Analysis of single-walled carbon nanotubes using spICP-MS with microsecond dwell time

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Abstract

Although catalytic metal nanoparticle (NP) impurities in carbon nanotube (CNT) structures are undesirable in a manufacturing context, they can be used as proxies to detect CNTs by single particle inductively coupled plasma – mass spectrometry (spICP-MS). The use of 100 microsecond dwell times, a recent advancement for spICP-MS, increases resolution between pulse events and reduces background signal, improving the detection of low metal-content CNTs. Serial filtration supports the assertion that metal NPs, containing yttrium in this study, are primarily incorporated in CNT structures and remain bound to the tubes throughout analysis. Analysis of both acid-digested and suspended CNTs confirmed that CNTs are fully ablated in the plasma and that all residual metals are ionized. This result, combined with good agreement with UV-Vis spectroscopy analysis, indicates that spICP-MS can provide quantitative CNT mass analysis. Though Nanoparticle Tracking Analysis (NTA) was less accurate than spICP-MS when counting Au-PVP NPs, it gave higher particle number concentrations than spICP-MS for CNTs. The likely undercounting of CNTs by sp-ICPMS is ascribed to the heterogeneous distribution of yttrium NPs among CNTs, and the small diameter of many yttrium NPs (~15 nm), nearing their detection limit. Despite this limitation, its sensitivity and elemental specificity make spICP-MS a powerful nanometrology method for environmental exposure assessment of CNTs. To illustrate this capability, CNT association with *Daphnia magna* was measured at µg/L CNT concentrations. After 48 hours of exposure to a CNT suspension, $3.48 \pm 0.32\%$ of CNTs were taken up by *D. magna* adults. This simple and direct application of spICP-MS to detect CNTs in organisms can be applied to toxicological investigations to help understand potential bioaccumulation of CNTs in aquatic ecosystems.

Keywords:

Nanometrology, yttrium nanoparticles, particle number concentration, *Daphnia magna*, biological exposure

1. Introduction

Nanotechnology has grown rapidly in recent decades, driven by the novel properties of nanoparticles (NPs)¹⁻³. Carbon nanotubes (CNTs) have unique mechanical, electrical, and thermal properties which are beneficial to diverse applications such as energy storage, electronic components, and new material development^{4, 5}. With their increasing and potential widespread use, CNT production exceeded several thousand tons in 2013⁶. In addition to beneficial applications, the potential for environmental and human health impacts of NPs have garnered attention⁷⁻⁹. The large amount of CNTs produced is likely to result in release to the environment during manufacturing, transportation, and use phases across their life cycle^{1, 9-11}. There are concerns over the safety of CNTs due to their very small size and high aspect ratio, as well as their graphitic properties¹². One of the major challenges in determining the health and safety effects posed by CNTs is detecting and quantifying the extremely low concentrations of CNTs that could be present in complex, heterogeneous environmental matrices¹³. For better understanding of potential CNT risk, sensitive and robust nanometrologies are therefore needed to evaluate exposure.

Most predictions of aqueous environmental concentrations of CNTs are in the ng/L range^{10, 11, 14}. However, the most commonly used characterization methods for CNTs, such as X-ray photoelectron spectroscopy, scanning electron microscopy, X-ray diffraction, transmission electron microscopy, UV-Vis and Raman spectroscopy, cannot efficiently detect or quantify CNTs in aqueous media at these low concentrations¹⁵. In contrast, inductively coupled plasma - mass spectrometry (ICP-MS) has detection limits of ng/L or sub-ng/L for most elements. Furthermore, detection of NPs is made possible by using ICP-MS in single-particle mode (spICP-MS)¹⁶⁻¹⁹.

Conventional ICP-MS analysis utilizes signal integration over 10s to 100s of milliseconds (ms) to average short-term instrumental noise or drift. In contrast, spICP-MS uses individual readings (counts/dwell time) taken at dwell times ≤ 10 ms to generate time-resolved data. This short dwell time allows measurement of dissolved metals, present as background signal, and simultaneous detection of NPs as "pulses". If the sample transport efficiency is determined²⁰, the intensities of pulse signals can be converted to analyte mass using a calibration curve constructed from dissolved ions of known concentrations. For chemically uniform (metal and metal oxide NPs) spherical NPs the measured masses can be used to obtain a particle size distribution¹⁷. The number of pulses can also be converted to particle number concentration²⁰. Direct measurement of CNTs by carbon analysis is not feasible using ICP-MS due to poor ionization and high background, particularly in realistic environmental settings due to the ubiquitous presence of carbon-containing compounds. However, residual metal NPs (e.g. Ni, Co, Y) are commonly embedded in CNT structures as an impurity from the catalytic synthesis, and these metals can be analyzed by spICP-MS¹⁸. Purification steps such as acid treatment are frequently applied to CNTs after synthesis to remove metal impurities^{15, 21-23}, but some metal NPs typically remain in the carbon structure. While these metal NPs allow for CNT detection, their quantity is likely to vary between individual CNTs, so size distributions of CNTs likely cannot be determined. Mass concentrations of CNTs can be measured by ICP-MS in samples if the metal content of the CNT is known^{24, 25}.

