ANALYSIS OF ENGINEERED NANOMATERIALS IN THE ENVIRONMENT

by

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ABSTRACT

With increasing incorporation of engineered nanoparticles (NPs) into consumer products, there is concern that these materials will be released to the environment with unknown ecological effects. Methods for detection and characterization of these materials at environmentally relevant concentrations are crucial to understanding this potential risk. A relatively new method, single particle inductively coupled plasma mass spectrometry (spICPMS), was applied to analysis of metal oxide NPs such as ZnO, CeO2, and TiO2, as well as silver nanowires and carbon nanotubes.

A lack of nanoparticulate “pulses” in spICPMS analysis of nano-ZnO led to a study on ZnO NP solubility in a variety of matrices. Dissolution of nano-ZnO was observed in nanopure water (7.18 – 7.40 mg/L dissolved Zn, as measured by filtration) and Roswell Park Memorial Institute medium (RPMI-1640) (~5 mg/L), but much more dissolution was observed in Dulbecco’s Modified Eagle’s Medium (DMEM), where the dissolved Zn concentration exceeded 34 mg/L. These results suggest that solution chemistry exerts a strong influence on ZnO NP dissolution and can result in limits on zinc solubility due to precipitation of less soluble solid phases.

Detection and sizing of metal-containing NPs was achieved at concentrations predicted for environmental samples (part-per trillion levels) using spICPMS. Sizing of silver nanowires, titanium dioxide and cerium oxide NPs was done by correlating ICP-MS response (pulses) from NPs entering the plasma to mass of metal in dissolved standards. The ratio of NP pulse detections to the total number of readings during analysis was optimized at 2.5% or less to minimize coincident pulses while still allowing definition of a size distribution.
Detection of single walled carbon nanotubes (CNTs) was performed using spICPMS. This study focuses on using trace catalytic metal nanoparticles intercalated in the CNT structure as proxies for the nanotubes. The small, variable, amount of trace metal in each CNT makes separation from instrumental background challenging, and multiple approaches to this problem were attempted. To highlight the potential of spICPMS in environmental studies the release of CNTs from polymer nanocomposites into solution was monitored, showcasing the technique’s ability to detect changes in released CNT concentrations as a function of CNT loading.
# TABLE OF CONTENTS

ABSTRACT ................................................................................................................................... iii

LIST OF FIGURES ..................................................................................................................... viii

LIST OF TABLES ....................................................................................................................... xi

LIST OF ABBREVIATIONS ....................................................................................................... xii

ACKNOWLEDGMENTS ............................................................................................................. xv

DEDICATION ............................................................................................................................. xvi

CHAPTER 1: INTRODUCTION

1.1 Consumer products containing NPs ................................................................. 1

1.2 Release scenarios for NPs ............................................................................. 2

1.3 Potential for ecotoxicity .............................................................................. 3

1.4 The need for advancements in nanometrology ........................................ 4

1.5 Thesis Organization .................................................................................. 6

Chapter 2: SOLUBILITY OF NANO-ZNO IN ENVIRONMENTALLY AND BIOLOGICALLY IMPORTANT MATRICES

2.1 Introduction ........................................................................................................ 8

2.2 Experimental .................................................................................................... 10

   2.2.1 Materials ................................................................................................. 10

   2.2.2 Characterization of NPs ........................................................................ 10

   2.2.3 Dissolution experiments ........................................................................ 13

   2.2.4 Equilibrium modeling .......................................................................... 15

   2.2.5 Ultrafiltration experiment .................................................................... 15

2.3 Results and Discussion ................................................................................ 16
2.3.1 Dissolution in nanopure and moderately hard water ...........................................16
2.3.2 Dissolution in cell culture media .................................................................20
2.3.3 Effect of temperature on dissolution .........................................................23
2.3.4 Separation of zinc species by ultrafiltration .............................................26

Chapter 3: OVERCOMING CHALLENGES IN ANALYSIS OF POLYDISPERSE METAL-
CONTAINING NANOPARTICLES BY SINGLE PARTICLE INDUCTIVELY
COUPLED PLASMA MASS SPECTROMETRY

3.1 Introduction ......................................................................................................29
3.2 Experimental ....................................................................................................32
  3.2.1 Materials ........................................................................................................32
  3.2.2 Characterization of NPs ....................................................................................33
  3.2.3 Sedimentation field-flow fractionation (SdFFF) .............................................36
  3.2.4 ICP-MS experimental details ........................................................................37
3.3 Results and Discussion ......................................................................................40
  3.3.1 Real-time data and analyte parameters .........................................................40
  3.3.2 Frequency counts of data for polydisperse metal-containing NPs..................42
  3.3.3 NP size calculation approaches .....................................................................43
  3.3.4 spICPMS size distributions of NPs .................................................................48
  3.3.5 Challenges in analyzing polydisperse NPs and coincidence of pulses ..........53

Chapter 4: DETECTION OF SINGLE WALLED CARBON NANOTUBES BY MONITORING 
EMBEDDED METAL NANOPARTICLES

4.1 Introduction ......................................................................................................58
4.2 Experimental ....................................................................................................61
  4.2.1 Preparation of CNT suspensions .................................................................61
  4.2.2 Characterization of CNTs ..............................................................................62
4.2.3 Comparison of spICPMS and NTA .................................................................63
4.2.4 Nanocomposite preparation for CNT release study .................................64
4.3 Results and discussion ..................................................................................65
  4.3.1 CNT characteristics ..................................................................................65
  4.3.2 Choice of element proxy ........................................................................66
  4.3.3 CNT pulse cut-off criteria ........................................................................69
  4.3.4 Quantification of CNT number concentration by spICPMS .....................74
  4.3.5 Comparison of spICPMS and NTA for CNT quantification .....................77
  4.3.6 Mass balance of analyte metal for varying CNT concentrations ..........80
  4.3.7 Detection of CNTs released from a polymer matrix ...............................86

Chapter 5: CONCLUSIONS
  5.1 Implications of results ..................................................................................91
  5.2 Quantification of ZnO NP dissolution ..........................................................91
  5.3 Use of spICPMS for analysis of polydisperse NPs ......................................92
  5.4 Detection of CNTs using spICPMS ..............................................................95
  5.5 Use of nanometrology in assessment of risk arising from NPs in the environment .................................................97

REFERENCES CITED ..........................................................................................98
APPENDIX ........................................................................................................107
LIST OF FIGURES

Figure 1.1 Number of listed products containing engineered NPs.................................1
Figure 1.2 Most commonly used NPs in consumer products, listed by element .............2
Figure 1.3 Traditional ICPMS vs single particle ICPMS analysis...............................5
Figure 2.1 SEM and TEM images of ZnO particles.......................................................12
Figure 2.2 Long-term dissolution of 100 mg/L bulk and nano ZnO in nanopure water and moderately hard water .................................................................16
Figure 2.3 EDS spectrum of precipitates formed in moderately hard water after addition of 80 mg/L Zn .........................................................................................19
Figure 2.4 Long-term dissolution of 100 mg/L bulk and nano ZnO (Alfa Aesar 44904) in DMEM and RPMI.................................................................20
Figure 2.5 EDS spectrum of precipitates formed in RPMI after addition of 80 mg/L Zn ....22
Figure 2.6 Dissolution of 100 mg/L ZnO NPs (Alfa Aesar 44904, consortium) in DMEM and RPMI after 0 and 24 h at both 20 and 37 °C.................................23
Figure 2.7 Dissolution of 10 mg/L ZnO NPs (Alfa Aesar 44904, consortium) in DMEM and RPMI after 0 and 24 h at both 20 and 37 °C.................................25
Figure 2.8 Particulate and dissolved zinc concentrations in various matrices at 20°C after 0 and 24 h using ~10 mg/L Alfa Aesar 44904 and consortium ZnO NPs ..........27
Figure 2.9 Permeate concentrations for Alfa Aesar 44904 and Consortium ZnO filtered with five ultrafilter membrane pore sizes at zero and 24 hours in nanopure water ....28
Figure 3.1 BBI Au NPs TEM image.............................................................................34
Figure 3.2 Inframat CeO2 NPs TEM image.................................................................34
Figure 3.3 Aldrich TiO2 NPs TEM image ..................................................................35
Figure 3.4 NanoComposix Ag nanowires TEM image ..............................................35
Figure 3.5 Time-resolved data for four NPs at 200 ppt in DI water .............................40
Figure 3.6 Number of NP pulse events from spICPMS analysis for TiO2, CeO2, and Ag nanowire NPs.................................................................43
Figure 3.7 Observed number of pulses vs. concentration for four separate analyses of Nanocomposix 100 nm Au NPs.

Figure 3.8 Size distributions for Aldrich TiO₂ and Inframat CeO₂ as determined by spICPMS and SdFFF analyses.

Figure 3.9 Manufacturer size distribution for Ag nanowires.

Figure 3.10 Length distributions for Ag nanowires at four different cross-sectional diameters.

Figure 3.11 The effect of NP concentration on size distribution for monodisperse (Au) and polydisperse (TiO₂ and Ag nanowires) systems in DI water.

Figure 3.12 Combinations of size distribution data for 60 ppt TiO₂.

Figure 4.1 Picture of chitosan and chitosan/SWNT nanocomposite.

Figure 4.2 spICPMS response data, for determination of the best analyte metal for CNTs used in this study.

Figure 4.3 Data for ICP-MS response from Figure 4.2, binned to show distribution of pulses above background.

Figure 4.4 Comparison of the spICPMS response for DI blanks and CNT supernatant.

Figure 4.5 Comparison of DI blank and 5 ng/L Southwest Nanotechnologies CNTs.

Figure 4.6 Relationship between CNT mass concentration (x) and number of CNT detection events (y) for NanoAmor CNTs.

Figure 4.7 Particle number concentrations as measured by NTA and spICPMS for three types of CNTs.

Figure 4.8 Ratio of measured to nominal metal mass for Carbon Solutions (yttrium) CNT at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to dissolved Y.

Figure 4.9 Ratio of measured to nominal metal mass for Carbon Solutions (yttrium) CNT at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to particulate Y.

Figure 4.10 Ratio of measured to nominal metal mass for NanoAmor (cobalt) CNT at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to dissolved Co.
Figure 4.11  Ratio of measured to nominal metal mass for NanoAmor (cobalt) CNT at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to particulate Co .................................................................84

Figure 4.12  Ratio of measured to nominal metal mass for Southwest (cobalt) CNTs at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to dissolved Co ...........................................................................................................85

Figure 4.13  Ratio of measured to nominal metal mass for Southwest (cobalt) CNTs at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to particulate Co .........................................................................................................86

Figure 4.14  Examination of the effect of loading on CNT release from CNT-chitosan nanocomposites ..............................................................................................................................89
# LIST OF TABLES

| Table 2.1 | Characterization of bulk and nanoparticulate ZnO by SEM, DLS, and zeta potential measurements ........................................................................................................11 |
| Table 2.2 | Concentrations of inorganic constituents in aqueous matrices: moderately hard water, DMEM (27), and RPMI ........................................................................................................13 |
| Table 2.3 | Linear model data fitting parameters for long-term dissolution of bulk ZnO in various matrices ................................................................................................................17 |
| Table 2.4 | Linear model data fitting parameters for long-term dissolution of Alfa Aesar 44904 nano-ZnO in various matrices ........................................................................................................17 |
| Table 2.5 | Linear model data fitting parameters for long-term dissolution of Alfa Aesar 45409 nano-ZnO in various matrices ........................................................................................................17 |
| Table 2.6 | Linear model data fitting parameters for long-term dissolution of Alfa Aesar 45588 nano-ZnO in various matrices ........................................................................................................17 |
| Table 2.7 | Visual MINTEQ simulation results for saturation indices of Zn-containing solids in DI, moderately hard water, DMEM, and RPMI ..........................................................................................18 |
| Table 3.1 | Characterization of NPs by dynamic light scattering and zeta potential ..............................................................................................................................33 |
| Table 3.2 | SdFFF channels dimensions ..................................................................................................................36 |
| Table 3.3 | Run parameters used to analyze TiO2 and CeO2 nanoparticle suspensions ..................................................................................................................37 |
| Table 3.4 | Instrument transport efficiency from comparing observed pulse number and theoretical particle number for Au NPs .............................................................................................44 |
| Table 4.1 | Size characterization and percent metal for all CNTs used ..................................................................................66 |
| Table 4.2 | Examination of different cut off criteria for differentiating CNT detection events from background signal ..................................................................................................................73 |
LIST OF ABBREVIATIONS USED

Bovine serum albumin .................................................................................................................. BSA
Brunauer-Emmett-Teller ............................................................................................................. BET
British Biocell, International .................................................................................................... BBI
Calcium carbonate ..................................................................................................................... CaCO₃
Carbon nanotube ...................................................................................................................... CNT
Celsius ......................................................................................................................................... °C
Cerium oxide ............................................................................................................................. CeO₂
Copper Kα.................................................................................................................................. Cu Kα
Cubic centimeter ....................................................................................................................... cm³
Dulbecco’s Modified Eagle’s Medium ................................................................................... DMEM
Dynamic light scattering ........................................................................................................... DLS
Energy dispersive x-ray spectroscopy ..................................................................................... EDS
Environmental Protection Agency ........................................................................................... EPA
Field-flow fractionation ............................................................................................................. FFF
Gravitational constant ............................................................................................................. G
Hour .......................................................................................................................................... h
Hydrochloric acid ..................................................................................................................... HCl
Hydrodynamic chromatography .............................................................................................. HDC
Inductively coupled plasma mass spectrometry ................................................................ ICP-MS
Inductively coupled plasma atomic emission spectroscopy ................................................ ICP-AES
Kilodalton ................................................................................................................................ kDa
Kilovolt ...................................................................................................................................... kV
Time

Titanium dioxide

Transmission electron microscopy

X-ray diffraction

Zinc carbonate

Zinc chloride

Zinc oxide
Acknowledgment

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Dedication

I dedicate this thesis to my parents, Bruce and Cathy Reed. My dad Bruce died in 2011 and I wish he could have been here to see me earn my doctorate. He was a great dad and I learned a lot from him. My mom has been loving and supportive my whole life, and always told me I could achieve anything I wanted to. She takes every opportunity to tell me I am a great writer – and she is happy to hear that people I have worked with share this view. My ability to write this thesis comes from my parents’ time spent reading to me as a child and instilling me with a love of reading and learning. Thank you both for everything you have given me in life!
CHAPTER 1
INTRODUCTION

1.1 Consumer products containing NPs

The special properties of NPs due to greater specific surface area and/or quantum confinement have made them very attractive for a variety of applications. Thus, there has been a large increase in consumer products listed as containing NPs in the last decade (Figure 1.1).

![Graph showing the number of listed products containing engineered NPs from 2005 to 2010. The number of products listed increases significantly from 2005 to 2010.](image)

Figure 1.1. Number of listed products containing engineered NPs. Adapted from Project on Emerging Nanotechnologies (1)

The NPs used in products cover a range of elemental composition, with silver, carbon, gold, and various metal oxides being the most common (Figure 1.2).

Silver is used most commonly for anti-microbial purposes, and can be found in bandages, sprays, and even drinks (1). CNTs are often touted as being a revolutionary new material with incredible tensile strength and conductivity, and are increasingly incorporated into polymers, bicycle frames, and tennis racquets (1, 2). Metal oxides are used for a variety of applications:
silica NPs are used as thickeners, anti-caking agents, and photocatalysts \((1, 3)\). Zinc and titanium oxide NPs are both used in sunscreens \((1)\), and titanium dioxide is used widely as a whitener in both food and personal care products \((4)\).

### 1.2 Release scenarios for NPs

With this increase in production of NPs comes an elevated probability of their release to the environment through manufacture, use, and disposal of consumer goods. Several studies have predicted probable environmental concentrations of various types of NPs using material flow analysis \((5-7)\). These studies typically predict that NP concentrations in various environmental compartments will be at or below the ppt range in surface waters, soils, and sediments. After incorporation into consumer products, regular use is likely to result in weathering and release of NPs to the environment \((8)\). After reaching the environment, a variety of transformations may occur which modify the original NP material, such as dissolution, adsorption to other NPs.
(homoaggregation) or natural particles (heteroaggregation), loss of original coating material and/or sorption of natural organic matter coating (8). Simulated laboratory weathering of nanocomposites showed CNTs protruding from the material as well as detectable release of CNTs, due to chemical degradation and mechanical stress (9). These processes may all occur after release and make detection and characterization of NPs in the environment a complicated task.

1.3 Potential for ecotoxicity

Although NPs are mostly not thought to pose a threat to human health (with a possible exception of CNT inhalation resulting in asbestos-like toxicity (10-12)), there are numerous studies showing increased toxicity to cells and microorganisms relative to larger particles of the same elemental composition. As silver NPs are incorporated into the most products specifically for their antimicrobial properties, they have been widely studied in toxicity tests to *Daphnia magna* (13-16) as well as human glioblastoma and lung fibroblast cells (17). The potential hazard of CNTs has been studied for pulmonary effects in mice (18), possible toxic effects of residual synthesis catalyst metal (19), and manufacturing workplace exposure to humans (20). Metal oxide NPs containing zinc, titanium, cerium, etc, have also been studied with regard to cell and animal toxicity in a wide range of studies (15, 21-29). The majority of studies find that NPs frequently have a greater toxic effect than corresponding larger particles with sizes on the order of microns. Although reasons for increased toxicity are often postulated (ROS production (21), dissolution to toxic metal ion (30-32)), the toxicity may vary greatly between different types of NPs. With the potential for increasing release to the environment and subsequent ecological impact, the demand for suitable detection methods is growing as well.
1.4 The need for advancements in nanometrology

The predicted environmental concentrations of NPs in the ppt range or below provide the need for advanced methods capable of detecting extremely small quantities of these materials. Many commonly used techniques for particle detection, quantification, and characterization have detection limits much higher, in the ppm or higher range.

