (d) DLS, (e) TEM based on counting >100 particles, and (f) TEM image of AuNPs.
Figure 4.2 also includes DLS (Figure 4.2d) and TEM (Figure 4.2e and f) analysis of the size distribution of 80 nm AuNPs for comparison with the spICP-MS results. The three analytical techniques were fairly consistent, and both DLS and TEM support the accuracy of spICP-MS results processed with the K-means algorithm value, which had a size range of approximately 70–110 nm and mean size of 85–90 nm (Table 4.1). All these results demonstrate that spICP-MS using the K-means algorithm as a signal processing method is a robust way to characterize the size distribution of nanoparticles.

It is noteworthy that we did not explore the applicability of the K-means algorithm to the case where the particle signals may potentially overlap with background signals. This can happen when the nanoparticle size is extremely small (e.g., < 10-20 nm for AuNPs) or there is a significant concentration of ionic metal species existing and increasing the background signals. Recently Cornelis and Hassellöv\textsuperscript{140} presented a signal deconvolution method to deal with this case using polygaussian distribution to describe the background signals and subtracting this component to obtain particle signals. The K-means algorithm functions based on seeking the least within cluster variation. The currently presented method takes the signal intensity magnitude as the only variable, or a feature, to calculate the within cluster variation. Thus we expect the current limitation for this method that when the particle signals lie below background signals, they will be more likely clustered to the background group. However, future work may add more features other than the signal magnitude to enable us achieve more robust and accurate estimation of background signals even for the case in which particle and background signals overlap. The other feature(s) may include the probability distribution of the signals as suggested by Cornelis and Hassellöv\textsuperscript{140}. A fusion of the two methodologies may accomplish more imperative
breakthrough for the signal processing of spICP-MS. The methodology we presented here can be the first step of exploring the applicability of K-means algorithm to spICP-MS and we demonstrated its advantage over the n×σ method of best accurately obtaining the particle signals for cases in which the signal intensity magnitude has the ability to differentiate the background and the particle signals, i.e., the overlap between the two is not taken into account.

4.5.2. Size resolution capability of spICP-MS compared with DLS

To evaluate the ability of spICP-MS to discriminate a small amount of a secondary impurity size nanoparticle from a primary size nanoparticle, a small amount of one size of AuNPs was spiked into a sample of primary-size AuNPs. Here 80-nm AuNPs were selected as the primary size for all mixtures because they are typically used in CMP slurries.

Figure 4.3 shows DLS and spICP-MS results for two mixtures. Figure 4.3a and c show samples of 80-nm primary size nanoparticles mixed with a 20-nm secondary impurity size that accounts for 5.6% of the sample based on mass. Figure 4.3b and d show samples of 80-nm dominant primary size sample nanoparticles mixed with a 100-nm secondary impurity size that accounts for 15.9% of the sample based on mass. Both DLS (Figure 4.3a) and spICP-MS (Figure 4.3c) are able to discriminate the two size groups in the 20-nm and 80-nm mixture (~60 nm size difference), as shown by the two main peaks in the size distribution histogram. However, DLS only partly detected the 20-nm impurity in the 20 nm to 30 nm range; on the basis of a comparison with DLS analysis of the 20-nm nanoparticles alone, most of the particles in the 20-nm size group were neglected in the mixture (Figure S4.1 of the Supplementary Information). In
contrast, spICP-MS (Figure 4.3c) can not only separate the primary size and secondary impurity size but correctly identifies the size distribution of the two groups given the individual sample analyses. Because spICP-MS produces the number-based size distribution on the basis of pulse frequency, the 5.6% (based on mass) of the sample that is the 20-nm secondary impurity-size particles accounts for a larger peak height and area than the 94.4% of the sample that is the 80-nm primary size, for mass is proportional to diameter cubed under spherical geometry and constant density assumptions. When 100-nm nanoparticles are the secondary impurity in a 80-nm primary-size sample, DLS is unable to discriminate between the two size groups, as shown in Figure 4.3b, because of the only ~20 nm difference between the two sizes, which is consistent with the previously reported DLS size resolution capability\(^{176}\). In contrast, spICP-MS with the K-means algorithm processing the raw signal can still give a boundary between the two size groups. In Figure 4.3c and d, the secondary impurity sizes (white bars) were quantitatively differentiated from the primary size (gray bars) by applying the K-means algorithm to the particle signals to cluster the data into two size groups after the algorithm had been used to remove background signals. In Figure 4.3d, there is no marked distance between two size group peaks because the 80-nm primary size (characterized as 88 nm) and the 100-nm secondary impurity size (characterized as 101 nm) are so close that some nanoparticles in the two original groups have overlapping diameters. The K-means algorithm assigns the overlapping diameters to one of the groups on the basis of the least within cluster variation principle. In comparing Figure 4.2c with Figure 4.3d, we find that some particles larger than 100 nm in the original 80-nm sample (Figure 4.2c) are clustered into the impurity size reasonably well (Figure 4.3d). Admittedly, we cannot
preclude the possibility that some of the larger particles detected in the 80-nm sample are caused by coincidence of spICP-MS pulses, which is a limitation and can lead to errors.

Figure 4.3. Particle size distributions of AuNP samples with 5.6% by mass of 20-nm nanoparticles (secondary impurity size) in 80-nm nanoparticles (a and c) and 15.9% by mass of 100-nm nanoparticles (secondary impurity size) in 80-nm nanoparticles (b and d), determined using spICP-MS and DLS analysis. The black and white bars in c and d differentiate two size groups.

When dealing with polydisperse particle samples, DLS calculates the multimodal size distribution (MSD) by collecting the decay rates of multiple particles, fitting them to the total autocorrelation function, and thereby producing a size distribution. During the
derivation from the initially detected light scattering intensity signal to the particle size, DLS exhibits a significant drawback in that a disproportionate intensity of response leads to massive domination of larger particle signals over smaller particle signals. Based on Rayleigh scattering theory, for a particle diameter $d < \lambda/20$, the scattering intensity $I \propto d^6$, whereas for $d > \lambda/20$, $I \propto d^2$. The laser light wavelength of the instrument used is 660 nm. When 20-nm particles mixed with 80-nm particles are analyzed by DLS, the scattering intensity of the 80-nm particles is more than 100 times higher than that of the 20 nm particles, which will result in the larger particles significantly masking the 20 nm particles. In addition, the multimodal size distribution output of DLS is actually an ill-posed mathematical conversion of the autocorrelation function to the diffusion coefficient in which small variations can cause large deviations\textsuperscript{132}. Even in the case in which the MSD output of DLS can resolve different sizes of nanoparticles, quantification is difficult to accomplish. Determination of the volume or number distribution output by DLS by mathematical means is based on good knowledge of the particle geometry, polydispersity, and underlying assumptions\textsuperscript{185}, which is often difficult to obtain.

4.5.3. \textbf{Quantifying by spICP-MS the percentage of secondary impurity-size nanoparticles mixed with primary-size nanoparticles}

Compared with other particle size characterization techniques, spICP-MS has a fundamental advantage owing to the measurement of elemental mass and concentration. The excellent sensitivity of mass spectrometry means that spICP-MS can not only highly resolve particle size but also quantify the amount of particles in different size groups. Herein the percentage of secondary impurity-size particles in AuNP mixtures was varied to test the ability of spICP-MS to quantitatively distinguish the secondary impurity size
from the primary size. Figure 4.4 shows the results from AuNP mixtures containing a 50-nm secondary impurity size in an 80-nm primary size. Different amounts of secondary impurity-size particles, which account for 1.6% (Figure 4.4a), 4.8% (Figure 4.4b), 7.9% (Figure 4.4c), and 12.4% (Figure 4.4d) of the mixtures by mass, were spiked into 80-nm primary size AuNPs. The K-means clustering algorithm was applied to differentiate the secondary impurity size from the primary. spICP-MS discriminates the 50-nm secondary impurity size from the 80-nm primary size well. After the K-means algorithm determined the boundary between the two size groups, the mean size for each group was calculated and is shown in Figure 4.4; the mean sizes are quite consistent with spICP-MS characterizations of the size of 50-nm and 80-nm AuNPs individually (Table 4.1). The two peaks corresponding to the two sizes as differentiated by the K-means algorithm can be contrasted with the individual size distribution results (Figure S4.1 and Figure S4.2c of the Supplementary Information). The size distribution is also fairly consistent, which suggests the K-means algorithm accurately discriminates the particle size groups. In Figures 4.4a, b, c and d, the increasing percentage of particles in the secondary impurity-size group is evident from the increasing height and area of the secondary impurity size peak. Similar experiments were carried out by spiking 20-nm and 100-nm secondary impurity-size particles into 80-nm primary-size AuNPs. The size distribution and resolution results of all AuNP mixtures can be found in Figure S4.2 of the Supplementary Information.
Figure 4.4. Particle size distributions determined by spICP-MS of AuNP mixture samples containing a 50-nm secondary impurity size (white) in an 80-nm primary size (gray); the percentages of secondary impurity-size particles in the various samples are: (a) 1.6%, (b) 4.8%, (c) 7.9%, and (d) 12.4%, based on mass. Legends indicate the mean diameters of the particles in each impurity size group and primary size group as determined by spICP-MS.

The percentage of particles in the secondary impurity size group was further quantified by counting the number of particles in the secondary and primary groups to evaluate their concentrations. Figure 4.5 shows the percentages of particles in 20-nm, 50-nm and 100-nm secondary impurity-size groups mixed with 80-nm primary-size AuNPs.
The expected impurity percentages on the x-axis are calculated from the amount of experimentally spiked secondary impurity-size particles as determined by acid digestion and typical ICP-MS analysis. The y-axis indicates the impurity percentage as determined by spICP-MS analysis with K-means algorithm signal processing. Linear fits of each sample show the correlations between the spICP-MS experimental data and expected data. For mixture samples in which the secondary impurity size group is 20 nm or 50 nm, all plots align closely along the 1:1 line, and the slope of the fitting line is nearly unity, which suggests that the spICP-MS technique can quantify with high accuracy a small percentage of impurity size particles in a monodisperse particle sample and that the achievable impurity size detection limit may be as small as 1–2% based on mass. Plots of mixture samples with 100-nm secondary impurities differ from the others by an intercept of the fitting line at ~5.0%. However, as the slope of the fitting line is still close to unity, the 5.0% intercept could be attributable to the original 80-nm monodisperse AuNP sample containing the ~100-nm impurity size. To confirm this, the K-means algorithm was used to discriminate the impurity size in the original 80-nm AuNPs; 5.8% of the nanoparticles were clustered in a 109 nm-mean-size group. Again, the chance of particle coincidence within a single dwell time exists, considering two or three 80-nm AuNPs can yield 101-nm or 115-nm single particle with the equivalent mass. Overall, spICP-MS with K-means algorithm signal processing is capable of quantifying a small percentage of impurity-size particles contained in a monodisperse particle system. The detection limit was ~1% of impurity-size nanoparticles in a sample based on mass, and the size difference that could be resolved was as small as 20 nm. Thus, spICP-MS is a promising technique to assist the QA/QC of nanoparticle products in industrial and
manufacturing processes, e.g., nanoparticle abrasive-based CMP slurries in the semiconductor industry. Compared with other techniques such as DLS, both the low detection limit and high size sensitivity of spICP-MS are advantageous when testing size homogeneity and monitoring particle aggregation in a CMP slurry.

Figure 4.5. Mass-based percentage of the impurity size group in AuNP samples determined by spICP-MS vs. the expected values determined by acid digestion with normal ICP-MS analysis. X error bars stand for one standard deviation based on duplicated samples; Y error bars stand for one standard deviation based on triplicated measurements. A linear fit was used to describe the data consistency of spICP-MS with expected values.

4.5.4. Quantitative size resolution of CeO₂ nanoparticles by spICP-MS

After testing the capability of spICP-MS with the K-means algorithm to quantitatively and sensitively resolve nanoparticle sizes with AuNPs, we applied this methodology to the CeO₂ nanoparticles that are commonly used in CMP slurries to demonstrate the
potential industrial applicability of this methodology. DLS measurement was also conducted for comparison.

Using a nominal 30-nm CeO\textsubscript{2} nanoparticle sample as an example, we measured the particle size by DLS and spICP-MS. Based on the data fitting of the autocorrelation function, DLS determined an effective diameter of 151.0 ± 1.4 nm, with the distribution half width and polydispersity estimated as 56.7 ± 0.2 nm and 0.141 ± 0.003, respectively. The effective diameter of 151 nm indicates that substantial agglomeration has occurred in the tested sample if the nominal 30 nm size reported by the manufacturer is reliable. Both the large half width (56.7 nm) and polydispersity (0.141) indicate the sample is hardly monodisperse (normally the polydispersity is less than 0.1 by DLS for a monodisperse sample). The DLS MSD result for the CeO\textsubscript{2} nanoparticle sample is shown in Figure S4.3a and b (Figure S4.3 in the Supplementary Information) and includes both intensity-weighted and number-weighted outputs. As expected, the intensity-weighted and number-weighted MSD results varied significantly for this polydisperse sample. The intensity-weighted result suggests that the most abundant size is in the 140–210 nm range, whereas the number-weighted result gives the dominant size as 40–60 nm. For a monodisperse particle sample (e.g., the AuNPs in the previous sections), DLS gives fairly consistent outputs regardless of data processing method, but its ability to handle polydisperse samples is low, and it provides insufficient information to accurately evaluate the size distribution. Figure S4.3c shows the size distribution determined by spICP-MS. Compared with DLS, spICP-MS detected particles in a wider size range, from 15 nm to 120 nm, which agrees with the 56.7 nm half width determined by DLS (unfortunately, DLS is unable to catch all sizes over the ~105-nm range). Thus, in terms
of QA/QC for nanoparticle size, spICP-MS does better than DLS to capture a wide size
distribution of a polydisperse sample. Although the sample gives a continuous size
distribution, K-means algorithm is able to cluster the sizes based on the least within
cluster variation principle. In Figure S4.3c, we applied the K-means algorithm to cluster
the nanoparticles into six groups and determined the mass percentage of each group.
Overall, spICP-MS analysis with the K-means clustering algorithm is a highly promising
technique for QA/QC work in nanotechnology in industry and manufacturing.

4.6. Conclusions
A new approach to processing spICP-MS data was originally tested by applying the K-
means clustering algorithm to the instrument signal data set. The K-means algorithm
substantially assists spICP-MS in two aspects: (1) compared to the traditional standard
deviation approach, the algorithm better differentiates particle signals from background
signals to more accurately determine particle concentration and size distribution and (2)
the algorithm enables the technique to quantitatively discriminate different sizes of
particles by clustering particle sizes into different groups based on the least within cluster
variation principle. spICP-MS resolves particle size more sensitively as compared with
DLS, as it is able to discriminate an impurity that makes up <2% by mass in a primary
size, and the size difference can be less than 20 nm. With the K-means algorithm, spICP-
MS is capable of measuring the particle size distribution and quantifying the percentages
of impurity and dominant sizes, which were demonstrated with mixtures of gold
nanoparticles. Nevertheless, the current application of K-means algorithm still has
limitations: (1) the methodology presented here may have issue with the case in which
the particles signals and background signals overlap; (2) the optimization of group
number k has not been addressed in this study, i.e., for an unknown sample k has to be selected arbitrarily. However, potential approaches to overcome this two current limitations are indicated, which may be achieved in the future. As an application example, spICP-MS with the K-means algorithm is highly promising for QA/QC work on nanotechnology in industry and manufacturing, where the sizes of nanoparticles are critical to applications.

4.7. Acknowledgements

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4.8. Supplementary Information

Figure S4.1 Particle size distribution histograms of BBI AuNPs determined by spICP-MS, DLS, and TEM.
20nm 1.1%

20nm 3.4%

20nm 5.6%

100nm 1.7%

100nm 5.0%

100nm 8.2%
Figure S4.2. Particle size distribution of AuNP samples with different size constituents.

All the percentages in the figure are based on mass.
CHAPTER 5

A DRY POWDER ASSAY RAPIDLY DETECTS METALLIC NANOPARTICLES IN WATER VIA MEASURING THE SURFACE CATALYTIC REACTIVITY

5.1. Abstract

A dry powder containing methylene blue (MB), sodium borohydride (NaBH₄), and HEPES buffer was designed to enable an assay that rapidly (2 minutes) detects metallic nanoparticles via measuring their surface catalytic reactivity. The method is termed the “Catalytic Reactivity to NanoParticle” assay (CRNP). Tested with standard gold (Au) NPs in water, the CRNP response measured as differential absorbance at 663 nm of the fine powder assay was linearly and reproducibly correlated to the NP surface area concentration. The CRNP detection limit was evaluated to be 51 µg/L for 50-nm Au NPs, or 0.32 m²/m³ of equivalent surface area of Au NPs, demonstrating its advantage for detecting small NPs at trace levels in water. I described the heterogeneous catalytic mechanisms on the NP surface by an electrochemistry-guided kinetic model, and simulated the overall kinetics of all identified reactions, including the hydrolysis of borohydride, the reduction of MB, and the oxidation of leuco methylene blue (LMB) by dissolved oxygen. The model revealed the key mechanism that the NPs store electrons as though electrodes on which MB is reduced. To demonstrate the environmental applications, CRNP was able to quantify multiple engineered NP species in water, including Au, silver (Ag), palladium (Pd), platinum (Pt), and copper oxide (CuO), and evaluate the surface catalytic reactivity order of them. In water samples containing known or unknown NP species, CRNP directly probes the NP reactivity potential that is
potentially relevant to the environmental fate or safety of nanomaterials because the assay responds to electron mediated stimuli important for NP surface reactions.

5.2. Introduction

Engineered nanoparticles (NPs) are widely used in industrial and commercial products, e.g., catalyst composites, coatings, and medicine\textsuperscript{11, 186, 187}, and their accidental releases into aqueous media in the environment are possible. Tracking NPs in water systems is a need for assessing their exposure and risks\textsuperscript{188, 189}. Existing techniques for detecting NPs in natural waters are often costly and/or time consuming\textsuperscript{132}. For example, techniques based on the inductively coupled plasma mass spectroscopy (ICP-MS), including single particle\textsuperscript{190}, flow field fractionation\textsuperscript{191}, and high pressure liquid chromatography (HPLC)-\textsuperscript{131}ICP-MS, have complicated and expensive setups, despite their sensitivity in analyzing metallic NPs in water. Transmission electron microscopy (TEM) can characterize NP size and morphology, but it is costly, laborious, and difficult to deduce low abundance or concentration in an environmental sample\textsuperscript{192, 193}. Some techniques have thus been employed to extract low-concentration NPs from liquids (e.g., “cloud-point extraction”\textsuperscript{65, 194}) prior to analysis. However, rapid and economic detection of NPs in water was not satisfactorily achieved.

Analyses of many environmental pollutants (e.g., chromium, chlorine, organic nitrate, chemical oxygen demand) are greatly facilitated by commercialized, rapid, and easy-to-use colorimeter-based assays. They are understood to be surrogates for more advanced instruments, and drawbacks of potential interferences are well understood. Such an assay exploits a pre-designed chemical mixture, typically in powder form, to react with a target compound, resulting in a colorimetric change (e.g., absorbance or fluorescence) that can
be measured and related to the target’s concentration. The assay often serves as reliable screening-level techniques to monitor contaminants in environmental wet laboratories or in the field. The same strategy can be applied to specific NP classes. Colorimetric probes based on fluorescence\textsuperscript{195} or absorbance\textsuperscript{196} exist to detect metallic NPs, but were used in wet-chemical forms often requiring precise and tedious laboratory handling. This motivated us to consider fundamental assay chemistry and develop a power assay kit for rapidly detecting metallic NPs in water.

Designing a powder assay kit requires a reaction and thus relies on NPs’ reactivity. Many metallic NPs, e.g., gold (Au), silver (Ag), iron (Fe), palladium (Pd), and platinum (Pt), are catalytic reactive and can invoke accelerated redox reactions on their surface relative to homogeneous reactions occurring in the liquid phase\textsuperscript{14, 197-201}. Therefore they are often applied in engineering fields including hydrogen generation\textsuperscript{202}, fuel cells\textsuperscript{65}, and pollutants degradation\textsuperscript{203}. When they catalyze a redox reaction, the NP surface mediates electron transfer from a reductant to an oxidant\textsuperscript{204}. To the extent metallic NPs are used to catalyze desired redox reactions, a well-controlled redox reaction can be used to detect the NPs from solutions relying on their surface catalytic reactivity.

The reduction of tetramethylthionine chloride (also known as methylene blue (MB)) by sodium borohydride (NaBH\textsubscript{4}) was selected as the powder assay reaction in this work. NaBH\textsubscript{4} is known to reduce chemicals including p-nitrophenol\textsuperscript{205}, ferrocyanate\textsuperscript{206}, and MB\textsuperscript{196}. I used MB in this study because it can be sensitively detected by a regular absorbance spectrometer (\(\lambda_{\text{max}}=663\) nm). Reduction of MB by NaBH\textsubscript{4} is extremely sluggish in the absence of any catalyst, providing a constant NP-free background. Using an NaBH\textsubscript{4}-MB redox reaction to detect Au NPs was demonstrated with solution reactions
by Corredor et al.\cite{196}. They showed linear correlations between the MB and Au NP concentrations when the reaction reached equilibrium. However, MB decay kinetics was only empirically fitted by an exponential function. Questions remain on the exact reaction scheme in the system. The observed non-zero MB concentration in reaction equilibrium indicated that certain reactions compete with the reduction of MB by \( \text{BH}_4^- \), which was not identified in Corredor’s work. Kinetics of the redox process was also not fully investigated. These factors are critical to accurately interpreting the outcome of the assay’s measurement and understanding the reaction mechanisms.

Herein I demonstrated state-of-the-art synthesis of a dry powder assay, namely the “Catalytic Reactivity to NanoParticle” assay (CRNP), which rapidly and inexpensively detects reactive NPs in water. I used well-characterized gold (Au) NPs of different sizes to validate the assay’s performance, and developed a kinetic model to investigate the reaction mechanisms. The model incorporated the key surface redox reaction and side reactions including borohydride hydrolysis and leuco methylene blue (LMB) oxidation, and revealed the role of NPs as heterogeneous catalysts. To evaluate the environmental applications of CRNP, I demonstrated its feasibility to different metallic NP species (Au, Ag, Pd, Pt, and copper oxide (CuO)) and to NPs in surface water, and revealed its powerful potential to quantify and evaluate the surface catalytic reactivity of metallic NPs in natural waters.
5.3. Materials and methods

5.3.1. Chemical reagents.

MB hydrate (≥95%, product #28514), sodium borohydride (NaBH₄, ≥98%, product #452173), HEPES (≥99.5%, product #H3375), and sodium hydroxide (NaOH, 99.99% trace metal basis, product #306576) were purchased from Sigma-Aldrich and used without further purification. Gold nanospheres (NanoXact, 0.05 mg/mL) with nominal sizes of 20 nm, 50 nm, and 80 nm were purchased from nanoComposix. According to the manufacturing information, these Au NPs are coated with tannic acid on the surface and suspended in solution with pH 5.1–5.4, giving negative zeta-potential (-44 mV for 20 nm and -54 mV for 50 nm and 80 nm)²⁰⁷. A 40-nm silver (Ag) NP sample was also purchased from nanoComposix (nanoXact, 0.02 mg/mL). The Ag NPs were coated with citrate and negatively charged (zeta-potential=-44 mV). Figure S5.1 shows TEM images and UV-VIS spectra of all the Au and Ag NP samples provided by the manufacture.

Palladium (Pd) and platinum (Pt) NP samples were adopted from two commercial products designed as human dietary supplement drinks. A full characterization of these two NP samples were performed in a previous study and used in this work. A copper oxide (CuO) nanopowder (Sigma-Aldrich, 544868, <50 nm) was dispersed in ultrapure water to make a stock solution (1 g/L). According to the manufacture, this CuO NP sample has an average size of 28 nm and specific surface area of 33 m²/g.

Ultrapure water (18.2 MΩ·cm, Barnstead GenPure xCAD Plus) was used to make all solutions unless stated otherwise. Surface water sample was collected from Colorado River and a portion was filtered (pore size 1.6 µm, Whatman GF/A, 1820-025). The filtered surface water contains 3.9 mg/L of dissolved organic carbon (DOC) and has a
UV absorbance of 0.074 at 254 nm (UV\textsuperscript{254}). A Suwannee River Natural Organic Matter (SRNOM) sample (2R101N, IHSS) was used as a model Natural Organic Matter (NOM) compound. The percentage of carbon (C) by mass of this SRNOM is 50.7 % according to IHSS, and 47.0% (used in this work) according to our total organic carbon (TOC) analysis.

5.3.2. CRNP assay powder synthesis and testing procedure.

The CRNP assay was designed to achieve a final working solution containing 20 μM MB, 10 mM HEPES buffer at pH 7, and 10 mM BH\textsubscript{4}-. This results in a NaBH\textsubscript{4}:HEPES:MB molar ratio of 500:500:1. To achieve a homogenous powder mixture, 0.06 g MB, 22.35 g HEPES, and 0.8 g NaOH (to raise the pH of HEPES buffer to 7 in the final solution) were dissolved into ~100 mL ultrapure water, and the resulting solution was frozen and then freeze dried (Labconco FreeZone Console Freeze Dry System, 6 Liter) under collector temperature < -40 °C and vacuum <0.45 mBar for 72 hours. After freeze drying, the powder was ground using mortar and pestle to achieve a visually uniform and fine powder. Because NaBH\textsubscript{4} reacts with water, it was not included in the freeze-dried mixture. Instead, NaBH\textsubscript{4} was directly ground in a mortar to achieve a fine powder. The two powders were well mixed at a mass ratio of 6.54:1 (converted from the required molarity ratio) in a glass vial using a vortex mixer, yielding the final reagent powder. To simulate a commercial assay kit, 0.28 g of the final powder was added to a clear glass vial designed for absorbance measurement (COD digestion vials; HACH product #2125815).

For each CRNP assay test, 10 mL sample was added to a prepared assay vial (described above), and a timer was started. Immediately after adding the sample to the
vial, the acute dissolution and hydrolysis of NaBH$_4$ in water formed hydrogen gas bubbles. The rapidly-generated bubbles assisted a fast diffusion of MB and achieved a homogeneous solution with a uniform blue color in a few seconds. The sample vial was gently and repeatedly inverted to maintain constant mechanical mixing and drive the hydrogen bubbles into the gas phase in the vial. After 2 min, the vial was placed in an absorbance spectrometer (DR5000, HACH) to measure the visible light absorbance at 663 nm.

**5.3.3. Kinetic analysis of the CRNP reaction in solution using time resolved UV-VIS spectrometry.**

The kinetics of the CRNP was investigated with separately prepared chemical solutions instead of powder to complement and validate the CRNP methodology. The solution-based assay method was adopted from Corredor’s work$^{196}$ with slight modifications. Briefly, 2.5 mL of prepared 10 mM HEPES buffer (pH=7) was added to a 1-cm polystyrene cuvette (perfector scientific, #9012). MB stock solution (4 mM) was added to reach a final concentration of 20 µM. The cuvette was then placed in a portable UV-VIS spectrometer (Ocean Optics, USB-ISS-UV/VIS and USB4000 light source), which was positioned on a magnetic stirring plate (IKA, Lab DISC). The solution in the cuvette was mixed with a magnetic micro stir bar (VWR Spinbar, 1.5×7.9 mm). While mixing, 12.5 µL of NaBH$_4$ stock solution (1 M, prepared in 1 mM NaOH in an ice bath to minimize hydrolysis) was added to the cuvette to achieve an initial BH$_4^-$ concentration of 5 mM. The spectrometer was operated in a time-resolved reading mode to acquire absorbance at 663 nm ($A_{663}^6$) and 760 nm ($A_{760}^7$) every 0.1 s. The analysis reaction was initiated directly in the cuvette by adding Au NP stock solution in a desired concentration.
The moment Au NP was added, absorbance at 663 nm started dropping. Time-resolved $A_{663}$ and $A_{760}$ data was saved for 20 min from the Au NP spiking point and exported for further analysis.

$A_{663}$ was recorded to quantify MB concentration, whereas $A_{760}$ was recorded to monitor light scattering caused by the hydrogen gas bubbles. Subtracting $A_{760}$ from $A_{663}$ corrected the $A_{663}$-time spectrum to reflect only MB reduction. A demonstration of this deconvolution process is given in Figure S5.2.

Specific modeling equations are developed in the subsequent section. Modeling used the corrected $A_{663}$-time spectrum for MB concentration versus time, using an experimentally assessed extinction coefficient of 74663 L·mol$^{-1}$·cm$^{-1}$ for MB. The kinetic data were fitted by a model using the non-linear regression toolbox of MATLAB (R2015b). All model calculations and simulations were also performed in MATLAB using its ordinary differential equation solver package.

5.4. Results and discussion

5.4.1. CRNP development and validation using Au NPs.

CRNP was applied to solutions of Au NPs with different sizes (20, 50, and 80 nm). Figure 5.1a shows, for each NP size, that the value of $A_{663}^{\text{remaining}}$, remaining MB concentration after the reaction, showing an inverse and linear correlation to the NP mass concentration ($C_{NP}^{\text{mass}}$). 2 min was selected as the recording time because a relatively stable absorbance in about 1.5-2.5 min was observed for all Au NP concentrations in our test. The corresponding slope (the absolute value) in Figure 5.1a varied among the three sizes in the order 20 nm >50 nm >80 nm, indicating the assay was more sensitive to smaller NPs.
Based on the principle heterogeneous catalysis, the steeper slope of smaller size NPs is attributed to the larger surface area. To verify this, I plotted (Figure 5.1b) $\Delta A^{663}$, the difference of $A^{663}_{2\text{min}}$ between a blank (i.e., ultrapure water without NPs) and a sample, over the surface area concentration (i.e., surface area per volume of solution) of equivalent Au NPs ($C_{\text{Au-avg}}$, in m²/m³). $\Delta A^{663}$ across different NP sizes shows a single linear correlation to $C_{\text{Au-avg}}^{\text{surface}}$, validating the principle of heterogeneous catalysis. A more mechanistic interpretation and validation is discussed later regarding the role of Au NP acting as heterogeneous catalyst.

![Figure 5.1](image_url)

**Figure 5.1.** Assay evaluation by Au NPs: $A^{663}_{2\text{min}}$ versus $C_{\text{NP}}^{\text{mass}}$ (a) and $\Delta A = (A^{663}_{2\text{min,blank}} - A^{663}_{2\text{min,sample}})$ versus $C_{\text{Au-avg}}^{\text{surface}}$ (b), for Au NPs of 20 nm, 50 nm, and 80 nm. Error bars show two times standard deviation of three samples.

A 50 nm AuNP solution (80 µg/L) was analyzed in 8× replication to determine the method detection limit (MDL) of the assay. The MDL was calculated using $2.998 (t_{0.99, n=7}) \times \sigma$ (standard deviation) and the experimental calibration curve (Figure 5.1b) following a method recommended by the United States Environmental Protection
Agency\textsuperscript{208}. The MDL was determined to be 51 $\mu$g/L for 50-nm Au NPs, or 0.31 $m^2/m^3$ as $C_{\text{Au-eq.}}$. In Figure S5.3, the MDL is converted to $C_{\text{NP}}^{\text{mass}}$ for different NP sizes for spherical Au NPs (density=19.3 g $\cdot$ cm$^{-3}$), assuming the intrinsic reactivity of the surface remains constant when size changes. The essential surface correlation of the assay determines that the method has lower mass-based MDL for smaller sizes. It is predicted that the assay can sensitively detect 1-nm Au NPs at $\sim$ 1 $\mu$g/L. This is a remarkable advantage over other analytical techniques, which do not have sufficiently low MDL or become limited to small size NPs. For example, the UV-VIS spectrometry gave a MDL of 400 $\mu$g/L evaluated with the 50 nm Au NPs (Figure S4), while the MDL for CRNP for the same size Au NPs is $\sim$50 $\mu$g/L; the single particle ICP-MS technique is able to detect Au NP at sub-ppb levels\textsuperscript{153}, yet only for sizes larger than $\sim$13 nm\textsuperscript{190}.