In a previous study, spICP-MS with 10 ms dwell times was used to detect single-walled CNTs (SWCNTs)

using ^{89}Y as the analyte isotope¹⁸. In this previous study a relationship between CNT mass concentration and particle number was established. Determining particle number was based on establishing an intensity cutoff, using the mean and standard deviation, above which particles are quantitatively counted. The effect of varying the cutoff on reported particle number versus CNT mass concentration was examined.

The very small size (~15 nm) of yttrium NPs in CNTs results in pulses of low intensity that must be detected above the background signal. Several spICP-MS studies have suggested that a single particle generates an ion cloud with duration in the ICP-MS quadrupole on the order of 300-500 microseconds (μs)²⁶⁻²⁹. Thus the background intensity contributes to the overall signal for nearly 95% of a 10 ms dwell time. Furthermore, the short duration of the pulse suggest that multiple particles could be detected (coincidence) during individual dwell times of about a microsecond or more. Coincidence is undesirable because it leads to undercounting of particle numbers and overestimation of particle sizes. Recent ICP-MS hardware and software advancements provide the means to shorten dwell times to the μs scale, which can achieve better characterization of NPs by reducing the effects of elevated background and coincidence³⁰.

Achieving improved analysis of CNTs, both in mass and particle number quantification, was the major goal of this study. We report the first use of 100 μs dwell times to collect spICP-MS data for CNTs. This study also goes beyond the previous work on spICP-MS analysis of CNTs by more fully confirming the association of metal NPs with CNTs and assessing the quantitative analysis of CNT-associated Y NPs. Finally, we demonstrate the ability of spICP-MS to measure biological exposure to CNTs by introducing *Daphnia magna* to a dilute aqueous CNT suspension, ultrasonically digesting the tissue, and measuring biological CNT association. The μs -spICP-MS method developed in this study provides a powerful new means of looking at part of the life cycle of CNTs and addresses some of the limitations of previous analytical approaches (i.e. use of radio-labeled CNTs and high CNT exposure concentrations).

2. Materials and methods

2.1 Reagents

The CNTs were obtained from Carbon Solutions (Riverside, CA) as a dry powder. Stock CNT solutions were prepared by suspending a known mass of CNTs in a known volume of ultrapure water (HPLC grade) containing 1 wt% sodium deoxycholate (Sigma-Aldrich, Saint Louis, MO). A bath Branson 1510 ultrasonicator (Danbury, CT) was used to suspend CNTs overnight. Stock solutions were diluted using 18.2 M Ω cm⁻¹ Nanopure water (Barnstead Nanopure Diamond, Lake Balboa, CA), on the order of 100 to 1000 times, to concentrations appropriate for spICP-MS. Diluted solutions were placed in 15 mL polypropylene centrifuge tubes and sonicated (Fisher FS60H bath sonicator, Pittsburgh, PA) for 15-20 minutes after each dilution. Dissolved Au calibration solutions (0, 2, 5, 10 $\mu\text{g/L}$) were prepared using Claritas PPT (SPEX Certiprep, Metuchen, NJ) ICP-MS standards and 2% hydrochloric acid (Optima, Fisher Chemical, Fair Lawn, NJ). Similarly, dissolved Y calibration solutions (0, 1, 2, 5 $\mu\text{g/L}$) were prepared using Claritas PPT ICP-MS Y standards and 2% nitric acid (Optima, Fisher Chemical, Fair Lawn, NJ). A gold NP standard (55 nm, National Institute of Standards and Technology (NIST) RM 8013, Gaithersburg, MD) was diluted to 1

µg/L from the stock 50 mg/L ($\sim 2.98 \pm 0.04 \times 10^{13}$ particles/mL) solution and analyzed to determine transport efficiency²⁰. Nanopure water was analyzed by spICP-MS in triplicate at the beginning of each experiment as a blank. Filtration of CNT suspensions was performed using 0.02 µm (Whatman Anotop 25 syringe filters, 6809-2002, Pittsburgh, PA), 0.2 µm (Whatman Anotop 25 syringe filters, 6809-2022, Pittsburgh, PA), and 2 µm (Millex syringe filter units, SLAP02550, Billerica, MA) filters. An Au-PVP NP (40nm, nanoComposix, AUPN40-25M, San Diego, CA) was obtained as a powder, suspended in HPLC water and used to determine the accuracy of spICP-MS for particle number-concentration analysis.