Electron microscopy methods such as TEM and SEM are excellent for providing characterization information on NPs, such as size, shape, and aspect ratio. These techniques can be coupled with EDS to obtain NP elemental composition as well. However, using these techniques to detect NPs at concentrations predicted in the environment would be prohibitively time-consuming, as the instrument user could spend hours to find a single particle in the microscope view.

DLS is a widely used technique for determining size distributions of particles, and is inexpensive and high throughput. However it requires concentrations at the ppm level or above, which makes it unsuitable for environmental analysis. In addition, the presence of a small fraction of large particles or aggregates can greatly skew the size distribution towards larger sizes, making it unreliable for environmental samples where transformations such as dissolution, aggregation, and adsorption of NPs is likely to create highly polydisperse size distributions (8).

The family of FFF techniques such as flow FFF and sedimentation FFF provide high resolution separation of a wide range of particle sizes and can generate size distributions via coupling to a variety of detectors. Use of ICP-MS as a detector provides elemental composition and ppt detection limits; however dilution in the FFF channel typically results in method detection limits in the ppb range. Sample pre-concentration can be used to improve this, however, making FFF-ICP-MS a potentially powerful technique for environmental analysis of
NPs. One drawback is sample run time: some types of FFF analyses, such as sedimentation FFF, can exceed one hour, making it potentially time-consuming for analysis of a large number of samples.

A technique that has recently seen a large increase in development and use for environmental detection of NPs is spICPMS. This technique uses an instrument present in most analytical labs, the ICP-MS, so little to no new instrumentation or equipment is needed for labs already containing one. It works by setting the dwell, or reading, time to 10 ms or less so the signal from only a single particle will be captured at a time. Figure 1.3 shows a schematic representation of the technique results and how it differs from a traditional ICP-MS analysis.

![Diagram](image)

Figure 1.3. Top half of diagram shows ICP-MS response for traditional analysis of dissolved metal, with relatively constant signal output. Lower half shows how spICPMS works by collecting data over very short dwell, or reading, times which may contain single NPs.
Chapter 3 describes in more detail the spICPMS method, including the equations for interpreting data and developing size distributions.

1.5 Thesis organization

This chapter is meant to give a brief overview of the current state of science with regard to NP incorporation into products, potential release to the environment, and motivation for developing methods for their detection, characterization, and quantification.

As NP stability/solubility is highly important for determining both environmental fate and potential toxicity, Chapter 2 details work performed on quantifying the dissolution of ZnO NPs in a variety of matrices. The original hypothesis was that ZnO NPs could be detected by spICPMS analysis. Attempts at spICPMS analysis of ZnO NPs merely showed elevated background signal indicative of particle dissolution. This provided the basis for the further study of ZnO solubility, with the hypothesis that ZnO NP dissolution rate and extent would vary with matrix as well as temperature. ZnO NPs are often cited as being toxic, possibly due to particle solubility. However previous studies have not thoroughly quantified this phenomenon, likely because they were only concerned with the toxicity results and not the NP stability. ZnO NP dissolution was analyzed in matrices relevant to the environment such as synthetic moderately hard water, and in matrices relevant to cellular toxicity testing such as DMEM and RPMI.

Chapter 3 deals with use of spICPMS in detection and characterization of NPs with non-uniform size distributions. The hypothesis of this study was that non-uniform NPs with a broad size distribution could be sized accurately with comparison to other characterization techniques, as this had not been demonstrated previously. Materials such as TiO$_2$, CeO$_2$, and Ag nanowires were detected and sized using this method, with results comparable to other methods such as
SdFFF and TEM. Recommendations on particle concentration ranges for spICPMS are made as well, to develop accurate size distributions.

The detection of CNTs using residual catalyst metals is the focus of Chapter 4. It is very challenging to detect CNTs in the environment due to the carbon background from cells, detritus, and in sediments and soils. The hypothesis was that detection of catalyst metals uncommon in the environment such as cobalt or yttrium could be used as a proxy for the CNTs. A CNT-nanocomposite was made and used for a product release scenario, with CNT release quantified and scaling with mass loading in the composite material.
CHAPTER 2
SOLUBILITY OF NANO-ZNO IN ENVIRONMENTALLY AND BIOLOGICALLY IMPORTANT MATRICES


2.1 Introduction

ZnO NPs have found use in a variety of products and applications such as semiconductors, catalysts, paints, and are increasingly found in consumer products such as sunscreen due to ZnO’s strong ultraviolet light absorption properties (33). Increased production and use of ZnO NPs suggests increased exposure potential for humans and other organisms. Although studies quantifying exposures of humans to engineered NPs are scarce, it is likely that increased industrial production will lead to human contact and NP exposures (dermal, inhalational, and ingestion) (34). These routes of exposure are well studied for various particles previously termed “sub-micron” in size (35-37), many of which are in the nanometer size regime. These previous risk assessments provide a starting point for analyzing risks associated with engineered NPs, but further studies are needed due to their novel properties.

Release of NPs into the environment (e.g. through wastewater treatment plant effluent) should increase ecosystem exposure, though this is difficult to quantify. Modeling efforts indicate that environmental ZnO NP concentrations may currently be high enough (0.3 µg/L in the U.S.; 0.432 µg/L in Europe) in wastewater treatment plant effluent to pose a toxicological
threat to aquatic organisms (5). Monitoring NP transformations in relevant matrices (38-40) enables an evaluation as to how long particles may persist in environmental or biological systems.

Release of NPs to the environment may create an ecological hazard. ZnO NP toxicity to animals has been studied using in vitro tests (10, 25, 41-43) and in vivo tests (22-24, 26, 44-46). When compared to other NPs, ZnO has often been found to be among the most toxic (10, 21, 22). Although ZnO NPs have been reported to have greater toxicity than dissolved Zn$^{2+}$ alone (43), the general consensus is that these particles dissolve relatively quickly and the Zn$^{2+}$ ion is the primary source of toxicity (22, 23, 25). A recent toxicology study by Poynton et al (47) suggests that both ZnO NPs and Zn$^{2+}$ are toxic, but have different modes of action. George et al (48) showed that iron-doping of ZnO NPs led to reduced dissolution and thereby decreased cytotoxicity, suggesting the possibility for manufacture of “safer” nanomaterials; however another study examining the effects of iron-doped ZnO on bacterial toxicity (49) showed that water chemistry influenced toxicity far more than doping.

With increasing human exposure to these engineered NPs, data reflecting their behavior in systems at physiological temperature and pH become more valuable. As Zn$^{2+}$ released by ZnO NPs is generally accepted as an important contributor to ZnO NP toxicity, it is crucial to quantify both the amount of Zn$^{2+}$ generated from a given dose of NPs as well as the rate of ZnO dissolution to Zn$^{2+}$. Here we compare dissolution of several ZnO NPs with bulk ZnO and a dissolved Zn reference (ZnCl$_2$) in biologically and environmentally relevant matrices. While previous studies have measured the amount of dissolved zinc released by ZnO NPs in simple solutions (23) and in cell culture media used for toxicity studies (43), to our knowledge, the present study is the first to quantify ZnO NP dissolution with multiple particle types and sizes in
a variety of matrices and temperatures. The goal of the present study was to examine how the extent and rate of ZnO NP dissolution varies with matrix, temperature, and particle source. In addition, a methodological comparison between 0.02-μm pore syringe filters and centrifugal ultrafilters was made to determine if one was more effective than the other at separating particulate from dissolved species.

2.2 Experimental

2.2.1 Materials

Zinc oxide particles used include a bulk powder (Alfa Aesar, 99.99% purity by metals basis) and three different aqueous NP suspensions (Alfa Aesar 44904, 45409, and 45588, purity not given by manufacturer). A fourth NP, in powder form, was obtained from the University of California, Los Angeles (UCLA) as a reference material for toxicology studies being concurrently performed by a consortium of research groups at several institutions in the United States. An ionic zinc source (ZnCl₂, reagent grade, Fischer Scientific) was used as a dissolved zinc control.

2.2.2 Characterization of NPs

A Zetasizer Nano ZS (Malvern) was used to determine the hydrodynamic diameter by dynamic light scattering. Samples of 100 mg/L ZnO dispersed in nanopure water (Nanopure Diamond, Barnstead) were analyzed in triplicate and the mean diameter ± standard deviation is provided for each particle in Table 2.1.
Table 2.1. Characterization of bulk and nanoparticulate ZnO by SEM, DLS, and zeta potential measurements. The Alfa Aesar NPs were observed to have polydisperse size distributions by SEM. Error in DLS and zeta potential values is the standard deviation of the mean values reported by the instrument. Additional analysis of all five particles by XRD confirmed materials as ZnO (Zincite).

<table>
<thead>
<tr>
<th>Manufacturer's reported size (nm)</th>
<th>Morphology by SEM</th>
<th>Size by DLS (nm), $n = 3$</th>
<th>Surface charge by Zeta at pH $\approx 8$ (mV), $n = 2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk</td>
<td>Irregular</td>
<td>1920±151</td>
<td>-14.8±1.2</td>
</tr>
<tr>
<td>Alfa Aesar 44904</td>
<td>Irregular Angular</td>
<td>274±8</td>
<td>-18.5±1.2</td>
</tr>
<tr>
<td>Alfa Aesar 45409</td>
<td>Rounded</td>
<td>159±4</td>
<td>-21.6±3.7</td>
</tr>
<tr>
<td>Alfa Aesar 45588</td>
<td>Angular</td>
<td>179±3</td>
<td>-23.4±3.1</td>
</tr>
<tr>
<td>Consortium</td>
<td>Spheroid</td>
<td>215±15</td>
<td>-29.2±1.0</td>
</tr>
</tbody>
</table>

Zeta potential measurements were also performed using the Malvern Zetasizer Nano ZS on 100 mg/L ZnO solutions in nanopure water. The measurements were taken in duplicate and reported as mean ± standard deviation. The pH of the solutions for the zeta potential determinations was measured to be approximately 8.

XRD with a Philips X’pert Pro Diffractometer (PANalytical) was performed to confirm the NPs’ chemical identity as ZnO. The solid powders were placed in sample holders and analyzed with Cu $K_\alpha$ radiation at $2\theta$ angles from 5° to 90°. The aqueous NP suspensions were prepared for analysis by placing a few drops of solution onto glass microscope slides and drying overnight to produce a solid ZnO sample. These were analyzed by the same method as the powders. Analysis of Zn precipitate formed in RPMI was also performed by XRD. A solution of ~80 mg/L Zn from aqueous ZnCl$_2$ stock solution in RPMI was centrifuged at 4000 rpm for 40 min to obtain precipitate. This was dried at 100°C and ground into a powder for XRD analysis.
A JSM-7000F (JEOL) field mission scanning electron microscope (SEM) was used to image the particles for general information on particle size and particle morphology. The samples were analyzed at 2.0 kV and imaged at 45-55,000x magnification, except the larger bulk ZnO, which was imaged at 25,000x. The samples were prepared as 100 mg/L ZnO solutions, spotted and vacuum dried on a 47mm 0.1 μm-pore size polycarbonate filter membrane (Nuclepore). This was then fixed to a sample mount with carbon tape and gold sputter-coated prior to analysis. This instrument was used in concert with EDS (EDAX Genesis Energy) to determine the elemental composition of precipitates formed in moderately hard water and RPMI. Zinc precipitates from moderately hard water and RPMI were collected as described for XRD analysis and fixed to carbon tape on a sample mount for analysis. Blank solutions of moderately hard water and RPMI (no Zn added) were centrifuged under the same conditions to ensure any precipitate observed was not solely a product of the matrix. No precipitates were observed in the blanks. The results of NP characterization are shown in Table 2.1 and Figure 2.1.

Figure 2.1. SEM and TEM images of ZnO particles: A) Bulk; B) Alfa Aesar 44904; C) Alfa Aesar 45409; D) Alfa Aesar 45588; E) Consortium (TEM, supplied by UCLA after synthesis)
2.2.3 Dissolution experiments

A long-term dissolution study was performed to investigate the equilibrium solubility of Zn\(^{2+}\) from dissolving 100 mg/L NP solutions in four matrices: nanopure water, EPA moderately hard water (50), DMEM (D5796, Sigma), and RPMI (R8758, Sigma) cell culture media. These media were chosen because of their use in toxicity studies and their differing constituents which may control solubility, particularly phosphate (0.906 mM in DMEM and 5.64 mM in RPMI; both buffered at pH 7.3; Table 2.2 for full constituent list).

Table 2.2. Concentrations of inorganic constituents in aqueous matrices: moderately hard water, DMEM (51), and RPMI (51). DMEM and RPMI matrices contain NaHCO\(_3\) in addition to constituents listed in references.

<table>
<thead>
<tr>
<th></th>
<th>Moderately hard water (mg/L)</th>
<th>DMEM (mg/L)</th>
<th>RPMI (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO(_3)</td>
<td>96</td>
<td>3700</td>
<td>2000</td>
</tr>
<tr>
<td>CaSO(_4)(\cdot)2H(_2)O</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KCl</td>
<td>4</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>MgSO(_4)</td>
<td>60</td>
<td>97.67</td>
<td>48.84</td>
</tr>
<tr>
<td>Ca(NO(_3))(\cdot)4H(_2)O</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>MgSO(_4)(\cdot)7H(_2)O</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>6400</td>
<td>6000</td>
<td></td>
</tr>
<tr>
<td>Na(_2)PO(_4)</td>
<td></td>
<td>800</td>
<td></td>
</tr>
<tr>
<td>Na(_2)HPO(_4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na(_2)PO(_4)(\cdot)(_2)H(_2)O</td>
<td>125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl(_2)</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe(NO(_3))(_3)(\cdot)9H(_2)O</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Additional matrices were created by adding 2 mg/ml BSA to DMEM and RPMI solutions to investigate possible effects of protein presence on NP dissolution. ZnO samples were prepared in 500 ml high-density polyethylene bottles which were first placed in a 5% HNO\(_3\) (Fischer Scientific) acid bath overnight and rinsed thoroughly with nanopure water to prevent residual zinc contamination. Preparation and filtration of solutions were performed in a filtered-air
laminar flow hood to prevent both zinc and microbial contamination. Care was taken to avoid touching bottle interiors while sampling; samples were poured directly into syringes, with no pipet tips being placed into bottles. No microbial growth was observed over the course of the experiment. All bottles were sealed at all times except when sampling. Between subsequent timepoints, all bottles were placed vertically on a shaker table running at approximately 80 RPM to prevent settling of NPs.

Background levels of zinc in the matrices were determined by filtering and analyzing a sample of each matrix prior to adding ZnO particles. Addition of ZnO to a bottle was followed by inversion to mix the solution and immediate collection and filtration of a subsample to obtain a $t = 0$ h value for dissolved $\text{Zn}^{2+}$. Syringe filters with 0.02 μm pores (Anotop 25mm, Whatman) were used to separate dissolved $\text{Zn}^{2+}$ from ZnO NPs. Dissolved $\text{Zn}^{2+}$ concentration was determined by ICP-MS (Perkin Elmer Elan 6100) and ICP-AES (Perkin Elmer Optima 5300 DV) after acidification to 2% HNO$_3$. Mixed-metal standards were used for instrument calibration prior to each analysis, and check standards were analyzed at the beginning and end of each run as well as after every ten samples for quality control. To calculate NP dissolution rates, long-term dissolution data for dissolved Zn vs. time were fit to linear models of the form $[\text{Zn}_{\text{dissolved}}] = \text{slope} \times \text{time} + \text{intercept}$ using OriginPro 8.1.

In addition to 100 mg/L ZnO, a particle concentration more relevant to toxicity exposure tests (10, 21, 24, 52, 53), 10 mg/L, was prepared for short-term dissolution studies in cell culture media. The effects of temperature on dissolution were also examined. Solutions of DMEM and RPMI were maintained at room temperature (20°C) and human body temperature (37°C) during dissolution of 100 mg/L and 10 mg/L ZnO (Alfa Aesar 44904 and consortium) and ZnCl$_2$. After addition of the zinc, the solution was inverted twice and a subsample collected and filtered.
immediately with Anotop 0.02-μm pore filters. The solutions were stored overnight on shaker
tables at both room temperature and in an incubator at 37°C and filtered again at 24 h. Zinc
concentration in the filtrate was determined by ICP-AES (Perkin Elmer Optima 5300 DV).

2.2.4 Equilibrium modeling

Chemical equilibrium modeling using Visual MINTEQ Version 3.0 was performed to
predict if Zn-containing precipitates would be formed after NP dissolution to Zn^{2+} in moderately
hard water and RPMI. The inorganic constituents of each matrix were entered into the model and
each used to calculate saturation indices in the presence of 8 and 80 mg/L Zn^{2+} (for 10 and 100
mg/L ZnO, respectively).

2.2.5 Ultrafiltration experiment

Complementary short-term dissolution experiments were performed using centrifugal
ultrafilters (Amicon Ultra, Millipore) to separate particles from ionic Zn^{2+}. Alfa Aesar 44904 and
consortium NPs were used to compare separation effectiveness of the 0.02-μm pore syringe and
centrifugal filters. For centrifugal filtration experiments: NP solutions were made in nanopure
water, EPA moderately hard water, DMEM, and RPMI at a concentration of 10 mg/L ZnO and
tested separately with 100 kDa membranes. ZnCl_{2} solutions at a concentration of 8.37 mg/L
(0.12 mM Zn, the same as the NP solutions) were made in the same matrices as a test for Zn
precipitation. Ten ml of NP suspension or ZnCl_{2} solution were added to the filtration device and
centrifuged for 30 min at 5000 g. The permeate was collected and saved for analysis. Nanopure
water was then added to the concentrate side of the filtration device and shaken by hand to
resuspend NPs collected on the membrane. The resuspended concentrate was weighed and
analyzed at the same time as the permeate. Analysis of dissolved zinc was performed by ICP-AES (iCAP 6300 Duo, Thermo) after acidification with 3% HNO₃. Because resuspension of the NPs from the centrifugal filter membrane allowed a systematic calculation of recovery, the rejection data complemented the filtrate concentration data. This approach to obtaining a mass balance was not possible with the sealed syringe filters, where sample handling for recovery determination was not feasible. In addition, five different ultrafilter MWCO (3, 10, 30, 50, and 100 kDa) were compared for separation effectiveness using Alfa Aesar 44904 and consortium NPs.