5.4.2. The CRNP reaction mechanism analysis.

In the scheme of Au NPs catalyzing the MB-$\text{NaBH}_4$ redox reaction, Corredor et al.\textsuperscript{196} supposed an equilibrium reached after $\sim$100 s of reaction when a stable MB concentration was observed. In both their assay and our powder assay, $\text{BH}_4^-$ (10 mM in a working solution) is in excess relative to MB (40 or 20 $\mu$M). If $\text{BH}_4^-$ reducing MB was the only dominant reaction, one would observe MB decaying to zero after sufficiently long reaction time; however, the observed reaction “equilibrium” suggested other reactions opposing MB reduction. To improve understanding of the reaction mechanism, I investigated the MB reduction kinetics using wet chemicals (i.e., as solutions) in solution-based and time-resolved UV-VIS spectrometry following Corredor et al.’s approach. MB decayed to a minimum in 50–100 seconds, remained in steady state at the
minimum for about tens of seconds, and appeared to reform until reaching an $A_{663}^\text{min}$ near the initial level (Figure 5.2). This temporary “steady state” is where the assay indicator should stand, and consistent with selecting $A_{663}^\text{min}$ in our assay test. Figure 5.2 also reveals that higher Au NP dosage leads to faster MB reduction rate and lower equilibrium level. However, despite faster reaction rate initially, it took longer time for higher Au NP dosage to reach the equilibrium, which opposes typical behavior of one-step reactions where faster reaction rates reach equilibrium in shorter time, thus suggesting the reaction(s) against MB reduction is not merely a back oxidation.

Figure 5.2. MB reaction kinetics (concentration versus time) in the presence of Au NP at (From top to bottom) 50 (red), 100 (pink), 160 (green), 200 (orange) and 250 (purple) µg/L, sampled every 0.1 s using time-resolved UV-VIS spectroscopy. MB concentration is converted from $A_{663}$ using its extinction coefficient of 74663 L · mol⁻¹ · cm⁻¹. All reaction are in conditions of $C_{0,BH_4} = 0.005$ M, $C_{0,MB} = 20$ µM, and 10 mM HEPES buffer (pH 7). Dashed lines present the model fitting.
MB was reduced to leuco methylene blue (LMB) in the assay reaction as confirmed by the formation of absorbance peak at about 254 nm\textsuperscript{209,210} (Figure S5.5). LMB can be oxidized by molecular oxygen and reform MB\textsuperscript{209,211,212}. BH\textsubscript{4}\textsuperscript{−} also reacts with water in room temperature, and the hydrolysis is favored in neutral and acidic conditions\textsuperscript{213}. I hypothesize these are the two major reactions going against MB reduction and formulate all reactions in the system as:

\begin{equation}
\text{BH}_4^- + 2\text{H}_2\text{O} \rightarrow \text{BO}_2^- + 4\text{H}_2, \quad (5.1)
\end{equation}

\begin{equation}
\text{BH}_4^- + 4\text{MB}^+ + 2\text{H}_2\text{O} \rightarrow \text{BO}_2^- + 4\text{LMB} + 4\text{H}^+, \quad (5.2)
\end{equation}

\begin{equation}
2\text{LMB} + \text{O}_2 \rightarrow 2\text{MB}^+ + 2\text{OH}^-, \quad (5.3)
\end{equation}

where MB\textsuperscript{+} is the cation species of MB (pK\textsubscript{a}=0)\textsuperscript{210}. Reaction (5.1) can happen both in bulk solution (homogeneous reaction) and on NP surfaces (heterogeneous reaction). Reaction (5.2) is the primary reaction enabling the assay and happens solely on NP surfaces.

Previous studies describe metal colloids catalyzed surface redox reaction via a electrochemistry scheme\textsuperscript{199,204,214-217}, which suggests that each metallic particle acts as a mixed (i.e., combined anode and cathode) electrode, withdrawing electrons from a reductant as an anode and discharging them to an oxidant as a cathode. Applying this scheme to our assay reaction, half reactions on Au NP surfaces composing reactions (5.1) and (5.2) include:

\begin{equation}
\text{BO}_2^- + 8\text{H}^+ + 8\text{e}^- \rightarrow \text{BH}_4^- + 2\text{H}_2\text{O}, \quad k_1^0, \quad E_1^0, \quad (5.4)
\end{equation}

\begin{equation}
2\text{H}_2\text{O} + 2\text{e}^- \rightarrow \text{H}_2 + 2\text{OH}^-, \quad k_2^0, \quad E_2^0, \quad (5.5)
\end{equation}
\[ \text{MB}^+ + 2e^- + H^+ \rightarrow \text{LMB}, \quad k_1^0, \quad E_1^0. \quad (5.6) \]

\(k_1^0, k_2^0,\) and \(k_3^0\) are standard rate constants, and \(E_1^0, E_2^0,\) and \(E_3^0\) are standard or formal potentials for reaction (5.4)-(5.6). Reaction (5.4) is anodic, and reactions (5.5) and (5.6) are cathodic.

Two reaction steps were hypothesized for our assay (Figure 5.3). In Step 1, Au NPs were instantly charged with electrons from the strong reductant, \(\text{BH}_4^-\), giving anodic current density of \(j_1\). This process is diffusion-controlled and takes place in a few milliseconds estimated from \(\text{BH}_4^-\)'s diffusion coefficient \(\left(1.6 \times 10^{-5} \text{ cm}^2 / \text{s}\right)\). Notably, Reaction (5.1) in solution is much slower (scale of minutes) than on NP surfaces (scale of milliseconds) and therefore dominates \(\text{BH}_4^-\) concentration. Meanwhile, electrons stored on Au NPs were discharged via reaction (5.5), giving cathodic current density of \(j_2\). When the charging and discharging rate counterbalanced each other (i.e., \(j_1 + j_2 = 0\)), a steady state potential \(E_S\) was established on the NP, establishing an electrode with potential \(E_S\). Contribution to \(j_2\) by reaction (5.6) is neglected in Step 1 because \(\text{H}_2\text{O}\) was in excess compared with MB and was not limited by mass transfer. In Step 2, the electrode reduction of MB (reaction (5.6)) takes place in response to \(E_S\), giving cathodic current density \(j_3\). The separation of Step 1 and Step 2 is validated because the time scale for establishing \(E_S\) (at milliseconds) was thousands of times shorter than that of reaction (5.6) (at tens of seconds estimated from Figure 5.3). A mathematical description of the kinetic steps is provided in the Supporting Information and leads to the reaction rate of MB reduction described by eq (5.7):
where \( C_{\text{NP}}^{\text{mass}} \) and \( A_m \) are the mass concentration and specific surface area (surface area per mass) of Au NPs; \( F \), \( R \), and \( T \) are Faradic constant (96485 C\( \cdot \)mol\(^{-1} \)), ideal gas constant (8.314 J\( \cdot \)K\(^{-1} \)\cdot\)mol\(^{-1} \)), and temperature (K). Eq (5.7) predicts that the rate of reaction (5.6) is first order to MB and half order to \( \text{BH}_4^- \). The outcome of the half order is based on the assumption that electron transfer coefficients of eq (5.4) to (5.6) all have a value of 0.5 (see Supporting Information), which may slightly deviate from the actual value (0.3–0.7 in most cases\(^{219} \)). A non-integer reaction order less than one with respect to \( \text{BH}_4^- \) from experimental data has also been reported for heterogeneously catalyzed \( \text{BH}_4^- \) hydrolysis\(^{220,221} \).

\[
\frac{dC_{\text{MB}}}{dt}_{(6)} = -2C_{\text{NP}}^{\text{mass}} A_m k_3^0 \exp \left[ -\frac{F}{4RT} \left( E_1^0 + E_2^0 - 2E_3^0 \right) \right] \left( \frac{k_1^0}{k_2^0 C_{\text{H}_2\text{O}}} \right)^{1/2} \cdot C_{\text{BH}_4^-}^{1/2} C_{\text{MB}}^-, \quad (5.7)
\]

**Figure 5.3.** Diagram for the proposed kinetic steps of surface reactions.

Kinetics including all reactions (eq (5.1) to (5.3)) are therefore readily formulated as in Table 5.1. Notably, because MB reduction is a surface reaction, its observed rate constant, \( k_{2,\text{obs}} \), is split, as given by eq (5.11), into a mass transfer component (\( k_{2, m} \)) and a surface electron transfer component (\( k_{2, e} \)), both of which are proportional to NP surface...
area in solution \(^{215,219}\). \(k_{2,e}\) corresponds to eq (5.6) and eq (5.7), with \(C_{BH_4}^{1/2}\) separated out. The kinetics model was numerically solved, with \(k_1, k_{2,m}, k_{2,e}, \) and \(k_3\) fitted using experimental data in Figure 5.2. The model (dashed lines in Figure 5.2) agrees well with experimental data except the highest Au NP concentration, where the discrepancy is likely due to the mass transfer limitation when \(BH_4^-\) is over consumed. The experimentally validated model supports the proposed “electron-transferring-through-NP” mechanisms, suggesting the assay works exclusively for materials with electron mobility (a feature of metallic materials).

**Table 5.1.** Kinetic reactions and initial conditions to fit experimental data.

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Initial conditions</th>
</tr>
</thead>
</table>
| \[
\frac{dC_{BH_4^-}(t)}{dt} = -k_1 C_{BH_4^-}(t) \]
| \[
C_{MB}(0) = C_{0,MB} \]
| \[
C_{LMB}(0) = 0 \]
| \[
C_{BH_4^-}(0) = C_{0,BH_4^-} \]
| \[
\frac{dC_{MB}(t)}{dt} = -k_{2,obs} C_{MB}^*(t) + k_3 C_{LMB}(t) \]
| \[
\frac{1}{k_{2,obs}} = \frac{1}{k_{2,m}} + \frac{1}{k_{2,e} \cdot C_{BH_4^-}^{1/2}(t)} \]

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**Figure 5.4.** Model fitted parameters—$k_1$ (a), $k_{2,e}$ (b), $k_{2,m}$ (c), and $k_3$ (d)—versus surface area concentration. Error bars represent two times the standard deviation of triplicate samples. Linear regression with equation $y(x)=kx$ was performed for $k_{2,e}$ and $k_{2,m}$, and the obtained slopes represent $k_{2,e}^S$ and $m_{MB}$, respectively.

Values of $k_1$, $k_{2,m}$, $k_{2,e}$, and $k_3$ are plotted versus $C_{NP}^{\text{surface}}$ (in m$^2$/m$^3$) in solution (Figure 5.4). $k_1$ (Figure 5.4a) remains constant ($p<0.05$) among all NP surface area concentrations and averages 0.029 s$^{-1}$, which is close to values (0.025 s$^{-1}$) in a previous study$^{213}$. $k_{2,m}$ is linearly correlated to $C_{NP}^{\text{surface}}$ (Figure 5.4c), where the slope (0.014 m/s) denotes the MB mass transfer coefficient in the system (denoted by $m_{MB}$, with the dimension of $\text{length} \times \text{time}^{-1}$). The mass transfer coefficient of a reactant to spherical
ultramicroelectrodes theoretically equals its diffusion coefficient \( (D_{MB}) \) (with the dimension of \( \text{length}^2 \times \text{time}^{-1} \)) divided by the radius of the electrode, for a diffusion-controlled process in steady state\(^{219}\). Applying this, \( m_{MB} \) of 0.014 m/s fitted by our model estimates the MB diffusion coefficient \( (D_{MB}) \) to be \( 2.8 \times 10^{-10} \) m\(^2\)/s. This value is the same order of magnitude as the reported \( D_{MB} \) (~5 \times 10^{-10} m\(^2\)/s) in a 10 mM NaCl solution\(^{222}\), yet the hydrodynamic (by stirring or shaking the solution during the reaction) mass transfer is not considered here. \( k_{2,e} \) is also linearly correlated to NP \( C_{\text{surface}}^{\text{NP}} \) (Figure 5.4b) in accordance with eq (5.7), which also shows that \( C_{\text{surface}}^{\text{NP}} \)-normalized \( k_{2,e} \) (the regression line slope, denoted by \( k_{2,e}^{0} \)) is jointly determined by \( k_{1,0}^{0}, k_{2,0}^{0}, k_{3,0}, E_{1,0}, E_{2,0}^{0}, \) and \( E_{3,0}^{0} \).

Figure 5.4d unexpectedly shows a decreasing trend for \( k_{3} \) versus \( C_{\text{surface}}^{\text{NP}} \). The possible reason is that, although reaction (5.3) presumably happens in the bulk solution only, one of its reactants, LMB, is a product of reaction (5.2), which is a surface reaction. The mass transfer of LMB from NP surfaces to the bulk solution may lead to the dependence of the reaction rate on surface area. The possibility that O\(_2\) diffuses to the NP surface to participate reaction (5.3) also exists, which is not distinguished at this stage without hurting the model’s efficacy.

Model simulations and experimental observations both corroborate that the overall reaction reaches pseudo-equilibrium because (5.1) the BH\(_4\)\(^{-}\) hydrolysis in bulk solution lowers \( E_{S} \) of NP phase and (5.2) the LMB oxidation creates a back reaction to MB reduction. In the beginning of the reaction, these two effects are trivial and do not limit the reaction rate. For validation, the first order MB reduction rate constant \( (k_{2,\text{obs}}) \) was calculated using the initial reaction rate data (i.e., in the first five seconds) in Figure 5.2.
Therefore, at the beginning of the reaction, the surface electron transfer is facile because of sufficiently charged NPs by adequate BH_4^−, and the overall reaction kinetics is controlled by the mass transfer of MB. The comprehensive model is also able to predict the influence of different parameters on the assay’s performance and to guide the assay’s design. A discussion is given in the Supporting Information (Figure S5.7 and thereafter).

5.4.3. Environmental applications of the CRNP assay.

CRNP is envisioned to facilitate the environmental analysis of metallic NPs with surface catalytic reactivity. First, it can quantify one species of NP in a consistent water matrix. For this purpose, a calibration curve correlating the assay’s response (i.e., ΔA_663) to the NP concentration is required. As examples, I analyzed for the calibration curves of Au, silver (Ag), palladium (Pd), platinum (Pt), and copper oxide (CuO) in ultrapure water matrix (Figure 5.5). C_{NP}^\text{surface}, which was previously showed to be directly related to the surface reaction rate, was estimated from C_{NP}^\text{mass} (providing known specific surface area) and chosen as the x-axis. For Au, Ag, Pt, and Pd NPs, the specific surface area was estimated by assuming all the NPs are spherical and have the density of the corresponding bulk materials. For the CuO NPs, the specific surface area was provided by the manufacture. The calibration curve varied, reflected from the different slope, among the five NP species, suggesting the catalytic surface reactivity order as Pd>Pt>Au>Ag>CuO. The selected five metallic (metal or metal-like) NPs demonstrated catalytic activity in specific reactions\textsuperscript{223-226}. A systematic comparison of the catalytic reactivity of the different metallic materials with the same reaction was not found. CuO
was expected to have the lowest surface catalytic reactivity because of being an oxide material, which has lower electron mobility than metals in general. Esumi et al. demonstrated that, in the reaction of reducing 4-Nitrophenol, the catalytic reactivity of different NP materials showed an order of Pd>Pt>Ag, agreeing with our finding\textsuperscript{225}. Elsewhere, Au NPs were more catalytically reactive than Ag NPs in solution using the reaction of borohydride reducing 4-Nitrophenol\textsuperscript{227}, yet another study suggested otherwise when they were attached onto reduced graphene oxide mat to catalyze the same reaction\textsuperscript{228}. However, neither study clarified the actual surface area in the catalytic reactions, whereas our findings suggested Au has higher surface-normalized catalytic reactivity than Ag. With each calibration curve in Figure 5.5, the concentration of one NP species can be estimated by measuring $\Delta A^{663}$ using CRNP.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Calibration curves ($\Delta A^{663}$ versus $C_{\text{NP}}^{\text{surface}}$) for Au, Ag, Pd, Pt and CuO NPs.}
\end{figure}

Many assays use \textit{equivalent units}. In biological systems, estrogenicity associated with potentially numerous specific, and often unknown compounds in wastewater or other
environmental samples, is expressed as ng/L of estradiol (E2) equivalents. Just as the COD assay expresses conversion of dichromate to chrome, due to unknown organics and inorganics in water, as an equivalent concentration of oxygen (mgO₂/L) needed to stoichiometrically achieve the same conversion, the CRNP can be normalized and expressed in equivalent units. Here I express the CRNP assay as the equivalent surface area of tannic acid coated Au NPs (C_{Au-eqv}^{surface}, in m²_{EQ-AuNP} / m³).

Second, CRNP can detect the catalytic reactivity of known or unknown NPs in an environmental water sample. To illustrate this, I compared the analyses of 150 µg/L Au NPs (50 nm) suspended in ultrapure water (Matrix 1), surface water (Matrix 2), filtered surface water (Matrix 3), and water containing SRNOM in the range of 0.5-10 mg/L as C (Matrix 4). Figure 5.6 presents the outcomes in ΔA_{663} and in C_{Au-eqv}^{surface} (calibrated by Figure 5.1b). Compared against Matrix 1, Matrix 2 showed higher (p<0.05) catalytic reactivity, suggesting the presence of other catalytically reactive compounds, which are likely also metallic colloids, other than the spiked Au NPs. Matrix 3 and Matrix 4 showed statistically insignificant (p>0.05) variance from Matrix 1, suggesting that the reactive compounds in the surface water were effectively removed by the glass fiber filter and the dissolved NOM puts negligible impact on the surface catalytic reactivity of Au NPs. This was further proved by assay tests with Au NPs suspended in water containing different levels of Suwanee River NOM (Figure S5.8). There are cases where the detected catalytic reactivity is contributed by “unknown” particles and other techniques are needed to identify the reactive species. For example, by CRNP I found in tap water certain constituent with a strong catalytic reactivity and further analysis showed it could be copper or compounds (Figure S5.9). CRNP may be able to pre-screen for the presence
of NP in environmental waters and because catalytic activity may be an indicator of potential adverse toxicity outcomes\textsuperscript{16,229}, the assay may be used as a screening test for the environmental health and safety (EHS) of nanomaterials. Clearly, more research directly correlating CRNP to toxicity would be needed to confirm this premise.

\textbf{Figure 5.6.} The catalytic reactivity of NPs analyzed by the powder assay for 150 \( \mu \text{g/L} \) Au NPs (50 nm) suspended in ultrapure water (Matrix 1), surface water (Matrix 2), filtered surface water (Matrix 3), and water containing SRNOM (0.5-10 mg/L as C, Matrix 4). Error bars stand for the standard deviation for triplicated measurements.

Overall, the fundamental insight gained by the assay suggests at least two important applications. First, the assay can be used as a rapid indicator to track known NPs (e.g., Au NPs) throughout reactors (e.g., environmental systems, biota). Second, the assay can be used to detect “unknown” particles in water by their catalytic reactivity, an indicator of the surface catalyzing potential of particles. Thus, the assay may also be useful to increase understanding of how environmental conditions (e.g., sulfidation or natural organic matter) passivate NP surfaces or, as illustrated by the difference between filtered
and unfiltered surface water, demonstrate the relative presence of surface catalyzing particles already in our water systems.

5.5. Acknowledgements

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5.6 Supporting Information

![TEM images and absorbance spectra](image)

**Figure S5.1.** TEM images and absorbance spectra for the three Au NP samples (20, 50 and 80 nm) and the Ag NP sample (40 nm). These images are directly requested from the material manufacture (nanoComposix, USA) and permitted to use without modification.
Figure S5.2. Deconvolution of MB absorbance-time spectrum. Light scattering caused by the reaction by-product, i.e., hydrogen gas, is recorded at 760 nm because this scattering is independent of wavelength. Therefore, subtracting the 760 nm spectrum from the 663 nm spectrum gives a deconvoluted MB absorbance spectrum versus time.
Figure S5.3. The predicted detection limit as NP mass concentration ($C_{DL}^{mass}$) as a function of NP diameter ($d_{NP}$) for Au NPs. The prediction is based on the well-defined mass-surface relationship ($C_{DL}^{mass} = \frac{C_{DL}^{surface} \cdot \rho_{NP} \cdot d_{NP}}{6}$), assuming the spherical geometry for Au NPs. $\rho_{NP}$ is the density of NPs. The inserted equation in the figure is generated given $C_{DL}^{surface} = 0.31 \text{ m}^2/\text{m}^3$ and $\rho_{NP} = 19.3 \text{ g/cm}^3$ for Au NPs.
**Figure S5.4.** Absorbance spectrum (400–800 nm) of 50 nm Au NPs in solution at different concentrations (left) and calibration by absorbance at 527 nm (A\text{527}, right). Using A\text{527}= 0.01 as the minimum reading threshold, the method detection limit was estimated to be 400 µg/L for 50 nm Au NP.

**Figure S5.5.** Absorbance-wavelength spectra at different times during an assay reaction. The decaying peak at 663 nm (MB, blue arrow) and the rising peak 254 nm (LMB, dark red arrow) indicate the reaction of MB→LMB.
Figure S5.6. Observed first order rate constant (k_{2,obs}) determined by taking the initial rate (using the first five seconds of data in Figure 5.3). Error bars represent two times the standard deviation of triplicate samples. Linear regression shows k_{obs} normalized to surface is 0.0144 (denoted by k_{2,obs}^S), which is very close to m_{MB}. The layout of k_{obs} also resembles k_{2,m} (Figure 5.5b). This implies that the initial reaction rate is controlled by MB mass transfer because surface electron transfer is much faster than mass transfer. To quantitatively demonstrate this, according to eq (5.11) (Table 5.1), one would reach

\[
\frac{1}{k_{2,obs}^S} = \frac{1}{m_{MB}} + \frac{1}{k_{2, e} C_{BH_4}^{1/2}} \text{ by multiplying each term of eq (5.11) by } C_{NP}^{Surface},
\]

accordingly, taking the initial BH_4^- equal to 5 mM and values of m_{MB} and k_{2, e}^S determined in Figure 5.5 one gets m_{MB} = 0.014 m \cdot s^{-1}, k_{2, e}^S \cdot C_{BH_4}^{1/2} = 0.07 m \cdot s^{-1},

and k_{2, obs}^S = 0.012 m \cdot s^{-1}. The calculated k_{2, obs}^S is very close to what is determined by initial rate herein (0.012 versus 0.0144 m \cdot s^{-1}). Also, the surface electron transfer rate is
evaluated to be five times \((k^5_{2,e} \cdot C_{BH_4}^{1/2}/m_{MB})\) of mass transfer rate, so the latter is the rate-limiting step.

**Figure S5.7.** Contour maps of MB concentration variance during assay reaction versus time and \(k_1\) (a), \(C_{0,BH_4}\) (b), \(k^5_{2,e}\) (c), or \(k_3\) (d), predicted by the model in this study. MB concentration is presented by color. The area of \(C_{MB} < 0.2\ M\) indicates the pseudo-equilibrium condition. In each panel, other than the varying parameter on y-axis, other parameters were set at values from Table 5.1 and Figure 5.4. *O_2* concentration was
calculated from $k_3$ using second order rate constant of reaction (5.3) ($k=89 \text{ M}^{-1}\text{s}^{-1}$) reported by ref$^{212}$.

**Influence of parameters variance on the assay’s performance.** Experimental data were well-described by the model (Table 5.1) simulations, indicating that $\Delta A_{663}$ read by the powder assay was linearly dependent on $C_{\text{NP}}^{\text{surface}}$ given initial conditions (Table 5.1) and kinetics parameters including $k_1$, $m_{MB}$, $k_{2,e}^S$, and $k_3$ (or $k_3/C_{\text{NP}}^{\text{surface}}$). The model can be used to select reactant initial conditions (i.e., $C_{0,MB}$, and $C_{0,BH_4}$) that should be used in the assay or to manage assay conditions, such as pH and dissolved oxygen, to design the reaction kinetics. $C_{0,MB}$ was set at 20 µM because this was the approximate maximum threshold of the linear dependence of $A_{663}^\text{MB}$ following Beer’s law and therefore was subject to limited variance. The value for $m_{MB}$ was dictated by MB diffusion in solution and mechanical solution mixing (i.e., repeated inversion by hand). $C_{0,BH_4}$ can be controlled to change the overall reaction kinetics. $k_1$ and $k_3$ can be varied by pH conditions and dissolved oxygen concentration$^{212}$. $k_{2,e}^S$ is intrinsically associated with the material and surface properties of metallic NPs to be detected.

Figure S5.7 depicts the influences of $k_1$, $C_{0,BH_4}$, $k_{2,e}^S$, and $k_3$ on the reaction kinetics predicted by our model; each contour plot maps MB concentration as a bivariate function of one parameter and time. Increasing $k_1$ or $k_3$, or decreasing $C_{0,BH_4}$ or $k_{2,e}^S$, leads to lower MB concentration and longer lasting time of the pseudo-equilibrium condition. When $k_1$ approaches zero (Figure S7a), MB achieves a nearly complete reduction after sufficient time, because $k_{2,\text{obs}}$ greatly surpasses $k_3$ in persistent presence of. Similarly, if
k₃ or oxygen concentration is zero (Figure S7d), MB will also be reduced completely given adequate BH₄⁻. The influence of increasing C₀,BH₄⁻ to the pseudo-equilibrium concentration becomes trivial above ~10 mM, as charging process reaches a limit determined by the NP’s electron capacitance. Therefore, redundant dosage of NaBH₄ in the assay should be avoided as it also induces more hydrogen gas bubbles, causing absorbance measurement interferences. By k₃'s (Figure S7c) one can evaluate the viability of the assay toward NPs being from highly reactive (high k₃) to inert (k₃ approaching zero). Overall, I demonstrated a sophisticated approach employing both experimental testing and semi-theoretical electrochemical modeling to design a powder assay kit that rapidly detects metallic NPs in aqueous solution.

Figure S5.8. The catalytic reactivity of 150 µg/L Au NPs in the presence of NOM at different concentration levels (0-9.4 mg/L). Catalytic reactivity is given in ΔA₆₅₃ (left axis) and C⁶₅₃ (right axis). Errors bars stand for one standard deviation on each side for
triplicated measurements. Paired two-sample t test suggests that samples containing 0.5, 2.4, or 9.4 mg-C/L NOM has insignificantly different mean values of catalytic reactivity comparing to the sample containing zero NOM, although the sample containing 4.7 mg-C/L NOM gave smaller measurement error. Overall, the influence of NOM on the catalytic reactivity of the Au NPs is negligible.

Figure S5.9. The catalytic reactivity as $C_{\text{Au-eq}}$ (left axis) and the total Cu concentration (right axis) of tap water and tap water after treatment by 0.45 µm filter (mixed cellulose esters, Millipore), 0.1 µm filter (Durapore PVDF membrane, Sigma-Aldrich), and activate carbon. Concentrations of Cu in all tap water was analyzed by ICP-MS (Thermo X series II). Cu$^{65}$ was used as the analyte isotope and argon was used as the carrying gas. A strong catalytic reactivity equivalent to ~1.3 m$^2$/m$^3$ gold surface, was found in the raw tap water (no treatment). This high reactivity was almost eliminated by carbon treatment, but not by regular filtration of 0.45 µm and 0.1 µm membranes. I analyzed the concentration of common metals, inlcuding Cr, Mn, Fe, Ni, Cu, An, Cd, and Pb in these
tap water samples, and only found the concentration of Cu is positively correlated to the observed catalytic reactivity. I excluded the possibility of free chlorine residual interfering with the reaction by analyzing an ultrapure water sample containing 1 mg/L free chlorine (i.e., slightly higher than 0.85 mg/L found in tap water in the experiment) by the DPD (N,N Diethyl-1,4 Phenylenediamine Sulfate) colorimetric method\textsuperscript{230}. The chlorinated sample did not show a notable MB reduction (data not shown). Therefore, I strongly suspect the catalytic reactivity in tap water is contributed by particulate copper or copper oxide, and it is also likely that the particulate copper is formed when copper ions are reduced by BH\textsubscript{4}\textsuperscript{-} in the assay, in which case an artifact is caused.

**Mathematical description of surface reactions.** As discussed, I assumed the surface reaction followed a mechanism as shown in Figure 5.3. The catalytic metallic NP acquires a steady-state potential (E\textsubscript{S}) within milliseconds through reaction (5.4) and (5.5). Following Butler-Volmer equation to describe kinetics of (5.4) and (5.5), one reaches:

\[
j_1 = -8Fk_1^0 \exp \left[ \beta \frac{F}{RT} (E - E_1^0) \right] C_{BH_4^-},
\]

\[
j_2 = 2Fk_2^0 \exp \left[ -\alpha \frac{F}{RT} (E - E_2^0) \right] C_{H_2O},
\]

where \( j_1 \) and \( j_2 \) are current densities (in C \cdot m\textsuperscript{-2} \cdot s\textsuperscript{-1}) of electrode reaction (5.4) and (5.5); \( \alpha \) and \( \beta \) are electron transfer coefficients for the anode and cathode reaction; and all other notations are the same to eq (5.7) in the main paper. When the steady potential is reached, one has:

\[
j_1 + j_2 = 0.
\]

Substituting eqs (s5.1) and (s5.2) into eq (s5.3), E at steady-state, i.e., E\textsubscript{S} can be solved:
\[ E_s = \frac{\beta E_1^0 + \alpha E_2^0}{\alpha + \beta} + \frac{RT}{(\alpha + \beta)F} \log \left( \frac{k_3^0 C_{H_2O}}{4k_4^0 C_{BH_4^-}} \right). \] (s5.4)

Eq (s5.4) appears similar to Nernst equation, but there are differences: (5.1) the standard reducing potentials of the two electrode reactions jointly determine \( E_s \) and (5.2) the standard rate constants of the two electrode reactions also play a role in the logarithm term.

As discussed in the main paper, I then assumed MB was reduced on the surface as adapted to \( E_s \), therefore the Butler-Volmer equation for reaction (5.6) is:

\[ j_3 = 2Fk_3^0 \exp\left[ -\alpha' \frac{F}{RT}(E_s - E_3^0) \right] C_{MB^+}^{\alpha'}, \] (s5.5)

where \( j_3 \) and \( \alpha' \) are the current density (in \( \text{C} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \)) and electron transfer coefficient of electrode reaction (5.6), respectively.

Accounting for stoichiometry, the MB reduction rate in reaction (5.6) is associated to \( j_3 \) as:

\[ \frac{dC_{MB^+}}{dt} = \frac{1}{2} \frac{dC_e}{dt} = -\frac{C_{NP}^{\text{mass}} A_m}{C_{MB}^{\text{mass}}} \frac{1}{2} \frac{j_3}{F}, \] (s5.6)

where \( C_e \) is a virtual electron concentration in a 3-D volume of the solution, with the unit of \( \text{mol/m}^3 \). All the other notations are the same as eq (5.7) in the main paper. Eq (5.7) in the main paper (and presented below) is then obtained by substituting eqs (s5.4) and (s5.5) into eq (s5.6) and assuming the transfer coefficients (\( \alpha, \alpha', \) or \( \beta \)) equal 0.5:

\[ \frac{dC_{MB^+}}{dt} \bigg|_{(6)} = -2C_{NP}^{\text{mass}} A_m k_3^0 \exp\left[ -\frac{F}{4RT} \left( E_1^0 + E_2^0 - 2E_3^0 \right) \right] \left( \frac{k_3^0}{k_2^0 C_{H_2O}} \right)^{1/2} C_{BH_4^-}^{1/2} \cdot C_{MB^+}. \] (5.7)
CHAPTER 6
FERRIC REDUCING ABILITY OF NANOPARTICLES: A
REACTIVITY ASSAY INFERRING DIFFERENT SURFACE
REACTION MECHANISMS

6.1. Abstract

I used a “Ferric Reducing Ability of Nanoparticles (FRAN)” assay to assess the surface reactivity of nanoparticles (NPs) suspended in water solutions. Derived from the well-established Ferric Reducing Ability of Plasma (FRAP) assay, the FRAN assay is based on the reduction of ferric (Fe$^{III}$) to ferrous (Fe$^{II}$) ions by electrons (e$^-$) transferred from the NP surface. In the present of 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), the concentration of Fe$^{II}$ ($C_{Fe^{II}}$) was obtained by measuring the absorbance at 593nm ($A_{593nm}$) every 0.2 s. In tests of 20-nm gold (Au) and silver (Ag) NPs, the surface reactivity was indicated by the plateaued $C_{Fe^{II}}$ ($C_{Fe^{II}}^p$), which showed a linear correlation to the NP concentration after a period of reaction time. Nevertheless, different patterns of the Fe$^{II}$ formation kinetics, which mathematically was approximated by an “exponential” function for Ag NPs and by a “logarithm” function for Au NPs, were observed. I developed two theoretical models that justified the observed different kinetic patterns, revealing two surface reaction mechanisms. Ag NPs followed a “sacrificial reducing” mechanism, in which the NPs consumed (being oxidized) themselves to reduce Fe$^{III}$. Ag$^+$ ions were formed as a result of Ag oxidation. Au NPs followed an “electrode discharging” mechanism, in which the NPs act as though small electrodes and transferred the stored electrons to Fe$^{III}$ when they are discharged. Overall, I demonstrated a method
to not only assess the magnitude of surface reactivity of NPs in water, but also distinguish two mechanisms of surface electron transfer.