Acid digestion was performed on the CNT suspension to determine total metal content. In this experiment, 5 mL of concentrated nitric acid (69.0-70.0%, Baker analyzed A.C.S. Reagent 9601, J.T. Baker, Phillipsburg, NJ) and 15 mL concentrated sulfuric acid (95.0-98.0%, Baker analyzed A.C.S. Reagent 9681, J.T. Baker, Phillipsburg, NJ) were added to ~100 mg CNTs with ~1 mg surfactant. The mixture was placed in an oil bath and refluxed at 70°C for 5 days. The temperature was raised to 90 °C and refluxing continued for an additional 30 days. The digested sample was diluted with Nanopure water and sonicated using a bath sonicator before being introduced into ICP-MS. All analyses were performed in triplicate.

2.2 Instrumentation

All spICP-MS data was collected on a NexION 300Q ICP-MS (Perkin Elmer, Waltham, MA) with an S10 autosampler. The analyte isotope was ⁸⁹Y and the dwell times used were 0.1, and 10 ms, with 60 s data acquisition time. The sample introduction flow rate was 0.3 mL/min. Data collection and spICP-MS calculations were performed using SyngistixTM Nano Application Module (more information on ICP-MS operating conditions is in SI Table 1). A Varian Cary 50 Conc UV-Visible Spectrophotometer (Palo Alto, CA) was used to obtain UV-Vis data with Cary Varian UV Scan Application 3.00. Imaging of CNTs was carried out using a Philips CN 300 Field Emission gun TEM operating at 300 kV. CNTs were dispersed in tetrahydrofuran/chloroform and followed by 4-hour bath sonication. The CNT was dip cast on a Lacey carbon grid (lacy, Ted Pella Inc., Redding, CA). Dip-coating was used for grid preparation with several seconds of association time, and then grids was allowed to air dry for approximately 10 min. Low electron voltages were used to minimize beam damage to the CNTs. Images were collected with a bottom mounted Gatan SC200 CCD camera. Elemental composition of the CNTs was measured simultaneously with the images by energy-dispersive X-ray spectroscopy (EDS). Nano Tracking Analysis (NTA) was used to measure particle number concentration for comparison with spICP-MS. A NanoSight LM 10 instrument (Ann Arbor, MI) equipped with a 405 nm laser source, a temperature-controlled chamber, and a scientific CMOS camera, was used¹⁸.

2.3 CNT association with *Daphnia magna*

The *D. magna* exposure experiment was performed following a modification of the OECD Guideline for the Testing of Chemicals, Test No. 202³¹. Reconstituted moderately hard water was prepared according to EPA methods³²⁻³⁴ (NaHCO₃, 96.0 mg/L; CaSO₄·2H₂O, 60.0 mg/L; MgSO₄, 60.0 mg/L; KCl, 4.0 mg/L). The CNT

stock suspension was diluted with moderately hard water and sonicated using Fisher FS60H bath sonicator for 20 min before *D. magna* addition. Triplicate experiments were performed in which 10 *D. magna* were added to 30 mL of a 5 µg/L CNT suspension. A set of *D. magna* in moderately hard water with no CNTs added was used as a control. After 48 hours, *D. magna* adults from each cup were collected and rinsed with Nanopure water, to remove adhered water, which contains dispersed CNTs, and loose, externally-attached CNTs, before being transferred into 10 mL Nanopure water. Sonication (Qsonica sonicator Q500, Newtown, CT) was used to homogenize *D. magna* (10 min probe sonication), and these homogenized samples were diluted 10:1 with Nanopure water and analyzed by spICP-MS.

3. Results and discussion

3.1 CNT Mass quantification

3.1.1 Association of Yttrium NPs with CNT

Figure 1 shows a TEM image of the Carbon Solutions CNTs used in this study. The dark circular objects are electron-dense materials that are metallic or metal-containing NPs contained in the CNT structure. EDS detected Ni, Y, and Fe in the CNTs (SI Figure 1). CNTs from different sources typically have different average metal content^{18, 24}. The metal isotope that produces the strongest spICP-MS signal relative to the background will be the one most useful for CNT analysis. The nickel content was higher than yttrium content (19.6% Ni vs 5% Y) for the Carbon Solutions CNTs. However, yttrium was chosen as the analyte in part because it is monoisotopic (100% as ⁸⁹Y) while nickel is distributed among five isotopes (most abundant at 68% as ⁵⁸Ni)¹⁸. Also, the pulses generated by Y were more distinct from the background than those for Ni, making it a better proxy analyte for the CNTs studied.