2.3 Results and Discussion

2.3.1 Dissolution in nanopure and moderately hard water

Quantification of Zn²⁺ following 0.02-μm pore filtration showed similar degrees of dissolution for each of the three Alfa Aesar ZnO particles in nanopure water, with all three solutions exceeding 7 mg/L of Zn²⁺ after 810 h (Figure 2.2a).

Figure 2.2. Long-term dissolution of 100 mg/L bulk and nano ZnO (Alfa Aesar 44904, 45409, and 45588) in nanopure water (a) and moderately hard water (b). Y-axis for all graphs is Zn concentration in filtrate. Error bars are 95% confidence intervals.
Comparing rates of particle dissolution, the bulk ZnO (5.56 $\mu$g L$^{-1}$ hr$^{-1}$) was slower to
dissolve than all three NPs (7.43 to 8.48 $\mu$g L$^{-1}$ hr$^{-1}$) (Tables 2.3-2.6).

Table 2.3. Linear model data fitting parameters for long-term dissolution of bulk ZnO in various matrices.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Slope ($\mu$g L$^{-1}$ hr$^{-1}$)</th>
<th>Intercept (mg L$^{-1}$)</th>
<th>Adjusted R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanopure</td>
<td>5.56</td>
<td>0.106</td>
<td>0.981</td>
</tr>
<tr>
<td>Mod. Hard</td>
<td>-0.0120</td>
<td>0.00762</td>
<td>0.190</td>
</tr>
<tr>
<td>DMEM</td>
<td>1.26</td>
<td>3.65</td>
<td>0.0535</td>
</tr>
<tr>
<td>DMEM + BSA</td>
<td>8.10</td>
<td>9.14</td>
<td>0.666</td>
</tr>
<tr>
<td>RPMI</td>
<td>0.617</td>
<td>0.319</td>
<td>0.170</td>
</tr>
<tr>
<td>RPMI + BSA</td>
<td>5.07</td>
<td>2.86</td>
<td>0.525</td>
</tr>
</tbody>
</table>

Table 2.4. Linear model data fitting parameters for long-term dissolution of Alfa Aesar 44904 nano-ZnO in various matrices.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Slope ($\mu$g L$^{-1}$ hr$^{-1}$)</th>
<th>Intercept (mg L$^{-1}$)</th>
<th>Adjusted R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanopure</td>
<td>8.13</td>
<td>1.06</td>
<td>0.986</td>
</tr>
<tr>
<td>Mod. Hard</td>
<td>-7.79*10$^{-9}$</td>
<td>0.0119</td>
<td>0.0433</td>
</tr>
<tr>
<td>DMEM</td>
<td>14.4</td>
<td>7.83</td>
<td>0.836</td>
</tr>
<tr>
<td>DMEM + BSA</td>
<td>8.84</td>
<td>12.6</td>
<td>0.558</td>
</tr>
<tr>
<td>RPMI</td>
<td>2.41</td>
<td>2.13</td>
<td>0.865</td>
</tr>
<tr>
<td>RPMI + BSA</td>
<td>1.15</td>
<td>8.99</td>
<td>0.147</td>
</tr>
</tbody>
</table>

Table 2.5. Linear model data fitting parameters for long-term dissolution of Alfa Aesar 45409 nano-ZnO in various matrices.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Slope ($\mu$g L$^{-1}$ hr$^{-1}$)</th>
<th>Intercept (mg L$^{-1}$)</th>
<th>Adjusted R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanopure</td>
<td>7.43</td>
<td>1.35</td>
<td>0.993</td>
</tr>
<tr>
<td>Mod. Hard</td>
<td>2.77</td>
<td>0.0781</td>
<td>0.849</td>
</tr>
</tbody>
</table>

Table 2.6. Linear model data fitting parameters for long-term dissolution of Alfa Aesar 45588 nano-ZnO in various matrices.

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Slope ($\mu$g L$^{-1}$ hr$^{-1}$)</th>
<th>Intercept (mg L$^{-1}$)</th>
<th>Adjusted R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanopure</td>
<td>8.48</td>
<td>1.13161</td>
<td>0.954</td>
</tr>
<tr>
<td>Mod. Hard</td>
<td>0.00210</td>
<td>0.00112</td>
<td>-0.138</td>
</tr>
</tbody>
</table>
This could be attributed to a greater specific surface area of the smaller NPs relative to the larger bulk-phase particles. In moderately hard water (Figure 2.2b) dissolution of the bulk and two of the NPs was minimal. Interestingly, the smallest particle (Alfa Aesar 45409, 40 NP) showed dissolution to 2.24 mg/L Zn$^{2+}$ at 810 h in moderately hard water, also suggesting a specific surface area effect. A relatively small amount of dissolution in moderately hard water suggests that ZnO NPs may persist in the environment once released.

To explain the observed differences in the amount of apparent dissolution, equilibrium chemical modeling was conducted to determine the potential for zinc that dissolved from NPs to reprecipitate. Modeling of dissolved zinc in moderately hard water by Visual MINTEQ suggests that dissolution of ZnO NPs results in oversaturation of Zn$^{2+}$ with respect to a solid phase of hydrozincite, Zn$_5$(CO$_3$)$_2$(OH)$_6$, with possible additional phases of ZnCO$_3$ and ZnO (zincite) (Table 2.7).

Table 2.7. Visual MINTEQ simulation results for saturation indices of Zn-containing solids in DI, moderately hard water, DMEM, and RPMI. Modeling input for [Zn$^{2+}$] added to each matrix was determined by experimental results for the maximum dissolved Zn observed in each matrix. Saturation index is calculated as SI = log IAP - log K$_{sp}$. Species with empty cells were not predicted to precipitate in the corresponding matrix.

<table>
<thead>
<tr>
<th>Solid species</th>
<th>DI</th>
<th>Moderately hard water</th>
<th>DMEM</th>
<th>RPMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrozincite</td>
<td>4.161</td>
<td>6.279</td>
<td>5.54</td>
<td>3.115</td>
</tr>
<tr>
<td>Smithsonite</td>
<td>0.531</td>
<td>0.924</td>
<td>2.25</td>
<td>1.609</td>
</tr>
<tr>
<td>ZnCO$_3$</td>
<td>0.431</td>
<td>0.824</td>
<td>2.15</td>
<td>1.509</td>
</tr>
<tr>
<td>Zincite (ZnO)</td>
<td></td>
<td>0.414</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZnCO$_3$·H$_2$O</td>
<td></td>
<td>0.284</td>
<td>1.61</td>
<td>0.969</td>
</tr>
<tr>
<td>Zn$_3$(PO$_4$)$_2$·4H$_2$O</td>
<td></td>
<td>5.276</td>
<td>5.804</td>
<td></td>
</tr>
</tbody>
</table>
Atomic weight percents by EDS analysis of precipitates collected from moderately hard water after addition of ZnCl₂ supported the modeling prediction of hydrozincite formation fairly well (Figure 2.3), though the small mass of precipitate obtained did not allow for further confirmation of this by XRD analysis.

![Figure 2.3](image.png)

Figure 2.3. Energy dispersive x-ray spectrum of precipitates formed in moderately hard water after addition of 80 mg/L Zn.

Zn solid phase changes from NPs have been reported: spontaneous formation of ZnCO₃ nanowire species from ZnO nanowires after exposure to ambient air levels of water and CO₂ was observed (54). Precipitates over 20 nm in particle diameter would be rejected by the largest filter pores (0.02 μm) used in this study and would not contribute to dissolved zinc concentration. Also, solid precipitates of CaCO₃ large enough to be rejected by filtration may adsorb zinc ions (55) and effectively remove them from solution as well.
2.3.2 Dissolution in cell culture media

In DMEM and RPMI cell culture media, a higher extent of dissolution was observed when compared to nanopure or moderately hard water. In DMEM, an apparent instantaneous dissolution was observed for all NPs, with slow subsequent increase in dissolved Zn (Figure 2.4a).

![Graph showing dissolution of ZnO in DMEM and RPMI](image)

Figure 2.4. Long-term dissolution of 100 mg/L bulk and nano ZnO (Alfa Aesar 44904) in Dulbecco’s Modified Eagle’s Medium (DMEM) (a) and Roswell Park Memorial Institute medium (RPMI) (b) (each with and without 2 mg/ml bovine serum albumin (BSA).) Error bars are 95% confidence intervals.

Dissolved zinc from bulk ZnO increased quickly over the first 24 h and remained relatively constant before reaching an eventual maximum of 8.45 mg/L. The dissolved zinc resulting from dissolution of Alfa Aesar 44904 showed an immediate Zn$^{2+}$ solubility over 10 mg/L, and eventually climbed to 34.2 mg/L at 1446 h. In this experiment, there was an excess of ZnO NPs added to the cell culture media (100 mg/L). However, the fast dissolution to reach a dissolved Zn concentration over 10 mg/L suggests that lower starting NP concentrations may completely dissolve to Zn$^{2+}$ in this medium.
A greater extent of dissolution (13.6 mg/L) was observed for the bulk ZnO in the presence of BSA. Particle-protein complexes that create a protein corona on the particle surface have been studied (56-59) and the extent of protein complexation may greatly affect how these particles interact with biological systems. These coronae could affect stability of the particles and cause a change in their dissolution. Although BSA-containing solutions quickly clogged the filter pores in this experiment, some amount of BSA (8 NP for an average monomer (60)) could be passed through the 0.02-μm pore filters with bound zinc (61), increasing the zinc concentration in the filtrate.

In RPMI, a smaller difference in dissolved zinc between bulk- and NP-containing solutions was observed (Figure 2.4b). The nanoparticulate ZnO appears to dissolve more than the bulk, but the difference between the phases is not as pronounced as in DMEM. A lower overall solubility for both phases was observed as well, with the nano ZnO reaching 4.66 mg/L at 1446 h. Modeling by Visual MINTEQ predicted Zn₅(PO₄)₂•4H₂O to precipitate in RPMI (Table 2.7; using inorganic substituents for modeling only; RPMI also contains vitamins not included in Visual MINTEQ’s library). Analysis of collected precipitates by EDX showed atomic ratios in agreement with this prediction (Figure 2.5).

A greater amount of solid precipitated in RPMI than in moderately hard water, and XRD analysis was performed to confirm the crystal structure with the closest library match being a zinc phosphate hydrate phase. A higher amount of phosphate is contained in RPMI than in DMEM (Table 2.2), which may account for much of the difference in solubility. Although we did not observe precipitates forming in NP-containing solutions themselves due to the large quantity of NPs already present, it can be inferred from precipitate analysis after addition of ZnCl₂ to these matrices that Zn²⁺ released by dissolving NPs in a given matrix may simply
Figure 2.5. Energy dispersive x-ray spectrum of precipitates formed in RPMI after addition of 80 mg/L Zn.

precipitate in another form and not be present as either NPs or dissolved Zn.

This long-term dissolution of ZnO NPs in cell culture media could be useful in predicting behavior in some biological systems. While ingestion and dermal exposures occur over shorter time scales, lodging of particles in the lungs (62) can have long-term effects. The observed solubility of ZnO NPs in simulated biological matrices suggests these particles could create very high local concentrations of dissolved Zn if lodged in the lungs. In addition, inhaled TiO₂ NPs have been found to be transported from the lungs to compartments such as the liver, heart, and nervous system (63). It is not known if ZnO NPs would persist in this environment long enough
to exhibit similar transport behavior due to their higher solubility, but exposure to ZnO would likely result in elevated dissolved Zn concentrations.

### 2.3.3 Effect of temperature on dissolution

For 100 mg/L ZnO samples in DMEM and RPMI, a consistent trend toward less zinc dissolution in a 24-hour period was observed at 37°C when compared to 20°C (Figure 2.6).

![Figure 2.6. Dissolution of 100 mg/L ZnO NPs (Alfa Aesar 44904, consortium) in Dulbecco’s Modified Eagle’s Medium (DMEM) and Roswell Park Memorial Institute medium (RPMI) after 0 and 24 h at both 20 and 37 °C. A dissolved zinc control (100 mg/L Zn from ZnCl₂) was also used to test for Zn precipitates in these cell culture matrices.](image-url)
A very consistent decrease in zinc solubility at 37°C was observed in DMEM, with dissolved Zn concentrations around 6-7 mg/L for both particles at 0 and 24 h, as opposed to ~12-13 mg/L at 20°C. As the enthalpy of ZnO dissolution is negative (64), it is expected that an increased temperature would result in lower Zn$^{2+}$ solubility. As observed in long-term experiments, immediate dissolution of ZnO in RPMI to ~2 mg/L occurred, and this was still evident after 24 h at 20°C for ZnO NPs. The difference in zinc solubility between temperatures in RPMI is not as large as in DMEM, but a slight decrease was observed for all NPs at 37°C. For ZnCl$_2$ in DMEM, a similar constancy at 0 and 24 h and difference in solubility is seen between 20°C and 37°C as in the NP solutions; however the concentrations are higher, approximately twenty-five and seventeen mg/L respectively.

At 10 mg/L ZnO, instantaneous dissolution behavior was also observed for the Alfa Aesar NP (Figure 2.7). However, at this concentration, the consortium NP was not observed to undergo the same immediate dissolution in DMEM as it exhibited at 100 mg/L. This could be due to a smaller degree of specific surface area for the consortium NP relative to the Alfa Aesar NP: the consortium NP is a powder which tends to remain as large aggregates of NPs in solution, reducing its available surface area, while the Alfa Aesar NP is well dispersed in aqueous solution. While aggregation may affect dissolution rates, the potential for this effect was not a focus of the present study.

Although this study was not designed to address the toxicity of the NPs examined, it has implications for toxicity research. In DMEM, the relatively high zinc solubility and instantaneous dissolution of some ZnO NPs raises questions about the ability to assess toxicity of these NPs when dosed in this medium. If this medium or another with similar zinc solubility were to be used in toxicity studies, it could be incorrectly concluded that the NPs are
Figure 2.7. Dissolution of 10 mg/L ZnO NPs (Alfa Aesar 44904, consortium) in Dulbecco’s Modified Eagle’s Medium (DMEM) and Roswell Park Memorial Institute medium (RPMI) after 0 and 24 h at both 20 and 37 °C. A dissolved zinc control (ZnCl₂) was also used to test for Zn precipitates in these cell culture matrices.

responsible for any observed toxic effect, although there may be no actual NP exposure due to their dissolution. Therefore, caution should be exercised in attributing toxicity to NPs if the solubility of Zn in the matrices used is not well known: solution chemistry appears to play a major role in the dissolution of ZnO NPs. In addition, particle type also appears to affect solubility. This is evidenced by the differences in behavior between bulk phase and nanoparticulate ZnO, ZnO NPs from different sources, and between NPs of different sizes.
Solution temperature also had an effect on NP dissolution in cell culture media, demonstrating that ZnO NPs may behave differently at human body temperature as opposed to room temperature.

2.3.4 Separation of zinc species by ultrafiltration

In an attempt to provide increased resolution of NP size and develop alternative filtration practices for NPs in biological testing, centrifugal ultrafiltration of samples was conducted and the results compared against 0.02-μm pore filtration. Syringe and centrifugal filters have been compared for quantification of dissolved Pb$^{2+}$ released from PbS NP aggregates with similar effectiveness (65). Overall, ZnO dissolution in the same four matrices (nanopure water, moderately hard water, DMEM, and RPMI) as determined by centrifugal filtration experiments exhibited similar behavior as when filtered by 0.02-μm pore filters (Figure 2.8).

Further, all five ultrafilter sizes (3 to 100 kDa) behaved similarly (Figure 2.9), suggesting they all adequately discriminated between nanoparticulate and dissolved zinc.

In the ultrafiltration experiments, the feed concentration was measured (and plotted in Figure 2.8 as the sum of dissolved and particulate zinc) for each replicate. The centrifugal filtration takes approximately 30 min, so a t = 0 data point is more likely to be between 0.5 and 1 h. This additional time could allow for a greater amount of NP dissolution relative to syringe filtration, where the first filtrate is collected approximately one minute after suspension of NPs. Compared with nanopure water, NPs in moderately hard water showed less dissolution after 24 h: 0.69 mg/L Zn for Alfa Aesar 44904 and 1.1 mg/L for the consortium particle. Moderately hard water also prevented the soluble zinc source (ZnCl$_2$) from passing through the membrane, again indicating formation of filterable precipitates from dissolved zinc.
Figure 2.8. Particulate and dissolved zinc concentrations in various matrices at 20°C after 0 and 24 h using ~10 mg/L Alfa Aesar 44904 (a) and consortium ZnO (b) NPs. ZnCl₂ (c) was used as a dissolved zinc control to test for possible Zn precipitation in solution. Dissolved concentration was measured directly in the filter permeate; particulate concentration is calculated as ([Zn]_{feed} - [Zn]_{permeate}). Total column height (black + white) represents measured feed Zn concentration.

Lower permeate Zn concentrations from the filtered ZnCl₂ solution after 24 h suggests continued precipitation over this time period. The culture fluids RPMI and DMEM again showed an immediate dissolution of NPs and little increase past that point after 24 h. The bulk of the data suggests relatively stable solubility maxima for aqueous zinc species in these matrices. However, the increase in solubility in DMEM after 600 h may indicate a more complex process may
control zinc solubility. These studies indicate that small pore filters and ultrafilters provide comparable data for ZnO NPs related to dissolution and potential reprecipitation.