6.2. Introduction

Benzie and Strain introduced the Ferric Reducing Ability of Plasma (FRAP) assay\textsuperscript{231}, which uses the degree to which the ferric (Fe\textsuperscript{III}) ions get reduced by any reducing agents in a plasma medium to suggest a total “antioxidant power” of the plasma. FRAP is easy to operate and has been widely used to assess the antioxidant power of dilatory product\textsuperscript{232}, biological fluids\textsuperscript{233}, vegetables, and plants\textsuperscript{234-236}. The principle of FRAP was also applied to serum media, giving the known “ferric reducing ability of serum (FRAS)\textsuperscript{22, 24}” assay. When in the serum is present a chemical with oxidative power, e.g., a nanomaterial\textsuperscript{237, 238} or an airborne particulate matter\textsuperscript{239}, the antioxidant power of the serum medium is reduced because of the oxidative stress caused by the chemical. Therefore, via evaluating the decrease of the antioxidant power of a serum caused by a certain added chemical, FRAS could indirectly assess the oxidative power of that chemical and imply the chemical’s toxicity by means of causing oxidative stress in cells\textsuperscript{24}. In both FRAP and FRAS, the assay functions relying the well-defined and easily-detectable Fe\textsuperscript{III} reduction.

Surface reactivity of NPs enables their applications in technologies including catalysis\textsuperscript{240}, nano-medicine\textsuperscript{18}, and nano-coatings\textsuperscript{241, 242}, but also invokes their hazard by means of, e.g., reacting with cell membranes\textsuperscript{243, 244} or causing oxidative stress\textsuperscript{245, 246} to living cells. Because most relevant surface reactions of NPs in water concern interfacial electron transfer processes, well-defined redox reactions were often used as models to evaluate NPs’ surface reactivity. Model reactions including the reduction of p-
nitrophenol, ferrocyanate, and methylene blue (MB) have been used. However, issues exist with many model reactions because of the side reactions occurring along with the intended redox reaction and causing interferences and ambiguity to the measurement. I previously showed that, when applying the model reaction of borohydride reducing MB to assess the surface reactivity of gold (Au) NPs, the reactions of borohydride with water and leuco methylene blue (LMB, the reduced form of MB) with oxygen (O₂) were coupled with the intended reaction and had to be resolved by comprehensive kinetic modeling (Chapter 5). Using MB, an organic dye, as the probe molecule may also lead to ambiguity when multiple electron transfer pathways occur simultaneously among different redox state of the molecule and give a complicated overall reaction scheme, which is unfavorable to precisely interpret the reactivity assessment outcome. The reduction of Fe(III) underlying FRAP is a one-electron transfer reaction, and no side reactions with it has been recognized in a water solution. I thus anticipated that the ferric reducing reaction would favor a more direct and unambiguous measurement of the surface reactivity of NPs in water, yet no existing studies have applied this model reaction to directly probe NP surface to our best knowledge.

To testify the feasibility of using the ferric reducing reaction to probe the surface reactivity of NPs in water, I prepared the solutions of the FRAP assay and applied them to NPs (Au and silver (Ag)) suspended in water, leading to the ferric reducing ability of NPs (FRAN) assay. The FRAN assay was advanced from the FRAP assay via recording the kinetics of Fe(II) formation with a time resolution of 0.2 s. I developed theoretical kinetic models to reveal the different surface electron transfer mechanisms reflected by Au and Ag NPs. As a result, FRAN was demonstrated to not only measure the
magnitude of the surface reactivity, but also identify the surface electron transfer mechanism.

6.3. Experimental Methods

6.3.1. Chemicals

Gold (NanoXact, 0.05 mg/mL) and silver (NanoXact, 0.02 mg/mL) nanospheres with nominal sizes of 20 nm were purchased from nanoComposix. Ferric Chloride (FeCl₃) solution was prepared from iron(III) chloride hexahydrate (FeCl₃•6H₂O, ≥98%, Sigma-Aldrich, F2877). 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was purchased from Sigma Aldrich (93285). Acetate buffer (pH 3.6) was prepared from acetic acid (Fisher Brand, BP2401C-212) and sodium acetate trihydrate (ACS reagent, ≥99%, Sigma Aldrich, 236500). Ultra pure water (18.2 MΩ·cm, Barnstead GenPure xCAD Plus) was used to make solutions.

6.3.2. FRAN assay operation

The formula of the solution for FRAN is adopted from the FRAP assay. Briefly, a final FRAN assay solution is obtained by mixing 2.5 mL TPTZ solution (10 mM in a 40 mM HCl solution), 2.5 mL FeCl₃ solution (20 mM in water), and 25 mL acetate buffer (0.3 M in water, pH=3.6) in a polystyrene centrifuge tube. For operation, 2.5 mL of the as-prepared FRAN solution was added into a 1-cm cuvette (perforctor scientific, #9012), which was then placed in a portable UV-VIS absorbance spectrometer (Ocean Optics, USB-ISS-UV/VIS and USB4000 light source). The analysis reaction was initiated by adding a NP stock solution into the cuvette to reach a desired concentration. The spectrometer was operated in a time-resolved reading mode, in which the absorbance at
593 nm ($A^{593}$, the primary absorbance peak of Fe$^{II}$-TPTZ) was recorded every 0.5 s. The recording was stopped when the increase of $A^{593}$ appeared to reach a plateau. The obtained kinetics data, i.e., $A^{593}$ versus time, was exported for further data analysis.

6.4. Results and discussion

6.4.1. FRAN assay performance

Figure 6.1 shows the kinetic profiles of Fe$^{II}$ formation for Au and Ag NPs. $A^{593}$ was converted to the concentration of Fe$^{II}$ ($C_{Fe^{II}}$) using the known extinction coefficient of Fe$^{II}$-TPTZ complex species ($\varepsilon=22600$ L $\cdot$ mol$^{-1}$ $\cdot$ cm$^{-1}$) in the solution. There are similar kinetic patterns between Au and Ag NPs. They both led to a continuous formation of Fe$^{II}$ after they were added to the FRAN solution, proving their surface reactivity toward reducing Fe$^{III}$. For both NPs, $C_{Fe^{II}}$ reached a plateau after about 100 s of increasing, and higher NP dosage yielded higher plateaued $C_{Fe^{II}}$ ($C_{Fe^{II}}^{p}$). In accordance with using the $A^{593}$ reaching steady state to indicate the antioxidant power in FRAP, $C_{Fe^{II}}^{p}$ can be used to indicate the surface reactivity. Figure 6.2 evidently shows that $C_{Fe^{II}}^{p}$ is linearly dependent on the NP concentration for both Au and Ag. The plateaued $C_{Fe^{II}}^{p}$ has been used to indicate the antioxidant power of a sample, i.e., the surface reactivity of NPs here. There are also dissimilar patterns between Au and Ag NPs. First, to get a comparable $C_{Fe^{II}}^{p}$, the required concentration of Au NPs is about one order of magnitude higher than that of Ag NPs. For example, to reach $C_{Fe^{II}}^{p}$ of ~2.5 µM, it requires 296 µg/L of Ag but 8300 µg/L of Au NPs (Figure 6.1). Second, the shape of the kinetic profile
differs between the two NP species. For Ag NPs, the increase of $C_{Fe^{II}}^{p}$ is approximately linear up to ~60 s (Figure 6.1a), and then gradually curved (~60-100s) to reach a plateau (>100s). For Au NPs, however, the increase of $C_{Fe^{II}}^{p}$ is very fast in the first 20s, and then became slower (~20-30s) until reaching a plateau. The flatness of the plateau also slightly varies. Comparing to the plateau of Ag, which is nearly constant by eyes (Figure 6.1a), the plateau of Au still had very small positive slope, i.e., an increasing (Figure 6.1b). Overall, by the criterion of using $C_{Fe^{II}}^{p}$ as the surface reactivity indicator, one may conclude that, for a comparable mass concentration and NP size (20 nm), Ag NPs has higher surface reactivity than Au NPs. However, the distinct kinetic profiles suggest that different electron transfer schemes may have occurred between Ag and Au.

**Figure 6.1.** Kinetics profiles for Ag (a) and Au (b) NPs tested by FRAN assay at different concentrations. Markers are experimental data and dashed lines are model simulation results.
Figure 6.2. The plateaued Fe$^{II}$ concentration ($C_{Fe^{II}}^p$) as a function of NP mass concentration ($C_{NP}^{mass}$) for Au and Ag NPs.

6.4.2. Theoretical description of kinetics

Based on the experimental observations (markers) in Figure 6.1, I first sought for mathematical descriptions of the distinct kinetic patterns. Empirical fitting showed that the kinetic profile of Ag was approximated by an exponential function ($exp$), which takes the form of $C(t) = P_1 \cdot (1 - \exp(1 - P_2 \cdot t))$, whereas that of Au was approximated by a natural logarithm function ($log$), which takes the form of $C(t) = P_1 \cdot \log(1 + P_2 \cdot t)$. This is clearly demonstrated in Figure 6.3, where the example data set of Ag-298 µg/L and Au-8.3 mg/L (with comparable $C_{Fe^{II}}^p$, Figure 1) were fitted by both $exp$ and $log$. For Ag, the $log$ overestimates the data at the initial and final period of time, and underestimates it in
the middle, while the exp fits the data better globally (Figure 6.3a). For Au, the exp underestimates the data at the initial and the final period of time, and overestimates it in the middle, while the log fits the data better globally (Figure 6.3b). The Residue plots (Figure 6.3c and d) for both further speak for the same conclusion. Without plotting, the same fitting comparison outcome was achieved for the rest of data set of other NP concentrations.

![Figure 6.3](image)

**Figure 6.3.** Comparison of the exp and log fitting for Ag NPs at 296 µg/L (a) and Au NPs at 6.9 mg/L (b), and the residuals of fitting ((c) and (d)) for all cases.

To mechanistically justify the observed different kinetic profiles, which are mathematically reflected as exp versus log, I developed two theoretical models reflecting
two surface reaction mechanisms, namely the “sacrificial reducing” and the “electrode discharging”.

Ag NPs follow the “sacrificial reducing” mechanism. In this scenario, Fe$^{III}$ is reduced by directly Ag$^{0}$ atoms constituting the Ag NPs, in an overall redox reaction:

$$\text{Ag}^{0} + \text{Fe}^{III} \rightarrow \text{Ag}^{+} + \text{Fe}^{II}.$$  (6.1)

Thus it is accompanied by the oxidation of Ag$^{0}$ to Ag$^{+}$, which is released into the solution. Oxidative dissolution of Ag NPs is a known mechanism for them to release Ag$^{+}$ ions. The word “sacrificial” emphasizes the fact that the NP material gets consumed or transformed as a result of reducing Fe$^{III}$ in the assay’s performance.

The redox occurs via the attacking of Fe$^{III}$ to the Ag$^{0}$ on the NP surface. Shown in Figure 6.4a, I envisage this reaction takes place at the NP-water interface, which is represented by an infinitesimal thickness of of $\delta$ around each Ag NP. This assumption leads to a basic electron transfer kinetic equation for reaction (6.1):

$$\frac{dC_{\text{Ag}^{0}, \text{exp}}}{dt} = k \cdot C_{\text{Fe}^{III}} \cdot C_{\text{Ag}^{0}, \text{exp}},$$  (6.2)

where the molar concentration relative to the total solution volume is used for both reactants. A list of symbols meaning and variable units is given in separate section to the end. With the assumption of spherical geometry and known material density, $C_{\text{Ag}^{0}, \text{exp}}$ is further described as:

$$C_{\text{Ag}^{0}, \text{exp}} = \rho_{\text{AgNP}} \cdot \pi \cdot D^2 \cdot \delta \cdot C_{\text{AgNP}}$$  (6.3)

Further derivations from eqs (6.2) and (6.3) leads to the formation kinetics of Fe$^{II}$ as:

$$C_{\text{Fe}^{II}}(t) = C_{\text{Fe}^{II}, 0} \left[1 - \exp(-k_{\text{obs}} \cdot t)\right].$$  (6.4)
Details on the derivation of eq (6.4) is given in the Supporting Information.

**Figure 6.4.** Reaction mechanisms diagram for sacrificial reducing by Ag NPs (a) and the electrode discharging by Au NPs (b).

Au NPs follow the “electrode discharging” mechanism. When a NP contact water, surface charge is developed essentially due to the chemical potential difference between the solid and water phases. This “charging process” can be imposed by the polar water molecules in a extremely short time-scale, and leads to a surface potential (which derives the known “zeta-potential”) between particle surface and the bulk water, i.e., the interface. In this scenario, I analogize a NP to a very small electrode, which carries an initial surface charge density \( q_0 \) and potential \( \Phi_0 \). Fe\(^{III} \) is reduced at the NP or the “electrode” surface by taking the stored “free” electrons (\( e^- \)) from the solid phase, leading to an “electrode discharging” process (Figure 6.4b). Differing from the “sacrificial reducing” mechanism, the NP material is not consumed in this case. The Fe\(^{III} \) reduction is described by a half reaction:

\[
Fe^{III} + e^- \rightarrow Fe^{II}.
\]  

The kinetics reaction (6.5) can be described by the Butler-Volmer equation:


\[ i = F \cdot A \cdot k_0 \cdot C_{Fe^{III}} \cdot \exp\left(-\frac{\alpha \cdot F \cdot \eta}{R \cdot T}\right). \]  \hspace{1cm} (6.6)

The meaning of all symbols is given at the end of the article. When dealing with the discharging process of an electrode, the interface of it with water can be treated as a capacitor model\(^\text{217, 219}\) (Figure 4b). With the basic relation of potential, charge and capacitance, \(\eta = Q/C\), eq (6) is converted to:

\[
\frac{dQ}{dt} = F \cdot A \cdot k_0 \cdot C_{Fe^{III}} \cdot \exp\left(-\frac{\alpha \cdot F \cdot Q}{R \cdot T \cdot C}\right). \hspace{1cm} (6.7)
\]

Eq (6.7) is a differential equation with respect to \(Q(t)\). Solving eq (7) plus further derivations gives rise to the \(F^{II}\) formation kinetic equation:

\[
C_{Fe^{III}}(t) = L \cdot C_{\text{mass}}^{\text{AgNPs}} \cdot A_{m, \text{AgNPs}} \cdot C_S \cdot \log\left(1 + K \cdot C_{Fe^{III}} \cdot t\right); \hspace{1cm} (6.8a)
\]

\[
L = \frac{R \cdot T}{\alpha \cdot F^2}; \hspace{1cm} (6.8b)
\]

\[
K = \frac{k_0}{L \cdot C_S} \cdot \exp\left(-\frac{q_0}{L \cdot F \cdot C_S}\right). \hspace{1cm} (6.8c)
\]

A full derivation of eq (6.8) is given in the Supporting Information.

Eqs (6.4) and (6.8a) justify the observed the \(exp\) kinetic profile for Ag and the \(log\) kinetic profile for Au. In Figure 6.1, dashed lines delineate all the fittings by the two equations for data sets of all NP concentrations. \(C_{\text{AgNPs}}^{\text{molar, exp}}\) and \(k_{\text{obs}}\) in the “sacrificial reducing” model, and \(C_S\) and \(K\) in the “electrode discharging” model, were treated as unknown parameters in fitting. Fitted parameter values were plotted over the concentration of NPs in Figure 5. Given excessive concentration of Fe\(^{III}\), \(k_{\text{obs}}\) is a parameter separated from the concentration of Ag. Indeed, Figure 5a confirms that \(k_{\text{obs}}\) is
independent on the dosage of Ag NPs. The model is further validated as the fitted
\[ C_{Ag^{0},exp}^{molar} \]
values fall on a 1:1 line with the experimental initial dosage of Ag NPs. In accordance with the assumed reaction (6.1), the final molar concentration of Fe\(^{II}\), i.e.,
\[ C_{Fe^{II}}^{p} \]
is also evidently equivalent to the total molar concentration of Ag, i.e.,
\[ C_{Ag^{0},exp}^{molar} \]
(Figure S1). This validates the one-electron transfer of reaction (6.1) and proves that all the Ag NPs were oxidized to Ag\(^{+}\) at the end of the reaction. In the case of Au NPs, the fitted \( C_S \) value is independent on the NP concentration (Figure 6.5b), suggesting that \( C_S \) remains constant in the solution despite different NP concentrations. This implies that \( C_S \) is an intensive property of the NP material. Classical models describing the colloidal surface charge distribution based on the Poisson-Boltzmann theory also suggest that the surface specific capacity of colloids in a solution is only dependent on the colloids material and solution constitution\(^{248}\), agreeing with our observation. The fitted K value increases with the dosage of Au NP (Figure 6.5b). Eq (6.8c) suggests that K is a function of \( k_0 \), \( q_0 \) and \( C_S \) (\( \alpha \) is assumed to be 0.5\(^{219}\)). Theoretically, I did not expect any of these three parameters would vary with Au NP concentration. The observed variance of K may be caused by the limitation of mass transfer in the reaction, which would lead to the observed \( k_0 \) increases with the NP concentration. Future studies should carry out investigation on the surface electron transfer rate, mass transfer rate, and surface charge density in the assay reaction system in order to justify the variance of K with NP concentration.
6.5. Conclusion

I applied FRAN to assess the surface reactivity of NPs. The degree of $\text{Fe}^{\text{III}}$ reduction on the NP surface, reflected as $C_{\text{Fe}^{\text{II}}}^{p}$, can serve as an indicator of the surface reactivity. However, revealed by the kinetic measurement and modeling, the surface reactivity can be based on distinct surface reaction mechanisms. Demonstrated with Ag and Au NPs, the “sacralificial reducing” and “electrode discharging” models were developed to distinguish two reaction mechanisms. For Ag NPs, $\text{Fe}^{\text{III}}$ is reduced directly by $\text{Ag}^{0}$ on the surface of Ag NPs via a one-electron transfer redox reaction, and the Ag NPs were consumed as a result. For Au NPs, the reduction of $\text{Fe}^{\text{III}}$ is analogized to a electrode reaction via the electrode discharging, as each Au NP is treated as a small electrode. Classical kinetic theories lead to two models, which describe the two mechanisms and well fit the experimental data. Overall, I show that different NP species may undergo different surface reaction mechanisms in the presence of the same reactants, calling the need for examining the electron transfer scheme for colloidal surface reactions that are comprehensively studied in many cases.

6.6. Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Meaning and unit in UI</th>
<th>Related equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mathbf{A}$</td>
<td>Total surface area of NPs in a solution, [m$^2$].</td>
<td>(6), (7), (s5), and (s6)</td>
</tr>
<tr>
<td>$\mathbf{A}_{\text{m,AuNP}}$</td>
<td>Specific surface area of Au NPs, [m$^2$·kg$^{-1}$].</td>
<td>(8)</td>
</tr>
<tr>
<td>$\mathbf{C}$</td>
<td>Total capacitance of NPs in a solution, [F].</td>
<td>(7), (s5), and (s6)</td>
</tr>
<tr>
<td>$\mathbf{C}_s$</td>
<td>Surface specific capacitance of NPs in a solution, [F·m$^{-2}$].</td>
<td>(8)</td>
</tr>
<tr>
<td>$\mathbf{C}_{\text{molar}}^{\text{Ag}^0,\text{exp}}$</td>
<td>Total molar concentration of Ag$^0$ added in an experiment, [mol·m$^{-3}$].</td>
<td>(2) and (3)</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Equation</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>----------</td>
</tr>
<tr>
<td>$C_{\text{AgNP}}^{\text{num}}$</td>
<td>Number concentration of Ag NPs in a solution, [m$^{-3}$].</td>
<td>(3), (s1), and (s4)</td>
</tr>
<tr>
<td>$C_{\text{AuNP}}^{\text{mass}}$</td>
<td>Mass concentration of Au NPs in a solution, [kg · m$^{-3}$].</td>
<td>(8)</td>
</tr>
<tr>
<td>$C_{\text{Fe}^{\text{III}}}$</td>
<td>Molar concentration of Fe$^{\text{III}}$ in a solution, [mol · m$^{-3}$].</td>
<td>(2), (6)-(8), (s1), (s5), and (s6)</td>
</tr>
<tr>
<td>$C_{\text{Fe}^{\text{II}}}$</td>
<td>Molar concentration of Fe$^{\text{II}}$ in a solution, [mol · m$^{-3}$].</td>
<td>(4), (8), and (s6)</td>
</tr>
<tr>
<td>$D$</td>
<td>Diameter of a NP, [m].</td>
<td>(3), (s2), (s3), and (s4)</td>
</tr>
<tr>
<td>$D_0$</td>
<td>Initial diameter of a NP, [m].</td>
<td>(s4)</td>
</tr>
<tr>
<td>$F$</td>
<td>Faraday constant, 96485 [C · mol$^{-3}$].</td>
<td>(6)-(8), (s5), and (s6)</td>
</tr>
<tr>
<td>$i$</td>
<td>Cathodic current, [A].</td>
<td>(6)</td>
</tr>
<tr>
<td>$k$</td>
<td>Second order reaction rate constant, [m$^3$ · mol$^{-1}$ · s$^{-1}$].</td>
<td>(2) and (s1)</td>
</tr>
<tr>
<td>$k_0$</td>
<td>Standard rate constant for an electrode reaction, [m · s$^{-1}$].</td>
<td>(6), (7), (s5), and (s6)</td>
</tr>
<tr>
<td>$k_{\text{obs}}$</td>
<td>Observed rate constant, [s$^{-1}$].</td>
<td>(4)</td>
</tr>
<tr>
<td>$Q$</td>
<td>Total charge on NPs’ surface, [C].</td>
<td>(7), (8), (s5), and (s6)</td>
</tr>
<tr>
<td>$Q_0$</td>
<td>Initial total charge on NPs’ surface, [C].</td>
<td>(s6)</td>
</tr>
<tr>
<td>$q_0$</td>
<td>Surface charge density of NPs in a solution, [C · m$^{-2}$].</td>
<td>(8)</td>
</tr>
<tr>
<td>$R$</td>
<td>Idea gas constant, 8.314 [J · mol$^{-1}$].</td>
<td>(6)-(8), (s5) and (s6)</td>
</tr>
<tr>
<td>$t$</td>
<td>Time, [s].</td>
<td>(2), (4), (7), (8), and (s1)-(s6)</td>
</tr>
<tr>
<td>$T$</td>
<td>Temperature, [K].</td>
<td>(6)-(8), (s5), and (s6)</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>Transfer coefficient, dimensionless.</td>
<td>(6)-(8), (s5), and (s6)</td>
</tr>
<tr>
<td>$\eta$</td>
<td>Over potential, [V].</td>
<td>(6)</td>
</tr>
<tr>
<td>$\rho_{\text{AgNP}}^{\text{molar}}$</td>
<td>Molar density of a Ag NP, [mol · m$^{-3}$].</td>
<td>(3), (s1) and (s4)</td>
</tr>
<tr>
<td>$\delta$</td>
<td>In infinitesimal thickness of the interfacial layer, [m].</td>
<td>(3) and (s1)</td>
</tr>
</tbody>
</table>
6.7. Acknowledgement

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6.8. Supporting Information

![Graph showing concentration (molar) of Ag atoms versus FeII concentration (molar)](image)

Figure S6.1. Concentration (molar) of Ag atoms versus the total formed FeII concentration (molar). The data lining up with a 1:1 ratio line proves the one-to-one stoichiometry of the reaction between Ag0 and FeIII, i.e., \( \text{Ag}^0 + \text{Fe}^{III} \rightarrow \text{Ag}^+ + \text{Fe}^{II} \).
Derivation of the “direct oxidation” kinetics model (eq (6.4))

The main text gave eqs (6.2) and (6.3):

\[ \frac{dC_{\text{molar}}^{\text{Ag}^0,\text{exp}}}{dt} = k_0 \cdot C_{\text{Fe}^{\text{III}}/\text{II},\text{s}} \cdot C_{\text{molar}}^{\text{Ag}^0,\text{exp}} \quad (6.2) \]

\[ C_{\text{molar}}^{\text{Ag}^0,\text{exp}} = \rho_{\text{AgNP}} \cdot \pi \cdot D^2 \cdot \delta \cdot C_{\text{AgNP}}^{\text{num}} \quad (6.3) \]

Substituting \( C_{\text{molar}}^{\text{Ag}^0,\text{exp}} \) in eq (6.2) with eq (6.3), one reaches,

\[ \rho_{\text{AgNP}} \cdot \pi \cdot \delta \cdot C_{\text{AgNP}}^{\text{num}} \cdot (2D) \frac{dD}{dt} = \left(k_0 \cdot C_{\text{Fe}^{\text{III}}/\text{II},\text{s}}\right) \cdot \rho_{\text{AgNP}} \cdot \pi \cdot D^2 \cdot \delta \cdot C_{\text{AgNP}}^{\text{num}} \quad (s6.1) \]

In the FRAN analysis, \( C_{\text{molar}}^{\text{Ag}^0,\text{exp}} \) (1.67 M) was controlled in excessive to \( C_{\text{molar}}^{\text{Ag}^0,\text{exp}} \) (at μM levels) and thus could be taken constant throughout the reaction. Let \( k_0' = k_0 \cdot C_{\text{Fe}^{\text{III}}/\text{II},\text{s}} \), and eq (s6.1) can be simplified to:

\[ \frac{dD}{dt} = \frac{1}{2} \cdot k_0' \cdot D \quad (s6.2) \]

Eq (s6.2) suggests that the diameter of the Ag NPs undergoing oxidative dissolution decays as first-order kinetics. Let \( D_0 \) be the initial size of Ag NPs, and eq (6.2) can be simply solved:

\[ D(t) = D_0 \cdot \exp\left(-\frac{1}{2} \cdot k_0' \cdot t\right) \quad (s6.3) \]

Following reaction (6.1) in the main text, the concentration of the formed Fe^{II} at time \( t \) is equivalent to the concentration of released Ag^{+} at the same time. Therefore, \( C_{\text{Fe}^{\text{II}}}(t) \) is described as:
\[ C_{Fe}^{ii}(t) = C_{AgNP}^{num} \cdot \rho_{AgNP}^{molar} \cdot \frac{1}{6} \cdot \pi \cdot \left( D_0^3 - D(t)^3 \right). \] (s6.4)

Substituting eq (s6.3) into (s6.4) and noticing that \[ C_{AgNP}^{num} \cdot \rho_{AgNP}^{molar} \cdot \frac{1}{6} \cdot \pi \cdot D_0^3 = C_{molar}^{molar}, \] one reaches eq (6.4) in the main text:

\[ C_{Fe}^{ii}(t) = C_{Ag}^{molar} \cdot \left[ 1 - \exp\left( -k_{obs} \cdot t \right) \right], \] (6.4)

where \[ k_{obs} = \frac{3}{2} k_0. \]

**Derivation of the “electrode reaction” kinetics model (eq (6.8))**

Let \( Q_0 \) be the total electronic charge on Au NPs surfaces in the solution at \( t_0 \), eq (6.7) in the main text can be solved:

\[ Q(t) = \frac{C \cdot R \cdot T}{F \cdot \alpha} \log \left( \exp \left( \frac{\alpha \cdot F \cdot Q_0}{C \cdot R \cdot T} \right) + \frac{k_o \cdot F^2 \cdot A \cdot \alpha \cdot C_{Fe}^{m} \cdot t}{C \cdot R \cdot T} \right). \] (s6.5)

Noticing the balance of charge in reaction (5) in the main text, one has:

\[ C_{Fe}^{ii}(t) = -\frac{1}{F \cdot V} \cdot (Q_0 - Q(t)); \] (s6.6a)

\[ C_{Fe}^{ii}(t) = \frac{C \cdot R \cdot T}{V \cdot F^2 \cdot \alpha} \log \left( \exp \left( \frac{\alpha \cdot F \cdot Q_0}{C \cdot R \cdot T} \right) + \frac{k_o \cdot F^2 \cdot A \cdot \alpha \cdot C_{Fe}^{m} \cdot t}{C \cdot R \cdot T} \right) - \frac{Q_0}{F \cdot V}. \] (s6.6b)

It is noteworthy that \( Q \) is the charge on NPs; when reaction (6.5) proceeds, \( Q \) becomes more positive and thus increases with \( t \). \( Q_0 - Q(t) \) is negative.

Eq (6.8) in the main text is nothing more than a series of mathematical simplification of eq (s6b). First, notice a few replacements of variables:
\[ C_S = \frac{C}{A}; \]

\[ A_{m,\text{AuNP}} \cdot C_{\text{AuNP}}^{\text{mass}} = \frac{A}{V}; \]

\[ q_0 = \frac{Q_0}{A}. \]

Eq (6b) becomes:

\[
C_{Fe}^n(t) = \frac{A_{m,\text{AuNP}} \cdot C_{\text{AuNP}}^{\text{mass}} \cdot C_S \cdot R \cdot T}{F^2 \cdot \alpha} \log \left( \exp \left( \frac{\alpha \cdot F \cdot q_0}{C_S \cdot R \cdot T} + \frac{k_0 \cdot F^2 \cdot \alpha \cdot C_{Fe}^{\text{II}} \cdot t}{C_S \cdot R \cdot T} \right) - \frac{q_0 \cdot A_{m,\text{AuNP}} \cdot C_{\text{AuNP}}^{\text{mass}}}{F} \right)
\]

\[
= \frac{A_{m,\text{AuNP}} \cdot C_{\text{AuNP}}^{\text{mass}} \cdot C_S \cdot R \cdot T}{F^2 \cdot \alpha} \log \left( \exp \left( \frac{\alpha \cdot F \cdot q_0}{C_S \cdot R \cdot T} + \frac{k_0 \cdot F^2 \cdot \alpha \cdot C_{Fe}^{\text{II}} \cdot t}{C_S \cdot R \cdot T} \right) - \frac{q_0 \cdot \alpha \cdot F}{C_S \cdot R \cdot T} \right) \]

\[
= \frac{A_{m,\text{AuNP}} \cdot C_{\text{AuNP}}^{\text{mass}} \cdot C_S \cdot R \cdot T}{F^2 \cdot \alpha} \log \left( \exp \left( - \frac{q_0 \cdot \alpha \cdot F}{C_S \cdot R \cdot T} \right) \cdot \left( \exp \left( \frac{\alpha \cdot F \cdot q_0}{C_S \cdot R \cdot T} + \frac{k_0 \cdot F^2 \cdot \alpha \cdot C_{Fe}^{\text{II}} \cdot t}{C_S \cdot R \cdot T} \right) \right) \right)
\]

\[
= \frac{A_{m,\text{AuNP}} \cdot C_{\text{AuNP}}^{\text{mass}} \cdot C_S \cdot R \cdot T}{F^2 \cdot \alpha} \log \left( 1 + \exp \left( - \frac{q_0 \cdot \alpha \cdot F}{C_S \cdot R \cdot T} \right) \cdot \left( \frac{k_0 \cdot F^2 \cdot \alpha \cdot C_{Fe}^{\text{II}} \cdot t}{C_S \cdot R \cdot T} \right) \right)
\]

\[
= L \cdot C_{Fe}^{\text{mass}} \cdot A_{m,\text{AuNP}} \cdot C_S \cdot \log \left( 1 + K \cdot C_{Fe}^{\text{II}} \cdot t \right),
\]

where, \( L = \frac{R \cdot T}{\alpha \cdot F^2} \), and \( K = \frac{k_0}{L \cdot C_S} \cdot \exp \left( - \frac{q_0}{L \cdot F \cdot C_S} \right) \). The derivation of eq (6.8) is therefore completed.
CHAPTER 7

CONTROL OF NANOPARTICLES USED IN CHEMICAL MECHANICAL POLISHING/PLANARIZATION SLURRIES DURING ON-SITE INDUSTRIAL AND MUNICIPAL BIOLOGICAL WASTEWATER TREATMENT


7.1. Abstract

Nanoparticles of silica (SiO$_2$), cerium (CeO$_2$) and alumina (Al$_2$O$_3$) are used in liquid slurries by a number of industrial applications, including chemical mechanical polishing/planarization (CMP) processes used multiple times during production of computer chips. These nanoparticles are designed to be dispersed in the CMP slurries, which are used once and subsequently discharged to sewers. The global production of these three nanoparticle CMP slurry materials exceeds 5000 tons/year, placing them among the highest nanomaterial production volumes worldwide. Industrial on-site treatment of nanoparticles is not part of most discharge permits, but some semi-conductor facilities apply on-site treatment for other limits related to metals (e.g., arsenic, copper), turbidity and/or biochemical oxygen demand. This chapter characterizes commonly used CMP nanoparticles, investigates removal efficiency using a representative industrial on-site treatment strategy (chemical softening and precipitation) and off-site treatment at biological wastewater treatment plants. We also demonstrate the use of techniques to characterize nanoparticles in liquid solutions, including the ability to separate dissolved from nanoparticle forms of silica. Overall, the results provide information on a large
production volume nanoparticle waste stream that has the potential to enter the environment.