Use of catalytic metal NPs to quantify CNTs requires that the NPs be associated with the CNTs. Several lines of evidence support that the NPs are primarily associated with CNTs. First, no metal NPs were observed as individual particles in TEM analysis; instead, they were all encapsulated in the CNT structure (Figure 1a, SI Figure 2). This observation can be somewhat subjective as it is difficult to obtain representative images of particles on the TEM grid, especially for particles of highly different shape and size. Most NPs shown in TEM images are ~15 nm in diameter (size distribution in Figure 1b, based on 122 particles measured) with a range of 5-35 nm. In unfiltered CNT suspensions (Figure 2a), yttrium spICP-MS analysis of CNTs measured pulses up to 200 counts. According to calculations based on the dissolved Y calibration standards, our size detection limit by spICP-MS for yttrium NPs sized as 100% Y is approximately 10 nm with an ⁸⁹Y response of 1 count/dwell time, while a 15 nm yttrium NP produces a pulse of 2 counts/dwell time, and a 35 nm NP produces a pulse on the order of 25 counts/dwell time. The pulses above 25 counts/dwell time shown in the spICP-MS data are very likely to be the result of multiple NPs embedded in the CNTs, or multiple bundled CNTs. Unfiltered CNT suspensions are also observed to contain a large number of pulses in the range of 1-25 counts. This could be evidence of CNTs that contain a

single NP, but the possibility of free metallic NPs cannot be completely excluded by the unfiltered CNT data.

To further demonstrate the association of metal NPs with the CNTs, filters of varying pore sizes (2, 0.2, 0.02 μm) were used to separate a 5 mg/L CNT suspension (Figure 2) which was prepared in triplicate. The 2 μm filtrate generated a similar response as the unfiltered suspension sample (among 2827 ± 71 particles, only 87 ± 19 were removed), indicating CNTs can pass through the 2 μm filter easily. These results are consistent with the manufacturer-reported size of CNTs of 3.8 ± 1.8 nm in diameter, and 1800 ± 1000 nm in length. In the 0.2 μm -filtered sample, well over half of the pulses observed in the unfiltered sample are eliminated (2032 ± 33 pulses were removed). The 0.2 μm filter is able to remove most of the CNTs, however there are still ^{89}Y pulses detected by spICP-MS in the filtrate. In contrast, the 0.02 μm filtration showed removal of nearly all pulses (only 20 ± 7 pulses detected in the filtrates) and simultaneously lowered the background ^{89}Y signal. Statistical analysis (t-test) of spICP-MS results suggest that the background signal (responses of 0 counts & 1 count) in the 0.02 μm filtrate is not significantly different from a Nanopure water blank ($p > 0.05$). Over 99% of the total instrument responses in these two samples are a signal of 0, with limited responses of 1 count and above in the real time data. The reduction of the background signal in the 0.02 μm filtrate indicates that there is no detectable dissolved yttrium in the CNT suspension. Bennett *et al.* also showed that a very limited amount of dissolved yttrium could be leached from the CNT structure when using deionized water as the experimental matrix³⁵. Results of a control study of filtration of dissolved ^{89}Y (shown in SI Figure 3) also strongly supported the absence Y sorption loss during filtration as a mechanism for background signal reduction. Thus the low-intensity (1-5 counts/dwell time) instrument responses observed in the present study are likely due to small yttrium particles, primarily encapsulated in CNTs.

In the yttrium NP size distribution (5-35 nm with mean size of 15 nm), yttrium NPs sized < 20 nm should generate ^{89}Y responses of 1-5 counts. Thus if yttrium exists as discrete particles in suspension, the 0.02 μm filtrate should have a greater number of responses between 1 and 5 counts compared to the Nanopure water blank. The similarity between spICP-MS signals of the 0.02 μm filtrate and the Nanopure water thus provides further evidence for the association of yttrium NPs with CNTs. However, yttrium particles < 20 nm may not effectively pass through the 0.02 μm filter. Thus the lack of pulses in the 1-5 counts/dwell time range in the 0.02 μm filtrate does not eliminate the possibility that some free yttrium particles are present. Overall, the effective removal of ^{89}Y responses by filtration at various pores sizes supports the assertion that yttrium particles are primarily associated with the CNT structures.

3.1.2 Yttrium NPs in CNTs are fully ionized in inductively coupled plasma

While the sp-ICPMS filtration results described in 3.1.1 demonstrate the ability to detect small yttrium NPs in CNTs, for quantitative analysis it is important to confirm that the plasma is able to fully ablate the CNT structure and ionize all the yttrium metal. To evaluate this, acid digestion and subsequent spICP-MS analysis was performed on CNTs, with the results compared to an undigested CNT dispersion. The digested CNT sample and the CNT suspension were diluted by the same factor before ^{89}Y spICP-MS analysis. In the sp-ICPMS data (see SI Figure 4), the acid digested CNT results showed no obvious pulses, only a background (dissolved) signal, indicating the CNTs were dissolved by the concentrated acid. In

contrast, analysis of the CNT suspension showed numerous pulses, consistent with the yttrium in the suspended CNTs being mainly in particle form. Integration of the instrument signal over the full 60 seconds of data collection for both samples, with subtraction of background (acid or Nanopure water signal) and quantification via a dissolved yttrium standard calibration allowed calculation of the concentrations of yttrium in both samples. The acid digested sample had $2.18 \pm 0.02 \mu\text{g/L}$ yttrium based on a triplicate ICP-MS analysis, while the suspension had $2.15 \pm 0.02 \mu\text{g/L}$ yttrium. The near identical measured values for yttrium concentrations for both samples supports the hypothesis that the plasma effectively ablates all of the Y NPs contained in the CNTs. Based on the CNT mass suspended, these results confirmed the as-received CNTs contain ~5 wt% yttrium.