Figure 2.9. Permeate concentrations for Alfa Aesar 44904 and Consortium ZnO filtered with five UF membrane pore sizes at zero and 24 hours in nanopure water. Feed concentrations at zero hours were measured to be 17 and 11 mg/L as Zn for Alfa Aesar 44904 and Consortium, respectively.
CHAPTER 3
OVERCOMING CHALLENGES IN ANALYSIS OF POLYDISPERSE METAL-
CONTAINING NANOPARTICLES BY SINGLE PARTICLE INDUCTIVELY COUPLED
PLASMA MASS SPECTROMETRY


3.1 Introduction

With the rapid rise in applications for engineered NPs, they have been increasingly incorporated into industrial and consumer products. Among the most widely used metal-containing NPs are Ag, TiO₂, ZnO, and CeO₂. Silver NPs are used in the greatest number of consumer products due to their antimicrobial properties, and can be found in items from athletic gear to pacifiers (66). A variety of products contain nano TiO₂, which has been investigated for a variety of applications such as photocatalysis (3), environmental remediation (67), and is used in consumer products such as toothpaste and sunscreen (68, 69) and is seen increasingly in food (4). Nano ZnO is also found in cosmetics like sunscreen (33) as well as being touted as an effective semiconductor (70). Cerium oxide NPs are utilized for polishing (71) and combustion emissions catalysis (72). With this increasing use comes concern regarding the potential ecological and human health risks of exposure to these materials. All of the NPs listed here have potential for release to the environment as a result of their varied applications. Predicted environmental concentrations from life-cycle assessments are typically in the ppt range (5, 73).

Although a myriad of laboratory toxicity studies have been performed (10, 21, 27, 29, 31, 74), and some studies have examined possible scenarios for modification of NPs in the
environment (8), little is known regarding how various environmental parameters actually modify NP behavior and toxicity. Silver NPs are used for beneficial antimicrobial purposes, but this very property may also cause them to be ecologically harmful when released to the environment (75). Nano TiO2 has been shown to be essentially nontoxic at concentrations relevant for environmental exposures (21, 24), but photocatalytic effects have not been fully investigated. While nano ZnO has been demonstrated to be more toxic than Zn²⁺ alone (43), it is generally thought that the NPs readily dissolve to Zn²⁺ (76) which is in turn toxic (23). Although some studies have found nano CeO₂ to be toxic (29), it has also been observed to have a protective effect for cells (41, 74). In a study comparing toxicity of various metal oxide NPs, Brunner et al found toxic response ordered as ZnO > CeO₂ ≈ TiO₂ to both MSTO and 3T3 cells (10). There is also concern about increased toxicity of NPs with high aspect ratios such as nanowires and nanotubes, as they are often morphologically similar to asbestos (77).

As the nanotechnology industry has grown, demand for methods of detecting, quantifying, and characterizing NPs has been increasing. Although an extensive array of methods exists for use in the laboratory setting (78), many of the commonly employed methods are quite limited when applied to real-world settings (i.e. complex solution matrices, background particles, low NP concentration, polydisperse and aggregating materials). DLS works well for sizing monodisperse samples but performs very poorly in complex matrices where aggregation and dissolution may induce polydispersity; it is also particle and element non-specific. Electron microscopy provides NP size information and can be coupled with EDS analysis for elemental composition information; however it is time consuming and may be ineffective when attempting to analyze environmentally relevant concentrations (5, 73) in the μg/L or ng/L range. Hyphenated separation and detection methods such as HDC-ICP-MS and FFF-ICP-MS are
useful for simultaneous determination of particle size and elemental composition of complex NPs (78), but these methods may also fail to reach the desired ng/L detection limit due to sample dilution in the separation step.

In this study we have examined spICPMS, a method for detecting and quantifying metal-containing NPs. The method has its origins in airborne particle analysis with concentrations in the femtogram range (79). The current spICPMS theory and development work was performed in a series of studies by Degueldre et al (80-82), and it has been suggested for use in Au NP detection for bioanalytics (83). Briefly, introduction of a dilute suspension of NPs into the ICP-MS will result in the generation of a pulse each time an individual particle enters the plasma, is ionized and detected as a packet of ions. By collecting time-resolved data both the number concentration and the size distribution of the NP sample can be examined.

Recent work by Mitrano et al (84) and Laborda et al (85) has further developed the method for use in silver NP detection and characterization, and Pace et al (86) provided detailed discussion of the importance of instrument transport efficiency in quantification and sizing of NPs. The multi-element capability of ICP-MS suggests that spICPMS could potentially be applied effectively to the analysis of many different metal-containing NPs, provided isobaric and matrix interferences can be avoided. Additional considerations with respect to successful application of spICPMS to the detection and quantification of any particular NP sample includes: NP size, mass percent and isotopic abundance of the analyte element in the NP, and the stability of the NP with respect to dissolution.

To the best of our knowledge, this technique has not been previously used for analysis of the NPs discussed in this work: Ag nanowires, TiO₂, ZnO, and CeO₂. The goal of the present study was to evaluate the feasibility of spICPMS for detecting and sizing these metal-containing
NPs. A particular aspect of this investigation is the use of the method for sizing of materials with broad size distributions, non-spherical shapes, and NPs for which monodisperse standards are not readily available. Because polydisperse materials were examined, and spICPMS is a particle counting technique, special consideration is given to the requirements for particle concentration and total number of instrument readings for robust detection, quantification, and size characterization.

3.2 Experimental

3.2.1 Materials

The NPs used in this study were either purchased from commercial sources or obtained from colleagues. The TiO$_2$ (Aldrich, #634662) and CeO$_2$ (Inframat, #58N-0802) were obtained as powders, and the polyvinylpyrrolidone-coated Ag nanowires (nanoComposix, #RKB3144) was received as an aqueous suspension. The ZnO NPs were synthesized as described in Wu et al (87) at the University of Iowa and were supplied as a powder. All dilutions for spICPMS analysis were performed with 18 MΩ nanopure water (Barnstead Nanopure,) henceforth referred to as DI water. FL70 surfactant (Fischer Scientific) was used to maintain a stable suspension of CeO$_2$ NPs for SdFFF analysis. Dissolved Ag, Ti, Zn, and Ce standards were Claritas PPT (SPEX Certiprep) ICP-MS grade standards and were diluted in 2% HNO$_3$ (Optima trace metal grade, Fisher). Dissolved Au standards were Claritas PPT (SPEX Certiprep) ICP-MS grade, diluted in 2% HCl (Optima trace metal grade, Fisher).
3.2.2 Characterization of NPs

A Zetasizer Nano ZS (Malvern) was used to determine hydrodynamic diameter by DLS. Samples of each NP were dispersed in DI water at 50 mg/L and analyzed in triplicate, with the mean diameters and standard deviations provided in Table 3.1. This instrument was also used to perform triplicate Zeta potential measurements on 100 mg/L NP solutions in DI water, pH unadjusted and in the range of 5.1 to 6.6, with mean values and standard deviations reported in Table 3.1. We recognize that for the high aspect ratio silver nanowires, the hydrodynamic diameter of the particles is approximate and may not accurately represent either length or width.

Table 3.1: Characterization of NPs by dynamic light scattering and zeta potential

<table>
<thead>
<tr>
<th></th>
<th>Size from manufacturer (nm)</th>
<th>DLS (nm)</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrich TiO₂</td>
<td>~100 (BET)</td>
<td>301±158</td>
<td>-27.4±3.6</td>
</tr>
<tr>
<td>Inframat CeO₂</td>
<td>50-80 (BET)</td>
<td>79±8</td>
<td>42.7±0.3</td>
</tr>
<tr>
<td>U of Iowa ZnO</td>
<td>130 (TEM)</td>
<td>130±14</td>
<td>29.1±0.4</td>
</tr>
<tr>
<td>NC Ag wires</td>
<td>2055 (L) x 33 (d) (TEM)</td>
<td>2351±63</td>
<td>-19.2±1.9</td>
</tr>
<tr>
<td>BBI Au</td>
<td>100</td>
<td>101±1</td>
<td>-41.4±3.3</td>
</tr>
</tbody>
</table>

Particle morphology information and confirmation of manufacturer reported size range was obtained using a FEI CM200 TEM. The NP samples were prepared in an ethanol solution to aid in drying. After immersion in a sonication bath for two minutes, small volumes (< 1 mL) were added dropwise onto a carbon-coated 300 mesh copper TEM grid and allowed to evaporate. The acceleration voltage used was 200 kV and magnification ranged depending on the size of the NP being analyzed.
Figure 3.1. BBI Au NPs TEM image

Figure 3.2. Inframat CeO$_2$ NPs TEM image
Figure 3.3. Aldrich TiO$_2$ NPs TEM image

Figure 3.4. NanoComposix Ag nanowires TEM image (from manufacturer spec sheet)
3.2.3 Sedimentation field-flow fractionation (SdFFF)

Two SdFFF systems (CF-2000 and S-1000, Postnova Analytics GmbH, Landsberg, Germany), were used to size the TiO$_2$ and CeO$_2$ NPs for comparison with spICPMS. Ultraviolet absorbance at 350 nm (Postnova PN3241, UV diode array detector) was used for sample detection. The TiO$_2$ samples were analyzed in DI water, whereas CeO$_2$ required analysis in 0.05 wt% FL-70 to maintain a stable suspension at the concentration required for SdFFF-UV/VIS analysis. Separate suspensions (5 mg mL$^{-1}$) of TiO$_2$ and CeO$_2$ NPs were made using TiO$_2$ and CeO$_2$ powders and the respective carrier solutions. The suspensions were sonicated for 30 minutes using an ultrasonic bath. The suspensions were then passed through a 0.45 µm filter to remove any large particles or aggregates that could not be dispersed during the sonication step. The sample injection volume was 100 µL. The SdFFF channel dimensions and run conditions are summarized in Tables 3.2 and 3.3.

Table 3.2: SdFFF channels dimensions

<table>
<thead>
<tr>
<th>System</th>
<th>Channel thickness (cm)</th>
<th>Channel volume (mL)</th>
<th>Channel radius (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0165</td>
<td>2.90</td>
<td>15.1</td>
</tr>
<tr>
<td>B</td>
<td>0.0200</td>
<td>2.26</td>
<td>10.2</td>
</tr>
</tbody>
</table>
Table 3.3: Run parameters used to analyze TiO$_2$ and CeO$_2$ nanoparticle suspensions

<table>
<thead>
<tr>
<th>Method</th>
<th>Initial RPM</th>
<th>Final RPM</th>
<th>$t_1$ (min)</th>
<th>$t_a$ (min)</th>
<th>Channel flow rate (mL/min)</th>
<th>Relaxation time (min)</th>
<th>Injection volume (µL)</th>
<th>Carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1800</td>
<td>100</td>
<td>5.6</td>
<td>-45.0</td>
<td>1.00</td>
<td>10</td>
<td>100</td>
<td>0.05% CTAB</td>
</tr>
<tr>
<td>II</td>
<td>2200</td>
<td>57</td>
<td>10.0</td>
<td>-80.0</td>
<td>1.00</td>
<td>10</td>
<td>100</td>
<td>0.05% FL-70 De-ionized water</td>
</tr>
<tr>
<td>III</td>
<td>3000</td>
<td>57</td>
<td>10.0</td>
<td>-80.0</td>
<td>1.00</td>
<td>3</td>
<td>100</td>
<td>0.05% FL-70 De-ionized water</td>
</tr>
</tbody>
</table>

The samples were allowed to equilibrate under an initial constant centrifugal field (5,360-10,057 m sec$^{-2}$ (1800-3000 rpm) for 3-10 min prior to the start of the experiment. After an additional 5-10 min, a power decay function (88) was used to bring the field to 50-100 rpm at the end of the analysis. The channel flow rate was set at 1 mL min$^{-1}$. The particle size distributions were constructed using FFF theory using the retention time and UV/VIS detector response (89).

3.2.4 ICP-MS Experimental Details

Solutions of each NP were made at mass concentrations in the ng/L range. The actual mass concentrations used were varied among NPs, as different-sized particles require different mass concentrations in order to have similar number concentrations, which are desired for effective analysis. Calculation of a nominal particle number concentration from mass concentration, particle density, size, and shape (generally assumed to be spherical) is a starting
point for analysis of each NP. This avoids concentrations that would result in pulse coincidence. The polydisperse nature of the NPs used here, and the non-spherical shape of the Ag nanowires, prevents initial calculation of an accurate particle number concentration. Rather, a number concentration was estimated from the manufacturer-supplied characterization data to select a starting mass concentration range. Based on the number of NP-generated pulses obtained from spICPMS analysis of the initial solution, the final concentration range was found. The optimal number concentration is directly related to the ratio of pulses to total readings, as discussed below. A DI blank for each element was included in all analyses. Each sample was run in duplicate to examine reproducibility, and comparison of sequential analyses ensured that NPs had not settled out of solution during analysis. Stock suspensions of NPs were diluted to the desired concentrations by serial dilution with approximately 10-minute bath sonication (Fisher, FS60H) periods between performing each dilution. All dilutions were made in 15 mL Falcon polypropylene centrifuge tubes. The ICP-MS used is a Perkin Elmer NexION 300q with an S10 autosampler. Dissolved Ag, Ti, Zn, and Ce standards in the range of 100 ng/L to 10 ug/L were used for creating metal calibration curves. The concentrations of dissolved standards used for each element were dependent on pulse intensities for each NP. The dissolved standard concentration range was chosen such that a typical NP pulse intensity value would be contained within the range of the calibration curve. Calibration blanks were included with all metal standard curves. Previous work has shown 10 ms dwell times (readings) to be the most effective for minimizing both measurement of partial pulses and particle coincidence (86, 90). In this work a typical single run consisted of 20,000 readings. Aside from monitoring \(^{49}\text{Ti}\) for TiO\(_2\) analysis due to SO\(^+\) interferences with \(^{48}\text{Ti}\), all elements were analyzed using the most abundant isotope: \(^{197}\text{Au}\) (100% abundant), \(^{140}\text{Ce}\) (88.48%), \(^{107}\text{Ag}\) (51.84%), and \(^{64}\text{Zn}\) (48.6%). The average sample
introduction flow rate was 0.97 mL min\(^{-1}\). Sample tubing was replaced before each day’s analysis to minimize sample contamination by carryover and to maintain consistent flow rates.

During ICP-MS operation, the majority of liquid sample flows to waste, while a small fraction (< 5-10 %) is aerosolized and reaches the plasma, where it is vaporized and the NPs are ionized for elemental detection by the mass spectrometer. The number of ions generated by disintegration of an NP in the plasma is proportional to the NP size, which, as will be shown, can be related to instrument signal intensity via a dissolved metal calibration curve. Although other factors affect the actual number of ions reaching the detector, including losses in the vacuum system, efficiency of ion transport in the quadrupole, and ablation efficiency in the plasma, it is assumed that these processes act equally upon ions generated from the NP and those from the dissolved standards. Thus, these additional factors are assumed not to affect the sizing of NPs by this method. Ascertaining the fraction of sample that enters the plasma (typically 5-10% as noted above) is crucial for quantitative analysis of NP size and number concentration. Well-characterized Au NPs of a narrow particle size distribution (BBI, 100 nm) were used as a particle standard to assess sample transport efficiency during spICPMS analysis using the calculations described by Pace et al (86) and later in this thesis (Page 52). Two approaches can be taken to determine and utilize the transport efficiency for sizing. In one case the observed pulse numbers are compared to the calculated number concentration. The approach used in this study adjusts the transport efficiency to make the computed particle size be equivalent to the reported size.
3.3 Results and Discussion

3.3.1 Real-time data and analyte parameters

The effectiveness of spICPMS analysis for different NPs varies with several parameters. A mass concentration detection limit for any given type of NP will be greatly influenced by particle size. The number of particles in solution diminishes rapidly when mass concentration is constant and particle diameter increases. This is illustrated by comparing Figure 3.5 (a) and (b).

![Graphs showing ICP-MS response for different NPs](image)

Figure 3.5. Time-resolved data for four NPs at 200 ppt in DI water: (a) Aldrich TiO$_2$ (~100 nm), (b) Inframat CeO$_2$ (50-80 nm), (c) synthesized ZnO (~130 nm), (d) Nanocomposix Ag nanowires (L = ~2000 nm).

The larger TiO$_2$ NP ((a), ~100 nm diameter) yields far fewer pulses above background than the smaller CeO$_2$ ((c), ~50-80 nm diameter). The ability to differentiate a pulse from the background depends on the number of ions reaching the detector and thus is affected by isotopic
abundance, mass percent of metal in metal oxide or mixed-metal NPs, and potential for NP dissolution leading to an elevated background.

For analysis of Ti, the most abundant Ti isotope, $^{48}$Ti, is essentially unusable for quadrupole ICP-MS analysis due to mass interferences causing an elevated background. In this case $^{49}$Ti was used to avoid this interference; however, its lower abundance results in less intense pulses. Although the TiO$_2$ NPs are larger than the CeO$_2$ used here, and would be expected to generate a larger packet of total ions in the plasma and thus a more intense pulse, the relative isotopic abundances influence this. The $^{140}$Ce isotope has an abundance of 88.48%, while $^{49}$Ti has an abundance of 5.41%. In addition, the percent metal in the particle affects pulse intensity. For TiO$_2$, 59.9% of particle mass is Ti, while 81.4% of CeO$_2$ is comprised of Ce. The Ag nanowires were analyzed using $^{107}$Ag, which is 51.84% abundant, but they are 100% Ag and have lengths on the order of microns, and therefore generate easily detectable pulses (3.5d). It is not surprising that ng/L concentrations of ZnO appear to completely dissolve to Zn$^{2+}$ and result in spICPMS data containing no pulses (Figure 3.5). Previous studies on dissolution of ZnO NPs found Zn$^{2+}$ concentrations reached the mg/L range rapidly after addition to various matrices (23, 76). The data in the present study further suggest that dissolution may be too rapid for ZnO NPs to be detected at environmentally relevant concentrations, predicted by a review of mass flow modeling studies to be in the ng/L range (approximately 0.1 to 100 ng/L) for surface water (6, 91).