7.2. Introduction to Nanoparticles in CMP Fluids

Chemical mechanical polishing/planarization (CMP) is a process that removes materials by a combination of chemical and mechanical (or abrasive) actions to achieve highly smooth and planar material surfaces. CMP can be used to planarize a variety of materials including dielectrics, semiconductors, metals, polymers, and composites, and in semiconductor production processes it is crucial for achieving the performance goals of modern microprocessor and memory chips\(^{62, 63}\). A typical CMP operation scheme is shown in Figure 7.1. Inorganic abrasive particles are an important component of CMP slurries. The three most commonly used abrasive particles in CMP slurries are the metal/metalloid oxide species alumina (\(\text{Al}_2\text{O}_3\)), silica (\(\text{SiO}_2\)) and ceria (\(\text{CeO}_2\))\(^{64}\). These three engineered nanomaterials are among the top 10 in production and use, and worldwide consumption amounts of 5,500 ton/year for silica and 55 ton/year for alumina and ceria have been reported\(^{11}\). The CMP process plays a significant role in utilizing the considerable amount of the three nanomaterials\(^{249, 250}\). CMP is estimated to be the second largest market for nanomaterials with a total annual usage of 9,400 ton\(^{186}\), and CMP nanoparticles constituted nearly 60% of the total $1 billion worldwide market for nanopowders by 2005\(^{57, 58}\). During the CMP process, a large volume of ultrapure water is used to clean the surface of polishing substrate (e.g., wafers), generating wastewater containing nanoparticles from the CMP slurry\(^{251}\). Thus CMP wastewater is potentially a major source of nanoparticles into sewer systems, and eventually into the environment. However, essentially no data exists on these materials because Si, Ce and Al are not
regulated metals in industrial discharges or at municipal wastewater treatment plants (WWTPs). Silica is the most commonly used abrasive and Si concentrations of 1000 - 2000 mg/L have been reported in CMP tools effluent\textsuperscript{252, 253}. Because they are used in lesser quantities than silica slurries, alumina and ceria concentrations in CMP tools effluent are expected to be lower than SiO\textsubscript{2} (e.g., 1-100 mg/L). Current industrial discharge limits into sewers from semiconductor fabrication facilities generally include turbidity, biochemical oxygen demand (BOD) and specific chemicals for which municipal WWTPs have limits (e.g., arsenic, copper, fluoride, zinc, etc.). Not all fabrication facilities require on-site industrial treatment; this depends on their flow contribution to the local sewer system and local regulations.

![Figure 7.1. Schematic of representative CMP operation.](image)

The abrasive particles in CMP slurries are usually roughly spherical. Depending on different applications, particle size in CMP slurries can vary from 50 nm to 1 \( \mu \text{m} \), and trends are toward CMP particles less than 100 nm in diameter to achieve highly polished surfaces\textsuperscript{64, 65}. The three most common oxide abrasives used in CMP slurries, SiO\textsubscript{2}, Al\textsubscript{2}O\textsubscript{3} and CeO\textsubscript{2} nanoparticles, usually have a relatively uniform shape and size. Silica can be
distinguished as fumed silica or colloidal silica based on the synthesis method. Fumed silica is formed in a pyrogenic process by oxidizing chlorisilane (SiCl\textsubscript{4}) at high temperature\textsuperscript{66}. Colloidal silica is formed in liquid phase from a Si precursor (e.g., Na\textsubscript{2}SiO\textsubscript{3})\textsuperscript{67}. A widely referenced method of synthesizing colloidal silica was presented by Stöber\textsuperscript{68}, and a tetraalkylsilicate was used as the Si precursor in this method. Ceria nanoparticles used in CMP slurries typically have a crystalline structure, thus often yielding sharp edges, corners and apexes\textsuperscript{69}. Alumina nanoparticles used in CMP slurries can be α-alumina, Υ-alumina, δ-alumina and fumed alumina\textsuperscript{70}. Alumina is softer than silica or ceria and is sometimes be coated with harder material such as silica\textsuperscript{64}. Nanoparticles below 100 nm can still be detected from CMP wastewater after the manufacturing lifetime\textsuperscript{253, 254}.

Considering the widespread use of nanomaterials in industrial CMP, it is important to balance their benefit to society with knowledge about the potential risks associated with release of these materials to the environment. Specifically, the ecological impacts and toxicity of CMP nanomaterials are of increasing interest to environmental researchers and agencies\textsuperscript{4, 6}. Figure 7.2 illustrates potential intervention points (i.e., onsite treatment, municipal wastewater treatment plants) to remove nanomaterials. The majority of on-site industrial treatments involve precipitation of metals, or sorption of pollutants (e.g., arsenic sorption) onto precipitates. For example, lime softening is commonly designed to remove dissolved and particulate copper from fabrication facilities waste streams. Precipitated materials are gravitationally settled and disposed of in landfills. Materials not removed are generally discharged to municipal sewer systems and enter municipal wastewater treatment plants (WWTPs) that often use biological treatment designed to
remove nutrients (carbon, nitrogen, phosphorous) but are also capable of removing nanomaterials.\textsuperscript{255-257}

\textbf{Figure 7.2}. Source, treatment and fate of CMP nanoparticles used in the semiconductor industry.

In this chapter, we present lab-scale experiments designed to simulate the fate and removal of CMP nanoparticles through wastewater water treatments including lime-softening and biomass adsorption processes. The objective is to investigate how well the CMP nanoparticles can be removed. We also present analytical techniques and limitations for quantifying engineered nanomaterials.
7.3. Materials and Methods

7.3.1. Selection of CMP slurries that constitute representative nanoparticle species

We obtained four CMP slurry products from Cabot Microelectronics (Aurora, IL) as part of a collaboration with the Semiconductor Research Corporation. CMP slurries generally contain suspended nanoparticles, surfactants, oxidants and other proprietary constituents. For use in this study, simplified slurries were provided absent of additives other than nanoparticles and acids or bases as pH adjusting agents. The nanoparticles were dispersed and stabilized in suspension using mechanical processes, by Cabot, and the nanoparticles purportedly remain stable in suspension for months to years. These four CMP slurry products contain different nanoparticle oxide species: colloidal silica, fumed silica, ceria, and alumina, which represent the most commonly used nanoparticles in CMP slurries. The manufacturer-provided information about the four CMP slurries is shown in Table 7.1 and their complete characterization is the focus of another paper by the chapter authors. Here we present measurements for nanoparticle size distribution and characterization by dynamic light scattering (DLS), scanning electron microscopy (SEM) and single particle ICP-MS (spICP-MS). Chemical compositions of the four CMP slurries were analyzed by ICP-MS after appropriate chemical and microwave digestion. For cases in which dissolved species are required to be distinguished from particulates, we used centrifugal ultrafilters to remove nanoparticles from solution.

Table 7.1. Characterization of four CMP slurries provided by the manufacturer

<table>
<thead>
<tr>
<th>Slurry</th>
<th>nanoparticle concentration</th>
<th>nanoparticle size (nm)</th>
<th>pH</th>
<th>Additives</th>
</tr>
</thead>
</table>

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<table>
<thead>
<tr>
<th>Material</th>
<th>Concentration</th>
<th>50-60</th>
<th>2.5-4.5</th>
<th>acetic acid &lt;1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colloidal silica</td>
<td>3 wt%</td>
<td>50-60</td>
<td>2.5-4.5</td>
<td>acetic acid &lt;1%</td>
</tr>
<tr>
<td>Fumed silica</td>
<td>5 wt%</td>
<td>120-140</td>
<td>10</td>
<td>KOH &lt;1%</td>
</tr>
<tr>
<td>Ceria</td>
<td>1 wt%</td>
<td>60-100</td>
<td>3-4</td>
<td>---</td>
</tr>
<tr>
<td>Alumina</td>
<td>3 wt%</td>
<td>80-100</td>
<td>4.5-5.0</td>
<td>nitric acid &lt;1%</td>
</tr>
</tbody>
</table>

### 7.3.2. Simulated Chemical Softening and Biological Treatment

To simulate the onsite lime softening treatment process, jar tests were performed with diluted CMP slurries with CaCl\(_2\) added under different pH conditions. The four types of CMP slurries were diluted 500-1000x to get around 20-30 mg/L metal concentrations of each nanoparticle species, which is fairly close to the corresponding concentrations in CMP wastewater. CaCl\(_2\) was selected as the softening agent instead of lime or Ca(OH)\(_2\) for the sake of better controlling pH conditions. All jar tests were operated in order of: (1) rapid mixing at 100 rpm for 2 min; (2) flocculation at 30 rpm for 30 min; and (3) sedimentation for 60 min. Samples were collected from the supernatant.

For the biomass adsorption test, we followed methods as previously published\(^{255}\). Fresh biomass suspension was cultured in lab-scale sequencing batch reactors (SBRs), which was inoculated with return activated sludge from a WWTP in the metro Phoenix area. The SBRs were operated in a 10 hour cycle mode, which consisted 8 hours of aeration, 90 minutes of sludge settling, and 30 minutes of feed solution replacement. The settled supernatant was discharged and 2 L fresh synthetic feed solution was added in each replacement. The cultured biomass was diluted to 1000 mg/L to be used for the nanoparticle adsorption experiment. All adsorption systems were buffered at pH 7 with 1 mM NaHCO\(_3\). The 4 CMP slurries were tested with two concentration levels: (1) a
concentration to match those used in the jar test, which is 26mg/L as Si for colloidal silica, 24 mg/L as Si for fumed silica, 20 mg/L for ceria and 31 mg/L for alumina; (2) a lower concentration level which was 4 mg/L for each nanoparticle. After addition of the CMP slurries into the biomass adsorption system, the solutions were shaken for 3 hours and then allowed to gravitationally settle for 30 minutes. The supernatants were sampled and their metal concentrations were analyzed by inductively coupled plasma - mass spectrometry (ICP-MS). In previous work, experiments with biomass from lab-scale SBRs and full-scale municipal WWTPs showed nearly identical nanoparticle removal. The benefit of the lab-scale SBR biomass is the low concentrations of trace metals compared with those often present in full-scale WWTP biomass.

7.4. Analytical Methods

7.4.1. DLS analysis

A Brookhaven ZetaPals Analyzer (Brookhaven Instruments, Holtsville, USA) was used to perform DLS analysis with the particle sizing function. The wavelength and the scattering angle were set at 660 nm and 90°, respectively. A multimodal size distribution (MSD) algorithm was used to characterize the particle size distribution. The MSD output format was set as intensity, the direct instrument measurement. Refractive indexes were used as: 1.765 for alumina, 2.200 for ceria, and 1.542 for silica.

7.4.2. Single Particle ICP-MS analysis for sizing nanoparticles

Single particle ICP-MS (spICP-MS) is an emerging technique used to size and quantify nanoparticles in aqueous matrices. Details about the operation of spICP-MS can be found in recent publications. Briefly, short signal integration (dwell) times are used to detect pulses of analyte above background, with the pulses representing particle detection events. A Thermo X series II ICP-MS was used for all analyses. The
instrument was placed in time-resolved analysis (TRA) mode in which the signal was recorded every dwell time (integration time of one reading by the detector) of 10 ms duration. A glass nebulizer and a conical spray chamber constitute the sample introduction system. The sample flow rate was set in the range of 0.6–0.7 ml•min⁻¹.

¹⁴⁰Ce and ²⁷Al were used as the analyte isotopes for ceria and alumina nanoparticles. The analysis of silica was not performed because the detection limit for Si by spICP-MS is relatively high (> 400 nm) ²⁵⁹.

7.4.3. CMP Nanoparticle Chemical Digestion

Digestion is performed for the purpose of transforming nanoparticles into ions, allowing robust quantification of metal concentrations by ICP-MS. Conventional digestion methods²⁶⁰ use acid (e.g., HNO₃, HCl, etc.) and heat the sample on hot plate or in a microwave digestion system. Herein we determined the digestion method for the four types of CMP nanoparticles as follows.

For colloidal silica and fumed silica, an alkaline solution, tetramethylammonium hydroxide (TMAH, 25%, J.T. Baker) was used to digest silica nanoparticles with microwave assistance. Specifically, 4 mL TMAH stock solution was added to each sample, with the total sample volume at 15 mL. The microwave was operated by ramping the temperature up to 150 °C in the first 15 min; ramping the temperature up to 180 °C in the second 15 min; holding the temperature at 180 °C for 30 min. For ceria and alumina, 2 mL hydrofluoric acid (HF) (50%, J.T. Baker), 2 mL HCl (30%, J.T. Baker) and 6 mL HNO₃ (70%, J.T. Baker) were added into the sample and total volume was adjusted to 15-20 mL. The microwave procedure was identical to that used for silica.
7.4.4. Separation of Nanoparticles from Dissolved Ions

Centrifugation is a traditional method to separate particulates from solution. Instead of relying solely upon mass density separation, which is ineffective for very small nanoparticles, a centrifugal ultrafiltration device with 30,000 Dalton nominal molecular weight limit (NMWL) (Millipore, Darmstadt, Germany) was used to separate nanoparticles and the ionic (dissolved) species for all 4 CMP slurries. Samples in centrifugal ultrafiltration devices were centrifuged at 5000 G for 30 min.

To demonstrate the effectiveness of the centrifugal ultrafiltration device to separate nanoparticles and dissolved species, we used a commercial SiO$_2$ nanoparticle (PM1040, Nissan Chemical, Houston) and ionic SiO$_2$ standard solution (HACH, Loveland). We made three solutions: (1) solution containing 1000 ppb (as SiO$_2$) ionic standard and 1000 ppb (as SiO$_2$) nanoparticle; (2) 1000 ppb nanoparticle; and (3) 1000 ppb ionic standard. 10 mL of each solution was pipetted into the centrifugal ultrafiltration devices. Thus the three solutions should give: (1) 4.7 µg ionic Si and 4.7 µg nanoparticle Si; (2) only 4.7 µg nanoparticle Si; and (3) only 4.7 µg ionic Si. Figure 7.3 shows the recovery for the filtrate and concentrate for the three solutions. The recoveries of both filtrate (i.e., constituents that flux through the filter) and concentrate (i.e., constituents that are rejected by the filter) for all cases are close to the spiked amount. For the mixture case (solution (1)), both ionic Si and nanoparticle Si can be nearly completely recovered (102±6% for ionic Si and 106±8% for nanoparticle Si, error bars are based on triplicate measurements by the instrument). For the solution containing only nanoparticle Si, 0.1 µg of Si (out of the total 4.7 µg) was detected from the filtrate (considered as ionic Si), which accounts for only ~2% of the Si mass. This indicates that some Si present in the
silica nanoparticle solution passed through the ultrafiltration membrane. It is likely that the filterable Si was present as dissolved Si species resulting from dissolution of the original silica nanoparticles. For the solution containing only ionic Si, it gives recovery of 94% (4.4 µg out of 4.7 µg), which is the minimum recovery of the three experimental permutations.

**Figure 7.3.** Test results of the centrifugal ultrafiltration devices for silica nanoparticles and ionic Si. Filtrate solution has passed through the ultrafiltration membrane, which represents dissolved Si species. Concentrate refers to solution rejected by the ultrafiltration membrane, which represents the particulate phase. In the three cases, solution containing 4.7 µg ionic Si and 4.7 µg nanoparticle (NP) Si, solution containing only 4.7 µg ionic Si, and solution containing only 4.7 µg NP Si were tested with the centrifugal ultrafiltration devices. The filtrate recovery and concentrate recovery were measured by ICP-MS and shown as Si mass in the figure. Recoveries of ≥ 94% can be obtained for all cases for both filtrate (dissolved Si) and concentrate (silica NP).
7.5. Results

7.5.1. Characterization of CMP nanoparticles

Table 7.2 summarizes different techniques that were used to characterize the nanoparticle size in the four CMP slurry samples. By comparing the results of each technique with the size provided by the manufacturer, we see the colloidal silica size measured by DLS or SEM is lower than the reported value. For the fumed silica nanoparticle, there is a big difference between the measured results of DLS and SEM, with DLS closer to the reported value. To understand this we need to refer to the SEM images of the four types of CMP nanoparticles, which are shown in Figure 7.4. Colloidal silica gives discrete spherical images under SEM whereas fumed silica shows clusters of particles. This confirms the known character of fumed silica that aggregation unavoidably occurs during the combustion synthesis process\textsuperscript{67}. In this case, size obtained by DLS is based on the Brownian motion of particles in water and gives the effective diameter closer to the cluster size. For the size obtained by SEM, we measured the diameters of discrete particles on the image. Similarly, with ceria and alumina, DLS analysis reports larger diameter than SEM for both nanoparticles. From the SEM image, ceria particles appear more like crystals with clear edges and corners, which may cause the overestimation of DLS based on their motions in water. Some aggregation occurred for alumina nanoparticles based on the SEM images, which may cause the larger observed size by DLS since DLS is easily biased toward larger particles\textsuperscript{176}. The aggregation states of fumed silica and alumina are also confirmed by TEM (data not shown) analysis.
Table 7.2. Nanoparticle size obtained by different analytical techniques and the manufacturer of the CMP slurries

<table>
<thead>
<tr>
<th>Technique</th>
<th>Colloidal silica</th>
<th>Fumed silica</th>
<th>Ceria</th>
<th>Alumina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (nm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DLS</td>
<td>45.1±13.4</td>
<td>183.5±60.8</td>
<td>184.9±76.2</td>
<td>156±60.3</td>
</tr>
<tr>
<td>SEM</td>
<td>37±7</td>
<td>38±14</td>
<td>42±16</td>
<td>84±21</td>
</tr>
<tr>
<td>spICP-MS</td>
<td>NA</td>
<td>NA</td>
<td>40±19</td>
<td>78±20</td>
</tr>
<tr>
<td>Manufacturer-</td>
<td>50-60</td>
<td>120-140</td>
<td>60-100</td>
<td>80-100</td>
</tr>
<tr>
<td>provided</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 7.4. SEM images of the four types of CMP nanoparticles.
Ceria and alumina nanoparticles were also characterized by spICP-MS. Figure 7.5 shows the time-resolved data and converted particle size distributions by spICP-MS for ceria and alumina nanoparticles in CMP slurries. The time-resolved data (Figure 7.5a and b) contains the instrument intensity versus time, in which each pulse represents a particle signal. By relating the pulse height to the particle size via a dissolved calibration curve\textsuperscript{75, 76}, particle size distributions (Figure 7.5c and d) can be converted from the time-resolved data. spICP-MS is a promising nano-analysis technique for environmental samples with the advantage of low detection limit (ng/L levels) and sensitive size resolution ability. Herein it is demonstrated that spICP-MS can be used to detect the ceria and alumina nanoparticles in CMP slurries. Due to mass spectrum interferences in ICP-MS analysis for Si, the size detection limit by spICP-MS analysis of silica nanoparticles is around a few hundred nanometers\textsuperscript{87}.

The surface charge of metal oxide nanoparticles influences their interaction with other surfaces, and hence their fate during different treatment processes\textsuperscript{18}. The iso-electric point ranges from 1.7 to 3.5 for silica, 6.7 to 8.6 for ceria, and 8 to 9 for alumina\textsuperscript{261}. Collaborators at Colorado School of Mines (J. Ranville) measured zeta potential for the CMP nanoparticles at pH 8.4 in 10 mM NaHCO\textsubscript{3} to be -16 mV for colloidal silica and more negative for fumed silica (> - 35 mV).
Figure 7.5. spICP-MS analysis for ceria and alumina nanoparticles in CMP slurries: a, b: time-resolved data (intensity versus time) of spICP-MS for ceria and alumina; c, d: converted particle size distributions for ceria and alumina.

7.5.2. Removal of nanoparticles in CMP slurries through calcium-based softening process

During the onsite treatment of semiconductor wastewater, lime softening process (e.g., adding lime to adjust pH to 10-12) is often adopted to remove ionic metal chemicals (e.g., Cu). Nanoparticles are potentially removed as well through this process. We used CaCl$_2$ as the calcium source to simulate the softening process. The Ca dosage was set at 1.9 mM and the removal of nanoparticles was examined under different pH conditions. The
concentration of Ca was selected according to the fact that equivalent amount of lime gives rise to a pH about 11, which is a desired pH for typical lime softening process\textsuperscript{262}. Four types of nanoparticles in CMP slurries were investigated separately and their concentrations in the supernatant were determined by ICP-MS after digestion. All concentrations are reported as the elements of Si, Ce and Al. Figure 5.6 shows the removal efficiencies for the four types of nanoparticle in CMP slurries under different pH conditions. Other than colloidal silica, all the tested pH conditions were in the basic range to mimic lime softening conditions. For colloidal silica, a wide pH range was examined. This showed that the lowest removal for colloidal silica occurred around pH 5 to 6, which is about equal to the original pH of the diluted slurry. Only \textasciitilde15\% removal of Si was observed around pH 2 to 4. This peculiar stability of silica in vicinity of the zero point of charge (~ pH 2) can be due to the lack of hydroxyl ions, which catalyzes the formation of siloxane bonds among particles and contributes to aggregation occurrence\textsuperscript{67}. In the pH range of 6 to 7, nanoparticle removal increased from <10\% to \textasciitilde90\%. Above pH 7, the removal of colloidal continue increasing and >95\% removal can be achieved at higher pH >10. Similar trends are observed for fumed silica under basic pH conditions. Above pH 8, \textasciitilde95\% removal efficiency can be achieved for fumed silica nanoparticles. High removal efficiency, greater than 90\%, was observed under all basic conditions tested for ceria nanoparticles. Alumina showed different behavior compared with the other three types of nanoparticles, which is reflected as a continuous and slow increase of the removal efficiency from pH \textasciitilde6.5 to 12.5. The removal efficiency was raised from 16\% (pH 6.6) to 92\% (pH 12.5).
Figure 7.6. Removal efficiencies of Si, Ce and Al for four types of nanoparticles in CMP slurries under different pH conditions. Ca dosage was 1.9 mM in all cases. The starting concentrations are: 26 mg/L as Si for colloidal silica, 24 mg/L as Si for fumed silica, 20 mg/L as Ce for ceria, and 31 mg/L as Al for alumina. Concentrations are based on total metal measurement by ICP-MS. pH values are obtained after jar test when reactions are considered in equilibrium.

For the colloidal and fumed silica nanoparticles, we assume the dissolved Si (e.g., silicate) plays a role in the removal of total Si. Silica particles can have solubility of ~100 mg/L under neutral pH and the solubility significantly increases when pH becomes higher\(^a\). By using the centrifugal ultrafiltration method, we found the dissolved Si concentrations in colloidal silica and fumed silica original slurries are 30.2±0.9 mg/L and 37.0±1.5 mg/L, respectively. Thus the dissolved Si concentrations in both slurries are lower than expected silica solubility. After the original slurries were diluted to simulate the wastewater concentrations, we detected lower dissolved Si concentrations before
performing the jar test. Figure 7.7 gives the details of dissolved Si concentration in colloidal and fumed silica CMP slurries under different pH conditions before and after jar test. In Figure 7.7a, a clear increase of the dissolved Si before jar test was observed for colloidal silica from pH 7 to 12.5. Dissolved Si also increased slightly when the pH was lower than 7. This trend is similar to the reported silica solubility versus pH\textsuperscript{67}. After the jar test, the dissolved Si generally remained at concentrations below 1 mg/L. This result indicates the dissolved Si after jar test may be dominated by, other than the dissolution of silica, other phenomena that are less influenced by the base pH conditions. Above pH 7, the removal efficiency increased with the increasing initial dissolved Si.

The observations lead to a possible mechanism for the removal of silica nanoparticles. The reaction of dissolved Si (e.g., silicate) and Ca\textsuperscript{2+} to form calcium silicate hydrate, may bridge silica nanoparticles to increase agglomeration and therefore particle settling. Previous work has reported this reaction in cement chemistry\textsuperscript{263}. In contrast, as seen in Figure 7.7b, the fumed silica nanoparticle slurry did not show an apparent trend as did colloidal silica. The dissolved Si increased with pH but not as rapidly as with colloidal silica. After the jar test, dissolved Si remained at \~ 1 mg/L under most pH conditions except for pH 7, which gave \~ 3.5 mg/L dissolved Si. Overall, chemical precipitation showed an ability to remove a very large percentage of CMP nanoparticles when practiced at elevated pH levels. While some nanoparticles appear in the effluent, most of the residual metal content of the settled water appears to be dissolved metals (Si, Ce, Al) rather than nanoparticles. Thus, chemical softening using lime appears a good strategy for on-site industrial treatment of CMP nanoparticles. Future work needs to conduct similar work in the presence of surfactants, oxidants and other additives also present in
mixed semiconductor waste streams – and jar tests similar to those employed here should be effective in assessing the impact of these additives.

![Graphs showing dissolved Si concentrations before and after jar test for CMP slurries containing colloidal silica and fumed silica.]

**Figure 7.7.** Dissolved Si concentrations before and after jar test for the CMP slurries containing: (a) colloidal silica and (b) fumed silica. Total Si removal efficiency (right axis) are shown as comparison.

Additional experiments were conducted by holding pH constant and varying the amount of added calcium. These experiments were conducted two different ways. First, colloidal silica was tested at pH 7.0±0.2 in the presence of zero to 4 mM Ca (Figure 7.8). Here, adding divalent calcium promoted aggregation and removal of the colloidal silica nanoparticles. At pH 7, colloidal silica is negatively charged and favorable interactions with calcium lead to aggregation. Second, colloidal silica was combined with ceria and alumina CMP nanoparticles to form a mixed slurry that may be more representative of mixed waste streams at a Fab (Figure 7.9). The pH was 7.0±0.5 and again calcium addition varied from zero to 4 mM Ca. Without any calcium, near complete removal of
the Si occurred – which is in contrast to experiments with colloidal silica alone (Figure 5.8). At pH 7, the alumina nanoparticles are likely positively charged and favorably aggregate with the negatively charged colloidal silica; the ceria nanoparticles will be near neutral near pH 7. Adding calcium to the mixed slurry only slightly decreased the nanoparticle removal efficiency, potentially by sorbing to the colloidal silica, increasing its surface charge (more positive) and decreasing its aggregation potential with the other nanoparticles.

**Figure 7.8.** Effect of calcium addition on the removal of colloidal silica at pH 7.0±0.2
Figure 7.9. Effect of calcium addition on removal of colloidal silica, ceria and alumina nanoparticles at pH 7.0±0.5 from a mixed slurry (each nanoparticle at an initial concentration of 10 mg/L).

7.5.3. Removal of nanoparticles in CMP slurries by biomass adsorption

In the biomass adsorption test, two concentration levels of nanoparticles were examined. The first concentration level was set the same as the jar test, i.e., ~20-30 mg/L as the corresponding metal for each type of nanoparticle. The second concentration level was set at 4 mg/L as the corresponding metal, thus taking into account that the nanoparticle concentrations will be lower when they enter a municipal wastewater treatment plant.

Figure 7.10 shows the removal of four types of nanoparticles in CMP slurries. At the higher concentration level (Figure 5.10a), greater than 60% nanoparticles in all CMP slurries can be removed by biomass. Colloidal silica shows the highest removal efficiency of 88.8%, followed by ceria nanoparticles with 82.7% removal efficiency. It is
interesting that fumed silica behaves quite differently than colloidal silica, and at 67.6% shows the minimum removal efficiency of the four slurries. With a lower starting concentration of 4 mg/L, which is shown in Figure 7.8b, slightly different behaviors were observed. In this case, ceria nanoparticles showed the highest removal efficiency of 86.8%. Colloidal silica, fumed silica, and alumina nanoparticles gave comparable removal efficiencies in the range of 60-70%. By cross-comparing the two concentration levels, lower removal efficiencies were obtained with the 4 mg/L concentration level than the higher concentration level for colloidal silica, fumed silica and alumina. For ceria, similar removal efficiencies were obtained for the two concentrations levels. Overall, nanoparticle removals of 60% to 80% were observed at the screening biomass dose of 1000 mg/L. However, compared to similar experiments conducted with silver, gold or titanium dioxide nanoparticles performed elsewhere the removals of CMP nanoparticles are much lower. It is likely that the mechanical stabilization process employed in preparing CMP slurries created nanoparticles that are more difficult to remove than commercially available, stock nanoparticles.
Figure 7.10. Removal of 4 types of nanoparticles in CMP slurries by biomass adsorption (1000 mg/L dry weight of biomass): (a) starting concentration at 20-30 mg/L as each element; (b) starting concentration at 4 mg/L as each element. Labeled percentage values on the white bar represent the removal efficiency of each type of nanoparticles by biomass.

7.6. Conclusions

As engineered nanomaterials are tested for their potential toxicity and adverse ecological effects, it is also important to understand the ability of existing industrial and municipal treatment processes to remove nanoparticles used in widely used applications such as CMP slurries. By simulating the lime softening and biomass treatment processes on a laboratory scale, the removal effectiveness of these two treatment processes on nanoparticles in CMP slurries were demonstrated. Testing with four representative types of nanoparticles, including colloidal silica, fumed silica, ceria and alumina, it shows that, in the calcium based softening process, greater than 90% removal efficiencies can be achieved for all types of nanoparticles by manipulating the pH conditions. Generally higher pH values (e.g., pH 11-12.5) give rise to higher removal efficiencies. For colloidal silica and fumed silica, dissolved Si concentration increases with pH and may contribute to the removal of total Si. After the softening treatment, dissolved Si generally remains at ~ 1 mg/L for both colloidal silica and fumed silica. In the biomass adsorption process, about 60-80% nanoparticles in CMP slurries can be removed. When the nanoparticle concentrations change from 20-30 mg/L to 4 mg/L (corresponding to the potential concentrations in fresh CMP wastewater and potential concentrations in municipal wastewater, respectively), removal efficiencies by the biomass adsorption
process decrease for colloidal silica, fumed silica and alumina, whereas for ceria a high removal efficiency (> 80%) can still be obtained.

Throughout this study, analysis techniques such as DLS, spICP-MS, microwave digestion, and centrifugal ultrafiltration demonstrated their viability for different data objectives. Perhaps the most novel analysis shown here was demonstrating the applicability of spICP-MS for detection and sizing of ceria and alumina nanoparticles contained in CMP slurries. The application of spICP-MS for silica nanoparticles is currently very limited by background noise, but is an area of active research. Despite this analytical challenge, we demonstrate quantification of dissolved Si and SiO$_2$ nanoparticles by centrifugal ultrafiltration, and recommend this method as an effective approach to separate ionic and particulate species.

7.7. Acknowledgement

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CHAPTER 8

ADSORPTION OF III/V IONS (In(III), Ga(III) AND As(V)) TO SiO₂, CeO₂ AND Al₂O₃ NANOPARTICLES USED IN THE SEMICONDUCTOR INDUSTRY

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8.1. Abstract

The semiconductor industry annually uses thousands of tons of nanoparticles (NPs) in chemical mechanical polishing (CMP) processes. III/V ions (e.g., In(III), Ga(III) and As(V)) are emerging in the industrial wastewater along with growing applications of III/V materials. CMP NPs (colloidal SiO₂ (C-SiO₂), fumed SiO₂ (F-SiO₂), CeO₂ and Al₂O₃) may interact with III/V ions (e.g., In(III), Ga(III) and As(V)) and facilitate their transport in aquatic systems. Across a range of pH levels, we found appreciable adsorptions occurs for: In(III) ion to CeO₂ or Al₂O₃ NPs; Ga(III) ion to C-SiO₂, F-SiO₂, CeO₂ or Al₂O₃ NPs; and As(V) ion to CeO₂ or Al₂O₃ NPs. We determined the intrinsic surface complexation constant (K_C,int) between ion and NP by fitting the experimental data with a surface complexation model (SCM). We then extended the SCM to calculate adsorption as a function of NP size. As size increases, surface site density (SSD) increases whereas specific surface area (SSA) decreases; both eventually reach a plateau where the bulk material stands. Nonetheless, mass site density (MSD), i.e., the site number per mass, increases with size reduction, indicating smaller particles give higher adsorption capacity based on the same mass. Upon a 1-nm size reduction of C-SiO₂ NPs, the model predicts MSD increases <1% for size >100nm, and >10% for size <10nm. For C-SiO₂ NP adsorbing Ga(III) ions, the model predicts that decreasing NP size enhances
the adsorption efficiency the most in the pH ranges of 2.5-3 and 8.5-11. This work
represents the first SCM capable of incorporating size-dependent NP properties to predict
adsorption of environmentally relevant ions.