3.1.3 Comparison of spICP-MS to UV-Vis spectroscopy

To further confirm the association of yttrium NPs with CNTs, spICP-MS analysis was compared with UV-Vis analysis. Although the sensitivity of UV-Vis is significantly less than spICP-MS, it is directly sensitive to the carbon structure of the CNTs, while spICP-MS uses the metal NPs as an indirect means for CNT detection. Filtered and unfiltered CNT suspensions were analyzed by both UV-Vis and spICP-MS to compare the measured concentrations by optical density (UV-Vis) to yttrium presence (spICP-MS). Similarity in quantitative results between the two methods would further validate the hypothesis that Y NPs are associated with CNTs.

UV-Vis analysis was performed on dilutions of an as-prepared 5 mg/L CNT suspension, while spICP-MS was performed on samples further diluted for yttrium quantification. It should be noted that the dilutions needed to place samples in the dynamic range for each instrument are very different and this was accounted for when calculating both initial and measured concentrations. To quantitatively measure CNT concentration in suspension with UV-Vis, the linear dependence of optical density (absorbance) on suspension concentration is utilized to establish a concentration absorptivity constant at a given wavelength. The UV-Vis spectroscopy absorption spectrum of the CNT is shown in SI Figure 5a and spans from 190 to 800 nm with a maximum observed at 230 nm. Although this is the maximum absorbance, and therefore provides the strongest instrument response for low concentrations of CNTs, for filtration studies 700nm was used to measure SWCNT concentration because this wavelength falls well outside the UV window where the sodium deoxycholate surfactant absorbs³⁶. From the established calibration curve (SI Figure 5b), it can be seen that at 700nm the concentration/absorptivity constant is $0.028/(\text{cm} \cdot \text{mg/L})$.

The mass concentration of yttrium obtained by integration of the spICP-MS data (Figure 2) and UV-Vis results for C/C_0 , where C_0 is the unfiltered sample response, and C is the filtered sample concentration, was in good qualitative agreement (Figure 3). For the $2 \mu\text{m}$ -filtered samples spICP-MS and UV-Vis measured $94.96 \pm 4.22\%$ and $87.96 \pm 0.42\%$ of the initial CNTs passing through the filter, respectively. Although the measured amounts of CNT removal by filtration are statistically different ($p < 0.05$) for the two analytical methods, it was confirmed that the majority of these CNTs could permeate a filter with a $2 \mu\text{m}$ pore size. The $0.2 \mu\text{m}$ filtrates were found to contain ~28% of the initial CNT concentration as measured by both techniques ($28.12 \pm 1.58\%$ detected by spICP-MS, and $28.60 \pm 0.66\%$ detected by UV-Vis). There is no significant difference between these two results ($p > 0.05$). A difference between the methods in measured

CNT removal was observed for the 0.02 μm filtrate. While spICP-MS measured $1.04 \pm 0.12\%$ of CNTs passing the filter, the UV-Vis analysis suggested $5.16 \pm 0.21\%$ of CNTs are passing the filter. Given the dimensions of the CNTs it seems unlikely the higher UV response is indicating CNT passage through the 0.02 filter μm . A possible alternative explanation for the higher UV response is that a minor amount of dissolved organic matter or fine particles of amorphous carbon are released from the CNTs. While similar, but not in perfect agreement, the quantitative results for these CNT samples as directly measured by UV-Vis (sensitive to the CNTs) and indirectly by spICP-MS (sensitive to Y NPs contained in the CNTs) provide further evidence that yttrium NPs are primarily encapsulated in the CNT structures.

3.2 Estimation of particle number

3.2.1 Reduction in number of coincident pulses

One of the major measurands of spICP-MS analysis is particle number concentration, quantified via the counting of pulse events. This assumes that each pulse corresponds to a single particle and so it is critical to quantify the detection events accurately to determine particle number concentration of a NP suspension. To achieve an accurate analysis, the first step is to determine the measureable dynamic range of NP concentration, that is: 1) not too dilute, to ensure that a sufficient number of pulses can be detected in a sample run time, and 2) is not too concentrated, to avoid incurring a significant number of coincident pulses. The advantages of μs dwell times for reducing coincidence have been previously demonstrated^{30, 37, 38}. Concentrations of 1 and 5 $\mu\text{g/L}$ CNTs were tested using a 100 μs dwell time to establish the upper concentration limit (Figure 4 a & b). The integrated yttrium mass of these samples scaled with CNT mass concentration ($47.14 \pm 0.98 \text{ ng/L}$ and $240.05 \pm 3.61 \text{ ng/L}$ respectively), but significant coincidence occurred during analysis of the 5 $\mu\text{g/L}$ CNT sample (illustrated in the highlight box in Figure 4a). An approximate upper limit of 1 $\mu\text{g/L}$ CNTs was established for a 100 μs dwell time, approximately 10-fold greater than for 10 ms³⁰.