DI blanks (no NPs) for each element of interest are run as part of every analysis. In the case of both TiO$_2$ and CeO$_2$, the mean of the baseline for the blanks and the NP-containing solutions are often significantly different, however the difference between the means is less than 1 count per dwell time in each case. This is too small of a difference to quantify dissolved metal
or size small particles. For Ag nanowire-containing solutions, the baseline mean is typically 5-20 counts above that of the DI blank, which correlates to a dissolved Ag concentration of 10 to 40 ppt. In the case of ZnO, the baseline is very different from the blank due to dissolution of ZnO NPs to dissolved Zn. A dissolved calibration curve could be used to quantify the Zn concentration here as well.

3.3.2 Frequency counts of data for polydisperse metal-containing NPs

The spICPMS data were binned based on pulse intensity (i.e., counts) for a clearer view of how pulse heights are distributed for a given sample, and to check for effective separation of NP pulses from any dissolved elemental signal or background interference. As can be seen from example data presented in Figure 3.6a and b, the metal oxide NPs have a portion of the distribution that backs up against the background, with the smaller NPs in the sample likely encountering size detection limits for the given elements. Size detection limits for NPs under 20 nm have been reported for relatively monodisperse NPs (85) that allow for clear differentiation of pulses from background. However, the metal oxide NPs studied here tend to be more polydisperse, with “small” particles present in the distribution, making clear separation of NP pulses from the background more difficult to observe. When converted to an intensity distribution, the consistently intense Ag nanowire pulses observed in Figure 3.5d are easily separated from background, as shown in the inset (Figure 3.6c).
Figure 3.6. Number of NP pulse events from spICPMS analysis for TiO$_2$, CeO$_2$, and Ag nanowire NPs, displayed here with bin size = 100. The inset for Ag nanowires (c) shows the first two bars re-binned with bin size = 10 and focused on the instrument response up to 200 counts to illustrate separation of NP pulses from instrumental background and dissolved Ag background from ~10 to ~50 counts.

3.3.3 NP size calculation approaches

During spICPMS operation, the majority of liquid sample flows to waste, while a small fraction is aerosolized, reaches the plasma, and is ionized for detection by the mass spectrometer. Ascertaining the fraction of sample that is analyzed is crucial for quantitative analysis of NP size and number concentration. The transport efficiency may depend on a variety of parameters, such as the nebulizer gas flow rate, sample flow rate, as well as the shape and position of both the
spray chamber and nebulizer. Each of these parameters may change slightly during instrument maintenance and tuning processes, so assessing transport efficiency for each day’s analysis is important for obtaining accurate size and number data on NP samples. BBI Au NPs (100 nm) were used to assess sample transport efficiency during spICPMS analysis by two different methods (86). The first method, based on particle number in solution, compares the number of observed pulses in a given run to the theoretical particle number in solution. The theoretical particle number at a given concentration is calculated assuming a spherical particle of known size (100 nm for the Au NP used) and a density of 19.3 g/cm³. Dividing the observed particle number/min by the theoretical particle number/min yields a transport efficiency for the instrument under a given set of flow parameters and sample introduction devices. Table 3.4 shows values obtained for this approach to efficiency determination.

Table 3.4. Instrument transport efficiency from comparing observed pulse number and theoretical particle number for Au NPs. *Theoretical particle number per minute is based on calculated particles/mL and measured at 0.974 mL/min sample flow rate.

<table>
<thead>
<tr>
<th>Au NP concentration (ppt)</th>
<th>Theoretical particle number/min*</th>
<th>Observed pulse number/min</th>
<th>Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>963.8</td>
<td>30.0</td>
<td>3.11</td>
</tr>
<tr>
<td>25</td>
<td>2409.6</td>
<td>78.2</td>
<td>3.25</td>
</tr>
<tr>
<td>50</td>
<td>4819.2</td>
<td>76.2</td>
<td>3.16</td>
</tr>
<tr>
<td>100</td>
<td>9638.4</td>
<td>150.9</td>
<td>3.13</td>
</tr>
<tr>
<td>150</td>
<td>14457.5</td>
<td>145.0</td>
<td>3.01</td>
</tr>
<tr>
<td>200</td>
<td>19276.7</td>
<td>317.0</td>
<td>3.29</td>
</tr>
<tr>
<td>Average:</td>
<td></td>
<td></td>
<td>3.15</td>
</tr>
</tbody>
</table>

These data are based on Au NP runs from a single day. Figure 3.7 shows the reproducibility of Au NP analyses on four different days. Although for this data set results are
quite reproducible, instrument performance can vary day-to-day, even with proper optimization, and thus the transport efficiency will vary as well. It is recommended that transport efficiency be determined each day of analysis.

![Graphs showing the relationship between pulses and concentration](image)

Figure 3.7. Observed number of pulses vs. concentration for four separate analyses of Nanocomposix 100 nm Au NPs. Although the slope is reproducible within ~10%, including a standard monodisperse NP in each spICPMS analysis is recommended for evaluating day-to-day variation in instrument efficiency.

A second method of ascertaining transport efficiency is a particle size-based approach. The number of ions generated by disintegration of an NP in the plasma is proportional to the NP size, and can be related to instrument signal intensity via a dissolved metal calibration curve. This has been shown previously by Degueldre et al (82) and Pace et al (86), who uses Equation
3.1 to transform a dissolved element calibration curve of instrument signal, I, vs. analyte concentration, C, to instrument signal vs. analyte mass per instrument reading, W.

\[
W = \left[ \eta_n \cdot q_{\text{liq}} \cdot t_{\text{dt}} \cdot C \right] \quad (3.1)
\]

The instrument dwell time setting, \( t_{\text{dt}} \), and flow rate, \( q_{\text{liq}} \), are set by the user, and the transport efficiency \( \eta_n \) is adjusted to make a calculated diameter fit the known NP diameter using the procedure as follows. By using a gold standard of a size sufficient to create a distribution well elevated above the background data, in this study 100 nm, a distribution of intensities is obtained. After transformation of the dissolved metal calibration curve to the desired units of ug/event (W) versus intensity/event, the intensity of each NP pulse is used in Equation 3.2 to calculate the mass of a single NP, \( m_{\text{ENP}} \).

\[
m_{\text{ENP}} = f_M^{-1} \cdot \left[ \frac{(I_{\text{pulse}} - I_{\text{Bkgd}})/\eta_i}{m} \right] \quad (3.2)
\]

Where the mass fraction of the analyte metal in the NP is given by \( f_M \), (1 for Au and Ag, 0.599 for TiO\(_2\), and 0.814 for CeO\(_2\)) and \( m \) is the slope of W versus intensity. \( I_{\text{Bkgd}} \) is composed of both the instrumental background, equivalent to the intercept of the dissolved calibration curve (b) and dissolved elemental background in the NP sample. \( I_{\text{Bkgd}} \) is subtracted from pulse intensity \( I_{\text{pulse}} \), to obtain the intensity due to the NP (\( I_{\text{NP}} \)). The degree to which the NP is ablated and ionized in the plasma is given by \( \eta_i \); this is assumed to be 1. The mass of an NP can be
related to a particle diameter ($d_{NP}$) via a given density, $\rho$, and assuming a spherical geometry by Equation 3.3.

$$d_{ENP} = \left[ \frac{6 \cdot m_{ENP}}{\pi \cdot \rho} \right]^{1/3} \quad (3.3)$$

The calibration standards and NPs are both assumed to be atomized and ionized to the same degree in the plasma. Should this not be the case, then different transport efficiencies will be obtained from the two methods. Since we have seen only small differences in efficiency calculated from the two different methods, we believe the assumption about ionization efficiency is correct for the particles studied here. To study the extent of atomization for other NPs such as TiO$_2$, a standard particle would need to be used to calculate a particle number concentration. A number-based efficiency would then be compared with a size-based efficiency. Since metal oxide NPs with narrow size distributions are not currently available to the best of our knowledge, this experiment has not been performed.

For unknown NPs, in some cases the distribution of pulse intensities are not clearly separated from the background. The situation can arise from the presence of very small NPs in the distribution and/or from a high dissolved metal background. To extract the intensities due to NPs from the background instrument response in a given run, iterative calculations are performed using Microsoft Excel. The entire dataset is used for calculation of a mean and standard deviation of measured intensity. Any data points above $\bar{x} + 5s$ are counted as a pulse arising from an NP, and are removed from the dataset. The resulting new dataset is used to recalculate the mean and standard deviation, any pulses above $\bar{x} + 5s$ are added to the number of
pulses from the first calculation and removed from the dataset, and the process is repeated until converging on mean value for the background response. The resulting pulses from the dataset are then processed by Equations 3.2 and 3.3. A less conservative approach can use 3s to detect the smaller NPs in the size distribution. This approach relies on the assumption that the dissolved background signal is normally distributed. This is not always the case, especially with analytes having very low background response on the order of 0-5 counts. This approach has the potential to overestimate particle number concentrations and include “false-positives” in the lowest size range of the distribution, but should not affect the size analysis of the NPs that are well-resolved from the background. Using the calculated instrument transport efficiency and the appropriate dissolved metal calibration curves, size distributions of TiO₂ and CeO₂ were determined (Figure 3.8).

3.3.4 spICPMS size distributions of NPs

Although the spICPMS size distribution mode for CeO₂ agreed well with nominal NP diameter (50-80 nm) and characterization data, further validation of results was desired. Analysis of NPs by SdFFF to obtain a complete size distribution for comparison to spICPMS sizing was performed, and the results are also shown in Figure 3.8. For TiO₂, particle diameter is reported as ~100 nm, the mode for spICPMS sizing was closer to 162 nm, with the SdFFF mode appearing around 122 nm. The full width at half max for the spICPMS peak was 175 nm, and 160 nm for the SdFFF analysis. The shape of the distribution was very similar between the two methods, although the spICPMS data had a longer tail representing larger NPs in the 300-500 nm range. In this particular SdFFF analysis a 0.45-um filtration step was used to remove larger aggregates.
prior to analysis, which may also have prevented analysis of NPs of ~350+ nm. Furthermore, while the spICPMS data represents a number-based size distribution, the UV response for

Figure 3.8. Size distributions for Aldrich TiO₂ and Inframat CeO₂ as determined by spICPMS (histograms) and SdFFF (line graphs) analyses. For spICPMS results, the TiO₂ was run at 60 ppt in DI water, and the CeO₂ was run at 10 ppt in DI water. The SdFFF for CeO₂ was run in 0.05% FL70 surfactant at 5 mg mL⁻¹ due to problems keeping a stable suspension in DI at the higher concentrations required for SdFFF.

the SdFFF is likely not directly related to the number of particles eluting at any given time but is related to the turbidity generated by the particles. Although this relationship is likely to be closely related to the mass concentration, light scattering is a highly complex phenomena that is
also dependent on many other factors. Coupling of FFF to mass sensitive detectors, such as ICP-MS, and conversion of the spICPMS data to mass-based distributions would facilitate comparison of the methods.

For CeO$_2$, the size distribution maximum obtained by both spICPMS and SdFFF is in the range of 50-80 nm, and in agreement with the diameter range provided by the manufacturer (BET analysis). As with the TiO$_2$ data, the SdFFF and spICPMS size distributions have largely the same shape. The mode of the distribution was 65 nm for the spICPMS results and 76 nm for SdFFF data. The full width at half max was 70 nm for spICPMS and 58 nm for SdFFF. Adjusting the particle transport efficiency parameter contained in the spICPMS sizing equation would allow for greater overlap between the two distributions, but this value is experimentally determined in this case by aligning the Au standard NP at 100 nm, and as such should not be modified when analyzing other NPs of unknown size. The similarity in the sizing results from the two techniques in fact suggests that our assumption that the ionization efficiency of the NP and the dissolved standards are the same is correct within the experimental uncertainty of the data. If the ionization efficiency was different for NPs and dissolved standards, the sizing results would not match those obtained from other techniques nearly as well. If this technique is used for analysis of unknown NPs such as those in environmental samples, confirmation of size distribution results by FFF or TEM will likely be impossible due to the low concentrations expected.

Analysis of Ag nanowires (nanoComposix) was also performed by spICPMS. Characterization data provided by the manufacturer (Figure 3.9) shows the distribution of wire lengths to be in the 1000-3000 nm range.
When analyzing the nanowire size by spICPMS, the assumption of spherical particle geometry for diameter calculation is not appropriate. Thus, after calculating a mass associated with a given pulse height and using density to obtain a total NP volume, a cross-sectional diameter was held constant to allow calculation of a wire length. This presents a problem in creating a size (length) distribution, as the cross-sectional diameter may vary as well. The manufacturer’s characterization data suggests that this diameter is 33.1+/− 5.7 nm. However, a small variation in this value when calculating a wire length can make a large change in a length distribution. This is illustrated in Figure 3.10, which shows length distributions for these Ag nanowires at four different (assumed) cross-sectional diameters. Some of the discontinuity between the manufacturer’s length distribution and the upper size obtained here by spICPMS may perhaps be explained by a transport phenomenon. Vieira et al (92) estimated aerosol droplet cut-off diameters for typical ICP spray chambers to be in the 2 – 4 um range. If some droplet sizes in a distribution are limited to this range, a portion of NPs having wire-like aspect ratios
may be unable to aerosolize and reach the plasma, leading to a distribution artificially weighted to the smaller end of calculated lengths. Another possible reason for error (on both the high and low end of the distribution) is assuming a constant cross-sectional diameter. This approach introduces error to the length calculation, but to obtain a length distribution it’s necessary since geometric information is not produced by spICPMS. The purpose of Figure 3.10 is partly to show the challenges in sizing such high aspect ratio materials accurately.

![Figure 3.10](image_url)

Figure 3.10. Length distributions for Ag nanowires at four different cross-sectional diameters: 20, 25, 33 (reported mean), and 40 nm.

In addition to variability in a rod or wire’s cross-section, the potential exists for this parameter to be completely unknown. This would be most easily determined by electron microscopy; however, in environmental samples where NP concentrations are expected in the ppt range, it is unrealistic to find enough particles to measure this quantity. This presents a
limitation of spICPMS for sizing: although an NP mass can be determined, assumptions must be made about the geometry, especially with respect to aspect ratio. The nanowires were not analyzed by SdFFF because of the non-ideal behavior of rods in the separation, which makes hydrodynamic size calculations difficult (93).

### 3.4.5 Challenges in analyzing polydisperse NPs and coincidence of pulses

As NP concentration in solution is increased, the potential for two or more NPs to simultaneously enter the plasma and be detected as a coincident pulse also increases. When analyzing larger NPs with a narrow size distribution (monodisperse) it is fairly obvious when this has occurred. To illustrate the differences in observing coincidence for a monodisperse system (Au), moderately polydisperse system (TiO$_2$), and extremely polydisperse system (Ag nanowires), these NPs were analyzed and sized at a given concentration for a single run. The resulting size distributions were compared with those obtained by running a tenfold dilution of the original concentration for ten times the number of readings (Figure 3.11). Figure 3.11a shows sizing results for analysis of ten spICPMS runs (200,000 readings) of a 50 ppt Au sample.

This shows a unimodal distribution centered on 100 nm. The same total number of particles, a single run (20,000 readings) of 500 ppt Au (Figure 3.11b), shows how an observed mass corresponding to two 100 nm Au NPs is calculated to be a single 126 nm NP, creating a bimodal distribution. This also illustrates the importance of selecting an appropriate concentration range when calculating transport efficiency by the particle number approach; each coincidence event reduces the number of observed particles by one or more and will alter the calculated transport efficiency. For Figures 3.11a and 3.11b, the total number of particles observed is expected to be the same; when accounting for the fact that each coincident pulse at
Figure 3.11. The effect of NP concentration on size distribution for monodisperse (Au) and polydisperse (TiO2 and Ag nanowires) systems in DI water. A single sample of 500 ppt Au was analyzed and compared to a 50 ppt Au sample run ten times. Similar analyses of TiO2 and Ag nanowires were performed, with single 600 ppt TiO2 and 800 ppt Ag samples run once and tenfold dilutions run ten times. The Ag lengths were calculated by holding the cross-sectional diameter constant at 33 nm.