8.2. Introduction

Nanomaterials enable tremendous benefits in industrial manufacturing processes but
may pose new risks as technology changes. The semiconductor industry uses thousands
of tons of nanoparticles (NPs) in the chemical mechanical polishing (CMP) process$^{11, 249, 250}$. Typically NPs at 1-5 wt% are suspended in aqueous CMP slurries, and their common
chemical compositions are SiO$_2$, CeO$_2$ and Al$_2$O$_3$$^{64}$. Worldwide, utilization quantities of
these three nanomaterials are preeminent ($\sim$5,500 ton/yr of SiO$_2$ and 55 ton/yr of CeO$_2$ or
Al$_2$O$_3$)$^{11}$ CMP, with a high consumption of NPs as abrasives, became the second largest
nanomaterial market, following the automotive catalysts$^{186}$. As shown in the CMP
schematic in Figure 8.1, NPs are applied with the CMP slurry between a rotating pad and
a wafer surface and subjected to down force through a wafer carrier. NPs polish the
wafer surface through physical abrasion and chemical reactions (e.g., bonding ions off
the wafer surface). After a polishing cycle, large volumes of ultrapure water flush CMP
debris, chemicals and NPs off the wafer$^{64, 264}$ and end in a CMP waste-stream.

Onsite treatment of NPs from CMP tools is not directly regulated, and CMP NPs can
be discharged to municipal sewers and potentially to aquatic environment$^{264}$. SiO$_2$, the
most prevalent CMP NPs, can occur at 1000-2000 mg-Si/L in a CMP waste-stream$^{84, 252}$. Concentrations of Al in a CMP waste-stream are lower due to a less consumption of
Al$_2$O$_3$ NPs; up to 12 mg-Al/L has been reported$^{265}$. Concentrations of Ce in real CMP
waste-streams have not been reported to our knowledge. Despite the lack of report on the
actual Ce concentration in waste-streams, it is likely at mg/L levels upon a regular CMP application. Human and ecological toxicity has been studied for SiO$_2^{17, 18, 266, 267}$ and CeO$_2^{18, 243, 268}$ NPs. Recent work suggests industrial NPs can pose “indirect” risks, for example, through releasing toxic ions, which is referred to as a “Trojan Horse” mechanism$^{269, 270}$. In fact, such mechanisms are widely used in biomedical applications to facilitate the delivery of hydrophobic drugs to target cells$^{271}$. It becomes meaningful to investigate whether the industrial CMP NPs can carry and deliver other toxic ions, thereby posing indirect risks to the environment and human health.

![Semiconductor chemical mechanical polishing (CMP) schematic.](image)

**Figure 8.1.** Semiconductor chemical mechanical polishing (CMP) schematic.

In recent years, III/V semiconductor materials (e.g., GaAs, InP, etc) find more applications in electronics and light emitting diodes (LEDs) due to their high electron mobility and charge transfer efficiency$^{32, 33}$. III/V elements have been detected in industry wastewater and aqueous environment close to electronic manufactures. Chen et al. detected 0.95-20.95 µg/L indium (compared to 0.01 µg/L background level) in the groundwater near a semiconductor manufacturing in Taiwan$^{272}$. Sturgill et al. found ~50 mg/L Ga in a wastewater sample originating from a GaAs polishing facility$^{273}$, and Torrance et al. estimated the Ga concentration in polishing wastewater can vary from 27
mg/L to 2000 mg/L. Torrance’s team also detected dissolved As in the range of 1800-2400 mg/L from polishing waste-streams generated by GaAs CMP facilities. Known as a carcinogenic and toxic matter, arsenic is regulated by the USEPA with a maximum contamination level (MCL) in drinking water of 10 µg/L. Indium and gallium are not regulated, but they do pose toxicity potentials. Indium tin oxide (ITO), indium chloride (InCl₃), indium arsenide (InAs) and indium phosphide (InP) can cause pulmonary toxicity, thus posing human health hazard. Gallium species including gallium arsenide (GaAs) and gallium oxide (Ga₂O₃) particles are retained in the lungs and cause inflammatory response and pneumonocyte hyperplasia after being instilled into rats. GaAs is soluble in vitro in different aqueous buffer solutions and in vivo in the blood of rats. Therefore, to assess the environmental health and safety (EHS) with III/V-CMP wastewater, improved knowledge regarding the fate and transformation of III/V species is necessary.

CMP NPs coexisting with III/V ions within the industrial environment may pose a synergistic EHS risk. Aquatic chemistry of ions determines their speciation in water. The mineral solubility of indium is low when complexing with OH⁻, S²⁻, CO₃²⁻ and PO₄³⁻, with solubility constants ranging from 10⁻⁷³ to 10⁻¹⁵. Gallium is quite insoluble in equilibrium with OH⁻, with a solubility constant of Ga(OH)₃ at 10⁻⁹-10⁻¹² depending on the solution ionic strength and temperature. Gallium also stably complexes with SO₄²⁻ and HPO₃²⁻, with stability constants of 2.99 and 7.33, respectively. In a naturally aerated water (i.e., an oxidizing condition) or a CMP wastewater in which H₂O₂ is often present, the prevailing oxidation state of arsenic is +5. As(V) species are in arsenic acid (H₃AsO₃) and arsenate (AsO₃³⁻), which are highly soluble in water. These data
indicate that in circum-neutral aquatic environment, In(III) and Ga(III) tend to precipitate as hydroxide compounds, and As(V) exists as anions. However, CMP NPs may change the thermodynamic equilibriums in water via adsorbing the ions onto their surface. For the most common CMP NP materials (SiO$_2$, CeO$_2$ and Al$_2$O$_3$), information on the complexation with III/V species is very scarce. Lin et al.\textsuperscript{289} investigated adsorptions of In(III) and Ga(III) ions onto $\gamma$-Al$_2$O$_3$ and applied a triple-layer surface complexation model to describe their experimental data; however, intrinsic complexation constants are obtained only under acidic experimental conditions. Adsorptions of As(V) to mesoporous silica\textsuperscript{290} and aluminum oxide\textsuperscript{291,292} were studied. However, these studies often just give empirical adsorption isotherms in specific conditions. To enable a good prediction of adsorptions in general conditions, the intrinsic surface complexation constants are needed for III/V ions and CMP NPs.

The aim of this paper is to quantify adsorption of In(III), Ga(III) and As(V) ions to the most commonly used four types of CMP NPs (colloidal SiO$_2$ (C-SiO$_2$), fumed SiO$_2$ (F-SiO$_2$), CeO$_2$ and Al$_2$O$_3$). We developed a surface complexation model (SCM) to describe the adsorption process, with the intrinsic surface complexation constant ($K_{C,\text{intr}}$) parameterized for ion and NP by fitting the adsorption efficiency data across a range of pH levels. We then expanded the SCM to account for the effect of NP size on adsorption and to understand difference between nano- and bulk- materials as adsorbents.
8.3. Experimental methods

8.3.1. Chemicals and materials

Four CMP slurry samples composed of C-SiO$_2$, F-SiO$_2$, CeO$_2$ or Al$_2$O$_3$ NPs were obtained from an industry CMP slurry vendor. Additional information and characterization data of these four CMP NP samples were published in a previous study$^{293}$. Indium(III) trichloride (InCl$_3$, 99.999% trace metals basis, Sigma Aldrich), gallium(III) nitrate hydrate (Ga(NO$_3$)$_3$·xH$_2$O, 99.9% trace metals basis, Sigma Aldrich) and sodium arsenate dibasic 7-hydrate (Na$_2$HAsO$_4$·7H$_2$O, A.C.S. Reagent, J.T. Baker) were used as the In(III), Ga(III) and As(V) sources for all adsorption experiments. Ga(NO$_3$)$_3$ was used instead of GaCl$_3$ because of a too violent hydrolysis reaction of GaCl$_3$ in water. Ultra pure hydrochloride acid (HCl, ~30%, Ultrex II, J.T. Baker) and trace-metals-based sodium hydroxide (NaOH, >99.99%, Sigma Aldrich) were used as pH adjusting agents. Sodium chloride (NaCl, >99.5%, Sigma Aldrich) was used to control a solution’s ionic strength. Ultra pure water (18.3 MΩ, NANOpure Infinity, LA, USA) was used to make solutions.

To separate NPs from a liquid, a centrifugal ultrafiltration device (NMWL=30K Da, EMD Millipore) assisted with a centrifuge (Sorvall, Legend X1, ThermoFisher) was used. Concentrations of In, Ga and As were detected on a ICP-MS (Thermo X-series II). Standards for the ICP-MS were purchased from VHG Labs (Manchester, USA).

8.3.2. Adsorption tests

CMP slurries were diluted to 100 mg/L (for experiments with indium and gallium) or 500 mg/L (for experiments with arsenic) of Si, Ce or Al. NaCl was added to fix ionic
strength at 0.1 M. The solution pH was adjusted by HCl or NaOH. Each adsorption test was conducted by transferring 50 mL of a CMP NP solution to a 125 mL wide mouth polyethylene bottle (Nalgene, Thermo Scientific) and adding a specific amount of In(III), Ga(III) or As(V) ion stock solution (with a \( \geq 100 \) dilution factor to minimize the total volume change). Sealed bottles were shaken (Eberbach, Model 6010) for 72 hours to reach adsorption equilibrium. Final pH of each sample after 72-hour adsorption was measured and used as the equilibrium pH condition. Then 9 mL of each sample was transferred to a centrifugal ultrafiltration tube to separate all NPs from the solution. The permeate was acidified prior to the ICP-MS analysis of In, Ga or As concentration. Control samples where ion samples were tested without adding any NPs were used to confirm insignificant ion losses through the testing procedures.

In natural water, In(III) and Ga(III) tend to precipitate into In(OH)_3 and GaO(OH), both of which have a very low solubility. Using the known solubility products\(^{294,295}\) and hydrolysis constants (Table S8.1 in the SI), solubility diagrams are plotted for solution in saturation with In(OH)_3 or GaO(OH) and shown in Figure 8.2. In(III) is nearly insoluble (<10\(^{-7}\) M) in pH 4-10. Ga(III) shows a minimum solubility about 10\(^{-7}\) M in pH 4-5. Therefore, we performed the adsorption experiment for indium with initial concentration at 1.0 \( \times \) 10\(^{-6}\) M under acidic conditions only, and for gallium for initial concentration at 1.5 \( \times \) 10\(^{-7}\) M under acidic, neutral and basic conditions. Because of the low solubility of In(III) or Ga(III), to maintain the same adsorbent to adsorbate ratios as other experiments we used lower NP concentration.
**Figure 8.2.** The solubility and speciation of In(III) (left) and Ga(III) versus pH. Figures are plotted using equilibrium data from Table S1 and solubility product data for In(OH)₃ ($K_{sp}a=10^{-36.9}$) and GaO(OH) ($GaO(OH)(c)+H_2O \rightleftharpoons Ga(OH)_3(aq)$, $log K = -7.4$).

**8.3.3. Zeta potential measurement**

Zeta potential of CeO₂ and Al₂O₃ NPs was measured (ZetaPALS, Brookhaven, Holtsville, USA). Solutions containing NPs were prepared in 1 mM KNO₃ solution (to control consistent ionic strength and conductivity of the working solution recommended by the manufacturer). NP concentration was adjusted to achieve a good instrument reading intensity (50-500 kcps). Zeta potential was read at multiple pH levels from 2 to 13. The obtained zeta potential data was plotted versus pH and fitted with the Gouy-Chapman theory based diffusive layer model.

**8.4. Modeling description**

**8.4.1. The surface complexation model**

Well-established SCM strategies were applied to the adsorption of III/V ions to CMP NPs. Hydroxyl groups (–OH) on the NP surface were assumed to be the adsorption sites.
for III/V ions. The –OH groups on NP surface are amphoteric and in acid-base equilibrium as:

$$\text{NP(OH)}_2^+ \rightleftharpoons \text{NP(OH)} + \text{H}^+ \quad K_{s,1} = \frac{[\text{NP(OH)}][\text{H}^+]}{[\text{NP(OH)}_2^+]} \quad \text{Eq}(8.1)$$

$$\text{NP(OH)} \rightleftharpoons \text{NP(O}^- + \text{H}^+ \quad K_{s,2} = \frac{[\text{NP(O}^- ][\text{H}^+]}{[\text{NP(OH}]} \quad \text{Eq}(8.2)$$

where “[ ]” represents the concentration of NP surface groups or the activity of ions (the activity is obtained by multiplying concentration with activity coefficient) in solution.

The same notation is applicable throughout the text.

SiO$_2$ has pK$_{s,2}$ (i.e., -logK$_{s,2}$) equal to 6.8 and the zero point of charge (pH$_{zpc}$) equal to ~2. This predicts pK$_{s,1}$ of SiO$_2$ is negative, as pH$_{zpc}$ is at the midpoint of the two pK$_s$ values. Therefore Eq(8.1) is excluded for SiO$_2$ NPs. The previous study$^{293}$ justified this rationale with electric mobility (an indicator of surface charge) of the CMP SiO$_2$ NPs.

We fit zeta data using diffusive layer model based on the Gouy-Chapman theory and obtained $K_s$ values for CeO$_2$ (pK$_{s,1}$= 5.6 and pK$_{s,2}$=8.0) and Al$_2$O$_3$ (pK$_{s,1}$= 6.8 and pK$_{s,2}$=12.1) NPs. Table 8.1 gives the equations of this model. The model fits zeta potential data as a function of pH (Figure S8.1 in the SI), with adjustable parameters including $K_{s,1}$, $K_{s,2}$ and the surface adsorption site density (SSD, nm$^{-2}$).
Table 8.1. The diffusive layer model for assessing $K_s$ values via fitting the zeta-potential data

$$K_{s,1} = \frac{[(NP)\text{OH}] [H^+]}{[(NP)\text{OH}_2^+]},$$

$$K_{s,2} = \frac{[(NP)\text{O}^-] [H^+]}{[(NP)\text{OH}]}$$

$$C_{NP} \cdot \text{SSA} \cdot \text{SSD} \cdot N_A^{-1} \cdot 10^{12} = \left[ (NP)\text{OH}_2^+ \right] + \left[ (NP)\text{OH} \right] + \left[ (NP)\text{O}^- \right]$$

$$\sigma_p = \frac{F}{C_{NP} \cdot \text{SSA} \cdot 10^{-6} \cdot \left[ (NP)\text{OH}_2^+ \right] - \left[ (NP)\text{O}^- \right] }$$

$$\sigma_p = (8RT \varepsilon_0 c \times 10^3)^{\frac{1}{2}} \sinh \left( \frac{2F\psi_0}{2RT} \right)$$

$$\psi_{zeta} = \psi_0 \cdot \exp(-\kappa \cdot x), \quad \kappa = \left( \frac{2F^2 I \times 10^3}{\varepsilon_0 RT} \right)^\frac{1}{2}$$

SSA $\equiv$ specific surface area, (m² $\cdot$ kg⁻¹); SSD $\equiv$ surface site density, (nm²); $N_A$ $\equiv$ the Avogadro number, (6.022 $\times$ 10²³ mol⁻¹); $C_{NP}$ $\equiv$ NP concentration, (mg $\cdot$ L⁻¹); $\varepsilon_0$ $\equiv$ permittivity of the vacuum (8.854 $\times$ 10⁻¹² C $\cdot$ V⁻¹ $\cdot$ m⁻¹); $\varepsilon$ $\equiv$ the dielectric constant of water, (78.5 at 25 °C); R $\equiv$ ideal gas constant, (8.314 C $\cdot$ V $\cdot$ mol⁻¹ $\cdot$ K⁻¹); T $\equiv$ temperature, (K); c $\equiv$ electrolyte concentration, (M); z $\equiv$ charge of the ion; F $\equiv$ Faraday constant, (76485 C $\cdot$ mol⁻¹); $\psi_0$ $\equiv$ potential on the surface, (V); x $\equiv$ the distance where the zeta potential is defined, (nm); $\psi_{zeta}$ $\equiv$ zeta potential, (V); I $\equiv$ the ionic strength, (M).

In the SCM, ions are adsorbed to NPs via complexing with –OH groups on the surface, with an equilibrium complexation constant defined$^{296}$. The apparent complexation constant ($K_{C,\text{app}}$) includes the intrinsic constant term ($K_{C,\text{intr}}$), which is attributed to the Gibbs energy of the sorption bond formation, and the coulomb term ($K_{C,\text{coul}}$), which is attributed to the electrostatic field near the NP surface$^{296, 297}$. The latter reflects the electrostatic energy change when an ion approaches the charged surface from the bulk solution. James and Healy$^{298}$ also considered the solvation energy, in addition to the chemical and electrostatic energy, in their adsorption model, which was used to explain
the adsorption of some metal ions to quartz surface\textsuperscript{299}. Our model does not isolate the solvation energy term but consider it within the overall Gibbs free energy of the surface complexation reaction.

Generally, an ion $M^{n+}$ complexing with NP surface –OH group is written as:

$$
(NP)OH + M^{n+} \rightleftharpoons (NP)OM^{(n-1)+} + H^+ \cdot K_{C,app} = K_{C,intr} \cdot K_{C,coul}
$$

Eq(8.3)

where, $K_{C,app}$ and $K_{C,coul}$ are calculated as:

$$
K_{C,app} = \frac{[\text{(NP)}OM^{(n-1)+}][H^+]}{[(\text{NP})OH][M^{n+}]}
$$

Eq(8.4)

$$
K_{C,coul} = \exp\left(-\frac{\Delta z F \psi_0}{RT}\right)
$$

Eq(8.5)

In Eq(8.5), $\Delta z$ is the change in NP surface charge, and all the other notations are the same as in Table 8.1.

Four components constitute the entire SCM in this study: speciation of free ions in solution (Table S1), dissociation of the NP surface –OH groups (Eq(8.1) and Eq(8.2)), complexation of ion species with –OH groups (Table 8.2), and the mass balance of adsorbate (ions) and adsorbent sites. We applied the activity coefficients derived from the Debye-Hückel approximation\textsuperscript{300,301} to concentrations of all free ions. The specific surface area (SSA) was predetermined by BET method (99.5 m$^2$/g for C-SiO$_2$, 51.0 m$^2$/g for F-SiO$_2$, 17.0 m$^2$/g for CeO$_2$ and 50.4 m$^2$/g for Al$_2$O$_3$). The model is essentially a multi-equation system, which we used to fit the experimental data (i.e., adsorption percentage of ion versus pH) and determine the adjustable $K_{C,intr}$ values.
Table 8.2. The complexation equations of III/V ions with CMP NPs.

<table>
<thead>
<tr>
<th>Reaction Equation</th>
<th>( K_{C,app} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>((\text{NP})\text{OH} + \text{In}^{3+} \rightleftharpoons (\text{NP})\text{OIn}^{2+} + \text{H}^+)</td>
<td>( \frac{[\text{(NP)}\text{OIn}^{2+}][\text{H}^+]}{[\text{(NP)}\text{OH}][\text{In}^{3+}]} )</td>
</tr>
<tr>
<td>((\text{NP})\text{OH} + \text{Ga}^{3+} \rightleftharpoons (\text{NP})\text{OGa}^{2+} + \text{H}^+)</td>
<td>( \frac{[\text{(NP)}\text{OGa}^{2+}][\text{H}^+]}{[\text{(NP)}\text{OH}][\text{Ga}^{3+}]} )</td>
</tr>
<tr>
<td>((\text{NP})\text{OH} + \text{Ga(OH)}_2^+ \rightleftharpoons (\text{NP})\text{OGaOHa} + \text{H}_2\text{O})</td>
<td>( \frac{[\text{(NP)}\text{OGaOHa}][\text{H}^+]}{[\text{(NP)}\text{OH}][\text{Ga}^{3+}]} )</td>
</tr>
<tr>
<td>((\text{NP})\text{OH} + \text{Ga(OH)}_3 \rightleftharpoons (\text{NP})\text{OGa(OH)}_2 + \text{H}_2\text{O})</td>
<td>( \frac{[\text{(NP)}\text{OGa(OH)}_2][\text{H}^+]}{[\text{(NP)}\text{OH}][\text{Ga}^{3+}]} )</td>
</tr>
<tr>
<td>((\text{NP})\text{OH} + \text{Ga(OH)}_4^- \rightleftharpoons (\text{NP})\text{OGa(OH)}_3^- + \text{H}_2\text{O})</td>
<td>( \frac{[\text{(NP)}\text{OGa(OH)}_3^-][\text{H}^+]}{[\text{(NP)}\text{OH}][\text{Ga}^{3+}]} )</td>
</tr>
<tr>
<td>((\text{NP})\text{OH} + \text{AsO}_4^{3-} \rightleftharpoons (\text{NP})\text{OHAsO}_4^{3-})</td>
<td>( \frac{[\text{(NP)}\text{OHAsO}_4^{3-}]}{[\text{(NP)}\text{OH}][\text{AsO}_4^{3-}]} )</td>
</tr>
<tr>
<td>((\text{NP})\text{OH} + \text{HAsO}_4^{2-} \rightleftharpoons (\text{NP})\text{AsO}_4^{2-} + \text{H}_2\text{O})</td>
<td>( \frac{[\text{(NP)}\text{AsO}_4^{2-}]}{[\text{(NP)}\text{OH}][\text{HAsO}_4^{2-}]} )</td>
</tr>
<tr>
<td>((\text{NP})\text{OH} + \text{H}_2\text{AsO}_4^- \rightleftharpoons (\text{NP})\text{HAsO}_4^- + \text{H}_2\text{O})</td>
<td>( \frac{[\text{(NP)}\text{HAsO}_4^-]}{[\text{(NP)}\text{OH}][\text{H}_2\text{AsO}_4^-]} )</td>
</tr>
<tr>
<td>((\text{NP})\text{OH} + \text{H}_3\text{AsO}_4 \rightleftharpoons (\text{NP})\text{H}_2\text{AsO}_4 + \text{H}_2\text{O})</td>
<td>( \frac{[\text{(NP)}\text{H}_2\text{AsO}_4]}{[\text{(NP)}\text{OH}][\text{H}_3\text{AsO}_4]} )</td>
</tr>
</tbody>
</table>

NP can be SiO₂, CeO₂ or Al₂O₃ NPs.
8.4.2. The surface complexation model taking account of NP size for colloidal SiO$_2$ NPs

In the above SCM, SSA and SSD are parameterized and experimentally predetermined. However, these two parameters depend on the particle size. Relating SSA and SSD to NP size enables the established SCM to calculate adsorption as a function of size.

For a spherical NP with known density, SSA can be calculated as:

$$\text{SSA} = \frac{A}{w_{NP}} = \frac{\pi \cdot d^2}{\rho \cdot \frac{1}{6} \pi \cdot d^3} = \frac{6}{\rho \cdot d}$$

\text{Eq}(8.6)

where $A$ is the NP surface area, $w_{NP}$ is the NP mass, $d$ is the NP diameter, and $\rho$ is the NP density. \text{Eq}(8.6) reads the SSA decreases as the NP size increases following a hyperbola trend.

To relate SSD to NP size, Iler$^{67}$ recommends a calculation method that assumes each atom on the particle surface holds one $-\text{OH}$ group. For the colloidal SiO$_2$ NPs, SSD depends NP size as:

$$\text{SSD} = 7.86 + \frac{1.33}{d^2} - \frac{5.61}{d}$$

\text{Eq}(8.7)

where SSD is in nm$^{-2}$ and $d$ is in nm. A derivation of \text{Eq}(8.7) is given in the SI.

8.5. Results and discussion

8.5.1. Adsorption occurrence screening for III/V ions and CMP NPs

Figure 8.3 shows data at representative acid, neutral and basic conditions for different ions with each CMP NP. Precipitation of In(III) ions in neutral to basic conditions limits the experiment below pH 5. Adsorption occurred for In(III) ion to CeO$_2$ and Al$_2$O$_3$ NPs;
Ga(III) ion to C-SiO₂, F-SiO₂, CeO₂ and Al₂O₃ NPs; and As(V) ion to CeO₂ and Al₂O₃ NPs. Adsorptions of In(III) or As(V) ions to C-SiO₂ or F-SiO₂ NPs were statistically insignificant in comparison to the control samples. For the rest, loss of ions by adsorption was significant comparing to the control samples and pH condition influenced ion adsorption.

**Figure 8.3.** A screening matrix chart for the adsorption of In(III), Ga(III) or As(V) ions to colloidal SiO₂ (C-SiO₂), fumed SiO₂ (F-SiO₂), CeO₂ or Al₂O₃ NPs, under acidic,
natural or basic conditions. White bars show the recoveries of ions (>95%) in control samples without any NP added, and shaded bars show the ion concentration in solution after the adsorption experiment. The difference of the shaded bar to the corresponding white bar gives the concentration of ions that are adsorbed to the NPs. The ion initial concentrations are at $1.0 \times 10^{-6}$ M for In(III), $1.5 \times 10^{-7}$ M for Ga(III) and $1.0 \times 10^{-4}$ M for As(V).

Using x-ray diffraction pattern, a previous study confirmed C-SiO$_2$ and F-SiO$_2$ NPs are both amorphous silica$.^{293}$ Amorphous silica has highly hydrated surface in aqueous matrices with a high coverage of $\text{–OH}$ groups (i.e., the silanol groups)$^{67,302}$. The $\text{–OH}$ groups are often active adsorption sites, making SiO$_2$ able to adsorb many metal ions, such as Fe$^{3+}$, Pb$^{2+}$, Cu$^{2+}$, Cd$^{2+}$ and others$^{67,303}$. In our study, C-SiO$_2$ and F-SiO$_2$ significantly adsorbed Ga(III) ions but not In(III) ions or As(V) ions. Wakatsuki et al. also observed a strong affinity of SiO$_2$ to Ga$^{3+}$ ions via specific adsorptions$^{304}$, which is attributed to a high ionic potential (the ratio of the ionic charge to the ionic radius) of Ga$^{3+}$. For a cation, a high ionic potential also correlates to a low general solubility in water. Because In(III) has a larger radius (0.81Å) than Ga(III) (0.62Å)$^{305}$, In(III) has a lower ionic potential and therefore less tendency to form covalent bonds with SiO$_2$ surface. In(III) also has a very low solubility (<$10^{-7}$ M) at pH 5-9. The ionic potential of AsO$_4^{3-}$ predicts a high tendency to be specifically adsorbed to surface like Fe(OH)$_3$$^{304}$. We attribute the low adsorption of AsO$_4^{3-}$ to SiO$_2$ NPs to the negatively charged surface (therefore repulsing anions) of SiO$_2$, whose zero point charge is low (~pH 2)$^{67}$.

CeO$_2$ and Al$_2$O$_3$ adsorb all the three ions with similar trends over acidic to basic conditions. In(III) ions are adsorbed to CeO$_2$ and Al$_2$O$_3$ NPs more effectively than SiO$_2$.
NPs but still much less than Ga(III) to the same NP absorbents, in accordance with the lower ionic potential of indium. The difference between In(III) adsorption to CeO$_2$ and Al$_2$O$_3$ from SiO$_2$ NPs indicates different bond formation energy of In(III) toward –OH groups on different NP surfaces. Because of pH dependent changes in surface charges of CeO$_2$ (pH$_{zpc}$ ~7-8)\textsuperscript{293,306} and Al$_2$O$_3$ (pH$_{zpc}$ ~10)\textsuperscript{293,306}, they effectively adsorb As(V) ions with a favorable electrostatic force approaching the NP surface. CeO$_2$ and Al$_2$O$_3$ also have higher adsorption capability to As(V) anions at lower pH conditions, in accordance with what has been observed for general anions’ adsorption based on complexation with –OH groups on a solid surface\textsuperscript{297}.

8.5.2. Surface complexation modeling

Figure 8.4 shows In(III) adsorption to CeO$_2$ or Al$_2$O$_3$ NPs below pH 5. We only consider the dominating In$^{3+}$ species in the model (Table 8.2). SCM with fitted K$_{C,intr}$ parameters draws a line that is consistent with the experimentally observed trend. The difference between experimental and modeled data for In(III) on CeO$_2$ NP below pH 3 may be due to the slight dissolution of CeO$_2$ at low pH. We detected 40 µg/L dissolved Ce in equilibrium with 100 mg/L CeO$_2$ NPs at pH 2.5. A different adsorption mechanism (e.g., a solid surface precipitation\textsuperscript{307}) other than the complexation with –OH groups may occur at low pH.
Figure 8.4. Complexation of In(III) ion to CeO$_2$ and Al$_2$O$_3$ NPs. The circle markers are experimental data and the solid line is the model fitting. $C_{0,\text{In(III)}}$ is $1.0 \times 10^{-6}$ M. NP concentration is 100 mg/L as Ce or Al.

Figure 8.5 shows the experimental and modeled data for Ga(III) adsorption as a function of pH for all four CMP NPs. In all cases, Ga(III) adsorption increases with pH at the lower range, reaches a plateau across the neutral range, and decreases at the higher range. However, the pH where the adsorption starts decreasing varies among different NPs: ~ pH 9 for both SiO$_2$, ~ pH 11 for CeO$_2$, and ~ pH 7 for Al$_2$O$_3$. The similar adsorption profiles for C-SiO$_2$ and F-SiO$_2$ suggest similar surface adsorption sites, despite different synthesis approaches; C-SiO$_2$ is synthesized through sol-gel process in a solution, and F-SiO$_2$ is synthesized through pyrogenic approaches in gas phase under high temperature$^{67}$. 
Figure 8.5. Complexation of Ga(III) to C-SiO$_2$, F-SiO$_2$, CeO$_2$ and Al$_2$O$_3$ NPs. The circle markers are experimental data, and the solid line is the model fitting. $C_{0,\text{Ga(III)}}$ is $1.5 \times 10^{-7}$ M. NP concentration is 100 mg/L as Si, Ce or Al.

Figure 8.6 shows the experimental and modeled data for surface complexation of As(V) ions to CeO$_2$ and Al$_2$O$_3$ NPs. For both NPs, As(V) shows a relatively stable adsorption in the acidic pH range and a decreasing trend around the neutral pH range. This agrees with the behavior of anions in a surface complexation model, in which the protons drive the complexation reaction to the right (see As(V) equations in Table 8.2). The model fitting curve captures the experimental data trend. Some deviation is observed
at high pH for Al$_2$O$_3$ NPs, and we suspect it can be attributed to a different adsorption mechanism related to the significant dissolution of Al$_2$O$_3$ in basic conditions (e.g., more than 1mg•L$^{-1}$ dissolved Al was detected at pH >10 in a solution containing 100 mg/L Al$_2$O$_3$ NPs).

Figure 8.6. Complexation of As(V) to CeO$_2$ and Al$_2$O$_3$ NPs. The circle markers are experimental data, and the solid line is the model fitting. C$_{0,\text{As(V)}}$ is 1.1×10$^{-4}$ M. NP concentration is 500 mg/L as Ce or Al.

The fitted K$_{C,\text{intr}}$ between different III/V ions and CMP NPs are reported in Table 8.3 as LogK$_{C,\text{intr}}$. K$_{C,\text{intr}}$ separates the Gibbs energy of complexing bond formation$^{297}$ from the electrostatic force attraction and therefore represents the potential of “chemsorption”. A high K$_{C,\text{intr}}$ (or LogK$_{C,\text{intr}}$) indicates a higher potential of the ion to be chemisorbed by the surface.
Table 8.3. The logarithm of Intrinsic surface complexation constants determined by model fitting for different III/V ions and CMP NPs.