3.2.2 Evaluation of particle detection cut-off criteria

In a previous study using 10 ms dwell times for analysis, two different cut-offs were investigated ($\bar{x}+3s$ and $\bar{x}+5s$). Repeating the use of a 10 ms dwell time gave a 1 count/dwell time average background, which resulted in cutoffs of 4.81 and 7.15 counts/dwell time (SI Figure 6). This high background results in difficult differentiation of particle-created signals from background noise, resulting in undercounting of detection events¹⁸. Only recently have dwell times shorter than 3-10 millisecond been used^{30, 37-39}, in part to reduce this background. Using a dwell time of 100 μs , the average background instrument response decreased to 0.002 counts (0.2 % non-zero readings) for MilliQ water (Figure 4c). Reducing the dwell time makes differentiating very small particle pulses from the background possible.

The method we applied in this study to determine various cut-off values, using 100 μs dwell time data

collection, is based on the Nanopure blank reference data^{18, 40}. In the time-resolved data for the Nanopure water blank (Figure 4 c), of the 600,000 readings collected in 60 sec, only about 1200 readings on average gave non-zero counts. This gives an average of 0.002 counts/dwell time, and with addition of 5s of the background signal, yields a possible cut off of 0.0812 counts/dwell time. Our procedure requires that two consecutive readings have responses greater than the cutoff in order to qualify as a particle detection event. This criterion is based on the fact that a pulse event should occur over several dwell times, given the typical pulse duration of a few hundred μ s. This approach eliminates short-term instrument noise. (see SI Figure 7 for a more detailed procedure description). This simple approach proved effective although more rigorous methods of sample deconvolution from the background have been proposed³⁸.

A small difference in cut-off can lead to different measured particle number for samples that contain significant numbers of particles near the mass detection limit. For example, in the highlighted area (Figure 4 a) of the 5 μ g/L CNTs time-resolved data, there is a “peak” comprised of 11 non-zero readings. If the cut-off is set to 0 counts/dwell time, two pulses will be reported, as the two larger pulses on the left will be considered as only one effective pulse because the data does not drop to the zero count/dwell time cut-off. When the cut-off is set to 1 count/dwell time, three pulses will be counted. Also the nearly last reading shown in Figure 4 a, having 2 counts/dwell time, will not be counted as only one reading is above the cutoff. If a cut-off of 4 or 5 counts/dwell is used only one pulse will be registered over the entire 5 ms shown.

To examine how particle detection cut-off affects quantification of CNTs across a range of particle concentrations, a series of samples with varying CNT mass concentration were analyzed using 2 different cut-off criteria. In Figure 5, the number of pulses (detection events, left Y-axis) was proportional to CNT mass concentration (X-axis) in the range of 0-1000 ng/L with a cut-off of 1 count. When the concentration is higher than 1000 ng/L, the non-linearity may be largely due to coincidence. But when using cut-off of 0 counts/dwell time, this linear relationship only occurs for samples with mass concentration of 200 ng/L or lower. It was discussed above that using 0 counts/dwell time as cut-off may result in some small “shoulder” peaks counted as the continuous tail of large pulses, which leads to the undercounting of pulse events. The number of detection events was divided by the measured transport efficiency, flow rate, and acquisition time to obtain the measured particle number concentration (Figure 5, right Y-axis). As the cut-off value is increased, the number of particle detection events decrease. With consideration of quantification accuracy, as well as coincidence, the ideal dynamic range of CNT mass concentration analysis was chosen to be 10 -1000 ng/L with a cut-off of 1 count for this study. The mass detection limit of yttrium was found to be 0.5 ng/L in Nanopure water, thus the detection limit for Carbon Solutions CNT (5 wt% Y) is about 10 ng/L, measured as 4.0×10^3 particles/mL. Samples with concentrations lower than this range may require multiple runs to collect enough particle detection events to be statistically different than a Nanopure water blank sample. Samples with higher concentrations will require dilution to avoid coincidence effects. Establishing the cut-off criterion and dynamic CNT concentration range for the CNTs used allowed further studies; 1) comparison of CNT quantification with Nano Tracking Analysis, and 2) quantification of CNT associated with *Daphnia magna*.