~126 nm actually represents two 100 nm NPs, the difference in the number of particles detected for the two samples only varies by ~3%. Particle coincidence was observed to increase with concentrations above 50 ppt Au; for 100 nm Au NPs this corresponds to a particle number concentration of $5 \times 10^6$ particles/L. Although this is a useful approximate particle concentration (for the flow rate used and transport efficiency calculated in this study) to use in order to avoid coincidence in a monodisperse sample, calculating the ratio of NP pulses to total readings is
more applicable to polydisperse systems such as those analyzed here. Repeated analysis of 50 ppt Au samples, which allowed clear definition of a size distribution and little to no coincidence peak, showed good reproducibility of this ratio, with 2.90±0.23 % (n = 16) of readings containing NP pulses. By comparison, a highly polydisperse system of NPs such as TiO₂ may not exhibit obvious coincidence in a size distribution, as seen in Figure 3.11 (c) and (d). Having a distribution that spans hundreds of nanometers allows for a much larger variety of possible combinations of particle coincidence. For the 60 ppt TiO₂ sample, <1% of the total readings were NP pulses, convincing us that coincidence was not occurring in this sample. For a 75 ppt sample of the 100 nm Au NP used, the beginning of a coincidence peak was clearly seen, which correlated to 3.95% NP pulses. The 600 ppt TiO₂ sample in Figure 3.11d was above this limit at 4.28% pulses. The most probable coincidence of two particles involves two particles of ~150 nm, as seen in Figure 3.11c and d as the distribution peak. The mass of two 150 nm particles would be detected as a single pulse and calculated to be equivalent to a single 216 nm particle. Similarly, a pair of 200 nm particles, which also have a high probability of being observed in the more dilute 60 ppt sample, would be equivalent to the mass of a single 252 nm particle. These and other similar coincidence events fall into the observed shoulder on the peak of the 600 ppt TiO₂ size distribution. Coincidence of other particle sizes and number of particles surely occur and contribute to the overall distribution of a more concentrated solution of NPs, but their contributions may tend to be lost in a very polydisperse system such as the TiO₂ analyzed here. An example of how coincidence can be reduced while still defining a size distribution by the method of running a dilution for a longer time is seen in Figure 3.12, with combinations of 2, 4, 6, and 10 sets of 60 ppt TiO₂ size distributions.
In the 80 ppt Ag sample in Figure 3.11e, 1.53% of readings contained an NP pulse, also below the ratio observed for the 50 ppt Au sample which did not exhibit coincidence. The 800 ppt Ag (Figure 3.11f) run had 14.9% of its readings as pulses, making it likely that coincident pulses occurred. Comparing the Ag nanowires’ length distributions, again it is not immediately obvious that coincidence has occurred in the more concentrated 800 ppt sample relative to the ten 80 ppt samples. These distributions were created by holding cross-sectional diameter constant at 33 nm. This introduces additional uncertainty about where on a length axis a coincidence of two NPs would occur. However, assuming this diameter is sufficiently constant so as to not create a large difference in length, any coincidence of two particles would simply
result in a length that is the sum of the two individual nanowires lengths. In Figure 3.11e, the most probable observed lengths are in the 300 – 1500 nm range. Combinations of two of these lengths would result in additional lengths being observed in the 600 – 3000 nm range; however much of this range is contained in the original 300 – 1500 nm range, and coincidence falling in this range would likely be completely masked for such a polydisperse system. For the 800 ppt Ag sample, a slight increase in the number of counts was observed across the range of lengths from 1500 - ~3300 nm, which may be due to coincidence of NPs. This highlights the difficulty of distinguishing between polydispersity and coincidence in NP samples with a broad size distribution. While polydisperse systems present challenges in analysis, coincidence of NPs will not perturb a size distribution nearly as much as it will for a monodisperse sample. This may be considered advantageous when analyzing environmental samples for NPs, as they are expected to be highly polydisperse systems, partially due to environmental modifications such as dissolution, weathering, and biotransformation (8).

For a well-defined monodisperse NP, the number of coincident pulse events may be predicted by a Poisson distribution. The probabilities of an instrument reading containing no NP pulse (P(0)), a pulse from a single NP (P(1)), or a pulse resulting from simultaneous detection of two NPs (P(2)) can be estimated and compared to empirical data. However, for the polydisperse NPs used in this study, the coincidence pulses are calculated to be the same mass as single particles that are actually contained in the distribution. Due to this difficulty in evaluating the probability of coincidence for polydisperse NPs by a statistical method, occurrence of coincidence was determined by the dilution scheme used here.

4.1 Introduction

Increased production and use of engineered NPs over the last 5 years (66) and growing concern about the potential hazards of these materials has prompted a need for improvements in detection and characterization methods. Carbon-based NPs, such as fullerenes and CNTs, are contained in more consumer products than any other NP except for Ag (66). Specifically, concern regarding exposure to CNTs in the manufacturing process, and their potential release into the environment from CNT-containing composites, drives the need for analytical techniques capable of detecting these materials. Mechanical agitation of CNTs during manufacturing has been shown to result in detectable concentrations of airborne particulates (20). In this case, CNTs may cause dermal toxicity (94), but more importantly they have the potential for pulmonary toxicity due to their morphological similarity to asbestos (11, 12). Airborne concentrations in the immediate vicinity of manufacturing processes may far exceed those predicted in the environment (µg m-3 vs. pg m-3 respectively (20, 91)), but there is still potential ecotoxicological risk associated with CNTs. Incorporation of CNTs into a variety of consumer products creates the potential for release of these materials to the environment with subsequent transport and transformation processes (8). Expected aqueous environmental concentrations of
CNTs (~ng L⁻¹) (7, 91) are currently well below concentrations used in laboratory toxicity tests, which are often in the mg L⁻¹ range (95-97). This “concentration gap” for particle detection provides strong motivation for development of metrology capable of detecting extremely small amounts of NPs.

Methods to detect environmentally relevant concentrations of CNTs are rare. Those commonly used for characterization of CNTs, such as TEM, STEM, UV-Vis and Raman spectroscopy (98), do not have the capability to efficiently detect the low concentrations expected in the environment, although near infrared fluorescence spectroscopy has been used to analyze CNTs in sediments and tissues (99). In contrast, ICP-MS has detection limits at the ng/L or sub – ng/L level for most elements. However, carbon is generally not detectable with standard ICP-MS methods although synthesis of CNTs typically utilizes metals such as Mo, Ni, Co, Y, and Fe (100, 101) for catalytic growth of the carbon structure. Consequently, residual metal catalyst particles frequently persist in the CNT structure after manufacture (102) and have been associated with toxicity to organisms via generation of reactive oxygen species (94). CNTs are often purified after synthesis to remove metal impurities (103), but even after acid purification metals intercalated within carbon structures typically account for several percent of the particle mass (19, 104). Quantification of metal impurities in CNTs has been performed by ICP-MS (105, 106) for analysis of bulk metal content; however, we are interested in the ability to use these metal nanoparticles as a route to detect and quantify CNTs at the extremely low concentrations likely to be encountered in the environment.

Specifically, the goal of this study was to evaluate the ability of spICPMS to detect trace catalytic metal nanoparticles intercalated in CNTs as proxies for the materials themselves. Initially developed for metal colloid analysis by Degueldre et al (107), spICPMS has recently
been used for detection, quantitation of particle number, and sizing of engineered nanoparticles such as Ag (84-86) and metal oxides such as TiO$_2$ and CeO$_2$ (108). An in-depth discussion of the theory behind this technique can be found in these previous studies. In brief, NPs entering the plasma are disintegrated to a packet of ions. Consequently, metal nanoparticles appear as individual pulses that are distinguished from the background, with the ion intensity being directly related to the number of analyte atoms in the NP. The instrument signal is reported as counts of the analyte isotope per dwell time or reading, e.g. $^{89}$Y counts per 10 ms. Size information on chemically uniform, (roughly) spherical NPs, such as metals or metal oxides, can be extracted from spICPMS (85, 86). For rod-like NPs length distributions can be determined if the minor dimensions have been determined from SEM/TEM data (108). One of the challenges inherent in using spICPMS for CNT analysis is that variable metal content among individual CNTs makes size/length estimation impossible unless the bulk metal content is applied to all CNTs, although sizing was not the focus of this study.

In contrast to sizing, a determination of particle number concentrations from spICPMS is more direct and relies on applying the transport efficiency to the observed pulse frequency. As in previous publications (86, 90), we define transport efficiency as the fraction of sample droplets, containing NP and/or dissolved analytes, which reach the plasma. Determination of number concentration is, however, strongly affected by what criterion is used for defining a pulse as opposed to background noise. This is especially true for polydisperse samples having a significant population of small particles that cannot be separated from the background. This will be an issue for single walled CNTs due to heterogeneity in both the size and number of metal nanoparticles that are embedded within the carbon cylinder.
Another important consideration in the design and implementation of appropriate analytical techniques for NP detection is the issue of selectivity. Aqueous environmental matrices are extremely heterogeneous, and NP concentrations are expected to be many orders of magnitude lower than that of other particles and soluble chemicals present. This issue is particularly important for CNTs due to the prevalence of naturally occurring carbon-containing species (i.e. cells, organic detritus, humics). This is exacerbated in the case of biological tissues where detection of CNTs by carbon analysis is clearly not possible. Therefore, using metals as a surrogate to detect the presence of CNTs with spICPMS could provide a superior means of differentiation of CNTs from other materials even when CNTs are present at extremely low concentrations.

The first goal of this study was to assess the ability of spICPMS to detect CNTs using trace metal catalysts as proxies. Further investigation of this method’s capabilities for detection and quantification of CNTs at environmentally relevant concentrations was carried out: the end goal of this work was to determine if spICPMS could be used to detect and quantify CNTs in a simulated release study. To the best of our knowledge, this is the first work to use spICPMS as a detection method for CNTs in any application.

4.2 Experimental

4.2.1 Preparation of CNT suspensions

The CNTs used for this study were acquired from Nanostructured and Amorphous Material (NanoAmor) (1280 NPG), Carbon Solutions (AP-SWNT), and SouthWest NanoTechnologies (SG65). The CNTs used were not surface modified. Stock CNT solutions were prepared (at Johns Hopkins University, JHU) by initially adding a known CNT mass to a
known volume of DI water that contained 1% w/w Triton™-X-100 (Sigma-Aldrich). The CNTs were suspended by sonicating the mixture overnight in a low power (70 W) Branson 1510 ultrasonicator. At this stage the stock solutions were sent from JHU to CSM for ICP-MS analysis. Prior to ICP-MS analysis all stock CNT solutions were diluted to concentrations in the ng/L range using 18Ω nanopure water (Barnstead Nanopure) in 15 mL polypropylene centrifuge tubes. Suspensions were homogenized after each dilution by immersion in a bath sonicator (Fisher, FS60H, 150 W) for 10 minutes. Optimal concentrations varied among the samples and were chosen to avoid coincidence while providing sufficient counts for statistical robustness ($10^8$). DI blanks were analyzed for each metal isotope used for CNT detection, and each sample was run in duplicate to evaluate reproducibility. To determine the amount of soluble metal released from the CNTs, a separate set of CNT suspensions were prepared in nanopure water, which were then centrifuged for 1 hour at 125000 g (Optima XL, Beckman) to remove the CNTs. This supernatant was used to compare to deionized water in determining the level of background counts. Dissolved Au, Co, and Y calibration standards were prepared using Claritas PPT (SPEX Certiprep) ICP-MS standard grade stock solutions. A gold nanoparticle standard (BBI, 100 nm) was used for quantification of instrument efficiency.

### 4.2.2 Characterization of CNTs

Bulk physical and chemical characterization involved Energy Dispersive Spectroscopy (EDS) analysis, which was performed using a cold cathode field emission scanning electron microscope (SEM; JEOL 6700F) equipped with an EDAX Genesis 4000 X-ray analysis system (detector resolution of 129 eV).
4.2.3 Comparison of spICPMS and NTA

A Perkin Elmer NexION 300q with an S10 autosampler, using 20,000 dwell times (10 ms dwell time, total data collection time = 200 seconds per sample), provided the spICPMS data for both CNT suspensions and dissolved standards and blanks. The sample introduction rate in all experiments was 0.97 mL/min. Given the ability of spICPMS to detect the presence of a very small number of metal nanoparticles, extra caution was needed to avoid sample carry over. Methods included rinsing of the nebulizer and spray chamber, as well as daily changes of the peristaltic pump tubing, which also minimized flow rate drift.

To compare particle number measurements from spICPMS and Nano Tracking Analysis (NTA), samples were prepared and split into two aliquots, one for analysis by each method. If samples were too concentrated for analysis by one method, they were diluted in the same manner as above, using nanopure water and 15 minute bath sonication. Reported particle concentrations were corrected for the degree of dilution needed. 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). The sample (350-400 µL) was injected into the sample cell via a latex- and oil-free 1 mL syringe. A video (30 s) of each sample was collected and analyzed using NTA 2.3 Build 011 software (NanoSight Ltd.). The sample cell was then evacuated, rinsed and disassembled for further cleaning. All components were dried completely prior to reassembly. These data collection and cleaning processes were repeated three times for each sample type. Results for number concentration (particles/mL) were averaged over the three replicates.
4.2.4 Nanocomposite preparation for CNT release study

To prepare CNT/polymer nanocomposites a 20 mg/mL aqueous stock solution of chitosan (Sigma-Aldrich, MW = 20,000) in 2 v/v% acetic acid (Fisher) was stirred and heated at 50°C for approximately four hours. Chitosan was chosen as a model product polymer and for its biodegradable properties. The stock solution was centrifuged with a Unico Powerspin LX at 4000 rpm to remove any undissolved solids. Carbon Solutions single-walled CNTs, known to contain trace yttrium catalyst, were then weighed into separate flasks and mixed in a 0.1, 0.5, 1, 2, 3, 4, and 5 wt/wt% with the chitosan stock solution to prepare well defined CNT-chitosan nanocomposites. Each suspension was stirred for approximately five minutes and sonicated for four hours with frequent stirring, creating a CNT suspension stabilized by chitosan macromolecules. After sonication, the suspension was centrifuged at 4000 rpm to remove any aggregates. 5 mL of each CNT-chitosan suspension and 5 mL of pure chitosan were then dried in aluminum dishes (44 mm diameter, 12.5 mm height, Fisherbrand) overnight; each composite was created from the same volume of the appropriate CNT/chitosan solution to ensure that they exhibited comparable size, shape and surface area. Once the coupons had dried, they were peeled from the aluminium dishes. The coupons were then soaked in a 1M NaOH (Fisher) bath for 1 hour to remove excess acetic acid and washed with copious amounts of DI water. Once prepared, the coupons were placed in 100 mL of DI water (Figure 4.1) and left for 7 days.

The CNT content of the nanocomposites varied from 0.1 wt% to 5 wt%, and included a control sample (0 % CNT, i.e. pure chitosan). At the end of the 7 day period samples were collected from the surrounding DI water. Once collected, solid sodium deoxycholate was added to each aliquot to keep the CNTs dispersed prior to spICPMS analysis.
4.3 Results and discussion

4.3.1 CNT characteristics

To determine which metals should be investigated for possible detection by spICPMS, characterization of CNTs was performed by SEM-EDS. Although metal contents were provided by the manufacturers for each CNT, this analysis was carried out to verify manufacturers’ claims and ensure there were no other metals which may be usable for spICPMS detection. Characterization data for size and metals content is given in Table 4.1.

Preliminary measurements using residual metal nanoparticles as a proxy for CNTs were done by monitoring multiple isotopes for each CNT studied, in order to ascertain the most effective analyte for each material. All metals which were identified in a particular CNT were examined by spICPMS. Based on these preliminary results, the most readily observed metal isotope was used for the remaining analyses. This was done to avoid switching isotopes during spICPMS analysis.
Table 4.1. Size characterization and percent metal for all CNTs used. Type of CNTs: SWNT = single-walled nanotube

<table>
<thead>
<tr>
<th>CNT brand and Materials (NanoAmor)</th>
<th>Type</th>
<th>Length (nm)</th>
<th>Diameter (nm)</th>
<th>Metal content (manufacturer)</th>
<th>Metal content by EDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nanostructured and Amorphous</td>
<td>SWNT</td>
<td>5000-15000</td>
<td>1.1</td>
<td>Co 0.6%, Mo 0.1%, Mg 1.2%</td>
<td>Co 0.5%, Mo 0.1%, Fe 0.1%</td>
</tr>
<tr>
<td>Carbon Solutions</td>
<td>SWNT</td>
<td>1800±1000</td>
<td>3.8±1.8</td>
<td>Ni, Y (1-30 wt%)</td>
<td>Ni 19.4%, Y 6.0% (at%)</td>
</tr>
<tr>
<td>Southwest Nanotechnologies</td>
<td>SWNT</td>
<td>578±358</td>
<td>0.8±0.1</td>
<td>Co, Mo (1-15 wt%)</td>
<td>Co 1.1%, Mo 3.7% (at%)</td>
</tr>
</tbody>
</table>

4.3.2 Choice of element proxy

Metal content (Table 4.1) is an important consideration in choosing the analyte isotope, although there are other relevant factors. Choice of an analyte will be influenced by its isotopic abundance, and the potential for isobaric or polyatomic interferences on the isotopes. For example, although Ni is more abundant than Y in Carbon Solutions CNTs (19.6 wt % Ni vs. 6.0 wt% Y) by EDS analysis, the most abundant Ni isotope, $^{58}\text{Ni}$, is essentially unusable for ICP-MS analysis due to mass interferences with ArO, CaO, and Fe. As Y is monoisotopic at mass 89, it also gives a stronger signal than $^{60}\text{Ni}$ (26.2% abundant) despite a greater relative amount of Ni in
the CNT. For these reasons, Y was considered to be a more viable analyte (Figure 4.2) for
determining the presence of Carbon Solutions CNTs. Similar factors were considered in
choosing $^{59}$Co as the analyte for NanoAmor and Southwest Nanotechnologies CNTs. Thus,
although pulses were observed for all Mo isotopes analyzed, their abundances are distributed
over seven isotopes, none being more than 25% abundant; the most viable Mo isotope, $^{98}$Mo, is
24.1% abundant. It has a higher background and fewer large pulses are observed than for $^{59}$Co,
which is also monoisotopic. For this reason $^{59}$Co was preferred as the metal isotope to analyze
Southwest Nanotechnologies and NanoAmor CNTs (see Figure 4.2)

![Graph](image)

Figure 4.2. spICPMS response data, for determination of the best analyte metal for CNTs used in
this study. The CNTs were at 1 ug/L to ensure enough CNTs would be in solution for analyte
comparison. Only data for one isotope each of Ni ($^{60}$Ni) and Mo ($^{98}$Mo) are shown here. Other
isotopes were not as usable due to mass interferences and lower isotopic abundances. For Carbon
Solutions CNT, $^{89}$Y appears to have more pulses and more intense pulses above background,
making it a better choice for detecting CNTs by this method. For NanoAmor and Southwest
CNTs, $^{59}$Co is the clear choice over $^{98}$Mo for the same reasons.
Contributors to the background include instrumental (electronic) noise, isobaric interferences on the analyte isotope, and dissolved analyte. In the current application a low background is a crucial consideration as many CNTs will generate small pulses, due to either low metal content or small particle size, that are close to the background. This becomes clear when intensity data are binned, and the output is presented as a histogram of number of events (counts) on the y-axis versus the spICPMS response (signal intensity) on the x-axis as shown in Figure 4.3.