<table>
<thead>
<tr>
<th>Log (K_{C,intr})</th>
<th>Colloidal SiO₂</th>
<th>Fumed SiO₂</th>
<th>CeO₂</th>
<th>Al₂O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>In³⁺</td>
<td>NA</td>
<td>NA</td>
<td>8.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Ga³⁺</td>
<td>-5.0</td>
<td>-5.4</td>
<td>5.1</td>
<td>12.1</td>
</tr>
<tr>
<td>Ga(OH)₂⁺</td>
<td>-15.0</td>
<td>-15.0</td>
<td>-0.6</td>
<td>12.2</td>
</tr>
<tr>
<td>Ga(OH)₃</td>
<td>5.5</td>
<td>6.7</td>
<td>-1.0</td>
<td>5.8</td>
</tr>
<tr>
<td>Ga(OH)₄⁻</td>
<td>10.2</td>
<td>10.5</td>
<td>11.5</td>
<td>-1.4</td>
</tr>
<tr>
<td>AsO₄³⁻</td>
<td>NA</td>
<td>NA</td>
<td>12.8</td>
<td>-10.0</td>
</tr>
<tr>
<td>HAsO₄²⁻</td>
<td>NA</td>
<td>NA</td>
<td>8.2</td>
<td>-21.6</td>
</tr>
<tr>
<td>H₂AsO₄⁻</td>
<td>NA</td>
<td>NA</td>
<td>-3.4</td>
<td>0.6</td>
</tr>
<tr>
<td>H₃AsO₄</td>
<td>NA</td>
<td>NA</td>
<td>12.4</td>
<td>8.2</td>
</tr>
</tbody>
</table>

C-SiO₂ and F-SiO₂ have very similar LogK_{C,intr} values toward all ions, indicating they have very similar adsorption sites on their surface. Despite a negatively charged surface of SiO₂ NPs over a wide pH range >2, they have high Log(K_{C,intr}) values toward Ga(OH)₃ and Ga(OH)₄⁻, which start dominating the Ga(III) speciation from pH 4, making them a good adsorbent for Ga(III) in about pH 4-10. At pH >10, the rejection of Ga(III) is due to the strong negative charge on the SiO₂ surface, where the coulomb term dominates over K_{C,intr}. Without distinguishing the ion speciation driven by pH, Wakatsuki et al. estimated the chemsorption stability constant (which is defined as the negative logarithm constant of the reverse reaction of Eq(8.3) and therefore is in line with the LogK_{C,intr} in...
this study) for SiO$_2$ and Ga(III) to be 5 at pH 4$^{304}$. In our study, Ga(OH)$_3$ present at pH 4 had Log$K_{c, intr}$ equal to 5.5 for C-SiO$_2$ and 6.7 for F-SiO$_2$, which agrees well with Wakatsuki’s estimation.

CeO$_2$ has high $K_{c, intr}$ to all three ions. Few studies exist on the adsorption capability of CeO$_2$ for In(III) and Ga(III), and this study fills that knowledge gap. Feng et al.$^{308}$ studied the adsorption of arsenate to CeO$_2$ NPs and reported the adsorption efficiency as a function of pH conditions similar to the trend observed in Figure 8.6. However, $K_{c, intr}$ values are not reported in their study while they are estimated in ours.

Al$_2$O$_3$ has high Log$K_{c, intr}$ with In$^{3+}$ in In(III), Ga$^{3+}$, Ga(OH)$_2^+$, Ga(OH)$_3$ in Ga(III), and H$_3$AsO$_4$ in As(V). Lin et al.$^{289}$ examined the adsorption of In(III) and Ga(III) to $\gamma$-Al$_2$O$_3$ and applied a triple-layer model. They reported the monodendate Log$K_{c, intr}$ of $\gamma$-Al$_2$O$_3$ to In$^{3+}$ and Ga$^{3+}$ are 8.87 and 10.3, respectively, whereas our model gives 13.0 and 12.1 for CMP Al$_2$O$_3$ NP to the two species. Both studies confirm the Al$_2$O$_3$ surface has a strong affinity to In(III) and Ga(III) ions. The difference can be attributed to the discrepancy of our model from their model, in which they added the bidendate complexation mechanism (whose Log$K_{c, intr}$ are 1.63 for In$^{3+}$ and 3.15 for Ga$^{3+}$), lowering the monodendate complexation constant. Plus, the CMP Al$_2$O$_3$ NP is not exactly the same as $\gamma$-Al$_2$O$_3$ from a comparison of their XRD patterns$^{293,309}$. The CMP Al$_2$O$_3$ does not show diffraction pattern when $2\theta$ is greater than 30, despite shifted diffractions of (111) and (220) surfaces (part of the $\gamma$-Al$_2$O$_3$ pattern)$^{293}$. Therefore, it is very likely the CMP Al$_2$O$_3$ NP is in a transition from $\gamma$-Al$_2$O$_3$ to the amorphous, resulting in different adsorption sites on the surface from $\gamma$-Al$_2$O$_3$. Goldberg and Johnston evaluated $K_{c, intr}$ for As(V) with amorphous Al$_2$O$_3$ and reported 9.39 for H$_3$AsO$_4$ and 6.35 for H$_2$AsO$_3^-$ (the
value was modified to account for different formulation of their complexation equation from ours), whereas our values are 8.2 and 0.6, respectively. The two studies agree that the As(V) species has stronger Al₂O₃ affinity, with the corresponding LogK_{C,intr} values larger than zero. Higher values of K_{C,intr} in their studies are possibly due to their smaller SSD (2.31 nm²) assumption than 19.6 nm² in our work. Also, the amorphous Al₂O₃ can be different from the CMP Al₂O₃ on the phase and surface.

8.5.3. The particle size effect on ion adsorption predicted by surface complexation modeling

Eq(8.6) and Eq(8.7) calculate SSA and SSD as a function of particle size for C-SiO₂ NPs and are plotted in Figure 8.7. SSA and SSD are intensive variables for a material and are independent of mass or number of particles in solution for a given size. As size grows, SSD increases and SSA decreases; the variance of both becomes dramatic below ~20 nm. Practically, NPs are quantified according to mass (i.e., mass concentration for a solution). For a given NP mass concentration, the mass site density (MSD, number of site per mass), which is the product of SSA (area per mass) and SSD (number of site per area), determines the adsorption capacity. As shown in Figure 8.8, MSD approaches zero with large particles and increases as particle size decreases. As size approaches the nano-range (i.e., <100 nm), the curve’s steeper tangent line indicates faster MSD increase and greater sensitivity to size. To quantify this, we define n as the MSD increase percentage per 1-nm size reduction as shown in Eq(8.8).

\[ n = \frac{\text{MSD}(d-1) - \text{MSD}(d)}{\text{MSD}(d)} \]
The NP size is divided into three regions arbitrarily using boundaries of \( n=1\% \) and \( n=10\% \). Consequently, the increase of MSD with size reduction is negligible for \( d > 100 \) nm \( (n<1\%) \), moderate for \( 10 \) nm \( < d < 100 \) nm \( (1\%<n<10\%) \) and dramatic for \( d < 10 \) nm \( (n>10\%) \).

**Figure 8.7.** Dependence of SSD (Left y-axis) and SSA (right y-axis) on size for C-SiO\(_2\) NPs, calculated by Eq\((8.6)\) and Eq\((8.7)\). The inset plot gives a wider size range covering \( >100 \) nm levels.
Figure 8.8. Mass site density (MSD) dependence on NP size for C-SiO$_2$ NPs. Three regions are identified. MSD increase caused by NP size reduction is dramatic (n>10%) in Region I, moderate (1%<n<10%) in Region II, and negligible (n<1%) in Region III.

Taking Eq(8.6) and Eq(8.7) into the SCM enables the model to give the adsorption efficiency as functions of pH and size for a fixed mass concentration of NPs. Figure 8.9 shows the result for C-SiO$_2$ NPs adsorbing Ga(III) ions in a color contour map over the axes of pH and NP size. At a specific NP size, the adsorption efficiency depends on pH with the same trend as Figure 8.5 (the C-SiO$_2$ panel). As NP size decreases, the high-adsorption plateau broadens. Smaller size C-SiO$_2$ NPs have higher adsorption capacity over a broader pH range than larger ones. Influence of size on adsorption efficiency is significant only in approximately pH ranges of 2.5-3 and 8.5-11. For example, at pH 10, 100 nm C-SiO$_2$ NPs give ~10% Ga(III) adsorption efficiency, whereas 10 nm C-SiO$_2$ NPs give ~60%. The influence of size on the adsorption efficiency is related to not only the MSD but also to the surface affinity to the ionic species at a pH condition. Too high
or too low affinity to the existing ionic species trivializes the effect of MSD and the size effect. As the NP size gets larger (e.g., >100 nm), adsorption is constant for all pH, reaching the property of the corresponding bulk material.

![Figure 8.9](image.png)

**Figure 8.9.** A contour plot of the adsorption efficiency of Ga(III) ($C_0=1.5\times10^{-6}$M) to C-SiO$_2$ NPs (100 mg-Si/L) over the axes of pH and NP diameter. The color variance reflects the magnitude of adsorption efficiency, shown in the colorbar on the right. Dark red indicates adsorption greater than 90%, and dark blue indicates adsorption less than 10%.

SCM assumes the adsorption happens only through complexing with surface groups and therefore is applicable to monolayer chemsorption. The extension to NP size effect is for spherical particles whose adsorption sites are atoms on the outmost surface layer. Some existing studies reported results consistent to what is predicted by our model. Madden et al.$^{310}$ found the adsorption of Cu$^{2+}$ ions to smaller hematite NPs (7 nm) is notably higher than to larger NPs (25 or 88 nm), however only at pH 5-7, agreeing with what is predicted by our model. Engates and Shipley$^{311}$ investigated the adsorption of Pb, Cd, Cu, Zn and Ni ions to TiO$_2$ NPs and compared the bulk- and nano-TiO$_2$. They found
the nano-TiO$_2$ adsorbed more than the bulk-TiO$_2$ based on the same mass but the opposite based on the same surface area. This is also observed in the work by Gao et al.$^{312}$, who found that with the same surface area concentration of ~100 m$^2$\cdot$L^{-1}$, 145-nm TiO$_2$ shows higher adsorption efficiency to Cd(II) ions than 39-nm TiO$_2$. MSD provides a good indication of adsorption capacity for a fixed mass concentration, whereas SSD indicates adsorption capacity for a fixed area concentration. Our model predicts that as size gets small, MSD increases but SSD decreases, thereby predicting what is observed in their experiments. Overall, this model demonstrates that the adsorption capability of NPs depends particle size in the “nano” range and is also influence by both surface area and site density.

### 8.6. Conclusions

This work shows that industrial NPs used in CMP slurries influence the fate of typical III/V ions in CMP wastewater via adsorption. Results demonstrate that SiO$_2$ NPs significantly adsorb Ga(III) ion but not In(III) and As(V) ions. CeO$_2$ and Al$_2$O$_3$ NPs significantly adsorb In(III), Ga(III) and As(V) ions. The adsorption increases with pH for cations (i.e., In(III) and Ga(III)) and decreases for the anion (i.e., As(V)). The SCM was applicable to the adsorption of III/V ions to CMP NPs, with $K_{C,intr}$ determined via fitting the experimental data. Adsorption efficiency depended on pH, which can be explained by the different $K_{C,intr}$ of different hydrolyzed species of one type of ion to a CMP NP. SCM is useful to predict the adsorption occurrence in other conditions. When NP size reduces, SSA increases but SSD decreases. However, for a fixed mass, NPs have higher adsorption capacity than the bulk material because of the increase of MSD with size reduction. For C-SiO$_2$ NPs, the MSD increase is most significant for sizes smaller than
~10 nm, insignificant for size larger than ~100 nm and moderate between 10 and 100 nm. The size effect on the adsorption efficiency predicted by the extended SCM, however, is significant only in specific pH range (e.g., 2.5-3 and 8.5-11 for C-SiO$_2$ NPs). This is because the adsorption is dominated by not only available sites but also the site affinity to the adsorbate ion (reflected by $K_{C,int}$).

8.7. Acknowledgements

This work is funded by the Semiconductor Research Corporation (SRC, Task # 425.052), the National Science Foundation (CBET #1507750, #1507446, and EEC#1449500) and the USEPA (RD835580). We thank Dr. Shyam Aravamudhan from North Carolina A&T State University for providing the measurement of specific surface area of the four CMP NPs.

8.8. Supporting Information

![Zeta-potential of CeO$_2$ and Al$_2$O$_3$ NPs as a function of pH.](image_url)

**Figure S8.1.** Zeta-potential of CeO$_2$ and Al$_2$O$_3$ NPs as a function of pH. The markers are the experimental measurement, and the solid line is the diffusive layer model fit (Table 1). The model fit parameterizes the pK$_s$ values of surface –OH groups and the surface site density (SSD), which are given with the charts.
Table S8.1. Hydrolysis of In(III), Ga(III) and As(V) ions and the equilibrium constants.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Equilibrium Constant (logK)</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAsO$_4^{2-}$ $\rightleftharpoons$ H$^+$ + AsO$_4^{3-}$</td>
<td>log($K_{A}^A$)</td>
<td>-11.6</td>
<td>Drever, 1997$^{288}$</td>
</tr>
<tr>
<td>H$_2$AsO$_4^-$ $\rightleftharpoons$ 2H$^+$ + AsO$_4^{3-}$</td>
<td>log($K_{A}^A$)</td>
<td>-18.36</td>
<td>Drever, 1997$^{288}$</td>
</tr>
<tr>
<td>H$_3$AsO$_4$ $\rightleftharpoons$ 3H$^+$ + AsO$_4^{3-}$</td>
<td>log($K_{A}^A$)</td>
<td>-20.6</td>
<td>Drever, 1997$^{288}$</td>
</tr>
<tr>
<td>In$^{3+}$ + H$_2$O $\rightleftharpoons$ In(OH)$^{2+}$ + H$^+$</td>
<td>log($K_{1}^In$)</td>
<td>-3.48</td>
<td>Biryuk et al., 1986$^{313}$</td>
</tr>
<tr>
<td>In$^{3+}$ + 2H$_2$O $\rightleftharpoons$ In(OH)$_2$$^+ + 2$H$^+$</td>
<td>log($K_{2}^In$)</td>
<td>-7.67</td>
<td>Biryuk et al., 1986$^{313}$</td>
</tr>
<tr>
<td>In$^{3+}$ + 3H$_2$O $\rightleftharpoons$ In(OH)$_3$$^+ + 3$H$^+$</td>
<td>log($K_{3}^In$)</td>
<td>-12.75</td>
<td>Biryuk et al., 1986$^{313}$</td>
</tr>
<tr>
<td>Ga$^{3+}$ + H$_2$O $\rightleftharpoons$ Ga(OH)$^{2+}$ + H$^+$</td>
<td>log($K_{1}^Ga$)</td>
<td>-2.87</td>
<td>Biryuk and Nazarenko, 1973$^{314}$</td>
</tr>
<tr>
<td>Ga$^{3+}$ + 2H$_2$O $\rightleftharpoons$ Ga(OH)$_2$$^+ + 2$H$^+$</td>
<td>log($K_{2}^Ga$)</td>
<td>-6.55</td>
<td>Biryuk and Nazarenko, 1973$^{314}$</td>
</tr>
<tr>
<td>Ga$^{3+}$ + 3H$_2$O $\rightleftharpoons$ Ga(OH)$_3$$^+ + 3$H$^+$</td>
<td>log($K_{3}^Ga$)</td>
<td>-11.07</td>
<td>Biryuk and Nazarenko, 1973$^{314}$</td>
</tr>
<tr>
<td>Ga$^{3+}$ + 4H$_2$O $\rightleftharpoons$ Ga(OH)$_4$$^-$ + 4H$^+$</td>
<td>log($K_{4}^Ga$)</td>
<td>-17.3</td>
<td>Vagramjan and Leshawa, 1967$^{315}$</td>
</tr>
</tbody>
</table>

Relate the surface site density (SSD) to the NP size for colloidal SiO$_2$ NPs

For a spherical colloidal SiO$_2$ NP, we denote p as the number of Si atoms across the diameter of the sphere, in the unit of nm$^{-1}$. The total number of Si atoms in this sphere is:
where, $N_t$ is the total number of Si in the sphere.

The weight of this SiO$_2$ NP is calculated as:

$$w_{NP} = \frac{N_t \cdot MW}{N_A} = \frac{\pi \cdot p^3 \cdot MW}{6N_A}$$  \hspace{1cm} \text{Eq(S2)}$$

where, $w_{NP}$ is the weight of the SiO$_2$ NP and MW is the molecular weight of SiO$_2$ (28.1 g·mol$^{-1}$).

$w_{NP}$ is also calculated as:

$$w_{NP} = \rho \cdot \frac{1}{6} \pi \cdot d^3$$  \hspace{1cm} \text{Eq(S3)}$$

where $\rho$ is the density of colloidal SiO$_2$ NPs and $d$ is the particle diameter.

Eq(S2) and Eq(S3) give:

$$p = \left( \frac{\rho \cdot N_A}{MW} \right)^\frac{1}{3} \cdot d$$  \hspace{1cm} \text{Eq(S4)}$$

For colloidal SiO$_2$ NPs, $\rho$ is 2.2 g·cm$^{-3}$ and MW is 28.1 g·mol$^{-1}$. Substituting these values into Eq(S4) gives:

$$p = 2.80d$$  \hspace{1cm} \text{Eq(S5)}$$

where $d$ is in nm.

Based on Eq(S1), the number of Si atom on the sphere surface ($N_s$) is calculated as:

$$N_s = \frac{1}{6} \pi \left(p^3 - (p - 2)^3 \right)$$  \hspace{1cm} \text{Eq(S6)}$$

Given the assumption that each Si atom on the surface carries one –OH group, SSD is calculated as:
SSD = \frac{N_s}{\pi \cdot d^2} \quad \text{Eq}(S7)

Substituting \text{Eq}(S5) \text{ and } \text{Eq}(S6) \text{ into } \text{Eq}(S7) \text{ gives:}

\begin{align*}
\text{SSD} &= 7.86 + \frac{1.33}{d^2} - \frac{5.61}{d} \\
\text{Eq}(S8)
\end{align*}

where \(d\) is in nm.
CHAPTER 9

ADSORPTION OF AS\textsuperscript{III} AND AS\textsuperscript{V} IONS ONTO CeO\textsubscript{2} NANO PARTICLES: IMPLICATIONS TO SURFACE REDOX STATE AND REACTIVITY

9.1. Abstract

Cerium oxide (CeO\textsubscript{2}) nanoparticles (NPs) may coexist with arsenic (As) ions in chemical mechanical polishing (CMP) wastewater at semiconductor manufacturing sites, creating circumstances in which As ions are adsorbed onto CeO\textsubscript{2} NPs. I found that both As\textsuperscript{III} and As\textsuperscript{V} ions were adsorbed onto CeO\textsubscript{2} NPs, but adsorption isotherms showed different adsorption energies and capacities between the two species. Ferric Reducing Ability for Nanoparticles (FRAN) assay analysis suggested that the adsorbed As\textsuperscript{III} and As\textsuperscript{V} ions mitigated the reducing ability of the CeO\textsubscript{2} NP surface. Kinetic modeling of the assay reaction further implied the electron transfer scheme varied as As ions covered the surface. The X-ray Photoelectron Spectroscopy (XPS) provided evidence that the adsorbed As\textsuperscript{III} ions filled the Ce 4f orbitals, caused the conversion of Ce\textsuperscript{IV} to Ce\textsuperscript{III} on the NP surface, and reduced the electron mobility in the NP solid phase, yet the adsorbed As\textsuperscript{V} did not show this effect. Electron Paramagnetic Resonance (EPR) analysis confirmed the formation of hydroxyl radicals (HO) when CeO\textsubscript{2} NPs react with H\textsubscript{2}O\textsubscript{2}. The adsorption of As\textsuperscript{III} and As\textsuperscript{V} ions mitigated CeO\textsubscript{2} NPs’ reactivity to induce HO in general. The reaction between CeO\textsubscript{2} NPs and H\textsubscript{2}O\textsubscript{2} was not fully justified by a regular Fenton/Haber-Weiss mechanism and the surface adsorption of As ions added more complexity to the reaction. As the surface redox state and reactivity are critical to CeO\textsubscript{2} NPs’ toxicity and environmental risks, this study provided methodologies to assess them and unraveled how they were influenced by As ion adsorption.

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9.2. Introduction

Engineered nanoparticles (NPs) that are derived from novel technologies or commercial products were concerned due to their potential environmental health and safety risks. Among many other engineered NPs, Cerium Oxide (CeO$_2$) is side used in the semiconductor polishing slurry products, fuel catalysis technologies, and UV-coatings or paints$^{11}$. The production amount of CeO$_2$ NPs by big companies is estimated to be tons per year in the US$^{316}$, and the total production can be as high as hundreds tons per year worldwide$^{11}$. The tremendous consumption of CeO$_2$ NPs implies high likelihood of their presence in water systems of the environment.

One of the potential risks of CeO$_2$ NPs is their ability to induce reactive oxygen species (ROS) when they are exposed to organisms$^{18}$ or cells$^{317}$, but there are also evidence that they can scavenge ROS and protected cells against oxidative stress$^{318-320}$. In either case, the potential of CeO$_2$ to participate ROS-involved reactions can be characterized generally by surface reactivity. Most studies used as-synthesized pristine CeO$_2$ NPs to study surface reactivity or toxicity$^{18,321-323}$, but little has been investigated on how the surface reactivity varies after the surface is transformed in changed environment throughout the material’s lifetime. For example, when CeO$_2$ NPs are used in the chemical mechanical polishing (CMP) process for wafer manufacturing, they interact with a semiconductor material (i.e., a wafer substrate) on the polishing pad in both mechanical and chemical means, and end in the waste stream along with the polishing debris (in solid or ion species). Depending on the semiconductor materials being polished, a variety of chemicals may exist in CMP wastewater, and a concerning one is ionic arsenic (As) derived from the modern III/V group semiconductor materials.
(e.g., gallium arsenide (GaAs) and indium phosphide (InP)). Adsorption of ionic As species onto NPs in CMP wastewater is anticipated. I previously demonstrated the significant adsorption capacity of CeO₂ NPs toward In^{III}, Ga^{III}, and As^{V} ions in water, and estimated the intrinsic adsorption constants for all ionic species using surface complexation models^{324}. The adsorption of redox active ions, such as As^{III} and As^{V}, likely influences the original surface reactivity of CeO₂ NPs, yet few studies have elucidated this effect.

This potential surface reactivity change caused by adsorption of redox active ions is related to the distinctive electronic structure of CeO₂ material. Computational^{325, 326} and experimental studies^{327, 328} both proved that between CeO₂’s wide band gap (~6 eV), which is between the O 2p and Ce 5d states, are localized Ce 4f states. The occupation of 4f states occurs when Ce^{IV} is reduced to Ce^{III} and simultaneously an oxygen vacancy forms, leading to the known oxygen buffering ability of cerium oxide material^{329}. This redox state swinging of CeO₂ plays a critical role in reaction involving ROS^{318, 323}. Adsorption of redox active As^{III/V} ions onto CeO₂ NPs creates chances that the ion’s orbital forms resonance with CeO₂’s electronic band, forming broadened adsorbate electronic states. Depending on the position and broadness of them, the adsorbate electronic states may overlap with CeO₂ 4f states, causing the reduction of Ce^{IV} to Ce^{III} and thus changing the surface redox reactivity. They may also serve as intermediate states mediating electron transfer on CeO₂ surface. Singh et al. showed that adsorption of phosphate shifted the redox state and the catalytic property of CeO₂ NPs^{330}. Considering remarkable adsorption of As ions onto CeO₂ NPs can happen in industrial wastewater, it
is critical to examine whether the adsorbed As^{III/V} ions affects the surface redox states and the reactivity of CeO$_2$ NPs.

In this work, I investigated the adsorption of As^{III} and As^{V} ions onto CeO$_2$ NPs used in a CMP slurry product, and the related impact on surface redox state and reactivity, by experiment and modeling. Adsorption isotherms of two As ions were examined under an acidic pH condition that is favorable to coulomb interaction. Two analyses, including the ferric reducing ability of NP (FRAN) and electron paramagnetic resonance spectroscopy (EPR) facilitated with spin trap (i.e., DMPO), were used to assess the surface reactivity of CeO$_2$ NPs with and without the adsorption of As ions. Kinetic modeling was performed on FRAN analysis to elucidate surface electron transfer schemes. X-ray photoelectron spectroscopy (XPS) was used to examine the variance of electronic structure and redox states of the CeO$_2$ NP surface caused by arsenic ion adsorption. All approaches jointly demonstrated the surface redox state and reactivity of CeO$_2$ NPs were changed by adsorption of As^{III} or As^{V} ions, however, in different mechanisms.

9.3. Experimental Methods

9.3.1. Chemicals

The CeO$_2$ NP material used in this work was adopted from a CMP slurry product. The slurry contains 1% of CeO$_2$ by weight, i.e., 10g/L. Information and characterization data of this CeO$_2$ NP sample in detail were published in a previous study$^{293}$. As^{III} and As^{V} ions were prepared from sodium (meta)arsenite (NaAsO$_2$, ≥99%, Sigma Aldrich) and sodium arsenate dibasic 7-hydrate (Na$_2$HAsO$_4$·7H$_2$O, A.C.S. Reagent, J.T. Baker), respectively. Ultra pure hydrochloride acid (HCl, ~30%, Ultrex II, J.T. Baker), trace-
metals-based sodium hydroxide (NaOH, >99.99%, Sigma Aldrich), and sodium chloride (NaCl, >99.5%, Sigma Aldrich) were used to adjust solution pH and ionic strength as needed. Ferric Chloride (FeCl₃) solution was prepared from iron(III) chloride hexahydrate (FeCl₃•6H₂O, ≥98%, Sigma-Aldrich, F2877). 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) was purchased from Sigma Aldrich (93285). Acetate buffer (pH 3.6) was prepared from acetic acid (Fisher Brand, BP2401C-212) and sodium acetate trihydrate (ACS reagent, ≥99%, Sigma Aldrich, 236500). Ultra pure water (18.2 MΩ-cm, Barnstead GenPure xCAD Plus) was used to make solutions.

9.3.2. Adsorption Experiments

The CeO₂ NP stock solution (10 g/L) was diluted to 5 by mass of CeO₂. The ionic strength of the solution was fixed by 0.1 M NaCl. pH was adjusted to 3.6 using HCl or NaOH. 50 mL of the prepared CeO₂ NP solution was used for an adsorption test at one condition. Each tested solution was stored a 125 mL wide mouth polyethylene bottle (Nalgene, Thermo Scientific), and a desired amount of As³⁺ or As⁵⁺ ions was added (with a ≥100 dilution factor to minimize the total volume change) to initiate the adsorption. Sealed bottles were shaken (Eberbach, Model 6010) for 72 hours to reach adsorption equilibrium. 9 mL of each sample was transferred to a centrifugal ultrafiltration device (NMWL=30K Da, EMD Millipore) to separate remaining ions in the solution from NPs with a centrifuge (Sorvall, Legend X1, ThermoFisher). The permeate was acidified and analyzed for the total concentration of As using ICP-MS (Thermo X-series II). 40 mL of each remained sample was centrifuged with swinging buckets (Thermo Scientific, IEC MULTI) to collect the precipitated NP powder. The collected NP powder was re-suspended in ultrapure water and centrifuged repeatedly for three times to wash away As
ion residues. The final collected CeO$_2$ NPs powder samples were further dried under 80 °C (Thermo Scientific, Heratherm OGS180), and then ready for the following experiments and analyses.

9.3.3. Ferric Reducing Ability of NPs (FRAN) analysis

A part of each collected CeO$_2$ powder sample was suspended into ultrapure water, mixed, and sonicated for 10 min to make a solution containing 10 CeO$_2$. Thus solutions of CeO$_2$ NPs covered different amount of As ions were obtained and used for FRAN (details in Chapter 6) analysis to assess their reducing activity. A FRAN assay solution was prepared by mixing 2.5 mL TPTZ solution (10 mM in a 40 mM HCl solution), 2.5 mL FeCl$_3$ solution (20 mM in water), and 25 mL acetate buffer (0.3 M in water, pH=3.6) in a polystyrene centrifuge tube. For operation, 2.5 mL the as-prepared FRAN solution was added into a 1-cm cuvette (perfector scientific, #9012), which was then located in a portable UV-VIS absorbance spectrometer (Ocean Optics, USB-ISS-UV/VIS and USB4000 light source). The analysis reaction was initiated by adding the CeO$_2$ NP stock solution into the cuvette to reach 100 of CeO$_2$. The spectrometer was operated in a time-resolved reading mode and acquired absorbance at 593 nm (A$_{593}$), where is the absorbance peak for Fe$^{II}$ product, every 0.5 s. The recording was stopped when the increase of A$_{593}$ appeared to reach a plateau. However, for CeO$_2$ covered with As$^V$ ions, the plateau was not well-defined (see details in the discussion). The obtained kinetics data, i.e., A$_{593}$ versus time, was exported for further data analysis.
9.3.4. X-ray Photoelectron Spectroscopy (XPS) analysis

XPS was performed on Vacuum Generator (220i-XL) with a base pressure of $5 \times 10^{-10}$ Torr. The X-ray source is monochromatic Al K-α with a power setting of 63 W. The beam spot size was 400 µm. CeO$_2$ NPs were pressed into clean and high purity indium foil and then loaded to the vacuum for analysis. The recorded data was exported and analyzed using CasaXPS (Version 2.3.17PR1.1).

9.3.5. Detection of hydroxyl radicals (HO) by Electron Paramagnetic Resonance (EPR) Spectroscopy analysis

Detection of HO generated by CeO$_2$ NPs in the presence of H$_2$O$_2$ was performed by EPR using a spin-trapping method. DMPO was used as a spin-trapping agent. A desired amount of a CeO$_2$ NP sample was mixed in a solution (with total volume 0.5 mL) containing 30 mM acetate buffer (pH 3.6), 0.1 mM DETAPAC, 1 mM H$_2$O$_2$, and 50 mM DMPO, and incubated in an eppendorf test tube (Eppendorf™ 022363221) located in an iced bath. After 5 min, 20 µL solution was transferred to a 2 mm Thin Wall Precision Quartz EPR Sample Tube (Wilmad-LabGlass 704-PQ-100M) and analyzed by EPR (Bruker ELEXSYS E580 CW X-band, Bruker, Rheinstetten, Germany). Continuous wave (CW) EPR spectra were recorded at room temperature. A cylindrical mode resonator (ER 4103TM) was used. The magnetic field modulation frequency was 100 kHz with a field modulation amplitude of 0.1 mT peak-to-peak. The microwave power was 4 mW, the microwave frequency was 9.74 GHz, and the sweep time was 84 seconds. Each spectrum was the average of 4 scans. The as-recorded EPR spectra were double integrated and calibrated by TEMPO standard to quantify the concentration of HO.
9.3.6. Dynamic Light Scattering (DLS) analysis.

DLS was performed on a Brookhaven ZetaPals Analyzer (Brookhaven Instruments, Holtsville, USA) to characterize the hydrodynamic sizes of CeO$_2$ NPs in solutions. The applied incident light has a wavelength of 659 nm and the scattering angle was set at 90°. The light scattering signal was fitted with parameters of “effective hydrodynamic diameter” and “polydispersity”, both of which were compared across different samples to ensure the NP size consistence after As ion adsorptions.

9.4. Results and Discussion

9.4.1. Adsorption of As$^{III}$ and As$^{V}$ ions onto CeO$_2$ NPs

I performed the adsorption test under acidic condition (pH=3.6), because not only low pH favors the columbic interaction of As anions to CeO$_2$ surfaces$^{324}$, but also many CMP slurries are acidic by design$^{293}$. The adsorption isotherms, i.e., the concentration of As adsorbed onto CeO$_2$ NPs (in) versus the concentration of As in the solution (C$_{As}$, in), for both As$^{III}$ and As$^{V}$ ions is given in Figure 9.1. Both As ions were adsorbed onto CeO$_2$ NPs but had different isotherm characters. $q_{As^{III}}^{CeO_2}$ steeply increased in a narrow range of C$_{As}$ (0-0.5) and then plateaued when C$_{As}$ is greater than ~2. $q_{As^{V}}^{CeO_2}$ also showed a sharp increase at lower C$_{As}$ (0-0.5 mmol/L), but continued to slowly increase with C$_{As}$ instead of showing an apparent plateau. Assuming the surface adsorption energy is homogeneous on the CeO$_2$ surface for both As ions, I fitted the two sets of data with a Langmuir Isotherm equation:

$$q_{As^{III}}^{CeO_2} = \frac{Q_M \cdot b \cdot C_{As}}{1 + b \cdot C_{As}}; \quad q_{As^{V}}^{CeO_2} = \frac{Q_M \cdot b \cdot C_{As}}{1 + b \cdot C_{As}}, \quad \text{(9.1)}$$
where $Q_M$ and $b$ are fitting parameters.

**Figure 9.1.** Adsorption isotherms for As$^{\text{III}}$ and As$^{\text{V}}$ ions being adsorbed onto CeO$_2$ NPs. All adsorption experiments were performed at pH 3.6. The initial concentration of CeO$_2$ NPs was 5 for all cases.

Figure 9.1 depicted the fitting line (dashed) and the fitting parameters are given in Table 9.1. In the Langmuir equation used here (eq 9.1), $Q_M$ (in ) suggests the maximum adsorption capacitance, and $b$ (in ) is a form of adsorption equilibrium constant and proportional to the exponential of the heat of adsorption ($\Delta H_a$):

$$
   b \propto \exp\left(-\frac{\Delta H_a}{RT}\right).
$$

(9.2)

Values in Table 9.1 suggest that the CeO$_2$ NPs have a larger maximum adsorption capacitance, i.e., more adsorption sites, to As$^{\text{III}}$ ions than As$^{\text{V}}$ ions, but the adsorption of As$^{\text{V}}$ ions is more thermodynamically favorable (i.e., more negative $\Delta H_a$) than that of As$^{\text{III}}$
ions. This indicates that the CeO$_2$-As$^V$ adsorbate state on the surface has a lower energy than the CeO$_2$-As(III).