3.2.3 Quantification of CNT number concentration by spICP-MS and NTA

Both spICP-MS and Nano Tracking Analysis (NTA), a laser-based particle counting method, were performed on multiple NP suspensions to compare particle number quantification. Numerous prior studies have investigated the use of NTA to examine Au NP dispersions⁴¹. Since in this study the CNTs were dispersed from a powder we chose to use a powder form of Au NPs. A known mass of Au NP (polyvinylpyrrolidone-coated) powder was suspended in HPLC-grade water and analyzed by both methods. The Au NP had a 38 nm gold core, which comprises 25% of the total mass (as stated by the manufacturer), the other 75% being the polyvinylpyrrolidone coating. The NTA measured $4.57 \pm 0.07 * 10^9$ particles/mL, while spICP-MS measured $10.67 \pm 0.58 * 10^9$ particles/mL. The mass concentration of Au-PVP NPs, computed from the NTA analysis, and accounting for the particle composition, was 10.43 ± 0.16 mg/L. The calculation based on the spICP-MS analysis gave a result of 24.35 ± 1.32 mg/L, much closer to the intended 25.13 mg/L Au-PVP mass concentration obtained by weighing out the sample. Both methods showed their comparability for counting particle numbers within less than an order of magnitude. For analysis of this particular NP, spICP-MS was found to count more particles than NTA (by a factor of 2) but appeared more accurate based on the computed mass concentration.

The CNT stock solution used in the method comparison had a mass concentration of 13 mg/L. In order to be in the dynamic range of each method the stock solution was diluted to 1 µg/L for spICP-MS measurement, while NTA samples were diluted by a factor of 10 from the stock solution. Accounting for the dilution used, the stock CNT solution was determined to contain $4.88 \pm 0.041 * 10^9$ particles/mL by spICP-MS. Measurements by NTA counted $80.8 \pm 0.14 * 10^9$ particles/mL, approximately 16 times more than measured by spICP-MS. Given that for the Au NP the two methods were in agreement within a factor of 2, this greater difference suggests undercounting of the CNTs. Variations in metal distribution among the CNTs could contribute to the difference in measured particle number by the two methods. Analysis by TEM (SI Figure 6) demonstrated the non-uniform metal distribution in the CNTs. It is very likely that there are CNTs containing little to no metal NPs, rendering them non-detectable by spICP-MS. The limited Y content of the metal NPs contained in the CNTs is another potential explanation, since these NPs are hard to distinguish from the background signal. The smallest Y NP that can currently be detected by spICP-MS with the ICP-MS used in this study is about 15 nm. As shown in Figure 1, a significant fraction of particles was smaller than this size limit.

3.3 Application: spICP-MS measurement of CNT association in *Daphnia magna*

The potential release of CNTs into surface waters is increasingly likely to occur as CNTs are used in more consumer products^{11, 42, 43}. Accumulation within increasing trophic levels has been observed for NPs other than CNTs⁴⁴. Thus, although CNT concentrations expected in surface water are generally quite low (ca. ng/L levels), bioaccumulation of CNTs may result in elevated quantities of CNTs in some organisms. The first step for bioaccumulation of CNT is their association with small aquatic organisms such as filter feeders

which serve as prey to larger species. One such organism, *Daphnia magna*, has a ubiquitous presence in freshwater systems around the world. It is a commonly used laboratory animal for ecotoxicology studies due to its ecological relevance and ease of culture. Acute toxicity tests in which *D. magna* were exposed to CNTs have been performed previously; however, these studies have required either CNT modification (surface treatment, radioactive labeling, etc.)⁴⁵⁻⁴⁷, or use of unrealistically high CNT concentrations to facilitate their detection *in vivo*. One of the goals of the present study is to demonstrate the ability of splCP-MS to detect µg/L concentrations of unmodified CNTs after biological association. In this study we have not made an attempt to differentiate CNTs ingested by the *D. magna* from those associated with their carapace. From an ecological perspective, predators are exposed to both forms of CNTs and thus differentiation between CNTs in the gut and from the organism surface is unimportant. However, previous studies have shown that CNTs associated with *D. magna* were ingested and resided in the organism's gut in greater amounts than attached externally to the carapace^{45, 46}.

In order to demonstrate the ability of splCP-MS to detect CNTs in organisms, *D. magna* were exposed in triplicate to a 5 µg/L CNT suspension in EPA moderately hard water (MHW). After CNT exposure, the organisms were homogenized by sonication for splCP-MS analysis. A 10-fold dilution factor was used to create CNT suspensions that were within the dynamic range of splCP-MS analysis. After 48 hours of exposure to moderately hard water (control, no CNTs added), *D. magna* adults show very limited yttrium content with an average ⁸⁹Y response of 0.005 counts/dwell time (Figure 6 a). However, as seen in Figure 6b, yttrium pulses clearly demonstrate that CNT association with *D. magna* occurs with 412 ± 19 pulses observed. Given the comparatively lower number of pulses detected for the non-exposed *D. magna* (7 ± 2 pulses) we can extrapolate a detection limit of about 1 µg/L for the experimental conditions used. The integrated ⁸⁹Y signal from the *D. magna*, when compared to the CNTs remaining in the MHW after 48-hours, gave a calculated value of 3.48 ± 0.32 % of the total initial CNT mass added to the test matrix. This study demonstrates that it is possible to detect CNTs taken up by organisms using splCP-MS at µg/L solution concentrations and even when a relatively small fraction of the CNTs become associated with the organism. In addition, modification of the procedures, including use of more *D. magna* (greater than the 10 adults that were used in this study), reducing or eliminating the dilution of the homogenate, or use of longer splCP-MS data collection could make it possible to detect CNTs in *D. magna* exposed to sub-µg/L concentrations. A manuscript detailing further experiments to examine dose-response relationships using splCP-MS is in preparation.