![Histograms showing data distribution](image)

Figure 4.3. Data for ICP-MS response from Figure 4.2 binned to show distribution of pulses above background. Binning of data is performed using OriginPro 8.5’s Frequency Count command on a column of real-time ICP-MS response data. The bin size is chosen to best display the data; in this case a bin size of 1 was used for maximum resolution of pulses from background.
This is a common approach used to interpret spICPMS data (86). For each CNT the metal analytes shown were chosen because of the low counts (< 5 counts/dwell time) indicative of low instrumental noise and lack of interferences for that isotope. For example, the binned data for the Carbon Solutions SWNTs shows that the spICPMS response for $^{89}$Y as compared to $^{60}$Ni has a greater number of large pulses (spICPMS response > 10) and a smaller number of “background” pulses (spICPMS response < 5). Both factors favor using $^{89}$Y as compared to $^{60}$Ni for indirect SWNT detection.

4.3.3 CNT pulse cut-off criteria

Once the most appropriate metal isotope had been identified for each CNT type it was important to determine the ICP-MS response for that particular analyte. This is necessary because the pulse intensity distribution from metal nanoparticles can often run up against the background, making it difficult to discriminate signals from smaller metal nanoparticles from the background. Conversely, the presence of dissolved ions can increase the background signal and cause underestimation of the particle number concentration, by masking the presence of small pulses arising from particles. We addressed this issue by determining if there was a difference in background counts between a DI blank and the supernatant of a CNT-containing solution. In these experiments, CNT samples were prepared and then centrifuged (1 hour, 125000 Gs) to remove any CNTs. The supernatant was then diluted by factors equivalent to diluting the original CNT solutions to 200 and 400 ng/L, so any dissolved metal ions which were released from the CNTs would be diluted by the same amount as that for CNT-containing solutions. Figure 4.4 shows data where the DI blank and the 200 ng/L equivalent dilutions of the supernatant are compared for each of the three CNT types (Nanostructured and Amorphous Materials, Carbon
Solutions, and Southwest Nanotechnologies). The blank and supernatant means for $^{89}$Y had no significant difference ($p<0.05$) for Carbon Solutions CNT. Although there was a significant difference between the means for the Southwest Nanotechnologies and NanoAmor solutions ($p > 0.05$), the upper tail of the signal distribution widths were similar enough that it can be assumed there is insufficient dissolved metal in CNT-containing solutions to affect discrimination of CNT pulses.

One of the main challenges in quantification of CNTs by spICPMS is the small fraction of metal nanoparticles in the materials. The low metal content means that there will be a less intense pulse for a given CNT than that for a material such as a gold nanoparticle, which is 100% Au on a per particle basis. This highlights the need to determine a pulse cut off criteria to discriminate pulses that correspond to CNTs from background signal. Fortunately, the similarity of the DI water and the supernatant spICPMS data for each of the three CNTs under investigations demonstrates that a statistical analysis of DI blank data has the potential to provide the parameters needed to determine the most appropriate pulse cut-off criteria in CNT-containing solutions. Experimentally, we addressed the need for a quantitative approach in differentiating a pulse from background by comparing multiple analyses of a DI blank and a 5 ng/L concentration of Southwest Nanotechnologies CNTs. The goal was to determine if we could establish a protocol for establishing a standard cut-off above any instrumental or dissolved background for quantifying nanoparticulate pulses which would simultaneously minimize false positives in the blank and maximize pulses in the CNT sample. Both the DI water blank and the 5 ng/L CNT samples were run 30 times, for a total of 600,000 data points each, with the raw data shown in Figure 4.5a and 4.5c. The reason for the low concentration was to have a small enough
Figure 4.4. Comparison of the spICPMS response for DI blanks and CNT supernatant at the different metal isotope masses used for detection of each CNT. The ICP-MS response values for the DI blank and corresponding supernatant data for NanoAmor and Southwest Nanotechnologies SWCNTs, using $^{59}$Co as the analyte, are distributed differently because the analyses were performed on different days.

number of CNTs in solution, particularly ones that may appear near the background, that most of the ICP-MS counts would be equivalent to those in the DI blank. At a very low concentration,
multiple runs (in this case 30) were performed to increase the volume of sample analyzed and allow for a larger number of CNT pulse events to be detected. The corresponding ICP-MS response distribution was binned up to 50 counts and is shown in Figure 4.5b and 4.5d and to 10 counts in the inset figures to show the background ICP-MS response. Figure 4.5 shows that differences between the DI blank and the CNT sample are only clearly seen above about 7-8 counts.

Figure 4.5. Comparison of DI blank and 5 ng/L Southwest Nanotechnologies CNTs. Data show the sum of 30 individual runs corresponding to 600,000 readings. Panels (a) and (c) show real-time data for analyses of DI and 5 ng/L CNTs, respectively, with (b) and (d) showing the ICP-MS response binned, illustrating where CNT pulses begin to become visible above the background. Insets in (b) and (d) show the similarity in the distribution of ICP-MS response for values $\leq 7$ ("background").
In Table 4.2 the mean ($\bar{x} = 1.30$) and standard deviation ($s = 1.17$) of the ICP-MS response were calculated by averaging all the data for the DI blank (Figure 4.5a). Although it is clear that the background signals are not normally distributed (Figure 4.5), we believe these statistical parameters provide the basis for differentiating small particle-created signals from the background noise. Indeed, this approach has been used by a number of researchers for studies of more uniform NPs (85, 90). Three different cut-off criteria for the ICP-MS response were examined to evaluate the number of false positives (apparent CNT detection events) in the blank and the ICP-MS response values (pulses) above the cut-off value which were considered to be CNTs.

Table 4.2. Examination of different cut-off criteria for differentiating CNT detection events from background signal. Values above each cut-off value were considered to be CNT detection events.

<table>
<thead>
<tr>
<th>Cut-off criterion</th>
<th>Calculated value from DI blank data (n=600,000)</th>
<th>False positives in blank</th>
<th>Percent of total blank readings (n = 600,000)</th>
<th>Pulses above cut-off in 5 ng/L Southwest Nanotechnologies CNT sample</th>
<th>Percent of total readings in 5 ng/L Southwest Nanotechnologies CNT sample (n = 600,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\bar{x} + 3s$</td>
<td>$4.81$</td>
<td>$7998$</td>
<td>$1.3%$</td>
<td>$22475$</td>
<td>$3.7%$</td>
</tr>
<tr>
<td>$\bar{x} + 5s$</td>
<td>$7.15$</td>
<td>$84$</td>
<td>$0.014%$</td>
<td>$786$</td>
<td>$0.13%$</td>
</tr>
<tr>
<td>$10$</td>
<td>10</td>
<td>2</td>
<td>$0.00033%$</td>
<td>81</td>
<td>$0.0135%$</td>
</tr>
</tbody>
</table>
Analysis of Table 4.2 reveals that with the lowest cut-off criterion ($\bar{x} + 3s$), although the number of pulses counted as CNT detection events in the CNT solution was greatest (22475), so was the number of false positives in the DI blank (7998, corresponding to 1.3% of the readings). The most conservative cut-off we tested was 10 counts, which was chosen after visual inspection of the CNT data, and showed that in the DI blank nearly all of the ICP-MS response values were below this level. Table 4.2 shows that when using this more stringent cut-off criterion only 2 of 600,000 readings in the DI blank counted as pulses, but the number of pulses counted in the CNT solution was also reduced drastically, from the 22475 observed with an $\bar{x} + 3s$ cut-off criterion, to 81. The $\bar{x} + 5s$ value (7.15) was deemed a good compromise between these cut-offs: 786 pulses were counted as CNT detection events in the CNT solution and only 84 in the DI blank. This corresponds to what is observed in the binned data (Figure 4.5 (b) and (d)), notably that the difference in the distributions between the DI blank and CNT solutions appears above 7 to 8 counts. Although in general we favor a statistical approach to defining the CNTs above background, we will demonstrate in the analysis of the CNT release studies that the choice of cut-off may be highly dependent on the experimental conditions.

4.3.4 Quantification of CNT number concentration by spICPMS

Having established these analytical and data evaluation protocols, the ability of spICPMS to determine variable CNT concentrations was explored by analyzing a DI blank and solutions containing 200-800 ng/L NanoAmor CNTs. To test the generality of this approach, we deliberately chose a different type of CNT from the Southwest Nanotechnology CNTs used to establish the cut-off criteria. To illustrate the effect that different cut-off criteria have on quantifying CNT concentrations, two values were used to discriminate nanoparticulate pulses
from the background signal as described in Table 2: $\bar{x} + 3s$ and $\bar{x} + 5s$ of the ICP-MS response values obtained from the DI blank. Analysis of Figure 4.6 shows that in the CNT concentration range of 0-800 ng/L, the number of pulses varies in proportion to the CNT concentration for both cut off criteria. However, note the number of pulses detected in the DI blank for the $\bar{x} + 3s$ cut-off criterion: 213 pulses out of 20,000 readings, for 1.07% of readings falsely identified as pulses. This is in line with the fraction of false positives reported in Table 4.2 for this criterion, and should be considered when selecting a cut-off. Although the increase in pulse number with increasing CNT concentration using this cut-off is clearly seen in Figure 4.6, the aforementioned high background, and low linear correlation ($r^2 = 0.732$) results in a weak proportional relationship. In contrast, the $r^2$ value for the $\bar{x} + 5s$ cut-off criterion ($r^2 = 0.959$) is significantly improved, and more importantly shows more clearly that the number of pulses counted as CNT detection events is directly proportional to CNT concentration.

The number of CNT detection events can also be used to estimate the measured CNT concentration. The first step in calculating particle numbers is to determine the sample transport efficiency; the fraction of a given sample which reaches the plasma and is analyzed. This was accomplished following a method developed by Pace et al (86) using a well-characterized, highly monodisperse Au nanoparticle (100 nm, BBI). Pulses generated during spICPMS analysis of this Au NP can be related to mass of Au by a calibration curve generated by using dissolved Au, and mass is transformed to particle diameter based on the known density and volume of Au NPs. The calibration curve enables the transport efficiency term to be estimated. The transport efficiency term must be calculated for each day’s analysis, as it has been observed to vary from day to day. For the data shown in Figure 4.6 the measured transport efficiency was 0.050 (5.0% of sample volume reached the plasma) assuming that the transport efficiency of CNTs and Au NPs
Figure 4.6. Relationship between CNT mass concentration (X) and number of CNT detection events (Y) for NanoAmor CNTs. Cut-offs of $\bar{X} + 3s$ and $\bar{X} + 5s$ based on the DI blank data were used to illustrate how different cut-off values affect the apparent number of CNT detection events. The number of pulses above the cut-off value was used to calculate a measured number concentration of CNTs in particles/mL using known flow rate, sample run time, and instrument transport efficiency. A predicted CNT number concentration for a given mass concentration is shown for comparison with the measured values, using data on the average CNT density, length, and diameter.

are comparable. For each mass concentration, the number of observed pulses was divided by the transport efficiency, flow rate (mL/min), and sample run time (min) to obtain a value for measured particle number concentration (right Y-axis, Figure 4.6).

To compare with the measured particle concentrations, the predicted CNT number concentrations for a given CNT mass concentration (ng/L) can also be calculated using CNT length and width characterization data supplied by the manufacturer (Table 4.1). In the first step of this analysis the average volume of a single CNT was determined by multiplying the average
length by the cross-sectional area, assuming a cylindrical geometry. This average volume was multiplied by the density (1.14 g/mL) to obtain the average mass of a single CNT. Finally, to determine the particle concentration (particles/mL) the mass concentration of a CNT solution (mg/mL) was divided by the average mass of a single CNT (mg/particle). It should be noted that uncertainties in the polydispersity in the dimensions (widths and lengths) of the CNTs and their degree of dispersion into individual tubes make this a gross approximation at best. Results from this analysis are shown in the top X-axis in Figure 4.6.

A comparison of the measured and predicted CNT number concentrations (Figure 4.6) reveals that the concentrations measured by spICPMS were consistently about four orders of magnitude less than those predicted. As mentioned previously this is most likely due to the significant variations in CNT metal content within CNT samples, with many CNT particles containing metal masses below instrument detection limit. Also, the CNTs may be present as bundles of tens to hundreds of particles, although the use of a surfactant to prepare the CNTs in solution was designed in part to maximize the number of individual CNTs present. In the spICPMS analysis, each bundle would only be counted as one pulse, resulting in severe undercounting of actual CNT concentrations.

4.3.5 Comparison of spICPMS and NTA for CNT quantification

To further examine the discrepancy between measured and computed particle number concentration, Nanoparticle Tracking Analysis (NTA) was performed to directly measure CNT number concentrations. Solutions of three CNTs from NanoAmor, Southwest Nanotechnologies and Carbon Solutions were prepared at concentrations of $10^{10}$ particles/mL calculated from size characterization data as previously described. These three samples were split for analysis by both
NTA and spICPMS. The results for the comparison are shown in Figure 4.7. The cut-off criterion for spICPMS analysis used for this experiment was \( \bar{x} + 5s \) of DI blank data.

![Graph showing particle number concentrations for NTA and spICPMS for different CNTs and materials.](image)

**Figure 4.7.** Particle number concentrations as measured by NTA and spICPMS for three types of CNTs. In addition to the CNTs, comparisons between the techniques were made using a highly monodisperse Au NP solution and a moderately polydisperse TiO\(_2\) NP solution. Horizontal black bars in columns indicate the diluted concentrations at which the measurements were made; these were then multiplied by the dilution factor to obtain the measured concentration of the undiluted solution. BDL – Below detection limit

The calculated particle number for a solution containing a known mass concentration of Au NPs matched well with both spICPMS and NTA measurements, providing confidence that
both techniques can be accurate for quantification of a solution containing monodisperse spherical particles. However, the accuracy decreased for the moderately polydisperse, irregularly spheroidal, TiO₂ (Sigma-Aldrich, measured size range ~40 - 400 nm), with values calculated from both NTA and spICPMS falling short of the calculated particle number. Consistent with the analysis presented in Figure 4.6, the measured CNT particle number concentrations determined by spICPMS in Figure 4.7 were 10³ – 10⁶ particles/mL lower than those predicted based on the assumed average physical characteristics and at least 10² lower than those values measured by NTA. As mentioned previously, the likely reasons for this discrepancy between predicted number concentrations and those measured by spICPMS are variations in metal content in individual CNTs, CNT bundling, as well as polydispersity in the CNT size/mass due primarily to length distributions. Bundling of CNTs may also explain the discrepancy between predicted and NTA-measured concentrations. As shown by Jurkschat et al (104), the size of metal catalyst nanoparticles used in CNT synthesis can vary widely with smaller metal particles, ~5 nm, intercalated non-uniformly in the CNT structure. We believe that the reason for the poor analytical sensitivity is because we are only observing CNTs which contain enough total metal mass to generate pulses above our chosen cut-off. This would correspond only to CNTs (or CNT bundles) which are large enough and contain a large number of metal nanoparticles and/or those which contain larger sized catalytic metal nanoparticles. In this respect, variations in the number and size of catalyst nanoparticles contained within individual CNTs will greatly affect the pulse height observed by spICPMS analysis, as the pulse height will be directly proportional to total mass of metal in a CNT. The variation in CNT size is also a factor; a smaller CNT containing the same percent metal as a larger CNT may not contain enough metal mass to generate a pulse which can be detected above the instrumental detection limit for that element.
Despite the undercounting of measured CNT number concentration, it is important to note that spICPMS exhibits superior particle number detection limit compared to other analytical techniques. For example, in the case of a 100 ng/L CNT solution made using Carbon Solutions CNT, the particle number measured by spICPMS is \( \sim 10^3 \) particles/mL lower than that predicted from size data. However, at this low concentration thought to be representative of potential environmental releases, spICPMS was able to detect CNTs, while NTA results registered below detection limit (BDL).

### 4.3.6 Mass balance of analyte metal for varying CNT concentrations

In an attempt to explain the discrepancy between predicted CNT concentrations and those measured by spICPMS, a mass balance calculation was performed for the analyte metals. The nominal mass concentration of CNTs was used along with flow rate, analysis time, and estimates of percent metal in the CNTs, to calculate a total metal mass expected in each sample run. This was compared to measured metal mass for each run obtained by integrating the ICPMS signal over the full analysis. The values used for the predicted metal content in the CNTs were the low and high estimates provided by the manufacturers, as well as our own EDS analysis (Table 4.1). Two extreme scenarios were analyzed to ascertain whether either could account for the nominal metal content in the samples. First we assumed all the metal detected was present as dissolved species. In this case the integrated signal was compared to the dissolved calibration curve directly. In the second approach we assumed all the metal detected was present as particles. For this calculation the mass computed by comparing the integrated signal to the dissolved calibration curve was corrected for the transport efficiency. This was needed because in the case of particles only a fraction of the particles are represented in each dwell time. The data analyzed
were chosen for the wide range of CNT concentrations used for all three CNTs found to be detectable by spICPMS in this study. The degree of agreement of the measured metal mass to that predicted by CNT mass concentration and CNT metal content is displayed as a ratio of measured to nominal metal mass in Figures 4.8-4.13.

Figure 4.8. Ratio of measured to nominal metal mass for Carbon Solutions (yttrium) CNT at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to dissolved Y.

The assumption that all metal detected is dissolved is likely incorrect, but was chosen to examine this extreme case. Due to the low background observed in all spICPMS analyses of CNTs, there is very little dissolved metal present. For the Carbon Solutions CNT, assuming all metal detected is dissolved yields very low measured/nominal yttrium ratios (Figure 4.8), with 1.5% or less of predicted metal in the sample being detected. Assuming all dwell times contain a particle
Figure 4.9. Ratio of measured to nominal metal mass for Carbon Solutions (yttrium) CNT at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to particulate Y.

detection event, as shown in Figure 4.9, measured/nominal ratios are closer to accounting for all the yttrium mass. For the manufacturer’s lower estimate (1% by weight Y), the measured mass/nominal mass for yttrium are within an order of magnitude of 1, much closer than the approach of comparing predicted and measured particle number concentrations (Figure 4.7). This could be seen as further evidence that the CNTs tend to bundle prior to analysis even in the presence of surfactants, and large pulses above background may represent bundles of hundreds of CNTs. The observed measured/nominal ratio below 1 could be a result of incomplete ablation of the CNT/metal particle in the plasma, with spICPMS pulses and therefore the total metal mass measured artificially smaller than would be expected for the full metal mass present in the CNT.
The measured/nominal ratio also tends to decrease with increasing CNT concentration. This is likely an effect of the background counts: for the lower concentrations, especially 50 and 100 ng/L CNT, the background may consist of more instrumental noise than dissolved metal signal, leading to an inflated measured metal mass. As more metal is contained in the samples, e.g. 1000 ng/L CNT, the elevated background signal is likely due more to the metal present than to any instrumental noise. Figure 4.10 shows the results for the analysis of Co in NanoAmor CNTs under the assumption that all metal detected is dissolved. As with Carbon Solutions, the NanoAmor measured metal mass is much lower than expected from the prepared CNT suspensions under this assumption.