**Table 9.1.** Fitted parameter values by the Langmuir Isotherm Equation (eq(9.1)).

<table>
<thead>
<tr>
<th>Adsorbate Ion</th>
<th>$Q_M$ (mmol/kg)</th>
<th>Standard Error of $Q_M$</th>
<th>$b$ (L/mmol)</th>
<th>Standard Error of $b$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>As(III)</td>
<td>451.6</td>
<td>43.3</td>
<td>0.467</td>
<td>0.123</td>
<td>0.954</td>
</tr>
<tr>
<td>As(V)</td>
<td>119.0</td>
<td>5.7</td>
<td>51.4</td>
<td>35.7</td>
<td>0.801</td>
</tr>
</tbody>
</table>

**9.4.2. FRAN analysis of surface reducing ability**

Both the original CeO$_2$ and CeO$_2$ with As covered on the surface were collected in powder form and then suspended in ultrapure water. The primary hydrodynamic sizes of all samples were checked by DLS to confirm insignificant aggregation has occurred throughout the experiment (Figure S9.1). All the samples were analyzed by FRAN to assess the surface reducing ability of the pristine (i.e., without As adsorption) and As-covered CeO$_2$ NPs. Figure 9.2 depicts $C_{Fe^{II}}^p$, a surface reactivity indicator of FRAN, versus $q_{CeO_2_{As^{III}}}^p$ for As(III)-covered CeO$_2$ NPs. A dramatic decrease of $C_{Fe^{II}}^p$ was observed when $q_{CeO_2_{As^{III}}}^p$ increased from 0 to ~110 mmol/kg. As $q_{CeO_2_{As^{III}}}^p$ increased from ~110 mmol/kg, $C_{Fe^{II}}^p$ showed a very slow increasing trend. For As(V)-covered CeO$_2$ NPs, however, FRAN could not give a regular reactivity indicator (i.e., $C_{Fe^{II}}$), because the kinetics of Fe$^{II}$ formation, instead of a monotonous increase, showed a short-time increase followed by a slow decrease (Figure S9.2), and $C_{Fe^{II}}^p$ was not defined.
In Chapter 6 have been demonstrated two different reaction mechanisms, depending on the role of NPs, that may happen in FRAN assay. In the “sacrificial reducing” mechanism, the NP material itself donates electrons to Fe$^{III}$ ions and the formation of Fe$^{II}$ shows an exponential trend. Alternatively, in the “electrode discharging” mechanism, the NP material serves as a mixed electrode, which can store electrons and reduce Fe$^{III}$ at the surface like a cathodic reaction. In this case, the Fe$^{II}$ formation shows a logarithm trend. Herein, to identify which mechanism is followed by CeO$_2$ (pristine or As$^{III}$-covered) NPs in FRAN assay, the kinetics of Fe$^{II}$ was modeled, which is shown in Figure 9.3. The kinetics for pristine CeO$_2$ NPs was best fitted by a logarithm function, while the exponential function underestimates the data in the initial time (<150 s) and overestimate in ~150-400s, suggesting pristine NPs follow the “electrode discharging” mechanism in
the FRAN reaction. The “electrode discharging” mechanism was still followed by CeO$_2$ NPs with a small amount of As$^{III}$ surface coverage (e.g., $q_{As}^{CeO_2}=19.1$ and 37.8). However, when As$^{III}$ surface coverage becomes large (i.e., $q_{As}^{CeO_2} > 100$), the logarithm trend of Fe$^{II}$ formation is replaced by the exponential trend. In this case (e.g., $q_{As}^{CeO_2}=109.5$ mmol/kg in Figure 9.3), the logarithm function overestimates the data in the initial time (<100 s) and underestimate in 100-400, suggesting that the “sacrificial reducing” mechanism replaces the “electrode discharging” mechanism when $q_{As}^{CeO_2}$ is high.

**Figure 9.3.** Fe$^{II}$ formation kinetics profiles for pristine and As(III)-covered CeO$_2$ NPs at different $q_{As}^{CeO_2}$. All dots are experimental data. Lines are the fitting of experimental data with a logarithm function (black and solid) or an exponential function (red and dashed).

Although the formula of CeO$_2$ suggests valence IV of Ce, the material is known to undergo rapid and repeatable interconversion between the Ce$^{IV}$ and Ce$^{III}$ state as the
redox condition changes\textsuperscript{332,333}. This property can lead to the actual “CeO\textsubscript{2}” material being non-stoichiometric (i.e., CeO\textsubscript{2-x}, 0 ≤ x ≤ 0.5), and is also the essential cause of its catalytic activity\textsuperscript{334,335}. The interconversion of Ce\textsuperscript{IV}/Ce\textsuperscript{III} states gives rise to the diffusion of small polarons in CeO\textsubscript{2-x} material following a hopping mechanism\textsuperscript{333,335,336}. Consequently, upon being activated, CeO\textsubscript{2-x} has electron mobility and thus conductivity\textsuperscript{333,335,336}, which leads to the capability of storing and transferring electrons in the “electrode discharging mechanism” of “CeO\textsubscript{2}” NPs in FRAN assay. Another knowledge is that hopping-based conductivity for oxide materials can be affected by doping\textsuperscript{337}. Therefore, it is reasonable to hypothesize that the observed different FRAN response of As-covered CeO\textsubscript{2} NPs from the pristine ones is caused by that the adsorbed As changes the redox state of Ce and the electron mobility in the particle phase.

XPS was used to probe the electronic states of CeO\textsubscript{2} NPs on the surface. The As 2p peak (binding energy = 1320-1330 eV) was identified for all As-covered NP samples and its area is well correlated to $q^{\text{CeO}_2}_{\text{As}^{\text{III}}}$ (Figure 9.4), providing As was adsorbed onto the CeO\textsubscript{2} and remains throughout the experiment. A well-established method\textsuperscript{338} based on fitting the Ce 3d spectrum with 8 peaks was followed to determine the oxidation states of Ce on the surface. The detail of the peak fitting process is given in the Supporting Information. After the peaking fitting, the percentage of peak u’’’ quantitatively suggests the amount of Ce\textsuperscript{IV} feature in the material\textsuperscript{338}. Figure 9.4 shows the variance of Ce\textsuperscript{IV} feature, i.e., the u’’’ area percentage, on the CeO\textsubscript{2} NP surface with $q^{\text{CeO}_2}_{\text{As}^{\text{III}}}$ . The increase of $q^{\text{CeO}_2}_{\text{As}^{\text{III}}}$ led to a decrease of Ce\textsuperscript{IV} state (Figure 9.4a), suggesting the conversion from Ce\textsuperscript{IV} to Ce\textsuperscript{III} occurred when As\textsuperscript{III} ions are adsorbed. The increase of did not lead to a decrease of Ce\textsuperscript{IV}, but the Ce\textsuperscript{IV} feature remained nearly constant when is small (<60
mmol/kg), and slightly increased when $q_{\text{As}^{\text{III}}}$ became greater than 80 mmol/kg (Figure 9.4b). This observation indicates that As$^{\text{V}}$ adsorption did not cause the conversion of Ce$^{\text{IV}}$ to Ce$^{\text{III}}$, but very slightly the vice versa.

**Figure 9.4.** Ce(IV) feature indicated by $u^{'+''}$ area percentage and As 2p area of CeO$_2$ NPs with different As (III) (a) or As (V) (b) coverage, analyzed by XPS.

Looking at the electronic structure, the conversion Ce$^{\text{IV}}$ to Ce$^{\text{III}}$ state is achieved by the filling of 4f orbitals$^{329,336}$. The XPS analysis result suggests that As(III) at the adsorbate sites can donate electrons to fill the 4f orbitals, in analogy to n-type doping effect. Thus, the actual As$^{\text{III}}$-adsorbed NPs are non-stoichiometric, i.e., CeO$_{2-x}$. $x$ increases as more Ce$^{\text{IV}}$ was reduced to Ce$^{\text{III}}$. The increase of $x$ can lead to the increase of the activation energy of polaron hopping in the material and thus the decrease of electron mobility$^{332}$. As a result, in the FRAN analysis, the reflected surface reactivity of “CeO$_2$” NPs decreased at small $q_{\text{As}^{\text{III}}}$ but maintained the “electrode discharging mechanism”. When $x$ raised to higher level, the “electrode discharging mechanism” was replace by the “sacrificial reducing mechanism”. This is mostly likely attributed to that the significant coverage of As$^{\text{III}}$ ions effectively protected “CeO$_2$” NPs from the attaching of Fe$^{\text{III}}$ ions,
insulating the “electrodes”. However, Fe$^{III}$ directly oxidized the As$^{III}$ adsorbate on NP surface, giving a regular first-order reaction (i.e., an exponential kinetics trend). From our experiment, Fe$^{III}$ reduction under the “sacrificial reducing mechanism” is slower than the “electrode discharging mechanism”, and its enhancement by higher is also small (Figure 9.2).

9.4.3. The surface reactivity leading to the formation of HO by reacting with H$_2$O$_2$

EPR was used to analyze the concentration of HO radicals (C$_{OH}$) induced by CeO$_2$ NPs in the presence of H$_2$O$_2$ (1 mM). The adsorbed As$^{III}$ and As$^{V}$ ions lead to different C$_{OH}$, when the reaction reached equilibrium in 5-20 min. For As$^{III}$ ion adsorption, C$_{HO}$ first dramatically declined with a small amount of As adsorption (q$^{\text{CeO}_2}$$_{As^{III}}$ =19.1 mmol/kg) (Figure 9.5). When q$^{\text{CeO}_2}$$_{As^{III}}$ continued to increase, however, C$_{HO}$ increased again and reached a plateau after q$^{\text{CeO}_2}$$_{As^{III}}$ reached ~110 mmol/kg. When q$^{\text{CeO}_2}$$_{As^{III}}$ became very high (>300 mmol/kg), C$_{HO}$ became smaller again. As$^{III}$ ions influenced HO formation differently: C$_{HO}$ continuously decreased with increasing q$^{\text{CeO}_2}$$_{As^{V}}$, and stabilized at ~0.6 mM after q$^{\text{CeO}_2}$$_{As^{V}}$ is greater than 100 mmol/kg.
Figure 9.5. HO concentration induced by pristine and As-covered CeO$_2$ NPs in the presence of 1 mM H$_2$O$_2$ in water. Dashed line standards for HO concentration detected from a NP-free blank sample.

Although previous works$^{18,323}$ have reported the reaction of CeO$_2$ NPs with H$_2$O$_2$, a clear reaction mechanism has not been reached. Heckert et al.$^{339}$ suggested a Fenton-like reaction mechanism imposed by a Ce$^{III}$/Ce$^{IV}$ cycle, in manner of the Haber-Weiss Reaction$^{340}$. This mechanism would require a first step of reduction of H$_2$O$_2$ by Ce$^{III}$, mimicking the role of Fe$^{II}$ in a Fenton reaction. However, the same team later reported that CeO$_2$ NPs with more Ce$^{III}$ states are less reactive with H$_2$O$_2$$^{323}$, conflicting with the Fenton/Haber-Weiss mechanism. Our EPR analysis agreed with this finding, as the As (III) adsorption increased the amount of Ce$^{III}$ state and significantly lowered HO production comparing to pristine CeO$_2$ NPs. In fact, some old studies suggested the Ce$^{IV}$
ion is able to oxidize $\text{H}_2\text{O}_2^{341,342}$, which possibly served as the first reaction step in the CeO$_2$ NP case.

CeO$_2$ NPs are also found to scavenge certain ROS (e.g., superoxide) and the scavenging ability is correlated to Ce$^{\text{III}}$ feature$^{318,319}$. Superoxide ($\text{O}_2^-$) and its protonated form hydroperoxyl (HO$_2$) are commonly involved in the decomposition of $\text{H}_2\text{O}_2^{340,343}$. HO$_2$ serves as a intermediate in the chain of Haber-Weiss reaction and promote the decomposition of $\text{H}_2\text{O}_2^{340}$. If the increase of Ce$^{\text{III}}$ terminated the chain of Haber-Weiss reaction via scavenging HO$_2$, it may also cause the decrease of HO formation as observed in our experiment. Another potential factor is the insulating effect of As adsorption to CeO$_2$ surfaces. This mechanism probably dominated the case of As(V) adsorption, where increasing As(V) coverage monotonously decreased HO formation, as the As(V) ions effectively protected CeO$_2$ from $\text{H}_2\text{O}_2$. Moreover, the redox cycle of As$^{\text{V}}$/As$^{\text{III}}$ may also have played roles in the reaction. The increase of CO$_{\text{OH}}$ when As$^{\text{III}}$-coverage is greater than $\sim$20 mmol/kg is probably dominated by the As species adsorbed on the surface, in a similar manner of what was found in the FRAN assay. Overall, the clear reaction mechanism of the reaction between As-covered CeO$_2$ NPs and $\text{H}_2\text{O}_2$ is not reached at this stage. However, the complexity of this reaction is raised here and worthy to be examined by future studies.

9.5. Acknowledgement

This work was jointly funded by the NSF (CBET1507750) as a GOALI project with the Semiconductor Research Corporation (SRC, 425.052), the US Environmental Protection Agency through the STAR program (RD83558001), and the NSF Nanosystems Engineering Research Center for Nanotechnology-Enabled Water Treatment
(EEC-1449500). I am thankful to Drs. Marco Flores and Timothy Karcher for their assistance and helpful discussions on EPR and XPS analysis.

### 9.6. Supporting Information

**Figure S9.1.** Effective hydrodynamic diameter of CeO$_2$ NP samples covered by different amount of As (III or V) ions. Results suggest the effective size of CeO$_2$ NPs after adsorbing As ion remained closely to that of the pristine CeO$_2$ NPs (i.e., the first dot in the graph at $q_{\text{As}^{\text{III/II}}}^{\text{CeO}_2} = 0$).
**Figure S9.2.** Kinetics profiles of Fe$^{II}$ formation in FRAN analysis for CeO$_2$ NPs with different As(V) ion coverage ($q_{As^{V}}^{CeO_2}$). The result shows that the As(V)-covered CeO$_2$ NPs did not give a regular monotonous increase of $C_{Fe^{II}}$. Instead, $C_{Fe^{II}}$ increased in a short amount of time to reach a peak, and then appeared to decrease until it reached zero. However, higher $q_{As^{V}}^{CeO_2}$ gave lower $C_{Fe^{II}}$ at the peak position.

**Peak fitting for the Ce 3d spectrum**

Peak fitting for the Ce 3d spectrum was performed in CasaXPS following a well-established method$^{338}$. 8 peaks were initialized as in Table S9.1.
Table S9.1. Initial peak parameters for fitting.

<table>
<thead>
<tr>
<th>Peak name</th>
<th>u''''</th>
<th>u''</th>
<th>u'</th>
<th>u</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.E.1 position (eV)</td>
<td>916.6</td>
<td>906.7</td>
<td>903.3</td>
<td>900.4</td>
</tr>
<tr>
<td>fwhm2 (eV)</td>
<td>2</td>
<td>3</td>
<td>2.8</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Peak name</th>
<th>v''''</th>
<th>v''</th>
<th>v'</th>
<th>v</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.E.1 position (eV)</td>
<td>898.0</td>
<td>888.1</td>
<td>884.8</td>
<td>881.8</td>
</tr>
<tr>
<td>fwhm2 (eV)</td>
<td>2</td>
<td>3</td>
<td>2.8</td>
<td>2</td>
</tr>
</tbody>
</table>

1“B.E.” stands for “binding energy”.

2“fwhm” stands for “full width at half maximum”.

During fitting, B.E. of all peaks were fixed and fwhm were allowed to vary ± 0.1 eV.

The derivation of all peaks above can be referred to a couple of previous literature.

An example of peak fitting for pristine CeO₂ NPs is given in Figure S9.3.

Figure S9.3. Peak fitting of the Ce 3d spectrum for pristine CeO₂ NPs. The percentage of u'''' peak area was then used to indicate the quantity of Ce⁴⁺ on a material surface.
CHAPTER 10
SYNTHESIS: ASSESSING SURFACE REACTIVITY OF NANOPARTICLES IN WATER: MUCH MORE THAN READING A NUMBER

10.1. Introduction

The rise of nanotechnology in many engineering and medicine fields, along with its merits, has also aroused some skepticism with regards to its safety, ethics and environmental impacts. Because of large uncertainties of nanomaterials’ exposure and toxicity, environmental health and safety (EHS) of nanomaterials are particularly concerned and have been actively studied in the past decades.

EHS studies of nanomaterials incorporate multiple sectors, e.g., toxicology, risk assessment, analytics, and environmental fate and transformation. Most researchers worked in a single sector. This dissertation suggested the surface reactivity of nanoparticles (NPs) is a key property that can interrelate these different sectors. The surface reactivity of NPs comprehensively implies their toxicity, fate and transformation, and facilitates nanomaterial analysis purpose, and thus the assessment of it is of paramount importance to EHS studies. Numerous assays were applied to probe the surface reactivity of NPs. Despite different names (e.g., the oxidative stress/potential assay or the reactive oxygen species (ROS) detection assay) it may have, a surface reactivity assay initiates a single or multiple surface reactions and employs an instrument (e.g., an absorbance or fluorescence spectrometer) to evaluate the reaction progression. The assay’s outcome is often a single instrumental reading, lacing further justifications of the inference of the reading.
Moreover, the concept of surface reactivity, the impetus by which the surface of NPs participates reactions, is still broad. In fields of NPs’ applications, different types of surface reactivity, e.g., the catalytic or redox reactivity, were known to enable different functions, yet they were not distinguished by many of the existing surface reactivity assays. Related questions are further raised. What type of surface reactivity is probed by an assay? Does a single instrumental reading by an assay suffice a precise assessment of the interested surface reactivity? How to design an assay capable of precisely measuring surface reactivity and identifying the reactivity type? Without answering these questions, the outcome of a NP surface reactivity assay can be perplexing.

In this chapter, I synthesize the findings on NP surface reactivity assessment in this dissertation, meta-analyze the analytical outcomes of different assays, and finally summarize strategies to precisely assess the NP surface reactivity of interest.

10.2. Selected NP surface reactivity assays and their principles

To answer the questions raised above, I chose four abiotic surface reactivity assays to discuss and compare. Three of them were used in this dissertation to assess the surface reactivity of NPs in water.

**Borohydride-methylene blue (BHMB) assay.** BHMB assay utilizes the model reaction of borohydride (BH$_4^-$) reducing methylene blue (MB) catalyzed by NPs. The detail of the electron transfer mechanism for this assay was discussed in Chapter 5. In the assay’s reaction, NPs act as a heterogeneous catalyst, and convey electrons from the donor (BH$_4^-$) to the accepter (MB). The variance of MB concentration (suggested by $A_{663nm}$) was measured as a function of time in the assay operation, giving a kinetics profile. An example for 50 nm Au NPs at 160 µg/L is given in Figure 10.1a. Initially,
MB concentration decreased over time as being reduced (to form leuco methylene blue (LMB)) by sufficient BH$_4^-$ catalyzed by Au NPs. After reaching a “pseudo-equilibrium” (i.e., approximately 80-120 s), MB appeared to reform due to significant hydrolysis of BH$_4^-$ and the back oxidation of LMB. The surface reactivity of NPs in this assay can be implied by the MB concentration in the “pseudo-equilibrium” (approach I) or by the MB surface reduction rate constant that is obtained by an inclusive kinetics model (approach II). Chapter 5 demonstrated and discussed the two approaches. Approach II represents a more direct reflection the surface reactivity.

**Ferric reducing ability of NPs (FRAN) assay.** FRAN assay is based on the reduction of ferric ions (Fe$^{III}$) by NPs to form ferrous ions (Fe$^{II}$). The generated Fe$^{II}$ ions are complexed with 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), giving a detectable absorbance at 593 nm ($A_{593\text{nm}}$). In the assay’s operation, Fe$^{II}$ concentration (\(\cdot\)) was measured overtime, giving its formation kinetics profile. An example for 20 nm AuNPs at 5.4 mg/L is shown in Figure 10.1b. When the tested NP sample was added into the assay, the concentration of Fe$^{II}$ continuously increased until it reached a plateau. The plateau level from the kinetics profile was taken to suggest the surface reactivity of the NPs (Figure 10.1b). Chapter 6 discussed this assay in details.

**Electron Paramagnetic Resonance (EPR) assay.** EPR spectroscopy is based on the interaction of magnetic field with the magnetic moment of unpaired electrons in radical species. The detection of hydroxyl radicals (HO), one of the most important ROS, is challenged by the extremely short lifetime of the radical in water\textsuperscript{351}. The “spin trapping” strategy was used in this work to overcome this difficulty. 5,5-dimethyl-pyrroline-N-oxide (DMPO) was applied to trap HO in a solution and form DMPO-HO spin-adduct,
which has a longer lifetime ($t_{1/2} = 2.6$ h)$^{352}$ and thus is directly measureable by EPR. A DMPO-HO spin-adduct gives an identifiable EPR spectrum ($A_N = A_H = 14.9$ G). To quantify the present DMPO-OH or HO radicals, the second derivative spectrum is doubly integrated and then calibrated using a radical standard 2,2,6,6-Tetramethyl-1-piperidinyloxy (TEMPO). EPR assay in this work quantified the concentration of HO after the tested NPs reacted with $\text{H}_2\text{O}_2$ (1 mM) at pH 3.6 buffered acetate (30 mM). $\text{H}_2\text{O}_2$ is widely used as a substrate to test NPs’ potential to form ROS, because of its common presence in cells and some water media. Figure 10.1c shows the EPR spectrum tested with 20 nm Au NPs at 500 µg/L.

**UV-illuminated methylene blue degradation (UVMB) assay.** UVMB assay was designed to reveal the surface reactivity that requires the activation of photo-energy. In this assay, a solution containing 40 µM MB and desired amount of tested NPs was illuminated by 312 nm UV light. To achieve a high throughput analysis, I performed the assay in a 96-wall plate, where each well served as a reactor. The decay of MB was monitored by absorbance at 650 nm. Figure 10.1d shows the example of applying UVMB assay to P25 TiO$_2$ NPs at 15 mg/L. Pseudo-first order kinetics was observed up to about 30 min. We took the pseudo-first order rate constant of MB decay as the indicator of NP surface reactivity by UVMB assay.
Figure 10.1. Typical measurement data of four NP surface reactivity assays: BHMB (a), FRAN (b), EPR (c) and UVMB (d). Quantification strategies are described by red notes in each figure panel.

10.3. Categorization of NP surface reactivity

Depending on the mechanism of surface reaction, the surface reactivity of NPs can be categorized. After examining the electron transfer processes and the required activation of reactions in all the assays, I identified three reactivity types: catalytic-, redox-, and photo-reactivity (Figure 10.2).
Figure 10.2. Diagram for three types of NP surface reactivity distinguished by electron transfer processes.

The surface catalytic reactivity indicates the NPs’ ability to catalyze redox reactions. In reactions by virtue of catalytic reactivity, the NP surface withdraws electrons from donors and delivers them to acceptors, acting as though an “electron terminal”. This essential principle determines that most NPs with high surface catalytic reactivity are materials with adequate electron mobility or conductivity. Indeed, most known NP catalysts are metals, e.g., gold (Au), iron (Fe), palladium (Pd), and platinum (Pt)\textsuperscript{14,197-201}, which have been applied in fuel cells\textsuperscript{65}, hydrogen generation\textsuperscript{353}, and pollutants degradation\textsuperscript{203}. The surface redox reactivity indicates the potential of NPs themselves to be oxidized or reduced by other chemicals. In reactions by virtue of redox reactivity, the redox state of elements on the NP surface changes (i.e., suggested by $M^{n+} \rightarrow M^{(n+1)+}$ in Figure 10.2) and the material on the surface is consumed or transformed as a result. NPs composed of redox-active elements often have high surface redox reactivity, which is utilized for certain functions. For example, Ag NPs have high surface redox reactivity, because Ag\textsuperscript{0} is readily oxidized to Ag\textsuperscript{+} by common oxidants (e.g., O\textsubscript{2}). As a result, Ag NPs can release silver ions (Ag\textsuperscript{+})\textsuperscript{65,354} after being oxidized and generate ROS (e.g., superoxide (O\textsubscript{2})\textsuperscript{−} and peroxide) via reducing O\textsubscript{2}\textsuperscript{65}. Both Ag\textsuperscript{+} ions and ROS can destroy
bacteria and thus Ag NPs are used as antibacterial agents in fabrics\textsuperscript{115, 241}, paints\textsuperscript{355}, wound dressing\textsuperscript{356}, etc. A simple way to predict a NP species’ redox reactivity is via the reducing potential of the elements constituting the NP. The surface photo reactivity indicates the potential of the NP surface to absorb photo-energy (i.e., photons) from light to undergo photo-catalytic or photo-redox processes. Reactions by virtue of photo reactivity essentially have the same electron transfer mechanism with catalytic or redox reactivity, but require the activation of photo-energy. Thus photo-catalytic and photo-redox reactivity can be further discriminated from it. NPs constituted by semiconductor material (e.g., TiO\textsubscript{2} and ZnO) commonly have surface photo-catalytic reactivity due to their band-gap electronic structure, and can be used as photo-catalysts\textsuperscript{357} and UV-blocking agents in sunscreen\textsuperscript{358}. Some NPs, e.g., quantum dots (QDs)\textsuperscript{359}, may have surface photo-redox reactivity.

10.4. Comparison of surface reactivity assay performance on different NP species

It was hypothesized that different reactivity assays can target for different reactivity types and thus give different analytical result to the same set of NP samples. To testify this hypothesis, I applied the four assays above to five NP species (Au, Ag, CeO\textsubscript{2}, SiO\textsubscript{2}, and TiO\textsubscript{2}) at multiple concentrations. Figure 10.3 shows the “reactivity indicator” (described in Figure 10.1) by each assay against the surface concentration ($C_{\text{NP, surface}}$, i.e., surface per volume of solution, in m\textsuperscript{2}/m\textsuperscript{3}) of each NP species. A valid surface reactivity indicator should give a linear correlation to $C_{\text{NP, surface}}$ and thus becomes constant after being normalized to surface area. This is true for the BHMB (Figure 3a), the FRAN (Figure
3b) and the UVMB (Figure 3d) assay, but not for the EPR assay (Figure 3c). As \( C_{\text{NP}}^{\text{surface}} \) increased, the indicator of EPR assay, \( C_{\text{HO}} \), showed a decreasing trend for Au and Ag NPs, but an increasing followed by a decreasing trend for CeO\(_2\), TiO\(_2\) and SiO\(_2\) NPs.

**Figure 10.3.** Surface reactivity indicator for five NPs (Au, Ag, CeO\(_2\), TiO\(_2\), and SiO\(_2\)) analyzed by BHMB (a), FRAN (b), EPR (c), and UVMB (d) assay. Dots placed at zeros suggest assay responses below the detection limit for those measurements. For each NP species, was calculated from mass concentration providing known specific surface area, which is estimated from NP geometry (for Au (15.54 m\(^2\)/g) and Ag (28.57 m\(^2\)/g)), provided by the manufacture (for TiO\(_2\) (55.55 m\(^2\)/g)), or experimentally measured by BET (for CeO\(_2\) (17.0 m\(^2\)/g) and SiO\(_2\) (99.5 m\(^2\)/g)).
Surface reactivity magnitude can be ranked among NP species via normalizing a valid “indicator” to $C^\text{surface}_{NP}$, i.e., the slope of the linear relationship in Figure 10.3a, b, and d. Some NPs did not show a detectable response in an assay, where the indicator value was plotted as zero. Table 10.1 summarized the ranking order of reactivity magnitude of the five NP species analyzed by each assay. Because of the invalid reactivity indicator, the ranking order is undeterminable by EPR assay. Obviously, the four reactivity assays gave different reactivity ranking orders among the five NP species, implicating that the four assays probed different types of surface reactivity.

**Table 10.1.** Reactivity magnitude order of five NP species assessed by four reactivity assays

<table>
<thead>
<tr>
<th>Assay Name</th>
<th>Order of reactivity magnitude of five NP species</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHMB</td>
<td>Au &gt; Ag &gt; CeO$_2$ = TiO$_2$ = SiO$_2$ = 0</td>
</tr>
<tr>
<td>FRAN</td>
<td>Ag &gt; Au &gt; CeO$_2$ &gt; TiO$_2$ = SiO$_2$ = 0</td>
</tr>
<tr>
<td>EPR</td>
<td>Undeterminable</td>
</tr>
<tr>
<td>UVMB</td>
<td>TiO$_2$ &gt; Au = Ag = CeO$_2$ = SiO$_2$ = 0</td>
</tr>
</tbody>
</table>

**10.5. Identification of reactivity types by reactivity assays**

Although a variety of surface reactivity assays$^{22}$ are being used in research or industry activities, under few circumstances has the reactivity type that is probed been identified. Users report “numbers” measured by assays, but often overlook the essential meaning of the numbers. In this dissertation, I emphasized the importance of distinguishing the surface reactivity types that are probed by assays, for the sake of better understanding
NP-induced reactions that are related to ecological toxicity. Schemes in Figure 10.2 suggest the surface reactivity type is determined by the electron transfer mechanisms. As such, to identify which reactivity type is probed by an assay needs the inspection of reaction mechanisms involved in the assay. Accordingly, Table 10.2 summarized the reactivity type probed by the aforementioned assays with the corresponding hypothetical reactions. Among the assays, BHMB probes exclusively the surface catalytic reactivity. FRAN and EPR assays may possibly probe the surface redox or catalytic reactivity, and UVMB probes the surface photo reactivity, which, however, can be further discriminated between photo-redox and photo-catalytic reactivity.

The assignment of reactivity types to the assays is partially proved with standard NPs in this dissertation. I undertook kinetic modeling to reveal the electron transfer scheme and reaction pathways in reactivity assays, which leads to the identification of the reactivity type probed by an assay. By kinetic modeling, BHMB assay was shown to detect the surface catalytic reactivity (Chapter 5), whereas FRAN assay was shown to detect the surface catalytic or redox reactivity depending on different electron transfer schemes imposed by different NP species (Chapter 6). The kinetic analysis for FRAN assay tells that the surface reactivity assay and type do not necessarily follow a one-to-one relationship. Thus one should carefully interpret the assay result to suggesting that cares should be taken when assigning the assay result to a specific reactivity type.

EPR assay used in this study is based on the reaction(s) between NPs and H$_2$O$_2$ to form HO. However, it is unclear yet the details of the all the electron transfer mechanism and all the reaction pathways. The formation of HO by NP reacting with H$_2$O$_2$ was studied for multiple NP species, e.g., Ag$^{360}$, SiO$_2^{17,18}$, and CeO$_2^{18}$. These reactions were
often referred to as “Fenton-like” reactions\textsuperscript{18, 317, 360}, and pathways following the Haber-Weiss\textsuperscript{340} mechanism were often proposed. That being the case, the reaction pathways can be generalized as the following:

\begin{align}
(NP^*)^{x+} + H_2O_2 \rightarrow (NP)^{(x+1)^+} + OH^- + HO & , \\
HO + H_2O_2 \rightarrow H_2O + HO_2 & , \\
HO_2 + H_2O_2 \rightarrow O_2 + H_2O + HO & , \\
HO + (NP^*)^{x+} \rightarrow (NP)^{(x+1)^+} + OH^- & .
\end{align}

In this scheme, NP surface can both induce (eq (10.1a)) and scavenge (eq (10.1d)) HO radicals, with the latter supporting the observed decrease of $C_{HO}$ in Figure 10.3c. However, coupled reaction pathways involving various intermediate oxygen species (i.e., hydroxyl, superoxide, peroxide, and molecular oxygen) make it difficult to isolate the surface electron transfer process. $C_{HO}$ is thus associated with the concentration of multiple species, and not linearly dependent on in Figure 10.3c. EPR assay may indicate the surface redox reactivity based on eq (10.1a). However, one cannot rule out the possibility that the reduction of $H_2O_2$ occurs via an “electrode discharging” mechanism (Table 10.2), in which case EPR would suggest the surface catalytic reactivity. Moreover, the Haber-Weiss mechanism, despite being widely proposed, remains hypothetical for NP-H$_2$O$_2$ systems. The UVMB assay is indicative for photo reactivity but unable to clearly distinguish photo-catalytic and photo-redox reactivity at the current stage. TiO$_2$ NPs should reflect the photo-catalytic reactivity in my experiment, as evidenced by existing studies on similar types of reactions\textsuperscript{361}. Examples of photo-redox reactivity was not demonstrated in my study but was reported with certain NPs (e.g., QDs)\textsuperscript{359}. Photo-
catalytic or photo-redox reactivity can be distinguished via examining whether the NP surface is consumed or transformed.
Table 10.2. A summary of “assay-reactivity type” relation according to the hypothetical reactions.