Conclusion

Use of residual catalyst metal as a proxy for CNTs for analysis by splCP-MS is effective for the detection and mass quantification of CNTs. Specifically, the use of ⁸⁹Y NPs for Carbon Solutions CNT is effective due to its mass loading in the CNT (> 5%), its low background, and its 100% isotopic abundance. Using splCP-MS it is possible to measure these CNTs in the concentration range predicted in surface waters (ca. ng/L level). Quantification of total metal in both acid - digested CNTs and a CNT suspension provided evidence for the full ablation and ionization in the ICP, a necessary requirement for quantitative analysis of CNT mass concentration. Results of filtration using various pore sizes suggest that no measurable

dissolved yttrium was leached from the CNTs and yttrium-containing particles were associated with the larger CNT structures. Results suggest that the integration of the total metal signal provides a quantitative measurement of CNT mass concentration.

Yttrium particle numbers detected by spICP-MS scaled with CNT mass concentration, although not all CNTs contain detectable levels of Y NPs, resulting in undercounting. Shortening the instrument dwell time from 10 ms to 100 μ s improves the resolution between NP-generated pulses and enables improved counting of particles. Further development of the technique is needed to achieve accurate quantification of CNT number concentrations.

Since CNTs are increasingly used as an additive in polymers, release from polymer nanocomposites is a major potential source of CNTs in aqueous systems⁴⁸⁻⁵¹. The investigation of biological uptake of CNTs released from nanocomposites should be a main focus of future work. The ability of spICP-MS to quantify both CNT mass, and if possible particle number, in biological tissues will be an important advance over traditional ICP-MS analysis. It is still unclear if particle mass or particle number (or some other metric such as surface area) is the appropriate dose metric for the ecotoxicology of nanomaterials. Thus the additional information gained from spICP-MS may help inform us of the relative importance of these characteristics. Finally, yttrium is a very rare element in environmental systems; so direct analysis of Y-containing CNTs in surface water may be possible using spICP-MS.

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Figures captions

Figure 1. a) Transmission electron microscopy (TEM) image of Carbon Solutions SWCNTs. Grey tubes represent graphitic carbon, and dark objects are metal nanoparticles. The association between metal NPs and CNT can be observed as grey graphitic carbon capsulate dark NPs. b) size distribution of metal nanoparticles in the CNT structure. Total of 122 particles were sized, with 98% of them less than 35 nm in equivalent spherical diameter.

Figure 2. Time-resolved ^{89}Y data for filtration results of 5 mg/L CNTs using 2, 0.2, and 0.02 μm pore size filters. Solutions were diluted to 1 $\mu\text{g/L}$ for spICP-MS analysis.

Figure 3. Ratio of the pre- and post- filtration concentrations of 5 mg/L CNTs as measured by both UV-Vis ($\lambda = 700 \text{ nm}$) and spICP-MS (mass concentration) and normalized to the unfiltered sample. 3 filters with different pore size (2, 0.2, and 0.02 μm) were used.

Figure 4. Time-resolved spICP-MS data for CNTs with mass concentrations of a) 5 $\mu\text{g/L}$, b) 1 $\mu\text{g/L}$ and c) 0 $\mu\text{g/L}$. Low background produced by microsecond dwell time allows for a particle detection cut-off of 1 count (red horizontal line) without introduction of false detections (Figure 4 c). Coincidence (illustrated by the boxed area in Figure 4 a) is observed at 5 $\mu\text{g/L}$ concentration

Figure 5. Particle detection events (left y-axis) and measured CNT particle number concentration (particles/mL, right y-axis) versus CNT mass concentrations (0, 0.1, 0.2, 0.5, 1.0, and 2.0 $\mu\text{g/L}$), with cut-offs of 0 and 1 counts/dwell time. Particle number concentration are calculated based on pulses counted, transport efficiency and sample flow rate.

Figure 6. Time-resolved spICP-MS data of homogenized *Daphnia magna* suspension after 48 hr exposure to either (a) moderately hard water (MHW) or (b) 5 $\mu\text{g/L}$ CNT suspension in MHW. Both were diluted by 10x prior to spICP-MS analysis.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6