Figure 4.10. Ratio of measured to nominal metal mass for NanoAmor (cobalt) CNT at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to dissolved Co.
Figure 4.11. Ratio of measured to nominal metal mass for NanoAmor (cobalt) CNT at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to particulate Co.

The measured/nominal metal mass ratio for NanoAmor CNT assuming all dwell times contain particle detection events is much closer to accounting for all metal mass in the suspensions. This is especially true for the nominal metal masses calculated using the EDS data (0.5 atomic % Co, 2.4 weight % Co). As with the other two CNTs’ measured/nominal ratio under the assumption that all metal is dissolved, the amount of metal measured is far short of that suggested by the CNT concentration and estimates of metal content for Southwest CNTs. The extreme case of assuming all residual catalyst metal is present in the dissolved form clearly underestimates the total mass of metal detected. This is not surprising, as the metal is expected to primarily be
As with the NanoAmor CNTs, the assumption that all readings contain a particle detection event is closer to predicted values than assuming all metal is dissolved for the Southwest CNTs. Whether the calculation is performed using the manufacturer’s reported metal content or our EDS results, the proximity of the measured to the predicted values are much closer for the metal mass balance than they are for comparing CNT number concentrations. For the Southwest CNTs, the measured/nominal ratio is closer to 1 than for the other CNTs (Figures...
Figure 4.13. Ratio of measured to nominal metal mass for Southwest (cobalt) CNTs at concentrations from 50 to 1000 ng/L, assuming all ICP-MS signal is due to particulate Co.

4.9 and 4.11). If ablation efficiency is a factor in recovery of total metal in solution by this mass balance approach, it is possible that the smaller Southwest CNT (Table 4.1) is more completely ablated in the plasma and therefore less affected by this. This mass balance approach for residual metal could be very useful for quantification of CNT recovery in experiments related to toxicity and environmental studies.

4.3.7 Detection of CNTs released from a polymer matrix

The incorporation of CNTs into polymer matrices to create a nanocomposite has been explored for a variety of materials, typically done to improve mechanical strength or
conductivity of the material \((2, 109, 110)\). Release of CNTs from nanocomposites due to polymer degradation is a possible route of entry into the environment \((111)\). To probe the ability of spICPMS to detect CNTs released from these nanocomposites well-defined CNT composites were created by combining a known mass of CNTs (Carbon Solutions) and chitosan and placed in DI water. The extent of CNT release was studied for nanocomposites with varying mass percent CNT (sampled at 7 days) by analyzing an aliquot of the surrounding DI water by spICPMS. In collecting spICPMS data it is necessary to strike a balance between low particle number concentration and sufficient analysis time to allow a statistically relevant amount of data to be collected, as was demonstrated for polydisperse metal oxide nanoparticles and Ag nanowires \((112)\). Furthermore it has been shown in Chapter 3 that the total number of readings containing a particle should be in the range of 5 -15 \% to avoid coincidence events, where multiple particles enter the plasma simultaneously and are detected as a single pulse \((90, 108)\).

Initial analysis of the release samples indicated that a 1:200 dilution in nanopure water would be ideal to maximize the number of CNT detection events while minimizing coincidence. Longer analysis times were also used to increase the number of CNTs detected, in order to improve confidence in any observed trends. Thus, 100,000 readings were taken instead of the 20,000 used in the CNT characterization studies. This increased the analysis time of a single sample from 3.4 minutes to 17 minutes.

Figure 4.14 shows the data acquired from spICPMS analysis of supernatant that contained different CNT loadings (0 - 5\% by weight) in CNT/chitosan composites. Based on the previously discussed statistical analysis of spICPMS blanks, applying the \(\bar{x} + 5s\) criterion to the chitosan control sample (0 \% CNT) suggests a cut off of 1.37 counts. This value was the result of a low background obtained from the control sample (pure chitosan, 0\% CNTs), with most
readings being 0 or 1 count per dwell time. Thus, any data points at 2 or more counts would be considered a CNT-generated pulse using this criterion. After evaluating the number of data points that were at or above 2 counts for each sample, it became clear that this cut-off is not appropriate for examining the effect of loading on CNT release to solution (Figure 4.14). This is particularly apparent in Figure 4.14(c), where a cut-off value of 1.37 would require us to conclude that every sampling event corresponds to a CNT detection event due to the elevated background level. Given that the previous analysis of Carbon Solutions CNT supernatants suggest no release of dissolved Y, the elevated backgrounds for the higher loading, especially the 5% sample, implies a dramatic, non-linear increase in the number of low-metal content CNTs, which is physically unreasonable. However, it is also clear from a visual inspection of the raw ICP-MS data that the number of CNTs released into solution does scale with the CNT loading in the polymer sample (compare Figures 4.14(a), (b) and (c)). Thus, it is apparent that the cut-off criteria established in simple solutions (Figure 4.6 and Table 4.2) are no longer valid in more complex aqueous conditions. Indeed, establishing appropriate cut-off criteria remains as a challenge which must be overcome if spICPMS is to be able to provide quantitative data on CNT concentrations in realistic environmental matrices.

Accepting that the choice of an appropriate cut-off criteria is somewhat arbitrary at the present time and represents an area for future research and refinement, a visual inspection of the data with the highest background (5% loading) informed the choice of a 20 count cut-off where clearly all data above this point was due to detection of a CNT (Figure 4.14 (c)). Using this cut-off criterion, Figure 4.14 (d) shows that there is a linear correlation between the number of CNT detection events and the CNT loading. This relationship is qualitatively consistent with the changes observed in the ICPMS data shown in Figure 4.14(a)-(c). We believe that these release
Figure 4.14. Examination of the effect of loading on CNT release from CNT-chitosan nanocomposites. Five samples were run for each analysis for a total of 100,000 readings. Real-time data for three individual CNT loadings of 0.5%, 2%, and 5% by mass are shown along with a plot of CNT detection events using a 20 count cut-off criterion.

studies provide a reasonable reflection of the current strengths and limitations of spICPMS in analyzing CNTs in environmentally relevant scenarios. In terms of strengths, spICPMS offers significant advantages over other techniques in terms of particle number detection limits, and can also provide qualitative insights into how external variables (e.g. CNT loading in a polymer composite) impact the number of CNTs released. However, the changes in background shown in Figure 4.14 also underscore the challenges of using spICPMS to provide unambiguous
quantitative information on the concentration of polydisperse materials in all but the simplest of solutions (e.g. situation represented in Figure 4.6), with CNTs representing an extreme example.
CHAPTER 5
CONCLUSIONS AND FUTURE WORK

5.1 Implications of results

The findings detailed in this thesis have been previously published and cited, and thus have already affected various areas of research related to nanotechnology. The implications of each study will be briefly discussed for each section in the conclusions.

5.2. Quantification of ZnO NP dissolution

The results of this study showed that ZnO NPs are often quick to dissolve in a variety of matrices relevant to nanotoxicity testing even at mg/L concentrations. One of the major implications of this work is in toxicity testing, especially in vitro where it is unlikely that ZnO NPs added to toxicity media at predicted environmental concentrations (ppt range) would result in NP exposure to test cells. Rather the data suggest dissolution would likely produce dissolved Zn exposures instead. Much lower extent of dissolution was observed for ZnO NPs in moderately hard water. This medium was used as a representative environmental freshwater, and the results suggest ZnO NPs released from products such as sunscreen may be more stable in the environment. Knowledge regarding particle stability is highly important to a variety of fields, and this study has been cited by a number of journal articles in the fields of nanotoxicity, environmental fate and transport, and nanoanalytics.

Further experiments to explore the possibility of environmental persistence of ZnO NPs in moderately hard water should be performed. In moderately hard water, dissolved Zn did not increase, except slightly for the smallest ZnO NP (Figure 2.2). Chemical equilibrium modeling
results suggest that any ZnO dissolution would result in supersaturation of a Zn-containing precipitate of a different composition than ZnO (hydrozincite). ZnO NPs could be suspended in moderately hard water and split into two fractions: one to be analyzed immediately and one to allow time for possible dissolution and precipitation. The solutions could each be run through filters with small enough pores to prevent particles from permeating, so they are collected on the filter surface. These filters can then be imaged by SEM in order to compare size distributions before and after the elapsed time, to test for any changes in NP size. Analysis by EDS on particles could test for potential precipitates such as hydrozincite. Experiments could be run in a wider variety of cell and animal culture media to determine the concentration of ZnO NPs which will dissolve almost immediately to Zn\(^{2+}\), in order to inform future toxicity studies on the extent of ZnO dissolution and potential re-precipitation of Zn in other mineral forms.

5.3 Use of spICPMS for analysis of polydisperse NPs

This work addressed the growing need for development of robust analysis methods for NPs in environmental samples. Concentrations in the environment have been predicted to be in the low part-per-trillion range (5, 73), which are below the detection limit of widely used existing analysis techniques such as FFF-ICP-MS, electron microscopy, or DLS. Qualitative detection and characterization of NPs by electron microscopy may be possible in this concentration range, but is tedious and non-quantitative. In addition to elemental specificity and ability to perform analyses in ppt concentration ranges, another strength of spICPMS analysis is its short run time per sample. In the examples presented here, this method has shown sizing capability comparable to SdFFF with UV-Vis detection. For the method parameters used in this study a single sample analyzed by spICPMS takes approximately 5 minutes; an SdFFF run is
typically closer to one hour in length. The high-throughput capability of this technique makes it quite powerful for applications such as detection and sizing of NPs in toxicity tests or environmental analysis.

The purpose of this study was to determine the applicability of spICPMS analysis to a variety of polydisperse NPs, as these systems are expected to be more representative of NPs in the environment. The types of NPs chosen have not previously been analyzed by this method to the best of our knowledge, and are among the more widely used, making them important for potential release to the environment and subsequent ecological impact. Dissolution of ZnO in DI water at ppt concentrations prevented analysis of these NPs by spICPMS. However, in Chapter 2 we reported that ZnO NPs exhibit reduced dissolution in moderately hard water, leading to concern that these particles may still persist in the environment, in which case detection by spICPMS may be possible. Titanium (49Ti), cerium (140Ce), and silver (107Ag) have low instrumental background levels when analyzed by spICPMS, making detection of NP pulses highly feasible. Sizing of these metal-containing NPs by spICPMS through determination of instrument transport efficiency and use of a dissolved calibration curve was found to be accurate when compared with results from other commonly used techniques. This illustrates the potential for spICPMS to be used as a tool for both detection and characterization of polydisperse NPs in environmental samples. In addition to polydispersity, environmental NP samples are expected to be more chemically diverse (multiple types of particles as well as particles containing multiple metals) than the single-NP samples analyzed in method development studies. Due to the short duration of the NP pulse, it is not currently possible to analyze a single particle for multiple elements. Our lab group at CSM is working with Perkin Elmer, the manufacturer of our ICPMS, in developing microsecond dwell time spICPMS to address this current shortcoming. In the case
of samples containing two or more types of NPs (e.g. CeO$_2$ and TiO$_2$), we recommend separate analyses for each element to avoid switching ICP-MS analyte mass between dwell times.

Our findings suggest that the best approach in deciding on an appropriate concentration range for analysis of polydisperse NPs by spICPMS may be to find a mass concentration that is near but below a certain particle coincidence level. This will allow for enough pulses to define a size distribution but avoid coincidence that will skew the distribution toward larger sizes. In this study, definite coincidence for a monodisperse particle was observed above an NP pulse/reading ratio of about 5%. We recommend this as a conservative guideline for spICPMS analysis, but a consequence of analyzing polydisperse samples is that the presence of a small amount of coincidence may not significantly degrade the size distribution. Further development of spICPMS is needed before it is to become the standard for environmental NP analysis, but it has been demonstrated here to be quite promising. The implications of this work are more specific to nanometrology, and move forward the state of science with regard to both detection and characterization of NPs in the environment.

Future work should include simulated natural waters to investigate how the presence of dissolved salts, often in the mg/L range, as well as natural organic matter, affects the ability of this technique to detect and size NPs at environmentally relevant concentrations. More soluble NPs such as ZnO or Ag may be detectable by spICPMS if they are in fact stable with respect to dissolution in matrices such as moderately hard water, as suggested for ZnO in Chapter 2. Although Chapter 3 showed ZnO NPs dissolving to Zn$^{2+}$ in nanopure water, resulting in elevated background Zn signal and no pulses by spICPMS analysis, these experiments could be repeated with ZnO in moderately hard water to investigate the ability of this technique to detect soluble NPs in an environmentally relevant matrix. A new variation on spICPMS analysis is the use of
very short dwell times, as low 0.1 ms, compared to 10 ms used for all results in this thesis. These short dwell times reduce the background from both instrumental and dissolved analyte sources, and decrease the probability of particle coincidence. Similar experiments on the same particles used in this study could be used to test the ability of this “fast scan” spICPMS to size polydisperse NPs, with comparison to the size distributions generated in Chapter 3. This may also allow detection of multiple elements in the same particle, which could enable differentiation of some engineered NPs from natural colloids.

5.4 Detection of CNTs using spICPMS

Detection of CNTs using residual catalyst metals was found to be possible using spICPMS. The most effective metal for analysis depends on its chemical identity, abundance in the CNT as well as its natural isotopic abundance, and needs to be determined for each CNT. Although spICPMS was unable to quantify CNTs at concentrations used for NTA, it has the ability to detect the presence of CNTs at ng/L levels as well as to monitor increasing CNT concentration by detecting increasing pulse number. No other technique currently has this capability at such low concentrations. In simple solutions and with appropriate cut-off criteria the number of CNT detection events was found to be directly proportional to the number of CNT particles in the ng/L concentration range. In a more environmentally relevant application, spICPMS was shown to be able to detect and qualitatively determine relative changes in the concentration of CNTs released from a nanocomposite material. We believe that the current shortcoming of this technique regarding quantification is rooted in the difficulties in establishing threshold values that can be applied in different situations and the inability of spICPMS to detect most of the comparatively small nanoparticles incorporated within individual CNTs. The ability
of spICPMS to differentiate a CNT pulse from background, however, could be improved with a greater efficiency of ion transport in the instrument quadrupole and a more sensitive mass detector. Even with the current situation which can best be described as a semi-quantitative ability to detect CNTs by metal proxy, there are potential applications. As environmental CNT concentrations have been predicted to be in the ng/L range, tracking of CNTs by this method would likely be possible in environmental and biological systems where the surrounding matrix would not be expected to contain pulses of metals such as Co, Y, Mo, or Ni. With our current ability to detect CNTs by spICPMS, we have demonstrated excellent concentration detection limit and ability to monitor relative increases in concentration, but are limited in accurate quantification of CNTs. As CNTs are one of the NPs cited most often as having potential human health impacts due to their morphological similarity to asbestos, proactively developing methods for their detection is paramount. Further development of this method for CNT analysis will focus on improving the accuracy of quantification. One possible solution to the background issue would be to determine a background cut-off criterion for each individual sample by comparing the ICP-MS response function before and after the sample was ultra-centrifuged to remove all of the CNT particles. By this approach sample-to-sample variations in the background signal could potentially be accounted for by subtracting the centrifuged sample’s background signal. Coupled with a mass balance for the analyte metal, this approach could help quantify both dissolved and CNT concentrations, especially CNTs generating a signal very close to background.

Although in this study it was assumed that there is no correlation between the size of a CNT and the amount of metal catalyst present in a CNT, a possible method for investigating this could be sedimentation FFF (SdFFF) with subsequent spICPMS analysis. The CNTs would be separated by size in the SdFFF step, and sample fractions would be collected for analysis by
spICPMS. If the different sizes of CNTs in SdFFF fractions generate spICPMS pulses that scale in intensity with CNT size, this would be evidence of a potential relationship between metal content and CNT size. This could allow sizing of CNTs based on spICPMS analysis of embedded metals. Further investigation into the ability of spICPMS to quantify CNTs could involve use of near-IR fluorescence. This method has been previously shown to obtain CNT recoveries up to 100% in water and estuarine sediments and could be used to validate further attempts at quantification by spICPMS. Experiments with human health implications could involve attempts to detect and quantify CNTs in simulated lung fluid by spICPMS and near-IR fluorescence, as CNTs have been compared to asbestos due to their high aspect ratio.

5.5 Use of nanometrology in assessment of risk arising from NPs in the environment

In risk assessment, Risk = Hazard x Exposure. Much work has already been performed in assessing the potential hazard of NPs, based on a wide range of toxicity testing studies. It is very often found that NPs are more toxic than larger particles of the same chemical composition. However the extent of NP exposure expected in the environment is still highly unknown. Modeling studies have predicted concentrations in various environmental compartments (e.g. surface water, sediment) but little information exists on the life cycle of these materials. The results presented in this thesis have focused on development and use of spICPMS, and it is expected that this technique will likely play a large role in studies designed to quantify NPs along various stages in their life cycle. Other methods commonly used for detection, characterization, and quantification, such FFF, DLS, and TEM/SEM, will also be crucial in this analysis. Further developing and applying advanced analytical techniques will allow accurate assessment of the potential exposure of these materials to both humans and the environment, thereby providing a measure of the risk associated with their use.
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