<table>
<thead>
<tr>
<th>Assay name</th>
<th>Reactivity type</th>
<th>Hypothetical surface reaction</th>
<th>Demonstrated NP species</th>
<th>Discussion chapter</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHMB</td>
<td>Catalytic</td>
<td>$BH_4 + 2H_2O \xrightarrow{NP^* (1)} BO_2 + 8H^+ + 8e^-$</td>
<td>Au</td>
<td>Chapter 5&amp;10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$MB^+ + 2e^- + H^+ \xrightarrow{NP^*} LMB$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Redox</td>
<td>$(NP^<em>)^{x+} + nFe^{3+} \rightarrow (NP^</em>)^{(x+n)+} + nFe^{2+}$</td>
<td>Ag, CeO$_2$</td>
<td>Chapter 6, 9&amp;10</td>
</tr>
<tr>
<td>FRAN</td>
<td>Catalytic</td>
<td>$Fe^{3+} + e^- \xrightarrow{NP^*} Fe^{2+}$</td>
<td>Au, As-covered CeO$_2$</td>
<td>Chapter 6, 9&amp;10</td>
</tr>
<tr>
<td></td>
<td>Redox</td>
<td>$(NP^<em>)^{x+} + H_2O_2 \rightarrow (NP^</em>)^{(x+1)+} + OH^- + HO$</td>
<td>Unclear$^2$</td>
<td>Chapter 9&amp;10</td>
</tr>
<tr>
<td>EPR</td>
<td>Catalytic</td>
<td>$H_2O_2 + e^- \xrightarrow{NP^*} OH^- + HO$</td>
<td>Unclear$^2$</td>
<td>Chapter 9&amp;10</td>
</tr>
<tr>
<td>UVMB</td>
<td>Photo-catalytic</td>
<td>$NP^* \xrightarrow{h^+ + e^-}$</td>
<td>TiO$_2$</td>
<td>Chapter 10</td>
</tr>
<tr>
<td></td>
<td>Photo-redox</td>
<td>$MB + h^+ \rightarrow P$ ; and/or $H_2O + h^+ \rightarrow HO + MB \rightarrow P$</td>
<td>Not demonstrated$^5$</td>
<td>Chapter 10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$(NP^<em>)^{x+} + MB + H_2O \xrightarrow{h^+}(NP^</em>)^{y+} + P^{(4)}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: (1) NP* stands for NP surface; (2) At this stage, redox and catalytic reactivity is not distinguishable in the EPR assay; (3) Oxidation of MB may be fulfilled by holes(h$^+$) and/or by ROS (e.g., HO) formed by the oxidation of H$_2$O by holes. The stoichiometry and specific reaction products are not determined at this stage; (4) Stoichiometry and product are not identified; (5) This reactivity type or reaction mechanism was not demonstrated in this work.
10.6. Generalization of reaction pathways in a surface reactivity assay: how to design or use an assay properly?

To design a surface reactivity assay, a surface reaction, the rate of which reflects the targeted surface reactivity, is first needed. Determined by the surface reactivity type, the surface reaction can be a redox or catalytic process:

\[ \text{NP}^* + R \rightarrow (\text{NP}^*)^i + P, \quad k_r^S; \quad \text{(10.2a)} \]
\[ R \xrightarrow{\text{NP}^*} P, \quad k_c^S; \quad \text{(10.2b)} \]

where \( R \) and \( P \) are reactant(s) and product(s), \( \text{NP}^* \) and \( (\text{NP}^*)^i \) are original and transformed (i.e., reduced or oxidized) NP surface, and \( k_r^S \) and \( k_c^S \) are the rate constants of the two reactions. \( k_r^S \) and \( k_c^S \) are controlled by the NP surface reactivity, and thus the probing target of an assay. Photo-reactivity differs from the other two reactivity types by the requirement of photo-energy input (i.e., an operation condition), and thus shares the same chemistry principles.

The discrimination of eq(10.2a) and eq (10.2b) requires additional reaction mechanism analysis, e.g., via theoretical modeling of kinetics (Chapter 6), but not different assay designs (e.g., FRAN can probe both redox and catalytic reactivity). Thus, they can be generalized as:

\[ R \xrightarrow{\text{NP}^*} P, \quad k^S. \quad \text{(10.3)} \]

In eq (10.3) \( \text{NP}^* \) can be a NP surface undergoing redox transformation or acting as a catalyst, and thus \( k_r^S \) and \( k_c^S \) are generalized as \( k^S \).

The second need for a surface reactivity assay is a “probe”, which also participates certain reaction (i.e., a probe reaction) and is measured by an instrument. The probe
reaction and the surface reaction can be identical or different, giving the “direct probe” and the “indirect probe” schemes, respectively (Table 10.3). In the “direct probe” case, the probe molecule is one of the reactants of the surface reaction, and thus directly probes the surface reactivity. In the “indirect probe” case, the probe molecule reacts with the product of the surface reaction, and thus indirectly inferring the NP surface reactivity via probing the procession of the surface reaction. An example of the “indirect probe” scheme is a group of assays based on detecting ROS formation induced by NPs in water. In that case, a probe reacts with a ROS species (e.g., HO, superoxide (O2-), and hydrogen peroxide (H2O2)), which is the product of the surface reaction caused by the surface reactivity.

**Table 10.3.** The scheme of the “direct probe” and the “indirect probe” assay.

<table>
<thead>
<tr>
<th>Probe type</th>
<th>Reaction scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct probe</td>
<td>Pr $\xrightarrow{NP^*} P^r$</td>
</tr>
<tr>
<td>Indirect probe</td>
<td>R $\xrightarrow{NP^*} P$, P + Pr $\rightarrow P^r$</td>
</tr>
</tbody>
</table>

Notations: Pr is the “Probe” molecule; $P^r$ is the product of Pr transformation.

$k^S$ is a precise surface reactivity indicator for assays using a direct probe. $k^S$ correlates with the concentration of Pr or $P^r$ only if the surface or probe reaction is in steady state. Examples of using a concentration parameter in steady state to indicate the surface reactivity are the $C_{MB}$ (Chapter 5) and $C_{Fe^3}$ (Chapter 6) for BHMB and FRAN assay.

However, the quality of the steady state should be examined, because it can be
approximated (e.g., FRAN detecting surface catalytic reactivity in Chapter 6) or temporary.

For assays using indirect probes, the concentration of the surface reaction product (i.e., P) is often used as the surface reactivity indicator. However, the concentration of the probe molecule should be properly designed in order to precisely probe the procession of the surface reaction. Two strategies are generally used: the “constant probe concentration” and the “varying probe concentration”.

The “constant probe concentration” strategy is based on applying an excessive dose of Pr relative to R, leading to a much faster probe reaction than the surface reaction, i.e.,

\[
R \xrightarrow{k^S} P + Pr \xrightarrow{k^P} Pr
\]  

(10.4)

The design of excessive dose of Pr and thus an approximately constant concentration of it in the probe reaction is to fulfill the condition of “\( k^{Pr} C_{Pr} C_R \gg k^S R \)”, under which reaction (10.5) can be approximated as:

\[
R + Pr \xrightarrow{k^S} Pr
\]  

(10.5)

In this case, the indirect probe approaches a direct probe, as P becomes a reaction intermediate whose lifetime is short. Thus, when the steady state of (10.5) is created, the concentration of \( Pr \) becomes a surface reactivity indicator. EPR assay in this work is based on this strategy, with P being HO, Pr being the DMPO spin trap, and \( Pr \) being the DMPO-HO spin adduct.

The “varying probe concentration” strategy is based on applying the probe molecule at a proper initial concentration, by which the concentration variance of Pr by reacting with
P is easily detected by an instrument. Accordingly, the surface reaction, probe reaction and side reaction are well separated, i.e.,

\[ \text{R} \xrightarrow{k^S} \text{P}, \]  
(10.6a)

\[ \text{S}^p + \text{P} \xrightarrow{k^{s,p}} \text{P}^{s,p}, \]  
(10.6b)

\[ \text{Pr} + \text{P} \xrightarrow{k^p} \text{P}^{pr}. \]  
(10.6c)

The kinetics of P is described as:

\[ \frac{dC_P}{dt} = k^S C_R - k^{s,p} C_{sp} C_P - k^{pr} C_{Pr} C_P. \]  
(10.7)

When P is in steady state, it gives:

\[ C_{Ps} = \frac{k^S C_R}{k^{s,p} C_{sp} + k^{pr} C_{Pr}}. \]  
(10.8)

Herein, the assay design should fulfill the condition of “k^{pr} C_{Pr} \ll k^{s,p} C_{sp}”, under which the following is reached:

\[ C_{Ps} = \frac{k^S C_R}{k^{s,p} C_{sp}}, \]  
(10.9a)

\[ \frac{dC_{Pr}}{dt} = -k^{pr} C_{Ps} C_{Pr} = -k_{obs} C_{Pr}. \]  
(10.9b)

The principle is to assess k_{obs} by measuring the pseudo first order kinetics of Pr decay. k_{obs} is linearly correlated to C_{Ps}^{ss}, which indicates the surface reactivity as discussed previously. Therefore, k_{obs} becomes an indirect indicator for the surface reactivity. To design an assay using the “varying probe concentration strategy”, C_{Pr} and k^{pr} should be controlled to ensure Pr does not perturb C_{Ps}^{ss} and thus the “pseudo-first order” is fulfilled in eq (10.9b). UVMB assay in this work is an example of this strategy, as it was
considered that MB decay is due to its reaction with ROS that is formed by surface reactions.

Apart from the surface and probe reaction(s) (Table 10.3), which were formulated to enable a surface reactivity assay, side reactions other than the intended reaction(s) often inevitably exist during the assay’s operation. Table 10.4 lists the general reactions that can be involved in a surface reactivity assay. Side reactions ((c)-(f)) can be coupled with the surface and probe reaction(s), creating difficulties in accurately analyzing the surface and probe reactions to extract the effective surface reactivity indicator, yet they are easily overlooked during performing an assay if one is satisfied with a single instrument reading as the assay’s outcome. Table 10.5 specifies the surface, probe and side reactions of the four assays in this work and three other assays widely used by others.

**Table 10.4.** A generic reaction scheme in a surface reactivity assay.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R \xrightarrow{NP^w} P$, $k^S$</td>
<td>(a) Surface reaction</td>
</tr>
<tr>
<td>$Pr \rightarrow P^Pr$, $k^{Pr}$</td>
<td>(b) Probe reaction</td>
</tr>
<tr>
<td>$S^R + R \rightarrow P^{S,R}$, $k^{S,R}$</td>
<td>(c) Side reaction(s) scavenging R</td>
</tr>
<tr>
<td>$S^P + P \rightarrow P^{S,P}$, $k^{S,P}$</td>
<td>(d) Side reaction(s) scavenging P</td>
</tr>
<tr>
<td>$S^{Pr} + Pr \rightarrow P^{S,Pr}$, $k^{S,Pr}$</td>
<td>(e) Side reaction(s) scavenging Pr</td>
</tr>
<tr>
<td>$S^{Pb} + P^Pr \rightarrow P^{S,Pb}$, $k^{S,Pb}$</td>
<td>(f) Side reaction(s) scavenging $P^Pr$</td>
</tr>
</tbody>
</table>

Notations: $S^R$, $S^P$, $S^{Pr}$, and $S^{Pb}$ are the scavengers of R, P, Pr and $P^Pr$, respectively. All $k$’s are reaction rate constants of corresponding reactions.
Table 10.5. Summary of surface, probe and side reactions, and probing information of common assays in this study and literature.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Identified reactions</th>
<th>Correspondence to Table 4</th>
<th>Probe molecule</th>
<th>Probing category/target</th>
<th>Comment(s) on side reactions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHMB</td>
<td>$\text{BH}_4^- + 4\text{MB}^+ + 2\text{H}_2\text{O} \xrightarrow{\text{NP}^*} \text{BO}_2^- + 4\text{LMB} + 4\text{H}^+$</td>
<td>(a) or (b) (c) (f)</td>
<td>MB</td>
<td>Direct/NP surface</td>
<td>Fully identified</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>$\text{BH}_4^- + 2\text{H}_2\text{O} \rightarrow \text{BO}_2^- + 4\text{H}_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>$2\text{LMB} + \text{O}_2 \rightarrow 2\text{MB}^+ + 2\text{OH}^-$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRAN</td>
<td>$\text{Fe}^{3+} \xrightarrow{\text{NP}^*} \text{Fe}^{2+}$</td>
<td>(a) or (b)</td>
<td>$\text{Fe}^{3+}$</td>
<td>Direct/NP surface</td>
<td>no side reactions</td>
<td>This study</td>
</tr>
<tr>
<td>EPR</td>
<td>$\text{H}_2\text{O} \xrightarrow{\text{NP}^*} \text{HO}$</td>
<td>(a) (b) (d) (f)</td>
<td>DMPO</td>
<td>Indirect/HO</td>
<td>Partially identified</td>
<td>17, 360</td>
</tr>
<tr>
<td></td>
<td>$\text{HO} + \text{DMPO} \rightarrow \text{DMPO-HO}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>$\text{S}^\text{P} + \text{HO} \rightarrow \text{P}^{\text{S,P}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{H}_2\text{O}_2 + \text{DMPO-HO} \rightarrow \text{P}^{\text{S,P}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UVMB</td>
<td>$\text{H}_2\text{O} \xrightarrow{\text{NP}^*} \text{HO}$</td>
<td>(a) (b)</td>
<td>MB</td>
<td>Indirect and direct/HO and NP surface</td>
<td>Not evaluated</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>$\text{HO} + \text{MB} \rightarrow \text{P}^{\text{p,CBA}}$, and</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$\text{MB} \xrightarrow{\text{NP}^*} \text{P}$</td>
<td>(a) or (b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XTT</td>
<td>$\text{O}_2 \xrightarrow{\text{NP}^*} \text{O}_2^-$</td>
<td>(a) (b) (d)</td>
<td>XTT</td>
<td>Indirect/O$_2^-$</td>
<td>Partially identified</td>
<td>16, 362</td>
</tr>
<tr>
<td></td>
<td>$2\text{O}_2^- + \text{XTT} + \text{H}^+ \rightarrow \text{XTT-formazan} + 2\text{O}_2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$2\text{O}_2^- \rightarrow \text{P}^{\text{S,P}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCFH</td>
<td>$\text{R} \xrightarrow{\text{NP}^*} \text{ROS}$</td>
<td>(a) (b)</td>
<td>DCFH</td>
<td>Indirect/ROS</td>
<td>Not evaluated</td>
<td>24, 245, 363</td>
</tr>
<tr>
<td></td>
<td>$\text{ROS} + \text{DCFH} \rightarrow \text{DCF}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pCBA</td>
<td>$\text{R} \xrightarrow{\text{NP}^*} \text{HO}$</td>
<td>(a) (b)</td>
<td>pCBA</td>
<td>Indirect/HO</td>
<td>Not evaluated</td>
<td>16, 362</td>
</tr>
<tr>
<td></td>
<td>$\text{HO} + \text{pCBA} \rightarrow \text{P}^{\text{p,CBA}}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 10.5 delivers useful guidance on designing and using surface reactivity assays. Frist, the most efficient reactivity assay is one with the minimum number of reactions and thus clear reaction mechanisms. FRAN assay, which is based on a single surface reaction, is an example of an efficient assay. The advantage of a small number of reactions is the convenience of extracting the surface reaction rate, i.e., the reactivity indicator. The kinetic modeling of FRAN assay data effectively discriminates and quantifies surface redox catalytic reactivity. Second, identifying and decoupling side reactions in an assay facilitate precise measures of surface reactivity. Demonstrated by BHMB assay, when all the side reactions were decoupled from the surface reaction via kinetic modeling, the surface electron transfer rate as obtained as a more precise reactivity indicator. Third, most assays based on indirect probes have interferences by side reactions. This is particularly true for assays designed for detecting ROS to suggest surface reactivity. For example, in EPR assay I observed H$_2$O$_2$ scavenging DMPO-OH and NP scavenging HO. These interferences led to the failure of maintaining steady state and the nonlinear correlation of HO concentration to the NP surface area. The consequences are difficult to eliminate when the side reactions are dependent on the NP species and the solution chemistry. Consequently, these assays are often unable to give equivalent reactions to different NP species, causing perplexity when comparisons of the assay outcomes of different NP species are attempted. Forth, assays with complex reaction pathways can have mixed (i.e., direct and indirect) probing schemes. For example, in the use of UVMB assay to TiO$_2$ NPs, MB, the probe, could target HO generated from H$_2$O oxidation (by holes) indirectly, or the holes on the NP surface directly.$^{361}$
To conclude, I recommend, in designing and using NP surface reactivity assays, a deeper inspection of assays’ reaction mechanisms beyond simply reading a number as the outcome, which may not suffice the needs of a study depending on the research objectives. This work demonstrated using theoretical kinetic modeling to identify the surface reaction transfer scheme and surface reactivity types, and to separate side reactions from surface and/or probe reactions.

10.7. Merits of revealing surface reactivity type and surface reaction mechanism in assays to NP toxicity assessment

Abiotic NP surface reactivity assays can infer NPs’ toxicity potential. Enormous studies used biotic assays to test NPs’ toxicity to living cells\(^{18}\) or organisms at multiple trophic levels\(^{19-21}\). A common issue for many biotic toxicity assays is the lack of full understanding of all reaction pathways involved in NP toxicity in in biological media. However, key mechanisms of NP toxicity were proposed, including the cause of oxidative stress via producing ROS\(^{18,364}\) and the release of toxic ions\(^{270}\) via dissolution. Abiotic surface reactivity assays can infer NP toxicity because the evaluated “reactivity” is associated with these key toxicity mechanisms. Some direct correlation between abiotic and biotic assays were demonstrated\(^{16}\).

Researchers tried to use the physicochemical properties of NPs to predict their toxicity. A primary approach is to develop Quantitative Structure-Activity Relationship (QSAR) models. QSAR models found the correlation between NP toxicity to the NP material’s band gap\(^{18,40}\) and the enthalpy of formation\(^{39}\). However, these correlations by QSAR models are empirical and cannot justify the “property-toxicity” relationship mechanistically.
Herein I propose a hierarchal relationship among the property, reactivity and toxicity of NP surface (Figure 10.4). This hierarchy highlights the importance of surface reactivity in linking the property on the bottom to the toxicity on the top. It nicely lines up with the “predictive toxicological paradigm for the safety assessment of nanomaterials” proposed by Nel’s research team\textsuperscript{365}, who suggested using “tired” assays, from “in-vitro” (reflecting the reactivity assay in Figure 10.4) to “in-vitro” (reflecting the biotic toxicity assay in Figure 10.4), to assess nanomaterials’ toxicity potential. However, they recommended the QSAR approach to seek for linkage among different tiers of assays.

![Diagram of the “property-reactivity-toxicity” scheme and interconnection among the components.](image)

**Figure 10.4.** A diagram of the “property-reactivity-toxicity” scheme and interconnection among the components.

This work explored an alternative path—analyzing reaction mechanisms in surface reactivity assays—in addition to QSAR, to seeking the connection among the three sectors in Figure 10.4. Between the “reactivity” and “toxicity”, the revelation of reaction mechanisms and thus the identification of reactivity types in reactivity assays can infer likely reactions the NPs participate in the biological environment. NPs with high surface catalytic reactivity will possibly catalyze redox reactions and modifying electron transfer
chains in cells. NPs with high surface redox reactivity can have higher potential to release toxicant or scavenge compounds in biological media. Between the “property” and “reactivity”, clarified reaction mechanism of reactivity assays can give clues on what NP properties control the observed reactivity. In Chapter 9, the observed surface catalytic reactivity was associated to the material’s electronic structure and elemental oxidation state. Overall, seeking the interconnection within the “property-reactivity-toxicity” relationship by analyzing reaction mechanisms of reactivity assays provides more solid understanding of the hierarchal structure of Figure 10.4 and thus more fundamental evaluation and management of the safety of nanomaterials.
CHAPTER 11

SUMMARY, CONCLUSION AND FUTURE RECOMMENDATIONS

11.1. Summary

The guiding goal of this dissertation is to advance the detection techniques and the knowledge of surface reactivity of engineered nanoparticles (NPs) in water. Each of the main chapters identified a specific objective to achieve and thus contributed to the overarching goal.

11.1.1. Specific objectives

1. Estimated the size detection limit of single particle ICP-MS (spICP-MS) for 40 metallic elements to guide the feasibility of spICP-MS toward a wide range of nanoparticle species. (Chapter 3)

2. Achieve more confident discrimination of particle signals from background noise and a method to quantitatively resolve NP sizes for spICP-MS technique. (Chapter 4)

3. Develop an efficient assay to detect and measure the surface catalytic reactivity of NPs in water. (Chapter 5)

4. Develop an assay to assess the surface reducing ability of NPs in water and identify the surface electron transfer mechanisms. (Chapter 6)

5. Investigate the removal efficacy of industry onsite and municipal wastewater treatment to NPs used in the chemical mechanical polishing (CMP) slurries. (Chapter 7)
6. Quantify and predict the adsorption of III/V ions (In\textsuperscript{III}, Ga\textsuperscript{III}, and As\textsuperscript{V}) onto NPs used in CMP slurries via experiments and surface complexation modeling. (Chapter 8)

7. Investigate whether the adsorption of arsenic ions onto CeO\textsubscript{2} NPs alters the NPs’ surface reactivity. (Chapter 9)

8. Distinguish surface reactivity assays by mechanisms and recommend sophisticated strategy of designing and using the assays properly. (Chapter 10)

11.1.2. Key findings

Chapter 3: Nanoparticle size detection limits by single particle ICP-MS for 40 elements

1. Size detection limits (D\textsubscript{min}) varied remarkably among the 40 elements: Ta, U, Ir, Rh, Th, Ce and Hf show the lowest D\textsubscript{min} values \( \leq 10 \) nm; Bi, W, In, Pb, Pt, Ag, Au, Tl, Pd, Y, Ru, Cd and Sb have D\textsubscript{min} in the range of 11-20 nm; D\textsubscript{min} values of Co, Sr, Sn, Zr, Ba, Te, Mo, Ni, V, Cu, Cr, Mg, Zn, Fe, Al, Li and Ti are located in 21-80 nm; and Se, Ca and Si show high D\textsubscript{min} values greater than 200 nm.

2. D\textsubscript{min} was influenced by the isotopic abundance of the analyte, the background noise in black and the instrument sensitivity specific to the analyte element.

3. Analyses of engineered NPs (Au, Ag and TiO\textsubscript{2}) and natural surface water confirmed the practical minimum detectable sizes are consistent with the evaluated D\textsubscript{min} values.

Chapter 4: Quantitative resolution of nanoparticle sizes using single particle inductively coupled plasma mass spectrometry with the K-means clustering algorithm
1. The K-means algorithm achieved more reliable discrimination of particle signals from background signals than the widely used “n × σ” threshold approach.

2. The K-means clustering algorithm can quantitatively discriminate secondary “impurity-size nanoparticles,” present at fractions of less than 2% by mass, from primary-size nanoparticles with the minimum resolvable size difference between the primary and secondary nanoparticles at ~20 nm.

3. spICP-MS with the K-means clustering algorithm gives more accurate particle size resolution than the dynamic light scattering (DLS).

Chapter 5: A dry powder assay rapidly detects nanoparticles in water via measuring the surface catalytic reactivity

1. The developed dry powder assay rapidly (2 minutes) detected Au NPs in aqueous solutions and gave linear calibration to Au concentration.

2. The assay’s detection limit for 50 nm Au NP was 51 µg/L, which converts to 0.32 m²/m³ as surface area per volume of solution.

3. An electrochemistry guided comprehensive kinetics model led to a mechanistic understanding of the assay’s reactions. Hydrolysis of BH₄⁻ and the back oxidation of leuco-methylene blue (LBM) are side reactions that compete with the reduction of MB.

4. Other than Au NPs, the assay also successfully detected Pd, Pt, and Ag NPs.

Chapter 6: Ferric reducing ability of nanoparticles: a reactivity assay inferring different surface reaction mechanisms
1. Both Au and Ag NPs showed notable surface reducing activity analyzed by the “Ferric Reducing Ability of Nanoparticles (FRAN)” assay, but different $\text{Fe}^{\text{II}}$ formation kinetics profiles in the assay.

2. Kinetics modeling revealed that in the assay’s reaction with Au NPs, an “electrode reaction mechanism” was followed, whereas in the reaction with Ag NPs, a “direct oxidation mechanism” was followed.

Chapter 7: Control of nanoparticles used in chemical mechanical polishing/planarization slurries during on-site industrial and municipal biological wastewater treatment

1. Simulation of calcium-based softening process showed greater than 90% removal efficiencies were achieved for $\text{SiO}_2$, $\text{CeO}_2$, and $\text{Al}_2\text{O}_3$ NPs used in CMP slurries, providing high pH conditions (e.g., >9).

2. Simulation of biomass adsorption process showed about 60-80% of NPs used in CMP slurries can be removed.

3. spICP-MS effectively characterized $\text{CeO}_2$ and $\text{Al}_2\text{O}_3$ NPs used in CMP slurries, but met challenges for $\text{SiO}_2$ NPs due to the high size detection limit for Si.

Chapter 8: Adsorption of III/V ions (In(III), Ga(III) and As(V)) to $\text{SiO}_2$, $\text{CeO}_2$ and $\text{Al}_2\text{O}_3$ nanoparticles used in the semiconductor industry

1. Across a range of pH levels, appreciable adsorptions occurred for: In(III) ion to $\text{CeO}_2$ or $\text{Al}_2\text{O}_3$ NPs; Ga(III) ion to C-$\text{SiO}_2$, F-$\text{SiO}_2$, $\text{CeO}_2$ or $\text{Al}_2\text{O}_3$ NPs; and As(V) ion to $\text{CeO}_2$ or $\text{Al}_2\text{O}_3$ NPs.
2. Surface complexation model could validate the experimental of III/V ion adsorption onto CMP NPs under different pH conditions.

3. Surface complexation model incorporated with NP size parameters showed that size increase caused surface site density (SSD) to increase but specific surface area (SSA) to decrease.

Chapter 9: Adsorption of As(III) and As(V) ions onto CeO$_2$ nanoparticles: implications to surface redox properties and reactivity

1. Both As(III) and As(V) ions were adsorbed on to CeO$_2$ NP surfaces, but they had different adsorption energies and capacities.

2. The Ferric Reducing Ability for Nanoparticles (FRAN) assay analysis suggested that the adsorption of As(III) and As(V) ions mitigated the reducing activity of CeO$_2$ NPs.

3. The adsorbed As(III) ions filled the Ce 4f orbitals, caused the conversion of Ce$^{IV}$ to Ce$^{III}$ on the NP surface, and thus reduced the electron mobility in the NP solid phase.

4. The adsorption of As(III) and As(V) ions also mitigated CeO$_2$ NPs’ reactivity to generate hydroxyl (HO) radical when interacting with H$_2$O$_2$.

Chapter 10: Synthesis: assessing surface reactivity of nanoparticles in water: much more than reading a number

1. Four different reactivity assays, including the BHMB, the FRAN, the EPR, and the UVMB, gave different reactivity orders of five NP species (Au, Ag, SiO$_2$, CeO$_2$, and TiO$_2$).

2. Different reactivity assays probed different types of NP surface reactivity.
3. Different reactivity assays are based on different “probing schemes” and different reaction pathways.

4. An assay based on the simplest reaction pathway most efficiently assesses the NP surface reactivity.

5. Most reactivity assays have side reactions coupled with the functioning reaction(s) when they are performed on NPs, causing interface that is possibly excluded by modeling strategies.

11.2. Conclusions

Detection of engineered NPs in natural waters is still challenged by the small sizes and low concentrations. spICP-MS held the promises in the past decade to address the challenges via in-situ detecting metallic NPs at low concentrations in water, but still faced issues to new NP species other than common standards (e.g., Au and Ag), which were frequently used for methodology development. This study provided an overarching guidance on the feasibility of this technique to a broad range of NP species via estimating the size detection limit (D_{min}) of 40 metallic elements. The result forecast elements for which spICP-MS would have an advantage owing to their low D_{min}, e.g., Ce and U. Meanwhile it pointed out the challenges posed by high D_{min} for a few common species, e.g., Ti, Ca and Si. These overall guided the future work on spICP-MS with the technique’s potentials and current limitations. Besides pursuing instrumentation revolutions, this study improved spICP-MS by applying a new signal processing method. The application of the K-means Clustering Algorithm discriminated the particle signals from the background noise more precisely than the traditional method, and quantitatively and sensitively resolved NP sizes. Apart from advanced instrument like spICP-MS, a
simple, rapid and economic powder assay based on NP surface catalysis was develop to achieve detection of surface-reactive NPs in water. A big advantage of this assay is its high sensitivity to small size NPs because of their larger surface area per mass. It was predicted that the assay could detect 1 nm Au NPs at 1 ppb in water.

This study greatly focused on the surface reactivity of NPs in water, because surface reactivity is closely associated with the use, fate, and toxicity of NPs. I revealed the alteration of surface reactivity of CeO$_2$ NPs, which are tremendously used by the semiconductor industry for wafer polishing, after they adsorbed arsenic ions on their surfaces. The reactivity alteration was explained via the change of surface electronic structure caused by the arsenic adsorption. Adsorption of ions onto surface is one of the most common surface transformations that may occur to engineered NPs when they enter water media. Indeed, I also confirmed the adsorption of typical III/V ions (Ga$^{III}$, In$^{III}$ and As$^{V}$), which may occur in the semiconductor wastewater, onto industrial CeO$_2$ and Al$_2$O$_3$ NPs. Surface complexation models were developed the NP-ion interaction under other conditions.

Even though enourmal surface reactivity assays are in market and used in various research activities, most of their outputs are in a manner of a single instrument reading and used without adequate knowledge of their meanings. I revealed the different reactivity types (including the catalytic, the redox, and the photo-reactivity) probed by different assays depending on the assay reaction mechanisms. Kinetics modeling enabled the identification of a specific reaction mechanism via decoupling multiple reactions in a system and simulating surface reactions. I generalized the principles of designing the probe in a chemical assays and recommended that, in order to achieve precise surface
reactivity measures, the reaction pathways of an assay should be comprehensively analyzed and adequate data should be acquired for identifying the reaction mechanism.

11.3. Future recommendations

I recommend future work on improving the spICP-MS technique should explore the applications on broader NPs species than frequently used standards (e.g., Au and Ag). As this study has laid out a explicit guidance of estimated size detection limit for 40 metallic elements, future work can test certain NP species which have been estimated to have low $D_{\text{min}}$ or focus on tackling the challegens of high $D_{\text{min}}$ for ubiquitous NP species, such as SiO$_2$, and CaO. The practical application of spICP-MS will be massively extended if it can be tested with more NP species.

To explore more rapid and easy-to-use sensing tools for NP detection, NP surface reactivity can be focused to design effective NP sensors (i.e., detection assays). Suggested by this study, a full understanding of the surface reactions, e.g., heterogeneous catalysis, can facilitate the design of such sensors. Future research toward this direction can thus pursue effective combination of theoretical and experimental approaches, using theories to guide the technology.

The principle of combining theories and experiments is also applicable to the development of NP surface reactivity assays. Future work on developing and using NP surface reactivity assays should consider the different reactivity types, which are in correspondence to the reaction mechanisms. The actual probing target and side interfering reactions in an assay’s performance should be examined in order to draw precise conclusions on what is actually implicated by the assay’s outcome. For assays designed for measuring reactive oxygen species (ROS), future developers or users should lay out
the exact reaction scheme and condition constrains that are required for validated measurement. To precisely and directly indicate NP surface reactivity, assays should be designed with the simplest reaction scheme or provided with modeling strategy to decouple the reaction complexity.

Many studies on assessing the safety of engineered nanomaterials worked with standard nanomaterial samples, I call for the attention to potential surface transformation that may occur when they enter different water matrices. Adsorption of ions or other molecules can commonly take place in natural waters. Future studies should examine the impact of such surface transformation on the nanomaterials’ risk potentials. A key research strategy would be examining the mechanistic interrelationship within the scheme of “property-reactivity-toxicity” (Figure 10.4). Research work should be laid out in a hierarchy according to this scheme, toward understanding the causality among different components.
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